White Paper – What Revelar™ Measures

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Each cell in our bodies is surrounded by a membrane made up of high molecular weight unsaturated fatty acid molecules called phospholipids. When the body does not have enough natural antioxidants to protect it from the reactive oxygen-based free radicals that we produce as a byproduct of breathing (collectively known as reactive oxygen species, or ROS), these phospholipids (as well as proteins and DNA) can be attacked by the excess free radicals present, resulting in a condition we call oxidative stress. Many diseases, as well as exposure to toxins and even some normal activities, are known to cause oxidative stress; these include heart disease, cancer, autoimmune and neurodegenerative diseases, infectious diseases such as HIV, hepatitis, chronic fatigue syndrome, as well as lifestyle choices such as smoking and even strenuous exercise.

Numerous studies over the past three decades using tissue or plasma samples have shown that certain low molecular weight compounds known as aldehydes are very effective biomarkers of oxidative stress. These compounds are the end products of a chemical reaction that starts with an attack on phospholipids by the oxygen based hydroxyl radical ($OH^{\cdot}$) to form unstable compounds known as lipid hydroperoxides and ends with aldehyde release.

Many of these studies have relied on the colorimetric TBARS[1] (thiobarbituric acid reactive substances) assay to detect aldehydes because of its ease of use. However, the TBARS assay measures primarily only one aldehyde, namely malondialdehyde (MDA), a byproduct of the lipid peroxidation of arachidonic acid (an omega-6 fatty acid), and studies using more sophisticated chromatographic methods for analysis have shown that as many as 33 different aldehydes are produced by lipid peroxidation,[2] of which MDA is a very minor component. In particular, the latter studies have shown that the C3 to C10 saturated n-alkanals, e.g., hexanal (C6), heptanal (C7), and nonanal (C9), breakdown products from the peroxidation of linoleic and arachidonic (omega-6), palmitoleic, and oleic acids, respectively, and some unsaturated 2-alkenals, e.g., acrolein, correlate strongly with oxidative stress levels.

More recently, new studies using cutting edge laboratory analytical methods have shown that many of the key aldehydes associated with the lipid peroxidation process are also exhaled in a person's breath, that the amount of aldehydes present in such a non-invasive (e.g., compared to tissue or plasma sampling) breath sample reflect the presence of disease states known to involve an oxidative stress pathway or the effects of lifestyle habits such as smoking, and that the levels of these aldehydes change after appropriate therapy.[3][4][5]

Given its broad impact, there is a clear need in many areas of health care and biological research for a method that can simply and accurately measure oxidative stress levels using such a non-invasive sampling method. However, the analytical methods cited above required very expensive equipment, highly skilled personnel, and a great deal of time to run each sample, rendering them impractical for widespread use.

Now, Pulse Health has developed a system using a specific and sensitive reagent and a small, portable reader device that can measure aldehydes in the same type of non-invasive breath sample – and do so quickly, accurately, precisely, and inexpensively. This novel and proprietary technology is called Revelar.

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[1] Kelly et al., 1998 Environmental Health Perspectives Volume 106, Number 7
[2] Kawai et al., 2007 Chemical Research in Toxicology, Volume 20(1), pp. 99-107
[3] Nowak et al., Free Radical Biology & Medicine, 2001,Vol. 30, No. 2, pp. 178–186
[4] Corradi et al., American Journal of Respiratory and Critical Care Medicine, 2003, VOL 167, pp 395-399
[5] Corradi et al., American Journal of Respiratory and Critical Care, 2003, VOL 167, pp 1380-1386

