**Clinical Histology**

**Procedure**

Histo25.01

**Hucker-Twort Gram Stain**

<table>
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<th>Final Approval: May 2010</th>
<th>Effective: May 2010</th>
<th>Next Review Date: May 2015</th>
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List all stakeholder(s) and dates of approval:

<table>
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<tr>
<th>Stakeholder Name(s):</th>
<th>Date</th>
<th>Reviewed</th>
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Describe briefly the most recent revision made to this policy, procedure or protocol & why:

New Procedure

**Purpose/Policy Statement:**

This procedure demonstrates the presence of gram-positive and gram-negative organisms in tissue sections for diagnostic purposes. This procedure will stain gram positive and negative bacteria without the toxic and dangerous chemicals, picric acid and ether. The green counterstain allows enhanced visualization of organisms.

**Definitions:**

- n/a

**STEPS / KEY POINTS**

**PROCEDURE:**

1. Deparaffinize and hydrate sections to DH₂O.
2. FLAT SLIDE: stain sections with **Crystal Violet-Oxalate (A)** for 30 sec.
3. Brief DH₂O rinse.
4. FLAT SLIDE: stain sections with **Weigert’s Iodine (B)** for 20 sec.
5. Differentiate sections individually by vertically dipping slides in **Acetone** until purple stain ceases to run from sections.
6. Immediate brief DH₂O rinse.
7. FLAT SLIDE: stain sections with **Working Twort Stain** for 2 min
8. Brief DH₂O rinse.
9. Agitate quickly in clean **Acetone** to dehydrate, clear through xylene or xylene substitute.
10. Coverslip with synthetic medium.

**CALCULATIONS:** N/A

**CALIBRATION:** N/A

**QUALITY CONTROL:** Each time the procedure is performed, a known gram-positive and gram-negative tissue slide labeled with the current date and “GRAM CONTROL” shall be stained simultaneously with the patient slide(s).

**PROFICIENCY TESTING:** College of American Pathologists HistoQIP.

**RESULTS:**

Gram positive bacteria – dark blue
Gram negative bacteria – red
Background – light green
RBC’s – green to greenish-blue
PROCEDURE NOTES:
1. Crystal Violet Oxalate Solution A is stable but may need to be filtered toward end of shelf life.
2. The Twort Stain, once mixed, loses its effectiveness within 30 min.
3. Double distilled water is preferred. If not available, you may adjust pH by adding 1 drop of glacial acetic acid.
4. Do not place slides in alcohol after Twort stain; the Neutral Red will be removed.
5. Modifications to formula per Newcomer Supply experience.

LIMITATIONS OF PROCEDURE:  Deionized H₂O is used in place of recommended double distilled water.

Equipment/Supplies (If Applicable):

SPECIMEN: Paraffin sections cut at 4 microns and mounted on glass microslides.

MATERIALS, REAGENTS:
Solution A Crystal Violet Oxalate
Solution B Weigert’s Iodine
Solution C Neutral Red
Solution D 1% Fast Green
Working Twort Stain Prepare fresh before each use and use within 30 min.

DH₂O . . . . . . . . . . . . 5 ml
Neutral Red (C) . . . . 1 ml
1% Fast Green . . . . . 0.5 ml

INSTRUMENTATION OR EQUIPMENT:
Leica Autostainer XL
Light Microscope

Form Name & Number or Attachment Name (If Applicable):
Histo25

Author Position:
Lead Histologist

Review/Revision Authority (Position Not Individual Name):
Lead Histologist

Expert Consultant Position/s (Not Individual Name/s):
N/A

References (Required for Clinical Documents):

MANUFACTURER’S PACKAGING BROCHURE/INSERT: Newcomer Supply Gram Stain Kit, Hucker-Twort Technical Memo, and Original October 2003, Catalog #9125A.

Is there a Regulatory Requirement? Yes ☐ No ☐
If yes, insert requirement information here:

Review History (No Changes):
Shelly M. Siegel HT Date: 8/25/2010 Reviewed ☒ Revised ☒
Mark Magliner MD Date: 8/2710 Reviewed ☒ Revised ☒
Shelly Siegel Date: 5/3/11 Reviewed ☒ Revised ☒

Revision History (Note changes in area under header):
N/A

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N/A

Policy, Procedure or Protocol Cross Reference Information:
N/A