Specimen Collection, Transport, and Processing

Parasitology Laboratory

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Learning Objectives

At the end of this session, students should be able to:

a. describe the proper stool specimen collection and transport;
b. explain the proper stool specimen processing and the use of different fixatives; and
c. describe the different stool screening methods.

COLLECTION AND TRANSPORT:

- Protozoan forms (trophozoites and cysts) and helminth stages (egg, larvae, proglottids and adult worms) are found in stool samples.
- Typical stool collection protocol:
  - 3 specimens in 10 days
- Amebiasis
  - 6 specimens in 14 days

Medications: Barium, Bismuth & Mineral oil

Antibiotics and Antimalarial (delayed 2 weeks following the therapy).

Samples are collected in a clean, water-tight container with tight-fitting lid.

<u>Sample required: 2 to 5g/ size of a walnut</u>

Urine should not contaminate the stool sample.

Stool should not be retrieved from the toilet bowl.

Water should not contaminate the stool sample.

Toilet paper should not be seen in the sample.

Sample Labelling:

Patient's name

Identification number

Physician's name

Date and time of collection

Age

Gender

FIXATIVE AS PRESERVATION:

Ideal sample: Freshly collected stool sample

Substances that preserve the morphology of protozoans and prevent development of helminth eggs and larvae.

Ratio is 3:1 (3 parts fixative; 1 part of stool)

FIXATIVES FOR PRESERVATION:

1. Formalin
2. Polyvinyl Alcohol (PVA)
3. Sodium Acetate Formalin (SAF)
4. Modified Polyvinyl Alcohol
5. Alternative Single Vial Systems

1. FORMALIN

- All-purpose fixative for helminths and protozoans.
- 2 concentrations:
  - 5% - protozoan cysts
  - 10% - helminth eggs and larvae.
- Used for routine direct examinations and concentration techniques but NOT for permanent smears.

ADVANTAGES:

- Easy to prepare
- Preserves specimen up to several years.
- Long shelf-life

DISADVANTAGES:

- Does not preserve parasite morphology adequately for permanent smears.
- Trophozoites may not be recovered.
- Morphology of cysts and eggs may fade with time.
- Potential health hazard.

2. POLYVINYL ALCOHOL (PVA)

- Combined with Schaudinn solution (Zinc Sulfate, Copper Sulfate or Mercuric Chloride).
- Trophozoites and cysts of protozoans, most helminth eggs can be detected.
- Can be used for preparation in a permanent stained smear.

ADVANTAGES:

- Long shelf-life when stored at room temperature.
- Concentration techniques can be performed (Two-vial system).

DISADVANTAGES:

- Potential health problems (Mercury in Schaudinn solution).

3. Sodium Acetate Formalin (SAF)

- Alternative fixative to PVA and Schaudinn fixative.
- Used in concentration techniques and permanent stained smears.

ADVANTAGES:	DISADVANTAGES:
• Easy to prepare.	• Addition of Albumin is necessary.
• Long-shelf life	
• Use for preparing smears for staining with Modified acid-fast stain for Coccidian cysts.	• Protozoan morphology is not clear in permanent smears.
	• Choice of permanent stain (Iron Hematoxylin)

4. Modified Polyvinyl Alcohol (PVA)

- Alternative fixative to Mercury-based PVA by using Copper Sulfate and Zinc Sulfate.
- Can be used for concentration methods and permanent smears.
- Do not provide the same quality of preservation of protozoan morphology.

5. Alternative Single-Vial System

- Single vial systems are free of Formalin and Mercury.
- Used for concentration techniques and permanent smears.
- Used in fecal immunoassays.
- Disadvantage:
  1. Do not provide the same quality as Mercury-based fixative.
  2. Organism identification will be more difficult.

PROCESSING ▸

- **MACROSCOPIC**
  - Consistency (degree of moisture)
  - Color (Normal: brown)
  - Gross abnormalities (adult worms, proglottids, pus and mucus)
- **MICROSCOPIC**
  - Direct methods
  - Concentration techniques
  - Permanently stained smears

Trophozoite motility – fresh sample is required.

