



# Overcoming diagnostic challenges in management of Leishmaniasis: a history of operational research and innovation

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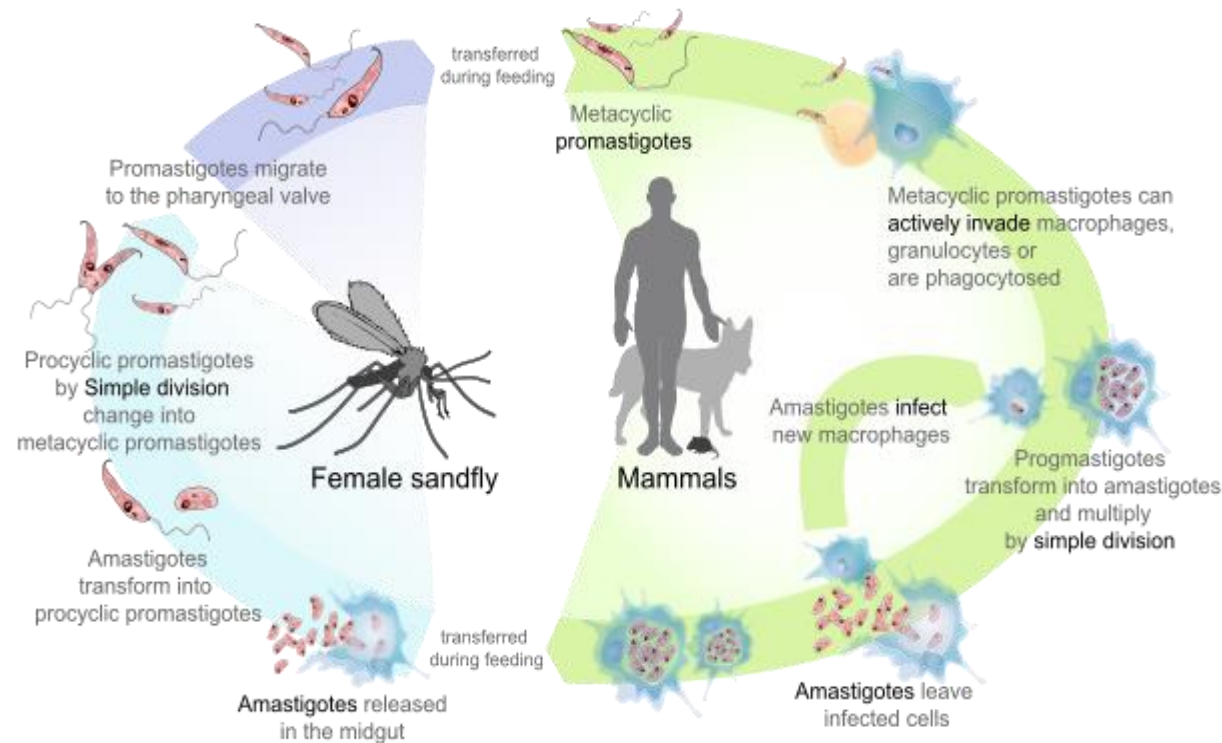
Diagnosics Symposium TU-Delft, LUMC, 13<sup>th</sup> January 2021

