

Near patient detection of anti-MDA5 antibodies using photonic ring immunoassays

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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.

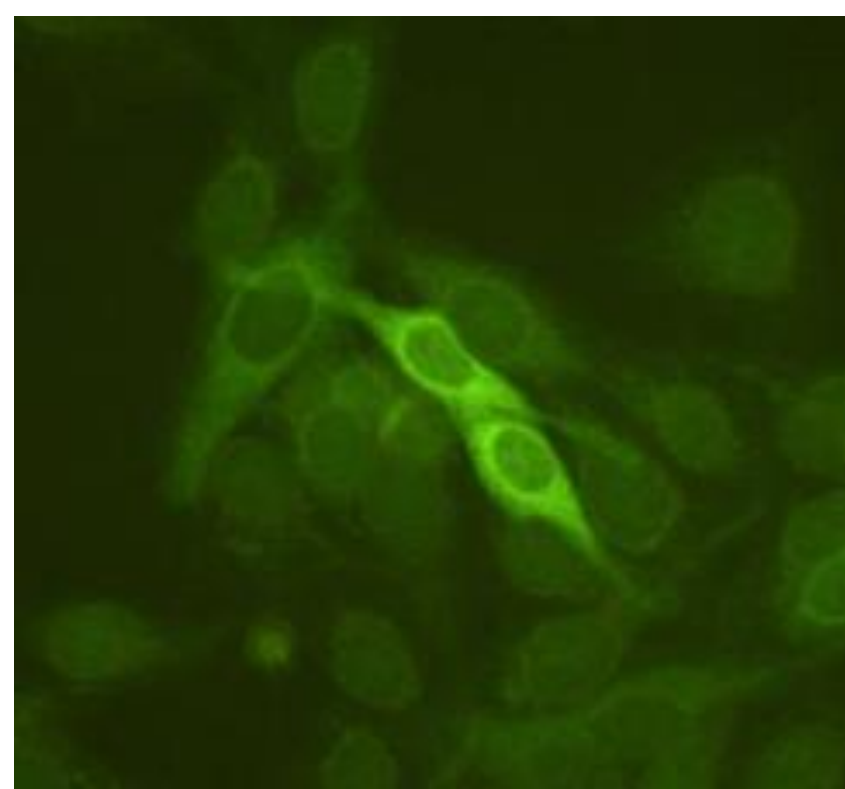


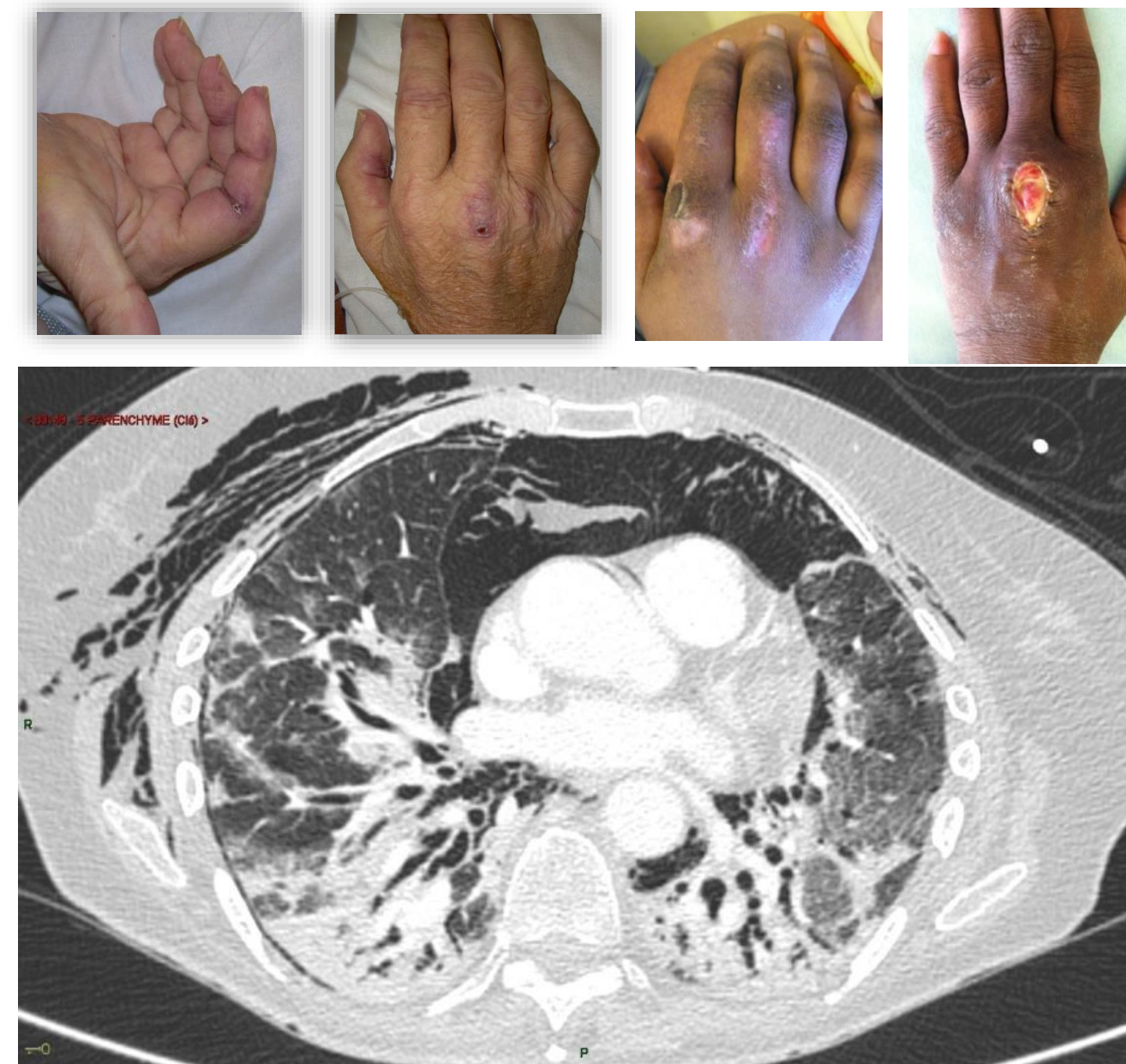
FIGURE 1
 Diffuse cytoplasmic fluorescence on Hep-2000 cells obtained with a serum sample containing anti-MDA5 antibodies.
 Serum dilution at 1:80

Background/Purpose:
 The presence of anti-MDA5 antibody is associated with amyopathic dermatomyositis and/or rapidly progressive interstitial lung disease that can be fatal (Figure 4). In the latter case, it is mandatory to confirm the diagnosis as soon as possible in order to initiate aggressive treatments and the presence of anti-MDA5 autoantibodies is an important part of the differential diagnosis. The immunoassay using photonic microrings is a new sensitive technology that enables rapid results in less than 15 minutes.

The aim of our study was to evaluate this technology for the detection of anti-MDA5 antibodies in sera with known positivity.

Methods:
 The system is based on immobilizing the antigenic target, MDA5, above the photonic rings in a silicon chip. We analyzed a total of 72 sera drawn from 40 patients known to be positive for anti-MDA5 antibody in our laboratory using immunoblotting assays and 4 negative controls. For some patients, several samples drawn during the follow-up could be analyzed.

FIGURE 4: Clinical and CT features of MDA 5 patients



Results and discussion
Practicality of Testing on the Maverick Instrument
