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Peripheral blood film on transparent polyvinyl chloride sheet: A newfangled haematological tool

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Abstract
The peripheral blood smear is a frontline diagnostic tool in cytological examinations of different blood cells. An attempt was made to replace conventional method of blood smear preparation by replacing glass slides with transparent polyvinyl chloride sheets to increase the shelf-life and quality of smears. A methanol resistant transparent polyvinyl chloride sheet was used and giemsa staining technique was employed. To score the merit of this technique, conventional method of blood smear on the glass slide was also done simultaneously. Microscopic examination of transparent polyvinyl chloride sheet blood smears after six months of storage revealed high quality images of different blood cells and also, they were devoid of fungal contamination. Statistical analysis revealed high equality of variance in leucogram obtained during different months of storage. Hence it is a cost effective ideal haematological tool to increases the shelf-life of high quality smears and reduce the continuous replacement of damaged blood films.

Keywords: Blood film, transparent polyvinyl chloride sheet, microscopy, haematological tool

1. Introduction
Peripheral blood film is an important and basic laboratory tool in diagnosis and monitoring of various clinical conditions and therapeutic response. The detailed study on the morphology of various cellular components of blood and haematological examination during different clinical conditions can be done with the help of peripheral blood film [1-3]. In spite of invention of high end automated haematological techniques and instruments, peripheral blood film examination remains the gold standard laboratory technique for various haematological investigations. In various educational and training institutions the blood films prepared on the glass slides were used for the teaching purpose to sensitize the learners about the basic morphology of various blood corpuscular components. But the initial inexperience of the beginners leads to the destruction of the valuable blood films prepared on the glass slide and which needs constant replacement. Blood smears on glass slides can get easily contaminated by fungus and their staining quality can get reduced on repeated usage.
To minimize these problems, based on the study by Mello *et al.* [4] we prepared peripheral blood films on the polyvinyl chloride sheets with the aim of producing more readily available teaching source for the haematological studies with high staining quality and long shelf-life. Polyvinyl chloride is a polymer of the vinyl chloride monomer, they are wrinkle resistant and flexible and long-lasting [5].

2. Materials and Method
2.1. Preparation of polyvinyl chloride transparent sheets
Polyvinyl chloride transparent sheets were obtained from Delhi Print linkers, Delhi, India and they were tested for resistant to methanol. Strips of transparent sheets were immersed in the methanol (Chemco Fine Chemicals, Bombay, India) for 30 minutes and then visually examined for any textural changes. To ensure the homogenous distribution of blood on the polyvinyl chloride transparent sheets, they were treated with absolute alcohol for few seconds. Polyvinyl chloride transparent sheets were cut into small pieces in the size of glass slide to ensure the equality in size between transparent sheets and glass slide (Figure – 1).