Table 1. Aldehyde Reactivity (in absorbance units) with the Revelar reagent

Aldehyde	5 min	10 min	30 min	60 min
20uM decanal (C10)	2.653, 2.681	-----, 2.684	2.66, 2.68	2.66, 2.707
10uM decanal (C10)	2.547	2.715	2.738	2.735
20uM nonanal (C9)	2.703, 2.707	-----, 2.733	2.723, 2.737	2.723, 2.773
10uM nonanal (C9)	2.699	2.697	2.719	2.715
20uM octanal (C8)	1.498(?), 2.75	-----, 2.757	2.71, 2.765	2.715, 2.816
10uM octanal (C8)	0.725	1.07	1.581	1.647
20uM heptanal (C7)	2.289, 2.74	-----, 2.742	2.701, 2.748	2.70, 2.792
10uM heptanal (C7)	0.841	1.192	1.533	1.409
20uM hexanal (C6)	1.913, 1.406	-----, 2.087	2.52, 2.437	1.706, 1.842
10uM hexanal (C6)	0.436	0.595	0.586	0.323
20uM pentanal (C5, valeraldehyde)	1.198	-----	2.255	1.468
20uM butanal (C4, butyraldehyde)	1.428	-----	2.35	1.551
20uM propanal (C3, propionaldehyde)	0.282	------	0.499	0.218
20uM acetaldehyde (C2)	0	0	0.001	0.001
20uM formaldehyde	0.002	0.003	0.005	0.007
10uM formaldehyde	0	0.001	0.001	0.002
20uM MDA (malondialdehyde)	0.024, 0.018	-----, 0.07	0.691, 0.679	2.054, 2.172
10uM MDA (malondialdehyde)	0.01	0.038	0.295	0.729
20uM HNE (4-hydroxynonenal)	0.069	-----	0.084	0.102
20uM HHE (4-hydroxyhexenal)	0.068	-----	0.082	0.104
20uM acrolein (2-propenal)	0.279	-----	1.622	1.723
20uM 2-furaldehyde (furfural)	0.004	-----	0.004	0.004
20uM salicylaldehyde	0.001	-----	0.001	0.001
20uM acetone	0.002	0	0	0
10uM acetone	0	0	0	0
20uM 2-butanone – 2	0.001	0.001	0.001	0.001
10uM 2-butanone – 2	0.001	0.001	0.001	0.002

Schiff base formation is a well-known chemical reaction of aldehydes. The Revelar reagent was chosen for its ready participation in this reaction, and for its distinctive color change in the process of aldehyde complex formation. A wide variety of aldehydes (dissolved in 10% aqueous DMSO) relevant to oxidative stress were tested for their reactivity with the Revelar reagent; complex formation, expressed as an increase in complex absorbance value, was monitored as a function of time (where an absorbance value of approximately 2.7 indicates essentially complete reaction of the aldehyde). Results are shown Table 1.

As shown by the data, the Revelar reagent reacts rapidly and completely (and in a concentration dependent manner) with most of the aldehydes relevant to oxidative stress. Although for technical reasons this experiment was carried out in aqueous solution, it is reasonable to assume that the reactivity profile of the Revelar reagent with the aldehydes in a breath sample is analogous, and that the Revelar score represents the total amount of complex formed from all of the reactive aldehydes present in a breath sample. There are several advantages to this approach: a single aldehyde in the breath would be extremely hard to see, but because Revelar measures a sum of aldehydes (that effectively provide a larger sample), the Revelar system has more than adequate sensitivity, and even small changes in aldehyde breath content can be precisely measured; and the results also give a more complete picture of the lipid peroxidation processes occurring in the body.[6]

[6] Alhamdani et al., 2006 American Journal of Nephrology Volume 26(3), pp. 299-303

Another advantage of the Revelar reagent is its specificity. Other reagents are known to react with aldehydes, but their reactivity is not specific only to the breakdown products of oxidative stress; that is, they also react with structurally related molecules like ketones (normally generated in the body by the breakdown of carbohydrates and amino acids) and thus would not provide results that are a specific measure of oxidative stress. In contrast, the Revelar reagent shows very low reactivity with such non-relevant compounds, especially those known to be present in exhaled breath.[7] For example, it shows almost no reactivity with the ketones 2-butanone or acetone (the latter is a natural metabolite that is normally present at low levels in breath, elevated in the breath of diabetics), or with aldehydes such as salicylaldehyde and acetaldehyde (the latter a natural metabolite that is elevated in the breath after drinking alcohol) that are not produced by oxidative stress.

In summary, because of its high degree of reactivity with most of the important aldehydes produced by oxidative stress and its measurement of all of these aldehydes simultaneously in a breath sample, the Revelar system has a high degree of sensitivity. The Revelar system also measures only the aldehydes in a person's exhaled breath that are specifically related to the lipid peroxidation process. Thus the noninvasive, simple, and rapid Revelar test provides a precise, sensitive, and specific method for measuring oxidative stress levels.

[7] Smith et al 2007 J. Breath Res. (electronic) Volume 1, Number 1, 14004 (12pp)