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1.0 Purpose

This SOP describes the testing procedures to determine the seal integrity of crimp-capped vials.

2.0 Scope

This test method defines materials, equipment, and a procedure that will detect a leak in the container closure system. The test is conducted in a vacuum desiccator large enough to hold the solution and the testing vials. The vials are placed in dye solution contained in a transparent vacuum desiccator and vacuum is applied for 30 minutes. The vacuum is released and the vials are held at atmospheric pressure for 30 minutes. The vials are then tested for the presence of dye in the vials. The testing includes both visual examination and spectrophotometric determination. The results are evaluated for meeting test specifications.

3.0 Authority and Responsibility

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

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

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

3.4 BQA is responsible for quality oversight of this procedure.

4.0 Materials and Equipment

4.1 Beckman Coulter DU800 Spectrophotometer, MEF 80400 or BDP approved equivalent.

4.2 Quartz Cuvettes

4.3 Balance.

4.4 Laboratory glassware (volumetric apparatus, beakers, etc.).

4.5 Any device or design that can keep the vials submerged in the solution.

4.6 Water for Injection (WFI) BDP PN 30295 or BDP approved equivalent

4.7 Ultrapure water.

4.8 Timer.

4.9 Disposable glass tubes, BDP PN 20144 or BDP PN 20143 or BDP approved equivalent.

4.10 Suitable supplies or equipment for the preparation and mixing of dye solution.

4.11 Alcohol wipes, BDP PN 21492 or BDP approved equivalent.

4.12 Kimwipes, 4.5 x 8.5, BDP PN 20091 or BDP approved equivalent.

4.13 Paper Towels, BDP PN 20341 or BDP approved equivalent.

4.14 DECON-AHOL, 70% Isopropyl Alcohol, BDP PN 30129 or BDP approved equivalent.

4.15 Testing vials.

4.16 Vacuum Desiccator.

4.17 Vacuum source or pump.

4.18 Vacuum gauge BDP MEF 72130 or BDP approved equivalent.

4.19 Three-way stopcock.

4.20 Methylene Blue (MB), BDP PN 30251.

4.21 Calibrated Pipettor (1 mL), Pipet tips, 1-1000 µL, BDP PN 20769.

4.22 Syringe with needle, BDP PN 21720 or BDP approved equivalent.

4.23 Gloves, Nitrile BDP PN: 20764, 20765, 20766, 20767 or BDP approved equivalent.

5.0 Procedures

5.1 Solutions Preparation

5.1.1 Dye Solution

5.1.1.1 Prepare MB Stock Solution by dissolving Methylene Blue in ultrapure water (1.5 g ± 0.1 g in 1 liter or equivalent ratio). Label this solution as MB Stock Solution (~1,500 µg/mL). Record the solution in the Solutions Logbook as per ***SOP 22702 - Solutions Used in Process Analytics*.**

Four dilutions are prepared from the MB Stock Solution. Label each appropriately. Dilutions of MB Stock Solution must be prepared daily. Different volumes of each dilution can be prepared as long as the ratios remain the same.

5.1.1.2 MB Dilution #1 = 1:100 dilution (~15 µg/mL)

Dilute 1 mL of MB Stock Solution to 100 mL with ultrapure water.

5.1.1.3 MB Dilution #2 = 1:10 dilution (~1,500 ng/mL)

Dilute 1 mL of MB Dilution #1 to 10 mL with WFI.

5.1.1.4 MB Dilution #3 = 1:10 dilution (~150 ng/mL)

Dilute 1 mL of MB Dilution #2 to 10 mL with WFI.

5.1.1.5 MB Dilution #4 = 1:10 dilution (~15 ng/mL)

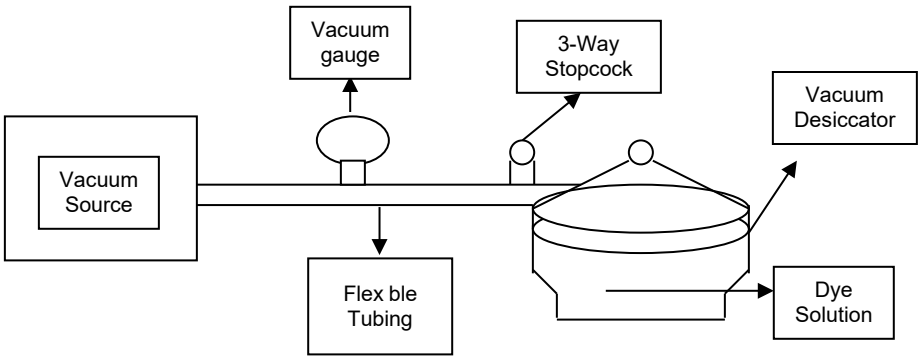
Dilute 1 mL of MB Dilution #3 to 10 mL with WFI.

5.1.2 Blank Solutions

5.1.2.1 Blank solutions are prepared using the contents of vials that have not been exposed to Methylene Blue dye ingress testing. Pull at least 2 vials or a quantity that will provide twice (2x) the volume needed to blank (zero) the spectrophotometer. Samples containing less than 0.4 mL will need to be pooled to ensure accurate analysis. Label the pulled vials as blanks. Blank solution will be used immediately prior to sample analysis in Section 5.4.3.

5.2 Apparatus Setup

5.2.1 Set up the test apparatus as shown in the following diagram.



5.2.2 Make sure the vacuum source is able to provide at least 25” Mercury vacuum.

5.3 Testing

5.3.1 Fill the vacuum container with a sufficient volume of MB Stock Solution to cover the vials. A suitably sized beaker or open vessel can be placed inside the container to reduce the volume of dye solution required to fully cover the vials.