# Leishmaniases

- Visceral leishmaniasis
- Cutaneous leishmaniasis
- Mucocutaneous leishmaniasis

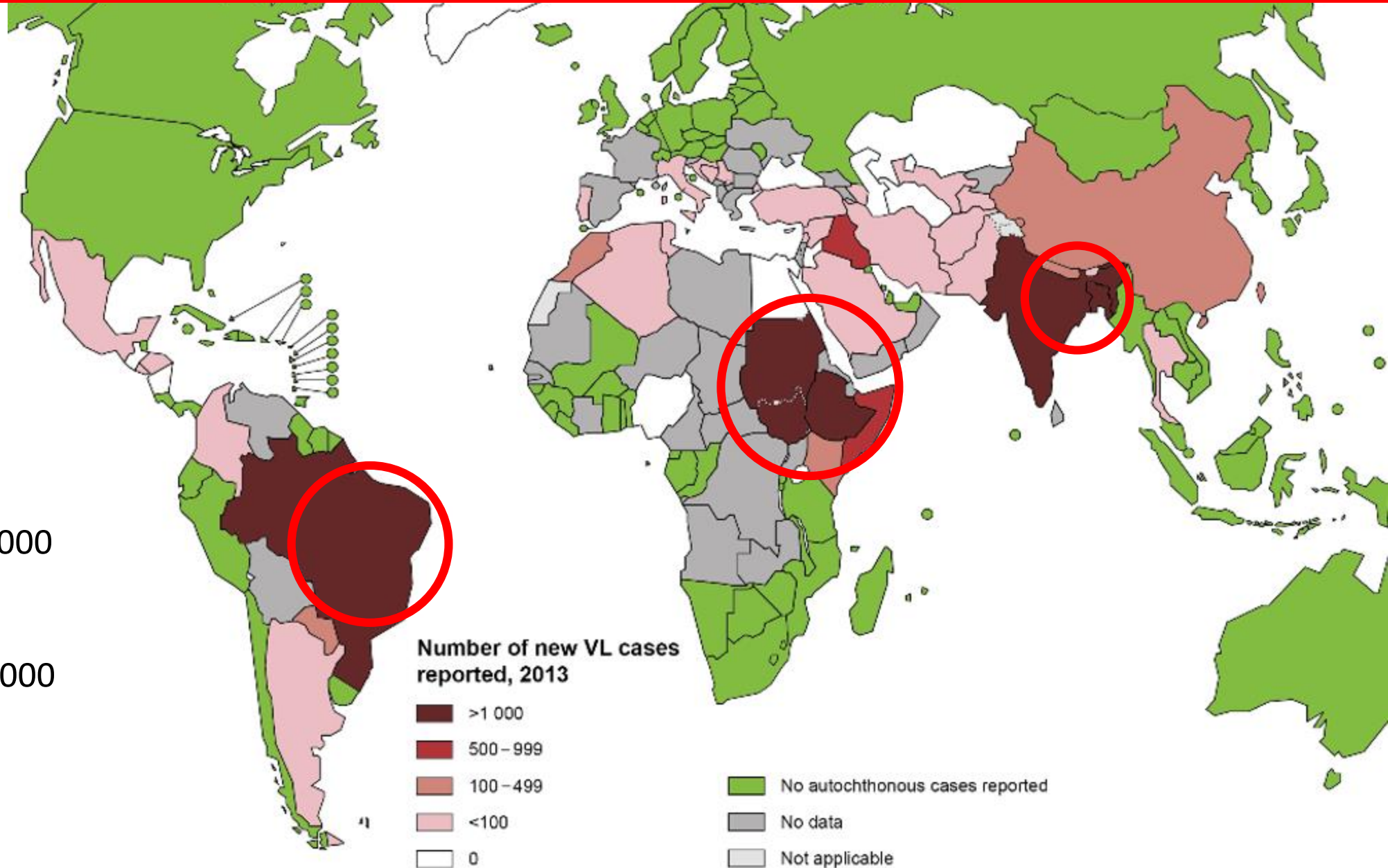


Leishmania promastigotes



Leishmania life cycle

# Geographical distribution of Visceral Leishmaniasis (2018)



78 countries

Incidence 50,000 - 90,000 cases per year

Mortality 10,000 – 20,000 deaths annually

# VL in East Africa and South Asia

**Parasite:** *Leishmania donovani*

**Vector:** *Phlebotomine* sandflies

**Transmission:** anthroponotic



**Systemic disease** of the viscera.

Leishmania amastigotes multiply in macrophages.

VL patients are severely immunocompromised.

**Signs and symptoms:**

fever, weight loss, cachexia, diarrhoea, hepatosplenomegaly, pancytopenia, epistaxis, anorexia, abdominal pain.

**Complications:** due to anaemia, malnutrition and co-infections (pneumonia, dysentery, malaria, TB, HIV).



**VL is fatal if not treated**



### Treatment of VL:

- Long (17-28d), toxic, expensive,

### Treatment of multiple complications:

- Opportunistic infections
- Anaemia
- Malnutrition
- Organ failure



## VL treated by MSF, 1989-2019

	Cases treated
<b>South Sudan</b>	66,398
<b>Sudan</b>	36,763
<b>Ethiopia</b>	17,626
<b>Somalia</b>	3,791
<b>Kenya</b>	3,083
<b>Uganda</b>	2,437
<b>India</b>	13,478
<b>Bangladesh</b>	3,581
<b>TOTAL</b>	<b>147,581</b>

Hospital (*Fulbaria, Bangladesh*)



Field Hospital (*Abdurafi, northern Ethiopia*)



**Under the trees  
(South Sudan)**







## VL in South Sudan

- Very poor health infrastructure
- Major physical barriers
- War / insecurity
- Population displacement



Access challenged  
for patients and  
health workers



# Diagnostic work-up

- Two steps:**
- Clinical suspicion
  - Diagnostic confirmation (tests)

## Clinical suspicion

- High sensitivity; compromise on specificity
- Clinical suspicion defines pre-test probability
- East Africa: prevalence VL in clinical suspects: 20-50%

	<b>Sudan</b>
Fever	95-100
Weight loss (wasting)	70-100
Epistaxis (nosebleed)	47-88
Anorexia (lack of appetite)	70-87
Abdominal pain	41-81
Cough	63-76
Diarrhoea	17-45
Nausea/vomiting	22-27
Splenomegaly	90-100
Lymph node enlargement	36-84
Hepatomegaly	56-100

**History of prolonged fever (2 weeks or more) with splenomegaly and/or lymphadenopathy and/or wasting**

# Differential Diagnosis

Diseases with similar clinical symptoms:

**Malaria**

**Hyperreactive malarial splenomegaly**

**Schistosomiasis**

**Brucellosis**

**Typhoid fever**

**Typhus**

**Tuberculosis**

**Splenic abscess**

**Chronic hepatitis with portal hypertension**

**HIV/AIDS**

**Hematological malignancies**

# Diagnosis: Parasitology

## Lymph node



- Lacks sensitivity ~50-80%

## Bone marrow



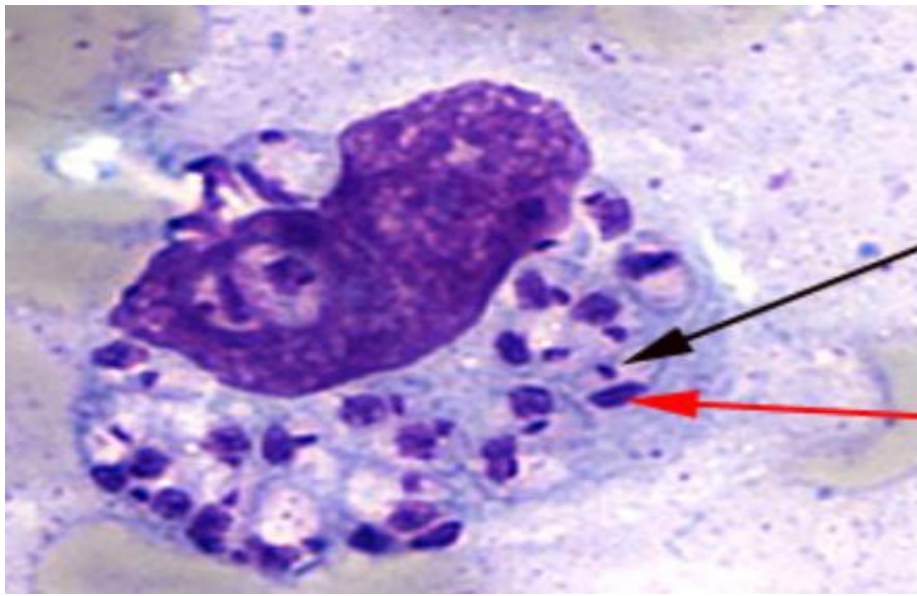
- Lacks sensitivity ~70-80%
- Painful
- Sterilisation
- Medical procedure