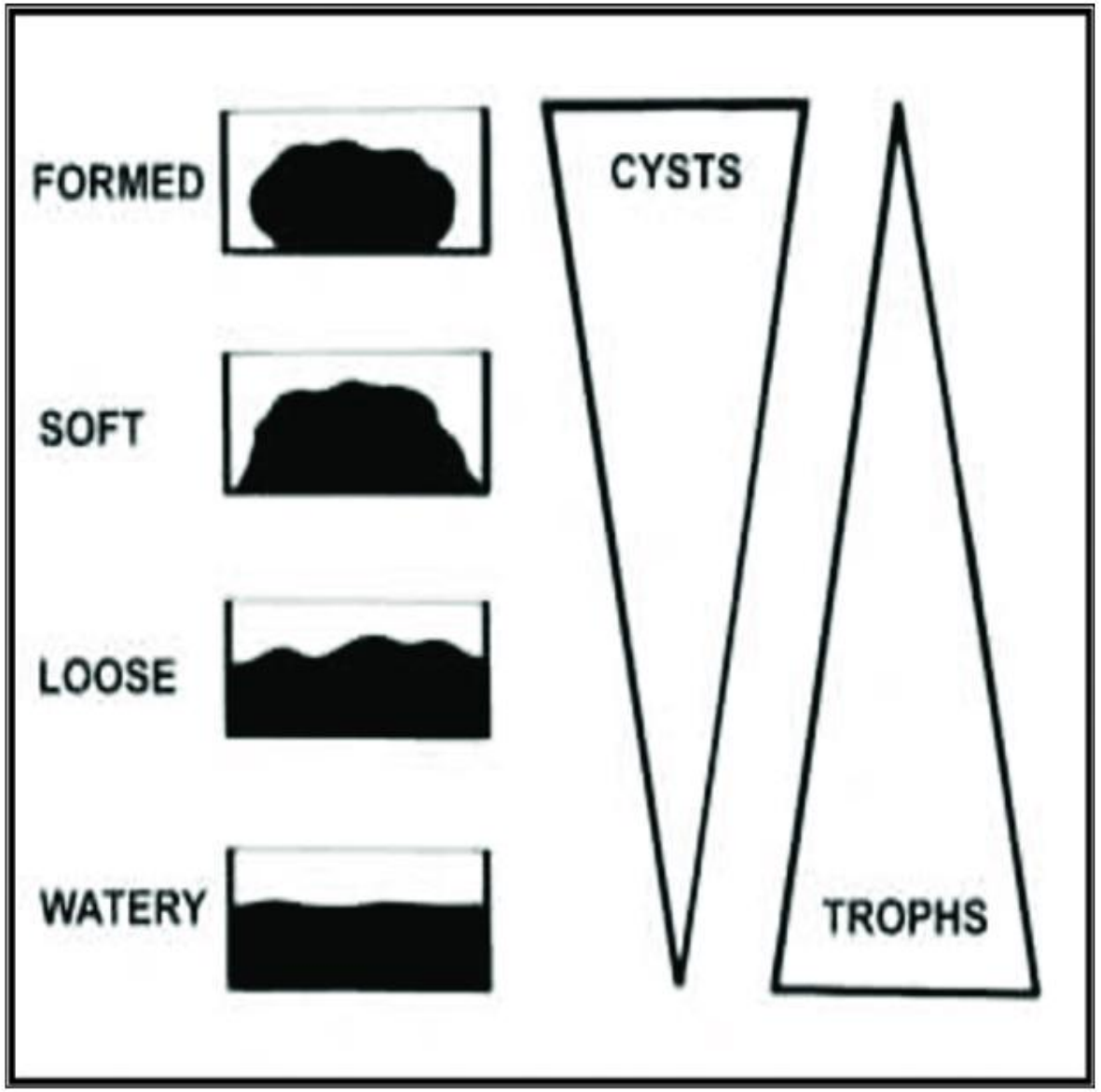
Protozoan cysts, helminth egg and larvae are not sensitive.

Liquid stool samples: Processed within 30 mins. (Presence of trophozoites)

Semi-formed stool samples: Evaluated within 1 hour (Presence of trophozoites and cysts)

Formed samples: Can be held within 24 hours.

Guidelines are not met; the samples must be preserved.



Brown: Color caused by chemical changes in bilirubin (a pigment resulting from the breakdown of red blood cells). All shades of brown are considered normal.

Green: Green vegetables, such as spinach, are common causes of green stool.

Red: Can indicate bleeding of the lower digestive tract or rectum.

Yellow: Can be caused by an infection known as Giardia or can ndicate bowel hyperactivity.

Blue: Can indicate illness in babies or can be the result of eating foods that contain blue dyes.

White: Ofen caused by a lack of bile (produced in the liver) and by certain medications.

