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Efficacy of three different Giemsa staining procedures for detection of *Leishmania* amastigotes in slit skin smears

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Background: Cutaneous Leishmaniasis (CL) is a neglected tropical disease. Parasitological diagnosis is routinely done by microscopy of Stained Slit Skin smears (SSS).

Objective: The aim was to assess the efficacy of three different stains for detection of *Leishmania* amastigotes in slit skin smears.

Method: Thirty SSS slides prepared from ten suspected patients presented to the Parasitology laboratory, University of Sri Jayewardenepura were used for the test. Blood smears prepared from five healthy volunteers were used as controls. Those slides were stained using three different Ramonowsky stains, namely Giemsa (GS), May Grunwarld (MG), and Wright's Giemsa (WG). Standard operating protocols were followed at all times for obtaining samples, preparation of SSS and stains, staining, and microscopy. Standard guideline was followed for the parasite count (WHO).

Results: In GS smears platelets and White Blood Cells (WBC) were stained in bright purple and Red Blood Cells (RBC) were stained in grey colour. In MG and WG, platelets and WBCs were stained in bright purple and RBCs were stained in brown or pink giving more contrast. However, intensity of pink colour was lower in WG than MG stained slide. The dot (nucleus) and dash (kinetoplast) of the amastigotes were seen in dark purple colour in slides stained with all three stains. Cytoplasm of the amastigote was stained in pink colour. It was more intense and contrasting in MG stained slides. Staining of the background and other cellular components in SSS were the same as in controls. Eighteen smears (six patients) were positive for *Leishmania* amstigotes by all three staining methods. Parasite counts were identical in all three stained methods.

Conclusion: MG stained slide had more contrasting background, making it easy to visualize. However, this enhanced visualization did not result in an increased sensitivity, especially when used by trained and experienced personnel.