Lab Technician's Guide to Troubleshooting: 20 Common Issues with Biological Stains

At Ethos Biosciences, we are known for providing exceptional service and quality to our customers. No matter the complexity of the issue, we get to the root of the problem and help you to solve it as quickly as possible, ensuring your lab is back up and running so you can serve patients again.

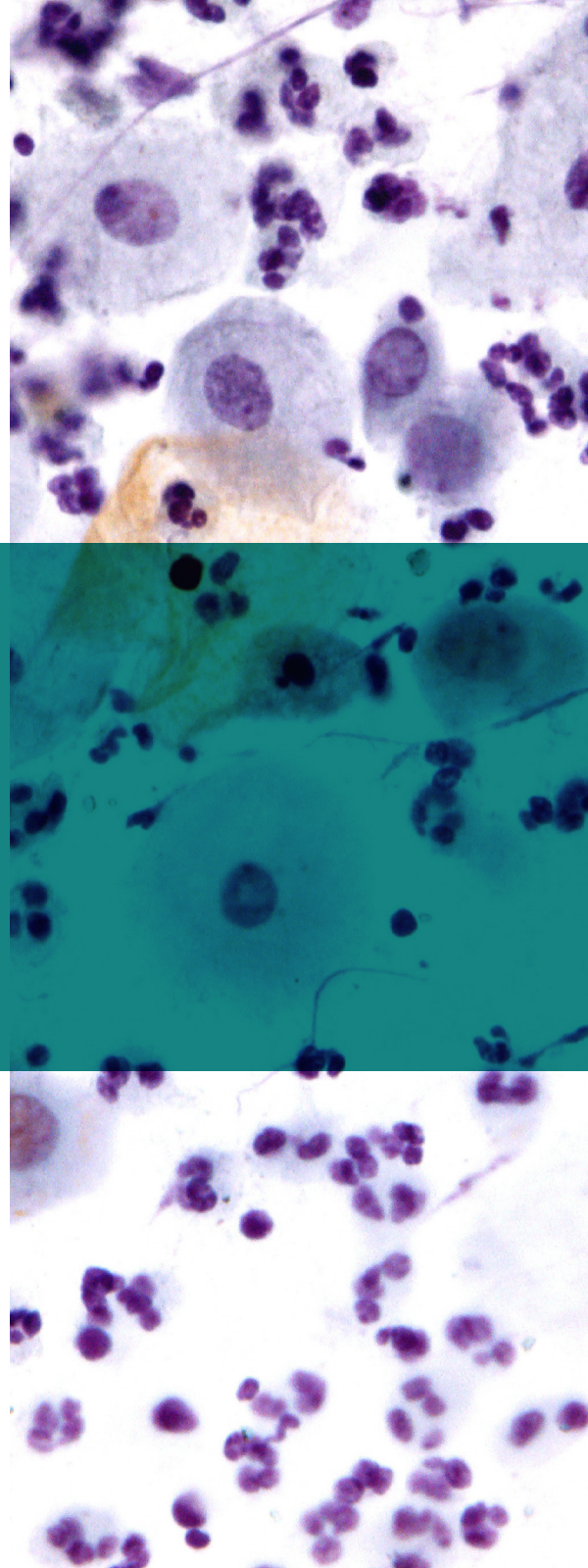
This eBook covers the main specimen types typically encountered by our lab technician customers: hematology, histology, cytology, and microbiology specimens.

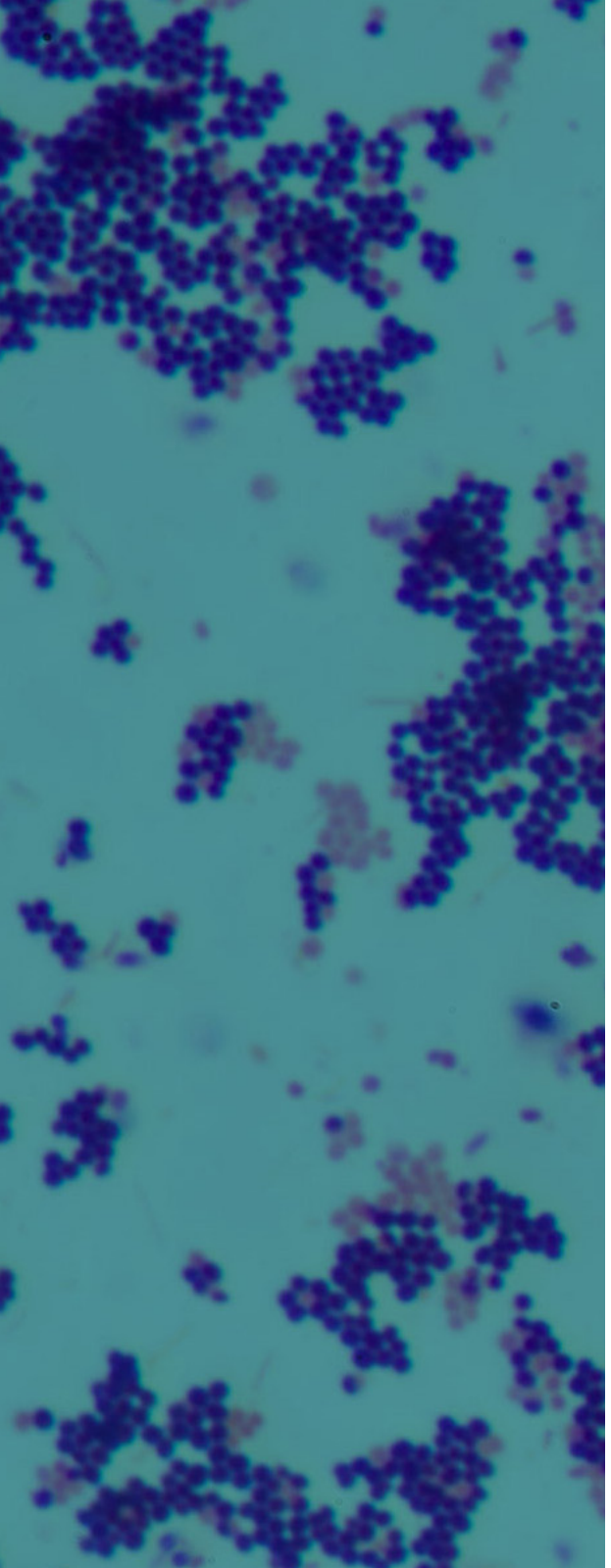
Here, we'll delve into problems you may encounter with routine stains for each of these common specimen types:

- Wright or Wright-Giemsa stains for hematology
- Harris or Gill Hematoxylin & Eosin stains for histology or cytology
- Gram or Acid Fast stains for microbiology

We also cover some common non-stain related issues.

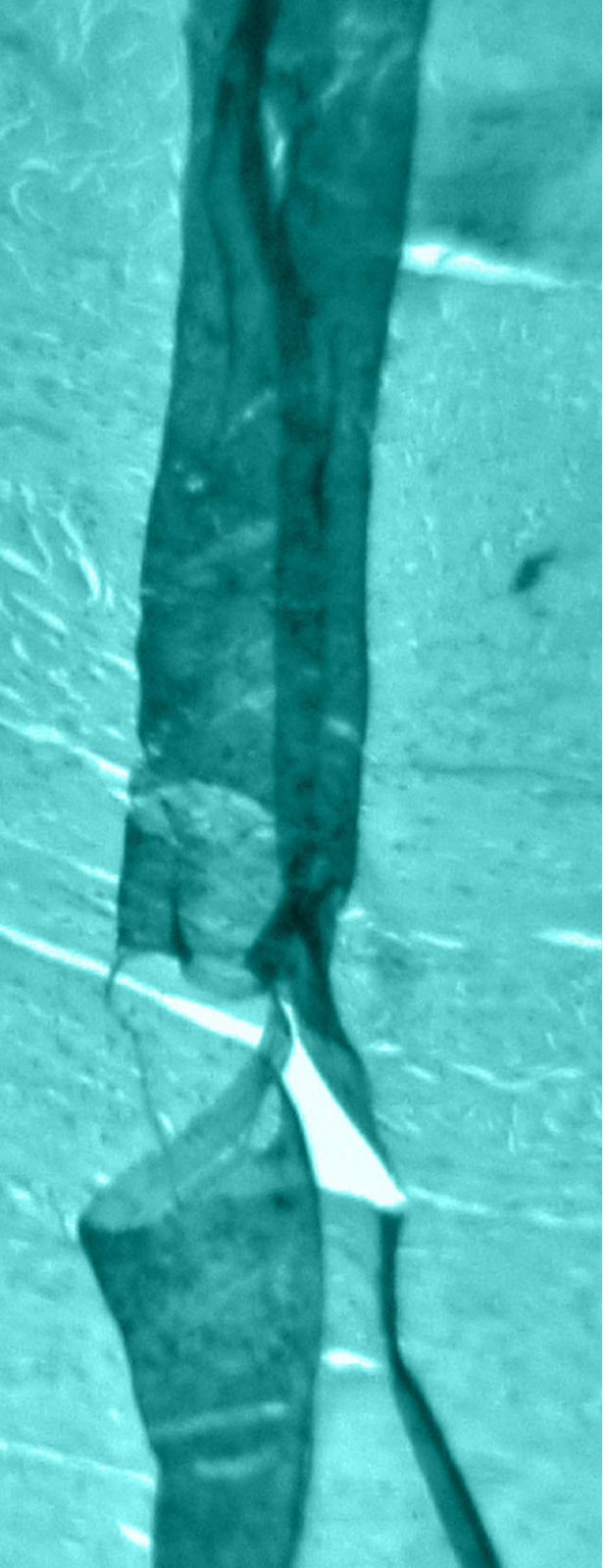
The ebook covers 20 common issues with biological stains you might experience in your lab. If you don't see the stain type or issue you're looking for here, we can still help. We'd love to hear from you. Call one of our application experts at 1-800-441-0366 or email us at info@ethosbiosciences.com



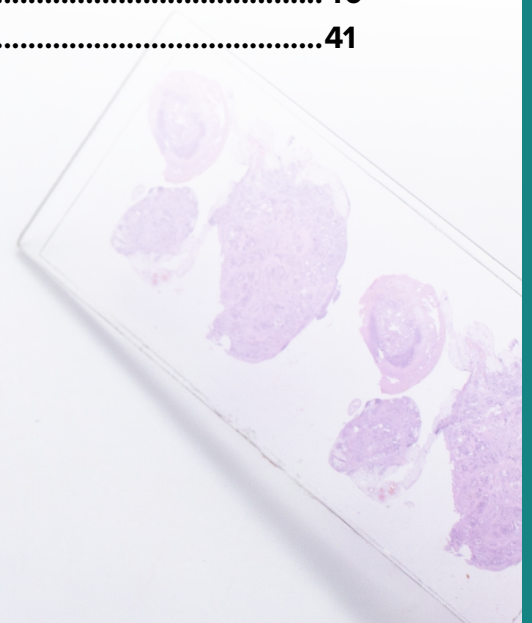


WHAT BIOLOGICAL STAINING ISSUES ARE YOU EXPERIENCING?

This section is a collection of effective solutions for some of the most common issues technicians experience in the lab. Read on to help determine the cause of these common quandaries with biological stains and what steps you can take immediately to aid in solving them.



NON-STAIN ISSUES



01 COMMON ISSUE 1: BASOPHILIC STAINING IS WEAK OR HAS POOR DEFINITION

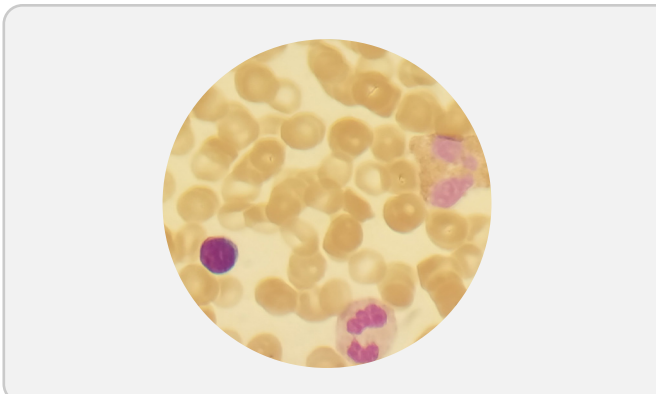
USING QUICK III™ OR 3-STEP STAIN

When lab technicians contact us with a "QUICK III," or 3-Step, hematology stain with poor definition in the specimen, there are three potential ways to solve this:

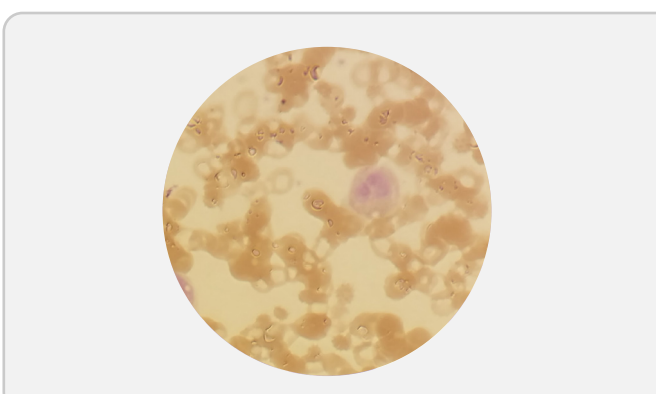
1. First try increasing the time in Solution II (blue, basophilic stain) to enhance the intensity of the basophilic/white blood cells, which are typically harder to stain.

