



Evaluation Report

NUMBER
MDA 02106

AUTOSTAT™ II Anti-Thyroid Peroxidase



Immunology Quality Services

MDA Evaluation Report
MDA 02106

WHAT YOU CAN EXPECT FROM MDA EVALUATION REPORTS

The Device Evaluation Service (DES) aims to provide independent and objective evaluations of medical devices available on the UK market. Specialist centres, mainly in NHS Trusts, do the evaluations under long-term contract to, and in accordance with protocols approved by, the MDA. The evaluations are usually of a unit supplied by the manufacturer. We would expect this unit to be representative of the product on the market but cannot guarantee this. Prospective purchasers should satisfy themselves with respect to any modifications that might be made to the product type after MDA's evaluation. The reports are intended to supplement, not replace, information already available to prospective purchasers.

© Crown Copyright 2002

Apart from any fair dealing for the purposes of research or private study, or criticism, or review, as permitted under the Copyright, Designs & Patents Act, **1988**, this publication may only be reproduced, stored or transmitted in any form or by any means with the prior permission, in writing, of the Controller of Her Majesty's Stationery Office (**HMSO**).

Enquiries concerning reproduction outside those terms should be sent to **HMSO** at the undermentioned address:

**The Copyright Unit,
Her Majesty's Stationery Office,
St. Clements House,
2 - 16 Colgate,
NORWICH,
NR3 1BQ.**

MDA Evaluation

of the

AUTOSTAT™ Anti-Thyroid Peroxidase

**D Patel
RR Sanderson
PAE White**

IMMQAS
UK NEQAS Immunology and Immunochemistry
Northern General Hospital
Sheffield

Contents

Summary	2
Introduction	3
Evaluation protocol	4
Product details	6
General information.....	6
Assay details.....	6
Packaging and labelling.....	8
Quality controls and calibrators.....	8
Assay procedure	10
Assay protocol.....	10
Ease of use.....	11
General comments.....	11
Results	12
Sensitivity and specificity.....	12
Imprecision.....	12
Accuracy.....	13
Linearity.....	14
Matrix effects.....	15
Conclusion	16
References	17
Appendix	18
IFCC guidelines on packaging and labelling.....	18
Manufacturer's comments.....	19
How to obtain MDA evaluation reports.....	20

Summary

The purpose of this evaluation was to assess the performance of the AUTOSTAT™ II Anti-Thyroid Peroxidase Kit in the detection and quantitation of TPO autoantibodies in serum. The AUTOSTAT™ II Anti-Thyroid Peroxidase assay is IgG specific and utilises purified human TPO as the antigen. Assays were performed in strict accordance with the manufacturer's instructions and the distributor was given the opportunity to train the operators prior to the commencement of the evaluation. The analytical procedures were carried out at IMMNAS during July 2002.

The evaluation included an assessment of packaging and labelling of the kit, clarity of operating instructions, safety and ease of use. The cost per test, based on the list price, is documented together with the technical requirements.

The status of all samples was confirmed using an established method, the ORGENTEC Anti-TPO assay. The comparator assay is IgG specific and utilises purified human TPO as the antigen. IgG anti-TPO sensitivity in relation to ORGENTEC was 100% with a specificity of 90%. The overall agreement between the two methods was 94%. Within-assay imprecision ranged from 5.4% to a very poor 33.4%. Between-assay imprecision ranged from 1.5% to a very poor 46.2%. Linearity of dose response was acceptable up to a concentration of 3939.2 U/ml. The AUTOSTAT™ II Anti-Thyroid Peroxidase assay is calibrated against the NIBSC reference preparation MRC 66/387, which is not recognised as the international reference preparation by the World Health Organisation, therefore results are reported in U/ml. A mean value of 1406.9 U/ml was obtained for the NIBSC reference preparation, MRC 66/387 against a target value of 1000 U/ml. This result indicates that the assay may have been calibrated incorrectly. The product is CE marked with reference to the IVD Directive (Directive 98/79/EC).

Using the information provided by the manufacturer, sample values of less than 35 U/ml were recorded as negative and values greater than 50 U/ml were recorded as positive. Samples of 35-50 U/ml were recorded as equivocal. There were 13 "false positive" results which comprised one sample from a female donor under the age of 50 years, one from a male donor over the age of 50 years, three lipaemic samples, four haemolysed samples and four icteric samples.

