Comparison of Results Between Binary and Gradient Point-Of-Care MMP/HNE Enzyme Detection Technologies

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INTRODUCTION

Proteases like MMP2, MMP9 and Human Neutrophil Elastase (HNE) are known to play important roles in normal wound healing, yet their elevated levels can lead to chronic, non-healing wounds. One point-of-care diagnostic that detects the presence of these proteases, offering a binary result (high vs. not high), is commercially available outside the United States. It is CE-marked, and used as the standard of care in Europe for measuring proteases. This binary assay (BA) approach provides a somewhat limited view of the actual level of proteases at the time of testing (i.e., how high above a normalized level) as well as movement from this level in subsequent testing (i.e., has the level increased or decreased, and by how much, since the previous assessment). A newer technology has been developed that provides a visual gradient-based, colorimetric assay (GBCA) and also an optional quantified outcome through a small, handheld measuring device. These results provide clinicians with more information regarding the direction the wound is heading after initial measurement, helping to better suggest clinically relevant therapeutic interventions to bring elevated protease levels to more normalized levels.

The GBCA itself is a fluid suspension containing gelatin-coated microspheres in a buffer. The method of action theorizes that as gelatinases are introduced to the assay, they flocculate/degrade the gelatin coating. The resultant flocculation results in both:

- An optically observable darkening of the color of the solution
- commensurate increase in turbidity of the solution due to the additional particulate matter from the lysed gelatin fragments released into solution.

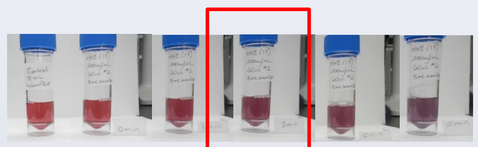
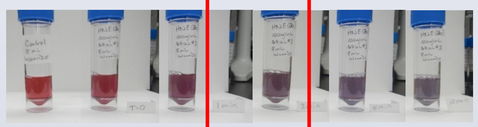
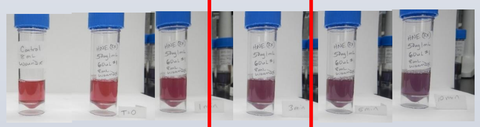
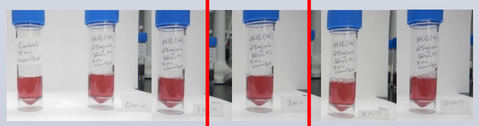
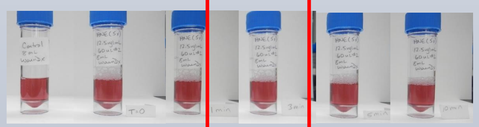
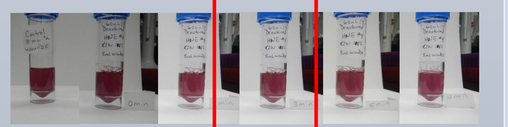
This study compared results of these two technologies.

MATERIALS AND METHODS

Six serial aliquots of serine proteinase Human Neutrophil Elastase (HNE) (Athens Research, Athens, GA) and one denatured sample were introduced into the novel GBCA to assess colorimetric changes at several time points (T=0, T=1 min, T=3 min, T=5 min, T=10 min). Images were taken to compare to baseline. GBCA analyte flocculation by HNE was also quantified using a handheld turbidimeter (Lutron Electronics Co., LTD, Taipei, Taiwan) for each dilution/time point.

These same sequential aliquots/dilutions were tested with the BA.

RESULTS

	GBCA Colorimetric (visual) Measure (Control, 0 mins, 1 min, 3 mins, 5 mins, 10 mins)	Turbidimetry Average Score at 3 minutes (variance from baseline)	BA result
HNE - Dilution 1x - 1X concentration		271	+
HNE - Dilution 2x – 0.5X concentration		493	+
HNE - Dilution 3x – 0.25X concentration		257	+
HNE - Dilution 4x – 0.125X concentration		5	–
HNE - Dilution 5x – 0.0625X concentration		0	–
HNE – DENATURED 1x		10	–

3 mins

CONCLUSION

In this lab study, varying concentrations of HNE in simulated wound fluid were tested with the GBCA (both visually for color changes as well as quantitatively with a turbidity meter) and with the BA. The BA – a binary assay – shows only whether the protease levels are above or below a set threshold.

For Dilutions #1X, 2X, and 3X, there were clear and significant visual color changes using the GBCA. The color change was variable dependent on the concentration of HNE in solution, and also as a factor of time. The one anomaly was the #2X concentration, which resulted in a darker color and higher turbidimetry score than the #1X concentration. This is believed to be the result of interaction with the buffer, which at that specific ratio allowed the HNE to reach its highest level of activity within the solution. Similarly, the Turbidimetry scores were significantly higher than baseline. Both the visual color result and the quantitative turbidimetry result align with the method of action of the GBCA. For these "elevated" concentrations, the BA tested positive, indicating that the level of HNE within solution is higher than the set cutoff threshold level for HNE within the assay.

For concentrations #4X, 5X, 6X and the denatured sample, there were no significant visual color changes using the GBCA, and the turbidimetry scores were not significantly changed from baseline. Similarly, the BA tested negative for these, indicating that the level of HNE within solution was lower than the set cutoff threshold level for HNE within the assay.

For varying high and low concentrations, the GBCA provides gradient level results (both visually and quantitatively), whereas the BA provides only a binary assessment. Interestingly, both devices showed "positive" results for dilutions #1X, 2X, and 3X, and "negative" results for #4X, 5X, and 6X. This result indicates that the GBCA has a roughly similar positive threshold level as the BA. However, in clinical practice, the gradient nature of the GBCA may prove to be more relevant and actionable to clinicians in repeat testing such as at the next wound assessment. These qualitative and quantitative measurements could prove to be highly insightful into wound biochemistry as clinicians seek to not only change their therapeutic interventions based on the presence of elevated levels of proteases, but also seek to assess the direction of the protease levels after the initial assessment.

Further study is required to assess the correlation between the GBCA's qualitative and quantitative scores and the healing trajectory of wounds as well as the clinical utility of gradient-based results when making treatment decisions.