Methods for Rapid Prototyping Novel Labware: Using CAD and Desktop 3D Printing in the Microbiology Laboratory

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Running title: **3D printing labware for microbiology**

Significance and Impact of the study

We present methods for designing and 3D printing microbiological labware offering alternatives to off-the-shelf consumables that allows low-cost rapid prototyping and customisation of microbial culture tools. We demonstrate customised 3D printed rapid prototype solid medium culture dishes and dip-slides, and modified inoculating loops and customisable replicating pins for plating bacteria. 3D printed labware can offer local production to avoid dependence on commercial suppliers, rapid customisation of existing designs, and rapid evaluation of entirely new tools. Using 3D printing to develop novel labware tailored to simplify routine work in the microbiology laboratory can save time and labour compared to relying on off-the-shelf mass-manufactured labware.

Abstract

Although the microbiology laboratory paradigm has increasingly changed from manual to automated procedures, and from functional to molecular methods, traditional culture methods remain vital. Using inexpensive desktop fused filament fabrication (FFF) 3D printing, we designed, produced and tested rapid prototypes of customised labware for microbial culture namely frames to make dip slides, inoculation loops, multi-pin replicators, and multi-well culture plates for solid medium. These customised components were used to plate out samples onto solid media in various formats, and we illustrate how they can be suitable for many microbiological methods such as minimum inhibitory concentration tests, or for directly detecting pathogens from mastitis samples, illustrating the flexibility of rapid prototyped culture consumable parts for streamlining microbiological methods. We describe the methodology needed for microbiologists to develop their own novel and unique tools, or to fabricate and customise existing consumables. A workflow is presented for designing and 3D printing labware and quickly producing easy-to-sterilise and re-useable plastic parts of great utility in the microbiology laboratory.

Keywords: 3D printing, fused filament fabrication, microbiology labware, dip slide, loop, culture dish.

1 Introduction

In the past, great steps forward in microbiology have been supported by methodological changes, often requiring custom labware, instruments or reagents produced in-house. The origins of modern microbiology lie with the development of tools such as the petri dish, developed in the 1880s (Shama 2019), which allowed pure isolates to be cultured from colonies on solid media, a method that has barely changed in over a century (Lagier, Edouard et al. 2015). Recently, the convenience of off-the-shelf products supplied by manufacturers has improved lab throughput, with the consequence that microbiology labs can become dependent on supply of conventional components from commercial suppliers. Innovative analytical techniques remain critical to human health and wealth, and underlying most analytical methods and microbial research lies microbiological labware. Rapid prototyping of plastic objects has never been simpler or more accessible, and the potential for 3D printing in research is expanding and expected to lead to a new approach where microbiology labs can adapt and customise labware for individual applications. Fused filament fabrication (FFF) 3D printers retailing for under £200 are reliable, easy to use, and widely available across the world. While more advanced rapid prototyping methods are becoming more common, this simple approach produces a wide range of different shaped plastic objects, suitable for many laboratory activities.

The material cost of parts runs around £20 per kg, plus labour cost associated with operating the printers. Custom labware offers opportunities to rapidly innovate to solve common problems in microbiology laboratories.

In-house designed and 3D printed labware can offer at least three benefits: Firstly, 3D printing offers the chance to locally make labware that is identical to standard consumables, but without being dependent on commercial suppliers/distributors and thus at lower cost, available faster, or avoiding delivery delays (for example during supply chain disruption). Secondly, modified versions of conventional/commercial products can be designed, and 3D printed that are customised to individual needs (for example, different shape agar plates to conventional petri dishes). Finally, entirely new tools can be created and evaluated rapidly, allowing rapid iterative development of new methods. Here, we focus on rapidly creating modified versions of conventional culture tools. For example, whilst round petri dishes are conventionally used and suitable for a wide range of methods, they are often inconvenient for example not being compatible with microplate well grids and multichannel pipetting. Alternative square dishes are less widely available and still don't correspond to the standard 12x8 grid and 9mm pitch of 96-well microwell plates. Dishes with multiple compartments are less common, partly because different assays need different sized compartments, but replica plating samples onto multiple agar types is a common procedure, requiring large stacks of petri dishes and large volumes of solid media. Whilst standardisation is extremely helpful for automation and instrument development (e.g.microplate readers), microwell plates themselves are only available in a limited range of configurations of well sizes, numbers, and arrangements. They are most commonly produced for eukaryotic tissue culture or molecular biology, rather than being configured specifically for microbiology methods such as culture and functional/phenotypic assays. (Maia Chagas, Prieto-Godino et al. 2017)As 3D printing allows rapid production of any shape or size of culture dish to be customised, it can support high throughput testing in multi-well solid agar plates configured for specific microbiology methods.

To create new microbiological labware for research and diagnostics fabricated commercially by typical mass-manufacture methods such as injection moulding or thermoforming, researchers would need close support from vendors, manufactures and suppliers. The cost for final mass-produced components and prototypes will be expensive for unique or unusual objects and designs because of the high capital cost of tooling plus cost of experts to redesign, and researchers may need to buy in bulk without being able to obtain one piece for a quick evaluation (Neches, Flynn et al. 2016).

