Validation of a New Testing Algorithm for Syphilis in Trinidad & Tobago

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Contents

§ Trinidad STI Clinic
§ Diagnosis of Syphilis in Trinidad & Tobago
§ Objective of Validation
§ Methodology
§ Results – QPCC&C/CDC
§ Implementation
§ Conclusion
§ Acknowledgement
STI Clinic – Trinidad & Tobago

• Queen’s Park Counselling Centre and Clinic (QPCC & C)
  • 2 main clinics (north & south)
  • 9 satellite clinics

• Clinic services include:
  • Diagnosis and treatment of STI patients and their partners
  • Walk-in rapid HIV testing and counselling
  • Syphilis testing for employment

• Laboratory services include:
  • Detection of syphilis infection in pregnant women, high risk groups, etc.
  • Operates as a Reference point for testing of Syphilis within national laboratory network

• Quality Assurance program
  • Oneworld Accuracy Program (3 test events per year)
## QPCC & C Statistics

<table>
<thead>
<tr>
<th>YEAR</th>
<th>TPHA NEGATIVE</th>
<th>TPHA POSITIVE</th>
<th>TPHA INDETERMINATE/INCONCLUSIVE</th>
<th>TPHA TOTAL</th>
<th>TPPA NEGATIVE</th>
<th>TPPA POSITIVE</th>
<th>TPPA TOTAL</th>
<th>LATENT SYphilis</th>
<th>INFECTIOUS SYphilis</th>
<th>TOTAL</th>
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<td>398</td>
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</table>
Diagnosis of Syphilis – Trinidad & Tobago

• Syphilis is a sexually transmitted infection caused by the spirochete treponema pallidum

• May also be transmitted from mother to fetus during pregnancy or at birth, resulting in congenital syphilis

• There are two commonly used approaches to serological diagnosis of syphilis:
  1. The traditional algorithm
  2. The reverse-sequence algorithm

• The decision to use either algorithm is based on syphilis prevalence, the expected workload, the requirement for automation, and the available budget for labour and consumables, as well as the EMTCT program which started in 2013.
Syphilis Tests

- Serological Tests
  - Nontreponemal Tests
    - VDRL
    - RPR
    - TPHA
  - Treponemal Tests
    - TPPA
    - ELISA
    - FTA-ABS
- Microscopic Examination
  - Dark field Microscopy
Diagnosing Syphilis – Trinidad & Tobago

• Currently QPCC&C uses serological testing following the traditional algorithm. The screening test is the VDRL test.
• Reactive samples are quantified, followed by confirmation with the TPHA assay.
• Result interpretation is as follows:

<table>
<thead>
<tr>
<th>VDRL</th>
<th>TPHA</th>
<th>Interpretation</th>
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<tbody>
<tr>
<td>Reactive</td>
<td>Positive</td>
<td>Current/past exposure to syphilis</td>
</tr>
<tr>
<td>Reactive</td>
<td>Negative</td>
<td>False positive</td>
</tr>
<tr>
<td>Non-Reactive</td>
<td>Positive</td>
<td>False Negative</td>
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</table>
Several limitations have been identified when applying the traditional algorithm in diagnosing syphilis:

- False positive VDRL test result due to various medical conditions, e.g. autoimmune diseases, pregnancy, etc.
- False negative VDRL test result as a result of the prozone phenomenon.
- Interpretation of test results is subjective and rely on staff competency.
- Difficulty in the identification of persons previously treated for syphilis, those with untreated or incomplete treated syphilis as these individuals may have non-reactive VDRL test result.
Objective of the Validation

• Due to significant limitations, an alternative approach for testing and diagnosis became necessary. The British Association of Sexual Health and HIV (BASHH) team suggested a different approach.
  • **The Reverse Sequence Algorithm**

• As such a validation protocol was developed and implemented.

• The objectives for validating this new algorithm were:
  • To decrease the ergonomic stress of pipetting large numbers of samples
  • To increase detection rate of early and latent syphilis
  • To increase sample processing
  • To decrease turnaround time
Methodology

**Test Kit Selection**

- Bio-Rad Syphilis EIA II test kit, the BD VDRL Antigen test kit, and the Serodia TPPA test kit

