New diagnostic tools for different challenges in VL elimination

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Senior Scientist

INFECTIOUS DISEASE RESEARCH INSTITUTE

Elimination challenges

Disease →

Cure/ Complication/Relapse

Elimination challenges

Disease →

Cure/ Complication/Relapse

Detection

Surrogates – Ab, biomarkers

Direct – Ag, NAAT

Practicality….

Ghosh et al. Parasites & Vectors (2016) 9:383
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Parasites & Vectors

RESEARCH Open Access

Evaluation of diagnostic performance of rK28 ELISA using urine for diagnosis of visceral leishmaniasis

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Abstract

Background: Recombinant fusion proteins are now commonly used to detect circulating antibodies for the serodiagnosis of visceral leishmaniasis (VL) in Asia, Africa and the Americas. Although simple, these tests still require blood collection and their use in remote settings can be limited due to the need of collection devices, serum fractionation instrument and generation of biohazardous waste. The development of an accurate and non-invasive diagnostic algorithm for VL, such as could be achieved with urine, is desirable.

Methods: We enrolled 87 VL patients and 81 non-VL individuals, including 33 healthy endemic controls, 16 healthy non-endemic controls, 16 disease controls and 16 tuberculosis (TB) patients. We compared the efficacy of recombinant antigens rK28, rK39 and rKRP42 for the diagnosis of VL when either serum or urine were used to develop antibody-detection ELISA.

Results: As expected, each of the antigens readily detected antibodies in the serum of VL patients. rK28 ELISA showed the highest sensitivity (98.9 %), followed by rK39 and rKRP42 ELISA (97.7 and 94.4 %, respectively); overall specificity was > 96 %. When urine was used as the test analyte, only a marginal drop in sensitivity was observed, with rK28 ELISA again demonstrating the greatest sensitivity (95.4 %), followed by rK39 and rKRP42 ELISA, respectively. Again, the overall specificity was > 96 %.

Conclusions: Our data indicate the potential for using urine in the diagnosis of VL. Detection of antibodies against rK28 demonstrated the greatest sensitivity. Together, our results indicate that rK28-based antibody detection tests using urine could provide a completely non-invasive tool amenable for diagnosis of VL in remote locations.

Keywords: Visceral leishmaniasis, Diagnosis, rK28, rK39, rKRP42, ELISA, Serum, Urine, Bangladesh

Table 4 Sensitivity and specificity of rK28 ELISA performed using serum and urine samples from VL patients and non-VL individuals for diagnosis of VL

Group	Serum					Urine			
	Subjects (*n*)	Positive (*n*)	Sensitivity N (%)	Specificity *n* (%)	95 % CI	Positive (*n*)	Sensitivity *n* (%)	Specificity *n* (%)	95 % CI
VL	87	86	86 (98.85)	na	93.76–99.97	83	83 (95.40)	na	88.64–98.73
All controls (EC + NEC + DC + TB)	81	3	na	78 (96.30)	89.56–99.23	3	na	78 (96.30)	89.56–99.23
EC	33	1	na	32 (96.97)	84.24–99.92	1	na	32 (96.97)	84.24–99.92
NEC	16	0	na	16 (100)	79.41–100	1	na	15 (93.75)	69.77–99.84
DC	16	1	na	15 (93.75)	69.77–99.84	0	na	16 (100)	79.41–100
TB	16	1	na	15 (93.75)	69.77–99.84	1	na	15 (93.75)	69.77–99.84

Abbreviations: *na*, not applicable; 95 % CI, sensitivity or specificity at 95 % confidence interval

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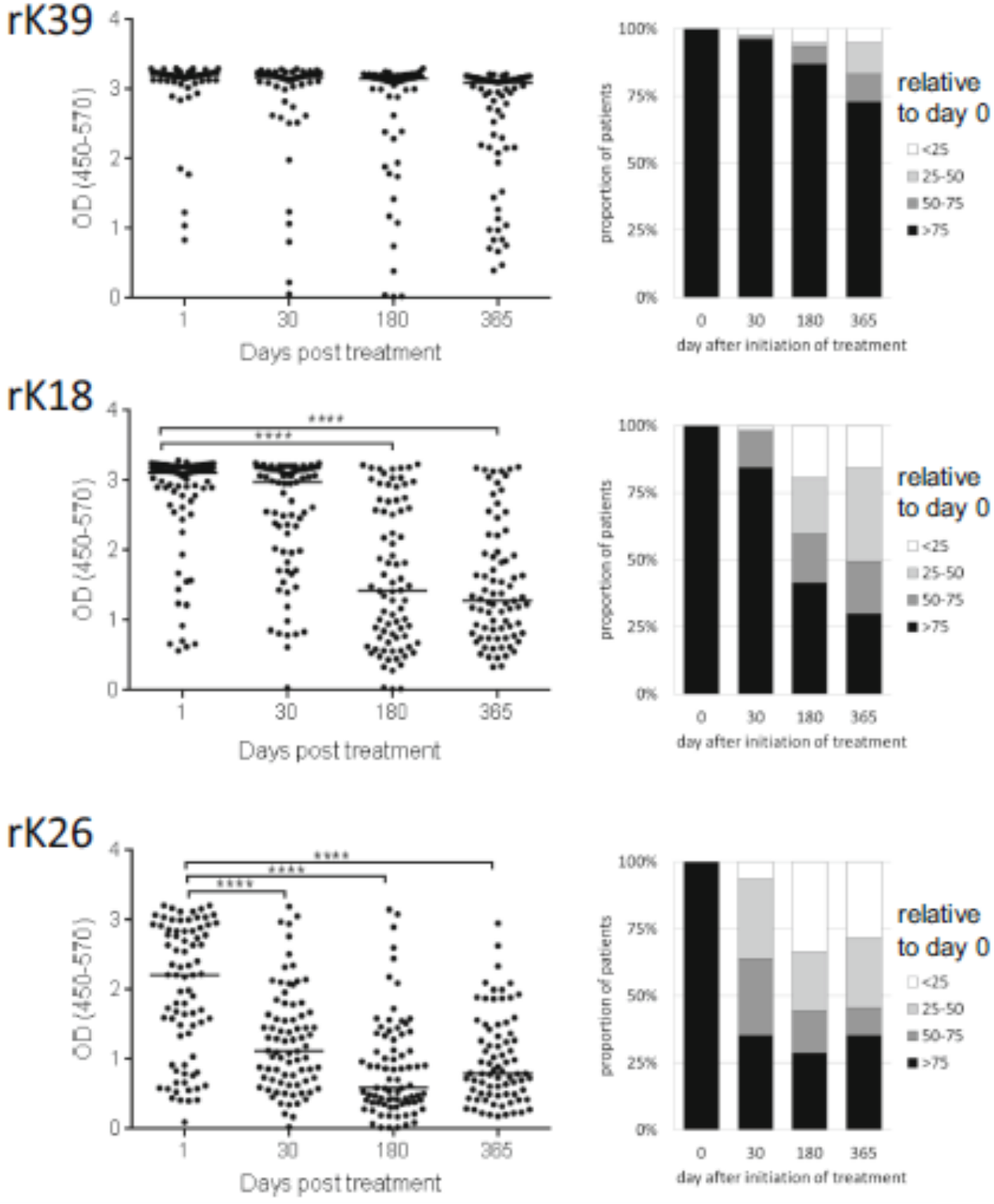
ARTICLE

Specific antibody responses as indicators of treatment efficacy for visceral leishmaniasis

A. C. Vallur · A. Hailu · D. Mondal · C. Reinhart · H. Wondimu · Y. Tutterrow · H. W. Ghalib · S. G. Reed · M. S. Duthie

Abstract Acute visceral leishmaniasis (VL) is caused by infection with parasites of the *Leishmania donovani* complex and may be fatal if not treated. Early diagnosis and efficacious treatment are the keys to effective VL management and control. Novel regimens are being developed to overcome limitations in VL treatment options, which are currently restricted by high costs, severe systemic side effects, and unresponsiveness. Although simple and accurate serological tests are available to help confirm VL, none are suitable to monitor treatment efficacy and cure. Here, we confirm that serum antibody responses to the diagnostic antigens rK39 and rK28 are unaltered by treatment, but demonstrate that antibodies produced against two antigens, rK26 and rK18, can be used as an indirect measure of parasite clearance. The levels of anti-rK18 and -rK26 antibodies were high in patients at initial diagnosis but declined in patients treated with either SSG (Ethiopia) or AmBisome™ (Bangladesh). Taken together, we propose that serological tests which measure antibodies to rK26 and rK18 merit consideration as potential markers of treatment success and cure.

