Near Patient Anti-Nuclear Antibody Multiplex Testing Using Whole Blood for the Diagnosis of Connective Tissue Diseases in a Tertiary Care Center

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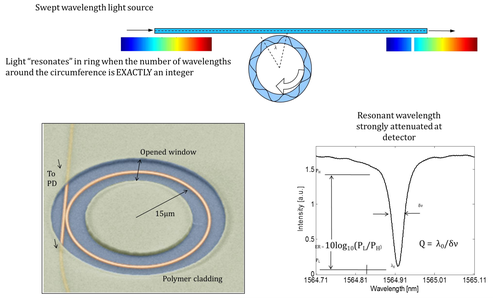
Introduction: Genalyte has developed a revolutionary multiplex detection technology based on silicon photonics that uses ring resonance to measure binding of macromolecules to sensors on a miniature silicon chip. The Maverick™ Detection System detects changes in resonance wavelength as macromolecules such as virus particles, proteins and nucleic acids bind to the sensors. An application for autoimmunity is the measurement of autoantibodies in serum and whole blood.

Principal of Operation



Ro60		FIDIS	
		+	-
Maverick	+	54	7
Blood	-	0	168
Total Agreement		97%	
Positive Agreement		100%	
Negative Agreement		96%	

Ro60		CONCLUSION	
		+	-
Maverick	+	60	2
Blood	-	0	169
Total Agreement		99%	
Positive Agreement		100%	
Negative Agreement		98%	

SSB		FIDIS	
		+	-
Maverick	+	14	8
Blood	-	3	204
Total Agreement		95%	
Positive Agreement		82%	
Negative Agreement		96%	

SSB		CONCLUSION	
		+	-
Maverick	+	16	7
Blood	-	2	206
Total Agreement		96%	
Positive Agreement		89%	
Negative Agreement		97%	

SM		FIDIS	
		+	-
Maverick	+	20	16
Blood	-	0	193
Total Agreement		93%	
Positive Agreement		100%	
Negative Agreement		92%	

SM		CONCLUSION	
		+	-
Maverick	+	23	1
Blood	-	0	194
Total Agreement		94%	
Positive Agreement		100%	
Negative Agreement		93%	

RNP		FIDIS	
		+	-
Maverick	+	32	37
Blood	-	0	160
Total Agreement		84%	
Positive Agreement		100%	
Negative Agreement		81%	

RNP		CONCLUSION	
		+	-
Maverick	+	50	20
Blood	-	0	161
Total Agreement		91%	
Positive Agreement		100%	
Negative Agreement		89%	

SCL70		FIDIS	
		+	-
Maverick	+	3	0
Blood	-	1	225
Total Agreement		100%	
Positive Agreement		75%	
Negative Agreement		100%	

SCL70		CONCLUSION	
		+	-
Maverick	+	3	0
Blood	-	0	227
Total Agreement		100%	
Positive Agreement		100%	
Negative Agreement		100%	

Background/Purpose:
Detection of anti-nuclear antibodies for the diagnosis of connective tissue diseases (CTD) often requires the patient sample to be sent to a clinical lab where complex algorithms to obtain conclusive results, including immunofluorescence on Hep2 cells, ELISA, multiplex analysis and immunoblotting, can delay the delivery of results to the physician and the patients.
The Maverick Detection System (Genalyte, Inc. USA) performs multiplexed detection of autoantibody binding events by measuring the shift in wavelength of ring resonance as the antibodies bind to the antigens on the surface above the rings. Individual clusters of 4 rings each on the ANA 12 Photonic Ring Immunoassay (PRI) Chips are functionalized with SSA/Ro-60, SS-B, Sm, RNP, Scl-70, PCNA, RiboP, dsDNA, nucleosome, Ku, Centromere B and Jo-1 antigens. Just 10 µL of whole blood is required and results are obtained in less than 15 minutes. The objectives of this study were to compare the results obtained in real time on the Maverick with those from the standard procedures in the lab, and to compare those results to the patient's diagnosis.

Methods:
Whole blood from 235 consecutive patients followed-up between March and June 2016 at the Pitié-Salpétrière hospital (Paris, France) was analyzed in the clinical lab on the ANA 12 PRI. 142 patients had systemic lupus erythematosus (SLE), 13 had Sjögren's syndrome, 10 had primary anti-phospholipid syndrome, 6 had ANCA associated vasculitis, 4 had Raynaud's phenomenon, 4 had rheumatoid arthritis, 5 had systemic sclerosis and 3 had myositis. Other patients had a final diagnosis different from CTD. Comparisons were made with results obtained on corresponding sera at the laboratory using IFA screening tests and confirmatory testing with FIDIS™ multiplex assays (THERADIAG) and when necessary Immunoblotting especially for the detection of anti-Ku antibodies (D-TEK) or anti-DNA ELISA (DiaSorin), Farr assay and anti-nucleosome ELISA (Werfen). Not all samples were tested on the FIDIS, which is why there are different total sample numbers in the tables.

