
Standard Procedure SP 0002-2:2005

Methods of preparing slides for microscopy – Part 2 Preparing a blood smear

1 Scope

This Standard Procedure can be used to prepare a blood smear for the purpose of microscopic examination. A thin smear allows the various types of blood cells to be seen and identified. Blood smears play an important role in diagnosing a wide range of illnesses.

2 Definitions

blood smear

a very thin layer of blood spread across a microscope slide

fixing

the process of preserving the blood smear, so that cell structures remain visible

Leishman's Stain (Eosin methylene blue)

a mixture of a red and a blue dye. It stains red blood cells pink, and stains platelets and the nuclei of white blood cells blue.

mountant

a thin layer of liquid placed between the smear and cover slip. It holds the cover slip in place. To help more light to pass through the slide, the mountant should have a refractive index similar to that of the glass.

3 Principle

Blood is smeared out thinly to give a single layer of cells spread across the slide. Staining the blood cells different colours helps to distinguish and identify different types of cell.

4 Apparatus

- specimen of fresh animal blood **CAUTION:** possible biohazard
- 2 microscope slides and a cover slip
- disposable dropper
- hair dryer or bench lamp (portable electrical appliance tested)
- 4 glass staining pots (or 100 cm³ beakers)
- forceps
- methanol **CAUTION:** highly flammable, toxic
- neat Leishman's Stain (1.5% solution in methanol) **CAUTION:** highly flammable, toxic, stains skin and clothing
- dilute Leishman's Stain (neat Stain diluted 1:10 with water buffered at pH6.8) **CAUTION:** harmful, stains skin and clothing
- mountant (e.g. DPX **CAUTION:** contains xylene [dimethylbenzene] – harmful, irritant, flammable)

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5 Test Specimens

The blood should have had an anticoagulant added while fresh, to prevent clotting.

6 Procedure

- The microscope slide must be clean and dry. To avoid finger marks, handle the slide and cover slip only by their edges.
- Using a disposable dropper, put one drop of blood towards one end of a glass slide. Hold another slide at 45° just touching the blood, as shown in figure 1. Quickly push it along the bottom slide to smear the blood into a thin layer.
- Dry the smear by holding the slide over a bench lamp, or by using a hair dryer.
- Fix and stain the smear as follows:
 - Using forceps, immerse the slide in four staining pots (or beakers) for 5 minutes in each pot:
 - methanol, to fix the smear;
 - neat Leishman's Stain;
 - dilute Leishman's Stain;
 - another pot of dilute Leishman's Stain.
- Remove the slide with forceps and hold under a slowly-running tap to rinse off surplus liquid Stain. Then dry it as above.
- Place a few drops of mountant onto the dried smear. Carefully lower a cover slip onto the slide. Press down gently with the forceps.

figure 1

