CHALLENGES FOR THE DIAGNOSIS OF VISCERAL LEISHMANIASIS IN THE KALA-AZAR ELIMINATION PROGRAMME IN THE INDIAN SUB-CONTINENT

IMMUNICON 2016

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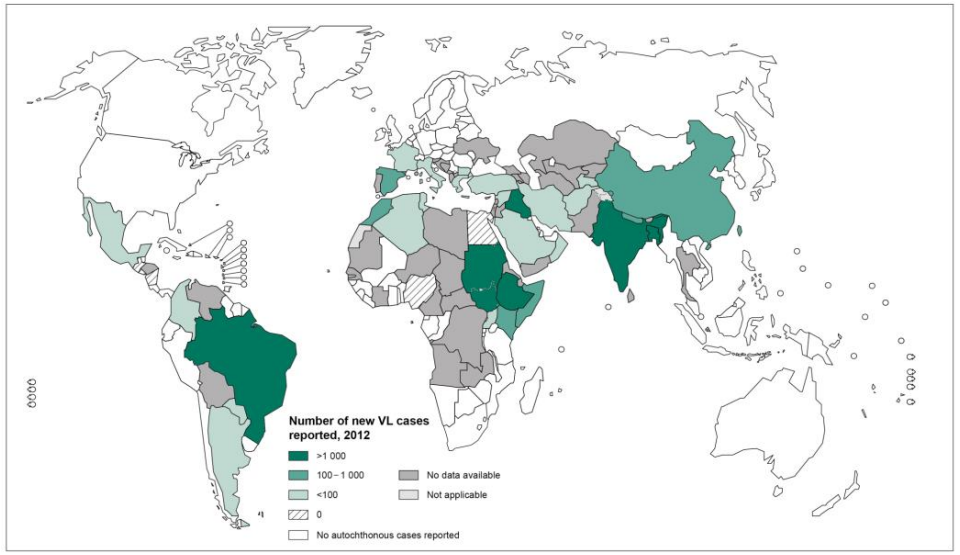
Dr Suman Rijal, Director, DNDi India

Leishmaniasis Global Situation

Visceral leishmaniasis (VL)

- The Indian subcontinent, Brazil & East Africa-highly endemic.
- Over 90% of new cases occur in six countries:
  - **Bangladesh, Brazil, Ethiopia, India, South Sudan, Sudan.**
- *Current estimate* - 200 000 to 400 000 new cases worldwide each year.

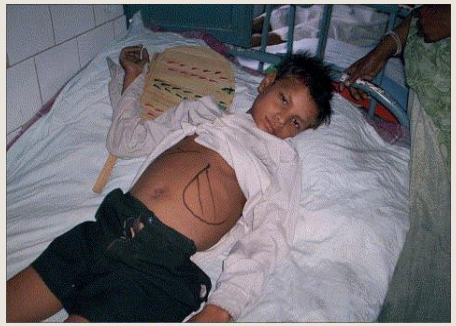
Status of endemicity of visceral leishmaniasis, worldwide, 2012



Data Source: World Health Organization
Map Production: Control of Neglected Tropical Diseases (NTD) World Health Organization

World Health Organization

Region	Estimated annual VL incidence
Americas	4,500 to 6,800
Sub-Saharan Africa	
East Africa	29,400 to 56,700
Mediteranean	1200 to 2000
Middle east to central Asia	5000 to 10,000
South Asia	162,100 to 313,600
Global Total	**202,200 to 389,100**



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Kala-azar: Background

- Disease of "Poorest of the poor"
- Early and accurate diagnosis crucial
  - Fatal disease if untreated
  - VL Drugs toxic
  - Reduce transmission of infections.
- Diagnostic tools should be ASSURED (Affordable, Sensitive, Specific, User Friendly, Rapid, Equipment free and Deliverable)
- Aim of target product profile (Boelaert et al 2007)

  - Sensitivity > 95%
  - Specificity > 90%

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Diagnostic tests in VL

- **Parasitology:**
  Microscopy, Culture
  Molecular diagnosis: PCR, LAMP

- **Serological:**
  Immuno florescence antibody test (IFAT)
  ELISA: rk 39
  Direct agglutination test (DAT)
  Immunochromatographic strip (ICT): rk39, rkE 16.

