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Improved diagnosis of syphilis at the point-of-care

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Thrasher Research Fund
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Diagnostic tests for syphilis in Resource constrained settings

A combination of **2 tests** to confirm syphilis infection
1) **Treponemal tests** (TPHA) for treponemal antibodies
2) **Non-treponemal tests** (RPR) distinguish **current** from **past** infection

- Require expensive lab equipment, technical expertise, seldom available outside reference labs.
- significant barrier to effective control syphilis in resource constrained settings.

Rapid-point-of care (RPOC) treponemal antibody tests
- currently used for on site screening in primary health care settings.
- Address lack of access to a laboratory and the low patient return rates
- **Cannot** be used to distinguish active infection from past/treated infection
- Cannot monitor effectiveness of treatment.
- Reluctance to implement these tests exists

Syphilis RPOC Target product profile

BOX 1: WHO target product profile – minimum and preferred assay performance for the screening (treponemal reference) and confirmation (non-treponemal/RPR reference) components of a dual screening/confirmation point of care test for active syphilis [4].

Performance	Treponemal component (screening)		Non-treponemal component (confirmation)	
Reference tech	TPPA or TPHA		RPR	
	Minimal	Optimal	Minimal	Optimal
Clinical Sensitivity	>80%	>90%	>95% high titre (1:8) specimens	>99% high titre (1:8) specimens
Clinical Specificity	>90%	>95%	>80%	>95%

World Health Organization. *Point of Care Tests - Target Product Profiles and Research Questions*. 2015 [cited 2016 November]; Available from: http://www.who.int/reproductivehealth/topics/rtis/POCTs-target-product-profiles.pdf.

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Diagnostic development pathway:

1. **Develop** a simple to use, low-cost ($2.50 per test), instrument-free, sensitive and specific POC diagnostic test for active syphilis that produces accurate results within 30 minutes

2. To **optimise** the prototype test until it meets minimum clinical sensitivity for active syphilis >95% and specificity of >80% when tested with the reference method (TPHA+/RPR tire ≥1/8)

3. To independently **evaluate** prototype test performance in the laboratory using stored patient samples.

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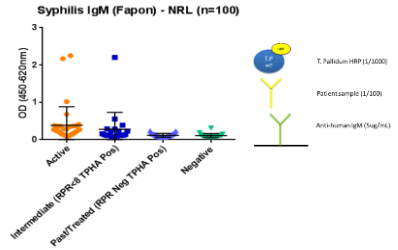
1. Evaluation of novel active syphilis biomarkers by ELISA

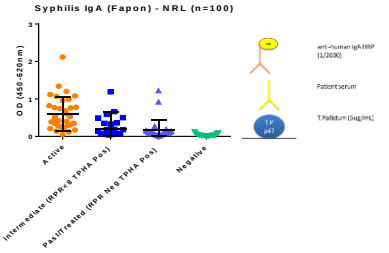
- Detection of anti-syphilis IgA and IgM were evaluated as potential biomarkers of active syphilis infection



	IgM pos	IgM neg
RPR + ≥ 8	23	11
RPR - < 8	12	54

total 100 patients

Sensitivity %	**67.65**
Specificity%	**81.82**
Predictive pos %	65.71
Predictive neg %	83.08
Test Efficiency %	77.00



	IgA pos	IgA neg
RPR + ≥ 8	29	5
RPR - < 8	13	53

total 100 patients

Sensitivity %	**85.29**
Specificity%	**80.30**
Predictive pos %	69.05
Predictive neg %	91.38
Test Efficiency %	82.00

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1. Improving IgA performance using 2 Tp antigens

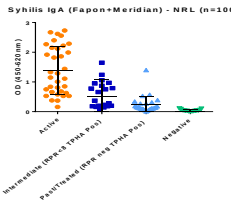
Combining two syphilis antigens in an ELISA assay increased the sensitivity of detecting active/early syphilis significantly but specificity has decreased marginally.

Enough data to proceed with transition from ELISA to rapid test

COMBINED

Tp1/Tp2	IgA pos	IgA neg
RPR + ≥ 8	33	1
RPR - < 8	16	50

Sensitivity %	**97.1**
Specificity%	**75.8**
Predictive pos %	67.3
Predictive neg %	98.04
Test Efficiency %	83.0



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The Syphilis IgA rapid Test

- Qualitative lateral flow assay
- *Treponema pallidum* antigens are immobilized onto Test line (T)
- A procedural control (C) is included in the test to determine that the assay has been run correctly and to indicate whether the sample is IgA deficient.
- Colloidal gold-labelled anti-human IgA antibody detects *T.pallidum* specific IgA in the patient's sample.
- Visual readout any visible line in Test area=positive result
- Test time is 30 minutes using 5ul of serum, plasma or whole blood.