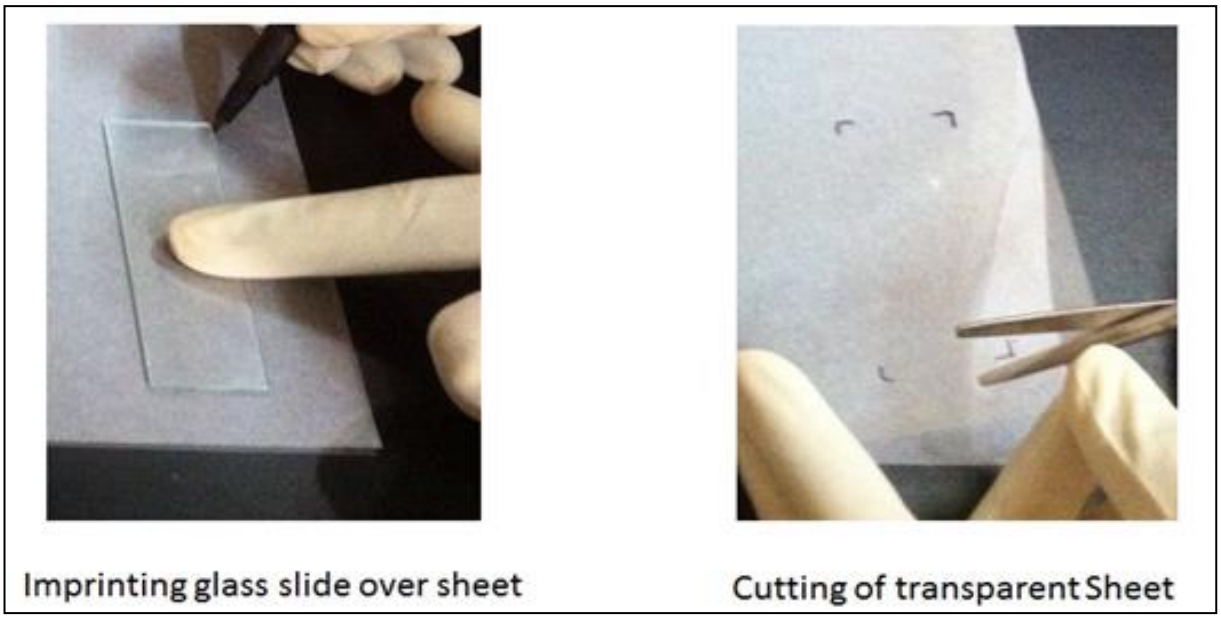

Fig 1: Preparation of Polyvinyl Chloride Sheets

2.2. Preparation of blood smears

Blood from healthy dogs was used to standardize the preparation method and to evaluate the quality of prepared blood films. To prevent the cross contamination during preparation, blood from individual animals were used. Two microliter of blood immediately after collection was used for preparation of the thin blood film. After dropping the blood on the sheets, a medium sized acrylic square was used to spread the blood to form the thin blood film (Figure – 2).