Outside the distribution network of the major suppliers of microbiology consumables even conventional labware is not always accessible. In many parts of the world, it can take 4 or more weeks to receive materials for experiments, further limiting the opportunity for rapid testing and innovation. Purchasing power parity distorts the price of scientific research materials, combining with high shipping costs, and significantly limiting the availability of microbiology labware and materials. This can delay uptake of the latest methodology, and restrict microbiology labs in low resource areas to older methods (e.g. round petri dishes). Even in well-resourced laboratories with rapid access to commercial labware, supply-chain disruption (e.g. shipping blockage) and depletion of critical components (e.g. during pandemic) can be overcome if individual labs can produce their own in-house labware. Yet the need for global analytical

microbiology both for local public health and for coordinated surveillance, especially for infectious agents and antimicrobial resistance genes that can spread rapidly across the world, continues to drive a need for more, better, analytical microbiology in all regions, especially those with lower resources for accessing innovative lab tools.

Another global microbiology pressure is that current gold-standard culture methods, are often time-consuming to simultaneously perform on many samples, making it challenging to address outbreaks or for surveillance. Theyremain labour intensive, placing high costs to microbiology laboratories of vital importance for public health. For example, at least four different media might be needed to identify critical types of pathogen in each sample. One petri dish contains 20 ml of each media type, therefore, 2000 ml of each media and a total of 8000 ml media will be prepared and sterilized to process 100 samples. It's estimated that 400 Petri dishes will be disposed of and take more than 200 minutes to complete just one step of the bacterial identification procedure. This could be streamlined if round plastic petri dishes were replaced with smaller dishes that combine multiple media, to significantly reduce the volume of media and associated labour. Similarly, inoculation loops, frequently used for subculture or streaking out colonies, remain unchanged for many decades. Single-use plastic loops are very similar in design to metal wire loops, yet plastic permits many different configurations. These could be customized in size to fit with other common lab formats, for example by combining with 96 well plates to process many samples simultaneously, without needing expensive multichannel pipettes for plating. Likewise, dip slides have in some applications replaced loops and petri dishes for plating, saving labour and time of staff in the laboratory, but these are only available from a few suppliers in simple configurations with only one agar type per dip-slide.

The accessibility of 3D printing driven by the rise of Rep-Rap FFF rapid prototyping (Gross, Erkal et al. 2014) now offers an opportunity to rapidly customise plastic microbiology labware. Rapid prototyping methods including additive manufacturing and three-dimensional printing have been widely applied for industry as an innovation tool, and these methods have increasingly spread into life science, medical and healthcare research. This was first introduced in 1986 by Charles Hull as a manufacturing tool, by designing bespoke objects created from 3D design software, then fabricating solid objects through layer-by-layer printing of stl files (Sharafeldin, Jones et al. 2018). Current biomedical applications include medicines, dentistry, pharmaceutical development, bioengineering, and medical devices (Awad, Trenfield et al. 2018, Sharafeldin, Jones et al. 2018, Gonzalez-Henriquez, Sarabia-Vallejos et al. 2019, Culmone, Henselmans et al. 2020). With such benefits as flexible design, cost saving and eco-friendly environmental material, 3D printing allows prototyping and manufacture of many research tools. Biocompatible parts similar to biological tissue such as bones, heart valve have also been explored (Culmone, Henselmans et al. 2020). In the microbiology field, examples have been published including a 3D printed motility assay device (Neches, Flynn et al. 2016), and digital microscopes for the microbiology laboratory (Neches, Flynn et al. 2016, Maia Chagas, Prieto-Godino et al. 2017, Del Rosario, S. Heil et al. 2021), however we do not yet see widespread uptake.

We believe many microbiology researchers and analytical laboratories can benefit from in-house rapid prototyped labware, by adopting CAD and desktop 3D printing methods. We propose that the latest desktop

3D printers and open-source CAD software are now accessible enough for non-engineers to adopt into their labs. In this paper, we describe practical methodology for introducing free open-source CAD plus low-cost desktop 3D printing into a microbiology research laboratory, to rapidly prototype and manufacture custom microbiology labware. We illustrate the power of 3D printing to replace petri dishes and pipettes by designing, fabricating, and testing novel customised frames to create multi-agar dip slides, customised inoculation loops, bespoke multi-channel dishes for replica plating onto a panel of agar types, and replicator pins in different configurations. For these designs, we maintain compatibility with 96-well microplates to simplify processing of multiple samples, and at the same time interface with current labware standards. For these examples, we outline the methodology of design, 3D printing by FFF rapid prototyping, and present qualitative validation in the microbiology laboratory.

2 Results and Discussion

Rapid prototyping methods for in-house design and fabrication of microbiology labware

