

# RAPID WRIGHT'S STAIN FOR THE DETECTION OF IMPORTED *LEISHMANIA TROPICA*

Vasant Khachonsaksumet and Mario Riganti

Department of Tropical Pathology, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

**Abstract.** *Leishmania tropica* (cutaneous leishmaniasis) can be detected easily, rapidly, and conveniently by the examination of a skin ulcer smear that is stained with a modified method of Wright staining of blood (*ie* that used for routine hematological examinations).

## INTRODUCTION

Nowadays, international travel is fast and convenient, many workers travel in order to find employment overseas. Some returning workers import infectious diseases. *Leishmania tropica* is one of the species of *Leishmania* that cause cutaneous leishmaniasis (Oriental sore). This organism has been reported from endemic areas (Asia Minor) by returning workers. The workers infected with this organism may not have their condition promptly diagnosed because their home countries do not have endemic leishmaniasis; doctors may simply not consider leishmaniasis as a differential diagnosis.

During 1989-1991, fifteen patients that had returned from Saudi Arabia with *Leishmania tropica* were admitted to the Hospital for Tropical Diseases, Bangkok. They had worked in endemic areas and had not been treated before. Since the treatment of these patients with skin ulcer is different from others skin diseases; so the laboratory confirmation is essential for the rapid and correct diagnosis.

## MATERIALS AND METHODS

### Preparation of Wright's stain

A working stock of Wright's stain was

---

Correspondence: Vasant Khachonsaksumet, Department of Tropical Pathology, Faculty of Tropical Medicine, Mahidol University, 420/6 Rajvithi Road, Bangkok 10400, Thailand.

Tel: 02-2469000, ext 1622; Fax: 660-2468340  
E-mail: tmwym@mahidol.ac.th

prepared by grinding 300 mg of certified powder of Wright's stain (KgaA64271; Merck, Darmstadt, Germany) with 3 ml of glycerine in a mortar; a small amount of absolute methyl alcohol (acetone free) was added to give a total volume of 100 ml. This stain is that used in routine general hematology.

### Preparation of the smear

We scraped the rim of the skin ulcers of two patients and prepared smears following a standard technique.

### Method of staining

The smears were left to dry and then flooded with 1 ml of working Wright's stain for three minutes. Three milliliters of distilled water were added and left in place on the slide for eight minutes, during which time the slides were gently agitated. The slides were then washed with water until the stain was removed. Finally, they were blotted dry or dried with a hair dryer. The slides were examined directly under oil immersion, or were first covered with balsam or permount etc and a cover-slip.

## RESULTS

In all others forms of cutaneous leishmaniasis the diagnosis is confirmed by the demonstration of amastigotes in a smear or skin section (Northcutt and Tschen, 1991). The organisms are best revealed by Giemsa's stain: they appear as round or ovoid structures that

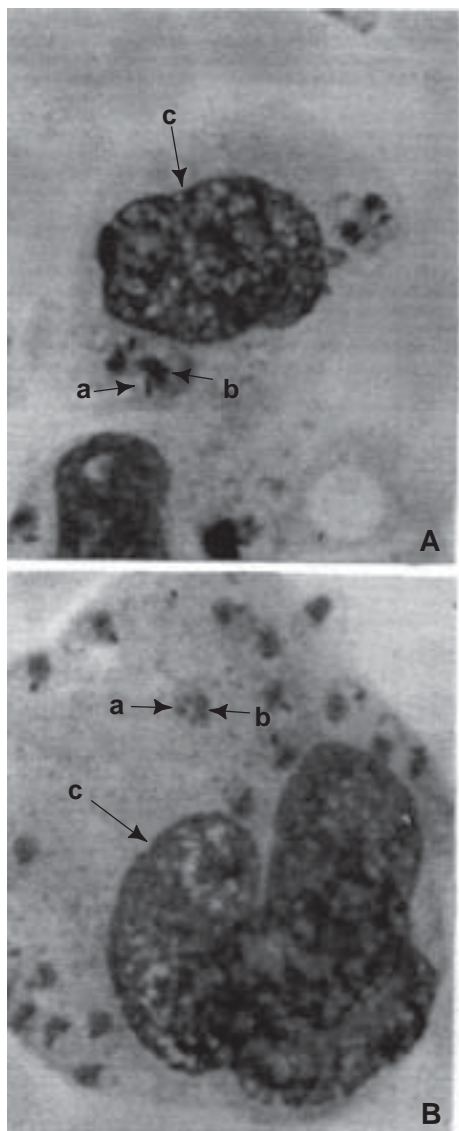


Fig 1—Giemsa's stain.

