Heat treatment of biochemical samples to inactivate Ebola virus: does it work in practice?

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To the Editor—We read with interest the article by Chong et al[1] on the effects of plasma heating procedures on common biochemical tests. Heat treatment at 60°C for 60 minutes has also been suggested by our Australian guidelines as a means to inactivate Ebola virus prior to routine laboratory processing.[2]

We recently performed a similar study to Chong et al[1] in our laboratory at the Royal Melbourne Hospital (Parkville, Australia). De-identified plasma (n=29) and serum (n=38) venous samples were collected in plastic BD vacutainer tubes (Becton, Dickinson and Company, Franklin Lakes [NJ], US) for electrolyte, liver function, and troponin testing on the Architect c16000 analyser (Abbott Laboratories, North Chicago [IL], US). After centrifugation, two 0.5-mL aliquots were obtained, one placed in a heat block at 60°C for 60 minutes, and the other paired sample left at room temperature. After heat inactivation, 24/27 (89%) plasma samples and 25/36 (69%) serum samples changed to a viscous jelly-like substance (Fig) and caused aspiration error on the Architect analyser. Centrifugation, manual stirring, and vortexing did not resolve the problem.

In serum samples that were not denatured by heating, electrolyte measurements had a strong correlation with results obtained by standard testing. Nonetheless, enzyme tests (liver function and troponin) showed a poor correlation (Table). Interestingly, these problems have not been previously reported.[2,3] The use of a heat block instead of a water bath[2] may have exposed our specimens to unequal heating, hotspots, or spikes in temperature, causing condensation or irreversible denaturing of proteins.[4]

We found that, in our hands, it was not possible to provide biochemical testing after heat treatment, as recommended by current national and international guidelines.

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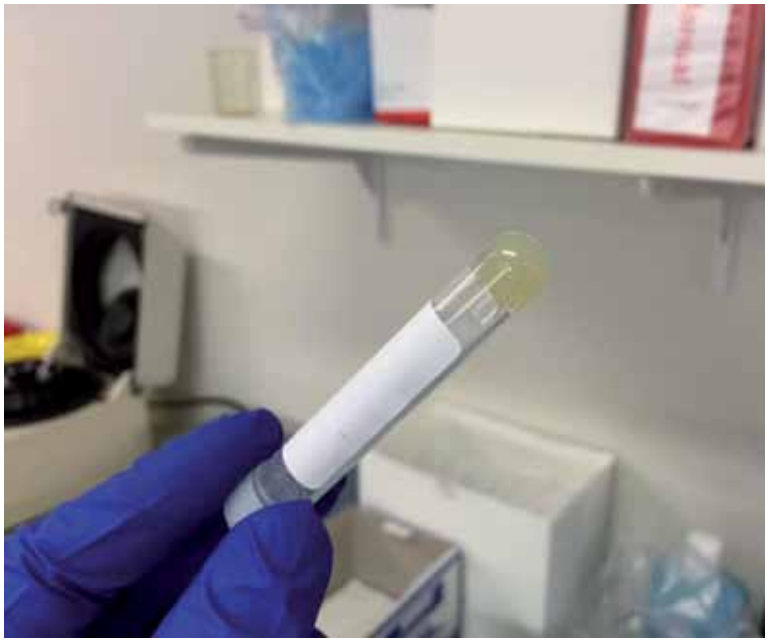

FIG. Heat inactivated plasma: this sample (0.5 mL) was heat inactivated at 60°C for 60 minutes

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Authors' Reply

To the Editor—We would like to thank Nguyen et al for their comments.

We concur with Nguyen et al that the use of heat blocks rather than a water bath may result in unequal heating, hotspots, or spikes in temperature. In our previous experience with heating procedures performed to determine alkaline phosphatase isoenzymes,[1] exposure of plasma to a temperature of 65°C caused gelling of most plasma specimens (unpublished observations). We suspect that the gelling temperature of normal human plasma is 60°C

TABLE. Heat inactivation of serum specimens (n=11) at 60°C for 60 minutes compared with matched room temperature controls. All statistical analyses were performed using the Wilcoxon signed rank test, which is the non-parametric method of comparing matched pairs (GraphPad Prism 6.0). A P value of <0.002 was considered significant after a Bonferroni adjustment for 21 multiple comparisons

Analyte	Room temperature		Heat inactivated		P value
	Median	95% CI	Median	95% CI	
Electrolytes					
Sodium (mmol/L)	141	137.8 - 143.8	142	138.4 - 145.7	0.168
Potassium (mmol/L)	4.15	3.78 - 4.48	4.25	3.83 - 4.54	0.07
Chloride (mmol/L)	104.5	101.4 - 108.7	104	102.0 - 110.0	0.119
Calcium (mmol/L)	2.25	2.00 - 2.284	2.04	1.85 - 2.16	0.014
Phosphate (mmol/L)	1.27	0.92 - 1.70	1.12	0.82 - 1.56	0.037
Magnesium (mmol/L)	0.85	0.78 - 1.01	0.89	0.72 - 0.99	0.016
Liver function tests					
Alkaline phosphatase (U/L)	80	58.73 - 190.9	5	4.54 - 7.15	<0.001*
Alanine aminotransferase (U/L)	31	10.62 - 122.1	6	5.7 - 6.8	0.001*
Aspartate aminotransferase (U/L)	32	23.38 - 62.08	17	6.33 - 32.22	<0.001*
Gamma-glutamyl transferase (U/L)	49.5	30.90 - 117.3	4	2.90 - 6.93	<0.001*
Bilirubin (μmol/L)	6.28	0 - 70.08	6.73	0 - 70.49	0.032
Renal function tests					
Creatinine (μmol/L)	94.45	80.31 - 201.1	97.9	82.66 - 204.0	0.065
Urea (mmol/L)	10.7	6.66 - 16.13	10.75	6.76 - 16.16	0.26
Bicarbonate (mmol/L)	21.65	18.18 - 25.28	19.25	16.82 - 23.74	0.005
Cardiac enzymes					
Troponin (μg/L)	295	0 - 1535	185	0 - 574.7	0.625
Creatine kinase (μmol/L)	80	16.96 - 300.9	7	6.78 - 7.59	0.001*
Total protein (g/L)	61	54.61 - 65.87	63	55.61 - 68.72	0.023
Albumin (g/L)	34	25.67 - 35.26	25	21.47 - 30.99	0.02
Glucose (mmol/L)	6.15	3.99 - 10.23	6.15	4.24 - 9.79	0.647
Indices					
Lipaemia	-0.01	-0.50 - 1.30	0.96	-0.17 - 3.55	0.001*
Icterus	13.3	-2.81 - 68.36	16.5	-19.32 - 81.05	0.054
Haemolysis	0.05	0.03 - 1.18	0.08	-0.20 - 0.15	0.008

Abbreviation: CI = confidence interval
* P<0.002

to 65°C.

In our experiments, we use a W14 water bath with 14 L capacity (Sheldon Manufacturing Inc, Cornelius [OR], US). The water bath has a much higher heat capacity than heat blocks, due to the large volume of water, as well as the considerably higher specific heat capacity of water (4.1813 J g^{-1} K^{-1}) when compared with aluminium (0.897 J g^{-1} K^{-1}),[2] a metal often used to manufacture heat blocks.

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