- **Antigen detection**
  Urine antigen detection agglutination test (KAtex)

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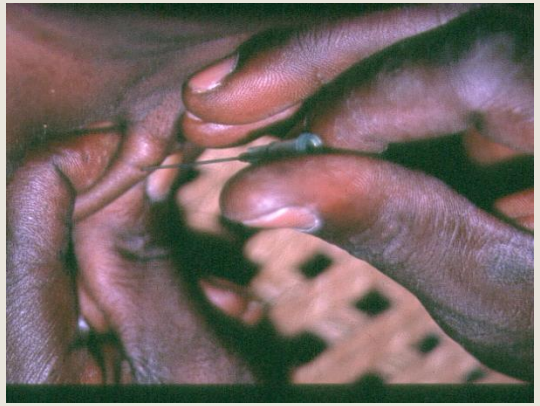
Microscopy

Lymph node

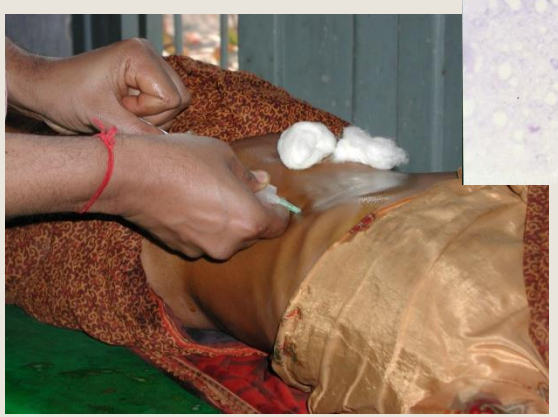


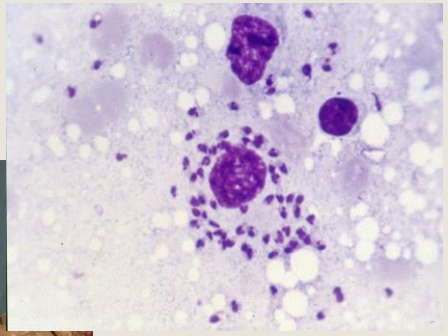
- Low sensitivity (53-65%)

Bone marrow

- Low sensitivity (53-86%)
- Painful
- Sterilisation!

Spleen





- Gold standard (93-99%)
- Needs expertise
  - Procedure
  - Reading
- Risk of bleeding