2. Be sure you are properly blotting the slide between stain Solution I and II, so each solution operates as intended. If you are not adequately blotting, Solution I (red, eosin stain) could be carried into Solution II (basophilic stain) thereby diluting Solution II or lead to further staining with Solution I.

3. Swap out your third solution (Solution II) for a fresh solution. If there is carryover from Solution I to Solution II, particularly if Solution II is quite low, it can result in more intense eosinophilic staining.



QUICK III: good stain example



QUICK III: basophilic staining is weak

01 COMMON ISSUE 1: BASOPHILIC STAINING IS WEAK OR HAS POOR DEFINITION

USING WRIGHT OR WRIGHT-GIEMSA STAIN

Reduce the time between preparation of the smear and fixation. If the slide is not fixed quickly, the intensity of the stain will decrease.

Change out the stain/buffer solution.
The most important part of the procedure for a well-defined basophilic stain is the stain/buffer mix, usually 1:5 (20% stain, 80% buffer). You might initially think to change out the 100% stain solution, however, the actual staining takes place in the stain/buffer solution. If the stain/buffer solution has not been changed in over six hours, it's time to change out the solution to ensure intensity in staining remains strong.

You can also increase the concentration of your stain/buffer solution.
Some labs prefer to use a 1:10 ratio (10% stain, 90% buffer). Increasing the ratio of stain to 1:5 (20% stain, 80% buffer) can often improve definition. Switch from pH 6.8 to pH 7.2 buffer. The stains and the buffer – in combination – make the definition between nuclei and cytoplasm more intense. Tweak the buffer combination based on what you want to see under each stain. If you're looking for a middle-of-the-road nucleus definition, choose a 6.8 pH buffer. If you're looking for more eosinophilic definition, choose a pH 7.2. If you're not getting the definition you seek, switch from a 6.8 pH to a 7.2 pH.

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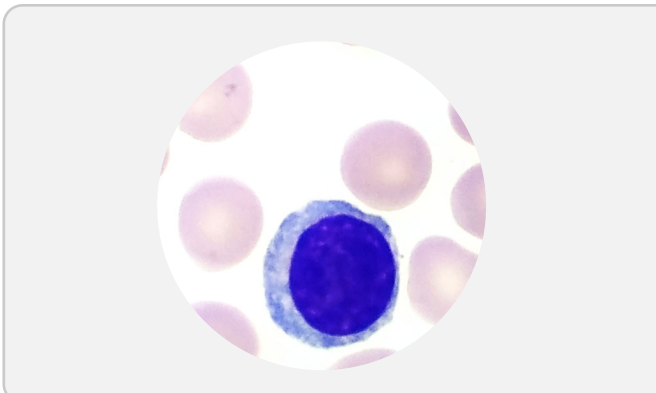
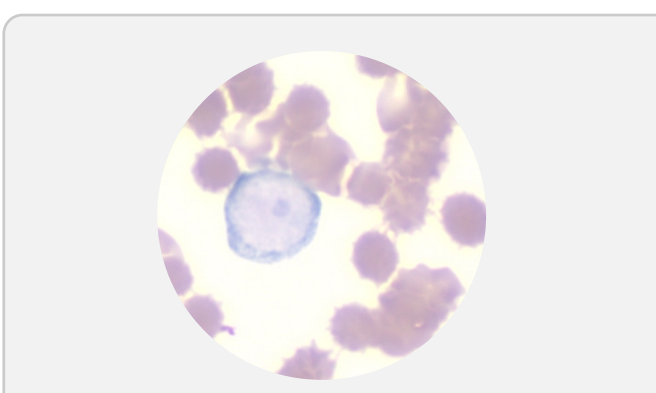


Wright or Wright-Giemsa stain: good stain example



Wright or Wright-Giemsa stain: basophilic staining is weak

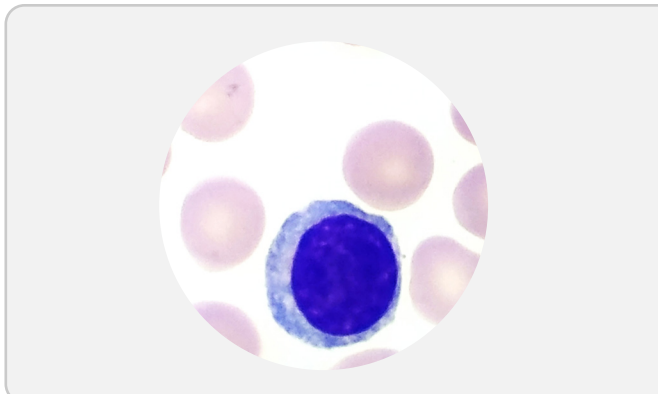
01 COMMON ISSUE 1: BASOPHILIC STAINING IS WEAK OR HAS POOR DEFINITION

USING WRIGHT OR WRIGHT-GIEMSA STAIN

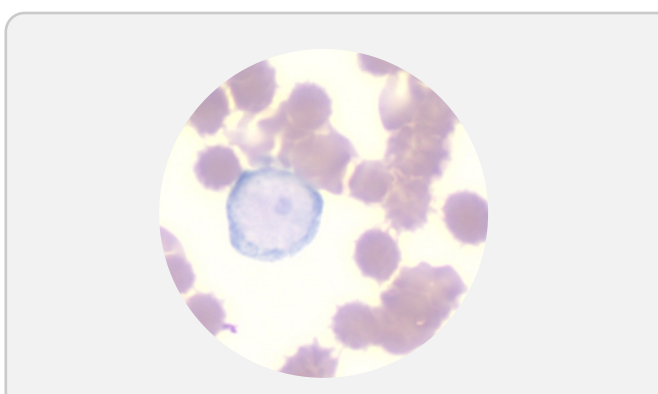
Continued from previous page

If that is unsuccessful, we recommend switching from Wright stain to a Wright-Giemsa stain. The Wright-Giemsa stain is formulated to produce more intense basophilic/nuclear staining, so making this change will enhance the definition in the proper direction.

Weak staining of white cell morphology or undefined neutrophils:
If you're not seeing a defined outline of white blood cells or if the purple nucleus within a neutrophil is not easily visible, you may be experiencing weak Wright or Wright-Giemsa stain causing a lack of definition within the specimen.



Wright or Wright-Giemsa stain: good stain example



Wright or Wright-Giemsa stain: basophilic staining is weak

01 COMMON ISSUE 1: BASOPHILIC STAINING IS WEAK OR HAS POOR DEFINITION

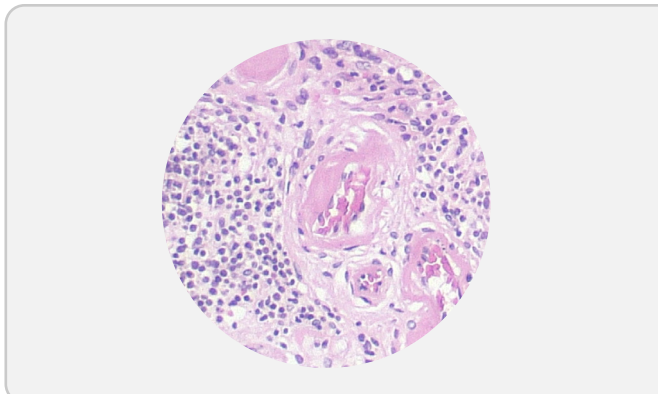
USING HARRIS H&E STAIN

Harris hematoxylin is a regressive staining technique, which means the tissue section is first overstained with hematoxylin, then excess dye is removed during the differentiation step in a weak acid alcohol. Weak basophilic (nuclear) staining in an H&E stain is caused when the hematoxylin is too weak or wasn't exposed to the specimen for adequate time. We recommend:

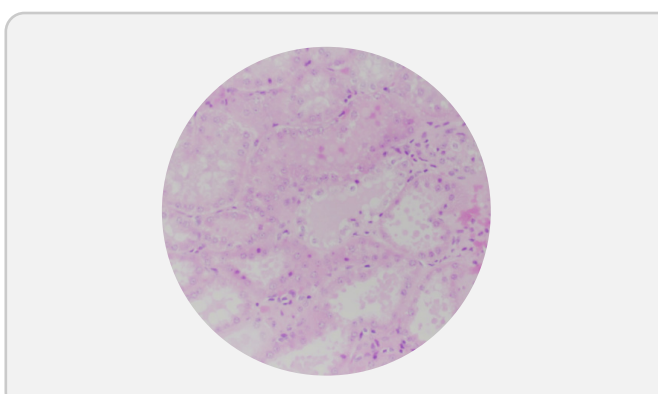
Exposure time in hematoxylin is too limited: To solve exposure time issues, simply adjust the length of time the slide is in the hematoxylin stain solution until you see the strength in staining you require.

Poor fixation or processing: Another cause of weak basophilic staining is poor fixation or tissue processing. This can mean that the stain doesn't bind to the tissue; this is an issue with preparing the tissue. See also page 32, Fixation Issues, for more help.

pH of hematoxylin out of range: If the hematoxylin pH is diluted past the ideal range provided by manufacturer, minimize any carry over of water to the hematoxylin solution during staining.



Harris hematoxylin: good stain example



Harris hematoxylin: basophilic staining is weak

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01 COMMON ISSUE 1: BASOPHILIC STAINING IS WEAK OR HAS POOR DEFINITION

USING HARRIS H&E STAIN

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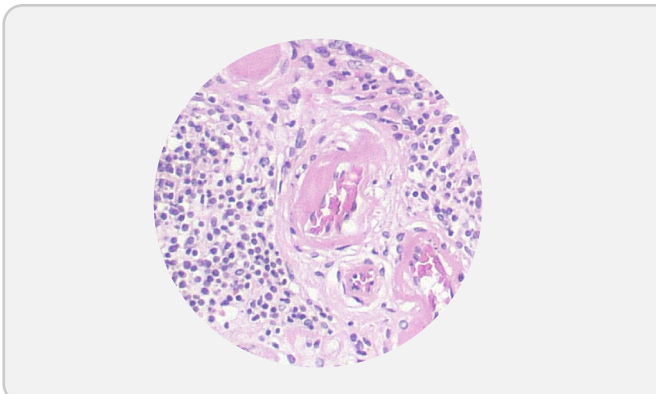
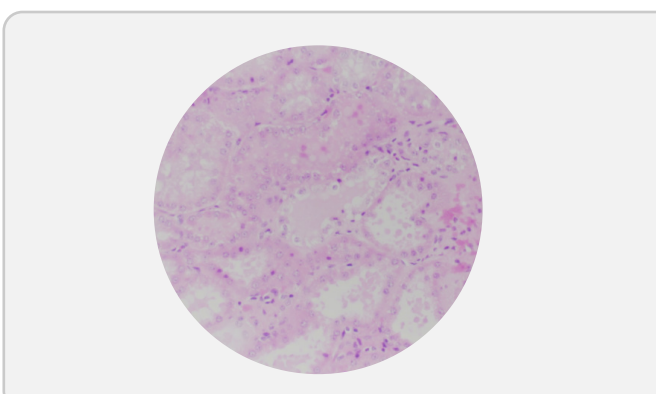
Weak solution penetration: For H&E stains on specimens that have been paraffin embedded, if the basophilic staining is too light, it may be that the paraffin didn't dissolve out of the tissue section completely. If there's wax remaining in the tissue section, the staining solution can't penetrate the specimen properly, which leads to weak staining. Return slide to fresh xylene to remove paraffin wax from section then proceed with procedure to re-stain tissue specimen.

Acid alcohol concentration is too high: If the acid alcohol is too strong for the differentiation step, or if the specimen spent too much time in acid alcohol, too much of the stain may have been removed using this regressive staining technique. To solve, select a differentiating solution with a lower concentration (i.e. change from 1% to 0.5% solution) or decrease the specimen's time in differentiating solution.



