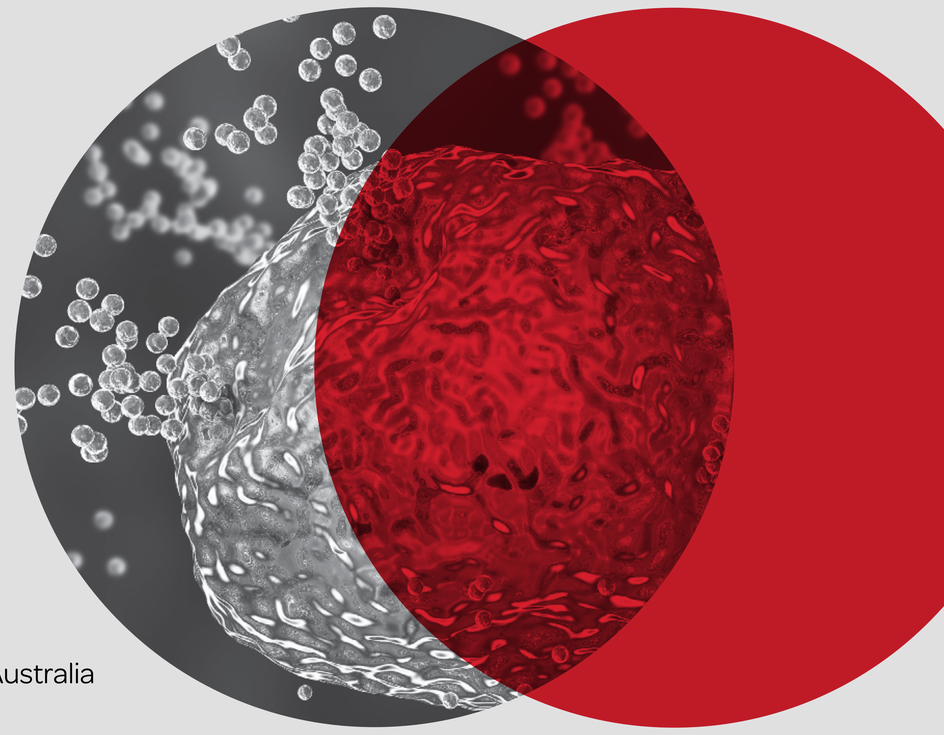
Review of lyophilisation of external quality assurance program material



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Introduction

Tryptase is an enzyme secreted from mast cell granules during degranulation at the onset of anaphylaxis, a severe, life-threatening, hypersensitivity reaction. Tryptase is a valuable biomarker to differentiate anaphylaxis from its mimics. Tryptase concentration rises sharply in the first 90 minutes from the onset of reaction and declines steadily following first order kinetics[1, 4]. With a half-life of ~2 hours, tryptase returns to baseline within 6 to 24 hours. Serial measurement of total serum tryptase is recommended from initial onset of acute symptoms (15 minutes to 2 hours) to baseline level[1]. In this context, the international consensus equation defines acutely elevated tryptase as greater than 1.2 × baseline tryptase + 2 µg/L[1,2,4].

Baseline tryptase can also be used as a marker of mast cell burden and is elevated in systemic mastocytosis. Higher baseline tryptase can lead to severe anaphylaxis, and a risk of osteoporosis. Tryptase can also be elevated in haematological neoplasms[2].

The RCPAQAP Tryptase external quality assurance (EQA) program comprises 8 samples tested throughout a program year. Sample integrity is a critical factor in provision of EQA programs. Ensuring stability at conditions samples are likely to be exposed to during shipping (temperatures and times) and storage until being tested is an ISO requirement (ISO/IEC 17043) for proficiency test providers.

Routine internal assessments of sample stability at the RCPAQAP conducted in 2018/2019 indicated that tryptase samples have reduced stability when exposed to 37°C for prolonged periods (7 days). These conditions are common during peak shipping periods, especially to international participants. Subsequent internal evaluations showed that sample stability could be improved by lyophilisation of samples. This study aims to assess performance with the RCPAQAP's Tryptase program following an initiative to improve the quality of the program material through lyophilisation.

