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.

Kwame Agyemang Adusei,Alex K. Owusu-Ofori

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, employed in this study are briefly described.

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Materials

- Monoclonal Antisera A,B,O by Contributed by users

Protocol

PREPARATION OF THICK SMEAR

Step 1.

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

PREPARATION OF THICK SMEAR

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

Step 3.

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

PREPARATION OF THICK SMEAR

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

Step 5.

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

PREPARATION OF THIN SMEAR

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

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

Step 8.

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

PREPARATION OF THIN SMEAR

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

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

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

Step 12.

The slides were then placed on a drying rack and allowed to air dry.

PREPARATION OF THICK AND THIN FILM FOR MICROSCOPY

Step 13.

An oil immersion was put on the prepared thick film and was examined using the X100 objective lens.

PREPARATION OF THICK AND THIN FILM FOR MICROSCOPY

Step 14.

Thin films were first examined using the X40 objective lens 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 was changed to X100 objective lens to examine the malaria parasites.

REPORTING OF MALARIA PARASITES

Step 15.

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 16.

NUMBEROF PARASITES/µL = NUMBER OF PARASITES COUNTED/NUMBER OF WHITE BLOOD CELLS COUNTED× 8000

PROCESSING OF BLOOD SAMPLES BY MALARIA RDT CASETTES (HRP-2)

Step 17.

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

PROCESSING OF BLOOD SAMPLES BY MALARIA RDT CASETTES (HRP-2)

Step 18.

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

PROCESSING OF BLOOD SAMPLES BY MALARIA RDT CASETTES (HRP-2)

Step 19.

Four drops of assay diluent were placed vertically into the square assay diluent well and the results were read after 30 minutes.

PROCESSING OF BLOOD SAMPLES BY MALARIA RDT CASETTES (HRP-2)

Step 20.

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

PROCESSING OF BLOOD SAMPLES BY MALARIA RDT CASETTES (HRP-2)

Step 21.

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 22.

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

BLOOD GROUPING

Step 23.

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

BLOOD GROUPING

Step 24.

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

BLOOD GROUPING

Step 25.

An applicator was used in mixing and the tile was rocked forward and backward for 3 minutes.

BLOOD GROUPING

Step 26.

The results were read macroscopically upon agglutination and in cases where the results were unclear, were read microscopically.

DESIGN OF QUESTIONNAIRE

Step 27.

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