 The detection of anti-MDA5 antibodies with the Maverick instrument could be performed by any member of the lab after a short training of less than 15 minutes, including the explanation of the technique and the manipulation of samples. As expected, results for samples could be retrieved with the MDA5 PRI assay within 15 minutes after the filling of wells with serum samples and insertion into the Maverick instrument. Because the consumable array occupies the read head of the instrument for the entire duration of the test, the throughput of the instrument is about 4 sets of MDA5 tests per hour.

Anti-MDA5 autoantibody immunofluorescence pattern of Hep-2000 cells
 The presence of anti-MDA-5 antibodies could be first suspected using indirect immunofluorescence on Hep-2000 cells. Diffuse cytoplasmic fluorescence pattern is observed on rare cells (FIGURE 1). This rare fluorescence can be overlooked by the pathologists if the whole well is not carefully studied indicating that alternative methods for the detection of anti-MDA5 antibodies are required, especially in emergency settings

photonic ring immunoassay for the detection of anti-MDA5 antibodies
 Using the photonic ring immunoassay, we could confirm the presence of anti-MDA5 antibody in all samples known as positive for anti-MDA5 antibody by other techniques. No false positive or false negative results were found. Moreover, high levels of MDA-5 antibodies measured at diagnosis are associated with interstitial lung disease with respiratory functional impairment at presentation (FIGURE 5). Interestingly, we observed a decrease in anti-MDA5 antibodies titers in followed-up patients.