Through FFF 3D printing, custom labware can be designed or redesigned to replace commercial consumables or to create new tools. The process of creating a novel consumable needs several steps from design to manufacture and testing (figure 1). We highlight key practical considerations that a microbiology laboratory would need to consider when starting to 3D print labware (table 1). An increasing number of repositories host open-source designs freely available to download and 3D print, so in-house design isn't always necessary although these are not always straightforward to use (Alcock, Hudson et al. 2016). For in-house development we use OpenSCAD open-source computer aided design (CAD) software (available from www.openscad.org), because it is freely available and the open source model permits anyone else to open and/or edit our design files. However, proprietary CAD software is also available, with many educational establishments having access to more advanced CAD packages for research and teaching. Using OpenSCAD software, we can create and edit a bespoke model (step 1) that can be openly shared for others to edit. This software is parametric, allowing critical dimensions to be defined and rapidly changed- for example size or number of wells in an agar dish. A 3D object file is then exported in "Standard Triangle Language" or "Standard Tessellation Language" (.slt) file format which then needs to be 'sliced' into many layers to be 3D printed using open source software configured for the desktop 3D printer. The sliced file is then transferred to the desktop 3D printer (Figure 1 step 2). In the step 2, all parameters for printing including temperature, z axis, layer resolutions, and speed, can be modified if desired, or generic settings used that are supplied with most 3D printers. Printing settings need optimisation for properties such as mechanical strength and stiffness, and for microbiology labware these often need tuning to ensure parts contain liquid without leaking. Many printing parameters will be selected to match the 3D printer material and printer used locally, so we cannot provide general rules for 3D printing microbiology labware, however we have successfully used generic settings supplied with the printer for PLA. We almost exclusively print microbiology research labware using poly lactic acids (PLA) plastic, despite some limitations of its material properties. Although many other options are available such as polyethylene tetraphtlatate (PET) or Acrylonitrile Butadiene Styrene (ABS) which are more stable at higher temperatures than PLA, we have never found significant advantages, and PLA is simpler to print using inexpensive 3D printers. To print smaller parts (e.g. <10 x 10x 1 cm objects)

producing the prototype parts just takes a few hours; one day or overnight is needed either for larger objects or to print a large batch of smaller objects.

Table 1: Key Considerations for rapid prototyping and 3D printing microbiology labware

Topic	Barrier	Cost	Evaluated in this manuscript	Alternative	Conclusion
CAD software	Can you access CAD package?	Free Open Source and free licenses to proprietary CAD packages available	OpenSCAD	FreeCAD or Fusion360. Some advanced software can be licensed but many academic institutions have license to more advanced CAD packages for research and teaching	CAD software now widely available, both free open source and commercial products
CAD skills	Can a microbiology researcher design 3D parts for rapid prototype testing?	Many online training resources available free of charge	New designs created and refined by doctoral research student	Can contract engineer to design parts. Can collaborate with engineering department.	Basic skills for simple models can be gained in a few days. More complex designs need more expertise
3D model design	Has what you need already been designed?	Growing libraries of open source 3D models available free of charge	In-house design by microbiology researcher	Can download many 3D designs; can collaborate with engineer or use consultancy	Design time for in-house CAD fits within scope of doctoral research
3D printing hardware	Can you access a 3D printer?	Basic desktop 3D printer costs significantly less than £200	Prusa i3 MK3 (£700 from Prusa Research, Prague in 2019) and Creality Ender 3 (£190 from Farnell, Leeds UK in 2020)	Many 3D print services offer fast prototyping. Makerspaces and universities have shared 3D printing facilities	Capital cost of basic 3D printers no longer a significant barrier
3D printing skills	Can you operate a desktop 3D printer	Many online training resources	Printed in-house by doctoral research student	3D printing facilities and services offer technical support for printing	Sufficient time is required for researcher to operate and troubleshoot 3D printer
3D printing materials	Are 3D printed parts compatible with microbiology experiments	£20 per Kg for poly-lactic acid (PLA) in 2020	Poly-lactic acid (PLA) from multiple local suppliers effective for the applications we tested	Polyethylene tetraphtalate (PET) and acrylonitrile butadiene styrene ABS (multiple suppliers) offer	Cost of 3D printing consumables lower than conventional microbiology lab consumables

	increased heat resistance

Sterilisation of 3D printed microbiology labware

Sterility is a crucial requirement in microbiological methodology and this is also the most important question for any protocol or material to be used in a microbiology laboratory (Neches, Flynn et al. 2016). PLA has a low melting temperature to simplify 3D printing, and softens in boiling water, but surprisingly we found it possible to autoclave PLA labware parts, if some care is taken during sterilisation. We found that autoclave could only be used for PLA parts when individual pieces were carefully wrapped with aluminium foil and placed on a flat surface during autoclaving, and allowed to fully cool before removal. Although some minor changes in shape occurred, for simple parts the functional shape was retained and overall dimensions remained within 2% of original (e.g. an 100mm long dip-slide frame only shrunk by 1mm after autoclaving). However, if parts were placed together in a pot for autoclaving, their shape changed after autoclaving, becoming unusable (Figure 2). The amount of distortion after autoclaving depended greatly on the shape and size of the parts; so every design must be checked for compatibility with autoclaving. We found ABS parts less sensitive to deforming as expected from its higher melting temperature, so alternative 3D printer materials such as PET and ABS may therefore be more tolerant of microbiology autoclaves. However, we found the convenience and speed of printing PLA parts outweighed this improved heat resistance.

The simplest option was sterilisation with 70% alcohol, followed by drying, which we found very effective and reliable to sterilise PLA labware with no loss of function or damage to this plastic. PLA parts couldn't be used for microbiology culture directly after printing, because although the melt-processing at above 200° C during printing will sterilise parts (Neches, Flynn et al. 2016), our laboratory 3D printer is not maintained in an aseptic environment and so prototype parts were unsurprisingly not sterile (figure 2). Installing the 3D printer into an aseptic cabinet would avoid this, but would dramatically increase the cost and space requirements, as laminar flow HEPA-sterilised workstations are larger and more expensive than the 3D printer within; furthermore the airflow would be likely to affect the printing by changing the airflow and cooling speed of polymer after extrusion- 3D printers are known to be sensitive to drafts. Using an enclosed 3D printer might also be beneficial for part sterility, although these can be more expensive than the cheapest desktop 3D printers without enclosure.