Fig. 2 Antigen-specific antibody responses of Bangladeshi VL patients. Antigen-specific IgG binding of matched sera from before and after the initiation of SDA treatment of Bangladeshi VL patients was measured by ELISA against rK39, rK18, and rK26. In the *left panels*, each point represents the OD of an individual serum and the median is represented by the *horizontal line*. ****$p < 0.001$ versus day 0 samples, as measured by paired one-way ANOVA. In the *right panels*, to objectively contrast the changes in each antigen-specific response, we normalized against the intake OD (day 0) and then expressed results as the proportion of patients demonstrating responses that were <25 %, 25–50 %, 50–75 %, or >75 % at each time point relative to day 0 (initiation of treatment)



PLOS | NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

The Severity of Visceral Leishmaniasis Correlates with Elevated Levels of Serum IL-6, IL-27 and sCD14

Priscila L. dos Santos[1], Fabrícia A. de Oliveira[1], Micheli Luize B. Santos[1], Luana Celina S. Cunha[1], Michelle T. B. Lino[1], Michelle F. S. de Oliveira[1], Manuela O. M. Bomfim[1], Angela Maria Silva[1], Tatiana R. de Moura[1], Amélia R. de Jesus[1,2,3], Malcolm S. Duthie[4], Steven G. Reed[4], Roque P. de Almeida[1,2,3]*

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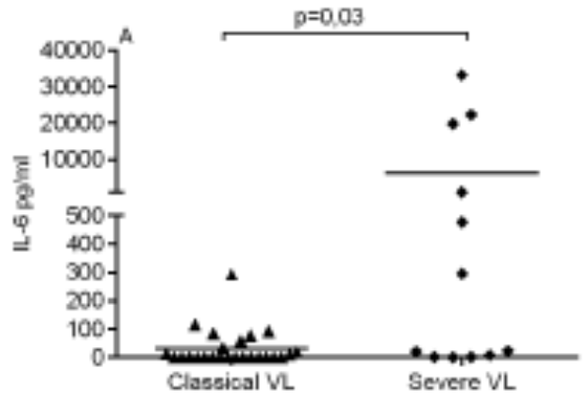

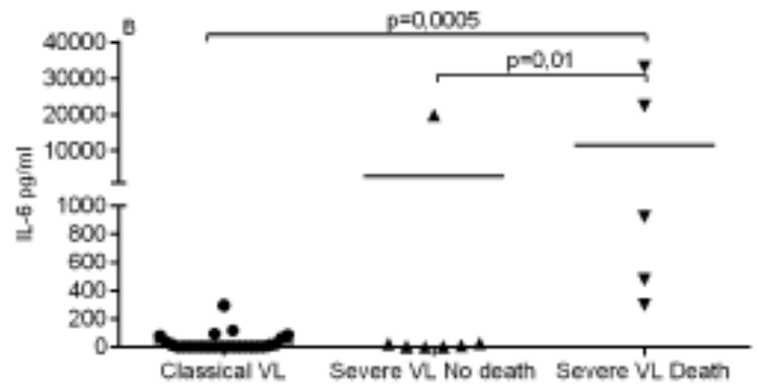

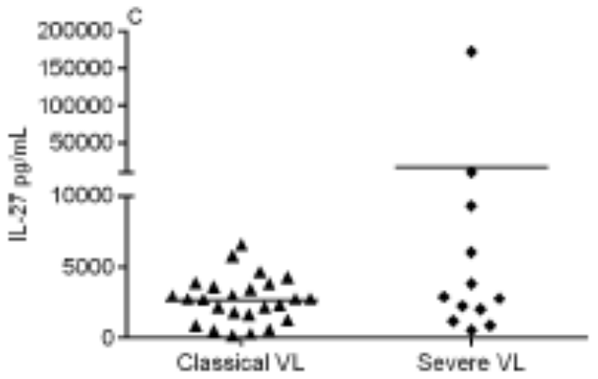

Fig 3. High IL-6 levels in serum is associated with disease severity. Serum levels of IL-6 and IL-27 measured before treatment by Luminex assay were compared in VL patients. (A) IL-6 levels in patients with classical VL (n = 25) and SVL (n = 12). (B) IL-6 levels in patients with classical VL (n = 25) and SVL that survived (n = 7) and SVL that died (n = 5). (C) IL-27 levels in patients with classical VL (n = 25) and SVL (n = 12).

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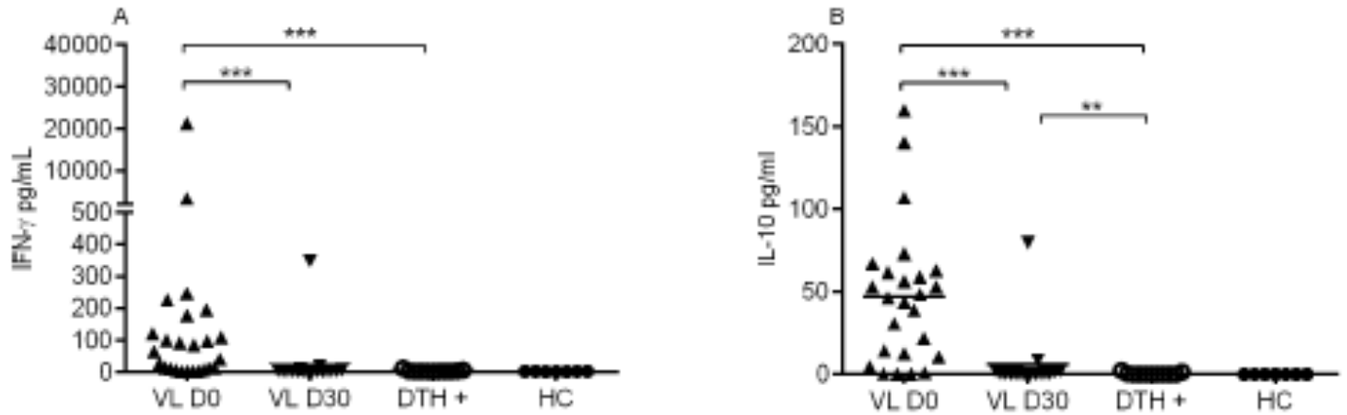

Fig 1. Serum levels of cytokines. Cytokines were measured by Luminex assay in sera of VL patients before (n = 25) and after treatment (n = 17) (D0 and D30, respectively), DTH+ (n = 11) and healthy control (n = 7). (A) IFN-γ (B) IL-10, (C) IL-6, (D) IL-27 and (E) TNF-α.

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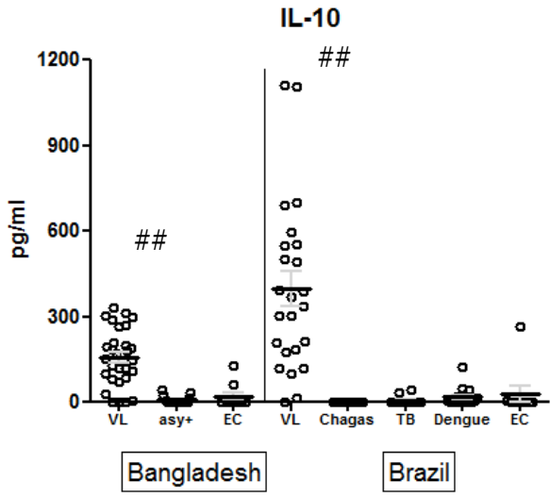
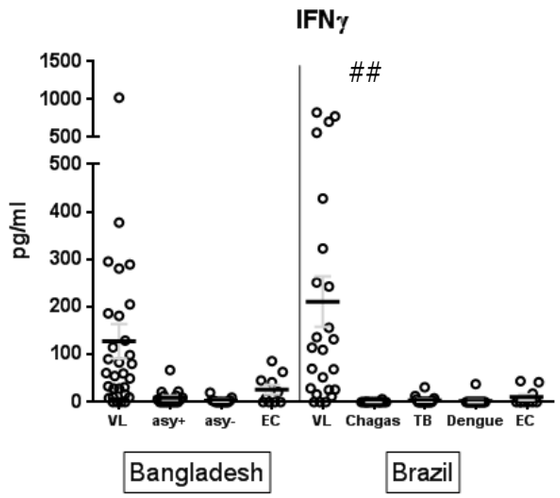
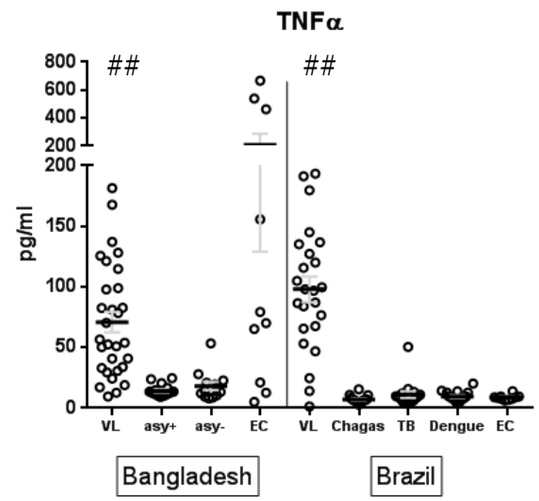
ARTICLE