- Test kits selected for use in the algorithm was based on:
  - Sensitivity
  - Specificity
  - Intended use of kit
  - Availability
<table>
<thead>
<tr>
<th>Name of Test Kit</th>
<th>What the kit detects</th>
<th>Type of Sample to be used</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
<th>Collection Medium</th>
<th>Objectivity of Test</th>
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<tbody>
<tr>
<td>BD VDRL Antigen with Buffered Saline</td>
<td>Reagin Antibodies</td>
<td>* Serum  * CSF</td>
<td>93.3 - 95.2</td>
<td>92.7 - 98.9</td>
<td>* Red top tubes  * Dry tube without anticoagulant</td>
<td>Screening and clinical monitoring</td>
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<td>Treponema pallidum Antibodies</td>
<td>* Serum  * Plasma</td>
<td>100</td>
<td>99.4</td>
<td>* Red top tubes  * Sodium citrate, heparin, or EDTA coagulated tubes</td>
<td>Confirmation</td>
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<td>Serodia - TP.PA</td>
<td>Treponema pallidum Antibodies</td>
<td>* Serum  * Plasma</td>
<td>90</td>
<td>100</td>
<td>* Red top tubes  * Sodium citrate, heparin, or EDTA coagulated tubes</td>
<td>Confirmation</td>
</tr>
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</table>
Methodology

Sample Selection

• Sample pool originated from high risk groups based on prevalence rate for the period February to July 2012
  • HIV patients – 6.1%
  • STI patients – 2.9%
• The ethics committee of the MOHTT gave ethical approval for the use of these samples for the process
• 65 samples were tested using the Bio-Rad Syphilis EIA II, VDRL, and TPPA assays
• As per CDC’s request 40 of those samples were shipped to the International Laboratory Branch, Centers for Disease Control and Prevention, Atlanta to be tested using Trep-Sure (EIA), RPR, and TPPA
  • 11 positives (EIA, VDRL, and/or TPPA reactive)
  • 29 negatives (EIA)
## Results – CDC and QPCC & C

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Trep-Sure</th>
<th>Od 450</th>
<th>TPPA</th>
<th>Qual-RPR</th>
<th>Quan-RPR</th>
<th>Trini-Elisa</th>
<th>Trini-VDRL</th>
<th>VDRL Titer</th>
<th>Trini-TPPA</th>
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</table>
### Results – CDC and QPCC & C

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Trep-Sure</th>
<th>Od 450</th>
<th>TPPA</th>
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<th>Trini-Elisa</th>
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</table>
Results – Interpretation

• International Laboratory Branch, Centers for Disease Control and Prevention, Atlanta confirmed that the results obtained were 100% concordant between both laboratories

• Validation process based on results obtained was successful
General Diagnosis of Syphilis

ELISA (IgG, IgM, IgA)

- Reactive
- Non-Reactive

Venereal Disease Research Laboratory (VDRL) (Quantitative)

- Reactive
- Non-Reactive

ELISA: Reactive
VDRL: Reactive

REPORT:
Consistent with treponemal infection. Record as treated case.

ELISA: Reactive
VDRL: Non-Reactive

REPORT:
Discordant results. Further testing and confirmation.

Perform Treponema pallidum particle agglutination (TPPA)

ELISA: Reactive
VDRL: Non-Reactive
TPPA: Negative

REPORT:
Syphilis unlikely. If at risk for infection, repeat after 2-4 weeks.

ELISA: Reactive
VDRL: Non-Reactive
TPPA: Positive

REPORT:
Previously treated/untreated syphilis infection.

For suspected cases of Neurosyphilis please send a CSF sample for VDRL in addition to serological samples.
Implementing New Syphilis Algorithm Nationally

- Obtain permission/sign off from the Chief Medical Officer in Trinidad & Tobago for the application of the newly developed algorithm to be used in health institutions across the country.
- Nationwide sensitization of medical staff in relation to the new validated syphilis algorithm.
- Validation and implementation of rapid syphilis testing in antenatal clinics, STI clinics, and labour wards.

- Biggest challenge during study
  - MOHTT procurement process
Conclusion

• The validation of these test kits and reverse algorithm in Trinidad and Tobago will allow the national STI reference laboratory to increase its throughput and offer an improved service to the population.

• The validation of these test kits and algorithm will provide for:
  • Higher throughput
  • Less occupational hazard
  • Probability of false negatives decreased significantly if not cease altogether
  • Validation and nationwide use of Rapid Syphilis tests in antenatal clinics, etc.
  • Foundation for development of a children’s diagnostic algorithm
  • An approach for diagnosis of suspected neurosyphilis
  • Possibility of decentralization some aspect of syphilis laboratory testing/screening
Acknowledgement

• Staff and Patients of Queen’s Park Counseling Centre & Clinic
• Staff of the HIV and AIDS Coordinating Unit
• CDC Caribbean Regional Office Laboratory Strengthening team
• EMTCT TWG, Pan American Health Organization
• Shawn Kirk, Technician at Trinidad Public Health Laboratory
• Medical Research Foundation, Trinidad & Tobago
• Ministry of Health, Trinidad and Tobago
• International Laboratory Branch, CDC Atlanta
THANK YOU
Questions

1. The test kits selected for use in the algorithm was based on:
   a) Specificity
   b) Sensitivity
   c) Intended use of kit
   d) Availability
   e) All of the above

2. Which of the following is NOT a treponemal test?
   a) TPHA
   b) RPR
   c) TPPA
   d) FTA-ABS