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1.0 Purpose

This SOP describes microbial sterile membrane filtration as stipulated by current United States Pharmacopeia (USP) Standards utilizing the Millipore Milliflex-100 Test System to determine the bioburden of a test sample.

2.0 Scope

This SOP applies to all Process Analytics/Quality Control (PA/QC) personnel that will be performing this procedure.

3.0 Authority and Responsibility

3.1 The Director, Technical Operations, Process Analytics/Quality Control (PA/QC) has the authority to define this procedure.

3.2 PA/QC is responsible for training laboratory personnel and documenting this training to Biopharmaceutical Quality Assurance (BQA).

3.3 PA/QC personnel are responsible for the performance of this procedure.

3.4 PA/QC is responsible for reviewing the data and documentation of the results of this procedure.

3.5 BQA is responsible for quality oversight of this procedure.

4.0 Materials

4.1 Milliflex PLUS Pump Single Head Unit

4.2 Milliflex Oasis Pump Dual Head Unit

4.3 Tubing-(inner), BDP PN 21550

4.4 Tubing-(outer), BDP PN 21505

4.5 Milliflex (0.45 µm) Filter Funnel Unit, BDP PN 21527 (White Grids) / BDP PN 22089 (Black Grids)

4.6 Milliflex Oasis (0.45 µm) Filter Funnel Unit, BDP PN 10784 (White Grids)

4.7 Sabouraud Dextrose Agar (SDA) media cassette, BDP PN 10389, 10787 or equivalent

4.8 Tryptic Soy Agar (TSA) media cassette, BDP PN 10390, 10785 or equivalent

4.9 Plate Count Agar (PCA) media cassette, BDP PN 10416

4.10 Milliflex Oasis Sanitization Kit Consumables, BDP PN 31377

4.11 Bleach, BDP PN 10579

4.12 PBS, BDP PN 30007

4.13 Sterile WFI, BDP PN 30295

4.11 70% IPA, BDP PN 30129

4.12 Cavicide, BDP PN 10168

4.13 Bleach Germicidal Disinfectant, BDP PN 10167

4.14 Sterile disposable spreaders, BDP PN 20670

4.15 Type 1 water

5.0 Procedure – Using Milliflex PLUS Pump Single Head Unit

Perform all manipulations in a unidirectional flow biosafety hood, per **SOP 19102 - *Routine Use and Disinfection of Biological Safety Cabinets, Incubators, Shakers and Centrifuges***. Observe all principles pertaining to sterile technique when performing this procedure. Ensure all samples are properly labeled prior to entry into the BSC.

NOTE: The BSC must be cleaned immediately prior to performing this assay.

5.1 Plug in and/or switch on the pump. Disinfect the pump head using a disinfectant-saturated wipe.

5.1.1 Remove the cover from the vacuum pump filter support. The display will show the main program screen, proceed to 5.1.6:

MFX100 - - -

Mil/100/100 mL

5.1.2 If pump fails to read this, select OK to go to main menu.

5.1.3 Scroll to sampling and select OK.

5.1.4 Scroll to auto sampling and select OK.

5.1.5 Scroll to MFX100 and select OK. The display will now read MFX100.

5.1.6 Place a Milliflex media cassette on the flat work surface of the biosafety hood.

5.1.7 Place the Milliflex filter onto the vacuum filter support.

NOTE: Make sure the filter membrane at the base of the unit is flush with the top of the vacuum pump filter support.

5.1.8 Remove the cover from the Milliflex filter unit. With the Milliflex filter unit placed and the cover removed, the display should read 0 mL. If the display does not read 0 mL, proceed to Step 5.1.11.

5.1.9 Pour 100 mL of sample into the funnel slowly. Once the unit detects ~95 mL, a beep will inform the operator to discontinue pouring. The pump will automatically start when the display reads 100 mL ± 0.5 mL.

NOTE: A 100 mL sample is to be filtered if adequate volume is provided. If a smaller sample is used, see step 7.0.

