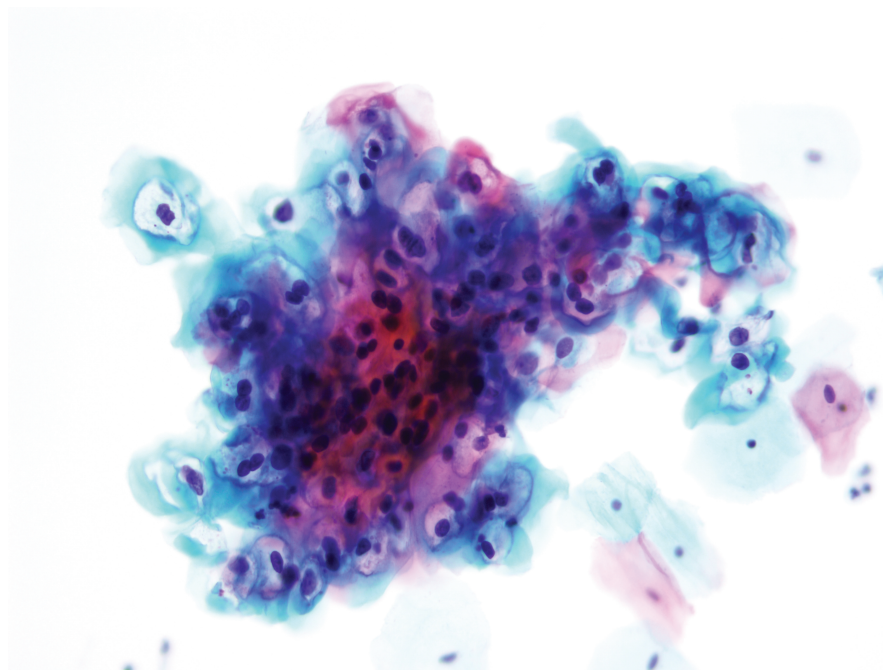


HOLOGIC®



ThinPrep™ Stain

User's Manual



ThinPrep™ Stain User's Manual

HOLOGIC®



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Introduction

Introduction

Introduction

This User's Manual is intended for customers using Hologic products to stain ThinPrep™ Pap Test slides for cytologic screening.

The ThinPrep Stain User's Manual provides our customers with standardized fixation and staining protocols using Hologic products in order to achieve consistent, high quality results. Technical information on reagents and protocols, basic troubleshooting techniques and Maintenance Schedules are included.

The ThinPrep Stain User's Manual is written in a format to make it convenient for our customers to incorporate this information into their individual Laboratory Procedure Manuals.

INTENDED USE

ThinPrep™ Stain Nuclear Stain

ThinPrep Stain Nuclear Stain is intended to be used in a Papanicolaou staining procedure in conjunction with any ThinPrep Stain Rinse Solution, ThinPrep Stain Bluing Solution, ThinPrep Stain Orange G Solution, and ThinPrep Stain EA Solution. For professional use.

ThinPrep™ Stain Rinse Solution

ThinPrep Stain Rinse Solution is intended to be used in a Papanicolaou staining procedure in conjunction with ThinPrep Stain Nuclear Stain, ThinPrep Stain Bluing Solution, ThinPrep Stain Orange G Solution, and ThinPrep Stain EA Solution. For professional use.

ThinPrep™ Stain Rinse II Solution

ThinPrep Stain Rinse II Solution is intended to be used in Papanicolaou staining procedure in conjunction with ThinPrep Stain Nuclear Stain, ThinPrep Stain Bluing II Solution, ThinPrep Stain Orange G Solution, and ThinPrep Stain EA Solution. For professional use.

ThinPrep™ Stain Bluing Solution

ThinPrep Stain Bluing Solution is intended to be used in a Papanicolaou staining procedure in conjunction with ThinPrep Stain Nuclear Stain, ThinPrep Stain Rinse Solution, ThinPrep Stain Orange G Solution, and ThinPrep Stain EA Solution. For professional use.



INTRODUCTION

ThinPrep™ Stain Bluing II Solution

ThinPrep Stain Bluing II Solution is intended to be used in a Papanicolaou staining procedure in conjunction with ThinPrep Stain Nuclear Stain, ThinPrep Stain Rinse II Solution, ThinPrep Stain Orange G Solution, and ThinPrep Stain EA Solution. For professional use.

ThinPrep™ Stain Orange G Solution

ThinPrep Stain Orange G Solution is intended to be used in a Papanicolaou staining procedure in conjunction with any ThinPrep Stain Nuclear Stain, ThinPrep Stain Rinse Solution, ThinPrep Stain Bluing Solution, and ThinPrep Stain EA Solution. For professional use.

ThinPrep™ Stain EA Solution

ThinPrep Stain EA Solution is intended to be used in a Papanicolaou staining procedure in conjunction with any ThinPrep Stain Nuclear Stain, ThinPrep Stain Rinse Solution, ThinPrep Stain Bluing Solution, and ThinPrep Stain Orange G Solution. For professional use.

WARNINGS/PRECAUTIONS

Refer to the Instructions for Use for each ThinPrep Stain solution for warnings and precautions and for a description of symbols that appear on the product labels.

For proper handling, storage and disposal of each chemical, refer to the recommendations on the manufacturer's Safety Data Sheet (SDS).

If any serious incident occurs related to this device or any components used with this device, report it to Hologic Technical Support and the competent authority local to the patient and/or user.

As with all laboratory procedures, universal precautions should be followed.



