



Liqui-PREP™

The Next Generation of Liquid Cytology

Manual Technical Tips

TIP Number: 008

Subject: Cytology Staining

TECHNICAL TIP OVERVIEW:

This Technical Tip is the result of many requests for Cytology Staining procedures. There are perhaps as many Cytology Staining procedures as there are Cytology processing laboratories. Before we offer the following information, please consider the following points:

- The Liqui-PREP™ prepared Slide can be stained using any Histology or Cytology Stain. The following staining information is **NOT** required or recommended by LGM International, Inc. This is simply the LGM International, Inc. research and QC laboratories sharing their experience, preferences and procedures with you, our valued clients.
- Four (4) Cytology Staining Procedures - 4 Staining procedures are presented. Each page contains procedure description, notes, procedure steps & timing, and discussion of the components used.
- Approximate Procedure Timing - The timing of each step are suggested and can be changed based on the resulting slide colors, etc. desired. Strength and quality of the stain have a huge effect on the slide intensity. Total Staining timing is the simple addition of the total time of each step. We do not include manipulation time which depends on the personnel, space, etc.
- Stability section - The Stability section are recommended time to change solutions (as recommended by the America Society of Clinical Pathologists). There will also be notes in this section from LGM's experience.
- LGM International, Inc.'s Preferred Stain Supplier - LGM has used most of the major suppliers of staining products. We have focused our staining with Richard Allan Scientific (website: rallansci.com) Staining Products. Richard Allan Scientific products are not the moderately high priced products, but in routine use, they gives us the **LOWEST COST** while giving us the best quality. Our selection of Richard Allan Scientific product reasons are as follows:
 - ◇ **PREMIXED STAINS** - The stains come premixed and every lot of stain is exactly the same. This saves us time in staining line preparation, but more importantly, **STAINED SLIDE COLORS AND DETAIL ARE EXTREMELY CONSISTANT FROM SLIDE TO SLIDE, DAY TO DAY, MONTH TO MONTH, AND YEAR TO YEAR.**
 - ◇ **STAIN STABILITY** - ALL the Richard Allan Scientific Stains last 6 to 8 times longer when in the staining line. Because of this, we make far fewer purchases of stains.
 - ◇ **FASTER STAINING PROCEDURES** - Because of the quality of the these stains, we have been able to reduce our staining times.
 - ◇ **QC AND LABOR REDUCTION** - Because we do not have to mix stains weekly we save the labor making stains and performing QC for the stain lots.

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

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PROCEDURE 1: “Cyto-Stain” Cytology Staining Procedure

DESCRIPTION & NOTES - This procedure is cytology staining procedure which substitutes Cyto-Stain for the OG-6 and EA50 stains. Cyto-Stain combines both OG-6 and EA-50 as counterstains. This procedure gives good hues to the colors and very good green colors.

APPROXIMATE STAINING TIME: 19 Minutes and 33 Seconds (15 Staining Stations before applying slide covers).

STAIN STATION NUMBER	REAGENT	~TIME	STABILITY & NOTES
1	95% Alcohol	2 Minutes	Change Every 500 Slides
2	95% Alcohol	2 Minutes	Change Every 500 Slides
3	Tap Water	1 Minute	Change Every 200 Slides
4	HEMATOXYLIN I	~1.5 Minutes	Change every 2 weeks or 2,500 Slides
5	Tap Water	1 Minute	Change Every 200 Slides
6	BLUING REAGENT	1 Minute	Change every 2 weeks or 2,500 Slides
7	Tap Water	1 Minute	Change Every 200 Slides
8	95% Alcohol	30 Seconds	Change Every 500 Slides
9	CYTO-STAIN	~3 Minute; 30 Seconds	Change every 2 weeks or 2,500 Slides
10	100% Alcohol	1 Minute	Change Every 500 Slides
11	100% Alcohol	1 Minute	Change Every 500 Slides
12	100% Alcohol	1 Minute	Change Every 500 Slides
13	Xylene	1 Minute	Change Every 500 Slides
14	Xylene	1 Minute	Change Every 500 Slides
15	Xylene	1 Minute	Change Every 500 Slides

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PROCEDURE 1: “Cyto-Stain” Cytology Staining Procedure

Reagent Information:

HEMATOXYLIN I :

A progressive stain developed to be used as a nuclear stain in the cytology staining procedure. This Richard Allan product requires no filtration prior to use and will not form crystals upon standing or exposure to cold weather. The staining intensities are similar to Gill Hematoxylin (1, 2 and 3). HEMATOXYLIN I gives different nuclear staining intensities based on the staining time.

