Syphilis Automation: *Updates on automated nontreponemal Rapid plasma Reagin (RPR) tests*

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Conflict of Interest

- No conflicts of interest or financial disclosures

Syphilis automation

- The diagnosis of syphilis is challenging and determining a correct stage requires “laboratory results” along with “clinical presentations/history of the disease”
- Serological tests are the most commonly used for syphilis and are of two types; nontreponemal (RPR, VDRL) and treponemal tests (EIA, TP-PA)
- Manual syphilis tests (e.g. RPR, TP-PA) require skilled staff to setup, perform and interpret results
- Automated syphilis tests reduce hands-on time and repetitive motion injuries, minimize subjectivity with result interpretation, and benefit labs with documentation

Syphilis automation

- High-volume laboratories seeking to improve workflow lean towards “automation”
  - Reverse algorithm; automated treponemal tests
- FDA-cleared automated treponemal tests are widely used for syphilis testing
- Now we have “automated nontreponemal RPR” tests

Automated nontreponemal RPR tests

- Automated nontreponemal RPR tests were recently introduced in the United States for syphilis
  - **BioPlex 2200 Syphilis Total & RPR** (Bio-Rad Laboratories, Inc., CA)
  - **AIX 1000 agglutination RPR analyzer** (Gold Standard Diagnostics, Inc., CA)
  - **ASI Evolution automated RPR syphilis test** (Arlington Scientific, Inc., UT)

Automated nontreponemal RPR tests

BioPlex 2200 syphilis Total and RPR (Bio-Rad, CA)

- BioPlex 2200 syphilis total & RPR is based on flow immunoassay principle
- Detects treponemal (TP47/TP17) and nontreponemal (cardiolipin) antibodies
- Offers qualitative results for treponemal assay and qualitative/titer for nontreponemal assay

Source: Respective manufacturer's website

Automated nontreponemal RPR tests

AIX 1000 RPR (Gold Standard Diagnostics, CA)

ASI Evolution (Arlington Scientific, UT)

- Based on flocculation reaction like RPR
- Use cameras and proprietary hardware/software tools
- Offers qualitative/titer for nontreponemal assay

Source: Respective manufacturer's website

Automated nontreponemal RPR tests

Table 1 Key features of the automated RPR tests

Parameters		Automated RPR		
		BioPlex RPR	**AIX 1000**	**ASI Evolution**
Turnaround (tests/hour)		200	192	190
Specimen vial	Type	Polystyrene / polypropylene	Polystyrene / polypropylene	Polystyrene / polypropylene
	Dimension (mm)	12 x 75	16 X 100	12 x 75
Specimen volume	Qualitative / Screening (µL)	240	305	300
	Quantitative low titer (µL)	285	394	290
	Quantitative high titer (µL)	405	220	140
Titer range	Low titer	1:4 to 1:64	1:1 to 1:16	1:1 to 1:64
	High titer	1:128 to 1:2048	1:16 to 1:256	1:128 to 1:2048

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Automated nontreponemal RPR tests

- These three platforms automate sample testing/reporting procedure, hence circumventing the subjectivity in interpretation
  - Record results electronically; BioPlex RPR provides antibody index value and AIX 1000/ASI Evolution capture test well images, offering added benefit with lab documentation (digital result output)
  - Minimize subjectivity in result interpretation
- Only a handful of studies are available evaluating automated RPR tests
- CDC and and APHL collaborated to evaluate the performance of three automated RPR test systems assessing reproducibility, qualitative and quantitative/titer reporting

Automated nontreponemal RPR tests

- Reproducibility panel testing
  - A reproducibility panel comprised of 15 nonreactive and reactive sera (RPR titer 1:1 to 1:64) was prepared
  - Each specimen was tested 10 times at participating public health labs
  - Data analyzed and Point estimate calculated; 69 to 95%

Table 2 Results of the reproducibility panel testing for the automated RPR tests

Parameters	Nonreactive (n=2)	Low titer (n=4)	Moderate titer (n=7)	High titer (n=2)	Point Estimate % [95%CI]
RPR Titer range	-	1:1 to 1:2	1:4 to 1:16	1:64	
Automated RPR tests	% Agreement to manual RPR				
BioPlex RPR	100	60 to 100	40 to 100*	100	68.7 (60.9 to 75.5)
AIX 1000	100	100	100**	90 to 100	94.7 (89.8 to 97.3)
ASI Evolution	100	30 to 100	70 to 100	80	86.0 (79.5 to 90.7)

*Two sera (1:16) were out of range (gave 4 to 8 fold higher titer) for all 10 repeated runs

**One serum (1:16) gave 4 fold lower titer for 7 out of 10 repeated runs

Automated nontreponemal RPR tests

- Quantitative Panel testing
  - A quantitative panel comprised of 50 syphilis reactive sera (RPR titer 1:64 to 1:1024) was prepared
  - For data analysis, following criteria were kept:
    - Within range: 2-fold (1 dilution) to manual RPR titer
    - Out of range: Not within 2-fold (1 dilution) to manual RPR titer

