# Performance of a Rapid, 60 Second Multiplex Test for Simultaneous Detection of Antibodies to HIV-1, HIV-2 and T. pallidum in Serum, Plasma and Whole Blood

*R. Galli, CDC Diagnostics Conference, Atlanta, March 2016*

bioLytical LABORATORIES

# Review of INSTI® Immunofiltration Platform Attributes

## INSTI HIV-1/HIV-2 Antibody Test


## INSTI HIV Assay Characteristics

| Performance Attribute | INSTI HIV-1/HIV-2 Antibody Test |
|---|---|
| HIV 1 | Yes |
| HIV 2 | Yes |
| Time to Results | 1 minute |
| FDA approved | Yes |
| CLIA Waived | Yes |
| Assay description | IgM/IgG antibody sensitive flow-through rapid immunoassay |
| Early Detection | 20-21 days post infection |
| Specimen Volume | 50 µL |

# INSTI Multiplex HIV-1/HIV-2/Syphilis Antibody Test Development Objectives

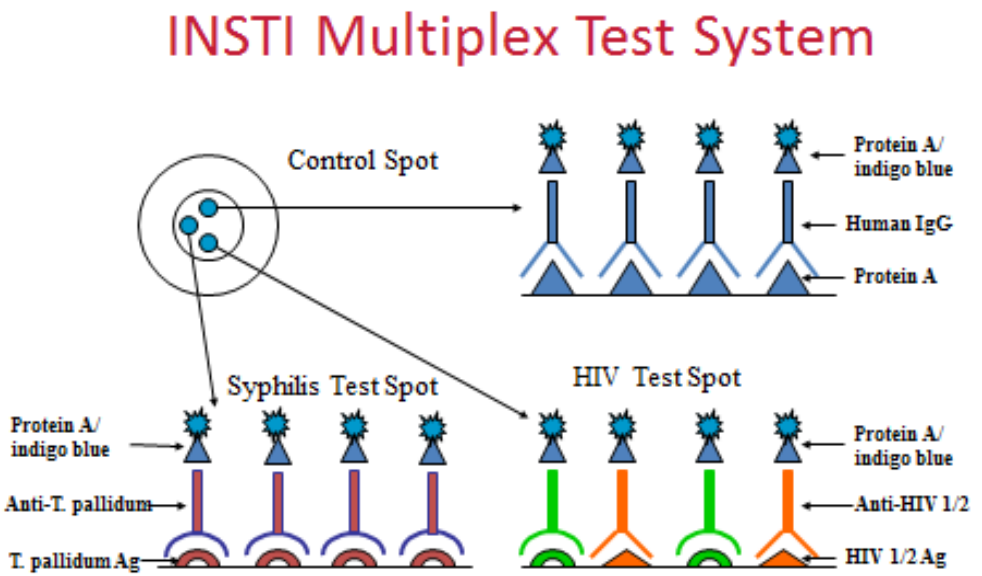
**Developed in response to growing need for integrated POC testing devices for sexually transmitted blood-borne infections (STBBI).**

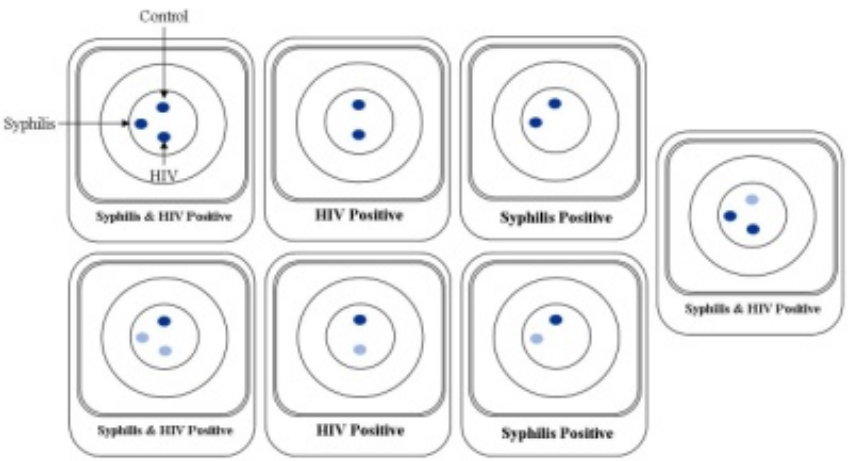

- **Determine the performance of the T. pallidum assay in the INSTI Multiplex compared to reference methods for detection of T. pallidum antibodies.**
- **Preserve all design and performance attributes of the INSTI HIV-1/HIV-2 antibody assay**
- **Achieve performance attributes for T. pallidum similar to HIV**

# INSTI MULTIPLEX HIV-1/HIV-2/Syphilis Antibody Test

The **INSTI MULTIPLEX HIV-1/HIV-2/Syphilis Antibody Test** is a single use, rapid, flow-through *in vitro* qualitative immunoassay for the detection of antibodies to Human Immunodeficiency Virus Type 1, Type 2 and Treponema Pallidum in human EDTA-whole blood, fingerstick blood, serum or EDTA-plasma.