5.3.2 Allow frozen vials to thaw fully prior to testing. Verify that blank vials have been pulled per Section 5.1.2. Place vials to be tested in the dye solution. If needed, add more dye solution to the container. Vials must be fully submerged in the dye solution. It may be necessary to keep the vials together by using a rubber band, tape or other means to prevent the vials from floating. Any other technique may be used to accomplish the same result. When using any technique, care must be exercised that the area around the cap or the neck of the vial is kept clear (not blocked or wrapped) to allow the dye solution to flow freely into gaps and potential leak points.

5.3.3 Vacuum is applied to the container to reduce the external pressure. After the desired vacuum (12-15 inches Hg) is reached, the container is maintained at these conditions for 30 minutes. Visually monitor the vacuum during this period.

5.3.4 After 30 minutes, release the vacuum gently and bring the container to atmospheric pressure. Keep the vial samples in dye solution for an additional 30 minutes at atmospheric pressure.

5.3.5 Open the vacuum container and remove the vials. Rinse the outside of each vial with Ultrapure water. Collect the blue rinse water and dispose of as hazardous waste. Remove the Flip-Off seal from each vial and continue to rinse the vials until visually free of methylene blue. Spray the vials with DECON-AHOL and wipe clean with alcohol wipes or Kimwipes. Repeat the cleaning process as needed to remove all visible methylene blue from the top of each vial. Allow any residual DECON-AHOL to dry prior to performing analysis.

5.4 Analysis

5.4.1 Physical: Perform visual examination for any coloration of the solution inside the vials. If any blue coloration is present in any of the vials run those at the end of the sample set to avoid contamination of the remaining samples. Record visual results on Attachment I.

5.4.2 Spectrophotometer Setup and System Suitability

5.4.2.1 Make sure the spectrophotometer is on and warmed. Refer to ***SOP 22941 - Operation of the Beckman Coulter 800 DU Spectrophotometer*****,** for operation of the instrument.

5.4.2.2 Set the wavelength to 664 nm. Perform the analysis using appropriate cuvettes according to sample size.

5.4.2.3 Fill the cuvettes with WFI and blank (autozero) the instrument. Read the absorbance of WFI three times (each time with fresh WFI).

Calculate the average (mean) and standard deviation. The division step in the standard deviation calculation uses (n-1), where n is the total number of readings (3). If using Microsoft Excel, the

equivalent standard deviation function is 'STDEV'. The specification calculation is:

(3 x Standard Deviation) + Average
Example:

Average (Mean):

$$\frac{(\text{WFI \#1}) + (\text{WFI \#2}) + (\text{WFI \#3})}{3}$$

Standard Deviation:

$$\sqrt{\left(\frac{(\text{Mean} - \text{WFI \#1})^2 + (\text{Mean} - \text{WFI \#2})^2 + (\text{Mean} - \text{WFI \#3})^2}{2}\right)}$$

Specification Calculation:

(3 x Standard Deviation) + Average

5.4.2.4 Read the absorbance of MB Dilution #4.

Specification: The absorbance value of MB Dilution #4 must be greater than 3 times the standard deviation + the average of the 3 WFI readings. If the absorbance value does not meet the specification prepare a new set of dilutions per section 5.1.1. If the new set of dilutions does not result in meeting the specification prepare new MB Stock Solution and perform the dilution steps. If new dilutions and new MB Stock Solution does not result in meeting the specification notify the area supervisor and request maintenance on the spectrophotometer – Do Not Proceed with sample analysis. Placard the spectrophotometer as Out of Service.

Save the file to the Scientific Data/Du 800 data network folder and print a copy.

5.4.2.5 Proceed to Section 5.4.3 if the specification in Section 5.4.2.4 is met.

5.4.3 Sample Analysis

Remove the Flip-Off seal from the blank vial(s). Transfer the vial contents to a disposable glass tube or directly into the cuvette. Blanks and samples can be transferred by syringe, keeping the crimp and stopper in place.

NOTE: Avoid creating air bubbles when transferring the blank and test samples to the cuvette. Monitor the absorbance after blanking (step 5.4.3.1) to ensure that the zeroed reading is stable, i.e., not fluctuating beyond the 4th decimal place. If excessive fluctuation is observed remove the cuvette and inspect for air bubbles. Tap the bottom of the cuvette gently to free any trapped air bubbles and re-blank the spectrophotometer.

5.4.3.1 Fill the cuvette with Blank solution (contents of vials from Section 5.1.2 - not subjected to vacuum test). Blank (autozero) the instrument.

5.4.3.2 Read the absorbance of the contents of the sample vials from Section 5.4.1 after performing the visual examination.

6.0 Specifications

6.1 The absorbance of each sample must be less than or equal to the absorbance of MB Dilution #4.

7.0 Recording and Reporting

7.1 The results including the system setup, suitability and sample analysis are recorded on Form 22105-01. Attach spectrophotometric reading printouts and completed Form 22105-01 to the QC test request.

7.2 Report any failures immediately by notifying the Supervisor or designee of the out of specification results in accordance with SOP 22004 - Managing Out-of-Specification Test Results or Unexpected Test Results.

8.0 References

SOP 22941 *Operation of the Beckman Coulter 800 DU Spectrophotometer.*

SOP 22702 *Solutions Used in Process Analytics*

SOP 22004 *Managing Out-of-Specification Test Results or Unexpected Test Results.*

[redacted]