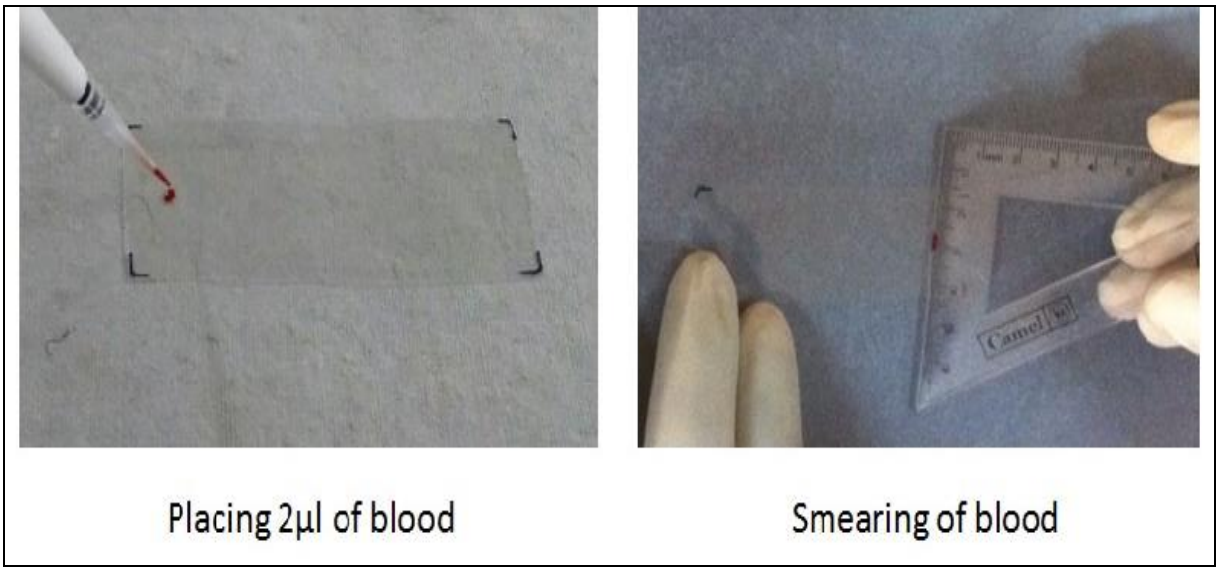

Fig 2: Preparation of blood smear

2.3. Fixing and staining of blood smear

Blood films were air dried and fixed with methanol by dipping the sheet into the container with methanol for two seconds. Thin blood film on the glass slide was prepared as per the method described by Adewoyin and Nwogoh (2014) [1]. For staining the blood films 10% diluted Giemsa staining (Sigma – Aldrich, catalogue No: G5637 – 5G) solution was used. Staining procedure was adopted as per the recommendation of World Health Organization (WHO, 2006) [6]. First the blood films were flooded with 10% Giemsa staining and allowed to stand for 45 minutes (Figure – 3). Then the blood film was washed with buffered saline to remove the iridescent scum and air dried.

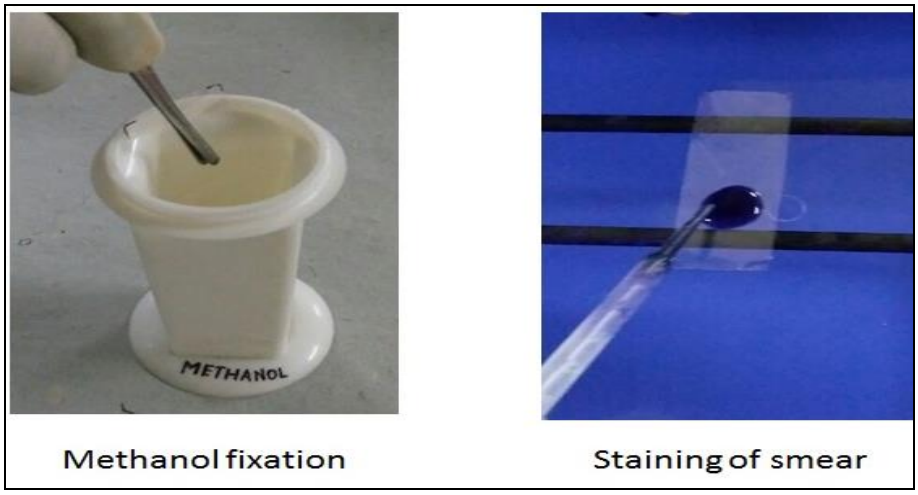

Fig 3: Fixing and staining of blood smear

2.4. Examination of blood smear

After staining the blood films were coated with varnish (Universal Paints and Corporation Chennai – 600 039, Tamilnadu, India) using 2 ml syringe attached with 24 gauge needle as shown in the figure -4 to protect the blood film from fungal and bacterial colonization.

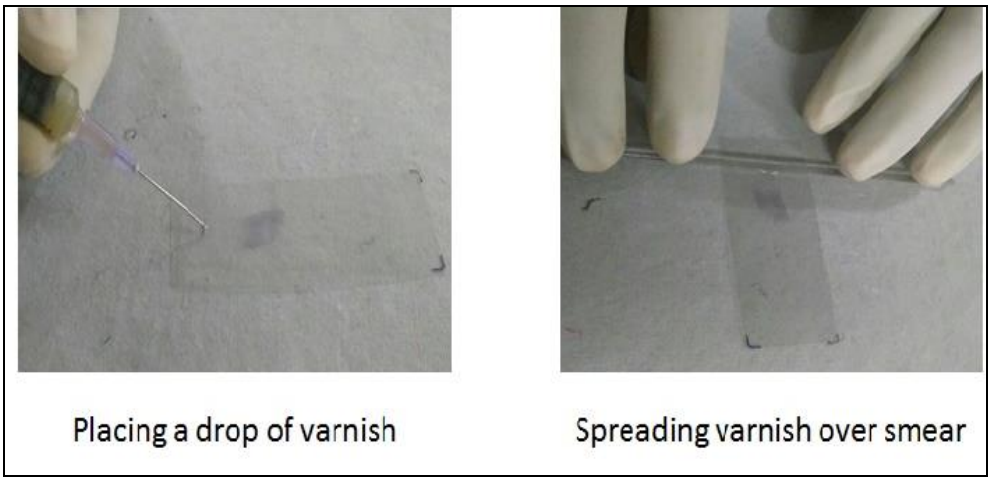

Fig 4: Placing varnish over blood smear

During examination of the blood films on sheets for the staining clarity and morphological characteristics of blood cells, they were mounted on to the glass slide by placing drop mineral oil (Sigma-Aldrich, catalogue No: M8410) (Figure - 5).

