

A Comparative Study - Manual Method against Automated Peripheral Smear Preparation

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Abstract: A blood film or peripheral blood smear is a thin layer of blood smeared on a microscope slide and then stained in such a way to allow the various blood cells to be examined microscopically. This can be done by manual method or with automated analysers. This study is based on comparative study of manual method and automation (SC 120 auto slide maker and stainer) in making of blood films for evaluation.

Keywords: blood film, peripheral blood smear, microscopically.

INTRODUCTION

Blood film the most widely used test since its introduction from 1800s. Antonie van Leeuwenhoek, in the seventeenth century was the first to describe blood cells using whole blood preparations. Later, introduction of aniline dye in 19th century which made a possible study of individual blood cell by smearing a drop of blood, drying, fixing and staining. Paul Ehrlich introduced eosin as the first of these dyes for staining blood films in 1856, followed by hematoxylin in 1865. Lot of new redefining of techniques of staining, slide preparation and quality of dye was made, but the basis of film making and analysis have not changed

METHOD

Slide for blood film preparation

- Sealed fresh box slides are preferably used to be free of dust and fingerprints
- Slide should be corrosive resistant
- Glass slides typically measure 75x25 mm, 1 mm in thickness, flat and free from distortions/ripples and must be clear and colourless
- Slides may be plain or with frosted area for writing

Type of Blood Sample Used

Venous (anticoagulated-EDTA most commonly used) or capillary blood, as obtained by skin puncture both are acceptable. Sample should be processed within 2hrs of collection for better results in case of delay samples to be stored between 2-8degree C.

Blood film preparation methodologies include

1. Manual (wedge) [1]

Small amount of blood is placed on to a glass slide and spread with a help of another slide, spreader. The blood drop should be placed approximately 1 cm from the end of the slide. As soon as a blood drop has been placed on the slide, move the spreader slide slowly at an angle of 30-45degree for making the smear. Minimum of 1-1.5 cm space should be present at the end, smear measuring approximately 3cm. smear has

three parts head, body and tail. The ideal place for viewing the smear is in between the body and tail [2].

Staining by Leishman stain

William Boog Leishman, a British pathologist, modified the original Romanowsky method and devised a stain which is widely known as Leishman's stain. This consists of methylene blue and eosin dissolved in absolute methyl alcohol[3]. Commercially available Leishman stain powder (0.6 gram) is mixed with water-free absolute methyl alcohol (400 ml). Prepared stain should be kept tightly stoppered in a brown bottle and stored in a cool, dark place at room temperature. Exposure to direct sunlight causes deterioration of the stain. After preparation, stain should be kept for 3-5 days before using since it improves the quality of the stain.

Drying of Blood Film

Normally, air-drying without forced air circulation is sufficient. Blood films should be labelled

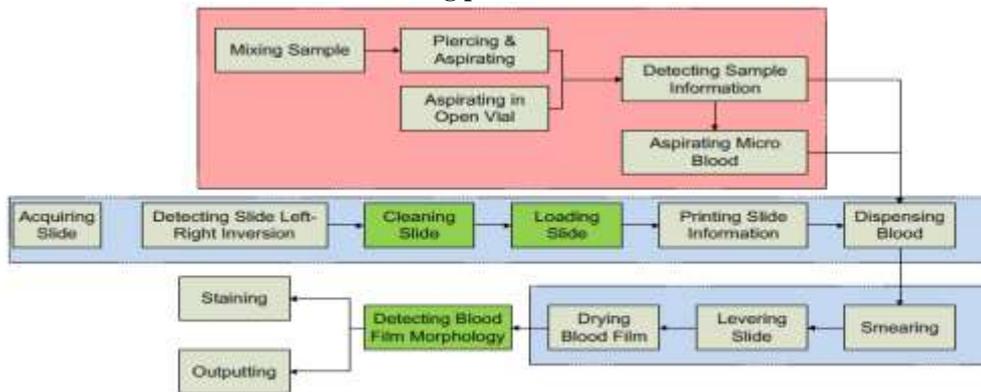
clearly and labels should be resistant to smudging and the other effects solvents. The label should contain minimum of patients sample number.