Not feasible for field use

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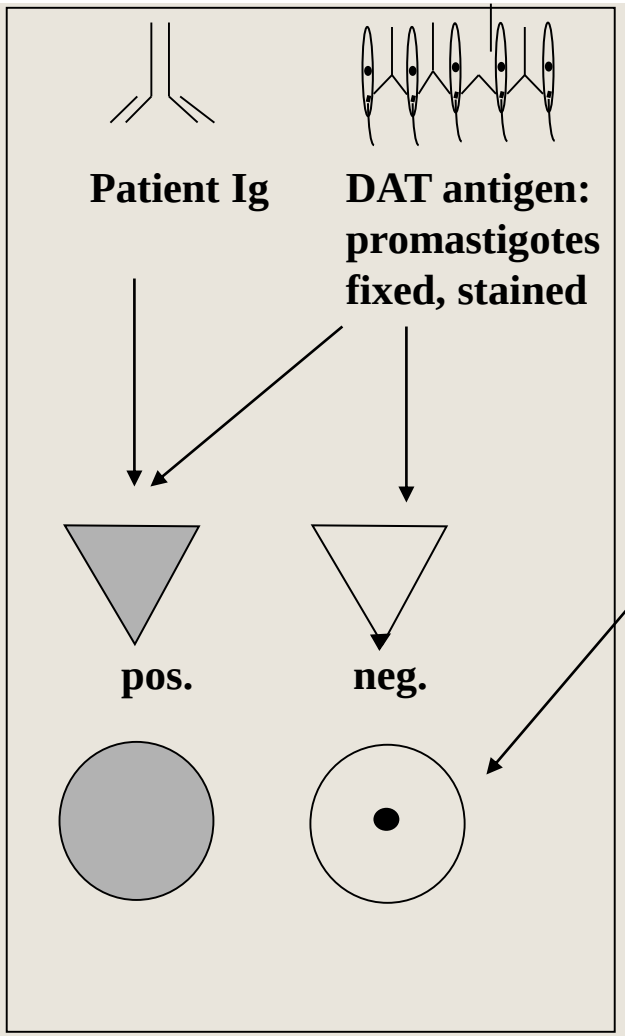
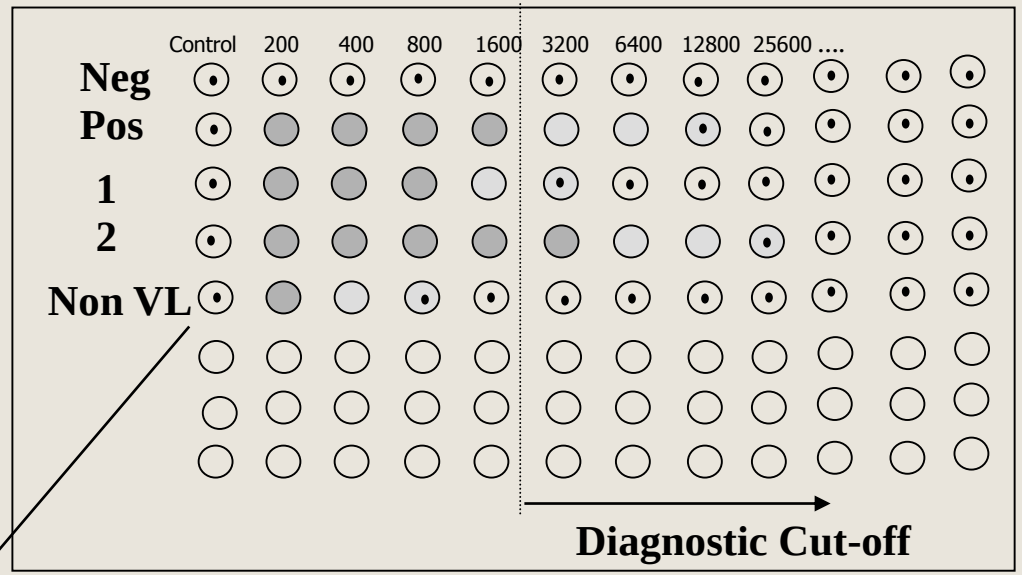
Direct Agglutination Test (DAT) El Harith *et al.* 1986





Microtitre plate with 96 V-shaped wells

LIMITATIONS

- Some technical expertise
- Involves several hours or overnight incubation

Reading after 18 hours only; Alternative "FAST" 1-titer (1:200) Schoone *et al* 2001

- Limited commercial access

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Direct Agglutination Test: meta-analysis

Table 2 Sensitivity and specificity of direct agglutination test in various subgroups

Subgroups	No of studies	Sensitivity (95% CI)	No of studies	Specificity (95% CI)
All studies (n=30)	29	94.8 (92.7 to 96.4)	27	97.1 (93.9 to 98.7)
Trial phase:				
I	20	94.3 (91.5 to 96.2)	17	98.1 (94.2 to 99.4)
II	5	97.7 (87.4 to 99.6)	5	97.2 (92.5 to 99.0)
III	4	94.3 (87.9 to 97.4)	5	90.9 (75.9 to 96.9)
Region:				
South Asia	11	97.1 (94.9 to 98.4)	10	95.7 (88.1 to 98.5)
East Africa	11	93.2 (89.1 to 95.8)	10	96.1 (89.2 to 98.6)
Elsewhere	7	92.8 (86.8 to 96.2)	7	99.8 (97.5 to 100)
Leishmania species:				
L donovani	23	95.1 (92.7 to 96.7)	21	96.4 (92.5 to 98.4)
Other	6	93.0 (85.1 to 96.9)	6	99.7 (94.6 to 100)
Type of antigen:				
Freeze dried	4	89.0 (84.1 to 92.5)	4	99.1 (74.4 to 100)
Aqueous	25	96.2 (94.2 to 97.5)	23	96.7 (93.0 to 98.5)

