

Lugol's Iodine

(Working Solution)

Code: 40129

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%)
- Lugol's iodine (Working Solution)

EQUIPMENTS

- 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.

PROCEDURE

- 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.
- 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.
- 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.
- Systematically scan the entire 22mm cover slip with overlapping fields with the 10x objective.
- Switch to high dry (40X objective) for more detailed study of any suspect eggs or Protozoa.

QUALITY CONTROL

For direct smear:

- Check the working iodine solution each time it is used
- Iodine should be the color of strong Orange Peko tea, discard if it is too light.
- 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.
- The microscope should be calibrated (within the last 12 months)
- All QC results should be appropriately recorded and any “out- of-control” results referred to the laboratory director for action.
- Ensure that reagents and chemicals used are not expired.
- Safety note: Universal precautions should be observed.

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.

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.

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REFERENCES

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