



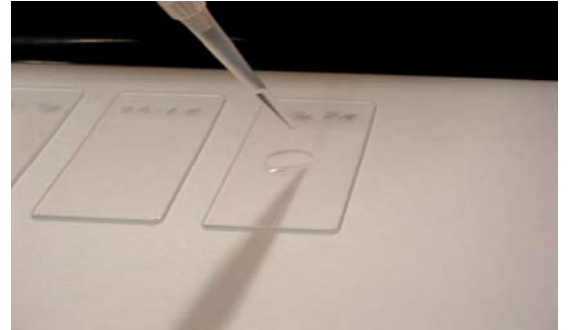
Liqui-PREP™

The Next Generation of Liquid Cytology

Technical Tips

Number: 00017

Date: 11/21/06



SUBJECT: Sputum and Bronchial Washing cytology preparations with **Liqui-PREP™**

TECHNICAL TIP OVERVIEW:

The Sputum specimen is considered to be a moderate volume specimen, which is usually extremely cellular. These specimens are also the most difficult specimen to process because of the high concentration of mucous. The challenge is to take the cells from the mucous with out major cellular damage. Once this is accomplished, making good diagnostic slides are simple. Bronchial washings are easier to process because the mucous is usually not as heavy as with a good induced sputum.

Specimen Transport:

SPUTUM:

To avoid bacterial over growth, reduce cellular degradation and begin mucous digestion the specimen should be collect and **Liqui-PREP™ Lytic Reagent** should be added to the specimen. Lytic Reagent should be added in a 1:20 ratio, i.e. 10 ml sputum to 100 ml of Lytic Reagent. These Sputum specimens should be delivered to the laboratory as soon as possible. Upon receipt in the laboratory, processing should be performed as soon as possible.

BRONCHIAL WASHINGS:

These specimens are collected in a clinic situation and therefore are generally delivered to the laboratory immediately after collection. Upon delivery to the laboratory, the specimen should be immediately poured into a container (50 ml centrifuge tube) and the **Liqui-PREP™ Lytic Reagent** can be used to wash the collection tube out into the container. The final Lytic Reagent to specimen should be approximately 1: 2. The specimen should be held with the Lytic Reagent for at least 2 hours before processing.

Specimen Preservation:

BRONCHIAL WASHINGS:

After the Bronchial Washing is poured and washed into the 50 ml centrifuge tube, the specimen is centrifuged at 1,000g for 10 minutes. The supernatant should be poured off. 3 ml of **Liqui-PREP™ Preservative Solution** or **Liqui-PREP™ Lytic Reagent** should be used to wash the pellet from the 50 ml centrifuge tube into a 15 ml centrifuge tube at least 3 times. The specimen should be mixed well using a vortex and allowed to fix for at least 1 hour.

Any Questions, Contact your local Liqui-PREPTM Representative or :

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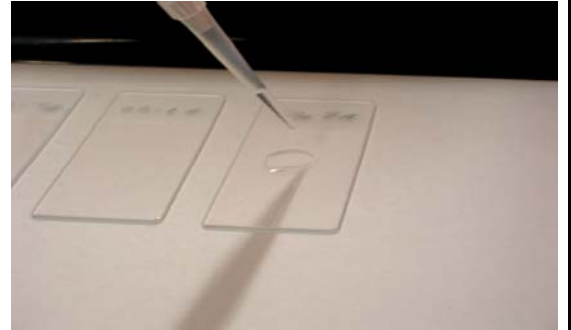
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Specimen Preservation: (continued)

SPUTUMS:

Chemical Mucous Digestion - There are numerous Chemical Mucous Digestion products on the market. If using these commercial products, it is extremely important to follow the manufacturers instructions for proper use. After digestion, the following procedure should be used:

- In most cases the specimen is larger than a 15 ml centrifuge can accommodate, it will have to be concentrated.
- Using 50 ml centrifuge tubes, centrifuge at 1,000g for 10 minutes.
- Pour off the supernatant.
- Using the **Liqui-PREP™ Lytic Reagent** in 3 ml aliquots, rinse the 50 ml centrifuge tube into a 15 ml centrifuge tube.
- Centrifuge @ 1,000 x g for 10 minutes.
- Pour off the Supernatant
- Pipette 8 ml of **Liqui-PREP™ Preservative Solution or Liqui-PREP™ Lytic Reagent** onto the specimen.
- Mix well using a vortex and allow the specimen to sit for 1 hour prior to processing.

Blender Digestion - There are as many Blender Digestions procedures as there are people. Blender procedures range from High speed for 30 seconds to low speed for 10 minutes. The challenge is to “cut” up the mucous while leaving as many cells intact as possible. Whatever blender protocol is decided on, the procedure is as follows:

- Use **Liqui-PREP™ Lytic Reagent** to rinse the sputum into the blender and blend according to the in-house determined procedure.
- Pour the blended sputum into 50 ml centrifuge tubes for concentration. (Use additional Lytic Reagent to rinse the blender into 50 ml centrifuge tube.
- centrifuge at 1,000g for 10 minutes.
- Pour off the supernatant.
- Using the **Liqui-PREP™ Lytic Reagent** in 3 ml aliquots, rinse the 50 ml centrifuge tube into a 15 ml centrifuge tube.
- Centrifuge @ 1,000 x g for 10 minutes.
- Pour off the Supernatant
- Pipette 8 ml of **Liqui-PREP™ Preservative Solution or Liqui-PREP™ Lytic Reagent** onto the specimen.



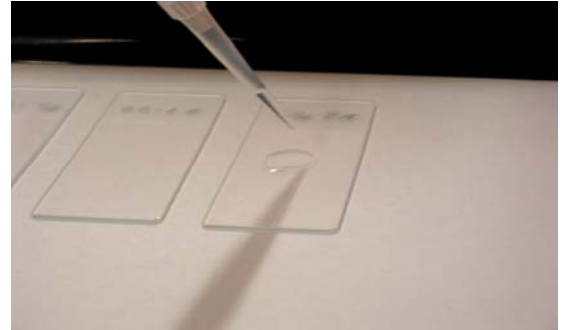
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Processing Concentration:

Whether using Chemical or Blender to break-up the mucous, at this point in the procedure, the specimen is fixed in a 15 ml centrifuge tube.

- Centrifuge @ 1,000 x g for 10 minutes.
- Pour off the supernatant
- Pipette 8 ml of **Liqui-PREP™ Preservative Solution** into the centrifuge tube
- Using a vortex, mix the specimens well.
- Pipette 4 ml of **Liqui-PREP™ Cleaning Solution** into the centrifuge tube.
- Centrifuge at 1,000g for 10 minutes.
- Pour off the supernatant and blot the tube on a dry paper towel to produce a reasonably dry pellet.

To dilute the cell pellet with **Liqui-PREP™ Cell Base** there are two options:

For specimens with a cell pellet under 0.5 ml dilute 1 part of cell pellet with 3 parts of Cell Base

Or

For Specimens with a cell pellet over 0.5 ml

- Mix the cell pellet very well using a vortex to produce a HOMOGENEOUS suspension.
- Using a pipette, take 100 µl of the HOMOGENEOUS suspension and place it into a new centrifuge tube.
- Into the 100µl HOMOGENEOUS suspension, pipette 300 or 400 µl of Cell Base.

Now matter which dilution technique is used:

- Mix the Pellet/Cell Base suspension well using a vortex.
- Using a 50µl pipette, place the 50µl sample onto a clean glass microscope slide.
- Allow the slide to dry completely prior to staining.
- Stain and read.