Clinical Application of New Treponemal Antibody Test in Blood Donors

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Introduction

- Syphilis – infectious disease - caused by the spirochete: *Treponema pallidum*

- Route of transmission
  - Sexual contact
  - Mother to fetus in utero
  - Blood transfusion
Reported case of transfusion-transmitted Syphilis

- Kilduffe and DeBakey (1942) – referenced more than 100 cases of transfusion-transmitted syphilis - from direct transfusion

- In 1966 – Lymphoma patient who received 25 units of fresh platelets and 5 units of RBC (non-reactive VDRL for all donations)

- Report in 1983 from The Netherlands

  The infant girl acquired Syphilis from exchange transfusion with fresh whole blood in 1977.

  Donor – seronegative at the time of donation and investigation showed that he became infected shortly before donation.

Ref. Schmidt P, Transfusion 2001;41:1069-1071
Prevention of transfusion transmitted syphilis

- Donor deferral for behaviors related to syphilis
- Physical exam- for signs of primary syphilis
- Screening test
- Stored blood – fastidiousness of syphilis

Spirochetemia – cleared before antibodies are detectable

Ref: Transfusion 2009;49:617-619
Concerned about Syphilis and blood transfusion

- Syphilis is concerned harmful to patient – from evidence of transfusion-transmitted syphilis reported cases (direct transfusion)

- Syphilis can be inactivated by refrigerated blood for 72 - 96 hours – fastidious of T. pallidum

- Syphilis as STD – concerned about HIV
Screening test – for syphilis

- **Wassermann test** – 1906
  Wassermann, Neisser and Bruck developed complement-fixation test

- VDRL -1946 Harris, Rosenberg and Riedel developed Venereal Disease Research Laboratory (VDRL) test

- RPR -1961 Portnoy et al. modified unheated serum reagin to create the rapid plasma reagin (RPR)
Treponemal tests

- **TPHA** - 1965 Rathlev developed the haemagglutination test for syphilis (TPHA)
- **TPPA** – 1998 Young et al. reported on a latex agglutination test using cloned treponemal antigens
- **EIA** – 1990 – EIA test for antibody to Treponema pallidum (Jean Claude Lefevre)
- **CMIA** -
Our blood bank

- We collect blood 100-200 units/day
- We test donated blood for HBsAg, HIV Ag/Ab, anti-HCV and syphilis screening test
- Then sero-negative donations – test for HIV/HBV/HCV Nucleic acid

We want to test all serological infectious markers on one automated system.

Test: HBsAg, Anti-HCV, HIV combo, Syphilis
ARCHITECT /2000<sub>sr</sub> Solution
( Immunoassay Module )

- **Adapts to different workloads**
  - High throughput
    - up to 200 tests/hour
    - With 5-hour walk-away time
  - Integration
    - Links with c8000 \(\rightarrow\) ci8200

- **Multi-Dimensional Sampling enhances laboratory workflow**
  - Fast TAT & High Throughput
  - Immediate STAT (35 samples)
  - Continuous Access to samples (135 samples)
  - Intuitive sample indicator lights
  - Carrier holds the majority of tube types (5 to 25 samples)

- **Patented technologies provide confidence in results**
  - Minimizes interferences with **Smartwash**
  - **Clot detection** delivers accurate sampling
  - **Chemflex<sup>TM</sup>** technology provides flexible protocols, linearity, sensitivity
  - **Software** is common to all platforms & easy to use
ARCHITECT Syphilis TP Assay Fundamentals

- Utilises 3 recombinant antigens which are attached to the exterior of the cytoplasmic membrane via lipid anchors and are dominant in the immune response to *T. pallidum*
  - TpN47
  - TpN17
  - TpN15
  
  During primary syphilis, the earliest responses are against TpN47 and some of the flagellar proteins, followed by TpN15 and TpN17.

- Anti-human format (anti-IgG/IgM)
- Sample (30 μl), ASD (90 μl), Microparticles (50 μl)
- Uses reagents common to other ARCHITECT immunoassays
ARCHITECT Syphilis TP Assay Protocol

2-Step sandwich Assay

Sample IgG, IgA ja IgM (black = Syphilis TP Ab)

Recombinant TpN47, TpN7 ja TpN15 antigen (E. coli) coated microparticles.

incubation 18 min

Syphilis antibodies bind to immobilised antigens.

Removal of non-bound sample.

Anti-human IgG/IgM: Acridinium ester conjugate (murine)

incubation 4 min

Conjugate binds to antibodies bound to solid phase.

