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A Novel Point-of-care Test for Screening and Diagnosis of Infectious Syphilis

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Background – The problem

- Syphilis A re-emerging global public health concern
  - 2020: Estimated **7.1 million** new infections
  - 2019: Global incident cases in 2019: **14.11 million**, an increase of 60% from 8.84 million in 1990
  - 1990-2019: Estimated annual percentage change: **+0.16%;** +0.09% (genital herpes); +0.06 (trichomoniasis), −0.21% (chlamydia), and −0.14% (gonorrhoea)
- Estimated global maternal syphilis prevalence in 2016: 0.69% (0.57-0.81)
  - Congenital syphilis: 661,000 cases (473/100,000 live births)
  - 355,000 ABOs: **>200,000** stillbirths & neonatal deaths; 41,000 pre-term/low-birth weight births; 109,000 clinical CS cases
- Of these ABOs: **73%** attending ANC but were not tested (57%) or tested but not treated (16%) for syphilis

The Challenge - Diagnosis of active syphilis in ANC settings in LMICs

- Combination of a treponemal (TPHA/TPPA) screening test (exposure to Treponema pallidum), and a non-treponemal (RPR) confirmatory test (active/infectious syphilis)
- Confirmatory tests: Require serum, laboratory-based => limited availability
- Point-of-care tests (POCT) for syphilis: Cannot effectively distinguish between active and past/treated infections
- Over- and/or under-diagnosis and treatment of active syphilis

The Need – An accurate, rapid POCT for confirmation of active syphilis

WHO (2016)

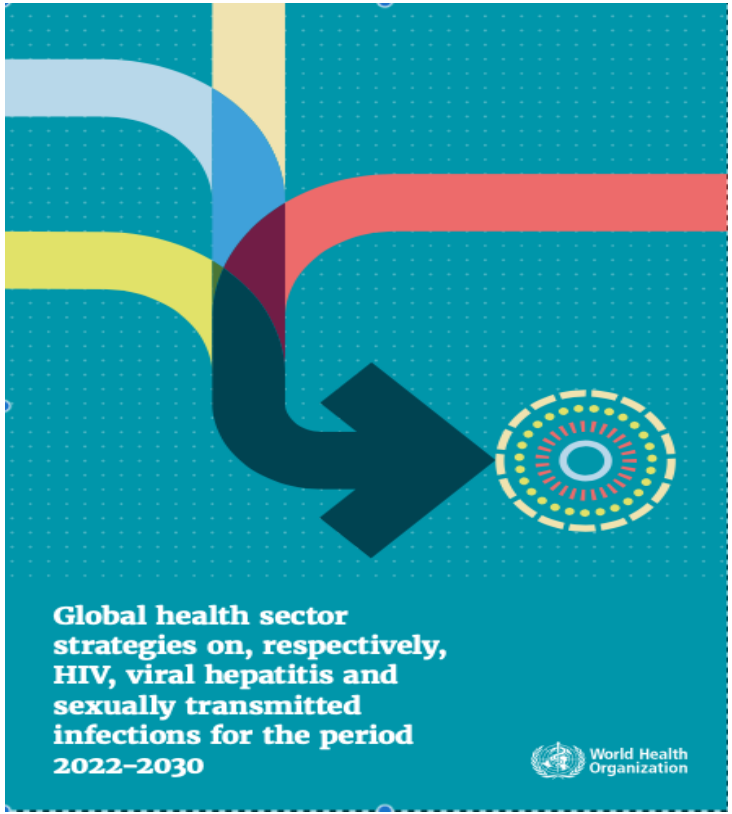
BOX 1: WHO target product profile – minimum and preferred assay performance for the screening (treponemal reference) and confirmation (non-treponemal/RPR reference) components of a dual screening/confirmation point of care test for active syphilis [4].

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%

Research Questions…

- New biomarkers for acute/active syphilis infection?