Conclusion
 The photonic ring immunoassay is a fast and reliable technology for the detection of anti-MDA5 antibodies especially suitable for patients in critical care conditions

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Principle of Operation

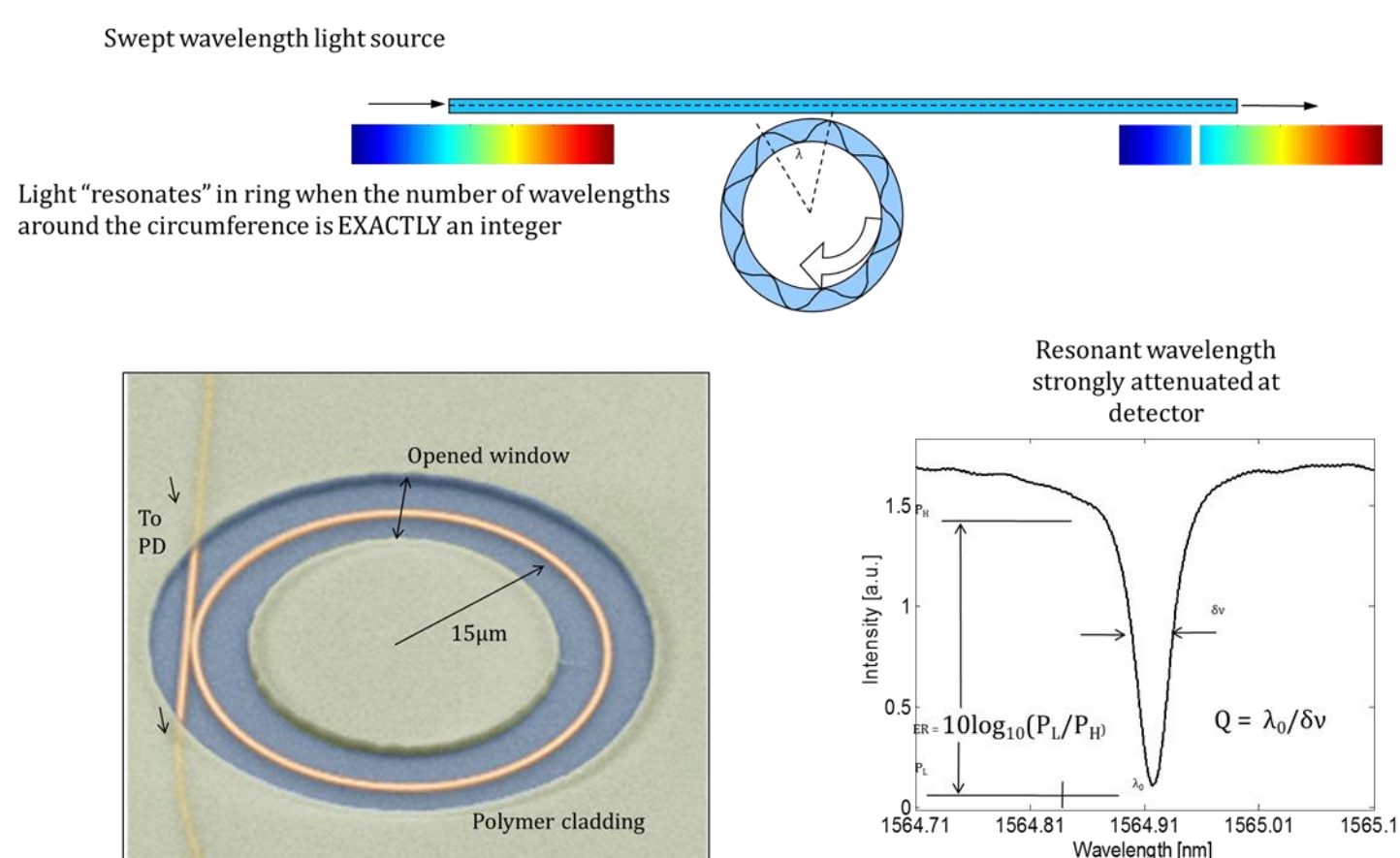


