

Acute Anaphylaxis Monitoring: Studies Using Mast Cell Tryptase

AIM

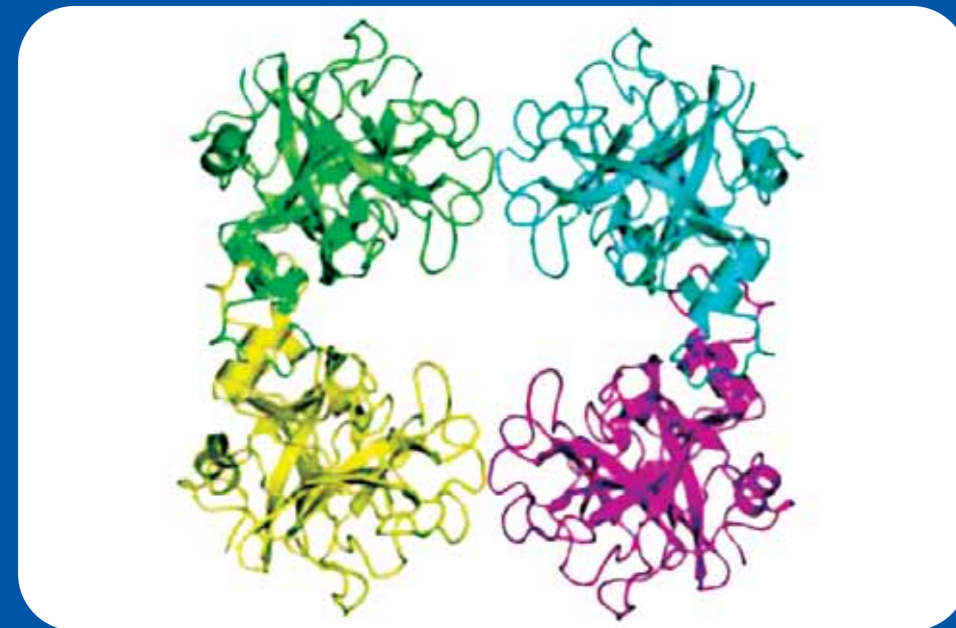
Mast cell tryptase is a specific biomarker for the acute IgE-mediated allergic response known as anaphylaxis. Currently, the only commercial sources of controls and standards for tryptase are a recombinant protein or patients' samples. Our objective was to prepare a stable and reproducible tryptase protein standard from human tissue and to evaluate its suitability when diluted in patient normal human serum for External Quality Assessment (EQA) distribution.

INTRODUCTION

Mast cells are the key effector cells in allergic responses. When stimulated by a specific allergen they release a number of mediators of inflammation, amongst which is the serine protease tryptase. Tryptase is released into the circulation after a patient suffers an anaphylactic reaction caused by drugs, insect venom or food. Measurements of tryptase can help identify and assess the extent of the reaction. Basal levels of tryptase are in the order of 0.2 to 14.0 µg/L, with peak levels of more than 40 µg/L following allergic stimulation.

There is evidence that mast cell tryptase comprises a multigene family, although the catalytically active β-tryptase is the form released during systemic anaphylaxis.

Mast Cell Tryptase Tetramer



THE PROBLEM

For EQA programs in particular, a native and reliable purified mast cell tryptase is required, with adequate stability when diluted in normal human serum.

STUDY DESIGN

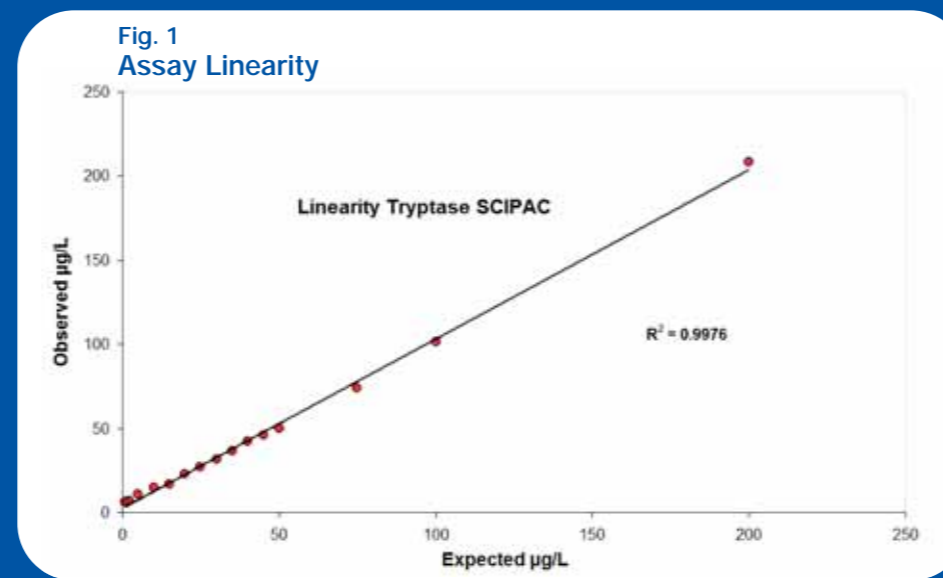
Mast cell tryptase from human lung tissue was purified at SCIPAC using heparin affinity chromatography. Purity was estimated as ~95%. Samples of two different concentrations were diluted in normal human serum and sent to testing laboratories throughout the world, 67% being in the UK.