Harris H&E stain: good stain example



Harris H&E stain: basophilic staining is too weak

01 COMMON ISSUE 1: BASOPHILIC STAINING IS WEAK OR HAS POOR DEFINITION

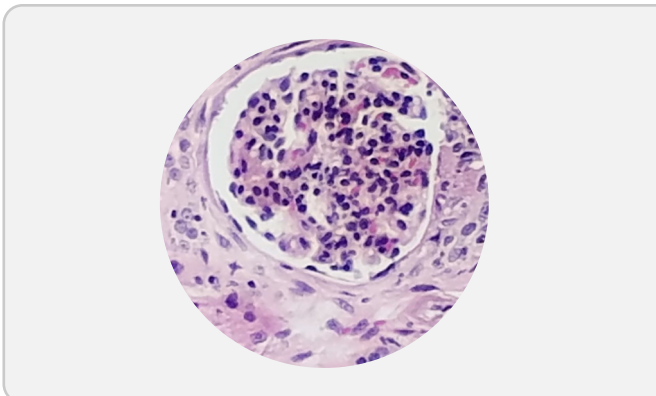
USING GILL H&E STAIN

Gill Hematoxylin is a progressive stain, which means the stain is administered to the tissue section without the need of a subsequent step in a differentiator (generally, acid alcohol) to remove excess basophilic stain.

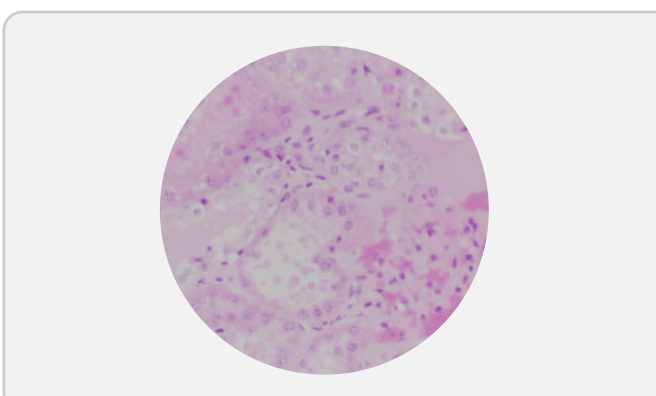
Exposure time in hematoxylin is too limited: To solve for inadequate exposure time, increase the length of time the slide is in the hematoxylin stain until you see the intensity in staining that's required.

pH of hematoxylin out of range: To solve for hematoxylin being diluted past the ideal range of pH 5-6 or other provided by the manufacturer, minimize any carry-over of water to the hematoxylin solution during staining.

pH of water out of range: Check pH of water prior to staining and use deionized or distilled water to replace tap water when possible. This can solve the issue of having too much chlorine in water, which can act as a bleaching agent and weaken the hematoxylin stain.



Gill H&E: good stain example



Gill H&E: basophilic staining is weak

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01 COMMON ISSUE 1: BASOPHILIC STAINING IS WEAK OR HAS POOR DEFINITION

USING GILL H&E STAIN

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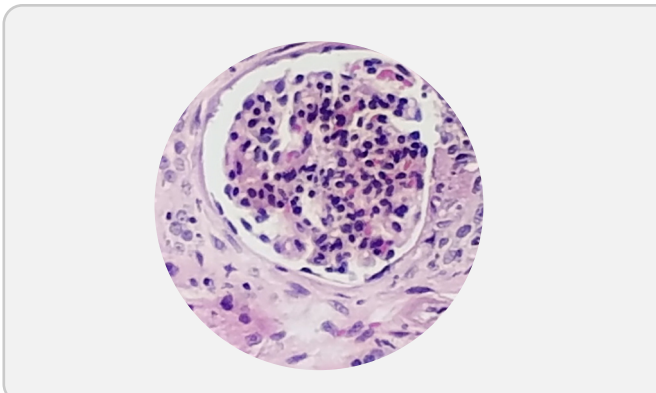
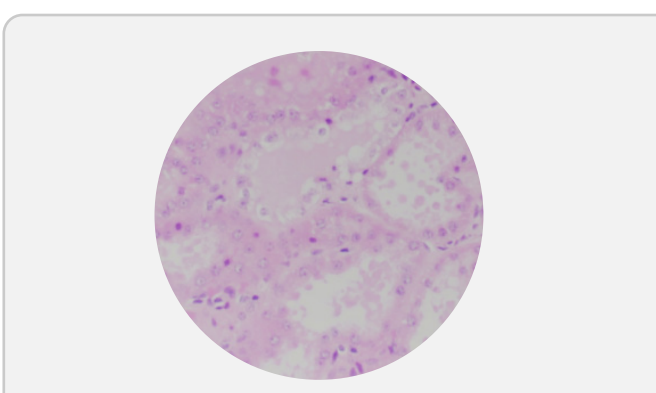
Another cause of weak basophilic staining is poor fixation or processing. This can mean that the stain doesn't bind to the tissue; this is an issue with preparing the tissue. See page 32 about fixation issues for more help.

Weak solution penetration: For H&E stains on specimens that have been paraffin embedded, if the basophilic staining is too light, perhaps the paraffin didn't dissolve out of the tissue section completely. If there's wax in the tissue section, the water-based staining solution can't penetrate the specimen properly, leading to weak staining. Return slide to fresh xylene to remove paraffin wax from section then proceed with procedure to re-stain tissue specimen.

Hematoxylin is the wrong strength: Consider changing the strength of your Gill Hematoxylin. Intensity of hematoxylin stain can be increased or decreased based on the strength of stain selected. For example: Gill 1x is the weakest stain, Gill 2x provides a stronger staining intensity than Gill 1X. Gill 3x is the strongest and provides the greatest staining intensity in comparison.



Gill H&E Stain: good stain example

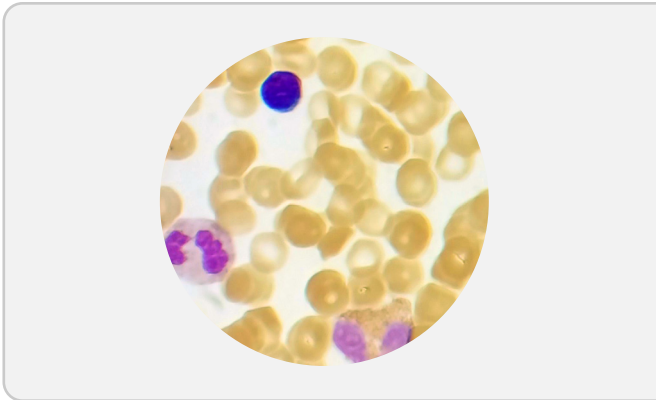


Gill H&E Stain: basophilic staining is weak

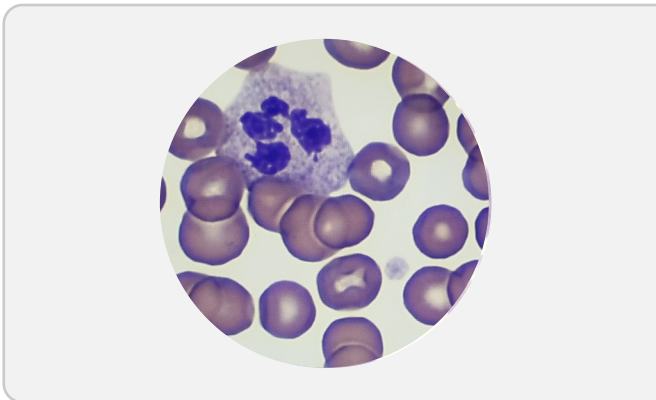
02 COMMON ISSUE 2: BASOPHILIC STAINING IS TOO STRONG OR "TOO BLUE"

USING QUICK™ OR 3-STEP STAIN

When you observe basophilic staining that is too strong when using a 3-step stain, replace with fresh solution of Solution I (red, eosin stain), which is the solution that stains eosinophilic components and red blood cells a reddish/pink color. You can also increase time in Solution I and decrease time in Solution II, which stains the basophilic cellular components blue.



QUICK III: good stain example



QUICK III: basophilic staining is too blue

02 COMMON ISSUE 2: BASOPHILIC STAINING IS TOO STRONG OR “TOO BLUE”

USING CLASSICAL WRIGHT OR WRIGHT-GIEMSA

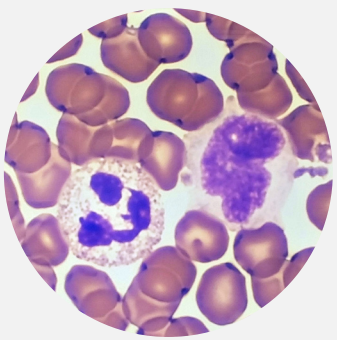
If you observe nuclear staining that is “too blue,” potential solutions are:

Reduce time in stain/buffer mix.

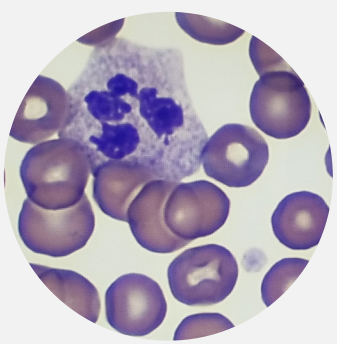
You might naturally think to reduce time in the 100% stain solution, but in reality, you should reduce time in the stain/buffer solution, as this is where the staining of the specimen actually occurs.

Try switching from 7.2 pH to 6.8 pH buffer.

If you’re using a pH 7.2 buffer, the higher pH might make the stain too blue. To resolve, try switching to 6.8 pH, which will increase the intensity of the eosinophilic (red) cellular components. Additionally, you can reduce the concentration of stain in the stain/buffer solution from 1:5 (20% stain, 80% buffer) to 1:10 (10% stain, 90% buffer).



Wright or Wright-Giemsa: good stain example



Wright or Wright-Giemsa : basophilic staining is too strong

02 COMMON ISSUE 2: BASOPHILIC STAINING IS TOO STRONG OR "TOO BLUE"

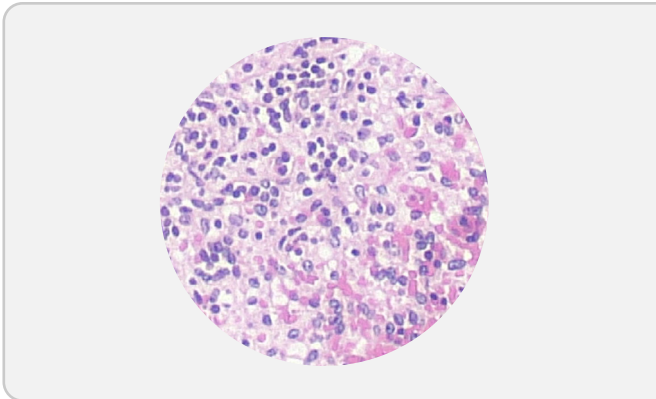
USING HARRIS H&E

Harris hematoxylin is a regressive stain. The tissue section is overstained, then excess dye is removed with acid alcohol until you reach the target intensity and differentiation. In an H&E stain where the basophilic staining is too strong, it might be that the hematoxylin is not being adequately removed in the acid alcohol or the specimen has been exposed for too long. To resolve this:

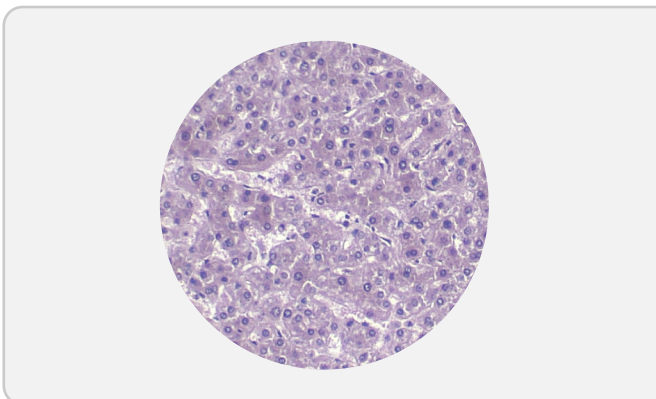
Reduce the time the specimen is in the Harris Hematoxylin stain. Adjust stain time until the correct color intensity is achieved.

Try increasing the differentiating time in acid alcohol or increasing the concentration of the acid alcohol solution used (i.e. change from 0.5% to 1%) to aid in the removal of excess hematoxylin stain from the tissue.

Change type of Harrison Hematoxylin to an 'acidified' Harris Hematoxylin," and use as a progressive rather than regressive stain. This will allow more consistent staining results, even for a beginner.



Harris hematoxylin : good stain example



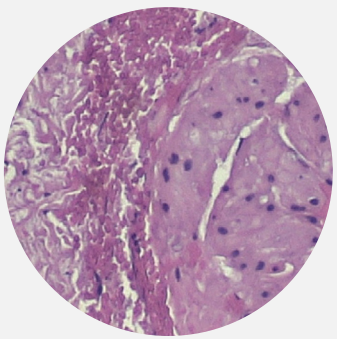
Harris hematoxylin : basophilic staining is too strong

02 COMMON ISSUE 2: BASOPHILIC STAINING IS TOO STRONG OR “TOO BLUE”

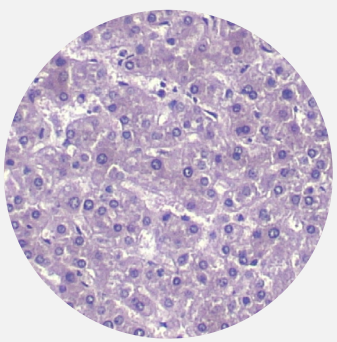
USING GILL H&E STAIN

Gill Hematoxylin is a progressive stain, which means the stain is administered to the tissue section without the need of a differentiator (generally, acid alcohol), to remove excess basophilic staining. In an H&E stain where the basophilic staining is too strong, it might be that the hematoxylin is too concentrated or the specimen has been exposed for too long. Try reducing the time the specimen is in the Gill Hematoxylin to reduce the staining intensity.

