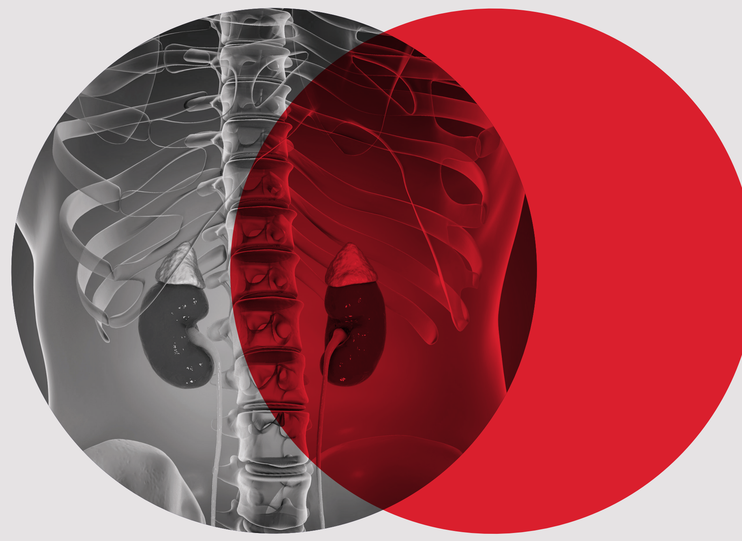
Challenging the robustness of plasma free metanephrine methods – Are laboratories hydrolyzing samples?



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Introduction

External quality assurance (EQA) is an essential tool for the provision of quality laboratory results. Ideally, EQA materials should reflect the composition of patient samples to ensure their clinical relevance and applicability. Where possible, RCPAQAP sources materials from real patients. However it is frequently necessary to supplement with analytes to achieve desired concentration ranges that represents both healthy and diseased states. This manipulation may render samples unrepresentative of actual patients (non-patient like).

Measurement of metanephrines is considered standard practice for the biochemical diagnosis of phaeochromocytoma and paraganglioma(1). Metanephrines are excreted in urine predominantly as sulphated conjugates. Urinary metanephrines are reported as total (free and conjugated) after deconjugation with acid hydrolysis. Plasma also contains free and conjugated metanephrines but they are reported exclusively as free (unconjugated) levels.

RCPAQAP Plasma Metanephrines program distributes lyophilised human plasma samples which are expressly spiked with free metanephrines to achieve the desired range of values in the linear program. Any unintentional hydrolysis of the unconjugated plasma components would give falsely raised levels.

In early 2019 ACBA introduced a challenge sample into the program. This plasma was spiked with urine to predetermined free metanephrine corresponding closely to level 2 of the linear set. In this way ACBA introduced a high level of conjugated metanephrines. The aim of this study was to observe if participants were causing inadvertent hydrolysis of conjugated metanephrines during preparation of plasma EQA samples.

Method

Normal human plasma was sourced, pooled, and spiked with urine containing both conjugated and free metanephrines. This challenge sample was lyophilised and included with the kits that were distributed to all participants enrolled in the 2019 RCPAQAP Plasma Metanephrines program.

Participants were not informed of the unique nature of these samples, and analysed the material as part of a routine survey conducted in January 2019. Statistical analysis of returned data was performed using RCPAQAP in-house software. Median recoveries and distribution of results (CV%) were compared to a previous survey sample with similar target value (March 2018). Subsequent review followed from the ACBA.

Results

A total of 47 laboratories participated in the survey, representing a variety of sample preparation techniques and liquid chromatography methods. Median and coefficient of variation (CV) results for both the challenge *patient-like* sample (22-02) and normal non *patient-like* sample (20-05) are displayed in table 1 and table 2 below respectively.

Table 1. Summary of results (pmol/L) for *challenge* sample spiked with free and conjugated metanephrines (22-02) by method

Column / Reagent Kit	n	Median	Mean	S.D.	CV(%)
All results	47	474	481.8	33.3	6.9
Own Reagent	20	473	481.3	28.5	5.9
Waters	7	470	466.6	28.5	6.1
Chromsystems	6	506	445	175.3	39.4
Phenomenex	2	481	481	67.9	14.1
Recipe	3	452	466.7	28.0	6.0
Labor Diagnostika Nord	3	420	412	119.2	28.9
Not Specified	8				

Table 2. Summary of results (pmol/L) for *normal* sample spiked only with free metanephrines (20-05) by method

Column / Reagent Kit	n	Median	Mean	S.D.	CV(%)
All results	45	457	455.9	38.8	8.5
Own Reagent	23	458	458.9	33.8	7.4
Waters	6	439	447.2	45.6	10.2
Chromsystems	5	482	474.6	256.9	54.1
Phenomenex	3	391	401.7	44.0	10.9
Recipe	4	471	473.8	24.2	5.1
Labor Diagnostika Nord	4	222	308.2	202.3	65.6
Not Specified	5				

Figure 1. Distribution of results for *challenge* sample spiked with free and conjugated metanephrines (22-02). Results ranged from 91 to 720 pmol/L.