The Papanicolaou Stain (Pap Stain)

PRINCIPLE

Papanicolaou staining is the universal method for staining gynecologic slides for cytologic diagnosis. When performed properly, it results in:

- Crisp nuclear detail
- Differential cytoplasmic staining
- Transparent cells

The ThinPrep™ Stain is used in conjunction with the ThinPrep Stain protocol and recommended reagents. The ThinPrep reagents and protocols have been developed by Hologic to produce consistent and standardized, high quality results for cytologic screening.

SPECIMEN REQUIREMENT

ThinPrep™ Pap Test slides processed on all approved ThinPrep™ Processors.



FIXATION

Fixation

PRINCIPLE

Fixatives are applied to specimens to preserve cytologic morphology. Immediate and proper fixation is critical to accurate cytologic diagnosis.

FIXATION PROCEDURE

Slides are deposited into a bath containing standard laboratory alcohol fixative (defined as 95% ethyl or reagent alcohol). For slides not intended for use with the ThinPrep™ Imaging System, certain laboratory procedures may require alternate fixatives in the bath or for it to remain empty.

For slides fixed in alcohol and intended for use with the Genius™ Digital Diagnostics System and ThinPrep Imaging System:

- Note that the alcohol in the fixative bath should be changed after 100 slides or daily, whichever comes first.
- Slides should be placed in a multi-slide holder (staining rack) immersed in a bath of 95% reagent alcohol or 95% ethyl alcohol.
- The slides must remain in the alcohol for at least 10 minutes prior to staining.
- Do not allow the slides to air-dry at any time prior to staining.
- Stainer bath solutions should be kept covered when not in use to minimize evaporation.

Staining

For storage temperatures and “opened bottle” limits of stain solutions, refer to the product label or its Instructions for Use.

Note: Filter ThinPrep™ Nuclear Stain before initial use and when topping up, per standard laboratory practice.

CAUTION: For slides intended for use with the ThinPrep Imaging System or the Genius Cervical AI algorithm, be sure to follow the staining instructions and applicable protocols exactly as stated. Do not substitute any solutions or stains required in the protocols, as this may compromise the performance of the ThinPrep Imaging System or the Genius Cervical AI algorithm.

REQUIRED MATERIALS FOR STAINING

WARNING

Poisonous Substances
Flammable Liquids

Refer to SDS section of this manual.

From Hologic

ThinPrep Stain Nuclear Stain

ThinPrep Stain Rinse Solution or ThinPrep Stain Rinse II Solution

ThinPrep Stain Bluing Solution or ThinPrep Stain Bluing II Solution

ThinPrep Stain Orange G Solution

ThinPrep Stain EA Solution

Follow local regulations or the manufacturer’s recommendations for handling and cleanup of spills. Refer to manufacturer’s SDS for more information.

From Other Suppliers

Alcohols -

- 50% reagent or ethyl alcohol
- 70% reagent or ethyl alcohol
- 95% reagent or ethyl alcohol
- 100% reagent or ethyl alcohol



STAINING

Clearing Agents -

- Xylene
- Shandon Xylene Substitute (Epredia)
- Pro-Par Clearant (Anatech, Ltd., a Div. of Cancer Diagnostics, Inc.)
- Histo-Clear™ HS-200 (National Diagnostics)
- Histolene Trajan and other Australia/New Zealand distributors

Water Quality

Distilled or deionized water that meets these Hologic specifications for Instrument Feed Water (CLSI, C3-A4 standard, 2006):

≤ 1.0 microSiemens/cm conductivity
or ≥ 1.0 megOhm-cm resistivity

REAGENTS

ThinPrep Stain Nuclear Stain

ThinPrep Stain Nuclear Stain is an aqueous solution that serves to stain the nuclei of cells on cytologic slide preparations while maintaining cytoplasmic morphology. Refer to page 1.1 for more information on the intended use of the product.

ThinPrep Stain Rinse Solution

ThinPrep Stain Rinse Solution is an aqueous solution that removes excess ThinPrep Nuclear Stain from cellular components prior to bluing in cytologic slide preparations, enhancing nuclear staining and clarifying cytoplasm. Refer to page 1.1 for more information on the intended use of the product.

ThinPrep Stain Rinse II Solution

ThinPrep Stain Rinse II Solution is an alcohol-based solution that removes excess ThinPrep Stain Nuclear Stain from cellular components on slide preparations for cytologic evaluation, enhancing nuclear staining and clarifying cytoplasm. Refer to page 1.1 for more information on the intended use of the product.

ThinPrep Stain Bluing Solution

ThinPrep Stain Bluing Solution is an aqueous, alkaline solution that causes cellular components stained with ThinPrep Stain Nuclear Stain to darken to blue/violet, enhancing nuclear morphology in cytologic slide preparations. Refer to page 1.1 for more information on the intended use of the product.

ThinPrep Stain Bluing II Solution

ThinPrep Stain Bluing II Solution is an aqueous, alkaline solution that darkens the cellular components stained with the ThinPrep Nuclear Stain to blue/violet, enhancing nuclear morphology in cytologic slide preparations. Refer to page 1.2 for more information on the intended use of the product.

ThinPrep Stain Orange G Solution

ThinPrep Stain Orange G Solution is an alcohol-based solution that serves to stain the cytoplasm of cells on cytologic slide preparations. It will stain keratin in the cytoplasm of cells while maintaining nuclear staining and nuclear morphology. Refer to page 1.2 for more information on the intended use of the product.

