Procedure

1. Obtaining of Samples

1.1. Sampling

Production personnel conduct all sampling in the following areas. Details of these sampling procedures are outlined in **MICLAB 095**.

- Terminally Sterilised Products

Samples are to be selected randomly from throughout the autoclave load.

- Aseptically Filled Products

Samples are to be selected from the beginning, middle and end of each batch, plus the first units filled after any prolonged downtime (greater than one hour). Samples are to be of saleable standard, not rejects.

1.2. Sample Size required for Initial Sterility Testing

Determine the sample size of the product type and batch size.

2. Setting up a Testing Session

2.1. Checking the testing requirements for each product code

Every product sampled must be documented properly in details when setting up a test session.
This will indicate what tests are required for each product code. Always cross-check the BPN on the sample container against the sample ID sheet.

2.1.1. Parametric Release

Not all terminally sterilised products require sterility testing. Check for Parametric Release status of any product code.

2.1.2. Sterility Test

These products require sterility testing. A standard sterility testing session is comprised of 4 products and a sterile control to be membrane filtered and any number of direct inoculations along with a suitable sterile control.

2.1.3. Bacterial Endotoxin

Check if a product requires Bacterial Endotoxin testing and also by which test method, either gel clot or KCA. If the product requires Bacterial Endotoxin testing, separate these samples from the sterility samples. Ensure that these samples are labelled with the Product Name & strength, batch, code and place these in the receptacle for the type of Endotoxin test required.

2.1.4. Biological Assay

If a biological assay is required separate the samples from the Sterility samples. Ensure these samples are labelled with the Product Name, batch and code and are given or communicated to the technician responsible for Assay testing.

2.2. Known Sterile Controls

The contents of the testing session including sterile controls, media and diluents used are to be recorded in the log book.

Add into the Batches form for the correct BPN if major stoppage samples have been tested for sterility.

Add into the 'Comments' section in the sterility test log book if the sterility test session was swabbed down by a technician other than the one performing the testing, also record the Steritest lot number from the Steritest, Sterility Testing device Certificate of Analysis. Equipment sterilisation details for the session should also be recorded in the comments section, including the autoclave used, the cycle number and the date of sterilisation.

After completing the sterility test session enter into the sterility test log book and any information that might impact on the test results.

3. Sterility Testing Procedures

3.1. Procedure –Always check batch number before starting test.

3.1.1. Aqueous Solutions

All aqueous injection solutions could be tested using the Steritest II canister (brand) Membrane Filtration system employing 0.45-micron filters. Products are to be sterility tested individually and each Autoclave Load is to be tested separately. However machines that run in tandem from the same Bulk Solution can be pooled.

The entire contents of the above mentioned number of containers or 20mL from each, whichever is least, is to be filtered through the sterile needle by piecing through the softest part of the container, this will vary depending on the container type.

The Steritest canisters and the filters are then washed 3 times with 100mL of Sterile Peptone Water. These washes should be individual. After the final wash the plugs are to be placed in the bottom of the individual canisters.

One canister is to be clamped and filled with 100mL of sterile Fluid Thioglycollate Medium (FTM) + 0.5% v/v Tween 80 and the other canister is to be clamped and filled with 50 mL of sterile Trypticase Soy Broth (TSB) + 0.5% v/v Tween 80. These are then sealed for incubation.

3.1.2. Non-Injectable, Non-Filterable Products

Example- 1 jelly form

Aseptically transfer 1mL from each unit into a 600mL Wheaten bottle containing 250mL of sterile Trypticase Soy Broth + 0.1% w/v Lecithin + 0.7% w/v Polysorbate 80.

Repeat dispensing from the remaining units into a second, third and fourth Wheaten bottle.

Aseptically transfer 1mL (about 3cm) from each of the units into a 600mL Wheaten bottle containing 500mL of sterile Fluid Thioglycollate Medium + 0.1% w/v Lecithin + 0.7% Polysorbate 80.

Repeat dispensing from the remaining units into a second Wheaten bottle.

Example- 2 suspension form

Aseptically insert a sterile air filter into a 600 mL bottle containing 600 mL sterile Fluid Thioglycollate Medium + 0.5% v/v Tween 80 (FTM).

Using a Liquid Transfer Kit, (e.g. Millipore TEA000010), push the "thick" needle through the rubber stopper of the bottle containing the first media type.

Push the "thin" needle through the bottom of the plastic container and transfer the whole contents of all containers into the FTM bottle.

