# UNIVERSITY OF SOUTHAMPTON

FACULTY OF HEALTH SCIENCES

**Specimen collection technique and standards for diagnosing urinary tract infections**

by

**Linda Collins**

Thesis for the degree of Doctor of Philosophy

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## ABSTRACT

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**Specimen collection technique and standards for diagnosing urinary tract infections**

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A urinary tract infection (UTI) is one of the leading reasons for treatment in primary healthcare. It is estimated that 50% of the female population in the UK will have least one occurrence of the infection in their lifetime. It is a debilitating condition and causes a variety of lower urinary tract symptoms (LUTS). The recommended practice for detecting a UTI is by analysing a urine specimen and culturing the sample for bacterial growth and antibiotic sensitivities. There are two main specimen collection methods: the midstream urine (MSU) and the catheter specimen of urine (CSU). The CSU is recognised as the gold standard, but requires an invasive procedure. The MSU which is the non-invasive clinical standard is regarded as insufficient because the method is frequently reported as contaminated with skin and vaginal flora, but the definitions of contamination in the literature varies. Drawing on the published body of knowledge, this study aimed to investigate and determine what constitutes contamination using microbiological culturing and the uroplakin-3 cell staining technique that detects the presence of cells that originate from the bladder.

A two phase, single blind, cross over design study was conducted, comparing four different urine specimen collection methods. Experiment one tested the hypothesis that a MSU has equal merits to a CSU when capturing urothelial cells that are indicative of a UTI. A total of 60 patients and 30 controls were recruited into the study. The MSU specimens were compared with the CSU specimens to determine urinary cell origin using uroplakin-3 staining. The findings proved that the cells found in the MSU were not contaminants as commonly assumed, but were inflammatory markers of infection invading the lower urinary tract. Experiment two tested the hypothesis that if a MSU has equal merits to a CSU, then a directly voided urine specimen (natural urination) will be the optimal method when capturing the majority of urothelial cells that have been exfoliated from the bladder. A total of 31 patients were recruited and the MSU specimens were compared with the directly voided urine to determine the proportion of cells that originate from the bladder. The findings demonstrated that the directly voided urine was the optimal method and had the ability to capture predominant urothelial cells.

A qualitative study of patient views and experiences of urine specimen collection was conducted. Thirty patients were interviewed and the data were analysed for recurrent themes. The study had shown an ideal urine specimen is that which is sensitive to the underlying pathology of a UTI. It is also a urine specimen that is easy to collect. The direct void is the recommended method of choice but should be accompanied with microscopy. Uroplakin staining should be initiated to further detect the positive presence of uroepithelial cells when distinguishing the difference between urinary contamination.

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# DECLARATION OF AUTHORSHIP

I, Linda Collins declare that this thesis and the work presented in it are my own and have been generated by me as the result of my own original research.

Specimen collection technique and standards for diagnosing urinary tract infections

I confirm that:

1. This work was done wholly or mainly while in candidature for a research degree at this University;
2. Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
3. Where I have consulted the published work of others, this is always clearly attributed;
4. Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
5. I have acknowledged all main sources of help;
6. Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
7. None of this work has been published before submission.

Signed: 

Date: Thursday 15th December 2016

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# Definitions of abbreviations

| | |
|---|---|
| µl | Microlitre |
| ATP | Adenosine triphosphate |
| CCU | Clean-Catch urine |
| CFU ml | Colony forming units per millilitre |
| CI | Confidence Intervals |
| Cm | Centimetres |
| CRF | Case Report Form |
| CSU | Catheter specimen of urine |
| DAPI | Florescent stain that binds to the nucleus and bacteria on a urothelial cell |
| Direct Void | Urinating directly into a specimen container with no technique |
| *E.coli* | Escherichia coli |
| Epc | Epithelial cells |
| FLUTS | Female lower urinary tract symptoms |
| GCP | Good Clinical Practice |
| IC | Interstitial Cystitis |
| ICA | Interstitial Cystitis Association |
| ICIQ | International Consultation on Incontinence Questionnaire |
| ICS | International Continence Society |
| LUTS | Lower urinary tract symptoms |
| Mls | Millilitres |
| MSU | Midstream urine sample |
| N | Number (typically refers to patients) |
| NICE | National Institute for Clinical Excellence |
| OAB | Overactive bladder |
| PBS | Painful Bladder Syndrome |
| PEEZY MSU® | Midstream urine collection device |
| PhD | Doctor of Philosophy |
| PICO | P= patient/ problem/population, I= intervention, C= comparison & O= outcome |
| QOL | Quality of life |
| R&D | Research and Development |
| RBC | Red Blood Cells |
| SD | Standard deviation |
| SOP | Standard Operating Procedure |
| SPSS | Statistical package for the social sciences |
| SPA | Supra Pubic Aspiration |
| SV | Simple voided urine in a pot with no technique |
| UPEC | Uropathogenic Escherichia coli |
| UP-3 | Uroplakin-3 |
| UTI | Urinary tract infection |
| WBC µl | White Blood cells per microliter |

# Chapter 1

# Urinary Tract Infection

## 1.1 Introduction and background

In primary and secondary healthcare, doctors and nurses are often presented with patients that have a urinary tract infection (UTI) (Chin et al., 2016, Subramaniam et al., 2016). A UTI is defined as a bacterial invasion of the urinary tract with pathogens commonly identified as *Escherichia coli* (*E.coli*), *Klebsiella pneumoniae, Proteus mirabilis, Enterococcus faecalis and Staphylococcus saprophyticus* (Flores-Mireles et al., 2015). Public Health England (2016) have reported that a UTI is not just defined as the presence of pathogens, but is also stipulated by the ways in which these pathogens invade bladder tissue. It is a common infection which manifests itself with various lower urinary tract symptoms (LUTS) including falls and fractures in the older population (Soliman et al., 2016). UTIs are detected by analysing a specimen of urine, but diagnosis of the infection is frequently missed when urine specimens are reported as negative or get dismissed for being contaminated (Frazee et al., 2015). This increases the number of chronic and complex UTI cases which become difficult to treat and patients bear the brunt of the debilitating condition. There are many claims that non-invasive urine specimens harbour contaminants in comparison to invasive methods (Wolfe et al., 2012, Collier et al., 2013, Hooton et al., 2013, Frazee et al., 2015). Thus, non-invasive urine specimen collection methods get discredited based on these assumptions and are not recognised for their diagnostic attributes. From clinical experience, patients frequently report that their quality of life has been blighted with a chronic UTI, and for many years have gone untreated due to their urine test results being reported as negative or contaminated. Patients often concluded that their urine specimens are contaminated, based upon what they have been informed by clinicians. Empirical research was required in order to address these matters and refute these assertions with evidence. This thesis includes a two-part experimental study that compares non-invasive urine specimen collection methods to an invasive method in order to determine the optimal method of collection. Experiment one tested the hypothesis that a non-invasive urine specimen has equal

merits to an invasive method when detecting the positive presence of a UTI. Subsequently, experiment two tested the hypothesis that a directly voided urine specimen (natural urination) with no technique would be the optimal non-invasive specimen collection method when compared against orthodox methods. Each of these experiments deployed florescent staining of bladder cells to determine the origin of the cells and contradict the claims that non-invasive urine specimens are greatly contaminated. It was also important to understand patient experiences of providing a urine specimen for diagnostic testing. Albeit a common clinical practice, donating a bodily fluid is an invasion of privacy. This led to the final study in this thesis which explored patient views and experiences of urine specimen collection methods and examined the recurring themes that emerged from the data.

This introductory chapter discusses the prevalence of a UTI, the process and characteristics of the infection, the different methods for detecting the presence of an infection and the various specimen collection methods. This chapter will also include a brief outline of the subsequent chapters that form the entire thesis. The goals of this chapter are to discuss:

- The patient population commonly identified with a UTI (Section 1.2)
- The pathophysiology of the bladder and bacterial invasion of the lower urinary tract (Section 1.3)
- The various methods of detecting the presence of an infection and current practice for urine specimen collection (Section 1.4)

## 1.2 Urinary tract infection: prevalence

A UTI is a common bacterial invasion of the urinary tract, and is the leading reason for treatment in primary care within the United Kingdom (NICE, 2013). Women are more affected than men, as 1 in 3 women will be treated with antibiotics for a UTI by the age of 24, 40% to 50% of women will experience one or more UTI in their lifetime with 10 to 15% experiencing recurrent infections (Foxman and Brown, 2003). It has been reported that a UTI is a common medical condition accounting for 7–8 million clinic visits per year

(Robinson et al., 2015), which results in extensive health care costs globally (Stamm and Norrby, 2001), due to the continual use of prescribed antibiotics (Foxman, 2010). The increased incidence of UTI's are found in post-menopausal women accompanied with other LUTS such as urinary incontinence, cystocele and post-void residual urine (Raz and Stamm, 1993).

Although post-menopausal women are frequently diagnosed with a UTI, it is commonly identified amongst young adolescents attending sexual health centres and accounts for 17% of treatment cases (Huppert et al., 2007). In the adult population of 65 years and older, a UTI is the second most common cause of infectious disease related to hospitalisations in the United States (Curns et al., 2005). However, earlier reports have highlighted the incidences of community and institutional acquired UTI prevalent among the elderly population nationally and internationally (Boscia and Kaye, 1987, Sandford, 1975).

The urinary tract is constantly exposed to the invasion of microorganisms from the exterior environment, particularly because of the anatomical placement of the urethra, in the vicinity of the rectum (Okragla et al., 2014). A UTI can be detected by testing a urine specimen using a dipstick to determine the positive presence of leukocytes esterase, nitrites, protein and blood, as well as microscopy to identify the presence of pyuria from a fresh urine specimen (Lunn et al., 2010). A UTI is also detected by the presence of lower urinary tract symptoms (LUTS) which can be manifested with symptoms such as hesitancy, reduced stream, intermittent stream and dysuria (Abrams et al., 2002). *E.coli* is the most common bacterial invader of the urinary tract, and accounts for approximately 80% of community-acquired uncomplicated UTIs, particularly in women under 50 years of age (Ronald, 2003).

## 1.3 Infection of the lower urinary tract and its identification

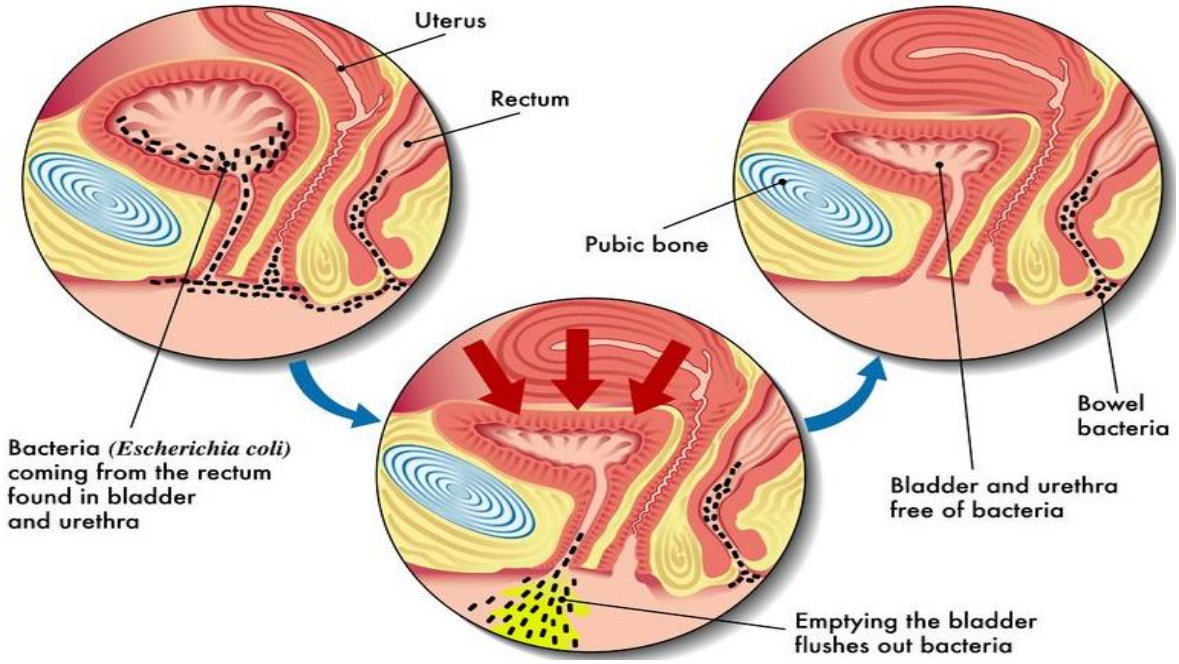
### Anatomy and physiology of the bladder

The female lower urinary tract consists of the bladder, the bladder neck, the trigone and the urethra (Watson, 2011). The bladder neck and the urethra are the lowest parts of the tract and are connected by tissue of the anterior vaginal wall (Swisher et al., 2014 ). The trigone is the lowest part of the bladder neck and is the least mobile part of the bladder as it is firmly adherent to the underlying muscle (Patel and Chapple, 2008). The bladder has three distinctive layers, the outer tissue layer which is known as serosa; the middle smooth layer is called the detrusor muscle which is responsible for the contractions that set off the sensation to void and the innermost lining layer is called the urothelium and comprises of transitional cell epithelium that provides an elastic barrier that is impervious to urine (Patel and Chapple, 2008).

### The process of infection

According to Mysorekar and Hultgren (2006) an infection of the lower urinary tract occurs when bacteria invades the urethra, migrates to the urothelium and colonises the cell epithelium of the bladder. This process is illustrated in image 1.

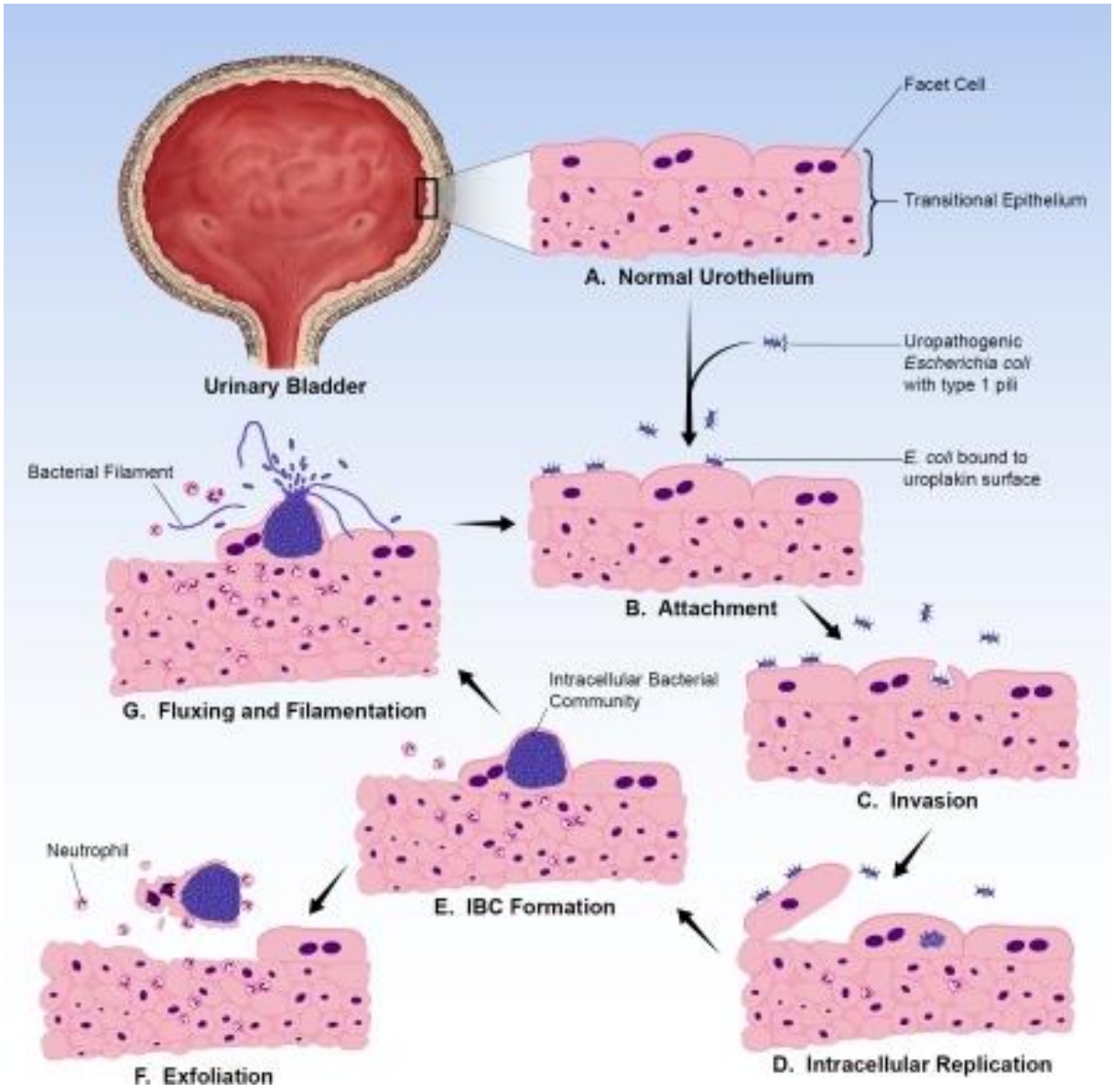
**Image 1: Urinary tract infection of the bladder**




(Health Navigator, 2016)

When the superficial cells have been invaded, the uropathogens rapidly replicate and form intracellular communities, also known as intracellular colonisation. Intracellular pathogens such as bacteria hijack cells which allow pathogens to reach an appropriate cellular niche for their survival and replication (Panek et al., 2014). Image 2 illustrates this process observed in a murine model which closely resembles the human bladder.

**Image 2: Uropathogenic Escherichia coli (UPEC) observed in the Murine Cystitis Model**




(Rosen et al., 2007)

Flores-Mireles et al., (2015) relates the process of intracellular colonisation to the development of urinary biofilms, the process in which infected bladder cells form a protective shield, hindering eradication and promoting multiplication. They report that colonisation and invasion of the superficial bladder cells set off the inflammatory responses (Image 2: B and C), including neutrophil infiltration, which attempt to eradicate extracellular bacteria. This then triggers intracellular replication (Image 2: D and E), the

infected cells multiply and form biofilms (Image 2: F and G). The multiplication of biofilms in the colonised cells hinder the function of the urothelium and epithelium triggering lower urinary tract symptoms like urinary hesitancy, reduced stream, incomplete bladder emptying and bladder pain (Rosen and Klumpp, 2014). When the urine flow from the bladder is impeded or interrupted this causes the urine to retain and the residual urine becomes static, allowing bacteria to ascend the urethra, move into the bladder mucosa and triggers the inflammatory process (Selius and Subedi, 2008). In severe urinary retention cases, stagnant urine can migrate back into the kidneys causing pyelonephritis, inflammation of the kidneys (Piccoli et al., 2014). An untreated infection can lead to chronic inflammation of the urethra causing narrowing, swelling and painful urination, this is known as urethritis (Selius and Subedi, 2008). An infection of the lower urinary tract can be detected through various different symptoms. The reporting of symptoms are indicative of an acute flare, and aids the diagnosis of an infection.

**Symptoms of a lower urinary tract infection**

Lower urinary tract symptoms (LUTS) are commonly recognised as storage, voiding and incontinence symptoms (Haylen et al., 2010), and these symptoms have proven to have positive associations with age (Bray et al., 2013) and obesity (Vaughan et al., 2013). Research on female LUTS has also looked at the 'overactive bladder' (OAB), which is a term used to define the sudden onset of the urgent need to void frequently, during the day or night, with or without urinary incontinence and is closely associated with storage symptoms (Lai et al., 2014). Similarly, these symptoms are often reported during an acute UTI. According to Bent et al., (2002) an acute UTI may also cause severe but transient LUTS, including dysuria, urgency, frequency, and urgency urinary incontinence. LUTS and UTI have overlapping symptoms, and earlier authors may argue that LUTS is a predefined condition that deals with the theories and pathophysiology of the bladder and the motor function of the detrusor muscle (Brading, 1997). More recent authors believe that the urothelium is the mediator of the bladder, and that urothelial inflammation and infection defines the etiology of LUTS (Hannan et al., 2012).

An acute UTI is often diagnosed when $\geq 10^5$ bacterial colony forming units (CFU)/ml of a single organism is present from a cultured urine sample (Kass, 1957). However, a recent study has suggested that the diagnostic threshold should be reduce to $\geq 10^3$ bacterial CFU/ml as this would leave fewer uropathogens in the UTI cohort untreated (Price et al., 2016). Studies have also reported that intracellular bacterial colonisation in the female bladder has been recognised as a pathological cause of urinary incontinence (Khasriya et al., 2010) and has precipitated OAB symptoms (Hessdoerfer et al., 2011). Authors have reported on the fastidious, anaerobic, and difficult-to-cultivate organisms that are microbiologically associated with LUTS (Latthe et al., 2008, Wolfe et al., 2012). There is a wealth of evidence that draws the conclusion that the symptoms of UTI are triggered by the bacterial invasion of the urothelium, which sets off the urothelial inflammatory mediators and alters the nerve activity of the bladder mucosa (Mulvey et al., 2001, Anderson et al., 2004, Nielubowicz and Mobley, 2010, Hannan et al., 2012). Thus, causes symptoms such as urgency, urge incontinence, nocturia, bladder pain, and nocturnal enuresis all of which can be defined as LUTS (Sorrentino et al., 2014).

## 1.4 Current methods of detecting a urinary tract infection

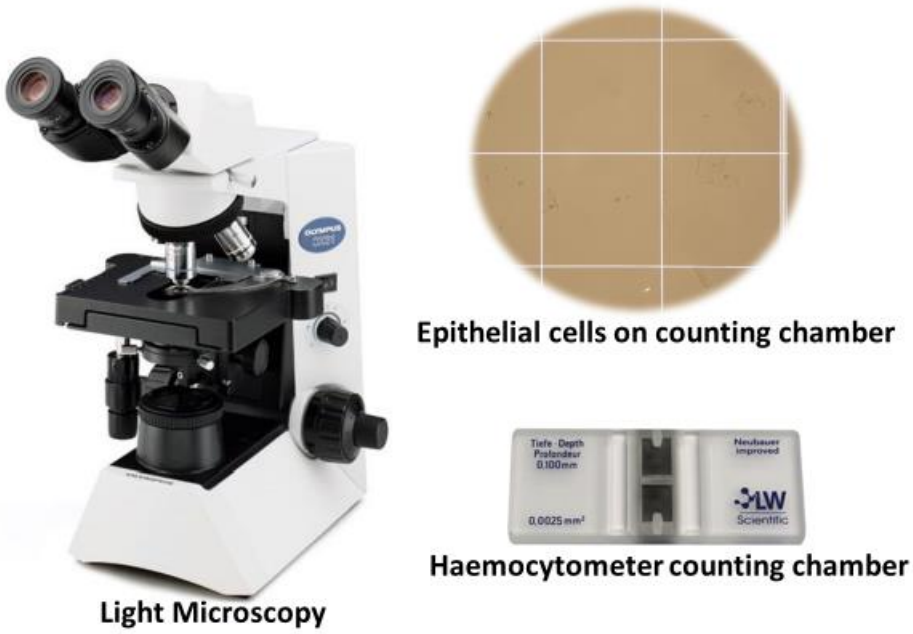
**Dipstick urinalysis**

A standard method of detecting a UTI is commonly done by using a dipstick urinalysis. A dipstick urinalysis is a rapid inexpensive diagnostic test which is used in conjunction with or in place of a urine culture (Huysal et al., 2013). The dipstick urinalysis measures the markers of pyuria and bacteriuria, the two diagnostic factors often associated with a UTI (Turner et al., 2014). On a dipstick urinalysis pyuria is the measurement of urinary leukocytes and bacteriuria is the presence of nitrites (Pappas, 1991). Not all dipstick urinalysis tests detect the presence of leukocytes and nitrites, but identifying any one of these markers increases the positive predictive value of the urinalysis (Raza-Khan et al., 2006). Although it is a standard method for routinely screening for the presence of infection, the dipstick has been discredited due to its inability to sensitively detect the presence of a UTI in comparison to urine microscopy (Khasriya et al., 2010).

**Urine microscopy**

Urine microscopy has been used to identify urinary leukocytes since the early 1890s. However, Dukes (1928) introduced another method of assessing urine using a counting chamber to evaluate the presence of urine cells from a fresh unspun specimen (Image 3). From then onwards newer studies investigated the use of urinary microscopy for identifying bacteriuria, as an alternative to relying on urine cultures (Hallstrom et al., 1975, Vickers et al., 1991, Hiraoka et al., 1993). Urine microscopy is also used to detect the presence of haematuria using a haemocytometer (Yeoh et al., 2013) and uroepithelial cells (Khasriya et al., 2013). More recent studies advocate the use of light microscopy as a standard screening practice for detecting bacteriuria in males and females (Sorrentino et al., 2014), and light microscopy should not be restricted to screening for bacteriuria in pregnant women who undergo genitourinary procedures as it has been recommended (Lewis et al., 2013).

**Image 3: Urine microscopy and haemocytometer counting chamber**



**Urine sediment culturing**

Although a UTI is common in women, it also affects neonates, males and the elderly (Tambekar et al., 2006). There are various different microorganisms that invade the lower urinary tract, and a urine culture is the gold standard for diagnosing the pathogens that are responsible for a UTI (Health Protection Agency, 2011). A variety of different organisms can cause a UTI such as protozoan's parasites and fungi. However, bacteria are the dominant offending organisms and are commonly identified in urine cultures (Singhal et al., 2014). Bacteria accounts for more than 95% of diagnosed UTIs (Arjunan et al., 2010), and it is often reported that these bacteria's originate from the anal region and identified as *E. coli* (Uwaezuoke and Ogbulie, 2006). Urine cultures frequently cultivate an *E. coli*, a bacterium recognised as the most common etiological agent of the disease, and accounts for 75 to 90% diagnosed cases of a UTI in both outpatient and inpatient settings (Kashef et al., 2010). Other organisms such as *Enterococcus, Proteus, Klebsiella and Staphylococcus* are also observed as cultivated uropathogens (Saperston et al., 2014). A UTI is often diagnosed when $\geq 10^5$ bacterial colony forming units (CFU)/ml of a single organism is present from a cultured urine sample (Kass, 1957), and the main purpose of a urine culture is to diagnose the bacteria and determine the antibiotic sensitivity for treating the infection (Ninan et al., 2014), but diagnosis of an infection is often deferred due to the frequent identification and reporting of epithelial cells and other bladder sediments found in the urine that are regarded as contaminants (Collier et al., 2013).

**Urothelial cell identification**

The innermost layer of the bladder is called the urothelium and comprises of transitional cell epithelium (Patel and Chapple, 2008). It is regarded as a protective barrier to the underlying nervous, muscular and vascular tissue from the potential harmful contents that are found in the urine (McDermott et al., 2012). Evidence has shown that a bacterial infection of the bladder can disrupt the function of the urothelium, triggering an inflammatory response that causes the intracellular colonised transitional cells to migrate to the base of the bladder and voided as part of the urinary stream (Anderson et al., 2003, Reigstad et al., 2007, Khasriya et al., 2013). Studies have also shown the

identification and origin of urothelial cells in the murine model by using a cell staining technique called DAPI which binds to cell DNA and bacteria (Dodmane et al., 2014), and uroplakin that binds to cell membranes (Wu et al., 1994, Thumbikat et al., 2009, Lee, 2011). Uroplakin comprises of four transmembrane proteins (1a, 1b, 2 and 3). However, uroplakin-3 specifically binds to the surface of human urothelial cells and has been regarded as a valuable marker of urothelial cell origin (Wu et al., 2009, Horsley et al., 2013).

The urothelial cell origin and its identification are unique and it is evident that these cells found in the urine are exfoliated as a result of an inflammatory response of the urothelium, and are regarded as important diagnostic urinary sediment. Such urothelial cells are predominantly found at the base of the bladder due to the storing mechanism of the bladder trigone which restricts the back flow of urine back into the bladder dome (Viana et al., 2007). Traditional methods of cell analysis would interpret these cells as a contaminants (Collier et al., 2013) but newer methods of cell identification have regarded these cells to be a marker of inflammation of the bladder (Khasriya et al., 2013).

**Urine specimen contamination**

The UK Standards for Microbiology Investigations (Public Health England, 2016) have defined contamination as mixed growth of urinary pathogens. Improper urine collection techniques have been considered the main reason for contaminated urine specimens (Frazee et al., 2012), thus reports of contaminants delay the confirmation of diagnosis which leads to delayed treatment (Shrestha et al., 2013). This matter is important because the standard methods of urine samples taken by the midstream urine method (MSU) are used routinely in the health services to screen for a UTI and to identify the causative microbes and their antibiotic sensitivities. More invasive methods e.g. the catheter specimen (CSU) are postulated as better and less prone to contamination, but are not recommended by NICE due to the invasiveness of the methods. A CSU is obtained when a hollow thin tube is inserted along the urethra and into the bladder, which bypasses the external genitalia.

Some authors have drawn attention to the pitfalls of contaminated urine specimens, but fail to define what are the microbes or sediments that cause specimen contamination (Blake and Doherty, 2006, Vaillancourt et al., 2007, Appannanavar et al., 2013, Shrestha et al., 2013). If urine specimens are thought to be contaminated because the sampling method is faulty, then patient harm is probable, as the sample may get dismissed, diagnosis of a UTI will not be determined and treatment for the infection will not proceed.

However, if contamination has not been defined then inflammation of the urothelium i.e. shedding of urothelial cells may be confused with skin and vaginal flora and urine specimens will then get dismissed as contaminants when they are actually revealing the presence of a UTI. Therefore, the first step to consider is the integrity of the sampling methods using a specific definition of contamination and adopting microbiological techniques that allow for a clearer discrimination between skin, vaginal and urothelial cells. Contamination is a broad term that covers the identification of vaginal and microbial cells (i.e. lactobacillus and Gardnerella vaginalis) found in the urine. It is important to access and identify urothelial cells and separate the differences between the skin and vaginal cells. An ideal urine specimen would be one that contains cells and sediments that are indicative of the bladder microbiome. Therefore, for the purpose of this thesis, urine contamination is defined as sediments or cells that do not reflect or resemble the cell pathology of the urinary tract.

**Specimen collection and current practice**

Routine screening for the presence of a suspected UTI is part of the initial clinical assessment when patients are admitted into hospital or community care services (Rahn, 2008). Nursing staff initiate the production of a urine specimen from patients by requesting a specimen of urine for testing (Simerville et al., 2005). A review of recent nursing textbooks and journals (Pellat, 2007, Iggulden et al., 2009, Dougherty and Lister, 2011) reveal four different methods which include non-invasive and invasive methods of specimen collection. Dougherty and Lister (2011) identify the most common non-invasive

method of urine sampling as a midstream urine specimen (MSU). The term clean-catch urine is frequently used synonymously with MSU, but this sampling method does not include cleaning of the urethral meatus (Pellat, 2007).

Iggulden et al., (2009) identified a catheter specimen of urine (CSU) as an invasive urine sampling method which involves a sterile catheter being inserted into the bladder to drain off a specimen of urine. Similarly, Titus and White (2006) described the suprapubic aspiration as another invasive urine collection method, as a urine specimen is aspirated straight from the bladder from the supra pubic region. The urine specimens that are obtained via these four different methods are then sent for a culture which will determine the pathogen invading the urinary tract, but it is well known that urine cultures are frequently blighted by reports of contaminated urine specimens (Bekeris et al., 2008). Contemporary methods of non-invasive urine collection techniques have been introduced to clinical practice such as the Peezy MSU device and a straight forward directly voided urine (natural urination). However, these methods have also been criticised for harbouring contaminants (Collier et al., 2013, Frazee et al., 2015).

## 1.5 Summary

Identifying a urinary tract infection requires a meticulous analytical process. Providing a urine specimen is the initial step for diagnosing the disease, but the correct urine collection method is vital when determining the causative pathogens that invade the urinary tract. Improper urine collection techniques continue to be the main reason for contaminated urine specimens and an accurate definition is yet to be validated. The overall aim for this thesis is to explore the qualitative and quantitative differences of the cells and sediments found in different methods of urine specimen collection, and to establish whether the urinary sediments found in urine specimens are contaminants as it has been reported. In addition, this thesis will also explore patient views and experiences of providing urine specimens by different collection methods.

## 1.6 Subsequent chapters

The next chapter of this thesis is the literature on urine specimen collection methods. The literature review will look at the aims and objectives of each of the studies, the sampled groups, methods used and their findings. Chapter three describes the experimental work that was carried out in order to refute the assumptions of urine specimen contamination as identified in the literature review. There were two experiments conducted. Experiment one hypothesised that a non-invasive urine collection method (MSU) has equal merits to an invasive method (CSU) when capturing cells that are indicative of an infection and compared a midstream urine specimen to a catheter specimen. Experiment two hypothesised that if a non-invasive urine collection method (MSU) is better at capturing cells, then a directly voided urine specimen (natural urination) will be superior. Three non-invasive methods were compared (MSU, Peezy MSU and directly voided urine). To validate these hypotheses, microbiological culturing and uroplakin cell staining was conducted to determine cell origin and to numerate cell proportions in each of the urine collection methods. Statistical test were deployed to determine relationships and differences between each of the urine collection methods.

Chapter four presents the results from experiment one and two and chapter five discusses the results from the experiments. Patient views and experiences of urine specimen collection are explored in chapter six along with the findings and discussion. In chapter seven the quantitative and qualitative components are assimilated in order to understand the relationships between findings and chapter eight concludes the entire thesis and highlights the implications the results have on clinical practice.

# Chapter 2

# Urine specimen collection methods: A review of the literature

## 2.1 Purpose of reviewing the literature

The purpose of this literature review was to examine evidence for the comparative reliability of non-invasive versus invasive methods of sampling urine for the detection of a urinary tract infection (UTI). A concluding summary at the end of this review will highlight the key findings identified, which will provide a justification for the selected methodological approaches for the experimental studies explained in chapter three.

## 2.2 Search methods

This literature review used the PICO search strategy (P= patient/ problem/population, I= intervention, C= comparison and O= outcome) to find relevant literature that focused on the four main methods of urine sampling. According Caldwell et al., (2012) PICO aids a systematic approach to search published literature, and enables clinicians to find the best literature to answer clinical questions. The clinical question for this review was: what is the reliability of non-invasive urine sampling methods compared to invasive methods in adult women? Table 1 illustrates how the clinical question was approached using the PICO strategy.

**Table 1: PICO Search Strategy**

| Patient/ population | Intervention Non-invasive | | Comparison Invasive | Outcome |
|---|---|---|---|---|
| Adult women | MSU | Versus | CSU | Specimen quality<br>Specimen contamination<br>Detecting urinary tract infection |
| | MSU | | SPA | |
| | CCU | | CSU | |
| | CCU | | SPA | |
| | Direct void | | CSU | |
| | Direct void | | SPA | |
| | Urine collection device | | CSU | |
| | Urine collection device | | SPA | |

The literature on urine specimen collection was obtained from a variety of databases. The Southampton University library, The British Library, Archway Healthcare Library and local regional libraries were visited and used to obtain literature. Electronic research databases and indexes such as Web of Knowledge, Medline, CINAHL, EMBASE, The Cochrane library, Pubmed and The British Nursing Index were visited. These databases and indexes were searched to examine the body of knowledge that focused on urine specimen collection methods. Various search terms were developed using the PICO strategy, and this has been illustrated in table 2.

**Table 2: Search Terms**

| Search terms | MeSH Terms | CINAHL terms | Databases |
|---|---|---|---|
| Catheter specimen of urine<br>CSU<br>Clean intermittent catheterisation<br>Supra pubic aspiration<br>Midstream urine<br>MSU<br>Clean-catch urine<br>CCU<br>Urine Specimen handling<br>Urine collection techniques/methods<br>Invasive/ non-invasive methods of urine collection<br>Urinary tract infection/detection/diagnosis<br>Women<br>Direct void<br>Collection device | Specimen handling<br>Urine specimen collection<br>Diagnoses | • Catheterisation<br>• Urinary tract infection<br>• Urine specimen care | • BNI,<br>• CINAHL<br>• EMBASE<br>• Medline<br>• Web of Science<br>• The Cochrane library<br>• Pubmed |

The search terms were refined further using the Boolean operators: (AND and OR) and Truncation: * in order to obtain literature specific and relevant for this review. CINAHL terms and Medical subheadings (MeSH terms) were also used in order to generate relevant literature within each database. The literature search initially started broad with the aim of obtaining various studies on different urine collection methods. The search was narrowed down further by using search terms specific to urine sampling methods illustrated in table 2 above.

## 2.3 Inclusion and exclusion criteria

The inclusion criteria were adult women, in-patient, out-patient and community settings and literature written in English and dated from 2002 to 2012 (2012 was the year the literature review was being conducted). The exclusion criteria were children, men, non-English literature and literature dated prior to 2002. These parameters were included so that the literature review focused solely on women identified with or without a UTI, within the community, who were presented to primary or secondary healthcare services

for urine specimen collection and to recognise recent urine sampling practice within the last 10 years. Table 3 list the inclusion and exclusion criteria and the rationale for each criterion.

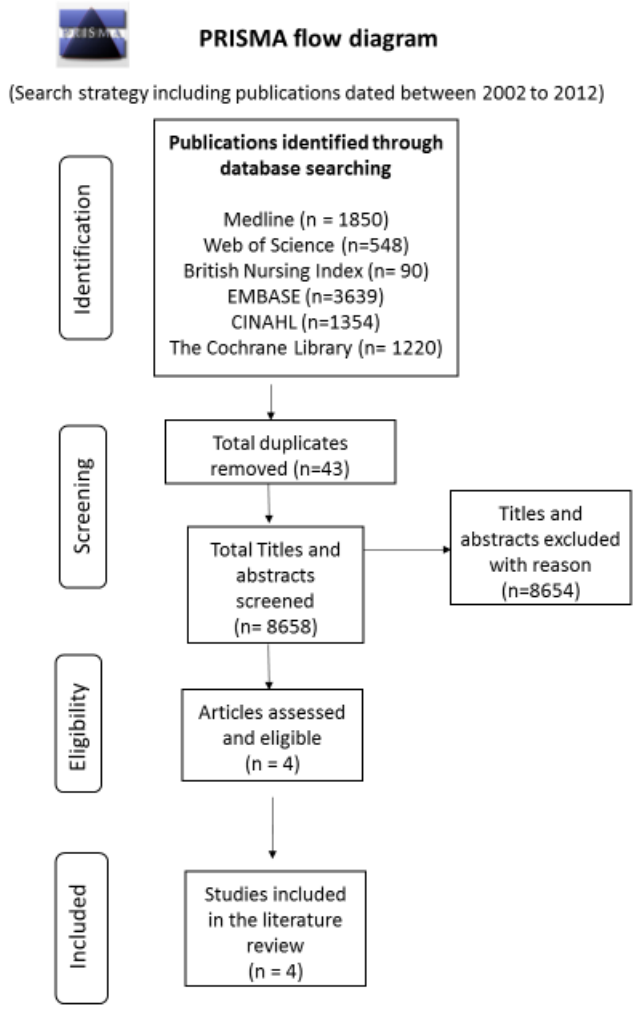
**Table 3: Inclusion and Exclusion Criteria**

| Inclusion criteria | Rationale | Exclusion criteria | Rationale |
|---|---|---|---|
| **Adult women** → | 1 in 3 women are treated for UTI (Foxman & Brown 2003). | **Children** → | Paediatric urine collection is difficult & diagnosing UTI is a continual challenge (NICE 2007). |
| **In- patient/ Outpatient & Community Settings** → | UTI is the leading reason for treatment in the UK (MeReC Bulletin 2006). | **Men** → | Men are treated for UTI less frequently than women (Matthews and Lancaster 2011). |
| **In English** → | Publications in other languages would not be comprehendible. | **Literature dated before 2002** → | Literature older than 10 years will limit knowledge on current urine collection methods. |

## 2.4 Literature retrieved

The flow diagram below adopted the PRISMA search strategy technique (Moher et al., 2009) and highlights the number of references found in each database based upon the search terms in table 2.

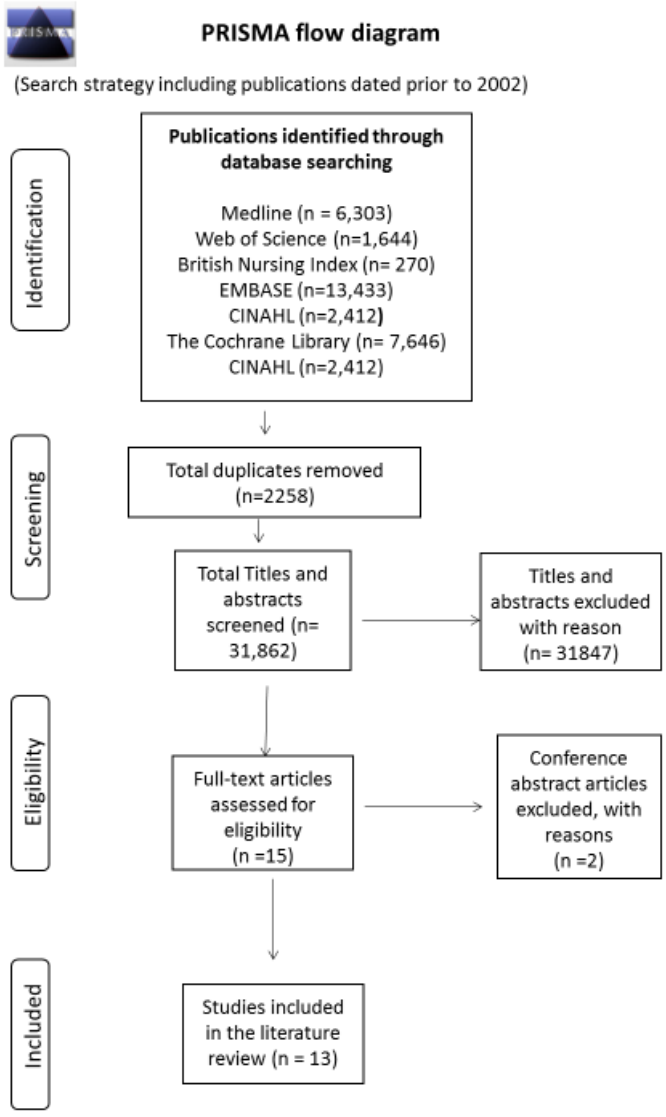
**Figure 1: Prisma flow diagram**




A total of 8658 titles and abstracts were screened with the intention of finding publications that focused on urine specimen collection methods. Although there were many titles that included the words 'urine' and 'specimen collection', the majority of the abstracts did not focus on urine specimen collection methods. Only four studies were relevant and therefore the search was conducted again without the 2002 to 2012 publication parameters. Subsequently, other relevant literature were retrieved, reviewed and added to the literature review including newer literature dated from 2012 to 2015. This was initiated so that additional studies could be found and reviewed. The flow diagram below highlights the number of studies found in each database based using the same search strategy that was carried out previously. Fifteen comparative studies on urine specimen collection methods were found.

