untitled protocol

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Abstract

LABORATORY PROTOCOLS FOR PREPARATION OF BLOOD SMEARS, REPORTING OF MALARIA PARASITES, PROCESSING OF BLOOD SAMPLES USING MALARIA RDT (HRP-2), BLOOD GROUPING AND DESIGN OF QUESTIONNAIRE (PREVALENCE OF *PLASMODIUM* PARASITAEMIA IN BLOOD DONORS AND A SURVEY OF THE KNOWLEDGE AND PRACTICES OF TRANSFUSION MALARIA AMONG HEALTH WORKERS IN A HOSPITAL IN KUMASI, GHANA

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Materials

- Monoclonal antisera A,B and D by Contributed by users

Protocol

PREPARATION OF THICK SMEAR FOR MICROSCOPY

Step 1.
A drop of well mixed blood (6ul) was placed on a clean grease free slide using a pipette.

PREPARATION OF THICK SMEAR FOR MICROSCOPY

Step 2.
An applicator was used to spread the drop of blood in a circular way to ensure the spread of blood with moderate thickness, covering evenly an area about 1cm diameters.

PREPARATION OF THICK SMEAR FOR MICROSCOPY

Step 3.
Slides were labeled with subject’s code number and allowed to air-dry.

PREPARATION OF THICK SMEAR FOR MICROSCOPY

Step 4.
The smears were stained with a freshly prepared 10% Giemsa stain for 15 minutes and washed with clean distilled water.

PREPARATION OF THICK SMEAR FOR MICROSCOPY

Step 5.
The slides were then placed on a drying rack in a vertical position and allowed to air dry.

PREPARATION OF THIN SMEAR FOR MICROSCOPY

Step 6.
A drop of well mixed blood (2ul) was placed about 1cm from the frosted end of a slide using a pipette.

PREPARATION OF THIN SMEAR FOR MICROSCOPY

Step 7.

A smooth clean edge of a second slide (spreader) was placed on the specimen slide, just in front of the blood drop.

PREPARATION OF THIN SMEAR FOR MICROSCOPY

Step 8.

The spreader slide was held at 30° angle and was drawn back against the drop of blood.

PREPARATION OF THIN SMEAR FOR MICROSCOPY

Step 9.

As the blood started to spread to the ends of the spreader, the spreader was pushed forward with a light, smooth, and quick steady motion.

PREPARATION OF THIN SMEAR FOR MICROSCOPY

Step 10.

The frosted edge was labeled with the subject's code number and the blood smear was dipped in a Coplin jar containing absolute methanol for 10 seconds and then air dried again.

PREPARATION OF THIN SMEAR FOR MICROSCOPY

Step 11.

The smear was then stained with a freshly prepared Giemsa stain solution for 30 minutes and washed with distilled water

PREPARATION OF THIN SMEAR FOR MICROSCOPY

Step 12.

The slides were then placed on a drying rack and allowed to air dry. An oil immersion was put on the prepared thick film and was examined using the X100 objective lens and the thin films were first examined using the X40 with an oil immersion placed to the feathery tip of the smear to check the staining, morphology and distribution of the cells and afterwards the objective lens is changed to X100 to examine the malaria parasites.

REPORTING OF MALARIA PARASITES

Step 13.

Number of parasites seen were counted in relation to a predetermined number of white blood cells and an average of 8000/µl was taken as standard. 200 white blood cells were counted in 100 fields. All parasite species and forms including both sexual and asexual forms were counted together.

REPORTING OF MALARIA PARASITES

Step 14.

NUMBEROF PARASITES/µL =Number of parasites counted/ Number of white blood cells counted × 8000

BLOOD SAMPLES PROCESSING FOR MALARIA DIAGNOSIS USING MALARIA RDT (HRP-2)

Step 15.

In the laboratory, 5ul of whole blood from the labeled EDTA tube, was taken with a pipette provided with the test kit.

BLOOD SAMPLES PROCESSING FOR MALARIA DIAGNOSIS USING MALARIA RDT (HRP-2)

Step 16.

The blood was pipetted into the round sample well of the RDT.

BLOOD SAMPLES PROCESSING FOR MALARIA DIAGNOSIS USING MALARIA RDT (HRP-2)

Step 17.

Four drops of assay diluent were placed vertically into the square assay diluent well and the results

were read after 30 minutes.

BLOOD SAMPLES PROCESSING FOR MALARIA DIAGNOSIS USING MALARIA RDT (HRP-2)

Step 18.

The same procedure was performed on samples collected from blood donors and non-donors.

BLOOD SAMPLES PROCESSING FOR MALARIA DIAGNOSIS USING MALARIA RDT (HRP-2)

Step 19.

A positive results was recorded when the test and control bands appeared after 30 minutes. A negative results was also recorded when only the control band showed.

BLOOD GROUPING

Step 20.

The tile method using the forward grouping was used for determining the blood groups of blood donors and non-donors (controls).

BLOOD GROUPING

Step 21.

5ul of blood donors and non-donors blood samples were put in the appropriate square of the tile.

BLOOD GROUPING

Step 22.

A drop of a known antisera was added to the sample.

BLOOD GROUPING

Step 23.

An applicator was used in mixing and the tile was rocked forward and backward for 3 minutes. The results were read macroscopically upon agglutination and in cases where the results were unclear, were read microscopically

QUESTIONNAIRE

Step 24.

The questionnaire was categorized into a three-part document with a socio-demographics section, knowledge section and a section on attitudes and practices. Responses to a total of 20 opened and closed complete questions were unspecified so as to obtain validated answers without fear of victimization. Health workers were given less than 24hours to fill the questionnaire and those who did not submit were followed up in person