Some changes to materials properties may occur after sterilisation either with autoclaving or 70% ethanol treatment, however although this class of polymer can be degraded by autoclaving (Rozema, Bos et al. 1991) we found that all parts retained adequate mechanical strength. Different materials (even the same filament type from different supplier) and other printing parameters also alter mechanical properties of 3D printed parts, and every lab must therefore check the suitability of parts in-house. Furthermore, potential interference with culture container material with microbiological experiments must be checked in-house, for example possible leaching of plastic additives that might interfere with antibiotic susceptibility or growth assays, in common with conventional labware. We explored if some 3D printed labware parts could be re-

used if needed as an alternative to single-use and disposal. While PLA is often labelled as a biodegradable polymer, re-use could also contribute to solving the overuse of single-use plastic labware recently which contributes to pollution globally (Chen, Awasthi et al. 2021). We found that careful washing followed by re-sterilisation using 70% ethanol was adequate to permit re-use, and simple PLA parts remained functional for containing agar medium when used at least 5 times before deteriorating. Beyond five wash - ethanol sterilisation -and re-use cycles, the dip-slide frames became too brittle to use.

Sterilisation of 3D printed parts has been explored elsewhere, not only with reference to microbiology (Neches, Flynn et al. 2016) but also in bioscience research and medical device fields. For example the effect of autoclaving on material properties of 3D printed medical devices and surgical implants has been extensively studied (Boursier, Fournet et al. 2018, Aguado-Maestro, De Frutos-Serna et al. 2021, Pérez Davila, González Rodríguez et al. 2021). Likewise 3D bioprinters have been established for cell culture where sterility is vital (Kahl, Gertig et al. 2019).

Preparation of custom solid medium configurations in dip slide frames for direct sample plating onto multiple media

Direct detection of pathogens from samples is a major concern for the microbiology laboratory, often tackled using culture methods including an array of identification media that select certain species and colour colonies. Conventional labware requires stacks of petri dishes, increasing labour and taking up space in refrigerators and incubators. We designed “dip slide frames” containing two different solid agars in multiple configurable wells (Figure 3A). Long rectangles provided larger areas of agar suitable for colony identification, and we added two rows of circular wells to permit use for comparing growth on smaller areas of other solid agars – for example with added serial dilutions of antibiotics to permit direct determination of minimum inhibitory concentration (MIC; agar dilution method, manuscript in preparation). To illustrate and evaluate the design concept, we used just two Chromoagar types (gram+ and gram-) in the whole frame and cultured a mixture of *E.coli* and *Klebsiella pneumoniae* illustrating the different colony colours on the media - pink for *E.coli*, and blue for *Klebsiella pneumoniae*. These frames are used in the same way as commercial dip-slides by just dipping directly into liquid samples and overnight incubation at 37^0C. These 3D printed frames allowed us to rapidly test the feasibility of including multiple solid media in a single dip-slide (Figure 3B); using multiple sizes to determine the smallest needed to detect growth of particular targets.

Using these prototypes, we discovered that the light sources and exact material of PLA used to print the parts were crucial factors to record and interpret the results (Figure 3C). In our data, natural transparent PLA was the best choice material to get a clear image recording colony growth, compared to other colours or materials. For example, it was possible to see the colonies by eye or digital photograph on the surface of solid agar under room lighting with blue or white PLA material. However, with white light illumination under the parts, the coloured and white PLA were opaque preventing illumination, making it hard to observe colonies on the lightbox. In contrast, the natural PLA was transparent allowing white backlight to illuminate the bacterial colonies, and giving good colour record of colony type, helpful for the identification of bacteria

on chromogenic identification media. We illustrated this with three dilutions of a mastitis milk sample (Figure 3D). Having established this design concept is feasible, we are working further to explore the performance of this design for rapid direct testing (manuscript in preparation).

Preparation of custom solid medium plates and plating loops in diverse formats

Processing multiple samples is simplified by microwell plates, yet the size of common solid media culture dishes does not match conventional 9mm pitch microwell plates. 3D printed cultureware can be configured to suit microwell plates, and forto specific test procedures. To illustrate this, we designed customized multi-well agar plates and loops (Figure 4A) allowing us to streak out panels of samples from standard microwell plates onto solid medium. A 3D printed row of loops (Figure 4B) with 9mm matching 96-well plate format simplified plating by allowing an entire row or column to be inoculated onto agar in one go (Figure 4C). We found that modifying the loop diameter (Figure 4D) allowed us to change the volumes of the sample inoculated onto agar, ranging from ~10 µl down to ~1 µl, clearly demonstrated by the similar numbers of colonies deposited by loop vs micropipette (Figure 4E). The 3D printed loops behaved similarly to commercial disposable plastic inoculation loops – but- it is worth noting that careful technique is still required as with any loop inoculation method to deposit equal quantities; loops are not a direct substitute for precise volume dispensed by pipettes. On the five-column multi-media plate, Baird Parker, Chromoagar for gram-negative and gram-positive, Mackonkey, and Mueller Hinton agar were prepared that would permit culture and identification of a wide range of species such as *Staphylococcus aureus, Klebsiella penumoniae, E.coli,* and *Pseudomonas aeruginosa*. Each medium type spreads into 10 compartments but with boundaries separating the individual plated samples, reducing chance of cross-contamination. We combined this multi-media plate with multi-well inoculation loops to plate out a set of reference strains in parallel (Figure 4C). Using the multi-well plate reduced the volume of media significantly compared to conventional petri dishes. The multi-well plate reduced the common problem of cross-contamination or fusion of droplets when plating multiple samples from 96-well plates onto agar in a petri-dish. Using smaller loop size could also reduce the volume of liquid deposited, further reducing the risk of cross-contamination. 3D printed labware is ideal for applications where droplet fusion or cross-contamination is a problem, as designs are not limited to standard 9mm pitch between microwells in wells for commercial products, so wider spacing is possible. Diluting wells, inoculation loops, and agar dishes be designed together to meet custom needs.