5.1.10 If the display does not read 0 mL, Tare the weight function. Press "ok" to display the menu. Press "ok" again to choose the TARE function. Follow the instructions on the display; remove filter and push START. The display will read "measuring…please wait…" After the tare process is complete, refer to Step 5.1.10.

5.1.11 As the pump filters, the display will show the exact amount of liquid being filtered. After filtration is complete, the pump will automatically stop, and the display will go back to the main program screen.

5.1.12 Remove the Milliflex filter from the vacuum pump. The membrane should have a convex shape as viewed from the bottom of the filter funnel.

5.1.13 Remove the white/opalescent cover from the solid cassette.

5.1.14 Place the funnel base down over the exposed medium cassette so the membrane touches the medium.

5.1.15 Press down on the top of the funnel with the palm of your hand. The funnel will shear off at the grove on the unit. Refer to 6.0, Culturing the Sample.

5.1.16 A negative control is prepared by filtering 100 mL of PBS.

5.2 Operating Pump in Manual Mode

5.2.1 Remove the cover from the vacuum pump filter support. The display will show the main program screen:

MFX100 _____

Mil/100/100 mL

NOTE: Refer to Step 5.1.12 if the pump fails to display this.

5.2.2 Press the OK button to display the menu. The display will show "TARE".

5.2.3 Scroll down until the display shows "MANUAL" and select OK. The display will show "volume OFF"; select OK again. The display now shows "MANUAL" and is now ready for manual samplings.

5.2.4 Press the START button to start filtration. During filtration, the display will show "FILTRATION".

5.2.5 When the sample is finished filtering, press the START button again and the display will show "DRY OUT".

5.2.6 When dry out is complete, the pump will automatically stop and the display will go back to "MANUAL". Refer to Step 5.1.13 thru 5.1.16 to complete preparation of the funnel unit.

NOTE: After processing each sample, the pump head can be wiped clean using wipes moistened with a disinfectant.

5.2.7 A negative control is prepared by filtering 100mL of PBS.

5.3 Sanitization of Milliflex PLUS Pump Single Head Unit

5.3.1 Make sure pump is on. The display will show the main program screen; if it does not, refer to Step 5.1.12.

5.3.2 Go to the main menu by pressing the OK button.

5.3.3 The display shows: TARE.

5.3.4 Press the down arrow until the display shows: SANITIZATION.

5.3.5 Press OK. The display shows “Put expendable press start”. Place a new Milliflex funnel onto the pump. Make sure that the funnel unit is flush with the top of the vacuum pump filter support. Press START.

5.3.6 The display shows:

FILL 100 mLS of agent

Press Start

NOTE: 250-ppm Bleach solution is prepared by adding 4.9 mL of Bleach to 1000 mL of Type 1 Water.

5.3.7 Fill the funnel with 100 mL of 250 ppm bleach and press start. If the pump detects not enough bleach, the display will show: ERROR. Press C and refill the funnel. The display will read: Sanitizing. The pump pulls through 50 mL of agent, pauses for 10 minutes, and then pulls through the remaining 50 mL. A bar will be displayed showing the progress of the sanitization.

5.3.8 After the pump has completed the sanitization, it will run a drying cycle displaying: Fill 100 mL of water, press start.

5.3.9 Fill the funnel with 100 mL of Type 1 water and press START. The display will show: Rinsing.

5.3.10 The pump pulls through all the water and runs a drying cycle. The display will show: Fill 100 mL of water and press START.

5.3.11 Fill the funnel with 100 mL of Type 1 water and press start. A total of four rinses are required.

5.3.12 After the fourth rinse has been completed, the display will show: Sanitization finished, press START.

5.3.13 Press START. Remove the funnel and discard it.

5.3.14 Press C to go back to the main program screen or press the ON/OFF button to turn the unit off.

5.3.15 Wipe off the top of the pump and place the cover back on. Unplug the unit when not in use.

6.0 Procedure – Using Milliflex Oasis Pump Dual Head Unit

Perform all manipulations in a unidirectional flow biosafety hood, per **SOP 19102 - *Routine Use and Disinfection of Biological Safety Cabinets, Incubators, Shakers and Centrifuges***. Observe all principles pertaining to sterile technique when performing this procedure. Ensure all samples are properly labeled prior to entry into the BSC.

