Side-by-Side Comparison:
DPP® HIV-Syphilis Multiplex Rapid Test and Syphilis Health Check Rapid Test

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BROTHERS HEALTH COLLECTIVE
BHC
MANY HANDS LIFT THE WEIGHT

DISCLOSURES

- Eugene Martin, PhD (Rutgers University – Robert Wood Johnson Medical School) (PI): No relevant disclosures.

PARTICIPANTS:

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- This project was a collaborative effort between Chembio Diagnostics AND the Brothers Health Collective – Chicago, IL and was funded by Chembio.

SIDE BY SIDE: SHC & DPP HIV-SYPH

BACKGROUND:

Syphilis is resurgent in the US and HIV co-infection is increasingly common

- 45.5% of all syphilis cases among MSM are HIV-positive

A simple, multiplex rapid test screening for HIV & SYPHILIS has much to offer 'at risk' communities:

→Simultaneous, efficient screening for two diseases that often occur together

OBJECTIVE:

- Assess the **FIELD PERFORMANCE** of both products against a blinded panel of well-characterized, plasma specimens (mostly weakly reactive) to Treponema pallidum (TP) in a CLIA-waived setting with naïve users.
- Evaluate the utility of the DPP Micro Reader to reduce subjectivity of operators in interpreting Treponemal test line reactivity.

The Rapid Screening Environment

- Laboratories that perform ONLY tests that are "simple" and that have an "insignificant risk of an erroneous result" may obtain a CLIA **certificate of waiver**.
- Over 1,400 test systems are currently classified as ‘waived’. One of these is the Syphilis Health Check.
- Test operators in waived settings have ‘limited or no training or hands-on experience in conducting laboratory testing” ---> so-called “untrained operators” or “waived users”

Quality Assurance Programs
– Little things matter! -

- In a RAPID TEST setting.. Little things DO matter!
  - Who tests
  - How they test
  - Where they test, how much light is available in the testing area
  - How the devices are handled (temperature/timing/expiration dates)
  - How operators are trained
  - What they understand about the test and its limits
  - Internal or external time pressures that operate on testers
- While CLIA WAIVED devices are: "simple laboratory examinations and procedures that have an insignificant risk of an erroneous result"! if you're on the other end of a falsely positive or negative result, it matters a whole lot

WHAT IS:
"an insignificant risk of an erroneous result"?

CLIA Waiver requires that an operator:

- Follow the manufacturer's instructions for performing a test means to follow ALL of the instructions in the **package insert** from "intended use" to "limitations of the procedure."
  - **The TRUTH IS: It rarely happens…**
- **Quick Reference Instructions (QRIs)**
- Some tests are, however, more *ROBUST* than others

What makes a GREAT Point-of-Care Test?

A robust test LIMITS what an operator can do, limits assumptions regarding what he/she knows and minimizes decision-making.

For example:

- **Vision** – If you have to read the result, it's a potential problem
- **Dilution** – If you have to dilute a specimen, it's a potential problem
- **Pipetting** – If you have to pipet a solution, it's a potential problem.
- **Reagents** – If temperature control is critical, it's a potential problem.
- **Timing** – In some settings expectations are unrealistic creating timing errors (assay runs too long)

A good POC test reduces the steps to reaching a definitive result.

We were curious…

- If you take experienced rapid HIV testers (22) without any familiarity with either rapid test --- AND
- You provide the operator with Quick Reference Instructions (QRIs) for both assays ---- AND
- You give them as much time as they need
- You alternate which assay is used first by half the testers
- You alternate the specimen order
- And they perform the assay under the eyes of an experienced MONITOR
- Evaluate a blinded panel of 9 unknown, but challenging specimens

How would the two assays perform?

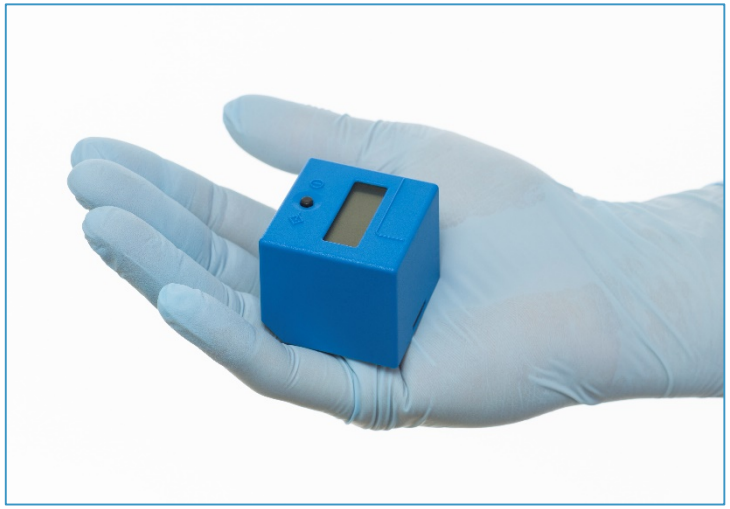
DPP® **HIV-Syphilis** Assay

- The Chembio DPP® HIV-Syphilis Assay is a single-use, immunochromatographic, rapid test for the simultaneous detection of antibodies to Human Immunodeficiency Virus Types 1 and 2 (HIV 1/2) and *Treponema pallidum* in fingerstick whole blood, venous whole blood, serum, and plasma.
- The test is currently undergoing FDA **premarket approval (PMA)**.
- Initially seeking approval as a non-waived device (CLIA Moderate Complexity)

Unique features:

1. Dual Pathway Platform (DPP)
2. DPP®SampleTainer® bottle contains a premeasured dilution buffer within a closed vial to serve as a dropper for performing the assay.