Customised pin replicator allows personalised configuration for plating onto antibiotic plates to measure Minimum Inhibitory Concentration (MIC)

While commercial petri-dishes and microplates may be as cheap as 3D printed versions, other labware and tools may be hard to access with a high initial purchase cost. A range of steel pin replicators are available but can cost £500 or more to purchase, which may not be justified for a small experiment or evaluation. Furthermore, only a few configurations are available. 3D printing allows a laboratory to evaluate the usefulness of a device such as a pin replicator prior to investing in commercial hardware, as well as rapid

customisation. Through open source design with open source software and FFF fabrication, two formats of pin replicators were rapid prototyped with 31 pins (designed to fit conventional round petri-dishes when square dishes are unavailable) and with 48 pins (for custom 3D printed square dishes), allowing replication of bacteria from 96-well microplates with a standard 9-mm pitch. We used these for agar dilution on conventional petri dishes, and for replicating onto 3D printed customised multi-well plates. 15 isolates including multiple mastitis sample isolates plus reference strains were replica plated from colonies prepared in a microwell plate, and found to have significant resistance to Cefoxitin and Streptomycin, with variable sensitivity to Gentamicin (Figure 4G and data not shown). These tools offer an alternative to plating using micropipette or multichannel pipettor, or to commercially available pin replicators that are only available at a high price in a few limited configurations. Custom configurations of pin replicators may be helpful to fit with different multiwell plates/petri dishes or to replica plate spots of different size or shapes of inoculum onto solid media.

Conclusions

Fused filament fabrication technology can become an essential tool to facilitate innovation in the microbiology laboratory. Whether producing in-house versions of existing labware to avoid supply problems, customising standard labware for specific methods, or developing entirely new components, our method guide and findings, can help researchers create or replace many types of labware without waiting for vendors to innovate or for delivery of supplies, as well as quickly iterating any parameters for experiments. With recent developments of FFF technology and inexpensive desktop 3D printers, it has become possible for researchers to design whatever they want and test in few hours. This will save time, ease research budgets, and ultimately reduce turnaround time to improve the quality of microbiology. Although we provide examples for plating and replication of bacterial samples to determine MIC on agar, plus dip-slides and plates for multi–channel agar media, many different labware components can be readily designed and tested. Other equipment in the microbiology laboratory has also been designed and 3D printed including digital microscopy (Sharkey, Foo et al. 2016), centrifuge (Byagathvalli, Pomerantz et al. 2019) and micropipettes (Brennan, Bokhari et al. 2018). The open publication of these designs will support a new wave of microbiology innovation across the globe.

Material and Methods

Design files for loop and frame dip slide, multi – channel rectangle dish, and pin replicator

All openscad and stl file of the labware in this study are available to download as electronic supplementary information and details of these files are available in table S1. All original OpenSCAD design files are available under an open source hardware license, for open re-use and modification, and as well as downloading these from ESI they can all be accessed via a GitLab repository (https://gitlab.com/AlEdwards/various-lab-designs).

Bacterial strains

E.coli ATCC 25922, *Pseudomonas aeruginosa* ATCC 10145 and *Klebsiella pneumoniae* ATCC 13883 and *S. aureus* NCTC 8355 were used for replication test and streak on agar media in this study. These strains were inoculated overnight at 37^0C on Mueller Hinton (MH) agar (Thermofisher, UK). Alongside reference strains, 13 isolates from mastitis using Chromagar was also used. Replica plated strains numbered in figure 4G were as follows: spot numbers 1, 2, 5, 6 *E. coli* isolates plus 8 ATCC 25922 reference; 3, 4 *K. pneumoniae* isolates and 9 ATCC 13883 reference; 7 P. aeruginosa ATCC 10145; 11-15 *S. aureus* isolates plus 15 NCTC 8355 reference.

Sterility testing

After printing, replicate labware samples were be divided three parts: one set was autoclaved by wrapped with aluminium paper either individually or put together in a pot, the second set were 70% alcohol sterilised and last set unsterilised. After sterilisation, all pieces were put into 10ml LB media in a 50mL tube and incubated at 37^0C to check for contamination. Successful sterilisation was achieved when no growth of bacteria was detected, with clear liquid media indicating passing the sterility test. In this 3D printing methodology study, sterility tests were performed overnight, to confirm parts were suitable for overnight culture of rapidly growing strains. For experiments requiring longer incubation, a longer sterility test would be more appropriate, to exclude possibility of contamination with slower-growing organisms.