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Chappuis et al. *BMJ 2006;* 333: 723-7

RDT in VL

- Rapid diagnostic tests (RDTs) are defined as equipment-free diagnostic devices that do not require highly skilled laboratory staff
- The recombinant form of the 39 amino-acid-sequence from *L. chagasi* is the most widely used and is known as rK39. Other recombinant antigens such as rK9, rK16, rK26 and rK28 have also been evaluated

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rK-39 Strip test for VL diagnosis

rk-39 strip test using blood samples:

(1) Healthy control
(2) Malaria case
(3) VL case

Advantages:

- **Easy, rapid and sensitive**
- **Based on blood sample**
- **Cost effective**
- **Field applicable**

Disadvantages:

- **Active VL or asymptomatic or past infection??**

  (due to persistence of Abs after VL)
- **Parasite clearance/ Assessment of cure??**
- **Efficacy low in immunocompromised**

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Cochrane Review: RDT for VL in clinical suspect patients - rk39 ICT

Population: Patients suspected to have visceral leishmaniasis disease

Reference standard: (1) direct smear test or culture of splenic aspirate; (2) composite reference standard based on one or more of the following: parasitology, serology, or response to treatment; or (3) latent class analysis

Region	No of participants (studies)	Pooled sensitivity (95% CI)	Pooled specificity (95% CI)
Indian subcontinent	1468 (6 studies)	0.97 (95% CI 0.90 to 1.00)	0.90 (95% CI 0.76 to 0.98)
East Africa	1692 (9 studies)	0.85 (95% CI 0.75 to 0.93)	0.91 (95% CI 0.80 to 0.97)