This included the four studies retrieved in the initial search. Two studies were not accessible in full text due to being conference abstracts. The remaining thirteen studies which were accessed with full text were included within this review. These studies were chosen based on a combination of factors which included the abstract, content and evidence of exploring non-invasive urine sampling methods versus invasive methods for detecting a UTI.

**Figure 2: Prisma flow diagram**



## 2.5 Process of critiquing the literature

The literature were reviewed using Holland and Rees (2010) critiquing frameworks for quantitative and qualitative studies. The Holland and Rees frameworks (Appendix 1) were chosen based upon the effective strategy to objectively analysis the strengths and weakness of the studies, and permitted the chosen studies to be evaluated through evidence based information. Each framework provided an effective structure to critically appraise the studies based upon the aspects of report which include the background, aims, sample, methods, results, discussion and research limitations. The germane data were extracted and are presented in the table shown below. The table was used for ease of comparison, starting with the earliest publication first. Each study was scrutinised critically to identify emergent trends and the outcomes. All of these data are included in Table 4.

**Table 4: Publications reviewed and included in the literature review**

| # | Studies included | Country of publication | Study aims | Design<br>Sample<br>Data collection<br>Analysis methods | Key findings |
|---|---|---|---|---|---|
| **1** | Linton Kb and Gillespie (1969) Collection of urine from women for bacteriological examination. Journal of Clinical Pathology 22(3): 376–377 | United Kingdom | To identify the reliable and convenient method for urine specimen collection. | **Design:** Comparative study<br>**Sample:** 81 Urological patients, 2043 Antenatal patients and 53 medical inpatients.<br>**Data collection:** catheter specimens of urine (CSU), midstream urine specimens (MSU), voided urine specimen and urinal funnel tube.<br>**Analysis methods:** Unknown<br>**Analysis of contamination:** Not stated. | More than 80% of catheter specimens showed no bacterial growth, more than 50% of MSU showed slight contamination.<br>Bacterial growth was the same in both comparisons with Staphylococcus as the dominant organism.<br>The tube funnel produced specimens with less contamination. |
| **2** | Lemieux and St-Martin (1968) Reliability of clean-voided mid-stream urine specimens for the diagnosis of significant bacteriuria in the female patient. Can. Med Assoc.J. 98(5): 241-245 | Canada | To compare Clean-voided specimens to specimens obtained by catheterization | **Design:** Comparative study<br>**Sample:** student nurses, asymptomatic female patients &symptomatic female patients<br>**Data collection:** MSU vs CSU<br>**Analysis methods:** Unknown<br>**Analysis of contamination:** The presence of *lactobacillus*, *Sarcinae* and *Gaffkya tetragena.* | Midstream urines showed bacterial growth (69%). Catheterized specimens showed no bacterial growth after 48.<br>Bacterial count and organism found correlated with the symptoms. |
| **3** | Eng, J, Torkildsen EM and Christensen A (1978) Bacteriuria in the puerperium: an evaluation of methods for collecting urine specimens. Am.J.Obstet.Gynecol. 131(7): 739-741 | Norway | To assess the usefulness of specimen collection in the diagnosis of bacteriuria in the puerperium of women post-partum. | **Design:** Comparative study<br>**Sample:** 518 post-partum women without symptoms of UTI<br>**Data collection:** MSU vs SPA (Suprapubic aspiration)<br>**Analysis methods:** independently on cultivation results.<br>**Analysis of contamination:** Mixed growth from the same bacterial species. | SPA urine specimens yielded a sterile result.<br>The level of confidence in the MSU specimen for bacteriologic cultivations is strongly affected by the technique of collection. |
| **4** | Gower P, R and Roberts A, P (1975) Qualitative assessment of midstream urine cultures in the detection of bacteriuria. Clin Nephrol 3(1): 10- 13 | United Kingdom | To assess the quality of midstream urine samples to suprapubic aspiration for the detection of bacteriuria. | **Design:** Comparative study<br>**Sample:** 3550 women attending cervical smear screening<br>**Data collection:** MSU vs SPA<br>**Analysis methods:** Unknown<br>**Analysis of contamination:** Not stated. | SPA is the most efficient way of determining whether patients have bacteriuria. |

| # | Studies included | Country of publication | Study aims | Design<br>Sample<br>Data collection<br>Analysis methods | Key findings |
|---|---|---|---|---|---|
| **5** | Stamm WE, Counts GW, Running KR, Fihn S, Turck M and Holmes KK (1982) Diagnosis of coliform infection in acutely dysuric women. The New England Journal of Medicine 307(8): 463-468 | United States of America | Establishing the best diagnostic criterion for coliform infection using various urine sampling methods. | **Design:** Comparative study<br>**Sample:** 187 female patients & 67 female controls.<br>**Data collection:** MSU, CSU & SPA<br>**Analysis methods:** Fishers exact test, Students t-test.<br>**Analysis of contamination:** Not stated. | MSU results correlated closely with the 1st and 2nd urine cultures with mix growth commonly reported. |
| **6** | Little PJ, Peddie BA and Sincock AR (1980) Significance of bacterial and white cell counts in midstream urines. J.Clin Pathol 33(1): 58-60 | New Zealand | To compare bacterial and white cell counts in midstream urines to Suprapubic aspiration. | **Design:** Comparative study<br>**Sample:** 903 Adult women.<br>840 asymptomatic women examined at the first attendance at an antenatal clinic.<br>53 asymptomatic student nurses.<br>10 women presenting with symptomatic UTI.<br>**Data collection:** MSU & SPA<br>**Analysis methods:** Kass 1955 concept of quantitative urine cultures.<br>**Analysis of contamination:** Not stated. | Infection was confirmed in 26 of 35 patients with Gram-negative bacilli in the MSU.<br>MSUs with mixed organisms were excluded.<br>SPAs confirmed infection in 88 % of cases. |
| **7** | Walter FG and Knopp RK (1989) Urine sampling in ambulatory women: midstream clean-catch versus catheterization. Ann.Emerg.Med 18(2): 166-172 | United States of America | To determine whether any clinically important or statistically significant differences exist between urine obtained by midstream clean-catch (MSCC) sampling & that obtained by Catheter. | **Design:** Comparative study<br>**Sample:** 105 women with symptomatic UTI.<br>**Data collection:** MSU & CSU<br>**Analysis methods:** McNemar's test for paired data.<br>**Analysis of contamination:** Mixed Flora in subsequent MSU specimens. | Escherichia coli (*E.coli*) were the dominant organism.<br>There were sterile catheter urine cultures & mixed flora in their corresponding MSU specimens which were regarded as contaminated. |
| **8** | Michielsen WJ, Geurs FJ, Verschraegen GL, Claeys GW and Afschrift MB (1997) A simple and efficient urine sampling method for bacteriological examination in elderly women. Age Ageing 26(6): 493-495 | Belgium | To determine how collecting urine voided directly into a container compares with urine obtained by Suprapubic aspiration. | **Design:** Comparative study<br>**Sample:** 58 women with symptomatic UTI.<br>**Data collection:**<br>MSU vs SPA<br>**Analysis methods:** Unknown<br>**Analysis of contamination:** $\leq 10^5$ or $\geq 10^5$ mixed bacteria growth. | UTI was diagnosed in 13 SPA and in 17 MSU.<br>13 out of 17 MSU specimens were considered to be contaminated.<br>In 3 patient's pyuria and bacteriuria were demonstrated in the MSU specimen but not in the SPA specimen. |

| # | Studies included | Country of publication | Study aims | Design<br>Sample<br>Data collection<br>Analysis methods | Key findings |
|---|---|---|---|---|---|
| **9** | Wolfe A J, Toh E, Shibata N, Rong R, Kenton K, FitzGerald M, Mueller E, Schreckenberger P, Dong Q, Nelson D and Brubaker L (2012) Evidence of Uncultivated Bacteria in the Adult Female Bladder. Journal of Clinical Microbiology 50(4): 1376-1383 | United States of America | To test if the bladders of women who do not meet the clinical definition for UTI contain uncultivated bacteria.<br>To identify and recommend the most suitable urine collection method for urine culture. | **Design:** Comparative study<br>**Sample:** Group 1: control group, composed of patients undergoing surgery for benign gynecologic conditions, reported no urinary symptoms. Group 2: The comparison group included patients undergoing surgery for treatment of common Urogynecology conditions.<br>**Data collection:** MSU vs SPA & CSU<br>**Analysis methods:** paired t -test, McNemar's test, the Kolmogorov-Smirnov test and principal coordinate analyses (PCA).<br>**Analysis of contamination:** The presence of vulvo-vaginal bacteria *Lactobacillus* and *Prevotella.* | The MSU produced colonies upon cultivation in aerobic and/or anaerobic conditions, and were contaminated with vulvo-vaginal bacteria None of the paired CSU samples produced colonies upon cultivation in either aerobic or anaerobic conditions.<br>Organisms detected in SPA samples were contaminants. |
| **10** | Hooton TM, Roberts PL, Cox ME and Stapleton AE (2013) Voided midstream urine culture and acute cystitis in premenopausal women. N Engl J Med 369(20): 1883-91 | United States of America | Paired comparison to analyse the organisms that are considered to be causative in acute uncomplicated cystitis | **Design:** Comparative study<br>**Sample:** 226 Adult women with symptoms of cystitis.<br>**Data collection:** MSU vs CSU<br>**Analysis methods:** Spearman's correlation coefficients.<br>**Analysis of contamination:** The presence of *Lactobacillus.* | More than one uropathogen grew in 4 cultures of catheter urine and in 35 cultures of midstream urine.<br>Among the 53 cultures without growth in catheter urine, 13 (25%) had uropathogens in the paired midstream urine. |
| **11** | Schneeberger C, van den Heuvel ER, Erwich JJ, Stolk RP, Visser CE and Geerlings SE (2013) Contamination rates of three urine-sampling methods to assess bacteriuria in pregnant women. Obstet Gynecol. 121(2 Pt 1): 299-305 | Amsterdam | To compare contamination rates of three different urine-sampling methods in pregnant women to assess bacteriuria | **Design:** Comparative study<br>**Sample:** 113 pregnant women collected three different midstream urine samples consecutively<br>**Data collection:**<br>First void, MSU & clean-catch urine<br>**Analysis methods:** McNemar<br>**Analysis of contamination:** The presence of epithelial cells, Gram-positive rods or mixed bacteria in the Gram stain, and mixed growth or skin flora in the urine culture. | The contamination rate of midstream samples is comparable with the contamination rates of morning and clean-catch samples. |

| # | Studies included | Country of publication | Study aims | Design<br>Sample<br>Data collection<br>Analysis methods | Key findings |
|---|---|---|---|---|---|
| **12** | Collier S, Matjiu F, Jones G, Harber M and Hopkins S (2013) A prospective study comparing contamination rates between a novel mid-stream urine collection device (Peezy) and a standard method in renal patients. Journal of Clinical Pathology. | United Kingdom | To compare an MSU device to the standard MSU. | **Design:** Comparative study<br>**Sample:** 420 female renal transplant recipients and the results were compared with 424 matched historical controls, who used the standard method of urine collection<br>**Data collection:** MSU vs Peezy MSU<br>**Analysis methods:** Statistical analysis was performed using STATA V.11.0.<br>**Analysis of contamination:** The presence of epithelial cells and mixed growth urine culture. | White blood cells and epithelial cells were present in significantly higher concentrations in those samples taken with the Peezy device compared to the standard MSU.<br>Peezy increased the rates of both epithelial cells and mixed growths in the urine samples when compared with the historical controls. |
| **13** | Frazee BW, Enriquez K, Ng V and Alter H (2015) Abnormal urinalysis results are common, regardless of specimen collection technique, in women without urinary tract infections. Journal of Emergency Medicine 48(6): 706-11 | United States of America | To examine the impact of voided urine specimens for indicators of UTI versus urine contamination | **Design:** Comparative study<br>**Sample:** 40 asymptomatic female Emergency Medicine residents, medical students, and ED nurses under age 40 years.<br>**Data collection:** MSU vs directly voided urine<br>**Analysis methods:** McNemar's chi-squared test for repeated measures.<br>**Analysis of contamination:** The presence of epithelial cells. | Specimen contamination was very common, occurring in 70% of both the non-clean and ideal technique groups.<br>Presence of epithelial cells (>trace), considered an indicator of specimen contamination, was more common in the non-clean technique group, though epithelial cells were still found in 30% of specimens collected by ideal technique. |

## 2.6 Aims and objectives of the studies

According to Polit and Beck (2012) researchers should first identify the research aims and objectives that they hope to accomplish. In each of the thirteen studies this was achieved. All of the studies compared different methods of urine collection. Four studies compared MSU with suprapubic aspiration (SPA); another four compared MSU with CSU, two compared SPA, MSU and CSU and three recent studies compared non-invasive methods which were the MSU compared to a Peezy MSU device, the MSU compared against the first voided urine and the clean-catch urine and the other compared the MSU to a direct void (non-clean method). The findings reported between each of the different studies were inconsistent and in every case the analysis was affected by conjectured bias. In all cases culture results were dismissed, and regarded as contamination because of mixed growths, colony counts below arbitrary threshold and the presence of microbes not believed to cause urinary infection. These views were based on historical beliefs that were not founded on empirical evidence. None of the studies sought to explore or compare the different urine sampling methods from the perspective of the patient.

## 2.7 Participants

All thirteen studies sampled adult women presenting to healthcare services with or without symptoms of a UTI. The authors justified this selection because 1 in 3 women will be treated with antibiotics for a UTI by the age of 24 (Foxman and Brown, 2003). The authors of each study reported that the sample that they investigated represented the key population affected by a UTI. The number of participants enrolled in each study, varied and none reported a sample size calculation. In any case, that would have been extremely difficult because there were no data available to inform this process. The participants in studies 9 and 10 were clearly defined but the sample size was not described whereas it was in the others (studies 1, 2, 3, 4,5,6,7, 8,12 and 13). Polit and Beck (2012) argue that the larger the sample, the more representative of the population it is likely to be. This is true provided that there is no overt or concealed bias in the sampling process. However, it is a difficult practical problem because patients presenting

to healthcare services are selective by definition. Study 4 reported the largest sample of 3550 adult women. Although the sample size was large, the numbers included in the analysis were reduced because of participant eligibility and compliance.

## 2.8 Research design

All the studies claimed to be comparative. According to Schneider et al., (2004), such studies enable a comparison between different groups, by allowing the researchers to quantify the strength of the relationship between the outcome measures. In these cases, an unbiased comparison was not achieved because so many samples were arbitrarily excluded on the basis that they were contaminated. Studies 1, 5 and 9 were the only studies to incorporate three urine sample comparisons. Comparing three urine sampling methods was an appropriate way of determining which urine collection method out of the three provided the most optimal specimen for testing.

## 2.9 Data collection

In each of the thirteen studies the non-invasive urine sampling method was the MSU. This sampling technique was described in different ways in the texts and agreement was not close. Although the principle of the technique remained consistent in each study, the methods of obtaining the specimens were very different. The assumption implied by all papers was that the MSU is a well-established technique. Studies 2, 4 and 7 were the only studies which clearly described the process of collecting the non-invasive and invasive urine specimens. These studies were comparing the results from different specimen collection methods so it was unhelpful that several of the reports neglected to describe their urine collection methods in detail.

## 2.10 Findings

In six of the studies (1, 7, 8, 9, 12 and 13) the incidence of specimens believed to be contaminated is recorded, but not in the others. In each of these papers the authors claimed that the MSU specimens were more often contaminated than in the invasively collected specimens. This should not be assumed as a fact as the true identification of

contamination has not been defined. A majority of the studies reported increased numbers of positive cultures from midstream specimens in contrast to invasive methods (1, 2, 3, 5, 8 and 9).

Each of these studies based their judgements on assumptions and beliefs that had not been validated. There was no justification as to how they determined which organisms were uropathogenic and which were not. There was also no explanation of a threshold number of microbes that should be isolated before they are considered pathological. An MSU may be more likely to contain contributions from the sediment lying at the base of the bladder, whereas the CSU will sample urine above the bladder neck where sedimentary cells are less likely to accumulate. Before discussing the comparative merits of the two specimen collection methods, it should be taken into account that there is a possibility that the specimen collection techniques could be sampling the upper or lower urine specimen.

## 2.11 What is contamination?

The term contamination arises frequently within the reviewed literature and has been commonly associated with midstream urine specimens. Some of the literature reviewed has defined contamination as bacterial colonies that belong to normal skin flora such as coryne-bacteria and staphylococcus or that have migrated from the lower urinary tract (studies 8 and 10), and other studies mention that urine samples were contaminated but did not define it (1 and 6). According to study 12, contaminated urine is defined as squamous (epithelial) cells visible in microscopic urine and is considered as indicators of contamination. This was also reported in study 13. Wilson and Gaido (2004) define contamination as more than two different types of organisms at $>10^5$/mL, more than two different types of organisms at $10^4$–$10^5$/mL and more than one different type of organism at $<10^4$/mL. It is clear that there are various different definitions of contamination which brings into question the reliability and validity of the literature to date. There is no clear definitive answer of how contamination is measured, nor is there a threshold for squamous epithelial cells that clearly indicate the level of infection. Before accepting

common assumptions, it is necessary to be enlightened on how contamination is measured.

## 2.12 Measurement of contamination

It is frequently observed in the literature that the measurement of contamination is not clearly defined. Within the publications, the reviewer is often brought back to the notion that a contaminated urine specimen consists of mixed growth urine cultures or epithelial cells found in the urine (studies 1, 6, 7, 11 and 12). It is notable that where authors describe using epithelial cell counts as a means of assessing contamination, they make no reference as to how they decide that the index cells come from the skin. The urothelial surface is a rich source of flattened cells not dissimilar to skin or vaginal cells. It is possible, nowadays, to identify cells that come from the urothelium by using a stain for uroplakin since urothelial cells are uroplakin positive. At no time in the literature reviewed, has the use of uroplakin been described in the analysis of cells found in the collected urine specimens.

## 2.13 Limitations

All the publications reported on comparative data between invasive and non-invasive methods, so it is surprising to find that only one study reported the acquisition of participant consent in their methods (study 7). The data collected in studies 6 and 8 must be questioned because the descriptions of the methods were ambiguous and the urine sampling techniques were not properly explained. Studies 2 and 8 did not describe their approaches to data analysis so it would be difficult to draw any conclusions with confidence. In the more recent publications that were reviewed (studies 9 and 10), newer methods of urine collection have not been explored.

Newer methods of collecting a urine specimen and reducing the risk of contamination have focused on a MSU device called Peezy MSU. In study 12, the rate of contamination in different non-invasive urine sampling methods was investigated. This comparison was between the standard MSU and a MSU Peezy device. The Peezy MSU device is a mid-

stream urine collection kit, designed by Dr Vincent Forte (an NHS GP) to address the problem of inaccurate collected urine specimens provided by his patients. Dr Forte intended the system to offer the benefit of reducing urine contamination (Medical, 2012). Study 12 reported that the rates of urinary contamination did not decrease whilst using this device. They used epithelial cell counts as their measure of contamination.

## 2.14 Overall trends and patterns in the literature

The key message that emerges from the literature is that there are inconsistences with the way in which urinary contamination has been analysed. Each of the authors have their own intellectual interpretation of contamination. The reader is drawn to the fact that there are different urine collection methods used when collecting a urine specimen and all the studies reveal a varying degree of contamination based upon the urine collection method. The varying depiction of contamination could be due to the authors using different microbiological techniques to discriminate between uropathogens and contamination, and the earlier studies would have been guided by the microbiological culture protocols appropriate during that period. The differences in urine culture practices would have impacted on the findings of each study, and this is evident through the variations of how contamination has been described. In each of the studies contamination was identified through urine culture; however, there was no mention of other techniques of experimental work that supported their findings.

All the studies included in this review focused on adult women with or without symptoms of a UTI. Additionally, eight of these papers were published over ten years ago, and recent years have witnessed limited research in this area. Five studies included SPA, which would not be considered normal practice these days because it is painful and depends on the presence of a palpable bladder which would be very hard for a patient with UTI to achieve. Considering that women will experience one or more UTIs in their lifetime, with a recurrent infection (Foxman & Brown 2003), it is perhaps surprising that there has been only four recent comparative studies on urine sampling methods in the adult female population (studies 9, 10, 12, 13). It has been reported that a UTI is the most

common indication for treatment in primary care in the United Kingdom (MeReC Bulletin 2006). Nevertheless, the United Kingdom seems to have contributed only three studies to the literature of sampling methods (1, 4 and 12).

## 2.15 Summary of gaps in the literature

This literature review has demonstrated that the optimal method of collecting a urine specimen for diagnosing a UTI remains unknown. This is a problem that not only delays the confirmation of diagnosis but also defers the initiation of treatment. The literature reveals two methods of sampling, the non-invasive and the invasive methods. The non-invasive MSU method is frequently associated with contamination and the invasive CSU and SPA methods are frequently referred to as producing samples with less contamination. Based on the common practices identified in the literature review, the preferred method of obtaining a urine sample is by non-invasive methods, despite their assumed limitations. In busy clinical settings, where urine samples are frequently requested for analysis, the most appropriate method for the collection of a urine sample is still a subject of debate (Tosif et al., 2012). Although the literature claims that invasive methods of urine sampling are optimal, the feasibility of obtaining such samples is less sure because of constraints on time and resources. This has resulted in a recommendation that a non-invasive urine collection method should be used for analysis (NICE, 2007).

It is also evident that newer methods of urine collection have not been explored in greater depth such as the MSU Peezy device which has been used for collecting a specimen of urine in GP centres and hospitals across the National Health Service (NHS). Whilst the publications in this review have been critically examined, it still stands that the scientific validation of the optimal method of urine specimen collection needs to be explored. This literature review has been useful in pointing to errors that must be avoided when conducting further enquiry and has informed the gaps in knowledge that require remediation. An important omission from the data reported was any consideration of the patients' perspective. Asking someone to provide a urine specimen is an intrusion of

privacy. Albeit routine, it may be that many find the process noxious. The purpose is to obtain a urine specimen for testing. However, patients should know and understand the importance of providing a urine specimen for diagnostic testing. Understanding patient experiences, their anxieties and to what extent they are assuaged should be explored. A review of the literature on patient experiences of urine specimen collection will be explored further in chapter six.

## 2.16 Rationale for further research

With a urinary tract infection being the most common bacterial invasion of the urinary tract, and the leading cause for treatment in primary care within the United Kingdom (NICE, 2013), it is crucial that accurate urine testing supports the facilitation of treatment. Urine sampling is an important facet of the diagnostic process and a contaminated urine specimen delays confirmation of diagnosis and defers the initiation of treatment (Shrestha et al., 2013). Therefore, the need to identify the optimal method of urine collection is vital in order to put aside the assumptions of what is regarded as contamination and what is not. Chapter three will describe the experiments used to determine which urine collection method provides the accurate pathology of a urinary tract infection; it will examine the bladder sediments and cells that are found in the urine specimens of four different urine collection methods using advanced staining/microbiological techniques.

# Chapter 3

# Comparing non-invasive versus invasive methods of urine specimen collection

## 3.1 Introduction

In chapter two the literature review established that the research necessary to ascertain the best method of collecting a urine specimen for diagnosing a UTI has not yet been undertaken. The literature frequently criticises the non-invasive methods of urine collection for being contaminated and advocates the invasive methods for avoiding contaminants. However, the definition of contamination is inconsistent and it is difficult to identify clear criteria for discriminating contaminated samples. There appears to be a widespread assumption that the detection of epithelial cells in the urine implies contamination. It is presumed that the epithelial cells originate from the perineal skin or vagina but this has never been tested. It has not been considered that the epithelial cells found in urine specimens could be urothelial cells that have been exfoliated from the urothelium and into the urine during the inflammatory process of the infection.

The overall objective for this study was to explore the qualitative and quantitative differences of the cells and sediments found in different methods of urine specimen collection (Figure 3). The common assumption from the literature was that urine specimens collected non-invasively were frequently contaminated in comparison to invasive methods. The purpose of using a comparative study design was to carry out experimental work that would identify the origin of cells and sediments found in the bladder and to disprove conjectures with scientific evidence.

**Figure 3: Overall study objectives**

This chapter will expand on the theoretical framework for the study and describe the two different experiments deployed for determining which urine collection method provides the most accurate reflection of the pathology of a UTI. Experiment one aimed to test the hypothesis that non-invasive methods of urine specimen collection could be of the same quality as an invasive method when seeking an optimal specimen. Following on from testing the hypothesis in experiment one, experiment two aimed to investigate which non-invasive specimen collection method was better at capturing urothelial cells which are indicative of an infection. The microbiological culturing in experiment one and advanced staining in both experiments will also be described.

## 3.2 Theoretical framework

In the literature reviewed, the pathophysiology of a UTI was infrequently featured and the reason as to why there was a constant presence of cells found in a specimen of urine was not known. Earlier on in chapter one, there was a clear description of the pathophysiology and the different stages of a UTI. It also looked at the origin of urothelial cells and its identification as a unique characteristic of an inflammatory response in the urothelium (Anderson et al., 2003, Reigstad et al., 2007, Khasriya et al., 2013). It is understood that these cells found in the urine are exfoliated from the urothelium because of an immune response to fighting an infection (Rosen et al., 2007) and are regarded as important diagnostic urinary sediment. With this being highlighted, it was important to refute the constant assumptions that these cells found in a urine specimen are contaminants floating around in sterile urine. According to the literature reviewed, non-invasive methods of collecting urine are constantly reported as harboring epithelial cells which is also referred to as contaminants; however; invasive methods of urine specimen collection are postulated as being superior in that their techniques reduce the numbers of contaminants that are reported.

It was feasible to embark on two different urine comparison studies that would examine the quantitative and qualitative differences of cells found in urine using non-invasive and invasive specimen collection methods. It was important to establish whether the cells

found in non-invasive urine collection methods were contaminants and to numerate its proportion in comparison to an invasive method; this was achieved in experiment one. Experiment one compared the cells and sediments captured in two non-invasive urine collection methods a midstream urine specimen (MSU) and a Peezy MSU collection device. These two methods were compared to an invasive method which was the catheter specimen of urine (CSU). Albeit non-invasive methods of urine specimen collection may capture more cells than an invasive method, it was essential to establish which non-invasive method was best and this was achieved in experiment two.

## 3.3 Experiment one: non-invasive urine specimen collection versus invasive methods

Table 5 describes the process and action taken for experiment one.

**Table 5: Study schedule: experiment one**

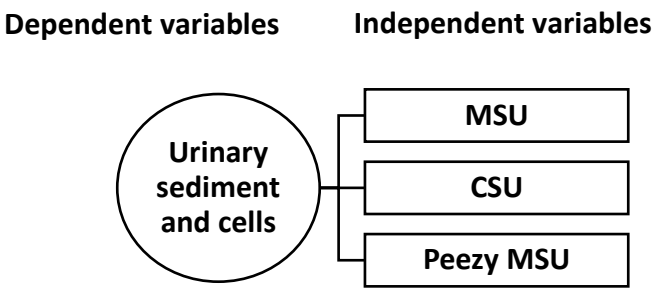
| Study Schedule | Patient actions | Control actions |
|---|---|---|
| **Sampling strategy** | Patients sent information sheet. | Controls given information sheet. |
| **Recruitment** | Patient Participant contacted to arrange a date for visit 1<br><br>Screening: Collection of demographic data to ensure eligibility | Control Participant contacted to arrange a date for visit 1<br><br>Screening: Collection of demographic data to ensure eligibility |
| **Visit 1 (Experiment One)** | 1. Informed consent<br>2. Patient completes questionnaires<br>3. MSU sample obtained<br>4. CSU sample obtained<br>5. Peezy MSU collected (subset only)<br>6. Urinalysis<br>♦ Microscopy for pyuria<br>♦ Routine culture<br>♦ Sediment culture<br>♦ Epithelial cell sediment analysis | 1. Informed consent<br>2. Control completes questionnaire<br>3. MSU sample obtained<br>4. Peezy MSU collected (subset only)<br>5. Urinalysis<br>♦ Microscopy for pyuria<br>♦ Routine culture<br>♦ Sediment culture<br>♦ Epithelial cell sediment analysis |

Experiment one aimed to test three hypotheses which have been listed below:

1. A MSU specimen has equal merits to a CSU specimen for collecting urothelial cells.
2. The majority of cells found in urine specimens collected by MSU or CSU are urothelial cells and not contaminating skin cells from the perineum of vagina.
3. The MSU Peezy device reduces urinary contamination in urine specimens compared to a standard MSU.

These hypotheses were being tested because the literature often reported on the assumptions that a MSU specimen was contaminated more so than the CSU specimen. Contrary to those assumptions, it could be said that the MSU specimen has equal merits to, or is more superior than a CSU, hence the need to test the hypotheses. This formed the theoretical basis for this investigation and the theoretical framework for experiment one is displayed in Figure 4 below.

**Figure 4: Theoretical framework for experiment one**




Thus, testing these hypotheses it was important to ask research questions that connected to what was commonly reported within the literature. It was often reported that epithelial cells found floating in urine specimens were contaminants, but these claims were not evidenced with microbiological tests or advanced staining techniques which in

most cases would have determined cellular origin. Yet, the term contamination was widely accepted when cells and sediments manifested in a urine specimen. The research questions for this experiment have been listed below.

1. Are the epithelial cells found in urine specimens collected by MSU or CSU 'contaminants'? *(As defined in section 2.11 of the literature review)* or are they urothelial cells?
2. Is a MSU specimen as reliable as a CSU specimen in capturing the maximum sample of urothelial cells?
3. Does an MSU Peezy device reduce 'contaminants' *(as defined in section 2.11 of the literature review)* in urine specimens in comparison to a standard MSU?

### 3.3.1 Aims

The aim of this experiment was to compare the MSU specimen collection method with CSU specimen collection method by differential quantification of urothelial cells using uroplakin- 3 staining. Urothelial cells stain positive for uroplakin and these cells will indicate that they originate from the bladder. This study also aimed to compare the MSU specimen collection method with MSU Peezy device by analysing the differences in the urothelial cell counts and uroplakin-negative cell counts.

### 3.3.2 Research design and methods

This study was a single blinded, cross over design comparing three different urine sampling methods utilising two participant groups a patient group and control group. The patient group were recruited from a medical urology centre and were diagnosed as having a symptomatic UTI. The control group were recruited from staff members that worked within the centre and did not exhibit any symptoms of an infection. The urine samples from the patient participant group with a diagnosed infection were compared to the urine samples from the control participant group that did not have an infection. The literature reviewed prior to conducting these experiments was dated from 1969 to 2012 and influenced the research designs for the two experiments. Subsequently, newer literature retrieved from 2012 to 2015 supported the discussion and findings of the study.

This study was presented to the National Research Ethics Service Committee (NRES) in London-Harrow in April 2013 (REC Reference 11/ LO/1096, Protocol Number 11/0157, IRAS Project ID 72929) in order to obtain approval to conduct the investigation. Accompanying documents that were sent to obtain approval were consent forms, participant information sheets, urinary symptoms questionnaires, case report form (CRF) (Appendix 2), and GP letters (Appendix 3) notifying of participant enrolment. Ethical approval was granted, which signified that this study complied with conditions that were favourable and worthy of safe research practice. Ethical approval was given subject to all clinicians in the study having undergone training in Good clinical practice (GCP).

### 3.3.3 Selection of participants

As 1 in 3 women will be treated with antibiotics for a UTI by the age of 24 and 40% to 50% of women will experience one or more UTIs in their lifetime (Foxman and Brown 2003), women were identified as the main population of investigation. Female patients that attended the medical urology centre in a north London trust and who were being treated for a UTI were selected for this study. These patients were of various ages, all known to have LUTS and each patient was on antibiotic treatment for their chronic bladder infection. Although all patients were on antibiotic treatment for the UTI, they still had cells present in their urine, which was identified when a fresh unspun urine sample was examined with light microscopy during routine follow up consultations. Inclusion and exclusion criteria were defined to ensure that the participants (patient group and control group) had the ability to comply with all aspects of the data collection process. Tables 6 and 7 describe the rationale for this process.

**Table 6: Inclusion and exclusion criteria for the patient group**

| Inclusion criteria | Rationale | Exclusion criteria | Rationale |
|---|---|---|---|
| **Adult women aged 18 years old and older** | 1 in 3 adult women will be treated with antibiotics for a UTI by the age of 24. | **Women aged less than 18 years old** | Women over 18 are more likely to experience a UTI than adolescents. |
| **Ability to complete a bladder symptoms questionnaire** | Competence to answer questions relating to their bladder symptoms. | **Inability to consent** | All participants must provide consent to participate. |
| **Diagnosed with LUTS which include overactive bladder (OAB), painful bladder syndrome (PBS), recurrent UTI, acute cystitis and on antibiotic treatment** | Patients diagnosed with any one of these symptoms in most cases would have an inflammatory signal in the bladder which would capture urinary sediments and cells during the disease process. | **Women with concurrent illnesses that may compromise the validity of the data** | Concurrent illnesses may impede findings, which could cause flawed results. |
| | | **Pregnant women** | Pregnant women are exempt from invasive procedures and urine samples would not reflect accurate results due to hormonal changes in the body. |

**Table 7: Inclusion and exclusion criteria for the control group**

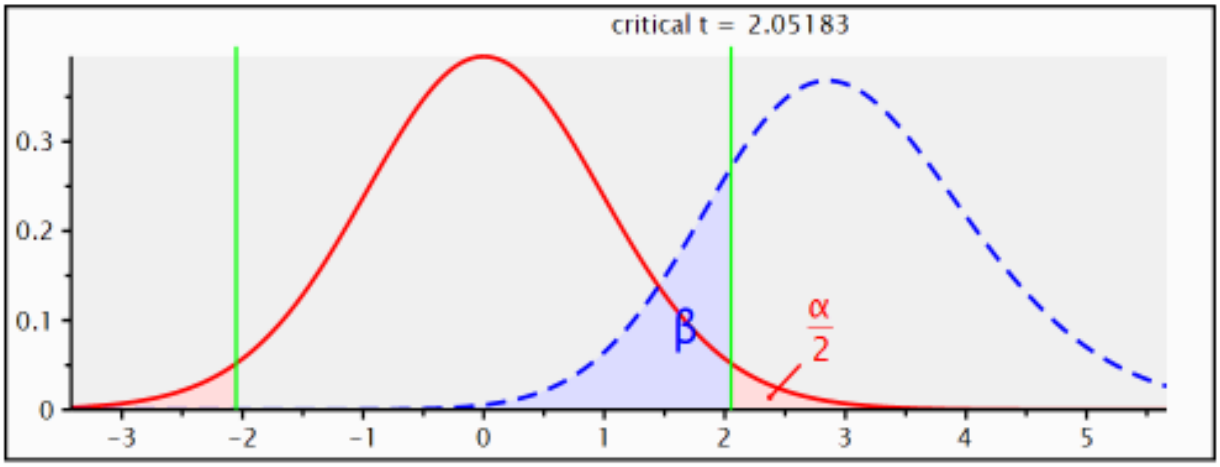
| Inclusion criteria | Rationale | Exclusion criteria | Rationale |
|---|---|---|---|
| **Adult women aged 18 years old and older** | To investigate the same population as the patient group. | **Women aged less than 18 years old** | Adult women were required as a control. |
| **Ability to complete a bladder symptoms questionnaire** | Competence to answer questions relating to their bladder to ensure they do not have any symptoms. | **Inability to consent** | All participants must provide consent to participate. |
| **Asymptomatic and not diagnosed with LUTS and not on antibiotic treatment** | A healthy bladder is needed to compare the difference between a bladder without an infection and a bladder infection. | **Women with concurrent illnesses that may compromise the validity of the data** | Healthy women without illnesses were required as controls. |
| | | **Pregnant women** | Urine samples from pregnant women would not reflect accurate results due to hormonal changes in the body. |

### 3.3.4 Participant sample size

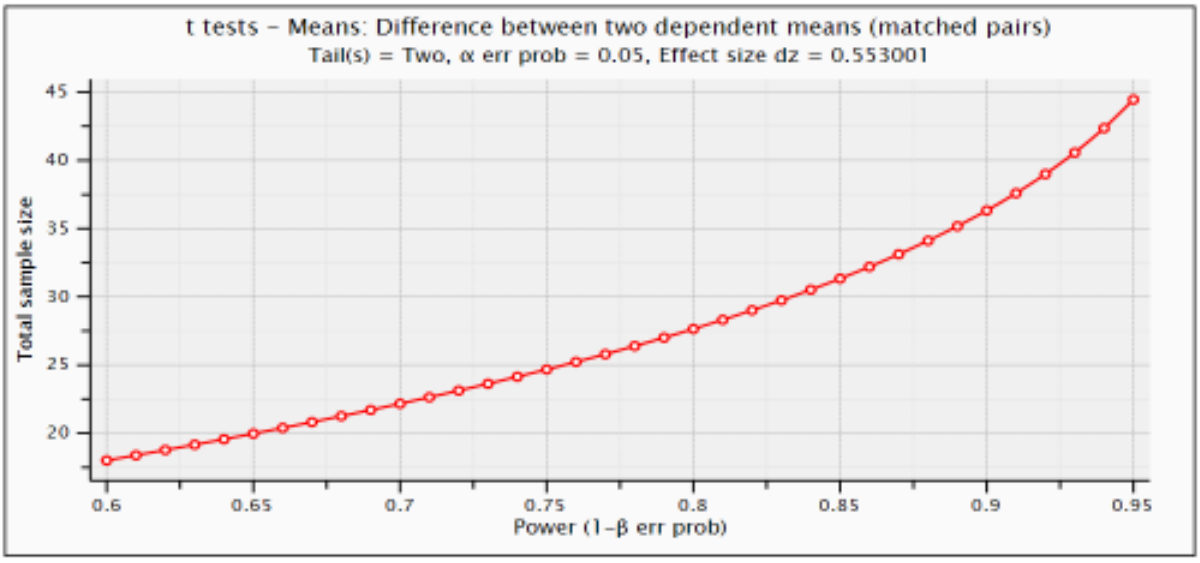
The sample size for this experiment (patient and controls) was calculated using the G Power software package. The G Power method calculated the difference between two dependent means because this study involved matched pairs. It was ascertained that there would be a clinically significant difference in the counts by obtaining data from an observational study. The observational study observed 201 women who were treated with antibiotics for UTI and diagnosed on account of pyuria on microscopy of a fresh unspun and unstained specimen of urine. The mean log wbc count $\mu l^{-1}$ at the start of treatment (mean =2.2, sd=1.9) was taken as well as the validated symptom scores that had a clinically significant response (mean = 1.2, sd=1.7). This gave an effect size (Cohen's d) of 0.55 which implies a medium effect size. Thus, a difference was estimated in the urine microscopy (10 wbc $\mu l^{-1}$) that would be clinically significant with the capacity to influence treatment decisions based on pyuria.

The t distributions (probability distribution when estimating the mean of a normally distributed population) for the two estimated outcomes is shown in figure 5. This assumed a two-tailed test. The plot of sample size against power is shown in figure 6. The effect size is Cohen's *d* =0.55; $\alpha$ = .05; power (1 – $\beta$ err probability) = .8; noncentrality parameter $\delta$ = 2.9; critical t= 2; df=27; sample size = 28; actual power = 0.8 or 80%. This means that it is necessary to keep sampling until at least 28 patients with pyuria have been recruited. It is expected that a majority will have zero pyuria and sampling will have to accommodate this phenomenon. It cannot be assumed that a zero pyuria will be reproduced across different specimen collection methods. Thus, it is not appropriate to reject patients found to have zero pyuria on the first analysis because that could introduce a selection bias. The practical implication is that the sample frame will be significantly larger than 28. Therefore, a sample size with a minimum of 30 was the aim.

## Figure 5: t distributions for the two estimated outcomes




## Figure 6: The plot of sample size against power



### 3.3.5 Sampling strategy for recruitment

The sampling strategy used to identify potential patient participants was convenience sampling of patients that attended the medical urology centre at a north London trust for treatment of a UTI. This sampling strategy was used because patients with known urinary symptoms were invariably asked to provide a urine sample for analysis when they presented and there was a high probability that their urinary sediments would contain key cells that were to be used in the outcome analyses (Lunn et al., 2010). The patients were approached by letter with a brief description of the study and a study information sheet. Each patient was provided with contact details and a response form with a stamped address envelope to report on their willingness to participate in the study. Controls were identified as staff and students from the north London trust. Posters and information sheets were distributed through the centre, informing potential controls of the study and what to do if they wanted to volunteer and participate. The telephone number and e-mail address of the research nurse were on the information sheets for direct contact.

### 3.3.6 Recruitment of patient and control participants

Patients who had read the information sheet and were happy to participate in the study expressed their interest by returning the response form in the stamped address envelope provided. The controls who were willing to participate in the study e-mailed the researcher notifying of their interest to participate in the study. Each participant who had contacted the researcher was given an appointment date and time for the screening process prior to enrolling in the study.

Patient and control participants were screened prior to study recruitment to determine eligibility to participate in the study. Demographic data were collected by asking patients and controls specific questions such as age, bladder symptoms, pregnancy and many other questions which would determine whether they were eligible for enrolment. The data were documented on a case report form (CRF) (Appendix 2) and reviewed twice to

ensure the participants were suitable. The recruitment schedule for both patient and control participants is displayed in table 5 earlier in this chapter.

