Tissue Digestion for Ferruginous Bodies for Light Microscopy

Margaret Gondo, Baylor College of Medicine

Asbestos fibers are commonly called ferruginous bodies due to the iron content of the fibers. After they are inhaled, the asbestos fibers become coated with glycoprotein and hemosiderin, deposited by macrophages.

This procedure may be used on either formalin fixed, paraffin embedded blocks or formalin fixed wet tissue. Because this procedure will result in complete destruction of the tissue sample used, it is very important to obtain written approval documenting this fact from the individual requesting this procedure, if the procedure is being done for someone else. The tissue should be from the lung parenchyma. Also, it is a good idea to use sterile, disposable tubes throughout this procedure to limit the possibility of contamination.

Deparaffinizing a Paraffin Block of Tissue:

Before a paraffin block of tissue can be digested, the paraffin must be removed and the tissue sample rehydrated through a graded alcohol series. This procedure is merely the reverse of processing the tissue into paraffin. (Carson 1997). Heat, at or below the temperature required for embedding tissue into paraffin, will increase diffusion, thus speeding up the entire process. There are points throughout the procedure where the deparaffinization act can be delayed.

1) Place the block on an embedder and melt down the paraffin surrounding the tissue. During this time, place 40 to 50 mL of fresh xylene in a 50 mL sterile centrifuge tube. Label the tube with a solvent resistant marker. (The tube of xylene may be warmed in either a 56° C oven or the warming section of the embedder.)

2) After the paraffin has melted from the tissue, place the tissue in the warmed tube of xylene for 2 hours. The tissue may remain in the xylene at room temperature either overnight or over the weekend. The purpose of the xylene is to remove all of the paraffin from the tissue, so that it won't clog the filter during the filtration process.

3) Change the xylene with the same amount of warmed, fresh xylene. Incubate for another 2 hours.

4) Carefully remove the xylene.

5) 2 changes of 100% ethanol, 15 minutes each change. (Again, if a little heat, less than 60° C is used, the process goes faster).

6) 2 changes of 95% ethanol 15 minutes each change.

7) 1 change 80% ethanol for 15 minutes.

8) Begin removing 5 mL of fluid from the tube and replacing it with 5 mL of double distilled water. Agitate tube. Allow tube to sit for 15 minutes. If tissue sinks to the bottom, remove 5 more mL and add another 5 mL of double distilled water. If tissue floats to the top, allow tissue to sit for 15 minutes. The tissue will sink to the bottom when all of the alcohol has been replaced with water.

Tissue digestion:

1) Fold 4 or 5 pieces of clean filter paper in half.

2) Place either the hydrated tissue from the paraffin block or formalin-fixed wet tissue on one side of the folded filter paper.

3) Place the opposite side of the filter paper on top of the tissue and press all of the liquid out of the specimen.

4) Obtain and record the weight of this specimen. This is the blotted wet weight of the sample and will be used to calculate the number of ferruginous bodies later on.

5) Mince the tissue with a surgical blade into approximately 1 mm pieces. Smaller pieces of tissue will result in a faster digestion time.

6) Place minced tissue into a new, sterile, 50 mL centrifuge tube.

7) Using a 0.2 micron size Acrodisc filter (Cat# 28142-340, VWR Scientific West Chester. PA 19380) or equivalent and a 6O mL syringe, carefully filter 30 mL of bleach (5.25% sodium hypochlorite) in with the sample. (I like using a 10 mL syringe because it is easier to 'push' fluid through a filter with a 10 mL syringe versus a 60 mL syringe. Using aseptic technique, the same syringe can be reused for one procedure since the filter will eliminate potential contaminants.)

8) Allow tissue to sit in bleach for approximately 1 hour. The bleach will digest the tissue. The solution is ready for filtration when all of the tissue has disappeared.

