Institut de recherche pour le développement

Agir avec le Sud
Acting with the South

Advances and challenges in current diagnostics for HAT

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Multi-step procedure

Diagnosis of HAT

- Suspicion of infection
  - Clinical, serological or molecular evidence
- Confirmation of infection: parasitology
  - Microscopic detection of the parasite
- Disease stage determination & treatment outcome
  - Examination of cerebrospinal fluid

Although the principles
of HAT diagnosis
hardly changed for
more than a century

…

important advances have occurred
in the last decade

Lyons, 1992. The colonial disease

Plate 5. Cuthbert Christy in Uganda, 1902.

Plate 1. Hassane Sakande in Guinea, 2013.

Evolving towards the ideal test:

Kettler, White & Hawkes.
Mapping the landscape of diagnostics
for sexually transmitted infection.
Geneva WHO/TDR 2004

A
S
S
U
R
E
D

Serology

- CATT/*T.b. gambiense* (Magnus, 1978):
  - In use since ’80-ies
  - Antigen: whole fixed trypanosomes

A
S
S
U
R
E
D

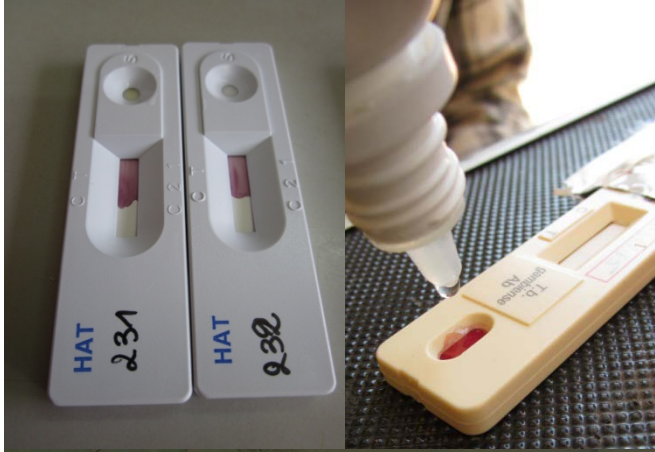
- 0.5€
- Sensitivity: 91.2% (78.1-99.8) (reviewed by Checchi, 2011)
- Specificity: 97.4% (93.8-99.2)
- Simple
- 5-10 minutes, storage 4°C
- Agitator, electricity
- Population screening for *T.b. gambiense*

Serology

- Rapid sero-Diagnostic Tests:
  - 1st generation tests commercialised
  - Antigen: purified native VSG

ASSURED

- 0.5$ - 1.5 €
- Sensitivity: 89.6-98.5% (Büscher, 2014; RDT test brochures)
- Specificity: 87-98.6%
- Simple
- 15 minutes, Robust
- Equipment free
- Individual, passive screening for *T.b. gambiense*



Serology

- Immune trypanolysis:
  - Reference test for *T.b. gambiense* specific antibodies
  - Antigen: live, cloned, bloodstream *T.b. gambiense*

A
- >8$

S
- Sensitivity: 95.1-100% (Van Meirvenne, 1995; Lutumba,2006; Jamonneau, 2010; Mumba, 2014)

S
- Specificity: 100% (Van Meirvenne, 1995)

U
- Live cloned *T.b. gambiense*

R
- 2.5-3 hours, live infective *T.b. gambiense*

E
- Liquid nitrogen, laboratory animals

D
- Surveillance, limited to reference labs Belgium, Burkina and DR Congo

Serology challenges

- Training of RDT users crucial, cfr malaria (Mukadi, 2013)
- Specificity of RDTs: further evaluation & improvement
  - Individual diagnosis: RDT Combination
  - Surveillance: RDT + trypanolysis: Specificity 99%
- Management of serological suspects?
  - Status: HAT, trypano-tolerant or false positive?
- 2nd generation RDTs:
  - Recombinant antigens: Easier & cheaper production
  - Inclusion of invariable antigens:
    Diagnosis of *T.b. rhodesiense*? (Sullivan, 2014)
  - Not yet commercialised & accuracy to be demonstrated