ThinPrep Stain EA Solution

ThinPrep Stain EA Solution is an alcohol-based solution that serves to stain the cytoplasm of cells on cytologic slide preparations. Refer to page 1.2 for more information on the intended use of the product.

Reagent alcohol or ethyl alcohol

Reagent alcohol (a blend of 90% ethyl, 4-6% methyl and 4-6% isopropyl alcohols) or ethyl alcohol may be used in the staining procedure. The 100% alcohol must be virgin, but 95% may be from approved recycling systems.

Note: For ThinPrep Imaging System applications and Genius Digital Diagnostics System applications, see the next section, RECYCLED SOLVENTS, for approved systems and guidelines.

WARNING

Poisonous Substance

Flammable Liquid

Refer to manufacturer's SDS for more information.



STAINING

Clearing Agents

Xylene

For ThinPrep applications or for slides intended for use with the ThinPrep Imaging System or the Genius Digital Diagnostics System, the following xylene alternatives are approved:

Shandon Xylene Substitute (Epredia)

Pro-Par Clearant (Anatech, Ltd., a Div. of Cancer Diagnostics, Inc.)

Histo-Clear HS-200 (National Diagnostics)

Histolene Trajan and other Australia/New Zealand distributors

CAUTION: ThinPrep Imaging System applications and Genius Digital Diagnostics System applications require the clearing agent to be matched to the coverslipping media. See Table 1.3 on page 1.19.

RECYCLED SOLVENTS

Systems and Guidelines

Alcohols recycled via the following systems are acceptable for use with the ThinPrep Imaging System and the Genius Digital Diagnostics System:

Filtration Systems—Alcohol

Creative Waste Solutions, Inc. (Bench Top Alcohol Recycling System)
www.cwsincorp.com or tel 888-795-8300

Distillation Systems—Alcohol and Xylene

CBG Biotech™
www.cbgbiochem.com or tel 800-941-9484

B/R Instrument
www.brinstrument.com or tel 410-820-8800

Recycled Alcohol Guidelines

General Guidelines (applies to all alcohol recycling)

- Laboratories must follow manufacturer's recommendations for alcohol recycling.
- Only Hologic-recommended alcohols should be used—reagent or ethyl alcohol.
- Laboratories are responsible for ensuring that the recycled alcohol concentration used in the ThinPrep staining protocol meets Hologic's recommendation.

Filtration Systems—Alcohol only

- Periodically have the output product independently tested (i.e., annually, at a minimum) to confirm proper function of the recycling system. Contact the manufacturer regarding this service.
- Only the 95% and 100% alcohols from the ThinPrep Stain setup should be put through the filtration system.
- 95% and 100% alcohols combined on the filtration system will result in an output alcohol concentration that will need to be adjusted to 95% prior to use in the ThinPrep Stain setup.

Distillation Systems—Alcohol

- Periodically have the output product independently tested (i.e., annually, at a minimum) to confirm proper function of the recycling system. Contact the manufacturer regarding this service.
- Only alcohols from the ThinPrep Stain setups can be distilled together.
- Alcohols from *non-ThinPrep stain* setups must not be co-mingled for recycling with ThinPrep alcohols.
- **ONLY 70%, 95% & 100% Reagent Alcohol or Ethyl Alcohol may be combined for recycling.** (**Note:** 50% alcohol must be discarded according to the laboratory's hazardous chemical waste disposal guidelines. If included for recycling, its high water content will reduce the recovery volume and significantly lower the output alcohol concentration.)

Testing:

Test the collected waste alcohol solution to ensure it is free of xylene prior to recycling. Otherwise, the quality of the output alcohol will be compromised.

Testing for Xylene Contaminant in Alcohol

1. Mix the contents of the alcohol collection container thoroughly.
2. Pour or pipette 20 mL into a clean, dry vessel (e.g., graduated cylinder)
3. Add 20 mL of tap water, cover, and invert several times to mix.
 - a. If any cloudiness appears within the solution (occurs almost immediately), xylene is present.
Then:
DISCARD (do not recycle) all the container's contents according to the laboratory's guidelines for hazardous chemical waste disposal.
Clean the container using fresh absolute alcohol and dry.
Begin new alcohol collection.
 - b. If no cloudiness is present, no xylene is present.
Proceed to recycle the collected alcohol.

Test the output alcohol

1. For xylene contaminant (See method above).



STAINING

2. Use a hydrometer to determine concentration.
3. Adjust with water or absolute alcohol to 95%, 70%, or 50% as needed.

Special note for CBG Biotech units which are used to recycle both alcohol and xylene:

- When alcohol recycling is to *follow* xylene recycling, assure the “System flush” is properly performed to prevent contaminating alcohol with residual xylene. Refer to the CBG Biotech “*Installation and Operating Instructions*” manual for details.
- After a “System flush,” test output from the first recycled alcohol run to ensure xylene is not present.

Caution: Failure to follow the recommendations for fractional distillation in this section may result in recycled product which is not acceptable for use with the ThinPrep Imaging System or the Genius Digital Diagnostics System.

Distillation Systems—Xylene

- Observe each manufacturer’s setup, maintenance and operation recommendations.
- Ensure the recycled xylene quality is suitable for use in ThinPrep staining protocols. Contact the respective manufacturer for their recommended laboratory test procedure.
- Periodically have the output product independently tested (i.e. annually, at minimum) to confirm proper function of the recycling system. Contact the manufacturer regarding this service.
- Only xylenes from ThinPrep Stain protocols can be distilled together. Xylenes from *non-ThinPrep stain setups* must not be co-mingled for recycling with ThinPrep xylenes.

