Technical Guide for Artisan™ Link Pro
Purpose
This document is intended to assist customers in troubleshooting stain protocols. Default instrument staining protocols have been validated by Dako. Any deviations from default protocols or package insert recommendations must be validated by the end user.

Distribution
This document is intended to be distributed to Dako Customers that have an Artisan™ Link Pro installed in their laboratory.

Scope & Delimitations
This guide contains information on advanced use and operation of the Dako instrument and assumes you have received basic training on the instrument. This guide does not provide instructions for the installation or upgrade of instrument software or hardware.
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Introduction

The Artisan™ Link Special Stains Smart Guide is to guide customers in troubleshooting the protocols for the Artisan™ Link Staining System.

How to Use

The Smart Guide is divided into the following sections for each stain:

- Introduction of the Stain Kit
  - Information on the staining protocol used in development of the kit
  - What component/bacteria/mineral is being demonstrated in the stain
- Control tissue type recommended to use with the stain kit
- Recommended tissue thickness for the stain
- Stain results for the different tissue components
- Pictures of the stain
- Description of reagents used and their purpose at each staining step
  - Time adjustment
  - Heat adjustment
- Troubleshooting suggestions for each protocol
  Suggestions can be used combined or as individual changes

Important:

- AR102 Working wash solution should be made fresh. Dako recommends wash solution made fresh when troubleshooting staining performance & rotate bulk liquids in regular use as indicated in the Artisan™ Link User Guide.
- Keep reagent packs stored per manufacturer’s instructions. Acclimate reagents completely to ambient temperature before each use. Colder reagent packs will significantly under develop tissue elements and prevent heat settings to adequately heat up solutions on the slide.
- Actual Time per Slide varies depending on a number of factors, including but not limited to: placement on carousel, quantity of slides on instrument, staining combination and staining protocol. Using onboard drying and deparaffinization will add additional time to the throughput.

Notes:

All changes made to the default protocol should be validated by the laboratory before using on patient tissue.
AR309  |  Artisan™ Clearing Solution

The Artisan™ Clearing Solution (ACS) is used to remove paraffin from processed tissue samples as a pre-staining step and is compatible with all of the Artisan™ Staining Kits* to be used on the Artisan™ Link Staining System. The Artisan Clearing Solution was validated using the following paraffin types:

- Fisher Brand Tissue Prep, Paraplast Plus and Richard Allen Type L.

Procedure

Drying Step

This step removes any residual water that may lie between the tissue section and the glass slides. The heat assists in preparing the slide for the application of the Artisan™ Clearing Solution. This step is used with the ACS and cannot be used alone.

Artisan™ Clearing Solution

The Artisan™ Clearing Solution is an environmentally safe heated solution that liquefies paraffin and removes paraffin from tissue sections. The ACS can be used with or without the Drying Step.

Bulk Fluid

The heated 100% Alcohol is used to remove the Artisan™ Clearing Solution from the tissue sections and to start the hydration steps.

Troubleshooting

The following are suggestions in resolving staining issues. These suggestions can be used together or as individual changes.

Tissue Section Loss

- Use charged slides. Do Not Use Salinized or Flex slides for Special Stains
- Do not place slides on the Artisan™ Link directly from the water bath
- Be sure to drain all excess water from slides
- Allow sections to air dry a minimum of 15 minutes before placing on the Artisan™ Link
- Increase temperature on Step 1 to 62 °C

Notes:

- The following paraffin types are not compatible with the ACS:
  - Paraffin using DMSO as an additive was not tested due to the carcinogenic risk associated with DMSO
  - Paraffin that have extra plastic polymers added to the paraffin
  - Beeswax
  - The melting point of the paraffin should be between 58-62 °C

*The use of the Artisan™ Clearing Solution in combination with the AFB Kit AR162 or AR362 may reduce the amount of target bacteria stained by the Acid Fast Bacteria Kit.
Paraffin Residue after Staining Run
There may be a slight residual of paraffin on the glass slide surrounding the tissue. This is normal.

Coverslipping with xylene/xylene substitutes will aid in removing residual paraffin. Onboard drying incorporated with clearing step may reduce residual paraffin.

Figure 1: Acceptable paraffin residual

Pre-drying slides in an off-line oven:
Uneven staining may occur if there is paraffin left on the slide due to placing the slides in an oven before placing on the Artisan™. As the paraffin melts in the oven it collects at the edge of the slide and solidifies at room temperature. This residue can cause the slide clip not to seal properly which can result in uneven staining. The residual paraffin can act as a barrier and not allow the reagents to spread or aspirate properly.

Recommendation: Air dry slides for at least 15 minutes or longer depending on specimen type i.e. bone, cartilage and use both drying and deparaffinization to achieve the optimal results.
AR162 | Acid-Fast Bacteria Stain Kit

Acid-Fast Bacteria (AFB) Stain Kit is used to demonstrate the presence of Acid-Fast Bacteria, which belongs to the bacterial genus of Mycobacterium.

**Control Tissue**
- Tissue infected with acid-fast bacteria

**Cut Thickness**
- 4 μm sections

**Time per Slide***
- 00:31:25

**Staining Interpretation**

<table>
<thead>
<tr>
<th>Tissue Component</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid-Fast Bacteria</td>
<td>Varying shades of red</td>
</tr>
<tr>
<td>Background</td>
<td>Light blue</td>
</tr>
</tbody>
</table>

* This time includes onboard drying and deparaffinization using the default protocol. Any adjustments to the protocol will alter the time per slide.

**Figure 2: Acid-Fast Bacteria.**

**Important:**
The use of the Artisan™ Clearing Solution (ACS) in combination with the AFB Kit AR162 or AR362 may reduce the amount of target bacteria stained by the Acid Fast Bacteria Kit. This optimized AFB protocol is not available on the Artisan™ Classic Instruments.
Procedure

**Carbol Fuchsin** stains the organisms red. The incubation time can be increased or decreased from 0 to 1800 seconds. The temperature can be decreased or increased from 23-50 ºC.

**Acid Alcohol** decolorizes the Carbol Fuchsin stained tissue. Acid-Fast Bacteria will decolorize less quickly. The incubation time can be decreased or increased from 0 to 1800 seconds.

**Wash Solution before Methylene Blue** dilutes the Methylene Blue. This step does not have adjustable incubation time or temperature.

**Methylene Blue** counterstains the tissue. The incubation time can be decreased or increased from 0 to 1800 seconds. No heat is applied.

Troubleshooting

The following are suggestions in resolving staining issues. These suggestions can be used together or as individual changes.

**Organisms Too Dark**
- Decrease incubation time in Carbol Fuchsin
- Increase Acid Alcohol incubation time

**Organisms Too Light**
- Decrease Acid Alcohol incubation time to 0 seconds
- Avoid long exposures to alcohol during deparaffinization steps

**Counterstain Too Dark**
- Increase sub washes to 6

**Counterstain Too Light**
- Increase incubation time in Methylene Blue

**Reagent Spreading Issues**
- Increase mixes on the reagents
Notes:

- For *Mycobacterium leprae* (leprosy), the Fites’ method is a preferred technique. The lipoid capsule of the leprosy organism is very sensitive to the alcohols and xylene used in routine acid-fast techniques, so special protective measures must be taken during steps that normally require alcohol and xylene (9). The Fites’ method uses peanut oil. Sample slides exposed to peanut oil prior to application to the Artisan™ have not been shown to cause any harm to the instrument operation. However, this technique has not been validated on the Artisan™ platform and must be optimized by the end user. Exposing the organism longer to Carbol Fuchsin may aid in organism identification.

- Carbol Fuchsin tends to fade if put through 95% Alcohol. It is recommended to air dry prior to Permanent Mount coverslipping to prevent over differentiation of organisms.

- Organisms found outside of the tissue section on slides. Check water quality used in wash solution. Water should be of laboratory quality. Avoid contact with tap water prior to staining. Tap water has been shown to contain acid fast bacteria that may contaminate staining results. A tissue section of uterus provides a good negative control when testing the water for organisms.
AR362 | Acid-Fast Bacteria Light Green Stain Kit

Acid-Fast Bacteria (AFB) Light Green Stain Kit is used to demonstrate the presence of Acid-Fast Bacteria, which belong to the bacterial genus of Mycobacterium.

Control Tissue
Tissue infected with acid-fast bacteria

Cut Thickness
4 μm sections

Time per Slide*
00:31:25

Staining Interpretation

<table>
<thead>
<tr>
<th>Tissue Component</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid-Fast Bacteria</td>
<td>Varying shades of red</td>
</tr>
<tr>
<td>Background</td>
<td>Light green</td>
</tr>
</tbody>
</table>

* This time includes onboard drying and deparaffinization using the default protocol. Any adjustments to the protocol will alter the time per slide.

Figure 3: Acid-Fast Bacteria Light Green.

Important:
The use of the Artisan™ Clearing Solution (ACS) in combination with the AFB Kit AR162 or AR362 may reduce the amount of target bacteria stained by the Acid Fast Bacillus Kit. This protocol is not available on the Artisan™ Classic Instruments...
**Procedure**

*Carbol Fuchsin* stains the organisms red. The incubation time can be decreased or increased from 0 to 1800 seconds. The temperature can be decreased or increased from 23-50 ºC.

*Acid Alcohol* decolorizes the Carbol Fuchsin stained tissue. Acid-Fast Bacteria will decolorize less quickly. The incubation time can be decreased or increased from 0 to 1800 seconds.

*Light Green* counterstains the tissue. The incubation time can be decreased or increased from 0 to 1800 seconds.

**Troubleshooting**

The following are suggestions in resolving staining issues. These suggestions can be used together or as individual changes.

**Organisms Too Dark**
- Decrease incubation time in Carbol Fuchsin
- Increase Acid Alcohol incubation time

**Organisms Too Light**
- Decrease Acid Alcohol incubation time to 0 seconds
- Avoid long exposures to alcohol during deparaffinization steps

**Counterstain Too Dark**
- Increase sub washes to 6

**Counterstain Too Light**
- Increase incubation time in Light Green
- Decrease sub washes

**Reagent Spreading Issues**
- Increase mixes on the reagents

**Notes:**
- For *mycobacterium leprae* (leprosy), the Fites’ method is a preferred technique. The lipoid capsule of the leprosy organism is very sensitive to the alcohols and xylene used in routine acid-fast techniques, so special protective measures must be taken during steps that normally require alcohol and Xylene (9). The Fites’ method uses peanut oil. Sample slides exposed to peanut oil prior to application to the Artisan™ have not been shown to cause any harm to the instrument operation. However, this technique has not been validated on the Artisan™ platform and must be optimized by the end user. Exposing the organism longer to Carbol Fuchsin may aid in organism identification.
- Carbol Fuchsin tends to fade if put through 95% Alcohol. It is recommended to air dry prior to Permanent Mount coverslipping to prevent over differentiation of organisms.
- If organisms are found outside of the tissue section on slides, check water quality used in wash solution. Water should be of laboratory quality. Avoid contact with tap water prior to staining. Tap water has been shown to contain acid fast bacteria that may contaminate staining results. A tissue section of uterus provides a good negative control when testing the water for organisms.
AR160 | Alcian Blue pH 2.5 Stain Kit

Alcian Blue pH 2.5 (AB 2.5) Stain Kit is used to identify weakly sulfated mucins. At pH 2.5, most acidic mucopolysaccharides stain a blue color. The tissue is counterstained with Nuclear Fast Red.

Control Tissue
Small intestine, umbilical cord or colon

Cut Thickness
4 μm sections

Time per Slide*
00:44:50

Staining Interpretation

<table>
<thead>
<tr>
<th>Tissue Component</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weakly sulfated acidic mucopolysaccharides, sulfomucins, hyaluronic acid, sialomucins:</td>
<td>Dark blue</td>
</tr>
<tr>
<td>Nuclei:</td>
<td>Red to pink</td>
</tr>
<tr>
<td>Cytoplasm:</td>
<td>Pale pink</td>
</tr>
</tbody>
</table>

* This time includes onboard drying and deparaffinization using the default protocol. Any adjustments to the protocol will alter the time per slide.

Figure 4: Alcian Blue pH 2.5.
Procedure

**Acetic Acid** allows secretion of the Alcian Blue into the tissue. The incubation time can be decreased or increased from 0 to 1800 seconds.

**Alcian Blue** stains the acidic mucins blue. The incubation time can be decreased or increased from 0 to 1800 seconds. The temperature can be decreased or increased from 23-50 °C.

**Nuclear Fast Red** counter stains the tissue. The incubation time can be decreased or increased from 0 to 1800 seconds.

Troubleshooting

The following are suggestions in resolving staining issues. These suggestions can be used together or as individual changes.

**Mucin Too Dark**
- Decrease temperature of Alcian Blue

**Mucin Too Light**
- Increase incubation time in the Alcian Blue
- Increase heat in Alcian Blue

**Counterstain Too Dark**
- Decrease incubation time in Nuclear Fast Red

**Counterstain Too Light**
- Increase incubation time in Nuclear Fast Red

**Reagent Spreading Issues**
- Increase mixes on the reagents

Notes:
Correct storage of the Nuclear Fast Red is critical. The NFR pack should **never** be stored in a refrigerator (2-8 °C) or allowed to cool below 15 °C. Cooling the pack will cause precipitate to appear. Optimal temperature is room temperature.
Alcian Blue/Periodic Acid-Schiff (AB/PAS) Stain Kit is used to distinguish all mucins in tissue sections. In this procedure, the acidic and neutral mucins vary by color.

**Control Tissue**
- Stomach, colon, or umbilical cord

**Cut Thickness**
- 4 μm sections

**Time per Slide**
- 00:40:50

**Staining Interpretation**

<table>
<thead>
<tr>
<th>Tissue Component</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutral mucins:</td>
<td>Magenta</td>
</tr>
<tr>
<td>Acidic mucins:</td>
<td>Blue</td>
</tr>
<tr>
<td>Mixtures of acidic and neutral mucins:</td>
<td>Color depends on the dominant entity and will range from blue-purple or purple through violet or mauve.</td>
</tr>
</tbody>
</table>

* This time includes onboard drying and deparaffinization using the default protocol. Any adjustments to the protocol will alter the time per slide.

