

Preparation of solutions for testing NHS respirators

Background

The fit of the respirators used by NHS workers in treating COVID-19 patients is tested to ensure they are properly protected. This is done by spraying a strong-tasting solution in the face of the person wearing a respirator. If they can taste the solution, the respirator does not fit properly or is otherwise faulty. Suitable solutions are sold commercially but there appears to be a shortage and some hospitals are struggling to secure the volumes needed to keep NHS staff protected. As solutions identical to the commercial solutions are easy to prepare and the materials required are cheap, the UoS have successfully prepared stocks for use in Southampton General Hospital and neighbouring NHS trusts and services. The solutions conform to British Standard BS ISO 16975-3:2017 and the US Occupational Safety and Health Administration (OSHA) in 29 CFR 1910.134 are rigorously quality checked before delivery.

In this document we have provided the QC form we used to validate our process and the procedures we used internally to ensure we produced solutions to the required specifications that were sterilised for storage and properly labelled for the end user. Please note these are not “instructions” – in undertaking this work you are to use your own expert knowledge and assess all risks and other requirements yourself. The University of Southampton does not accept any legal liability.

To carry out this work we required:

Equipment

Autoclaves capable of sterilisation under steam at 120 degrees

Low flow fume cupboards suitable for work under sterile conditions

Balances capable of measuring large masses (over 1 kg)

Balances capable of accurately measuring smaller masses (0 – 10 g)

5 L Schott bottles

High capacity measuring cylinders (1 L)

Magnetic stirrer plates

Drying ovens (75 °C)

HPLC-MS for quality control analysis

Consumables (see attached spreadsheet for sources of consumables and more details)

Bitrex (denatonium benzoate)

Sodium saccharine

NaCl

High purity water

125 mL or 60 mL plastic bottles that can be autoclaved to 120 °C

22 micron sterile filters

Waterproof labels

Ethanol

Decontamination of ALL glassware

All glassware and magnetic stirrers that have not been decontaminated previously to be washed as follows. Bottle brush (unused in chemistry) to be used to agitate.

- 1) 1 x acetone
- 2) 1 x 1 M HCl
- 3) 1 x 1 M NaOH
- 4) 3 x high purity DI water
- 5) 4 x EtOH and dry in oven @ 75 °C for 1 h.

General guidelines

There are four solutions: Bitrex (sensitivity), Bitrex (test), saccharine (sensitivity), saccharine (test). These should be prepared and bottled in batches to avoid cross over and mislabelling – **DO NOT ATTEMPT TO PREPARE MULTIPLE SOLUTIONS IN PARALLEL**. When preparing solutions, all glassware to be clearly labelled with contents and batch number BEFORE solutions are added. Each Schott bottle corresponds to a batch. Batch number, date, co-worker carrying out the transfer to be included on Schott bottle labels. QC to be carried out at all stages as specified in preparation below. Certificate to be issued specifying outcome of QC, co-workers who prepared solutions and bottled the products. Note: a given piece of glassware to be used for a same specific task when preparing the various batches. For example: a Schott bottle used for preparing “sensitivity” Bitrex solution will be used for the same exact task when preparing the next batch. All solution preparations should be performed wearing laboratory gloves sterilised with standard concentrated aqueous ethanol or equivalent.

Label glassware and MS vials (date, batch, co-worker) before beginning work. DO NOT prepare any other solution in parallel to avoid mixing of batches. Decide on scale (i.e. number of Schott bottles) before starting. 1 Schott bottle = 1 batch.

Equipment required

5 L Schott glass bottles

1 L measuring cylinder

Magnetic stirrer bars (unused for chemistry)

Stirrer plate

NaCl

Solid bitrex

Solid saccharine

22 micron sterile filters (single use)

Pasteur pipette (washed and oven dried)

MS vials

A sample of pure DI water (ca. 1 mL in MS vial) should be transferred for QC along with batch samples each day.

Example Saccharine Sensitivity Solution Preparation (~5 L scale)

Weigh 41.5 g of saccharine. Add solid to 5 L Schott bottle. Add magnetic stirrer. Add 5000 mL of DI water. Cap bottle loosely and stir until all solids dissolve (~15 min). Filter through a sterile 0.22 micron filter under reduced pressure (membrane pump, 500 mbar, 20% power) into a clear 5 L Schott bottle. Use Pasteur pipette to transfer ca. 1 mL aliquot to MS vial labelled with identity and batch number. Cap bottle firmly.

Example Saccharine Test Solution Preparation (~5 L scale)

Weigh 2490 g of saccharine. Add solid to 5 L Schott bottle. Add magnetic stirrer. Add 3000 mL of DI water. Cap bottle loosely and stir with gentle heating until all solids dissolve (~15 min). Filter through a sterile 0.22 micron filter under reduced pressure (membrane pump, 500 mbar, 20% power) into a clear 5 L Schott bottle. Use Pasteur pipette to transfer ca. 1 mL aliquot to MS vial labelled with identity and batch number. Cap bottle firmly once cool.

