Characterizing the Protein Content in Tears for Early Detection of Disease

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There is a critical need in civilian military applications for early (pre-symptomatic) detection of infectious diseases. For example, early detection of pathogens of upper respiratory infections can be extremely useful in identifying personnel who will become ill before they embark on a mission. This will help prevent those individuals from jeopardizing the mission and infecting others. The complexity of most biological fluids, e.g., blood and urine, necessitates time-consuming purification and isolation procedures for accurate analyses.

Our new method uses mass spectrometry and advanced proteomics to identify wellness biomarkers and pathogen biomarkers present in tear fluid obtained from healthy and diseased animals (Fig. 1). Tear samples are ideal specimens for rapid diagnosis because of their relatively clean background (compared to blood and urine) and non-invasive collection. Many types of systemic diseases give rise to ocular manifestations from either direct or indirect infection. Recent literature reports the up- and/or down-regulation of several proteins found in diseased tear samples when compared to healthy tear fluid.

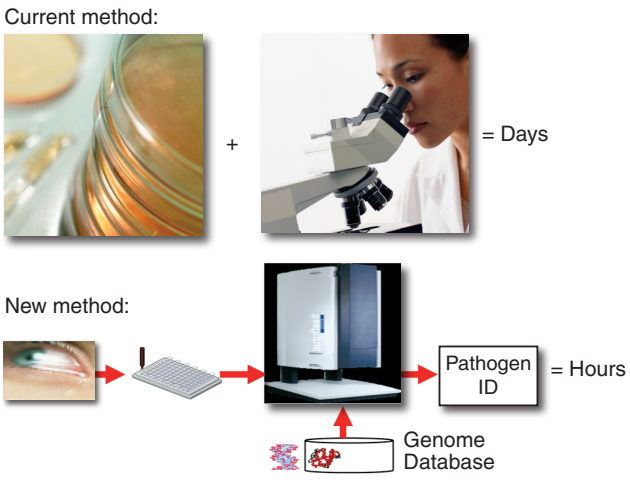

Figure 1. Our new method of detecting pathogens is much faster than the current method.

Monitoring these proteins is useful in recognizing an unhealthy state; however, knowledge pertaining to the cause may not be as revealing. The technology presented here will not only be applicable in identifying individuals who may have been exposed to a pathogenic organism but also aid in detecting and identifying the pathogen.

The overall objective of this project is to determine the feasibility of detecting and identifying biomarkers indicative of pathogen exposure and/or infection in tear fluid in real time. In our approach, the baseline healthy protein content of tear samples is first established by using advanced analytical proteomics technologies [Fig. 2, 0(c)]: matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and tandem mass spectrometry (MS/MS). In an initial proof-of-concept experiment, samples infected with a pathogen were examined, and the appropriate bio-

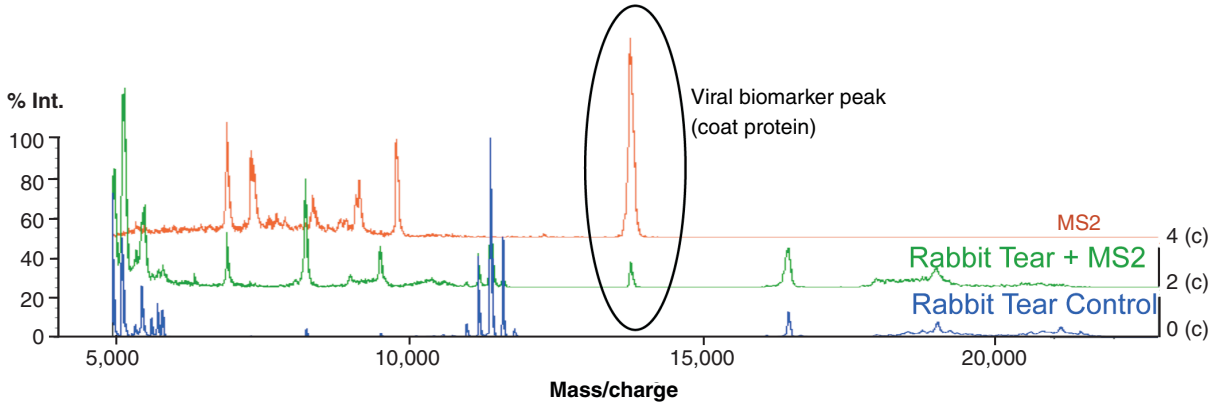

Figure 2. Biomarkers can be detected in rabbit tears by mass spectrometry.

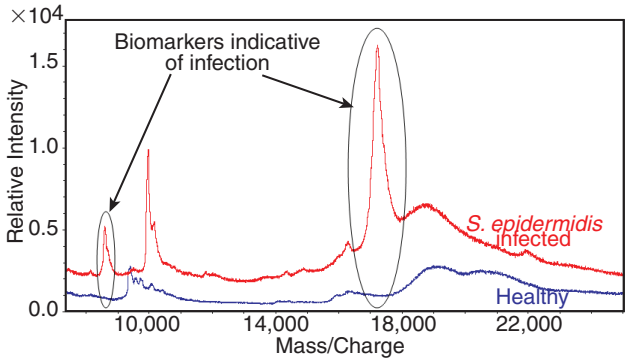

Figure 3. Biomarkers indicative of infection were detected.

markers characteristic of the pathogenic organism were detected and identified [Fig. 2, 2(c)].

Subsequent analysis of tear fluid from animals infected with a pathogen were investigated, and biomarkers indicative of bacterial exposure were observed (Fig. 3). We currently are determining whether these biomarkers are attributable to the host's response or representative of the pathogen.

A research team from APL and the Wilmer Eye Institute at the JHU School of Medicine has developed rapid MS-based technologies for detecting and identifying biomarkers obtained from healthy animals and animals exposed to select pathogens.

Future plans include the following:

- Confirm the identity of biomarkers in rabbit tears using tandem mass spectrometric methodologies.
- Conduct time-course measurements for determination of pre-symptomatic biomarkers.
- Identify biomarkers in tears associated with animal exposure to other pathogens (and possibly broaden to include exposure to chemicals and explosives).

For further information on the work reported here, see the references below or contact miquel.antoine@jhuapl.edu.

[1]Loon, S. C., Teoh, S. C., Oon, L. L., Se-Thoe, S. Y., Ling, A. E., Leo, Y. S., and Leong, H. N., "Severe Acute Respiratory Syndrome Coronavirus in Tears," *Br. J. Ophthalmol.* 88(7), 861–863 (2004).

[2]Li, N., Wang, N., Zheng, J., Liu, X. M., Lever, O. W., Erickson, P. M., and Li, L., "Characterization of Human Tear Proteome Using Multiple Proteomic Analysis Techniques," *J. Proteome Res.* 4(6), 2052–2061 (2005).