![Figure 5: Alcian Blue/PAS.](image)

![Figure 6: Alcian Blue/PAS with Alpha Amylase.](image)

**Important:**
There are two procedures for Alcian Blue/Periodic Acid-Schiff: AB/PAS and AB/PAS with Alpha-Amylase Digestion. Alpha-Amylase is sold as a separate pack and is used in conjunction with the AB/PAS kit to digest glycogen. The Alpha Amylase protocol is not available on the Artisan™ Classic Instrument.
Procedure
AB/PAS and AB/PAS with Alpha Amylase

Alpha-Amylase digests glycogen. The incubation time can be decreased or increased from 0 to 1800 seconds. The temperature can be decreased or increased from 32-60 °C.

Alcian Blue stains the acidic mucins blue. The incubation time can be decreased or increased from 0 to 2000 seconds. The temperature can be decreased or increased from 23-60 °C.

Two Wash Solutions after Alcian Blue rinses off the Alcian Blue. The incubation time can be decreased or increased from 0 to 1800 seconds.

Periodic Acid oxidizes tissue proteins to produce dialdehyde groups. The incubation time can be decreased or increased from 0 to 1800 seconds. The temperature can be decreased or increased from 23-60 °C.

Schiff’s reagent stains the neutral mucins. Schiff's reacts with the dialdehyde groups, created by the periodic acid. The incubation time can be decreased or increased from 0 to 1800 seconds. The temperature can be decreased or increased between the ranges of 23-60 °C.

Wash Solution after Schiff’s reagent intensifies the pink color imparted by the Schiff’s reagent.

Troubleshooting
The following are suggestions in resolving staining issues. These suggestions can be used together or as individual changes.

Proper fixation and processing is critical for this stain. Stomach and colon contain large amounts of enzymes that promote autolysis. If the tissue isn't properly fixed (i.e. preserved soon after biopsy), staining performance will be compromised.

Schiff’s Too Dark
- Decrease incubation time on the Schiff’s Reagent

Schiff’s Too Light
- Increase incubation time in Schiff’s Reagent
- Increase temperature to 37 °C in the Schiff’s Reagent
- Increase temperature to 40 °C in the Periodic Acid

Mucins Too Dark
- Decrease incubation time in Alcian Blue Solution
Mucins Too Light
- Increase incubation time in Alcian Blue Solution
- Increase the heat temperature in Alcian Blue Solution

Digestion Too Weak, Undesired Glycogen Background
- Increase incubation time in Alpha-Amylase

Reagent Spreading Issues
- Increase mixes on the reagents

Notes:
Stability of Schiff’s reagent
- Schiff’s reagent stability is directly influenced by the sulphite to sulphate gas exchange of sulphurous acid Schiff’s content. If the Schiff’s reagent is kept at 2-8 °C, the gas exchange is slowed, hence preserving the reactivity of the Schiff’s reagent (10). Correct storage temperature is critical.
- When use of the Schiff’s reagent is required, acclimating Schiff’s reagent to ambient temperature prior to instrument loading is necessary for specification staining.
**AR178 | Alcian Blue/Periodic Acid-Schiff/Hematoxylin Stain Kit**

Alcian Blue/Periodic Acid-Schiff/Hematoxylin (AB/PASH) Stain Kit is used to distinguish all mucins in tissue sections. In this procedure, the acidic and neutral mucins vary by color. The Hematoxylin provides nuclear counter stain.

**Control Tissue**


**Cut Thickness**

4 μm sections

**Time per Slide\***

00:55:08

**Staining Interpretation**

<table>
<thead>
<tr>
<th>Tissue Component</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutral mucins:</td>
<td>Magenta</td>
</tr>
<tr>
<td>Acidic mucins:</td>
<td>Blue</td>
</tr>
<tr>
<td>Mixtures of acidic and neutral mucins:</td>
<td>Color depends on the dominant entity and will range from blue-purple to violet or mauve</td>
</tr>
<tr>
<td>Nucleus:</td>
<td>Black to blue</td>
</tr>
</tbody>
</table>

* This time includes onboard drying and deparaffinization using the default protocol. Any adjustments to the protocol will alter the time per slide.

Figure 7: Alcian Blue/PAS Hematoxylin.
Procedure

**Alcian Blue** stains the acidic mucins blue. The incubation time can be decreased or increased from 0 to 2000 seconds. The temperature can be decreased or increased from 23-60 ºC.

**Two Wash Solutions after Alcian Blue** rinses off the Alcian Blue. The incubation time can be decreased or increased from 0 to 1800 seconds.

**Periodic Acid** oxidizes tissue proteins to produce dialdehyde groups. The incubation time can be decreased or increased from 0 to 1800 seconds. The temperature can be decreased or increased from 23-60 ºC.

**Schiff’s** reagent stains the neutral mucins. Schiff’s reacts with the dialdehyde groups, created by the periodic acid. The incubation time can be decreased or increased from 0 to 1800 seconds. The temperature can be decreased or increased from 23-60 ºC.

**Mayer’s Hematoxylin** counter stains the tissue. The incubation time can be decreased or increased from 0 to 1800 seconds. The temperature can be decreased or increased from 23-60 ºC.

**Wash Solution after Mayer’s Hematoxylin** washes off the Mayer’s Hematoxylin. The incubation time can be decreased or increased from 0 to 1800 seconds. The temperature can be increased or decreased from 23-60 ºC.

**Bluing Reagent** changes the hue of the Mayer’s Hematoxylin from purple to blue. The incubation time can be decreased or increased from 0 to 1800 seconds.

Troubleshooting

The following are suggestions in resolving staining issues. These suggestions can be used together or as individual changes.

Proper fixation and processing is critical for this stain. Stomach and colon contain large amounts of enzymes that promote autolysis. If the tissue isn’t properly fixed (ie. preserved soon after biopsy), staining performance will be compromised.

**Schiff’s Too Dark**
- Decrease incubation time on the Schiff’s Reagent
- Increase time in the Periodic Acid

**Schiff’s Too Light**
- Increase incubation time in Schiff’s Reagent
- Increase temperature to 37 ºC in the Schiff’s Reagent
- Increase temperature to 40 ºC in the Periodic Acid

**Mucins Too Dark**
- Decrease incubation time in Alcian Blue Solution
Mucins Too Light
- Increase incubation time in Alcian Blue Solution
- Increase temperature in Alcian Blue Solution

Nuclei Too Dark
- Decrease incubation time in Mayer’s Hematoxylin

Nuclei Too Light
- Increase incubation time in Mayer’s Hematoxylin

Nuclei Too Purple
- Increase incubation time in Bluing Reagent

Reagent Spreading Issues
- Increase mixes on the reagents

Notes:
Stability of Schiff’s reagent
- Schiff’s reagent stability is directly influenced by the sulphite to sulphate gas exchange of sulphurous acid Schiff’s content. If the Schiff’s reagent is kept at 2-8 ºC, the gas exchange is slowed, hence preserving the reactivity of the Schiff’s reagent (10). Correct storage temperature is critical.
- When use of the Schiff’s reagent is required, acclimating Schiff’s reagent to ambient temperature prior to instrument loading is necessary for specification staining.
The Congo Red Stain Kit procedure utilizes an Alkaline Alcoholic Congo Red solution to identify amyloid. Amyloid should be assessed using both a polarized and light microscope.

**Control Tissue**
- Tissue with amyloid or heart tissue

**Cut Thickness**
- 8 μm sections

**Time per Slide***
- 00:50:17

**Staining Interpretation**

<table>
<thead>
<tr>
<th>Tissue Component</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polarized Light Microscopy</td>
<td></td>
</tr>
<tr>
<td>Amyloid</td>
<td>Apple green</td>
</tr>
<tr>
<td>Light Microscopy</td>
<td></td>
</tr>
<tr>
<td>Amyloid, collagen, other fibrous materials:</td>
<td>Pale pink/salmon</td>
</tr>
<tr>
<td>Nuclei</td>
<td>Blue</td>
</tr>
</tbody>
</table>

* This time includes onboard drying and deparaffinization using the default protocol. Any adjustments to the protocol will alter the time per slide.

**Important:**
This protocol has been optimized for the Artisan™ Link instrument only.
Procedure

*Mayer's* stains the nuclei in the tissue. The incubation time can be increased or decreased from 0 to 1800 seconds. The temperature can be increased or decreased from 23-45 ºC.

**Alkaline Alcohol** suppresses the background staining. The time can be increased or decreased from 0 to 1800 seconds.

**Alcoholic Congo Red** this is an anionic dye that allows hydrogen bonding to the amyloid protein. The incubation time can be increased or decreased from 0 to 1800 seconds. The temperature can be increased or decreased from 23-45 ºC.

Troubleshooting
The following are suggestions in resolving staining issues. These suggestions can be used together or as individual changes.

**Amyloid Too Light (by light microscopy)**
- Increase incubation time in Alcoholic Congo Red
- Use thick tissue sections (8 µm)
- Decrease the sub rinses to 1 after the Alkaline Alcohol

**Amyloid Too Dark (by light microscopy)**
- Decrease the time in Congo Red
- Reduce heat to 23 ºC
- Change the bulk fluid on the rinses after the Congo Red to the following:
  - Rinse 1 – 95% Alcohol
  - Rinse 2 – 95% Alcohol
  - Rinse 3 – 100% Alcohol
  - Rinse 4 – 100% Alcohol
  - Eliminate rinse 5

**Reagent Spreading Issues**
Increase mixes on the reagents
Notes:
- 8 µm sections are recommended. (9) Thicker tissue sections will interfere with the polarized results. Sections that are not 8µm may not show the green birefringence.
- Sections cut thicker than 8µm will fluoresce yellow and not apple green
- Thinner sections will fluoresce with faint red colors (1)

Staining Intensity
- Avoid prolonged fixation in formalin as diminished staining may occur
- Tissue containing massive deposits, presumably of long standing, give less intense histochemical reactions than small newly formed deposits (1)
- Gross deposits may be easily seen in stained sections, early minimal deposits are often missed and differentiation between Amyloid is impossible (1)
- The Congo Red solution is placed on the tissue after being treated with the Alkaline Alcohol. The Alkaline Alcohol helps to diminish the background staining; it is not used as a differentiator.

Birefringence
- Literature supports specified cut thickness will cause variance in the birefringence. Bancroft and Stevens states, “green polarization color shown by amyloid under crossed polar was a function of the parallel alignment of amyloid fibrils and dye molecules and that the thickness of section is critical, for it is only at about 8-10 µm that the necessary… ‘red light is absorbed allowing the green light to pass’… too thin sections show faint red colors while yellow birefringent colors can be seen if the section (amyloid deposit) is too thick”. (1)
AR307 | Colloidal Iron Stain Kit

The Colloidal Iron stains acid mucopolysaccharides, acid glycoprotein and Cryptococcus neoformans in tissue. Colloidal ferric ions are, at a low pH, absorbed principally by carboxylated and sulfated mucosubstances. The excess reagent is washed out and the classic Prussian blue reaction is used to demonstrate iron bound in Gastro Intestinal mucins. This stain is not recommended for Dermatology specimens. The stain has been optimized for use with 4 µm thick tissue sections.

<table>
<thead>
<tr>
<th>Control Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small intestine</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cut Thickness</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 µm sections</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Time per Slide*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0:58:57</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Staining Interpretation</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Tissue Component</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carboxylated and sulfated mucopolysaccharides</td>
<td>Dark blue</td>
</tr>
<tr>
<td>and glycoprotein mucin</td>
<td></td>
</tr>
<tr>
<td>Nuclei:</td>
<td>Light pink</td>
</tr>
<tr>
<td>Cytomplasm:</td>
<td>Light pink</td>
</tr>
</tbody>
</table>

* This time includes onboard drying and deparaffinization using the default protocol. Any adjustments to the protocol will alter the time per slide.

**Important:**
This protocol is not available on the Artisan™ Classic instrument.
Procedure

Acetic Acid prepares the slides for the Colloidal Iron solution. Incubation times can be increased or decreased from 0 to 1800 seconds.

Colloidal Iron Solution, Wash Solution and Acetic Acid are combined to make the working Colloidal Iron Solution. This stains the acid mucopolysaccharides, acid glycoprotein and Cryptococcus neoformans. The incubation times can be decreased or increased from 0 to 2000 seconds. The temperature can be decreased or increased from 23-62 °C.

Acetic Acids (4 reagent packs) are used to remove excess colloidal iron working solution. Incubation times can be decreased or increased from 0 to 1800 seconds.

Potassium Ferrocyanide/Hydrochloric Acid are combined and mixed together to demonstrate the bound ferric iron with a Prussian blue reaction. The incubation time can be increased or decreased from 0 to 1800 seconds.

Nuclear Fast Red stains the nuclei and background tissue. The incubation time can be decreased or increased from 0 to 1800 seconds.

Troubleshooting

The following are suggestions in resolving staining issues. These suggestions can be used together or as individual changes

Background too blue
- Decrease time in Potassium Ferrocyanide/Hydrochloric Acid

Mucin too light
- Increase time in the Colloidal Iron Solution
- Increase heat in the Colloidal Iron Solution

Mucin too dark
- Decrease time in the Colloidal Iron Solution
AR163 | Elastic Stain Kit

The Elastic Stain Kit (EVG) utilizes Alcoholic Hematoxylin, Ferric Chloride and Lugol’s Iodine solutions for staining elastin fibers. Van Gieson’s solution is added to differentiate collagen from elastin.