Gill Hematoxylin is available in three strengths: 1X, 2X, and 3X. Intensity increases as numbers increase. Make sure you are selecting the appropriate strength for your specimen type. Gill 1X is ideal for cytology specimens, whereas Gill 3X is ideal for histology specimens. Gill 2X is in the middle making it a good choice for either cytology or histology specimen.



Gill H&E stain : good stain example



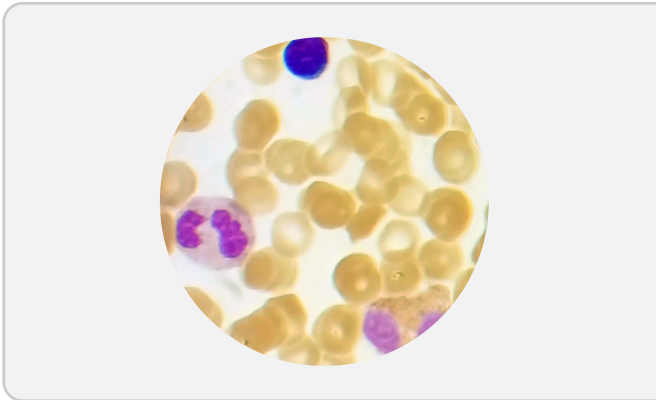
Gill H&E stain: basophilic staining is too strong

03 COMMON ISSUE 3: EOSINOPHILIC STAINING IS WEAK OR INTENSITY IS POOR

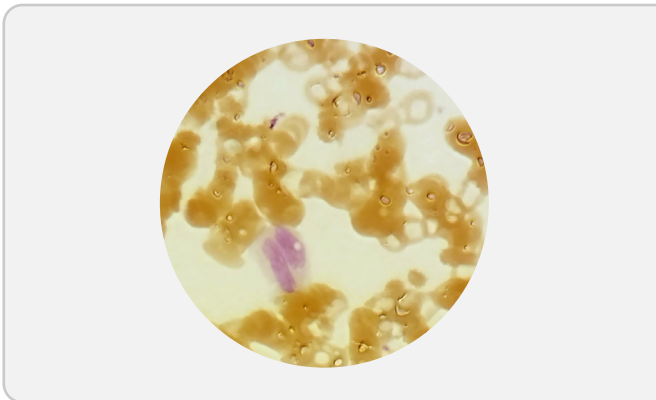
USING QUICK™ STAIN

As eosin provides the pink color to the cytoplasm within cells, weak eosinophilic staining would cause the specimen features to blend together, making it difficult to differentiate between the cell structure, collagen, muscle, and red cells within the specimen. There are some simple procedural changes to make to solve this problem.

To solve this issue, replace Solution I (red, eosin stain), which is the solution that conducts the staining of eosinophilic components and red blood cells a reddish/pink color. You can also increase time in Solution I and decrease time in Solution II, which stains the basophilic cellular components blue.



QUICK III: good stain example



QUICK III: eosinophilic staining is weak

03 COMMON ISSUE 3: EOSINOPHILIC STAINING IS WEAK OR INTENSITY IS POOR (red blood cell/eosinophilic intensity)

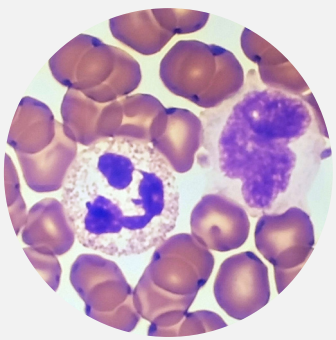
CLASSICAL WRIGHT OR WRIGHT-GIEMSA STAIN

This classical hematology stain, often an automated procedure, can require some specific adjustments to ensure the proper visual intensity in your specimens. If staining is sub-optimal there are typically three common issues we walk through to identify the cause and solution.

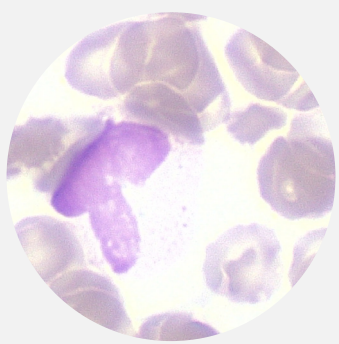
1. The specimen structure was degraded. If degradation of the specimen causes poor intensity due to a delay between fixation and staining, proceed by fixing a fresh smear and then move forward with your normal staining protocol. Adjust your procedure to fix immediately after performing the smear to avoid this issue.

2. The pH of the buffer needs to be more eosinophilic. Switching from a 7.2 pH buffer to 6.8 pH buffer will strengthen the buffer to be more eosinophilic, leading to greater intensity or distinction between in the red blood cells and eosinophilic morphology.

3. The buffer ratio has been compromised. Ensure that the buffer/stain mix ratio has not been altered by over-use and to be safe, swap the solution for a new mix. This should be done every eight hours to ensure strong intensity.



Wright or Wright-Giemsa stain: good stain example



Wright or Wright-Giemsa stain: eosinophilic staining is weak

03 COMMON ISSUE 3: EOSINOPHILIC STAINING IS WEAK OR INTENSITY IS POOR

USING H&E STAIN

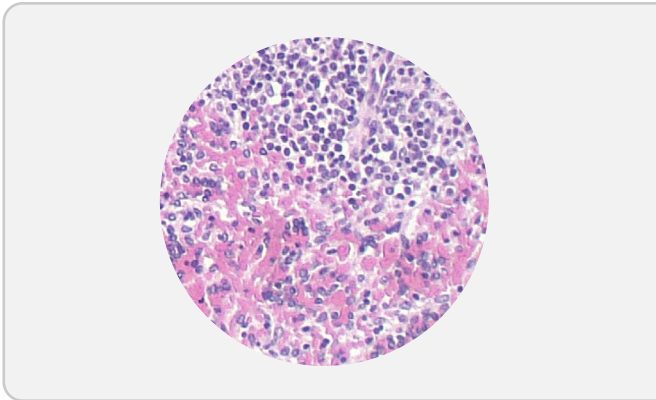
There may be several reasons for weak eosinophilic staining in your specimens.

The specimen was not exposed to the eosin for enough time.
Typically this would not be the case considering the rapid staining ability of eosin; nevertheless, a lack of sufficient dye exposure could cause staining to appear too light, or weak. To correct this issue, adjust your procedure to extend the exposure time in the eosin solution.

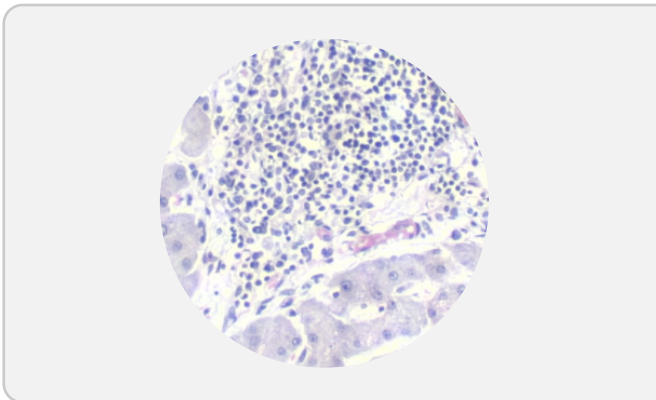
The specimen was subject to an exhausted eosin solution. The eosin solution will need to be discarded and replaced with fresh eosin.

The alcohol used for rinsing was not selected appropriately for the staining procedure. If a lower concentration of alcohol was used (i.e. 70% ethanol) it will extract too much eosin from the tissue section than expected. Replace with a 95% ethanol rinse after staining in eosin solution.

The specimen was exposed to an eosin with an increased pH (greater than 4.5). Confirm pH is between 4.0 and 4.5. If needed, pH can be adjusted by adding fresh eosin or adding concentrated acetic acid.



H&E stain: good stain example



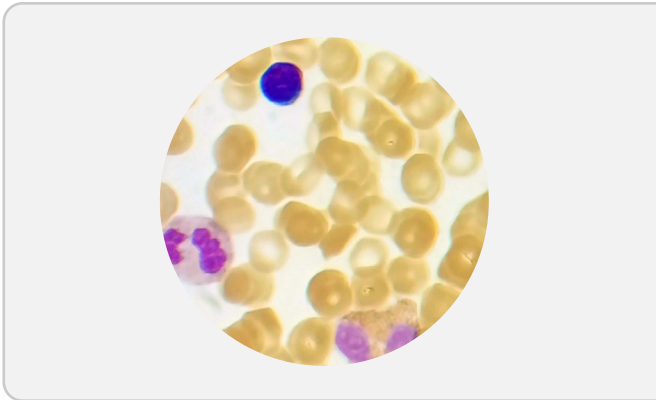
H&E stain: eosinophilic staining is weak

04 COMMON ISSUE 4: EOSINOPHILIC STAINING IS TOO STRONG, TOO INTENSE, OR "TOO PINK"

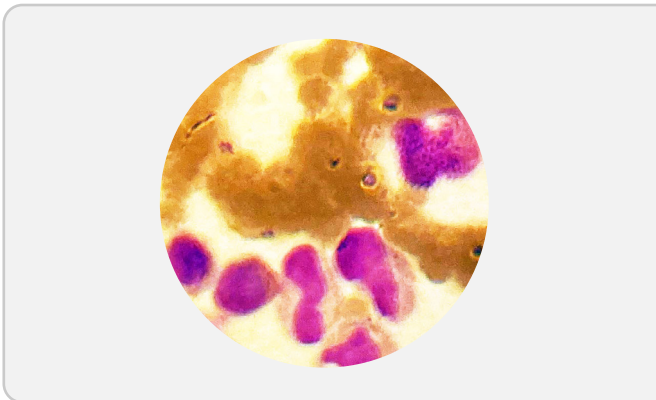
Try these two ways to prevent eosinophilic staining that is too intense using your QUICK™ stain procedure.

First, be sure you are properly blotting the slide between Solution I and II (reagents two and three) so each solution operates as intended. If you are not adequately blotting, Solution I (red, eosin stain) could be carried into Solution II (blue, basophilic stain) resulting in dilution of Solution II or further staining of Solution I.

Second, swap out your Solution I (red, eosin stain) for a new bath. If there is carryover from Solution I to Solution II, particularly if Solution II is quite low, it can result in more intense eosinophilic staining.



QUICK stain: good stain example



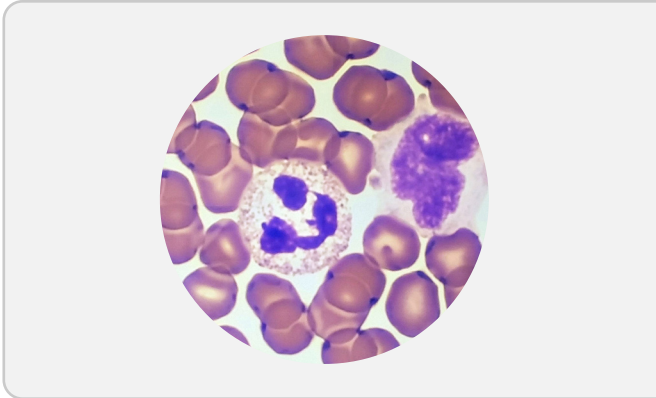
QUICK stain: eosinophilic staining is too strong

04 COMMON ISSUE 4: EOSINOPHILIC STAINING IS TOO STRONG, TOO INTENSE, OR "TOO PINK"

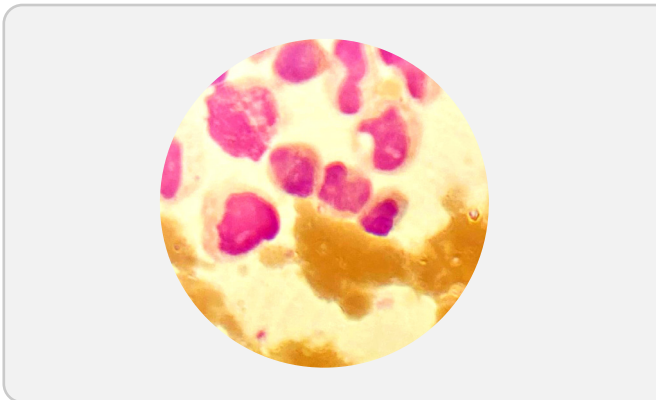
USING CLASSICAL WRIGHT OR WRIGHT-GIEMSA STAIN

If you find that your specimen's stain intensity is too strong when using a Wright-Giemsa stain in an autostainer, try switching to a 7.2 pH buffer from a 6.8 pH buffer to cut back on the eosinophilic properties of the lower-pH level buffer.