**Multiplex is currently CE marked for sale and distribution in EU member countries; undergoing full dossier review in the WHO prequalification process.**



# INSTI Multiplex Performance Data

# Sensitivity of INSTI Multiplex for Detection of T. pallidum antibodies in a commercial syphilis mixed titre panel*.

| | Panel Member ID Number | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| COMPARATOR ASSAYS | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** |
| Phoenix Biotech Trep Sure | P | P | P | N | N | N | N | N | P | P |
| Trinity Captia IgG | P | P | P | N | N | N | N | N | P | P |
| Trinity Captia IgM | N | N | N | N | N | N | N | N | P | N |
| Fisher Sureview RPR | P | P | P | N | P | P | P | N | P | P |
| Serodia-TP-PA | P | P | P | N | N | N | N | N | P | P |
| **INSTI Multiplex HIV-1/HIV-2/Syphilis Antibody Test** | **P** | **P** | **P** | **N** | **N** | **N** | **N** | **N** | **P** | **P** |

* ZeptoMetrix Corporation

INSTI T. pallidum antibody detection was identical to other T. pallidum reference assays

# INSTI Multiplex Performance Data

## INSTI Multiplex T. pallidum antibody positive percent agreement with TP-PA assay, n=145 serum/plasma

| INSTI Multiplex T. pallidum Antibody | TP-PA Final Interpretation | | |
|---|---|---|---|
| | Positive | Negative | Totals |
| Reactive | 138 | 0 | 138 |
| Non-Reactive | 7 | 0 | 7 |
| Totals | 145 | 0 | 145 |

The calculated positive percent agreement between INSTI Multiplex T. pallidum antibody detection and the approved TP-PA assay is **95.2%** (138/145 = 95.2%, with 95% CI 90.4-97.6%).

## INSTI Multiplex T. pallidum antibody negative percent agreement with TP-PA, n=379 serum/plasma

| INSTI Multiplex T. pallidum Antibody | TP-PA Final Interpretation | | |
|---|---|---|---|
| | Positive | Negative | Totals |
| Reactive | 0 | 5 | 5 |
| Non-Reactive | 0 | 374 | 374 |
| Totals | 0 | 379 | 379 |

The calculated negative percent agreement between INSTI Multiplex T. pallidum antibody detection and the approved TP-PA assay is **98.7%** (374/379 = 95.2%, with 95% CI 90.4-97.6%).

# Performance of the INSTI Multiplex HIV 1/HIV2/Syphilis Antibody Test compared to TP-PA for Contrived Whole Blood Specimens (n=169).

| INSTI Multiplex T. pallidum Antibody | TP-PA Final Interpretation | | |
|---|---|---|---|
| | Positive | Negative | Percent Agreement |
| Reactive | 62 | 0 | Positive percent agreement **96.9%** (62/64) |
| Non-Reactive | 2 | 105 | Negative percent agreement **100%** (105/105) |
| Totals | 64 | 105 | 169 |

# Multiplex T. pallidum antibody sensitivity in clinically staged syphilis serum/plasma samples

**Sensitivity of INSTI Multiplex for serum samples from patients with clinical syphilis diagnosis (n=96)***

| Study Panel | INSTI T. pallidum Reactive | INSTI T. pallidum Non-Reactive | Sensitivity |
|---|---|---|---|
| Primary syphilis (n=33) | 29 | 4 | **87.9%** (29/33) |
| Secondary syphilis (n=41) | 41 | 0 | **100%** (41/41) |
| Latent syphilis (n=22) | 21 | 1 | **95.5%** (21/22) |
| Totals | 91 | 5 | **94.8%** (91/96) |

* *Dr. Anna Bianchi, Laboratoire départemental de Seine-Saint-Denis, Paris, France, 2011, personal communication*