### 3.3.7 Attendance at the medical urology centre

The patient participants attended the medical urology centre for their first visit. Upon arrival, they reported to the reception desk to notify of their attendance and met with the researcher. At the study visit, detailed verbal descriptions of the potential risks and benefits of the study were explained to the participants and their families who were present. Participants who wished to be enrolled in the study were given the opportunity to read through the patient information sheet again (see Appendix 4). Participants had the opportunity to discuss with the researcher any queries or concerns that had risen whilst reading through the information sheet.

The control participants attended the medical urology centre for their first and only visit. They were familiar with the centre and therefore notified the researcher by telephone of their presence. At the study visit, detailed verbal descriptions of the potential risks and benefits of the study were explained to the control participants. Those who wished to be enrolled in the study were given the opportunity to read through the control information sheet again (Appendix 5). They also had the opportunity to discuss with the researcher any queries or concerns that had risen whilst reading through the information sheet. Attendance to the medical urology centre is displayed in in table 5 earlier in this chapter.

### 3.3.8 Informed consent and anonymity

Patient and control participants provided written consent on a printed consent form (Appendix 6), were given a copy for their records, a copy was filed in the site research file and a copy in clinical hospital notes. Both patient and control participants were informed of their rights to withdraw consent from the study at any time. To ensure patient and control participant anonymity and confidentiality all participants were given a study ID number that was not identifiably referenced to the participant. This unique number was used throughout the study and incorporated on the CRF and all other documents relating

to study participants. The chief investigator and the primary researcher were the only two clinicians that had access to the results of the study for analysis.

### 3.3.9 Symptoms' questionnaires

Each patient and control participant enrolled into the study completed an international consultation on incontinence questionnaire for female lower urinary tract symptoms (ICIQ-FLUTS) (Appendix 7). The 10-item scale questionnaire is a validated tool used to measure LUTS scores (Al Buheissi et al., 2008). The questionnaire was subdivided to measure pain and urgency symptoms. Pain and urinary urgency symptoms were also measured using a 10-item scale (Peters et al., 2008), and the symptoms' scores were later enumerated. Control participants were also required to complete the questionnaire as a standard comparison to the patient group, even though the control group were asymptomatic. The patient group also completed an international consultation on incontinence quality of life questionnaire (ICIQ-LUTSqol) (Appendix 8). This questionnaire was used to measure the impact of symptoms on activities of daily living and the wider quality of life. The controls were not required to complete this questionnaire as they did not have symptoms.

### 3.3.10 Process of urine specimen collection

The order of urine collection was randomised using a simple randomisation technique. A random sequence was generated using a Microsoft office excel spread sheet which allocated the order of sampling assignments for each patient (Altman and Bland, 1999). This approach to randomisation was straightforward and eliminated selection bias (Suresh, 2011).

Three different urine collection methods were used as comparators and these methods have been described further along in this chapter. The patient participants provided a urine sample by MSU, CSU, and in a subset, a Peezy MSU sample. The Peezy MSU samples were included as a subset to compare it against two common methods of urine specimen collection. The patient group were symptomatic and benefitted from urine sediment

culture results obtained from both non-invasive and invasive urine collection methods. The urine samples were collected one hour apart and the order in which the urine samples were collected was determined by simple randomisation which is explained further later in this chapter.

Controls provided urine samples by MSU only, and in a subset, the Peezy MSU to compare it against a common method of specimen collection. The subset Peezy specimens were chosen by random allocation. The control group provided one or two non-invasive urine specimens. The control group did not have any bladder symptoms and would not have merited a specimen obtained from an invasive method. This group was an important comparator, as they were regarded as the 'healthy non-infected bladder'. It was necessary to compare the urinary sediments from the healthy bladder to that of an infected bladder, to distinguish what constitutes contamination found in urine specimens. Table 8 describes the selection of urine collection methods. When all samples were collected, the participants were free to leave the centre.

**Table 8: Selection of urine collection methods**

| **Specimen collection method** | **Patient participants** | **Control participants** |
|---|---|---|
| **MSU** | Standard method of urine specimen collection | Standard method of urine specimen collection |
| **CSU** | Symptomatic and would merit a specimen collected from an invasive technique. | Asymptomatic and would not merit a specimen collected from an invasive technique. |
| **Peezy MSU** | To compare it against two common methods of urine specimen collection. | To compare it against one common method of urine specimen collection. |

**Midstream urine (MSU)**

Based on standard techniques of non-invasive urine sampling (Dougherty and Lister, 2011), the urine collection method used within the medical urology centre was the midstream urine sampling technique. This technique is described below:

- The participant washes their hands.
- Using a wet wipe, she thoroughly cleanses the genital area. She will be instructed to hold the outer edges of the labia apart and cleanse the introitus from front to back with the wet wipe.
- Without interrupting urinary flow, she passes the collecting bowl into the urine stream and collects a suitable specimen.
- As the stream comes to the end, the collecting bowl is moved away and the rest of the void is passed into the toilet.
- The participant washes their hands again at the end of sample collection.

**Catheter specimen of urine (CSU)**

The second urine collection method was accomplished by inserting a urinary catheter into the urethra to obtain the urine sample. The technique of collecting this urine sample is described below:

- The clinician washes her hands and opens a sterile dressing pack onto a clean trolley and prepares an aseptic field.
- She ensures that the woman is in a comfortable position on the bed with legs apart, knees bent and the perineal area exposed.
- The clinician pours some water based sterile lubricating jelly into the galipot, without touching the aseptic field, and empties the sterile Nelaton Plastic Pennine Catheter, CH12 female length catheter onto the aseptic field.
- She washes her hands again and then puts on the sterile gloves from the dressing pack, avoiding touching the outside of the gloves.
- The clinician places the sterile dressing towel and receptacle on the bed in front of the woman's perineal area and, using the gauze provided in the dressing pack, the clinician holds apart the labia.

- With her other hand, she dips the catheter in the lubricating jelly and by using a sterile non-touch technique inserts the catheter into the bladder via the urethra.
- A specimen is drained into the sterile container.
- The catheter is then removed.

**Peezy MSU**

The third urine collection method was obtained by urinating into the external device called Peezy MSU. The technique has been described below:

- The participant washes their hands.
- Using the wipe provided, she thoroughly cleanses the genital area.
- She then attaches the collection bottle to the Peezy, and positions the Peezy device against the body, and passes urine.
- As the patient begins to pass urine, the first part of the stream enters the funnel and begins to cause a piece of sponge to swell, thereby blocking the flow through the funnel.
- The midstream specimen is then channelled into the universal container, and the remainder is channelled through an overflow duct and into the toilet.
- The Peezy device is then discarded into the clinical waste bin.

**Random selection for the Peezy MSU**

This method of randomisation was generated using a Microsoft office excel spread sheet which allowed a simple sequence of random assignments of ‘yes’ and ‘no’ beside each of the Peezy collection allocations.

### 3.3.11 Blinding and laboratory procedures

This study was not blinded to the specimen collection methods used. This meant that the investigator and participants knew which urine specimen was being collected; however, the investigator was blinded when analysing the specimen and did not know the order in which the specimen was collected. It was intended to prevent the researcher from having

preconceived ideas of specimen collection order which could influence bias (Polit and Beck, 2012). The laboratory procedures that were used in this experiment have been described in table 9. A detailed explanation for why these procedures were utilised has been provided later in this chapter.

**Table 9: Laboratory procedures**

| Procedure | Rationale |
|---|---|
| Dipstick analysis | A standard method in the NHS to detect the presence of a UTI |
| Urine microscopy | To evaluate the presence of urine cells from a fresh unspun specimen |
| Routine culture | A standard routine method at the north London Trust for all urine samples obtained from in-patients and community patients |
| Sediment culture | A sediment culture is the gold standard for diagnosing the pathogens that are responsible for a UTI |
| Epithelial cell sediment | To identify whether the cells found in the urine are urothelial cells using the UP3 staining technique |

**Dipstick urinalysis**

All urine samples were analysed using the Siemens Multistix 10 Sg Reagent Strips. This urinalysis test is commonly used by clinicians to detect the presence of leukocyte esterase, red blood cells, protein, glucose and nitrites using the colorimetric strips.

The Multistix were dipped into the urine samples and left to sit for one minute before analysing the results. A positive Multistix test for leukocytes esterase and nitrites is considered a true diagnosis of a UTI (Mody and Juthani-Mehta, 2014). Although the dipstick method of analysis is flawed by its insensitivity (Khasriya et al., 2010), it was incorporated in the laboratory methods because it is a standard urinalysis test.

**Urine microscopy**

Based on Dukes' (1928) method of microscopic analysis, all fresh unspun urine samples were examined microscopically for the presence of pyuria and urothelial cells after the sample was collected. Microscopic urinalysis is an important aspect of diagnosing UTI as it

is used to look for formed cellular elements, casts, bacteria, yeast, parasites, and crystals (Burd and Kehl, 2011). A disposable 1ml pipette was loaded with a urine sample and the sample was placed on a clean haemocytometer and examined on an Olympus CX41 microscope with a magnification of x200. The leucocytes, red blood cells and epithelial cells found in each urine sample were enumerated.

**Routine culture**

All urine samples were sent to the north London Trust for routine culture and sensitivities. Diagnosing a UTI from a urine culture is currently based upon the criteria described by Kass (1957). Kass studied the MSU samples in women with chills, fever, flank pain and dysuria and who had grown more than $10^6$ bacteria colony forming unit's $ml^{-1}$ (cfu $ml^{-1}$). Kass came to the conclusion that $10^5$ cfu $ml^{-1}$ of a known urinary pathogen should be the threshold between true bacteriuria and contamination. This enumeration has become widely adopted and applied to a broad spectrum of different disease states in the National Health Service (NHS).

**Urine sediment culture**

A sediment extraction culture assay for the evidence of urine infection was carried out. All urine samples were stored in a 30 mls container and spun down in a Denley refrigerated centrifuge machine. This enabled the sediment in the sample to migrate to the base of the container so that it could be extracted using a 50 microlitre (uL) pipette. The sediment extract was diluted to five times the volume with phosphate buffered saline (PBS) serial dilution. 50 uL of the diluted urinary sediment (this is called the "neat" suspension which can be confusing) was plated onto an aerobic chromo genic agar plate (CPS3) and dispersed using a polypropylene cell L-shaped spreader. The culture plates were stored in an incubator set at 37$^o$C. Following a 24 hour incubation period, the organisms present on the culture plate were identified using the Chrom ID™ colour chart (Biomerieux, 2013) and quantified (Appendix 9). All organisms were enumerated by the researcher and double counted by a laboratory assistant to ensure there were no omissions or mistakes.

**Epithelial cell sediment**

According to Cipressa et al. (2014) DAPI cell staining is a key technique in cell biology that allows the stain to bind to cell DNA, and provides a direct visualisation of the localised patterns of cellular DNA content in order to help comprehend the cellular function. The DAPI stain technique was adapted by incorporating the seminal works of Webber et al., (1978). Their protocol for immunofluorescent tissue cell microscopy provided a comprehensive method for tissue fixation and fluorescent staining. Table 10 describes the techniques used.

**Table 10: Materials and methods for immunofluorescent cell microscopy**

**(Webber et al., 1978)**

**Preparation of Samples**

1. Procedure 1: Fix cells in 4% formaldehyde onto slide.
2. Permeabolise cells
3. Rinse four times with Pi/NaCl, phosphate-buffered saline.
4. Add primary antibody (0.05 mg/ml in Pi/NaCl) and incubate 45 minutes then wash well with Pi/NaCl.
5. Add fluorescein-labelled goat anti-rabbit antibody and incubate 45 minutes then wash well with Pi/NaCl.
6. Add 2 mls of deionized water to the 10mg vial of DAPI (5mg/ml solution)
7. Extract 2 microliter (µl) of DAPI solution and add to 100 µl of PBS creating a 300 micrometre (µM) DAPI intermediate dilution
8. Extract 1 µl of the 300 µM and add to 100 µl of PBS to create a 300 nanometre (uM) DAPI solution 1:100 per slide

**For immunofluorescence**

1. Mount microscopy coverslips to slide
2. Place slide in fluorescent microscope chamber for viewing.

This cell staining technique was suitable for identifying cell morphology, the nucleus and bacteria adherent to or inside cells. Whilst morphological characteristics can give the origin of some cells away, it is not always that clear and a staining method is necessary to bring some precision to the task. However, morphological analysis with DAPI staining was not enough; it binds via a non-intercalative mechanism to adenine and thymine rich regions of DNA (Portugal and Waring, 1988) so it shows up DNA but other cell characteristics are not so clear. It was found, later in the project, that a much better

option was to stain the cells for uroplakin, which is only present in cells of urothelial origin. Thus, from the tenth patient uroplakin staining proved to be a very important step. The uroplakin staining and linked florescence microscopy technique enable a confident identification of cells as coming from the urinary tract.

The protocol for this technique has been described by Esposito et al., (2009) and was followed with improving adaptations described by Horsley et al., (2013). Table 11 describes the laboratory protocol that was used. All urine specimens collected were stained with uroplakin on the same day. The uroplakin staining process was carried out in eleven separate stages.

**Step 1:** A glass slide was prepared by writing the patients study number, their initials, the urine sample method and the date of which the sample was obtained.

**Step 2:** A cuvette was assembled. A cuvette is made up of four components. The first component is the metal frame that locks and holds together the three supporting components of the cuvette. The second component is a plastic funnel chamber that channels the urinary cells so that they migrate on to the glass slide. The third component is the filter paper that filters excess urine away from the migrated cells which are on the glass slide, and the fourth component is the glass slide that collects the flattened cells that have migrated. A cryopen is then used to draw a circle at the base of the cuvette, to indicate where the cells will be placed once they have been collected on the glass slide.

**Step 3:** The cuvette was then placed in the Thermo Scientific Shandon Cytospin Cytocentrifuge machine and 80μl of a urine sample was pipetted into the funnel that was placed in a Cytospin Cytocentrifuge for 5 minutes at 800rpm (centrifugal force ~75 RCF). Thermo Scientific (2008), the manufacturing company of the Shandon Cytospin, explains that the machine aids the deposit of cells onto a clearly-defined area of a glass slide and allows for the absorption of the residual fluid into the sample chamber's filter card. Cytocentrifugation also constructively flattens cells for excellent nuclear presentation. During operation, the instrument's spinning action tilts the Shandon Cytofunnels upright and centrifuges cells onto the deposition area of the slide, giving all cell types equal

opportunity for presentation.

**Step 4:** Once the Cytospin Cytocentrifuge had stopped the cuvette was carefully removed from the machine and dissembled. The urinary sediment on the glass slide was then circumscribed with a hydrophobic barrier pen which prevented the urinary sediment from bypassing onto other parts of the slide.

**Step 5:** The cellular deposit on the slide was then fixed and preserved with 4% formaldehyde in Phosphate Buffered Saline (PBS) for 15 minutes and followed by washing three times with PBS.

**Step 6:** The next step was to permeabolise the cells (poke holes into the cell membrane) with 0.2% Triton X for 5 minutes. This process allows the stain to enter the cells and bind to intracellular in addition to extracellular binding sites enabling the stain to adhere to the uroplakin in the cell (Jamur and Oliver, 2010). There is a possibility that the Triton X may reduce the effectiveness of the hydrophobic barrier pen, so active monitoring of the slide was essential. After 5 minutes the cells were washed three times with PBS.

**Step 7:** 100 µl of 10% normal goat serum was then applied to the fixed cells for 30 minutes. The normal goat serum was applied in order to block non-specific binding sites. This had to be from the same species as the secondary antibody was raised in.

**Step 8:** After 30 minutes the normal goat serum was aspirated and replaced with 50µl of primary antibody mouse anti uroplakin 3 (raised in a mouse). The primary antibody was applied for 1 hour and later washed three times.

**Step 9:** The secondary antibody (goat anti mouse conjugated to Alexa-flour 488) was applied to the urinary cell sediment on each of the slides. The cells were incubated for 40 minutes in order that the goat antibody, that carried the fluorescein, adhered to and bound to the mouse antibodies on the uroplakin. After 40 minutes the cells were washed three times with PBS.

**Step 10:** At the end of the incubation period, a blue DAPI stain was applied to the cells. This was used in order to ensure that DNA in the cell nucleus be clarified. DAPI will stain

DNA in any bacteria that might be present in or on the cells. Identifying cell-associated microbes was not part of this project but making the nucleus highly visible helped in the counting of cells. The DAPI stain was left on the cells for 10-20 minutes. The cells were later washed three times with PBS.

**Step 11:** The cells on the slides were carefully mounted using a small amount of FluorSave reagent. This reagent was applied to preserve the cell fixation and enable a good visualisation of cells when fluorescent microscopy was to be used. A small square cover slip was applied on top of the reagent, and the four corners of the cover slip were sealed with clear nail varnish. The slides were kept in the dark shade, to avoid premature activation of the fluorescein, until examination with the fluorescent microscope was ready.

| **Table 11** | **Uroplakin-3 immunofluorescent cell staining protocol** |
|---|---|

1. Label the glass slide with the study number of the sample, date and researcher's initials.

2. Assemble the cuvette, placing the components in the following order; microscope slide, filter paper, funnel (filter paper fuzzy side up, slide frosted side up), marking the spot on the base of the slide using a cryopen.

3. Place the cuvette into the cytospin, ensuring that it is counterbalanced with another sample or a blank cuvette.

4. Pipette 80µl of the sample into the funnel and cytospin it for 5 minutes at 800rpm.

5. Carefully remove the cuvette from the cytospin and dissemble it, circumscribing the urinary sediment with a hydrophobic barrier pen.

6. Fix the cellular deposit with 4% formaldehyde in PBS for 15 minutes. Wash three times with PBS

7. Permeabolise (pokes holes into the cell membrane) with 0.2% Triton X for 5 minutes (note – this may reduce the effectiveness of the hydrophobic barrier pen). Wash three times with PBS. **Diluting 100% Triton X: get 99.8µl PBS +0.2µl Triton X = 100µl per slide.**

8. Block with normal goat serum 10% for 30 minutes (must be same species as your secondary was raised!) **Diluting 100% Goat Serum: 90µl PBS +10µl Goat Serum =100µl of 10% Goat serum**

9. Aspirate the blocking agent and replace it with 50µl of a 1:10 dilution of primary anti-UP3 antibody in PBS. Incubate for 1 hour. Wash three times with PBS. **Diluting 100% UP3: Take 5 µl UP3 +45 µl PBS= 50 µl per slide**.

10. Apply 50µl of a 1:250 dilution of fluorescein secondary antibody in 1% BSA to the sample and incubate for 40 minutes. Fluorescein in fridge (FITC) and labelled antibody goat anti mouse No: 488. Wash three times with PBS. **Mix 8µl of Fluorescein+ 42 µl BSA= 50 µl per slide**

11. Stain the deposit with a 1:100 dilution of DAPI in PBS for 10-20 minutes. Wash thrice with PBS. Carefully mount the slide using a small amount of FluorSave reagent. Seal with clear nail varnish. keep the slide in the dark at 4C until it can be examined with a fluorescence microscope.

**Florescent microscopy**

Each slide was examined using epi-fluorescent microscopy on an Olympus CX-41 and Leica DM4000B upright microscope in a dark room. The dark room was important, as slides exposed to direct light were at risk of light damage. All images were processed and analysed using Infinity Capture and Analyze V6.2.0, ImageJ 1.46r and the Leica Application Suite, Advanced Fluorescence 3.1.0 build 8587 software. During the staining process a cryopen had been used to draw a circle at the base of the glass slide, to identify where the cells would be located prior to florescent microscopy. The entire circle where the cells had been deposited was examined. The sensitivity of visualising the uroplakin positive cells was based on using the lowest visual scope magnification of 10x and gradually increasing the magnification as soon as the cell images on the slides were in focus. Once cell images on the slides were in focus the magnification was increased to 40x and the images of the cells were centralised to fit in the centre of the florescent microscope. Images of particular cells were electronically captured and stored for future reporting. All cells were enumerated by the researcher and double counted by a laboratory assistant to ensure there were no omissions or mistakes.

### 3.3.12 Statistics and data analysis

SPSS, a computerized statistical programme that calculates the statistics of data captured from quantitative research (Ghasemi and Zahediasl, 2012), was used to analyse the entire data collected. This programme supported the production of descriptive statistics and a frequency distribution of urinary symptoms, uroepithelial cell sediment and uropathogenic organisms which were considered to originate from lower urinary tract. Nonparametric tests were carried out to measure the statistical relationships. Agreement between test results was examined by the Bland-Altman method. The data were analysed using the several statistical methods. The Wilcoxon matched pairs is a nonparametric statistical test that compares two related samples to assess whether the mean ranks differ, if the pairs have a similar pattern, the positive and negative ranks should appear equal (Field, 2013). This is an alternative to the t-test when the population analysed is not normally distributed. A t-test was also used in order to measure the equality of means as

well as a Levene's test for equality of variances. These preliminary data were small and not normally distributed; therefore, the Wilcoxon matched pairs test was the appropriate statistical test to use. The Spearman's rank correlation coefficient (Spearman's R) is another nonparametric test that measures the relationship between two variables using a monotonic (continuous/ordinal) function (Field, 2013). The data distributions are tabled for each variable and these data include the 95% confidence intervals so that comparisons can be easily inferred. This test was used to understand the comparisons between groups. Cohen's Kappa coefficient is a statistical measure of agreement (Field, 2013) and where appropriate in this study the Bland-Altman analysis was used to examine agreement rigorously. Similarly, the Bland-Altman plot is a method of plotting data to measure the agreement between two different variables (Field, 2013), and clearly shows the arrangement of each variable.

**Rationale for using these statistical methods**

The purpose of reporting correlations (Spearman R), agreement (Cohen's Kappa and the Bland Altman Plot) and difference between pairs (Wilcoxon Matched pairs and the t-test) was to cover the confusions that can arise when these analyses are deployed. If two tests agree they must correlate but the reverse is not true; if they correlate they do not necessarily agree. Similarly, if pairs agree they should not differ on a matched pairs test, but data that do not agree could still fail to show a statistically significant difference. The limits of Cohen's Kappa are also illustrated by these data and the unquestioning reliance on a single statistical perspective is well shown.

## 3.4 Experiment two: comparing three different non-invasive urine specimen collection methods

**Introduction**

Experiment one compared non-invasive urine specimen collection methods with an invasive method. Experiment two was an important follow up component of the empirical study. In order to validate that a non-invasive method of urine specimen

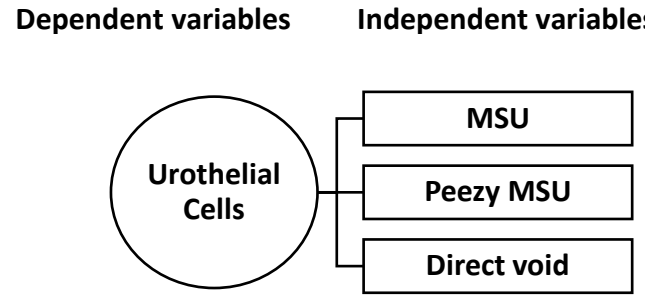
collection has equal merits to an invasive method, it was necessary to identify the different non-invasive methods used in clinical practice, which method is superior and has the ability to capture the majority of urothelial cells that are indicative of a UTI. The processes deployed to achieve this have been described in experiment two below. Table 12 describes the process and action taken for experiment two.

**Table 12: Study schedule for experiment two**

| Study Schedule | Patient actions |
|---|---|
| **Sampling strategy** | Patients sent information sheet. |
| **Recruitment** | Participant contacted to arrange a date for visit 2, 3 &4 |
| **Visit 2 (Experiment Two)** | 1. Informed consent<br>2. Non-invasive urine specimen collected<br>♦ Microscopy for epithelial cells<br>♦ Epithelial cell sediment analysis |
| **Visit 3 (Experiment Two)** | 3. Non-invasive urine specimen collected<br>♦ Microscopy for epithelial cells<br>♦ Epithelial cell sediment |
| **Visit 4 (Experiment Two)** | 4. Non-invasive urine specimen collected<br>♦ Microscopy for epithelial cells<br>♦ Epithelial cell sediment |

Experiment two aimed to test the hypothesis that a urine specimen collected freely by direct void is a superior method of specimen collection for capturing urothelial cells, compared to the MSU and the MSU Peezy specimens. This hypothesis was being tested because it was reported in the literature that urine specimens collected by a straight forward direct void is more likely to harbour contaminants (Frazee et al., 2015). Contrary to these reports, it could be said that directly voided urine (natural urination) captures the true pathology of a UTI, encompassing a majority of the cells and sediments that are indicative of an infection, hence the need to test this hypothesis. This formed the theoretical basis for this investigation and the theoretical framework for experiment two is displayed in Figure 7.

**Figure 7: Theoretical framework for experiment two**




In testing this hypothesis, it was essential to ask a research question that connected to what was commonly reported within the literature. As previously mentioned, it was often reported that epithelial cells found floating in urine specimens were contaminants, but these claims were not evidenced with advanced staining techniques which in most cases would have determined cellular origin and refuted the assumptions of contamination. The term contamination was widely accepted when cells were identified in a specimen of urine. The research question for this experiment has been identified as:

'Does a directly voided urine specimen (natural urination), using no special technique, exhibit more contamination *(as defined in section 2.11 of the literature review)* than a standard MSU or a MSU collection device?

### 3.4.1 Aims

The aim of experiment two was to compare three non-invasive urine collection methods: the standard MSU, MSU Peezy urine collection device and directly voided urine (natural urination) by urothelial cell count and urothelial cell staining technique using uroplakin- 3. Urothelial cells stain positive for uroplakin and these cells will indicate that they originate from the bladder.

### 3.4.2 Research design and methods

This study was a single blinded, cross over design comparing three different non-invasive urine specimen collection methods involving the patient participants only from the first experiment. As in the first experiment, the patient participants were recruited from the medical urology centre where they had been diagnosed as having a symptomatic UTI and were undergoing treatment for the infection. This second phase of the study was presented to the National Research Ethics Service Committee (NRES) in London-Harrow a year later in April 2014 (REC Reference 11/ LO/1096, Protocol Number 11/0157, IRAS Project ID 72929), in order to obtain approval to conduct the investigation. Accompanying documents that were sent to obtain approval included a consent form (Appendix 10) and participant information sheet (Appendix 11) for experiment two. Ethical approval was granted, which signified that this study complied with conditions that were favourable and worthy of safe research practice in the second part of the investigation.

### 3.4.3 Selection of participants and sample size

The patient participants were known to have LUTS and were also being treated in the specialist medical urology centre. Since that they were eligible for experiment one, they were also suitable participants to continue to experiment two. The sample size for experiment two was calculated using the G Power software package as it was used in experiment one. The principle of sample size calculation described earlier in this chapter remained the same for experiment two.

### 3.4.4 Sampling strategy and recruitment of patient participants

The patient participants were approached by letter with a brief description of the second study and a study information sheet. They were identified as suitable participants because they were eligible for the first study and active patients receiving treatment for their UTI. Each patient was provided with telephone contact details and a response form with a stamped addressed envelope to report on their willingness to participate. Patients who had read the information sheet and were happy to participate in the study expressed their interest by returning the response form in the stamped address envelope provided.

Each patient participant who had contacted the researcher and expressed their willingness to participate in the study, was given an appointment date and time to attend the medical urology centre for enrolment.

### 3.4.5 Attendance at the medical urology centre

The patient participants attended the medical urology centre for their second visit. Upon arrival, they reported to the reception desk to notify their attendance and to meet with the researcher. During the second study visit, detailed verbal descriptions of the potential risks and benefits of the study were explained to the participants. Patient participants who wished to be enrolled in the study were given the opportunity to read through the patient information sheet again (Appendix 11). They had the opportunity to discuss with the researcher any queries or concerns that had risen whilst reading through the information sheet. The following day patient participants attended the medical urology centre for their third visit. Having reported to the reception desk and met with the researcher, they were given the specimen collection bowl or device to provide the sample, depending on which method they had been randomly selected. Lastly, the patient participants attended the medical urology centre for their fourth and final visit. Everything they had done in the previous visits was repeated, but the method of urine collection differed according to the specimen collection method selected. Attendance at the medical urology centre is shown in table 12 earlier in this chapter. Patient participants were informed of their rights to withdraw consent from the study at any time. They provided written consent on a printed consent form (Appendix 10), were given a copy for their records, a copy was filed in the site research file and a copy in clinical hospital notes. The patient participants were given a study ID number when they had enrolled in the first study and this unique number was used throughout.

### 3.4.6 Process of urine specimen collection

The order of urine collection was randomised using the simple randomisation technique explained earlier in this chapter. This approach to randomisation was used again as it was straightforward and eliminated selection bias (Suresh, 2011). Three non-invasive urine

collection methods were used as comparators. These methods were MSU, Peezy MSU and direct void and were collected on three different consecutive days. The purpose of each collection method was to determine which technique captured the majority of cells and sediments that imply a UTI. The MSU and Peezy MSU techniques were explained earlier in this chapter. The direct void method is described below.

**Direct void with no technique**

- The participant is given a collection bowl
- The participant washes their hands
- She then urinates the entire volume of urine directly into collection bowl with no technique

Each patient participant was given the specimen collection bowl or urine specimen device to provide the sample. When each sample had been collected, the participants were free to leave the centre.

### 3.4.7 Laboratory procedures

The laboratory procedures that were used for experiment two have been described in table 13. A detailed explanation for why these procedures were used has been provided below.

**Table 13: Laboratory procedures experiment two**

| Procedure | Rationale |
|---|---|
| Urine microscopy | To evaluate the presence of urine cells from a fresh unspun specimen |
| Epithelial cell sediment | To identify whether the cells found in the urine are urothelial cells using the UP3 staining technique |

**Urine microscopy**

Based on Dukes' (1928) method of microscopic analysis, all fresh unspun urine samples were examined microscopically for the presence of urothelial cells after the sample was collected. Microscopic urinalysis is an important aspect of diagnosing a UTI as it is used to

look for formed cellular elements (Burd and Kehl, 2011). A disposable 1ml pipette was loaded with a urine sample and the sample was placed on a clean haemocytometer and examined on an Olympus CX41 microscope with a magnification of x200. The leucocytes, red blood cells and epithelial cells found in each urine sample were enumerated.

**Epithelial cell sediment**

The uroplakin staining and linked florescence microscopy technique enable a confident identification of cells as coming from the urinary tract. The protocol for this technique has been described by Esposito et al., (2009) and was followed with improving adaptations described by Horsley et al., (2013). Table 14 describes the laboratory protocol that was used. All urine specimens collected were stained with uroplakin on the same day. The uroplakin staining process was carried out in eleven separate stages as it was done in experiment one.

**Step 1:** A glass slide was prepared by writing the patients study number, their initials, the urine sample method and the date of which the sample was obtained.

**Step 2:** A cuvette was assembled. A cuvette is made up of four components. The first component is the metal frame that locks and holds together the three supporting components of the cuvette. The second component is a plastic funnel chamber that channels the urinary cells so that they migrate on to the glass slide. The third component is the filter paper that filters excess urine from the migrated cells which are on the glass slide, and the fourth component is the glass slide that collects the flattened cells that have migrated. A cryopen is then used to draw a circle at the base of the cuvette, to indicate where the cells will be placed once they have been collected on the glass slide.

**Step 3:** The cuvette was then placed in the Thermo Scientific Shandon Cytospin Cytocentrifuge machine and 80µl of a urine sample was pipetted into the funnel that was placed in a Cytospin Cytocentrifuge for 5 minutes at 800rpm (centrifugal force ~75 RCF). Thermo Scientific (2008), the manufacturing company of the Shandon Cytospin, explains that the machine aids the deposit of cells onto a clearly-defined area of a glass slide and allows for the absorption of the residual fluid into the sample chamber's filter card.

Cytocentrifugation also constructively flattens cells for excellent nuclear presentation. During operation, the instrument's spinning action tilts the Shandon Cytofunnel upright and centrifuges cells onto the deposition area of the slide, giving all cell types equal opportunity for presentation.

**Step 4:** Once the Cytospin Cytocentrifuge had stopped, the cuvette was carefully removed from the machine and dissembled. The urinary sediment on the glass slide was then circumscribed with a hydrophobic barrier pen which prevented the urinary sediment from bypassing onto other parts of the slide.

**Step 5:** The cellular deposit on the slide was then fixed and preserved with 4% formaldehyde in Phosphate Buffered Saline (PBS) for 15 minutes and followed by washing three times with PBS.

**Step 6:** The next step was to permeabolise the cells (poke holes into the cell membrane) with 0.2% Triton X for 5 minutes. This process allows the stain to enter the cells and bind to intracellular in addition to extracellular binding sites enabling the stain to adhere to the uroplakin in the cell (Jamur and Oliver, 2010). There is a possibility that the Triton X may reduce the effectiveness of the hydrophobic barrier pen, so active monitoring of the slide was essential. After 5 minutes the cells were washed three times with PBS.

**Step 7:** 100 µl of 10% normal goat serum was then applied to the fixed cells for 30 minutes. The normal goat serum was applied in order to block non-specific binding sites. This had to be from the same species as the secondary antibody was raised in.

**Step 8:** After 30 minutes the normal goat serum was aspirated and replaced with 50µl of primary antibody mouse anti uroplakin 3 (raised in a mouse). The primary antibody was applied for 1 hour and later washed three times.

**Step 9:** The secondary antibody (goat anti mouse conjugated to Alexa-flour 488) was applied to the urinary cell sediment on each of the slides. The cells were incubated for 40 minutes in order that the goat antibody, that carried the fluorescein, adhered to and bound to the mouse antibodies on the uroplakin. After 40 minutes the cells were washed three times with PBS.

**Step 10:** At the end of the incubation period, a blue DAPI stain was applied to the cells. This was used in order to ensure that DNA in the cell nucleus be clarified. DAPI will stain DNA in any bacteria that might be present in or on the cells. Identifying cell-associated microbes was not part of this project but making the nucleus highly visible helped in the counting of cells. The DAPI stain was left on the cells for 10-20 minutes. The cells were later washed three times with PBS.

**Step 11:** The cells on the slides were carefully mounted using a small amount of FluorSave reagent. This reagent was applied to preserve the cell fixation and enable a good visualisation of cells when fluorescent microscopy was to be used. A small square cover slip was applied on top of the reagent, and the four corners of the cover slip were sealed with clear nail varnish. The slides were kept in the dark shade, to avoid premature activation of the fluorescein, until I was ready to examine with the fluorescence microscope.

| **Table 14.** | **Uroplakin-3 Immunofluorescent cell staining protocol** |
|---|---|

1. Label the glass slide with the study number of the sample, date and researcher's initials.

2. Assemble the cuvette, placing the components in the following order; microscope slide, filter paper, funnel (filter paper fuzzy side up, slide frosted side up), marking the spot on the base of the slide using a cryopen.

3. Place the cuvette into the cytospin, ensuring that it is counterbalanced with another sample or a blank cuvette.

4. Pipette 80µl of the sample into the funnel and cytospin it for 5 minutes at 800rpm.

5. Carefully remove the cuvette from the cytospin and dissemble it, circumscribing the urinary sediment with a hydrophobic barrier pen.

6. Fix the cellular deposit with 4% formaldehyde in PBS for 15 minutes. Wash three times with PBS

7. Permeabolise (pokes holes into the cell membrane) with 0.2% Triton X for 5 minutes (note – this may reduce the effectiveness of the hydrophobic barrier pen). Wash three times with PBS. **Diluting 100% Triton X: get 99.8µl PBS +0.2µl Triton X = 100µl per slide.**

8. Block with normal goat serum 10% for 30 minutes (must be same species as your secondary was raised!) **Diluting 100% Goat Serum: 90µl PBS +10µl Goat Serum =100µl of 10% Goat serum**

9. Aspirate the blocking agent and replace it with 50µl of a 1:10 dilution of primary anti-UP3 antibody in PBS. Incubate for 1 hour. Wash three times with PBS. **Diluting 100% UP3: Take 5 µl UP3 +45 µl PBS= 50 µl per slide**.

10. Apply 50µl of a 1:250 dilution of fluorescein secondary antibody in 1% BSA to the sample and incubate for 40 minutes. Fluorescein in fridge (FITC) and labelled antibody goat anti mouse No: 488. Wash three times with PBS. **Mix 8µl of Fluorescein+ 42 µl BSA= 50 µl per slide**

11. Stain the deposit with a 1:100 dilution of DAPI in PBS for 10-20 minutes. Wash three times with PBS. Carefully mount the slide using a small amount of FluorSave reagent. Seal with clear nail varnish. keep the slide in the dark at 4C until it can be examined with a fluorescence microscope.

**Florescent microscopy**

Each slide was examined using epi-fluorescent microscopy on an Olympus CX-41 and Leica DM4000B upright microscope in a dark room. The dark room was important, to prevent the slides being exposed to direct light damage. All images were processed and analysed using Infinity Capture and Analyze V6.2.0, ImageJ 1.46r and the Leica Application Suite, Advanced Fluorescence 3.1.0 build 8587 software. Images of particular cells were electronically captured and stored for future reporting. All cells were enumerated by the researcher and double counted by a laboratory assistant to ensure there were no omissions or mistakes.

### 3.4.8 Statistics and data analysis

As in experiment one, SPSS was used to analyse the entire data collected. This programme supported the production of descriptive statistics which was used to look at differences in groups.

### 3.4.9 Summary

Two theoretical frameworks were developed and used as the underpinning basis for this investigation. The experimental techniques used for experiment one and two are significant and have an important role when answering the research questions. The data were analysed using SPSS and the results have been explored in chapter four.

# Chapter 4
# Results: Comparing non-invasive versus invasive methods of urine specimen collection

## 4.1 Introduction

This chapter presents the results from experiment one and experiment two. Experiment one was a comparison study which recruited a patient group and a control group. It aimed to test the hypothesis that a non-invasive urine collection method such as the MSU has equal merits to an invasive urine collection method the CSU, when examining the quantitative and qualitative cells and sediments found in a specimen of urine in each method. Experiment one deployed microbiological culturing and cell staining techniques using DAPI staining. DAPI cell staining binds to cell DNA which enables a visualisation of the cell nucleus and localised bacteria on the cells, but does not differentiate between the cells that originate from the bladder. Uroplakin-3 staining was later established. This cell staining technique enables visualisation of cells that originate from the bladder, also known as urothelial cells. Urothelial cells contain uroplakin and when stained, will show positive for uroplakin.

Experiment two was also a comparison study which recruited a patient group only. It aimed to test the hypothesis that if a non-invasive collection method such as the MSU is better at capturing cells and sediments found in a specimen of urine, then directly voided urine would be superior in that the whole volume of urine is voided and maximises the number of cells that are captured. In the same way as experiment one, experiment two deployed uroplakin-3 staining to establish a clear image of cells that originated from the bladder. The cells were enumerated to establish which urine collection method captured the majority of urothelial cells.

## 4.2 Experiment one: non-invasive urine specimen collection versus invasive methods

### 4.2.1 Participant groups

Of the 90 participants enrolled into the study there were 60 patients and 30 controls. The mean age of the patients was 60 (sd= 12) and for the controls 44 years (sd= 15). There was thus difference in age between the two groups. The patient group was an aging population that were being treated for a chronic UTI. The control group were workers from the medical urology centre that were a younger population and without a UTI. The association was that a healthy bladder (Controls) was to be compared against an infected bladder (Patients) in order to measure the qualitative and quantitative differences between groups.

### 4.2.2 Analysis of patient group with LUTS- symptom scores

With the 60 patients that had severe LUTS their mean urgency score was 16 (sd=11.1), with the normal score being zero and the highest score possible was 36. The mean pain score was 10.2 (sd=7.8). Similarly, a normal score was zero and the maximum possible score was 26. The mean score for the ICIQ-LUTS (without urgency and pain) was 64.3 (sd= 40.5). A normal person should score zero and the maximum possible score was 239. The mean score for the ICIQ-LUTSqol score was 151.5 (sd= 75.2). A normal person would score 15 with a maximum potential score of 324.The urgency, pain, ICIQ-LUTS and ICIQ-LUTSqol score distributions are all illustrated in the Figures 8, 9, 10 and 11. The highest scores for urgency, pain and LUTS would indicate a patient with severe symptoms.

**Figure 8: Distribution of urgency scores**

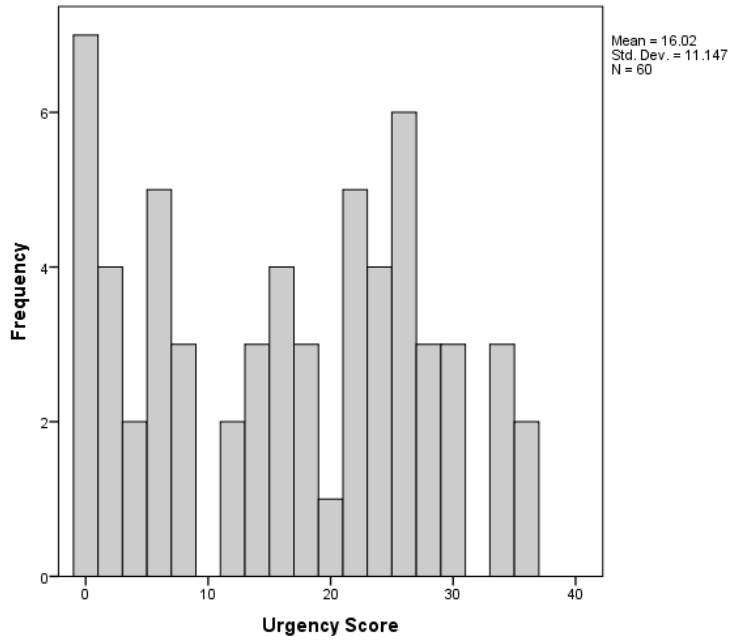
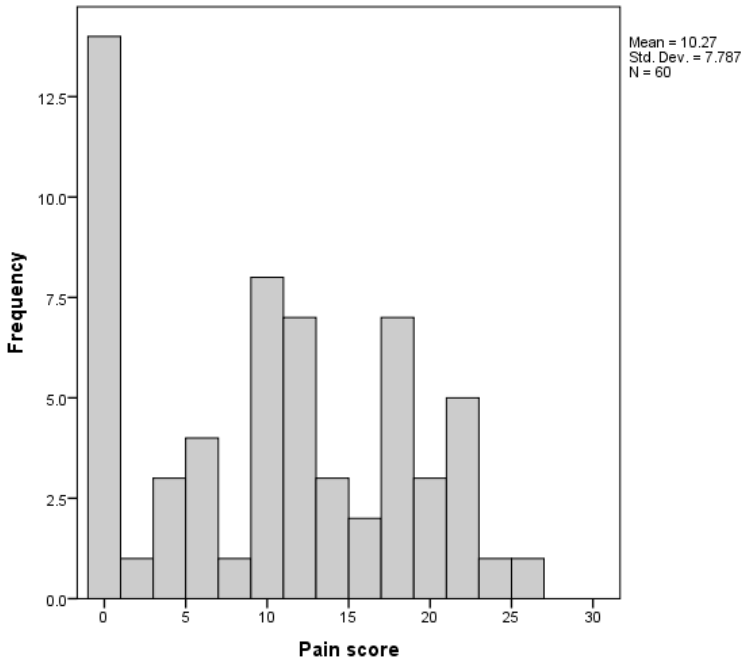



**Figure 9: Distribution of pain scores**




The absence of pain and urgency symptoms was reported by the majority of patients. Asymptomatic UTI is often not diagnosed due to the absence of symptoms, and not all patients exhibit symptoms. The presence of urinary pathogens is often the qualifying indicator for the diagnosis of a UTI.