Control Tissue
Cross section of artery, appendix and skin

Cut Thickness
4 μm sections

Time per Slide*
00:37:58

Staining Interpretation

<table>
<thead>
<tr>
<th>Tissue Component</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elastin fibers:</td>
<td>Black</td>
</tr>
<tr>
<td>Nuclei:</td>
<td>Blue to black</td>
</tr>
<tr>
<td>Collagen:</td>
<td>Red</td>
</tr>
<tr>
<td>Other tissue elements:</td>
<td>Yellow</td>
</tr>
</tbody>
</table>

* This time includes onboard drying and deparaffinization using the default protocol. Any adjustments to the protocol will alter the time per slide.

Figure 11: Elastic.
Procedure

Alcoholic Hematoxylin, Ferric Chloride and Lugol’s Iodine form a “lake”, which is used to over stain the tissue. The Ferric Chloride and Lugol’s Iodine are mordants. These mordants also oxidize the Alcoholic Hematoxylin to hematein. The incubation time can be decreased or increased from 0 to 1800 seconds.

Three Wash Solutions after Alcoholic Hematoxylin, Ferric Chloride and Lugol’s Iodine washes off the “lake”. These three steps do not have adjustable incubation time or temperature. Additional sub washes may be added to the last wash step.

Wash Solution before Ferric Chloride dilutes the Ferric Chloride – Elastic. This step does not have adjustable incubation time or temperature.

Ferric Chloride differentiates the elastin fibers. The incubation time can be decreased or increased from 0 to 1800 seconds.

Van Gieson’s Solution counterstains the tissue. The incubation time can be decreased or increased from 0 to 1800 seconds.

Wash Solution after the Van Gieson’s Solution washes off the Van Gieson’s Solution – Elastic. This step does not have adjustable incubation time or temperature. Additional wash steps can be added or decreased.

Troubleshooting

The following are suggestions in resolving staining issues. These suggestions can be used together or as individual changes.

Elastin Fibers Too Dark

- Decrease incubation time in Lugol’s Iodine to 600 seconds
- Increase incubation time in Ferric Chloride to 30 seconds

Elastin Fibers Too Light

- Increase incubation time in Lugol’s Iodine
- Reagent Spreading Issues
- Increase mixes on the reagents

Van Gieson’s too dark

- Increase wash steps
Lugol’s Iodine

Iodine has weak intermolecular forces, so the forces are broken easily. So instead of turning into a liquid, they turn into a gas. This is what is observed as “out gassing” from Dako’s Iodine packs in the AR163 Elastic Stain Lugol’s iodine. Discoloration (shown by circle) of the pack labels or box packaging may occur but performance has been proven to not be compromised.

![Image](image.png)

Figure 12: Lugol’s Iodine discoloration.

Notes:

Van Giesion’s solution is made with Picric Acid which has the tendency to leach out of the stained tissue. The leaching of the picric acid does not affect the diagnostic utility of the stain.

Using a non-toluene based mounting media may slow down the leaching, but not necessarily stop it from happening.
AR164 | Giemsa Stain Kit

The Giemsa Stain Kit is used to identify hematopoietic cells in tissue sections (ie: mast cells, basophils, polymorph nuclear leukocytes, etc.).

Control Tissue

<table>
<thead>
<tr>
<th>Tissue Component</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue containing mast cells</td>
<td></td>
</tr>
</tbody>
</table>

Cut Thickness

<table>
<thead>
<tr>
<th>Cut Thickness</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 μm sections</td>
</tr>
</tbody>
</table>

Time per Slide*

<table>
<thead>
<tr>
<th>Time per Slide*</th>
</tr>
</thead>
<tbody>
<tr>
<td>01:20:32</td>
</tr>
</tbody>
</table>

Staining Interpretation

<table>
<thead>
<tr>
<th>Tissue Component</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mast cell granules:</td>
<td>Purple</td>
</tr>
<tr>
<td>Basophiles:</td>
<td>Purple</td>
</tr>
<tr>
<td>Eosinophiles:</td>
<td>Bright pink</td>
</tr>
<tr>
<td>Lymphocytes:</td>
<td>Blue</td>
</tr>
</tbody>
</table>

* This time includes onboard drying and deparaffinization using the default protocol. Any adjustments to the protocol will alter the time per slide.
Procedure
Wash Solution and Giemsa Solution stains the hematopoietic cells. The incubation time can be decreased or increased from 0 to 4800 seconds. The temperature can be decreased or increased from 23-48ºC.

Acetic Acid Decolorizer differentiates the hematopoietic cells. The incubation time can be decreased or increased from 0 to 1800 seconds.

Troubleshooting
The following are suggestions in resolving staining issues. These suggestions can be used together or as individual changes.

Staining Too Dark
- Increase incubation time in Acetic Acid Decolorizer

Staining Too Light
- Increase incubation time in Giemsa Solution
- Decrease incubation time in Acetic Acid

Reagent Spreading Issues
- Increase mixes on the reagents
AR308 | Jenner-Wright Giemsa Stain Kit

The Jenner-Wright Giemsa (JW Giemsa) is used to permit differentiation of cells present in bone marrow specimens. Polychromatic stains are used as routine nuclear and Cytoplasmic stains for bone marrow biopsy sections because of the range of Cytoplasmic staining obtained.

### Control Tissue
Bone marrow or spleen

### Cut Thickness
2-3 μm sections

### Time per Slide*
00:35:23

### Staining Interpretation

<table>
<thead>
<tr>
<th>Tissue Component</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclei:</td>
<td>Blue</td>
</tr>
<tr>
<td>Eosinophiles:</td>
<td>Bright pink</td>
</tr>
<tr>
<td>Leukocytes:</td>
<td>Shades of pink, gray, or blue depending on cell type and development</td>
</tr>
</tbody>
</table>

* This time includes onboard drying and deparaffinization using the default protocol. Any adjustments to the protocol will alter the time per slide.

![Figure 14: Jenner-Wright Giemsa](image)

**Important:**
This stain is not intended for use on bone marrow smears or peripheral smears. This protocol is not available for the Artisan™ Classic Instrument.
Procedure

**Methanol** is used to mordent cells for the Jenner Solution. Incubation times can be increased or decreased from 0-1800 seconds.

**Jenner Solution and Wash Solution** are mixed together on board. This reagent stains the hematopoietic cells. Incubation times can be increased or decreased from 0-1800 seconds.

**Wright Giemsa Solution/Buffer** mixes on board and stain the hematopoietic cells. Incubation times can be increased or decreased from 0-1800 seconds.

**Acetic Acid** is used to differentiate the Jenner/Giemsa Stain. Incubation times can be increased or decreased from 0-1800 seconds.

Troubleshooting

The following are suggestions in resolving staining issues. These suggestions can be used together or as individual changes.

- A well-fixed, properly decalcified specimen will show excellent preservation of the nuclear detail, lack of calcium in the bony spicules and good cytoplasmic differentiation (1)
- Over decalcification can cause poor staining results
- Bone marrow biopsies should use a gentle decal solution mixed with a fixative
- Formic acid and ETDA solutions are mild formulations
- Monitor the time left in the decal solution

- If a more alkaline (blue) stain is desired, decrease the staining time the Wright Giemsa step
- If a more acid (red) stain is desired, increase the staining time in the Wright Giemsa step

Stain too dark

- Decrease the time in step 4 (Jenner solution) to 120 seconds
- Make sure the sections have been cut at 2-3 microns
- If a more alkaline (blue) stain is desired, decrease the staining time the Wright Giemsa step
- If a more acid (red) stain is desired, increase the staining time in the Wright Giemsa step

Notes:

The slides will have residual stain after completion of run. After removing slides from the Artisan™ Link do the following (see package insert) to remove residual stain:

- Dip 3-5 times in 95% alcohol
- Dip 3-5 times in 100% alcohol
- Dip 3-5 times in Xylene or Xylene substitute
- Repeat in Xylene or Xylene substitute
- Permanently mount

Excessive alcohol rinses may reduce staining results.
AR167 | Gomori’s Blue Trichrome Stain Kit

The Gomori’s Blue Trichrome Stain Kit is used to identify collagen fiber in liver and kidney tissue.

Control Tissue
Uterus, kidney and liver

Cut Thickness
4 μm sections

Time per Slide*
01:03:41

Staining Interpretation

<table>
<thead>
<tr>
<th>Tissue Component</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytoplasm:</td>
<td>Red</td>
</tr>
<tr>
<td>Fibrin:</td>
<td>Pink</td>
</tr>
<tr>
<td>Collagen:</td>
<td>Blue</td>
</tr>
<tr>
<td>Nuclei:</td>
<td>Blue or Black</td>
</tr>
<tr>
<td>Erythrocytes:</td>
<td>Red</td>
</tr>
</tbody>
</table>

* This time includes onboard drying and deparaffinization using the default protocol. Any adjustments to the protocol will alter the time per slide.

Figure 15: Gomori’s Blue Trichrome.
Procedure

**Bouin's Solution** acts as a mordant. The incubation time can be increased or decreased from 0 to 1800 seconds. The temperature can be decreased or increased from 37-60 ºC.

**Wash Solution after Bouin's Solution (STEP 3):** Clears the Bouin's Solution from the tissue.

**Wash Solutions after Bouin’s Solution (Step 4 & 5)** removes excess Bouin’s Solution from the tissue. The incubation time can be decreased or increased from 0 to 1800 seconds. The temperature can be decreased or increased from 23-60 ºC.

**Weigert's A and Weigert's B** are combined to make a working Weigert’s Hematoxylin Solution, which is used to stain nuclear chromatin. The incubation time can be decreased or increased from 0 to 1800 seconds. The temperature can be decreased or increased from 23-60 ºC.

**Gomori's Trichrome** stains the various tissue elements (cytoplasm, fibrin, collagen, erythrocytes, etc.). The incubation time can be decreased or increased from 0 to 1800 seconds.

**Acetic Acid** differentiates the tissue elements removing excess stain. The incubation time can be decreased or increased from 0 to 1800 seconds.

**Troubleshooting**

The following are suggestions in resolving staining issues. These suggestions can be used together or as individual changes.

Because this is a “One Step” Trichrome procedure, it is difficult to increase the intensity of the blue staining without also increasing the intensity of the red staining and vice versa.

**Staining Too Dark**
- Decrease incubation time in Gomori's Trichrome
- Increase incubation time in Acetic Acid

**Staining Too Light**
- Increase the mixes in the Bouins step
- Increase incubation time in Gomori's Trichrome
- Decrease incubation time in Acetic Acid
- Decrease the amount of rinses after Step 9

**Nuclei Too Light**
- Increase the mixes in the Bouins step
- Increase incubation time in Weigert’s B
- Decrease the amount of rinses after Step 7
The Gomori’s Green Trichrome Stain Kit is used to identify collagen fibers in liver and kidney tissue.

**Control Tissue**

Uterus, small intestine, liver, appendix and fallopian tube

**Cut Thickness**

4 μm sections

**Time per Slide**

00:47:54

**Staining Interpretation**

<table>
<thead>
<tr>
<th>Tissue Component</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytoplasm:</td>
<td>Red</td>
</tr>
<tr>
<td>Fibrin:</td>
<td>Pink</td>
</tr>
<tr>
<td>Collagen:</td>
<td>Green</td>
</tr>
<tr>
<td>Nuclei:</td>
<td>Blue or Black</td>
</tr>
<tr>
<td>Erythrocytes:</td>
<td>Red</td>
</tr>
</tbody>
</table>

*This time includes onboard drying and deparaffinization using the default protocol. Any adjustments to the protocol will alter the time per slide.*

Figure 16: Gomori’s Green Trichrome.
Procedure

**Bouin's Solution** acts as a mordant. The incubation time can be increased or decreased from 0 to 1800 seconds. The temperature can be decreased or increased from 37-60ºC.

**Wash Solution after Bouin's Solution (step 3):** Clears the Bouin's Solution from the tissue. Wash Solutions after Bouin's Solution (step 4 & 5) remove excess Bouin's Solution from the tissue. The incubation time can be decreased or increased from 0 to 1800 seconds. The temperature can be decreased or increased from 23-60 ºC.

**Weigert's A and Weigert's B** are combined to make a working Weigert's Hematoxylin Solution, which is used to stain nuclear chromatin. The incubation time can be decreased or increased from 0 to 1800 seconds. The temperature can be decreased or increased from 23-60 ºC.

**Gomori's Trichrome** stains the various tissue elements (cytoplasm, fibrin, collagen, erythrocytes, etc.). The incubation time can be decreased or increased from 0 to 1800 seconds.

**Acetic Acid** differentiates the tissue elements removing excess stain. The incubation time can be decreased or increased from 0 to 1800 seconds.

Troubleshooting

The following are suggestions in resolving staining issues. These suggestions can be used together or as individual changes.

Because this is a “One Step” Trichrome procedure, it is difficult to increase the intensity of the blue staining without also increasing the intensity of the red staining and vice versa.

**Staining Too Dark**
- Decrease incubation time in Gomori’s Trichrome
- Increase incubation time in Acetic Acid

**Staining Too Light**
- Increase the mixes on the Bouins Step 1
- Increase incubation time in Gomori’s Trichrome
- Decrease incubation time in Acetic Acid
- Decrease the amount of rinses after Step 9
The Grocott’s Methenamine Silver (GMS) Stain Kit is used to identify fungal organisms in tissue sections. The most widely used application of this technique is to identify Pneumocystis jiroveci.

**Control Tissue**
- Tissue with Aspergillus, Candida or *Pneumocystis*

**Cut Thickness**
- 4 μm sections

**Time per Slide**
- 01:14:08

**Staining Interpretation**

<table>
<thead>
<tr>
<th>Tissue Component</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fungi:</td>
<td>Black</td>
</tr>
<tr>
<td>Pneumocystis:</td>
<td>Black</td>
</tr>
<tr>
<td>Background:</td>
<td>Light green</td>
</tr>
</tbody>
</table>

* This time includes onboard drying and deparaffinization using the default protocol. Any adjustments to the protocol will alter the time per slide.