Routine testing was performed and the results were returned to UK NEQAS in Sheffield for data analysis. The analytical methods used were Phadia ImmunoCAP 100, 250 and 1000, which measure both α- and β-tryptase. Other data collected in addition to the tryptase result were the conditions of storage and the time lapse between receipt and analysis.

RESULTS

Linearity

Excellent linearity was obtained beyond the highest value of tryptase expected to occur in patients' samples, up to 200 µg/L ($r^2 = 0.9976$).



DATA ANALYSIS

Histograms for two different assay samples 1021 (nominally 30 µg/L) and 1022 (nominally 60 µg/L) are shown in Fig. 2 and Fig. 3 respectively and the statistical data in the tables. Outlier data points were removed from the analysis where there was evidence of poor sample storage or an inordinately long lag prior to analysis (5 data points in total for both samples). The results indicate agreement within 6% (sample 1021) and 3% (sample 1022) of the expected values and a coefficient of variance below 10% for all the laboratories in the analysis. There was no significant difference between results from the Phadia 100 and Phadia 250 analysers.

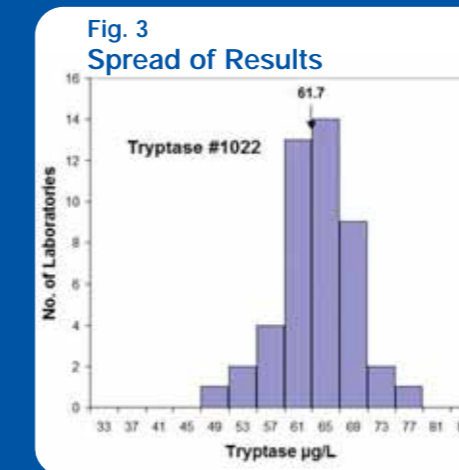
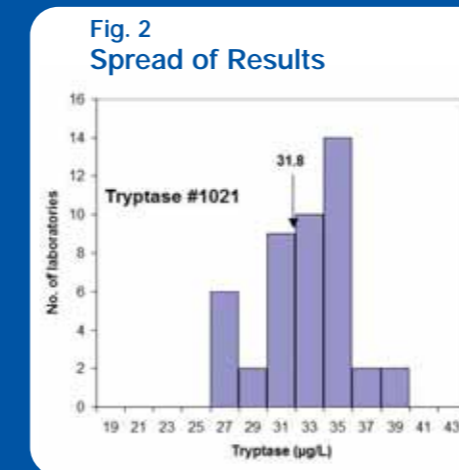


Table 1: All Methods: Statistics (Trimmed of poorly stored samples)

Assay Sample	Target Value µg/L	n=	Mean	±SD	±CV (%)
1021	30	45	31.8	3.2	9.9
1022	60	46	61.7	5.7	9.2

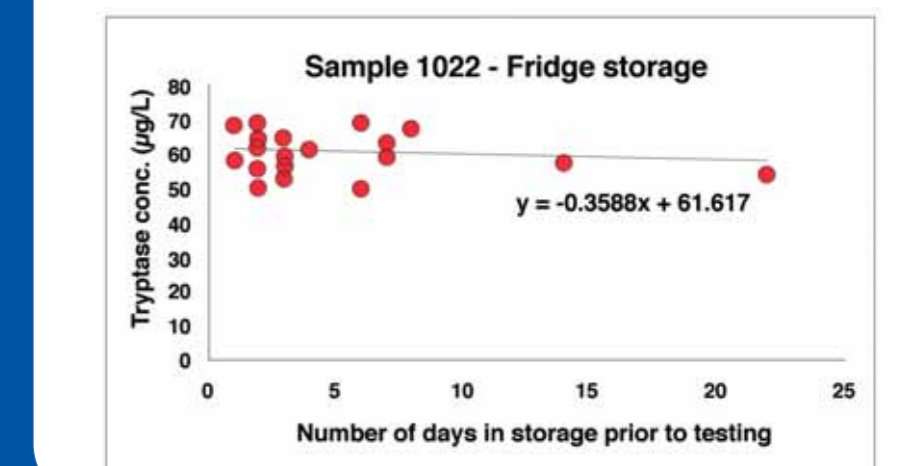
Table 2: Comparison of Phadia 100 and Phadia 250

Assay Format	Assay Sample	n=	Mean	±SD	P value
Phadia 100	1021	6	29.2	6.4	0.10
Phadia 250	1021	40	31.9	3.2	
Phadia 100	1022	6	62.2	3.4	0.67
Phadia 250	1022	42	61.0	6.4	

STABILITY

Initial studies on the stability of the serum-diluted samples (Fig. 4) indicate acceptable stability over a 3 week period. Freezer storage (<-15°C) gave similar stability graphs to those obtained using fridge storage.

Fig. 4
MCT Stability in fridge (2-8°C)



CONCLUSIONS

The mast cell tryptase prepared and purified from human lung tissue, diluted in serum, shows excellent results as a control and standard for EQA distribution. Further work during 2010 will include a much larger laboratory analysis trial and further assessment of stability.

AUTHORS

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