Caution: Failure to follow all recommendations for fractional distillation in this section may result in recycled product which is not acceptable for use with the ThinPrep Imaging System.

Procedures For Staining

PROCEDURE NOTES FOR AUTOMATED STAINING

The following information is to be used in conjunction with the applicable staining protocol.

WARNING

Poisonous Substances

Flammable Liquids

Refer to the SDS for more information.

Bath solution heights should completely cover the slides at full immersion. Add fresh reagent as needed to maintain bath heights. It is recommended that the final clearing agent bath be filled to a level above the cell spot but below the frosted area of the slide. This will reduce the amount of mounting media overspreading the label area after the coverslip is applied.

Staining baths should be covered when not in use to minimize evaporation of solutions and oxidative effects on the stains.

Change all solutions when the number of slides stained equals 1 slide for every 1 mL of bath volume, or once a week, whichever comes first. (For example if your bath volume is 450 mL, then change the bath after 450 slides, or once a week, whichever comes first.)

Note: A list of the staining protocols including stain station maps for automated stainers is located at the “Protocols” tab of this manual. The stainers listed are the ones that have been validated for use with the ThinPrep™ Stain Solutions and ThinPrep Pap slides processed on a ThinPrep Processor.

Recommended Stain Bath Washing Procedure

1. After emptying all stain baths, separate the baths for the clearing agent (Xylene, ProPar, etc.) from the others.
2. Clean clearing agent baths by wiping out any debris with a paper towel and letting them air-dry in a hood. **Do not use water in these baths.**
3. Wash out all of the other baths with a brush and water.
 - a. For baths that do not come clean with a brush and water:
Use a solution of 1% hydrochloric acid in 70% reagent or ethyl alcohol. This works particularly well for baths stained with Orange G and EA Solutions.
 - b. If step 4a does not clean the stain baths containing nuclear stain and those immediately following the nuclear stain, use a 10% solution of bleach in water. **Do not exceed a 10% bleach solution.**
4. Rinse out each bath several times with water and follow with a final rinse using distilled water. **Extra care must be taken to thoroughly rinse containers washed using the procedure in 4b, since any residual bleach can adversely affect the quality of subsequent staining.**
5. Allow the baths to air-dry or wipe them dry with paper towels before filling them for the next use.



Staining Troubleshooting

The following table is intended to guide the laboratory through standard troubleshooting practices if they are experiencing issues with the stain quality of slides stained with the ThinPrep Stain Solutions.

Table 1.1: Troubleshooting

Category		Clarifying Questions	Areas to Investigate
General	Frequency, onset, duration	What percentage of your work is being affected?	Is there a noticeable pattern in when the problems occur?
		When was the issue initially seen?	Does it happen when the stainer is changed? Is there anyone new performing staining?
		Imager information	Are you seeing Imager slide events?
Staining	Stains	Check Solution Lot numbers	Are any of your stain solutions expired?
			Is your Nuclear Stain solution open for 60 days or less?
			Did you filter your Nuclear Stain solution before use?
	Stainer	Are you following required stainer/ staining protocols?	Verify staining protocol. (Refer to the protocol.)
			Verify stain bath layout. (Refer to the protocol.)
			Verify solutions in bath are at the appropriate fill level.
			Verify slides are fully submerged in the post-stain solution baths throughout the process.
			Are you within the maximum allowable throughput limits for the stain table?
			Are you changing your post Nuclear Stain water per protocol?
			Are you cleaning stainer baths per recommended procedure? (Refer to the “Recommended Stain Bath Washing Procedure” on page 1.11.)

Table 1.1: Troubleshooting

Category		Clarifying Questions	Areas to Investigate
Staining	Other Solutions	Water	Water quality /Source/ How often or last time the system was verified?
		Alcohol	Do you make alcohols or buy alcohols?
			If you make alcohols, did you check the concentration of alcohols before use?
			Are you using recycled alcohol and following manufacturer's guidelines?
		Clearing Agents and Mounting Medium	Are you using an approved mounting medium / clearing agent combination? (Refer to tables 2 and 3 of this section.)
			Are you following the slide handling / drying guidelines?

Sample Biology

Certain Imaging System slide events (for example, 6615 or 6630) may be associated with patient biology when slides have excessive amounts of inflammation, bacteria, certain lubricants or blood. Contact Hologic Technical Support for guidance if there is an increase in such slide events.

Commonly Asked Questions and Answers

- Does Hologic have a recommended stainer bath washing procedure?
Yes, please see page 1.11 of this manual for details.
- Can a lab destain/re-stain and then image slides if an applications issue arises which would normally call for this process?
Hologic does not support destaining and re-staining slides intended for imaging with the ThinPrep Imaging System or Genius Cervical AI.
- Does Hologic support staining slides by hand that are intended for imaging?
Hologic does not support hand staining slides that are intended for imaging.
- Does Hologic have a Non-Gyn protocol which uses the ThinPrep stain solutions?
The ThinPrep Stain may be used for Non-Gyn slides, however, Hologic does not provide a protocol for this.



STAINING TROUBLESHOOTING

5. Can I substitute non-Hologic stain solutions in stainers for slides that are intended for imaging?
Hologic does not recommend using other stain solutions for slides intended for imaging with the ThinPrep Imaging System or Genius Cervical AI.

