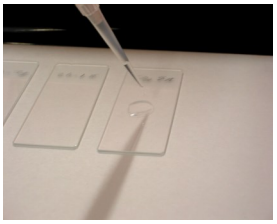
Liqui-PREP®
The Next Generation of Liquid Cytology

Technical Tips



Number: 00012

Date: 07/09/22

SUBJECT: Adjusting Staining Protocols

TECHNICAL TIP OVERVIEW:

This Technical Tip is the result of many requests for information of how to Adjust Staining Protocols. Before going into the adjusting protocol, there is some information we need to address:

- ◊ The dried **Liqui-PREP®** Slide is nothing more than an alcohol fixed specimen.
- ◊ There is no formaldehyde or other chemicals that are not friendly to staining.
- ◊ The Cell Base Capsule is completely inert and has no chemical effect on staining.

OVERVIEW:

This Technical Tip is a document to address the mysteries of staining. We have written this review to be used as a tool for persons wanting to improve or adjust their staining for Cytology slides. Some of the information in this document can be of value in all staining, but our focus is for Cytology Staining.

Before we begin, it is important to understand there is NO STANDARDIZED STAINING ATTRIBUTE. Pathologists from one area of the world like very lightly stained cells. When these "PERFECT" slides are reviewed by Pathologists from other parts of the world, these same EXCELLENT SLIDES are not acceptable. Some Pathologists want to see red or green cytoplasm on their slides. Other pathologists have no interest in cytoplasm and are firm in their opinion that Nuclear detain is all that is important. What is important is what the individual Pathologist prefers to make good differential diagnosis.

There are as many opinions about staining as there are professionals. This is because staining is a mix of art and science. Therefore, it is important to understand that staining is, for the most part, an individual preference.

Add to this, the many classical staining protocols, using several types of Hematoxylin as listed below:

Gill 1 Hematoxylin
Gill 2 Hematoxylin
Gill 3 Hematoxylin
Harris Hematoxylin
Modified Harris Hematoxylin
Mayer's Hematoxylin
Modified Mayer's Hematoxylin

All of these Hematoxylins have different staining characteristics as well as protocol induced staining characteristics. Add to all these classical stains, the over 100 Commercial Manufacturing Houses of Hematoxylins variations such as Hematoxylin 7211, Hematoxylin 1 and Hematoxylin 2 by Richard Allan Scientific.

Liqui-PREP®
The Next Generation of Liquid Cytology

Technical Tips **Number:** 00012

Here are some important points that should be used to get consistently stained slides:

Staining QC - After filling the staining line and before running the first patient slide, it is important to Quality Control the staining line. This requires having both unstained and stained reference slides. Simply, these unstained reference slides are run on the fresh staining line and then compared microscopically to the stained reference slides. This is Good Laboratory Practice.

Changing Staining Lines – Staining lines have a "shelf-life" and need to be changed. Attributes of expired staining lines vary by changes in intensity, hue, and color. Depending on the medium being stained, expired staining lines may be detected earlier or later. As an example, **Liqui-PREP®** processed slides are very sensitive to stain age. If the staining line is getting old, it will be seen earlier with these slides. Time to change, depends on the Type of stains used in the staining line and the manufacturer. LGM has used several manufactured stains and have found Richard Allan Stains to last the longest "shelf-life" between stain changes.

The following Table is an example of Staining Line Change Recommendations. **AGAIN, THE ACTUAL STAINING LINE CHANGE REQUIREMENTS DEPEND ON THE MANUFACTURER OF THE STAINS.** The following is only an example:

REAGENT	WHEN TO CHANGE
Hematoxylin 1 (RAS)	Change Every 2 weeks or after 2,500 slides stained
OG-6	Change Every 2 weeks or after 2,500 slides stained
EA-50	Change Every 2 weeks or after 2,500 slides stained
Cyto-Stain	Change Every 2 weeks or after 2,500 slides stained
Ethanols	Change Every 500 slides stained
Xylene	Change Every 500 slides stained
Tap Water Bath	Change Every 200 slides stained

Daily QC Review – It is suggested that QC slides should be run daily. At minimum weekly QC should be run and a few slides should be selected randomly and staining should be compared with the stained reference slides. If there are changes in the staining results, the staining line should be changed. This is Good Laboratory Practice.

Finally, new types of specimens and processing methods may require modification in the staining procedures. This is quite normal. Cytology and Histology stains want to be in alcoholic medium. This is the reason most staining protocols begin with a series of alcohol baths. The major effects on staining are time, reagent age and pH.

