DETECTION OF HELMINTH INFECTIONS IN STOOL AND BLOOD

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INTRODUCTION

• Helminth infection is still a major public health problem in Indonesia.

• Helminth infection:
  - Intestinal nematode such as *Ascaris lumbricoides*, *Trichuris trichiura*, Hookworms.
    • Prevalence in rural areas: ranging from 40%-70% and urban areas: ranging from 5%-10%.
  
  - Tissue nematode such as lymphatic filariasis caused by *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori*
    • Prevalence in endemic areas: ranging from 0.5%-19.64%. 
INTRODUCTION

- Detection of intestinal helminth infection:
  - Direct stool examination (qualitative)
  - Kato-katz technique (quantitative)
  - Formalin-ether concentration technique (quantitative)
INTRODUCTION

• Lymphatic filariasis:
  - Night blood examination:
    • Blood smear (qualitative)
    • Membrane filtration (quantitative)
  
  - Serology detection (commercially available):
    • Antigen detection for *Wuchereria bancrofti*
    • Antibody detection for *Brugia sp.*
METHODOLOGY

• Detection of intestinal helminth infection:
  - Stool sample preserved in 10% formaldehyde

• Detection of filariasis infection:
  - Blood sample preserved in anticoagulant (heparin or EDTA)
METHODOLOGY 1

• INTESTINAL HELMINTH INFECTION:
  - Direct stool examination:

  • Materials:
    - applicator stick,
    - clean slide,
    - pipette,
    - cover slip

  • Reagent: saline solution

  • Method: An applicator stick is used to mix about 50 mg of stool with one or two drops of normal saline placed on a clean slide. A uniform thin suspension is made and covered with a 22 mm square cover slip. The entire film is screened systematically for the presence of helminth ova and larvae or protozoan cysts and trophozoites.
METHODOLOGY 2

• Kato-katz technique
  - Materials:
    • Scrap paper,
    • nylon screen,
    • spatula,
    • cellophane strip
  - Reagents:
    • Solution used to soak cellophane strip:
      - 100 parts aquades
      - 100 parts glycerol
      - 1 part malachit green 3%
Kato-Katz technique

Materials:

- Wooden stick
- Cellophane soaked in malachit green-glycerol
- Antiseptic solution
- Kertas karton berlubang
- Nylon screen
- Scrap paper
- Filter paper
- faeces
- Prop or spatula
- Slide

Materials:
Kato-Katz Technique

Sieving the faecal material.

A small amount of faecal material is placed on newspaper or scrap paper and a piece of nylon screen (the sieve) is pressed on top so that some of the faeces is sieved through and accumulates on top.

A flat-sided spatula is used to take some of the sieved faecal samples on the upper surface of the screen.
Kato-Katz Technique

A template (cardboard with punched hole of specific size) is placed on the slide & the sieved faeces is added with the spatula so that the hole in the template is completely filled. The spatula is passed over the filled template to remove excess faeces from the edge of the hole. The template is removed carefully so that a cylinder of faeces is left on the slide. Now there is 40mg of feces left on the slide.
Kato-Katz Technique

The faecal material is covered with a pre-soaked cellophane strip.
Kato-Katz Technique

The slide and the faecal sample is pressed firmly against the hydrophilic cellophane strip to spread evenly. The slide is placed inverted on to filter paper to let the excessive fluid absorbed.
Kato-Katz Technique

The slide is placed on the bench with cellophane upwards to enable the evaporation of water while glycerol clear the faeces.