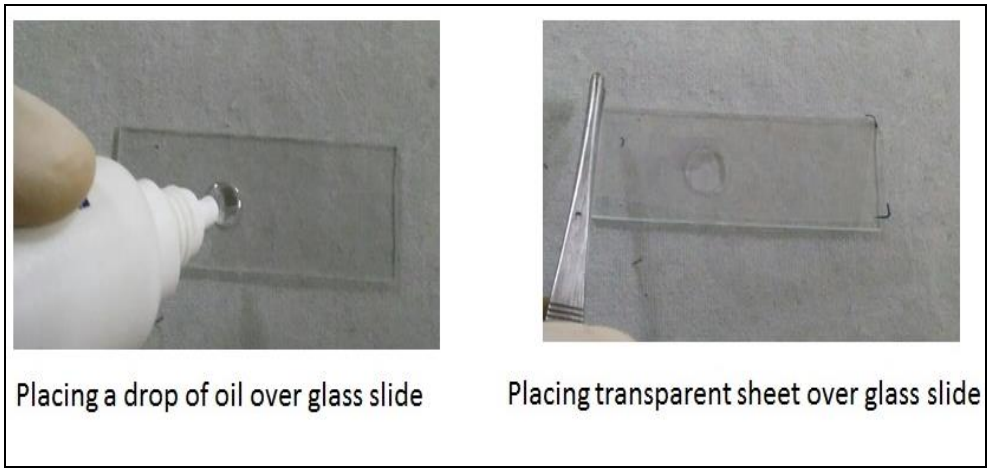

Fig 5: Mounting of transparent sheet over glass slide using immersion oil

To evaluate the durability and quality of the blood film on transparent sheets, they were compared with conventional glass slide blood film and photo documentation of the blood cells were also done by using Magnus XL Plus Tri-nocular camera microscope for six months period from the date of preparation (Figure-6).