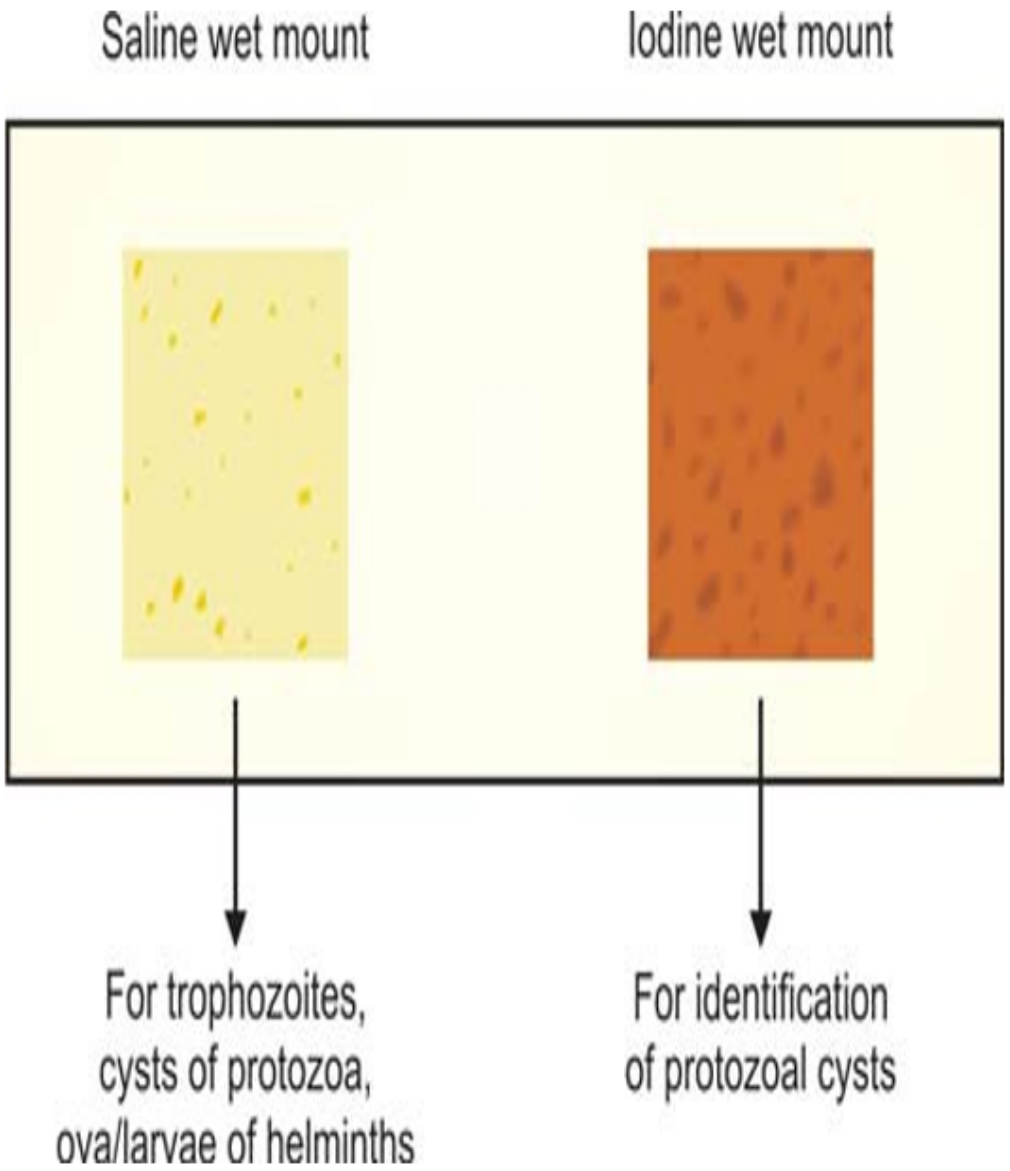
Black: May be caused by bleeding in the upper digestive tract.

MICROSCOPIC EXAMINATION:

1. Direct Wet Preparation

2. Concentration methods

3. Permanent Stained slides



1. DIRECT WET PREPARATION

- "Direct Wet Mount"
- Slide made by mixing a small portion of unfixed stool with saline or Iodine.
- To detect the presence of motile protozoan trophozoites (fresh samples).
- Direct Saline Wet Preparation
- Direct Iodine Wet Preparation

2. CONCENTRATION TECHNIQUES:

Formalin-Ethyl Acetate Sedimentation

- Principle: Specific gravity
- Ethyl Acetate is added to a saline-washed formalin-fixed sample.
- Parasites heavier than the solution settles in the sediment of the tube.
- Fecal debris are lighter and rises to the upper layer of the tube.

ADVANTAGES:
• Easy to prepare
• Good recovery of parasites
DISADVANTAGES:
• More fecal debris
• Challenging to the microscopist.

Zinc Sulfate Flotation

- Principle: Specific gravity
- Debris sinks at the bottom of the tube.
- Parasites are lighter thus rises on the top of the tube.
- Zinc sulfate (1.18-1.20) is used as the concentrating solution.

ADVANTAGES:

- More fecal debris is removed.
- Cleaner preparation, easier for microscopic examination.

DISADVANTAGE:

- Some helminths are dense and will not float.

3. PERMANENT STAINS

Final procedure in O&P examination.

Microscope slides that contains a fixed sample that has been allowed to dry and stained.

Critical portion of the O&P examination.

Sample of choice is PVA-prepared sample. SAF samples can be used but the stain must be Iron Hematoxylin.

Slides can be prepared from fresh samples but must not be allowed to dry and place immediately into a fixative.

Two common stains:

- Wheatly Trichrome
- Iron Hematoxylin

STOOL SCREENING METHODS

- "Rapid methods" (obtained as kits that contain monoclonal antibody).
- Commercial antibody is used to detect antigens in patient's samples.
- Enzyme Immunoassay, DFA & Membrane Flow Cartridge Technique.
- *E. histolytica, G. intestinalis* & *Cryptosporidum spp.*
- Highly sensitive and specific but detects one pathogen at a time.