Introduction

There are a number of autoantibodies associated with the autoimmune thyroid diseases, which are characterised as either primary or secondary antibodies. Primary antibodies are directly pathogenic and often directed against cell membrane receptors, whilst secondary antibodies do not appear to be involved in pathogenesis but can serve as a useful diagnostic marker for the presence of autoimmune thyroid disease. Thyroid peroxidase (TPO) antibodies are one of the major secondary antibodies associated with autoimmune thyroid disease [1].

TPO was previously known as thyroid microsomal antigen [2]. It is a 107 KD enzyme which is involved in thyroid hormone synthesis. TPO is located both on the cell surface and within the cytoplasm of thyroid acinar cells, bound to the vesicle which transports newly synthesised thyroglobulin, where it is involved in the iodination of thyroglobulin. High affinity antibodies (predominantly IgG) directed against TPO are found at elevated levels in the serum of patients with autoimmune thyroid disease such as Graves disease, Hashimoto's thyroiditis and myxoedema [3]. Diagnostics companies have recognised the importance of this antibody and have developed assays using either recombinant or purified human TPO as the antigen source.

Serum panel

A panel of 220 sera was tested, in duplicate, using a total of seven kits. Four were drawn from lot number AQE 202, expiry August 2003 and three from lot number AQE203, expiry October 2003.

The serum panel comprised the following samples:

- sera from normal blood donors under 50 years old (n=48)
- sera from normal blood donors over 50 years old (n=47)
- sera from hypothyroid patients with detectable anti-TPO antibodies as determined by the ORGENTEC Anti-TPO ELISA (n=47)
- sera from hyperthyroid patients with detectable anti-TPO antibodies as determined by the ORGENTEC Anti-TPO ELISA (n=25)
- sera from euthyroid patients with detectable anti-TPO antibodies as determined by the ORGENTEC Anti-TPO ELISA (n=13)
- sera to investigate the possible matrix effects of:
 - lipaemia (n=10)
 - haemolysis (n=10)
 - hyperproteinaemia (n=10)
 - hyperbilirubinaemia (n=10)

Accuracy

Accuracy was assessed with reference to the NIBSC reference preparation, MRC 66/387.

Imprecision

Sera with high, medium and low levels of anti-TPO antibodies were included in each assay run to determine between-assay imprecision.

The high, medium and low samples were tested twenty times in a single assay run to determine within-assay imprecision.

Sensitivity and specificity

Relative sensitivity and specificity of the assay were assessed in relation to the ORGENTEC assay. The overall agreement between the two methods is also documented.

Matrix effects

The effects of some potentially interfering substances were assessed by including appropriate samples as detailed in *Serum panel*.

Linearity

Two samples with known high concentrations of anti-TPO antibodies when previously assayed using the AUTOSTAT™ II Anti-Thyroid Peroxidase kit were diluted to give a final dilution of 1/10. These dilutions were assayed in duplicate and the results shown on a graph (Figure 1).

General

The evaluation included an assessment of:

- packaging and labelling in accordance with IFCC guidelines and COSHH regulations
- contents of the kit insert and clarity of operating instructions
- ease of use of the kit
- cost per test and time taken
- compatibility with existing laboratory work.

Product details

■ General information

Product: AUTOSTAT™ II Anti-Thyroid Peroxidase

Product code: DIH 002

Manufacturer: Hycor Biomedical Ltd
Pentlands Science Park
Bush Loan
Penicuik
EH26 0PL
UK

Distributor: As above

Tel: 0131 445 7111

Cost: The list price for a 96 well kit is given as £105.59
After due consideration for calibrators and controls, each kit provides sufficient reagents for 41 determinations in duplicate at a cost of £2.58 per sample. This cost does not take into account operator time and is exclusive of additional reagent costs, consumables and VAT.

■ Assay details

Description: The manufacturer's description of the kit and its intended use is given below:

"Enzyme-linked immunosorbent assay method for the quantitative determination of specific IgG autoantibodies to TPO in human serum. The results of the anti-TPO assay can be used as an aid in the diagnosis of auto-immune diseases including Hashimoto's Thyroiditis and Graves' disease. Levels of these autoantibodies are one indicator in a multi-factorial diagnostic regime."