## Spleen



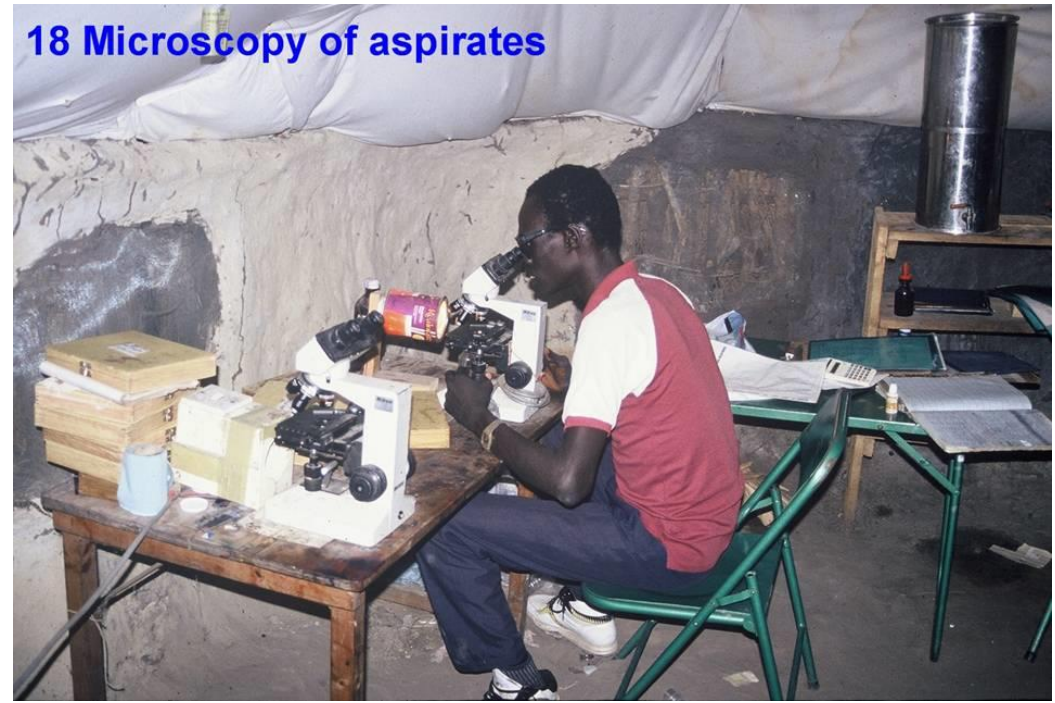
- Sensitivity ~95%
- Needs expertise
- Medical procedure
- Risk of bleeding

**Not suitable for remote field use**



Amastigote with nucleus + kinetoplast

## 18 Microscopy of aspirates

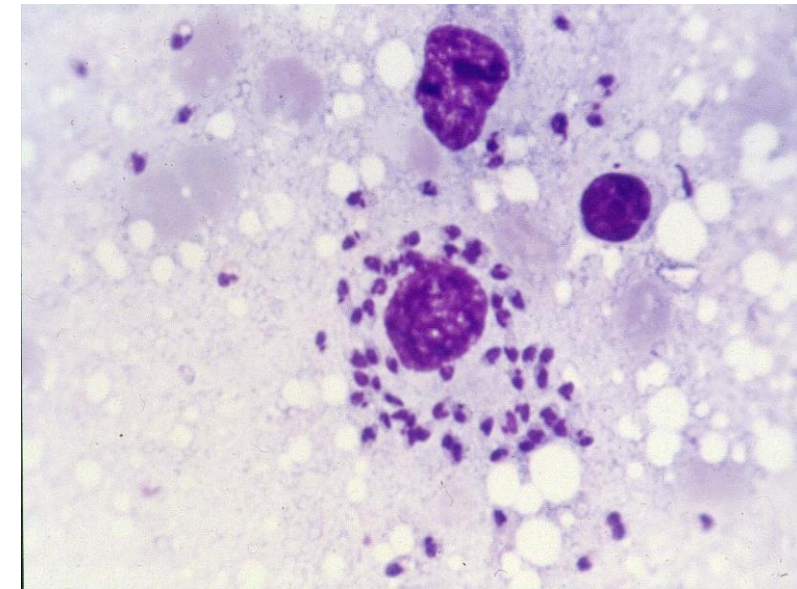


*Am. J. Trop. Med. Hyg.*, 76(4), 2007, pp. 689–693  
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### UTILITY OF LYMPH NODE ASPIRATION IN THE DIAGNOSIS OF VISCERAL LEISHMANIASIS IN SUDAN

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Manchester General Hospital, Manchester, United Kingdom; Department of Infection and Tropical Medicine, Northwick Park  
Hospital, Harrow, United Kingdom*