NOTE: The BSC must be cleaned immediately prior to performing this assay.

6.1 Plug in and/or turn on the pump. Remove the cover and disinfect the pump head using a disinfectant-saturated wipe.

6.2 Place all samples, filters, cassettes, and supplies into the BSC.

6.3 Sample Filtration

6.3.1 Place the Oasis filtration funnel on the pump head, lining up one of the frosted strips with the colored background on the pump. Open the lid on the funnel

6.3.2 Pour 100mL of the sample into the funnel and flip down the lid, but do not lock it.

NOTE: A 100 mL sample is to be filtered if adequate volume is provided. If a smaller sample is used, see step 7.0.

6.3.3 Tap the touch-sensitive switch corresponding to the pump being used to start the filtration.

NOTE: Tapping the switch three times within two seconds will start filtration on both pumps simultaneously.

6.3.4 When all liquid has been filtered, tap the switch again to start the dry-out cycle.

6.3.5 Once the dry-out cycle is complete, the pump will stop and the switch will stop blinking. Firmly press down on the funnel lid to lock it into place.

6.3.6 Carefully remove the funnel from the pump and remove the clear cover from the desired media plate, being careful not to touch the agar when removing the cover.

6.3.7 Place the funnel onto the media cassette and push down to ensure full contact between the membrane and the media. Open the lid and pinch the funnel to separate it from the membrane assembly. Tilt the lid sideways to remove it and place it onto the media cassette/membrane assembly and lock it into place. The cassette is now ready for incubation as directed in Section 7.

6.3.8 Remove the membrane support that remained on the pump by twisting it and dispose of the support.

NOTE: After processing each sample, the pump head can be wiped clean using wipes moistened with a disinfectant.

6.3.9 A negative control is prepared by filtering 100mL of PBS.

6.4 Sanitization of the Oasis Pump

6.4.1 Collect the sanitization kit pump head, consumables, and 250ppm bleach solution.

NOTE: 250-ppm Bleach solution is prepared by adding 4.9 mL of Bleach to 1000 mL of Type 1 Water.

6.4.2 Remove both pump heads by turning counterclockwise. Place the sanitization kit pump head on the pump to be sanitized and secure it into place by turning it clockwise. Place the attached plug on the other pump head and lock into place by turning counterclockwise. Rotate the trigger on the bottom of the kit counterclockwise to lock it. The light on the switch will turn green to indicate a lock.

6.4.3 Take one sanitization kit consumable funnel and place on the kit. Take one of the included syringes, remove its plunger and place on the luer connector on the sanitization kit.

6.4.4 Pour 60mL of the 250ppm bleach solution into the syringe and place the plunger back in the syringe.

6.4.5 Push the plunger down until the liquid level in the syringe is 50mL.

6.4.6 Open the funnel lid and fill with the bleach solution up to the 250mL mark. Replace the funnel lid.

6.4.7 Tap the switch to start the sanitization cycle.

6.4.8 Sanitization takes about 15 minutes and will progress automatically. Once complete, the sanitization kit and consumables can be removed. Wipe the kit dry of any remaining liquid and discard the consumables. Repeat section 6.4 for the other pump head or replace both standard pump heads if finished.

NOTE: It is possible to sanitize both pump heads at once if two sanitization kits are used. The process is the same as above, just done in duplicate and without plugging the opposite side.

7.0 Culturing the Sample

7.1 If a sample has insufficient volume to filter 100mL, a smaller volume may be used. This volume should be diluted up to 50mL in the funnel using the appropriate sterile diluent (PBS or WFI). The volume of the actual sample used should be recorded on **Form 22133-01**. Results are then calculated per 7.5.1.

7.2 Plate Count Agar (PCA) is used for Water Monitoring samples only. If Plate Count Agar is not available, Tryptic Soy Agar (TSA) plates can be used for water monitoring samples.

