Crystal Violet-Oxalate Stain, Alcoholic for Gram Stain, Brown-Brenn - Technical Memo

**SOLUTION:**

<table>
<thead>
<tr>
<th>Crystal Violet-Oxalate Stain, Alcoholic</th>
<th>250 ml</th>
<th>500 ml</th>
</tr>
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</table>
| Part 10422A                             | Part 10422B

**Additionally Needed:**

- Gram, Multi-Tissue, Artificial Control Slides: Part 4256 or Part 10448
- Xylene, ACS: Part 1445
- Alcohol, Ethyl Denatured, 100%: Part 10841
- Alcohol, Ethyl Denatured, 95%: Part 10842
- Iodine, Gram, Aqueous: Part 1140
- Acetone-Alcohol 1:1: Part 10016
- Basic Fuchsin Stain 0.25%, Aqueous: Part 1011
- Acetone, ACS: Part 10014
- Picric Acid-Acetone 0.1%: Part 1335
- Acetone-Xylene 1:1: Part 10015

**For storage requirements and expiration date refer to individual product labels.**

**APPLICATION:**

Newcomer Supply Gram Stain, Brown-Brenn is the traditional method used for differential staining of gram-positive and gram-negative bacteria in tissue sections, cultures and smears.

**METHOD:**

- **Fixation:** Formalin 10%, Phosphate Buffered (Part 1090)
- **Technique:** Paraffin sections cut at 5 microns and cultures/smears.
  - a. See Procedure Note #1.
- **Solutions:** All solutions are manufactured by Newcomer Supply, Inc.

All Newcomer Supply stain procedures are designed to be used with Coplin jars filled to 40 ml following the staining procedure provided below.

**STAINING PROCEDURE:**

1. Filter Crystal Violet-Oxalate Stain, Alcoholic with high quality filter paper.
2. Deparaffinize sections thoroughly in three changes of xylene, 3 minutes each. Hydrate through two changes each of 100% and 95% ethyl alcohol, 10 dips each. Wash well with distilled water.
   - a. See Procedure Notes #2 and #3.
4. Rinse well in several changes of distilled water, ensuring excess Crystal Violet-Oxalate Stain is removed.
5. Mordant in Iodine, Gram, Aqueous (1140) for 1 minute.
6. Rinse well in distilled water, ensuring excess iodine is removed. Blot excess water from slide, but not from the tissue section.
7. Decolorize one slide at a time by dipping in Acetone-Alcohol 1:1 (10016) until blue color stops running. Approximately 1-3 dips.
8. Counterstain in Basic Fuchsin Stain 0.25%, Aqueous (1011) for 3 minutes.
9. Rinse in distilled water and blot excess water from slide, but not from the tissue section.
   - a. Proceed with Steps #10 to #13 one slide at a time.
10. Dip once in Acetone (10014).
11. Dip in Picric Acid-Acetone 0.1% (1335) until sections have a yellowish-pink color, 3-10 dips. Agitate slides until desired intensity is achieved.
12. Dip in Acetone-Xylene 1:1 (10015), 5-10 dips. Check the control microscopically for proper differentiation.
   - a. Repeat Step #11 if additional differentiation is needed.
13. Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.

**RESULTS:**

- Gram-positive bacteria: Blue
- Gram-negative bacteria: Red
- Nuclei: Red
- Background tissue: Yellow

**PROCEDURE NOTES:**

1. For cultures/smears: Prepare within an accepted time frame a well-made culture/smear per your laboratories protocol with a focus on uniform cell distribution. The timings offered in this protocol are based on paraffin sections and may need to be altered for optimal culture/smear staining.
2. Drain staining rack/slides after each step to prevent solution carry over.
3. Do not allow sections to dry out at any point during staining procedure.
4. If using a xylene substitute, closely follow the manufacturer’s recommendations for deparaffinization and clearing steps.