BLUING REAGENT :

This Richard Allan product is used to enhance the color of the nuclei after hematoxylin staining. It changes the nuclear chromatin stain from a reddish-blue to a crisp blue-purple. The reaction is pH-dependent, the result of chelation. BLUING REAGENT is a buffered product that ensures the proper alkalinity (pH - 8.0), therefore pH shifts will not occur.

CYTO-STAIN:

This Richard Allan product is a single-solution counterstain which is a direct replacement for the conventional Eosin Azure (EA-50) and Orange G-6. Using CYTO-STAIN yields the full range of familiar colors and tints.

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PROCEDURE 2: “Cyto-Stain” RAPID Cytology Staining Procedure

DESCRIPTION & NOTES - This procedure is a rapid method of the cytology staining procedure which substitutes Cyto-Stain for the OG-6 and EA50 stains. This procedure gives more orange and less pink colors.

APPROXIMATE STAINING TIME - 15 Minutes and 30 Seconds (14 Staining Stations before applying slide covers)

STAIN STATION NUMBER	REAGENT	TIME	STABILITY & NOTES
1	95% Alcohol	2 Minutes	Change Every 500 Slides
2	95% Alcohol	2 Minutes	Change Every 500 Slides
3	Tap Water	1 Minute	Change Every 200 Slides
4	HEMATOXYLIN 7211	1 Minute; 30 Seconds	Change every 2 weeks or 2,500 Slides
5	Tap Water (trickle)	2 Minute	Change Every 200 Slides
6	95% Alcohol	30 Seconds	Change Every 500 Slides
7	CYTO-STAIN	1 Minute	Change every 2 weeks or 2,500 Slides
8	95% Alcohol	15 Seconds	Change Every 500 Slides
9	95% Alcohol	15 Seconds	Change Every 500 Slides
10	100% Alcohol	1 Minute	Change Every 500 Slides
11	100% Alcohol	1 Minute	Change Every 500 Slides
12	100% Alcohol	1 Minute	Change Every 500 Slides
13	Xylene	1 Minute	Change Every 500 Slides
14	Xylene	1 Minute	Change Every 500 Slides

HEMATOXYLIN 7211 :

Is a rapid progressive stain developed to be used as a nuclear stain in the cytology staining procedure. This Richard Allan product requires no filtration prior to use, however it will form crystals when exposure to cold weather.

CYTO-STAIN :

This Richard Allan product is a single-solution counterstain which is a direct replacement for the conventional Eosin Azure (EA-50) and Orange G-6. Using CYTO-STAIN yields the full range of familiar colors.

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PROCEDURE 3: CONVENTIONAL Cytology Staining Procedure

DESCRIPTION & NOTES - This procedure is the conventional cytology staining procedure using the OG-6 and EA50 stains.

APPROXIMATE STAINING TIME: 21 Minutes and 15 Seconds (19 Staining Stations before applying slide covers)

STAIN STATION NUMBER	REAGENT	TIME	STABILITY & NOTES
1	95% Alcohol	2 Minutes	Change Every 500 Slides
2	95% Alcohol	2 Minutes	Change Every 500 Slides
3	Tap Water	1 Minute	Change Every 200 Slides
4	HEMATOXYLIN 7211	1 Minute	Change every 2 weeks or 2,500 Slides
5	Tap Water (trickle)	1 Minute	Change Every 200 Slides
6	BLUING REAGENT	30 Seconds	Change every 2 weeks or 2,500 Slides
7	Tap Water	1 Minute	Change Every 200 Slides
8	95% Alcohol	30 Seconds	Change Every 500 Slides
9	** OG-6	2 Minute	Change every 2 weeks or 2,500 Slides
10	95% Alcohol	15 Seconds	Change Every 500 Slides
11	*** EA-50	3 Minute	Change every 2 weeks or 2,500 Slides
12	95% Alcohol	30 Seconds	Change Every 500 Slides
13	95% Alcohol	30 Seconds	Change Every 500 Slides
14	100% Alcohol	1 Minute	Change Every 500 Slides
15	100% Alcohol	1 Minute	Change Every 500 Slides
16	100% Alcohol	1 Minute	Change Every 500 Slides
17	Xylene	1 Minute	Change Every 500 Slides
18	Xylene	1 Minute	Change Every 500 Slides
19	Xylene	1 Minute	Change Every 500 Slides