FIGURE 2: Principles of immuno-analysis with photonic rings



FIGURE 3: Genalyte detection system

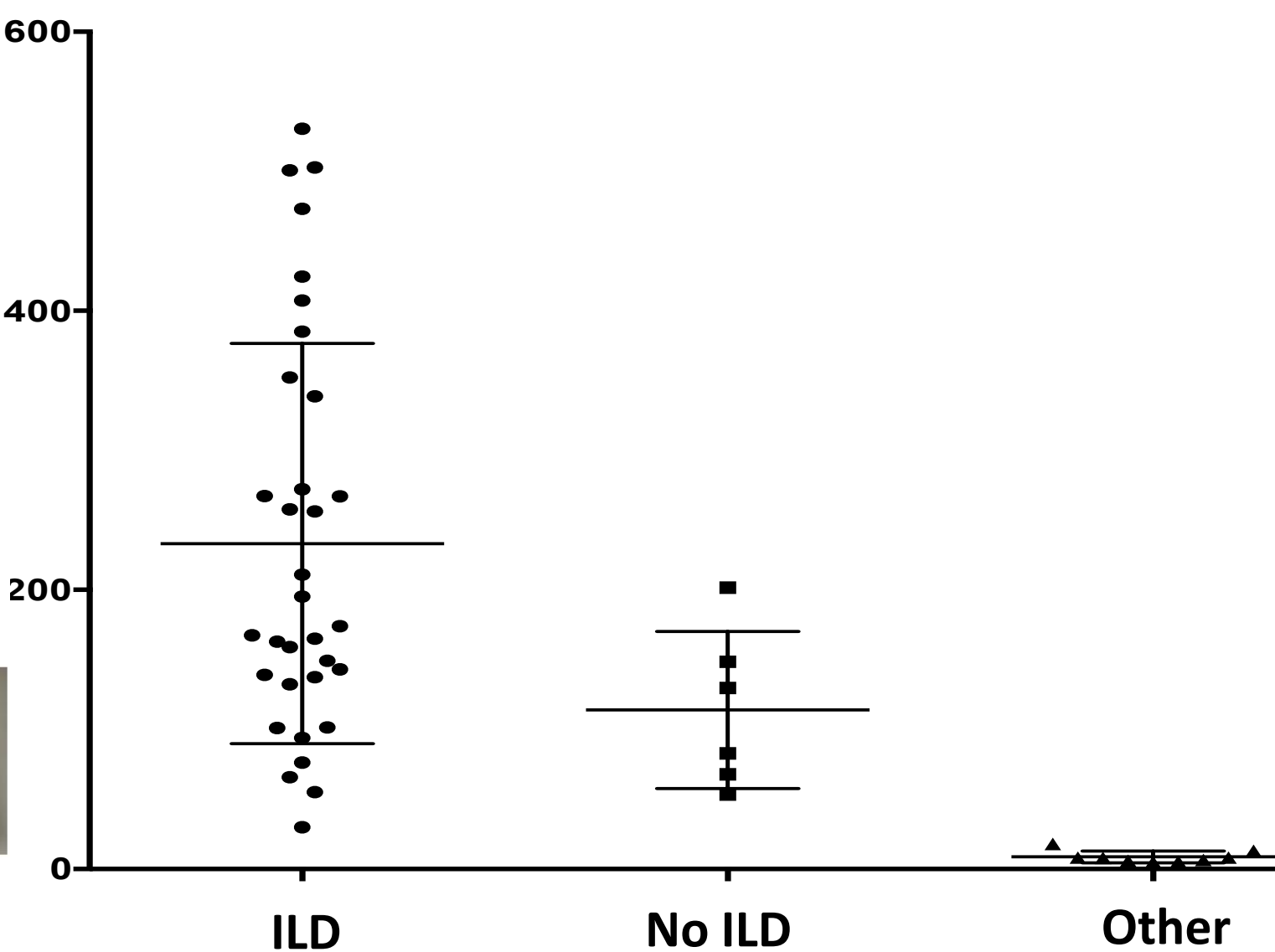


FIGURE 5: Results in 3 categories of patients.
 First group : patients with MDA5 antibodies and ILD
 Second group: patients with MDA5 antibodies without ILD
 Third group: patients with other diseases