---

**Important:**
This stain is not intended to be used on cytology smears, cytospins or thin prep specimens due to possible cross contamination. The GMS plus was developed for pneumocystis which uses a lower heat in the silver solution which eliminates background stain. The GMS has a higher heat and less time and works well for fungus.
Procedure
GMS and GMS Plus

Sodium Chromate and Perchloric Acid combine to form an instable form of Chromic Acid at elevated temperatures. "Chromic Acid" oxidizes the tissue, making the fungal cell walls reactive with silver. The incubation time can be decreased or increased from 0 to 1800 seconds. The temperature can be decreased or increased from 23-65 °C.

Sodium Bisulfite removes traces of the "Chromic Acid" left in the tissue. The incubation time can be decreased or increased from 0 to 1800 seconds.

Silver Nitrate and Methenamine Borate combine to form a Methenamine Silver solution. This alkaline silver solution reacts with the oxidized fungal walls. The addition of methenamine borate renders them visible. The incubation time can be decreased or increased from 0 to 1800 seconds. The temperature can be decreased or increased from 23-65 °C.

Wash Solution after Silver Nitrate and Methenamine Borate cools the solution and stops the chemical reaction occurring on the tissue. This step does not have adjustable incubation time or temperature.

Gold Chloride tones the silver to a black color. The incubation time can be decreased or increased from 0 to 1800 seconds.

Sodium Thiosulfate removes unreduced silver. The incubation time can be decreased or increased from 0 to 1800 seconds.

Light Green counterstains the tissue. The incubation time can be increased or decreased from 0 to 1800 seconds.

Troubleshooting
The following are suggestions in resolving staining issues. These suggestions can be used together or as individual changes.

For the best results, it is recommended not to change the default temperature settings. The chemical reactions that are required are extremely dependent on appropriate temperatures.

Fungi Too Dark
  - Decrease incubation time in Methenamine Borate

Fungi Too Light
  - Increase incubation time in Methenamine Borate
Fungi Not Black
- Add additional mixes to the Gold Chloride
- Increase incubation time in Gold Chloride

Other tissue elements staining
- Lower the Methenamine Borate/Silver to 300 seconds

Counterstain Too Light
- Increase incubation time in Light Green

Notes:
- The GMS plus was developed for pneumocystis which uses a lower heat in the silver solution which eliminates background stain.
- AR102 Wash Solution should be made fresh; light development of fungus can be symptomatic of a contaminated wash solution. Dako recommends wash solution made fresh when troubleshooting staining performance and rotate bulk liquids in regular use as indicated in the Artisan™ Link User Guide.
- Keep reagent packs stored per manufacturer’s instructions. Acclimate reagents completely to ambient temperature before each use. Colder reagent packs will significantly under develop tissue elements and prevent heat settings to adequately heat up solutions on the slide.
- Avoid heavily charged slides, especially covalently coated silanized slides or the Dako Flex slides. The charged coating will bind the silver nitrate solution rendering the slide indistinguishable from the tissue.
- Water quality is imperative for all silver stains. The resistivity is inversely proportional to the ionic content of water, the higher the water’s ion concentration, the lower its resistivity. Therefore, resistivity measurements are useful to assess the ion content of the water. The measurement is sensitive to the point where the only ionized species are the hydrogen and hydroxyl ions contributed by the water itself. A resistivity of 15 MΩ/cm at 25 °C is the minimum for purified reagent water, the concentration of ionic species is less than 10 gram equivalents per liter. (6) At this minimum, background caused by ionic binding with the ions in the water is at a minimum.
AR376 | Grocott’s Methenamine Silver Eosin Stain Kit

The Grocott’s Methenamine Silver (GMS) Eosin Stain Kit is used to identify fungal organisms in tissue sections. The most widely used application of this technique is to identify Pneumocystis jiroveci.

Control Tissue

| Tissue with Aspergillus, Candida or Pneumocystis |

Cut Thickness

4 μm sections

Time per Slide*

01:14:58

Staining Interpretation

<table>
<thead>
<tr>
<th>Tissue Component</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fungi:</td>
<td>Black</td>
</tr>
<tr>
<td>Pneumocystis:</td>
<td>Black</td>
</tr>
<tr>
<td>Background:</td>
<td>Pink</td>
</tr>
</tbody>
</table>

* This time includes onboard drying and deparaffinization using the default protocol. Any adjustments to the protocol will alter the time per slide.

Figure 19: GMS Eosin.

**Important:**
This stain is not intended to be used on cytology smears, cytopsins or thin prep specimens due to possible cross contamination. This protocol is not available on the Artisan™ Classic Instrument.
Procedure

**Sodium Chromate and Perchloric Acid** combine to form an instable form of Chromic Acid at elevated temperatures. “Chromic Acid” oxidizes the tissue, making the fungal cell walls reactive with silver. The incubation time can be decreased or increased from 0 to 1800 seconds. The temperature can be decreased or increased from 23-65 ºC.

**Sodium Bisulfite** removes traces of the “Chromic Acid” left in the tissue. The incubation time can be decreased or increased from 0 to 1800 seconds.

**Silver Nitrate and Methenamine Borate** combine to form a Methenamine Silver solution. This alkaline silver solution reacts with the oxidized fungal walls. The addition of methenamine borate renders them visible. The incubation time can be decreased or increased from 0 to 1800 seconds. The temperature can be decreased or increased from 23-65 ºC.

**Wash Solution after Silver Nitrate and Methenamine Borate** cools the solution and stops the chemical reaction occurring on the tissue. This step does not have adjustable incubation time or temperature.

**Gold Chloride** tones the silver to a black color. The incubation time can be decreased or increased from 0 to 1800 seconds.

**Sodium Thiosulfate** removes unreduced silver. The incubation time can be decreased or increased from 0 to 1800 seconds.

**Eosin** counterstains the tissue. The incubation time can be increased or decreased from 0 to 1800 seconds.

Troubleshooting

The following are suggestions in resolving staining issues. These suggestions can be used together or as individual changes.

For the best results, it is recommended not to change the default temperature settings. The chemical reactions that are required are extremely dependent on appropriate temperatures.

**Fungi Too Dark**
- Decrease incubation time in Methenamine Borate

**Fungi Too Light**
- Increase incubation time in Methenamine Borate

**Fungi Not Black**
- Add additional mixes to the Gold Chloride
- Increase incubation time in Gold Chloride
Other tissue elements staining

- Lower the Methenamine Borate/Silver to 300 seconds

Counterstain Too Light

- Increase time in Eosin

Notes:

- AR102 Wash Solution should be made fresh; light development of fungus can be symptomatic of a contaminated wash solution. Dako recommends wash solution made fresh when troubleshooting staining performance and rotate bulk liquids in regular use as indicated in the Artisan™ Link User Guide.
- Keep reagent packs stored per manufacturer’s instructions. Acclimate reagents completely to ambient temperature before each use. Colder reagent packs will significantly under develop tissue elements and prevent heat settings to adequately heat up solutions on the slide.
- Avoid heavily charged slides, especially covalently coated silanized slides or the Dako Flex slides. The charged coating will bind the silver nitrate solution rendering the slide indistinguishable from the tissue.
- Water quality is imperative for all silver stains. The resistivity is inversely proportional to the ionic content of water, the higher the water’s ion concentration, the lower its resistivity. Therefore, resistivity measurements are useful to assess the ion content of the water. The measurement is sensitive to the point where the only ionized species are the hydrogen and hydroxyl ions contributed by the water itself. A resistivity of 15 MΩ/cm at 25 °C is the minimum for purified reagent water, the concentration of ionic species is less than 10 gram equivalents per liter. At this minimum, background caused by ionic binding with the ions in the water is at a minimum.
AR175 | Gram Stain Kit

The Gram Stain Kit utilizes a stable Crystal Violet solution that aids in the differentiation of organisms.

Control Tissue

Tissue containing both Gram-positive and Gram-negative bacteria

Cut Thickness

4 μm sections

Time per Slide*

00:42:32

Staining Interpretation

<table>
<thead>
<tr>
<th>Tissue Component</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram-positive organisms:</td>
<td>Blue</td>
</tr>
<tr>
<td>Gram-negative organisms:</td>
<td>Red</td>
</tr>
<tr>
<td>Background:</td>
<td>Varying shades of blue/green</td>
</tr>
</tbody>
</table>

* This time includes onboard drying and deparaffinization using the default protocol. Any adjustments to the protocol will alter the time per slide.

Figure 20: Gram.

Important:

If the Gram positive cell walls have been damaged/disrupted for being treated with antibiotics the bacteria will not be able to retain the CV/Iodine complex and may appear gram negative.
Procedure

**Crystal Violet** is the primary dye used to identify Gram-positive organisms. The incubation time can be decreased or increased from 0 to 1800 seconds.

**Two Wash Solutions after Crystal Violet** further rinses the Crystal Violet to allow for more efficient rinsing of this dye from the slide. This step does not have adjustable incubation time or temperature.

**Wash Solution before Lugol’s Iodine** dilutes the Lugol’s Iodine. This step does not have adjustable incubation time and temperature.

**Lugol’s Iodine** acts as a mordant. The incubation time can be decreased or increased from 0 to 1800 seconds.

**Wash Solution after Lugol’s Iodine** rinses off the Lugol’s Iodine. This step does not have adjustable incubation time and temperature.

**Two Ethanol 100%** decolorizes the Crystal Violet from the Gram-negative organisms due to the chemistry and structure of the organism’s cell walls. The incubation time can be decreased or increased from 0 to 1800 seconds.

**Wash Solution after Ethanol 100%** rinses off the Ethanol 100%. This step does not have adjustable incubation time and temperature.

**Twort’s Fast Green – Gram and Twort’s Neutral Red** are combined to counterstain tissue and stain the Gram-negative organisms. The incubation time can be increased or decreased between the ranges of 0 to 1800 seconds.

**None Step** allows the slide to dry after the stain is complete so that the slide can be placed directly into Xylene from the instrument. The incubation time can be decreased or increased from 0 to 1800 seconds.

Troubleshooting

The following are suggestions in resolving staining issues. These suggestions can be used together or as individual changes.

Fresh 100% alcohol must be used in the Bulk Liquid Containers on the Artisan™ Link for this stain. **Do not** dehydrate after staining; this will cause further decolorization of the organism. Let slides air dry then dip in clearing agent and cover slip.

This stain is not intended for use on aspirates or smears.

**Gram-Negative Organisms Too Light**
- Increase incubation time in Twort’s Neutral Red
  Be aware that this may intensify the background staining as well
Gram-Negative Organisms Do Not Stain
- Increase incubation time in Ethanol 100%

Gram positive Organisms not Staining
- Increase the time in the Crystal Violet and the Iodine solution
- Reduce the amount of rinses after the Iodine

Crystal Contaminants
If crystals develop, this may be a result of inadvertent neglect to invert and mix solutions prior to staining. Since crystals vary, it is critical to identify the type of crystal observed: crystal violet, Lugol’s, etc. Fresh, correctly constituted wash solution and fresh alcohol solution may aid in solubility of chemicals mixed on board (procedure steps 3, 4, & 5).

Lugol’s Iodine
Iodine has weak intermolecular forces, so the forces are broken easily. So instead of turning into a liquid, they turn into a gas. This is what is observed as “out gassing” from Dako Iodine packs in the AR175 Gram Stain Lugol’s Iodine. Discoloration of the pack labels or box packaging will occur and performance from the iodine chemical in this procedure has been proven to not be compromised and can be used.

Figure 21 A: 20x Crystal Violet Crystals.  B: 20x Lugol’s Iodine Crystals.

Figure 22: Lugol’s Iodine discoloration.
Notes:
This may intensify the background staining as well.
The Gram Yellow stain is used in demonstrating gram-negative and gram-positive bacteria in tissue. Crystal Violet is applied first and then followed by an iodine mordant forming a dye lake. At this point, all cells are stained blue-black. Differences in the cell wall account for differentiation in the way the bacteria will decolorize. Gram-positive, will retain the Crystal Violet Iodine complex*; after decolorization, a counterstain is applied and gram-negative, light pink to magenta are stained.

Control Tissue
Tissue containing both Gram-positive and Gram-negative bacteria

Cut Thickness
4 μm sections

Time per Slide*
00:41:01

Staining Interpretation

<table>
<thead>
<tr>
<th>Tissue Component</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram-positive organisms:</td>
<td>Dark blue</td>
</tr>
<tr>
<td>Gram-negative organisms:</td>
<td>Light Pink to Magenta</td>
</tr>
<tr>
<td>Background:</td>
<td>Yellow</td>
</tr>
</tbody>
</table>

* This time includes onboard drying and deparaffinization using the default protocol. Any adjustments to the protocol will alter the time per slide.

Figure 23: Gram Yellow.

*If the Gram positive cell walls have been damaged/disrupted or have been treated with antibiotics the bacteria will not be able to retain the CV/Iodine complex and may appear gram negative.

Important:
This protocol is not available for the Artisan™ Classic Instrument.
Procedure

**Crystal Violet** is the primary dye used to identify Gram-positive organisms. The incubation time can be decreased or increased from 0 to 1800 seconds.

**Wash Solution before Lugol’s Iodine** dilutes the Lugol’s Iodine. This step does not have adjustable incubation time and temperature.

**Lugol’s Iodine** acts as a mordant. The incubation time can be decreased or increased from 0 to 1800 seconds.

**100% Ethanol after Lugol's Iodine** acts as a differentiator for the Crystal Violet to remove stain from the Gram negative bacteria. This does not have adjustable incubation time or temperature.

**Wash Solution after 100% Alcohol** further rinses to remove Crystal Violet. Additional rinse steps can be added or deleted.

**Basic Fuchsin** stains the Gram-negative bacteria. Incubation time can be increased or decreased from 0-1800 seconds.

**Decolorizing Solution (Gallegos Solution)** is used to fix the basic fuchsin dye in the gram-negative bacteria and to remove the basic fuchsin from the background which also has the effect of changing the red color of the Gram-negative bacteria to a reddish-violet color (15). Incubation time can be increased or decreased from 0-350 seconds.