Principle: phase The AUTOSTAT™ II assay for detection of autoantibodies is a solid immunosorbent assay (ELISA) in which the analyte is indicated by a colour reaction of an enzyme and substrate. The Autostat™ II assay wells are coated with purified antigen. On adding diluted serum to the wells the antibodies present bind to the antigens. After incubating at room temperature and washing away unbound material, horseradish peroxidase conjugated anti-IgG monoclonal antibody is added, which binds to the immobilised antibodies. Following further incubation and washing tetra-methyl benzidine substrate (TMB) is added to each well. A dark blue colour develops. Addition of the stop solution turns the colour to yellow. The colour intensity is proportional to the anti-TPO concentration in the original serum sample.

End point measurement: Photometric measurement at 450nm (and 600-650nm for dual wavelength readings).

Isotype specificity: IgG

Antigen source: Purified human thyroid peroxidase

Specimen type: Human serum

Required sample size: 5 µl of sample to be diluted 1/100 with sample diluent

Additional reagents required: .Distilled and deionised water

Major equipment required:

- 96-well microplate reader with 450 nm filter
- Automatic plate washer (optional)
- Data reduction software (optional)

Other equipment required:

- Appropriate tubes for sample dilution
- Precision pipettes to deliver volumes of 5 µl, 100 µl and 495 µl
- Repeating dispenser or multichannel pipette to deliver volumes of 50µl and 100 µl
- 30 minute timer

■ Packaging and labelling

The packaging and labelling of the kits were assessed in accordance with a modified form of the IFCC guidelines (detailed in the *Appendix*) and with COSHH regulations. The degree of conformity to these criteria is shown in table 1.

Packaging

Packaging consists of a cardboard box. Reagent containers are secured in wells that are cut into an inner cardboard insert. Microstrips are stored in a plastic frame in a resealable foil sachet.

Labelling

All components are clearly labelled in English, French, German, Italian and Spanish with details of the contents, lot number, storage requirements and expiry date. The conjugate is colour-coded pink. The outer package bears a label stating the name of the kit with a list of its contents, storage requirements, lot number and expiry date. The product is CE marked with reference to the IVD Directive (Directive 98/79/EC).

■ Quality controls and calibrators

Four prediluted calibrators at concentrations of 50-5000 U/ml are provided in the kit. A positive control with an acceptable range of 70-300 U/ml and a negative control with an acceptable value of < 30 U/ml are also provided. Results are interpreted as follows:

< 35	U/ml	negative
35-50	U/ml	equivocal
> 50	U/ml	positive

Table 1. Conformity to IFCC guidelines on packaging and labelling

1. Description of test and method	Y
2. Principles of test	Y
3. Reagents supplied	Y
4. Quality control sera supplied with kit	Y
5. Additional reagents required	Y
6. Equipment required	Y
7. Hazard warnings	Y
8. Examples of results	Y
9. Reference ranges	Y
10. Working range	Y
11. Within-assay imprecision	Y
12. Between-assay imprecision	Y
13. Specificity	Y
14. Sensitivity	Y
15. Accuracy	N
16. Effects of anticoagulants	N
17. High dose hook effect	N/A
18. Matrix effects	Y
19. Collection and storage of samples	Y
20. Kit storage and shelf life	Y
21. COSHH information	Y
22. Pack dimensions (height x depth x width)	110 x 135 x 170 mm

Assay procedure

■ Assay protocol

The assay protocol described in the kit insert was strictly followed, without modification. A summary of the procedure is given below together with the approximate times taken to complete each step when a full plate was tested.

