The Hain test: new reports from South Africa and Tanzania

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GenoType® MTBDRplus test procedure

1) DNA extraction

2) Amplification by PCR

3) Hybridization
   Reverse hybridization of amplified nucleic acids to specific DNA probes bound on strips

4) Evaluation

Specimen Free DNA DNA•STRIP® with specific probes

DNA Isolation

Amplification

DNA•STRIP® with ensuing color formation
Reaction zones of GenoType® MTBDRplus (examples)
FIND-MRC-NHLS South Africa demonstration project

Location of participating NHLS laboratories
South Africa demonstration project

- Prospective cohort of cases/suspects at high risk for MDR-TB
- Four provinces, each with one referral laboratory performing rapid assays
- Rapid assays compared with gold standard (MGIT culture + DST)
- 20,000 sputum specimens from high risk MDR-TB suspects
- Patient management based on rapid assay result
- Conventional DST remains gold standard if discrepancy with rapid assay result

- Validation data from 2 laboratories (WC and Gauteng)
Western Cape

- Estimated TB incidence 932 per 100,000 and TB-HIV co-infection 28% (2001 – 2002)
- MDR rate 3.9% in previously treated cases

- Cape Town NHLS, Greenpoint laboratory
  - Very high lab workload; approx. 400,000 smears, 150,000 MGIT cultures, 50,000 FL DST per annum
  - Up to 48 samples tested per day during Hain validation study
  - Molecular biologist + technologist
  - No previous experience with routine TB molecular testing
MTBDRplus validation in Cape Town

Pre-demonstration validation study in Cape Town laboratory (n = 536)

<table>
<thead>
<tr>
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<th>Rifampicin</th>
<th>Isoniazid</th>
<th>Multidrug-resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>98.9% (94/95)</td>
<td>94.2% (114/121)</td>
<td>98.8% (85/86)</td>
</tr>
<tr>
<td>Specificity</td>
<td>99.4% (357/359)</td>
<td>99.7% (330/331)</td>
<td>100% (362/362)</td>
</tr>
<tr>
<td>Overall accuracy</td>
<td>99.3% (451/454)</td>
<td>98.2% (444/452)</td>
<td>99.8% (447/448)</td>
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<tr>
<td>PPV</td>
<td>97.9% (94/96)</td>
<td>99.1% (114/115)</td>
<td>100% (85/85)</td>
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<tr>
<td>NPV</td>
<td>99.7% (357/358)</td>
<td>97.9% (330/337)</td>
<td>99.7% (362/363)</td>
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97% specimens gave interpretable results, including 95% of specimens with contaminated MGIT cultures
Gauteng

- Estimated TB incidence 670 per 100,000 and TB-HIV co-infection 64% (2001 – 2002)
- MDR rate 5.5% in previously treated cases
- Johannesburg NHLS, Braamfontein
  - High workload; approx. 100,000 smears, 125,000 cultures and 12,000 FL DST per annum
  - Molecular biologist + technologist
  - Previous routine molecular testing
MTBDR<em>plus</em> validation in Johannesburg

- Pre-demonstration validation study in Johannesburg laboratory (n = 576)
  - Results of Hain and conventional DST for 460 specimens

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<td>Sensitivity</td>
<td>91.2% (52/57)</td>
<td>90.8% (59/65)</td>
<td>98.0% (49/50)</td>
</tr>
<tr>
<td>Specificity</td>
<td>98.7% (293/297)</td>
<td>99.7% (288/289)</td>
<td>99.7% (297/298)</td>
</tr>
<tr>
<td>Overall accuracy</td>
<td>97.5% (345/354)</td>
<td>98.0% (347/354)</td>
<td>99.4% (346/348)</td>
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<tr>
<td>PPV</td>
<td>92.9% (52/56)</td>
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<td>NPV</td>
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- 86% specimens overall and 93% of specimens with interpretable conventional DST gave interpretable results
  - 9% specimens contained MOTTs
KwaZulu Natal province: IALCH NHLS Laboratory, Durban
• High level MDR-TB and HIV
• 90,000 smear + MGIT and 45,000 DST pa
• Training and validation of Hain test in progress

Northern Cape province: Kimberley NHLS Laboratory
• Very large rural province
• Approx 75,000 smears, 35,000 MGIT and 8000 FL DST pa)
• Training and validation of Hain test in progress
SA demonstration project - enrolment

- >5000 patients at risk for MDR-TB have been enrolled to date
- Approx 15% MDR in specimens tested
- Preliminary performance cf. conventional DST is similar to validation studies
Evaluation of the Hain Lifescience MTBDRplus test

• Tanzania samples
  – WHO, Tanzania NTP, KNCV, ITM
Methods

• Prospective collection
  – all smear-positives from drug resistance survey
  – 6 drops sputum in 1.5 ml ethanol 50% in water
  – besides sputum sent for culture and DST

• Selection forwarded to Antwerp SRL for testing
  – original sputum from strains sent for survey quality assurance: all retreatment cases + all resistant to FLD + equal number susceptible
  – here discussed: first batch only

• Kit protocol followed, except Boom extraction
  – better bands in pilot
Results

• 104 sputa analysed
  – very small volumes
  – 2 DNA extraction failure, 11 PCR failure
  – 91 (88%) good result
• rpoB: 100% agreement with strains received
  – 59/59 wildtype
  – 2/2 mutations (Mut 2; Mut 3)
• katG, inhA: 92% agreement
  – 50/55 katG & inhA wildtype
  – 6/6 with katG mutation
  – (1 inhA mutation, no DST result)
Progress of FIND Demonstration Projects - Hain MTBDRPlus

Completed
Validation studies in South Africa (Cape Town and Johannesburg)

Hain MTBDRPlus demonstration projects:
Training and validation in Durban and Kimberley in progress
Four sites in South Africa; 20,000 MDR suspects
Planning for implementation in Kenya and Russia.
Discussing possible studies in Nepal, Uzbekistan, Philippines, Indonesia, Thailand, Vietnam.

Completed
Preparation of interim study report for WHO
Presentation to *ad hoc* review board of WHO sub working group on laboratory capacity strengthening

Ongoing
Formal STAG review
WHO endorsement
ROLL OUT
Conclusions

• Overall performance characteristics of MTBDRplus assay are superior to conventional culture and DST in validation studies

• Assay can be successfully implemented routinely in high burden settings

• Assay is amenable to high throughput testing

• Laboratory capacity strengthening is critical

• Demonstration projects will assess feasibility, impact and cost-effectiveness

• Rapid MDR assays have the potential to revolutionize TB diagnostics
Acknowledgements

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