UvA-DARE (Digital Academic Repository)

The direct agglutination test for diagnosis of visceral leishmaniasis under field conditions in Sudan: comparison of aqueous and freeze-dried antigens

Zijlstra, E.E.; Osman, O.F.; Hofland, H.W.C.; Oskam, L.; Ghalib, H.W.; El-Hassan, A.M.; Kager, P.A.; Meredith, S.E.O.

DOI

[10.1016/S0035-9203\(97\)90518-6](https://doi.org/10.1016/S0035-9203(97)90518-6)

Publication date

1997

Published in

Transactions of the Royal Society of Tropical Medicine and Hygiene

[Link to publication](#)

Citation for published version (APA):

Zijlstra, E. E., Osman, O. F., Hofland, H. W. C., Oskam, L., Ghalib, H. W., El-Hassan, A. M., Kager, P. A., & Meredith, S. E. O. (1997). The direct agglutination test for diagnosis of visceral leishmaniasis under field conditions in Sudan: comparison of aqueous and freeze-dried antigens. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 91, 671-673. [https://doi.org/10.1016/S0035-9203\(97\)90518-6](https://doi.org/10.1016/S0035-9203(97)90518-6)

General rights

It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations

If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: <https://uba.uva.nl/en/contact>, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.

UvA-DARE is a service provided by the library of the University of Amsterdam (<https://dare.uva.nl>)

Download date: 26 Apr 2023

The direct agglutination test for diagnosis of visceral leishmaniasis under field conditions in Sudan: comparison of aqueous and freeze-dried antigens

E. E. Zijlstra¹*, O. F. Osman², H. W. Chr. Hofland², L. Oskam³, H. W. Ghalib^{2†}, A. M. El-Hassan², P. A. Kager¹ and S. E. O. Meredith³ ¹Department of Infectious Diseases, Tropical Medicine and AIDS, Academic Medical Centre, Amsterdam, The Netherlands; ²Institute of Endemic Diseases, University of Khartoum, Sudan; ³Department of Biomedical Research, Royal Tropical Institute, Amsterdam, The Netherlands

Abstract

The performance of the direct agglutination test (DAT) was evaluated under field conditions in an endemic area of visceral leishmaniasis in eastern Sudan, using aqueous (Aq) antigen which has to be kept refrigerated and a newly developed freeze-dried (FD) antigen which is stable at ambient temperature. Both antigens compared well, with 92–98% of readings being identical or only with one dilution difference in titre. FD antigen gave titres that were identical with Aq antigen in 73% of samples, higher in 19%, and lower in 8%. Owing to high ambient temperatures and low humidity, microtitre plate wells dried out during the standard procedures for elution and incubation. However, shortening the elution time from 12 to 4 h proved possible for both antigens; incubation could be reduced from 24 to 10 h for Aq antigen, after which the plates could still be read. Incubation with FD antigen required 18 h and the plates needed to be kept cool because of evaporation. Despite the longer procedure with the FD antigen, the DAT can be completed in 24 h and the use of this stable antigen, that does not require refrigeration, is a major improvement in performing the DAT under unfavourable field conditions.

Keywords: leishmaniasis, visceral leishmaniasis, *Leishmania donovani*, diagnosis, direct agglutination test, freeze-dried antigen, Sudan

Introduction

The diagnosis of visceral leishmaniasis (VL; kala-azar) classically rests on demonstration of *Leishmania* amastigotes in tissue aspirates. In addition, several serological tests have been developed, of which the direct agglutination test (DAT) appears to be a simple and economical test with high sensitivity and specificity (EL-HARITH *et al.*, 1988). In Sudan, the DAT proved useful for screening, with good sensitivity and negative predictive value (ZIJLSTRA *et al.*, 1991). Any diagnostic test should be evaluated in the area where it is to be used and, as VL is a rural disease, ideally it should be possible to perform the test under the most unfavourable conditions. An important problem with DAT is the limited stability of the currently used aqueous (Aq) antigen, for which a 'cold chain' is required. Recently, a freeze-dried (FD) antigen was developed that remains stable at ambient temperature and compared well with standard Aq antigen in the laboratory (MEREDITH *et al.*, 1995). In this study, we compared the performance of Aq and FD antigens in an endemic focus of VL in eastern Sudan.

Patients and Methods

Patients

The patients were taking part in a field study on the incidence of VL and post kala-azar dermal leishmaniasis in the village of Um-Salala in Rahad Province, Gedaref State, eastern Sudan (ZIJLSTRA *et al.*, 1994). There is no laboratory facility in the area and no electricity. The study was carried out in December 1993 (average outdoor temperature during the day 30–35°C; at night 20–25°C) and in April 1994 (average day temperature 40–45°C; at night 25–30°C). The humidity varied between 10% and 20%. A field laboratory was set up and the Aq antigen was stored in a solar powered field refrigerator. During the study period, the temperature inside the laboratory did not fall below 35°C, even at night. Finger-prick blood was collected on filter paper, air-dried and processed the same day. A random sample

was taken from the following patient groups. (i) Parasitologically confirmed active VL, (ii) treated VL, (iii) subclinical VL as shown by positive DAT and/or leishmanin skin results, in the absence of clinical symptoms, (iv) post kala-azar dermal leishmaniasis patients, (v) patients with leishmaniomas (the ulcer that may develop at the site of the sandfly bite), and (vi) endemic uninfected control subjects. In a previous study, 3 strains of *Leishmania* were identified in the area; 2 strains of *L. donovani* (zymodemes MON-18 and MON-82) and one of *L. infantum* (zymodeme MON-30) (typed by isoenzymes); using the Southern blotting technique, the *L. infantum* strain was indistinguishable from the *L. donovani* strains (L. Oskam, personal communication).