**Figure 10: Distribution of LUTS scores**

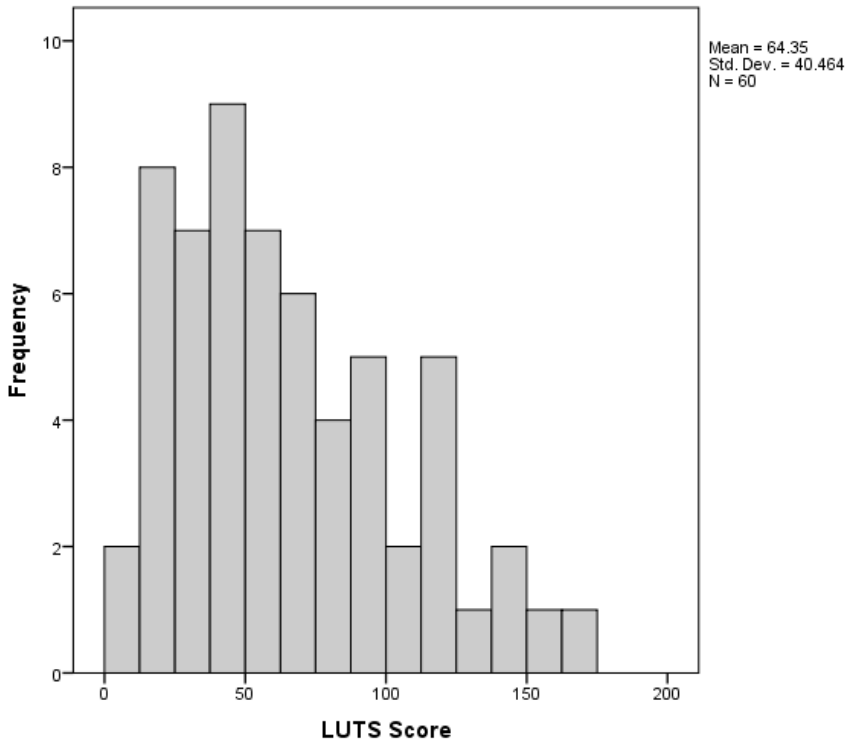
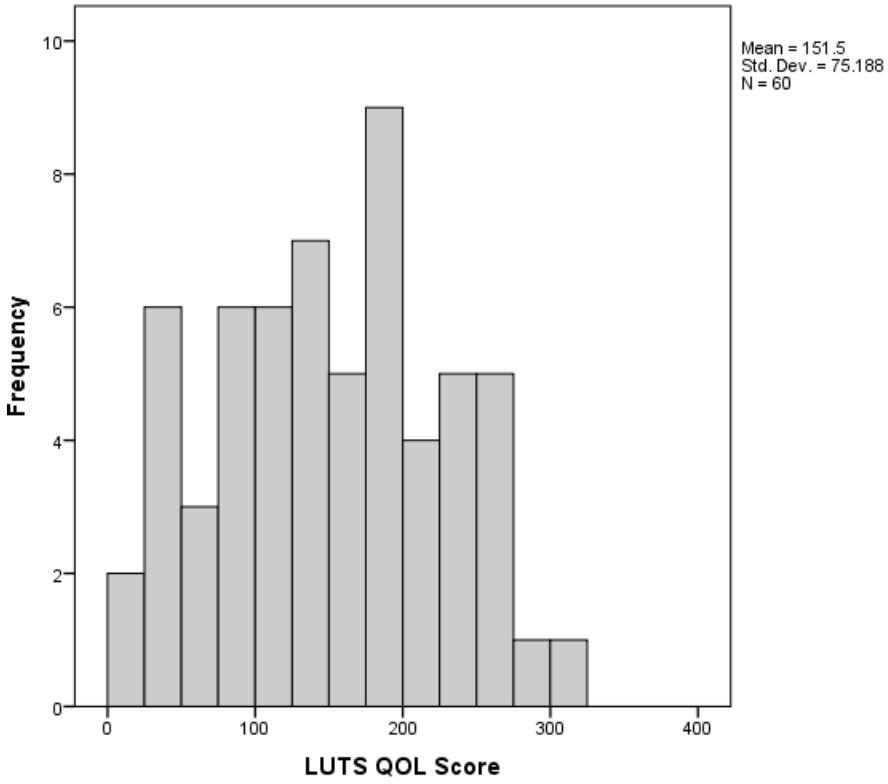



**Figure 11: Distribution of Quality of Life scores**



The overall LUTS and LUTS QOL scores was reflective of the patient group population symptoms which did not include the pain and urgency scores. It is evident from figure 10 and 11 that the patient's quality of life was affected by UTI and the accompanied symptoms.

### 4.2.3 Urine sampling and urinalysis results

The total number of urine samples is shown in Table 15. The patient group provided a MSU, CSU and a subset provided a Peezy MSU sample. The control group provided a MSU sample only, a subset of Peezy MSU samples were also obtained.

**Table 15: Urine sampling proportion across the patient and control group**

| Participants | MSU | CSU | Peezy MSU |
|---|---|---|---|
| **Patient** | 60 | 58 | 11 |
| **Controls** | 30 | 0 | 14 |
| **Total** | 90 | 58 | 25 |

### 4.2.4 Comparing MSU and CSU- Nitrites on dipsticks

These data were collected from patients who provided samples from MSU and CSU. The analysis used contingency tables and the measure of agreement is Cohen's Kappa. The first analysis scrutinises CSU/MSU pairs, measuring nitrite. The data were binary coded 0 = negative and 1 = positive; SPSS was able to translate these numbers into the words "Negative" and "Positive".

**Table 16: Case Processing Summary**

| | Cases | | | | | |
|---|---|---|---|---|---|---|
| | Valid | | Missing | | Total | |
| | N | Percent | N | Percent | N | Percent |
| MSU STIX NITRITES * CSU STIX NITRITES | 58 | 100.0% | 0 | 0.0% | 58 | 100.0% |

**Table 17: MSU STIX NITRITES * CSU STIX NITRITES Cross tabulation**

Count

| | | CSU STIX NITRITES | | |
|---|---|---|---|---|
| | | Neg | Positive | Total |
| MSU STIX NITRITES | Neg | 56 | 0 | 56 |
| | Positive | 0 | 2 | 2 |
| Total | | 56 | 2 | 58 |

It can be seen from table 17 that the MSU and CSU were in agreement when detecting nitrites.

### 4.2.5 Comparing MSU and CSU- Leucocyte esterase on dipsticks

The next analysis scrutinises MSU/CSU pairs, measuring leucocyte esterase on the dipstick analysis.

**Table 18: MSU STIX WBCs * CSU STIX WBCs Cross tabulation**

Count

| | | CSU STIX WBCs | | | | |
|---|---|---|---|---|---|---|
| | | 1+ | 2+ | Neg | Trace | Total |
| MSU STIX WBCs | 1+ | 1 | 0 | 0 | 0 | 1 |
| | 2+ | 0 | 2 | 0 | 0 | 2 |
| | Neg | 0 | 0 | 54 | 0 | 54 |
| | Trace | 0 | 0 | 0 | 1 | 1 |
| Total | | 1 | 2 | 54 | 1 | 58 |

**Table 19: Symmetric measures**

| | | Value |
|---|---|---|
| Ordinal by Ordinal | Spearman Correlation | .192 |
| Measure of Agreement | Kappa | .222 |
| N of Valid Cases | | 58 |

In this case, there are an increased number of options to analyse and the dispersion of probabilities is more balanced. However, there were limitations with the Kappa analysis as the Kappa test failed to detect agreement with the two sampling methods based on most of the results being negative. This is shown with the weak correlation of .192 seen with the Spearman correlation. These are ordinal data so a Bland-Altman analysis of agreement would not have been appropriate either.

### 4.2.6 Comparing CSU and MSU on urine microscopy for white blood cells

In this case, nonparametric data were scrutinised. As the data were not normally distributed it was important to analyse the paired data by different methods. Firstly, the paired data were compared using the nonparametric Wilcoxon test as seen in Table 20.

**Table 20: Wilcoxon Signed Ranks Test on urine microscopy for white blood cells in MSU/CSU**

**Ranks**

| | | N | Mean Rank |
|---|---|---|---|
| CSU microscopy wbc - MSU microscopy wbc | Negative Ranks | 25[a] | 15.70 |
| | Positive Ranks | 4[b] | 10.63 |
| | Ties | 29[c] | |
| | Total | 58 | |

The number of times white blood cells were not found in the MSU was paired against the number of times they were not found in the CSU. The non-occurrence of white blood cells in each specimen collection method was ranked. This analysis measured the mean rank differences between the two specimen collection methods. This test shows that the mean negative ranks (non-occurrence of white blood cells) were higher than the positive ranks (occurrence of white blood cells) in the CSU method when compared to the MSU method. The CSU had a greater number of negative results when detecting the presence of white blood cells.

It can be seen in the descriptive statistics in Table 21 that there is a statistically significant difference between the MSU and CSU in the pyuria (wbc) counts. The data were positively skewed so that both counts demonstrated a median of 0. However, scrutiny of the mean and 95% confidence intervals shows that the CSU pyuria was lower than MSU count when the counts were greater than zero.

**Table 21: The descriptive statistics for Pyuria counts: MSU versus CSU**

| | | | Statistic |
|---|---|---|---|
| MSU microscopy wbc | Mean | | 67.28 |
| | 95% Confidence Interval for Mean | Lower Bound | -18.66 |
| | | Upper Bound | 153.21 |
| | Median | | .00 |
| | Std. Deviation | | 326.832 |
| | Minimum | | 0 |
| | Maximum | | 2400 |
| | Interquartile Range | | 12 |
| | Skewness | | 6.721 |
| CSU microscopy wbc | Mean | | 24.93 |
| | 95% Confidence Interval for Mean | Lower Bound | -14.10 |
| | | Upper Bound | 63.96 |
| | Median | | .00 |
| | Std. Deviation | | 148.433 |
| | Minimum | | 0 |
| | Maximum | | 1120 |
| | Interquartile Range | | 0 |
| | Skewness | | 7.308 |

The two variables showed a correlation with a Spearman R of .430 which is not what should be seen. There should be a greater correlation if two the methods are thought to be measuring the same variable.

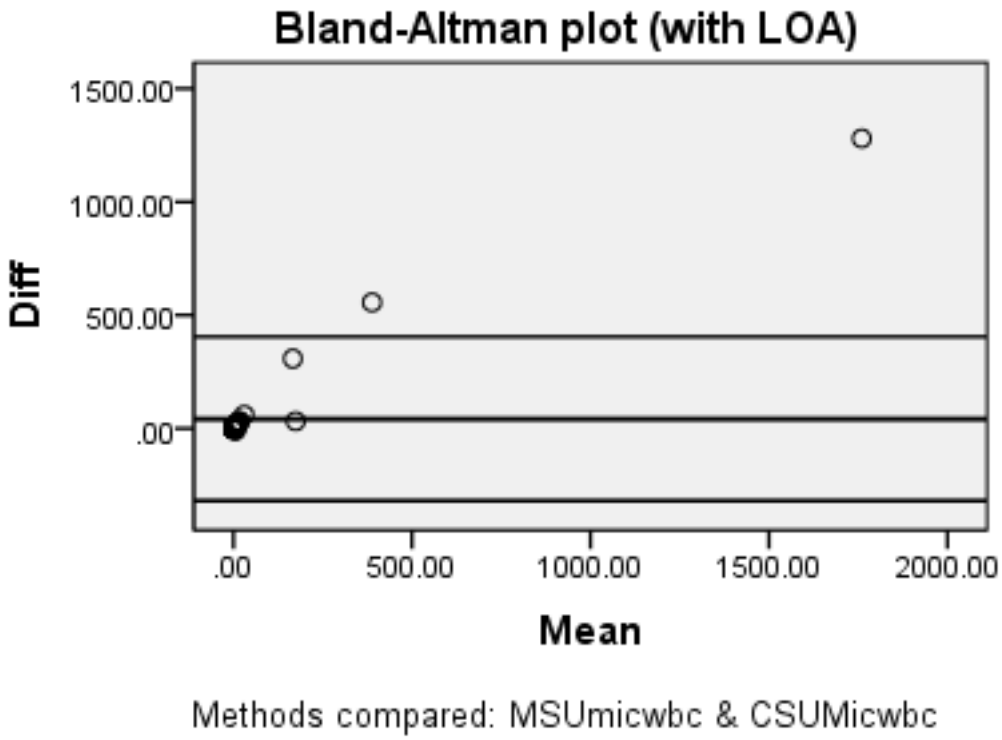
**Table 22: Correlations**

| | | | MSU microscopy wbc | CSU microscopy wbc |
|---|---|---|---|---|
| Spearman's rho | MSU microscopy wbc | Correlation Coefficient | 1.000 | .430** |
| | | Sig. (2-tailed) | . | .001 |
| | | N | 58 | 58 |
| | CSU microscopy wbc | Correlation Coefficient | .430** | 1.000 |
| | | Sig. (2-tailed) | .001 | . |
| | | N | 58 | 58 |

**. Correlation is significant at the 0.01 level (2-tailed).

The Bland-Altman analysis in Figure 11 shows the disagreement between the two analyses when the counts are greater than zero. Given the detection of differences using this nonparametric test, the Bland-Altman analysis is unnecessary because agreement is impossible in the presence of significant paired differences. The plot does however illustrate graphically the scale of the disagreement.

**Figure 12: Bland-Atman Plot 1: MSU/CSU microscopic Wbc**




**4.2.7 Comparing MSU and CSU on urine microscopy for epithelial cell counts**

On turning to the epithelial cell count, a very similar situation presents to that which occurred with the pyuria. In this case the epithelial cell counts were lower in the CSU analysis (median=0) compared to the MSU (median=2). For the reasons stated above, a Bland-Altman analysis is not necessary because the comparative and correlative analyses preclude the presence of agreement. Bland-Altman is useful when comparison of means, or medians, imply similarity and there is a bivariate correlation. In that situation, the Bland-Altman analysis checks for real agreement. The number of times epithelial cells were not found in the MSU was paired against the number of times they were not found in the CSU. The non-occurrence of epithelial cells in each specimen collection method was ranked. This analysis measured the mean rank differences between the two specimen collection methods. The Wilcoxon test below shows that the mean negative ranks (non-occurrence of epithelial cells) was higher than the positive ranks (occurrence of epithelial cells) in the CSU method compared to MSU.

**Table 23: Wilcoxon Signed Ranks Test on urine microscopy for epithelial cells in MSU/CSU**

**Ranks**

| | | N | Mean Rank |
|---|---|---|---|
| CSU microscopy epithelial - MSU microscopy epithelial | Negative Ranks | 26[a] | 18.46 |
| | Positive Ranks | 8[b] | 14.38 |
| | Ties | 24[c] | |
| | Total | 58 | |

The descriptive statistics in Table 24 show that the epithelial cells found in the MSU samples (mean= 22.2) exceeded greatly the numbers found in the CSU samples (mean= 2.7).

**Table 24: The descriptive statistics for epithelial counts MSU versus CSU**

| | | | Statistic |
|---|---|---|---|
| MSU microscopy epithelial | Mean | | 22.21 |
| | 95% Confidence Interval for Mean | Lower Bound | -5.50 |
| | | Upper Bound | 49.91 |
| | Median | | 2.00 |
| | Std. Deviation | | 105.377 |
| | Minimum | | 0 |
| | Maximum | | 800 |
| CSU microscopy epithelial | Mean | | 2.76 |
| | 95% Confidence Interval for Mean | Lower Bound | 1.47 |
| | | Upper Bound | 4.05 |
| | Median | | .00 |
| | Std. Deviation | | 4.897 |
| | Minimum | | 0 |
| | Maximum | | 18 |

### 4.2.8 CSU / MSU order effect- White Blood Cells

A t-test was performed to confirm whether there was an order effect with the number of Wbc's found in the CSU and MSU specimens that were collected first or second. The group statistics in Table 25 descriptively reveal that there was a difference between means when the Wbc's were counted from samples that were collected first in comparison to samples that were collected second. However, the t-test shown in Table 26 confirms that there was not a statistical significant difference. The Levene's test for equality of variances in the second and third column of Table 26 determines whether the two means have the same or different variability based on the Sig value. A Sig value greater than .05 indicates that the top row of the t-test should be examined and a value less than .05 indicates that the bottom row of the t-test should be read. In Table 26, the Sig. (2-tailed) values, also known as the p values in the t-test for equality of means, were greater than .05 which suggests that the population means were equal and that there was not a statistically significant difference. If the p values were less than .05 it would have suggested that there was a statistically significant difference.

**Table 25: CSU / MSU order effect- White Blood Cells**

**Group Statistics**

| CSU/MSU Order | | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| CSU microscopy wbc | 2nd | 26 | 43.94 | 199.175 | 35.209 |
| MSU microscopy wbc | 1st | 32 | 109.00 | 435.032 | 76.903 |

**Table 26: t-test for equality of means**

**Independent Samples Test**

| | | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | 95% Confidence Interval of the Difference | |
| | | F | Sig. | t | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | Lower | Upper |
| CSU microscopy wbc | Equal variances assumed | 4.510 | .038 | 1.084 | 56 | .283 | 42.399 | 39.131 | -35.990 | 120.788 |
| | Equal variances not assumed | | | 1.204 | 31.021 | .238 | 42.399 | 35.215 | -29.421 | 114.219 |
| MSU microscopy wbc | Equal variances assumed | 4.127 | .047 | 1.080 | 56 | .285 | 93.077 | 86.167 | -79.537 | 265.691 |
| | Equal variances not assumed | | | 1.195 | 32.568 | .241 | 93.077 | 77.873 | -65.438 | 251.591 |

### 4.2.9 CSU / MSU order effect- epithelial cells

A t-test was performed to confirm whether there was an order effect with the number of epithelial cells found in the CSU and MSU specimen collection methods. The group statistics in Table 27 descriptively show that there was a difference between means when the epithelial cells were counted from samples collected first in comparison to samples that were collected second. However, the t-test shown in Table 28 confirms that there was not a statistically significant difference as the Sig. (2-tailed) value was greater than .05 which suggests that the population means were equal.

## Table 27: CSU / MSU order effect- epithelial cells

**Group Statistics**

| CSU Order | | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| CSU microscopy epithelial | 2nd | 26 | 3.62 | 5.601 | 1.098 |
| MSU microscopy epithelial | 1st | 32 | 11.19 | 21.390 | 3.781 |

## Table 28: T-test for equality of means

**Independent Samples Test**

| | | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | 95% Confidence Interval of the Difference | |
| | | F | Sig. | t | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | Lower | Upper |
| CSU microscopy epithelial | Equal variances assumed | 3.931 | .052 | -1.206 | 56 | .233 | -1.553 | 1.288 | -4.132 | 1.027 |
| | Equal variances not assumed | | | -1.171 | 45.448 | .248 | -1.553 | 1.326 | -4.223 | 1.117 |
| MSU microscopy epithelial | Equal variances assumed | 3.235 | .077 | -.882 | 56 | .382 | -24.582 | 27.877 | -80.426 | 31.263 |
| | Equal variances not assumed | | | -.796 | 25.763 | .433 | -24.582 | 30.869 | -88.063 | 38.899 |

### 4.2.10 Peezy / MSU order effect- epithelial cells

A t-test was performed again to confirm whether there was an order effect with the number of epithelial cells found in the Peezy and MSU specimen collection methods. The group statistics in Table 29 descriptively reveal that there was a difference between means when the epithelial cells were counted from samples collected second in comparison to samples that were collected first. However, the t-test shown in Table 30 confirms that there was not a statistically significant difference as the Sig. (2-tailed) value was greater than .05 which suggests that the population means were equal.

**Table 29: Peezy / MSU order effect- epithelial cells**

**Group Statistics**

| Peezy Order | | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| PEEZY microscopy epithelial | 2nd | 12 | 6.22 | 11.465 | 3.822 |
| MSU microscopy epithelial | 1st | 13 | 8.20 | 15.985 | 5.055 |

**Table 30: T-test for equality of means**

**Independent Samples Test**

| | | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | 95% Confidence Interval of the Difference | |
| | | F | Sig. | t | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | Lower | Upper |
| PEEZY microscopy epithelial | Equal variances assumed | 1.266 | .276 | -.862 | 17 | .401 | -3.422 | 3.970 | -11.798 | 4.954 |
| | Equal variances not assumed | | | -.829 | 10.610 | .425 | -3.422 | 4.126 | -12.544 | 5.700 |
| MSU microscopy epithelial | Equal variances assumed | .208 | .654 | -.328 | 17 | .747 | -2.911 | 8.885 | -21.656 | 15.834 |
| | Equal variances not assumed | | | -.322 | 14.289 | .752 | -2.911 | 9.050 | -22.284 | 16.462 |

### 4.2.11 Comparing MSU and Peezy - Nitrites on dipsticks

A paired t-test was conducted to confirm whether the Nitrites test results from the MSU and Peezy MSU dipstick method correlated. Table 31 shows that there was a correlation as the results from both urine collection methods were negative with only one positive result.

**Table 31: MSU Stix Nit * PEEZY Stix Nit Cross tabulation**

Count

| | | PEEZY stix nit | | |
|---|---|---|---|---|
| | | Neg | Positive | Total |
| MSU stix nit | Neg | 24 | 0 | 24 |
| | Positive | 0 | 1 | 1 |
| Total | | 24 | 1 | 25 |

### 4.2.12 Comparing MSU and Peezy - Leucocyte esterase on dipsticks

A table was generated to descriptively confirm whether the Leucocyte esterase test results from the MSU and Peezy MSU dipstick method correlated. Table 32 shows that there was an agreement based on the negative test results, but of limited applicability because of the number of negative results.

**Table 32: MSU Stix Wbc * PEEZY Stix Wbc Cross tabulation**

Count

| | | PEEZY stix wbc | | | |
|---|---|---|---|---|---|
| | | 1+ | Neg | Trace | Total |
| MSU stix wbc | 1+ | 3 | 0 | 0 | 3 |
| | Neg | 0 | 20 | 0 | 20 |
| | Trace | 0 | 0 | 2 | 2 |
| Total | | 3 | 20 | 2 | 25 |

### 4.2.13 Comparing Peezy and MSU urine microscopy for white blood cell counts

A Bland-Altman Limits of Agreement (LOA) analysis was performed to measure the agreement of white blood cells found in the Peezy and MSU sampling methods (Table 33). The Bland-Altman plot shown in Figure 12 indicates that there was a substantial agreement between the two sampling methods. Because the data were not normally distributed, a Spearman's correlations test was carried out to assess the relationship between the two variables. Table 34 shows that there was no difference between the two groups because of the positive correlations of .950**.

**Table 33: Bland-Altman LOA Analysis**

| **Statistics (*)** | | |
|---|---|---|
| **Mean Diff** | **SD (Diff)** | **G-Mean** |
| -6.833 | 41.004 | 7.750 |
| Methods compared | | |
| **Method 1**<br>Peezy Microscopic Pyuria | **Method 2**<br>MSU Microscopic Pyuria | |

**Figure 13: Bland-Atman Plot 2: Peezy/MSU microscopic Pyuria**

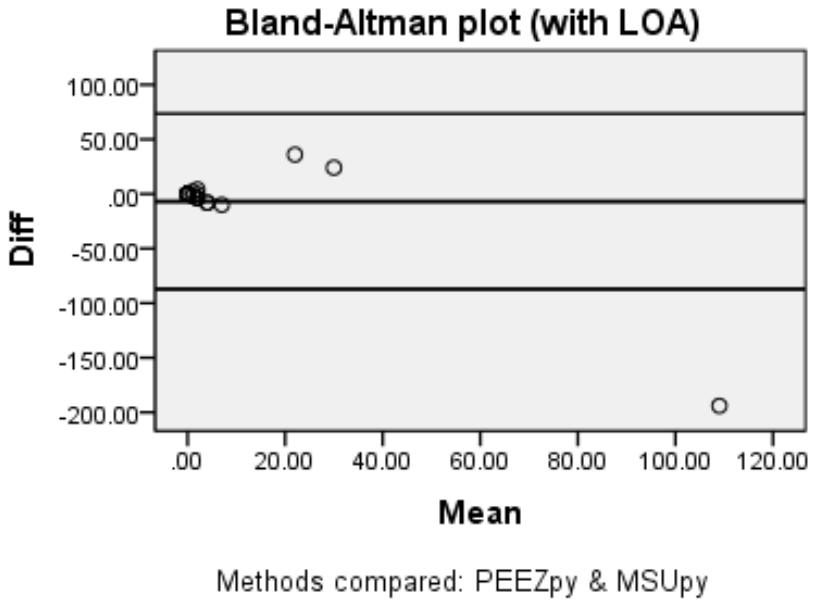



**Table 34: Nonparametric Correlations**

| | | | Mean | Abs. differences |
|---|---|---|---|---|
| Spearman's rho | Mean | Correlation Coefficient | 1.000 | .950** |
| | | Sig. (2-tailed) | . | .000 |
| | | N | 24 | 24 |
| | Abs. differences | Correlation Coefficient | .950** | 1.000 |
| | | Sig. (2-tailed) | .000 | . |
| | | N | 24 | 24 |

### 4.2.14 Proportion of uroplakin 3 cells in the MSU, CSU and Peezy specimens

Urothelial cells were a unique indicator for identifying urothelial inflammation. The proportions and numbers of uroplakin positive and negative cells were statistically quantified. Table 35 shows that the mean proportion of positive cells found in the MSU

was .92 which was closely matched to .93 found in the CSU. Similarly, there was no significant difference between the mean numbers of cells found in the Peezy method, as the mean proportion of .97 closely matched the MSU. The Wilcoxon test shown in Table 36 proves that there was no statistically significant difference between the mean ranks for the uroplakin 3 cells found in the MSU, CSU and Peezy.

**Table 35: Proportion of uroplakin 3 cells in the MSU, CSU and Peezy specimens**

**Descriptives**

| | | | Statistic |
|---|---|---|---|
| Proportion MSU UP3 | Mean | | .9277 |
| | 95% Confidence Interval for Mean | Lower Bound | .7459 |
| | | Upper Bound | 1.1095 |
| | Median | | 1.0000 |
| | Std. Deviation | | .14642 |
| | Minimum | | .67 |
| | Maximum | | 1.00 |
| | Interquartile Range | | .18 |
| Proportion CSU UP3 | Mean | | .9333 |
| | 95% Confidence Interval for Mean | Lower Bound | .7482 |
| | | Upper Bound | 1.1184 |
| | Median | | 1.0000 |
| | Std. Deviation | | .14907 |
| | Minimum | | .67 |
| | Maximum | | 1.00 |
| | Interquartile Range | | .17 |

**Table 35 Continued….**

| | | | |
|---|---|---|---|
| Proportion PEEZY UP3 | Mean | | .9790 |
| | 95% Confidence Interval for Mean | Lower Bound | .9411 |
| | | Upper Bound | 1.0168 |
| | Median | | 1.0000 |
| | Std. Deviation | | .03047 |
| | Minimum | | .93 |
| | Maximum | | 1.00 |
| | Interquartile Range | | .05 |

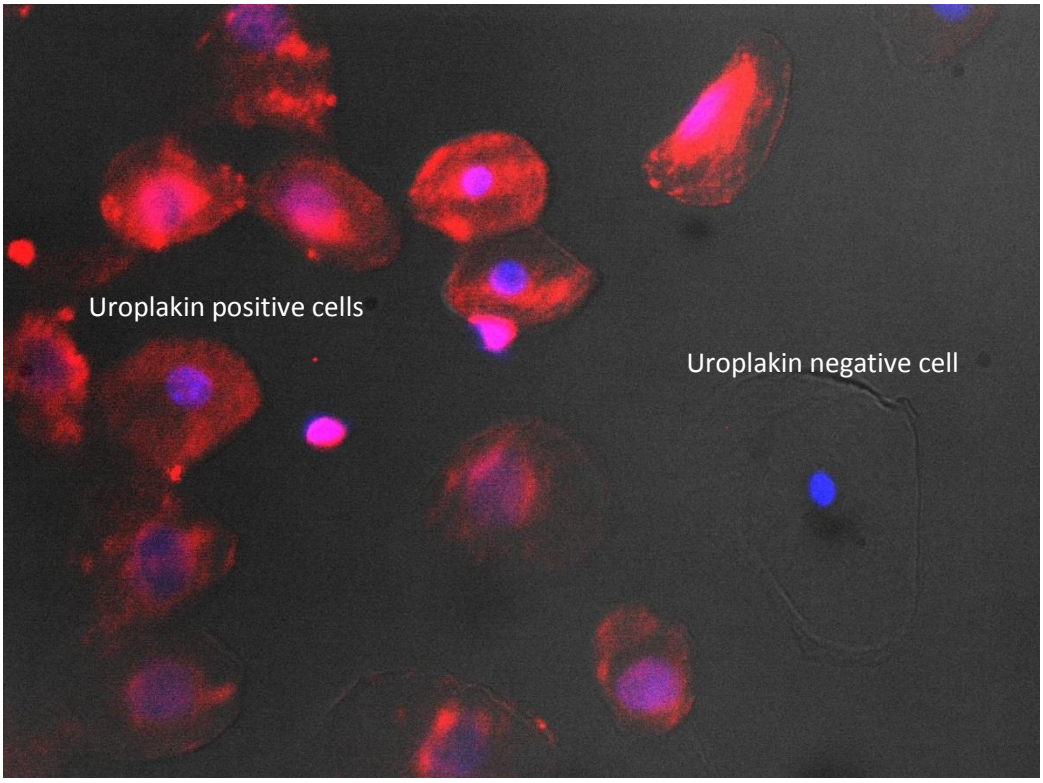
**Table 36: Wilcoxon Signed Ranks Test on the number of uroplakin 3 positive and negative cells in the MSU, CSU and Peezy samples**

**Ranks**

| | | N | Mean Rank |
|---|---|---|---|
| PropCSUup – PropMSUup | Negative Ranks | 4[a] | 5.25 |
| | Positive Ranks | 8[b] | 7.13 |
| | Ties | 13[c] | |
| | Total | 25 | |
| PropPEEZup – PropMSUup | Negative Ranks | 2[d] | 3.50 |
| | Positive Ranks | 7[e] | 5.43 |
| | Ties | 6[f] | |
| | Total | 15 | |

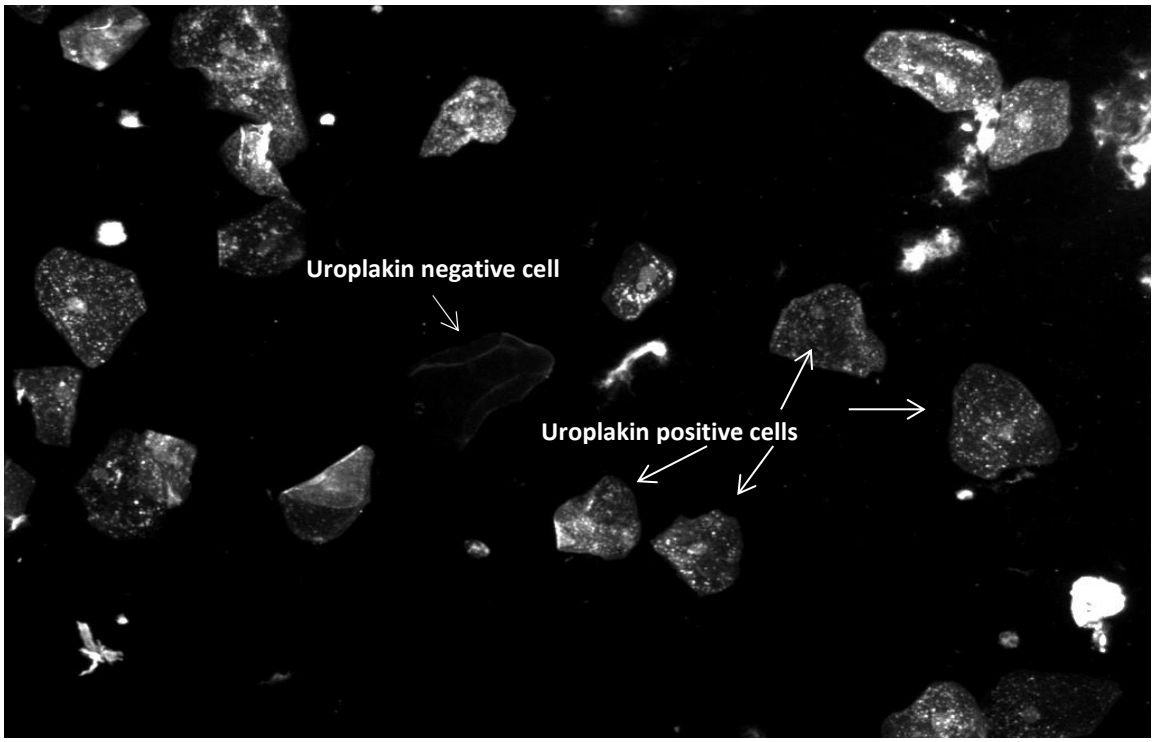
The unique distinction between the positive and negative uroplakin cells was easily distinguished upon florescent microscopy. Image 4 shows the microscopic difference where the cells viewed in florescent red with a blue nucleus were uroplakin positive, whereas the cell without the florescent red but with a blue nucleus was a negative cell.

**Image 4: Uroplakin-3 stained Urothelial Cells**




Similarly, image 5 shows the unique distinction between the positive and negative uroplakin cells upon florescent microscopy in grayscale, as it was captured from the Infinity Capture and Analyze application software. Uroplakin staining was an important technique to distinguish cellular origin. The DAPI staining technique used for the first ten urine samples was only able to detect the morphology of the cells which included the cellular outline and the cell nucleus. Therefore, the analysis of DAPI stained cells did not proceed and uroplakin cells were examined only.

**Image 5: Uroplakin-3 stained Urothelial Cells**




### 4.2.15 Sediment culture results

The sediment cultures performed on the urine samples obtained by MSU and Peezy MSU grew greater numbers of microorganisms compared to samples collected by CSU and this occurred in every dilution of the substrate including undiluted sediment. In all the three urine collection methods used, the two dominant bacteria isolated were *enterococcus* and *Escherichia coli*, which are Gram positive cocci and Gram negative bacilli commonly incriminated in causing a UTI (Fisher and Phillips, 2009). The urine samples from all three collection methods showed polymicrobial growths. The pie charts (Figures 14 to 18) illustrate the microbial distributions of raw bacterial colony counts at x5 dilution. The three collection methods are reported and in each case the data compare patients and controls as cross-sectional analyses. These data confirm previous findings that refute the idea of a sterile bladder and they reproduce the genus overlap between patients and controls. Thus, the pathogenicity of a microbe depends on the context which is probably influenced by several factors, known and unknown. Thus, the presence of a particular microbe does not necessarily imply pathology.

**Figure 14**

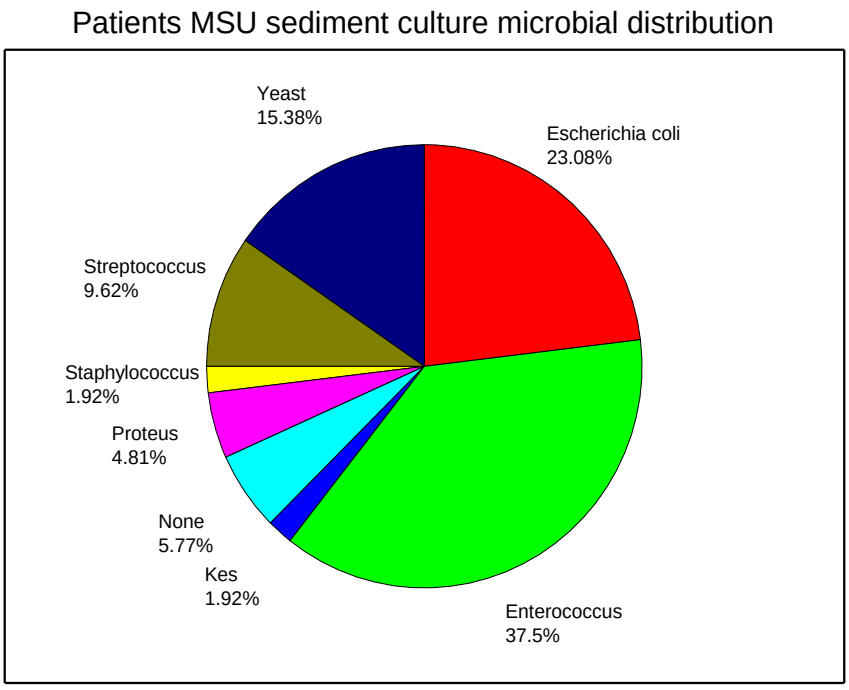
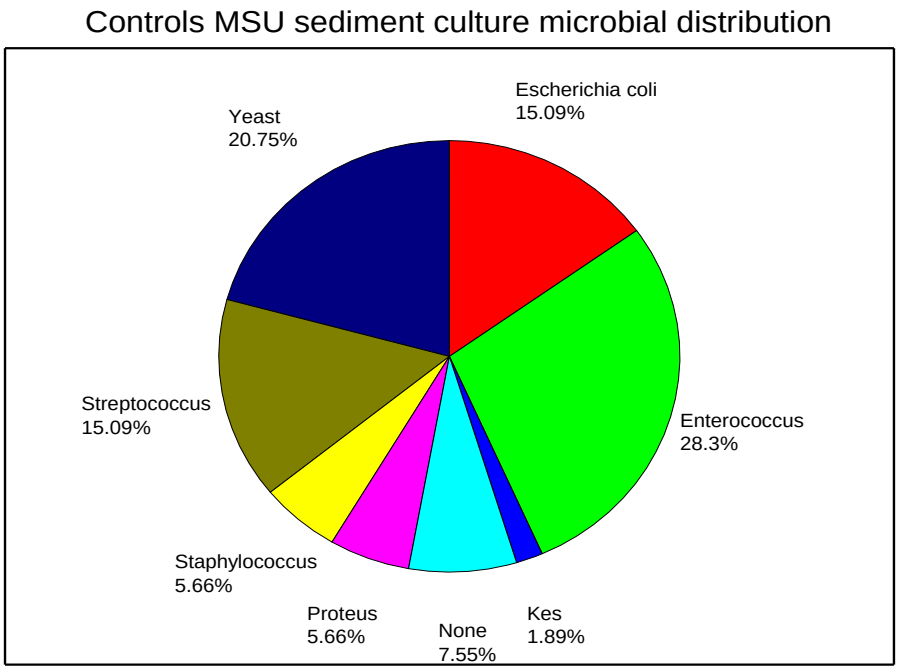
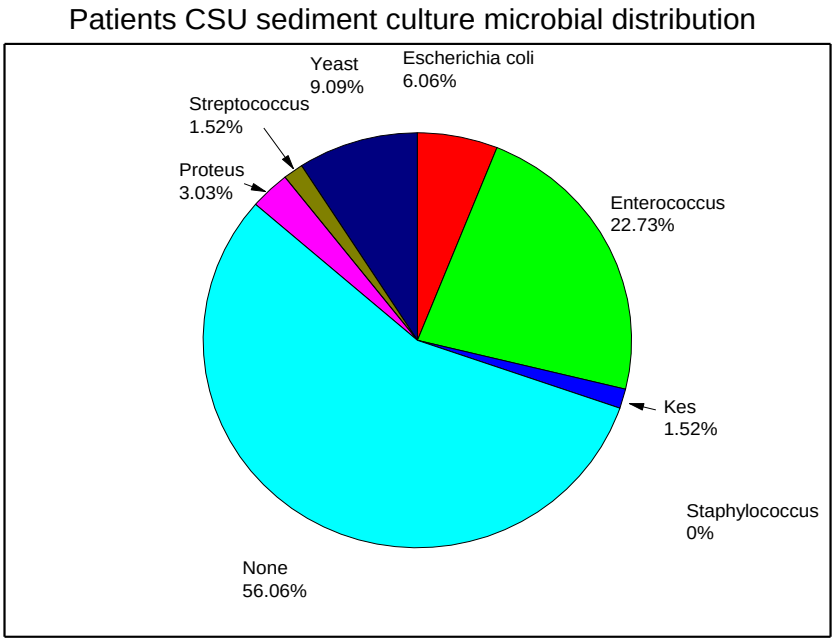



**Figure 15**



It is remarkable that the CSU resulted in fewer isolates than the other methods. An immediate conjecture, which historically has been accepted without question, is that the CSU is less contaminated. Serendipitously, a large number of studies conducted at the medical urology centre have demonstrated that the isolates from sediment cultures conducted on MSU samples are linked to pathology through symptoms, pyuria, urothelial shedding, cytokine expression and treatment response (Sathiananthamoorthy et al., 2012). This encourages the counter-factual proposition that the CSU technique is missing pathology.

**Figure 16**




Given that the urinary sediment of shed urothelial cells is the substrate for analysis, and that gravity will cause this to accumulate at the base of the bladder, it is plausible that the catheter is inserted into the urine above this sediment which it does not then sample. A corollary of this hypothesis is that a non-CSU and non-MSU sample may be a superior substrate because the first part of the void is likely to contain the greatest amount of sediment. The Peezy method similarly produced results like the MSU which exceeded the microbial counts from the CSU method.

