Liqui-PREP™
The Next Generation of Liquid Cytology

Manual Technical Tips

TIP Number: 010

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 ethanol 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 effect on staining.

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. Some of the information in this document can be of value in all staining, but our focus is for Cytology Staining (PAP Stain).

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 little interest in cytoplasm and are firm in their opinion that Nuclear detail is all that is important. What is important is what the individual Pathologist prefers.

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 Manufacturer House Hematoxylins by over 100 manufacturers such as Hematoxylin 7211, Hematoxylin 1 and Hematoxylin 2 by Richard Allan Scientific.

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

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When the results of staining are less than desired, there are numerous places to investigate. If the stains are made in the laboratory, the technologist bears the brunt of the problem. If the stains are pre-mixed, the manufacturer can be suspect. However, most times an issue can be resolved by adjusting staining times to resolve a specific problem.

Define the Issue - Prior to making any adjustments, clearly define the issue. If the nuclear stain is too light or dark, Hematoxylin and the chemicals before and immediately after can be a focus, with Hematoxylin timing being the first thought. If the cytoplasm is too light or heavy, the counter stains (OG-6 and or EA-50) should be the focus.

Having defined the issue, The following actions should be followed prior to adjusting any stains.

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 slides are run on the fresh staining line and then compared microscopically to the stained reference slides. This is Good Laboratory Practice. If there is an issue, this will confirm the issue.

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 example, Liqui-PREP™ Processed slides are 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. We are LGM have used several manufactured stains and have found Richard Allan Stains to last the longest “shelf-life” between stain changes. When adjusting stains, always start out with a fresh staining line.

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.*

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

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ADJUSTING STAINS:
Before evaluating staining characteristics and adjusting staining protocol, the following points should be addressed:

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 - After successful adjustment, validate the change by 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 5 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, decolorizing and to a lesser extent pH. The art of staining is the eye of the reader and the science of staining is controlled increasing or decreasing times to get the desired art.

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Desired Trait	Gill Hematoxylin Time	Acid Alcohol Clarifier (if using)	OG-6/EA-50 Time Adjust together	Post EA-50 Alcohol	Post EA-50 Alcohol
Nuclei Lighter, Cytoplasm unchanged	Decrease Stain Time	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 Soak time increased	No Change	No Change	No Change
Nuclei Darker, Cytoplasm Blue	Increase Stain Time	Decrease Soak Time	No Change	No Change	No Change
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 Orange	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 Nucleoli	Decrease Stain Time	Increase Soak Time	No Change	Decrease Soak Time	Decrease Soak Time

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