Method

RCPAQAP Tryptase program data for the 2018/2019 (non-lyophilised material) was compared to the 2020/2021 (lyophilised material). For the 2018 and 2019 program years, sample material was comprised of human serum samples shipped at ambient temperature and then stored frozen until testing. The 2020 and 2021 program material was comprised of lyophilised human serum samples which were shipped at ambient temperature, to be stored refrigerated and then reconstituted at the time of testing. Data for all program years were assessed using a one-way analysis of variance with Tukey's multiple comparison test to determine if there were any significant differences in performance year by year or as a result of changes to sample preparation.

Results and Discussion

A total of 32 samples were surveyed between 2018–2021 and the number of results submitted per survey ranged from 27-33. All laboratories used the Thermo Fisher Scientific ImmunoCAP™ Tryptase fluorescence enzyme immunoassay (FEIA) performed on the ImmunoCAP™ 100, 250, or 1000 platforms.

Serum tryptase values during these years ranged from 5–170 µg/L. Note that in general, patients with systemic mastocytosis have a persistently elevated serum tryptase baseline level exceeding 20 µg/L[1-3]. Figure 1 shows the mean and standard deviations for all surveys in 2018–2021.

The mean CV% for tryptase measurement in 2018, 2019, 2020 and 2021 were 8.1%, 9.7%, 7.6% and 8.6% respectively. Comparison of the CV% for all program years 2018–2021 (Figure 2) showed no significant variation (*p* values ranged from 0.32–0.99). This indicates no significant change in performance between programs years and no difference in performance with changes to the sample preparation (frozen vs. lyophilised serum).

The mean CV% calculated within the RCPAQAP program also reflect the average inter-laboratory variation for this assay, indicating good reproducibility as the manufacturer indicates an expected between run CV of 3–4%[3].

A lack of variation between assay CVs for the two sample preparations indicates that sample stability was unlikely to be an issue for this program previously. Increases in enrolments from international laboratories and global shipping delays during the COVID-19 pandemic would have potentially compromised the continued use of frozen samples.

Conclusion

This review of the RCPAQAP 2018–2021 Tryptase program data confirms that assay performance was not affected by a change in sample preparation (frozen vs. lyophilised serum). Sample stability did not appear to play a significant role in this program previously however moving to a lyophilised product ensures sample integrity to accommodate growing international participation and unavoidable global shipping delays.

