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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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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 screen 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.

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Anaemia testing by hemocue, kato-katz, children 6 to 24 months, Burundi, RDT malaria screening

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 [Materials required.pdf](#)

 [S2_File.Study questionnaire English.pdf](#)

 [S3_File.Study questionnaire local language Kirundi.pdf](#)

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

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 4th 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.