For all helminths, except hookworm eggs, the slide is kept for one or more hours at room temperature to clear the faecal material, prior to microscopic examination.
METHODOLOGY 3

Formalin-ether concentration (FEC) technique

- Materials: aplicator stick, Conical tube, Beaker
- Reagents: formalin saline, Ether, Iodine
- Method: Using an applicator stick, about 1 g of faeces was placed in a clean 15 ml conical centrifuge tube containing 7 ml formalin saline. The sample was dissolved and mixed thoroughly with a vortex mixer. The resulting suspension was filtered through a sieve into a beaker and the filtrate was poured back into the same tube. The debris trapped on the sieve was discarded. After adding 3 ml of diethyl ether to the formalized solution, the contents were centrifuged at 3,200 rpm for 3 minutes. The supernatant was poured away and the tube was replaced in its rack. Iodine stain preparation was made. The entire area under the cover slip was systematically examined using ×40 objective lenses.
**Table 1. Number of parasites recovered from 100 faecal samples using five different techniques**

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Techniques used for parasite identification</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total cases</td>
<td>WP</td>
<td>FEC</td>
<td>TRICH</td>
<td>AURAM</td>
<td>KATO</td>
</tr>
<tr>
<td><em>A. lumbricoides</em></td>
<td>24</td>
<td>9</td>
<td>15</td>
<td>-</td>
<td>-</td>
<td>24</td>
</tr>
<tr>
<td><em>T. trichuria</em></td>
<td>9</td>
<td>3</td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>9</td>
</tr>
<tr>
<td><em>Hookworm</em></td>
<td>7</td>
<td>1</td>
<td>7</td>
<td>-</td>
<td>-</td>
<td>7</td>
</tr>
<tr>
<td><em>G. lamblia</em></td>
<td>6</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>E. histolytica</em></td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>6</td>
<td>4</td>
<td>4</td>
<td>6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>I. butschlii</em></td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>B. hominis</em></td>
<td>17</td>
<td>1</td>
<td>0</td>
<td>17</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>D. fragilis</em></td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>E. nana</em></td>
<td>6</td>
<td>0</td>
<td>1</td>
<td>6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>C. parvum</em></td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>-</td>
</tr>
</tbody>
</table>

WP: Direct saline and iodine mount (wet preparation), FEC: Formalin-ether concentration technique, TRICH: Trichrome staining of faeces, AURAM: Auramine-phenol staining, KATO: Kato-Katz technique

**Kato-Katz** technique is more sensitive for detecting helminthic parasites, followed by the FEC technique & the smear wet preparation.

**Kato-Katz** is superior for detecting helminth infection parasites.

**Trichrome stain** is superior for detecting protozoan infection.
METHODOLOGY

• FILARIAL INFECTION
  - Thick blood smear:
    • Materials:

  ![Materials](image)
METHODOLOGY

- Clean finger with cotton-alcohol,
- Prick finger using blood lancet
METHODOLOGY

• Put two drops of blood (20ul) onto the slide - or take by capillary tube
METHODOLOGY

• Spread blood on the slide until becoming oval shape, with diameter about 1.5 cm
• Let the blood slide dry on room temperature and then put the slide on the water for hemolisa process.
• Add one drop methanol to the slide for fixation.
METHODOLOGY

Blood slide is ready for giemsa staining.

Stained by Giemsa for 15 minutes
METHODOLOGY

• Ready for microscopic examination
FILARIAL INFECTION

- **Membrane filtration:**
  - Materials:
METHODOLOGY

- Filter the blood using 5 um nucleopore
- Hemolysis the blood by adding aquadest
- Mf will be trapped in the nucleopore
- Let the blood slide dry on room temperature
- Add one drop of methanol to the filter on the slide for fixation before staining by giemsa
METHODOLOGY

• Ready for microscopic examination
Wuchereria bancrofti microfilaria

- Head
  - length
  - width
- Body nuclei
- Tail: no terminal nuclei
- Sheath: pale or unstained
Brugia malayi microfilaria

Sheath: read

Tail: terminal nuclei
Microfilaria

*W. bancrofti*  
*B. malayi*
Immuno Chromatographic Test (ICT)

- antigen detection for *Wuchereria bancrofti*
  - 50 µl plasma/blood dropped on absorbent pad
  - Read the card after 15’
  - Positive: 2 red lines
Brugia rapid (BR)

- antibody detection for *Brugia malayi*
  
  - 25 μl plasma / 100 ul dropped on *absorbent pad*
  
  - Read the card after 15’
  
  - Positive: 2 red lines