Introduction of Revelar™ and Oxidative Stress – The Development of Revelar

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Pulse Health

In 2005, Pulse Health decided to develop a non-invasive test for the measurement of the effects of reactive oxygen species (ROS or "free radicals"), also called oxidative stress. The Free Radical Theory of Aging holds that free radicals, in excess, contribute to a number of health conditions, including increased risk of cardiac disease, cancer and other effects of premature aging, just as the damage caused by ROS is also known to be the mechanism of expression after exposure to toxins.

Pulse selected Akers Biosciences, Inc. to work with Pulse's Science Advisory Board (SAB) and engineers to develop such a test, as Akers had experience in measuring other analytes in breath condensate, has numerous patents, and has developed 510(k) registered diagnostic devices. Beginning in 2006, Pulse and Akers began testing breath pathways for the detection of free radical effects based on peroxide by-products, namely aldehydes. By modifying and combining two existing platforms that identify other analytes in breath, Pulse and Akers were able to develop a rapid method to generate a numeric score representing aldehyde levels.

The Scientific Basis for Revelar

As noted, oxidative stress has been associated with various physiological and pathological processes. Production of ROS contributes to the oxidation of cell macromolecules such as lipids, proteins, and nucleic acids. Products of these reactions include alkanes, aldehydes, and other oxidized macromolecules. Lipid peroxidation is the most extensively investigated of these processes. Oxidation of cell membrane phospholipids produces a chain reaction of ROS targeted to polyunsaturated fatty acids, which produce unstable lipid hydroperoxides, and eventually aldehydes.

The Revelar system uses the well-known chemical reaction of an aldehyde with a primary amine to form what is called a Schiff base. Schiff base formation in the Revelar system is observed as the production of a purple colored substance. The amount of purple color produced in the Revelar reagent is proportional to the amount of aldehydes present in the sample. The Revelar device can detect the shift in color from pure Revelar reagent caused by the production of purple aldehyde complex. Quantification of the amount of shift against color standards leads to the calculation of the Revelar score.

Aldehydes are carried by the water vapor in exhaled breath. The Revelar reagent is produced by the deposition of reagent, acid and enhancer substances onto an inert, hygroscopic particulate support. The amount of this support is designed to have a specific loading capacity for water vapor, the principal component of exhaled breath, and is carefully measured out into each Revelar tube. The Revelar reagent system has appropriate sensitivity for aldehydes in breath because the reaction of reagent has been optimized for the specific level of water vapor that can be absorbed by the hygroscopic support.

Device Technology

The Pulse Health Revelar device employs a digital reflectance spectrometer platform to identify and measure the color change that takes place in the Revelar tube after exposure to a breath sample. This detection system can interpret the shift in color produced by the Schiff base formation of the reagent in the tube on exposure to aldehydes in a quantitative fashion. As a result the Revelar breath test provides a rapid, real-time snapshot for the user that other types of samples, such as urine, cannot.

Prototype Revelar devices have been tested to determine intra- and inter-day reproducibility, as well as long-term stability. Results have shown CV's of less than 3% for intra- and inter-day reproducibility, with stability of at least six months with the existing choice of battery and power consumption.

Please see the papers, '*What Revelar Measures*' and '*Summary of Revelar Performance Characteristics*' for more specifics on performance characteristic.