Molecular detection

		Trypanozoon	*T.b.g.*	*T.b.r.*	Sens	Spec	
DNA	PCR	x	x	x	99.0% (92.8-99.9)	97.7% (93.0-99.3)	Reviewed by Mugasa, 2012
	PCR-oligo	x					
	RT-PCR	x					
	LAMP	x	x	x	76.9-93%	92.8-100%	Matovu, 2010; Mitashi, 2013; Mugasa, 2014
RNA	NASBA	x			93.9%	100%	Mugasa, 2014
	SL-RT-PCR	x			92%	96%	Gonzalez-Andrade,2014

Molecular detection

- LAMP (Loop mediated isothermal amplification)
  - Random insertion mobile element (RIME)
  - *Trypanozoon*

A
- 5$

S
- Sensitivity: 77-93%

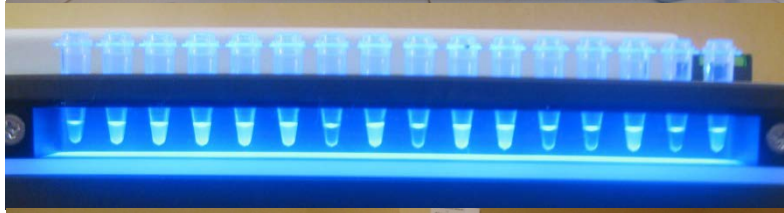
S
- Specificity: 93-100%

U
- Trained technician, micropipettes

R
- 1 hour, thermostable

E
- Incubator, electricity

D
- District/reference hospital level



Pictures: B. Bucheton

Molecular detection challenges

- Training of users is crucial
  - LAMP performers
  - Sample preparation (avoid contamination)
- Improved sensitivity: use of buffy coat
  - Experienced technician for isolation of buffy coat
  - Centrifuge
- Accuracy: to be documented
  - Published: reference labs & on extracted DNA
  - Field evaluation studies ongoing
- Management of molecular suspects?
  - Status: HAT, trypano-tolerant of false positive
- Future role of LAMP
  - Remote testing
  - Diagnostic algorithm?

Parasitology sensitivity (*T.b. gambiense*)

	LNA (lymph node aspirate)	WBF (wet blood film)	TBF (thick blood film)	mHCT (micro hematcrite centrifugation)	mAECT (mini anion exchange centrifugation)	mAECT-BC mAECT on buffy coat)
Miézan, 1994	59%	22%	35%	48%	85%	
Lutumba, 2006	19%	4%	27%	57%	75%	
Camara, 2010	77%				79%	97%
Mumba, 2014	39%			48%	80%	91%
	19-77%	4-22%	27-35%	48-57%	75-85%	91-97%

Parasitology

- Mini-Anion Exchange Centrifugation on Buffy Coat
  - Concentrate trypanosomes in buffy coat
  - Gel retains RBC, not trypanosomes
  - 2 models (Ivory Coast, DR Congo)

A – 6 €
S – Sensitivity: 91-97%
S – Specificity: 100% (assumed)
U – Trained technician
R – 1 hour, thermostable
E – Centrifuge, microscope
D – Mobile team, district/reference hospital level

Parasitology challenges

- Concentration methods recommended
  - Equipment limits implementation in peripheral health centres
  - mAECT:
    - Price (DEAE gel)
    - Vulnerable production: RDC + CI
- Quality control systems needed
  - Recordings

Stage determination

MSC (Miézan, 2000)	0-5 WBC /µl	≥ 6WBC/µl
Trypanosome -	Hemo-lymphatic (1)	Meningo-encephalitic (2)
Trypanosome +	Meningo-encephalitic (2)	Meningo-encephalitic (2)

A
- <0.5€

S
- Sensitivity 93-98%?