2. Automated (wedge) [4] SC-120 is an automated slide preparation instrument which accomplishes automatic

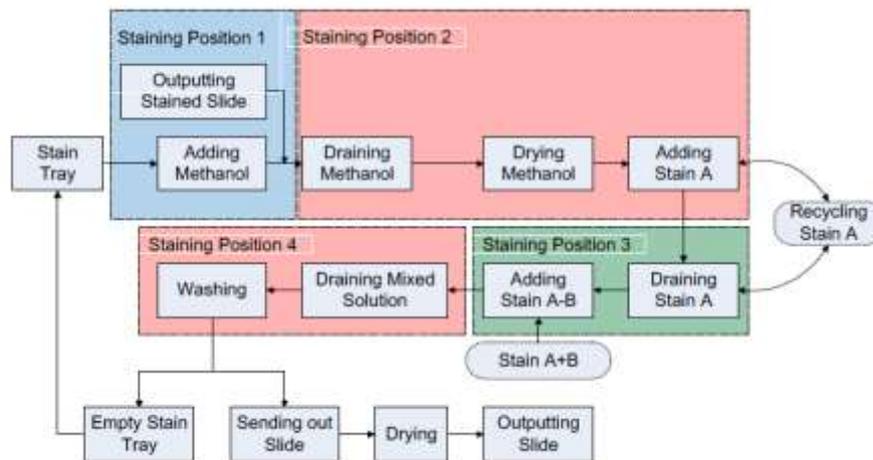
slide making and staining with clinical samples in the below mentioned principal

The workflow of slide making is shown in the figure below, where the steps with the red

Background color are all sample preparation procedures, and those with the blue background color are slide making procedures



The workflow of slide staining is shown in the figure below



EVALUATION OF STAINED BLOOD FILMS

Macroscopically a properly prepared and stained blood film should be pink in its thin part and show a purple-blue tint in the thicker parts. Microscopically, the red blood cells should be pink and the nuclei of the white blood cells more purple than blue. There should be no or minimal precipitation and staining should be uniform throughout the slide. The blood cells should be free from vacuoles and other artefacts.

DISCUSSION

Peripheral smear, diagnostic aid in evaluation of patients condition, decision of treatment and diagnosis of blood disorder. For maximum information to be gained from a smear it is necessary for a pathologist or haematologist to review the slide before

despatching the reports. This also helps in assessing the advantage and disadvantage of evaluating the manual method with automated analyser for slide making and staining process.

Need of faster blood smear study is usually a response to perceived clinical features or to an abnormality shown in a complete blood count. In these cases automated analyser helps by sooner and accurate making of smear for evaluation. In addition it directly prints the barcode on slides ensuring patients identification and reduces the risk of transcription errors which usually occurs in manual method. In automation uniformity (fig2) is attained in all samples over manual (fig1) as it is time framed in all process, from smearing till staining.

In manual method the major drawback being preparation artifacts [3] -The most common artefacts are caused by poor spreading techniques, slow drying in humid conditions, insufficient or delayed fixation, and fixing solutions that contain water. Slides that are not completely dry result in poorly or irregularly spread blood films, often with poor morphology as a result. Automated staining devices have their own specific

staining procedures as provided by the device manufacturers

There are many challenges to master the art of the manual smear that starts with uniform smear making with feathered edge. A quicker push at a higher angle results in a thicker smear. A larger drop of blood results in a longer smear[5].

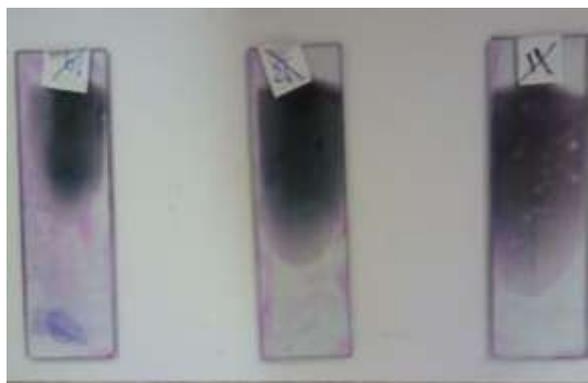


Fig-1: Manually made smear – note the uneven spread and staining with vacuoles

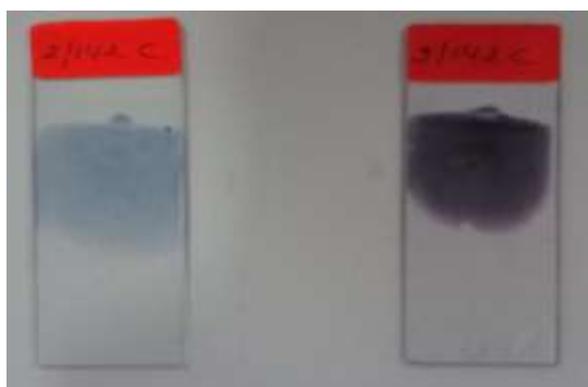


Fig-2: Automated analyser- Even smearing and staining

CONCLUSION

Peripheral smear, worldwide technique for evaluation of abnormal blood count and morphology can be done by manual and automated method. Automated method of PS preparation is quicker and accurate method and technical errors can be avoided. Manual method can be done at any places needed as the reagents can be easily transportable and repeatability is comfortable.

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