6. Can a lab self-validate stainers, protocols and mounting medium/clearing agent combinations for slides intended for imaging with the ThinPrep Imaging System or Genius Digital Diagnostics System?
Hologic does not recommend that; self-validating does not provide the level of quality management that good lab practices recommend. Hologic validates stainers, stain protocols and mounting medium/clearing agent combinations for slides intended for imaging with the ThinPrep Imaging System and Genius Cervical AI.

Coverslipping

PURPOSE

The mounting medium creates a permanent bond between the slide and the coverslip. It protects the cellular material from air-drying and shrinkage and acts as a seal to deter fading of the stain over time.

COVERSLIPPING REQUIREMENTS

It is recommended that the bath in which the slides are held prior to coverslipping has the clearing agent filled to a level above the cell spot but below the frosted area of the slide. This will reduce the amount of mounting media overspreading the label area after the coverslip is applied.

Various mounting media have been evaluated by Hologic for use with ThinPrep slides. These are found in the table on page 1.17.

Glass coverslips #1 thickness, 24 mm x 50 mm are recommended. Note that ThinPrep slides for use on the ThinPrep Imaging System require coverslips long enough to cover the area including the fiducial marks that are printed on the microscope slide.

Hologic-approved film or tape coverslip material for use with automated coverslipping instrumentation is also acceptable, with the same note for length required for ThinPrep plus Imaging slides.

Required Materials

Table 1.2 lists the mounting media, clearing agents, coverslips and combinations of these items that have been evaluated and approved for use with the ThinPrep Imaging System. Table 1.3 lists coverslipping films approved for use with the ThinPrep Imaging System.

Automated Glass Coverslipping

Refer to manufacturer's instructions for use of automated coverslipping instruments.

Note: Refer to Table 1.4 for recommendations for setting up a **Sakura Tissue-Tek™ FILM™** coverslipper for use with a ThinPrep Imaging System.

Note: Refer to detailed instructions for use of coverslipping tape/film on page 1.19.



COVERSLIPPING

Manual Glass Coverslipping

1. Quickly drain excess clearing agent from the slide.
2. Immediately apply sufficient mounting medium to the slide to minimize cornflaking, air bubbles or retraction. Avoid excessive medium, which may ooze out from under the coverslip and run down the edges of the slide.
3. Apply the coverslip using standard laboratory practice.
4. Using a lint-free wipe moistened in clearing agent, wipe the edges of the coverslip to clear any residual mounting medium.
5. Allow slides to dry completely (a minimum of 16 hours at room temperature or in a slide oven set at $37^{\circ}\text{C} \pm 3^{\circ}\text{C}$ for the dry time duration shown in Table 1.2) before placing in Imager slide cassettes or Genius Digital Imager slide carriers.

CAUTION: Make sure the coverslips are centrally placed and do not overhang any edges of the slide.

Glass Coverslip Drying Time

After coverslipping, slides must be allowed to adequately “dry” before being placed in the ThinPrep Imaging System or Genius Digital Imager. A slide can be considered “dry” when the medium has cured sufficiently so there is no movement of the coverslip in response to normal laboratory handling.

Table 1.2 illustrates drying times for slides placed in an oven set at $37^{\circ}\text{C} \pm 3^{\circ}\text{C}$ after automatic coverslipping.

Note: Drying times will differ depending upon variables such as the type of mounting medium, quantity of medium used, mounting method, temperature, and humidity.

Manually coverslipped slides may have drying times longer than those indicated in Table 1.3.

It is the responsibility of the laboratory to monitor and ensure that adequate, but not excessive, medium is applied to the slides and that they “dry” sufficiently prior to being placed on the ThinPrep Imaging System.

CAUTION: Failure to allow slides to dry sufficiently may compromise the performance of the Imager.

Table 1.2 Mounting Medium / Clearing Agent Validation Summary

Mounting Media			Clearing Agent	Minimum Dry Time @ 37°C +/- 3°C
Name / Part Number	Mounting Media Solvent	Distributor or Manufacturer		
Tissue-Tek™ Glas™ (6419)	Xylene	Sakura Finetek	Xylene	1 hour
Epredia Richard-Allan Scientific™ Mounting Medium 4111 also available as ClearVue™ Mountant 4211	Toluene	Epredia, Fisher Scientific	Xylene	1 hour
			Pro-Par Clearant	1 hour
			Shandon™ Xylene Substitute	1 hour
CoverSafe™ Mounting Medium	d-Limonene	StatLab Medical Products	Shandon™ Xylene Substitute	1 hour
			Pro-Par Clearant	1 hour
Pertex™ ¹ also available as CV Mount	Xylene	VWR or Leica Biosystems and other international distributors	Xylene	1 hour
			Histo-Clear™	1 hour
			Histolene	1 hour
DPX Mountant	Xylene	EMS (Electron Microscopy Sciences), Leica Biosystems or Merck (Int'l)	Xylene	1 hour
Micromount™	Xylene	Leica Biosystems	Xylene	1 hour
Neutral Mounting Medium ²	Xylene-Substitute	Wuxi Jiangyuan Industrial Trade Co. (China)	Environmentally Safe Clearing Agent ²	1 hour
Neutral Balsam ²	Xylene	Sinopharm Chemical Reagent Co. (China)	Xylene	1.5 hours
Malinol Mounting Medium	Xylene	Muto Pure Chemicals Co., Ltd. (Japan)	Xylene	2 hours
1 - Pertex is manufactured by HistoLab™ Products AB, Göteborg, Sweden. This product is not readily available for purchase in the USA.				
2 - Product available only in People's Republic of China.				
3 - Distributed in People's Republic of China. Validation conducted using 2 drops of mountant per slide.				