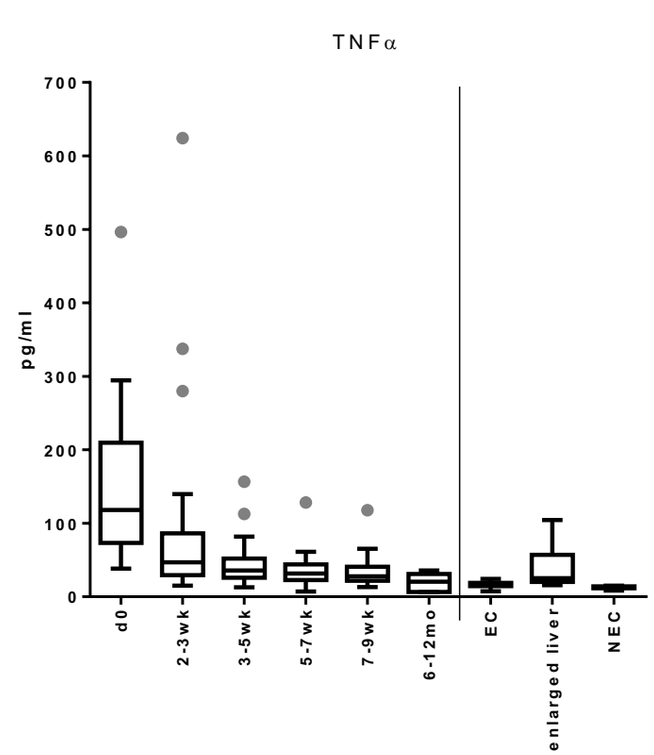
Alteration of the serum biomarker profiles of visceral leishmaniasis during treatment

M. S. Duthie · J. Guderian · A. Vallur · A. Bhatia · P. Lima dos Santos · E. Vieira de Melo · A. Ribeiro de Jesus · M. Todt · D. Mondal R. Almeida · S. G. Reed









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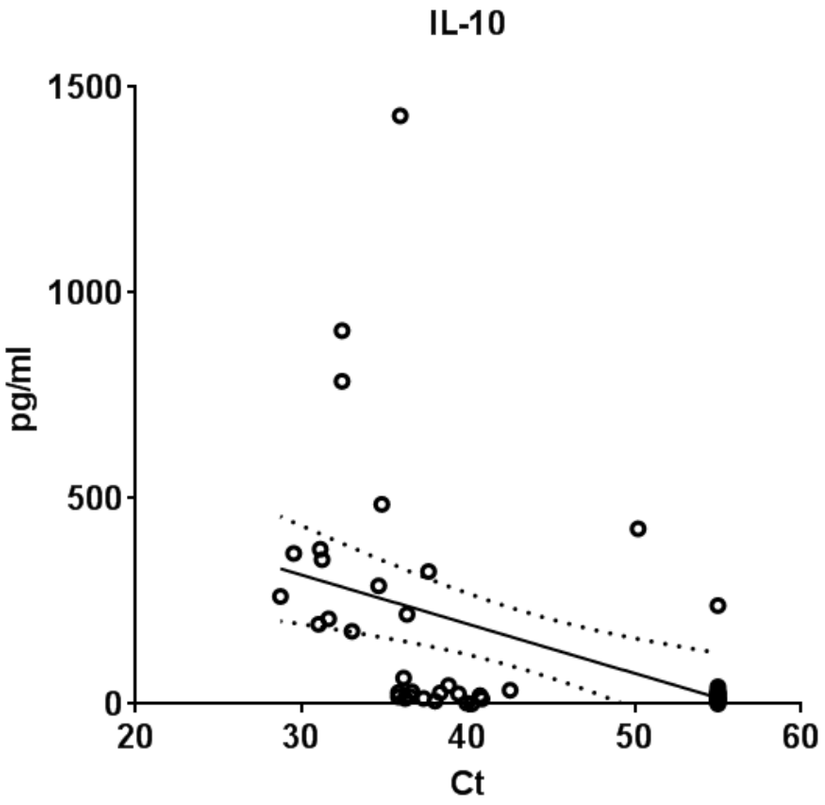

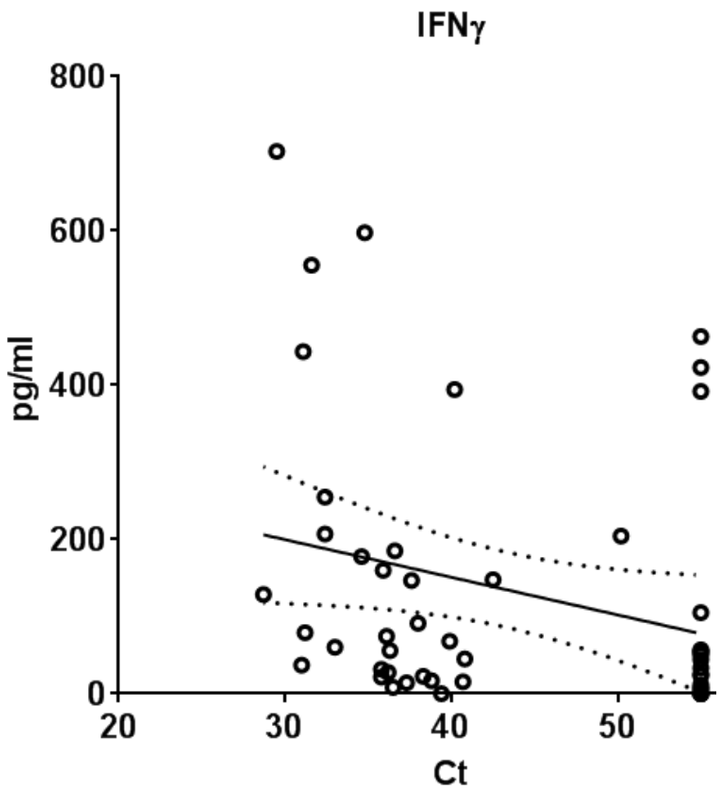

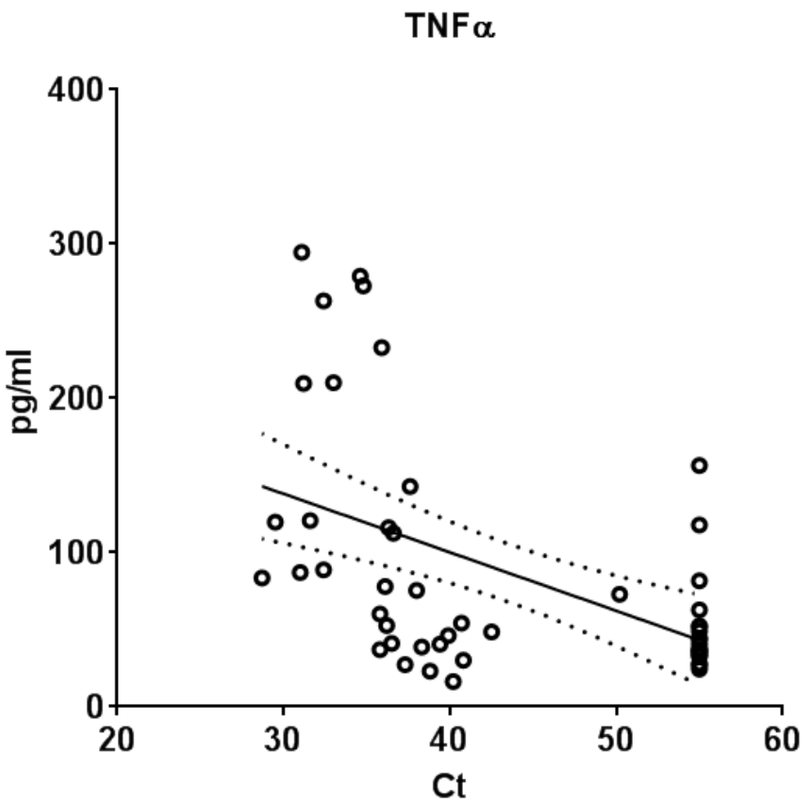

Vallur et al. BMC Infectious Diseases (2015) 15:384
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BMC Infectious Diseases

RESEARCH ARTICLE Open Access

Development and comparative evaluation of two antigen detection tests for Visceral Leishmaniasis

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Abstract

Background: Visceral leishmaniasis (VL) can be fatal without timely diagnosis and treatment. Treatment efficacies vary due to drug resistance, drug toxicity and co-morbidities. It is important to monitor treatment responsiveness to confirm cure and curtail relapse. Currently, microscopy of spleen, bone marrow or lymph node biopsies is the only definitive method to evaluate cure. A less invasive test for treatment success is a high priority for VL management.