The Need – Continues…



Key strategic and operational shifts required to end sexually transmitted infections (STIs) as public health concerns by 2030

*"Support accelerated research and development on prevention technologies, **diagnostics**, treatment and vaccines for sexually transmitted infections"*

Strategic direction 5: *Foster innovation for impact*. Action 104: *New diagnostics and testing strategies for STIs*

*"There is a pressing need for …. a rapid low-cost test that can **differentiate** active syphilis from ever infected…"*

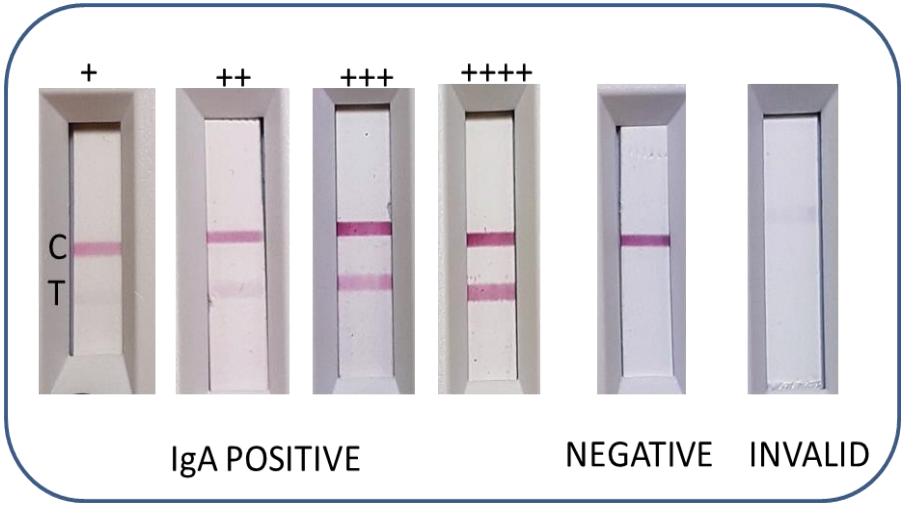
A Burnet's Diagnostic Solution – The *prototype* TP-IgA rapid POCT

1.
5 µl blood sample

2.
1 drop buffer/wait 10'

3.
4 drop buffer/wait 20'

4.
Read result



Treponema pallidum (TP) Immunoglobulin-A Antibodies (IgA)

Test performance in 79 pre-characterized samples from NRL, Melbourne

TP-IgA	TPHA +/RPR≥ 1:8 (active syphilis)	TPHA +/RPR – (past/treated)	TPHA –/RPR – (no syphilis)	Analytical performance % (95%CI)
				Sensitivity: 94.1% (95%CI = 80.3%-99.3%)
Positive	32	6	1	Specificity: 84.4%. (95%CI = 70.5%-93.5%)
Negative	2	19	19	Positive Predictive Value: 82.1% (95%CI = 66.5%-92.5%)
Total	34	25	20	Negative Predictive Value: 95% (95%CI = 83.1%-99.4%)

Test performance in 454 pre-characterized samples from NCSC, China

TP-IgA	RPR reference			Analytical performance % (95%CI)
	RPR ≥ 1:8	Negative	Total	Sensitivity: 96·1% (91·7%-98·5%)
Positive	147	46	193	Specificity: 84·7% (80·1%-88·6%)
Negative	6	255	261	Positive Predictive Value: 76·2% (69·5%-82%)
Total	153	301	454	Negative Predictive Value: 97·7% (95·1%-99·2%)