If that is unsuccessful, we recommend switching from Wright stain to a Wright-Giemsa stain. The Wright-Giemsa stain is formulated to produce more intense basophilic/nuclear staining, so making this change will enhance the definition in the proper direction.



Wright or Wright-Giemsa stain: good stain example



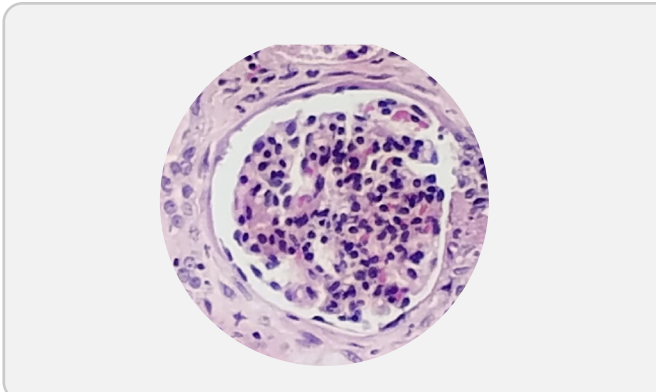
Wright or Wright-Giemsa stain: eosinophilic staining is too strong

04 COMMON ISSUE 4: EOSINOPHILIC STAINING IS TOO STRONG, TOO INTENSE, OR "TOO PINK"

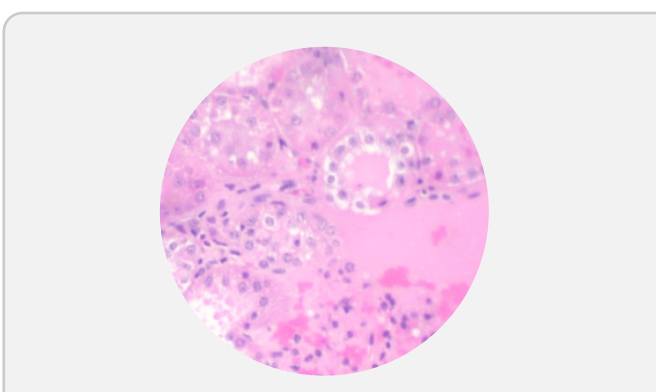
USING H&E STAIN

As eosin provides the pink color to the cytoplasm within cells, excess eosinophilic staining would cause the specimen features to blend together, making it difficult to differentiate between the cell structure, collagen, muscle, and red blood cells within the specimen. There are some simple procedural changes you can make to solve this problem.

Most frequently, this problem is caused when the specimen sections are exposed to the eosin for too long. This may mean that the current staining schedule is too long for the eosin you've purchased. To solve this, the stainer needs to be set to the appropriate time. The eosin product you have selected likely comes with staining recommendations; check these recommendations to help adjust to the appropriate exposure time in the stain.



H&E stain: good stain example



H&E stain: eosinophilic staining is too strong

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04 COMMON ISSUE 4: EOSINOPHILIC STAINING IS TOO STRONG, TOO INTENSE, OR "TOO PINK"

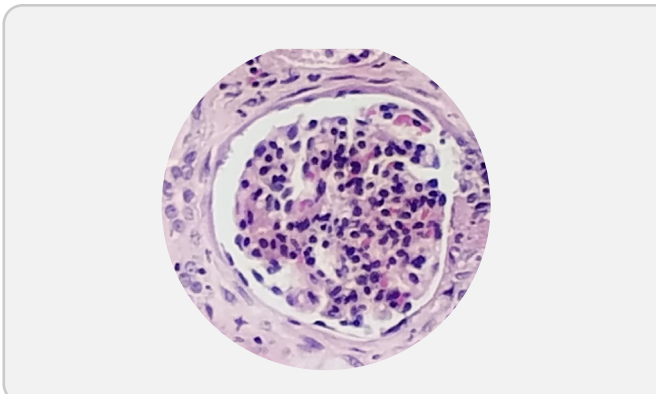
USING H&E STAIN

Continued from previous page

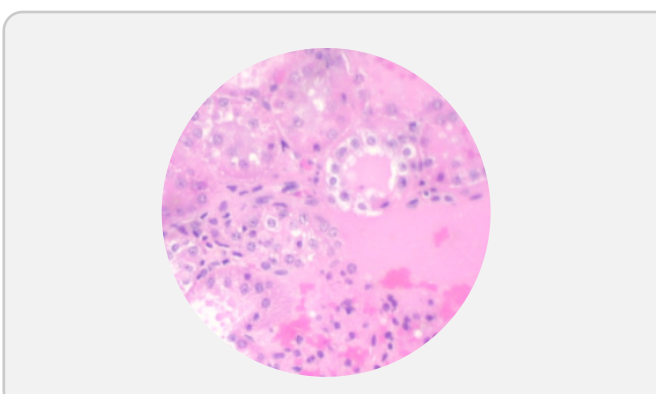
It's also possible that you've inadvertently selected the improper alcohol for the H&E stain. For example, isopropyl alcohol doesn't differentiate the eosin like ethanol does, so if isopropyl is being used, simply swap for an ethanol alcohol.

Improper alcohol concentration will also cause a pink color that is too strong because it's not pulling out the eosin effectively. The sweet spot is typically a 95% ethanol solution for a quick rinse followed by rinses in 100% ethanol. Using only 100% ethanol rinse is not effective at removing the eosin from the specimen.

Finally, check to see what concentration of eosin you are using. Eosin Y 1% solution is a more concentrated solution. If you are using the 1% solution and showing up too intensely, you may want to try Eosin Y 0.5% instead.



H&E stain: good stain example



H&E stain: eosinophilic staining is too strong

05 COMMON ISSUE 5: ACID-FAST ORGANISM NOT STAINING PROPERLY

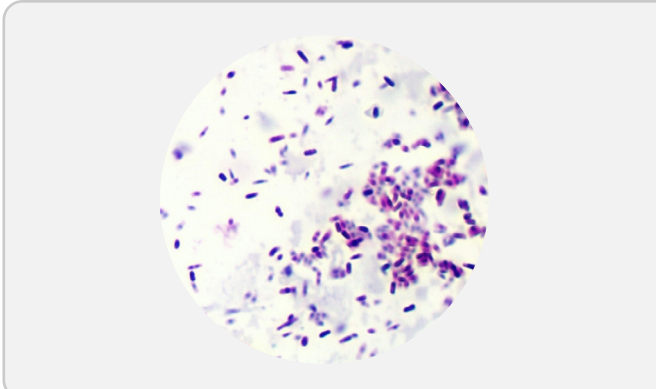
Acid-fast organisms have cell walls that are resistant to conventional staining. Acid-fast stains work to promote the dye uptake. Additionally, once stained, these organisms are not easily decolorized (even with alcohol or acid acetone solutions). This is where they get the name “acid-fast.” The decolorizer is used to remove the dye from any non-acid-fast material in the specimen.

The common issue that occurs with rapid growers, acid-fast organisms, is that the organism doesn’t retain the acid-fast dye and it fails to stain properly. As a result, the organism shows little-to-no carbol fuchsin coloring.

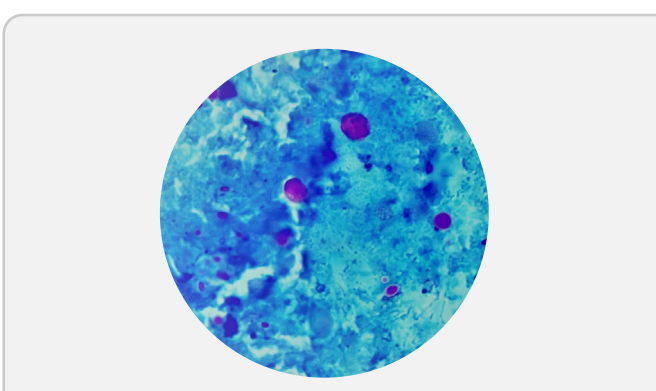
If the acid-fast organism isn’t retaining the dye as expected, perform a Modified Kinyoun stain protocol instead. This modifies the stain as a weak acid-fast stain, using sulfuric acid instead of hydrochloric acid.

- Flood the slide with Kinyoun’s carbol fuchsin for 5 minutes.
- Rinse the slide briefly (3-5 seconds) with 50% ethanol.
- Decolorize with 1% sulfuric acid for 2 minutes, or until no more color runs from the slide.
- Rinse the slide with water, then drain.
- Counterstain with methylene blue or brilliant green for 1-3 minutes.
- Rinse with water, then air dry.

The weak acid-fast stain will stain organisms that were not able to maintain the carbol fuchsin after decolorizing.



Acid-fast positive *Mycobacterium*



Acid-fast positive *Cryptosporidium* in fecal sample

06 COMMON ISSUE 6: GRAM STAIN DECOLORIZING EXCESSIVELY

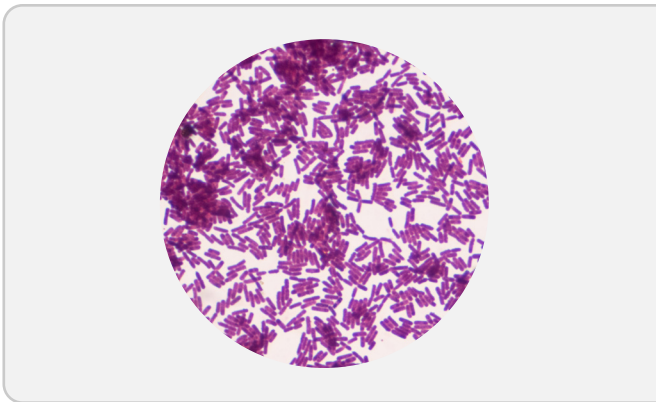
Typically, misdiagnosis of Gram stains are due to issues with the stain protocol and Gram stains decolorizing too quickly (suggesting Gram-positive bacteria) or too slowly (suggesting Gram negative bacteria). Try these tips to solve the issue of excessive decolorizing:

Reduce heat: Excessive heat alters cell morphology and breaks down cell walls, making cells more easily decolorized. Reduce the heat to reduce the risk of speedy decolorization.

Reduce water rinse time: Excessive washing can cause too much decolorization. Crystal Violet is susceptible to washout with water before binding to Iodine. Do not use more than a 5 second water rinse at any stage of the procedure.

Increase iodine exposure: Insufficient iodine exposure can negatively affect decolorization. Keep iodine bottles closed when not in use. Closed bottles lose more than 50% of available iodine in 30 days, while open bottles lose more than 90%.

Counterstain: Safranin and Crystal Violet are both basic dyes; Safranin can replace the Crystal Violet-Iodine complex when specimen is overexposed to the counterstain.



Gram stain: positive

Gram stain: negative

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06 COMMON ISSUE 6: GRAM STAIN DECOLORIZING EXCESSIVELY

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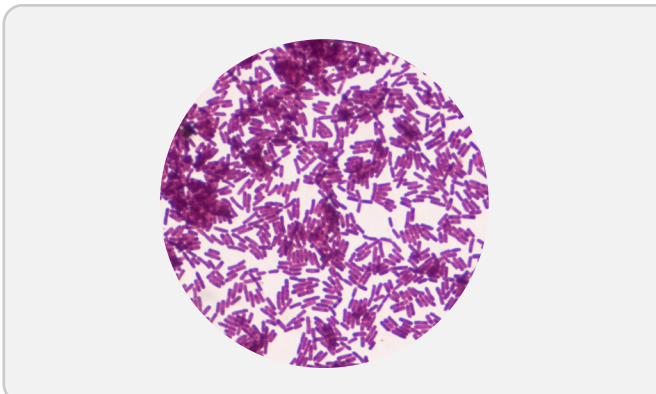
Change your decolorization length of time: Reduce the time in the solvent and rinse quickly, or use a decolorizing solution that is easier to control for the decolorizing step. The acetone percentage determines rate of decolorizing - 25% acetone is the slowest, and 75% acetone is the fastest.

Check the control slides: If the control slide is over-decolorized, then most likely the specimen will exhibit the same issue.

Change provider: The main factor for decolorizer is the acetone. A 50% acetone will decolorize much quicker than the 25/75. If you switch suppliers or make your own, it can impact performance. You will need to adjust the procedure accordingly.

Practice: Each type of specimen retains dyes differently than others. If there's a lot of mucus or if there are a lot of bacteria (stool sample), the specimen could hold on to color much more. Experience and practice over time will make these differences more apparent, so that you're able to adjust your expectations.