Methods: In this study, we describe the development of a capture ELISA based on detecting *Leishmania donovani* antigens in urine samples and comparison with the *Leishmania* Antigen ELISA, also developed for the same purpose. Both were developed as prototype kits and tested on patient urine samples from Sudan, Ethiopia, Bangladesh and Brazil, along with appropriate control samples from endemic and non-endemic regions. Sensitivity and specificity were assessed based on accurate detection of patients compared to control samples. One- Way ANOVA was used to assess the discrimination capacity of the tests and Cohen's kappa was used to assess their correlation.

Results: The *Leishmania* Antigen Detect™ ELISA demonstrated >90 % sensitivity on VL patient samples from Sudan, Bangladesh and Ethiopia and 88 % on samples from Brazil. The *Leishmania* Antigen ELISA was comparable in performance except for lower sensitivity on Sudanese samples. Both were highly specific. To confirm utility in monitoring treatment, urine samples were collected from VL patients at days 0, 30 and 180 post- treatment. For the *Leishmania* Antigen Detect™ ELISA, positivity was high at day 0 at 95 %, falling to 21 % at day 30. At day 180, all samples were negative, corresponding well with clinical cure. A similar trend was also seen for the *Leishmania* Antigen ELISA albeit; with lower positivity of 91 % at Day 0 and more patients, remaining positive at Days 30 and 180.

Discussion: The *Leishmania* Antigen Detect™ and the *Leishmania* Antigen ELISAs are standardized, user- friendly, quantitative and direct tests to detect *Leishmania* during acute VL as well as to monitor parasite clearance during treatment. They are a clear improvement over existing options.

Conclusion: The ELISAs provide a non-invasive method to detect parasite antigens during acute infection and monitor its clearance upon cure, filling an unmet need in VL management. Further refinement of the tests with more samples from endemic regions will define their utility in monitoring treatment.

Keywords: Diagnosis, *Leishmania*, Antigen, Treatment, Antibody, Kala azar, Infection

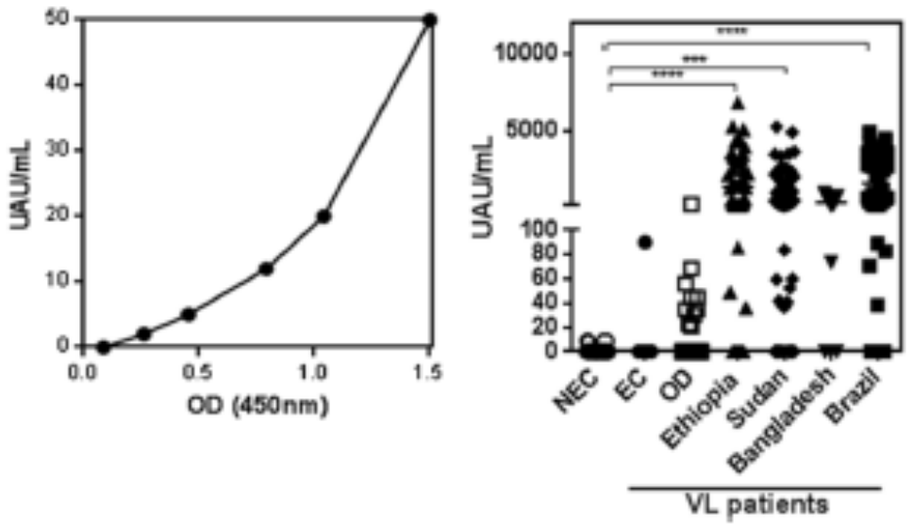

Fig. 3 Performance of *Leishmania* Antigen ELISA. **a**. Standard curve representing means of the signals obtained in the Leishmania Antigen ELISA using the calibrated standards provided with the kit. **b**. Performance of *Leishmania* Antigen ELISA on urine samples from VL patients in Ethiopia ($n = 46$), Sudan ($n = 64$), Bangladesh ($n = 13$) and Brazil ($n = 43$) compared to NEC ($n = 49$), EC from Bangladesh ($n = 10$) and OD ($n = 30$). OD consists of samples from patients with HAT ($n = 10$), *P. falciparum* malaria ($n = 10$) and tuberculosis ($n = 10$) in UAU/mL. Lines represent median

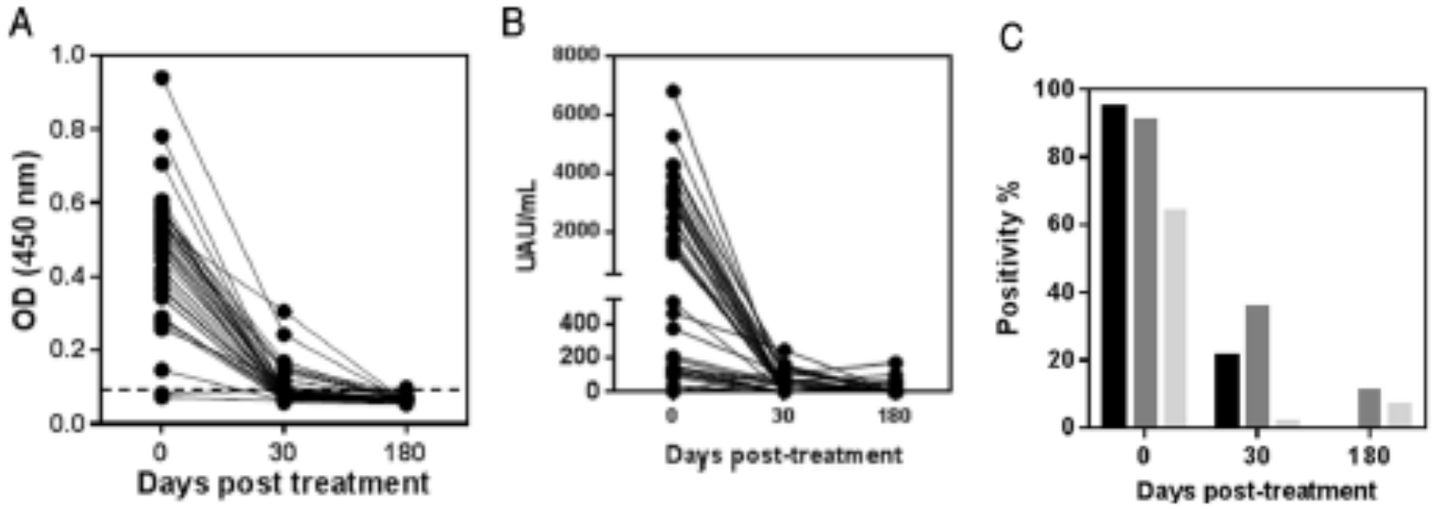

Fig. 4 Performance of *Leishmania* Antigen Detect™ ELISA and *Leishmania* Antigen ELISA on treatment follow- up urine samples from Ethiopia. **a**. Mean ELISA signals of 42 VL patients undergoing SSG, MEG or L-AmB treatment in Ethiopia at initiation (Day 0) and post initiation (Days 30 and 180) of treatment as measured by the *Leishmania* Antigen Detect™ ELISA. Dotted line indicates cut-off value for positivity as calculated from the mean of negative controls added 3 standard deviations. **b**. UAU/mL for 42 VL patients undergoing SSG, MEG or L-AmB treatment in Ethiopia at initiation (Day 0) and post initiation (Days 30 and 180) of treatment as measured by the *Leishmania* Antigen ELISA **c**. Positivity percentage of the *Leishmania* Antigen Detect™ ELISA (Black bars) and *Leishmania* Antigen ELISA (dark gray bars) compared to KAtex (light gray bars) on VL patient urine samples from Ethiopia at initiation (Day 0) and post initiation (Days 30 and 180) of treatment

Elimination challenges

Disease →

Cure/ Complication/Relapse

Infection →

Evaluation *en masse*

(practical, objective)

Intervention

(short-term, repetitive *versus* long-term, sustainable)

AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology

Accurate Serodetection of Asymptomatic *Leishmania donovani* Infection by Use of Defined Antigens

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Infection with *Leishmania donovani* is typically asymptomatic, but a significant number of individuals may progress to visceral leishmaniasis (VL), a deadly disease that threatens 200 million people in areas where it is endemic. While diagnosis of acute VL has been simplified by the use of cost-effective confirmatory serological tests, similar standardized tools are not widely available for detecting asymptomatic infection, which can be 4 to 20 times more prevalent than active disease. A simple and accurate serological test that is capable of detecting asymptomatic *L. donovani* infection will be useful for surveillance programs targeting VL control and elimination. To address this unmet need, we evaluated recombinant antigens for their ability to detect serum antibodies in 104 asymptomatic *L. donovani*-infected individuals (qualified as positive for *L. donovani*-specific antibodies by direct agglutination test [DAT]) from the Mymensingh district of Bangladesh where VL is hyperendemic. The novel proteins rKR95 and rTR18 possessed the greatest potential and detected 69% of DAT-positive individuals, with rKR95 being more robust in reactivity. Agreement in results for individuals with high DAT responses, who are more likely to progress to VL disease, was 74%. When considered along with rK39, a gold standard antigen that is used to confirm clinical diagnosis of VL but that is now becoming widely used for surveillance, rKR95 and rTR18 conferred a sensitivity of 84% based on a theoretical combined estimate. Our data indicate that incorporating rKR95 and rTR18 with rK39 in serological tests amenable to rapid or high-throughput screening may enable simple and accurate detection of asymptomatic infection. Such tests will be important tools to measure *L. donovani* infection rates, a primary goal in surveillance and a critical measurement with which to assess elimination programs.

