

| | | |
|--|---|-------------|
| MSH/TML Shared Microbiology Service Policy & Procedure Manual | Policy # MI\PAR\05\03\v01 | Page 1 of 4 |
| Section: Parasitology Manual | Subject Title: Laboratory Procedures for Stool Examination | |
| Issued by: LABORATORY MANAGER | Original Date: March 13, 2000 | |
| Approved by: Laboratory Director | Revision Date: | |

Direct Wet Preparation Of Fresh Stools

PRINCIPLE

The value of wet preparations lies in the fact that certain protozoa trophozoites retain their motility which may aid in their identification. Definitive identification however may not be possible, especially for amoeba, since the nuclei of trophozoites and cysts are often not clearly visible. Wet preparations on fresh unpreserved liquid stool should be performed and examined as soon as possible (within 30 minutes of passage) and on soft/formed stool within 60 minutes of passage provided that prior arrangements have been made with the lab. Wet preps can also be used to determine schistosome egg viability (“flame” cells). (Wet preps can also be performed on SAF fixed specimens but motility is lost since the fixative kills the parasites.)

SPECIMEN

- Fresh liquid stool (within 30 minutes of passage - by prior arrangement with lab)
- Duodenal or small bowel aspirate in SAF or if fresh - within 30 minutes of collection by prior arrangement with lab.
- Abscess sample
- Respiratory sample
- CSF sample
- Urine
- Stool in SAF (no motility possible)
- External QC samples

MATERIALS

Reagents

Normal saline (0.85%)

Lugols iodine (commercial product—Snap N’ Stain).

Preparation:

| | |
|--------------------------|---------|
| potassium iodide | 10 gms |
| powdered iodine crystals | 5 gms |
| distilled water | 100 mls |

| | | |
|--|----------------------------------|-------------|
| MSH/TML Shared Microbiology Service Policy & Procedure Manual | Policy # MI\PAR\05\03\v01 | Page 2 of 4 |
| Parasitology Manual | | |

1. Dissolve potassium iodide and iodine crystals in distilled water in a flask or bottle using a magnetic stirrer.
2. The potassium iodide solution should be saturated with iodine with some excess crystals left on the bottom.
3. Store in a tightly stoppered brown bottle protected from the light.
4. Label the bottle with the expiration date of one year.
5. Dilute a portion 1:5 with distilled water for routine use (working solution). Place this working solution in a dropper bottle and discard when the color lightens (within 14 days).

Equipment:

Pasteur pipettes
Glass microscope slides
Applicator sticks
Glass cover slips, (22 x 22 mm)
Sharps disposal container
Light microscope with ocular micrometer and set for Kohler illumination.

QUALITY CONTROL

For direct smear:

1. check the working iodine solution each time it is used
2. Iodine should be the color of strong Orange Peko tea, discard if it is too light.
3. Protozoan stained with iodine should contain yellow gold cytoplasm, brown glycogen material and paler refractile nuclei. The chromatoidal bodies may not be as clearly visible as in a saline mount.
4. The microscope should be calibrated (within the last 12 months)
5. All QC results should be appropriately recorded and any “out- of-control” results referred to the laboratory director for action.
6. Ensure that reagents and chemicals used are not expired.
Safety note: Universal precautions should be observed.

PROCEDURE

1. Place one drop of 0.85% NaCl on the left side of the slide and one drop of iodine (working solution) on the right side of the slide.

| | | |
|--|----------------------------------|-------------|
| MSH/TML Shared Microbiology Service Policy & Procedure Manual | Policy # MI\PAR\05\03\v01 | Page 3 of 4 |
| Parasitology Manual | | |

2. Take a small amount of fecal specimen and thoroughly emulsify the stool in saline and iodine using an applicator stick. The sample should be spread thinly enough that newsprint can barely be read when the slide is placed on top of text.
3. Slide a 22mm cover slip at an angle into the edge of the emulsified fecal drop. Push the cover slip across the drop before allowing it to fall into place.
4. Systematically scan the entire 22mm cover slip with overlapping fields with the 10x objective.
5. Switch to high dry (40X objective) for more detailed study of any suspect eggs or protozoa.

PROCEDURE NOTES

1. If a fresh or unpreserved sample is received:
 - Only process if the duration from passage is known. This is usually only done by prior arrangement with the lab.
 - perform a wet direct mount on liquid samples if received within 30 minutes of passage and on soft/formed stools within 60 minutes of passage and examine for motile trophozoites.
 - describe the consistency of the specimen, e.g. bloody, watery, loose, soft or formed.
 - request a repeat specimen in SAF if only a fresh specimen was sent.
2. Examine specimens macroscopically for the presence of adult worms, proglottids, scoleces and other abnormal conditions. Use applicator sticks to break up the stools as necessary.
3. The sample on the slide should be spread thinly enough that newsprint can barely be read when the slide is placed on top of text.
4. The microscope light should be reduced for low power observation since most organisms will be overlooked with a bright light illumination should be regulated so that some of the cellular elements and feces should be refractile. Iodine solution will help make the nuclei more visible. However it is not useful for confirming motility since it kills trophozoites. In preserved specimens the SAF replaces the saline and can be used in the direct smear; however no motility will be visible since the organisms are killed in SAF.
5. To prevent contamination of iodine solution, the drops of iodine working solution should be placed on the slide before the specimen is added.
6. Duodenal aspirates can be examined directly with or without added saline.
7. If the slide is to be kept for any period of time, the edges of the cover slip may be sealed with Vaseline in order to prevent evaporation.

| | | |
|--|----------------------------------|-------------|
| MSH/TML Shared Microbiology Service Policy & Procedure Manual | Policy # MI\PAR\05\03\v01 | Page 4 of 4 |
| Parasitology Manual | | |

REPORTING

Protozoan stained with iodine should contain yellow gold cytoplasm, brown glycogen material and paler refractile nuclei. The chromatoidal bodies may not be as clearly visible as in a saline mount. Protozoal trophozoites, cysts and helminth eggs and larva can be seen and identified. However results from the direct smear should be considered presumptive and should be definitively confirmed with concentrates and direct smears. Use “*Bench Aids in the Diagnosis of Intestinal Parasites*” or the tables found in the section on Permanent Smears to aid in species identification of the observed parasites.

LIMITATIONS

1. Once iodine is added the organisms will be killed and motility will be lost.
2. Specimens that arrive in the lab already preserved do not require a direct smear examination. Concentration and permanent stain smears should be performed instead.
3. Direct smears are normally examined at low (x100) and high dry (x400) power. Oil immersion examination (x1000) is not recommended since organism morphology is often not clear.

AUTHOR

Kevin C. Kain

REFERENCES

1. LPTP Broadsheet #11. Methodology update. Section 4.3. Oct 11, 1990
2. Garcia LS, Bruckner DA. Diagnostic Medical Parasitology. 3rd Edition. ASM Press, Washington DC. 1997.
3. Neva FA, Brown HW. Basic Clinical Parasitology. 6th Edition. Appleton and Lange, Norwalk Connecticut. 1994.
4. Ash LR, Oreil TC. Atlas of Human Parsitology. 4th Edition. ASCp Press, Chicago. 1997.
5. Yang, J., Scholten, T.H. Afixative for intestinal parasites permitting the use of concentration and permanent staining procedures. Am. J. Clin. Pathol. 67:300-304 1977