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LABORATORY PROTOCOLS OF ANAEMIA TESTING USING PORTABLE HEMOCUE, MALARIA SCREENING USING RDT (HRP-2), PROCESSING OF WET PREPARATION, KATO-KATZ AND EXAMINATION OF STOOL SAMPLES, REPORTING OF SOIL-TRANSMITTED HELMINTHES AND FAECAL PARASITES V.2

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Abstract

To determine the prevalence of anaemia in children aged 6-24 months living in a high malaria transmission setting in Burundi, we used laboratory protocols to test anaemia using a portable hemocue analyser and screend for malaria using a Malaria Ag Pf/Pan rapid test; a rapid, qualitative test for the detection of HRP-II (Histidine rich protein II- HRP-2). We also detected Soil-Transmitted Helminthiasis and other faecal parasites microscopically by wet preparation and Kato-Katz technique. Laboratory protocols were used in cross-sectional that determined the prevalence and factors associated with anaemia in children aged 6-24 months living in a high malaria transmission setting in northern Burundi.

Guidelines

1.WHO: **Bench aids for the diagnosis of intestinal parasites**. Geneva: World Health Organization, 1994.
2.WHO: **Public report, Product: SD Bioline Malaria Ag P.f and SD BIOLINE Malaria Ag P.f POCT.** In *Prequalification of Diagnostics Programme*; 2016.
3.Clinical and Laboratory Standards Institute: **Reference and Selected Procedures for the Quantitative Determination of Hemoglobin in Blood; Approved Standard**—Third Edition, H15-A3, Vol. 20 No. 28, Pennsylvania, USA, 2000; [(accessed on 28 January 2021); Available online: https://webstore.ansi.org/preview-pages/CLSI/preview_H15-A3.pdf]
4.Swiss Tropical Institute: **KATO-Katz technique for helminth eggs**. Basel, 2005

Materials

Materials required.pdf
S2_File.Study questionnaire Englis...
S3_File.Study questionnaire local la...

Troubleshooting

Safety warnings

Disposal all used materials and remaining stool samples according to the laboratory safety rules

Before start

1. Wear protective gloves before handling blood or stool specimens
2. Collect stool in a dry, sterilized, wide mouthed container/vial

ANAEMIA TESTING USING PORTABLE HEMOCUE

1 1.Switch-on the HemoCue Hb 301 analyser. Check if the vial of HemoCue Hb 301 is empty.

2 Preparation of the patient's site to prick

2.1 Open the alcohol swab

2.2 Clean the patient's 4th finger towards the side of the pulp (if the subject is right-handed, choose the 4th finger on the left hand and vice-versa)or a heel prick in the case of children age 6-11 months, and allow it to air dry.

2.3 After using the alcohol swab, place it on its wrapper and set it aside, it may be used again to stop the bleeding after collecting the subject's' blood.

3 Open the sterile lancet and prick the 4^{th} finger (or a heel prick in the case of children age 6-11 months).

4 After wiping away the first 2 or 3 drops of blood. The fingertip is lightly pressed until a collect amount of blood appears

5 Fill the microcuvette completely with blood in one step and make sure that no specimen is drawn out from the open end by wiping off specimen from the outside

6 Haemoglobin was measured by placing the microcuvette in the cuvette holder, gently sliding the cuvette holder to measuring position.

7 The recorded result was the hemoglobin value is displayed after 5 seconds.

MALARIA SCREENING USING RDT (HRP-2)

8 After the test packet opened, the test cassette was removed and labelled with subject's code number

9 Open the alcohol swab and Clean the patient's 4th finger towards the side of the pulp (if the subject is right-handed, choose the 4th finger on the left hand and vice-versa) or a

heel prick in the case of children age 6-11 months, and allow it to air dry.

10 After using the alcohol swab, place it on its wrapper and set it aside, it may be used again to stop the bleeding after collecting the subject's' blood.

11 Open the sterile lancet and prick the 4th finger (or a heel prick in the case of children age 6-11 months).

12 A collect amount of blood was collected using the capillary tube.

13 The blood sample was pipetted into the round hole of the RDT.

14 Place four drops of assay diluent vertically into the square hole of the cassette.

15 Wait for 15 minutes after adding assay diluent and read test results

16 A positive results was recorded when the test and control bands appeared after 15 minutes and a negative results when only the control band showed.

WET MOUNT PREPARATION OF STOOL SAMPLE FOR MICROSCOPY

17 Take samples with a wooden applicator from different places in the faeces sample (collected in a sterile container).

18 Place a drop of saline solution and one drop of Lugol's Iodine solution on a labelled and clean microscopy slide (subject's code number, date and hour)

19 Place the collected faeces specimen on the drops of solution.

20 Dissociate the faeces and remove harmful debris with an applicator if necessary.

21 Cover with a coverslip, pressing lightly to avoid air bubbles.

READING AND REPORTING FAECAL PARASITES FROM WET PREPARATION

22 Analyze the stool on the day of production and collection

23 Macroscopic examination: colour, consistency, blood, mucous, parts of parasite and adult parasite

24 Microscope calibration using ocular micrometer disk

25 10X magnification objective: systematic examination of the preparation to detect helminthes.

26 40X magnification objective: accurate identification of faecal parasites found at the 10X objective.

27 Microscopic examination to detect ova (eggs) or larvae of Soil-Transmitted Helminthiasis (STH) or any faecal parasite.

28 Report the nature of sol-transmitted helminthiasis or any faecal parasite on the laboratory report form. Report the result “POSITIVE” or “NEGATIVE”. If the result is POSITIVE, report the species of the detected parasites.

KATO-KATZ

29 PREPARATION OF THIN SMEAR

29.1 Cut hydrophilic cellophane into 25mm x 30mm pieces and soak them in 50% Glycerol-Malachite Green (or Methylene Blue solution) for at least 24 hours before use.

29.2 Label microscopic glass slide with the subject’s code number.

29.3 Transfer a small amount of stool onto a piece of scrap paper

29.4 Press nylon screen on top of faecal sample.

29.5 Using flat-sided of a wooden applicator stick, scrape across the upper surface of the screen the sifted faecal material so that only debris remains.

29.6 Place a template on a labelled microscope slide and transfer a small amount of sieved faecal material through the template and carefully fill the hole. Level with the applicator stick.

29.7 Remove the template vertically and carefully (avoid any horizontal movement) so that all the faecal material is left on the slide and none is left sticking to the template.

29.8 Cover the faecal sample on the slide with a glycerol-soaked cellophane strip, wipe off any excess of glycerol-malachite green solution on the upper surface of the cellophane with a small piece of absorbent tissue.

29.9 Invert the microscope slide and press the faecal sample against the cellophane on a smooth surface (a second clean microscope slide or a clean applicator) to spread the sample evenly.

29.10 Do not lift the slide straight up. The cellophane may separate. Gently slide the microscope slide sideways holding the cellophane.

30 READING AND REPORTING OF FAECAL PARASITES

30.1 The slide should be read within 30-60 minutes. To read the slide, place it under the microscope using the 40 and 100x magnification objectives.

30.2 Read all fields on the slide using the vertical 'zig zag' system and the tally counter to record how many eggs are seen under the slide as it is read.

30.3 Record the number and nature of each egg on a recording form next to the sample number. Multiply by the appropriate number to give number of eggs per gram of faeces: by 24 for a 41.7mg template. If there are no eggs, score "0".

30.4 Report the result “POSITIVE” or “NEGATIVE”. If the result is POSITIVE, report the species of the detected parasites.