Signal is related to Syphilis specific antibodies in sample. Results exceeding the calibrator signal are considered positive.
### ARCHITECT Syphilis TP
Summary of Performance Data

<table>
<thead>
<tr>
<th></th>
<th>Product development study</th>
<th>Feasibility/Verification Study</th>
<th>Package insert</th>
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</thead>
<tbody>
<tr>
<td><strong>Specificity</strong></td>
<td></td>
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<tr>
<td>Donor population</td>
<td><strong>99.75%</strong> <em>(8144/8165)</em></td>
<td><strong>99.80%</strong> <em>(4990/5000)</em></td>
<td><strong>99.78%</strong> <em>(1796/1800)</em> vs. TPPA &amp; FTA-ABS</td>
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<tr>
<td></td>
<td></td>
<td><strong>IRR – 0.19</strong></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td><strong>RRR – 0.18</strong></td>
<td></td>
</tr>
<tr>
<td>Diagnostic population</td>
<td><strong>99.81%</strong> <em>(536/537)</em></td>
<td><strong>99.6%</strong> <em>(562/564)</em> vs TPPA</td>
<td><strong>99.71%</strong> <em>(694/696)</em> vs. TPPA &amp; FTA-ABS</td>
</tr>
<tr>
<td><strong>Sensitivity</strong></td>
<td></td>
<td><strong>100%</strong> <em>(426/426)</em> vs TPPA</td>
<td><strong>100%</strong> <em>(131/131)</em> vs. TPPA &amp; FTA-ABS</td>
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## Prevalence of positive syphilis screening test

<table>
<thead>
<tr>
<th>Year</th>
<th>Test</th>
<th>Prevalence</th>
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<tbody>
<tr>
<td>2541</td>
<td>VDRL</td>
<td>0.91 (TPHA + 0.26%)</td>
</tr>
<tr>
<td>2542</td>
<td>VDRL</td>
<td>0.62 (TPHA + 0.34%)</td>
</tr>
<tr>
<td>2543</td>
<td>VDRL</td>
<td>0.67 (TPHA + 0.63%)</td>
</tr>
<tr>
<td>2544</td>
<td>Treponemal antibody</td>
<td>1.28 (TPHA + 0.92%)</td>
</tr>
<tr>
<td>2545</td>
<td>Treponemal antibody</td>
<td>0.88 (TPHA + 0.68%)</td>
</tr>
<tr>
<td>2546</td>
<td>Treponemal antibody</td>
<td>0.66 (TPHA + 0.60%)</td>
</tr>
<tr>
<td>2547</td>
<td>Treponemal antibody</td>
<td>0.85</td>
</tr>
<tr>
<td>2548</td>
<td>Treponemal antibody</td>
<td>0.68</td>
</tr>
<tr>
<td>2549</td>
<td>Treponemal antibody</td>
<td>0.85</td>
</tr>
<tr>
<td>2550</td>
<td>Treponemal antibody</td>
<td>0.70</td>
</tr>
<tr>
<td>2551</td>
<td>Treponemal antibody</td>
<td>0.64</td>
</tr>
<tr>
<td>2552</td>
<td>Treponemal antibody</td>
<td>0.50</td>
</tr>
</tbody>
</table>

*ARCHITECT Syphilis TP*
Correlation with history

- From 50 cases that came for follow up during 2008-2009

  Benefit to 44 from 50 cases
  Only 6 from 50 cases – known infection and completely treated
Special situation in Thailand

- 20 years ago - men had sex with commercial sex worker, got sexually transmitted disease (STD)
- They seek for medical treatment if they ever had symptoms of STD
- After married – no more commercial sex and become family men…
- All pregnant women were screened for Syphilis infection.
- Majority of these men’s wives were treated for Syphilis if they were infected before pregnant.
Problems

- Angry wives
- Cannot accept these donors for further donation
- Discordance with syphilis testing result from other institutes (if they use VDRL or RPR)
Our experiences

- New tests – benefit for epidemiologic study
- Must implement with counseling service
- Re-entry protocol – must be possible, we don’t want to lose good donors.
- We do lose good donors.
Meeting and discussion about Syphilis screening test for blood donors
January 2009

organized by – National Blood Centre Thai red cross society

- Sharing opinion and data from several blood centers and clinician (STD expert)
- We discuss about donor re-entry if they were completely treated.
- Cooperate with Bangrak Hospital for Syphilis treatment.
Re-entry protocol

- Criteria
  - positive treponemal antibody screening test with TPHA/VDRL
  - Treat - complete course
  - letter from physician
Syphilis – concern of blood center

- Loss of safe donor – past syphilis infection
- Screening test positive – infectious for syphilis or not
- Benefit
  - donor / family
  - Country – control syphilis
  - Blood safety ??
- Workload for notification and counseling
Conclusion

- Syphilis screening test – need to do in Thailand
- Data from donation .... benefit...
  - epidemiologic study
  - control of syphilis in Thailand - find untreated cases/partially treated

- We had to trade off
  - Loss of good donor
  - Angry donor/wife
  - Counseling effort
Thank you

- Mr. Tarin Pakdee, for photo of tests
- Khun Payom Darepatra – Donor manager
- Khun Wariya Panchavinnin and team (Infectious Screening Laboratory – Siriraj Blood Bank)
- Abbott Laboratories Limited
Thank you for your attention