7.3 Tryptic Soy Agar and Sabouraud Dextrose Agar are used for all other bioburden assay samples.

7.3.1 In the event that either of these media cassettes are not available for use, 100uL of the sample should be spread onto a full-size agar plate of the same media using sterile disposable spreaders and incubated as directed below. This process can be repeated if more than 100uL is to be sampled. The resulting calculation in step 6.5.1 should reflect the total number of organisms recovered from the total volume of sample spread across **all** media plates of the same type. Follow the incubation parameters in the steps below. Include a negative plate for each type of alternative agar plate used.

7.4 Incubate all bacterial bioburden cassettes (PCA and TSA) in the inverted position at 30-35□C for 48-72 hours (2-3 days). Samples may be incubated up to 120 hours (5 days) toallow colonies to develop.

7.4.1 If “extended bioburden” is indicated, cassettes should be incubated for 96-120 hours (4-5 days). If the 4-5 day window results in an assay being completed on a holiday or weekend, the assay may be read on the next working day and a comment made on the form to explain the extended incubation. The plates do not dry out and any result that was positive at day 4 or 5 will still show that positive result on day 6 or 7.

7.5 Incubate all fungal bioburden cassettes in the inverted position at 20-25□C for 48-120 hours. These plates may be read at any time during this interval to prevent any isolates from overgrowing the filter to render enumeration impossible. If no growth or only minute colonies are apparent at 48 hours, additional incubation shall be allowed for at least 120 hours (5 days) to allow fungal colonies to develop.

7.6 Colony Enumeration

After the required incubation period, count the number of bacterial or fungal colonies and record the data on **Form 22133-01**. White gridded or black gridded funnel units may be used interchangeably to enumerate any colonies isolated.

NOTE: Plates that exhibit a bacterial lawn, are substantially confluent, or have >**150** CFU’s should be recorded as TNTC on the form and entered in the results database as **151**.

7.6.1 If a volume of fluid different from the standard 100 mL was filtered, calculate the number of CFU/100 mL as follows.

$$\frac{100}{\text{Volume filtered (mL)}} \times (\text{CFU Counted}) = \text{CFU/100 mL}$$

7.7 Identification of Isolates

7.7.1 All positive bioburden samples and plates (R&D and GMP) must be retained until a determination is made by the Director of PA/QC (or designee) whether speciation or additional testing will be required.

7.7.2 Colonies obtained from GMP in-process or product release samples, final vialed R&D product samples, and validation samples must be speciated. Speciation can also be requested in other cases (such as process development samples) by the project scientist, Manufacturing, PA/QC Director, or BQA.

7.7.3 For speciation of bacterial isolates, streak each morphologically unique isolate onto a TSA plate and incubate at the original isolation incubation temperature overnight. The isolate may be sent to a vendor supplying identification services (such as Accugenix) or identified in-house.

8.0 Documentation

8.1 Record all results on **Form 22133-01**.

8.2 cGMP, GLP, and vialed final R&D product requests for bioburden testing must always have defined pass/fail criteria for all downstream process steps and product stages following culture harvest. Only in-process R&D and upstream culture samples can be accepted as FIO with a bioburden specification of “Report Results.” Any bioburden sample taken immediately following a sterile filtration step is expected to have a specification of “No Growth.” All results are reviewed by PA/QC (Technical Operations) and BQA.

8.3 The original data is archived with the QC Test Request Form in BQA per ***SOP 21402 – Document Storage and Archival Process***.

8.4 Record use, calibration, and maintenance activities in the equipment logbook.

9.0 References and Related Documents

SOP 19102 *Routine Use and Disinfection of Biological Safety Cabinets, Incubators, Shakers, and Centrifuges*

SOP 21402 *Document Storage and Archival Process*

Form 22133-01 *Bioburden Assay Data Sheet*

Current USP

Milliflex PLUS Single Head Unit Test System Operation, Sanitation, and Maintenance Instructions.
Milliflex Oasis User Guide

10.0 Change Summary

[redacted]