COVERSLIPPING

Mounting Media			Clearing Agent	Minimum Dry Time @ 37°C +/- 3°C
Name / Part Number	Mounting Media Solvent	Distributor or Manufacturer		
Fischer Chemical Permount™	Toluene	Fischer Scientific and other distributors	Xylene	3 hours
Fischer Chemical GS-Mounting Medium ³	Butyl Alcohol, Terpinol, Cyclohexane	Haerbin Gelin (China)	GS-Clearing Agent ³	17 hours
1 - Pertex is manufactured by HistoLab™ Products AB, Göteborg, Sweden. This product is not readily available for purchase in the USA.				
2 - Product available only in People's Republic of China.				
3 - Distributed in People's Republic of China. Validation conducted using 2 drops of mountant per slide.				

Use of Coverslipping Tape/Film

1. Follow manufacturer's recommendations for proper storage and operating conditions of the coverslipping product.
2. Place coverslipped slides flat in cardboard slide folders for a five-minute dry time at room temperature. Slides are then ready to be labeled (if needed) and imaged.
3. Slides may be imaged up to twenty-eight (28) days after coverslipping.
4. If using the Sakura Tissue-Tek FILM Coverslipper:
 - Refer to Table 1.3 for setup guidelines
 - Remove staining racks from the instrument immediately after coverslipping is complete. Do not let them remain in the output carousel.
 - Remove excess xylene by blotting the base of the stain rack on a lint-free wipe or paper towel. Then stand the rack up so slides are vertical.
 - Promptly remove slides from the rack, one at a time, wiping excess xylene from the back of each slide with a lint-free wipe.
 - Place slides flat in slide tray for drying.

Table 1.3: Coverslipping Film/Clearing Agent Validation Summary

Coverslip	Coverslip Solvent	Distributor or Manufacturer	Clearing Agent	Minimum Dry Time at Room Temperature
Tissue-Tek Coverslipping Film (4770)	Xylene activated	Sakura Finetek	Xylene	5 min
CrystalView Coverslipping Film	Xylene activated	StatLab Medical Products	Xylene	5 min



COVERSLIPPING

Table 1.4: Sakura Tissue-Tek FILM Coverslipper Settings for Use with the ThinPrep Imaging System

Setting	Tissue-Tek FILM Automated Coverslipper
Coverslip Length	45 mm
Xylene Dispensing (Range 1 - 5)	Setting #1
Xylene Holding Bath	Full Bath
Spring Tension Setting	Adjust to maximum tension
Slide Drying	<ul style="list-style-type: none">• Remove staining racks from instrument immediately after coverslipping is complete. Do not let them sit in the output carousel.• Remove excess xylene - dab base of stain rack on Kimwipe™ or paper towel and stand the rack up so the slides are vertical.• Immediately remove slides from rack and wipe excess xylene from back if slide with Kimwipe. Slides are now ready to label (if needed).

Coverslip Review

If any of the following are noted prior to imaging of the slide, the slide should be re-coverslipped per standard laboratory practice:

- Air bubbles

CAUTION: Large or numerous air bubbles can obscure cellular material.

- Retraction of mounting medium
- Coverslip is not straight or overhangs the edge of the slide.

Reagents

Follow manufacturer's recommendations for handling and cleaning up spills. Refer to manufacturer's SDS for further information.



Limitations of Fixation, Staining and Coverslipping

The ThinPrep™ reagents and protocols have been developed by Hologic to produce consistent and standardized, high quality results for cytologic screening. Substitution of reagents and/or changes to the Hologic staining protocols may produce less-than-optimal staining results.

If protocols are not followed or reagents are substituted, stain quality may be compromised.

Inadequately fixed specimens will not stain well.



Quality Control for the Papanicolaou Stain

PURPOSE

Regular monitoring of stain characteristics to:

- Ensure consistent well-stained slides for cytodiagnosis
- Detect stain quality issues prior to screening
- Comply with regulatory and accrediting agencies' Quality Control requirements

PROCEDURE

Microscopic review of stained slides based on your laboratory guidelines.

EXPECTED RESULTS

General Expected Results

- Well-stained cells that are transparent
- Distinct chromatin
- Good differential cytoplasmic staining
- Even staining throughout the slide
- Reproducible and consistent results

Standard for ThinPrep™ Nuclear Stain

Nuclear stain blue to blue/black

Threads between nuclear segments are visible in flattened polymorphonuclear leukocytes (PMNs).

Standard for ThinPrep Orange G Solution

Pale orange to yellow tint in the cytoplasm of non-keratinizing superficial squamous cells

Bright orange in keratinized superficial cells.

Standard for ThinPrep EA Solution

Non-keratinizing superficial and intermediate cells vibrant pinks and greens.

Nuclei and edges of overlapping cells are visible through the cytoplasm.



PROCEDURE NOTES

- Record results of microscopic review.
- Record problems/discrepancies and the remedial action taken.



Bibliography

Allen, K, editor, *ASCT A Guide to Cytopreparation*, 1998.

Atkinson, B, *Atlas of Diagnostic Cytopathology*, WB Saunders Company, 1992.

Bibbo, M and Day, L, editors, *Comprehensive Cytopathology*, WB Saunders Company, 2nd edition, 1997.

CLSI, *Preparation and Testing of Reagent Water in the Clinical Laboratory: Approved Guideline - Fourth edition*, C3-A4, 2006.