Filtering:

The filtering of the dissolved tissue is accomplished by using the setup listed below, or their equivalents:

Analytical Filter Holder 25 mm (cat# XX1002540, Millipore Corporation, Bedford MA. 01730-2271),

2 filter flasks (cat# XX10-047-05, Millipore Corporation, Bedford, MA 01730-2271)

Silicone tubing (cat# XX71-000-12, Millipore Corporation, Bedford. MA 01730-2271)

Vacuum Pressure Pump (cat# 54905-202, VWR Scientific, West Chester. PA 19380).

0.45 µm gridded filter (cat# 28148-675, VWR Scientific. West Chester, PA 19380)

Filtration Procedure:

1) Place the filter gridded side up in the filter assembly.

2) Fill one 50 mL centrifuge tube with double distilled water, fill another 50 mL centrifuge tube with double distilled water and add a few milliliters of ionic detergent. Larger volumes may be used.

3) *Warm tubes in a microwave (approximately 30 seconds). Temperature* of the tubes should be below 70° C.

4) Place a few milliliters of the tissue digest solution into the analytical filter apparatus.

5) Allow this to pass through the filter.

6) Flush the filter by adding a few milliliters of warm double distilled water.

7) Follow by adding a few milliliters of the soapy distilled water.

8) Follow by flushing with a few more milliliters of plain distilled water.

9) If these solutions pass through the filter, add more tissue digest solution. If the solutions do not pass through the filter, add more soapy water followed by distilled water. If the solutions still will not pass, remove the solution which will not pass with a clean transfer pipette, place this solution in a sterile 50 mL tube, and change filters.

10) Continue this process until all of the tissue digest solution has been passed through the filter.

11) Place approximately 25 mL of warm double distilled water into the tube which held the tissue digest solution, cap the tube, and shake vigorously. (Fibers will adhere to the side of the tube. This removes the fibers).

12) Repeat step 11 three times.

13) Flush some more warm double distilled water through the filter.

Removing Filter:

After the filtering the tissue digest solution, the filter must be dried and subsequently mounted onto a glass slide. The filter is somewhat delicate when wet. It is very brittle and delicate when dried. Care must be taken not to either tear the filter, or accidentally remove the fibers during transfer. The filter assembly clamps the filter, thereby leaving a ring around the area which is actually used to collect the filtrate. Unless the sample was extremely small, it should be apparent where the tissue digest solution was passed.

1) Remove filter from the filter assembly. Handle the filter so that the gridded side is always facing up.

2) Place the filter in a sterile petri dish.

3) Tape the edges of the filter down to the petri dish with Scotch tape, being careful not to tape over the area where the tissue digestion solution was passed.

4) The filter can be dried for a few days at room temperature or dried for an hour In a 56° C oven. (60° C will warp the petri dish causing the filter to curl).

5) When the filter is completely dry, it may be cut out of the tape with a surgical blade.

6) Place approximately 500 mL of toluene based mounting media in the center of a clean microscope slide.

7) Place the filter grid side up onto the mounting media.

8) Place a few drops of mounting media on top of the filter and a few drops of mounting media on either side of the filter.

9) Carefully place a coverslip onto the filter. Once it is placed onto the filter the coverslip cannot be removed as it will remove fibers.

10) Any air bubbles may be removed after the outer edges of the mounting media has dried. Then the air bubbles may be 'pushed' off of the filter. It does not matter if there are air bubbles under the coverslip. The important thing is not to have any air bubbles on the filter as they will make counting fibers difficult. ■

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King, J.C. and S.W. Wong. 1996. Autopsy Evaluation of Asbestos Exposure: Retrospective Study of 135 Cases with Quantitation of Ferruginous Bodies in Digested Lung Tissue. South. Med. J. 89(4):380-385.

Rogers. A.J. 1984. Determination of Mineral Fibre in Human Lung Tissue by Light Microscopy and Transmission Electron Microscopy. Ann. occup. Hyg. 28(1):1-12.

Roggli, V.L., S.D. Greenburg, L.H. Seitzman, M.H. McGavran, G .A. Hurst, C.G. Spivey, K.G. Nelson, L.R. Hieger. 1980. Pulmonary Fibrosis, Carcinoma, and Ferruginous Body Counts in Amosite Asbestos Workers. Am. J. Clin. Pathol. 73(4):496-503.