CENPB		FIDIS	
		+	-
Maverick	+	8	2
Blood	-	0	215
Total Agreement		99%	
Positive		100%	
Negative Agreement		99%	

CENPB		CONCLUSION	
		+	-
Maverick	+	8	2
Blood	-	1	215
Total Agreement		99%	
Positive Agreement		89%	
Negative Agreement		99%	

JO1		FIDIS	
		+	-
Maverick	+	1	0
Blood	-	2	226
Total Agreement		99%	
Positive Agreement		33%	
Negative Agreement		100%	

JO1		CONCLUSION	
		+	-
Maverick	+	1	0
Blood	-	0	229
Total Agreement		100%	
Positive Agreement		100%	
Negative Agreement		100%	

RIBOP		FIDIS	
		+	-
Maverick	+	10	2
Blood	-	9	103
Total Agreement		91%	
Positive Agreement		53%	
Negative Agreement		98%	

RIBOP		CONCLUSION	
		+	-
Maverick	+	11	1
Blood	-	8	105
Total Agreement		93%	
Positive Agreement		58%	
Negative Agreement		99%	

PCNA		FIDIS	
		+	-
Maverick	+	0	14
Blood	-	3	110
Total Agreement		87%	
Positive Agreement		0%	
Negative Agreement		89%	

PCNA		CONCLUSION	
		+	-
Maverick	+	5	10
Blood	-	0	113
Total Agreement		92%	
Positive Agreement		100%	
Negative Agreement		92%	

DNA		FIDIS	
		+	-
Maverick	+	47	23
Blood	-	10	50
Total Agreement		75%	
Positive Agreement		82%	
Negative Agreement		68%	

DNA		FARR	
		+	-
Maverick	+	43	24
Blood	-	9	65
Total Agreement		77%	
Positive Agreement		83%	
Negative Agreement		73%	

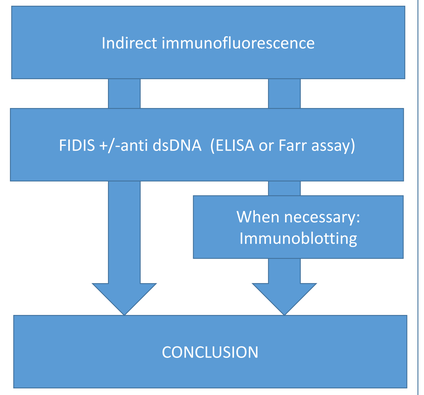
NUCLEOSOME		ELISA	
		+	-
Maverick	+	38	13
Blood	-	8	55
Total Agreement		82%	
Positive Agreement		83%	
Negative Agreement		81%	

Ku		CONCLUSION	
		+	-
Maverick	+	3	0
Blood	-	0	1
Total Agreement		100%	
Positive Agreement		100%	
Negative Agreement		100%	

Results
The Maverick Detection System showed excellent total, positive and negative percent agreement when compared to the final conclusion of the laboratory for Sm, Scl-70, Jo-1, SS-A/Ro 60, and Ku antigens with total, positive and negative percent agreement all above 93%. PCNA was above 92% and Centromere and SS-B were above 89%. For RNP, total agreement was 91%, positive was 100% and negative was 89%. For Ribosome P, the overall agreement and specificity were greater than 90%, but the sensitivity was lower. For anti-nucleosome and anti-DNA the ANA 12 PRI displayed diagnostic performances close to commonly used ELISA systems.

Resolution of discrepant results
Interestingly, 15 of 16 samples that were positive for Sm by Maverick but negative by the lab test were from patients diagnosed with SLE. All 15 of the Sm positive lupus patients were also positive for RNP, but the other was not. Thus, there was only 1 clinical false positive for Sm. The same was true for RNP. Nineteen of the 20 samples that were positive for RNP by Maverick but negative by the lab test were diagnosed with lupus and the other sample was from a Sjögren's syndrome patient who was positive for anti-RNP by dot-blot. There were only 2 discrepant results for SS-A/Ro 60, all positive for Maverick but negative by lab tests, and all had lupus. Similar results were found for SS-B/La, CenpB, Ribo-P and PCNA where all 10, 3, 9 and 12 discrepant samples, respectively, had lupus. For all cases with false negative results for RiboP with the ANA12 PRI, other specific autoantibodies were present and detected with the ANA12 PRI. Therefore, no diagnosis of CTD would have been missed by using the ANA PRI 12. There were no discrepant results between the Maverick and the conclusion of the lab for Jo-1, Scl-70 and Ku. As expected when anti-dsDNA tests are performed on different technologies, there were more discrepant results than found in the other tests. All 10 samples that were positive for the conclusion of the lab but negative on Maverick had lupus, while 19 of the 23 samples that were positive on Maverick but negative by the conclusion of the lab had lupus. For nucleosome, all 8 samples positive on the lab conclusion but negative on the Maverick had lupus, and 9 of 13 that were positive on Maverick but negative by lab conclusion had lupus. The other 4 had Sjögren's syndrome.

Diagnosis procedure



Conclusion
The Maverick detection system, which uses whole blood as the matrix and gives results in under 15 minutes, offers a reliable and rapid diagnostic solution to the search for autoantibodies in CTD. There was very good correlation between the results on Maverick and the lab conclusion for the 12 autoantibodies detected in this study. When there were differences, the results on the Maverick were in agreement with the diagnosis the vast majority of the time, particularly for Sm and RNP. The next step will be to perform the tests near the patient so that results can be given to the doctor in real time.

Disclosure: M. Miyara: Genalyte Inc., J.L. Charuel : none, S. Mudumba: Genalyte Inc., A. Wu : Genalyte Inc., P. Ghillani-Dalbin: none, Z. Amoura: none, R.W. Burlingame: Genalyte Inc., L. Musset: none