**MICRO.STAIN.3.0 POLYMORPHONUCLEAR LEUKOCYTES IN STOOL SPECIMENS**

**STATEMENT OF PURPOSE**

Fecal material is Gram stained to allow microscopic examination for the presence of

polymorphonuclear leukocytes (polys). Knowledge of the presence or absence of leukocytes is useful to the physician or clinician in the differentiation of enteric diseases. The presence of fecal leukocytes is suggestive of infection by an invasive pathogen.

**OWNER:**

Regional Microbiology Manager
Microbiology & Molecular Best Practice Team

**SPECIMEN**

Stool must be submitted in Total Fix preservative or PVA fixative at room temperature.Stool submitted on a swab or in a clean container, without preservative ≤ 1 hour after collection is acceptable. The smear should be prepared promptly, processed, stained and read at the Hospital-based lab if STAT. Only preserved specimens will be accepted by Regional Microbiology.

NOTE: If the amount of stool received is less than the required volume indicatedon the Total Fix transport container, a 1 part stool to 3 parts preservative (1 to 4 dilution) can be made. Pour out the appropriate volume from the transport container and add the appropriate amount of stool. Label container as “1 to 4 dil made”.

**REAGENTS**

Gram stain reagents (See MICRO.STAIN.1.0)

**EQUIPMENT**

1. Glass slides
2. Sterile cotton swabs
3. Bacticinerator
4. Immersion oil
5. Microscope

**CALIBRATION**

Not applicable

**QUALITY CONTROL**

None required – See MICRO.STAIN.1.0

**PROCEDURE**

NOTE: Perform procedure under biological safety hood.

|  |  |
| --- | --- |
| **Step** | **Action** |
| 1. | Label the frosted edge of a glass slide with the patient’s name, accession number, test (STPLY), and the date.  |
| 2. | Use a sterile cotton swab to make a thin smear of the stool on the slide, sampling from areas where blood and/or mucus is present. (If the stool is formed, it may be necessary to emulsify a small amount in a drop of sterile saline. Make a thin smear and allow to air dry.) |
| 3. | Heat fix the slide by one of two methods. Hold the back of the slide to the opening of a heat bacticinerator for 3-4 seconds or fix slide by placing a few drops of methanol on the air dried slide for 1 minute.  |
| 4. | Gram stain the smear. (See Gram stain procedure.) |
| 5. | Examine the stained smear for polymorphonuclear leukocytes (polys), using the 100X oil immersion objective on the microscope.  |
| 6. | If polys are observed, assign the appropriate quantitation according to the guidelines below.* Rare = < 1 per oil immersion field
* Few = 1-5 per oil immersion field
* Moderate = 6-25 per oil immersion field
* Many = greater than 25 per oil immersion field
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**CALCULATIONS**

**REPORTING RESULTS**

|  |  |  |  |
| --- | --- | --- | --- |
| **If** | **Then** | **Misys****Gateway Entry** | **QLS****(choose either pathway depending on site)** |
| No polys are seen | Report: No polys seen. | 1. Click on **Micro** **Result Entry**.2. Type Accession  number in the  **Value** field.3. Click on the test  desired and  Click on **Select**.4. Click on  **Observations**.5. Hit F8 to bring up  the Micro keyboard.6. Click on # 9 key  (NPOLY) under  observation # 1.7. Arrow or click to # 2.8. Hit shift and ~ key to finalize result.9. Click on OK.10. Double click on  **SAVE**.11. Click on **EXIT**. | 1. Function: QAENT2. Enter result code: NLEU in the STWBC test field in the FWC workcard3. File, release final report, and verify results. | 1. Function: 3,3,12. Allow release,  and return.3. At worklist,  type site  specific  worklist.4. Type accession  number.5. At field 3  Source:  (should read  Stool)6. At field 14,  type # and  #JINPOLY. |
| Polys are seen | Report: (Quantity)Polys seen. | 1. Click on Micro  Result Entry.2. Type Accession  number in the  Value field.3. Click on the test desired and  Click on Select.4. Click on  Observations.5. Hit F8 to bring up the Micro  keyboard.6. Click on the key  that corresponds to the amount of  polys present  (rare, few, mod,  many) and then  the H (POLYS)  key under  observation #1.7. Arrow or click to  # 2.8. Hit shift and ~ key to finalize result.9. Click on OK.10. Double click on SAVE.11. Click on EXIT. | 1. Function: QAENT2. Enter result codes: [QAUNTITY],LEU in the STWBC test field in the FWC workcard3. File, release final report, and verify results. | 1. Function: 3,3,12. Allow release,  and return.3. At worklist,  type site  specific  worklist.4. Type accession  number.5. At field 3,  Source:  (should read  Stool).6. At field 14,  type the  appropriate  code: #JIRPOLYS (rare polys); #JIFPOLYS (few polys);  #JIMOPOLYS  (mod polys) #JIMAPOLYS (many polys) |

**INTERPRETATION**

The presence of fecal leukocytes may indicate intestinal infection with *Salmonella*, *Shigella, Yersinia, Campylobacter, Edwardsiella, Plesiomonas,* or invasive *E coli*. It is especially significant in cases of pseudomembranous colitis associated with *Clostridium* *difficile.* It may also indicate non-bacterial inflammatory processes such as ulcerative colitis. Leukocytes are usually not present in diarrheal stools resulting from infection with viruses, toxogenic bacteria (i.e. *Staphylococcus, Clostridium perfringens, Vibrio cholera*, Shiga-like toxin producing *E coli*) and most parasites.

**REFERENCES**

 A. Henry, J.B., Clinical Diagnosis and Management by Laboratory Methods, 18th ed., 1991,

 W.B. Saunders Co., pp 536, 541-542.

 B. Isenberg, HD. Ed., Clinical Microbiology Procedures Handbook, 1.10.4, American

 Society for Microbiology, 1992.