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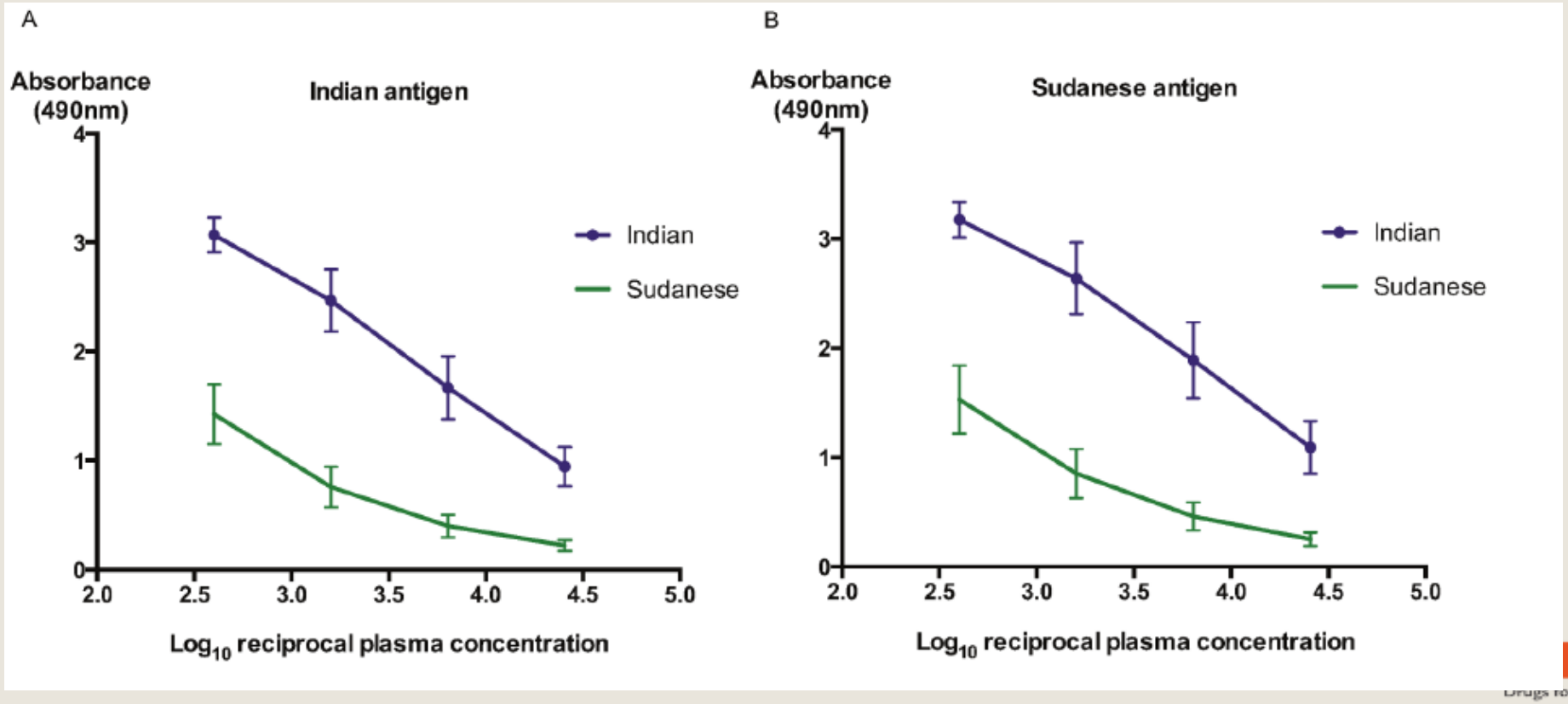
PLOS | NEGLECTED TROPICAL DISEASES

Significantly Lower Anti-*Leishmania* IgG Responses in Sudanese versus Indian Visceral Leishmaniasis

Tapan Bhattacharyya[1☯*], Duncan E. Bowes[1☯], Sayda El-Safi[2], Shyam Sundar[3], Andrew K. Falconar[4], Om Prakash Singh[3], Rajiv Kumar[3,5], Osman Ahmed[2,6], Marleen Boelaert[7], Michael A. Miles[1]



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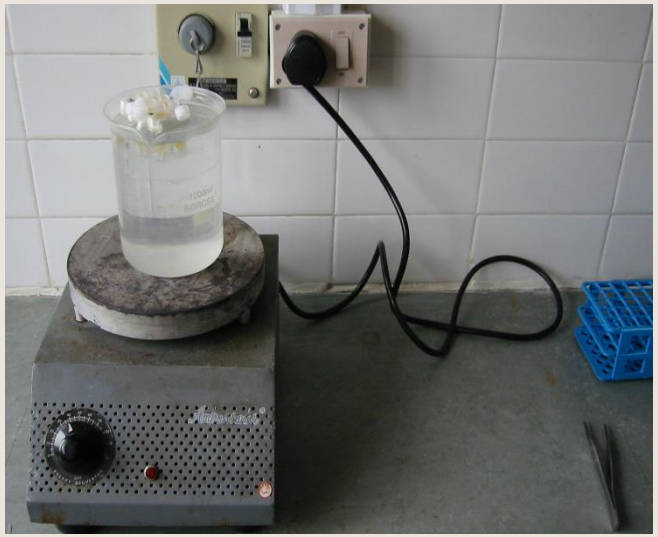
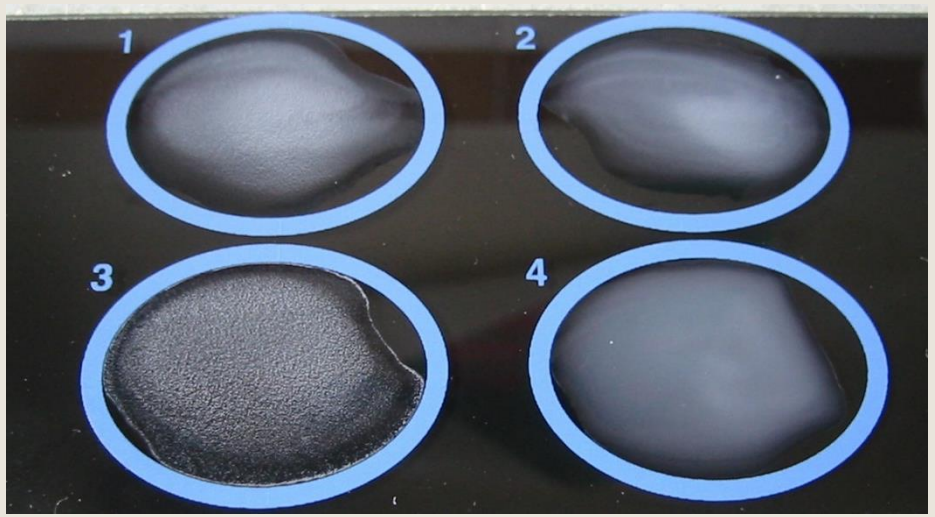
Urine antigen detection test Attar *et al.* 2000