Current CLIA Regulations page. CDC web site. Available at: www.cdc.gov/clia/regs/toc.aspx. Accessed November 7, 2011. Refer to <https://www.cdc.gov/clia/>

Commission on Laboratory Accreditation, Laboratory Accreditation Program, Cytopathology Checklist, 2001.

Hologic, Inc. *ThinPrep™ 2000 Processor Operator's Manual*, Part Number MAN-09270-001.

Hologic, Inc. *ThinPrep™ 5000 Processor Operator's Manual*, Part Number MAN-07493-002.

Hologic, Inc. *ThinPrep™ Genesis Processor Operator's Manual*, Part Number MAN-08098-002.

Hologic, Inc. *ThinPrep™ Imaging System Image Processor Operator's Manual*, Part Number MAN-09273-001.

Keebler, CM, Somrak, TM, editors. *The Manual of Cytotechnology*, 7th Edition, 1997.

Hologic, Inc. *Genius Digital Imager Operator's Manual*, Part Number MAN-08801-001

Regulations (Standards - 29 CFR), National Research Council Recommendations Concerning Chemical Hygiene in Laboratories (Non-Mandatory) - 1910.1450 App A.

Gill, Gary W. 1999. "The Papanicolaou Stain, Quality Control and Quality Assurance" *SCAN* 10(2):18-9, 21.

Gill, Gary W. 2001. "WHAT IS THE PAPANICOLAOU STAIN ANYWAY?" *SCAN* 12 (2): 33-60.

Triol, JH and Goodell, RM, editors. *ASCT Cytopathology Quality Assurance Guide*, Second Edition, Volume 1, 1992.



BIBLIOGRAPHY

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**Maintenance
Schedules**

**Maintenance
Schedules**



Maintenance Schedules

The maintenance schedules on the following pages may be reproduced for laboratory use.

- ThinPrep Stain™ Maintenance
- ThinPrep Stain Quality Control Check
- ThinPrep Stain Solutions - Inventory



MAINTENANCE SCHEDULES

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MAINTENANCE SCHEDULES

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MAINTENANCE SCHEDULES

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MAINTENANCE SCHEDULES

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Ordering Information

Ordering Information



Ordering Information

Mailing Address

Hologic, Inc.
250 Campus Drive
Marlborough, MA 01752 USA

Remittance Address

Hologic, Inc.
PO Box 3009
Boston, MA 02241-3009 USA

Business Hours

Hologic's business hours are 8:30 a.m. to 5:30 p.m. EST Monday through Friday, excluding holidays.

Customer Service

Product orders are placed through Customer Service by phone during business hours at 1-800-442-9892 Option 5.

Orders can also be faxed to the attention of Customer Service at 1-800-409-7591.

A copy of Hologic's limited warranty and other terms and conditions of sale may be obtained by contacting Customer Service at the numbers listed above.



ORDERING INFORMATION

Technical Support

For questions about ThinPrep Stains and related applications, representatives from Technical Support are available by phone 7:00 a.m. to 7:00 p.m. EST Monday through Friday at 1-800-442-9892 Option 6 (USA and Canada).

For Technical Support outside USA and Canada, contact your local Hologic Technical Solutions office or your local distributor.

For questions about ThinPrep Stains and related applications, representatives from Technical Support are available in Europe and the UK by phone 8:00 to 18:00 CET Monday through Friday, at TScytology@hologic.com and via the toll-free numbers listed here:

Asia	+852 3526 0718	Netherlands	0800 022 6782
Australia	+61 2 9888 8000	Portugal	800 841 034
Austria	0800 291 919	Spain	900 994 197
Belgium	0800 773 78	South Africa	0800 980 731
Denmark	8088 1378	Sweden	020 797 943
Finland	0800 114 829	Switzerland	0800 298 921
France	0800 913 659	UK	0800 032 3318
Germany	0800 183 0227	EMEA	0800 8002 9892
Ireland (Rep)	1 800 554 144	Additional countries	0041.21.633.39.26
Italy	800 786 308	Intl Fax number	0041.21.633.39.10
Luxembourg	8002 7708		

Protocol for Returned Goods

For returns on warranty-covered ThinPrep Stain items, contact Technical Support.

Reordering ThinPrep Stain Supply Items

Item	Quantity	Part Number
ThinPrep Stain Nuclear Stain	One 4-liter bottle	70780-001
ThinPrep Stain Rinse Solution	One 4-liter bottle	70779-001
ThinPrep Stain Bluing Solution	One 4-liter bottle	70793-001
ThinPrep Stain Orange G Solution	One 4-liter bottle	70781-002
ThinPrep Stain EA Solution	One 4-liter bottle	70782-002