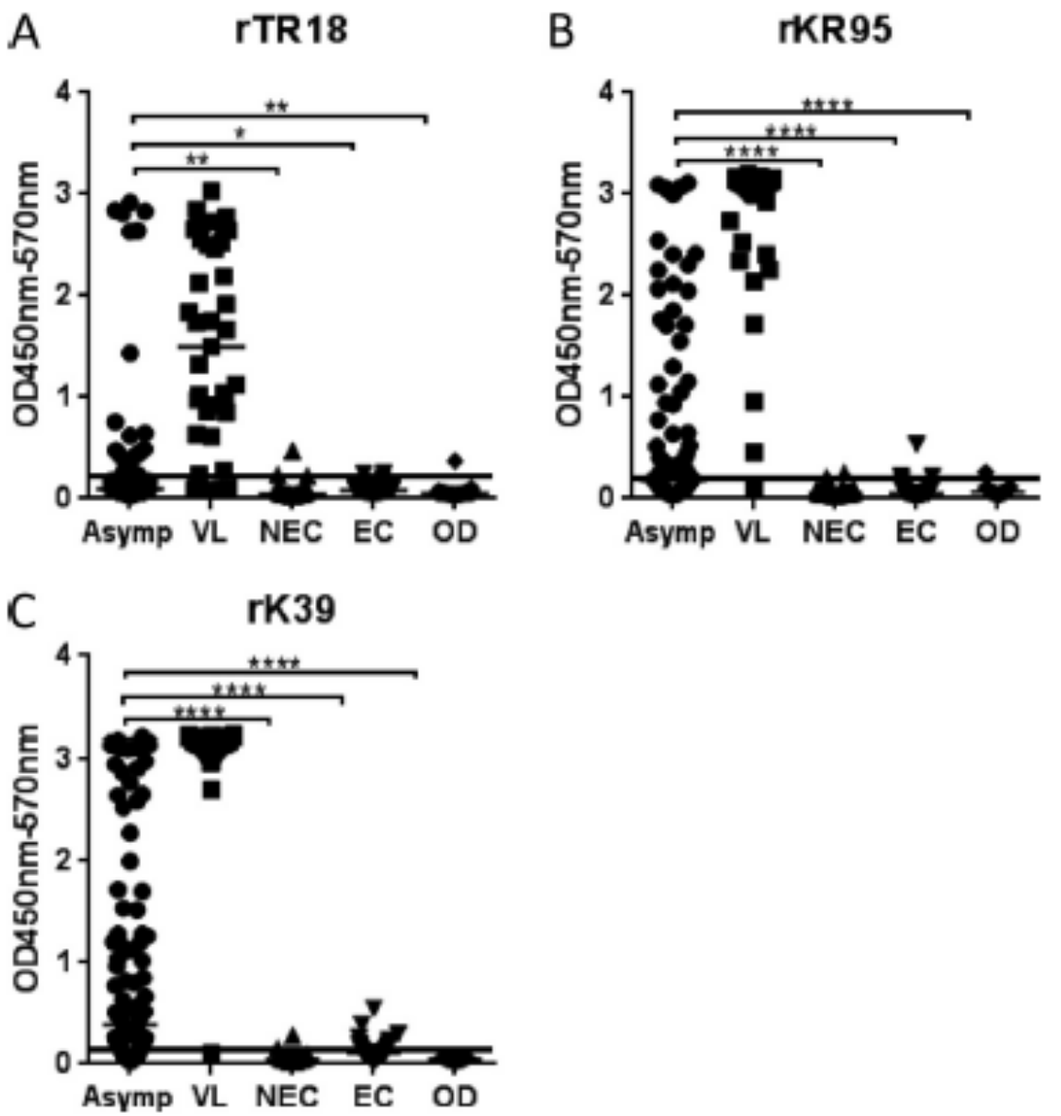
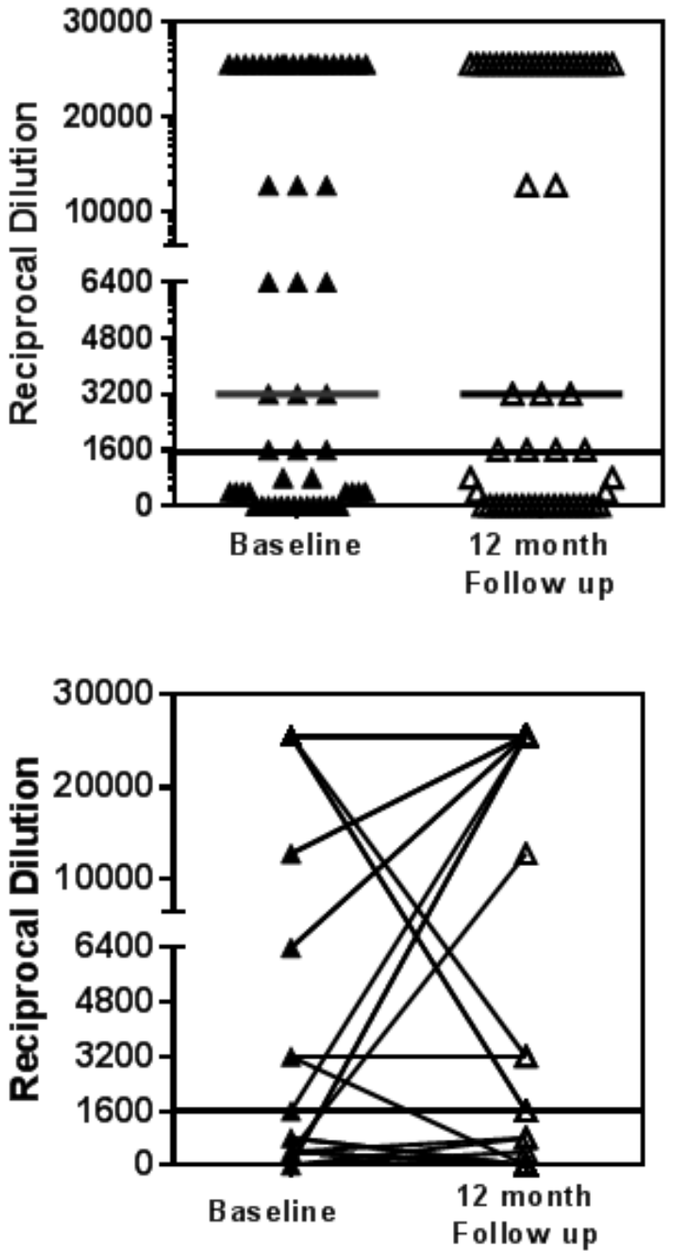