• Dilute wash buffer and prepare standard curve	10 mins
• Dilute patient samples 1/100 in sample diluent	15 mins
• Pipette 100 µl calibrators, controls and diluted patient samples to appropriate wells	5 mins
• Incubate at room temperature	30 mins
• Wash 3 times in wash buffer	5 mins
• Add 100 µl conjugate to all wells	2 mins
• Incubate at room temperature	15 mins
• Wash 3 times in wash buffer	5 mins
• Add 100 µl substrate solution to all wells	2 mins
• Incubate at room temperature	15 mins
• Add 50 µl stop solution to all wells	2 mins
• Read absorbance at 450 nm	5 mins
Total number of steps	12
Number of wash steps	2
Total assay time	111 mins
Estimated operator hands-on time	51 mins
Sample volume	5 µl

■ Ease of use

The clarity of the operating instructions and the ease of use of the kit were assessed by two independent operators. The criteria used were scored on a scale of 1 to 5. The findings are recorded in table 2.

Table 2. Ease of use

Labelling	5
Reagent colour coding	5
Clarity of written instructions	5
Ease of reagent preparation	5
Ease of sample preparation	4
Ease of test procedure	4
Ease of use of required equipment	5
Result interpretation	4
Overall test time	5
Compatibility with other laboratory work	5
COSHH information	4
TOTAL	51/55
Key: 1 - Very poor or very difficult 2 - Poor or difficult 3 - Adequate 4 - Good or easy 5 - Excellent or very easy	

■ General comments

- All assay components must be allowed to warm to room temperature before use.
- Samples for linearity and any beyond the assay range were diluted in the sample diluent provided in the kit.
- Reagents were added using a Biohit Proline electronic repeating dispenser.
- Optical densities were measured using the DYNEX plate reader in conjunction with Revelation data reduction software.
- Plates were washed using a programmable automatic washer (DYNEX MRW 4 bottle plate washer).

■ Sensitivity and specificity

Table 3a summarises the results obtained by ORGENTEC Anti-TPO and AUTOSTAT™ II Anti-Thyroid Peroxidase assay with sera from patients with raised TPO antibodies and normal blood donors. Relative sensitivity and specificity were calculated from the data and are recorded in table 3b.

Table 3a. AUTOSTAT™ II Anti-Thyroid Peroxidase vs ORGENTEC Anti-TPO

		AUTOSTAT™ II Anti-Thyroid Peroxidase		Total
		Positive	Negative	
ORGENTEC Anti-TPO	Positive	85	0	85
	Negative	13	121	134
Total		98	121	219

Table 3b. Relative sensitivity and specificity

Sensitivity (%)	100
Specificity (%)	90
Overall agreement (%)	94

■ Imprecision

Within-assay imprecision

Three samples containing high, medium and low levels of TPO antibodies were assayed 20 times each in a single assay run. The results are summarised in table 4.

Table 4. Within-assay imprecision (n=20)

	Sample 1	Sample 2	Sample 3
Mean (U/ml)	3233.8	1098.6	117.0
SD	1078.6	146.1	6.3
CV%	33.4	13.3	5.4

Between-assay imprecision

Table 5. Between-assay imprecision

	Sample 1	Sample 2	Sample 3
Mean (U/ml)	3066.4	1182.2	229.2
SD	368.4	102.1	105.8
CV%	12.0	8.6	46.2

Table 6. Between-assay imprecision (lot AQE202)

	Sample 1	Sample 2	Sample 3
Mean (U/ml)	2796.6	1159.5	324.7
SD	148.0	35.4	4.9
CV%	5.3	3.1	1.5

Table 7. Between-assay imprecision (lot AQE 203)

	Sample 1	Sample 2	Sample 3
Mean (U/ml)	3426.2	1212.4	133.7
SD	185.9	164.3	24.0
3CV%	5.4	13.6	18.0

■ Accuracy

Accuracy was assessed in relation to the NIBSC reference preparation MRC 66/387, assigned value 1000 U/ml. This sample was included in all assay runs. Results are recorded in table 8.