Methods

The antigens were prepared as described previously using *L. donovani* strain MHOM/SD/68/1S (EL-HARITH *et al.*, 1988; MEREDITH *et al.*, 1995) and the DAT was performed essentially as described by EL-HARITH *et al.* (1988). In brief, disks corresponding to 5 µL of blood were punched out of the filter papers, placed in a microtitre plate with V-shaped wells, and normal saline was added for elution (step 1). The plates were left at ambient temperature overnight (12 h). The next morning a fresh solution of normal saline containing 0.2% gelatine was prepared by heating in hot water on a charcoal fire to 56°C; after cooling, 2-mercaptoethanol (0.1 M) was added. The solution (75 µL aliquots) was pipetted into the microtitre plate and 50 µL of eluate were added to each well, followed by 50 µL of antigen (step 2: incubation). The FD antigen was reconstituted in 5 mL of normal saline, and thoroughly shaken before use. Initially, for screening purposes, serial dilutions were made from 1:200 to 1:1600. Negative control wells (antigen only; on each plate) and known negative and positive controls were tested daily. The titre was defined as the highest dilution at which agglutination was still visible, as a blue mat, enlarged dot with frayed edges, or enlarged blue dot, compared with negative control wells which had clear blue dots. We used known European control serum samples on filter paper (negative control ≤1:200, positive control 1:819200) and freeze-dried serum of Sudanese endemic controls (negative ≤1:200, positive 1:3200, 1:12800, and 1:819200). Incubation was for 18 h, after which samples with titres ≥1:3200 were diluted further to give end-point titres ≥1:102400. The tests were read independently by 2 individuals.

Address for correspondence: E. E. Zijlstra, Institute of Endemic Diseases, P.O. Box 102, Khartoum, Sudan.

*Present address: Institute of Endemic Diseases, Khartoum, Sudan.

†Present address: College of Medicine, King Saud University Abha Branch, Kingdom of Saudi Arabia.

‡Present address: The Mectizan Donation Program, Atlanta, GA, USA.

Table. Comparison of the use of aqueous and freeze-dried antigens in the direct agglutination test for visceral leishmaniasis under field conditions in eastern Sudan

Patients and date	Difference in titre			
	None	±1 dilution	±2 dilutions	±3 dilutions
December 1993				
Visceral leishmaniasis				
Active	13	6	1	—
Treated	15	14	1	—
Subclinical	2	10	—	—
PKDL ^a	11	4	—	—
Leishmanioma	—	2	—	—
Endemic controls	183	21	4	—
Total	224 (78%)	57 (20%)	6 (2%)	—
April 1994				
Visceral leishmaniasis				
Active	2	1	—	—
Treated	17	6	—	—
Subclinical	10	5	—	—
PKDL ^a	—	1	—	—
Leishmanioma	—	1	—	—
Endemic controls	74	29	11	1
Total	103 (65%)	43 (27%)	11 (7%)	1 (1%)

^aPost kala-azar dermal leishmaniasis.

Results

Totals of 287 and 158 samples were collected and tested, using both antigens, in December 1993 and in April 1994, respectively (Table). In 98% of the December samples, and 92% of the April samples, there was no difference in titre, or only one dilution difference, between readings obtained using the 2 antigens on the same serum samples; none and only 1%, respectively, differed by more than 2 dilutions.

Among the total 445 samples, readings obtained using FD antigen were identical in 327 (73%), higher in 83 (19%), and lower in 35 (8%) samples compared to the Aq antigen. The results obtained with all 445 samples are shown in the Figure.

The dots produced with the FD antigen were somewhat smaller than those obtained with Aq antigen, but this did not interfere with reading. It was necessary to

shake the FD antigen thoroughly after resuspension, after which it was left for 20 min and then shaken again.

In April 1994, the ambient temperature inside the field laboratory did not fall below 35°C, even at night, causing evaporation and thus concentration of the eluate (during step 1). After the standard incubation period (step 2), most wells were dry and reading was impossible. To overcome this problem we used a cool box kept in a shaded place outside the laboratory; temperatures inside the box ranged from 28°C to 35°C. To address further the problem of evaporation, we attempted to shorten the procedures. Elution (step 1) could be shortened to 4 h with samples kept at high ambient temperatures; aliquots of 50 samples eluted for 10 h in the cool box, gave identical results. Samples incubated (step 2) at ambient temperature for 10 h (after which the wells could still be read) were compared with aliquots left for the standard 18 h in the cool box. Identical results were obtained with Aq antigen after both procedures; with the FD antigen, the dots were still weak after 10 h at ambient temperature, but clear after 18 h when kept in the cool box.

