Liqui-PREP™
The Next Generation of Liquid Cytology

Manual Technical Tips

TIP Number: 012

Subject: Low Cellular Specimen Processing

TECHNICAL TIP OVERVIEW:

Liqui-PREP™ TIPS are intended to be guidelines for processing cytology specimens. The Laboratory Professional may use these guidelines or make modifications as needed to process a given specimen. In this section, each of the Liqui-PREP™ suggested basic steps will be addressed in detail along with some supporting publications.

These are specimens that are characterized by having extremely low cellularity. The main processing objective for these specimens is to ensure minimum cellular loss. These specimens may be collected in non-LGM container, such as cerebrospinal fluid, collected directly into a Liqui-PREP™ Collection Vials or directly into Liqui-PREP™ Preservative Solution in a container of the facility choice.

SUGGESTED LGM INTERNATIONAL PRODUCTS FOR PROCESSING:

- 30-100B - Liqui-PREP™ Cytology Processing Kit
  - ◊ 2 Bottles Liqui-PREP™ Preservative Solution (Fill: 990ml per bottle)
  - ◊ 2 Bottles Liqui-PREP™ Cellular Base Solution (Fill: 26ml per bottle)
  - ◊ 1 Box of 15ml Plastic Centrifuge Tubes (100 tubes)

- 30-100B1 - Liqui-PREP™ Cytology Processing Kit.
  - ◊ 2 Bottles Liqui-PREP™ Preservative Solution (Fill: 990ml per bottle)
  - ◊ 2 Bottles Liqui-PREP™ Cellular Base Solution (Fill: 26ml per bottle)

- Liqui-PREP™ Useful Accessories
  - ◊ 60-010: Liqui-PREPTM Collection Vials - 200 vials per case (10ml per vial)
  - ◊ 60-1000: Liqui-PREP™ Preservative Solution - 4 bottles (990ml per bottle)
  - ◊ 60-2000: Liqui-PREP™ Preservative Solution - 4 bottles (1,880ml per bottle)
  - ◊ 60-4000: Liqui-PREP™ Preservative Solution - 4 bottles (3,750ml per bottle)
  - ◊ 80-1000: Liqui-PREP™ Lytic Reagent - 4 bottles (990ml per bottle)

Any questions, contact your local Liqui-PREP™ representative or:

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SPECIMEN COLLECTION: Example of Specimen Types

- Cerebral Spinal Fluid (CSF) Specimens - These specimens are usually collected in 2 to 5 small test tubes. The Clinical Laboratory usually share the collected specimens, one tube to Hematology, one tube to Chemistry, one tube to Microbiology and one tube to Cytology/Histology. The fluid is collected and delivered "STAT" to the laboratory.
- Ocular and other micro-specimens - These specimens are collected and dispensed or rinsed into the Liqui-PREP™ Preservative Solution.
- Fine Needle Aspirates (FNA) Specimens - These specimens are best collected directly into a Liqui-PREP™ Preservative Solution. After collection, the specimen is dispensed and rinsed into the Liqui-PREP™ Preservative Solution.

NOTE: The collection syringe is rinsed by aspirating and dispensing several times to remove the specimen from the syringe.

- Heparin Saline Collection - It should be noted that other solution may be preferred such as saline, Heparin saline, etc.
- Bloody Specimens - Recommended Collection into a solution of 50% Liqui-PREP™ Preservative Solution and 50% Liqui-PREP™ Lytic Reagent is to minimize RBC presence for processing.

Cerebral Spinal Fluid (CSF) Specimens

Specimen Collection:

Cerebral Spinal Fluid (CSF) Specimens - These specimens are usually collected in 2 to 5 small test tubes. The Clinical Laboratory usually share the collected specimens, one tube to Hematology, one tube to Chemistry, one tube to Microbiology and one tube to Cytology/Histology. The fluid is collected and delivered "STAT" to the laboratory.

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Cerebral Spinal Fluid (CSF) Specimens

Specimen Preparation:

- Add equal amounts of Liqui-PREP™ Preservative Solution to the Cerebral Spinal Fluid in the collection tube.

NOTE: If the specimen volume is too large to add Liqui-PREP™ Preservative Solution, pour the contents of the collection specimen into a 15ml centrifuge tube. Rinse the specimen collection tube several times using Liqui-PREP™ Preservative Solution into the centrifuge tube. Allow the specimen to preserve for at least 1 hour prior to processing.

- Mix the tube well and allow to preserve for at least 1 hour prior to processing.
- After the specimen is preserved, pour the specimen into a 15ml centrifuge tube. Use additional aliquots of Liqui-PREP™ Preservative Solution to rinse the residual cells from the collection tube into the 15ml centrifuge tube.

