Advances and challenges in current diagnostics for HAT

Veerle LEJON
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
Evolving towards the ideal test:

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

Affordable, Sensitive, Specific, User friendly, Rapid & robust, Equipment free, Deliverable
Serology

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

- 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

  - 0.5$ - 1.5 €
  - Sensitivity: 89.6-98.5%
  - Specificity: 87-98.6%
  - Simple
  - 15 minutes, Robust
  - Equipment free
  - Individual, passive screening for *T. b. gambiense*

(Büscher, 2014; RDT test brochures)
Serology

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

  > 8$
  – Sensitivity: 95.1-100%  
    (Van Meirvenne, 1995; Lutumba, 2006; Jamonneau, 2010; Mumba, 2014)
  – Specificity: 100% (Van Meirvenne, 1995)

  – Live cloned *T.b. gambiense*
  – 2.5-3 hours, live infective *T.b. gambiense*
  – Liquid nitrogen, laboratory animals

  – Surveillance, limited to reference labs
    Belgium, Burkina and DR Congo

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Serology challenges

• Training of RDT users crucial, cfr malaria  
(Mukadi, 2013)

• Specificity of RDTs: further evaluation & improvement
  – Individual diagnosis: RDT Combination
  – Surveillance: RDT + trypanalysis: 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

<table>
<thead>
<tr>
<th>Method</th>
<th>Trypanozoon</th>
<th>T.b.g.</th>
<th>T.b.r.</th>
<th>Sens</th>
<th>Spec</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA PCR</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td></td>
<td></td>
<td>Reviewed by Mugasa, 2012</td>
</tr>
<tr>
<td>PCR-oligo</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Matovu, 2010; Mitashi, 2013; Mugasa, 2014</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LAMP</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>76.9-93%</td>
<td>92.8-100%</td>
<td>Matovu, 2010; Mitashi, 2013; Mugasa, 2014</td>
</tr>
<tr>
<td>RNA NASBA</td>
<td>x</td>
<td></td>
<td></td>
<td>93.9%</td>
<td>100%</td>
<td>Mugasa, 2014</td>
</tr>
<tr>
<td>SL-RT-PCR</td>
<td>x</td>
<td></td>
<td></td>
<td>92%</td>
<td>96%</td>
<td>Gonzalez-Andrade, 2014</td>
</tr>
</tbody>
</table>
Molecular detection

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

- 5$
- Sensitivity: 77-93%
- Specificity: 93-100%
- Trained technician, micropipettes
- 1 hour, thermostable
- Incubator, electricity
- District/reference hospital level
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)*

<table>
<thead>
<tr>
<th></th>
<th>LNA (lymph node aspirate)</th>
<th>WBF (wet blood film)</th>
<th>TBF (thick blood film)</th>
<th>mHCT (micro hematocrit centrifugation)</th>
<th>mAECT (mini anion exchange centrifugation)</th>
<th>mAECT-BC (mAECT on buffy coat)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Miézan, 1994</td>
<td>59%</td>
<td>22%</td>
<td>35%</td>
<td>48%</td>
<td>85%</td>
<td></td>
</tr>
<tr>
<td>Lutumba, 2006</td>
<td>19%</td>
<td>4%</td>
<td>27%</td>
<td>57%</td>
<td>75%</td>
<td></td>
</tr>
<tr>
<td>Camara, 2010</td>
<td>77%</td>
<td>77%</td>
<td>79%</td>
<td>97%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mumba, 2014</td>
<td>39%</td>
<td>48%</td>
<td>80%</td>
<td>91%</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Range</strong></td>
<td><strong>19-77%</strong></td>
<td><strong>4-22%</strong></td>
<td><strong>27-35%</strong></td>
<td><strong>48-57%</strong></td>
<td><strong>75-85%</strong></td>
<td><strong>91-97%</strong></td>
</tr>
</tbody>
</table>
Parasitology

• Mini-Anion Exchange Centrifugation on Buffy Coat
  – Concentrate trypanosomes in buffy coat
  – Gel retains RBC, not trypanosomes
  – 2 models (Ivory Coast, DR Congo)
  – 6 €
  – Sensitivity: 91-97%
  – Specificity: 100% (assumed)
  – Trained technician
  – 1 hour, thermostable
  – Centrifuge, microscope
  – 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

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<table>
<thead>
<tr>
<th>MSC (Miézan, 2000)</th>
<th>0-5 WBC /µl</th>
<th>≥ 6WBC/µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trypanosome -</td>
<td>Hemo-lymphatic (1)</td>
<td>Meningo-encephalitic (2)</td>
</tr>
<tr>
<td>Trypanosome +</td>
<td>Meningo-encephalitic (2)</td>
<td>Meningo-encephalitic (2)</td>
</tr>
</tbody>
</table>

- <0.5€
- Sensitivity 93-98%?
- Specificity
  - Invasive
- 30 minutes
- Microscope
- 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)


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

<table>
<thead>
<tr>
<th>Time of follow-up</th>
<th>First-stage (adapted from reference 102)</th>
<th>Second-stage (references 127 and 122)</th>
</tr>
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<tbody>
<tr>
<td>3 months (0–4)</td>
<td>T+: Relapse. Treat.</td>
<td>T+: Relapse. Treat.</td>
</tr>
<tr>
<td>6 months (5–9)</td>
<td>0–5 CSF–WBC/μL, T+: Cure</td>
<td>0–5 CSF–WBC/μL, T+: Cure</td>
</tr>
<tr>
<td></td>
<td>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</td>
<td>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</td>
</tr>
<tr>
<td>≥ 12 months (10–…)</td>
<td>0–5 CSF–WBC/μL, T+: Cure</td>
<td>0–20 CSF–WBC/μL, T+: Cure</td>
</tr>
</tbody>
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Mumba 2010; Priotto, 2012
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:

<table>
<thead>
<tr>
<th>Test specificity</th>
<th>Prevalence</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>0.1%</td>
</tr>
<tr>
<td>90%</td>
<td>1%</td>
</tr>
<tr>
<td>95%</td>
<td>2%</td>
</tr>
<tr>
<td>97%</td>
<td>3%</td>
</tr>
<tr>
<td>99%</td>
<td>9%</td>
</tr>
</tbody>
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HAT elimination context (WHO, 2012)