The development of new diagnostic tools for sleeping sickness

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WHO CC for Research and Training on Human African Trypanosomiasis Diagnosis
Distribution of human African trypanosomosis

- Human African trypanosomosis (HAT) or sleeping sickness is an infectious disease transmitted by tsetse flies
- Sleeping sickness has caused millions of deaths in sub-Saharan Africa in the 20th century

Number of HAT cases detected

Key to success: availability of point-of-care (sero)diagnostics
Traditional diagnostic workup (g-HAT)

Every test can be done at point-of-care but not by everyone and not everywhere

Checchi et al. (2011) PLoS NTD 5: e1233
Futuristic diagnostic workup (g-HAT + r-HAT)

Assumptions

- RDT very specific (whatever biomarker: Ab, Ag, DNA, RNA, metabolite...)
- RDT detects g-HAT and r-HAT
- functional and accessible primary health care centers
- oral treatment is safe and cures both stages of both subspecies
Rapid serodiagnostic tests

- Ab-detection
- only for *T.b. gambiense*
- 1st generation tests
  - native antigens
  - developed for use in fixed health centers
  - commercially available
  - **not appropriate for large scale screening**
    - suboptimal specificity
    - high volume and weight of kits

HAT Sero K-SeT,
Coris BioConcept, Belgium
http://www.corisbio.com/Products/Human-Field/Human-African-Trypanosomiasis.php

SD Bioline HAT,
Standard Diagnostics, South Korea
Rapid serodiagnostic tests

- 2\textsuperscript{nd} generation tests
  - SD and Coris: strip format
  - recombinant antigens
  - \textit{E. coli, S. frugiperda, P. pastoris, L. tarentolae}
  - SD: combination test for HAT and malaria
  - performance in the field yet unknown
  - presentation 0.3.2.16.002 on the recHAT Sero-Strip from Coris BioConcept
Parasitological diagnosis

• **Lymph node aspiration**
  – rapid (± 15 min), cheap, detects HAT cases without trypanosomes in blood

• **micro Hematocrit Centrifugation Technique**
  – rapid (± 25 min), cheap, detects <500 tryps/ml of blood, special reading chamber available, Se ~ 50%

• **mini Anion Exchange Centrifugation Technique**
  – rapid (± 30 min), detects < 30 tryps/ml of blood, Se ~ 80%

• **RBC lysis and AO staining of buffy coat**
  – slow (1 hour), cheap, detects <50 tryps/ml of blood, Se ~50%

• **Modified Single Centrifugation**
  – rapid, relatively cheap, applicable on 4 ml of CSF
LED microscopes

- Battery powered
- Relatively cheap
- Long lifetime of LEDs
- Blue and UV LEDs available for fluorescent microscopy
## Molecular diagnosis

<table>
<thead>
<tr>
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<th>Trypanozoon</th>
<th>T. b. gambiense</th>
<th>T. b. rhodesiense</th>
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<tbody>
<tr>
<td>PCR</td>
<td>TBR, 18S</td>
<td>TgsGP</td>
<td>SRA</td>
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<tr>
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<td>18S</td>
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<td>LAMP</td>
<td>RIME</td>
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<td>RT-PCR</td>
<td>SL-RNA</td>
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- High specificity (at least in theory)
- Limited sensitivity on clinical specimens
- None does better than traditional parasitological examination in microscopy
- **None is applicable at point-of-care**
Stage determination and follow-up

- Still based on cell count and parasite detection in CSF
- Most alternative biomarkers are not specific for HAT
  - CXCL10, osteopontin, neopterin.....
- Only neopterin and SL-RNA bear potential as test-of-cure but
  - follow-up is not recommended anymore in routine practice
  - techniques are not yet applicable at point-of-care
  - both require invasive lumbar puncture
Monitoring elimination and post-elimination

- Immune trypanolysis test (*gambiense*-specific Ab detection)

  - High specificity: positive result = contact with *T.b. gambiense*
  - High analytical sensitivity
  - Limitations: only for *T.b. gambiense*, only done at 3 reference laboratories, time to result: minimum 3 days
  - Useful to monitor *gambiense* parasite presence in humans and reservoir animals

Engstler et al., 2007 *Cell* 131: 1, 505-515
Challenges in development of diagnostics

• How to evaluate sensitivity of new diagnostics when prevalence is close to zero?
• Establishing diagnostic algorithms adapted on current situation in a HAT focus.
• How to reduce the volume of RDTs?
• Developing high throughput molecular tests including nucleic acid extraction at affordable price.
• Improving parasite detection
  – vital fluorescent staining compatible with mAECT
  – microfluidics
• Non-invasive test-of-cure
Challenges in deployment of diagnostics

- Low patient number => loss of expertise
- Accessibility and performance of health centers
- Quality control of diagnostic reagents
- Quality control of diagnostic workup

Diagnosis alone can't do the job
- Vector control
- Drugs
- Sustained funding for HAT control
- Living standard

Courtesy of Epco Hasker, ITM Antwerp
Selected references

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