Heat treatment for endocrinological investigations on plasma positive for human immunodeficiency virus (HIV)

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SUMMARY The effects of heat treatment of serum samples on the hormone analyses used in this laboratory were studied. Total T₄, testosterone, progesterone, and growth hormone were not systematically affected by heat treatment over the whole range of analyte concentrations studied; for thyroid stimulating hormone, no effect was noted on serum samples with concentrations of less than 10 mU/l. Significant changes occurred in total T₃, cortisol, follicle stimulating hormone, lutenising hormone, and prolactin.

It is suggested that with appropriate preliminary study, heat treated plasma samples may be used in endocrinological investigations without adversely affecting the diagnostic validity of the results.

The incidence of acquired immunodeficiency syndrome (AIDS) is increasing, with a prolonged survival period after detection of seroconversion, and a consequent increasing need for pathology investigations. Endocrinological analyses are particularly unsuited to the grade III containment facilities recommended for processing human immunodeficiency virus (HIV) positive samples. It has been suggested that heat inactivation (56°C)¹ of the virus may be used to reduce the risk of infection and allow analyses to be carried out under normal conditions.^{2–4}

In these studies, scant attention was paid to hormone analyses, yet within the laboratory such tests are least amenable to containment due to the expense both of duplicating equipment and of carrying out small batch analyses.

The present study was undertaken to examine the effect of heat treatment on the hormone analyses carried out in our department.

Material and methods

For each assay 16 to 52 previously analysed plasma samples were selected to cover a wide range of concentrations. The samples, which had been stored at –20°C, were thawed and a 0.6 ml aliquot heated at 56°C for 30 minutes and then cooled on ice for five

minutes. Both the untreated (A) and heat treated (B) portions were stored at –20°C until analysis. Just before assay all aliquots were centrifuged at 2500 rpm for five minutes to remove turbidity. The untreated and treated portions of each sample were assayed in duplicate in the same batch (table 1). Appropriate human control sera were included at the beginning and end of each assay. Analyses were repeated if controls were outside acceptable limits (± 2 SD)

Results were analysed by Student's paired *t* test.

Results

Table 2 shows the comparison of results obtained.

Total T₄, testosterone, progesterone, and growth hormone were not systematically affected by heat treatment over the whole range of analyte concentrations studied. The standard deviation of the percentage difference in results after heat treatment, however, exceeded the interbatch coefficient of variation for testosterone and growth hormone by a factor of more than three, indicating an increased variability of results.

For thyroid stimulating hormone, no apparent effect of heat treatment was noted in samples with concentrations of less than 10 mU/l, although a small, but significant, increase in thyroid stimulating hormone was noted after heat treatment in samples containing more than 10 mU/l.

In the remaining hormone assays there were

Table 1 *Methods of hormone analysis*

Analyte	Method	Supplier	Interbatch coefficient of variation (%)
Total T ₄	Single antibody RIA-charcoal separation	(in house)	8.2
Total T ₃	Double antibody RIA	NETRIA	4.7
Thyroid stimulating hormone	Immunoradiometric assay	NETRIA	4.0
Cortisol	Single antibody RIA-magnetic separation	SERONO Diagnostic	13.0
Testosterone	Double antibody RIA-preliminary extraction	St Thomas's Hospital	6.9
Progesterone	Single antibody RIA-magnetic separation	Amersham UK	12.4
Follicle stimulating hormone	Double antibody RIA	Chelsea Hospital	4.7
Lutenising hormone	Double antibody RIA	Chelsea Hospital	4.7
Prolactin	Double antibody RIA	Guildhay UK	7.7
Growth hormone	Double antibody RIA	SAPU	8.1

NETRIA, North East Thames Regional Immunoassay; SAPU, Scottish Antibody Production Unit.

significant changes in results after heat treatment throughout the range of analyte concentrations studied. With the exception of prolactin, the differences were relatively small (less than 20%).

Discussion

Although it is accepted that results of hormone assays are dependent to some extent on methodology, the radioimmunoassay or immunoradiometric assay used in this study is typical of that used in other laboratories, and it is therefore likely that our findings are applicable to most other laboratories using these techniques.

The revised Advisory Committee on Dangerous Pathogens guidelines on the safe handling of HIV specimens now permit the use of non-dedicated equipment and laboratory space for occasional, manual, analyses of these samples.⁵ Heat treatment sub-

stantially reduces the risk of infection.^{6,7} There is still controversy, however, as to whether heat treatment at 56°C for 30 minutes inactivates the HIV virus completely.⁵

This study shows that most hormone assays, with the exception of prolactin, are either unaffected by heat treatment or are affected to a relatively minor extent that would not radically change clinical decision making.

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Table 2 *Summary of changes in plasma hormone concentration after heat treatment*

Analyte (n =)	Units	Untreated		Mean difference after heat treatment (%)	SD of difference after heat treatment (%)	p value
		Range	Mean			
Total T ₄ (27)	nmol/l	9-395	135	+ 1.5	8.1	NS
Total T ₃ (19)	nmol/l	0.8-9.9	4.1	+ 9.8	9.8	<0.05
Thyroid stimulating hormone (52)	mU/l	<0.1-10.0	2.8	+ 3.6	7.1	NS
		10.1-54.5	33.7	+ 7.7	8.6	<0.05
Cortisol (24)	nmol/l	66-1045	438	+ 17.8	19.0	<0.05
Testosterone (25)	nmol/l	1.3-25.3	8.5	- 3.5	18.8	NS
Progesterone (21)	pmol/l	5.7-138.4	58.9	+ 8.0	22.9	NS
Follicle stimulating hormone (20)	mU/l	3.0-40.0	16.1	- 5.0	5.0	<0.05
Lutenising hormone (16)	mU/l	2.6-50.0	14.1	- 16.3	19.9	<0.05
Prolactin (20)	mU/l	291-1664	696	- 29.9	18.0	<0.05
Growth hormone (20)	mU/l	0.6-48.5	11.6	- 16.4	30.2	NS

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