**Figure 17**

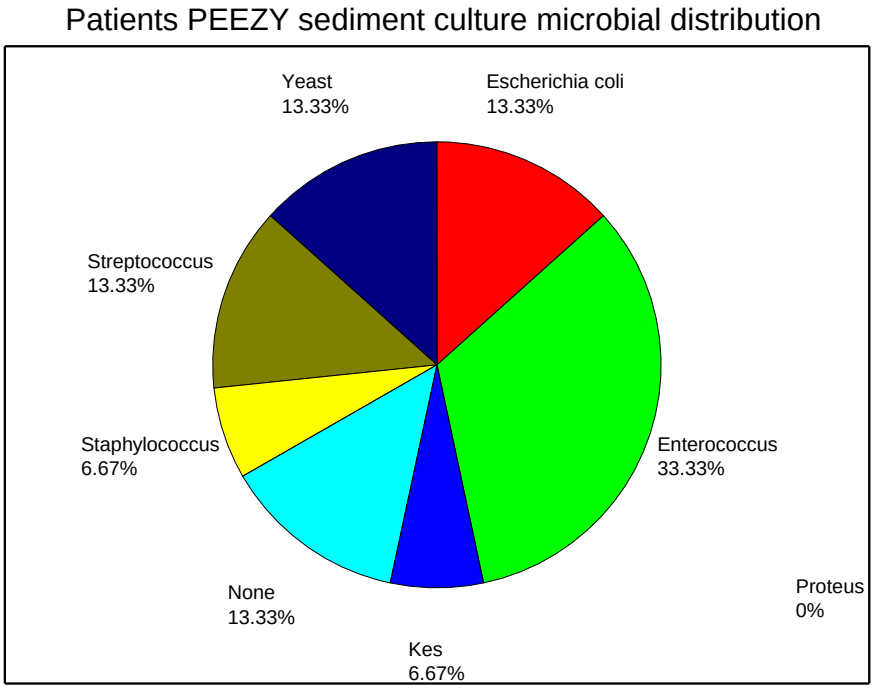
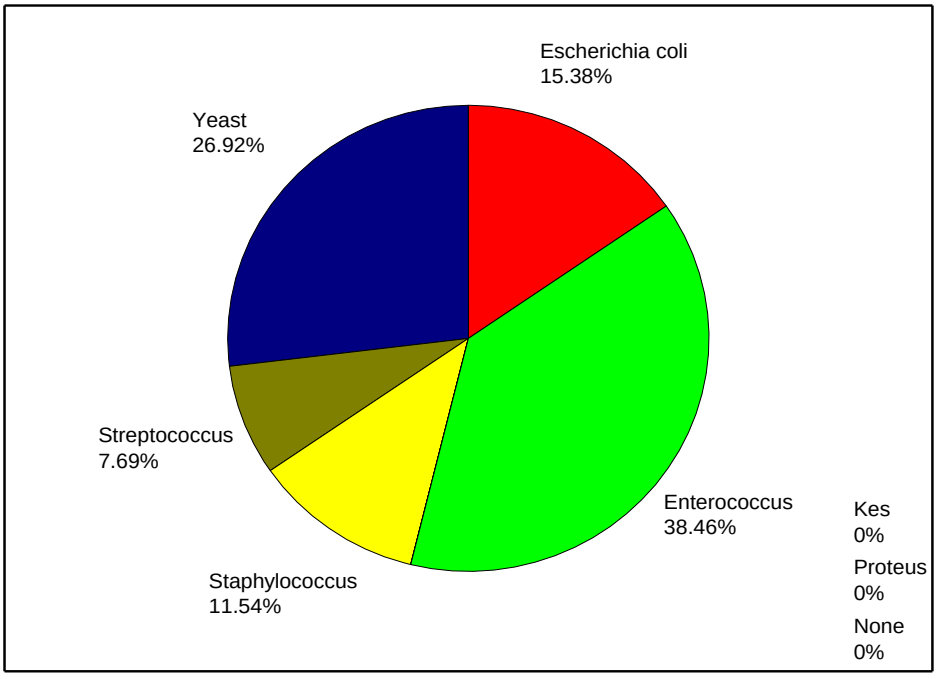



**Figure 18**



Evidence of the mixed organisms has been shown in image 6. This image shows a culture plate with various organisms from a patient's MSU specimen. The different organisms illustrated in Figure 14 are representative of what was observed on the culture plate in image 6. In contrast, image 7 displays a plate with a CSU sample which grew fewer organisms. Similarly, the CSU sediment culture results in Figure 16 illustrates what has been observed in image 7. The images below were urine cultures from the same patient.

**Image 6: Mixed growth MSU sediment culture**


**Image 7: CSU culture plate**


The results from experiment one have highlighted the patient demographics, symptoms, urine characteristics, microbiological cultures and uroplakin positive and negative cells. The data could have been supported with a correlation between the reporting of symptoms and the severity of urine culture results and the proportion urothelial cells. These data would have provided a link between pathogens and urothelial cells during the symptomatic stages of a urinary tract infection.

## 4.3 Experiment two: comparing three different non-invasive urine specimen collection methods

### 4.3.1 Introduction

This section of chapter four presents the results of the urine comparison study using three non-invasive urine collection methods. Descriptive analysis was used to look at the proportion differences of the numbers of epithelial cells and positive uroplakin cells found in all three non-invasive urine collection methods.

### 4.3.2 Participant groups

Thirty one participants from the first experiment were enrolled into the comparison study. The mean age of the patients was 62 (sd= 10).

### 4.3.3 Comparing epithelial cell counts mean from MSU, Peezy and direct void

Descriptive statistics were used to look at the differences between the means and the proportion of epithelial cells found in all three non-invasive urine collection methods. Table 37 shows that there was a difference with the direct void mean which was greater in comparison to the MSU and Peezy methods.

**Table 37: Comparing epithelial cell counts from MSU, Peezy and direct void**

| | N | Mean | Std. Deviation | Minimum | Maximum |
|---|---|---|---|---|---|
| MSU microscopy Epc | 31 | 6.00 | 9.893 | 0 | 38 |
| PEEZY microscopy Epc | 31 | 4.32 | 5.115 | 0 | 20 |
| DV microscopy Epc | 31 | 9.52 | 15.451 | 0 | 62 |

### 4.3.4 Comparing positive uroplakin cell counts from MSU, Peezy and direct void

Descriptive statistics were used again to look at the differences between the means and the proportion of uroplakin positive cells found in all three non-invasive urine collection methods. Similarly, Table 38 shows that there was a difference with the direct void mean which was greater in comparison to the MSU and Peezy methods.

**Table 38: Comparing positive uroplakin cell counts from MSU, Peezy and direct void**

| | N | Mean | Std. Deviation | Minimum | Maximum |
|---|---|---|---|---|---|
| MSU UP3 Positive cells | 31 | 9.16 | 17.388 | 0 | 89 |
| PEEZY UP3 Positive cells | 31 | 3.90 | 8.055 | 0 | 39 |
| DV UP3 Positive cells | 31 | 17.32 | 27.631 | 0 | 112 |

### 4.3.5 Comparing negative uroplakin cell counts from MSU, Peezy and direct void

The descriptive statistics in Table 39 reveal that the Peezy method had the lowest mean of negative cells compared to the MSU and direct void.

**Table 39: Comparing negative uroplakin cell counts from MSU, Peezy and direct void**

| | N | Mean | Std. Deviation | Minimum | Maximum |
|---|---|---|---|---|---|
| MSU UP3 Negative cells | 31 | 1.32 | 2.982 | 0 | 13 |
| PEEZY UP3 Negative cells | 31 | .87 | 2.473 | 0 | 13 |
| DV UP3 Negative cells | 31 | 3.71 | 9.267 | 0 | 37 |

# Chapter 5

# Discussion: Comparing non-invasive versus invasive methods of urine specimen collection

## 5.1 Introduction

This chapter will discuss the results that have emerged from experiment one and experiment two. Experiment one examined three different urine collection methods; two were non-invasive urine collection methods (MSU and Peezy MSU) and one was an invasive method (CSU). The discussion about experiment one will explore the demographics of the patient and control groups, the record of symptoms, urine sampling for urinalysis, the sediment cultures and the epithelial cell sediments. The discussion regarding experiment two will look at the characteristics of three different non-invasive urine collection methods which were the MSU, Peezy MSU and directly voided urine. It will also highlight the significance of the results that have emerged from examining three non-invasive urine collection methods and the proportion of the urothelial cells found in each of these sampling methods. The theoretical frameworks for experiment one and experiment two are presented in Figure 4 and 7 to remind us of the independent and dependent variables that form the theoretical basis for the discussions.

**Figure 4: Theoretical framework for experiment one**

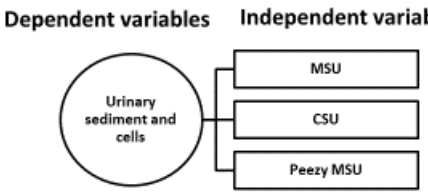
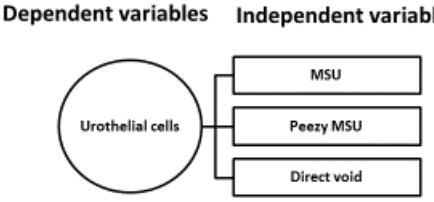



**Figure 7: Theoretical framework for experiment two**



## 5.2 Experiment one: non-invasive urine specimen collection versus invasive methods

### 5.2.1 Participant groups and urinary symptoms

The results have shown that the patient group was exhibiting higher levels of lower urinary tract symptoms and a majority tested positive for a UTI. The pain, urgency, FLUTS and LUTSqol scores were good descriptors of the symptoms that were described by the patient group. The symptoms distribution graphs show that patients describing pain symptoms also had symptoms of urinary urgency. Castro-Diaz et al., (2014) have recognised these combined symptoms as a result of a chronic inflammatory response in the bladder of patients with recalcitrant UTI. The urgency and pain scores described by the patient group were indicative of infection because they were associated with activation of innate immune response which is known to be most commonly stimulated by bacterial cell adhesion. All of the patients were on antibiotic treatment which would have had an effect on the eradication of urinary pathogens thus, may have had an impact on the severity of symptoms. Correspondingly, the symptoms scores may have been greater than what has been reported and analysed. The control group was asymptomatic and did not report any lower tract symptoms.

### 5.2.2 MSU and Peezy MSU versus the CSU

The MSU and Peezy MSU samples were more sensitive in identifying the pathology of the disease process. These sampling methods captured more urothelial cells which are a key part of the bladder sediments formed in the urine during the inflammatory response to infection. There were greater numbers of wbc and epithelial cells found in the MSU and Peezy MSU samples on urine microscopy in comparison to CSU samples. To date, most authors interpret the epithelial cells found in the MSU and Peezy MSU as contaminants shed from the skin surface of the perineum and the vagina (Collier et al., 2013). This has fed the assumption that the CSU is a better sampling method as it provides a clearer specimen. It is true that a CSU sample is clearer but the sampling method used for a CSU differs fundamentally to the MSU and Peezy MSU in a critical manner. When a catheter is inserted in to the bladder, it travels along the urethra and enters the bladder with the eye

of the catheter rising well above the base where the greater part of the sediment is likely to accumulate. The bladder base is formed by the trigone which Viana et al., (2007) described as a muscle with an anti-reflux mechanism that prevents the back flow of urine into the ureters and kidneys. The prevention of urine back flow encourages urinary sediment with urothelial cells to accumulate at the base of the bladder. These cells will be eliminated through the urethral orifice during micturition, but the sediment will not enter a catheter that is inserted above the level of the trigone.

The majority of epithelial cells in the urine are not contaminants (Khasriya et al., 2013), but are proven to originate from the bladder and prove to be crucial markers of bladder pathology (Horsley et al., 2013). Although the catheter appears to provide a cleaner and non-contaminated specimen (Wolfe et al., 2012), this method of urine collection misses the key pathological sediment that is required for achieving a reliable diagnosis. There did not appear to be a significant difference with the proportion of wbc and epithelial cells identified on urine microscopy. The cells found in the urine samples did not differ in the samples that were collected first or second.

### 5.2.3 Sediment cultures

The sediment cultures carried out on the urine samples collected by MSU, CSU and Peezy MSU grew polymicrobial organisms on chromogenic agar plates. It is now known that these polymicrobial isolates should be considered as potentially pathological and should not be dismissed as mixed growth of doubtful significance (Sathiananthamoorthy et al., 2012). In the patient group an increased quantity of bacterial isolates was identified. Although patients were symptomatic and grew polymicrobial organisms from their MSU, CSU and Peezy MSU samples, the CSU sediment cultures showed quantitative and qualitative differences to the other two methods. It is questionable whether the CSU method of sampling provides a reliable result as has previously been claimed (Wilson and Gaido, 2004). These data suggest that the CSU leads to an underestimation of the severity of the infection. There could have been a possibility that the urinary sediment was too

thick to pass through the eye of the catheter, hindering the maximum number of sediments obtained and cultured.

The control group were asymptomatic at the time they were enrolled into the study. However, bacterial growth was found to have cultivated on some of the MSU and Peezy MSU samples. The bacterial quantities did not measure up to the amounts observed in the patient group, but these microbes illustrate that the "healthy bladder" can also produce a positive sediment culture. This supports the modern contention that there is no such thing as a sterile bladder and all urine samples have the capability of growing polymicrobial isolates. The MSU and Peezy MSU samples cultured from the patient and control groups grew similar organisms such as *Enterococcus* and *Escherichia coli,* both of which are commonly associated with invasion of the lower urinary tract (Chapman et al., 2014).

### 5.2.4 Epithelial cells sediment using uroplakin-3

Within the literature there is a constant reiteration of the claim that epithelial cells found in MSU samples are contaminants and their detection results in many urine samples being rejected by central laboratories (Collier et al., 2013). Using the uroplakin-3 staining technique it was possible to establish that a very high proportion of cells found in the urine samples originated from the urinary tract. The number of MSU and Peezy MSU positive cells exceeded the number of positive cells found by the CSU sampling method. This provided further evidence that the CSU underestimates the infection. Evidence presented in this study reveal that, epithelial cells found in the urine are not contaminants, as the literature assumes, but are urothelial cells that have been exfoliated from the bladder during the inflammatory process of metaplasia and bladder urothelium regeneration. The notion that urinary epithelial cells imply contamination is a conjecture that was never supported by evidence, and yet it is widely accepted without questioning. This has a detrimental effect on urinary diagnostics, and continues to influence microbiological testing and urine handling in current practice (Linder et al., 2014, Basak and Uzun, 2013, Shrestha et al., 2013). Florescent microscopy and uroplakin-3 cell

staining is an important method for analysing cell origin, and has proved extremely successful in testing the historical assumptions that were challenged by these experiments.

## 5.3 Experiment two: comparing three different non-invasive urine specimen collection methods

### 5.3.1 Epithelial cells found in the MSU, Peezy and direct void

In the first experiment, the MSU and Peezy MSU methods produced samples with more of the pathological substrate than the CSU. In the second study, the simple direct void proved to be the superior collection method, providing a much greater concentration of the pathological substrate. Thus, the MSU and Peezy MSU methods underrepresented the disease process. The MSU method collects from the middle part of the urinary stream and misses the first part of the stream which contains a greater proportion of the sediment that has collected at the bladder neck. Similarly, the sponge bung at the base of Peezy MSU device inflates as the first part of the urinary stream is voided, in order to catch the middle part of the stream into the attached container. Thus, the bulk of the sediment from the deposit at the bladder neck is trapped in the sponge. However, the method, with adaptation, might offer a superior means of collecting the deposit and separating it from the rest of the void. It would seem that the direct void is the better collection method when it comes to urinary diagnostics. It is without technique, does not require the genital area to be cleansed prior to voiding and it is plausible to claim that it is a non-invasive urine collection method that captures the true pathology of a UTI.

### 5.3.2 Positive versus negative: The uroplakin cells found in the MSU, Peezy and direct void

Urinary contamination with the presence of epithelial cells continues to be the main reason why specimens get discarded (Collier et al., 2013). A recent meta-analysis reported on the processes that prevent urinary contamination and it was highlighted that a MSU without cleansing was not recommended, nor was collection of the first part of

urinary stream (LaRocco et al., 2016). The results reported from the second experiment challenge these claims. A very high proportion of these cells stained as uroploakin-3 positive and so they must have originated from the bladder. There is a widespread assumption that epithelial cells in the urine imply contamination but no evidence has ever been presented to prove this claim. These findings contradict strongly held, long-term beliefs and so they may struggle to gain acceptance.

When examining the mean proportion of epithelial cells that stained negative to uroplakin, the Peezy MSU specimens had the least. This probably confirms the assumption that this method traps fewer non-bladder epithelial cells and therefore succeeds in its goal. However, there is a price to pay in that a smaller proportion of the key pathological sediment from the bladder is sampled and that may lead to a reduction in sensitivity which the microscopy and culture data imply. These results imply that the better sample for urinalysis is the direct void method which seems to provide a good representation of the pathological cells. A key point is that the qualities of a urine specimen cannot be determined unless the uroplakin staining method has been included as part of the analysis. Understanding where urothelial cells originate from provides evidence and facts.

**5.4 Methodological considerations**

There were two laboratory limitations that should be reflected upon for future research. The first limitation was the time frame in which the urine specimens were collected, to when they were stained with uroplakin in the laboratory. All urine specimens had to be transported to the laboratory in a private car, increasing the waiting period to approximately four hours. The cells in the urine could have depleted, thus reducing the number of cells for preservation and staining. The second limitation was the large number of urine specimens stained with uroplakin-3 on the same day. Uroplakin staining was a significant part of the study. Although stained cells were enumerated by the researcher and double counted by a laboratory assistant; processing fewer urine specimens in a day

would have reduced the risk of omissions and mistakes at each stage of the staining process.

### 5.5 Summary

Assumptions about the quality of a urine specimen have led to non-invasive methods being discredited. Without validation, the MSU method has been referred to as the method that harbours contaminants. Notwithstanding the beliefs of clinicians that a MSU is the best method for collecting a urine specimen, patients are the primary donators of urine specimens and exploring their experiences on urine specimen collection should provide insight into whether they are accustomed to these conjectures. In the next chapter, the literature on patient experiences of urine specimen collection methods will be reviewed. It will then continue with a description of the process used for conducting the qualitative study that examined patients' views and experiences of providing a urine specimen for diagnostic testing.

# Chapter 6

# Patient views and experiences of urine specimen collection methods

## 6.1 Introduction

In chapter three, the two experiments relied heavily on examining urine specimens given by the participants. Although the participants consented to taking part in the study, it was not known how they felt or what their experiences were on providing a non-invasive and invasive urine specimen for testing. It was considered important to find out more about their views on each of the urine collection methods, and to discover their preferences. Thus, a qualitative study using interviews was conducted to examine patients' views and experiences of providing a urine specimen for diagnostic testing.

This chapter includes a review of the literature on patient experiences of urine collection methods and a description of the process used for conducting the qualitative study that examined patients' views and experiences of urine specimen collection. The findings and discussion form the last part of this chapter and a summary at the end highlights key aspects identified within this chapter.

## 6.2 A review of the literature

The purpose of this literature review was to examine studies that investigated patients' experiences, preferences and choice towards urine testing for genitourinary infections. The inclusion criteria for the review was research related to adult patients and adolescents, as opposed to children under 12 years old, with experience of urine specimen collection in in-patient, out-patient and community settings and literature written in English. These parameters were included so that the literature review focused solely on patient experiences of urine specimen collection in a health care setting.

The literature on patient experience was reviewed from the same databases that were used to access publications on urine sampling methods. Thus, the electronic research databases and indexes such as Web of Science, PubMed, EMBASE and CINAHL were explored. There were no date restrictions on the literature search; this was because there was a need to gather as many relevant papers that addressed three main aspects which were patient experience, satisfaction and preferences. As there were a large number of publications found relating to these three aspects, the search was refined further using specimen collection, urinary testing and urinary samples as a method of extracting specific literature. Boolean operators: (AND and OR) and Truncation: (*) were applied to the search in order to obtain literature specific and relevant for this review. CINAHL terms and Medical subheadings (MeSH terms) were also used in order to generate relevant literature within each database. Table 40 shows the number of publications found based on the search terms and refined terms. The subsequent table (Table 41) lists all the relevant literature starting with the earliest publication first.

## Table 40: Literature search strategy

| Database/ Indexes | Main search terms | Numbers found | Relevant studies with refined search terms:<br>• **Specimen collection**<br>• **Urinary testing**<br>• **Urinary samples** |
|---|---|---|---|
| **PubMed** | | | |
| | Patient information | 260,834 | 2 |
| | Patient preference | 12,231 | 3 |
| | Patient satisfaction | 87,915 | 5 |
| **Web of science** | | | |
| | Patient information | 10,000 | 0 |
| | Patient preference | 8,966 | 0 |
| | Patient satisfaction | 10,000 | 400 |
| **EMBASE** | | | |
| | Patient information | 55,854 | 4 |
| | Patient preference | 25,647 | 6 |
| | Patient satisfaction | 215,713 | 245 |
| **CINAHL** | | | |
| | Patient information | 6,252 | 10 |
| | Patient preference | 875 | 4 |
| | Patient satisfaction | 35,708 | 587 |

**Duplicates removed**

**70%** were duplicates and removed leaving **379** titles to screen.

**Abstracts screened**

**100** abstracts screened. **89** were irrelevant and dismissed.

**Literature eligible**

**11** papers were relevant and reviewed.

**Table 41: Publications reviewed and included in the literature**

| # | Studies included | Country of publication | Study aims<br>Clinical Speciality | Design<br>Sample<br>Data collection<br>Analysis methods | Key findings |
|---|---|---|---|---|---|
| **1** | Vriesema JLJ, Poucki MH, Kiemeney L and Witjes JA (2000) Patient opinion of urinary tests versus flexible urethrocystoscopy in follow-up examination for superficial bladder cancer: A utility analysis. Urology 56(5): 793-797 | Netherlands | To examine patients' opinions about the required validity of non-invasive diagnostic tools.<br><br>**Clinical Speciality:** Lower urinary tract symptoms/Bladder Microbiology | **Design:** Survey<br>**Sample:** 102 patients who were at least 1 year in follow-up for superficial bladder cancer. Antenatal patients and 53 medical inpatients.<br>**Data collection:** Questionnaire Telephone interview<br>**Analysis methods:** Standard gamble technique | Patients prefer flexible UCS as the diagnostic method in the follow-up of superficial bladder cancer if a urinary test is less than 90% sensitive. |
| **2** | Serlin M, Shafer MA, Tebb K, Gyamfi AA, Moncada J, Schachter J and Wibbelsman C (2002) What sexually transmitted disease screening method does the adolescent prefer? Adolescents' attitudes toward first-void urine, self-collected vaginal swab, and pelvic examination. Archives of Pediatrics & Adolescent Medicine 156(6): 588-591 | United States of America | To assess sexually active adolescents' attitudes toward 3 screening collection techniques.<br><br>**Clinical Speciality:** Sexual health | **Design:** Comparative study<br>**Sample:** A convenience sample of 155 ethnically diverse females aged 12 to 21 years.<br>**Data collection:** First-void urine (FVU), Self-collected vaginal swab specimens, pelvic examination with clinician-collected endocervical swab specimens.<br>**Analysis methods:** The Friedman test<br>Wilcoxon rank sum follow-up tests. | Most sexually active adolescents attending clinics for pelvic examination prefer to be screened for sexually transmitted diseases first by the FVU.<br><br>Second by the self-collected vaginal swab test.<br><br>Last by the pelvic examination. |

| | | | | | |
|---|---|---|---|---|---|
| **3** | Hsieh YH, Howell MR, Gaydos JC, McKee KT, Quinn TC and Gaydos CA (2003)Preference among female army recruits for use of self-administrated vaginal swabs or urine to screen for Chlamydia trachomatis genital infections. Sexually Transmitted Diseases 30(10): 769-773 | United States of America | To investigate the preference and comfort level of military women for the collection of self-administered vaginal swabs compared with urine, for the diagnosis of genital chlamydial infections.<br><br>**Clinical Speciality:** Sexual health | **Design:** Survey<br>**Sample:** 2785 Female Army recruits from Fort Jackson South Carolina.<br>**Data collection:** 1403 completed questionnaires 1382 provided both specimen and questionnaire.<br>**Analysis methods:** independently on cultivation results. | A study of preferences for urine versus self-administered vaginal swabs for the detection of Chlamydia trachomatis showed that women generally found self-administered vaginal swabs acceptable.<br><br>Self-administered vaginal swabs should be a feasible alternative to urine collection in situations in which specimen storage or transport is an issue. |
| **4** | Newman SB, Nelson MB, Gaydos CA and Friedman HB (2003) Female prisoners' preferences of collection methods for testing for Chlamydia trachomatis and Neisseria gonorrhoeae infection. Sexually Transmitted Diseases 30(4): 306-309 | United States of America | To compare female prisoners' preferences for collection of specimens (self-collected vaginal swab specimens, urine collection, or pelvic examination) for detection of Chlamydia trachomatis and Neisseria gonorrhoeae.<br><br>**Clinical Speciality:** Sexual health | **Design:** Comparative study<br>**Sample:** 535 women between the ages of 18 and 52 years of inmates in a large federal prison.<br>**Data collection:** Self-collected vaginal swab specimens, urine specimen & pelvic examination.<br><br>A questionnaire regarding the ease of each method and their preferences for future specimen collection<br>**Analysis methods:** Unknown | The study population of female federal prisoners expressed no aversion to the self-collection of either vaginal swab or urine specimens for STD testing.<br><br>Most participants expressed a preference for non-invasive techniques rather than a pelvic examination. |
| **5** | Pimenta JM, Catchpole M, Rogers PA, Perkins E, Jackson N, Carlisle C, Randall S, Hopwood J, Hewitt G, Underhill G, Mallinson H, McLean L, Gleave T, Tobin J, Harindra V and Ghosh A (2003) (2003) Opportunistic screening for genital chlamydial infection. I: Acceptability of urine testing in primary and secondary healthcare settings. Sexually Transmitted Infections 79(1): 16-21 | United Kingdom | To determine the acceptability of opportunistic screening for Chlamydia trachomatis in young people in a range of healthcare settings.<br><br>**Clinical Speciality:** Sexual health | **Design:** Observational study<br>**Sample:** Sexually active women aged between 16 and 24 years attending healthcare settings for any reason. | An opportunistic model of urine screening for chlamydial infection is a practical, universally acceptable method of screening. |

| | | | | | |
|---|---|---|---|---|---|
| | | | | **Data collection:** Urine samples tested by ligase chain reaction & In-depth interviews<br>**Analysis methods:** Evaluation data: participants' attitudes and views towards opportunistic screening and urine testing. | |
| **6** | Tebb KP, Paukku MH, Pai-Dhungat MR, Gyamfi AA and Shafer MAB (2004) Home STI testing: The adolescent female's opinion. Journal of Adolescent Health 35(6): 462-467 | United States of America | To assess sexually active adolescent females' attitudes of home tests for sexually transmitted infections.<br><br>**Clinical Speciality:** Sexual health | **Design:** Longitudinal study<br>**Sample:** Adolescents aged between 13-20-years-old<br>**Data collection:** first void urine (FVU), self-collected vaginal swab samples, a pelvic examination with STI screening by endocervical swabs pre-examination health survey<br>**Analysis methods:** Friedman tests. | The young women preferred home STI testing. Adolescent preferences may be heavily influenced by the pelvic examination experienced. Multiple screening options (clinical and home-based) need to be available to increase access to care. |
| **7** | Chernesky MA, Hook EW, Martin DH, Lane J, Johnson R, Jordan JA, Fuller D, Willis DE, Fine PM, Janda WM and Schachter J (2005) Women find it easy and refer to collect their own vaginal swabs to diagnose Chlamydia trachomatis or Neisseria gonorrhoeae infections. Sexually Transmitted Diseases 32(12): 729-733 | Canada | To survey women's opinions on ease and preferences as to sampling after collecting their own vaginal swab and urine and a physician collection of vaginal swab and cervical swab.<br><br>**Clinical Speciality:** Sexual health | **Design:** Survey<br>**Sample:** 1,090 women consenting to gynecologic sampling for Chlamydia trachomatis completed a survey.<br>**Data collection:** Questionnaire<br>**Analysis methods:** They analysed the data for ease of self-collection and preferences for a vaginal swab, urine, or cervical swab. | Self-collected vaginal swabs were easy to collect and patients preferred them over urine and cervical swabs. |

| | | | | | |
|---|---|---|---|---|---|
| **8** | Jackson SR, Dryden M, Gillett P, Kearney P and Weatherall R (2005) A novel midstream urine-collection device reduces contamination rates in urine cultures amongst women. BJU Int 96(3): 360-364 | United Kingdom | To evaluate a novel urine-collection device (UCD) that automatically collects midstream urine (MSU) sample, and compare contamination rates to those of the conventional MSU sampling method.<br><br>To evaluate patient/staff preferences between the two methods.<br><br>**Clinical Speciality:**<br>Lower urinary tract symptoms/ Bladder Microbiology. | **Design:** Comparative study<br>**Sample:** 20–35 years Women attending outpatient clinics in four different centres.<br>**Data collection:**<br>2823 samples collected for culture **Analysis methods:** SPSS | The data indicate that the UCD was easy to use and more acceptable to the patients providing the samples. Reasons for this may include the reduction of spillage during collection.<br><br>Clinical staff supervising the use of the UCD preferred it to conventional MSU sampling because they thought it reduced the time taken to collect and process samples. |
| **9** | Bilardi JE, De Guingand DL, Temple-Smith MJ, Garland S, Fairley CK, Grover S, Wallace E, Hocking JS, Tabrizi S, Pirotta M and Chen MY (2010) Young pregnant women's views on the acceptability of screening for chlamydia as part of routine antenatal care. Bmc Public Health 10 | Australia | To determine the acceptability of screening pregnant women aged 16-25 years for chlamydia as part of routine antenatal care.<br><br>**Clinical Speciality:** Sexual health | **Design:** Cross-sectional study<br>**Sample:** 100 Pregnant women aged 16-25 years.<br>**Data collection:** Semi structured interview<br>**Analysis methods:** NVivo | Chlamydia testing using urine specimens was highly acceptable among young pregnant women in this study.<br><br>For antenatal chlamydia testing to be broadly accepted it is imperative pregnant women are well informed about chlamydia and the benefits of screening. |
| **10** | Howard EJ, Xu F, Taylor SN, Stoner BP, Mena L, Nsuami MJ, Powell S, Lillis R and Martin DH (2011) Screening methods for Chlamydia trachomatis and Neisseria gonorrhoeae infections in sexually transmitted infection clinics: what do patients prefer? Sex Transm Infect 87(2): 149-51 | United States of America | To determine self-obtained vaginal swabs and first-catch urine samples testing for Chlamydia trachomatis and Neisseria gonorrhoeae screening are acceptable replacements for a traditional provider visit.<br><br>**Clinical Speciality:** Sexual health | **Design:** Survey<br>**Sample:** 2887 patients seen at STI clinics in three US cities.<br>**Data collection:** Questionnaire<br>**Analysis methods:** Unknown | If there was a long clinic wait, 58% of the survey participants preferred to wait to see a doctor.<br><br>If the clinic had to turn patients away, 41% of patients preferred to come back the next business day.<br><br>46% preferred to self-collect a sample. |

| | | | | | |
|---|---|---|---|---|---|
| **11** | Collier S, Matjiu F, Jones G, Harber M and Hopkins S (2013) A prospective study comparing contamination rates between a novel mid-stream urine collection device (Peezy) and a standard method in renal patients. J Clin Pathol. | United Kingdom | To investigate a novel urine collection device (Peezy) in a renal outpatient clinic to determine whether it reduced contamination of urine samples.<br><br>To evaluate patient experiences of urine sampling.<br><br>**Clinical Speciality:**<br>Lower urinary tract symptoms/ Bladder Microbiology. | **Design:** Comparative study<br>**Sample:** 848 Female patients, 424 historical patients (control group)<br>& 420 Peezy users (study group)<br>**Data collection:** Standard MSU into a sterile jug, Peezy device & Questionnaire (for Peezy users only)<br>**Analysis methods:** STATA V.11.0 | Peezy: Patients complained of urine being spilt and the instructions not being clear despite the staff in the unit explaining the method and posters and information leaflets being freely available. |

### 6.2.1 Literature retrieved

In total eleven publications were retrieved, all dated within the last thirteen years and related to urine testing for genitourinary infections. Of the eleven studies, five were conducted in the USA, three were carried out in the UK and the remaining three were carried out in Canada, Australia and the Netherlands. There were a higher number of published articles on patient preference and satisfaction towards urinary sexual health screening. It was obvious from the literature retrieved that patient views and experiences of urine specimen collection for diagnosis of a UTI were not explored. All the studies were reviewed to identify common themes that emerged from the findings in each publication.

### 6.2.2 Patient experiences of urine specimen collection for sexual health screening

In all of the papers that focused on sexual health screening, the ultimate study objectives were to examine what patients thought was the best method of obtaining a specimen, and whether these preferred techniques could be implemented as a standard practice (studies 2, 3, 4, 5, 6, 7, 9 and 10). It was frequently observed that the main specimen collection methods were first voided urine and vaginal swabs. Four out of eight papers reported that the participants preferred the urine collection method compared to the vaginal swab method (studies 2, 4, 5 and 9). This technique of specimen collection was highly accepted due to it being a non-invasive; however two papers reported that self-collected swabs were a preferred method over a urine specimen as the technique was simple and there was no difficulty in the collection process (studies 3 and 7). The findings in each of these studies were very important, as they have helped to develop new approaches to clinical and home based screening practices.

Patient views and experiences should take precedence in clinical policies and guidelines and, to some degree this has strengthened patient autonomy when it comes to healthcare provision (Sheppard, 2014). Study 6 examined patients' preferred method of providing three specimens for sexual health screening. These three specimens were the first part of voided urine, self-collected swab and pelvic examination with endocervical swab by a clinician. It was reported that the participants in the study preferred home sexual health tests, which in most cases are vaginal swab kits or urine specimens which are posted to a specialist screening unit (Wayal et al., 2011). The choice of having a pelvic examination and endocervical swab was heavily influenced by previous experiences of an

internal examination. Study 6 identified that it was necessary to provide patients with the opportunity of having multiple screening options, clinical and home based, in order that patient choice and preference were incorporated into their screening practices.

### 6.2.3 Patient experiences of urine specimen collection for microbiology

Three studies explored patient satisfaction regarding non-invasive urine sampling techniques (studies 1, 8 and 11). Study 8 compared the MSU sampling method against a urine collection device. The purpose of their investigation was to explore the rates of contamination between each sampling method and to evaluate patient preferences between the two techniques. A total of 2823 women were enrolled into the study and had the opportunity to provide two urine samples for testing. They reported that the urine collection device was the preferred method by the patients and the clinical staff who supervised the use of the device. This sampling technique was highly acceptable compared to the standard MSU, because urinary spillage was not experienced and the time it took to collect and process the specimen was reduced.

Similarly, study 11 investigated the suitability of a non-invasive urine collection device in order to reduce contamination in urine specimens. At the same time, they also wanted to explore what patients experienced whilst using the device. A total of 420 patients and 424 historic patients (controls) provided a urine sample by urinating into the Peezy MSU device. They evaluated patients' experience by sending out a questionnaire survey that measured patient satisfaction. With only a 16% return rate they were unable to draw a generalised conclusion to the findings. However, they did report that there was patient dissatisfaction towards urine spillage and unclear instructions on how to use the device. In contrast, study 1 did not examine patient experiences of urine collection methods, but explored patients' opinions on urinary testing versus urethrocystoscopy for diagnosing superficial bladder cancer. Urethrocystoscopy is an invasive procedure whereas obtaining a urine specimen is not. Patients were followed up one year after treatment for superficial bladder cancer. Questionnaires were given out and telephone interviews were conducted in order to gather data on patient experiences of urethrocystoscopy as opposed to a urine test for detecting bladder cancer sensitivity. They reported that patients would much rather have the urethrocystoscopy compared to the urine test, if the urine test was 90% less sensitive in diagnosing superficial bladder cancer. Patient

experiences of both the standard MSU method and the urine collection devices in each study were mixed, and there were no similarities in the findings of all three studies.

### 6.2.4 Common themes

Each study explored attitudes and perceptions towards specimen collection for diagnostic testing. Within the literature, it is frequently observed that patients prefer self-sampling methods as opposed to a healthcare professional carrying out the task (studies 2, 4 and 6). It is understood that patients' choices and preferences promote change in standard practice (study 6), which strengthens patient autonomy with care provision and screening management (Sheppard, 2014). The literature identifies that self-screening practices appear to take place more frequently in the sexual health settings, and even though self-screening occurs when patients provide a urine sample for testing, it is not always recognised as a convenient screening practice (study 1). There are various reasons why urine sampling has not been regarded as the preferred method of diagnostic testing. The literature has identified the difficulties that patients report about urine sampling such as urine spillage and the inability to carry out the correct technique (study 11), as well as patient uncertainty towards the accuracy of urine testing (study 1). However, understanding patient experiences of specimen collection for urinary diagnostics is important knowledge for future practice.

### 6.2.5 Conclusions from the literature review

Understanding patient satisfaction broadens the knowledge that clinicians have about patient preferences and choice. It also shows that patients' views are important when organising management of care. Screening programmes and specimen testing have changed, as patient experiences have modernised clinical policies and guidelines, and this has enabled patients to become more autonomous in healthcare provision. There are various thoughts and opinions when it comes to urinary diagnostics, which has been observed within the literature reviewed. Understanding why urine specimen collection is least preferred compared to other screening practices will require further exploration. However, there is some insight into the difficulties that patients are faced with when asked to provide a specimen of urine (studies 8 and 11).

Based on this literature review, it was essential to gain knowledge of, and insight into, patient experiences of collecting a urine specimen for diagnosing a UTI. It is common practice in clinical outpatient settings to request a urine specimen from patients. However, it is not common practice to evaluate a patient's experience of their perceptions of a urine collection method or technique. Some studies have shown the importance of obtaining patient feedback on different methods of urine sampling (studies 8 and 11) whereas others have omitted this consideration (Unlu et al., 2007, Shrestha et al., 2013). Qualitative studies are necessary when a researcher seeks to understand the meaning of experiences (Al-Busaidi, 2008). Qualitative research is the essential method of enquiry when gathering information about personal views and experiences in order to identify the true meaning of an unknown phenomenon (Denzin and Lincoln, 2011).

## 6.3 Eliciting patient views and experiences of urine specimen collection methods

### 6.3.1 Aims

Patients are often approached by a clinician requesting a urine specimen for testing when attending urological outpatient appointments. Providing a urine specimen for testing is an invasion of privacy and understanding how patients feel about donating a bodily fluid is important. Eliciting patient views and experiences of urine specimen collection was the fundamental aspect of bringing together patient views and experiences of providing a specimen of urine. The aim of this study was to explore the experiences patients have when providing a urine specimen for diagnostic testing and to examine recurring themes that emerged from the use of non-invasive and invasive urine collection methods.

### 6.3.2 Research design and methods

A qualitative approach was the most suited for this study. Denzin and Lincoln (2011) refer to the theoretical importance of qualitative research when exploring views and experiences. Qualitative research explores perceptions through lived experiences (Denzin and Lincoln, 2011) and has been used in many other studies to explore sensitive topics (Spiers and Smith, 2015, Smith and Osborn, 2015, Wawrziczny et al., 2015). This study was presented to the national research ethics service committee (NRES) London-Harrow

in April 2014, in order to obtain approval to carry out the investigation. Additional documentation was included, a consent form (Appendix 12), a participant information sheet (Appendix 13) and a list of the interview questions (Appendix 14). Ethical approval was granted as the study complied with conditions that were favourable and worthy of safe research practice. The study was managed in accordance with the Royal College of Nursing Research Ethics guidelines (RCN, 2011).

### 6.3.3 Sampling strategy

The focus of this study was to investigate patients' views and experiences of providing a non-invasive and invasive urine specimen. It was intended that all 31 patients' who had participated in experiment two would be interviewed. They were identified as suitable candidates as they had first-hand experience of providing non-invasive urine specimens in experiment two as well as an invasive urine specimen in experiment one. Each of these participants was approached by letter (Appendix 15) with a brief description of the study and a study information sheet. Each participant was provided with contact details and a response form with a stamped address envelope to indicate their willingness to participate in the study. They were also given the opportunity to contact the medical urology centre by e-mail or telephone at their convenience.

The participants that expressed an interest in taking part in the study were invited to the medical urology centre in writing and, at attendance, were fully informed about the study and what was involved. Information regarding why the study was being conducted was explained in detail, the importance of exploring patient experiences was discussed and why they were an eligible participant was explained. They also had the opportunity to read through the information sheet with the researcher and ask questions relating to the study. When the participants were satisfied, fully informed of the study and agreed to take part, they signed a consent form. The participants were given a copy for their records, a copy was filed in the site research file and a copy in clinical hospital notes. Participants were also informed of their rights to withdraw consent from the study at any time.

### 6.3.4 Interview questions

The interviews were conducted by the clinical research nurse who usually asked patients in the clinic to provide a urine specimen for testing. A semi-structured interview schedule was devised which included a set of four interview questions. Semi-structured interviewing was chosen because it is an open method that allows new ideas to emerge during the interviewing process (Bruce and Lune, 2014). The open-ended questions were based on the research aims which sought to explore patients' views and experiences of providing a urine specimen for diagnostic testing. Four different urine collection methods were used as described in Table 42.

**Table 42: Urine collection methods**

| Midstream Urine (MSU) | Catheter Specimen (CSU) | MSU Peezy Device | Direct Void |
|---|---|---|---|
| **Concept** | | | |
| Catching the middle part of the urine stream. | Inserting a catheter to drain a specimen of urine. | Urinating into a device that collects the middle part of the urine stream. | Urinating directly into a specimen bowl with no technique. |

As no publications were found that had explored patient experiences on urine collection methods for diagnosing a UTI, the interview questions were devised to provide insight regarding patients' experiences when asked to perform the task. At the beginning of each interview, an open question was asked about their experiences of providing a urine specimen for testing. The open discussion started with….*So tell me about your experiences of providing the different urine specimens at the previous study visits.* This opening was to remind the participants of the different urine collection methods and give them the opportunity to speak freely about their experiences.

