Microfilaria Isolation on Nucleopore Filters

PRINCIPLE

The Nucleopore filter method is gaining in popularity because large volumes of blood can be examined (many mls) and therefore it can detect the presence of microfilaria down to less than one organism per ml.

SPECIMEN

Blood sample in a yellow or purple top tube

SAFETY

Make sure that the filter unit is assembled correctly and never force fluid through the filter unit at a high pressure-- this is an invitation to spraying the sample.

PROCEDURE

This is considered to be a non-routine procedure therefore it should only be performed by experienced personnel.

1. Place a nucelopore filter membrane (25 mm, 0.3 μm) in a Swinex filter being careful not to damage the membrane. (The membrane is pale white, the wax paper between is pale blue.)

2. Assemble the filter unit and tighten until snug.

3. Place a 12 ml syringe, a glass slide, a beaker of water and a beaker for waste material in a sterile cabinet.

4. Draw 1 ml of blood into the syringe, followed by 10 ml of water.

5. Swirl the contents of the syringe for 30 seconds to lyse the red cells.

6. Connect the syringe to the filter unit and gently press the contents of the syringe through the filter. Refill the syringe with 10 ml of water and gently push that through the filter. If the filter alone is being examined for microfilaria, then try to push about 2 ml of air through the filter. If the microfilaria will be examined after release from the filter, omit the air step since it "sets" the microfilaria onto the filter and makes

dislodging them difficult.

7. Disconnect the water filter from the syringe, and open the filter unit.
   If the filter alone is being examined, then remove the membrane from the unit and place it on a glass slide. A "wet mount" examination of the filter can be made at this time. Place a generous drop of 100% methanol on the filter, wait 30 seconds and then let the methanol run off the slide. Stain the filter with Giemsa (30 minutes/5% stain works well), gently rinse with tap water and examine. Finer microfilaria require higher magnification and slide can be mounted to permit oil immersion.

   If the microfilaria are to be removed from the filter, then the filter can be agitated with a minimal amount of water to release the microfilaria. The liquid sample can then be transferred to a slide to check for the presence and species of microfilaria. Not all of the microfilaria release with this method so it is important to examine the filter (as above) to determine if microfilaria are present.

8. Properly dispose of the liquid waste and the syringe and wash the filter unit.

QUALITY CONTROL

- White cell debris should be present on the filter membrane. The integrity of the filter unit can be determined at the point in the assay where air is forced into the filter unit--if air passes through the membrane easily then the procedure needs to be repeated with a new filter unit.
- Ensure that the microscope has been calibrated in the last year and that the results of the calibration are displayed on the microscope base.

REPORT

Any microfilaria or other parasites seen. Speciation requires haematoxylin staining of the observed microfilaria (see **Hematoxylin Stain for Microfilaria**).

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REFERENCES

Denis, D.T. and Kean, B.H. *J. Parasitol*. **57:** 1146 1971