**Tartrazine** stains the background yellow. Incubation time can be increased or decreased from 0-1800 seconds.

Troubleshooting

The following are suggestions in resolving staining issues. These suggestions can be used together or as individual changes.

**Gram positive bacteria control not Staining**
- Increase incubation time in Crystal Violet
- Decrease the amount of rinses after step 4 to 3

**Gram negative bacteria control not Staining**
- Decreased the amount of rinses to 3 in step 4 (Lugol's Iodine)
- In step 5, change the third rinse from 95% alcohol to 100% alcohol, the fifth rinse to 95% alcohol
- In Step 7, decrease the rinses to 3
- In Step 8, increase the incubation time to 350 seconds
- In Step 9, decrease the rinses to 1
AR158 | Iron Stain Kit

The Iron Stain Kit is used to identify iron pigments. Loss of iron stores in bone marrow is indicative of anemia. An excess of iron deposited in organs, such as liver, spleen, kidney, and bone marrow may be a result of Hemachromatosis.

Control Tissue
Spleen or liver with hemosiderosis

Cut Thickness
4 μm sections

Time per Slide*
00:28:45

Staining Interpretation

<table>
<thead>
<tr>
<th>Tissue Component</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferric iron:</td>
<td>Blue</td>
</tr>
<tr>
<td>Nuclei:</td>
<td>Red</td>
</tr>
<tr>
<td>Background:</td>
<td>Pink</td>
</tr>
</tbody>
</table>

* This time includes onboard drying and deparaffinization using the default protocol. Any adjustments to the protocol will alter the time per slide.

Figure 24: Iron.
Procedure
Potassium Ferrocyanide and Hydrochloric Acid is combined and reacts with any ferrous ion present in the tissue, resulting in the formation of a stable bright blue pigment. The incubation time can be decreased or increased from 0 to 1800 seconds. The temperature can be decreased or increased from 23-50 °C.

Nuclear Fast Red counterstains the tissue. The incubation time can be decreased or increased from 0 to 1800 seconds. The temperature can be increased or decreased from 23-50 °C.

Troubleshooting
The following are suggestions in resolving staining issues. These suggestions can be used together or as individual changes.

Iron Too Dark
- Decrease temperature and/or incubation time in Hydrochloric Acid

Iron Too Light
- Increase incubation time in Hydrochloric Acid

Counter stain Too Dark
- Decrease temperature and/or incubation time in Nuclear Fast Red

Counter stain Too Light
- Increase incubation time in Nuclear Fast Red

Notes:
If precipitate of Nuclear Fast Red (NFR) appears at the conclusion of staining, rinse vertically in several changes of deionized water until desired results are observed.

Check recommended storage conditions of NFR on Dako labeled packaging.

Correct storage of the Nuclear Fast Red is critical. The NFR pack should never be stored in a refrigerator (2-8 °C) or allowed to cool below 15 °C. Cooling the pack will cause precipitation reaction to commence and potential visible precipitate observed on the slide. Optimal temperature is room temperature.
The Jones’ Basement Membrane (Jones’ or PAS-M) Stain Kit is used to identify glomerular and tubular basement membranes.

**Control Tissue**

Kidney with glomeri

**Cut Thickness**

2 μm sections

**Time per Slide**

01:11:28

**Staining Interpretation**

<table>
<thead>
<tr>
<th>Tissue Component</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basement membrane:</td>
<td>Black</td>
</tr>
<tr>
<td>Nuclei:</td>
<td>Red</td>
</tr>
<tr>
<td>Background:</td>
<td>Pink</td>
</tr>
</tbody>
</table>

* This time includes onboard drying and deparaffinization using the default protocol. Any adjustments to the protocol will alter the time per slide.

**Important:**

If the Gram-positive cell walls have been damaged/disrupted for being treated with antibiotics the bacteria will not be able to retain the CV/Iodine complex and may appear Gram-negative.
Procedure

**Periodic Acid** oxidizes tissue proteins to form dialdehyde groups. The incubation time can be decreased or increased from the ranges of 0 to 1800 seconds.

**Silver Enhancer** adheres to oxidized basement membranes and attracts the silver. The incubation time can be decreased or increased from 0 to 1800 seconds.

**Wash Solution after Silver Enhancer** preheats the slide and removes any excess Silver Enhancer. The incubation time can be decreased or increased from 0 to 1800 seconds. The temperature can be decreased or increased from 50-65 ºC.

**Methenamine Borate and Silver Nitrate** are combined to form a Methenamine Silver solution. This alkaline silver solution then reacts with the oxidized membrane walls rendering them visible. The incubation time can be decreased or increased from 0 to 1800 seconds. The temperature can be decreased or increased from 48-65 ºC.

**Wash Solution after Methenamine Borate and Silver Nitrate** rinses the slide and removes any excess silver. The incubation time can be decreased or increased from 0 to 1800 seconds. The temperature can be decreased or increased from 48-65 ºC.

**Gold Chloride** tones the silver to a black color. The incubation time can be decreased or increased from 0 to 1800 seconds.

**Sodium Thiosulfate** removes unreduced silver. The incubation time can be decreased or increased from 0 to 1800 seconds.

**Nuclear Fast Red** counterstains the tissue. The incubation time can be increased or decreased from 0 to 1800 seconds.

Troubleshooting

The following are suggestions in resolving staining issues. These suggestions can be used together or as individual changes.

For the best results, it is advisable to not change the default temperature settings, because the chemical reactions required are extremely dependent on appropriate temperatures.

**Basement Membranes Too Dark**
- Decrease incubation time in Silver Nitrate and/or Wash Solution after Silver Enhancer

**Basement Membranes Too Light**
- Increase incubation time in Silver Nitrate or increase incubation time in Periodic Acid. Be aware that over hydrolysis (Step 2) will result in a less than optimal stain.
Basement Membranes Too Brown
- Increase incubation time in Gold Chloride

Counterstain Too Dark
- Decrease incubation time in Nuclear Fast Red

Counterstain Too Light
- Increase incubation time in Nuclear Fast Red

Notes:
- If precipitate of NFR appears upon staining conclusion, rinse slide vertically in DI water until desired results appears.
- Correct storage of the Nuclear Fast Red is critical. The NFR pack should never be stored in a refrigerator (2-8 °C) or allowed to cool below 15 °C. Cooling the pack will cause precipitation reaction to commence and potential visible precipitate observed on the slide. Optimal temperature is room temperature.
- Keep reagent packs stored per manufacturer’s instructions. Acclimate reagent completely to ambient temperature before each use. Colder reagent packs will significantly under develop tissue elements.
- Avoid heavily charged slides, especially covalently coated silanized slides or Dako’s Flex Slides. The charged coating will bind to the silver nitrate solution rendering the slide indistinguishable from the tissue.
- Water quality is imperative for all silver stains. The resistivity is inversely proportional to the ionic content of water, the higher the water’s ion concentration, the lower its resistivity. Therefore, resistivity measurements are useful to assess the ion content of the water. The measurement is sensitive to the point where the only ionized species are the hydrogen and hydroxyl ions contributed by the water itself. A resistivity of 15 MΩ/cm at 25 °C is the minimum for purified reagent water, the concentration of ionic species is less than 10 gram equivalents per liter. (6) At this minimum, background caused by ionic binding with the ions in the water is at a minimum.
AR480 | Jones’ Basement Membrane H&E Stain Kit

The Jones’ Basement Membrane (Jones’ or PAS-M) H&E Stain Kit is used to identify glomerular and tubular basement membranes.

Control Tissue
Kidney with glomeri

Cut Thickness
2 μm sections

Time per Slide*
01:04:02

Staining Interpretation

<table>
<thead>
<tr>
<th>Tissue Component</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basement membrane:</td>
<td>Black</td>
</tr>
<tr>
<td>Nuclei:</td>
<td>Blue</td>
</tr>
<tr>
<td>Background:</td>
<td>Pink</td>
</tr>
</tbody>
</table>

* This time includes onboard drying and deparaffinization using the default protocol. Any adjustments to the protocol will alter the time per slide.

Fig. 26: Jones’ H&E.

Important:
This protocol is not available on the Artisan™ Classic Instrument.
Procedure

**Periodic Acid** oxidizes tissue proteins to form dialdehyde groups. The incubation time can be decreased or increased from the ranges of 0 to 1800 seconds.

**Silver Enhancer** adheres to oxidized basement membranes and attracts the silver. The incubation time can be decreased or increased from 0 to 1800 seconds.

**Wash Solution after Silver Enhancer** preheats the slide and removes any excess Silver Enhancer. The incubation time can be decreased or increased from 0 to 1800 seconds. The temperature can be decreased or increased from 50-65 °C.

**Methenamine Borate and Silver Nitrate** are combined to form a Methenamine Silver solution. This alkaline silver solution then reacts with the oxidized membrane walls rendering them visible. The incubation time can be decreased or increased from 0 to 1800 seconds. The temperature can be decreased or increased from 48-65 °C.

**Wash Solution after Methenamine Borate and Silver Nitrate** rinses the slide and removes any excess silver. The incubation time can be decreased or increased from 0 to 1800 seconds. The temperature can be decreased or increased from 48-65 °C.

**Gold Chloride** tones the silver to a black color. The incubation time can be decreased or increased from 0 to 1800 seconds.

**Sodium Thiosulfate** removes unreduced silver. The incubation time can be decreased or increased from 0 to 1800 seconds.

**Hematoxylin** stains the nuclei. The incubation time can be increased or decreased from 0 to 1800 seconds.

**Eosin** stains the background. The incubation time can be increased or decreased from 0 to 1800 seconds.

Troubleshooting

The following are suggestions in resolving staining issues. These suggestions can be used together or as individual changes.

For the best results, it is advisable to not change the default temperature settings, because the chemical reactions required are extremely dependent on appropriate temperatures.

**Basement Membranes Too Dark**

- Decrease incubation time in Silver Nitrate and Wash Solution after Silver Enhancer
Basement Membranes Too Light
- Increase incubation time in Silver Nitrate or increase incubation time in Periodic Acid.
  Be aware that over hydrolysis (step 2) will result in a less than optimal stain.

Basement Membranes Too Brown
- Increase incubation time in Gold Chloride

Notes:
Keep reagent packs stored per manufacturer’s instructions. Acclimate reagent completely to ambient temperature before each use. Colder reagent packs will significantly under develop tissue elements.

Avoid heavily charged slides, especially covalently coated silanized slides or Dako’s Flex Slides. The charged coating will bind to the silver nitrate solution rendering the slide indistinguishable from the tissue.

Water quality is imperative for all silver stains. The resistivity is inversely proportional to the ionic content of water, the higher the water’s ion concentration, the lower its resistivity. Therefore, resistivity measurements are useful to assess the ion content of the water. The measurement is sensitive to the point where the only ionized species are the hydrogen and hydroxyl ions contributed by the water itself. A resistivity of 15 MΩ/cm at 25 °C is the minimum for purified reagent water, the concentration of ionic species is less than 10 gram equivalents per liter. At this minimum, background caused by ionic binding with the ions in the water is at a minimum.
The Jones’ Basement Membrane (Jones’ or PAS-M) Light Green Stain Kit is used to identify glomerular and tubular basement membranes.

**Control Tissue**
- Kidney with glomeri

**Cut Thickness**
- 2 μm sections

**Time per Slide**
- 01:01:36

**Staining Interpretation**

<table>
<thead>
<tr>
<th>Tissue Component</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basement membrane:</td>
<td>Black</td>
</tr>
<tr>
<td>Background:</td>
<td>Light green</td>
</tr>
</tbody>
</table>

* This time includes onboard drying and deparaffinization using the default protocol. Any adjustments to the protocol will alter the time per slide.

**Figure 27: Jones Light Green.**

**Important:**
This protocol is not available on the Artisan™ Classic Instrument.
Procedure

**Periodic Acid** oxidizes tissue proteins to form dialdehyde groups. The incubation time can be decreased or increased from the ranges of 0 to 1800 seconds.

**Silver Enhancer** adheres to oxidized basement membranes and attracts the silver. The incubation time can be decreased or increased from 0 to 1800 seconds.

**Wash Solution after Silver Enhancer** preheats the slide and removes any excess Silver Enhancer. The incubation time can be decreased or increased from 0 to 1800 seconds. The temperature can be decreased or increased from 50-65 °C.

**Methenamine Borate and Silver Nitrate** are combined to form a Methenamine Silver solution. This alkaline silver solution then reacts with the oxidized membrane walls rendering them visible. The incubation time can be decreased or increased from 0 to 1800 seconds. The temperature can be decreased or increased from 48-65 °C.

**Wash Solution after Methenamine Borate and Silver Nitrate** rinses the slide and removes any excess silver. The incubation time can be decreased or increased from 0 to 1800 seconds. The temperature can be decreased or increased from 48-65 °C.

**Gold Chloride** tones the silver to a black color. The incubation time can be decreased or increased from 0 to 1800 seconds.

**Sodium Thiosulfate** removes unreduced silver. The incubation time can be decreased or increased from 0 to 1800 seconds.

**Light Green** stains the background. The incubation time can be decreased or increased from 0 to 1800 seconds.

Troubleshooting

The following are suggestions in resolving staining issues. These suggestions can be used together or as individual changes.

For the best results, it is advisable to not change the default temperature settings, because the chemical reactions required are extremely dependent on appropriate temperatures.

**Basement Membranes Too Dark**
- Decrease incubation time in Silver Nitrate and/or Wash Solution after Silver Enhancer

**Basement Membranes Too Light**
- Increase incubation time in Silver Nitrate or increase incubation time in Periodic Acid. Be aware that over hydrolysis (Step 2) will result in a less than optimal stain.

**Basement Membranes Too Brown**
- Increase incubation in Gold Chloride
Notes:
Keep reagent packs stored per manufacturer's instructions. Acclimate reagent completely to ambient temperature before each use. Colder reagent packs will significantly under develop tissue elements.

