# Application of a Novel Anti-Nuclear Antibody Multiplex Test Using Fingerstick and Venous Whole Blood in a Rheumatology Clinic Demonstration of Feasibility

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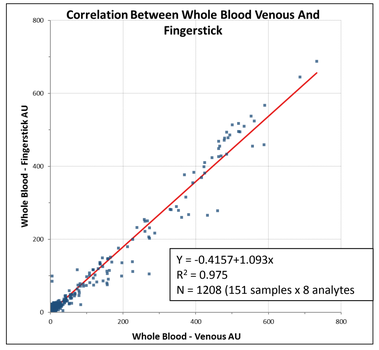
**Background/Purpose:** Multiplex assays that measure anti-nuclear autoantibodies (ANA) in real time using whole blood were developed on the Maverick™ instrument (Genalyte, Inc., USA). Because the assay takes less than 10 minutes to complete, it would be possible to perform the test in a near patient setting in an outpatient clinic. The purpose of this study was to evaluate the feasibility of using this novel instrument to perform tests on 8 ANA markers in the clinic and to compare those results to the same sample tested in Genalyte's CLIA registered laboratory.
**Methods:** An Institutional Review Board (IRB) application was approved so that patients who were seeing a rheumatologist and were going to be tested for ANA using the clinics' standard lab testing procedures could also volunteer to donate additional venous and/or finger-stick blood for further testing. Both the venous and finger-stick blood were immediately tested on the ANA 8 on the Maverick instrument in the clinic. This multiplex immunoassay measures autoantibodies to SS-A 60, SS-B, Sm, Sm/RNP, Scl-70, Jo-1, centromere B and dsDNA. The cutoff between negative and positive was standardized so that 40 AU and greater are positive. Even though results were available in real time, the protocol specified that the doctor would not be given results until the end of the study. 151 samples of whole blood (venous and fingerstick) were collected at 3 study sites, tested on the Maverick and then returned to the Genalyte CLIA lab for processing into serum for comparison testing on the FIDIS™ Connective 10 (Theradiag, France). In addition, clinical diagnosis was available for 88 patient samples (46 with SLE and 42 with other inflammatory diseases).
**Results:** The results between whole blood finger stick (WBFS) and whole blood venous (WBVN) tested on the Maverick, and serum tested on the FIDIS for positive, negative and total agreement are shown in the tables 1 to 3.
The $r^2$ correlation between the values for all of the 8 ANA markers from finger stick (153 times 8 yields 1,224 results) versus venous whole blood is 0.98 as shown in Figure 1.
**Conclusion:** This pilot study demonstrated the feasibility of performing multiplex ANA testing on whole blood in a near patient setting in an outpatient clinic. There is extremely high correlation for absolute value between venous blood and finger-stick blood, and between positive and negative results seen with whole blood on the Maverick and serum on the FIDIS. While further studies are required to quantify the impact such a diagnostic system might have on quality of care, there is potential for such a capability to improve timeliness of diagnosis, increase patient centricity and reduce overall healthcare resource utilization

**Fig 1. Correlation Between Whole Blood Venous and Fingerstick**




**Introduction:** Genalyte has developed a novel 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.

Comparison of the results on the Maverick between 2 samples types, Whole Blood Venous (WBVN) and Whole Blood Finger Stick (WBFS), are shown in Table 1. Comparisons of those results to the results of testing serum on the FIDIS are shown in Tables 2 and 3.

## Principle of Operation

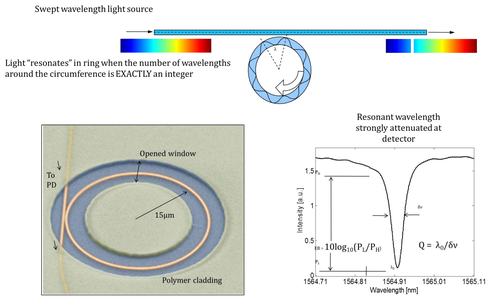



**Table 1. Agreement Between Maverick Whole Blood Fingerstick (WBFS) And Venous Blood (WBVN)**

| WBFS Vs WBVN (N=151) | Centromere B | dsDNA | Jo1 | Sm/RNP | Scl70 | Sm | SS-A 60 | SS-B |
|---|---|---|---|---|---|---|---|---|
| True Positive (++) | 9 | 17 | 0 | 28 | 1 | 11 | 27 | 12 |
| False Negative (+-) | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 |
| False Positive (-+) | 4 | 1 | 1 | 2 | 1 | 1 | 3 | 0 |
| True Negative (--) | 138 | 132 | 150 | 120 | 149 | 138 | 121 | 139 |
| | | | | | | | | |
| Positive Agreement | 100.0% | 94.4% | | 96.6% | 100.0% | 91.7% | 100.0% | 100.0% |
| Negative Agreement | 97.2% | 99.2% | 99.3% | 98.4% | 99.3% | 99.3% | 97.6% | 100.0% |
| Overall Agreement | 97.4% | 98.7% | 99.3% | 98.0% | 99.3% | 98.7% | 98.0% | 100.0% |