Specimen Cleaning and/or Concentration:

After specimen preparation, the specimens are in 15ml centrifuge tubes. The specimen centrifuge tubes are centrifuged at ~1000xg for approximately 10 minutes. After centrifugation, the supernatant is decanted by inverting the centrifuge tube. It is quite normal for no visible pellet to be observed after decanting, however there are cells present.

Cellular Encapsulation and Slide Production:

Because of the extremely low number of cells, it is best to encapsulate the cells in the processing centrifuge tube. Use 50µl to 100µl of Liqui-PREP™ Cellular Base Solution for the cellular encapsulation.

- Pipette 50µl to 100µl of Liqui-PREP™ Cellular Base Solution directly into the decanted processing specimen centrifuge tube.
- Mix the specimen centrifuge tube well using a vortex mixer.
- Aspirate 50µl of the well mixed specimen centrifuge tube and dispense or inoculate it on a microscope slide.
- Allow the specimen microscope slide to dry, stain the slide and read.

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Ocular and Other Micro-specimens

Specimen Collection:

Ocular and other micro-specimens - These specimens are collected and dispensed or rinsed into the Liqui-PREP™ Preservative Solution.

Specimen Preparation:

- Allow the samples to preserve for at least 1 hour prior to processing.
- After the specimen is preserved, pour the specimen into a 15ml centrifuge tube. Use additional aliquots of Liqui-PREP™ Preservative Solution to rinse the collection container into the 15ml centrifuge tube.

Specimen Cleaning and/or Concentration:

After specimen preparation, the specimens are in 15ml centrifuge tubes. The specimen centrifuge tubes are centrifuged at ~1000xg for approximately 10 minutes. After centrifugation, the supernatant is decanted by inverting the centrifuge tube. It is quite normal for no visible pellet to be observed after decanting, however there are cells present.

Cellular Encapsulation and Slide Production:

Because of the extremely low number of cells, it is best to encapsulate the cells in the processing centrifuge tube. Use 50µl to 100µl of Liqui-PREP™ Cellular Base Solution for the cellular encapsulation.

- Pipette 50µl to 100µl of Liqui-PREP™ Cellular Base Solution directly into the decanted processing specimen centrifuge tube.
- Mix the specimen centrifuge tube well using a vortex mixer.
- Aspirate 50µl of the well mixed specimen centrifuge tube and dispense or inoculate it on a microscope slide.
- Allow the specimen microscope slide to dry, stain the slide and read.

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Fine Needle Aspirates (FNA) Specimens

Specimen Collection:

Fine Needle Aspirates (FNA) Specimens - These specimens are best collected directly into a Liqui-PREP™ Preservative Solution. After collection, the specimen is dispensed and rinsed into the Liqui-PREP™ Preservative Solution.

NOTE: The collection syringe is rinsed by aspirating and dispensing several times to remove the specimen from the syringe.

- Heparin Saline Collection - It should be noted that other solution may be preferred such as saline, Heparin saline, etc.
- Bloody Specimens - Recommended Collection into a solution of 50% Liqui-PREP™ Preservative Solution and 50% Liqui-PREP™ Lytic Reagent is to minimize RBC presence for processing.

Specimen Preparation:

- If these specimens are collected in Liqui-PREP™ Preservative Solution, allow the samples to preserve for at least 1 hour prior to processing.
- After the specimen is preserved, pour the specimen into a 15ml centrifuge tube. Use additional aliquots of Liqui-PREP™ Preservative Solution to wash the collection tube into the 15ml centrifuge tube.

NOTE: Using Heparin Saline Solution - Upon arrival in the laboratory, the specimen should be poured into a 15ml centrifuge tube. The specimen tube should be rinsed using aliquots of Liqui-PREP™ Preservative Solution into the 15ml centrifuge tube.

The centrifuge tube should be immediately centrifuged @ 1,000g for approximately 10 minutes.

The centrifuge tube is decanted and ~8 ml of the Liqui-PREP™ Preservative Solution is added to the remaining pellet. Allow at least 30 minutes for preservation prior to processing.

NOTE: Bloody Specimens Collected in 50% Liqui-PREP™ Lytic Reagent - Upon arrival in the laboratory, the specimen should be poured into a 15ml centrifuge tube, rinsed using Liqui-PREP™ Preservative Solution and centrifuged 1,000xg for ~10 minutes. The centrifuge tube is decanted and ~8 ml of the Liqui-PREP™ Preservative Solution is added to the remaining pellet. Allow at least 15 minutes for preservation prior to processing.