Direct detection pathogens from milk samples

Mastitis milk samples were collected by the University of Reading farm and transported to laboratory to perform test. These were diluted at three concentration (10^{-1} to 10^{-3}) for testing. Dip slide frames, designed with 10 round or squared shape was 72 mm length and 24 mm wide and dived two parts: one set of small chambers suitable for determining minimum inhibitory concentration (Taga and Bassler 2003) and the long part for identification of pathogens. Next, selective media was filled up into wells formed by the frame, and the dip-slides were then used by dipping directly into milk samples at the three dilution and overnight incubation at 37^0C. Dip-slides were imaged by digital photography either with ambient room light or on a USB white light tracing box (Amazon, UK).

Plating bacterial samples with loops and multi-well plate

The Baird Parker agar (BP), MacConkey's agar (MC), Selective Chromoagar for gram negative and gram positive, Mueller Hinton agar were prepared according to manufacturer instructions then poured into multi – channel rectangle dish with 11 ml of media for each row. Then, sterile loops were applied to take bacterial solution from 96 well plate to streak or drop bacteria into media, and incubated overnight at 37^0C.

Replica plating for Minimum Inhibitory Concentration (MIC) agar dilution

The protocol to perform MIC - agar dilution followed CLSI guidelines. Strains isolated from mastitis samples and reference strains were prepared at standard inoculum density in 96-well plates. Cefoxitin, Gentamycin, and Streptomycin (Sigma, UK) were prepared as stock concentration, then diluted into Mueller

Hinton agar and filled into round petri dishes. The pin replicators were used to transferring the inoculum from 96-well plates onto the surface of Mueller Hinton agar, followed by incubation at 37^0C overnight.

Acknowledgements

We would like to thank Barney Jones for providing spare mastitis milk samples from the University of Reading farm.

Conflicts of Interest

None.

Figure Legends

Figure 1: Method and workflow for rapidly prototyping custom microbiology consumables using CAD and desktop FFF 3D printing.

Figure 2: Sterilisation of 3D printed PLA labware. a) Illustrates set of parts unchanged by sterilisation with 70% ethanol. b) When bulk autoclaved in a pot, the heat-softened parts were badly distorted. c) However, if carefully positioned flat on a tray in individual foil wrappers, minimal distortion was seen and parts could be used after autoclaving in spite of softening during heating. d) Parts were either added to broth medium either without sterilisation, or after autoclaving or 70% ethanol sterilisation; after overnight incubation in broth, the culture medium remained clear only following sterilisation, indicating printed parts were not sterile directly after fabrication.

Figure 3: Customisable dip-slides for direct sample plating onto multiple agar types. A illustrates customisation options. B operation of custom dip-slides for sample plating. C Frames printed with the indicated PLA colours were illuminated from above or below, and imaged to show how natural PLA with backlight makes colonies most clearly visible. D Example of mastitis milk sample plated onto custom dip-slides.

Figure 4: Fully configurable multi-chamber plates, inoculation loops, and pin replicator allows customised plating onto custom shapes of solid media. A multi-chamber dishes for agar allow multiple samples to be separated but plated onto the same agar, poured into each connected column. B 96-well plate compatible loops were designed, printed and used to plate panels of samples onto 5 agar types in the custom plates. C growth of bacteria streaked onto 5 different solid media. D loop size can be customised allowing different volumes to be plated. E illustration of loops for plating a series of culture volumes using variable-diameter loops (top 3 rows of spots) vs micropipette spots (lower 3 rows, comprising 1, 2, 4, 6, 8 and 10 µl culture medium respectively from left to right). F Illustration of pin replicator designed for 96-well plate and round petri-dish. G Example of 15 bacterial strains replica plated on Mueller Hinton (MH) vs differing concentrations of gentamicin in conventional petri dishes, to determine MIC by agar dilution.