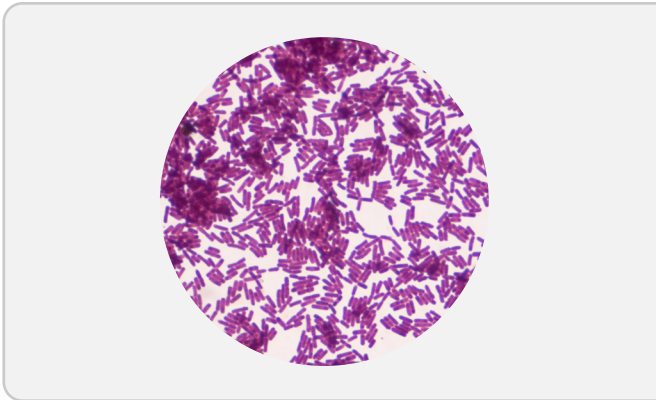


Gram stain: positive

Gram stain: negative

07 COMMON ISSUE 7: GRAM STAIN DECOLORIZING TOO SLOWLY

If the Gram stain is falsely negative, it is decolorizing too slowly. If you don't decolorize quickly enough, or conversely, if you keep the colorizer on too long, you'll get a false negative for the Gram positives. Adjust the time the specimen is in the colorizer and remove it sooner to avoid this issue.



Gram stain: positive

Gram stain: negative

08 COMMON ISSUE 8: PRECIPITATE ON THE SLIDE

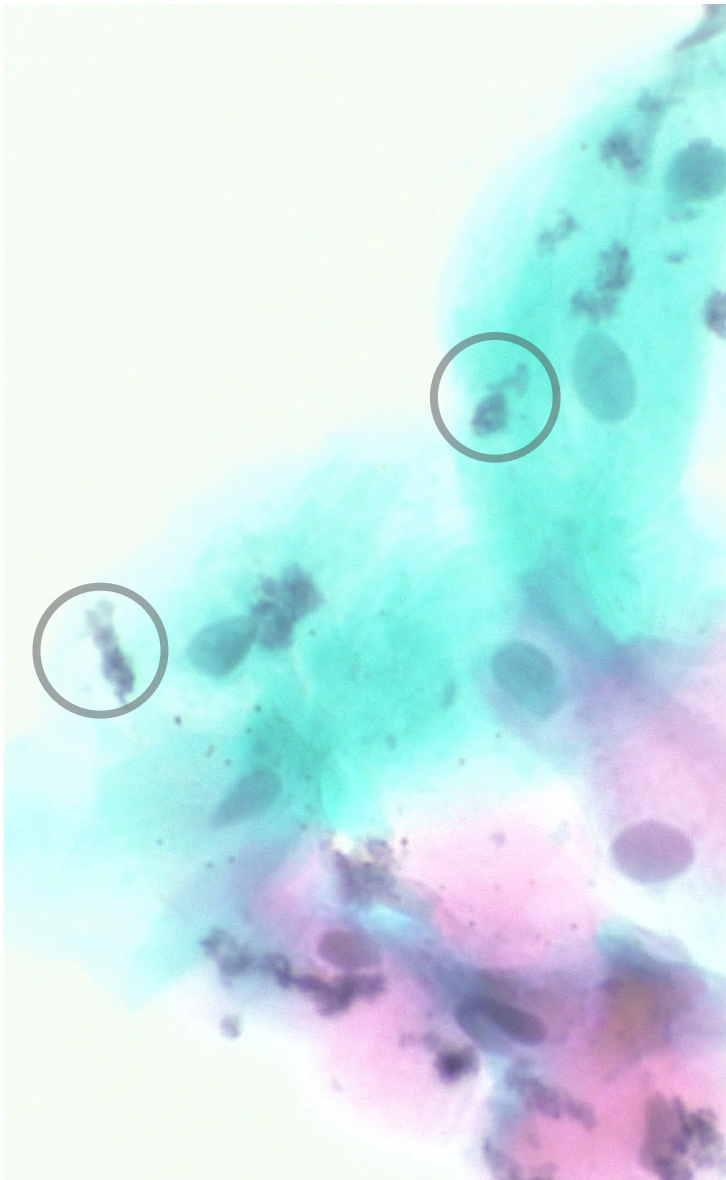
Precipitate on the slide can be caused by a few different procedural issues. Check your procedures to find if any of these remedies would be appropriate for your slides.

Time to swap solutions out and clean equipment: If the stain, stain/buffer and buffer have been sitting for a while, they canform a precipitate. To address, run a cleaning cycle on your autostainer and start with fresh solutions. Maintaining a cleaning schedule can improve consistency of slide staining.

Filter the stain: The stain solution may require filtration if precipitation is showing in the slide. Sometimes the stain has been sitting too long and can separate out of solution. Though manufacturers filter the stain, the laboratory's SOP should include a step to re-filter prior to use.

Dilute the stain: If you have the intensity that is required but also have precipitate, try diluting the stain with the carrying matrix (typically methanol or water). Dilute the stain 5%, shake well and then filter.

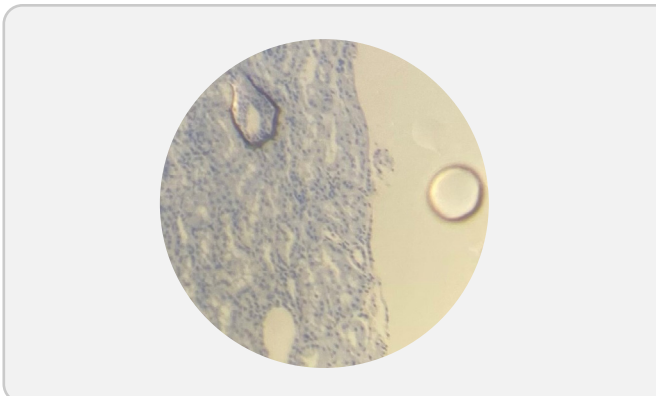
Keep at room temperature: If the stain is cold (from transit or storage), warm it to room temperature and then shake well to ensure it hasn't separated.



09 COMMON ISSUE 9: CLOUDINESS IN THE SLIDE

Cloudiness in the slide is caused by insufficient clearing after the staining process using an H&E stain.

H&E staining requires a series of dehydration and clearing steps prior to slide coverslipping in preparation for microscopic analysis. The slides are first placed into a 95% alcohol solution after staining, which begins the dehydration series of the tissue specimens on the slides. The slides are then transferred to 100% alcohol, which removes any remaining water from the specimens. After this series of alcohol rinses, the slides are placed into a solvent like xylene as a clearant to remove the alcohol and prepare the slides for mounting. If any slide containing the specimen has any water residue at this stage, the slide will appear cloudy and will not be suitable for microscopic analysis.



Water droplets

Cloudiness

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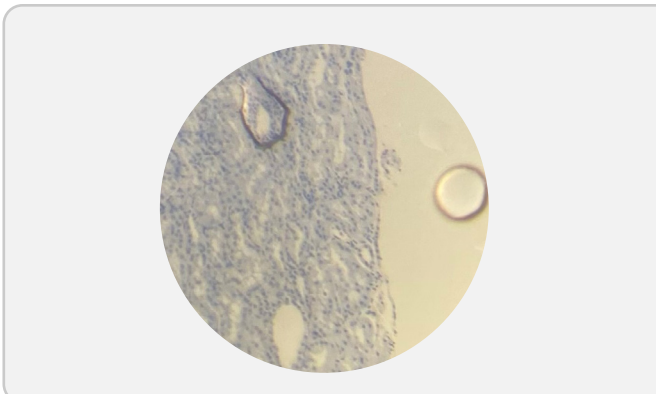
09 COMMON ISSUE 9: CLOUDINESS IN THE SLIDE

Cloudiness in the slide is caused by insufficient clearing after the staining process using an H&E stain.

There are two main causes:

Insufficient clearing through the alcohol (insufficient dehydration): Refresh the alcohol solutions to provide dehydration of the specimen, or leave the specimen in the series of alcohol solutions for a longer period of time to dehydrate it further.

Inconsistent solution volumes in staining containers: Cloudiness can also occur if the alcohol solution depths are not sufficient to cover the slide surface. In this instance, the alcohol rinses will not properly remove all moisture from the tissue and surface of the slide that remains from previous staining steps. For example, if residual water remains on the tissue slide when it is transferred from the alcohol to xylene, it will cause water droplets in the xylene, and subsequently cause cloudiness on the slide as water and xylene are not miscible. The dehydrating process (series of alcohol rinses) prepares the slide for coverslipping. If the slide contains any water residue at the final clearing (xylene) step, the slide will appear cloudy. The cloudiness will appear immediately and will present a blotchy cloudy appearance if coverslipped. Correct this issue by returning the slide to xylene, then place it in fresh 100% alcohol. Finally, reverse back to fresh xylene and coverslip the slide using a synthetic resin.



Water droplets

Cloudiness

10 COMMON ISSUE 10: PARTICULATE IN THE STAIN

Particulates in the stain solution can occur if the stain, stain/buffer and buffer have been sitting for a while. Over time, the the dye components of the stain can precipitate. To resolve, run a cleaning cycle and start with fresh solutions.

Artifacts in Gram stains

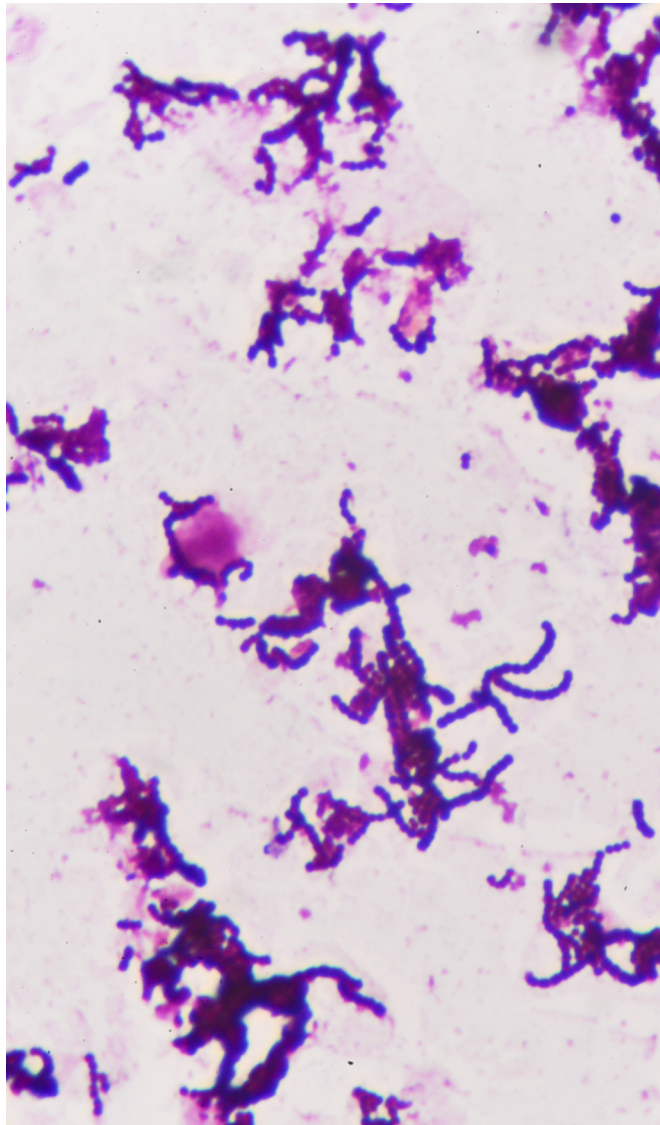
Artifacts in Gram stains can occur when the Crystal Violet dye leaves particulates that can be mistaken as Gram-positive organisms. This may be caused by excessive staining or insufficient rinsing. Artifacts tend to have a homogenous deep purple color and vary in shape and size.

Reduce the time in the stain to reduce excessive staining, or increase rinse times to remove more of the dye.

Fixation, decalcification and processing issues

Some issues that seem to be a problem with a stain, are actually problems with specimen handling and prep (including fixation, decalcification, processing and deparaffinization). These issues may be addressed by reviewing the procedure to find where errors might have occurred.

Particulates in the stain



11 COMMON ISSUE 11: INCOMPLETE FIXATION

Incomplete fixation of the specimen to the slide can cause a host of issues. Use these remedies to fix this common issue.