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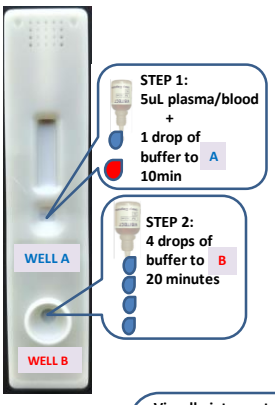
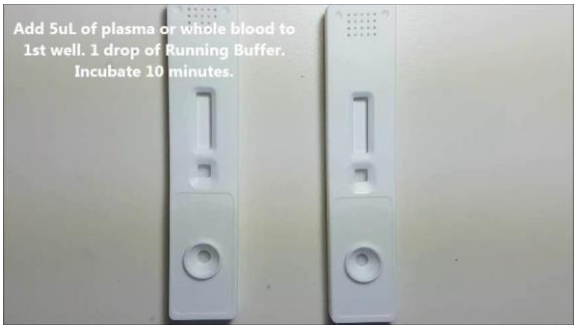
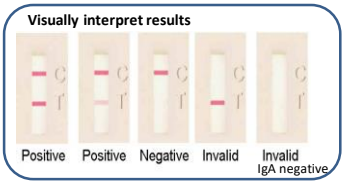
Test Procedure







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Laboratory evaluation of the rapid IgA RPOCT

- Preliminary laboratory evaluation to assess its ability to identify active syphilis from a population of syphilis antibody positive and negative serum samples.
- National Center for Sexually Transmitted Disease Control, Nanjing, China in a 'blinded' study using (n=458) stored serum samples.
- Classified by rapid plasma reagin (RPR) and *Treponema pallidum* Haemagglutination (TPHA) serology
- HREC approval granted by Alfred Health and NCSTD Nanjing

458 serum samples were classified into the following groups:

- 154 active syphilis samples (TPHA positive +RPR titre ≥8)
- 153 past treated syphilis infection (TPHA positive, RPR negative)
- 151 healthy controls (TPHA and RPR negative)

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IgA RPOCT differentiates Past/treated from active syphilis

	IgA Confirm RPOCT		
Reference test	**Positive**	**Negative**	**Total**
Active infection (TPHA + RPR≥8)	148	6	154
Past/treated (TPHA+/RPR-)	43	107	150
Negative (TPHA-/RPR-)	3	148	151
		*Three results were indeterminate	455*

	Percent	**(95% CI)**
Sensitivity	96.1%	(91.6-98.4)
Specificity	84.7%	(80.2-88.6)
Specificity (past/treated)	71.3%	(63.4-78.4)
Specificity (negative)	98.0%	(94.3-99.6)
Positive predictive value	76.3%	(69.8-81.8)
Negative predictive value	97.7%	(95.0-99.1)

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Combining IgA RPOC + Determine™ Syphilis TP RPOC

IgA + total Ab RPOC classifies active, past/treated & negative

IgA RPOC + Determine™ Syphilis TP RPOC

Reference	active +/+	Past/treated -/+	negative -/-	TOTAL
TPHA + RPR ≥8 active	148	6	0	154
TPHA+/RPR-ve Past/treated	43	107	0	150
RPR-ve /TPHA-ve negative	3	1	146	150
		(95% CI)	*4 indeterminates	454
% Sensitivity active + Past/treated	100.0	97.1 - 100		
% Specificity Past/treated	71.3	63.6 - 78.0		
% Specificity negative	97.3	93.1 - 99.2		

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In summary

- Anti-**treponemal IgA** is a potential marker for syphilis infections
- Can be converted to a RPOC device
- Met the WHO TPP performance requirement
- Used in combination with a rapid screening syphilis test, it can further classify 71.3% (107/150) of TP antibody positive samples as past/treated
- Immediate access to diagnosis and increased syphilis treatment uptake

- Further studies need to be undertaken using whole blood on high risk populations in a clinical setting.
- Low incidence hard to acquire performance data on blood
- Is IgA is detectable in newborn samples
- Validation using fingerprick blood

- Proof of concept to product requires resources, and long term investment. (>5years)
- ISO13485 accredited facilities for design phase for commercialisation(time/$$)
- Extensive clinical trials to meet regulatory requirements.

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Thank you to everyone….

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