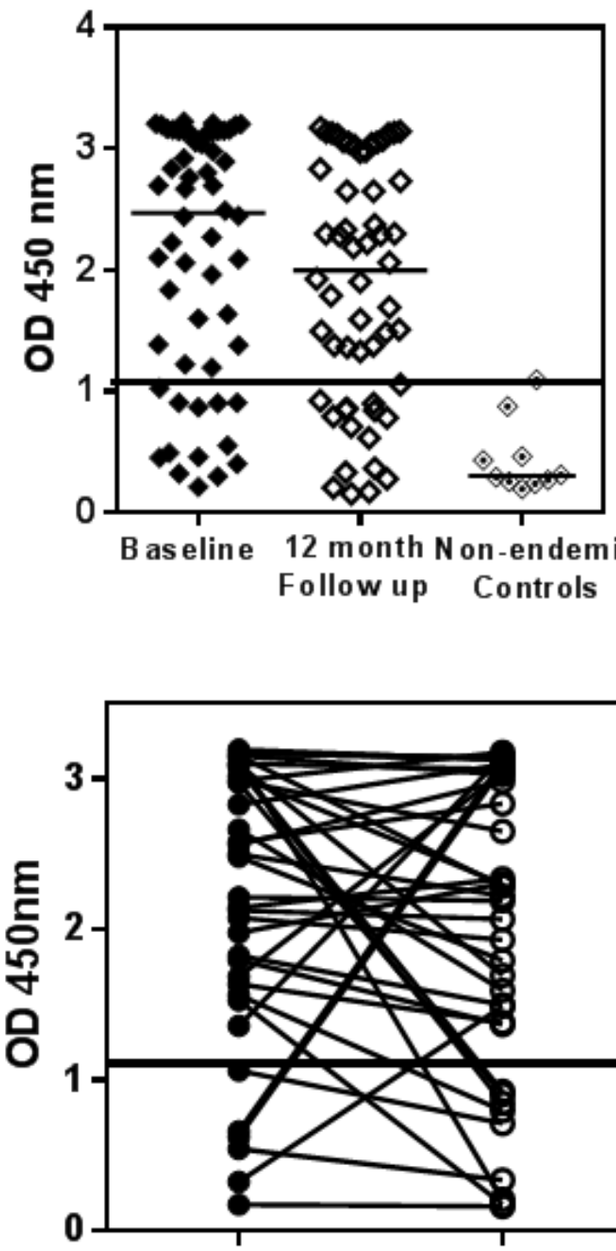
FIG 1 Assessment of the ability of a serological test comprising recombinant proteins to detect antibodies in the serum of asymptomatic infected individuals. Asymptomatic infected individuals were defined as DAT positive, and serum antibodies against the indicated antigens were measured by ELISA. The optical density of each serum sample from asymptomatic individuals (Asymp) (circles), VL patients (VL) (squares), healthy nonendemic controls (NEC) (triangles), DAT-negative healthy endemic controls (EC) (inverted triangles), and controls with other diseases (OD) (diamonds) is plotted. Median optical density is indicated for each group (black bars). The black line intersecting each plot identifies the cutoff above which samples were considered positive. ****, *P* value of <0.0001; * and **, *P* value of <0.05 and <0.01, respectively, as measured by one way ANOVA.

Asymptomatic infected individuals

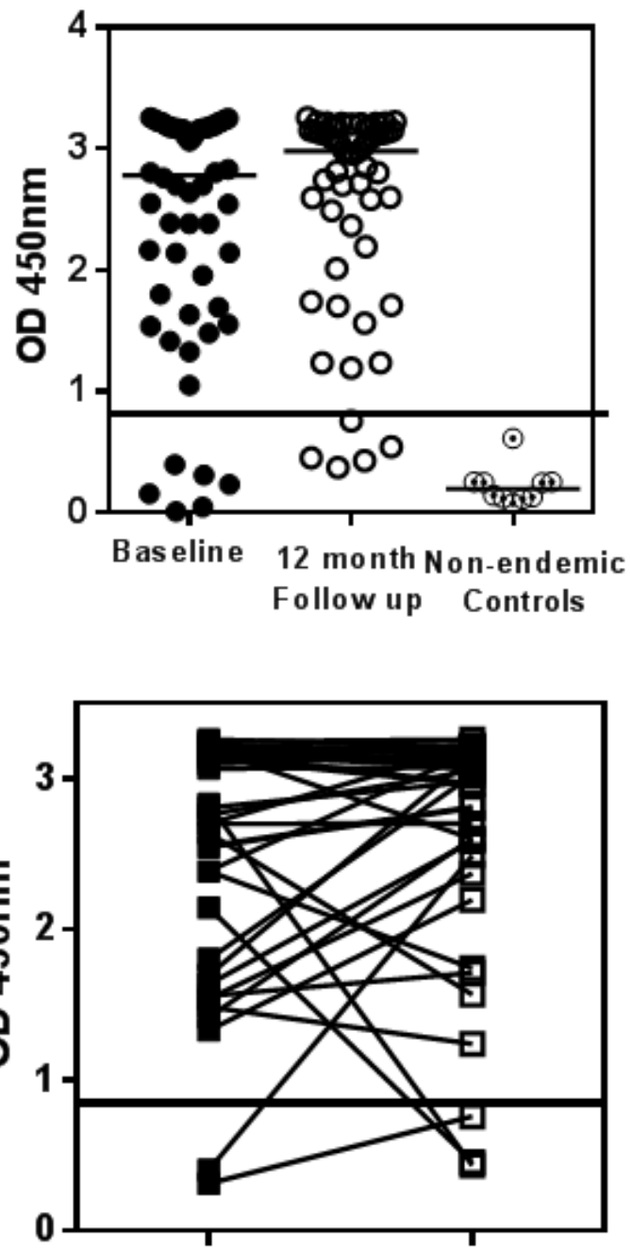
Direct Agglutination Test (DAT)



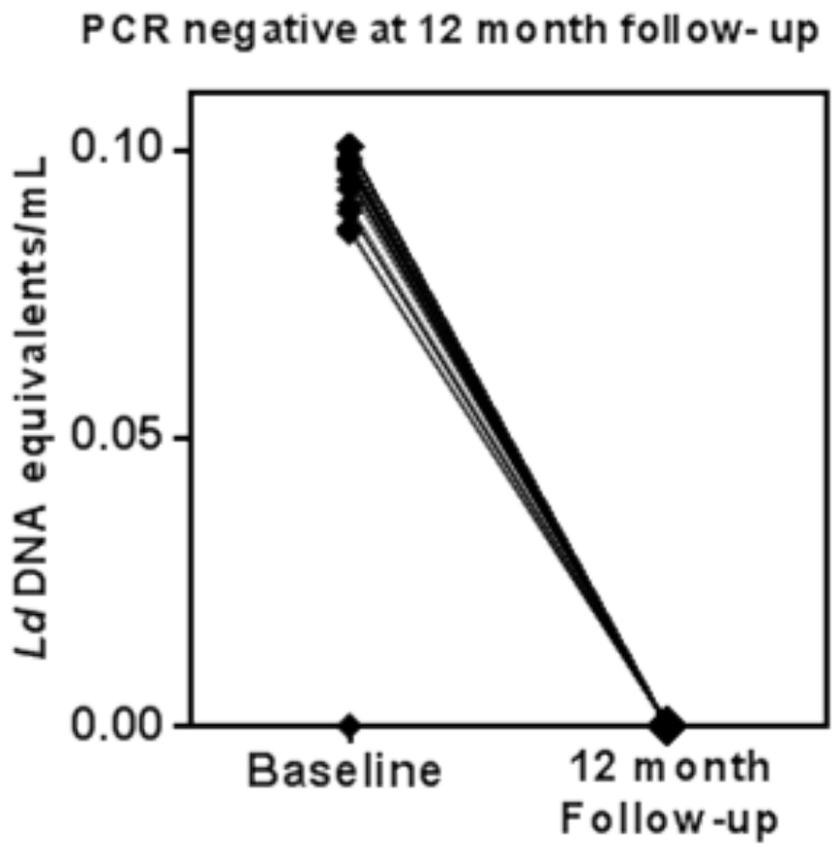
Lysate ELISA

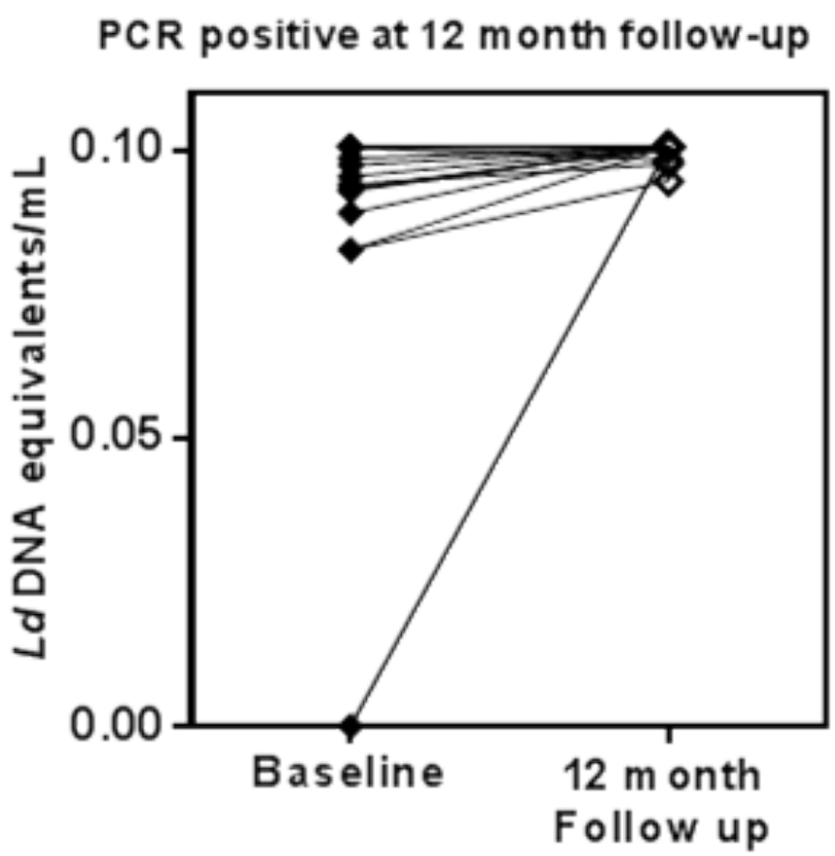


rK39 ELISA



Asymptomatic infected individuals





Progress to clinical study (F3+GLA-SE)