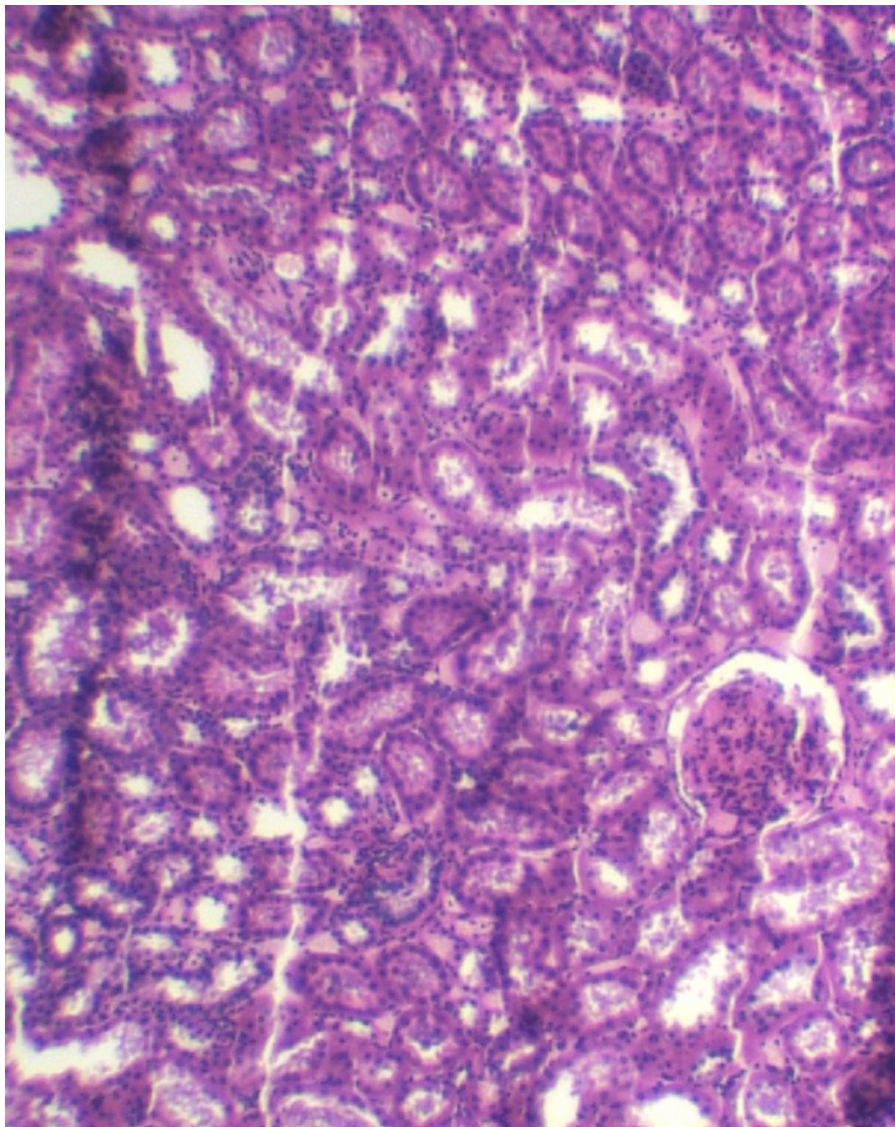
Confirm fixative is appropriate for tissue type. Incomplete fixation can be caused by inadequate penetration of the fixative into the tissue. When using 10% neutral buffer formalin (# 3320), you can score large specimens to allow the fixative to fully penetrate.

Reduce time between collection and fixation (or accommodate a delay). The whole purpose of fixation is to preserve the specimen while it is fresh, and any delayed or incomplete fixation leads to tissue degradation and compromised cells. Minimize time between tissue collection and placement of the specimen in fixative. If fixative will not be used immediately after collection then transfer specimens to a transport medium, such as Michel's Transport Medium (# 7850) . This is a tissue transport medium used for preservation of biopsy specimens prior to further processing.

12 COMMON ISSUE 12: EXCESSIVE DEHYDRATION

Exposing a specimen to alcohol for too lengthy a time may cause excessive dehydration. Review the tissue processing schedule to determine if a change is needed to shorten the time in the alcohol. Multiple processing schedules may also be created to satisfy various tissue types and sizes (e.g. large bone schedule, soft tissue schedule, biopsy schedule).

Excessive dehydration



13 COMMON ISSUE 13: INADEQUATE DECALCIFICATION

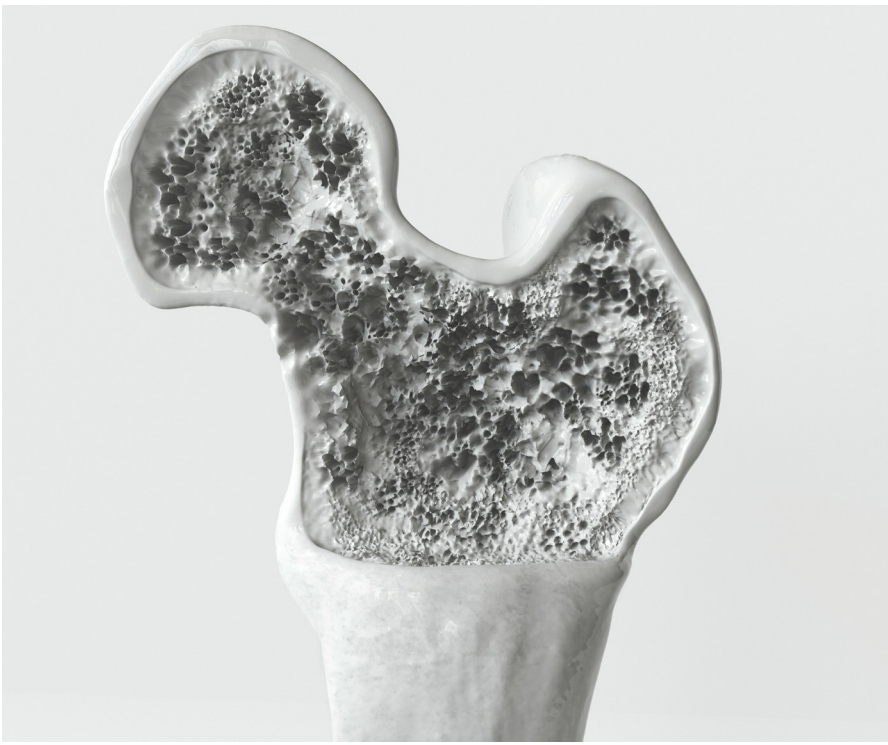
Some specimens contain calcium deposits, whether natural or induced. For ease of processing, sectioning and staining of such specimens, it is recommended to first begin with a process of decalcification. A good decalcification procedure will remove calcium from the specimens, and enable them to be properly processed. This results in very good staining and tissue morphology. Improper decalcification can lead to issues such as bone debris, microtomy issues or dark hematoxylin staining of tissue on the slide. Check the suggested decalcification times for the specimen and adjust your protocol as needed to achieve adequate results. You may need to extend the specimens' time in decalcification solution. Test the specimen for complete decalcification prior to proceeding with tissue processing.

The decalcification process is easily controlled. A hydrochloric acid based decalcifier (# 3363) is stronger and will therefore remove the calcium from the bone at a faster rate but must be monitored closely.

14 COMMON ISSUE 14: EXCESSIVE DECALCIFICATION

Excessive decalcification can lead to loss of nuclear detail or complete loss of nuclear staining. Consequently, you must adjust the time in the decalcification solutions or select the appropriate type of decalcifier prior to tissue processing.

A formic acid decalcifier (#3360) is mild and the decalcification process is easily controlled. A hydrochloric-acid-based decalcifier (#3363) is stronger and will therefore remove the calcium from the specimen at a faster rate, but must be monitored closely. For example, if decalcifying a large canine femur, a hydrochloric acid decalcifier may be appropriate to decrease overall decalcification time. However, if decalcifying a mouse femur or sternum, a milder formic acid decalcifyer would provide better control.



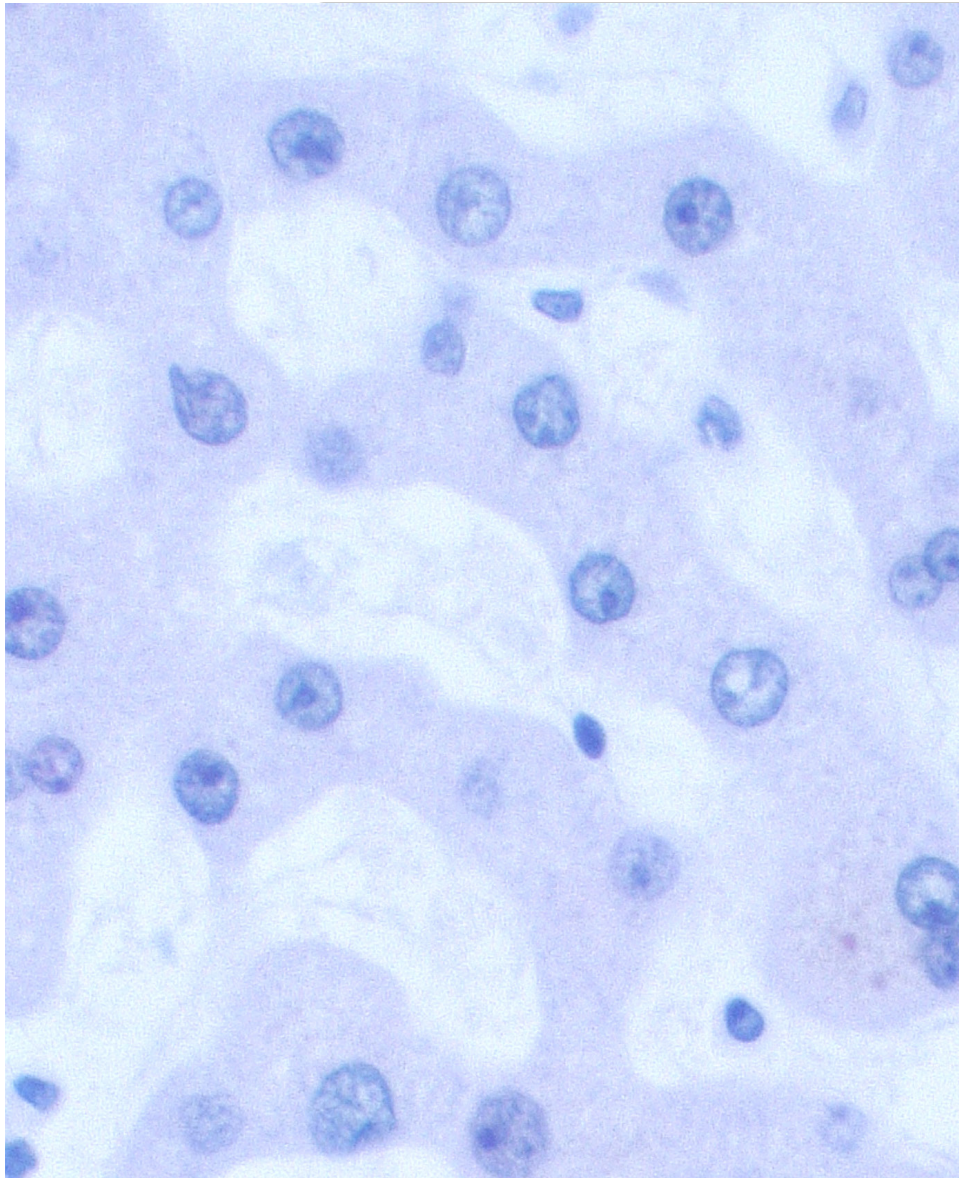
15 COMMON ISSUE 15: INCOMPLETE DEPARAFFINIZATION

If basophilic staining is too light in specimens that have undergone paraffin-H&E staining / paraffin embedding, this is often a sign of incomplete deparaffinization.

If the paraffin was not completely removed from the tissue section. The subsequent staining solutions will not be able to penetrate the specimen properly, leading to the weak/light basophilic and/or cytoplasmic staining.

We recommend returning the slide to xylene (#3346) to remove the excess paraffin wax and conducting the stain procedure again.