The DPP® Micro Reader



- Results generated by the rapid test are algorithmically evaluated and interpreted with a microreader to provide definitive diagnostic results for low analyte concentrations, which may otherwise result in faint or ambiguous test results.
- The assay IS NOT read visually.
- Each reader is good for 3000 reads. Battery operated.

SHC vs. DPP HIV-SYPH

METHODS:

- Blinded, 9-member panels were provided to 22 experienced rapid test operators untrained on either SHC or DPP HIV-SYP.
- Operators were asked to follow the manufacturer's quick reference guide (QRI).
- Experienced assay monitors observed rapid test performance and visual reads by all operators.
- Lighting conditions in the testing area were optimal.

LIMITATIONS:

1. Operators utilized fixed volume pipettes to apply specimens instead of the provided transfer pipettes
2. TP specimens were chosen to provide a challenging range of reactivity
3. The DPP HIV-SYPH test is currently undergoing FDA review for PMA approval

OPERATOR INTERPRETATION OF BLINDED SPECIMENS

Syphilis Health Check			DPP® HIV-Syphilis Assay System			
TP Results			TP Results		HIV Results	
Positive	Negative	Interpretation	Positive	Negative	Positive	Negative
	100.0% (43/43)	*Called a negative - negative*		97.7% (43/44)		
13.1% (20/153)		*Correctly called a positive TP positive*	94.1% (143/152)			
	86.9% (133/153)	*Missed a positive TP*		5.9% (9/152)		
Not applicable		*Correctly called a positive HIV positive*			95.5% (21/22)	
Not applicable		*Missed a positive HIV*				4.5% (1/22)

1. SHC RT *experienced* monitors re-interpreted 28 SHC results (14.1%) as reactive compared to the operator visual read. The microreader used in the DPP assay re-interpreted 3/196 results (1.5%).
2. Invalid assays were not counted in the denominator

Inexperienced Operators Compared to Unblinded Truth

KEY FINDINGS

Controls:

SHC SYPH: 43/44 – 1 Invalid
DPP SYPH: 43/44 – 1 Positive
DPP HIV+: 21/22 – 1 Negative

Treponemal Ab+: 154 REACTIVE

SHC SYPH – 20 Positive
SHC SYPH – 133 Negative
SHC SYPH – 1 INVALID

DPP SYPH – 143 Positive
DPP SYPH – 9 Negative
DPP SYPH - 2 INVALID

The DPP HIV-SYP assay agreed with the characterized result >98% of the time. Experienced monitors re-classified 3/154 reactive results for DPP HIV-SYP (1.9%) and 28/154 (11.7%) of SHC reactive results.

	SHC RESULTS			DPP RESULTS					
	SYPHILIS Test Line Interpretation			SYPHILIS Test Line Interpretation			HIV Test Line Interpretation		
PANEL- SAMPLE DESCRIPTION	Invalid	Positive	Negative	Invalid	Positive	Negative	Invalid	Positive	Negative
[1]TP & HIV Nonreactive	0	0	22	0	1	21	0	0	22
[2]TP Nonreactive & HIV-1 Reactive	1	0	21	0	0	22	0	21	1
[3]TP High Reactive (Syph G - 4.028) & HIV Nonreactive #1	0	1	21	0	19	3	0	0	22
[3,4]TP *Low Reactive (*Syph G - 1.67*) & HIV Nonreactive #1*	1	9	12	1	21	0	1	0	21
[3,4]TP Low Reactive (Syph G 1.3418) & HIV Nonreactive #2	0	2	20	1	18	3	1	0	21
[3,4]TP Low Reactive (Syph G - 0.9185) & HIV Nonreactive #3	0	0	22	0	22	0	0	0	22
[3,4]TP Low Reactive (Syph G - 1.3023) & HIV Nonreactive #4	0	3	19	0	21	1	0	0	22
[3,4]TP Low Reactive (Syph G - 0.95) & HIV Nonreactive #5	0	1	21	0	21	1	0	0	22
[3,4]TP Low Reactive (Syph G - 1.92) & HIV Nonreactive #6	0	4	18	0	21	1	0	0	22
TOTALS	**2**	**20**	**176**	**2**	**144**	**52**	**2**	**21**	**175**

All TP specimens: Two sources: Medical Research Networx and Zeptometrix.
All specimens TPPA Positive and RPR Reactive. All specimens characterized by CAPTIA™ Syphilis (T. Pallidum)-G signal/cutoff ratio (S/C) ratios (Syph-G). HIV negative plasma. All TP Low Reactive specimens characterized by CAPTIA Syphilis (T. Pallidum)-G signal/cutoff ratio (S/C) ratios between 0.9 – 1.9.

Summary of Agreement between Experienced Monitors and Inexperienced Operators

		SHC: Health Check - TP		DPP - TP	
Result	**Truth**	**Operator**	**Monitor**	**Operator**	**Monitor**
Reactive	154	20	48	144	147
NonReactive	44	176	150	52	49
TOTAL	*198*	*196*[2]	*198*[2]	*196*[1]	*196*[1]

SHC RT *experienced* monitors re-interpreted 28 SHC results (14.1%) as reactive compared to the operator visual read. The microreader used in the DPP assay limited re-interpretation to 3/196 results (1.5%).

CONCLUSIONS

- Simple and easy … is not always so!
- Visual interpretation of rapid tests by inexperienced operators is often challenged by more experienced users;
- Errors in rapid test performance can be reduced by designing tests that minimize operational missteps (pipetting and dilution) and by standardizing the read and interpretation process;
- Readers that standardize the interpretation of an assay are less prone to subsequent re-interpretation;
- **NOTE:** This study was designed to compare inexperienced operators gaining familiarity with two different syphilis detecting rapid tests. It was not designed to challenge either assay under optimal performance conditions!

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