

PERFORMANCE CHARACTERISTICS OF THE REVELAR™ FREE RADICAL BREATH CONDENSATE ASSAY

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The Revelar Free Radical Breath Condensate Assay (“Revelar Assay”) has been developed to detect free radicals in the form of aldehydes in breath condensate. This home-use assay is comprised of two components: a digital, handheld electronic analyzer, and a single-use disposable tube. The reagents contained in the Revelar tube act to absorb breath condensate, and react this condensate with a reagent that produces a color. The tube is then inserted into the Revelar analyzer, and the color is then read and interpreted. The results are displayed as Revelar units, ranging from 0-99,999. Increasing Revelar units indicate increased levels of aldehydes in breath condensate.

Numerous studies have been performed to establish the performance characteristics of the Revelar Assay. In fact, although the Revelar Assay is intended for informational and nutritional uses, these studies have been performed according to the Food and Drug Administration’s Good Manufacturing Practices guidelines. Moreover, the extent and number of studies performed on the Revelar Assay exceed those studies contained in the 510(k) Premarket Notification for the .02 Breath Alcohol Check Detection System by Akers Biosciences, Inc., which has been cleared by FDA.

This paper will describe the core studies that have been performed on the Revelar Assay related to performance characteristics. These studies include: 1.) inter- and intra-day reproducibility; 2.) accuracy and precision; 3.) reactivity to various aldehydes; 4.) population range studies; and 5.) correlation of serum aldehyde to breath condensate aldehyde levels.

Reproducibility

Ten Revelar analyzers were used to determine inter- and intra-day reproducibility. 20 low and 20 high Revelar standards were run on each analyzer every day for 5 days. Intra-day coefficients of variation ranged from 2.97 - 8.23 % for the low standards, and 1.02 - 2.47% for the high standards. Inter-day coefficients of variation ranged from 4.27 - 6.80% for the low standards, and 1.55 - 2.10% for the high standards. Coefficients of variation throughout the 5 day study were 5.46% for the low standards, and 1.84% for the high standards.

Accuracy and Precision

Five Revelar analyzers were used to determine accuracy and precision. 10 Revelar tubes were charged with standard solutions of aldehydes using a simulated human lung device (Akers Biosciences, Inc.). For tests run in early December, 2007, accuracy was determined to be +/- 12.4% in the low end of the range (18,000 Revelar units), and +/- 1.7 % in the mid range (58,000 Revelar units). Precision was determined by coefficients of variation, which ranged from 7.09 - 10.6% in the low range, and 1.89 - 3.62% in the mid range.

Precision was further assessed through the use of eleven different human subjects to charge Revelar tubes with multiple breath condensate samples. Coefficients of variation for these tests completely on 11/4/07 ranged from 3.82 (47,409 Revelar units) to 10.71% (70,336 Revelar units).

Reactivity to Aldehydes

The reactivity of the Revelar Assay to various aldehydes is listed in Table 1.

Table 1. Relative Reactivity of the Revelar Assay to Various Aldehydes

	5 MIN.
octanal (C8)	2.75
nonanal (C9)	2.703
decanal (C10)	2.681
heptanal (C7)	2.289
butanal (C4, butyraldehyde)	1.428
hexanal (C6)	1.406
pentanal (C5, valeraldehyde)	1.198
propionaldehyde (C3)	0.282
acrolein (2-propenal)	0.279
HNE (4-hydroxynonenal)	0.069
HHE (4-hydroxyhexenal)	0.068
MDA (malondialdehyde)	0.024
2-furaldehyde (furfural, furan-2-aldehyde)	0.004
Formaldehyde	0.002
salicylaldehyde	0.001
2-butanone	0.001
Acetone	0.001
acetaldehyde (C2)	0

Population Range Studies

Revelar Assay results were obtained from 98 human subjects. The Revelar unit results ranged from 27,991 to 99,999. The data were grouped in units of 10,000, and the frequencies are detailed in Table 2.

Table 2. Frequency of Revelar unit results In a Normal Population

Revelar UNIT	FREQUENCY
20-29,999	2
30-39,999	2
40-49,999	18
50-59,999	22
60-69,999	18
70-79,999	17
80-89,999	14
90-99,999	5

Correlation of Aldehydes in Serum and Breath Condensate

A correlation study was performed on 16 human subjects to determine the relationship between aldehyde levels in serum, using a modified Revelar reagent system, and aldehyde levels in breath condensate, using the Revelar Assay. Serum samples were obtained immediately after obtaining a Revelar breath condensate result, and assayed the same day. Using a Pearson's parametric analysis, there was a significant positive correlation between serum and breath condensate aldehyde levels ($p < .01$).

Conclusion

The Revelar Breath Condensate Assay System has been extensively shown to be an accurate and precise measurement of free radicals (as aldehydes) in human breath condensate. It is significant that aldehyde levels in blood serum correlate with aldehyde levels in breath condensate. Breath condensate levels of aldehydes can thus be used as a reliable source of information during a nutritional assessment of an individual.