Avoid heavily charged slides, especially covalently coated silanized slides or Dako's Flex Slides. The charged coating will bind to the silver nitrate solution rendering the slide indistinguishable from the tissue.

Water quality is imperative for all silver stains. The resistivity is inversely proportional to the ionic content of water, the higher the water's ion concentration, the lower its resistivity. Therefore, resistivity measurements are useful to assess the ion content of the water. The measurement is sensitive to the point where the only ionized species are the hydrogen and hydroxyl ions contributed by the water itself. A resistivity of 15 MΩ/cm at 25 °C is the minimum for purified reagent water, the concentration of ionic species is less than 10 gram equivalents per liter. At this minimum, background caused by ionic binding with the ions in the water is at a minimum.
AR173 | Masson’s Trichrome Stain Kit

The Masson’s Trichrome Stain Kit is used to identify muscle, collagen fiber, fibrin and erythrocytes in tissue.

**Control Tissue**

| Uterus, small intestine, liver, appendix or fallopian tube |

**Cut Thickness**

| 4 μm sections |

**Time per Slide**

| 01:15:56 |

**Staining Interpretation**

<table>
<thead>
<tr>
<th>Tissue Component</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle fibers:</td>
<td>Red</td>
</tr>
<tr>
<td>Fibrin:</td>
<td>Pink</td>
</tr>
<tr>
<td>Collagen:</td>
<td>Blue</td>
</tr>
<tr>
<td>Nuclei:</td>
<td>Blue or black</td>
</tr>
<tr>
<td>Erythrocytes:</td>
<td>Red</td>
</tr>
</tbody>
</table>

* This time includes onboard drying and deparaffinization using the default protocol. Any adjustments to the protocol will alter the time per slide.

**Figure 28:** Kidney stained with Masson’s Trichrome.

**Figure 29:** Liver stained with Masson’s Trichrome.

**Important:**

This protocol is not available on the Artisan™ Classic Instrument.
**Procedure**

**Bouin’s Solution** acts as a mordant. The incubation time can be decreased or increased from 0 to 1800 seconds. The temperature can be decreased or increased from 37-60 °C.

**Wash Solution after Bouin’s Solution** rinses the Bouin’s Solution from the tissue. Extra rinses may be added.

**Second and Third Wash Solutions after Bouin’s Solution** rinses the Bouin’s Solution from the tissue. The incubation time can be decreased or increased from 0 to 1800 seconds. The temperature can be decreased or increased from 23-60 °C.

**Weigert’s A – Masson’s and Weigert’s B** are combined to make a working Weigert’s Hematoxylin solution, which is used to stain nuclear chromatin. The incubation time can be decreased or increased from 100 to 900 seconds.

**Wash Solution after Weigert’s A and Weigert’s B** rinses the working Weigert’s Hematoxylin solution from the tissue.

**Biebrich Scarlet Acid Fuchsin and Wash Solution** are combined and stain the acidophilic cytoplasm and muscle fibers. The incubation time can be decreased or increased from 0 to 1800 seconds.

**Phosphotungstic- Phosphomolybdic Acid** creates an environment for a substitution reaction on the tissue. Phosphotungstic Phosphomolybdic Acid is taken up by the connective tissue, which allows it to be replaced by Aniline Blue. The incubation time can be decreased or increased from 0 to 1800 seconds. The temperature can be decreased or increased from 23-48 °C.

**Wash Solution and Aniline Blue** are combined to stain collagen fibers. The incubation time can be decreased or increased from 0 to 1800 seconds. The temperature can be decreased or increased from 23-60 °C.

**Wash Solution and Acetic Acid** are combined to allow differentiation between the muscle and collagen fibers. The incubation time can be decreased or increased from 0 to 1800 seconds.

**Troubleshooting**
The following are suggestions in resolving staining issues. These suggestions can be used together or as individual changes.

**Nuclei Too Light**
- Increase the mixes in the Bouins Step.
- Decrease the amount of rinses after Step 7 & 8.
Red Too Dark
- Decrease incubation time in the Bouin's Solution
  Be aware that this will lessen the tissue’s ability to retain the red dye

Red Too Light
- Increase incubation time in Biebrich Scarlet Acid Fuchsin
- Increase incubation (and/or heat) in Bouin's Solution
- Increase the mixes in the Bouins Solution

Blue Too Dark
- Decrease temperature (and/or incubation) in Aniline Blue
- Decrease Phos/Phos incubation and/or heat until desired blue achieved

Blue Too Light
- Increase incubation time in Aniline Blue
- Increase the mixes in the Bouins Solution
- Decrease the amount of rinses after Step 13

Notes:
Any extreme procedural changes to the red or blue will create an overall purple color. The theory behind the purple observation is that the Biebrich Scarlet is washed out of the tissue section when sub-optimal or alternative fixatives to 10% NBF are utilized. Consequently, the aniline blue takes over these areas deeming them purple in appearance. A qualified pathologist should interpret these results since some disease processes can mimic these affects also. Please review Dako package insert for tissue recommendations. Using a predetermined control tissue recommended in the Dako package insert specifications will aid in determining quality control of unknown fixed specimens.
AR168 | Mucicarmine Stain Kit

The Mucicarmine Stain Kit is used to identify epithelial mucins.

**Control Tissue**
Small intestine, colon or appendix

**Cut Thickness**
4 μm sections

**Time per Slide**
01:09:39

**Staining Interpretation**

<table>
<thead>
<tr>
<th>Tissue Component</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mucin:</td>
<td>Pink</td>
</tr>
<tr>
<td>Nuclei:</td>
<td>Black</td>
</tr>
<tr>
<td>Background:</td>
<td>Yellow</td>
</tr>
</tbody>
</table>

* This time includes onboard drying and deparaffinization using the default protocol. Any adjustments to the protocol will alter the time per slide.

Figure 30: Mucicarmine.
Procedure

Weigert's A and Weigert's B are combined to make a working Weigert's Hematoxylin solution, which is used to stain nuclear chromatin. The incubation time can be decreased or increased from 0 to 1800 seconds.

Acid Alcohol decolorizes by removing excess Weigert's Hematoxylin solution from the background. The incubation time can be decreased or increased from 0 to 1800 seconds.

Wash Solution after Acid Alcohol rinses the Acid Alcohol completely from the tissue. The incubation time can be decreased or increased from 0 to 1800 seconds.

Wash Solution and Mucicarmine Solution are combined to stain epithelial mucins. The incubation time can be decreased or increased from 0 to 3000 seconds. The temperature can be decreased or increased from 23-45 °C.

Tartrazine counterstains the tissue. The incubation time can be decreased or increased from 0 to 1800 seconds.

Troubleshooting

The following are suggestions in resolving staining issues. These suggestions can be used together or as individual changes.

Nuclei Too Light
- Increase incubation time in Weigert's B

Mucin Too Light
- Increase incubation time in Mucicarmine Solution
- Increase temperature in the Mucicarmine Solution

Mucin Too Dark
- Decrease incubation time in Mucicarmine Solution

Notes:

Tartrazine, an anionic dye binds to cationic tissue elements that are minimally impacted by heat, time, or concentration. The pH is the most significant influence on the staining reaction.
**AR313 | Orcein Stain Kit**

Virus particles inside host cells are called viral inclusion bodies. A hepatitis B virus lies on the surface of the virus particle. Hepatitis B Surface Antigen (HBsAG) can be detected by using the Orcein staining method. The antigen appears as fine granules either diffusely spread throughout the cytoplasm or concentrated in the cytoplasm peripheral to the sinusoid space. Copper associated proteins when in excessive pathologic amounts, such as Wilson’s disease and some forms of cirrhosis, can be detected by using the Orcein staining method.

<table>
<thead>
<tr>
<th>Control Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver tissue containing positive HBsAG. Fetal liver can be used for Copper or liver with Wilson’s disease. A multi-block containing positive liver for HBsAG and Copper with normal liver is recommended.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cut Thickness</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 μm sections</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time per Slide*</th>
</tr>
</thead>
<tbody>
<tr>
<td>00:34:25</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Staining Interpretation</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBsAG/Elastin fibers:</td>
<td>Dark reddish brown</td>
</tr>
<tr>
<td>Copper associated protein:</td>
<td>Dark red to purple</td>
</tr>
<tr>
<td>Background:</td>
<td>Pale pink to pink</td>
</tr>
</tbody>
</table>

* This time includes onboard drying and deparaffinization using the default protocol. Any adjustments to the protocol will alter the time per slide.

**Important:**

This protocol is not available on the Artisan™ Classic Instrument.

**Figure 31: Orcein - Copper Associated Protein.**

**Figure 32: Orcein - HBs/AG.**
**Procedure**

*Potassium Permanganate and Sulfuric Acid* are mixed together to make acid permanganate to oxidize the tissue. Incubation time can be increased or decreased from 0-1800 seconds.

*Oxalic Acid* clears the acid permanganate completely from the tissue. Incubation time can be increased or decreased from 0 to 1800 seconds.

*Orcein Solution* stains the Hepatitis B Surface Antigen, Elastic Fibers and the Copper associated proteins. Incubation time can be increased or decreased from 0 to 3600 seconds. Heat can be increased or decreased from 23-52 °C

**Troubleshooting**
The following are suggestions in resolving staining issues. These suggestions can be used together or as individual changes.

**Background to dark**
- Reduce the Orcein time to 300 seconds in step 5
- Change the last two bulk liquid rinses from wash solution to 95% Alcohol in step 5

**To remove residual stain from slides**
- After removing slides from the Artisan™ Link, dehydrate starting with 3-5 dips in 95% alcohol, 100% Alcohol then Xylene or Xylene substitute

  or

- After removing slides from Artisan™ Link, dehydrate starting with 3 dips in 70% alcohol, 3 dips in 95% alcohol, 100% Alcohol (2 changes) then Xylene

**Notes:**
Excessive alcohol rinses may reduce staining results.
The Periodic Acid-Schiff (PAS) Stain Kit is used for demonstrating glycogen in the liver. Duplicate sections are stained with and without a pretreatment of the glycogen-digesting enzyme, Alpha-Amylase, followed by the PAS procedure.

**Control Tissue**

Liver containing glycogen. Kidney for basement membrane

**Cut Thickness**

4 μm sections

**Time per Slide***

01:18:39

**Staining Interpretation**

<table>
<thead>
<tr>
<th>Tissue Component</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAS-positive structures:</td>
<td>Magenta</td>
</tr>
<tr>
<td>Nuclei:</td>
<td>Blue</td>
</tr>
<tr>
<td>Background:</td>
<td>Pink</td>
</tr>
</tbody>
</table>

* This time includes onboard drying and deparaffinization using the default protocol. Any adjustments to the protocol will alter the time per slide.

Figure 33: PAS with Alpha Amylase.

Figure 34: PAS without Alpha Amylase.
Procedure
There are two procedures for Periodic Acid-Schiff: PAS and PAS with Alpha-Amylase Digestion. Alpha-Amylase is sold as a separate pack and is used in conjunction with the PAS kit to digest glycogen.

PAS and PAS with Alpha-Amylase Digestion

Alpha-Amylase digests glycogen in tissue. The incubation time can be decreased or increased from 0 to 1800 seconds. The temperature can be decreased or increased from 32-60 °C.

Periodic Acid oxidizes tissue proteins to produce dialdehyde groups. The incubation time can be decreased or increased from 0 to 1800 seconds. The temperature can be decreased or increased from 23-60 °C.

Schiff’s Reagent reacts with the dialdehyde groups. The incubation time can be decreased or increased from 0 to 1800 seconds. The temperature can be decreased or increased from 23-60 °C.

Wash Solution after Schiff’s Reagent intensifies the pink color imparted by the Schiff’s Reagent. The incubation time can be decreased or increased from 0 to 1800 seconds. The temperature can be decreased or increased from 23-60 °C.

Mayer’s Hematoxylin counterstains the tissue. The incubation time can be decreased or increased from 0 to 1800 seconds.

Bluing Reagent changes the hue of the Mayer’s Hematoxylin from purple to blue. The incubation time can be decreased or increased from 0 to 1800 seconds.

Troubleshooting
The following are suggestions in resolving staining issues. These suggestions can be used together or as individual changes.

Digestion Too Weak, undesired glycogen background
- Increase incubation time in Alpha-Amylase

Schiff’s Too Dark
- Decrease temperature of Periodic Acid
- Decrease temperature of the Schiff’s reagent
Schiff's Too Light
- Add additional mixes to the Periodic Acid
- Increase incubation time in either Schiff’s reagent
- Increase Wash Solution after Schiff’s reagent

Counterstain Too Dark
- Decrease incubation time in Mayer’s Hematoxylin

Counter stain Too Light
- Increase incubation time in Mayer’s Hematoxylin

Nuclei Too Purple
- Increase incubation time in Bluing Reagent

Notes:
Stability of Schiff's reagent
Schiff's reagent stability is directly influenced by the sulphite to sulphate gas exchange of sulphurous acid Schiff's content. If the Schiff's reagent is kept at 2-8 °C, the gas exchange is slowed, hence preserving the reactivity of the Schiff's reagent (10). Correct storage temperature is critical.

When use of the Schiff's reagent is required, acclimating Schiff's reagent to ambient temperature prior to instrument loading is necessary for specification staining.
The Periodic Acid-Schiff/Green (PAS/Green) Stain Kit is used to identify fungi in tissue samples.

Control Tissue
Skin tissue with *Candida albicans*

Cut Thickness
4 μm sections

Time per Slide*
00:43:54

Staining Interpretation

<table>
<thead>
<tr>
<th>Tissue Component</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fungi:</td>
<td>Magenta</td>
</tr>
<tr>
<td>Background:</td>
<td>Blue to green</td>
</tr>
</tbody>
</table>

* This time includes onboard drying and deparaffinization using the default protocol. Any adjustments to the protocol will alter the time per slide.
Procedure

Periodic Acid oxidizes adjacent hydroxyl groups in the mucopolysaccharides components of the fungal cell wall, which creates dialdehyde groups. The incubation time can be decreased or increased from 0 to 1800 seconds. The temperature can be decreased or increased from 23-50 ºC.