As the participants started to remember how they performed the urine collection methods, interview question 1 was asked. Each question has been listed in the Table 43 along with the rationale for that question.

**Table 43: Interview questions with rationale**

| Question number | Question asked | Rationale for question |
|---|---|---|
| **Interview question 1** | Which method of urine collection did you prefer and why? | This question was asked because of one of the themes rising from the literature review on patient experiences was that participants remember sampling methods they prefer rather than sampling methods that are least preferred. |
| **Interview question 2** | Which method of urine collection did you not like and why? | This question was asked to establish which method of urine specimen collection was least preferred and what their reasons were. |
| **Interview question 3** | What method of urine collection do you think provided the most clean sample and why? | The importance of urine sampling was addressed in chapter three. The patients attending the medical urology centre are aware that a clean uncontaminated urine specimen is required when diagnosing a UTI. At the beginning of this question, the participants were reminded of the importance of a clean uncontaminated specimen. This question aimed to explore the participants' perception of optimal specimen collection. |
| **Interview question 4** | Which method of urine collection do you think should be used as a standard method and why? | The literature review on patient experiences of urine sampling highlighted that patient preferences should influence the way screening and diagnostic tests are to be implemented. This question was included to explore how patient experiences might influence change in the future of specimen collection and diagnostic testing. |

All of the questions were tested out by conducting mock interviews with colleagues at the medical urology centre. This was undertaken to check the wording of the questions, the understanding of what was being asked and whether the appropriate interview skills had been deployed. Medical terms were excluded from the interviews so that the participants could fully understand the questions being asked. The researcher had the opportunity to talk about the interview questions with colleagues to clarify the meaning of each question.

### 6.3.5 Interview procedure

Each participant was given an appointment to attend the interview. They were welcomed at the front reception of the medical urology centre upon arrival. They were offered a hot or cold beverage prior to starting the interview to make them feel comfortable and later

taken to the clinical room where the interview took place. Each participant kept the same study number they had been given in experiment one and experiment two. This unique number was used throughout the interviewing process.

The interviews were conducted by the clinical research nurse. The research nurse was also a member of staff at the medical urology centre and had an active role in looking after, treating and following up the patients that attended the clinic. The participants were familiar with the nurse which made them feel comfortable and relaxed during the interview process. All interviews were conducted face to face and undertaken in the same clinical room where the participants were located when they had participated in the urine comparison study. It was intended that the clinical room would be used as a reminder to bring back the memory of when they had the invasive and non-invasive urine samples collected, and to prompt their thought process of what they had experienced during that time. The importance of participants visiting the medical urology centre for interviewing was to ensure the correct participant was being interviewed and vocal narratives were clearly being heard (Hennink et al., 2011).

Before embarking on each of the interviews participants were asked whether they would be prepared for the interviews to be recorded as part of the data collection process. They were reassured that patient confidentiality would be guarded throughout the study. In the event, a majority of the participants did not want their voices recorded as they felt that their bladder condition was a sensitive matter and they did not feel comfortable having their voices stored as data. Typically, in this type of research, interviews would be digitally recorded and transcribed *verbatim*. However, owing to the sensitivity of the topic and the feelings of the majority of the participants, a digital recorder was not used. Instead the interviewer was able to type responses of participants *verbatim* whilst the participant answered the questions. The participants were encouraged to speak freely whilst their answers were being typed. The researcher was a skilled typist, and had the expertise to accurately type whilst the interviews were being conducted. A good interviewer will adopt the skills to ask questions that are clear, short and open-ended, using appropriate follow-up probes and listening carefully to what has been said (Bruce and Lune, 2014). The *verbatim* data were typed carefully whilst each participant responded to the questions, and answers were enhanced by implementing the skill of

probing which included words such as why?, what else? and how? It was intended that thirty one interviews would be conducted in order to capture the views and experiences of each participant from experiment two. One participant did not want to participate in the interviews and refused consent. It was decided that the subsequent interview was not required and that thirty interviews would be sufficient for data analysis. At the end of each interview the participants were thanked for their time and were asked if there was anything they would like to add to their comments. This gave the participants an opportunity to bring up points that they had thought about during the interview, or that were important but had not been raised as one of the interview questions.

### 6.3.6 Analysis

All interview data were entered into NVivo which is a programme that organises, analyses and provides insight from unstructured data (QSR, 2016). The NVivo software grades the most frequently used words at the top of the analysis grid and gradually descends down to the least frequent word. This method of analysis was also supported with a numerical figure pertaining to how many times the word occurred, thus helping to establish recurring patterns, trends and themes, a process known as thematic analysis. Thematic analysis is the most common method of analysis in qualitative research (Guest et al., 2012). It favours the pinpointing, examining and recording of patterns and themes in the data (Braun and Clarke, 2014). It is particularly useful when exploring opinions and thoughts on matters that are not commonly researched. Thematic analysis was used in this study to explore the varied experiences patients have when providing a urine specimen for diagnostic testing. The data were analysed for repetitive words, phrases and themes that each participant used to describe their views and experiences. Text searching was used to find the occurrences of words, phrases or concepts that related to a particular interview question. This method of analysis proved very useful in identifying repetitive words that were commonly verbalised by each participant.

Word frequencies were also used to explore the numerical occurrence of commonly used words and concepts. Word clouds and word trees were used to present the data. A word cloud is a participant-generated combination of words which enables the data collected to be visualised through free listings, diagrams and rank ordering (Douma et al., 2015). Word clouds are dependent upon participant perceptions and reinforce word recognition as the principle mechanism of generating results (Bletzer, 2015). A word cloud is the best

method for visualising patient-generated data because it interprets a deeper and more detailed understanding of participant's perspectives on a certain subject or situation based on the frequency of words and themes identified (Hennink et al., 2011). Word clouds have been incorporated in many health and social science research studies in order to present themes (Weiwei et al., 2010), words or concepts that relate to biological themes (Baroukh et al., 2011) as well as words or concepts that relate to different levels of satisfaction and personal experiences (Bletzer, 2015). A word tree displays the results of the data as a tree with branches that represent the various contexts in which the word or phrase occurs (QSR, 2016). This visualisation helps with finding recurring themes or phrases arising from the root term.

### 6.3.7 Credibility of analysis

The distinctive nature of thematic analysis is that it attempts to ensure credibity of the analytical process (Guest et al., 2012). The data were audited at various stages of the analysis. The process of auditing is shown in Table 44. The realibity of interpretation of themes was determined by consensus between the researcher and an NVivo educator specialist. This is considered a trustworthy and transparent method for identifying themes in qualitative analysis (Bazeley and Jackson, 2013).

**Table 44: Credibility of analysis: auditing process**

| Stage | Audit Process | Amendments |
|---|---|---|
| **Analysis of first five interviews** | Analysis checked and reviewed by NVivo educator specialist | Corrected errors generated through data inputting |
| **Review of the emergent themes** | Checked by the researcher to ensure the thematic analysis process was correct | Looked at other techniques to extract themes. |
| **Analysis of the subsequent ten interviews** | The interviews were thoroughly examined by NVivo educator specialist | Emergent themes were considered an accurate interpretation of finding and supported the generation of themes. |
| **Themes were finalised** | Time was spent with NVivo educator specialist to discuss how to present the data descriptively. | The researcher continued to work on organising and creating the final themes. |

## 6.4 Patients' views and experiences of urine specimen collection methods: findings and discussion

### 6.4.1 Introduction

The findings from this study indicated patients' views and experiences of providing four different urine specimens using invasive and non-invasive methods. Thirty interviews were conducted and analysed using NVivo which helped identify recurring themes that emerged from the interview data. Following on from the findings, the main themes that were identified are discussed and a concluding summary highlights the significance of these themes along with the methodological considerations.

### 6.4.2 Findings: patient experiences of urine specimen collection methods

Thirty interviews were conducted and four themes were established which were:

**Theme 1:** Straightforward urine collection

**Theme 2:** Painful urine specimen collection

**Theme 3:** The optimal specimen collection

**Theme 4:** Simple urine collection methods as standard practice

Each of these themes will be presented in this chapter and will incorporate participants' quotes and comments in order to illustrate their views on non-invasive and invasive urine specimen collection methods. Participants will remain anonymous and quotes and comments are assigned to the four-digit study ID number that was given to participants in the first study.

### Theme 1: Straight forward urine collection

With the first interview question '*which method of urine collection did you prefer and why?* Each of the participants spoke about being able to provide a urine sample that was easy and straightforward. The directly voided method was repeatedly referred to and was regarded as being the most straightforward of all of the sampling methods. Many of the participants talked about being able to provide a urine specimen that was not labour

intensive or painful. Participants shared their views on the directly voided urine by saying *''its easy''* (Participant 3867). One participant was more explicit in saying *'' it's not a problem you just open your legs and let it all out''* (Participant 3458). Another participant continued said: *''I prefer urinating into the pot. It's straightforward''* (Participant 2365).

The participants often mentioned being able to carry out a task that was quick and easy, especially as bladder troubles were the primary reason for them attending the centre. The word tree in Appendix 16 shows the results of this theme in more detail and the word cloud (Appendix 17) provides a visual representation of the results. The MSU and the Peezy method were not considered straightforward compared to the direct void. There were mixed views towards the MSU as some participants found the MSU method challenging and expressed concerns with comments such as:

> *''You wonder whether you have done it right''* (Participant 2906).
>
> *''the midstream is awkward because you don't know when you catching the midstream''* (Participant 2287).
>
> '' *The midstream I don't like because if you lose the urine whilst getting the midstream you lose it all over your hands'' (*Participant 3121).
>
> *''The MSU can be tricky and a bit messy and it has caught me out a couple of times''* (Participant 2977).

Straightforward urine collection was considered the best way for obtaining a urine specimen. The Peezy MSU was regrded as complex and was not the easiest method for obtaining a urine sample and participants frequency expressed their views on the complexities of the device with comments such as:

> *'' I wasn't keen on the peezy as I needed an extra pair of hands, and I didn't trust the instruction provided in the pack''* (Participant 3600).
>
> *'' I didn't like the peezy because I couldn't work out how it worked and I was worried it was going to disconnect and I would end up peeing all over the floor''* (Participant 4014).
>
> *''The MSU peezy; It was confusing; I needed a demonstration of how to use the device. I wasn't sure whether I was to stand up or sit down when using the device''* (Participant 4265).

It was clearly established that participants were more enthusiatic about providing a urine specimen that was easy and straightforward to perfom. Although they were familiar with the MSU, there remained uncertainties with the collection technique.

**Theme 2: Painful urine specimen collection**

As a majority of the participants attending the clinic had painful bladder symptoms, the experience of having the catheter inserted into the bladder brought back the memory of that pain. Thefore, participants commonly described their experiences of being catheterised as painful and uncomfortable. The pain symptoms were described only in the catheter method and not in the MSU, Peezy or direct void methods as these methods were all non-invasive. Participants described their dislike towards the catheter method with comments such as:

> *''I don't particularly like the catheter because it's scary even though it's not and feels as though you might get an infection''*(Participant 3499).
> *'' It's slightly uncomfortable. When it's taken out it has a strange sensation''* (Participant 2584).

The majority of the participants experienced a great deal of pain with the catheter collection method. In addition it infringed on their privacy due to the process of the collection method. One participant highlihted this point by saying *''The catheter was a bit invasive and it hurt''* (Participant 2977). As the catheter technique was believed to produce the optimal specimen, the pain associated with the method was endured for the purpose of obtaining an uncontaminated specimen. The word tree in Appendix 18 shows the results of this theme in greater detail and the word cloud Appendix 19 provides an illustration of the results.

**Theme 3: The optimal specimen collection**

The catheter was regarded as the urine collection method that produced the best specimen for diagnostic testing. When asked 'what method of urine collection do you think provided the cleanest sample and why?' the participants responded with answers such as:

*''The catheter of course. Because it's not touching anything to pick up any germs''* (Participant 3458).

''Probably the catheter because your introducing it straight into the urethra and there are no chances of germs entering''(Participant 3963).

A majority of the participants were adamant that the catheter was a better method and could not compare to the non-invasive methods. Although there were no overt claims that the catheter specimen was less likely to contain contaminants, there were subtle remarks suggesting that the catheter is a superior method with comments such as:

*''The catheter went straight in and eliminated areas where it could get contaminated''* (Participant 3600).

''*The catheter provided the cleanest sample as it is all internal and it's the cleanest way of collecting the sample''*(Participant 4014).

As more interviews were conducted it emerged that participants thought that the method of how the catheter was inserted into the bladder would bypass the chances of specimen contamination and because of this, strongly believed that the catheter sampling method was the optimal specimen for identifying their UTI. One participant confirmed this by saying:

*''I would have thought that the catheter would provide the opportunity to be uncontaminated, and provide the cleanest sample''* (Participant 3456).

Some of the participants did not consider the other three methods could compete with the catheter and shared their experiences by saying:

*'' Surely the other methods were not as good at getting a cleaner sample like the catheter?''* (Participant 4300).

*'' There is a likelihood of contamination from the other methods''* (Participant 2131).

*'' The other methods would most probably pick up germs, which the catheter wouldn't''* (Participant 3458).

There was a strong consensus that the catheter specimen was a superior specimen collection method and this was evident through the comments of each participant.

**Theme 4: Simple urine collection methods as standard practice**

Participants' agreed that they would like a urine collection method that would be easy and straightforward to perform. There was a further theme that demonstrated that the majority of the participants considered that the direct void should be the standard method in clinical practice for patients without a complex bladder, unlike them who require specialist treatment interventions. The participants were probed to extract more details on their thoughts, and the reasons why they suggested the direct void method. The participants responded with answers such as:

*''I think the pee in the pot should be the standard method, its straightforward''* (Participant 3965).

''*Because it is less time and takes less resources, it's something that is understandable by all''* (Participant 2211).

*''I would go for just straightforward peeing as in a doctor's office you would not have the time to do it any other way. I find that a majority of toilets are not designed for collecting midstream urine. It's difficult to get a midstream because it's hard to judge when you actually get the midstream, and you sometimes end up urinating on your hands''* (Participant 3963).

The participants highly reccomended a straightforward direct void as the best way for obtaining a urine specimen in general clinical practice for patients without a complex bladder. The word tree (Appendix 20) shows the results of this theme in greater detail.

### 6.4.3 Discussion: patient experiences of urine specimen collection methods

The participants were known to have a chronic UTI and a complex bladder. The interview data that emerged from this study revealed how important it was for them to have an uncontaminated urine specimen that would show the true pathology of their disease.

The participants thought that because the catheter was inserted directly into the bladder it provided a true identification of the causative pathogens unlike the non-invasive methods. The catheter specimen was considered the optimal method for obtaining a urine specimen free from external contamination and was the best method for them as they had a chronic urine infection accompanied with complex bladder symptoms. The reassurance of knowing that a urine collection method would provide an accurate identification of a UTI was considered an important factor, and in this case the catheter was the best method. The participants did not believe that the other non-invasive methods were of the same calibre, and were not trusted to scrutinise the microbes that were invading the urinary tract. Chapter three reveals that this was not the case, and that the catheter specimen actually bypassed the cells and sediments that were at the base of the bladder, which were crucial for diagnosing the offending microbes.

Despite the pain, there was a strong belief that the catheter method was best for identifying the UTI. Although pain and discomfort were closely associated with having a urine specimen collected by the catheter, the pain was the ultimate price to pay for an uncontaminated specimen. The participants were known to have a urinary tract infection and some had urethral inflammation, as a secondary symptom, at the time the catheter was being inserted into the bladder. Urethritis is the inflammation of the urethra and is commonly diagnosed in patients with a urinary tract and sexually transmitted infection (Moi et al., 2015). Having the catheter inserted with the presence of urethritis triggered further pain symptoms. Although the participants may not have had a comprehensive understanding of the pathophysiological aspects of a urinary tract infection, they were more aware of the pain and discomfort associated the catheter method compared to the other three non-invasive methods. The participants focused on having a urine specimen that was uncontaminated and that would identify the true pathology of a urinary tract infection. With the intention of obtaining an optimal specimen, the participants would rather endure the pain associated with the catheter method, than to have a contaminated specimen from the MSU, Peezy MSU and direct void methods. The direct void was the most desired urine collection method as it was straightforward, but was not considered the appropriate method for those who had a complex bladder, a UTI and required an accurate pathology of their infection.

The participants expressed that the direct void was straightforward and very easy to perform. They were not required to wipe the perineal area prior to voiding which many considered was a natural way to urinate. The direct void method was considered effortless and a well-suited urine collection method for anybody providing a urine specimen. The participants felt the need to express their views and experiences of urine collection methods that were not straightforward. They were vocal when describing the problems they faced when collecting a midstream specimen and when using the Peezy MSU device. The difficulty with the midstream method was that participants often reported that they were unsure which part of the urinary stream was the middle, and accurate judgement of this remained a constant concern. That was not the only unease related to the midstream method; fear of soiling hands during the collection process and having to wipe the perineal area brought on anxieties of whether the wiping method was correct or not. There were apprehensions towards the Peezy MSU device as participants often reported their lack of understanding on how to use it, as well as discussing their fears of the device being disconnected during the voiding process. These were anxieties and concerns raised when it came to providing a specimen in ways other than the direct void method.

With an increased number of patients asked to provide a urine specimen in busy outpatient settings for urinary diagnostics, the direct void method undoubtedly would seem to ease the pressures on clinical staff who explain urine collection techniques to patients. The importance of patient experiences is that it influences changes in clinical practice (Tebb et al., 2004). The interview data revealed that participants collectively believed that the direct void was a well-suited method for urine collection in standard general practice. The ability to provide a urine specimen that was simple and without a technique was favoured. The participants thought the direct void method had these attributes and believed that it should be implemented as standard general practice for specimen collection. Being able to provide a specimen that was easy to perform was the specimen method of choice. However, the direct void method was regarded a better option for patients who did not have a chronic urinary tract infection or a complex bladder.

### 6.4.4 Summary

This qualitative study has shown that patients with a UTI and a complex bladder believe that an uncontaminated urine specimen will identify the true pathology of their infection. The catheter specimen was believed to be the correct method and was regarded as a superior technique over the MSU, Peezy MSU and direct void. Despite pain being an associated consequence with the catheter method, pain symptoms were tolerated with the intention of obtaining an uncontaminated specimen. A straightforward urine collection technique was highly desired, but for those who did not require a specialist urine specimen for urinary diagnostics. The participants strongly believed that the direct void method should be the future of urine collection in general practice, and complicated urine collection techniques should be avoided.

### 6.4.5 Methodological considerations

There were a number of noted limitations that should be reflected upon for future research. The first limitation was the number of interview questions. Although the questions were relevant to the area of enquiry, an assortment of different questions may have contributed to a broader dialogue between the participants and the interviewer and may have contributed to the generation of more emergent themes. Questions such as:

1. What has been your experience of urine collection in the past?
2. What are the challenges you faced when providing a urine sample for testing?
3. Are early morning urine samples the best specimens for diagnosing a UTI or can urine specimens be collected at any time of the day?

These types of questions may have extracted a series of unexplored data and developed into hierarchical themes. The second possible limitation was that the participants were recruited from the medical urology centre where they received treatment. Despite being suitable candidates with experience in urine specimen collection, it could have been that some of the participants felt it was required of them to participate in the study in order to show gratitude and appreciation for the care they had received at the centre. In addition, they may have felt reluctant to withdraw consent from the study even if they wanted to.

# Chapter 7
# Urine specimen collection methods and patients' experiences

## 7.1 Introduction

Urine specimen collection is an important task and patients are instructed to provide a urine specimen for diagnostic testing. In chapter five it was revealed that urine specimens contain sediments and cells that are indicative of a UTI, and the urine specimen collection methods are the key factor facilitating the diagnostic process. In chapter six it was evident that patient views on, and experiences of urine specimen collection varied, but it was certain that a straightforward urine collection method was much more appreciated compared to the pain and complexities experienced in the other methods. In order to establish the association between the quantitative and qualitative results it is essential to describe and highlight the key findings. This chapter will look at how the findings discussed in chapter five on non-invasive versus invasive urine collection methods integrates with chapter six on patient views and experiences towards urine specimen collection. It will highlight the key findings, assimilate the quantitative and qualitative components and discuss the new knowledge that has emerged from the combined studies.

## 7.2 Key findings from experiment one: non-invasive urine specimen collection versus invasive methods

Experiment one tested the hypothesis that a MSU specimen has equal merits to a CSU specimen for collecting urothelial cells when diagnosing a UTI. It compared two non-invasive urine collection methods (MSU and Peezy MSU) to an invasive method (CSU). Non-invasive methods were frequently criticised for harbouring contaminants such as epithelial cells, whereas the invasive method was regarded as the collection methods that had very few cells. Using uroplakin staining and florescent microscopy, it was identified that the cells found in the in urine were in fact urothelial cells that originated from the bladder and were exfoliated into the urine during the innate inflammatory response of an infection in the bladder. The majority of these cells were found in the MSU and Peezy MSU and very few were identified in the CSU. The catheter insertion through the urethra

bypassed these cells which lay at the base of the bladder. There was also the possibility that the urinary sediment was to thick to pass through the eye of the catheter, hindering the maximum number of cells and sediments obtained and cultured. However, the MSU and Peezy MSU methods were able to capture these cells during the urinary void. This justifies the greater proportion of cells found in non-invasive urine specimens compared to invasive methods, and refutes the assumptions of urine specimen contamination.

## 7.3 Key findings from experiment two: comparing three different non-invasive urine specimen collection methods

Experiment two tested the hypothesis that a urine specimen collected freely by direct void is a superior method of specimen collection for capturing urothelial cells compared to the MSU and the Peezy MSU specimens. It compared three non-invasive methods which were the MSU, Peezy MSU and direct void. Using uroplakin staining and florescent microscopy, it was identified that the majority of the urothelial cells were found in the directly voided urine specimen compared to the MSU and Peezy MSU. The MSU technique discards the first part of the urinary stream which contains the majority of the cells and catches the middle part of the stream which contains fewer cells. This justifies the greater proportion of cells found in the directly voided urine (natural urination) compared to MSU methods, and again refutes the assumptions of urine specimen contamination in a straightforward voided urine.

## 7.4 Key findings from patient experiences on urine specimen collection methods

This study aimed to explore the views and experiences patients have when providing a urine specimen for diagnostic testing. Interview questions were conducted which included four main questions, and the responses from these questions generated themes. The questions and themes are displayed in Table 45.

**Table 45: Interview questions and emergent themes**

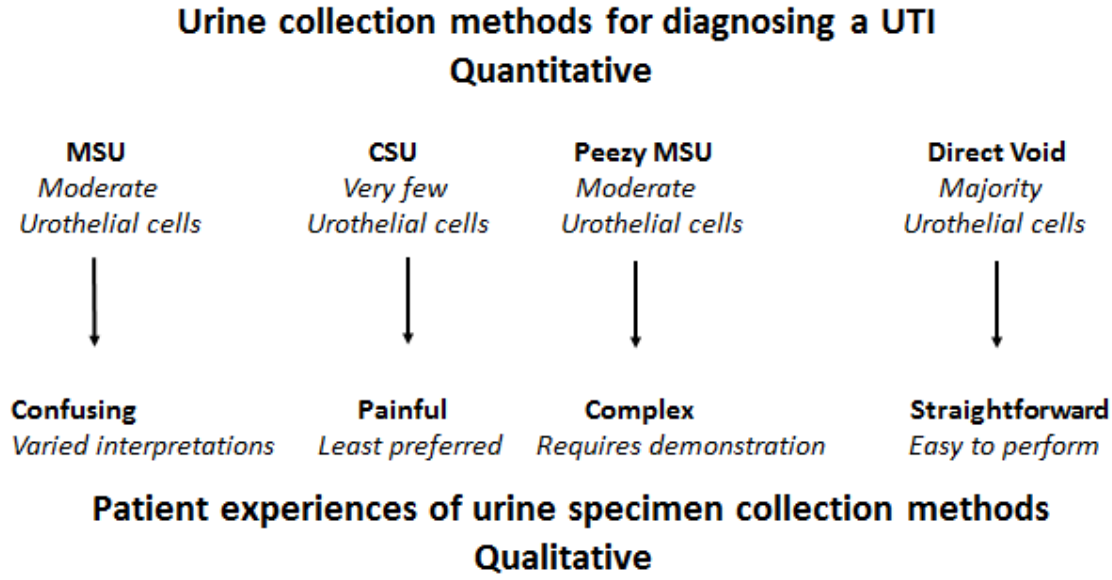
| Interview questions | Themes |
|---|---|
| **Interview question 1:** Which method of urine collection did you prefer and why? | **Theme 1:** Straightforward urine collection |
| **Interview question 2:** Which method of urine collection did you not like and why? | **Theme 2:** Painful urine specimen collection |
| **Interview question 3:** What method of urine collection do you think provided the most clean sample and why? | **Theme 3:** The optimal specimen collection |
| **Interview question 4:** Which method of urine collection do you think should be used as a standard method and why? | **Theme 4:** Simple urine collection methods as standard practice |

A majority of the participants believed that the invasive urine collection method (CSU) was less contaminated compared to the three non-invasive methods (MSU, Peezy MSU and direct void). The catheter method was highly disliked due to the pain and discomfort associated with it. However, the participants were prepared to bear the pain with the intention of obtaining an uncontaminated urine specimen. The participants preferred the direct void method in comparison to the other three methods. It was desired based on its simplicity and was recommended as the standard method of urine specimen collection in general practice.

## 7.5 Assimilating quantitative and qualitative findings

Figure 19 presents the relationships between the quantitative and qualitative results and highlights distinct findings.

**Figure 19: The relationships between the quantitative and qualitative results**




The MSU is the standard method of urine specimen collection in clinical practice. Although it has been criticised for harbouring contaminants, experiment one refuted these claims and revealed it was urothelial cells that were identified in these specimens. Experiment two later confirmed that the MSU was not the best method for capturing these important cells in comparison to the other specimen collection methods. Participants found the MSU to be confusing with variations of the midpoint of the stream. The results from the quantitative and qualitative studies revealed that the MSU was not favoured.

The CSU is not the standard method of urine specimen collection in clinical practice, but is requested when a patient is unable to void and when an uncontaminated urine specimen is required. It is often assumed the CSU contains less contamination and is the optimal specimen for detecting the presence of an infection. Experiment one refuted these claims and revealed the catheter method failed to diagnose the presence of an infection, as it bypassed the urothelial cells that lay at the base of the bladder and sampled the upper urine which produced a cleaner specimen but provided a false diagnosis. Participants reported the CSU as painful, but would endure the pain for a clean or uncontaminated specimen. The results from the quantitative and qualitative studies did not favour the CSU either, and the findings from both studies justified this.

The Peezy MSU is a new urine collection device, recently introduced to clinical practice and has been criticised for harbouring contaminants. However, experiment one refuted these claims and revealed it was urothelial cells that were identified in these specimens. Experiment two later confirmed that the Peezy MSU was not the best method for capturing these important cells in comparison to the other methods. Participants found the Peezy MSU to be complex, required a demonstration and not suitable for those who need to provide a quick urine specimen. Again, the results from the quantitative and qualitative study did not favour the Peezy MSU method.

The directly voided urine is not a standard method of urine specimen collection in clinical practice. The literature revealed it has been criticised for harbouring contaminants, but experiment one refuted these claims and yet again revealed it was urothelial cells that were identified in these specimens. Experiment two later confirmed that the directly voided urine technique was the optimal method for capturing the majority of these important cells in comparison to the other specimen collection methods. Participants found the direct void method easy, straightforward and recommended that it should be adopted as standard clinical practice. The findings from the quantitative and qualitative studies favoured the directly voided urine specimen collection method.

## 7.6 Overall findings

Assimilating the findings from the qualitative and quantitative studies has generated new knowledge. There were persistent assumptions in the literature (Chapter two) that indicted non-invasive urine specimen collection methods for harbouring contaminants and that a painful invasive collection method provided an optimal specimen. The findings discussed in chapter five refuted these assumptions, and it was proven that the sediments and cells found in urine specimens were indicative of a UTI. These assumptions were believed to be true by the patient participants that were interviewed in chapter six, hence their rationale for not refusing and wanting to undergo an agonising specimen collection in the attempt to achieve an uncontaminated specimen. This has highlighted that patients have preconceived notions from published literature, which have conditioned their thoughts to believing in ‘no pain, no gain’. In actual fact, the pain that they had incurred had no benefit to them or the diagnosis of their infection. The

specimen collection method that was renowned for being ‘easy’, but believed to have contain a plethora of contaminants (direct void), was demonstrated as providing the optimal specimen. This finding was not reflected in the interview data, as the patient participants were assuaged by non-evidenced claims as seen in the reviewed literature (Chapter two).

The quantitative and qualitative studies in this thesis have shown the implications of assumptions, the impact it has on patient diagnosis and the importance of refuting invalid norms. The outcomes from the quantitative study have addressed the significance of correct urine specimen collection and the importance of cells that are found in urine specimens. In chapter one contamination was defined by the UK Standards for Microbiology Investigations (Public Health England, 2016) as mixed growth of urinary pathogens, and other authors described it as the presence of epithelial cells found in the urine (Frazee et al., 2012). It is now known that what was regarded as contamination was the vital urinary substrate providing the primary diagnosis of the infection. Based upon the experimental work conducted, contamination can now be defined as sediments or cells that do not reflect or resemble the cell pathology of the urinary tract. Similarly, the qualitative study has shown the detriment of preconceived notions and the harm incurred rather than the benefit perceived. It is now known that patients in clinical settings have been subjected to an array of urine specimen collection methods, in the attempt to achieve an uncontaminated specimen. These combined studies have distinctly shown the importance of refuting assumptions and the significance of examining patient views and experiences that have been influenced by invalid claims. The body of knowledge that focuses on urine specimen collection is yet to deploy microbiological culturing and advanced staining techniques as used in experiment one and experiment two. Utilising these advancements not only will support the validity of their findings, but will contradict the disapproval of non-invasive urine specimen collection methods. The condemnation of non-invasive urine specimen collection methods has not been judged by evidence, but has been criticised based on assumptions. Nonetheless, awareness of patient views and experiences is also a requirement that should be infiltrated in the body of knowledge. Likewise, a qualitative integration (Chapter six), should be assimilated in other studies so that researchers are able shed light on preconceived notions and identify the primary triggers of patient attitudes and philosophies.

## 7.7 General considerations and impact

There are general points to consider which were not explored in this research and can be identified as a limitation in this study. It is known that symptomatic UTI's such as urinary incontinence and terminal urinary leakage are prevalent among the elderly population (Boscia and Kaye, 1987), and there is a rise in the use of incontinence pads to manage these symptoms (Dykes and Bradbury, 2016). Obtaining an optimal urine specimen from elderly patients who are incontinent of urine and unable to spontaneously void was not explored. There is a need for further investigation on the future of urine specimen collection in this population group. The findings from experiment two revealed that a majority of the urothelial cells were found in the directly voided urine (natural urination), and in the elderly population who are incontinent, these important cells would be trapped in the fibres of the incontinence pads, hindering the diagnosis of cellular origin.

There are clinical and academic impacts that will occur as a result of publishing the findings from this thesis. Publishing the experimental findings will influence change in the way urine specimens are collected in the future which will have a clinical impact on current practices. Peer reviewed publications are subjected to expert scrutiny (Holland and Rees, 2010), and clinicians will gain a better understanding of how to interpret and understand the characteristics of a urine specimens from the published findings from this thesis. However, this impact does not transfer to the future of urine specimen collection in the elderly population who are incontinent and using incontinence pads, as further exploration is required. Academics will have the opportunity to transfer knowledge from this thesis that will impact on learning by providing a scientific rationale for urine specimen collection methods.

# Chapter 8

# Conclusions, implications and recommendations

Establishing the origin of cells found in urine specimens formed the theoretical basis of this thesis. Non-invasive urine collection methods were criticised for harbouring contaminants and invasive methods were postulated as having fewer contaminants. These two assumptions had never been verified. In chapter three, it was hypothesised that a non-invasive collected urine specimen (MSU) has equal merits to an invasively collected specimen (CSU) when capturing urothelial cells that reveal the true pathology of a urinary tract infection. Comparing the MSU to the CSU had shown that a majority of the urothelial cells were found in the non-invasive methods. The catheter method bypassed these cells which lay at the base of the bladder, thus producing a specimen that had fewer cells. It was also hypothesised that a urine specimen collected freely by direct void was a superior method of specimen collection for capturing urothelial cells compared to the MSU methods. Comparing the directly voided urine specimens to the MSU and Peezy MSU specimens had shown that a majority of the urothelial cells were found in the directly voided urine. Uroplakin-3 staining revealed that the cells found in urine specimens originated from the bladder and were exfoliated into the urinary stream during the inflammatory process of an infection.

Patient views and experiences of urine specimen collection have shown that an invasive urine collection method was believed to contain fewer contaminants compared to the non-invasive methods. The directly voided urine specimen was the desired method and the catheter method was least preferred as patients incurred pain and discomfort as a result. A significant finding was that patients would undergo the agony of a catheter specimen in the attempt to obtain a urine specimen that was uncontaminated. This was a finding based on the assumptions that the catheter specimen was the optimal method.

## 8.1 Recommendations for further enquiry on urine specimen collection methods

There remain some gaps in the body of knowledge on specimen collection. There are very few studies that focus on the importance of urine specimen collection for diagnostic testing. It emerged from this thesis that urinary contamination has been over estimated and the cells found in the urine specimens are shed as part of the innate immune response to the disease process in the bladder. It is recommended that further enquiry on specimen collection methods should be conducted. It is important that an investigation is carried out during the inflammatory stages in the bladder in order to identify the most appropriate sampling technique to deploy for an acute urinary tract infection.

1. **''The characteristics of a urine specimen from patients with an acute symptom flare''.** Using the techniques deployed in this thesis, further investigation should explore the pathogens and proportions of epithelial cells during the acute stages of a urinary tract infection.

2. **''The importance of urine specimen when patient symptoms have settled from an acute flare''.** This would be a useful investigation to explore the nature of a urine specimen post inflammatory stages, examining the contents found in the specimen when an aggravated bladder has subsided.

This thesis has shown the importance of examining the qualitative and quantitative microbiology of four different urine collection methods. It has helped to challenge a belief founded on conjecture. The results of this thesis have shown that symptomatic patients do exhibit a positive urinary sediment culture despite the negative routine analyses. Polymicrobial isolates are the norm and mono-cultures should not be regarded as the main importance. This thesis has generated a number of contentious propositions that should be considered when planning diagnostic urine testing.

1. Urinary microscopy should be used to detect the presence of Wbcs, epithelial cells and other bladder sediments instead of urinary dipstick when patients present with lower urinary tract symptoms.
2. The direct void urine collection method should be used as a standard collection method when detecting the true pathology of a urinary tract infection.
3. The first part of the urinary stream should be regarded as significant when patients are asked to provide a urine specimen.
4. Epithelial cells found in non-invasive urine specimens should not be dismissed as contamination but should be recognised as part of the inflammatory manifestations in the urinary deposit.
5. Urinary specimen contamination should not be assessed unless the uroplakin-3 staining technique has been deployed during the analysis process. Some of these methods can be implemented, but laboratory procedures will need to be adapted to incorporate the uroplakin staining technique.

## 8.2 Recommendations for further enquiry on patient experiences

There also remains a gap in the literature on patient views and experiences of urine specimen collection and a limited number of studies actually focus on patient experiences regarding diagnostic testing for a urinary tract infection. A significant theme that emerged from the findings in this thesis was that straightforward urine specimen collection was preferred. This area of enquiry would benefit from further research on patients experiences such as:

1. Patient experiences of how they are instructed to give a urine specimen at different clinics and outpatient centres.
2. Patient experiences of the kind of urine collection method they have been asked to provide.
3. Patients understanding of a urinary tract infection and the importance of urine specimen collection.

This may help to predict whether clinicians are instructing patients to provide a urine sample using a standard techique. It is particularly important to explore the levels of

understanding patients have regarding a urinary tract infection and the need for a quality urine specimen. Further research on this subject would contribute to a deeper undersatnding of patient experiences on a wider scale.

## 8.3 Implications for future urine collection methods

This thesis has also shown the importance of examining patient experiences, as it helps to understand the views concerning a subject matter (Bell and Leite, 2016). The results of this thesis have generated several implications to support the future of urine collection methods for urinary diagnostic testing.

1. Patient preference and choices should be taken into consideration when implementing policies and protocols that influence their care. Patients with chronic bladder conditions understand the mechanism of their bladders, and should have their views considered when expressing the need for change regarding their screening practices.

2. Some patients with lower urinary tract symptoms may require the toilet urgently and frequently at any time during the day. This could also be the situation when patients attend a clinic or specialist centre for diagnostic testing. This suggests that clinicians should ask patients to provide a urine specimen that is quick, easy and straightforward such as the direct void method.

3. The direct void method captures urinary cells and sediments that have been expelled from the bladder. Clinicians must be aware of the pathophysiological importance of bladder sediments and cells when it comes to diagnostic testing. They must understand the difference between urinary sediment and urinary contamination, and possibly share this knowledge with patients when requesting a urine specimen, so that patients are informed of the details regarding urine collection.

4. An ideal urine specimen is that which is sensitive to the underlying pathology of a UTI i.e. detects cells that have been shed into the urinary stream. It is also a urine specimen that is easy to collect. The direct void is the recommended method of choice but should be accompanied with microscopy and uroplakin staining to detect the positive presence of an infection.

## 8.4 Summary

This thesis has highlighted the importance of urine specimen collection and the significance of urinary cells and sediments when diagnosing a UTI. The belief that urine specimens were contaminated was founded on clinical assumptions, and scientific evidence has proven to refute these conjectures. Patient experiences of urine specimen collection are valuable and this thesis has revealed that patients are taught to believe that invasive methods of urine specimen collection are the best however, this has been proven otherwise and patients should be made aware of scientific evidence and not conditioned to assumptions.

