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**Microscopic examination of blood films for malaria parasites
(English Translation)**

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Foreword

This standard was drafted in accordance with the rules given in GB/T 1.1-2009.

Drafting organizations of this standard. Hainan Provincial Center for Disease Control and Prevention, Jiangsu Provincial Institute of Parasitic Disease Control, and Chinese Center for Disease Control and Prevention Parasitic Disease Prevention and Control Institute, Yunnan Provincial Parasitic Disease Prevention and Control Institute, Hainan Provincial Agricultural Reclamation General Administration Hospital.

1 Scope

This standard specifies the technical specifications for the detection of Plasmodium by blood smear microscopy.

This standard is applicable to the microscopic examination of malaria parasites by disease prevention and control institutions and medical institutions at all levels.

2 Terms and definitions

The following terms and definitions apply to this document.

Plasmodium spp

Plasmodium is a single-celled, parasitic eukaryotic animal that is the pathogen of malaria (malaria). The host of malaria parasites Plasmodium falciparum (Plasmodium falciparum),

Plasmodium vivax (Plasmodium vivax), Plasmodium vivax (Plasmodium vivax) malariae) and Plasmodium ovale, etc.

Blood films

A smear made by spreading blood on a glass slide. Blood smears for microscopic examination of plasmodium include thick blood film smears and thin blood film smears Two kinds.

3 Apparatus and equipment

3.1

Biological microscope (100x oil immersion objective lens, 5x or 10x eyepiece).

3.2

Counter.

3.3

Glass slide (clean glass slide without scratches and oil stains).

3.4

Push the film.

3.5

Staining rack for blood slices.

3.6

Blood slice drying rack.

3.7

Slide box.

3.8

Dyeing plate and dyeing tank.

4 Reagents and materials

4.1

Ji's staining stock solution.

4.2

pH7.2 phosphate buffered saline (PBS).

4.3

Methanol (analytical grade).

4.4

Cedar oil or special immersion oil (refractive index ≥ 1.5).

4.5

Xylene (analytical grade).

4.6 75% alcohol.

4.7

One-time blood sampling needle.

4.8

Disposable gloves.

5.1.1 Blood collection site and blood collection method

Sterilize the blood collection site with 75% ethanol. After it dries, puncture the earlobe or fingertip with a disposable blood collection needle to get the blood. The baby can get the blood from the hallux or heel Puncture the blood. Take a sterile push film, hold the middle of the side edge of the push film with your thumb and index finger, scrape 4 μ L~5 μ L of blood with the lower left corner of the push film A thick blood film is made, and then 1 μ L to 1.5 μ L of blood is scraped from the middle of the end to make a thin blood film.

5.1.2 Thick blood film production

Take a glass slide, apply a drop of blood from the lower left corner of the push slide to the center of the slide to the left, draw a circle from the inside to the outside to form a diameter of 0.8cm~1.0cm the thickness of the thick round blood film is that 5-10 white blood cells can be seen in the field of an oil microscope.

5.1.3 Thin blood film production

Use a dry cotton ball to wipe off the blood stains on the lower left corner of the slide, and then flatten the lower edge of the slide against the midline of the slide. When the blood is between the slide and the slide, when the time is extended to about 2cm wide on both sides, keep the two glass slides at 25°~35°, and quickly push forward from right to left to form a thin tongue-like blood film. Each slide 1 thick blood film and 1 thin blood film (see Appendix A).

5.1.4 Number

After the blood film is prepared, place it horizontally, and after it is fully dried, use a pencil to number on the ground glass on one side of the slide or on the thin blood film.

5.2.1 Thin blood film fixation

Place one end of the thin blood film facing down at 45°, dip a cotton swab into the methanol solution, and wipe it evenly on the surface of the thin blood film to avoid touching the thick blood film.

5.2.2 Thick blood film hemolysis

Add a few drops of distilled water to the dry thick blood film to completely cover the blood film and hemolyze for a few minutes. When the blood film turns light gray, pour out the blood to dissolve it.

Staining does not require hemolysis within 1d after the thick blood film is made, and hemolysis is required for more than 1d.

5.3.1 Preparation of Ji's stain solution (see Appendix B)

Ji's stain includes Ji's stain stock solution and Ji's stain working solution. The stock solution of Ji's dye solution is fully ground from 5g Ji's powder and 250mL glycerin Add 250mL methanol to prepare. The stock solution of Ji's dye solution can be stored for a long time under dark conditions. Commonly used Ji's staining solution includes 2%, 3% and There are three kinds of 10% concentration, which are respectively prepared in proportions from the stock solution of Ji's stain solution and pH 7.2 phosphate buffered saline (PBS). Ji's dye solution working solution can only Prepare fresh when used.

5.3.2 Staining of a single blood smear (see Appendix C)

A single blood smear is commonly used for microscopic malaria detection in clinical malaria patients. Conventional dyeing method using 3% Ji's dye solution Blood smears have good staining quality and can be stored for a long time. The staining time is about 30 minutes. The fast dyeing method with 10% Ji's dye solution takes longer dyeing time Short, 8 min to 10 min, but blood smears are not suitable for long-term storage.

5.3.3 Staining of batches of blood smears (see Appendix C)

Gee's staining of batches of blood smears is often used for microscopic plasmodium detection in population epidemiological investigations, and 2% Gee's stain is often used for batching Stain the blood smear.