Acrobat Document

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Cochrane Review: RDT for VL in clinical suspect patients - Latex agglutination test in urine

Population: Patients suspected to have visceral leishmaniasis disease

Reference standard: (1) direct smear test or culture of splenic aspirate; (2) composite reference standard based on one or more of the following: parasitology, serology, or response to treatment; or (3) latent class analysis

Region	No of participants (studies)	Pooled sensitivity (95% CI)	Pooled specificity (95% CI)
VL endemic regions	1374 (6 studies)	0.64 (95% CI 0.41 to 0.86)	0.93 (95% CI 0.77 to 0.99)

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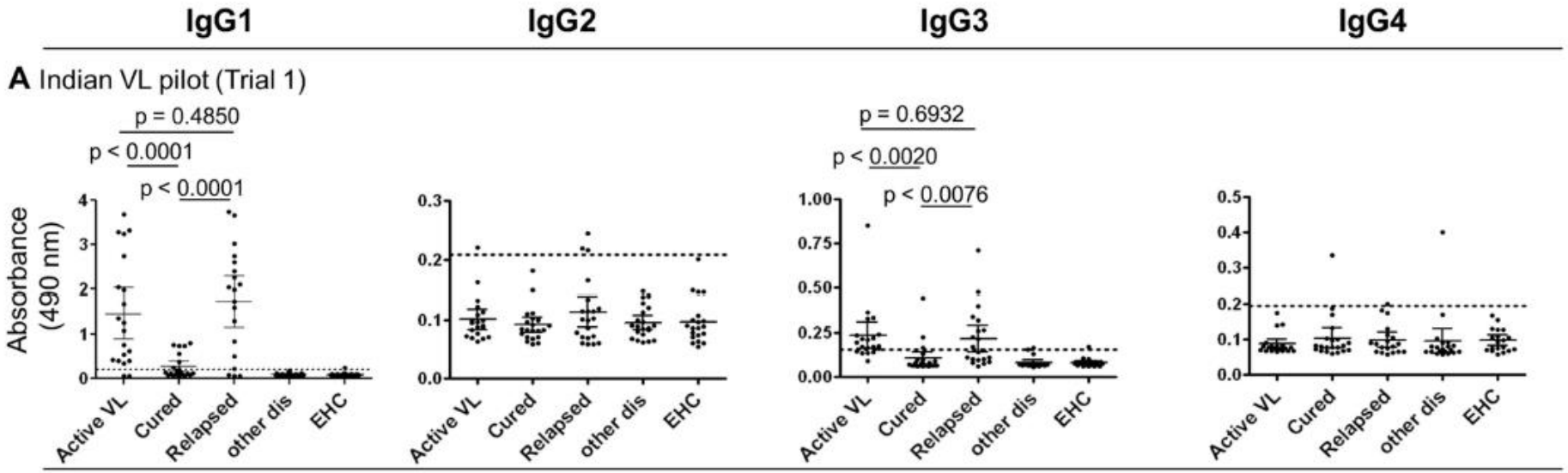
IgG1 as a biomarker for VL