OPEN

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ORIGINAL ARTICLE

From mouse to man: safety, immunogenicity and efficacy of a candidate leishmaniasis vaccine LEISH-F3+GLA-SE

Rhea N Coler[1], Malcolm S Duthie[1], Kimberly A Hofmeyer[1], Jeffery Guderian[1], Lakshmi Jayashankar[1], Julie Vergara[1], Tom Rolf[1], Ayesha Misquith[1], John D Laurance[1], Vanitha S Raman[1], H. Remy Bailor[1], Natasha Dubois Cauwelaert[1], Steven J Reed[1], Aarthy Vallur[1], Michelle Favila[1], Mark T Orr[1], Jill Ashman[1], Prakash Ghosh[2], Dinesh Mondal[2] and Steven G Reed[1]

Key antigens of *Leishmania* species identified in the context of host responses in *Leishmania*-exposed individuals from disease-endemic areas were prioritized for the development of a subunit vaccine against visceral leishmaniasis (VL), the most deadly form of leishmaniasis. Two *Leishmania* proteins—nucleoside hydrolase and a sterol 24-c-methyltransferase, each of which are protective in animal models of VL when properly adjuvanted— were produced as a single recombinant fusion protein NS (LEISH-F3) for ease of antigen production and broad coverage of a heterogeneous major histocompatibility complex population. When formulated with glucopyranosyl lipid A-stable oil-in-water nanoemulsion (GLA-SE), a Toll-like receptor 4 T_H1 (T helper 1) promoting nanoemulsion adjuvant, the LEISH-F3 polyprotein induced potent protection against both *L. donovani* and *L. infantum* in mice, measured as significant reductions in liver parasite burdens. A robust immune response to each component of the vaccine with polyfunctional CD4 T_H1 cell responses characterized by production of antigen-specific interferon-γ, tumor necrosis factor and interleukin-2 (IL-2), and low levels of IL-5 and IL-10 was induced in immunized mice. We also demonstrate that CD4 T cells, but not CD8 T cells, are sufficient for protection against *L. donovani* infection in immunized mice. Based on the sum of preclinical data, we prepared GMP materials and performed a phase 1 clinical study with LEISH-F3+GLA-SE in healthy, uninfected adults in the United States. The vaccine candidate was shown to be safe and induced a strong antigen-specific immune response, as evidenced by cytokine and immunoglobulin subclass data. These data provide a strong rationale for additional trials in *Leishmania*-endemic countries in populations vulnerable to VL.
Clinical & Translational Immunology (2015) **4**, e00; doi:10.1038/cti.2015.6; published online xx xxx 2015