Incomplete deparaffinization



16 COMMON ISSUE 16: WRINKLES AND FOLDS/ COMPRESSION

MICROTOMY ISSUES

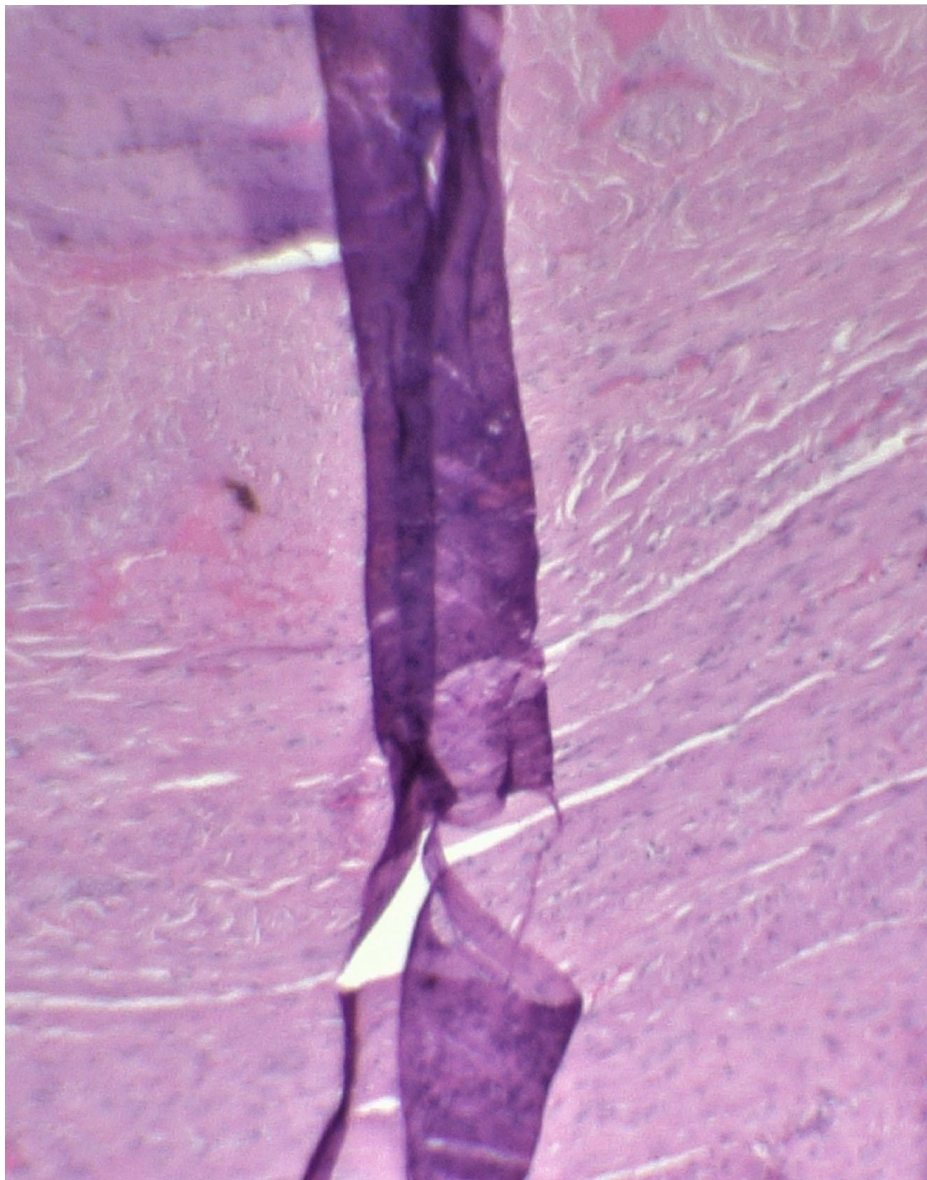
Wrinkles and folds (or compression) in a section can be caused by tools or practices. Here are a few suggestions to tackle these issues:

Adjust temperature: Paraffin blocks: Ice paraffin blocks before cutting on the microtome. Frozen blocks: Set the appropriate temperature in the cryostat for the tissue specimen and allow the specimen block to acclimate prior to cutting frozen blocks.

Check knife/blade: Change to a new blade, and keep the blade surface clear and clean from any excess paraffin or frozen sections.

Check angle: Improper angle of knife holder can cause compression. Angle setting is generally five to ten degrees; however, consult your equipment vendor for the suggested angle for your cutting device.

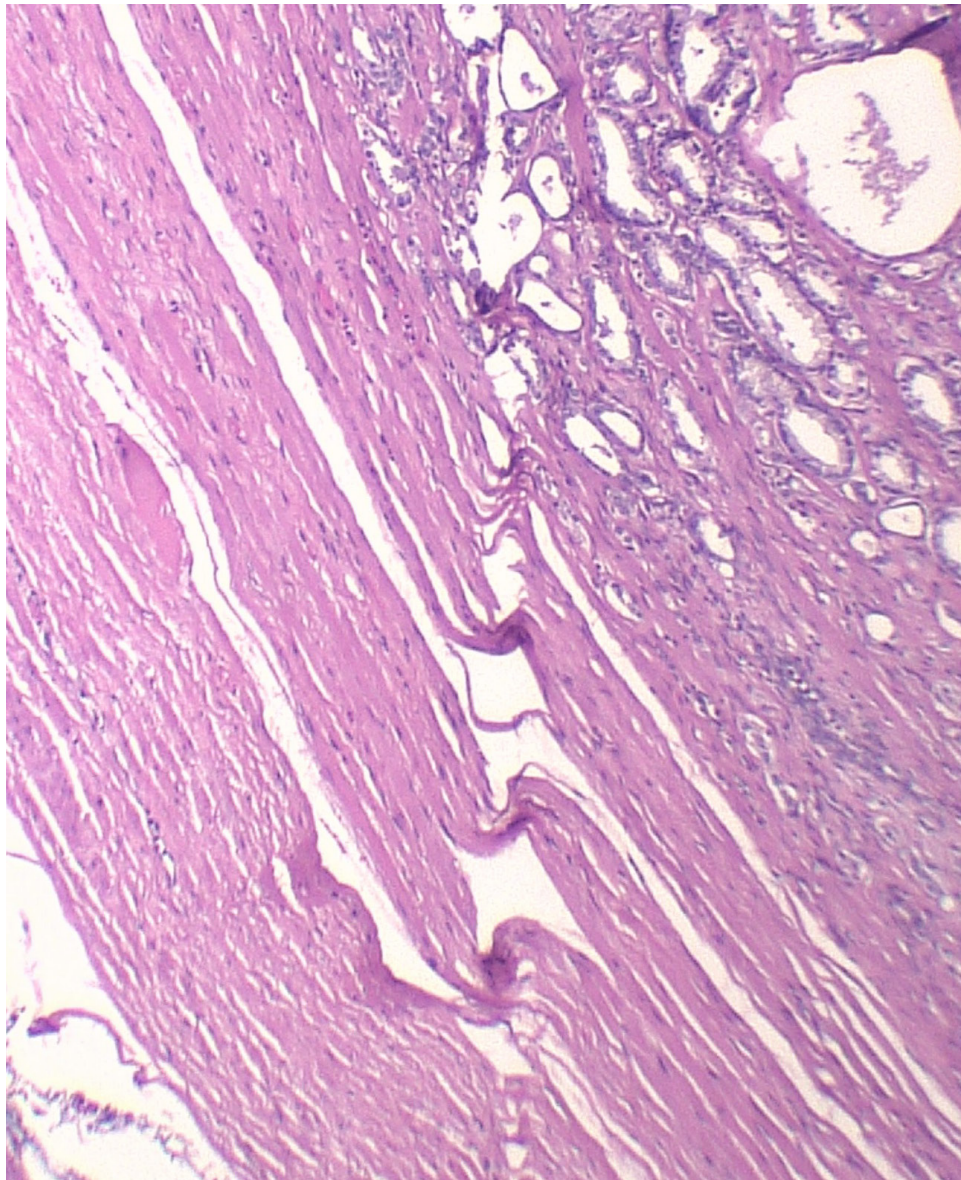
Fold in tissue



17 COMMON ISSUE 17: KNIFE LINES OR SCRATCHES THROUGH TISSUE SPECIMENS

Address the problem of knife lines or scratches through tissue specimens by inserting a new blade on the microtome or slide blade over to an unused area to cut. Knicks in the blade can cause knife lines in your tissue sections.

Knife in tissue



18 COMMON ISSUE 18: THICK/THIN SECTIONS

Uneven sections in a specimen can be resolved by adjusting the microtome angle from five to ten for a more even cut. Check the tightness of all the locks on your cutting device to confirm the issue is not due to a loose specimen chuck or loose blade holder.

19 COMMON ISSUE 19: INCOMPLETE TISSUE SECTIONS

Incomplete tissue sections can lead to incomplete analysis. There are two solutions: either adjust the angle of the specimen block, or adjust the block holder on the microtome.

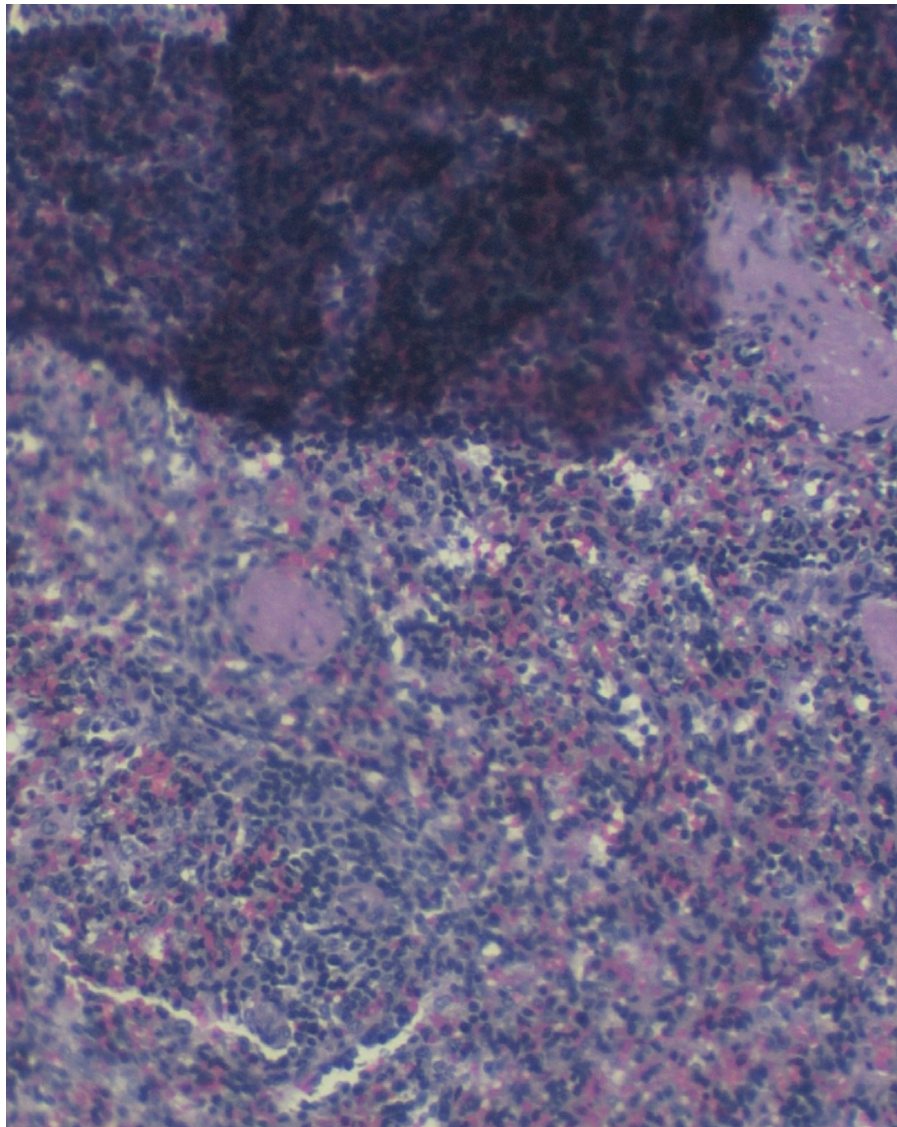
If the specimen was embedded on a flat plane, then you will need to face into the tissue block further to get a “complete section.” Otherwise, adjust the block holder on the microtome to obtain a complete section.

If the specimen is not leveled in the block (paraffin block), consult with supervisor to find if it can be re-embedded.

20 COMMON ISSUE 20: FLOATERS/DEBRIS

Floaters are caused by residual debris from a previously cut specimen. To remedy, clean the water bath in between sections to remove any debris left from the prior sample. This is easily performed by wiping a piece of Kimwipe® across the surface of the water bath until free of debris.

Floaters / Debris



Our commitment to service

Astral is uniquely positioned to offer highest-level technical support and troubleshooting assistance. As a manufacturer, we have a deep understanding of dye and stain formulations; this also enables us to achieve highest quality standards for our products. Astral products are supported by fully-equipped, on-site laboratories that are staffed with experienced technicians. We understand the laboratory ecosystem, and are able to provide truly authentic support to our customers. We're happy to assist with product selection or protocol review, and will take our troubleshooting to the bench if a deeper investigation is required.

This eBook is intended to provide support in navigating some of the most common stains and specimen types. We invite you to contact one of our highly-knowledgeable application experts (800-441-0366, info@ethosbiosciences.com) if a more in-depth discussion or assistance for one of our other stains is needed.

Our commitment to quality

At Astral, quality extends far beyond basic dye formulation and concentration checks. Our fully-integrated program ensures control from start to finish—it begins with raw materials, and encompasses dye and reagent formulation, manufacturing and quality control. Product QC is comprehensive, with performance testing that features actual specimens to ensure that dyes meet expected intensity and cellular component differentiation criteria. For Astral, Quality isn't simply a claim—it's the cornerstone of our philosophy and our commitment to you.

Our commitment to you

At the end of the day, we do all of these things for one purpose: to serve our valued customers. We hope that this collection of common histologic staining issues and troubleshooting tips has been helpful. However, if you didn't find what you need within these pages, we invite you to browse protocols on our website EthosBiosciences.com or to contact one of our application experts (800-441-0366; info@ethosbiosciences.com).
We're happy to help!

Thank you for downloading our E book.

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