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# Appendix 1

## A framework for critiquing qualitative research articles

This is Table 7.3 found in chapter 7 and provides a framework for critiquing qualitative research articles. This has many of the same aspects of quantitative framework found in 7.2, but has some differences that relate to the principles of qualitative, or 'interpretative', research. You might like to print this off to use on qualitative articles you would like to critique.

| Aspect | Questions |
|---|---|
| **Focus** | What topic is the concern of this article? Is this an important topic? The focus here will be broader than that of quantitative research and may emphasise experience of a condition or situation. |
| **Background** | How does the researcher argue that the topic is worthwhile? How widespread or big a problem is it? Is the seriousness of the topic reinforced by the previous studies? Is there a thorough review of the literature outlining current knowledge on this topic?<br><br>The background may make the qualitative approach a logical choice. |
| **Aim** | What is the statement of the aim of the data collection? This usually begins with the word 'to' and may concentrate on an exploration of a situation, e.g. 'The aim of this study is to explore the lived experience of chronic illness. |
| **Methodology or Broad approach** | Within a broad qualitative approach is it phenomenological, ethnographic, grounded theory, or broad qualitative design? Does this match the statement of the aim? |
| **Tool of data collection** | What was the method used to collect the data? Had this tool been used in previous studies of this type? A qualitative tool will not be piloted to check accuracy but may be used firstly on a small scale to give the researcher experience of its use in this situation. There may be mention of credibility where the researcher attempts to give clear details on the circumstances and environment in which data gathering took place. The descriptions of such things as individual interviews may be extensive to allow you to feel almost as though you were there. Do you feel this tool worked well or might an alternative have been more effective? |
| **Method of data analysis and presentation** | This is one of the most important steps in qualitative approach where the researcher's understanding emerges inductively from the data and their interpretation of what is going on with those involved. To make sense of large amounts of text the researcher may mention specific systems for analysing the data either in the form of computer programs such as NUDIST and NVivo, or systems designed by other qualitative analysts such as Colaizzi or Van Manon. There may be reference to immersion in the data where the researcher reads over and over the details of what people have said or done. Codes to categorised themes may be mentioned and illustrations of the way this was done may be presented to form an 'audit trial' to allow you to follow the way the researcher managed the data from transcript to coded themes. The data will be in the form of observed descriptions or verbal comments and statements from those involved. These may be quite powerful in their description of feelings and emotions where the researcher is attempting to provide evidence of 'credibility' so we can believe in the accuracy of the findings and the interpretation of them. |

| | |
|---|---|
| **Sample** | Here the numbers of participants will be low, perhaps under 10 and often not more than 20. Data collection may have stopped once 'saturation' was reached, that is, where no new categories emerged from the findings. Were there inclusion and exclusion criteria stated? Were these reasonable given the research question and the nature of the sample? Do the selection criteria limit to whom the results may apply? What method was used to select who got into the study (the sampling strategy)? Is this appropriate for this research question and approach? Does the sample suffer from any kind of bias? |
| **Ethical considerations** | Did an ethics committee (LREC, or in US an Institutional Review Board 'IRB') approve the study? Was informed consent gained and mention made of confidentiality? Could the study be said to be ethically rigorous? |

| | |
|---|---|
| **Main Findings** | What themes or categories arose from the findings in answer to their aim? Was there an attempt to ensure that the accuracy of these themes was checked in some way, for example by peer checking with<br><br>others not involved in the study, or more than one member of the team involved in interpretation of the findings? |
| **Conclusion and Recommendation** | Did they give a clear answer to their aim? Is this well argued and supported? Were clear recommendations made (who should do what, how, now)? If grounded theory, is there an attempt to explain what might lie behind the findings? |
| **Overall strengths and limitations** | What would you say were the aspects of the study they did well? What aspects were less successful? Did they acknowledge any limitations to the study? |
| **Application to practice** | How do the findings relate to practice? Should any changes be considered? |

## A framework for critiquing quantitative research articles

Below is Table 7.2, the critiquing framework you will see in chapter 7 in the book. This is best used with quantitative research articles. You might like to print this off to keep with you when you critique quantitative articles.

| Aspect | Questions |
|---|---|
| **Focus** | What topic is the concern of this article? Can you identify measurable 'variables' in the title or researcher's statement concerning their main interest? Is this an important topic for research? |
| **Background** | How does the researcher argue that the topic is worthwhile? How widespread or big a problem is it? Is the seriousness of the topic reinforced by the previous studies? Is there a thorough review of the literature outlining current knowledge on this topic? Are the key variables defined and an attempt made to consider how they can be measured? E.g. definitions of 'pain' or 'anxiety' and descriptions of scales frequently used to measure them. |
| **Aim** | What is the statement of the aim of the data collection? This usually begins with the word 'to', e.g. 'The aim of this study is 'to examine/determine/ establish/compare/etc'. If it is a randomised control trial there may be a hypothesis. |
| **Methodology or Broad approach** | Within a quantitative approach, is it a survey, experimental (RCT), or correlation study? Does seem suitable given the aim of the study? |
| **Tool of data collection** | What was the method used to collect the data? Had this been used in previous studies and so may be regarded as reliable or accurate? If not, was it piloted? Is there any mention of reliability or validity? Is there a rationale given for the choice of tool? Could an alternative tool have been considered? |
| **Method of data analysis and presentation** | Is the method of processing and analysing the results described in the methods section, such as statistical process through SPSS computer analysis, and are the results clearly presented in the results/findings section? Does the researcher clearly explain any statistical techniques or methods of presentation such as tables, graphs, pie charts? |
| **Sample** | On how many people, events, or things are the results based? If questionnaires were used, what was the response rate? If it was a randomised control trial, what was the dropout rate? Is either of these likely to have an impact on the results? Were there inclusion and exclusion criteria stated? Were these reasonable given the research question and the nature of the sample? Do they limit to whom the results may apply? What method was used to select who were included in the study (the sampling strategy)? Does the sample suffer from any kind of bias? |
| **Ethical considerations** | Did an ethics committee (LREC, or in US an Institutional Review Board 'IRB') approve the study? Was informed consent gained and mention made of confidentiality? Could the study be said to be ethically rigorous? |
| **Main Findings** | What did they find in answer to their aim? What were the large results that relate to the aim of the study? |
| **Conclusion and Recommendations** | Did they give a clear answer to their aim? If they stated a hypothesis, did they say if this was supported or rejected? Were clear recommendations made (who should do what, how, now)? |
| **Overall strengths and limitations** | What would you say were the aspects of the study they did well? What aspects were less successful? Did they acknowledge any limitations to the study? |
| **Application to practice** | How do the results relate to practice? Should any changes be considered? |

# Appendix 2

# CRF

**Investigator** xxxxx xxxxx

**1. Visit details**

Date of visit _ _/_ _ _/_ _ _ _

**2. Patient demographics**

Initials ___ Date of Birth _ _/_ _ _/_ _ _ _

**3. Inclusion criteria (tick)**

□ Female

□ Adult (age 18 years or over)

□ Able to give informed consent

□ OAB

□ PBS

□ Acute cystitis

□ Recurrent UTI

□ Lower urinary tract symptoms

□ Controls

□ Women using reliable contraception or postmenopausal women

**4. Exclusion criteria**

Male

Age less than 18 years old

Inability to consent

Women with concurrent illness

Pregnant women (Pregnancy test positive □ Pregnancy test negative □)

**5. Informed consent**

Have the inclusion and exclusion criteria been satisfied? (y/n)

Informed consent obtained (y/n)

Assign study number (_ _ _ _)

**6. Have you taken the following study samples?**

| □ **MSU sample 1st 2nd 3rd** | □ **CSU sample 1st 2nd 3rd** | □ **MSU Peezy® 1st 2nd 3rd** |
|---|---|---|
| Urine Microscopy (y/n) | Urine Microscopy (y/n) | Urine Microscopy (y/n) |
| Urine Dipstick (y/n) | Urine Dipstick (y/n) | Urine Dipstick (y/n) |
| Routine Culture (y/n) | Routine Culture (y/n) | Routine Culture (y/n) |
| Sediment culture (y/n) | Sediment culture (y/n) | Sediment culture (y/n) |
| Sediment adhesion analysis – (y/n) | Sediment adhesion analysis – (y/n) | Sediment adhesion analysis – (y/n) |
| Urinary creatinine (y/n) | Urinary creatinine (y/n) | Urinary creatinine (y/n) |
| ATP studies – aliquots frozen (y/n) | ATP studies – aliquots frozen (y/n) | ATP studies – aliquots frozen (y/n) |
| IL6 studies – aliquots frozen (y/n) | IL6 studies – aliquots frozen (y/n) | IL6 studies – aliquots frozen (y/n) |

**Study number:** **Initials:** **Visit date:**

***Urinalysis results***

| **Dipstick analysis** | **MSU** | **CSU** | **MSU Peezy®** |
|---|---|---|---|
| *Glucose* | | | |
| *Ketones* | | | |
| *Red blood cells* | | | |
| *Protein* | | | |
| *Nitrite* | | | |
| *White blood cells* | | | |

| **Microscopic analysis** | **MSU** | **CSU** | **MSU Peezy®** |
|---|---|---|---|
| *Mean white blood cell count (wbc/µl)* | | | |
| *Mean red blood cell count (rbc/µl)* | | | |
| *Mean epithelial cell count (epc/µl)* | | | |

| | **MSU** | **CSU** | **MSU Peezy®** |
|---|---|---|---|
| *Benchtop ATP (RLU)* | | | |

***Questionnaire data***

| | |
|---|---|
| Urgency score | |
| Pain score | |
| ICIQ-LUTS score | |
| ICIQ-LUTSqol score | |

*(Please file questionnaires in notes)*

***Routine urine culture data***

| Result | **MSU** | **CSU** | **MSU Peezy®** |
|---|---|---|---|
| Organism 1 | | | |
| Organism 2 | | | |
| Organism 3 | | | |
| Clinically significant | | | |

| | |
|---|---|
| Action | |

***Sediment culture data*** ***MSU*** ***CSU*** ***MSU Peezy®***

| Total mean bacterial growth (cfu $ml^{-1}$) | | | |
|---|---|---|---|

***Bladder cell analysis*** ***MSU Pos*** ***MSU Neg*** ***CSU Pos*** ***CSU Neg*** ***Peezy Pos*** ***Peezy Neg***

| Mean proportion of clue cells (%) | | | | | | |
|---|---|---|---|---|---|---|

***Urinary creatinine concentration*** ***MSU*** ***CSU*** ***MSU Peezy®***

| Creatinine concentration (mmol $l^{-1}$) | | | |
|---|---|---|---|

***Urinary ATP concentration*** ***MSU*** ***CSU*** ***MSU Peezy®***

| Mean ATP concentration (mol*$10^{8}$) | | | |
|---|---|---|---|

***Urinary IL-6 concentration*** ***MSU*** ***CSU*** ***MSU Peezy®***

| Mean IL-6 concentration (pg $ml^{-1}$) | | | |
|---|---|---|---|

Whittington Health NHS
Community Lower Urinary Tract Service
Hornsey Central Neighbourhood Health Centre
2nd Floor
151 Park Road
London
N8 8JD

4th *February 2014*

# Appendix 3

Dear Dr,

I am writing to you regarding a patient registered in your care.

**Name:**

**Address:**

**D.O.B:**

The above named individual has agreed to take part in a study designed to compare the experimental quality of the results obtained by midstream urine specimens compared with catheter specimens of urine. The samples will be evaluated for a multitude of markers of urinary tract infection

This study will involve one visit

Short title of study: MSU CSU Comparison

**Chief Investigator name:** Professor James Malone-Lee

**Sponsor:** University College London

I enclose the patient information sheet for your records.

If you have any questions please do not hesitate to contact me.

Yours sincerely,

Linda Collins BSc, MSc, RN

Whittington Health NHS

**Community Lower Urinary Tract Service**
**Hornsey Central Neighbourhood Health Centre**
**2nd Floor**
**151 Park Road**
**London**
**N8 8JD**

# Appendix 4

**Participant Information Sheet (Patients) Part 1**

## Short title: MSU CSU Comparison

**A randomised, single blind comparative study of urine sampling methods**

Protocol Reference Version, **2.1, 1st October 2013**

We would like to invite you to take part in our research study. Before you decide to enrol it is important that you to understand why the research is being done and what it would involve for you. One of our team will go through the information sheet with you and answer any questions you have. We suggest that this should take about 20 minutes. Talk to others about the study if you wish.

Part 1 tells you the purpose of this study and what will happen to you if you take part.

Part 2 gives you more detailed information about the conduct of the study.

Ask us if there is anything that is not clear. Take time to decide whether or not you wish to take part.

## What is the purpose of the project?

Many people suffer with lower urinary tract problems causing a variety of bladder symptoms that can include urinary incontinence (leaking from the bladder). There may be problems with bladder emptying and with recurrent urinary infection. We recognise that the symptoms are very unpleasant.

We have been working with patients who suffer from bladder troubles for many years. We have discovered that most of these people demonstrate a previously unrecognised inflammatory reaction in the urine that is evident provided that the urine is examined very fresh by a light microscope. When these specimens are sent to the ordinary laboratory more than half are reported as not showing infection, termed “culture negative”. We have developed a better method for analysing the samples that uses very sensitive microbiological methods and found that we can identify bacterial infection in over 80%.

Whilst we are working on newer better methods for diagnosis of urine infections, we need to discover the best ways of obtaining samples from our patients.

There are three methods we can use:

- Midstream urine sample (MSU) where we ask patients to pass urine into a collecting bowl
- Catheter specimen of urine (CSU) where we obtain a specimen by passing a catheter (small plastic tube) into the bladder.
- Peezy Midstream Urine (Peezy MSU) where we ask patients to pass urine using a specially designed collection device.

We know that the catheter method provides a very pure urine sample, but it is not the most convenient method and it would be much more acceptable to our patients if we could use the midstream method instead. However, we have to be sure that the MSU and the Peezy MSU can provide as pure a urine sample as the CSU.

The purpose of this study is to compare these three methods. We describe all the methods at the end of this information sheet.

### Why have I been invited?

We are looking at different groups of women, some of whom have urinary tract infection problems, and others who are called ‘controls’; that is that they don’t have any urinary problems. Men are rarely sampled by CSU, and so we are not inviting them to take part in this study.

**You have been chosen as you have urinary symptoms**

There will be 7 groups in total, and we hope to include 30 persons in each group, giving a total of 210 participants.

**Do I have to take part?**

It is up to you if you decide whether or not to take part. If you do decide to take part you will be given a copy of this information sheet to keep. You will be asked to sign a consent form, a copy of which will be given to you. If you decide to take part you are still free to withdraw at any time and without giving a reason. This will not affect the standard of care you receive.

**What will happen to me if I take part?**

If you decide to take part in the project the research clinician will arrange to see you. You will be asked some questions about your bladder symptoms. Your answers will be recorded on a form stored on a computer. This will take about 30 minutes. The computer record is closely guarded by the NHS security system so there is no unauthorised access to you record.

We will then take the first urine sample. Which method we do first will be decided by a random code similar to tossing a coin. We shall then wait about an hour, after which we shall take the second sample using the opposite method.

If you are not in the subset, then only two urine specimens will be taken by the midstream method (MSU) and a catheter specimen of urine

- MSU followed by CSU

Or

- CSU followed by MSU

If you have been randomly assigned to Peezy MSU group you will also be asked to provide a third sample an hour later

So it will either be:

- CSU followed by MSU followed by Peezy MSU

Or

- MSU followed by Peezy MSU followed by CSU

Or

- Peezy MSU followed by CS followed by MSU

The CSU collection will be done by a qualified female member of our clinical staff.

Part of the sample will be sent to the hospital laboratory to check for any bacteria in your urine, and the remainder will stay in our department for a number of tests. Immediately after the sample is provided, it will be looked at under the microscope by the research staff to detect small 'white blood cells,' which can signify an infection in the urine.

A lot of the time, tests performed in the hospital laboratory can come back as negative, and looking for white blood cells under the microscope straight away is a way of detecting infection that may otherwise have been missed. We would like to record the results of these tests in your research records. In addition, we will perform our own specialised bacterial culture on the urine, and store the remaining urine in our secure freezer for later analysis. This will include testing for substances that the bladder releases when it is distressed due to infection, known as Interleukin-6 (IL-6), and Adenosine Triphosphate (ATP).

If we find evidence of infection in your urine we shall treat this for you.

## Payments

We are not in a position to pay you for participation in the study, however, We shall be able to reimburse you for travel expenses incurred from attending the study visit. We would be grateful for receipts describing the expenses for our auditors. We are not in a position to pay you for participation in the study.

### Other studies

It would not be advisable for you to participate in this study if you are already a subject in another study.

### Pregnancy

Pregnant women are excluded from participation in this study. We shall do a pregnancy test to check this for you where necessary.

## What will I have to do?

You will have to:

1. Give your consent
2. Attend the our department for one visit
3. Provide two or three urine specimens at this visit.

| **Day Identification and visit** | **Actions** |
|---|---|
| Pre-study | You will be given a Patient Information Sheet |
| At least 48 hours later | You will be contacted to ask if you are interested in taking part in the study.<br><br>If yes, an appointment will be made for a Visit |
| Day 0 Visit 1 | ♦ Screening to check eligibility<br>♦ Study explained and any questions answered<br>♦ Consent form signedRandomised to CSU/MSU group only or subset of CSU/MSU?Peezy MSU<br>♦ Complete symptom questionnaires<br>♦ Provide MSU or CSU<br>♦ Wait 1 hour<br>♦ Provide MSU or CSUIf in subset provide third urine sample after one hour<br>♦ Analysis of urine samples |

## What about my current treatments for other conditions?

You will be able to continue all of your normal treatments and these will not be affected by this study.

## What are the alternatives to participating in the study?

It is entirely your decision if you wish to take part in the study and it will not affect your future care if you do not wish to do so. If you do not wish to take part you will have your assessment in the usual way and your condition will be managed as is done routinely by your consultant.

**What are the possible disadvantages and risks of taking part?**

We will be collecting one of the urine samples using a catheter. This is a tiny flexible tube, which is passed gently through into the opening of the urethra for a few centimetres until the bladder is entered.

There are some very minor risks associated with collecting a catheter specimen of urine. We use a very small catheter and the process is rarely painful. It tends to evoke a sensation as if you were about to start passing urine. The precise risk of infection cannot be estimated since it is so low and so unlikely.

**What are the possible benefits of taking part?**

You will have access to unusually close monitoring of your bladder symptoms and urine to detect infection. As with any research study, there is a possibility that you will experience no benefit from taking part.

**What if there is a problem?**

Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. The detailed information concerning this is given in Part 2 of this information sheet. If you have any concerns or complaints you should contact your study doctor in the first instance.

## Will my taking part in the study be kept confidential?

Yes. We will follow ethical and legal practice and all information about you will be handled in confidence. The details are included in Part 2.

## Contact Details

Your Doctor

**Professor James Malone-Lee** Tel. Number: **020 3074 2250**

This completes Part 1 of the Information Sheet. If the information in Part 1 has interested you and you are considering participation, please read the additional information in Part 2 before making any decision.

## What if new information becomes available?

As this study only involves one visit it is unlikely that this will occur.

### What will happen if I don't want to carry on with the study?

You are free to withdraw from the study at any time before and after signing the consent form without needing to give any explanations. The study may be ended at any time with or without your consent.

Unless you tell us otherwise, the information collected from you up to the point at which you leave the study will be used in the analysis. If you feel strongly that you would not wish to be the case, please let the investigator know.

### What if there is a problem?

Every care will be taken to ensure your safety during the course of the study. If you have a concern about any aspect of this study, you should ask to speak with the researchers who will do their best to answer your questions. Please contact **Professor Malone-Lee** in the first instance

### Harm

In the event that something does go wrong and you are harmed as a result of taking part in the approved research study, University College London (UCL), the Research Sponsor, has insurance arrangements in place for non-negligent harm. If you are harmed and this is due to someone's negligence then you may have grounds for legal action for compensation against the Trust but you may have to pay your legal costs.

Every care will be taken in the course of this clinical trial. However in the unlikely event that you are injured by taking part, compensation may be available.

If you suspect that the injury is the result of the Sponsor's (UCL) or the hospital's negligence then you may be able to claim compensation. After discussing with your study

doctor, please make the claim in writing to Professor Malone-Lee who is the Chief Investigator for the clinical trial and is based at **Community Lower Urinary Tract Service, Hornsey Central Neighbourhood Health Centre, 2nd Floor, 151 Park Road, London N8 8JD**. The Chief Investigator will then pass the claim to the Sponsor's Insurers, via the Sponsor's office. You may have to bear the costs of the legal action initially, and you should consult a lawyer about this.

Participants may also be able to claim compensation for injury caused by participation in this clinical trial without the need to prove negligence on the part of University College London or another party. You should discuss this possibility with your study doctor in the same way as above.

**Complaints**

If you have any questions about your rights as a research subject or have a complaint about the way in which the study has been carried out, please contact: Professor Malone-Lee in the first instance.

If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure. Details can be obtained from the hospital or from the Department of Health website: http://www.dh.gov.uk. You may obtain the necessary guidance from the hospital Patient Advice and Liaison Service (PALS), Whittington Health, Tel 020 7288 5956 or 020 7288 5957

**Will my taking part in this study be kept confidential?**

If you consent to take part in this study, the records obtained while you are in this study as well as related health records will remain strictly confidential at all times. The information will be held securely on paper and electronically at the hospital site managing this research under the provisions of the 1998 Data Protection Act. Your name will not be passed to anyone else outside the research team or the Sponsor (UCL), who is not involved in the study. You will be allocated a study number, which will be used as a code to identify you on all study forms. Any information about you which leaves the hospital will have your name and address removed so that you cannot be recognised (if it is applicable to your research).

Your records will be available to people authorised to work on the study but may also need to be made available to people authorised by the Sponsor, which is the organisation responsible for ensuring that the study is carried out correctly. By signing the consent

form you agree to this access for the current study and any further research that may be conducted in relation to it, even if you withdraw from the current study.

If you withdraw consent from the study, unless you object, your data and samples will remain on file and will be included in the final study analysis.

In line with the regulations, at the end of the study your data will be securely archived for a minimum of 20 years. Arrangements for confidential destruction will then be made.

**Will my GP be informed of my involvement?**

With your permission, your GP, and other doctors who may be treating you, will be notified that you are taking part in this study

**What will happen to any samples I give?**

Two urine specimens will be taken (CSU and MSU) and part of each sample will be sent to the hospital laboratory to check for any bacteria in your urine and another portion will be looked at under the microscope to detect small 'white blood cells', which can signify an infection in the urine.

A portion of the urine sample will be frozen and stored for further analysis of two other important substances that are of particular interest. Interlukin-6 (IL-6) is a chemical messenger that is detected in the urine in response to infection. It helps to activate the immune system to fight off the infection. Adenosine triphosphate (ATP) is released into the bladder in large amounts during infections and it appears to play an important role in the development of bladder symptoms.

You are being asked to gift the urine samples to UCL to be stored, anonymously and used in subsequent ethically approved research studies. There will be no identifying details on

the sample other than a study number that you will be assigned when you join the study, meaning that all samples will be anonymised. Samples will be stored in a monitored freezer in a secure laboratory in the Department of Medicine,. The samples will be analysed by researchers within the Division of Medicine at UCL. Once analysed the urine sample will be disposed of as per UCL regulations.

**Will any genetic tests be done?**

No. Genetic tests will not be done.

**What will happen to the results of the research?**

We shall use the data to make decisions on how we should plan our future discovery research

**Who is organising and funding the research?**

This study is organised by the Department of Medicine at the Whittington Health and is being paid for from Academic funds at UCL. The study is being sponsored by University College London.

**Who has reviewed the study?**

All research in the NHS is looked at by independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and approved by the _____ Research Ethics Committee.

**Further information and contact details**

You are encouraged to ask any questions you wish, before, during or after your treatment. If you have any questions about the study, please speak to your study nurse or doctor, who will be able to provide you with up to date information about the procedure(s) involved. If you wish to read the research on which this study is based, please ask your study nurse or doctor. If you require any further information or have any concerns while taking part in the study please contact.

**Your Doctor**

Name: Professor James Malone-Lee Tel. Number: **020 3704 2250**

If you decide you would like to take part then please read and sign the consent form. You will be given a copy of this information sheet and the consent form to keep. A copy of the consent form will be filed in your patient notes, one will be filed with the study records and one may be sent to the Research Sponsor.

You can have more time to think this over if you are at all unsure.

Thank you for taking the time to read this information sheet and to consider this study.

## Midstream Urine Specimen Method

You will be given a plastic container with lid, and 2 wet-wipes

### Cleansing before collecting the urine specimen

1. You will be asked to wipe your hands with 1 of the wet-wipes. You should then throw this into the Yellow waste bin.

2. You will be asked to thoroughly cleanse the entire genital area using the second wet-wipe and then throw this into the Yellow Waste bin.

3. You will be asked to hold the outer edges of labia apart and cleanse from front to back with the wipe.

**Please do not throw the wet-wipes into the toilet bowl, but into the yellow bin**

### Collecting the urine specimen

You will be asked to continue to hold labia apart while urinating and then follow the following instructions

1. Urinate (pee) a small amount of urine into the toilet.
2. Then without stopping, catch some urine into the plastic container by passing it into the urine stream.
3. As the stream comes to the end move the plastic container away and urinate (pee) the rest into the toilet
4. Put the lid onto the plastic container
5. container and screw the lid down

## Catheter Specimen of Urine Method

We use a very small plastic catheter, the width of a small straw, to collect the sample of urine directly from your bladder. Because the catheter is very small the process is rarely painful. It tends to evoke a sensation as if you were about to start passing urine.

- The clinician washes her hands using soap and water and prepares an aseptic (clean) area on a clean trolley.

- You will be asked to remove your underpants and lie on the bed on your back, in a slightly upright position with your legs apart, knees bent and the perineal (genital) area exposed.

- The clinician prepares the equipment on the clean field, and then wash her hands again and put on the sterile gloves to minimise the risk of infection.

- The Clinician then places the sterile dressing towel and receptacle on the bed in between your legs, and using gauze provided in the dressing pack, holds apart your labia with one hand, and with her other hand she dips the catheter in the lubricating jelly and, using a sterile non-touch technique, inserts the catheter into your bladder via the urethra. A specimen of urine is then drained into the sterile container. The catheter is removed, and you are able to get dressed.

- The Clinician will pour the urine into a container with a lid. She will then remove the gloves and wash her hands again.

- There is a very low risk of introducing new infection into the bladder, but we shall be monitoring our patients for infection anyway. The risk of infection is about 1 out of 100 catheterisations

## Peezy Midstream Urine Specimen Method

You will be given a Peezy Urine collection device, and 1 wet-wipes

### Cleansing before collecting the urine specimen

1. You will be asked to wipe your hands with 1 of the wet-wipes. You should then throw this into the Yellow waste bin.

2. You will be asked to thoroughly cleanse the entire genital area using the wipe provided in the Peezy MSU and then throw this into the Yellow Waste bin

Collecting the urine sample:

1. You attach the collection bottle to the Peezy,
2. Position the Peezy against the body
3. Sit well back on the toilet
4. Pass urine.

   As you begin to pass urine, the first part of the stream passes through the funnel and begins to cause a piece of sponge to swell, thereby blocking the flow through the funnel. The midstream specimen is then channelled into the universal container, and the remainder is channelled through an overflow duct and into the toilet.
5. Wait ---seconds to ensure all urine has been passed
6. Screw the top onto the urine collection bottle
7. Discard the Peezy into the bin.

Whittington Health NHS

**Community Lower Urinary Tract Service**
**Hornsey Central Neighbourhood Health Centre**
**2nd Floor**
**151 Park Road**
**London**
**N8 8JD**

# Appendix 5

Participant Information Sheet (Controls) Part 1

**Short title: MSU CSU Comparison**

**A randomised, single blind comparative study of urine sampling methods**

Protocol Reference **Version 2.1, 1st October 2013**

We would like to invite you to take part in our research study. Before you decide to enrol it is important that you to understand why the research is being done and what it would involve for you. One of our team will go through the information sheet with you and answer any questions you have. We suggest that this should take about 20 minutes. Talk to others about the study if you wish.

Part 1 tells you the purpose of this study and what will happen to you if you take part.

Part 2 gives you more detailed information about the conduct of the study.

Ask us if there is anything that is not clear. Take time to decide whether or not you wish to take part.

**What is the purpose of the project?**

Many people suffer with lower urinary tract problems causing a variety of bladder symptoms that can include urinary incontinence (leaking from the bladder). There may be problems with bladder emptying and with recurrent urinary infection. We recognise that the symptoms are very unpleasant.

We have been working with patients who suffer from bladder troubles for many years. We have discovered that most of these people demonstrate a previously unrecognised inflammatory reaction in the urine that is evident provided that the urine is examined very fresh by a light microscope. When these specimens are sent to the ordinary laboratory more than half are reported as not showing infection, termed “culture negative”. We have

developed a better method for analysing the samples that uses very sensitive microbiological methods and found that we can identify bacterial infection in over 80%.

Whilst we are working on newer better methods for diagnosis of urine infections we need to decide which is the best method of obtaining urine samples from our patients.

There are three methods we can use:

- Midstream urine sample (MSU) where we ask patients to pass urine into a collecting bowl
- Catheter specimen of urine (CSU) where we obtain a specimen by passing a catheter (small plastic tube) into the bladder.
- Peezy Midstream Urine (Peezy MSU) where we ask patients to pass urine using a specially designed collection device.

We know that the catheter method provides a very pure urine sample, but it is not the most convenient method and it would be much more acceptable to our patients if we could use the midstream method instead. However, we have to be sure that the MSU and the Peezy MSU can provide as pure a urine sample as the CSU.

The purpose of this study is to compare these three methods.

You will only be asked to provide a sample using the midstream urine sample method and you may be asked to provide one using the Peezy MSU method as well. Both of these are described in detail at the end of this information sheet.

**Why have I been invited?**

We are looking at different groups of women, some of whom have urinary tract infection problems, and others who are called ‘controls’; that is that they don’t have any urinary problems.

**You have been chosen as you have no urinary problems but would like to volunteer as a normal control.**

There will be 7 groups in total, and we hope to include 30 persons in each group, giving a total of 210 participants.

**Do I have to take part?**

It is up to you if you decide whether or not to take part. If you do decide to take part you will be given a copy of this information sheet to keep. You will be asked to sign a consent form, a copy of which will be given to you. If you decide to take part you are still free to

withdraw at any time and without giving a reason. This will not affect the standard of care you receive.

**What will happen to me if I take part?**

If you decide to take part in the project the research clinician will arrange to see you. You will be asked some questions about your bladder symptoms. Your answers will be recorded on a form stored on a computer. This will take about 30 minutes. The computer record is closely guarded by the NHS security system so there is no unauthorised access to you record.

If you have been randomly assigned to Peezy MSU group you will also be asked to provide two samples, one hour apart, either:

- MSU first, followed by Peezy MSU
- or Peezy MSU followed by MSU

If you are not in the subset, then only one urine specimen will be taken by the midstream method (MSU) and you will not be asked to provide a catheter specimen of urine or a Peezy MSU.

Part of the sample will be sent to the hospital laboratory to check for any bacteria in your urine, and the remainder will stay in our department for a number of tests. Immediately after the sample is provided, it will be looked at under the microscope by the research staff to detect small ‘white blood cells,’ which can signify an infection in the urine.

A lot of the time, tests performed in the hospital laboratory can come back as negative, and looking for white blood cells under the microscope straight away is a way of detecting infection that may otherwise have been missed. We would like to record the results of these tests in your research records. In addition, we will perform our own specialised bacterial culture on the urine, and store the remaining urine in our secure freezer for later analysis. This will include testing for substances that the bladder releases when it is distressed due to infection, known as Interleukin-6 (IL-6), and Adenosine Triphosphate (ATP).

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**Payments**

We are not in a position to pay you for participation in the study, however, We shall be able to reimburse you for travel expenses incurred from attending the study visit. We would be grateful for receipts describing the expenses for our auditors.

**Other studies**

It would not be advisable for you to participate in this study if you are already a subject in another study.

**Pregnancy**

Pregnant women are excluded from participation in this study. We shall do a pregnancy test to check this for you where necessary.

**What will I have to do?**

You will have to:

Give your consent

Attend the our department for one visit

Provide one or two urine specimens at this visit.

| **Day Identification and visit** | **Actions** |
|---|---|
| Pre-study | You will be given a Participant Information Sheet |
| At least 48 hours later | You will be contacted to ask if you are interested in taking part in the study.<br>If yes, an appointment will be made for a Visit |
| Day 0 Visit 1 | Screening to check eligibility<br>Study explained and any questions answered<br>Informed consent form signed<br>Randomised to MSU group only or subset of MSU and Peezy MSU<br>Complete symptom questionnaires<br>Provide a midstream urine sample (MSU)<br>If in subset provide second urine sample after one hour<br>Analysis of urine samples |

**What about my current treatments for other conditions?**

You will be able to continue all of your normal treatments and these will not be affected by this study.

**What are the alternatives to participating in the study?**

It is entirely your decision if you wish to take part in the study

**What are the possible disadvantages and risks of taking part?**

As a volunteer without bladder symptoms, you will be simply asked to attend for your study visit, complete some short questionnaires and provide a midstream sample of urine. There are no potential risks.

**What are the possible benefits of taking part?**

There is no benefit to you from taking part.

**What if there is a problem?**

Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. The detailed information concerning this is given in Part 2 of this information sheet. If you have any concerns or complaints you should contact your study doctor in the first instance.

**Will my taking part in the study be kept confidential?**

Yes. We will follow ethical and legal practice and all information about you will be handled in confidence. The details are included in Part 2.

**Contact Details**

Your Doctor

**Professor James Malone-Lee** **Tel. Number: 020 3074 2250**

This completes Part 1 of the Information Sheet. If the information in Part 1 has interested you and you are considering participation, please read the additional information in Part 2 before making any decision.

**Participant Information Sheet – Part 2**

**What if new information becomes available?**

As this study only involves one visit it is unlikely that this will occur.

**What will happen if I don't want to carry on with the study?**

You are free to withdraw from the study at any time before and after signing the consent form without needing to give any explanations. The study may be ended at any time with or without your consent.

Unless you tell us otherwise, the information collected from you up to the point at which you leave the study will be used in the analysis. If you feel strongly that you would not wish to be the case, please let the investigator know.

**What if there is a problem?**

Every care will be taken to ensure your safety during the course of the study. If you have a concern about any aspect of this study, you should ask to speak with the researchers who will do their best to answer your questions. Please contact **Professor Malone-Lee** in the first instance

**Harm**

In the event that something does go wrong and you are harmed as a result of taking part in the approved research study, University College London (UCL), the Research Sponsor, has insurance arrangements in place for non-negligent harm. If you are harmed and this is due to someone's negligence, then you may have grounds for legal action for compensation against the Trust but you may have to pay your legal costs.

Every care will be taken in the course of this clinical study. However, in the unlikely event that you are injured by taking part, compensation may be available.

If you suspect that the injury is the result of the Sponsor's (UCL) or the hospital's negligence then you may be able to claim compensation. After discussing with your study doctor, please make the claim in writing to Professor Malone-Lee who is the Chief Investigator for the clinical trial and is based at **Community Lower Urinary Tract Service, Hornsey Central Neighbourhood Health Centre, 2nd Floor, 151 Park Road,**

**London N8 8JD**. The Chief Investigator will then pass the claim to the Sponsor's Insurers, via the Sponsor's office. You may have to bear the costs of the legal action initially, and you should consult a lawyer about this.

Participants may also be able to claim compensation for injury caused by participation in this clinical study without the need to prove negligence on the part of University College London or another party. You should discuss this possibility with your study doctor in the same way as above.

**Complaints**

If you have any questions about your rights as a research subject or have a complaint about the way in which the study has been carried out, please contact: Professor Malone-Lee in the first instance.

If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure. Details can be obtained from the hospital or from the Department of Health website: http://www.dh.gov.uk. You may obtain the necessary guidance from the hospital Patient Advice and Liaison Service (PALS), Whittington Health, Tel 020 7288 5956 or 020 7288 5957

**Will my taking part in this study be kept confidential?**

If you consent to take part in this study, the records obtained while you are in this study as well as related health records will remain strictly confidential at all times. The information will be held securely on paper and electronically at the hospital site managing this research under the provisions of the 1998 Data Protection Act. Your name will not be passed to anyone else outside the research team or the Sponsor (UCL), who is not involved in the study. You will be allocated a study number, which will be used as a code to identify you on all study forms. Any information about you which leaves the hospital will have your name and address removed so that you cannot be recognised (if it is applicable to your research).

Your records will be available to people authorised to work on the study but may also need to be made available to people authorised by the Sponsor, which is the organisation responsible for ensuring that the study is carried out correctly. By signing the consent form you agree to this access for the current study and any further research that may be conducted in relation to it, even if you withdraw from the current study.

If you withdraw consent from the study, unless you object, your data and samples will remain on file and will be included in the final study analysis.

In line with the regulations, at the end of the study your data will be securely archived for a minimum of 20 years. Arrangements for confidential destruction will then be made.

**Will my GP be informed of my involvement?**

No

**What will happen to any samples I give?**

A urine specimen will be taken by and part of it will be sent to the hospital laboratory to check for any bacteria in your urine and another portion will be looked at under the microscope to detect small 'white blood cells', which can signify an infection in the urine.

A portion of the urine sample will be frozen and stored for further analysis of two other important substances that are of particular interest. Interlukin-6 (IL-6) is a chemical messenger that is detected in the urine in response to infection. It helps to activate the immune system to fight off the infection. Adenosine triphosphate (ATP) is released into the bladder in large amounts during infections and it appears to play an important role in the development of bladder symptoms.

You are being asked to gift the urine samples to UCL to be stored, anonymously and used in subsequent ethically approved research studies. There will be no identifying details on the sample other than a study number that you will be assigned when you join the trial, meaning that all samples will be anonymised. Samples will be stored in a monitored freezer in a secure laboratory in the Department of Medicine, where the samples will be analysed by researchers within the Division of Medicine at UCL. Once analysed the urine sample will be disposed of as per UCL regulations.

**Will any genetic tests be done?**

No. Genetic tests will not be done.

**What will happen to the results of the research?**

We shall use the data to make decisions on how we should plan our future discovery research.

**Who is organising and funding the research?**

This study is organised by the Department of Medicine at the Whittington Health and is being paid for from Academic funds at UCL. The study is being sponsored by University College London.

**Who has reviewed the study?**

All research in the NHS is looked at by independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and approved by the _____ Research Ethics Committee.

**Further information and contact details**

You are encouraged to ask any questions you wish, before, during or after your treatment. If you have any questions about the study, please speak to your study nurse or doctor, who will be able to provide you with up to date information about the procedure(s) involved. If you wish to read the research on which this study is based, please ask your study nurse or doctor. If you require any further information or have any concerns while taking part in the study, please contact:

**Your Doctor**

Name: Professor James Malone-Lee **Tel. Number: 020 3704 2250**

If you decide you would like to take part, then please read and sign the consent form. You will be given a copy of this information sheet and the consent form to keep. A copy of the consent form will be filed in your patient notes, one will be filed with the study records and one may be sent to the Research Sponsor.

You can have more time to think this over if you are at all unsure.

Thank you for taking the time to read this information sheet and to consider this study.

## Midstream Urine Specimen Method

You will be given a plastic container with lid, and 2 wet-wipes

### Cleansing before collecting the urine specimen

You will be asked to wipe your hands with 1 of the wet-wipes. You should then throw this into the Yellow waste bin.

You will be asked to thoroughly cleanse the entire genital area using the second wet-wipe and then throw this into the Yellow Waste bin

You will be asked to hold the outer edges of labia apart and cleanse from front to back with the wipe.

**Please do not throw the wet-wipes into the toilet bowl, but into the yellow bin**

### Collecting the urine specimen

You will be asked to continue to hold labia apart while urinating and then follow the following instructions

1. Urinate (pee) a small amount of urine into the toilet.
2. Then without stopping, catch some urine into the plastic container by passing it into the urine stream.
3. As the stream comes to the end move the plastic container away and urinate (pee) the rest into the toilet
4. Put the lid onto the plastic container

## Peezy Midstream Urine Specimen Method

You will be given a Peezy Urine collection device, and 1 wet-wipes

### Cleansing before collecting the urine specimen

You will be asked to wipe your hands with 1 of the wet-wipes. You should then throw this into the Yellow waste bin.

You will be asked to thoroughly cleanse the entire genital area using the wipe provided in the Peezy MSU and then throw this into the Yellow Waste bin

Collecting the urine sample:

1. You attach the collection bottle to the Peezy,
2. Position the Peezy against the body
3. Sit well back on the toilet
4. Pass urine.

   As you begin to pass urine, the first part of the stream passes through the funnel and begins to cause a piece of sponge to swell, thereby blocking the flow through the funnel. The midstream specimen is then channelled into the universal container, and the remainder is channelled through an overflow duct and into the toilet.

5. Wait ---seconds to ensure all urine has been passed
6. Screw the top onto the urine collection bottle
7. Discard the Peezy into the bin.

## Appendix 6

Whittington Health NHS

**Community Lower Urinary Tract Service**
**Hornsey Central Neighbourhood Health Centre**
**2nd Floor**
**151 Park Road**
**London**
**N8 8JD**
**Participant Identification Number for this trial:**

# CONSENT FORM (Patients)

## A randomised, single blind comparative study of urine sampling methods

**Name of Researcher: Linda Collins**

*Please initial box*

1. I confirm that I have read and understand the information sheet dated **1st October 2013 (version.3.1)** for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐

3. I understand that relevant sections of my medical notes and data collected during the study, may be looked at by individuals from the sponsor of the trial (UCL), from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐

4. I agree to my GP being informed of my participation in the study. ☐

5. I agree to take part in the above study. ☐

6. I agree my samples being gifted to UCL to be stored, anonymously and used in subsequent ethically approved research studies ☐

| | | |
|---|---|---|
| Name of Patient | Date | Signature |
| Name of Person taking consent | Date | Signature |
| Name of Chief Investigator *(if different to the person taking consent)* | Date | Signature |

When completed: 1 for participant; 1 (original) for researcher site file; 1 to be kept in medical notes.

# Appendix 7

MSU CSU Comparison

**Patient Number_____ Patient Initials_____**
**Date_____**

## Urinary Symptoms

We should be grateful if you would answer the following questions, thinking about how you have been, on average, over the **PAST FOUR WEEKS.**

1. **Please write your date of birth**_____

2A. **How often do you pass urine during the day**?

| | | |
|---|---|---|
| One to six times | 0 | |
| Seven to eight times | 1 | |
| Nine to ten times | 2 | |
| Eleven to twelve times | 3 | |
| Thirteen times or more times | 4 | |

2B. **How much does this bother you?**
(Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 10
**Not at all** **A great deal**

3A. **During the night, how many times do you have to get up to urinate, on average**?

| | | |
|---|---|---|
| None | 0 | |
| One | 1 | |
| Two | 2 | |
| Three | 3 | |
| Four or more | 4 | |

3B. **How much does this bother you?**
(Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 10
**Not at all** **A great deal**

4A. **Do you have a sudden need to rush to the toilet to urinate**?

| | | |
|---|---|---|
| Never | 0 | |
| Occasionally | 1 | |
| Sometimes | 2 | |
| Most of the time | 3 | |
| All of the time | 4 | |

4B. **How much does this bother you?**
(Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 10
**Not at all** **A great deal**

4C. **If you <u>do</u> experience urgency, a sudden need to rush to the toilet to urinate,……**

| | Tick if Yes |
|---|---|
| Does cold weather make matters worse? | |
| Does the sound of running water cause urgency? | |
| Does putting a key in your door or nearing home cause urgency? | |
| Does getting out of bed in the morning cause urgency | |
| Does fatigue or anxiety make matters worse? | |

5A. **Does urine leak before you can get to the toilet**?

| | | |
|---|---|---|
| Never | 0 | |
| Occasionally | 1 | |
| Sometimes | 2 | |
| Most of the time | 3 | |
| All of the time | 4 | |

5B. **How much does this bother you?**
(Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 10
**Not at all** **A great deal**

5C. **If you <u>do</u> experience urge incontinence, a urine leak before you can get to the toilet,……**

| | Tick if Yes |
|---|---|
| Does the sound of running water cause incontinence? | |
| Does putting a key in your door or nearing home cause incontinence? | |
| Does getting out of bed in the morning cause incontinence? | |

6A. **Do you have pain in your bladder**?

| | | |
|---|---|---|
| Never | 0 | |
| Occasionally | 1 | |
| Sometimes | 2 | |
| Most of the time | 3 | |
| All of the time | 4 | |

6B. **How much does this bother you?**
(Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 10
**Not at all** **A great deal**

6C. **If you <u>do</u> experience bladder pain,……**

| | **Tick if Yes** |
|---|---|
| Do you experience discomfort or pain on bladder filling? | |
| Is there pain or discomfort in or over the pubic area? | |
| Is this pain or discomfort relieved completely by passing urine? | |

| | |
|---|---|
| Is this pain or discomfort partially relieved by passing urine? | |
| Is this pain or discomfort unrelieved by passing urine? | |
| Is there bladder pain or discomfort whilst passing urine? | |
| Is there pain or discomfort after passing urine? | |
| Is there pain in your back over your kidneys? | |
| Is there pain referred to or felt in the genitals? | |
| Is there pain on the left or right side of the lower abdomen? | |
| Is there general abdominal pain? | |
| Is there pain radiating down your legs? | |

7A. **How often do you leak urine**?

| | | |
|---|---|---|
| Never | 0 | |
| Once or less per week | 1 | |
| Two or three times per week | 2 | |
| Once per day | 3 | |
| Several times per day | 4 | |

7B. **How much does this bother you?**
(Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 10
**Not at all** **A great deal**

8A. **Does urine leak when you are physically active, exert yourself, cough or sneeze**?

| | | |
|---|---|---|
| Never | 0 | |
| Occasionally | 1 | |
| Sometimes | 2 | |
| Most of the time | 3 | |
| All of the time | 4 | |

8B. **How much does this bother you?**
(Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 10
**Not at all** **A great deal**

9A **Do you ever leak for no obvious reason and without feeling that you want to go?**

| | | |
|---|---|---|
| Never | 0 | |
| Occasionally | 1 | |
| Sometimes | 2 | |
| Most of the time | 3 | |
| All of the time | 4 | |

9B. **How much does this bother you?**
(Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 10
**Not at all** **A great deal**

10 **How much urinary leakage occurs?**

| | | |
|---|---|---|
| No leakage | 0 | |
| Drops/pants damp | 1 | |
| Dribble/pants wet | 2 | |
| Floods, soaking through to outer clothing | 3 | |
| Floods running down legs onto the floor | 4 | |

11A. **Is there a delay before you can't start to urinate**?

| | | |
|---|---|---|
| Never | 0 | |
| Occasionally | 1 | |
| Sometimes | 2 | |
| Most of the time | 3 | |
| All of the time | 4 | |

11B. **How much does this bother you?**
(Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 10
**Not at all** **A great deal**

12A. **Do you have to strain to urinate**?

| | | |
|---|---|---|
| Never | 0 | |
| Occasionally | 1 | |
| Sometimes | 2 | |
| Most of the time | 3 | |
| All of the time | 4 | |

12B. **How much does this bother you?**
(Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 10
**Not at all** **A great deal**

13A. **Do you stop and start more than once while you urinate**?

| | | |
|---|---|---|
| Never | 0 | |
| Occasionally | 1 | |
| Sometimes | 2 | |
| Most of the time | 3 | |
| All of the time | 4 | |

13B. **How much does this bother you?**
(Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 10
**Not at all** **A great deal**

14A. **Do you leak urine when you are asleep**?

| | | |
|---|---|---|
| Never | 0 | |
| Occasionally | 1 | |
| Sometimes | 2 | |
| Most of the time | 3 | |
| All of the time | 4 | |

14B. **How much does this bother you?**
(Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 10
**Not at all** **A great deal**

15A. **Would you say that the strength of you urinary stream is……..**?

| | | |
|---|---|---|
| Not reduced | 0 | |
| Reduced a little | 1 | |
| Quite reduced | 2 | |
| Reduced a great deal | 3 | |
| No steam | 4 | |

15B. **How much does this bother you?**
(Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 10
**Not at all** **A great deal**

16. **Have you ever blocked up completely so that you could not urinate at all and had to have a catheter to drain the bladder**?

| | | |
|---|---|---|
| No | 0 | |
| Yes, once | 1 | |
| Yes, twice | 2 | |
| Yes, more than twice | 3 | |

17A. **Do you have a burning feeling when you urinate**?

| | | |
|---|---|---|
| Never | 0 | |
| Occasionally | 1 | |
| Sometimes | 2 | |
| Most of the time | 3 | |
| All of the time | 4 | |

17B. **How much does this bother you?**
(Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 10
**Not at all** **A great deal**

18A. **How often do you feel that your bladder has not emptied properly after you have urinated**?

| | | |
|---|---|---|
| Never | 0 | |
| Occasionally | 1 | |
| Sometimes | 2 | |
| Most of the time | 3 | |
| All of the time | 4 | |

18B. **How much does this bother you?**
(Please ring a number between 0 (not at all) and 10 (a great deal)

| 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| **Not at all** | | | | | | | | | | **A great deal** |

19. **Can you stop the flow of urine if you try while you are urinating**?

| | | |
|---|---|---|
| Yes, easily | 0 | |
| Yes, with difficulty | 1 | |
| No, cannot stop it flowing | 2 | |

# Appendix 8

☐☐ ☐☐ ☐☐ ☐☐☐
Initial number

ICIQ-LUTSqol 08/04

**CONFIDENTIAL**

☐☐ ☐☐ ☐☐
DAY MONTH YEAR
**Today's date**

## Quality of life

Below are some daily activities that can be affected by urinary problems. How much does your urinary problem affect you? We would like you to answer every question. Simply tick the box that applies to you.