Table 8. Accuracy

Mean (U/ml)	1406.9
SD	186.9
GSD	1.1
CV%	13.3

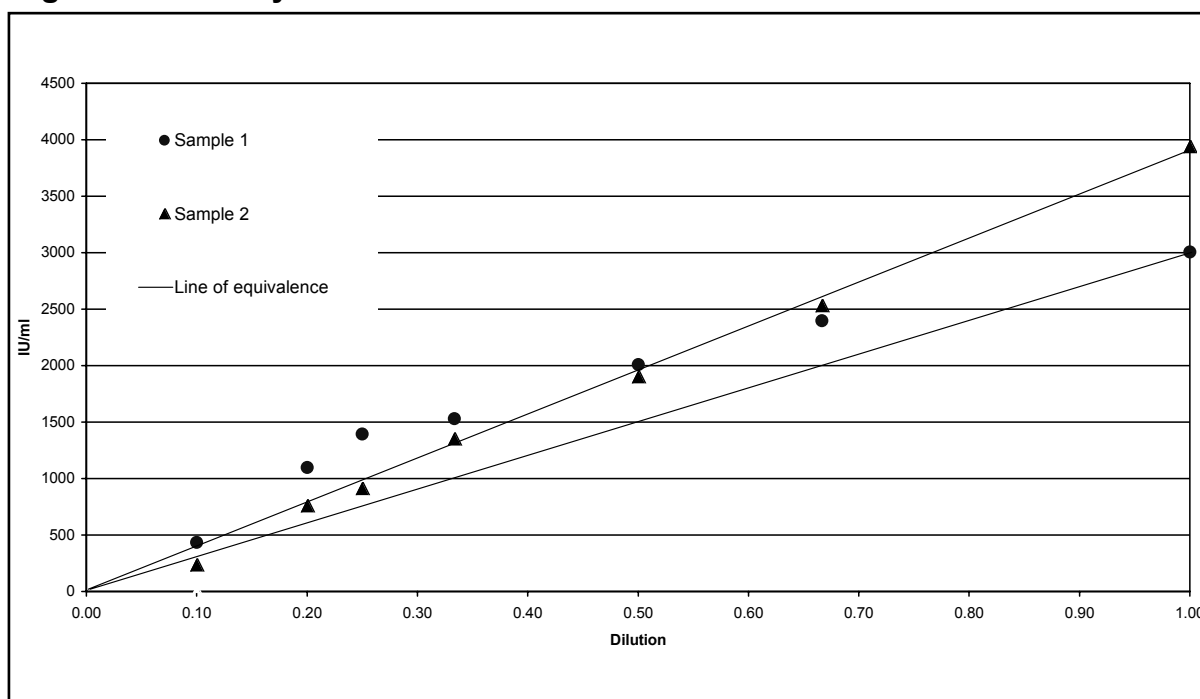
■ Linearity

Two samples containing high levels of anti-TPO antibodies when previously assayed using the AUTOSTAT™ II Anti-Thyroid Peroxidase kit were diluted in sample diluent to a final dilution of 1/10. These dilutions were assayed in duplicate. Results are shown in table 9. Figure 1 shows the line of equivalence for each sample.

Table 9. Linearity

Dilution	Sample 1 IU/ml	Sample 2 IU/ml
Neat	3008.0	3939.2
2/3	2396.7	2529.6
1/2	2011.1	1902.1
1/3	1529.3	1349.5
1/4	1394.7	911.4
1/5	1098.5	757.2
1/10	436.5	236.2