Found 65% sensitivity of lymphnode aspirate microscopy



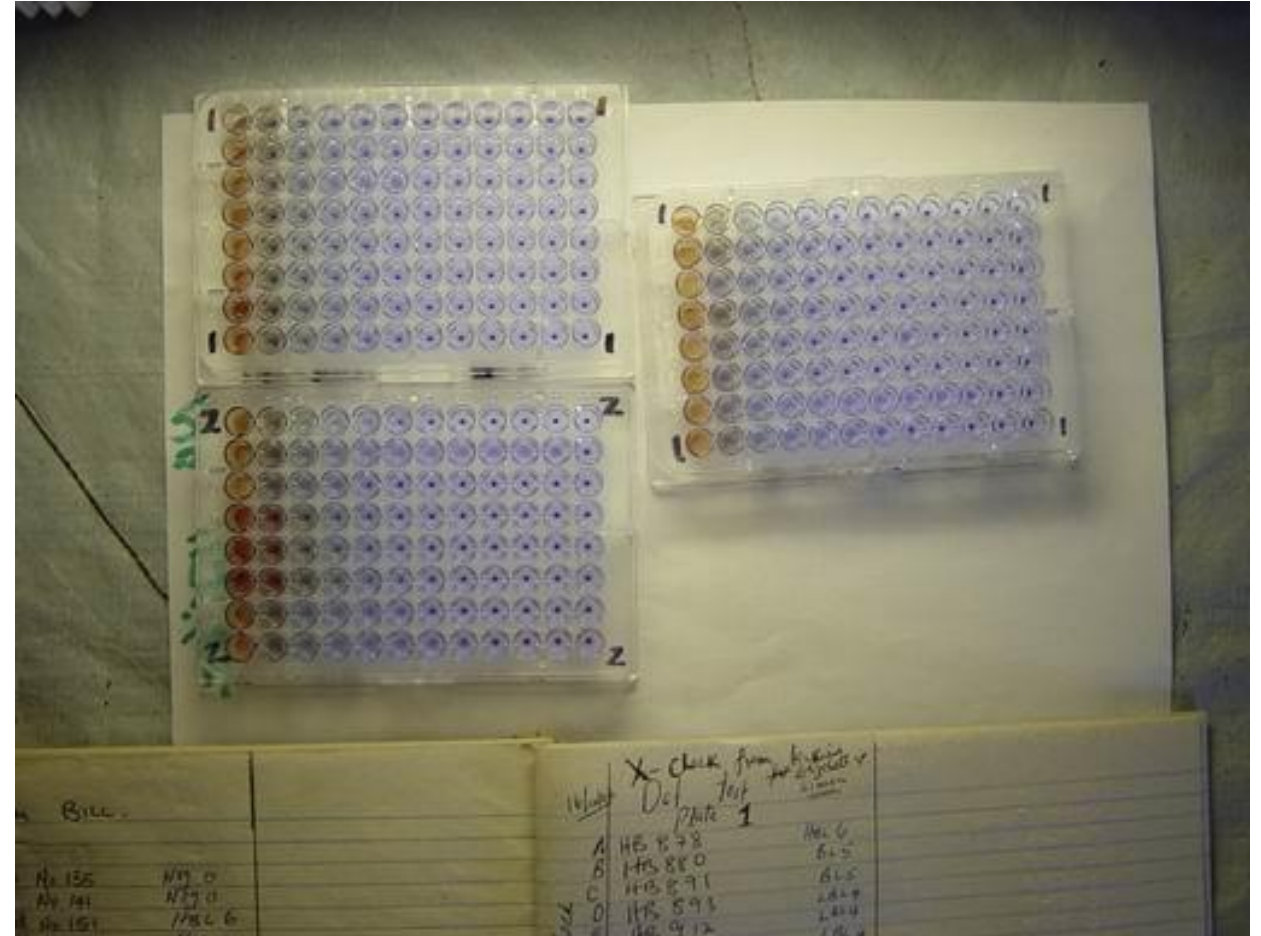
# Serology: DAT

## DAT (Direct Agglutination Test):

- Sensitive & specific
- Well validated
- Robust (FD Ag)

### But:

- Quite technical
- Time demanding ( $\geq 18h$ )
- ++ material needed
- Relatively expensive



**Not widely applicable without external support**

# Freeze-Dried DAT

JOURNAL OF CLINICAL MICROBIOLOGY, July 1995, p. 1742-1745  
0095-1137/95/\$04.00+0  
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## *Leish*-KIT, a Stable Direct Agglutination Test Based on Freeze-Dried Antigen for Serodiagnosis of Visceral Leishmaniasis

STEFANIE E. O. MEREDITH,<sup>1†</sup> NEL C. M. KROON,<sup>1</sup> EGBERT SONDRP,<sup>2</sup> JILL SEAMAN,<sup>2</sup>  
MARGA G. A. GORIS,<sup>2</sup> COR W. VAN INGEN,<sup>3</sup> HANS OOSTING,<sup>4</sup> GERARD J. SCHOONE,<sup>1</sup>  
WIEPKO J. TERPSTRA,<sup>1</sup> AND LINDA OSKAM<sup>1\*</sup>

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- More robust antigen, less sensitive to shock, not requiring cold chain
- Less wastage

# Serological RDT: simple, fast, low cost



2003





# Serological lateral flow RDT



Field test



Bedside test

# rK39 lateral flow RDT

*Am. J. Trop. Med. Hyg.*, 74(1), 2006, pp. 76–80  
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## EVALUATION OF A NEW RECOMBINANT K39 RAPID DIAGNOSTIC TEST FOR SUDANESE VISCERAL LEISHMANIASIS

KOERT RITMEIJER,\* YOSEPH MELAKU, MARIUS MUELLER, SAMMY KIPNGETICH, CAROLINE O'KEEFFE, AND ROBERT N. DAVIDSON

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JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 2005, p. 5973–5977  
0095-1137/05/\$08.00+0 doi:10.1128/JCM.43.12.5973-5977.2005  
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## Diagnostic Accuracy of Two rK39 Antigen-Based Dipsticks and the Formol Gel Test for Rapid Diagnosis of Visceral Leishmaniasis in Northeastern Uganda

François Chappuis,<sup>1,2\*</sup> Yolanda Mueller,<sup>1</sup> Alexandre Nguimfack,<sup>1</sup> John Bosco Rwakimari,<sup>3</sup> Sophie Couffignal,<sup>1</sup> Marleen Boelaert,<sup>4</sup> Philippe Cavailler,<sup>5</sup> Louis Loutan,<sup>2</sup> and Patrice Piola<sup>5</sup>

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Tropical Medicine and International Health

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## Comparison of an rK39 dipstick rapid test with direct agglutination test and splenic aspiration for the diagnosis of kala-azar in Sudan

Hans Veecken<sup>1</sup>, Koert Ritmeijer<sup>1</sup>, Jill Seaman<sup>1</sup> and Robert Davidson<sup>1,2</sup>

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*Am. J. Trop. Med. Hyg.*, 80(6), 2009, pp. 929–934  
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## Field Evaluation of rK39 Test and Direct Agglutination Test for Diagnosis of Visceral Leishmaniasis in a Population with High Prevalence of Human Immunodeficiency Virus in Ethiopia

Rachel ter Horst, Tewodros Tefera, Gessesse Assefa, Abdurazik Z. Ebrahim, Robert N. Davidson, and Koert Ritmeijer\*