**REFERENCES:**

4. Modifications developed by Newcomer Supply Laboratory.
Crystal Violet-Oxalate Stain, Alcoholic for Gram Stain, Hucker-Twort - Technical Memo

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**Additionally Needed:**

| Gram, Multi-Tissue, Artificial Control Slides | Part 4256 |
| Xylene, ACS | Part 1445 |
| Alcohol, Ethyl Denatured, 100% | Part 10841 |
| Alcohol, Ethyl Denatured, 95% | Part 10842 |
| Iodine, Weigert & Lugol, Aqueous | Part 12092 |
| Acetone, ACS | Part 10014 |
| Twort's Gram Stain Set | Part 14034 |

Solution A: Neutral Red Stain 1%, Alcohol
Solution B: Fast Green Stain 1%, Alcohol

**APPLICATION:**

Newcomer Supply Gram Stain, Hucker-Twort is a rapid and simple procedure that stains gram-positive and gram-negative bacteria without the use of picric acid. The Fast Green secondary counterstain provides the green background for clear detection of any red gram-negative bacteria present.

**METHOD:**

Fixation: Formalin 10%, Phosphate Buffered (Part 1090)

**Technique:** Paraffin sections cut at 5 microns

**Solutions:** All solutions are manufactured by Newcomer Supply, Inc.

All Newcomer Supply stain procedures are designed to be used with Coplin jars filled to 40 ml following the staining procedure provided below.

**STAINING PROCEDURE:**

1. Filter Crystal Violet-Oxalate Stain, Alcoholic with high quality filter paper.
2. Deparaffinize sections thoroughly in three changes of xylene, 3 minutes each. Hydrate through two changes each of 100% and 95% ethyl alcohols, 10 dips each. Wash well with distilled water.
   a. See Procedure Note #1.
4. Rinse quickly in distilled water.
5. Mordant in Iodine, Weigert & Lugol, Aqueous (12092) for 20 seconds.
6. Rinse quickly in distilled water.
7. Decolorize one slide at a time with Acetone (10014) until majority of the purple stain is removed, and tissue remains light gray. Approximately 2 quick dips.
8. Rinse quickly in distilled water.
9. Prepare fresh Twort Stain (12034); combine and mix well. Use within 30 minutes of preparation:
   a. Neutral Red Stain 1%, Alcoholic 9 ml
   b. Fast Green Stain 1%, Alcoholic 3 ml
   c. Distilled Water 30 ml
10. Stain in fresh Twort Stain for 2 minutes.
11. Rinse quickly in distilled water and carefully blot dry.
12. Agitate slides quickly in clean Acetone to dehydrate; do not use any alcohols.
   a. See Procedure Notes #2 and #3.
13. Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.

**RESULTS:**

Gram-positive bacteria | Dark blue
Gram-negative bacteria | Red
Cytoplasm and red blood cells | Shades of green
Nuclei | Red

**PROCEDURE NOTES:**

1. Drain staining rack/slides after each step to prevent solution carry over.
2. To tone down excessive red staining, add extra dips in acetone to differentiate and dehydrate the section. Check microscopically to ensure that over-differentiation does not occur.
3. Do not use any alcohol dehydration steps. The Neutral Red will be removed with alcohol exposure.
4. If using a xylene substitute, closely follow the manufacturer’s recommendations for deparaffinization and clearing steps.

**REFERENCES:**

4. Modifications developed by Newcomer Supply Laboratory.

**SUPPORT/WARRANTY:** For assistance regarding this product contact Newcomer Supply at 800-383-7799 or newly@newcomersupply.com. The information presented in this technical memo is to the best of our knowledge accurate. No warranty is expressed or implied. The user is responsible for determining the suitability of this product for their use and upon receipt assumes all liability for its use and responsibility for compliance with any laws or regulations. Please refer to www.newcomersupply.com for complete warranty information. © Newcomer Supply, Inc., September 2014