Figure 1. Linearity



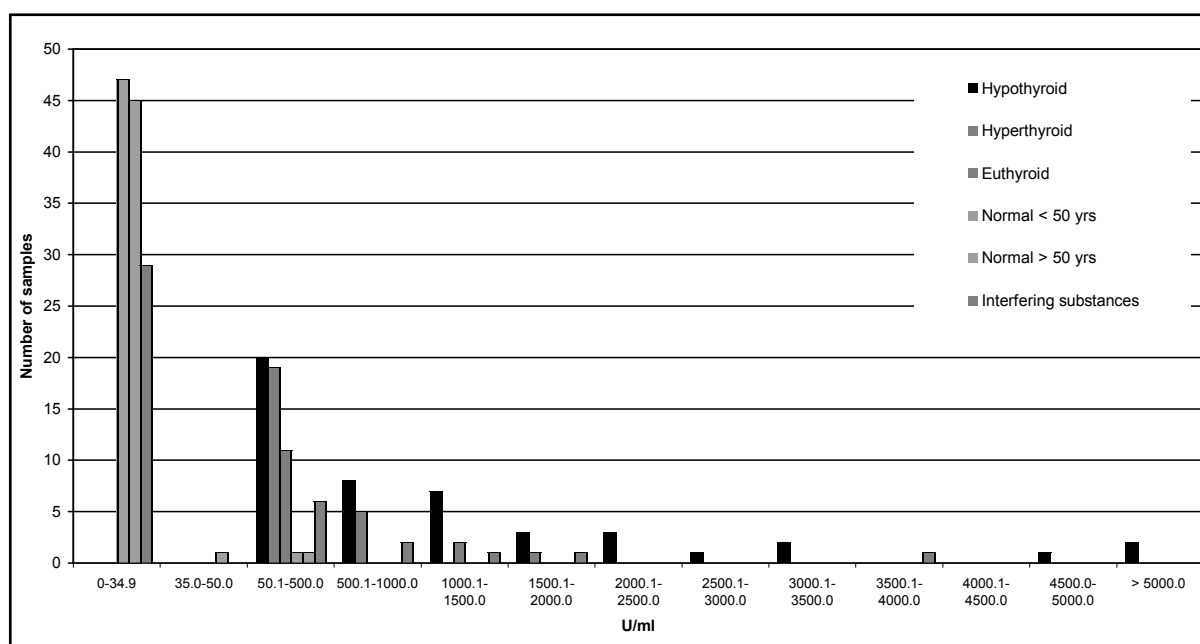
■ Matrix effects

“False positive” results were obtained from thirteen samples: one from a female donor under the age of 50 years, one from a male donor over the age of 50 years, three lipaemic samples, four haemolysed samples and four icteric samples.

Table 10. Sample distribution of anti-TPO antibody levels

TPO IU/ml	Hypo thyroid	Hyper thyroid	Euthyroid	Normal < 50 yrs	Normal > 50 yrs	Interfering substances
0-34.9	0	0	0	47	45	29
35.0-50.0	0	0	0	0	1	0
50.1-500.0	20	19	11	1	1	6
500.1-1000.0	8	5	0	0	0	2
1000.1-1500.0	7	0	2	0	0	1
1500.1-2000.0	3	1	0	0	0	1
2000.1-2500.0	3	0	0	0	0	0
2500.1-3000.0	1	0	0	0	0	0
3000.1-3500.0	2	0	0	0	0	0
3500.1-4000.0	0	0	0	0	0	1
4000.1-4500.0	0	0	0	0	0	0
4500.1-5000.0	1	0	0	0	0	0
> 5000.0	2	0	0	0	0	0

Figure 2. Sample distribution of anti-TPO antibody levels



Conclusion

The AUTOSTAT™ II Anti-Thyroid Peroxidase kit is presented in a standard 96-well microplate format familiar to most immunology laboratories, providing an assay which is easy to use. The kit packaging provided adequate protection for transit and storage. The written instructions are clear and easy to follow.

Sensitivity of the assay in relation to the ORGENTEC Anti-TPO assay was found to be 100% with a specificity of 90%. The overall agreement between the two methods was 94%. “False positive” results were seen in 13 samples: one from a female donor under the age of 50 years, one from a male donor over the age of 50 years, three lipaemic samples, four haemolysed samples and four icteric samples. A matrix effect was observed in the groups of sera which contained lipids, haemoglobin and bilirubin. The manufacturer indicates that samples containing elevated levels of haemoglobin, bilirubin, lipids or EDTA should not be used.

Within-assay imprecision ranged from 5.4% to a very poor 33.4%. Between-assay imprecision ranged from 1.5% to a very poor 46.2%. Linearity of dose response was acceptable up to a concentration of 3939.2 U/ml. Accuracy was assessed in relation to the NIBSC standard preparation MRC 66/387. A mean value of 1406.9 U/ml was recorded against the assigned value of 1000 U/ml. This result indicates that the assay may have been calibrated incorrectly.

A total assay time of approximately 2 hours gives rise to a rapid and absolute result. Since the assay is presented in a standard 96-well format, it is likely to be compatible with existing work in most immunology laboratories. Special instrumentation is not required.