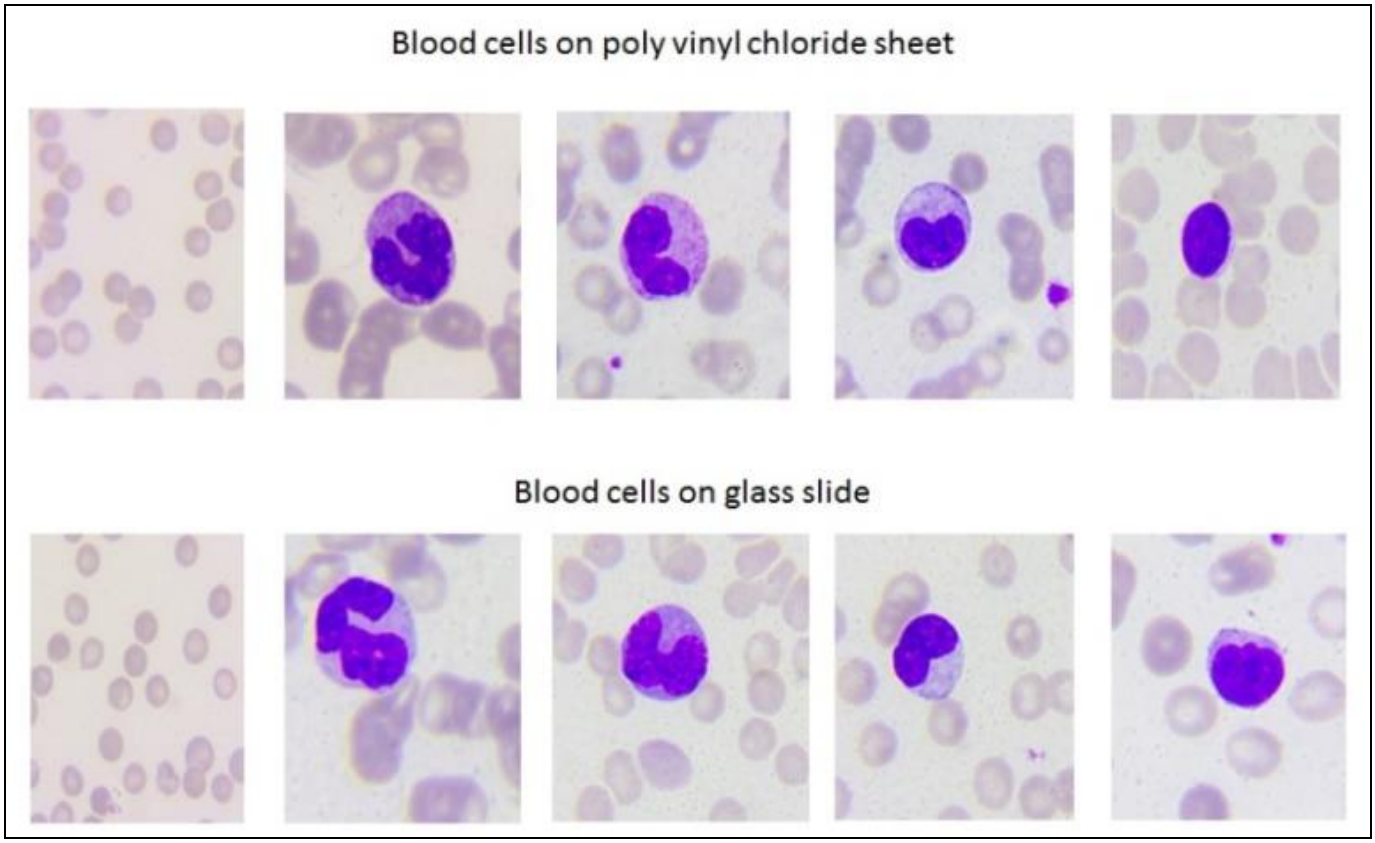

Fig 6: Comparison of blood cell morphology in blood film on both polyvinyl chloride sheet and glass slide (images are taken during the sixth month of storage) in 100X magnification.

2.5. Statistical analysis

The blood smear was examined monthly by three different microscopists during a period of six months and the leucograms obtained from blood smears prepared in different materials were compared for their equality of variance by using Analysis of Variance test and Levene's test.

3. Results

Transparent polyvinyl chloride sheets obtained from Delhi Print linkers, Delhi, India used for the preparation of the blood film was tested for the resistant to methanol which showed wrinkling resistant and no textural changes was found after treatment with methanol. Varnish obtained from Universal Paints and Corporation, Chennai – 600 039, Tamil Nadu, India did not affect the morphology of the blood cells in the blood film and also had the properties like rapid drying and non-reactiveness with the immersion oil. 32 trips were prepared from a single transparent sheet. Microscopic examination on high power magnification of blood film prepared on the transparent sheet revealed high clarity images of different blood cell types. Blood films on transparent sheets had minimal dye precipitation and blood cells were seen with clear background. It was easy to identify the different cell types based on their clearly stained nuclear morphology and cytoplasmic granules present in the granulocytes like neutrophils, eosinophils and basophils. Morphology of the erythrocytes was also clearly identified with the clean background. The blood smears were examined in a monthly basis up to six months for leucogram, staining clarity and presence of contamination. After six months of storage at room temperature there was no evidence of fungal or bacterial contamination and also there was no change in the image quality. Statistical analysis revealed leucogram obtained during different months had high equality of variance regardless the type of material used for blood film preparation (Table -1).

Table 1: Comparison of leucogram of blood cells in blood smears prepared in different materials according to the microscopists.