Schiff’s Reagent reacts with the dialdehyde groups. The incubation time can be decreased or increased from 0 to 1800 seconds. The temperature can be decreased or increased from 23-50 ºC.

Wash Solution after Schiff’s Reagent intensifies the pink color imparted by the Schiff’s Reagent. The incubation time can be decreased or increased from 0 to 1800 seconds. The temperature can be decreased or increased from 23-58 ºC.

Light Green counterstains the tissue. The incubation time can be increased or decreased from 0 to 1800 seconds.

Troubleshooting

The following are suggestions in resolving staining issues. These suggestions can be used together or as individual changes.

Schiff’s Too Dark
- Decrease the temperature of the Periodic Acid
- Decrease the temperature of Schiff’s Reagent

Schiff’s Too Light
- Add additional mixes to the Periodic Acid
- Increase the incubation time in Schiff’s reagent
- Increase the incubation time in the Wash Solution after Schiff’s reagent

Counterstain Too Dark
- Decrease the incubation time in Light Green

Counterstain Too Light
- Increase the incubation time in Light Green

Notes:
- Stability of Schiff’s reagent: Schiff’s reagent stability is directly influenced by the sulphite to sulphate gas exchange of sulphurous acid Schiff’s content. If the Schiff’s reagent is kept at 2-8 ºC, the gas exchange is slowed, hence preserving the reactivity of the Schiff’s reagent (10). Correct storage temperature is critical.
- When use of the Schiff’s reagent is required, acclimating Schiff’s reagent to ambient temperature prior to instrument loading is necessary for specification staining.
- Dead fungus (fungus that has died before the biopsy was procured) will not stain adequately. Quality control of unknown tissues is possible by staining predetermined positive control tissue simultaneously.
- The most common use is to demonstrate dermatophytic fungi found in skin lesions. Unlike the Grocott’s Methenamine Silver (GMS) Stain Kit, the PAS/Green stain will not identify all fungi and yeast (9).
AR182 | Reticulin/No Counterstain Kit

The Reticulin/No Counterstain (Retic/NCS) Kit is used to identify a primitive form of connective tissue called Reticulin.

Control Tissue
Liver, spleen and lymph node

Cut Thickness
4 μm sections

Time per Slide*
00:57:32

Staining Interpretation

<table>
<thead>
<tr>
<th>Tissue Component</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reticulin fibers:</td>
<td>Black</td>
</tr>
</tbody>
</table>

* This time includes onboard drying and deparaffinization using the default protocol. Any adjustments to the protocol will alter the time per slide.

Figure 36: Reticulin fibers.
Procedure

Potassium Permanganate oxidizes the tissue. The incubation time can be increased or decreased from 0 to 1800 seconds.

Oxalic Acid removes excess Potassium Permanganate from the tissue. The incubation time can be increased or decreased from 0 to 1800 seconds.

Ferric Ammonium Sulfate is used to sensitize the tissue to allow for replacement of the metal-organic compounds with the silver from the silver solution. The incubation time can be decreased or increased from 0 to 1800 seconds.

Wash Solution and Ammoniacal Silver Nitrate is combined to make a working silver solution. The incubation time can be decreased or increased from 0 to 1800 seconds.

Wash Solution after Ammoniacal Silver Nitrate further dilutes the Ammoniacal Silver Nitrate solution. This step does not have adjustable incubation time and temperature.

Alcoholic Formalin and Ethanol 95% reduces the silver deposited on the oxidized tissue to visible metallic silver. The Alcoholic Formalin incubation time cannot be adjusted. The Ethanol 95% incubation time can be decreased or increased from 0 to 1800 seconds.

Gold Chloride tones the silver to a black color. The incubation time can be decreased or increased from 0 to 1800 seconds.

Sodium Thiosulfate removes unreduced silver. The incubation time can be decreased or increased from 0 to 1800 seconds.

Troubleshooting

The following are suggestions in resolving staining issues. These suggestions can be used together or as individual changes.

Reticulin Too Light

- Increase incubation time in Ammoniacal Silver Nitrate and/or Ethanol 95%
- Increase incubation time in the Potassium Permanganate.

Notes:

Keep reagent packs stored per manufacturer's instructions. Acclimate reagent completely to ambient temperature before each use. Colder reagent packs will significantly under develop tissue elements.

Avoid heavily charged slides, especially covalently coated silanized slides or Dako's Flex Slides. The charged coating will bind to the silver nitrate solution rendering the slide indistinguishable from the tissue.

Water quality is imperative for all silver stains. The resistivity is inversely proportional to the ionic content of water, the higher the water's ion concentration, the lower its resistivity. Therefore, resistivity measurements are useful to assess the ion content of the water. The measurement is sensitive to the point where the only ionized species are the hydrogen and hydroxyl ions contributed by the water itself. A resistivity of 15 MΩ/cm at 25 °C is the minimum for purified reagent water, the concentration of ionic species is less than 10 gram equivalents per liter.(6) At this minimum, background caused by ionic binding with the ions in the water is at a minimum.
AR179 | Reticulin/Nuclear Fast Red Stain Kit

The Reticulin/Nuclear Fast Red (Retic/NFR) Stain Kit is used to identify a primitive form of connective tissue called reticulin. The tissue is counterstained with Nuclear Fast Red.

Control Tissue
Liver, spleen and lymph node

Cut Thickness
4 μm sections

Time per Slide*
01:02:59

Staining Interpretation

<table>
<thead>
<tr>
<th>Tissue Component</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reticulin fibers:</td>
<td>Black</td>
</tr>
<tr>
<td>Background:</td>
<td>Red</td>
</tr>
</tbody>
</table>

* This time includes onboard drying and deparaffinization using the default protocol. Any adjustments to the protocol will alter the time per slide.

Figure 37: Reticulin Nuclear Fast Red.
**Procedure**

**Potassium Permanganate** oxidizes the tissue. The incubation time can be increased or decreased from 0 to 1800 seconds.

**Oxalic Acid** removes excess Potassium Permanganate from the tissue. The incubation time can be increased or decreased from 0 to 1800 seconds.

**Ferric Ammonium Sulfate** is used to sensitize the tissue to allow for replacement of the metal-organic compounds with the silver from the silver solution. The incubation time can be decreased or increased from 0 to 1800 seconds.

**Wash Solution and Ammoniacal Silver Nitrate** are combined to make a working silver solution. The incubation time can be decreased or increased from 0 to 1800 seconds.

**Wash Solution after Ammoniacal Silver Nitrate** further dilutes the Ammoniacal Silver Nitrate solution. This step does not have adjustable incubation time and temperature.

**Alcoholic Formalin and Ethanol 95%** reduce the silver deposited on the oxidized tissue to visible metallic silver. The Alcoholic Formalin incubation time cannot be adjusted. The Ethanol 95% incubation time can be decreased or increased from 0 to 1800 seconds.

**Gold Chloride** tones the silver to a black color. The incubation time can be decreased or increased from 0 to 1800 seconds.

**Sodium Thiosulfate** removes unreduced silver. The incubation time can be decreased or increased from 0 to 1800 seconds.

**Nuclear Fast Red** counterstains the tissue. The incubation time can be increased or decreased from 0 to 1800 seconds.

**Troubleshooting**

The following are suggestions in resolving staining issues. These suggestions can be used together or as individual changes.

- **Reticulin Too Light**
  - Increase incubation time in Ammoniacal Silver Nitrate and/or Ethanol 95%

- **Counterstain Too Dark**
  - Decrease incubation time in Nuclear Fast Red

- **Counterstain Too Light**
  - Increase incubation time in Nuclear Fast Red
Notes:

If precipitate of NFR appears at the conclusion of staining, rinse vertically in several changes of deionized water until desired results are observed.

Correct storage of the Nuclear Fast Red is critical. Verify storage conditions on the Dako labeled packaging. The NFR pack should never be stored in a refrigerator or allowed to cool below 15 °C. Cooling the pack will cause precipitation reaction to commence and potential visible precipitate observed on the slide. Optimal temperature is room temperature.

Keep reagent packs stored per manufacturer’s instructions. Acclimate reagent completely to ambient temperature before each use. Colder reagent packs will significantly under develop tissue elements.

Avoid heavily charged slides, especially covalently coated silanized slides or Dako’s Flex slides. The charged coating will bind to the silver nitrate solution rendering the slide indistinguishable from the tissue.

Water quality is imperative for all silver stains. The resistivity is inversely proportional to the ionic content of water, the higher the water’s ion concentration, the lower its resistivity. Therefore, resistivity measurements are useful to assess the ion content of the water. The measurement is sensitive to the point where the only ionized species are the hydrogen and hydroxyl ions contributed by the water itself. A resistivity of 15 MΩ/cm at 25 °C is the minimum for purified reagent water, the concentration of ionic species is less than 10 gram equivalents per liter. At this minimum, background caused by ionic binding with the ions in the water is at a minimum.
**AR181 | Warthin-Starry Stain Kit**

The Warthin-Starry (W-S) Stain Kit is used to identify *Helicobacter pylori* and Spirochete.

<table>
<thead>
<tr>
<th>Control Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue with helicobacter and spirochetes</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cut Thickness</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 μm sections</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time per Slide*</th>
</tr>
</thead>
<tbody>
<tr>
<td>00:26:21</td>
</tr>
</tbody>
</table>

**Staining Interpretation**

<table>
<thead>
<tr>
<th>Tissue Component</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Helicobacter pylori</em></td>
<td>Black</td>
</tr>
<tr>
<td>Spirochetes:</td>
<td>Black</td>
</tr>
<tr>
<td>Background</td>
<td>Golden yellow</td>
</tr>
</tbody>
</table>

* This time includes onboard drying and deparaffinization using the default protocol. Any adjustments to the protocol will alter the time per slide.

![Figure 38: Warthin Starry – Spirochetes.](image1)

![Figure 39: Warthin Starry – Helicobacter pylori.](image2)
Procedure

**Pretreatment** prevents non-specific silver staining. The incubation time can be decreased or increased from 0 to 1800 seconds. The temperature can be decreased or increased from 35-65 ºC.

**Silver Nitrate** contains the silver used to impregnate the tissue. The incubation time can be increased or decreased from 0 to 1800 seconds. The temperature can be increased or decreased from 35-65 ºC.

**Hydroquinone** reduces the silver to its metallic, visible form. The incubation time can be decreased or increased from 0 to 1800 seconds. The temperature can be decreased or increased from 35-65 ºC.

**Troubleshooting**
The following are suggestions in resolving staining issues. These suggestions can be used together or as individual changes.

**Organisms Too Light**
- Increase heat on silver Nitrate step
- Increase heat on hydroquinone step

**Background Too Dark**
- Decrease heat on silver Nitrate step
- Decrease heat on Hydroquinone step

**Notes:**
Keep reagent packs stored per manufacturer’s instructions. Acclimate reagent completely to ambient temperature before each use. Colder reagent packs will significantly under develop tissue elements and undermine the instrument heat procedure settings to develop the tissue consistently.

Avoid heavily charged slides, especially covalently coated silanized slides or Dako’s Flex Slides. The charged coating will bind to the silver nitrate solution rendering the slide indistinguishable from the tissue.

Water quality is imperative for all silver stains. The resistivity is inversely proportional to the ionic content of water, the higher the water’s ion concentration, the lower its resistivity. Therefore, resistivity measurements are useful to assess the ion content of the water. The measurement is sensitive to the point where the only ionized species are the hydrogen and hydroxyl ions contributed by the water itself. A resistivity of 15 MΩ/cm at 25 ºC is the minimum for purified reagent water, the concentration of ionic species is less than 10 gram equivalents per liter.(6) At this minimum, background caused by ionic binding with the ions in the water is at a minimum.
Cleaning and Maintenance

For current detailed Maintenance recommendations, consult the latest revision of Dako Artisan™ Link Basic User Guide.