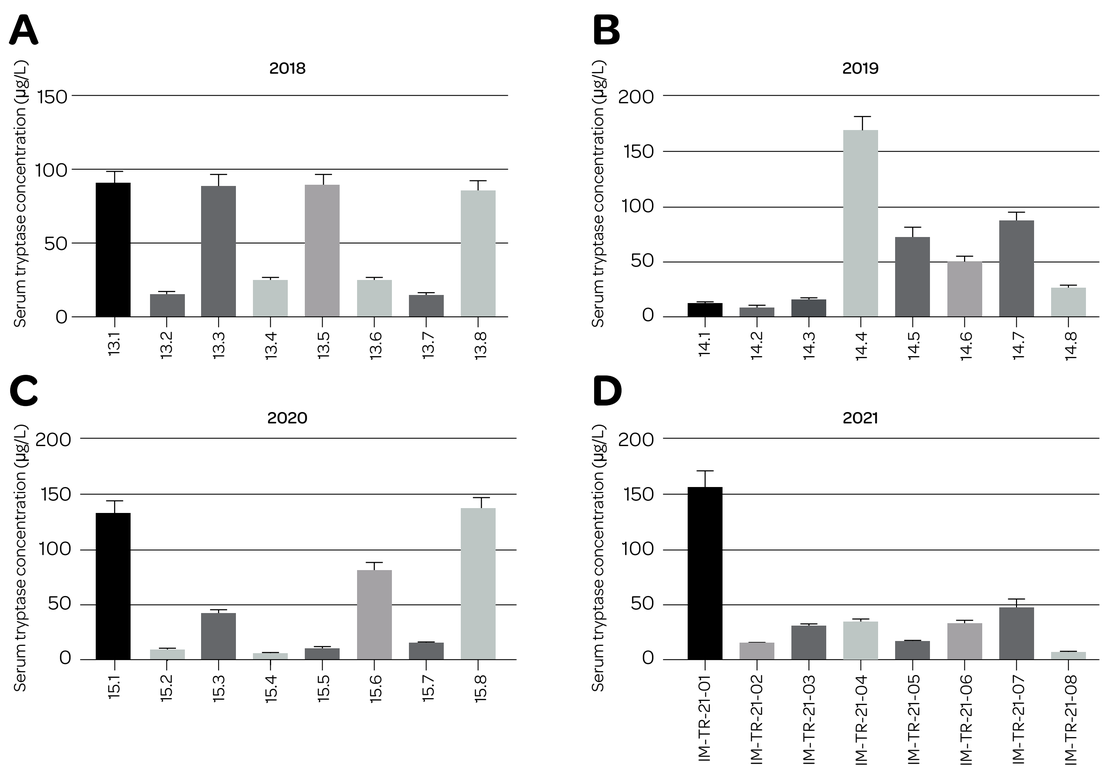

Figure 1. Results from the Tryptase program provided by the RCPAQAP. A-B: Results generated in 2018 and 2019 by participating laboratories using frozen serum samples. C-D: Results generated in 2020 and 2021 by participating laboratories using lyophilised serum that is then reconstituted prior to testing. Histograms display mean 1 SD.

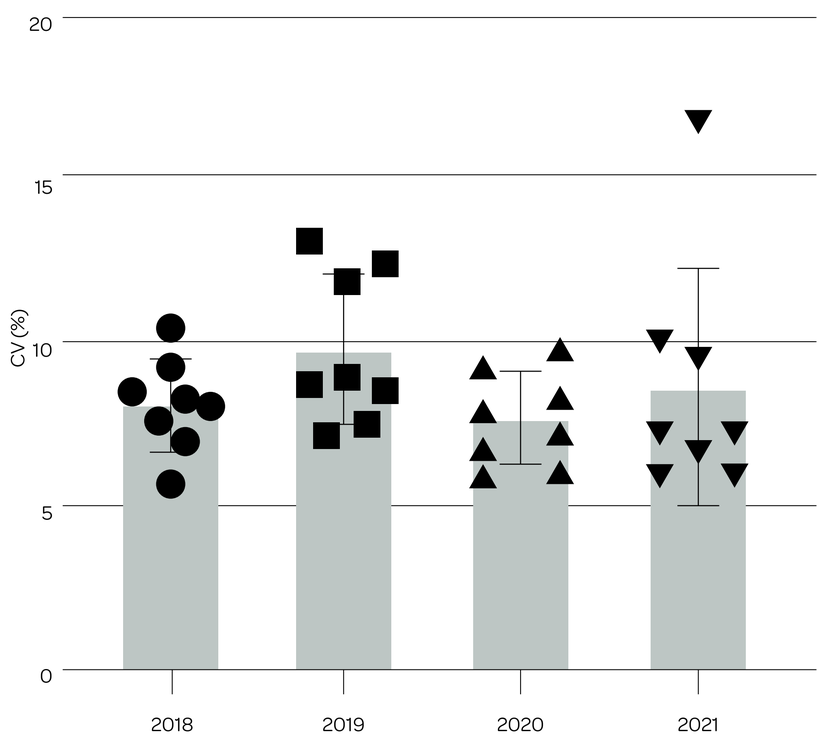

Figure 2. Analysis of CV for Tryptase programs of the RCPAQAP. Data points represent the mean CV(%) for the 8 surveys evaluated during each year. Data was analysed using a one-way ANOVA with Tukey's multiple comparison test.

References:

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