A rapid stain for the diagnosis of granuloma inguinale

N O'Farrell, A A Hoosen, K Coetzee, J van den Ende

Abstract

Tissue smears were prepared from 55 men and eight women with genital ulceration using two staining techniques and examined by direct microscopy for the presence of Donovan bodies. Twenty three smears were positive using the May-Grunwald-Giemsa staining method and 23 were positive using a rapid technique, the RapiDiff stain. The RapiDiff technique is suitable for use in the diagnosis of granuloma inguinale (donovanosis) in busy sexually transmitted diseases clinics in the developing world.

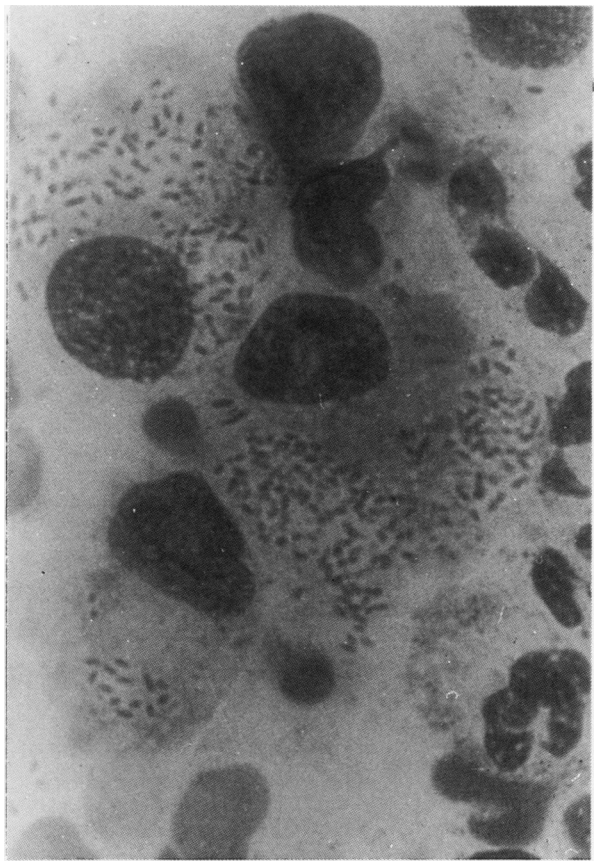
Donovan first described inclusion bodies in mononuclear cells as being characteristic of granuloma inguinale (donovanosis) in 1905. The condition is generally regarded as a sexually transmitted disease (STD) and is found more commonly in tropical climates where poor socio-economic conditions prevail, but has been thought to occur infrequently in Africa.[1] The causative organism *Calymmatobacterium granulomatis* has not been successfully cultured since the early 1960s,[2] and the diagnosis is made by the demonstration of Donovan bodies in tissue smears or biopsy specimens using the Giemsa Stain. We evaluated and compared tissue smears, from patients in a busy STD clinic for the presence of Donovan bodies, stained with the May Grunewald Giemsa (MGG) method and a rapid technique using the RapiDiff method and found the RapiDiff method to be quicker with similar efficacy and thus suitable for use in a busy clinic.

Patients and methods

Fifty five males and eight females attending the STD Clinic at King Edward VIII Hospital, Durban with lesions clinically suggestive of granuloma inguinale and who had not received antibiotics during the previous three weeks, were examined over a two month period in 1988.

A cotton tipped swab was gently rolled across the largest ulcer to remove debris. A second swab was rolled firmly across the edge of the lesion and smears made on two glass slides. One smear was stained with MGG (Clinical Science Diagnostics Ltd, Booysens 2016, SA) in the standard manner[3] and the other using RapiDiff (Clinical Science Diagnostics Ltd, Booysens 2016, SA) as follows:

(1) the smear was first placed in RapiDiff fixative for 15 seconds (6 dips), then



Donovan bodies in mononuclear cells stained with RapiDiff (× 1000).

City Health STD Department, King Edward VIII Hospital, Congella, Durban, South Africa
N O'Farrell

Department of Medical Microbiology, University of Natal Medical School, Congella, Durban, South Africa
A A Hoosen, K Coetzee, J van den Ende

Table Comparison of staining techniques

	Donovan bodies present		*Donovan bodies absent*	
	Male	*Female*	*Male*	*Female*
MGG Stain N = 60	20	3	32	5
RapiDiff Stain N = 60	20	3	32	5

(2) placed in solution RapiDiff 1 (Eosin Y) for 15 seconds (6 dips) and thereafter
(3) placed in solution RapiDiff 2 (Thiazine Dye Mixture) for 15 seconds (6 dips) and finally rinsed in phosphate buffer pH 6·8.

Stained slides were air dried and examined by light microscopy under oil immersion (1000 × magnification).

Results

Donovan bodies were detected in the same 23 of 60 patients (38·3%) using both RapiDiff and MGG methods (table). Smears from three patients were unavailable for evaluation with RapiDiff and therefore excluded from analysis.

Discussion

The microscopic appearance of Donovan bodies stained by RapiDiff differed slightly from that in MGG stained smears. RapiDiff stained bodies appeared slightly larger with an irregular outline, and unstained capsule and a pink-purple bacillary body. MGG stained bodies were smaller with characteristic bipolar condensation, staining a blue-purple colour.

The RapiDiff stain has been used for white blood cell differential counts and is identical to the Diff-Quik stain used for Tzanck smears.[4] The staining technique is simple and takes approximately one minute, far less than the 25–30 minutes required for the MGG method.

With genital ulceration and other sexually transmitted diseases assuming an increasing importance in the transmission of HIV in Africa,[5] prevention strategies must be linked to the control of genital ulcer disease. Granuloma inguinale in particular, may cause prolonged genital ulceration, and may therefore pose an added risk in the transmission of HIV. We found the rapid technique described above to be an effective method for the diagnosis of granuloma inguinale and consider it is suitable for routine use in STD clinics.

Address for correspondence: Dr N O'Farrell, Jefferiss Wing, St Mary's Hospital, London W2 1NY, UK.

1 Osoba AO. Sexually transmitted disease in tropical Africa. A review of the present situation. *Br J Venereal Dis* 1981;57: 89–94.
2 Sehgal VN, Prasad AL. Donovanosis—Current concepts. *Int J Dermatol* 1986;5:8–16.
3 Dacie J. Lewis SM. *Practical Haematology*. Edinburgh: Churchill Livingstone, 1984:52.
4 O'Keefe EJ, Burke WA, Steinbaugh JR. Diff-Quik Stain for Tzanck Smears. *J Am Acad Dermatol* 1985;13:148–9.
5 Piot P, Laga M. Genital ulcers, other sexually transmitted diseases, and the sexual transmission of HIV. *Br Med J* 1989;298:623–4.