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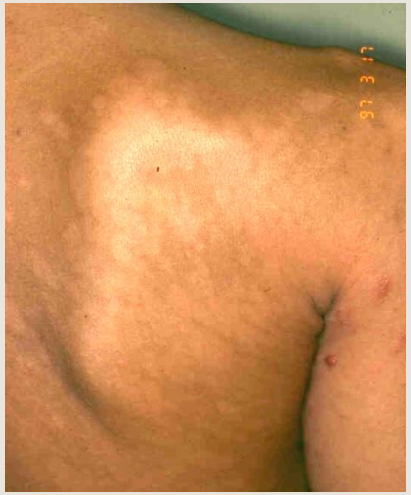
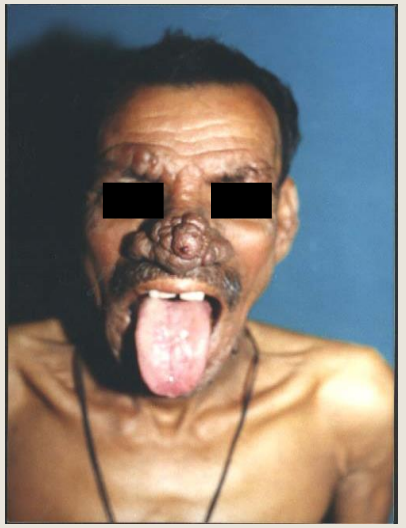
IgG1 as a Potential Biomarker of Post-chemotherapeutic Relapse in Visceral Leishmaniasis, and Adaptation to a Rapid Diagnostic Test

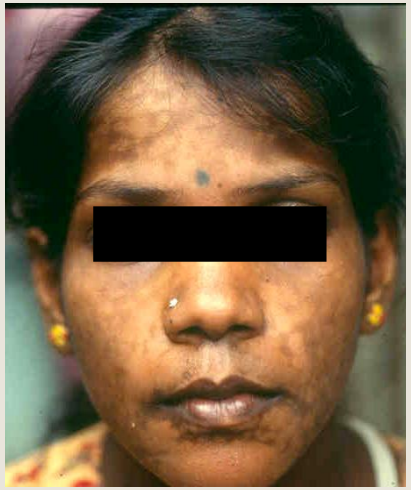
Tapan Bhattacharyya[1]*, Armon Ayandeh[1], Andrew K. Falconar[2], Shyam Sundar[3], Sayda El-Safi[4],

Challenges in diagnosis of PKDL

- Potential reservoir especially inter epidemic period
- Up to 10–20% of VL develop PKDL
- Clinical presentation varies, making diagnosis difficult
- Accurate diagnosis is important: long and toxic treatment
- Confirmation of diagnosis: skin slit smear (SSS) microscopy or histopathology, PCR
- Sn 40–60% in nodular lesions and even lower in patients with macular lesions







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Use of RDT in the field

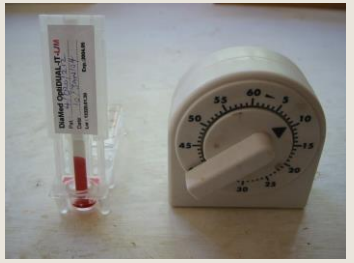
- Products in circulation:

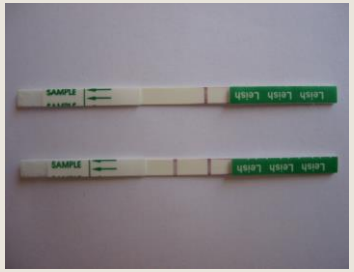
- Many generic brands in circulation: most, no peer reviewed scientific validation results