Example Bitrex Sensitivity Solution Preparation (~5 L scale)

Weigh 263 g of NaCl. Weigh 0.687 g of bitrex. Add solid to 5 L Schott bottle. Add magnetic stirrer. Add 5000 mL of DI water. Cap bottle loosely and stir until all solids dissolve (~15 min). Filter through a sterile 0.22 micron filter under reduced pressure (membrane pump, 500 mbar, 20% power) into a clear 5 L Schott bottle. Use Pasteur pipette to transfer ca. 1 mL aliquot to MS vial labelled with identity and batch number. Cap bottle firmly.

Example Bitrex Test Solution Preparation (~5 L scale)

Weigh 263 g of NaCl. Weigh 8.59 g of bitrex. Add solid to 5 L Schott bottle. Add magnetic stirrer. Add 5000 mL of DI water. Cap bottle loosely and stir until all solids dissolve (~15 min). Filter through a 0.22 micron filter under reduced pressure (membrane pump, 500 mbar, 20% power) into a clear 5 L Schott bottle. Use Pasteur pipette to transfer ca. 1 mL aliquot to MS vial labelled with identity and batch number. Cap bottle firmly.

Bottling and sterilisation

Bottling

125ml autoclavable bottles should first be washed using 2 washes of deionised water to remove any plastic particles. All work involves removing the lids from Schott bottles or 125ml bottles after this point should be performed in a clean MSC to avoid contamination from particulate or living materials, this includes spraying down all items that enter the MSC with 70% EtOH spray. Gloves should be worn whenever handling the solutions/ bottles. **Never working with different solutions in the same hood to avoid cross contamination, clean the hood fully with 70%EtOH between each solution.**

Aliquot the solution from the 5 L bottle into the 125 ml bottles using a clean and sterile bottle top dispenser. If this is not available sterile auto-pipettor tips can be used. Only aliquot 100 ml into each bottle to allow room for expansion during autoclaving. Once all solution has been dispensed partially tighten the screw caps of the 125 ml bottles and loosely tape with autoclave tape. Label a few bottles from the batch with the details of the solution.

Autoclaving/sterilisation

Once all bottles have been taped load into the autoclave and run on a cycle suitable for sterilising liquids, this will often be called the “fixed media” cycle but should have parameters as follows:

- Steri Temp: 121 °C
- Steri Time: 15 mins
- Dry Time: 20 mins
- Pulses: 3
- End Temp: 80 °C

Only run one solution in the autoclave at one time to avoid cross contamination.

The run will take approximately 2 hours, when complete remove the bottles and allow to cool slightly with the lids still loose. Randomly choose 5 bottles for quality control and take these to the MSC, using a 1 ml pipette and sterile tips take a 1 ml aliquot from each of the bottles and dispense each into a separate clean Eppendorf tube. Clearly label each Eppendorf with the solution and the sample number and deliver for QC testing. Once the bottles are at room temperature, tighten the caps fully while wearing sterilised nitrile gloves. Note the number of bottles in the supply chain document.

Labelling

Label the bottles using pre-printed stickers that include the type of solution, batch, bottle and QC pass/fail. Do not distribute until the samples have passed QC.

Quality Control (LC-MS)

Pre-Autoclave Sample Analysis

Create a concentration calibration curve from pre-made standards (include appropriate internal standard) in the linear dynamic range for the instrument and appropriate LC-MS analysis method. Create this on the day of sample analysis and acquire before the sample data.

Take a sample from each batch of prepared solution (SENSITIVITY and TEST) pre-autoclave (1-2 mL).

Diluted each sample using the appropriate internal standard to a concentration that is approximately in the middle of the calibration curve.

Analyse the batch sample in triplicate with solvent blank a between each sample to monitor/prevent carry over.

Calculate the concentration of the batch sample against the calibration curve and report the concentration of each analysis and standard deviation.

To pass QC, average sample concentration should be within 10% of the calculated concentration.

If QC is passed, the batch solution is then transferred to bottling and autoclave team.

Post-Autoclave Sample Analysis

Create a concentration calibration curve from pre-made standards (include appropriate internal standard) in the linear dynamic range for the instrument and appropriate LC-MS analysis method. Create this on the day of sample analysis and acquire before the sample data.

1 mL of sample solution is collected from a random selection of 10% of bottles in the batch.

Each sample is diluted using the appropriate internal standard to a concentration that sits in the middle of the appropriate calibration curve.

Analyse the samples in triplicate with blank analysis in between to prevent carry over.

Calculate the concentration of each sample against the calibration curve and report the concentration of each analysis with average and standard deviation.

To pass QC, average sample concentration should be within 10% of the calculated concentration.

If QC is passed, bottles can then be sent out for distribution.