References

1. Milford Ward A, Wild G, Riches P G and Sheldon J (1999). *SAS Protein Reference Units Handbook of Autoimmunity* 1st Ed. PRU Publications, Sheffield. **34-36**
2. Czarnocka B, Ruf J, Ferrand M, Carayon P and Lissitzky S (1985). Purification of the human thyroid peroxidase and its identification as the microsomal antigen involved in autoimmune thyroid diseases. *FEBS Letters*. **190**: 147-152
3. Whitham K, Patel D and Milford Ward A (2000). Epitope Expression in Nine Commercial Kits for the Determination of Anti-Thyroid Peroxidase (TPO) Antibodies. *J Clin Lab Immunol*. **51**: 21-38

■ IFCC guidelines on packaging and labelling

IFCC guidelines, modified for autoimmune serology kits, suggest that the following details be included in the kit insert:

1. Description of test and method.
2. Principle of the assay.
3. Reagents supplied (contents and concentrations)
4. Extra reagents required but not supplied.
5. Equipment required.
6. Hazard warnings.
7. Example of results.
8. Reference ranges
 - i) sample size
 - ii) description of population.
9. Working range.
10. Within-assay imprecision.
11. Between-assay imprecision.
12. Sensitivity.
13. Specificity.
14. Accuracy.
15. Effects of anticoagulants.
16. High dose hook or prozone effects.
17. Matrix effects.

■ Manufacturer's comments**HYCOR**

HYCOR BIOMEDICAL LTD
Pentlands Science Park
Bush Loan
Penicuik EH26 0PL
United Kingdom

Mrs. Rachael Sanderson
Biomedical Scientist
Kit Evaluation Unit
Department of Immunology
P.O. Box 894
Sheffield S5 7YT

Tel: +44 (0)131 445 7111
Fax: +44 (0)131 445 7112
Email: cs@hycorbiomedical.com
Web: www.hycorbiomedical.com

11th October 2002

Report on the Autostat™ II Anti-Thyroid Peroxidase Kit

Dear Rachael,

Thank you for the report on the Autostat™ II Anti-Thyroid Peroxidase Kit and for allowing us to give comments. We would like to make the following comments.

1. We are pleased that the confusion over the status of the reference preparation MRC 66/387 has been cleared. We have always reported results in U/ml and will continue to do so until the status of this reference is changed.
2. We are extremely disappointed in the precision studies as these are not consistent with our own data and seem to be restricted to individual sample results and not a general phenomenon.
3. The number of samples with interfering substances that have given positive responses is interesting. Previous studies have not indicated interference to such an extent. We clearly state that samples which are lipaemic, or haemolysed should not be used but the number of positives is surprising. It would be useful to know the thyroid status of these samples.
4. The report assessment of the accuracy against the reference preparation has been noted and we will review our calibration internally.
5. The Thyroid status of the "normal" panel and the "interference panel" would make any comparison more scientifically valid.
6. The above comments notwithstanding we feel that the report demonstrates the utility and performance of the Autostat™ II Anti-Thyroid Peroxidase Kit

Thank you once again for allowing us to comment on this report and I look forward to the final report and the eventual all company comparative report.

Yours sincerely
Kind regards



Dr. Jim Weston
General Manager
Hycor Biomedical Ltd

■ How to obtain MDA evaluation reports

MDA evaluation reports are published by the Medical Devices Agency, an Executive Agency of the Department of Health. They are available free of charge to the UK National Health Service (NHS), and are for sale to commercial organisations and other interested parties. A free catalogue of available reports can be obtained from the Orders Department, or downloaded from the MDA web site:

www.medical-devices.gov.uk

Ordering

Send your order to the address given below, stating the number, title and quantity of each report required. Your reports will be dispatched by second class post the following working day. If you are not a representative of the NHS, you will be invoiced separately. Non-NHS customers are reminded that it is not possible to offer refunds for reports ordered in error.

Orders Department
Room 1207
Medical Devices Agency
Hannibal House
Elephant and Castle
London
SE1 6TQ

Tel: 020 8972 8181
Fax: 020 8972 8105
E-mail: des@medical-devices.gov.uk

Enquiries

General enquiries should be directed to the Orders Department, as above. Technical enquiries should be addressed to Mrs. Dina Patel at IMMQAS:

Tel: 0114 271 5716
Fax: 0114 261 7691
E-mail: dpatel@immqas.org.uk

ISBN 1 84182 608 1

36 29097 1