Figures

Figure 1

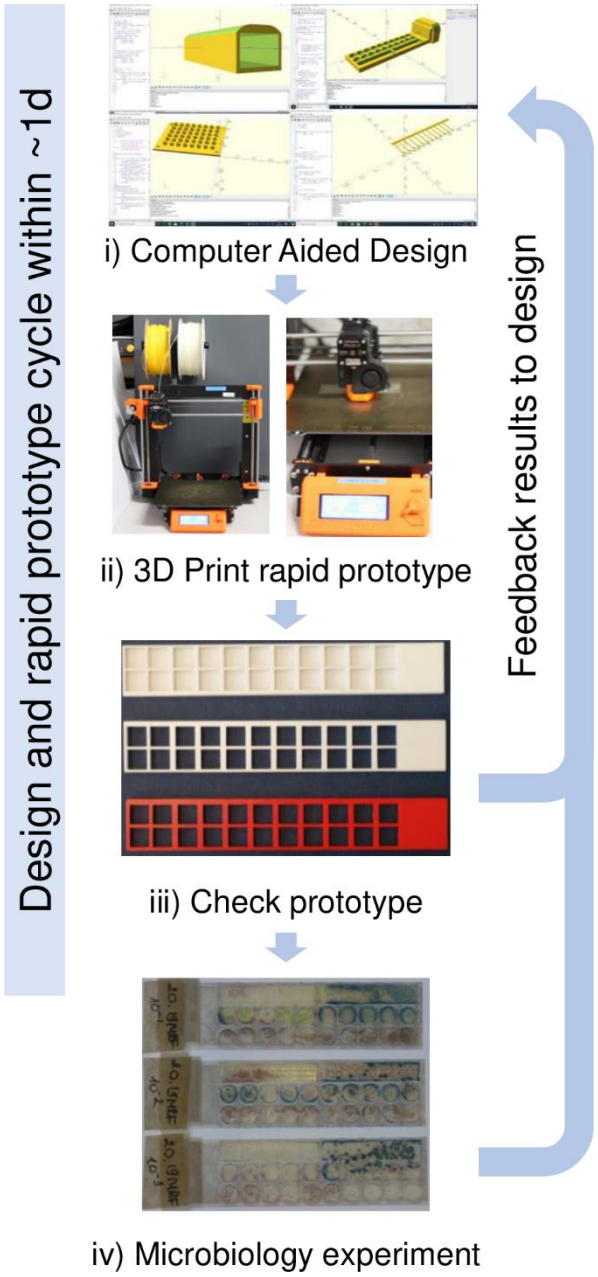
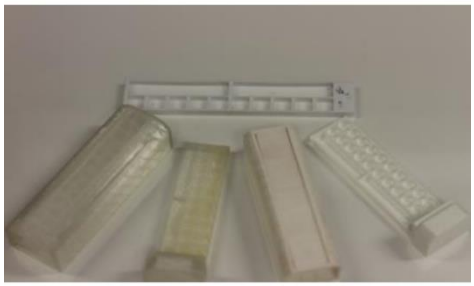


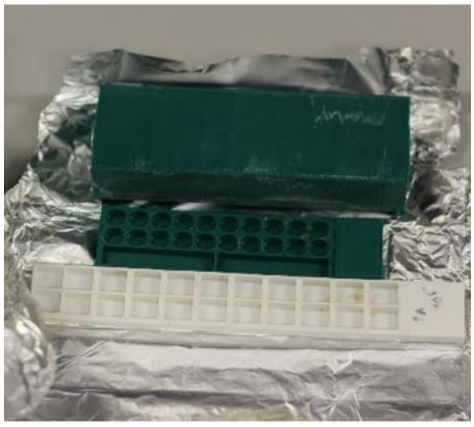
Figure 2

a) 70% Ethanol



b) 121^{0}C in pot

c) 121^{0}C individually wrapped



d)