Thus, the DAT using Aq antigen can be completed in one day during daytime, even at high ambient temperatures; using the FD antigen it takes somewhat longer, but still can be done in 24 h, and the samples need to be stored in a cool box or otherwise kept cool.

The European control sera (on filter paper) gave unchanged titres in December 1993, but showed gradually decreasing titres in April 1994. This could have been due to higher temperatures and/or accumulation of moisture in the cool box, resulting in bacterial or fungal contamination of the samples. The Sudanese freeze-dried control sera remained stable at all times.

Discussion

Both antigens performed equally well, giving identical titres with most samples and a difference of only plus or minus one dilution in 92–98% of the samples, which, for practical purposes in a test that is read by eye, is entirely acceptable. This is in agreement with earlier findings under field conditions (MEREDITH *et al.*, 1995).

The high ambient temperature had a major impact on the performance of the DAT. When temperatures are likely to be above 30°C, we advise shortening the elution step to 4 h. The incubation step may be shortened to 10 h when using Aq antigen, but FD antigen requires 18 h and samples should be kept cool. The advantage of

Figure. Comparison of aqueous and freeze-dried antigens (reciprocal titres) in the direct agglutination test for visceral leishmaniasis. The numbers between the solid lines indicate the number of observations in which the freeze-dried antigen gave titres which were identical with, or only one dilution higher or lower than, those obtained with the aqueous antigen.

the FD antigen is that it does not require refrigerating and remains stable, which outweighs these drawbacks. As had already been demonstrated in a well-equipped laboratory setting (EL-HARITH *et al.*, 1988), gelatine can also be used under field conditions with good results, obviating the need for fetal calf serum, which is expensive and requires refrigerating. The use of freeze-dried control sera, which are stable under adverse climatological conditions, is a major improvement.

In conclusion, use of the FD antigen and freeze-dried control sera contributes considerably to the performance and dependability of the DAT under basic field conditions.

Acknowledgements

We thank *Médecins sans Frontières* for logistical support. The study was funded in part by EEC grant no. TS3*-CT93-0245.

References

El-Harith, A., Kolk, A. H. J., Leeuwenburg, J., Muigai, R., Huigen, E., Jelsma, T. & Kager, P. A. (1988). Improvement

of a direct agglutination test for field studies of visceral leishmaniasis. *Journal of Clinical Microbiology*, **26**, 1321–1325.

Meredith, S. E. O., Kroon, N. C. M., Sondorp, E., Seaman, J., Goris, M. G. A., van Ingen, C. W., Oosting, H., Schoone, G. J., Terpstra, W. J. & Oskam, L. (1995). *Leish-KIT*, a stable direct agglutination test based on freeze-dried antigen for serodiagnosis of visceral leishmaniasis. *Journal of Clinical Microbiology*, **33**, 1742–1745.

Zijlstra, E. E., Siddig Ali, M., El-Hassan, A. M., El-Toum, I. A., Satti M., Ghalib, H. W. & Kager, P. A. (1991). Direct agglutination test for diagnosis and sero-epidemiological survey of kala-azar in the Sudan. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **85**, 474–476.

Zijlstra, E. E., El-Hassan, A. M., Ghalib, H. W. & Ismael, A. (1994). Endemic kala-azar in eastern Sudan: a longitudinal study on the incidence of clinical and subclinical disease and post-kala-azar dermal leishmaniasis. *American Journal of Tropical Medicine and Hygiene*, **51**, 826–836.

Received 30 April 1997; revised 21 July 1997; accepted for publication 22 July 1997

Announcements

Fourth International Conference of the Hospital Infection Society Edinburgh, Scotland, UK 13–17 September 1998

The deadline for submission of abstracts to this meeting is 18 May 1998. Further information and registration forms can be obtained from the Conference Secretariat, 4th International HIS Conference, Index Communications Meeting Services Ltd, Crown House, 28 Winchester Road, Romsey, Hampshire, SO51 8AA, UK; phone +44 (0)1794 511331/511332, fax +44 (0)1794 511455, e-mail his.icms@dial.pipex.com

Ninth International Training Course on Identification of Helminth Parasites of Economic Importance The Royal Veterinary College, Hatfield, UK 6 July–14 August 1998

Further details can be obtained from Dr L. M. Gibbons, The Royal Veterinary College, Hawkshead Lane, North Mymms, Hatfield, Hertfordshire, AL9 7TA, UK; phone +44 (0)1707 666208, fax +44 (0)1707 661464, e-mail djacobs@rvc.ac.uk

Fifteenth Congress of the International Society for Geographical and Epidemiological Ophthalmology Amsterdam, The Netherlands 26–27 June 1998

This conference will follow the International Congress of Ophthalmology. Further details can be obtained from Dr Paul Courtright, BC Centre for Epidemiologic and International Ophthalmology, University of British Columbia, St Paul's Hospital, 1081 Burrard Street, Vancouver, BC, V6Z 1Y6, Canada; e-mail pcourtright@stpaulshosp.bc.ca