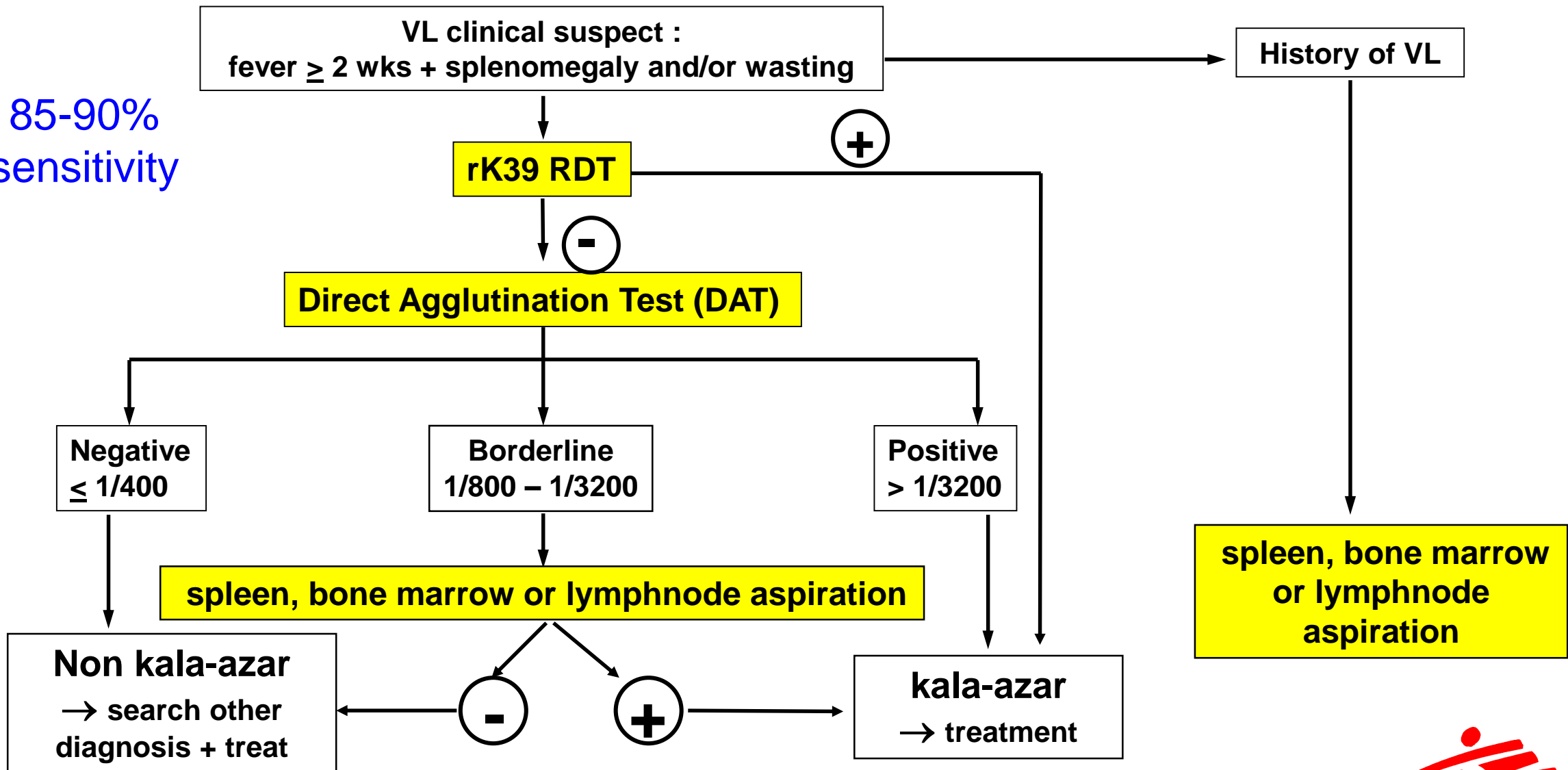
*Médecins Sans Frontières, Humera, Ethiopia; Kahsay Abera Hospital, Humera, Ethiopia; Ethiopian Health and Nutrition Research Institute, Addis Ababa, Ethiopia; Department of Infection and Tropical Medicine, Northwick Park Hospital, Harrow, United Kingdom; Médecins Sans Frontières, Amsterdam, The Netherlands*

- Various field validation studies with different formats of rK39 RDT's
- **IT-Leish** (BioRad) is the best performing: 90% sensitivity / 99% specificity in clinical suspects



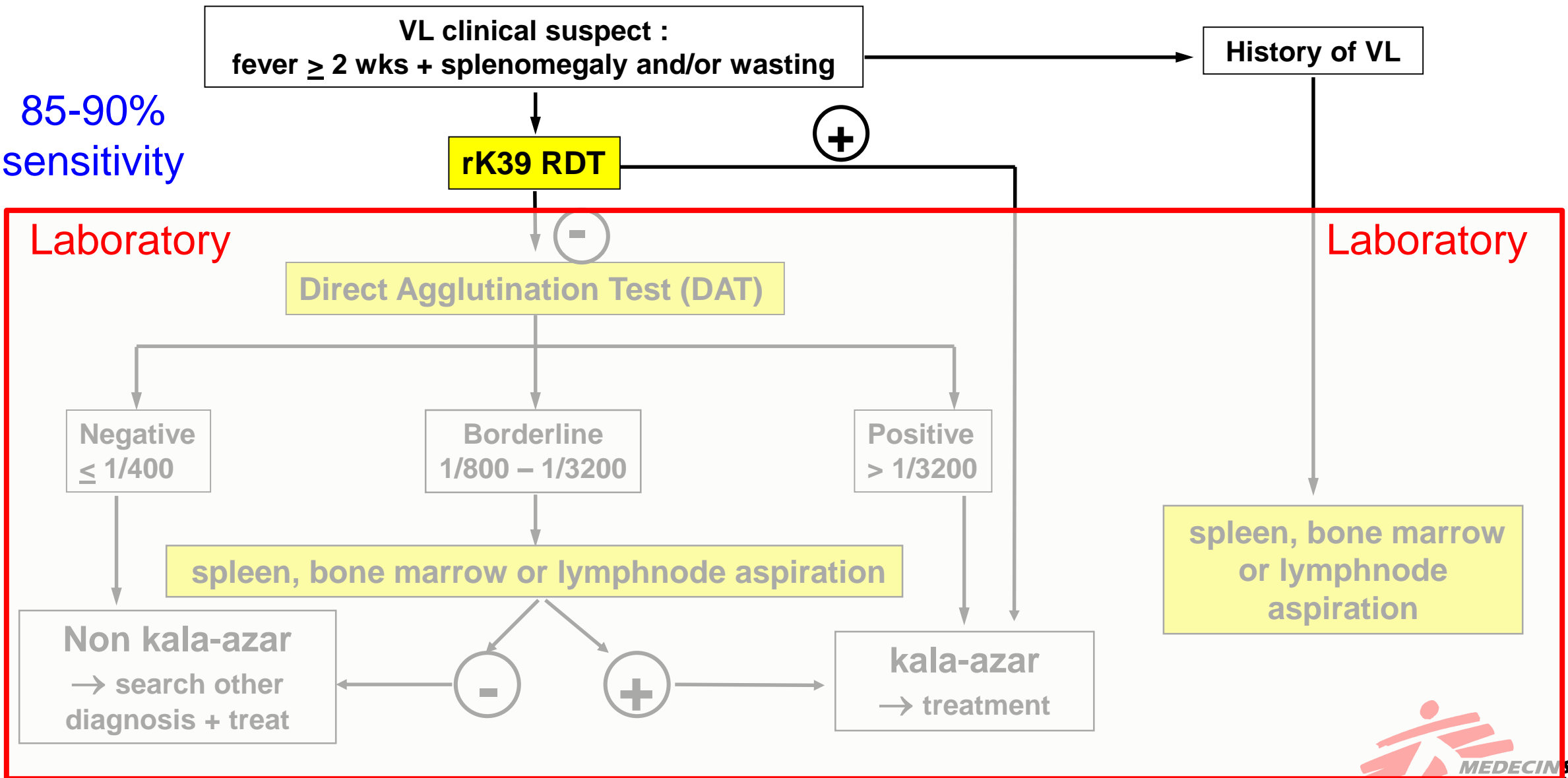
# Diagnostic Algorithm for VL in East Africa