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PROCEDURE 3: CONVENTIONAL Cytology Staining Procedure

Reagent Information:

HEMATOXYLIN 7211:

Is a rapid progressive stain developed to be used as a nuclear stain in the cytology staining procedure. This Richard Allan product requires no filtration prior to use, however it will form crystals when exposure to cold weather.

BLUING REAGENT:

This Richard Allan product is used to enhance the color of the nuclei after hematoxylin staining. It changes the nuclear chromatin stain from a reddish-blue to a crisp blue-purple. The reaction is pH-dependent, the result of chelation. BLUING REAGENT is a buffered product that ensures the proper alkalinity (pH - 8.0), therefore pH shifts will not occur.

** Papanicolaou Stain Modified OG is substituted for NON-GYN specimens where Papanicolaou Stain OG-6 is used for GYN specimens.

*** EA-36 or EA-50 can be used for GYN specimens. EA-65 and Modified EA are at times used for NON-GYN.

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PROCEDURE 4: LGM Modified Cytology Staining Procedure

DESCRIPTION & NOTES - This procedure LGM's modified rapid method of the cytology staining procedure which substitutes OG-6 and EA50 stains for Cyto-Stain. This procedure gives good nuclear definition..

APPROXIMATE STAINING TIME: 18 Minutes and 30 Seconds (17 Staining Stations before applying slide covers)

STAIN STATION NUMBER	REAGENT	TIME	STABILITY & NOTES
1	95% Alcohol	1 Minutes	Change Every 500 Slides
2	95% Alcohol	1 Minutes	Change Every 500 Slides
3	Tap Water	1 Minute	Change Every 200 Slides
4	HEMATOXYLIN I	1 Minute; 30 Seconds	** Change every 2 weeks or 2,500 Slides
5	Tap Water	30 Seconds	Change Every 200 Slides
6	Tap Water	30 Seconds	Change Every 200 Slides
7	Tap Water	1 Minute	Change Every 200 Slides
8	95% Alcohol	30 Seconds	Change Every 500 Slides
9	** OG-6	2 Minute; 30 Seconds	** Change every 2 weeks or 2,500 Slides
10	95% Alcohol	30 Seconds	Change Every 500 Slides
11	*** EA-50	3 Minute	Change Every 500 Slides
12	70% Alcohol	15 Seconds	Change Every 500 Slides
13	95% Alcohol	15 Seconds	Change Every 500 Slides
12	100% Alcohol	1 Minute	Change Every 500 Slides
14	100% Alcohol	1 Minute	Change Every 500 Slides
15	100% Alcohol	1 Minute	Change Every 500 Slides
16	Xylene	1 Minute	Change Every 500 Slides
17	Xylene	1 Minute	Change Every 500 Slides

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PROCEDURE 4: LGM Modified Cytology Staining Procedure

Reagent Information:

HEMATOXYLIN I :

A progressive stain developed to be used as a nuclear stain in the cytology staining procedure. This Richard Allan product requires no filtration prior to use and will not form crystals upon standing or exposure to cold weather. The staining intensities are similar to Gill Hematoxylin (1, 2 and 3). HEMATOXYLIN I gives different nuclear staining intensities based on the staining time.

LGM NOTE: STABILITY:

We change our Hematoxylin I and Cyto-Stain every 3 to 4 months and still get well stained slides.

** Papanicolaou Stain Modified OG is substituted for NON-GYN specimens where Papanicolaou Stain OG-6 is used for GYN specimens.

*** EA-36 or EA-50 can be used for GYN specimens. EA-65 and Modified EA are at times used for NON-GYN.

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