



Validation Procedure for ProtoCOL

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Validation Procedure

Requirements

ProtoCOL System

Pour Plate, Two Sector Spiral Plate, Whole Frame Spiral Plate and Zone Measurement Plate

ProtoCOL User Manual

Familiarity with ProtoCOL software

Pour Plate Validation

1. Create a New Study, enter a Study Name and click OK. In the resulting Create New Batch dialogue box enter a Name, select Pour Plate for the Measurement Type and select the '[Pour Plate Validation]' configuration from the Based on drop down list. Click OK.
2. Remove all dust from the Pour Plate Validation Plate; then mount it in the ProtoCOL in place of the dish holder.
3. If the entire plate cannot be seen use the Zoom In or Out icons.
4. Ensure that the 'colonies' are wholly within the frame boundary.
5. For the Zone Appearance choose the icon showing black colonies on a white background.
6. Set an exposure time of approximately 46ms for the SR camera or 70ms for the HR camera and move the Sensitivity Slider to approximately 129.
7. Count the plate by pressing the spacebar. Spatial calibration of the system is not required.
8. If the count per frame is 100 the system is working correctly.

Two Sector Spiral Plate Validation

1. Create a New Study, enter a Study Name and click OK. In the resulting Create New Batch dialogue box enter a Name, select Spiral Plate for the Measurement Type and select the '[Two Sector Validation]' configuration from the Based on drop down list. Click OK.
2. Remove all dust from the Two Sector Spiral Validation Plate; then mount it in the ProtoCOL in place of the dish holder.
3. If the entire plate cannot be seen use the Zoom In or Out icons.
4. Move the spiral frame to ensure that the 'colonies' do not touch any of the measuring grid lines and that all the 'colonies' are within the top and bottom sectors.
5. For the Zone Appearance choose the icon showing black colonies on a white background.
6. Set an exposure time of approximately 33ms and move the Sensitivity Slider to approximately 129.
7. Count the plate by pressing the spacebar. Spatial calibration of the system is not required.
8. If the count per frame is 48 the system is working correctly.

Whole Frame Spiral Plate Validation

1. Create a New Study, enter a Study Name and click OK. In the resulting Create New Batch dialogue box enter a Name, select Spiral Plate for the Measurement Type and select the '[Whole Frame Validation]' configuration from the Based on drop down list. Click OK.
2. Remove all dust from the Whole Frame Spiral Validation Plate; then mount it in the ProtoCOL in place of the dish holder.
3. If the entire plate cannot be seen use the Zoom In or Out icons.
4. Move the spiral frame to ensure that the 'colonies' do not touch any of the measuring grid lines.
5. For the Zone Appearance choose the icon showing black colonies on a white background.
6. Set an exposure time of approximately 33ms and move the Sensitivity Slider to approximately 129.
7. Count the plate by pressing the spacebar. Spatial calibration of the system is not required.
8. If the count per frame is 180 the system is working correctly.

Zone Measurement Plate Validation

1. Create a New Study enter a Study Name and click OK. In the resulting Create New Batch dialogue box enter a Name, select Inhibition Zones for the Measurement Type and choose Multiple zones read together. Click OK. In the resulting Inhibition Zones dialogue box enter 6 columns and 1 row for the Grid Size. Click OK.
2. Remove all dust from the Zone Measurement Validation Plate; then mount it in the ProtoCOL in place of the dish holder.
3. If the entire plate cannot be seen use the Zoom In or Out icons.
4. Ensure that all the zones are wholly within the six defined zone regions.
5. For the Zone Appearance choose the icon showing black colonies on a white background.
6. Set an exposure time of approximately 110ms and move the Sensitivity Slider to approximately 129.
7. Perform a spatial calibration by clicking on the Calibrate the Study icon, drawing a line (this line will represent the diameter of the circle) from one edge of the black circle to the other ensuring the 'line' passes through the central black dot. Enter "86" in the Distance box and "mm" in the Units box.
8. Measure the zones by pressing the spacebar.
9. If the measured zone diameters are within 0.5mm of the known measurement of 11mm, 13mm, 15mm, 17mm, 19mm and 21mm, respectively (smallest to largest) the system is working correctly.