85-90%  
sensitivity

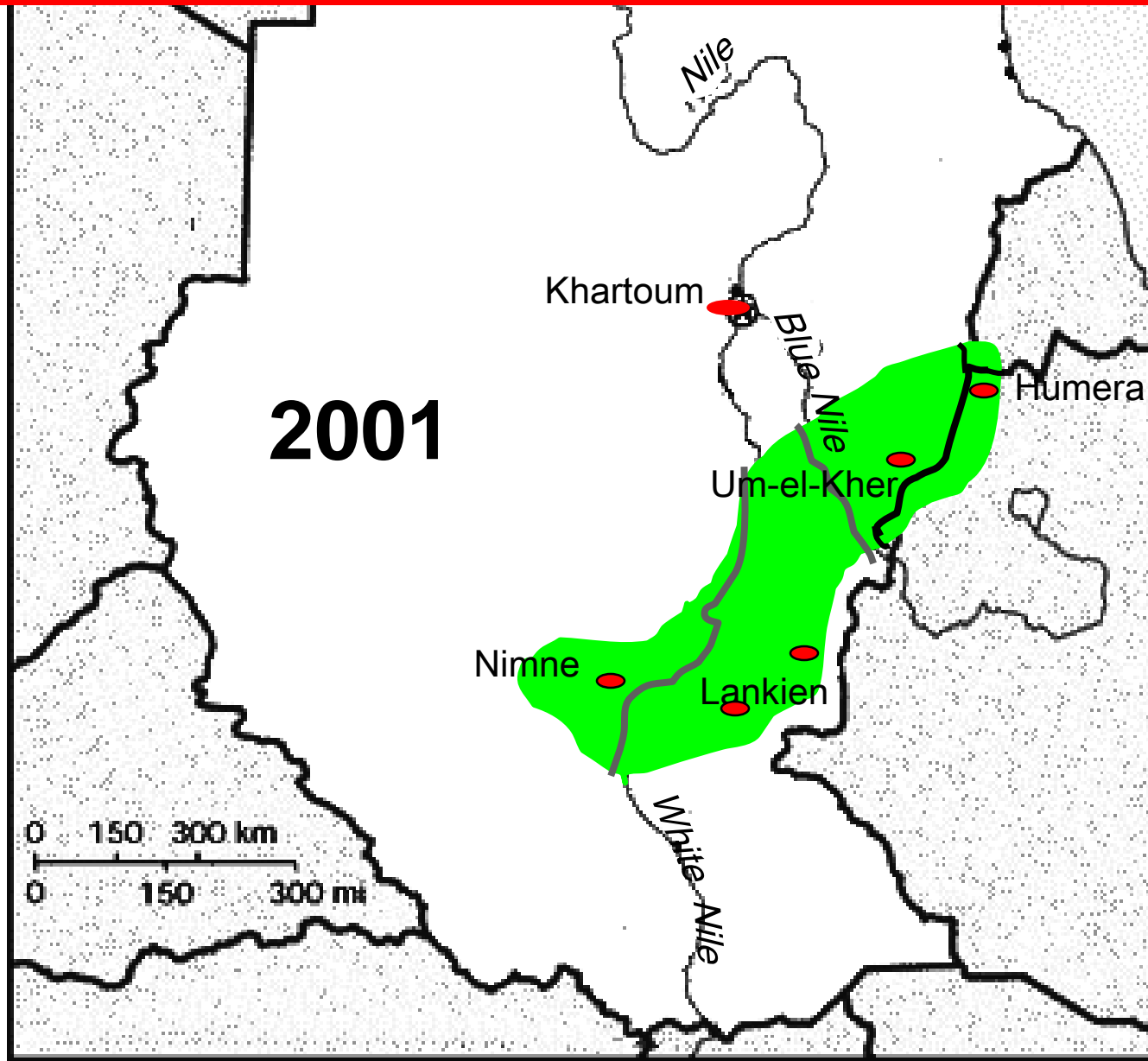


# Diagnostic Algorithm for VL in East Africa

85-90%  
sensitivity

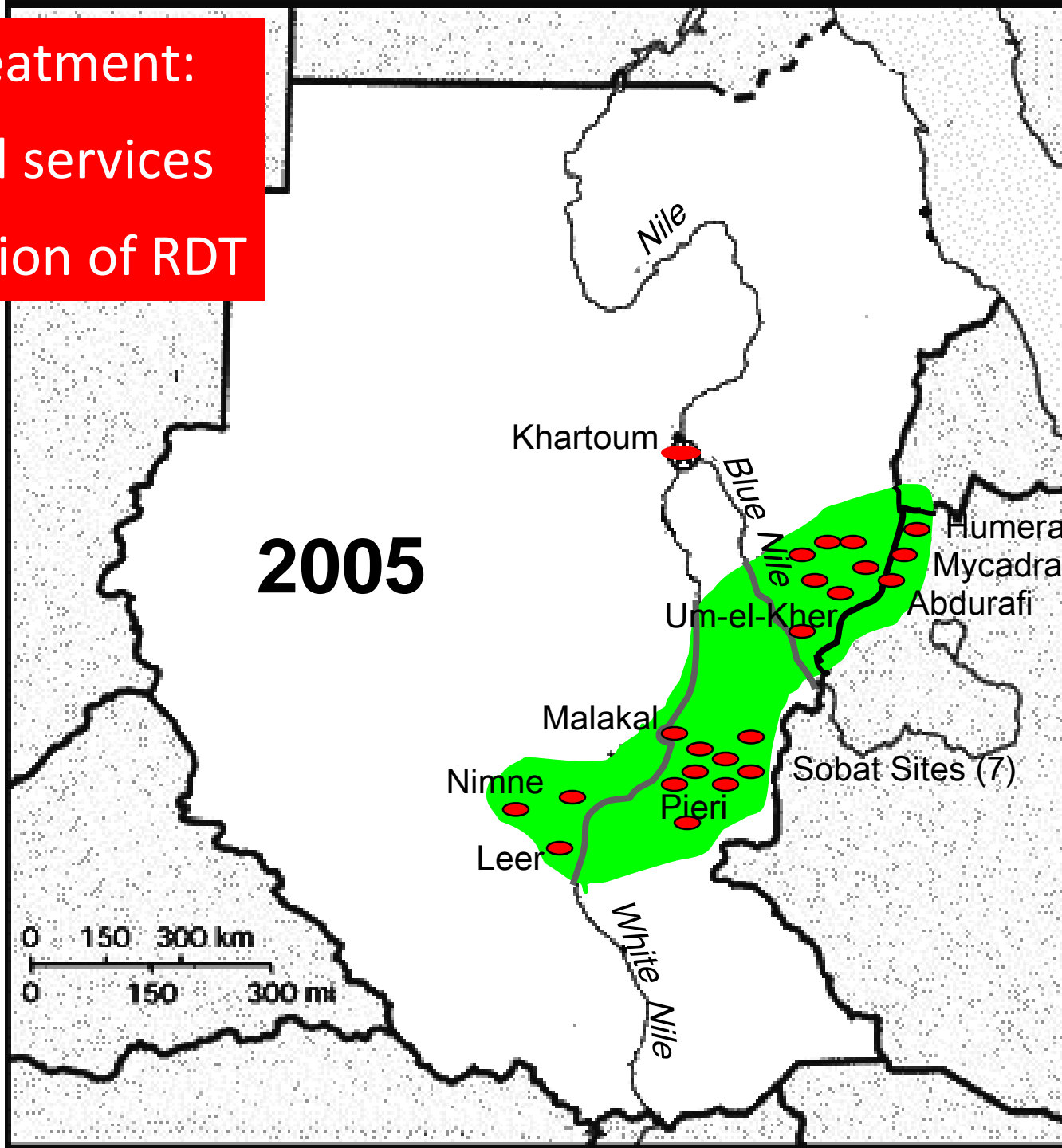


# Easy access to free VL care



Sudan  
South Sudan  
Ethiopia

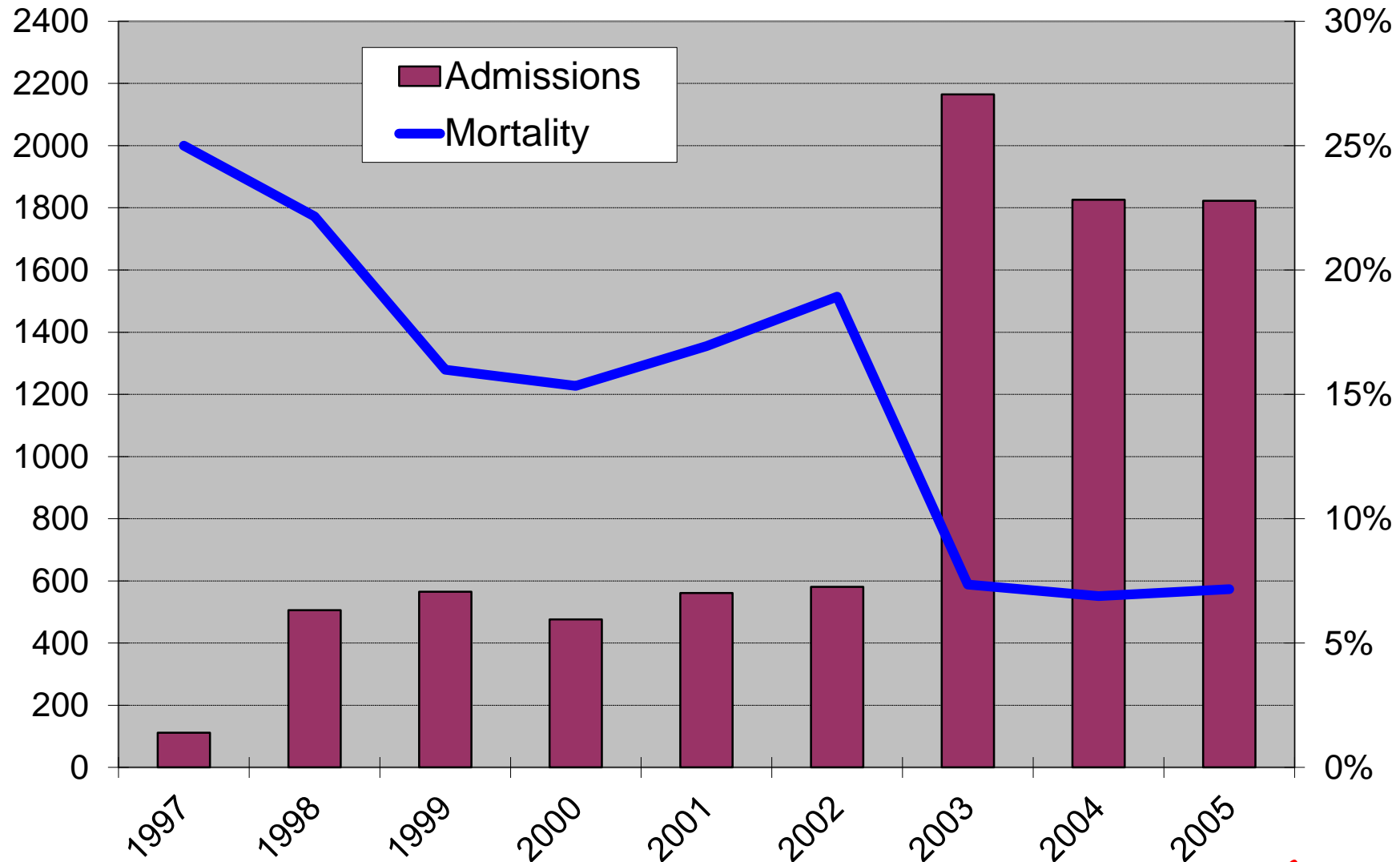
Access to treatment:  
Decentralised services  
after introduction of RDT



Sudan  
South Sudan  
Ethiopia



# VL patients treated and mortality Northwest Ethiopia (1997-2005)



Impact of introduction of RDT: access to diagnosis

# Other diagnostic developments

## Antigen-detection test


- High potential to monitor treatment efficacy and diagnose relapse (DD active versus past infection)
- Prototype: **KAtex urine test**
- Well-validated
- Low to moderate sensitivity
- Moderate to high specificity
- Reading of agglutination not always easy (subjective)
- Need for fresh & boiled urine
- Development of RDT (dipstick) format with increased sensitivity by FIND, but unsuccessful so far

*Am. J. Trop. Med. Hyg.*, 99(4), 2018, pp. 957–966  
doi:10.4269/ajtmh.18-0042  
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Antigen Detection in Urine for Noninvasive Diagnosis and Treatment Monitoring of Visceral Leishmaniasis in Human Immunodeficiency Virus Coinfected Patients: An Exploratory Analysis from Ethiopia