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Fine Needle Aspirates (FNA) Specimens

Specimen Cleaning and/or Concentration:

After specimen preparation, the specimens are in 15ml centrifuge tubes. The specimen centrifuge tubes are centrifuged at ~1000xg for approximately 10 minutes. After centrifugation, the supernatant is decanted by inverting the centrifuge tube. It is quite normal for no visible pellet to be observed after decanting, however there are cells present.

Cellular Encapsulation and Slide Production:

Because of the extremely low number of cells, it is best to encapsulate the cells in the processing centrifuge tube. Use 50µl to 100µl of Liqui-PREP™ Cellular Base Solution for the cellular encapsulation.

- Pipette 50µl to 100µl of Liqui-PREP™ Cellular Base Solution directly into the decanted processing specimen centrifuge tube.
- Mix the specimen centrifuge tube well using a vortex mixer.
- Aspirate 50µl of the well mixed specimen centrifuge tube and dispense or inoculate it on a microscope slide.
- Allow the specimen microscope slide to dry, stain the slide and read.

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Additional Processing Note:

FNA Red Blood Cell Removal:

- When the specimen arrives in the laboratory, pour and rinse the specimen into a centrifuge tube.
- Centrifuge the specimen at 1000xg for approximately 10 minutes. Pour off the supernatant.
- Pipette 4ml to 6ml of Liqui-PREP™ Lytic Reagent and an equal amount of Liqui-PREP™ Preservative Solution into the specimen centrifuge tube.
- Allow the specimen to lyse red blood cells for 10 to 15 minutes.
- Centrifuge the specimen at 1000xg for approximately 10 minutes. Pour off the supernatant.
- Pipette 4ml to 8ml of Liqui-PREP™ Preservative Solution into the centrifuge tube. Mix well using a vortex mixer.
- Allow to preserve for at least 30 minutes.
- Proceed to Specimen Cleaning and/or Concentration step, then to the Cellular Encapsulation and Slide Production Step.

References:

- "Evaluation for Cyto-preservability of Manual Liquid-Based Cytology Liqui-PREP™ and its Application to Cerebrospinal Fluid Cytology: Comparative Study with Cytospin", G. Park, M.D.; K. Lee, M.D.; C. Jung, M.D.; D. Lee, M.D.; B. Cho, M.D.; Y. Lee, M.D.; S. Shim, M.D.; K-Y. Lee, M.D.; C. Kang, M.D. - Department of Hospital Pathology and Pediatrics, College of Medicine, The Catholic University of Korea, Seoul, Korea
- "Comparison of Liqui-PREP™ and Conventional Preparations in Thyroid Fine Needle Aspirates", E. Park, M.D.; E. Cho, M.D.; I. Do, M.D.; S. Kim, M.D.; J. Shin, M.D.; B. Han, M.D.; Y. Oh, M.D. - Departments of Pathology and Radiology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea.
- "Significance of Estrogen Receptor 1 (ESR-1) Gene Imbalances in Colon and Hepatocellular Carcinomas based on Tissue Microarray Analysis", E. Tsiambas, M.D. - Department of Pathology, Medical School, University of Athens, Athens, Greece; S. Georgiannos, M.D. - Department of Brease Cancer Surgery, Blue Cross Hospital, Athens, Greece; D. Alexopoulou, M.D., S. Lambropoulou, M.D., D. Dimo, M.D., I Ioannidis, M.D. - Department of Cytology, Evangelismos Hospital, Athens, Greece'C. Kravvartis, M.D. - Department of Forensic Services, Larisa, Greece; N. Salemis, M.D., A. Karameris, M.D. - Department of Pathology, V.A. Hospital, Athens, Greece; S. Dourakis, M.D. - Department of Internal Medicine, Hipokration Hospital, Medical School, University of Athens, Athens, Greece.
- "Liquid Base Cytology in Evaluation of Thyroid Nodules", E. Keyhani, M.D. - Genetic Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran; S. Sharghi, M.D. - Endocrinology and Metabolism Research center, Tehran University of Medical Sciences, Tehran, Iran.; R. Amini, M.D. - Sepid Pathobiology Laboratory, Karaj, Iran; S. Sharghi, M.D. - Iran University of Medical Sciences, Tehran, Iran; M. Karimlou, M.D. - Social Department of Health Research Center, Department of Biostatistics, University of Social Welfare and Rehabilitation Services, Tehran, Iran; F. Moghaddam, M.D. - Genetic Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran; B. Larijani, M.D. - Endocrinology and Metabolism Research center, Tehran University of Medical Sciences, Tehran, Iran.

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