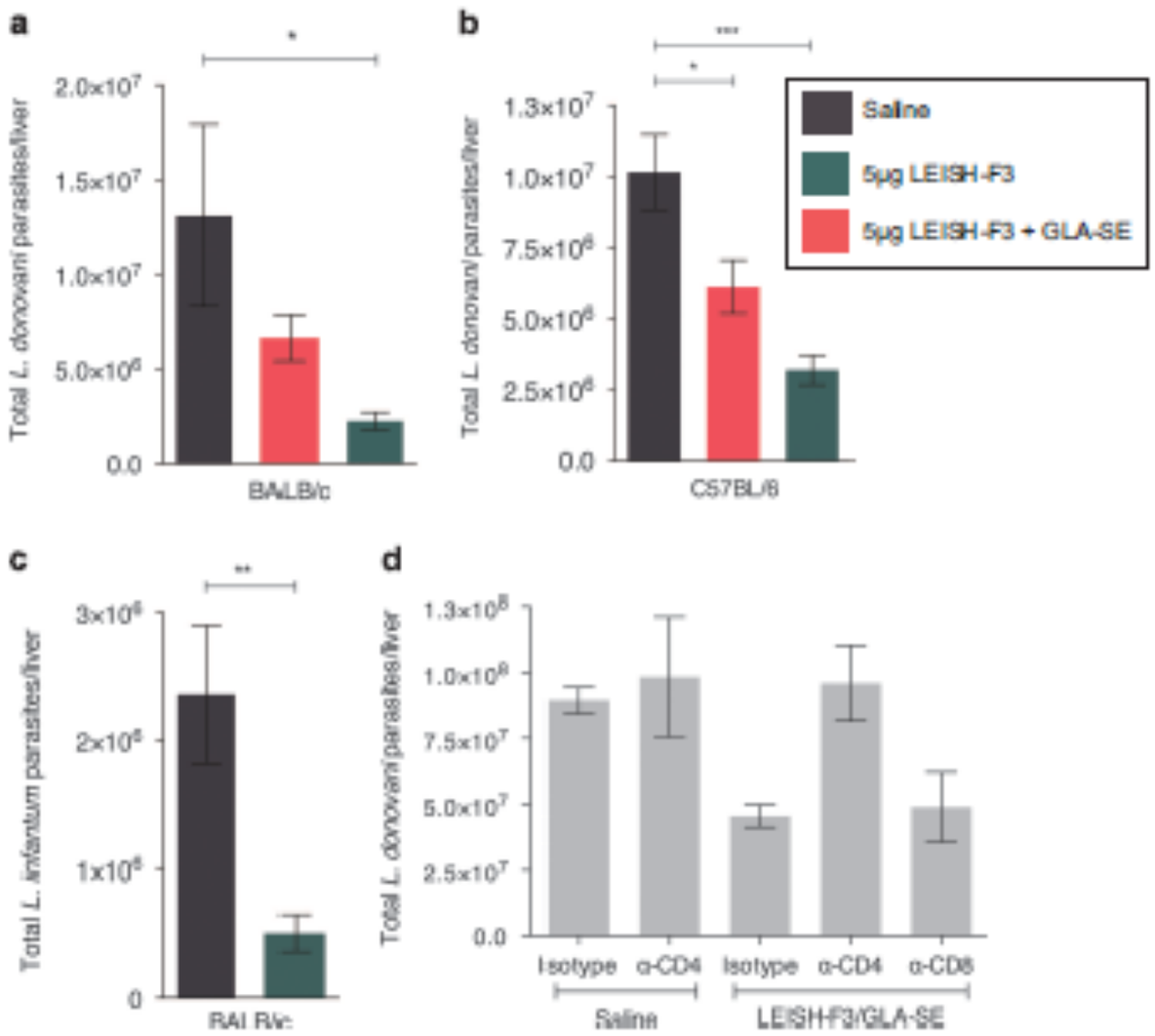


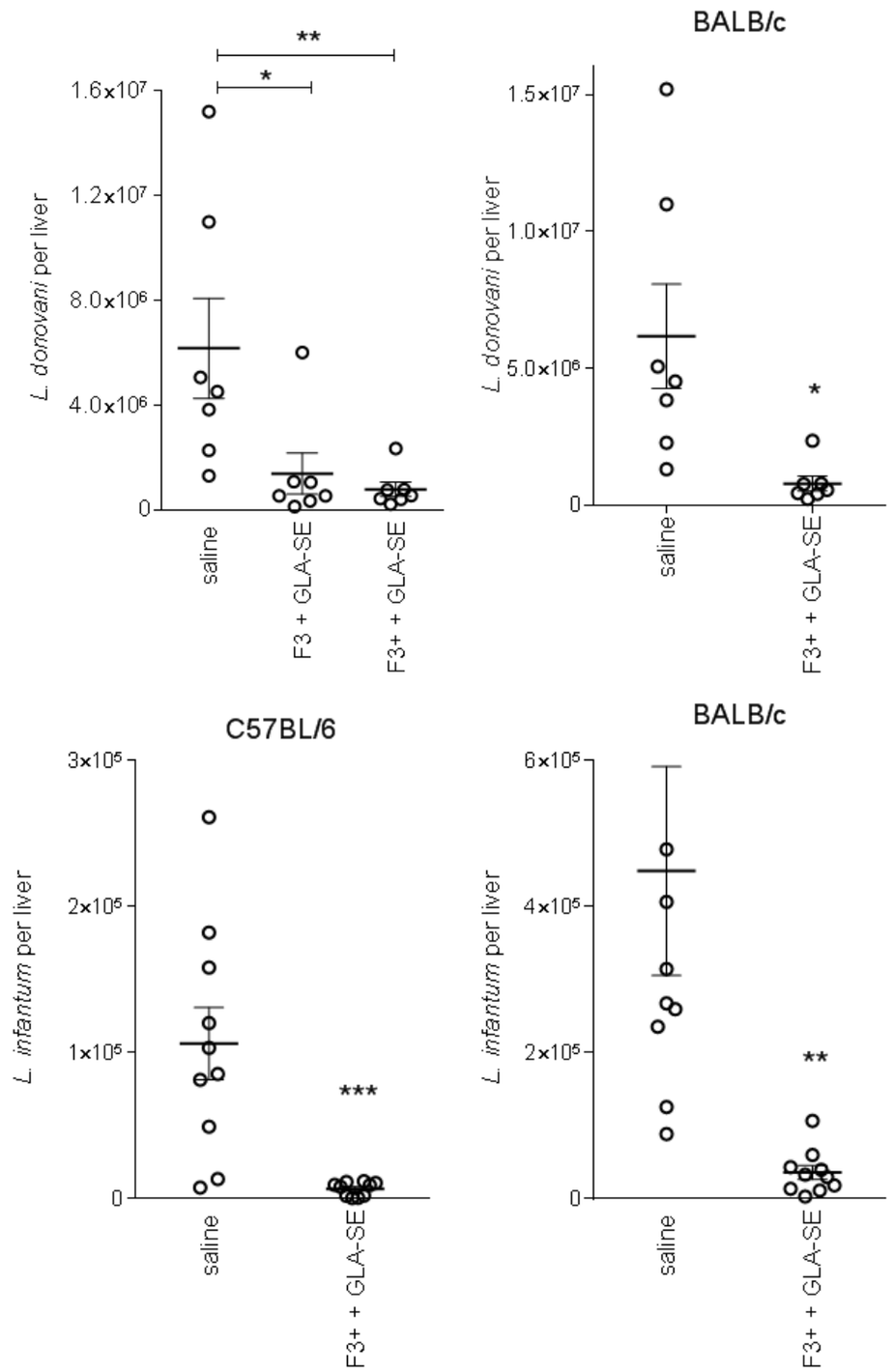
Figure 4 CD4 memory T cells generated by vaccination with LEISH-F3+GLA-SE protect against experimental VL infection. (**a** and **c**) BALB/c and (**b** and **d**) C57BL/6 mice were immunized with the indicated vaccines three times, at 3-week intervals. (**a**–**c**) Four weeks after the final immunization, mice were infected with (**a** and **b**) 1×10^6 *L. donovani* or (**c**) 5×10^6 *L. infantum* parasites. (**d**) Three weeks after the final immunization, mice received three intraperitoneal injections, on 3 consecutive days, of 0.5 mg of the indicated depleting antibody; 3 weeks later, mice were infected with 1×10^6 *L. donovani* parasites intravenously. (**a**–**d**) Livers were harvested 3 weeks after infection and analyzed by reverse transcription-PCR (RT-PCR) to determine the total parasite burden. (**a** and **b**) Statistics by Tukey's multiple comparison test. $^*P<0.05$ and $^{***}P<0.005$. (**c** and **d**) Statistics by unpaired *t*-test between indicated groups. $^*P<0.05$, $^{**}P<0.01$ and $^{****}P<0.001$.

Transition to F3+ antigen

Immunization with NSΔC (F3+) reduces *Leishmania* infection. Mice were subcutaneously injected a total of 3 times with 5ug protein formulated with GLA-SE, then one month after the final immunization were infected by intravenous injection of *Leishmania* promastigotes. Livers were removed one month after inoculation and parasite burdens were determined by qPCR. In (A) and (B), C57BL/6 and BALB/c mice, respectively, were infected with *L. donovani*. In the lower panels, C57BL/6 and BALB/c mice were infected with *L. infantum*. Each point represents the burden of each individual mouse, with the bars indicating the mean and SE for each group with 7-10 mice per group. Data are representative of results obtained with each protein in 2-3 independent experiments.

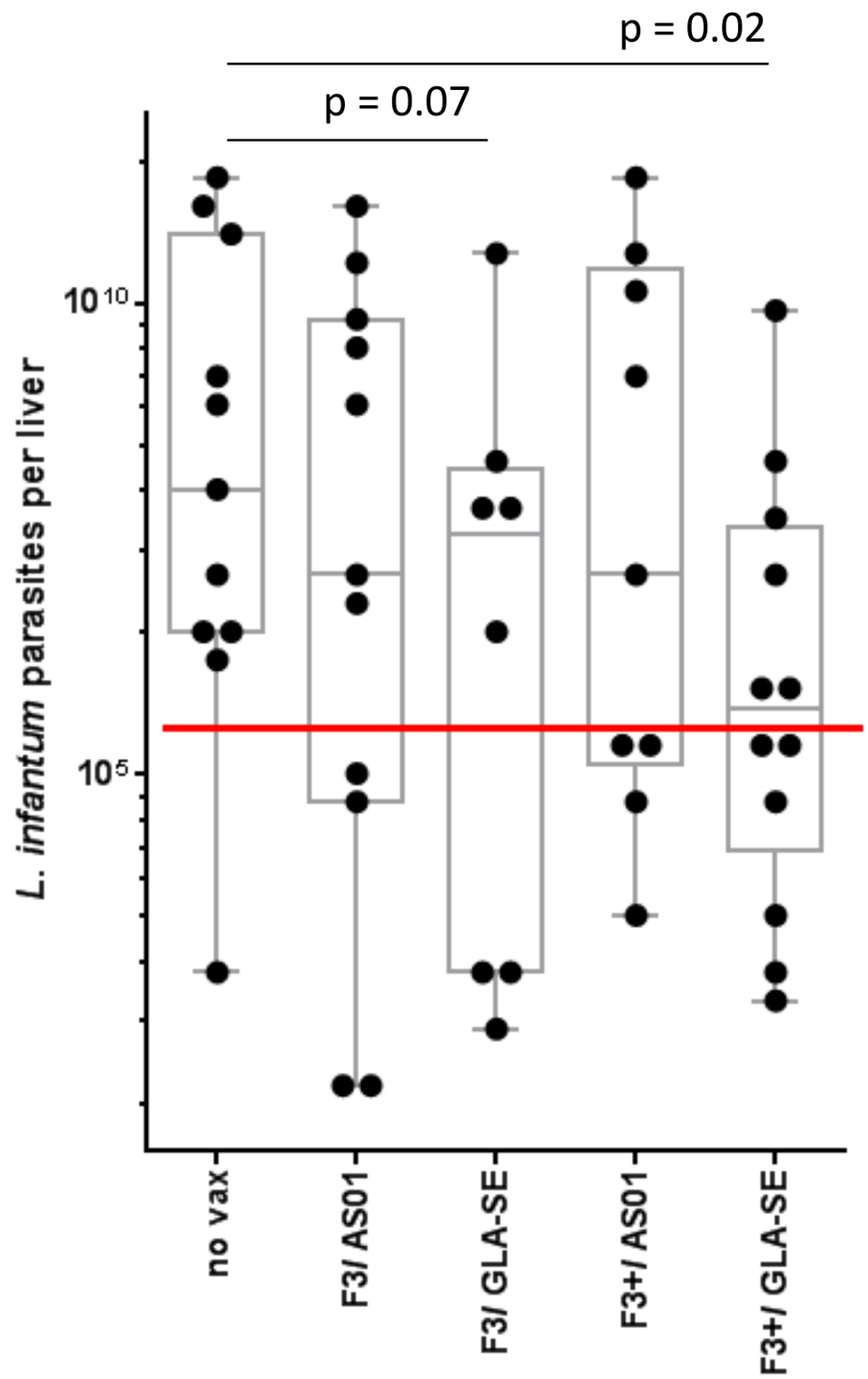
CONCLUSION –

F3+ candidate is highly and robustly protective against *Leishmania* infection



Challenge by sand fly

Immunization with NSΔC reduces parasite burden in hamsters infected with *L. infantum* during sandfly bloodmeals.



CONCLUSIONS –

a. F3+ candidate provides more robust, statistically significant reduction in parasite burden.

b. GLA-SE adjuvanted F3+ promotes protective response.

Elimination challenges

rK39 RDT →

(biomarkers/ Ag/ Ag/ PCR)

Ab/ Ag/ PCR →

Screening – high-throughput lab based/ portable, field-applicable

Intervention – IRS, vaccine

BILL & MELINDA GATES foundation

National Institute of Allergy and Infectious Diseases

IDRI

INFECTIOUS DISEASE RESEARCH INSTITUTE