

Limitation of the Vitros™ dry-slide technique in measuring high concentrations of salicylate in plasma

S A Iversen¹, A Walker¹, T Y Avades¹ and J F Wilson²

From the ¹Department of Chemical Pathology, Royal Sussex County Hospital, Brighton BN2 5BE, UK and the ²Heathcontrol External Quality Assessment Scheme, Department of Pharmacology, Therapeutics and Toxicology, University of Wales College of Medicine, Cardiff CF14 4XN, Wales

SUMMARY. The linearity of the Vitros™ dry-slide method for plasma salicylate was assessed in two ways: serial concentrations of sodium salicylate were added to fresh lithium heparin plasma, and the salicylate was determined both neat and in dilution. Vitros salicylate results submitted to the Heathcontrol External Quality Assessment Scheme were compared to the spike value. Similar loss of linearity was observed in both cases. Serious salicylate overdosage requiring specific clinical treatment may have been underestimated.

After a salicylate overdose plasma concentration may continue to rise for up to 24 h. Six hours after an overdose, a plasma concentration between 300 and 500 mg/L is associated with only mild toxicity, 500–700 mg/L with moderate toxicity, and those in excess of 700 mg/L are associated with severe toxicity.¹ The management of severe salicylism includes alkalinization of the urine if plasma salicylate concentration is 7–500 mg/L and haemodialysis if plasma concentrations are 7–700 mg/L. Therefore, the correct treatment of patients is directly dependent on good laboratory support.

A dry multilayered slide² (Vitros-Sali, Ortho-Clinical Diagnostics, Amersham, Bucks, UK) has been produced to measure plasma salicylate based on the following reactions:

Salicylate + NADH + H⁺ + O₂ $\xrightarrow{\text{salicylate hydroxylase}}$ catechol + CO₂ + NAD⁺ + H₂O
catechol $\xrightarrow{\text{tyrosinase}}$ 3-methyl-2-benzothiazolinone hydrazone (MBTH) + catechol $\xrightarrow{\text{tyrosinase}}$ red dye.

As shown above, salicylate in the sample is oxidatively decarboxylated to catechol, which is oxidized and coupled with MBTH, and the intensity of red dye that is formed is quantified by reflectance spectrophotometry (wavelength = 540 nm).³

In this technical note we describe the limitation of the dry-slide technology in measuring high plasma concentrations of salicylate. We

also describe the results from Vitros users participating in an External Quality Assessment (EQA) Scheme where the limitation described resulted in a high frequency of outliers.

Serial concentrations of sodium salicylate were spiked into six aliquots of 1 mL of fresh lithium heparin plasma collected from a healthy drug-free subject to achieve 1000, 800, 600, 400, 200 and 0 mg salicylate/L plasma. Salicylate analysis was carried out according to the Vitros method in duplicate on three Vitros analysers. The experiment was carried out twice: first using neat samples and second using samples diluted 1:3 (v/v) with 7 g/100 mL bovine serum albumin (BSA, Ortho-Clinical Diagnostics). This showed that the upper end of the dynamic range was 400 mg/L (Fig. 1), with samples above 400 mg/L showing marked underestimates.

Comparable work was carried out by the Heathcontrol EQA Scheme, where 12 spiked samples with nine different concentrations prepared from commercial human serum (Scipac Ltd, Sittingbourne, Kent, UK) and containing 0.05% w/v 'Bronidox', which consists of 10% 5-bromo-5-nitro-1, 3-dioxane dissolved in 1,2 propylene glycol as preservative, were distributed to laboratories within the scheme. Results from 15 laboratories using the Vitros technique are shown in Fig. 2. Those remaining, after rejecting measurements greater than 3 standard deviations (SD) from the all methods' consensus mean,⁴ showed a good straight-line relationship between Vitros measurements and the spike