Roggli, V.L., M.H. McGavran, J. Subach, H.D. Sybers, S.D. Greenberg. 1982. Pulmonary Asbestos Body Counts and Electron Probe Analysis of Asbestos Body Cores in Patients with Mesothelioma. Cancer 50:2423-2432.

While I established this protocol myself, I did have assistance from Dr. Joiner Cartwright, Jr. - and the work was done in Dr. Phil Cagle's laboratory

Margaret Gondo. .

Photoshop Distilled II

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Units).

- Enter the page **Height.**
- Enter **Resolution** in pixels/inch.
- Select **Mode** (*e.g.*, grayscale).
- Set the **Contents** (background) to white.
- Click OK.

2. Now **File → Open** all the images you want to combine in the plate.

3. **Crop** and **Adjust Levels** if necessary, then size it to fit in your figure with **Image → Image Size...**

- In 3.0 make sure Constrain: Proportions is checked and File Size is not. Enter Width and Height values.
- In 4.0 make sure Constrain Proportions box is checked and Resample Image is not.
- You may choose to resample (change the resolution) at this time. Remember that resampling up to a higher resolution means that pixels will be invented by Photoshop! Resampling to a lower resolution means losing pixels, but at least data is not invented. In either case, further image adjustments, such as gamma and sharpening, may be needed.

4. Set up your Grids and Guides:

- Go to **Window → Show Grid** and set up the number of lines and divisions.
- Go to **File → Preferences → Guides and Grids** and select a line type and color for guides
- Guides are made by depressing the mouse button and dragging from a ruler line to the position on the grid you want the guide line(s). Make a left vertical guide and a top horizontal guide.

5. Go to the image you want on the top left of the plate. **Select → All** then click on the **Move Tool** and drag and drop the image to the new file. The image will have become a new Layer and can be moved with the Move cursor and/or keyboard arrows as long as that layer is selected in the **Window → Palettes → Show Layers** palette. Place the image against the upper left guide of the blank white sheet. When you are near the guide line, it will "snap" to it.

6. Position more guides as needed to define the gutters between the individual images. Continue to select and move images to the new plate and position them as desired.

7. In 4.0 each part of the plate is still a separate layer, and each may be operated on individually by selecting that layer. To operate on the plate as a whole, the layers all need to be **Merged,** or the image needs to be **Flattened,** as in method I.

Method III: Select and Fill gutters.

- Use **Rectangular Selection Tool** to select and define an area you want to be a gutter, then **Edit → Fill** with white. This is useful to border inserts.

Method IV: The eyeball method. This method tends to give less precise results unless you really work on it, but allows for more freedom of placement of images.

- Drag and drop images and eyeball their positions. Don't worry about adding gutters; just space them as well as you can. Keep images as individual layers for greatest flexibility. ■

Royal Microscopical Society
1998 Programme of Forthcoming Meetings and Courses

24 Feb	National Cell Sorting Workshop
27 Feb	Microscopy of Interfaces - Joint EMAG/RMS Meeting
4 March	Annual Immunocytochemistry Meeting
6/8 April	Microscopy of Composite Materials - in Materials
14/18 April	Focus on Multidimensional Microscopy
20/24 April	Spring School in Electron Microscopy
June	Food Microstructure
June	Immunophenotyping Meeting
7/9 July	MICRO98 - Microscopy Conference and Exhibition
20/24 July	Light Microscopy Summer School, Principles & Applications
27/31 July	Light Microscopy Summer School, Confocal & Stereology
28/30 July	Bench-top Flow Cytometry Workshop
September	Lectins Workshop
September	Immunocytochemistry Course
September	Flow Cytometry Course
November	Ultrastructural Immunocytochemistry Course

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Tel.: +44 (0) 1865 248768
Fax: +44 (0) 1865 791237
eMail: meetings@rms.org.uk