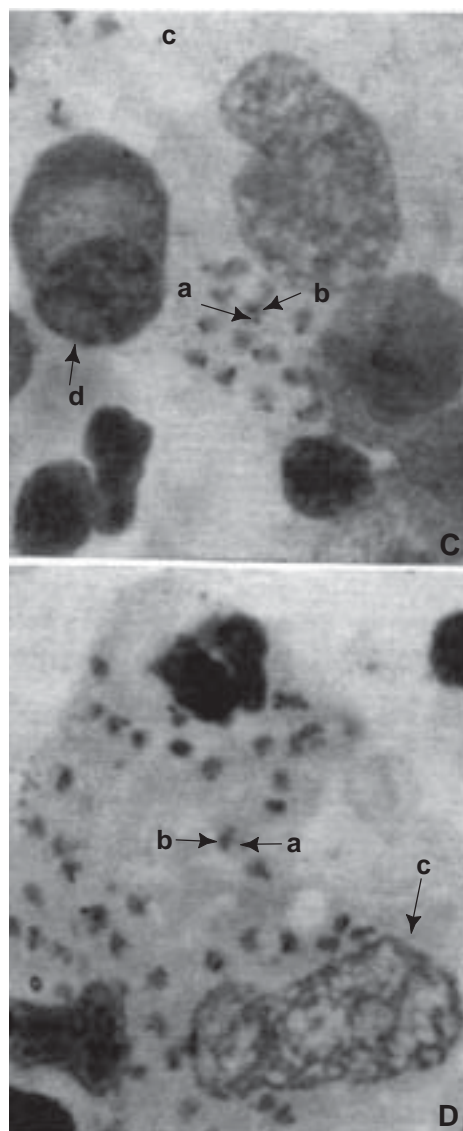


Fig 2—Wright's stain.

The smears from skin ulcer infected with *Leishmania tropica*. The magnification is x 1,000 in all cases. Images A and C feature blue light filtration: B and D are without light filtration. a = kinetoplasts; b = nuclei of *L. tropica*; c = nuclei of macrophages; d = nuclei of plasma cell.

measure 1.5 x 2.5  $\mu\text{m}$ ; the cytoplasm is reddish (Kubba and Al-Ginda, 1989). The spherical nucleus and the spherical or short, rod-like kinetoplast stain reddish-violet. Most of the organisms are within macrophages, but some are extracellular.

In this study, the smears from the two patients who came back from Saudi Arabia and who were admitted to the Hospital for Tropical Diseases, Faculty of Tropical Medicine, Bangkok, Thailand, in 1991, were stained with Giemsa staining (Fig 1); this method was com-

pared with Wright's stain (Fig 2). The short rod-like kinetoplasts took on a reddish stain, while the nuclei were blue to bluish-violet.

#### DISCUSSION

There are many ways of diagnosing cutaneous leishmaniasis: by clinical features, which are usually distinctive, by skin testing, by serology, or by culture of the organisms (McKee, 1996). This paper describes a staining technique for the microscopic diagnosis of cutaneous leishmaniasis that is simple, convenient and quick. Because the Wright's stain that is used to stain the smear is available in all hematology laboratories; the method may be adopted easily and with minimal expense. The basic stain is standard; the staining time is longer. Patients exposed in the endemic areas and suspected of having cutaneous leishmaniasis should have skin smear stained by this method.

#### ACKNOWLEDGEMENTS

The reagents used in this study were provided by the Department of Tropical Pathology, Mahidol University; the specimens were provided by the Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand. The assistance of Ms Preeya Srisopak is gratefully acknowledged.

#### REFERENCES

- Kubba RS, Al-Ginda Y. Leishmaniasis. *Dermatol Clin* 1989; 7: 331-51.
- McKee PH. Pathology of the skin with clinical correlations, 2<sup>nd</sup> ed. UK: Mosby-Wolfe, 1996: 4.53-4.55.
- Northcutt AD, Tschen JA. New ways to demonstrate pathogenic organisms. *Clin Dermatol* 1991; 9: 205-15.