**Performance of the INSTI Multiplex for detection of *Treponema pallidum* antibodies in patients with primary syphilis****

| | | T. pallidum reference test* | | Total | Sensitivity |
|---|---|---|---|---|---|
| | | Pos | Neg | | |
| INSTI T. pallidum | Pos | 11 | 0 | 11 | **78.6%** |
| | Neg | 3 | 0 | 3 | |
| Total | | 14 | 0 | 14 | |

***Reference test was conducted using TP PCR DNA detection from lesions.**

***Jeffrey D. Klausner, MD, MPH*
*Professor of Medicine and Public Health*
*UCLA David Geffen School of Medicine and Fielding School of Public Health, 2016, personal communication*

# INSTI Multiplex T. pallidum and HIV Results from a Field Study Conducted in Bangalore, India, 2012-2013, n=1010

## T. Pallidum antibody performance of the INSTI Multiplex Test against the reference T. pallidum antibody status in a prospective field study population, n=1010*.

| T. Pallidum antibody status | INSTI Multiplex T. pallidum Antibody Positive | INSTI Multiplex T. pallidum Antibody Negative |
|---|---|---|
| Positive | 14 | 2 |
| Negative | 1 | 993 |
| Total | 15 | 995 |

*study population had a low prevalence of syphilis: 1.6% (16/1010)

## HIV Performance of the INSTI Multiplex HIV-1/HIV-2/Syphilis Antibody Test against the reference HIV antibody status in prospective field study population, n=1010*

| Panel | INSTI Multiplex HIV-1/HIV-2 Positive | INSTI Multiplex HIV-1/HIV-2 Negative |
|---|---|---|
| HIV antibody positive (n=136) | 136 | 0 |
| HIV negative (n=874) | 874 | 0 |
| Total | 1010 | 0 |

HIV sensitivity: 100% (136/136)
HIV specificity: 100% (874/874)
*same study population had a high prevalence of HIV: 13.5%

# Comparison of INSTI Syphilis kits before and after IgM removal for IgM + and IgG-/+ samples

- INSTI has been shown to have affinity to HIV gp41 IgM antibodies: *"Sensitivity of a rapid point of care assay for early HIV antibody detection is enhanced by its ability to detect HIV gp41 IgM antibodies." N. Mosgabadi et al/Journal of Clinical Virology 71 (2015) 67-72*
- Recent data shows INSTI Multiplex also has affinity to T. pallidum p17/47 IgM antibodies:





| Panel Member | Bleed Date | Days Since 1st Bleed | Abbott Architect Syphilis Total (s/co)[1] | Trinity Biotech CAPTIA™ Syphilis IgG (Ab Index)[2] | Trinity Biotech CAPTIA™ Syphilis IgM Capture (Ab Index)[3] |
|---|---|---|---|---|---|
| PSS901-06 | 07 Apr 12 | 45 | 5.04 | 0.503 | 1.17 |
| PSS901-07 | 10 Apr 12 | 48 | 9.11 | 0.798 | 1.26 |
| PSS901-08 | 14 Apr 12 | 52 | 14.1 | 0.976[2] | 1.51 |
| PSS901-09 | 21 Apr 12 | 59 | 27.4 | 1.32 | 1.87 |

# Multiplex T. pallidum antibody assay replicates desirable performance attributes of INSTI HIV without affecting HIV performance

| Performance Attribute | INSTI HIV-1/HIV-2 Antibody Test | INSTI Multiplex |
|---|---|---|
| HIV 1 | Yes | Yes |
| HIV 2 | Yes | Yes |
| T. pallidum | No | Yes |
| Time to Results | 1 minute | 1 minute |
| FDA Approved | Yes | No |
| CLIA Waived | Yes | No |
| Assay Description | HIV IgM/IgG antibody sensitive flow-through rapid immunoassay | HIV and T. Pallidum IgM/IgG antibody sensitive flow-through rapid immunoassay |
| Early Detection | 20-21 days post HIV infection | 85% sensitivity in primary syphilis |
| Specimen Volume | 50 µL | 50 µL |

# Summary and Conclusions

- **Performance of the INSTI Multiplex HIV-1/HIV-2/Syphilis Antibody Test for T. pallidum was similar to other T. pallidum antibody methods.**

- **As with other T. pallidum assays, lower sensitivity was observed in primary syphilis (85%) compared to secondary or latent syphilis (100%, 95.5% respectively).**

- **Field studies indicate that the performance for Multiplex HIV was not affected by the addition of the syphilis test spot (100% HIV sensitivity and specificity).**

- **INSTI Multiplex can detect IgM antibodies to T. pallidum p17/47 antigen epitopes.**