Item	Quantity	Part Number
Stain Set	1 gallon bottle of each of the five stain solutions	70897-002
ThinPrep Stain Rinse II Solution*	One 4-liter bottle	ASY-04875
ThinPrep Stain Bluing II Solution*	One 4-liter bottle	ASY-04876
ThinPrep Stain Set II*	One 4-liter bottle each of Nuclear Stain Solution, Rinse II Solution, Bluing II Solution, Orange G Solution and EA Solution	PRD-03753
*Note, ThinPrep Rinse II and Bluing II Solutions are designed to work together and cannot be mixed with, or used in conjunction with, the original ThinPrep Rinse and Bluing Solutions.		
ThinPrep Stain User's Manual	Additional User's Manual	MAN-09267-001
Stain Protocol: Hologic Compass Stainer using 95% alcohol fixed slides	One protocol	AW-19464-001
Stain Protocol: Sakura Tissue-Tek DRS 2000 using 95% alcohol fixed slides	One protocol	AW-19453-001
Stain Protocol: Sakura Tissue-Tek Prisma or Prisma PLUS using 95% alcohol fixed slides	One protocol	AW-19456-001
Expansion Stain Protocol: Sakura Tissue-Tek Prisma or Prisma PLUS using 95% alcohol fixed slides	One protocol	AW-19462-001
Stain Protocol: Leica Autostainer XL using 95% alcohol fixed slides	One protocol	AW-19452-001
Stain Protocol: Leica ST5020 Multistainer using 95% alcohol fixed slides	One protocol	AW-19458-001
Stain Protocol: Thermo Shandon Varistain Gemini autostainer using 95% alcohol fixed slides	One protocol	AW-19455-001
Stain Protocol: Medite TST 44 using 95% alcohol fixed slides	One protocol	AW-19460-001
Stain Protocol using Rinse II and Bluing II Solutions on the Leica Autostainer XL	One protocol	AW-19466-001
Stain Protocol using Rinse II and Bluing II Solutions on the Leica ST5020 Multistainer	One protocol	AW-19465-001
Stain Protocol using the Rinse II and Bluing II Solutions on the Sakura Tissue-Tek DRS 2000 Stainer	One protocol	AW-19467-001



ORDERING INFORMATION

Item	Quantity	Part Number
Stain Protocol using the Rinse II and Bluing II Solutions on the Sakura Tissue-Tek Prisma Autostainer	One protocol	AW-19468-001
Stain Protocol using the Rinse II and Bluing II Solutions on the Sakura Tissue-Tek Prisma or Prisma PLUS Stainer in Expansion Configuration	One protocol	AW-19469-001
Stain Protocol using the Rinse II and Bluing II Solutions on the Medite TST 44 Stainer	One protocol	AW-19470-001
Stain Protocol using the Rinse II and Bluing II Solutions on the Hologic Compass Stainer using 95% alcohol fixed slides	One protocol	AW-19471-001



Safety Data Sheets

ThinPrep Stain Nuclear Stain Solution

ThinPrep Stain Rinse Solution

ThinPrep Stain Bluing Solution

ThinPrep Stain Orange G Solution

ThinPrep Stain EA Solution

ThinPrep Stain Rinse II Solution

ThinPrep Stain Bluing II Solution

The Safety Data Sheet (SDS) for each solution may be requested from Hologic Technical Support, or found on-line at www.hologicsds.com.



SAFETY DATA SHEETS

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Staining Protocols

The following staining protocols are available from Hologic:

Stainer	Protocol Description	Protocol Number
Hologic Compass Stainer	Stain Protocol: Hologic Compass Stainer using 95% alcohol fixed slides	AW-19464-001
Sakura Tissue-Tek DRS 2000 Slide Stainer	Stain Protocol: Sakura Tissue-Tek DRS 2000 using 95% alcohol fixed slides	AW-19453-001
Sakura Tissue-Tek Prisma Automated Slide Stainer	Stain Protocol: Sakura Tissue-Tek Prisma or Prisma PLUS using 95% alcohol fixed slides	AW-19456-001
Sakura Tissue-Tek Prisma Automated Slide Stainer	Expansion Stain Protocol: Sakura Tissue-Tek Prisma or Prisma PLUS using 95% alcohol fixed slides	AW-19462-001
Leica Autostainer XL	Stain Protocol: Leica Autostainer XL using 95% alcohol fixed slides	AW-19452-001
Leica ST5020 Multistainer	Stain Protocol: Leica ST5020 Multistainer using 95% alcohol fixed slides	AW-19458-001
Thermo Shandon Varistain Gemini Slide Stainer	Stain Protocol: Thermo Shandon Varistain Gemini autostainer using 95% alcohol fixed slides	AW-19455-001
Medite TST 44 Slide Stainer	Stain Protocol: Medite TST 44 using 95% alcohol fixed slides	AW-19460-001
Leica Autostainer XL	Stain Protocol using Rinse II and Bluing II Solutions on the Leica Autostainer XL	AW-19466-001
Leica ST5020 Multistainer	Stain Protocol using Rinse II and Bluing II Solutions on the Leica ST5020 Multistainer	AW-19465-001
Sakura Tissue-Tek DRS 2000 Slide Stainer	Stain Protocol using the Rinse II and Bluing II Solutions on the Sakura Tissue-Tek DRS 2000 Stainer	AW-19467-001
Sakura Tissue-Tek Prisma Automated Slide Stainer	Stain Protocol using the Rinse II and Bluing II Solutions on the Sakura Tissue-Tek Prisma Autostainer	AW-19468-001



STAINING PROTOCOLS

Stainer	Protocol Description	Protocol Number
Sakura Tissue-Tek Prisma Automated Slide Stainer	Stain Protocol using the Rinse II and Bluing II Solutions on the Sakura Tissue-Tek Prisma or Prisma PLUS Stainer in Expansion Configuration	AW-19469-001
Medite TST 44 Slide Stainer	Stain Protocol using the Rinse II and Bluing II Solutions on the Medite TST 44 Stainer	AW-19470-001
Hologic Compass Stainer	Stain Protocol using the Rinse II and Bluing II Solutions on the Hologic Compass Stainer using 95% alcohol fixed slides	AW-19471-001

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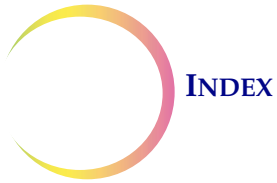
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User's Manual



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