121^{0}C Autoclaved	Ethanol 70%	No sterilisation

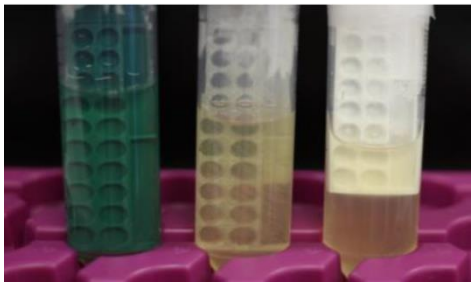


Figure 3

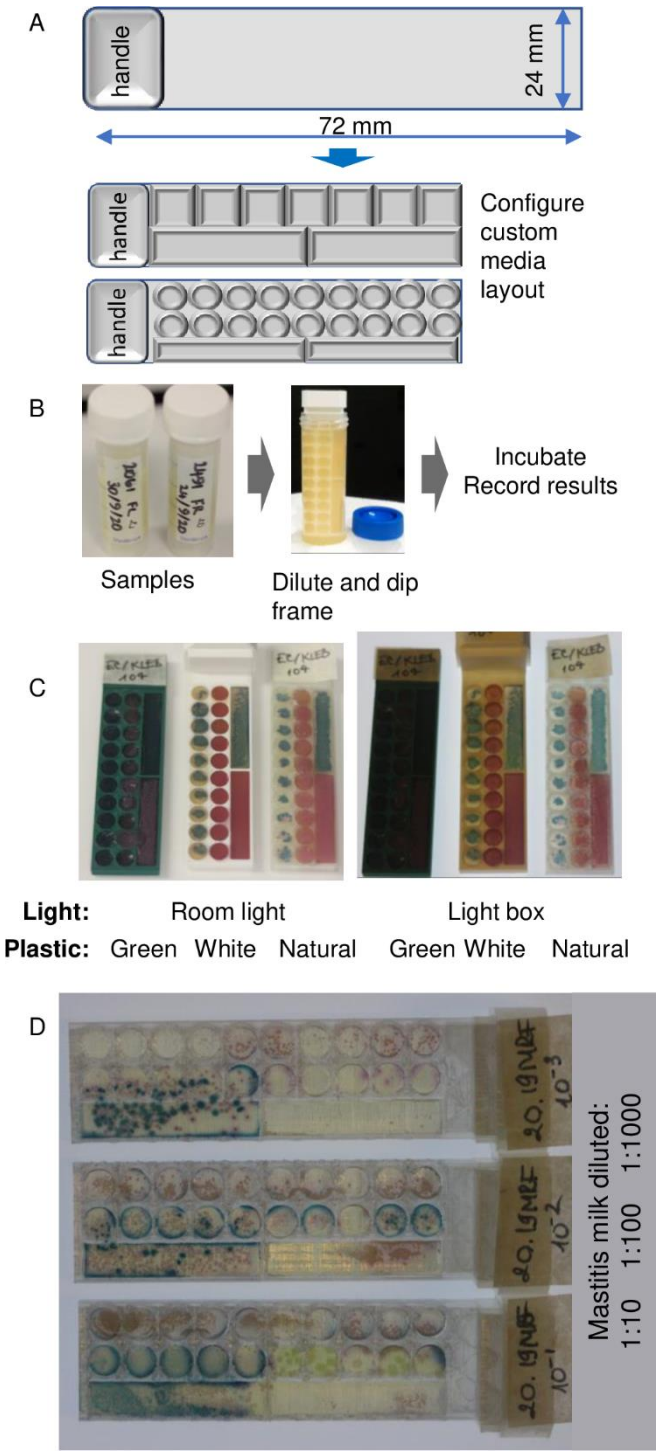
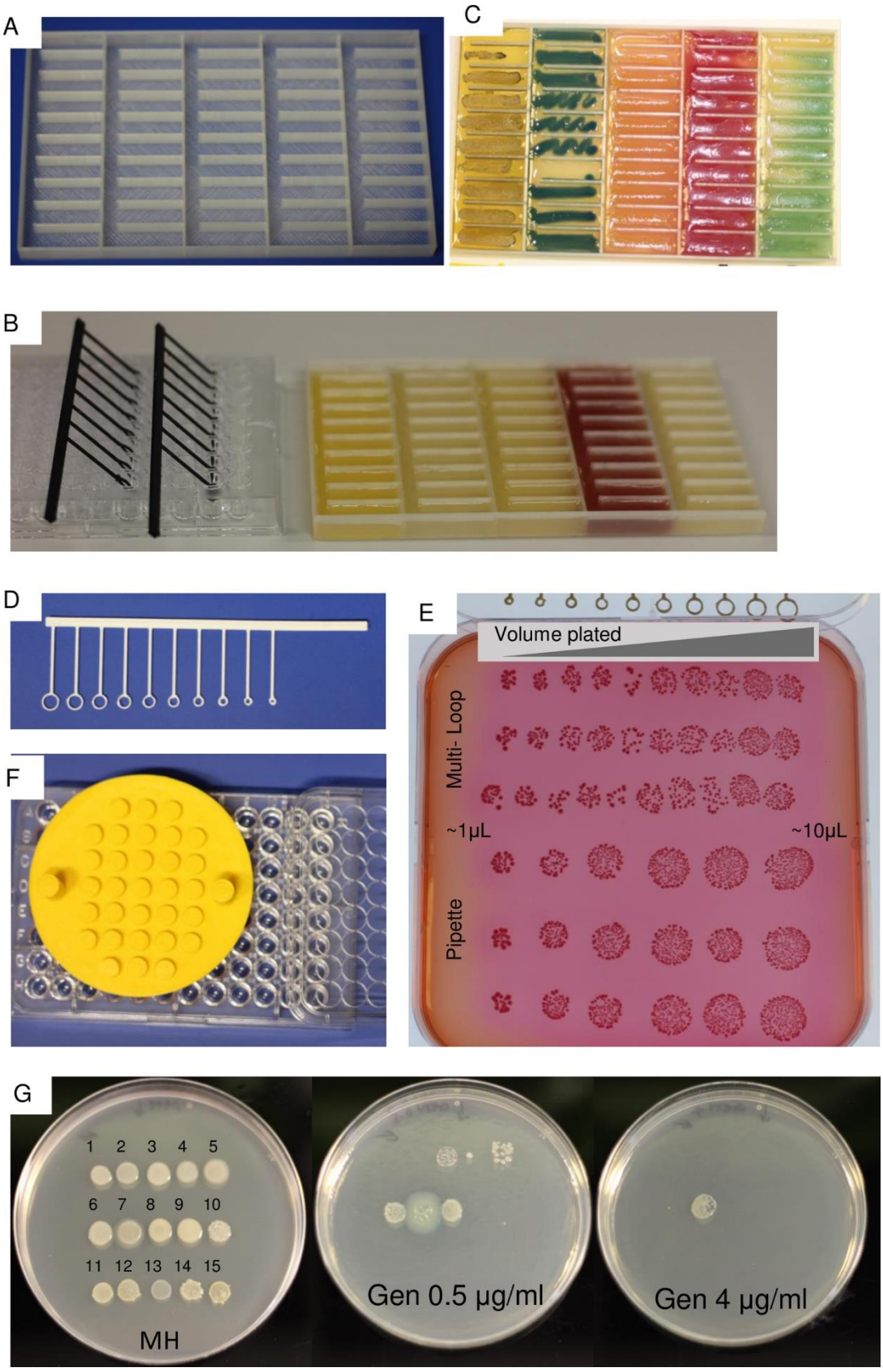

A
handle
24 mm
72 mm
handle
handle
Configure
custom
media
layout
B
Samples
Dilute and dip
frame
Incubate
Record results
C
Light:
Room light
Light box
Plastic:
Green White Natural
Green White Natural
D
Mastitis milk diluted:
1:10 1:100 1:1000

Figure 4


A
C
B
D
E
Volume plated
Multi- Loop
~1µL
~10µL
Pipette
F
G
1 2 3 4 5
6 7 8 9 10
11 12 13 14 15
MH
Gen 0.5 µg/ml
Gen 4 µg/ml

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