Development and Evaluation of Point-of-Care Diagnostic Tool for Human Brucellosis

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What’s Brucellosis?

- Facultative, gram-negative coccibacillus
- Zoonotic
  - *B. melitensis*
  - *B. suis*
  - *B. canis*
  - *B. abortus*
- Highly contagious
  - Fluids from animals
  - Aerosol inhalation
  - Consumption of unpasteurized cheese, milk, and undercooked meat
  - Poses occupational risk for shepherds, abattoir workers, veterinarians, dairy industry, and lab personnel
- Endemic in Mediterranean basin, Middle East, Central Asia, China, the Indian subcontinent, sub-Saharan Africa, and parts of Mexico and Central and South America.
Clinical Considerations

- Incubation period: 2-4 weeks (but can be several months)
- Acute: fever, myalgias, malaise, night sweats (malodorous), headache, abdominal pain, cough, arthralgias, anorexia
  - Lymphadenopathy, hepatomegaly, splenomegaly
  - Labs: transaminitis, leukopenia, lymphocytosis, mild anemia, thrombocytopenia
- Focal complications: arthritis, sacroiliitis, spondylitis, osteomyelitis, epididymo-orchitis, spontaneous abortion, meningitis, brain abscess, endocarditis (main cause of mortality)
- Chronic: clinical manifestations >1 year after diagnosis is established
  - Usually localized infection
- Relapse following treatment: 5-15%
Current Diagnostic Methods

- **Gold standard: blood or bone marrow culture**
  - Need BSL-3 for culture, variable sensitivity, long incubation
  - Usually made using clinical symptoms + serology

- **Serology**
  - E.g. qualitative slide agglutination test
  - No differentiation between acute, relapsed, chronic, or past resolved infection
  - Cross-reacts with other bacteria
  - Immune response in humans is highly variable

- **Nucleic acid amplification tests (NAATs) – most sensitive and specific**
  - May also stay positive despite antibiotic therapy
  - Generally require a reference laboratory
  - No standardization, no sufficiently validated commercial tests.

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Recombinase Polymerase Amplification (RPA)

- Isothermal (37-42 °C) DNA amplification technique
- Rapid (15-30 min)
- Sensitivity comparable to PCR
- Adaptable to lateral flow (LF) platforms

RPA Cycle, TwistDx
RPA Cycle

- Recombinases associate with primers
RPA Cycle

- Recombinases associate with primers
- Recombinases catalyze strand exchange
RPA Cycle

- Recombinases associate with primers
- Recombinases catalyze strand exchange
- Polymerases synthesize daughter strands while single-stranded binding proteins (SSB) stabilize ssDNA
Optimization for Lateral Flow

- Fluorophore-tagged probe included in reaction

Li et. al. Analyst (2019)
Optimization for Lateral Flow

- Fluorophore-tagged probe included in reaction
- Biotin-tagged primer

Li et. al. *Analyst* (2019)
Optimization for Lateral Flow

- Fluorophore-tagged probe included in reaction
- Biotin-tagged primer
- Fluorophore-tagged probe forms a truncated amplicon along with biotin-tagged primer
  - Truncated, double-tagged amplicons exit the amplification cycle and accumulate

Li et. al. Analyst (2019)
Optimization for Lateral Flow

- Dilute RPA reaction in buffer containing gold nanoparticles coated with anti-fluorophore antibodies
Optimization for Lateral Flow

- Dilute RPA reaction in buffer containing gold nanoparticles coated with anti-fluorophore antibodies
- Run on a lateral flow strip with anti-biotin test and control lines
Optimization for Lateral Flow

- Dilute RPA reaction in buffer containing gold nanoparticles coated with anti-fluorophore antibodies
- Run on a lateral flow strip with anti-biotin test and control lines
- Total time: < 1 hour

RPA nfo, TwistDx
Test results, UStar biotechnologies
Real Time RPA (RT-RPA)

TwistAmp™ exo Probe
Exonuclease cuts THF residue

THF residue
Quencher
3’ block
Fluorophore
Nuclease

RPA exo, TwistDx
**Brucella spp. RPA**

- **Target:** *bcsp31* gene
- **18 Brucella spp. tested positive:** *B. abortus, B. melitensis, B. suis, B. canis, B. neotomae, B. ovis*, and four Brucella vaccine strains
- **4 non-Brucella species tested negative:** *E. coli, P. multocida, S. suis*, and *P. aeruginosa*

**Table 4 RPA primers and probes used in this study.**

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence (5’–3’)</th>
<th>Genome location(CP007763.1)</th>
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</thead>
<tbody>
<tr>
<td>Bru. RPA F4</td>
<td>TGCATCCCGGCGCAGAACGCTTTTACAAGGAA</td>
<td>639988–639957</td>
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<tr>
<td>Bru. RPA R1</td>
<td>ATAACGAGCTGCGCAAATGTCAACCTCTCTAA</td>
<td>639873–639904</td>
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<tr>
<td>Bru. RPA P1</td>
<td>GCGGGCGTGACTGAATAAATCCCTCAATGA-(FAM-dT)-THF-GG-(BHQ1-dT)-TCCTGATATCTTA (C3 Spacer)</td>
<td>639956–639910</td>
</tr>
</tbody>
</table>

**Brucella spp. RPA-LF Does Not Detect Serologically Cross-Reactive Bacteria**

<table>
<thead>
<tr>
<th>Negative Control</th>
<th>B. suis biovar 1</th>
<th>B. suis biovar 2</th>
<th>B. abortus biotype 4</th>
<th>B. abortus biotype 9</th>
<th>B. abortus RB51</th>
<th>B. melitensis biotype 1</th>
<th>E. coli</th>
<th>E. chaffeensis</th>
<th>Y. enterocolitica</th>
<th>F. tularensis</th>
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</thead>
<tbody>
<tr>
<td>Positive Control</td>
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<td>+</td>
<td>-</td>
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</tr>
</tbody>
</table>
**Brucella spp.** RPA-LF can detect a single *Brucella spp.* genome
**Lower Limit of Detection**

**B. abortus genomes**  

<table>
<thead>
<tr>
<th>B. abortus genomes</th>
<th>Test Positive</th>
<th>Test Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
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<tr>
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<td>9</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>0</td>
</tr>
</tbody>
</table>

Limitations and Future Development

Limitations

- Optimization for DNA Extraction for use in assay will take longer to complete
- Lack of a BSL 3 lab means clinical validation must be done elsewhere

Future Experimentation

- Potential DNA Extraction Techniques
  - Optimized DNA extraction with Oscillating multi-tool with 3D printed adaptor
- Clinical Validation
  - Possible future work with Epicentre Uganda with facilitating pilot studies in rural southwestern districts

Distribution of bovine livestock in Uganda. Blue boxes highlight the proposed study districts.

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