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Effects of centrifugation on human saliva samples in reducing sample variability in specific pepsin measurement

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Introduction: Reflux of gastric contents is a causative factor in conditions such as chronic cough and Laryngopharyngeal Reflux. Currently the "gold standard" for diagnosis of reflux disease is 24 hour pH monitoring, an invasive technique which affects patient lifestyle and reflux frequency. However, pepsin is the major damaging agent within the gastric refluxate [1-2] and so it is more appropriate to use this as a marker for reflux than acid.

Aim: Here we present improvements in a novel, non-invasive, diagnostic test for reflux disease using pepsin as the marker in sputum/saliva samples.

Methods: Human sputum/saliva samples were obtained into 0.5 ml 100 mM citric acid from chronic cough patients. Pepsin activity was measured using the N-terminal assay [3]. Samples were defrosted, vortex mixed and assayed without processing. Then samples were centrifuged for 10 minutes at 10,000 rpm and the supernatant assayed. All samples were tested in triplicate and the variability of these triplicate values assessed by calculation, of the coefficient of variation, CV, determined by (SD/mean) x 100. Statistical significance was measured using the two-tailed unpaired t-test and considered significant if $p < 0.05$.

Results: 86 saliva samples were tested without processing. The variability between replicates was high with a mean (±SE) CV of 39 (±9)%. 131 saliva samples were tested with the centrifugation processing method. The variability between replicates was significantly reduced to a mean (±SE) CV of 20 (±2)%, nearly half that of the non-centrifuged samples.

Conclusion: We have significantly improved the methodology for the detection of specific pepsin activity in human saliva samples for the N-terminal assay, using simple processing techniques. Therefore we can more reliably detect and quantify pepsin activity for the diagnosis of reflux disease.

References:

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