- Antigens:rk39, rkE 16, recombinant antigens

- Performance of different brands may not be similar: e.g study from Uganda (Chappuis 2005)

Brand of rk39 ICT	Sens.% (95% CI)	Spec.% (95% CI)
Diamed IT Leish	97 (92-99)	97 (92-99)
Kala-azar detect	82 (74-87)	99 (95-100)





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Global Comparative Evaluation of Commercial ICT RDT Diagnostic Tests for VL Cunningham et al 2012

Product	Manufacturer	Catalog No.	Lateral Flow Format	Lot No.	Storage Temperature	Shelf Life	Control Line	Test Line, Bound Antigen
CrystalKA	Span Diagnostics, Ltd	56FT101	Dipstick	R2009004	4°C–30°C	18 mo	Yes	rKE16
				R2009003				
DiaMed-IT LEISH	Bio-Rad Laboratories	710124	Cassette	46240.27.01	2°C–30°C	16 mo	Yes	rK39
				46240.28.01				
Kalazar Detect	InBios International, Inc	INS105	Dipstick	KE 2108	RT (20°C–28°C)	24 mo	Yes	rK39
				KE 1047				
Signal–KA	Span Diagnostics Ltd	56FT100	Cassette	4000002561	2°C–8°C	12 mo	Yes	rKE16
				4000002604				
OnSite Leishmania Ab Rapid Test	CTK Biotech Inc	RO122S	Dipstick	F0317G2	2°C–30°C	18 mo	Yes	rK39
				F0318G2				

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Sensitivity, specificity of RDT : Indian sub-continent

Category Product	Manufacturer	Sample number Cases	Sensitivity (95% CI)	Sample number Controls	Specificity (95% CI)
Crystal KA (rKE16)	Span Diagnostics Ltd.	n = 250	92.8% (88.9-95.4%)	n = 499	99.2% (97.1-99.7%)
Diamed-IT Leish	DiaMed AG	n = 250	98.8% (96.5-99.6%)	n = 499	97.6% (94.8-98.9%)
Kalazar detect	InBios International, Inc.	n = 250	99.6% (97.8-99.9%)	n = 499	96.0% (92.8-97.8%)
Signal – KA (rkE 16)	Span Diagnostics Ltd.	n = 175	100% (97.9-100%)	n = 345	100% (97.8-100%)
Onsite Leishmania Ab Rapid *	CTK Biotech. Inc.	n = 250	99.6% (97.8-99.9%)	n = 499	96.8% (93.8-98.4%)

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Thermal stability of RDT: Indian SC

Product	Manufacturer	Proportion positive results among cases (n=20)			Proportion negative results among controls(n=4)			Proportion invalid results (n=24)		
		4ºC	37ºC	45ºC	4ºC	37ºC	45ºC	4ºC	37ºC	45ºC
Crystal KA	Span Diagnostics Ltd.	95%	90%	90%	100%	100%	100%	0%	0%	0%
Diamed-IT Leish	DiaMed AG.	100%	100%	0%	100%	100%	100%	0%	0%	100%
Kalazar detect	InBios International, Inc	100%	100%	100%	100%	100%	100%	0%	0%	0%
Onsite Leishmania Ab Rapid	CTK Biotech. Inc.	100%	100%	100%	100%	100%	100%	0%	0%	0%

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Summary

- Diagnostic tools for VL will only have an impact if they are widely available to patients
- Currently antibody detecting ICT are the only Rapid diagnostic test available as a point of care test for the Indian
- Rk39 ICT well validated and fulfills the ASSURED criteria
- Variability of different brands may be seen
- Ideally there is a need of RDT:

  - to assess cure and relapse (IgG1 shows looks promising).
- PKDL diagnosis: limitations
- Quality control mechanisms need to be standardized for selection, procurement and use in the field

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