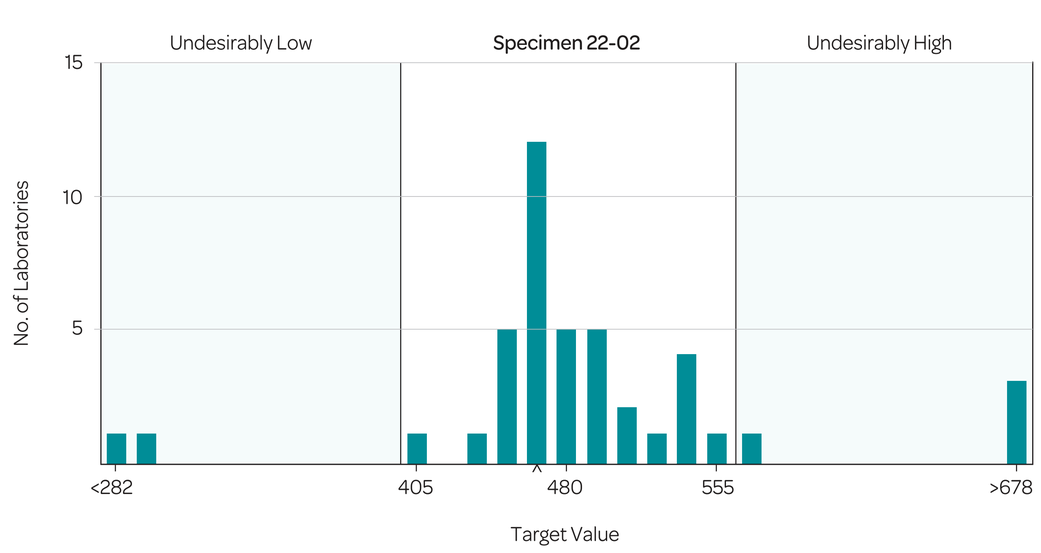
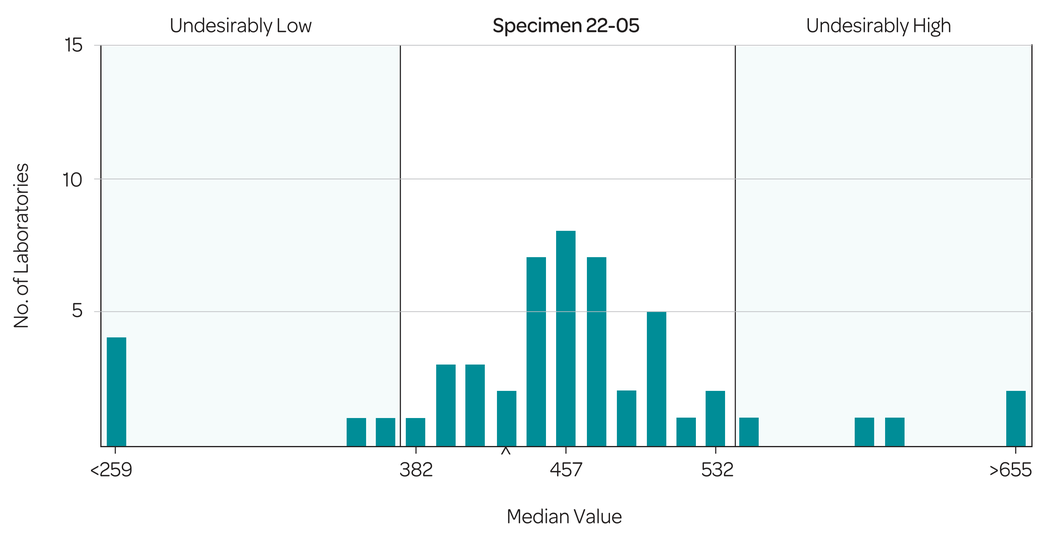


Figure 2. Distribution of results for *normal* sample spiked only with free metanephrines (20-05). Results ranged from 90-990 pmol/L.



Discussion

Testing for biogenic amines is a highly specialised process, which involves a number of technical steps to prepare specimens for analysis. Where possible, it is desirable for EQA schemes to capture information on sample preparation, as errors that occur during the this phase may contribute to diagnostic error and patient harm.

The results from this study demonstrate correlation in the distribution of results for both *patient-like* sample and the routine survey material. The All Results variability (CV) between the *patient-like* and equivalent normal sample was 6.9% and 8.5% respectively. An improved CV was observed across all methods for the *patient-like* group.

As the concentration of total plasma metanephrines is 20 to 30 times higher than free plasma metanephrines(2), it would be expected that hydrolysis of samples would cause a marked increase in free metanephrine recovery (>9000 pmol/L), and consequently an increased CV. Further, if this was occurring in a number of laboratories, the distribution of results would display bi-modality.

Conclusion

The findings of this study indicate that laboratories are not inadvertently hydrolyzing plasma free metanephrine samples. This is reassuring, as hydrolysis of “real” patient samples could potentially result in a false positive outcome.

References

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2. Eisenhofer G. Free or total metanephrines for diagnosis of pheochromocytoma: what is the difference? 2001;47(6):988-9.