As you read this paper remember the **Liqui-PREP®** Slide starts out in an alcohol based preservative and end in an alcohol based medium on the slide. The pH of the resulting medium on the slide is between 6.9 and 7.5. The Cell Base Capsule is extremely porous and therefore staining times will be reduced in most applications.

Liqui-PREP®
The Next Generation of Liquid Cytology

Technical Tips **Number:** 00012

ADJUSTING STAINS:

Before evaluating staining characteristics and adjusting staining protocol, the following points must be addressed: (This takes out variables when evaluating and adjusting staining protocols)

Fresh Staining Line – Always change the reagents in the staining line before evaluation staining characteristics and adjusting a staining protocol

QC The Staining Line – QC the staining line to ensure it is performing as expected.

Validate adjustment - Re-stain some slides confirming the adjustments to be made. Only a few slides are needed.

Slides for Staining – Make several slides of each specimen. We generally make 10 or more slides of each specimen. This allows several adjustment runs and a few confirmatory staining runs.

After performing the above items, proceed to adjusting the staining protocol. We suggest making a staining chart for this process and only making 1 change at a time in order to understand clearly the effects of the protocol change. The **chart ON THE NEXT PAGE** will give a good guide for this adjustment. Remember to use your current staining protocol as the base and adjust from there.

Finally:

Remember to track your changes to the staining protocol. After success with finding the correct staining protocol which satisfies the eye, make a few more staining runs with unknown slides to confirm the results.

Staining is both an art and science. If using alcohol baths at the beginning of the protocol, the original preservative has no effect on the staining. The major effects are staining times and pH. The art of staining is the eye of the reader and the science of staining is controlled increasing or decreasing times and pH to get the desired art.

LGM International, Inc.
Technical Services

Any Questions, Contact your local Liqui-PREP® Representative

LGM International, Inc.
Melbourne, FL 32934 USA
Telephone: (321) 254-0480; Fax: (321) 254-0481
Email: sales@lpalp.com

Liqui-PREP®
The Next Generation of Liquid Cytology

Technical Tips

Number: 00012

Desired Trait	Gill Hematoxylin Time	Acid Alcohol Clarifier	OG-6/ EA-50 Time Adjust to-gether	Post EA-50 Alcohol	Post EA-50 Alcohol
NUCLEI & CYTOPLASM					
Nuclei Lighter, Cytoplasm unchanged	Decrease Stain Time	If using, very slight time reduction	No Change	No Change	No Change
Nuclei Lighter, Cytoplasm more Green	Decrease Stain Time	Increase Soak Time	No Change	No Change	No Change
Nuclei Lighter, Cytoplasm Blue	Decrease Stain Time	Decrease Soak Time	No Change	No Change	No Change
Nuclei Unchanged, Cytoplasm more Green	No Change	Increase Soak Time	No Change	No Change	No Change
Nuclei Unchanged, Cytoplasm Blue	No Change	Decrease Soak Time	No Change	No Change	No Change
Nuclei Darker, Cytoplasm unchanged	Increase Stain Time	Increase Soak Time	No Change	No Change	No Change
Nuclei Darker, Cytoplasm more Green	Increase Stain Time	Slight in-creased Soak time	No Change	No Change	No Change
Nuclei Darker, Cytoplasm Blue	Increase Stain Time	Decrease Soak Time	No Change	No Change	No Change
CYTOPLASMA & NUCLEOLI					
Cytoplasm Lighter, More Red	No Change	No Change	Decrease 2 stain times Equally	Decrease Soak Time	Decrease Soak Time
Cytoplasm Lighter, Same Hue	No Change	No Change	Decrease 2 stain times Equally	No Change	No Change
Cytoplasm Lighter, Less Red	No Change	No Change	Decrease 2 stain times Equally	Increase Soak Time	Increase Soak Time
Cytoplasm Unchanged, More Red	No Change	No Change	No Change	Decrease Soak Time	Decrease Soak Time
Cytoplasm Unchanged, Less Red & More Or-ange	No Change	No Change	No Change	Increase Soak Time	Increase Soak Time
Cytoplasm Darker, More Red	No Change	No Change	Increase 2 stain times Equally	Decrease Soak Time	Decrease Soak Time
Cytoplasm Darker, Same Hue	No Change	No Change	Increase 2 stain times Equally	No Change	No Change
Cytoplasm Darker, Less Red	No Change	No Change	Increase 2 stain times Equally	Increase Soak Time	Increase Soak Time
More Red Necleoli	Decrease Stain Time	Increase Soak Time	No Change	Decrease Soak Time	Decrease Soak Time