We would be grateful if you could answer the following questions, thinking about how you have been, on average, over the PAST FOUR WEEKS.

**1. Please write in your date of birth:**

☐☐ ☐☐ ☐☐
DAY MONTH YEAR

**2. Are you** *(tick one)*: Female ☐ Male ☐

**3a. To what extent does your urinary problem affect your household tasks (e.g. cleaning, shopping, etc.)**

not at all ☐ 1
slightly ☐ 2
moderately ☐ 3
a lot ☐ 4

**3b. How much does this bother you?**
*Please ring a number between 0 (not at all) and 10 (a great deal)*

0 1 2 3 4 5 6 7 8 9 10
not at all — a great deal

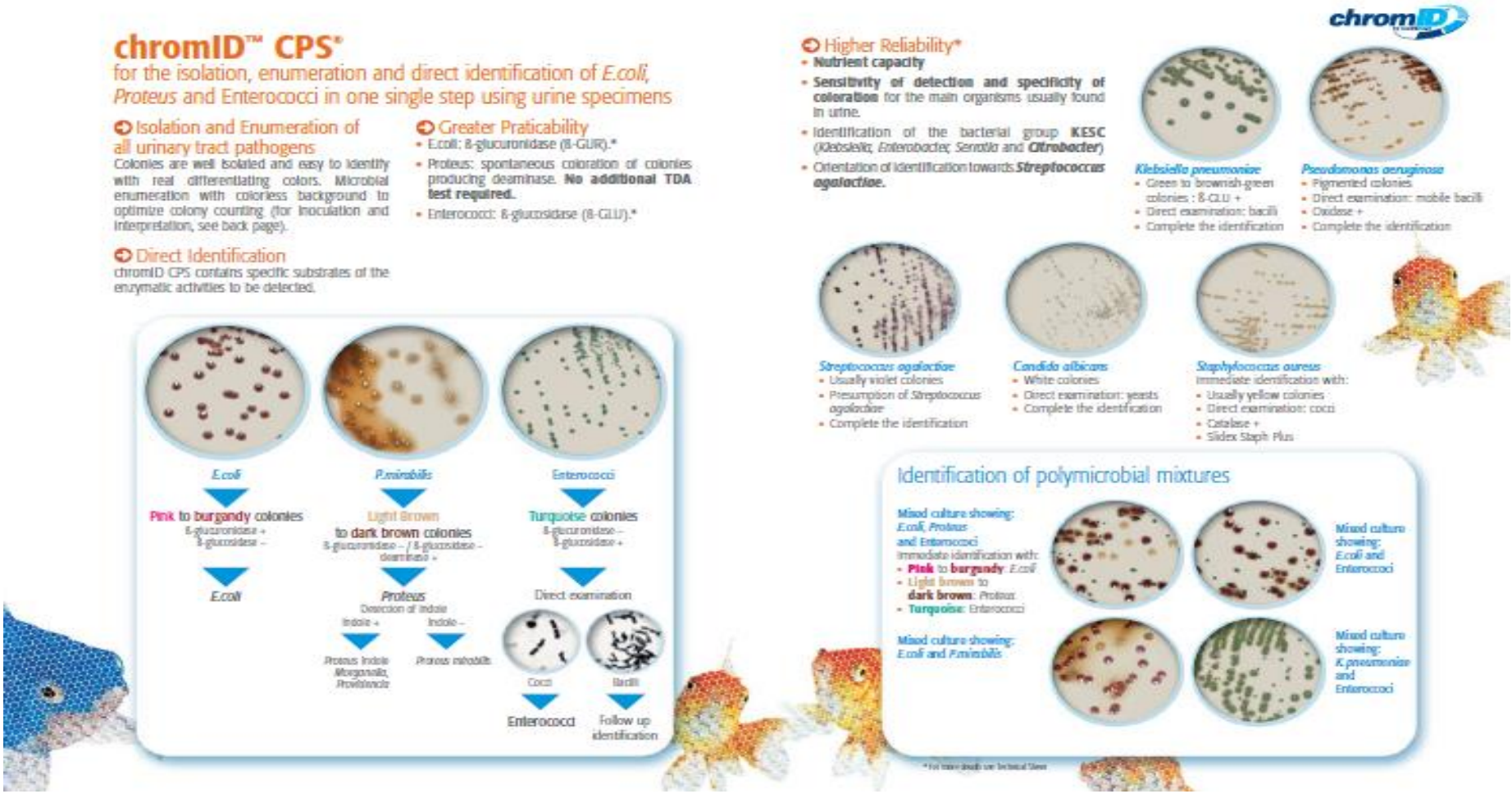
**4a. Does your urinary problem affect your job, or your normal daily activities outside the home?**

not at all ☐ 1
slightly ☐ 2
moderately ☐ 3
a lot ☐ 4

**4b. How much does this bother you?**
*Please ring a number between 0 (not at all) and 10 (a great deal)*

0 1 2 3 4 5 6 7 8 9 10
not at all — a great deal

# Appendix 9


chromID™ CPS®
for the isolation, enumeration and direct identification of E.coli, Proteus and Enterococci in one single step using urine specimens
Isolation and Enumeration of all urinary tract pathogens
Colonies are well isolated and easy to identify with real differentiating colors. Microbial enumeration with colorless background to optimize colony counting (for inoculation and interpretation, see back page).
Greater Praticability
E.coli: ß-glucuronidase (ß-GUR).*
Proteus: spontaneous coloration of colonies producing deaminase. No additional TDA test required.
Enterococci: ß-glucosidase (ß-GLU).*
Direct Identification
chromID CPS contains specific substrates of the enzymatic activities to be detected.
E.coli
Pink to burgandy colonies
ß-glucuronidase +
ß-glucosidase –
E.coli
P.mirabilis
Light Brown to dark brown colonies
ß-glucuronidase – / ß-glucosidase – deaminase +
Proteus
Detection of Indole
Indole +
Indole –
Proteus Indole Morganella, Providencia
Proteus mirabilis
Enterococci
Turquoise colonies
ß-glucuronidase –
ß-glucosidase +
Direct examination
Cocci
Bacilli
Enterococci
Follow up identification
Higher Reliability*
Nutrient capacity
Sensitivity of detection and specificity of coloration for the main organisms usually found in urine.
Identification of the bacterial group KESC (Klebsiella, Enterobacter, Serratia and Citrobacter)
Orientation of identification towards Streptococcus agalactiae.
chromID
Klebsiella pneumoniae
Green to brownish-green colonies : ß-GLU +
Direct examination: bacilli
Complete the identification
Pseudomonas aeruginosa
Pigmented colonies
Direct examination: mobile bacilli
Oxidase +
Complete the identification
Streptococcus agalactiae
Usually violet colonies
Presumption of Streptococcus agalactiae
Complete the identification
Candida albicans
White colonies
Direct examination: yeasts
Complete the identification
Staphylococcus aureus
Immediate identification with:
Usually yellow colonies
Direct examination: cocci
Catalase +
Slidex Staph Plus
Identification of polymicrobial mixtures
Mixed culture showing: E.coli, Proteus and Enterococci
Immediate identification with:
Pink to burgandy: E.coli
Light brown to dark brown: Proteus
Turquoise: Enterococci
Mixed culture showing: E.coli and P.mirabilis
Mixed culture showing: E.coli and Enterococci
Mixed culture showing: K.pneumoniae and Enterococci

## Appendix 10

Whittington Health NHS

**Community Lower Urinary Tract Service**
**Hornsey Central Neighbourhood Health Centre**
**2nd Floor**
**151 Park Road**
**London**
**N8 8JD**

**Participant Identification Number for this trial:**

# CONSENT FORM (Patients)

## A randomised, single blind comparative study of three non-invasive urine sampling methods

**Name of Researcher: Linda Collins**

*Please initial box*

1. I confirm that I have read and understand the information sheet dated **28th April 2014 (version.3.1)** for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐

3. I understand that relevant sections of my medical notes and data collected during the study, may be looked at by individuals from the sponsor of the trial (UCL), from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐

4. I agree to my GP being informed of my participation in the study. ☐

5. I agree to take part in the above study. ☐

6. I agree my samples being gifted to UCL to be stored, anonymously and used in subsequent ethically approved research studies ☐

| | | |
|---|---|---|
| Name of Patient | Date | Signature |
| Name of Person taking consent | Date | Signature |
| Name of Chief Investigator *(if different to the person taking consent)* | Date | Signature |

# Appendix 11

Whittington Health NHS

**Community Lower Urinary Tract Service**
**Hornsey Central Neighbourhood Health Centre**
**2nd Floor**
**151 Park Road**
**London**
**N8 8JD**

Participant Information Sheet (Patients) Part 2

**Short title: A randomised, single blind comparative study of three non-invasive urine sampling methods**

Protocol Reference **Version 3.1, 28th April 2014**

I would like to invite you to take part in our follow up segment of our research study. Before you decide to enrol it is important that you understand why this segment of the research is being done and what it would involve for you. We are happy go through the information sheet with you and answer any questions you have.

## What is the purpose of the project?

The patients that were enrolled into the MSU CSU study had provided two or three urine samples via different methods of producing urine samples. It was important to analyse further urine specimens by collecting three more non-invasive urine specimens.

## Why have I been invited?

You have been invited because you have been enrolled into the MSU CSU study and are eligible for take part in this part of the research.

## Do I have to take part?

It is up to you if you decide whether or not to take part. If you do decide to take part you will be given a copy of this information sheet to keep. You will be asked to sign a consent form, a copy of which will be given to you. If you decide to take part you are still free to withdraw at any time and without giving a reason. This will not affect the standard of care you receive.

**What will happen to me if I take part?**

If you decide to take part in the project, the research clinician will arrange to see you. You will be asked to visit the Hornsey Central Health Centre where you will be asked to provide a non-invasive urine sample on three different days. There are three methods we can use:

Midstream urine sample (MSU) where we ask patients to pass urine into a collecting bowl.

Peezy Midstream Urine (Peezy MSU) where we ask patients to pass urine using a specially designed collection device.

Directly voided urine, which is a straightforward natural urination into a bowl.

**Payments**

We are not in a position to pay you for participation in the study; however, we shall be able to reimburse you for travel expenses incurred from attending the study visit. We would be grateful for receipts describing the expenses for our auditors.

**What will I have to do?**

You will have to:

Give your consent

Attend our department for a short interview.

| Day Identification and visit | Actions |
|---|---|
| **Pre-study** | You will be given/sent an Participant Information Sheet |
| **At least 48 hours later** | You will be contacted to ask if you are interested in taking part in this segment of the study. If yes, an appointment will be made for a Visit |
| **Day of visit** | • Sign a consent form<br>• Asked to provide a urine specimen either by MSU, Peezy MSU or direct void (natural urination. |

**What are the alternatives to participating in the study?**

It is entirely your decision if you wish to take part in the study

**What if there is a problem?**

Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. The detailed information concerning this will be given at the later of this information sheet. If you have any concerns or complaints you should contact your study doctor in the first instance.

**Will my taking part in the study be kept confidential?**

Yes. We will follow ethical and legal practice and all information about you will be handled in confidence. The details are included later in the information sheet.

**Contact Details**

Your Doctor

**Professor James Malone-Lee** **Tel. Number: 020 3074 2256**

**What will happen if I don't want to carry on with the study?**

You are free to withdraw from the study at any time before and after signing the consent form without needing to give any explanations. The study may be ended at any time with or without your consent.

Unless you tell us otherwise, the information collected from you up to the point at which you leave the study will be used in the analysis. If you feel strongly that you would not wish to be the case, please let the investigator know.

**Complaints**

If you have any questions about your rights as a research subject or have a complaint about the way in which the study has been carried out, please contact: Professor Malone-Lee in the first instance.

If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure. Details can be obtained from the hospital or from the Department of Health website: http://www.dh.gov.uk. You may obtain the necessary guidance from the hospital Patient Advice and Liaison Service (PALS), Whittington Health, Tel 020 7288 5956 or 020 7288 5957

**Will my taking part in this study be kept confidential?**

If you consent to take part in this study, the records obtained while you are in this study as well as related health records will remain strictly confidential at all times. The information will be held securely on paper and electronically at the hospital site managing this research under the provisions of the 1998 Data Protection Act. Your name will not be passed to anyone else outside the research team or the Sponsor (UCL), who is not involved in the study. You will be allocated a study number, which will be used as a code to identify you on all study forms. Any information about you which leaves the hospital will have your name and address removed so that you cannot be recognised (if it is applicable to your research).

Your records will be available to people authorised to work on the study but may also need to be made available to people authorised by the Sponsor, which is the organisation responsible for ensuring that the study is carried out correctly. By signing the consent form you agree to this access for the current study and any further research that may be conducted in relation to it, even if you withdraw from the current study.

If you withdraw consent from the study, unless you object, your data will remain on file and will be included in the final study analysis.

In line with the regulations, at the end of the study your data will be securely archived for a minimum of 20 years. Arrangements for confidential destruction will then be made.

**What will happen to the results of the research?**

We shall use the data to make decisions on how we should plan our future discovery research.

**Who is organising and funding the research?**

This study is organised by the lower urinary tract symptoms service at the Whittington Health and is being paid for from Academic funds at UCL. The study is being sponsored by University College London.

**Who has reviewed the study?**

All research in the NHS is looked at by independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and approved by the Research Ethics Committee.

Thank you for taking the time to read this information sheet and to consider this segment of the study.

## Appendix 12

Whittington Health NHS

**Community Lower Urinary Tract Service**
**Hornsey Central Neighbourhood Health Centre**
**2nd Floor**
**151 Park Road**
**London**
**N8 8JD**

**Participant Identification Number for this trial:**

# CONSENT FORM (Patients)

### MSU CSU Patient experiences of providing a urine sample

**Name of Researcher: Linda Collins**

*Please initial box*

I confirm that I have read and understand the information sheet dated **28th April 2014** for **Version 3.1,** the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐

I understand that relevant sections of data collected during the study may be looked at by individuals from the sponsor of the trial (UCL), from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐

I agree to take part in the above study. ☐

| | | |
|---|---|---|
| Name of Patient | Date | Signature |
| Name of Person taking consent | Date | Signature |
| Name of Chief Investigator | Date | Signature |

# Appendix 13

Whittington Health NHS

**Community Lower Urinary Tract Service**
**Hornsey Central Neighbourhood Health Centre**
**2nd Floor**
**151 Park Road**
**London**
**N8 8JD**

Participant Information Sheet (Patients) Part 2

**Short title: MSU CSU patient perceptions of producing urine samples**

Protocol Reference **Version 3.1, 28th April 2014**

I would like to invite you to take part in our follow up segment of our research study. Before you decide to enrol it is important that you understand why this segment of the research is being done and what it would involve for you. We are happy go through the information sheet with you and answer any questions you have.

**What is the purpose of the project?**

The patients that were enrolled into the MSU CSU study had provided two or three urine samples via different methods of producing urine samples. After the urine samples were obtained, there wasn't an opportunity to find out about your experience of producing the samples.

Therefore, I would like to know more about your experience of producing a urine sample via the different methods, your perceptions towards the urine sampling process and a general insight of your thoughts and feelings.

**Why have I been invited?**

You have been invited because you have been enrolled into the MSU CSU study and are eligible for take part in this part of the research.

**Do I have to take part?**

It is up to you if you decide whether or not to take part. If you do decide to take part you will be given a copy of this information sheet to keep. You will be asked to sign a consent form, a copy of which will be given to you. If you decide to take part you are still free to withdraw at any time and without giving a reason. This will not affect the standard of care you receive.

**What will happen to me if I take part?**

If you decide to take part in the project, the research clinician will arrange to see you. You will be asked to visit the Hornsey Central Health Centre where you will be asked some questions about your experience of providing the urine samples via different methods. Your answers will be documented and later analysed. This will take no more than 30 minutes. The data collected is closely guarded by the NHS security system so there is no unauthorised access to you record.

**Payments**

We are not in a position to pay you for participation in the study; however, we shall be able to reimburse you for travel expenses incurred from attending the study visit. We would be grateful for receipts describing the expenses for our auditors.

**What will I have to do?**

You will have to:

Give your consent

Attend our department for a short interview.

| **Day Identification and visit** | **Actions** |
|---|---|
| **Pre-study** | You will be given/sent an Participant Information Sheet |
| **At least 48 hours later** | You will be contacted to ask if you are interested in taking part in this segment of the study.<br>If yes, an appointment will be made for a Visit |
| **Day of visit** | • Sign a consent form<br>• You will be asked some questions about your opinions of the different urine collection methods<br>• The interview will be documented so that data can later be analysed.<br>• The visit should last approximately 30 minutes. |

**What are the alternatives to participating in the study?**

It is entirely your decision if you wish to take part in the study

**What if there is a problem?**

Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. The detailed information concerning this will be given at the later of this information sheet. If you have any concerns or complaints, you should contact your study doctor in the first instance.

**Will my taking part in the study be kept confidential?**

Yes. We will follow ethical and legal practice and all information about you will be handled in confidence. The details are included later in the information sheet.

**Contact Details**

Your Doctor

**Professor James Malone-Lee** **Tel. Number: 020 3074 2256**

**What will happen if I don't want to carry on with the study?**

You are free to withdraw from the study at any time before and after signing the consent form without needing to give any explanations. The study may be ended at any time with or without your consent.

Unless you tell us otherwise, the information collected from you up to the point at which you leave the study will be used in the analysis. If you feel strongly that you would not wish to be the case, please let the investigator know.

**Complaints**

If you have any questions about your rights as a research subject or have a complaint about the way in which the study has been carried out, please contact: Professor Malone-Lee in the first instance.

If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure. Details can be obtained from the hospital or from the Department of Health website: http://www.dh.gov.uk. You may obtain the necessary guidance from the hospital Patient Advice and Liaison Service (PALS), Whittington Health, Tel 020 7288 5956 or 020 7288 5957

**Will my taking part in this study be kept confidential?**

If you consent to take part in this study, the records obtained while you are in this study as well as related health records will remain strictly confidential at all times. The information will be held securely on paper and electronically at the hospital site managing this research under the provisions of the 1998 Data Protection Act. Your name will not be passed to anyone else outside the research team or the Sponsor (UCL), who is not involved in the study. You will be allocated a study number, which will be used as a code to identify you on all study forms. Any information about you which leaves the hospital will have your name and address removed so that you cannot be recognised (if it is applicable to your research).

Your records will be available to people authorised to work on the study but may also need to be made available to people authorised by the Sponsor, which is the organisation responsible for ensuring that the study is carried out correctly. By signing the consent form you agree to this access for the current study and any further research that may be conducted in relation to it, even if you withdraw from the current study.

If you withdraw consent from the study, unless you object, your data will remain on file and will be included in the final study analysis.

In line with the regulations, at the end of the study your data will be securely archived for a minimum of 20 years. Arrangements for confidential destruction will then be made.

**What will happen to the results of the research?**

We shall use the data to make decisions on how we should plan our future discovery research.

**Who is organising and funding the research?**

This study is organised by the lower urinary tract symptoms service at the Whittington Health and is being paid for from Academic funds at UCL. The study is being sponsored by University College London.

**Who has reviewed the study?**

All research in the NHS is looked at by independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and approved by the Research Ethics Committee.

Thank you for taking the time to read this information sheet and to consider this segment of the study.

## Appendix 14

Whittington Health NHS

**Community Lower Urinary Tract Service**
**Hornsey Central Neighbourhood Health Centre**
**2nd Floor**
**151 Park Road**
**London**
**N8 8JD**

### List of interview questions

1. Which method of urine collection did you prefer and why?
2. Which method of urine collection did you not like and why?
3. What method of urine collection do you think provided the most clean sample and why?
4. Which method of urine collection do you think should be used as a standard method and why?

# Appendix 15

Whittington Health NHS

**Community Lower Urinary Tract Service**
**Hornsey Central Neighbourhood Health Centre**
**2nd Floor**
**151 Park Road**
**London**
**N8 8JD**

Dear,

Many thanks for taking part in the MSU CSU comparison study. Your participation in the study was very much appreciated by me and Professor James Malone-Lee. The data collected has yielded important data for analysis.

In order to validate the first part of the study it is important to gain insight on patient experiences of producing urine samples via the different methods of urine collection.

I would very much appreciate your participation in a short interview, which will allow me to find out your preferred method of producing a urine sample for testing and how your choice and preferences may facilitate urine collection methods in our future practice.

The interview should take no longer than 30 minutes. An information sheet explaining the details of the interview has been attached with this letter.

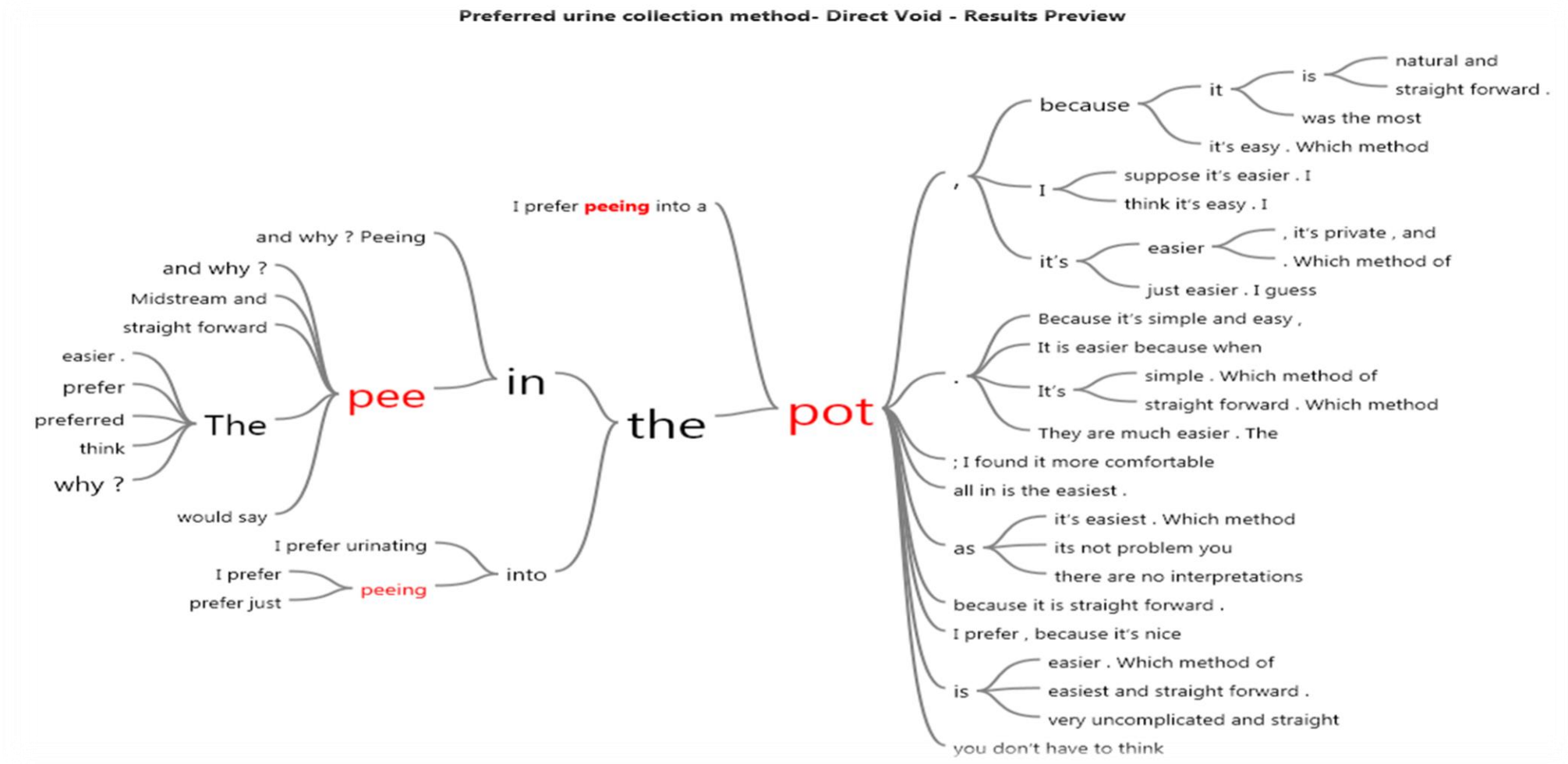
On the interview day two urine samples will be collected which will be a normal MSU sample and a urine sample collected in a pot with no instructed technique. These samples will be tested to see whether they produced the same diagnostics results.

Please indicate your willingness to participate in this part of the study by filing in the response form and mailing back to me in the envelope provided. Alternatively, you may contact me via e-mail (linda.collins7@nhs.net) or telephone (02030742252). I will be more than happy to answer any questions you may have.

Many thanks again for your participation and I look forward to seeing you again.

Linda Collins Bsc, MSc, RN
Community Lower Urinary Tract Service
Hornsey Central Neighbourhood Health Centre
2nd Floor
151 Park Road
London
N8 8JD

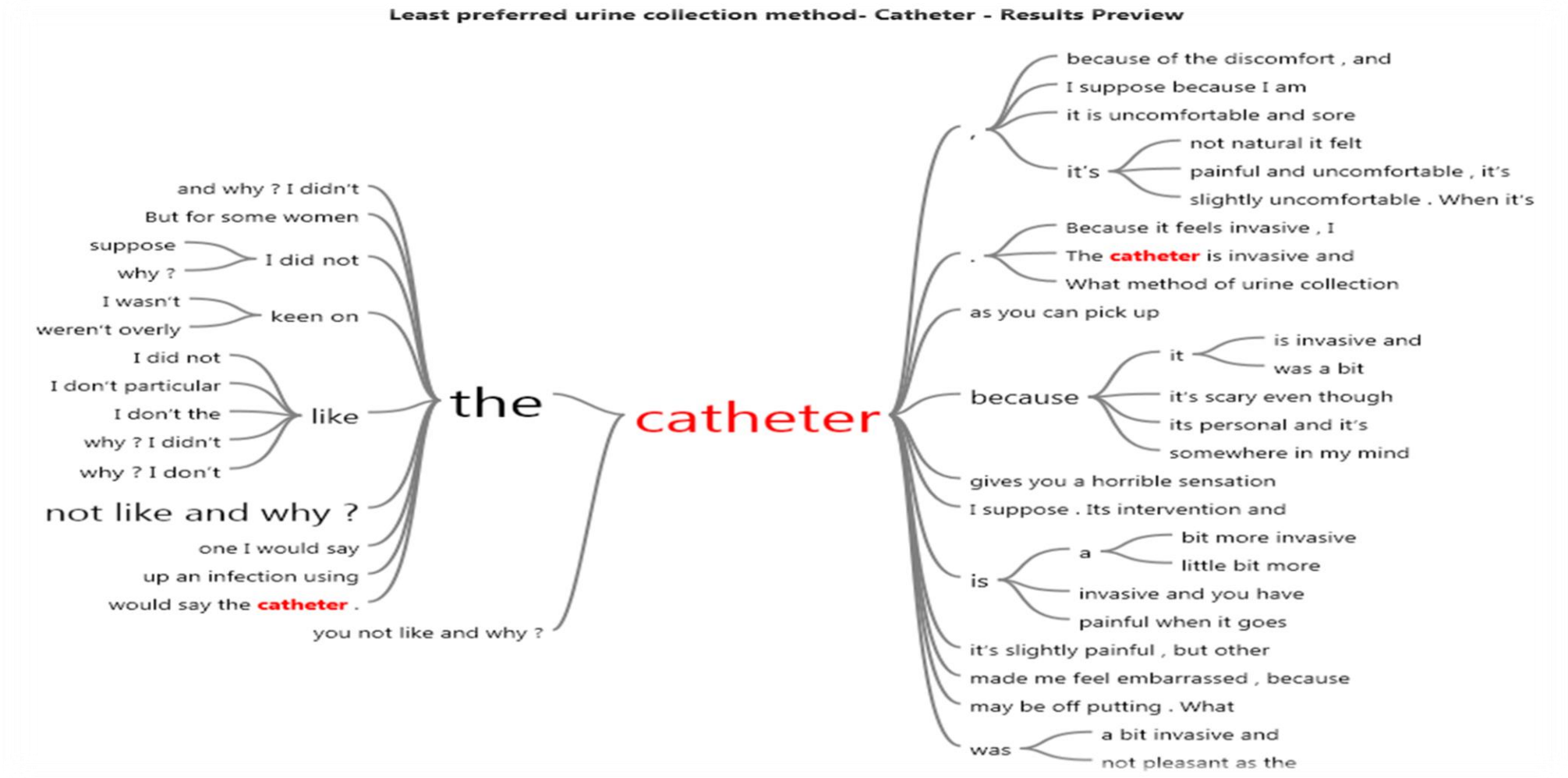
**Appendix 16**


Preferred urine collection method- Direct Void - Results Preview
The
easier .
prefer
preferred
think
why ?
pee
and why ? Peeing
and why ?
Midstream and
straight forward
would say
peeing
I prefer urinating
I prefer
prefer just
in
into
the
I prefer peeing into a
pot
,
because
it
is
natural and
straight forward .
was the most
it's easy . Which method
I
suppose it's easier . I
think it's easy . I
it's
easier
, it's private , and
. Which method of
just easier . I guess
.
Because it's simple and easy ,
It is easier because when
It's
simple . Which method of
straight forward . Which method
They are much easier . The
; I found it more comfortable
all in is the easiest .
as
it's easiest . Which method
its not problem you
there are no interpretations
because it is straight forward .
I prefer , because it's nice
is
easier . Which method of
easiest and straight forward .
very uncomplicated and straight
you don't have to think

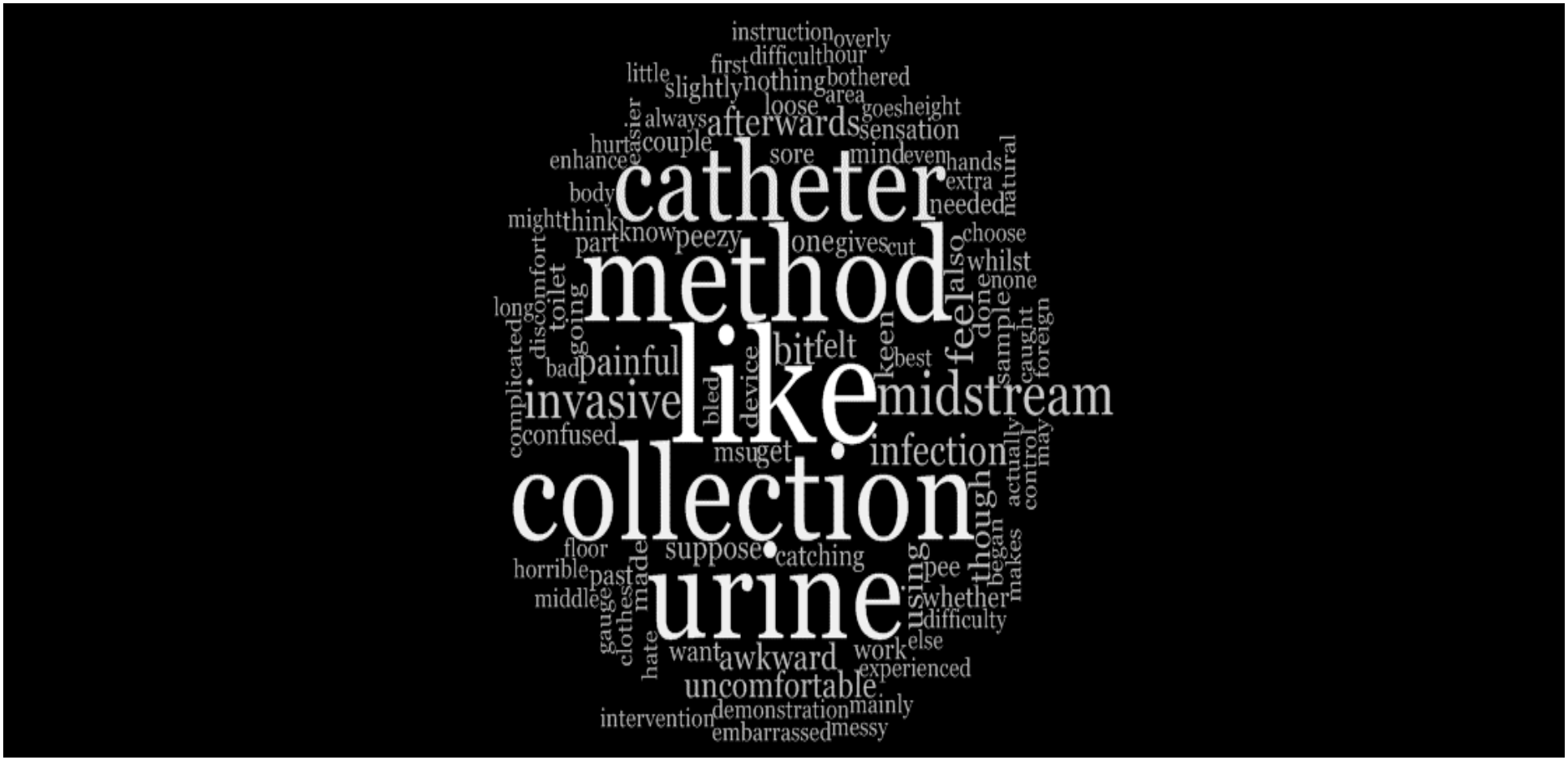
## Appendix 17


interpretations
effortless
desperate
open
procedure
collecting
ease
middle
legs
cleaner
stop
easier
easiest
easy
sample
problem
guess
amount
nice
know
difficult
familiar
alright
pot
forward
collect
relaxed
detect
process
control
clean
collection
quick
need
speezy
next
straight
like
think
msu
better
prefer
liked
seconds
reassuring
dignified
preferred
catheter
feel
let
come
one
peeing
methods
stream
delays
find
always
just
well
much
pee
seems
urinating
use
urine
wee
direct
ready
likely
found
bladder
private
also
anything
key
give
part
suppose
thing
simple
certain
idea
mind
start
method
looking
comfortable
infection
painful
empties
natural
prepare
wiping
uncomplicated

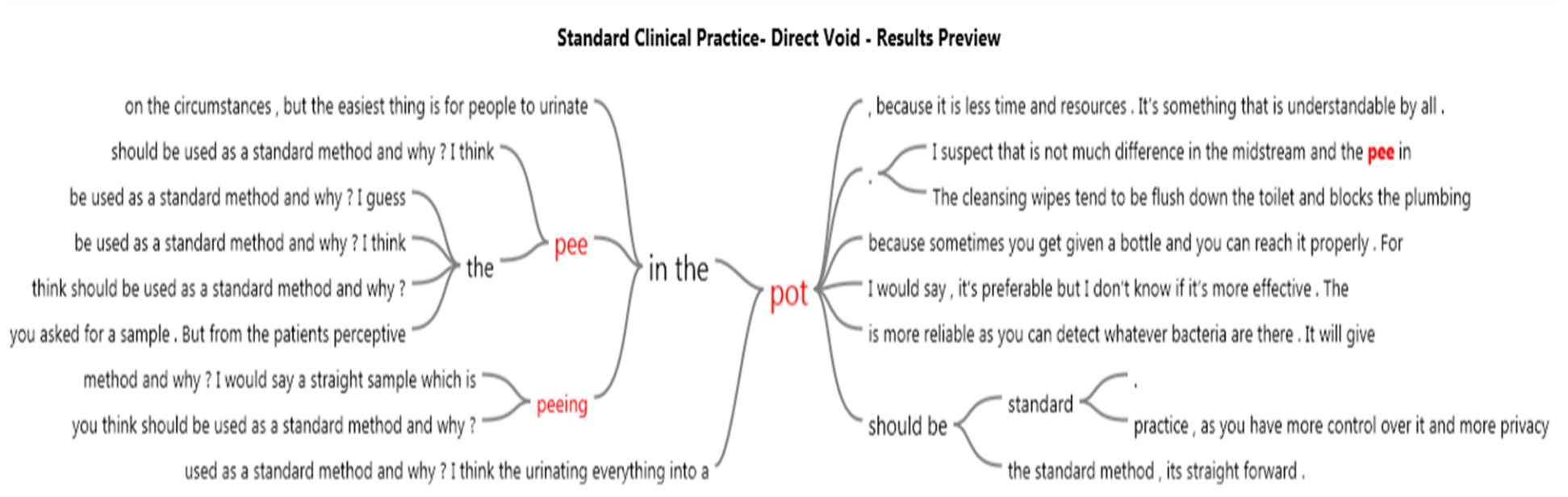
## Appendix 18


Least preferred urine collection method- Catheter - Results Preview
and why ? I didn't
But for some women
suppose
why ?
I did not
I wasn't
weren't overly
keen on
I did not
I don't particular
I don't the
why ? I didn't
why ? I don't
like
not like and why ?
one I would say
up an infection using
would say the catheter .
you not like and why ?
the
catheter
because of the discomfort , and
I suppose because I am
it is uncomfortable and sore
not natural it felt
it's
painful and uncomfortable , it's
slightly uncomfortable . When it's
Because it feels invasive , I
The catheter is invasive and
What method of urine collection
as you can pick up
it
is invasive and
was a bit
because
it's scary even though
its personal and it's
somewhere in my mind
gives you a horrible sensation
I suppose . Its intervention and
a
bit more invasive
little bit more
is
invasive and you have
painful when it goes
it's slightly painful , but other
made me feel embarrassed , because
may be off putting . What
was
a bit invasive and
not pleasant as the

## Appendix 19


instruction
overly
difficult
hour
first
little
slightly
nothing
bothered
area
loose
goes
height
always
afterwards
sensation
easier
hurt
couple
enhance
sore
mind
even
hands
catheter
extra
natural
body
needed
might
think
know
peezy
one
gives
cut
choose
part
method
also
whilst
toilet
discomfort
going
done
none
long
like
feel
sample
caught
foreign
complicated
bit
felt
keen
best
bad
painful
bled
device
midstream
invasive
confused
infection
msu
get
collection
actually
control
may
though
began
makes
floor
suppose
catching
using
horrible
past
made
pee
middle
whether
gauge
clothes
difficulty
hate
want
awkward
work
else
experienced
uncomfortable
intervention
demonstration
mainly
embarrassed
messy
urine

# Appendix 20


Standard Clinical Practice- Direct Void - Results Preview
on the circumstances , but the easiest thing is for people to urinate
should be used as a standard method and why ? I think
be used as a standard method and why ? I guess
be used as a standard method and why ? I think
think should be used as a standard method and why ?
you asked for a sample . But from the patients perceptive
the
pee
method and why ? I would say a straight sample which is
you think should be used as a standard method and why ?
peeing
used as a standard method and why ? I think the urinating everything into a
in the
pot
, because it is less time and resources . It's something that is understandable by all .
I suspect that is not much difference in the midstream and the pee in
The cleansing wipes tend to be flush down the toilet and blocks the plumbing
because sometimes you get given a bottle and you can reach it properly . For
I would say , it's preferable but I don't know if it's more effective . The
is more reliable as you can detect whatever bacteria are there . It will give
should be
standard
practice , as you have more control over it and more privacy
the standard method , its straight forward .