Reply to a letter from Jackson J et al: Effect of pH on thrombin activity measured by calibrated automated thrombinography

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AUTHOR RESPONSE

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We wish to thank Joseph Jackson and coworkers for their interest in our paper and for elaborating and commenting on effects of pH on thrombin generation (TG). We agree that it is possible to choose other buffers, and lactic acid has been used for investigation of thrombin-induced fibrin formation.[1] An advantage is that it is a physiological component in blood, although using the highest suggested concentration,[1] it is a much higher concentration than normal levels. We chose 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer because it is the buffer used in the reagents, and adding this to plasma as described in the paper does not change osmolality.

It is correct that pH increases during the initial procedures before measurement, but we do not think that this fully explains the effect of the HEPES buffer increasing TG since our measurements of pH changes during these procedures indicate that the buffer in the reagents is capable of keeping pH neutral in fresh plasma with an initially normal pH. Nevertheless, we have no other explanation for this effect of HEPES. An ideal solution to avoid escape of CO_2 would be to perform the assay in a chamber with 5% CO_2 atmosphere, but this is probably impractical.

It is an interesting figure (Figure 1 in Jackson et al[2]) showing the effect of pH on thrombin activity toward the fluorogenic substrate, which is very distinct—data that we did not know. However, we do not think that it is possible to overcome the effect of a high pH in the plasma sample using a calibrator with a matching pH. In the assay, we mix 80 µL of plasma with either 20 µL of trigger reagent or 20 µL of calibrator reagent; that is, in both instances plasma with a high pH is added, and consequently the wells with trigger and calibrator are pH matched (we expect that both of these reagents contain 20 mM of HEPES). We imagine that the activity of several coagulation factors is affected by the abnormal pH as also stated by Jackson et al,[2] and this will reduce the measured TG.

We also agree that measurements of samples from patients with abnormal pH preferably should be measured at the actual pH in the patient, but this is usually not possible in our assays. Interestingly, however, Mitrophanov et al[3] have in a very recent paper shown that the effect of acidosis induced in vitro in plasma samples on TG is minimal, although a potential effect of escape of CO_2 was not controlled in these experiments. We will also stress that the intention with our paper was to describe a preanalytical problem that can be minimized by keeping plasma samples in closed and filled tubes.

Finally, we want to thank Jackson et al once again for their comments and can only agree with their final remark that there are many considerations when performing the CAT analysis.

RELATIONSHIP DISCLOSURE

The authors declare nothing to report.

AUTHOR CONTRIBUTIONS

SRK wrote the reply. JN and SP read and reviewed the manuscript.

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