Table 3 Results of the quantitative panel testing for the automated RPR tests

Parameters	Automated RPR test		
	BioPlex RPR	AIX 1000	ASI Evolution
RPR titer range		1:64 to 1:1024 (n=50)	
Within range n(%)	32 (64)	47 (94)	34 (68)
Out of range n(%)	18 (36)	3 (6)	16 (32)
Spearman's correlation coefficient (p-value)	0.746 ($p = 5.2 \times 10^{-10}$)	0.893 ($p = 3.1 \times 10^{-18}$)	0.162 ($p = 0.262$)

Automated nontreponemal RPR tests

- Qualitative Panel testing

Table 4 Results of the qualitative panel testing for the automated RPR tests and comparison to manual RPR data

Status		Specimen n	Manual RPR		Automated RPR tests					
			Status	n	BioPlex RPR		AIX 1000		ASI Evolution	
					R n(%)	NR n(%)	R n(%)	NR n(%)	R n(%)	NR n(%)
Syphilis staged specimens (n=185)	Primary	24	R	19	18 (100)	0 (0)	18 (94.7)	1 (5.3)	15 (78.9)	4 (21.1)
			NR	5	1 (25)	3 (75)	3 (60)	2 (40)	1 (20)	4 (80)
	Secondary	43	R	41	37 (100)	0 (0)	40 (97.6)	1 (2.4)	39 (95.1)	2 (4.9)
			NR	2	0 (0)	2 (100)	2 (100)	0 (0)	1 (50)	1 (50)
	Early Latent	38	R	34	33 (97.1)	1 (2.9)	33 (97.1)	1 (2.9)	28 (82.4)	6 (17.6)
			NR	4	1 (25)	3 (75)	1 (25)	3 (75)	1 (25)	3 (75)
	Early NPNS	14	R	14	11 (84.6)	2 (15.4)	14 (100)	0 (0)	13 (92.9)	1 (7.1)
	Late Latent	66	R	57	47 (83.9)	9 (16.1)	55 (96.5)	2 (3.5)	53 (93)	4 (7)
			NR	9	1 (11.1)	8 (88.9)	1 (11.1)	8 (88.9)	0 (0)	9 (100)
Syphilis specimens with unknown stage		140	R	64	41 (82)	9 (18)	64 (100)	0 (0)	60 (93.8)	4 (6.2)
			NR	76	7 (14)	43 (86)	16 (21.1)	60 (78.9)	11 (14.5)	65 (85.5)
Non-reactive or not diagnosed with syphilis		409	R	2	1 (100)	0 (0)	1 (50)	1 (50)	2 (100)	0 (0)
			NR	407	8 (3.2)	240 (96.8)	1 (0.2)	406 (99.8)	5 (1.2)	402 (98.8)
Total		734	-	-	206 (39.2)	320 (60.8)	249 (33.9)	485 (66.1)	229 (31.2)	505 (68.8)
Overall Concordance % (95% CI)					92.6 (90.3 to 94.8)		95.9 (94.5 to 97.3)		94.6 (92.9 to 96.2)	
PPA % (95% CI)					90.6 (87.7 to 93.5)		93.6 (91.5 to 96)		91.3 (88.6 to 94)	
NPA % (95% CI)					93.9 (92 to 95.8)		97 (95.9 to 98)		96.0 (94.8 to 97.2)	
Kappa correlation % (95% CI)					84.5 (79.8 to 89.2)		90.7 (87.5 to 94)		87.3 (83.5 to 91.2)	

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Summary

Summary

- Automated RPR tests have a relatively rapid turnaround of approximately 200 tests per hour (qualitative testing)
- A higher test reproducibility was recorded for AIX 1000 compared to ASI Evolution and BioPlex RPR
- Our evaluation and prior published reports collectively show promising performance of automated RPR tests, particularly for qualitative testing
- For quantitative testing, variabilities in titer reporting were recorded for automated RPR tests
- A variability in RPR titer of 4-fold or greater could have significant clinical implications

Summary

- A laboratory should know the “titer capabilities” for automated RPR tests as it varies from one automated RPR test to another
- All reactive specimens should be diluted until an endpoint titer is achieved;
  - either by automated RPR test, or manual RPR if cannot be tittered on an automated RPR test due to instrument’s titer range limitations
- Inaccuracy with titer reporting as < 1:4 or > 1:256 could lead to unnecessary treatment, or follow-up lab visits

Summary

- Titer between an automated and a manual RPR should concord
- More robust the correlation between these two RPR methods, the greater confidence clinicians will have in the results when managing syphilis
- Laboratories considering a switch from manual to automated RPR should ensure that they communicate with clinicians about the potential differences between automated and manual RPR titers

Limitations

- Frozen sera (two freeze-thaw cycles) were used; warrants further testing using freshly collected sera
- Syphilis staging based on records from submitting judications, and accuracy could not be verified
- Prozone specimens were not available at the time of testing
- COVID-19 vaccines were not available at the time of testing, hence its potential interference on assay performance is negligible
- BioPlex RPR's reagent stability issue was not evaluated (FDA Class 2 device recall 2021)

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Q&A

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