Troubleshooting Artisan™ Link System

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Cause/Course of Action</th>
</tr>
</thead>
</table>
| Poor staining                                | ■ Reagent packs not properly primed  
■ Prime the reagent pack by inverting the reagent pack 2-3 times and point dispenser tip into a proper waste receptacle. Squirt 1 steady stream. Repeat if steady stream is not observed.  
■ Check dispenser assemblies for precipitate in test dispense stream and remove if present. If the problem persists, contact Technical Service Group.  
■ Verify working solution of Wash Solution and alcohol shelf life and reagent constitution. Remake fresh wash solution and/or alcohol if unsure. Rotate and clean bulk bottles routinely (refer to User Guide).  
■ Bulk bottles were not primed before session start. Prime all bulk bottles and perform “valve rinse” from fluid screen.  
■ Reagent pack chemicals were not acclimated to specific onboard ambient temperature before use. Repeat staining run.  
■ Check the stain notes in previous section for custom troubleshooting |
<p>| Slide clip not properly seated on the slide glass surface | Retry the procedure with new tissue and slide clip. Do not use slide similar to Figure 1. Make sure the clip gasket is not seated on a label surface like Figure 2 (red). If the problem persists, contact Technical Service Group. |
| Reagent pack empty (weighs less than 97mg)    | Replace the reagent pack.                                                                                                                                       |
| Slide not properly positioned                | Reseat the slide, ensuring that it lies flat between the guide rails. After snapping the slide clip in place, gently push forward to secure the gasket to the glass surface. |
| 2D label on slide won’t scan                 | Attempt to scan with hand held 2D scanner. Re-apply a reprinted label, making sure no bubbles are between the flap and the label surface. Apply even pressure to smooth label surface. Re-assign new patient ID and retry. If persists, call Dako Technical Support Group. |</p>
<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Cause/Course of Action</th>
</tr>
</thead>
</table>
| Green light is not illuminated on the instrument display or Artisan™ does not respond to user commands. | - Turn the switch on the power supply box or rear of Artisan™ (Pro) to the “|” (On) position  
- Instrument power cord not plugged in. Ensure the power cord is plugged securely into the power supply box or the rear of Artisan™ (Pro).  
- Instrument access door is open  
- Close the access door  
- Network cable unplugged  
- Plug the network cable securely into the instrument and CPU |
| Waste trap is full | - Empty waste bottles and waste trap, replace with caps secure; perform “rinse valves” from fluid screen observe steady fluid stream. Upon completion, verify there is no waste remaining in the 49/50 slide carousel positions.  
- O-ring missing from waste cap. Replace O-ring. |
| Instrument pressurization error | Bulk liquid bottles **not** completely sealed. Ensure the cap is properly seated on the bulk liquid bottles and tighten all waste and bulk liquid caps. |
| Computer monitor screen blank at system startup. Orange indicator light on monitor. | - Monitor not powered on or “sleep”  
- Press the monitor power button located on  
- The front of the monitor |
| Slide carousel will not move | - Slide clip improperly seated  
- Inspect the loaded slide clips within guide rails  
- Object is caught inside the instrument, causing the jam. Attempt to depress all loaded slide clips to ensure that they are properly seated. Inspect for loose objects. Make sure the reagent packs are loaded properly. |
| Door slightly ajar, causing CSA switch to open. | Close the door tightly to close the CSA switch. |
| Session not started | Click Begin Staining. You may enable System Configuration settings to have the run begin automatically upon START. |
| Slide clip leak/dirty ring residue remains at completion of staining | - Remove any fluid around guide  
- Slide improperly seated within guide rails that may have leaked  
- Insert a new slide onto the slide carousel, ensuring that the slide is within the guide rail. Add a fresh slide clip and start a new run.  
- Depress the slide clip until an audible click is heard. Gently push the clip forward to remove any gap between the gasket seal and glass. Make sure S3393 labels are being used and the label is not resting under the slide clip gasket. Do not re-use slide clips. |
<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Cause/Course of Action</th>
</tr>
</thead>
</table>
| Fluid left on slide at end of a run | - Waste bottle caps not tight  
- Tighten the waste bottle caps, perform “rinse valves” from fluid screen  
- Slide clip not properly seated within guide rails, interfering with aspirator  
- An audible click should be heard when the slide clip is properly seated. Gently push the clip forward to remove any slop between the gasket seal and glass.  
- Waste trap sensor is seated crooked. Realign the waste float sensor in the waste trap to seat properly (back of instrument). Tighten the waste trap cap. Perform “rinse valves” from fluid screen and observe no fluid remaining on slide carousel. |
| Instrument not properly initialized | Initialize the Artisan™ in ArtiCS software, report to Dako Technical Support immediately. |
| Hand held 2D bar code not scanning | Retry holding the scanner closer or further away so the target + is hovered over target 2D bar code. |
| Bottle detail window does not appear upon 2D scanning of a reagent pack | - Enter the serial number on the side of the pack in ADMIN/Reagents/Bottles tab  
- Re-boot Windows to re-activate the USB port that the 2D scanner is plugged  
- Re-engage the 2D scanner device into an alternate USB port  
- If all fails, call Technical Support Group for assistance |
| Tissue not stained evenly | - Pre-soak the slides with freshly made Wash Solution prior to staining run at least 5 minutes. Do not re-use slide clips  
- Tissue sections mounted close to slide clip gasket (or edge) will repel some stain from adhesion; avoid this practice  
- Ensure the slide surface is clean and free of debris or fingerprints. During microtomy, repeat submersion of the slide into the waterbath to capture serial sections can remove the manufacture slide coating. If the slide is coated with a charge, an unevenly charged surface can result influencing the ability of the reagent to spread evenly. Try using plain or uncharged slides to see if problem persists.  
- Avoid using slide labels that reside under the slide gasket seal. Use Dako labels, S3393 for best results  
- Remake Wash Solution, AR102 and replace on the Artisan™ instrument  
- Check fluid dispense from bulk liquid lines by priming and perform WVR  
- Revert to default procedure and use customized notes in this document for the respective stain being troubleshooted |
Frequently Asked Questions

How are the reagent packs to be oriented during storage of the kit packs?
The packs should be stored with the dispense tip down upon or after use. The protective caps may optionally be kept on the pack tips after use. The pack labeling has pack temperature storage conditions. The red stripe indicates Ambient and the blue stripe indicates 2-8 °C. Shipment conditions are different than end-user storage in some chemicals.

If I want to adjust a protocol, what should I adjust first, the time or the temperature? Why is temperature sometimes “off”?
Adjusting the incubation time to the maximum or minimum setting prior to adjusting the temperature will fluctuate run times and will give a less aggressive net change to staining; however, for some chemical steps (i.e. silver steps), heat is critical for an optimal chemical reaction. Remember that if several reagents are mixed on the slide, the incubation time and temperature will be reflected on the step of the last reagent added.

The “Off” denoted in the LINK procedure report denotes that the heat is programmed to remain OFF during the execution of the protocol during the staining session. In the Artisan™ Link procedure editing screen, “Off” in green boxes denotes that the end user may adjust the heat set point. If “Off” appears in a white box, the end user may not edit.

How can I track what is my workload volume?
- You can find your slide throughput by viewing COMPLETED tab
- You can find your bulk liquid throughput by viewing ADMIN/bulk liquid usage
- You can find your reagent usage throughput by viewing ADMIN/Reagent reports or by scanning any bottle’s serial number and view BOTTLE DETAIL

Do I need to prime the cartridge before every run?
No, the reagent packs should be primed daily as long as the following is performed:
Packs that have been inverted or mixed will introduce air into the dispense tip and will require a 1x steady stream confirmation before loading to the instrument. Since all packs require “MIX”, a prime will certainly follow since air has been introduced. MIX is labeled on the pack spine to indicate that reagents may have come out of saturation during rest between uses and will require an inversion of the pack to ensure a consistent quality dispense of the chemical. The packs have an overfill volume by the manufacture that allow for priming. A faulty prime should be reported immediately to your local Technical Support Representative.
If I prime will I get 100 or 50 tests out of the kit?
Yes, the cartridges are overfilled to allow for priming. The bar code tracks all inventory on the Dako Artisan™ Link instruments. Reagent inventory can be reviewed in the software using Bottle detail or Reagent reports; see user guide for instruction. A manual prime is approximately equal to 250 µL per steady stream for each dispense. Runs performing minimum of 1 control slide + 1 test slide yields the expected prime volume usage:
- **100 test pack:**
  - Test slide + 1 control slide = < 2 slides per run
  - (1) 250 µL/prime per run
  - 50 runs = 12.5 mL
  - The overfill volume is 20 mL, thus 20 mL - 12.5 mL = 7.5 mL to spare
- **50 test pack:**
  - Test slide + 1 control slide = < 2 slides per run
  - (1) 250 µL/prime
  - 25 runs = 6.25 mL
  - The overfill volume is 10 mL, thus 10 mL - 6.25 mL = 3.75 mL to spare

When using kits that have Silver Nitrate reagents I get a gray background on the slide, how do I avoid this?
The amount of charge that is applied to the slides will dictate the amount of background left on the slide. Dako has no control over this. Adjustments in the protocol will have no bearing on eliminating the gray background.
<table>
<thead>
<tr>
<th>Special Stain Protocol Complete with onboard Drying and Deparaffinization</th>
<th>Time for one Slide</th>
<th>Max. Slides in Nesting Group</th>
<th>Time for Single Nesting Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid-Fast Bacteria (AFB)</td>
<td>00:31:25</td>
<td>8</td>
<td>00:55:28</td>
</tr>
<tr>
<td>Acid-Fast Bacteria/Green (AFB/Green)</td>
<td>00:31:25</td>
<td>8</td>
<td>00:55:28</td>
</tr>
<tr>
<td>Alcian Blue pH 2.5 (AB 2.5)</td>
<td>00:44:50</td>
<td>14</td>
<td>01:03:49</td>
</tr>
<tr>
<td>Alcian Blue/Periodic Acid-Schiff (AB/PAS)</td>
<td>00:40:50</td>
<td>10</td>
<td>01:18:27</td>
</tr>
<tr>
<td>Alcian Blue/Periodic Acid-Schiff (AB/PAS) with Alpha Amylase</td>
<td>00:56:26</td>
<td>10</td>
<td>01:38:38</td>
</tr>
<tr>
<td>Alcian Blue/Periodic Acid-Schiff with Hematoxylin (AB/PAS/Hem)</td>
<td>00:55:08</td>
<td>10</td>
<td>01:50:24</td>
</tr>
<tr>
<td>Colloidal Iron</td>
<td>00:58:57</td>
<td>8</td>
<td>01:51:31</td>
</tr>
<tr>
<td>Congo Red</td>
<td>00:50:17</td>
<td>19</td>
<td>01:46:28</td>
</tr>
<tr>
<td>Elastic</td>
<td>00:37:58</td>
<td>8</td>
<td>01:15:27</td>
</tr>
<tr>
<td>Giemsa</td>
<td>01:20:32</td>
<td>15</td>
<td>01:48:05</td>
</tr>
<tr>
<td>Gomori’s Blue Trichrome</td>
<td>01:03:41</td>
<td>10</td>
<td>01:50:29</td>
</tr>
<tr>
<td>Gomori’s Green Trichrome</td>
<td>00:47:54</td>
<td>10</td>
<td>01:43:57</td>
</tr>
<tr>
<td>Gram</td>
<td>00:42:32</td>
<td>10</td>
<td>01:41:24</td>
</tr>
<tr>
<td>Gram Yellow</td>
<td>00:41:01</td>
<td>10</td>
<td>01:56:59</td>
</tr>
<tr>
<td>Grocott’s Methenamine Silver (GMS)</td>
<td>01:14:08</td>
<td>15</td>
<td>01:45:53</td>
</tr>
<tr>
<td>Grocott’s Methenamine Silver Plus (GMS +)</td>
<td>01:14:08</td>
<td>15</td>
<td>01:45:53</td>
</tr>
<tr>
<td>Grocott’s Methenamine Silver/Eosin (GMS/Eosin)</td>
<td>01:14:58</td>
<td>10</td>
<td>01:31:39</td>
</tr>
<tr>
<td>Iron</td>
<td>00:28:45</td>
<td>20</td>
<td>01:22:44</td>
</tr>
<tr>
<td>Jenner-Wright Giemsa (JW Giemsa)</td>
<td>00:35:23</td>
<td>15</td>
<td>01:22:11</td>
</tr>
<tr>
<td>Jones’ Basement Membrane (Jones’ or PAS-M)</td>
<td>01:11:28</td>
<td>10</td>
<td>02:00:03</td>
</tr>
<tr>
<td>Jones’ Basement Membrane Light Green (Jones’ or PAS-M/ Light Green)</td>
<td>01:01:36</td>
<td>10</td>
<td>01:58:03</td>
</tr>
<tr>
<td>Jones’ Basement Membrane H&amp;E (Jones’ or PAS-M/ H&amp;E)</td>
<td>01:04:02</td>
<td>7</td>
<td>01:54:14</td>
</tr>
<tr>
<td>Masson’s Trichrome</td>
<td>01:15:56</td>
<td>5</td>
<td>01:35:49</td>
</tr>
<tr>
<td>Mucicarmine</td>
<td>01:09:39</td>
<td>15</td>
<td>01:50:08</td>
</tr>
<tr>
<td>Orcein</td>
<td>00:34:25</td>
<td>15</td>
<td>01:32:50</td>
</tr>
<tr>
<td>Periodic Acid-Schiff (PAS)</td>
<td>01:18:39</td>
<td>20</td>
<td>01:54:15</td>
</tr>
<tr>
<td>Periodic Acid-Schiff with Alpha-Amylase</td>
<td>01:34:02</td>
<td>15</td>
<td>01:52:21</td>
</tr>
<tr>
<td>Periodic Acid-Schiff Green (PAS/Green)</td>
<td>00:43:54</td>
<td>15</td>
<td>01:38:55</td>
</tr>
<tr>
<td>Reticulin/No Counterstain (Retic/NCS)</td>
<td>00:57:32</td>
<td>10</td>
<td>01:45:54</td>
</tr>
<tr>
<td>Reticulin/Nuclear Fast Red (Retic/NFR)</td>
<td>01:02:59</td>
<td>9</td>
<td>01:46:02</td>
</tr>
<tr>
<td>Warthin-Starkey (WS)</td>
<td>00:26:21</td>
<td>24</td>
<td>01:33:26</td>
</tr>
</tbody>
</table>
Panel Examples

The following pages are examples of run times when combining several protocols in one run. The “panels” are only examples of what may be ordered for certain tissue types but can be modified by the laboratories discretion.

### Liver Panel

Special Stain Protocol Complete with onboard Drying and Deparaffinization

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<tr>
<td>Iron</td>
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<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Masson's Trichrome</td>
<td>1</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Reticulin/Nuclear Fast Red</td>
<td>1</td>
<td>2</td>
<td>8</td>
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<td>Orcein</td>
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<tr>
<td>Periodic Acid-Schiff (PAS)</td>
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<td>Periodic Acid-Schiff (PAS) with Alpha-Amylase (D)</td>
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Run Times

01:36:33 01:58:15 28

### Bone Marrow Panel

Special Stain Protocol Complete with onboard Drying and Deparaffinization

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Run Times

01:03:59 01:06:29 17

### Gastrointestinal Panel

Special Stain Protocol Complete with onboard Drying and Deparaffinization

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<tr>
<td>Alcian Blue/PAS</td>
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Run Times

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### Kidney Panel

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<td>Jones’ Basement Membrane (PAS-M) H&amp;E</td>
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### Lung Panel

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<td>Acid-Fast Bacteria (AFB)</td>
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<td><strong>01:15:36</strong></td>
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References


5. Churukian CJ. Manual of the Special Stains Laboratory. Department of Pathology and Laboratory Medicine, University of Rochester Medical Center, Rochester, New York, 1997.


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