**Table 2. Agreement Between FIDIS And Maverick Whole Blood Fingerstick (WBFS)**

| FIDIS Vs WBFS (N=151) | Centromere B | dsDNA | Jo1 | Sm/RNP | Scl70 | Sm | SS-A 60 | SS-B |
|---|---|---|---|---|---|---|---|---|
| True Positive (++) | 9 | 12 | 0 | 13 | 1 | 1 | 20 | 8 |
| False Negative (+-) | 2 | 3 | 0 | 0 | 2 | 0 | 0 | 1 |
| False Positive (-+) | 0 | 6 | 0 | 16 | 0 | 11 | 7 | 4 |
| True Negative (--) | 140 | 130 | 151 | 122 | 148 | 139 | 124 | 138 |
| | | | | | | | | |
| Positive Agreement | 81.8% | 80.0% | | 100.0% | 33.3% | 100.0% | 100.0% | 88.9% |
| Negative Agreement | 100.0% | 95.6% | 100.0% | 88.4% | 100.0% | 92.7% | 94.7% | 97.2% |
| Overall Agreement | 98.7% | 94.0% | 100.0% | 89.4% | 98.7% | 92.7% | 95.4% | 96.7% |

**Table 3. Agreement Between FIDIS And Maverick Whole Blood Venous Blood (WBVN)**

| FIDIS Vs WBVN (N=151) | Centromere B | dsDNA | Jo1 | Sm/RNP | Scl70 | Sm | SS-A 60 | SS-B |
|---|---|---|---|---|---|---|---|---|
| True Positive (++) | 11 | 12 | 0 | 13 | 1 | 1 | 20 | 8 |
| False Negative (+-) | 0 | 3 | 0 | 0 | 2 | 0 | 0 | 1 |
| False Positive (-+) | 2 | 6 | 1 | 17 | 1 | 11 | 10 | 4 |
| True Negative (--) | 138 | 130 | 150 | 121 | 147 | 139 | 121 | 138 |
| | | | | | | | | |
| Positive Agreement | 100.0% | 80.0% | | 100.0% | 33.3% | 100.0% | 100.0% | 88.9% |
| Negative Agreement | 98.6% | 95.6% | 99.3% | 87.7% | 99.3% | 92.7% | 92.4% | 97.2% |
| Overall Agreement | 98.7% | 94.0% | 99.3% | 88.7% | 98.0% | 92.7% | 93.4% | 96.7% |

Tables 4 and 5 below show clinical sensitivity and specificity calculations for both methods. A total of 88 patient samples (46 were diagnosed positive with lupus and other connective tissue diseases; 42 were diagnosed with other inflammatory diseases). A sample was considered positive if any of the 8 markers was positive and a sample was treated as negative if all of the 8 markers were negative for analyzing data for both methods. The results were compared against the clinical diagnosis to perform clinical data analysis.

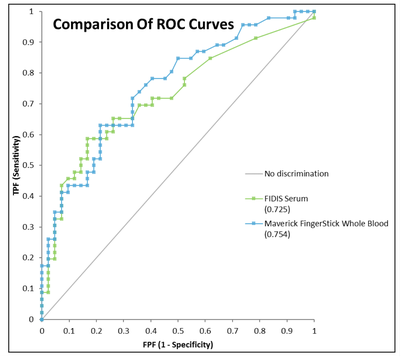
**Table 4. Clinical Performance of Maverick**

| N=88 | Maverick Fingerstick Blood | | |
|---|---|---|---|
| **Clinical Diagnosis** | + | - | Total |
| + | 29 | 17 | 46 |
| - | 10 | 32 | 42 |
| Total | 39 | 49 | 88 |
| | | 95% CI | |
| **TP (Sensitivity)** | 0.630 | 0.486 to 0.755 | |
| **TN (Specificity)** | 0.762 | 0.615 to 0.865 | |

**Table 5. Clinical Performance of FIDIS**

| N=88 | FIDIS Serum | | |
|---|---|---|---|
| **Clinical Diagnosis** | + | - | Total |
| + | 26 | 20 | 46 |
| - | 7 | 35 | 42 |
| Total | 33 | 55 | 88 |
| | | 95% CI | |
| TP (Sensitivity) | 0.565 | 0.422 to 0.698 | |
| TN (Specificity) | 0.833 | 0.694 to 0.917 | |

**Fig 2. ROC curve Comparison**




**Conclusion:** The Maverick instrument allows multiplex immunoassays to be performed on whole blood in a doctor's office, with results returned in less than 15 minutes from the time the blood is drawn. Whole blood from both a finger stick and a venous draw, as well as serum yield virtually the same results as each other. There is excellent correlation between the results on the Maverick and those from an FDA cleared multiplex test system, the Connective 10 on the FIDIS. In fact, there was equal correlation with diagnosis on the Maverick compared to the FIDIS in the group that had a diagnosis.

**Next Steps:**
- Optimize instrument and assay design based on feedback from clinical study
- Independently validate system performance in a larger study with results available to the ordering physician at the point of care

**Disclosure: S. Reddy**, Genalyte, Inc.; **D. Copland Reddy**, Abbvie, Genalyte, Inc.; **R. Burlingame**, Genalyte, Inc.; **V. Nelson**, Genalyte, Inc.; **C. Buchner**, Genalyte, Inc.; **J. Stewart**, Genalyte, Inc.; **S. Mudumba**, Genalyte, Inc.; **J. Custodio**, Genalyte, Inc.; **J. Wang**, Genalyte, Inc.; **R. Romero**, Genalyte, 3; **A. Wu**, Genalyte, Inc.; **C. Cherwein**, Genalyte, Inc.; **S. Smith**, Genalyte, Inc.; **M. A. Gleeson**, Genalyte, Inc.