Florian Vogt,<sup>1\*</sup> Bewketu Mengesha,<sup>2</sup> Helen Asmamaw,<sup>3</sup> Tigist Mekonnen,<sup>2</sup> Helina Fikre,<sup>2</sup> Yegnasew Takele,<sup>2</sup> Emebet Adem,<sup>2</sup> Rezika Mohammed,<sup>2</sup> Koert Ritmeijer,<sup>4</sup> Wim Adriaensen,<sup>1</sup> Yayehirad Melsew,<sup>5</sup> Johan van Griensven,<sup>1†</sup> and Ermias Diro<sup>2†</sup>

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 **frontiers**  
in Cellular and Infection Microbiology

ORIGINAL RESEARCH  
published: 29 March 2018  
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***Leishmania* Antigenuria to Predict Initial Treatment Failure and Relapse in Visceral Leishmaniasis/HIV Coinfected Patients: An Exploratory Study Nested Within a Clinical Trial in Ethiopia**

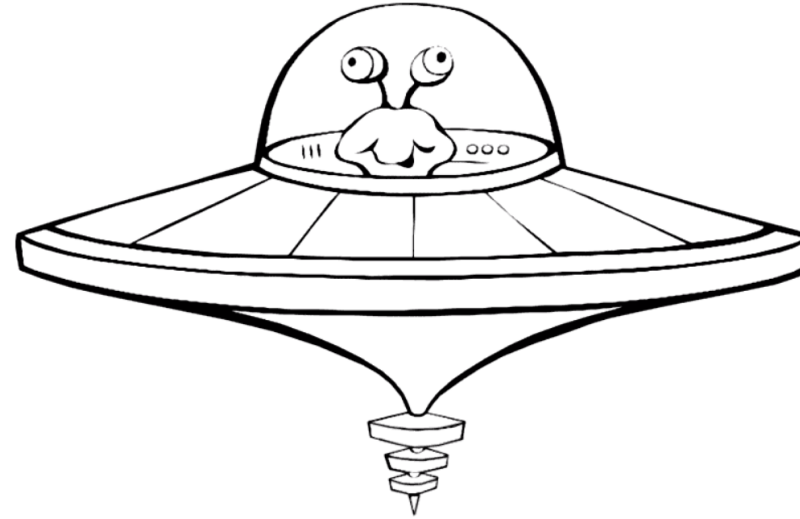
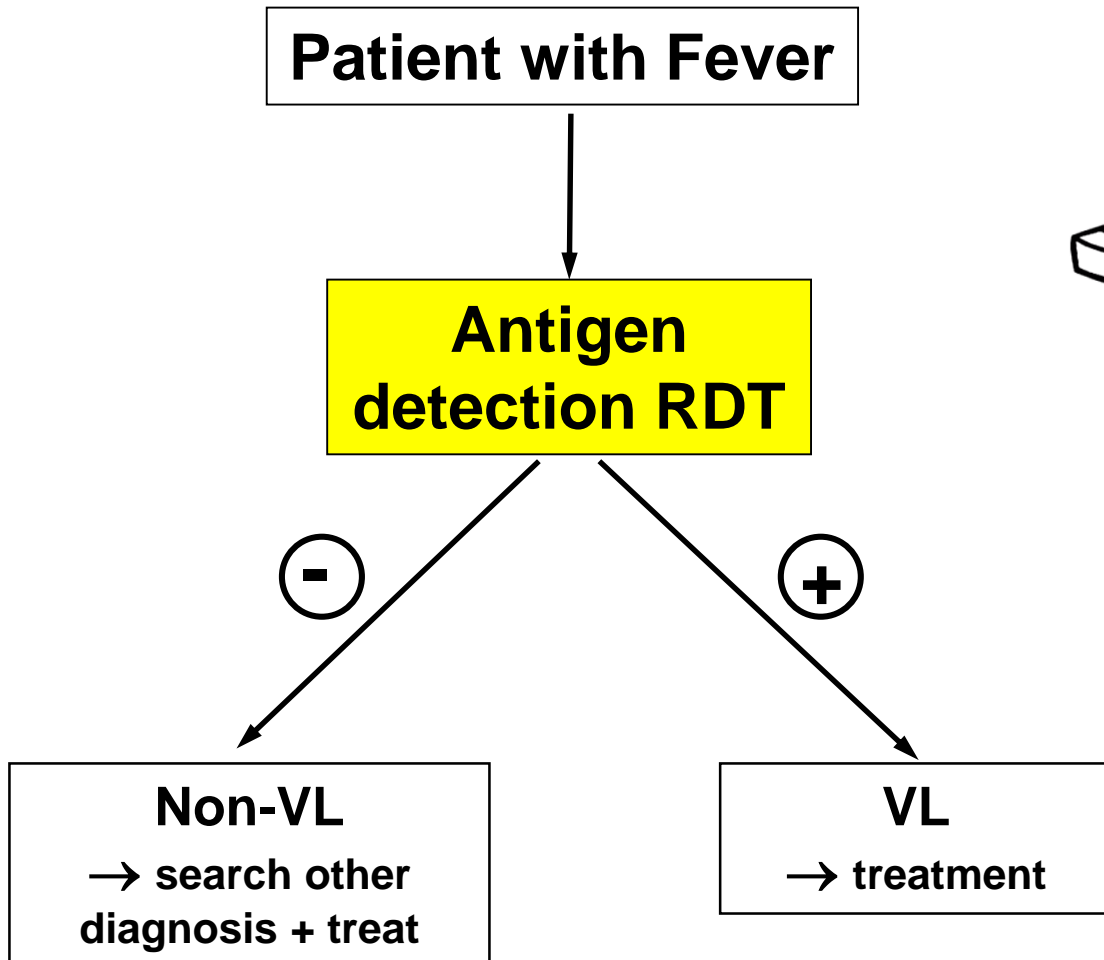
Johan van Griensven<sup>1\*</sup>, Bewketu Mengesha<sup>2</sup>, Tigist Mekonnen<sup>2</sup>, Helina Fikre<sup>2</sup>, Yegnasew Takele<sup>2</sup>, Emebet Adem<sup>2</sup>, Rezika Mohammed<sup>2</sup>, Koert Ritmeijer<sup>3</sup>, Florian Vogt<sup>1</sup>, Wim Adriaensen<sup>1</sup> and Ermias Diro<sup>1</sup>

“Low-Tech” PCR: **LAMP** (Loop-mediated isothermal amplification)





# What we need !



- Diagnosis of primary and relapse VL
- Assessment of treatment efficacy

Field lab  
South Sudan  
2019





**THANK  
YOU**