Blood Cells		Microscopists								
		A			B			C		
		Mean±SE	Variance	P Value	Mean±SE	Variance	P Value	Mean±S.E	Variance	P Value
Neutrophils	GS	63.66±0.49	1.466	0.290*	63.66±0.33	0.665	0.201*	64.33±0.42	1.065	1.000*
	TS	64.00±0.36	0.799	0.590**	64.50±0.61	2.280	0.263**	64.66±0.42	1.065	0.588**
Lymphocytes	GS	27.50±0.99	5.895	0.052*	26.50±0.56	1.898	0.479*	25.16±0.47	1.366	0.761*
	TS	26.83±0.47	1.366	0.558**	25.33±0.66	2.663	0.211**	24.66±0.42	1.065	0.451**
Eosinophil's	GS	5.33±0.49	1.065	0.670*	5.66±0.49	1.466	0.215*	7.16±0.30	0.565	0.661*
	TS	5.66±0.31	1.466	0.619**	6.50±0.22	0.299	0.156**	7.50±0.34	0.698	0.485**
Basophils	GS	0.66±0.21	0.266	0.234*	1.50±0.22	0.299	0.730*	1.16±0.30	0.094	0.220*
	TS	0.83±0.16	0.166	0.549**	1.16±0.16	0.166	0.260**	0.83±0.16	0.163	0.363**
Monocytes	GS	2.83±0.16	0.166	0.290*	2.66±0.21	0.266	0.247*	2.16±0.47	0.266	0.135*
	TS	2.66±0.33	0.665	0.664**	2.50±0.34	0.698	0.687**	2.33±0.21	0.749	0.756**

* - Levene's Test, ** - Analysis of Variance, GS – Glass slide, TS – Transparent sheet

4. Discussion

The haemato-morphologist mainly uses the blood film for the diagnosis of different morphological changes in the blood cells to identify the underlying pathology [7]. Microscopy needs adept approach to study the morphology of blood cell lineage. Normally thin blood smears can be assessed within 3 minutes by the trained experts but abnormal blood films requires longer time [8]. Evanescent of peripheral blood film examination was due to advent of high end automated instruments for the blood cell counting. In resource deprived areas, blood film examination is a valid option and hence the value of blood film examination for understanding the morphology of different blood cells cannot be down played.

In this study the method of preparation of the blood film on transparent sheets to identify the morphological changes in the blood cells was described. The transparent sheets were prepared from the polyvinyl chloride, which is normally used for printing and photocopying purposes. Initially, the method of preparation of blood smears on the transparent acetate sheets for the diagnosis of the malarial parasite was done by Mello *et al.* (2014) [4]. The blood film prepared on the transparent sheets did not jeopardize the image quality of different blood cells. The intra-cytoplasmic granular morphology of the blood cells were clearly visible and they aid in the differentiation of blood cells. Leucogram obtained from the transparent sheet blood film was highly consistent and this proved there was no change in cell staining characters.

The availability of blood film with good blood cell morphology is one of the determining factors for training the learners. This procedure aids in the preparation of a large number of blood film with less quantity of blood sample and also minimizes the loss of blood films with unique blood cell morphology by breakage, fungal and bacterial contamination because of repeated usage for the training purpose. So, this method reduces the restocking of the blood films. Discernible properties like simple method of preparation, convenience for transportation in normal envelope covers without any shipping damage made this method as a new-fangled haematological tool. In a single transparent sheet we can prepare 32 number of strips weighing less than 10 grams but the same number of glass slides weighs about 160 grams, so this property abate the economic loss during shipping of good blood films. The light weight and quality of the blood film in the transparent sheet made this as an effective tool in haematological studies. This method sores its merit due to high durability. Amount of stain or the dye consumed for the preparation of 32 blood film was less than the amount of the staining solution required for the staining of the same number of glass slides as per the recommendation of World Health Organization [6]. So, this also considered to be one of the main factors contributing to the financial cost of blood film preparation.

5. Conclusion

Blood film examination is a frontline diagnostic tool for unravelling the causes behind the symptoms of haemopathies. To prepare such effective tool, polyvinyl chloride transparent sheets proved to be an alternative platform for their preparation instead of using the conventional techniques. This material enables the low-cost and simplest method of preparation of the blood films with long shelf-life without compromising the image quality, staining characteristics and the morphology of the different blood cells. Considering the advantages of this method over glass slide blood smear preparation, it can be used as an ideal tool for various cytological examinations of blood cells and as a teaching aid for learners.

6. Acknowledgement

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