Identifying and differentiating active syphilis from past/treated infections

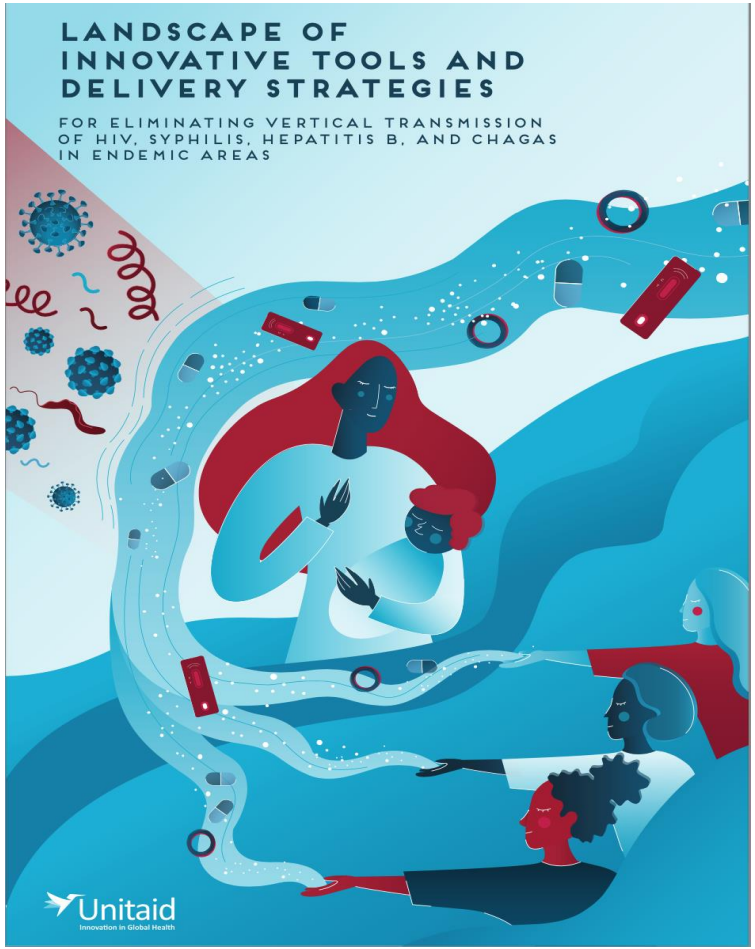
Assay/Reference		Active Syphilis TPHA+/RPR>=8	Past/treated TPHA+/RPR-	No Syphilis TPHA-/RPR-	Total
TP-IgA	Positive	147	43	3	193
	Negative	6	**107**	148	261
	Total	153*	150*	151	454
Determine ™ Syphilis	Positive	154	153	1	308
	Negative	0	**0**	149	149
	Total	154	153	150*	457
Visitect® Syphilis	Positive	154	133	4	291
	Negative	0	**12**	145	157
	Total	154	145*	149*	448

4 TP-IgA; 1 Determine ™; 10 Visitect® indeterminate test results were excluded

Test performance in fresh plasma samples from 503 pregnant women in South Africa

TP-IgA	TPHA+/RPR+ (Active syphilis)		TPHA+/RPR-	TPHA-/RPR-	TPHA-/RPR+	Total
	RPR≥1:8 (high titer)	RPR<1:8 (low titer)	Past/treated syphilis	No syphilis	Biological false positive	
Positive	4	1	0	3	2	10
Negative	0	0	9	484	0	493
Total	4	1	9	487	2	503

TP-IgA	RPR reference				Analytical Performance % (95%CI)
	Positive		Negative	Total	Sensitivity: 100% (59%-100%)
	≥8	<8			Specificity: 99·4% (98·2%-99·9%)
Positive	4*	3*	3	10	Positive Predictive Value: 70% (34·8%-93·3%)
Negative	0	0	493	493	Negative Predictive Value: 100% (99·3%-100%)
	4	3	496	503	



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Research Paper

Improving the coverage and accuracy of syphilis testing: The development of a novel rapid, point-of-care test for confirmatory testing of active syphilis infection and its early evaluation in China and South Africa

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TP-IgA						
In-house TP-IgA POCT prototype (Australia)[115]	Lateral flow, in-house manufactured immunochromatographic test designed to detect TP-specific IgA class anti- bodies Whole blood	Blood + buffer lateral flow	30 min	Unavailable	In-house assay	In-house assay

Clinical implications - Test performance data gaps

- On-site by non-laboratory technicians/clinical staff
- Screening for/Diagnosis of congenital syphilis
- High-risk/high-prevalence populations (e.g. MSM)

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Thank you for your attentions!

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