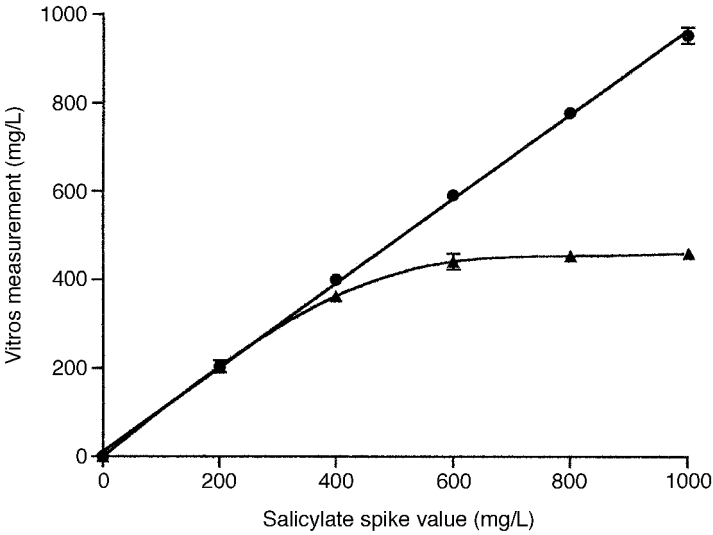


FIGURE 1. Salicylate measurements before and after plasma dilution. Samples without dilution (▲); samples after dilution 1:3 (●); each point represents the mean salicylate concentration standard deviation (SD) measured by three Vitros™ analysers in duplicate (see text for manufacturer's details).

value. The slope indicated a consistent negative bias of 9.7%. At spiked concentrations below 500 mg/L only occasional measurements were rejected as outliers. They were slightly low compared to the concentration expected, with percentage rejection rates of 0–13%. On the other hand, at concentrations above 500 mg/L the number of rejects increased to 19–33%. Interestingly, these latter rejected measurements

were similar to one another, falling in the range 314–497 mg/L.

Direct contact with a sample of the scheme participants confirmed that measurements of 7 500 mg/L that were good estimates of the spike value were the result of assays performed with diluted samples. By contrast, the rejected measurements resulted from the use of undiluted material. In two of the latter cases the laboratory

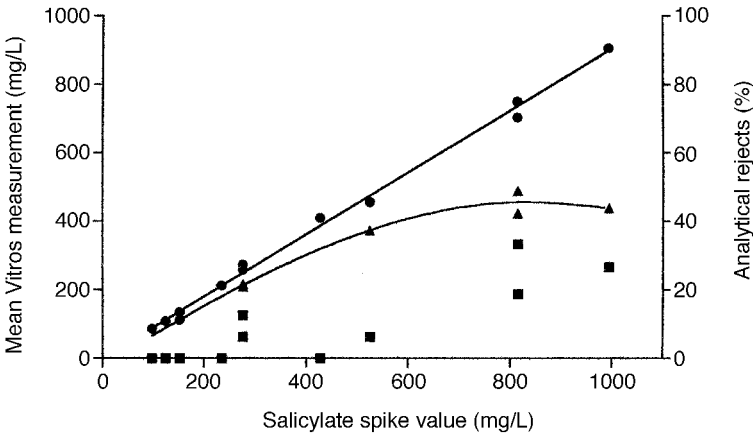


FIGURE 2. Mean serum salicylate measurement of non-rejects and rejects that were measured by the Vitros technique with the per cent of measurements rejected. Non-rejects (●); rejects (▲); and % analytical rejects (■).

reassayed the original sample after dilution when prompted by the Scheme Organizer, and produced correct high readings.

Prior to these data being available, the manufacturer claimed that the persistent negative method bias on EQA was due to a 'matrix effect' of the preservative in EQA material. Users were assured that patients' samples were not affected and that only samples with an initial result of 500 mg/L or more need to be diluted. This was reported to the Medical Devices Agency and, following replication of these data, the manufacturer issued a Product Correction for all users, notifying them of the need to dilute samples with an initial result of 400 mg/L or greater (Ortho-Clinical Diagnostics® technical bulletin circulated on 20 October 2000). The upper quality control material (Liquid Performance Verifier II Ortho-Clinical Diagnostics, Amersham, Bucks, UK) has a salicylate concentration of 240 mg/L. We therefore recommend that the limit for dilution should be reduced further to 300 mg/L in order to limit extrapolation.

This investigation showed that the marked loss of linearity demonstrated by EQA was not a

matrix effect and did indeed reflect the results in patients' samples, being demonstrated in fresh human plasma free of preservatives. We are concerned that serious salicylate overdose requiring specific clinical treatment may have been underestimated when this method was used according to the manufacturer's earlier instructions.

REFERENCES

- 1 Vale JA, Proudfoot AT. Poisoning with drugs and chemicals. In: Ledingham JGG, Warrell DA, eds. *Concise Oxford Textbook of Medicine*. Oxford: Oxford University Press, 2000: 1885-6
- 2 Arter T, Ferris R, Harris B, Schaeffer J, Shanklin N, Warren K. Development of the Kodak Ektachem clinical chemistry slide for salicylate. *Clin Chem* 1990; **36**: 1198
- 3 You K, Bittikofer JA. Quantification of salicylate in serum by use of salicylate hydroxylase. *Clin Chem* 1984; **30**: 1549-51
- 4 Healy MJR. Outliers in clinical chemistry quality-control schemes. *Clin Chem* 1979; **25**: 675-7

Accepted for publication 10 July 2001