S
- Specificity

U
- Invasive

R
- 30 minutes

E
- Microscope

D
- All levels

Treatment outcome

- ~~Systematic follow-up visits with LP at 6, 12, 18 and 24 months post-treatment~~
  - Pentamidine & NECT: low relapse rates
  - Low compliance to FU
- Focus FU on symptomatic patients
  - Trypanosome detection
    - CSF: modified single centrifugation
    - Blood: not to be neglected
  - Evolution of CSF WBC count: 2 step algorithm (Mumba 2010; Priotto, 2012)

ASSURED

WHO (2013): Technical Report Series 984

Criteria for assessing the outcome of treatment for human African trypanosomiasis in patients treated with drugs of known efficacy who present for follow-up

	Stage before initial treatment	
Time of follow-up[a]	First-stage (adapted from reference *102*)	Second-stage (references *121* and *122*)
3 months (0–4)	• T+: Relapse. Treat.	• T+: Relapse. Treat.
6 months (5–9)	• 0–5 CSF–WBC/µl, T–: Cure • 6–20 CSF–WBC/µl, T–: Uncertain evolution Follow–up at 12 months or treatment at the discretion of the clinician, taking into account clinical presentation • > 20 CSF–WBC/µl, T–: Relapse. Treat • T+: Relapse. Treat.	• 0–5 CSF–WBC/µl, T–: Cure • 6–49 CSF–WBC/µl, T–: Uncertain evolution Follow-up at 12 months or treatment at the discretion of the clinician, taking into account clinical presentation • ≥ 50 CSF–WBC/µl, T–: Relapse. Treat. • T+: Relapse. Treat.
≥ 12 months (10– …)	• 0–5 CSF–WBC/µl, T–: Cure • 6–20 CSF–WBC/µl, T–: Uncertain evolution Further follow-up or treatment at the discretion of the clinician, taking into account clinical presentation • > 20 CSF–WBC/µl, T–: Relapse. Treat. • T+: Relapse. Treat.	• 0–20 CSF–WBC/µl, T–: Cure • > 20 CSF–WBC/µl, T–: Relapse. Treat. • T+: Relapse. Treat.

Challenges in staging & treatment outcome

- New markers: CSF neopterin (Tiberti, 2012, 2013)
  - RDT being developed
  - Role in patient management?
- Staging: Decreasing importance
  - Lower toxicity of NECT
  - New drugs
- Diagnosing relapse:
  - Based on lumbar puncture
  - Invasive
- Research on blood markers ongoing

Conclusion

- Rhodesiense HAT: little progress
  - Antigens: sub-optimal accuracy
- Gambiense HAT:
  - Development RDT:
    Facilitate integration of HAT control
    - Performance, cost-effectiveness, implementation strategy
  - Molecular tests:
    - LAMP: approaching patient care level
    - RNA: more accurate
  - Staging: decreasing importance
  - Follow-up: simplified

Conclusion

- Be aware for over-optimism: Not yet «test & treat» scenario:
  - Syndromic algorithms to be evaluated (Palmer,2013)
  - PPV:

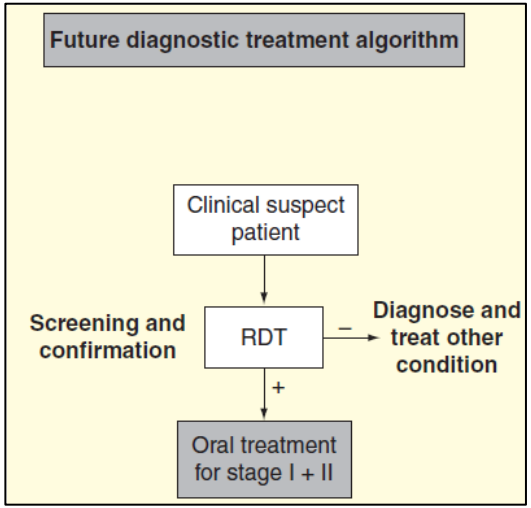
	Prevalence		
Test specificity	0.1%	0.01%	0.001%
90%	1%	0.1%	0.01%
95%	2%	0.2%	0.02%
97%	3%	0.3%	0.03%
99%	9%	1%	0.1%

Moderate intensity transmission

Low to very low intensity transmission

HAT elimination context (WHO,2012)

(Eperon, 2014)



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