Repeat the procedure above with a 600mL bottle containing 600mL sterile TSB+ 0.5% v/v Tween 80 (TSB).

4.2.4. **Record of Results:**

The Microbiology Manager or senior technician can record the results for the 7 day and 15 day checks, (or 14 full days), into the work book.

If either media is found to be contaminated, the following data should be recorded:

- Media involved
- Microscopic and morphological appearance of the organism/s
- Number of days of incubation
- Identity of the contaminant to at least Genus level.

4.2.5. Any contaminant/s found in an initial sterility test are to be retained in the refrigerator. The contaminants should be retained for a minium of 6 months on a TSA slope. If the test is found to be invalid one repeat test may be performed.

5. Interpretation of Results

5.1. The product meets requirements for sterility if no growth is evident in either the FTM or the TSB media after 14 full days incubation. This interpretation applies even if growth occurs in the control canisters.

5.2. If contamination has occurred in the initial sterility test, The Sterility Test Out Of Specification Result Investigation form, (**Form 680**) is to be filled out, a Deviation Report is to be raised and an Incident / investigation started, including a meeting held with all relevant parties. The **Form 680** is to be used as a minimum guide and does not limit the extent of the investigation. From the incident investigation the Microbiology Manager is to decide if the product is to be Repeat sterility tested or rejected depending on the out come of the incident investigation findings. The decision is to be based on the current compendial guidelines, Observation and Interpretation of Results.

See Appendix 1 (Flowchart of Sterility Test Results - Interpretation and Repeat Test), can be used as a guideline.

5.3. In the event of an initial sterility test failure the test may be considered invalid only when one or more of the following conditions are fulfilled:

1. The data of the microbiological monitoring of the sterility testing facility shows a fault;
2. A review of the testing procedure used during the test in question reveals a fault;
3. Microbial growth is found in the negative controls;
4. After determination of the identity of the microorganisms isolated from the test the growth of this species or these species maybe ascribed unequivocally to faults with respect to the material and/or the technique used in conducting the sterility test procedure.

5.4. If the test is found NOT to be invalid, then the product is to be rejected.

6. Repeat Testing of Sterile Products

6.1. Sample Size

If the above review justifies that a repeat sterility test can be conducted on a product, the sample size and the test method used are to be as for an initial sterility test, (Section 1).

6.2. Sampling

Samples must be collected at random by trained sampling staff. As the product requiring sampling may be at various stages of production, consultation with the Microbiology Manager will be necessary to decide the sampling plan to be conducted.

6.3. Repeat test Procedure

8.2. Documentation

8.2.1 Documentation must be completed for each product undergoing initial validation or revalidation.

8.2.2 Following the entry of test results, the completed validation form must be reviewed and authorised by Microbiology Manager, prior to the method being used in routine testing.

8.3. Test Methods

8.3.1. Test Articles

8.3.1.1. Aqueous Solutions

For aqueous solution validations the process is identical to routine product sterility testing except for the following two conditions:

1) The product volume filtered is 3 (three) times the routine test volume.

2) The final 100mL peptone water rinse must contain 10-100cfu of a viable challenge organism. The peptone water is to be inoculated immediately prior to the validation procedure. The required challenge organisms should be listed and must be used to validate each method.

8.3.1.2. Non-Injectable, Non-Filterable Products

For non-injectable, non-filterable product validations the process is identical to routine product sterility testing except for the following condition;

1) The final containers prior to incubation are inoculated with 10-100cfu of a viable challenge organism. The required challenge organisms should be listed and must be used to validate each method.

8.3.2. Control Articles

8.3.2.1. Positive controls must be performed each day validations are performed. The preparation of positive control units is identical to test units, however, no test product is filtered, (liquids) or added directly to the media, (non-filterables).

8.3.2.2. A minimum of one (1) positive control canister must be prepared for each organism used in the validation. The required organism is introduced into the test either by inoculating the final rinse (liquids), or direct inoculation (non-filterables).

8.3.2.3. Control canisters are incubated at the same time and under the same conditions as test canisters.

8.4. Incubation and Inspection

8.4.1. All test and control units are to be incubated and inspected as per the following table:

Canister Media & Organism	Incubation conditions	Inspection interval
FTM + *Staph aureus*	32±2°C	Not more than 5 days
FTM + *Ps aeruginosa*	32±2°C	Not more than 5 days
FTM + *Cl. Sporogenes*	32±2°C	Not more than 5 days
TSB + *B. subtilis*	22±2°C	Not more than 5 days
TSM + *C. albicans*	22±2°C	Not more than 5 days

