

STANDARD OPERATING PROCEDURE

TITLE: **TOTAL IGE EIA (HYTEC 288)**

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AREA OF APPLICATION: IMMUNOCHEMISTRY

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HEALTH & SAFETY

Good laboratory practice is the standard in all assays involving body fluids. Disposable gloves should be worn when handling serum samples, but this does not preclude washing hands regularly.

All potentially contaminated material generated must be disposed of in accordance with Hospital Policy.

COSHH assessment available. (COSHH REF 16).

Remember in the event of any accident or compromise to health and safety always inform a senior member of staff.

PRINCIPLE OF TEST

IgE immunoglobulin is important in the mediation of the allergic response. The mechanism involves an antigenic stimulation of immuno-competent B-lymphocytes by a specific antigen, which induces the lymphocyte to respond by producing specific antibody of several classes.

One class, IgE antibody, becomes partially bound via its Fc portion to receptors on the surface of mast cells and basophils to release various vasoactive amines in the blood and surrounding tissue. These substances cause smooth muscle constriction and ultimately lead to allergic conditions such as wheal and flare reactions, hives, dermatitis, rhinitis, hay fever, asthma, and anaphylactic shock.

Quantitative determination of IgE in human serum is valuable in the diagnostic assessment of patients with established or suspected allergic disease.

In the HYTEC Total IgE Enzyme Immunoassay (EIA), a total IgE disc is incubated with IgE from the patient's serum. The disc is washed, incubated with enzyme labelled anti-IgE, and then washed again. The resulting solid phase matrix with bound enzyme is proportional to the amount of total IgE in the patient sample. Substrate solution containing p-nitro-phenyl-phosphate (PNPP) is added and incubated, with the development of a yellow colour, which is measured spectrophotometrically. The concentration of total IgE is directly proportional to the colour intensity.

PERSONNEL OR TRAINING REQUIREMENTS

This assay can be carried out by all trained BMS staff and by MLA staff under supervision (with the exception of interpretation and reporting). Operators must be familiar with use of the Hytec 288 analyser.

SPECIMEN REQUIREMENTS

Separate serum, aliquot and store at – 20 °C in immunochemistry freezer until testing.

NB. EDTA plasma is **not** suitable.

Only serum samples have been validated for this assay.

EQUIPMENT

HYTEC 288
Measuring cylinder

REAGENTS

Total IgE kit containing: - (Cat. No. 74123)
Total IgE Conjugate – 13.5ml
IgE Antibody Discs
Total IgE Sample Diluent
EIA Wash solution Concentrate – 125ml 15x conc.
(Add 1750ml distilled water)
Substrate Diluent
PNPP Tablets
Stop solution – 300ml
Tubes – for assay blanks and dilutions

Reagents Required But Not Included: -

Total IgE Control (Cat. No. 73020)
Total IgE Calibrator Set (Cat. No. 72002)

PROCEDURE / METHODOLOGY

1. Allow all reagents to reach room temperature (20-25°C) before use. Mix each reagent bottle gently to avoid foaming.
2. Start up HyTec 288 using “*Start up*” SOP.
- 3. Check and empty waste and condensation bottles.**
4. Click on ‘**Configure**’, then ‘**change login**’ to own ID i.e. person performing this assay.
5. Create a worklist in Apex using “*IgE Worklist*” SOP. Maximum number of samples is 48.
6. Make any dilutions from previous batch, where patients’ results >2000 U/ml. (Usually 1/20 or 1/50 dilutions with PBS).

Patient requests can be entered via **INTERFACE** or **MANUALLY**.

Use of INTERFACE TEST REQUEST ENTRY: -

(see also, separate interface protocol)

- a) Click ‘**Import requests**’ A short program will execute. When finished, view imported requests to check that correct batch is available.
- b) For any alterations, click on ‘**Test Request**’. Find request to be altered. Any additions, deletions can be done at this point.

For MANUAL TEST REQUEST ENTRY: -

- c) Click on ‘**Test Request**’.
- d) Click on ‘**Assay Class**’ and choose ‘**Total IgE**’ assay.

e) Load patient details

E.g. Lab ID number ENTER

Alt F..... **F**astselect...Total IgE and **ENTER** or **Alt L**

Alt W.....**N**ew **p**atient

f) Click '**Done**'

ASSAY PREPARATION: -

1. Click on '**Prepare new assay'**

In 'assay data' click on '**Master assay**'..... Choose **Total IgE**

Blank rpt 2

Cal rpt 2

Sample rpt 1

Control rpt 2 is shown.

2. Click on '**Samples**'

3. Select Tests...usually **Select All**.

4. Check Lot. Numbers of reagents.

5. Click on **stored curve**, use stored curve if there is suitable curve available & recalibration is not necessary. (Curves usually valid for 1 month).

6. Click on **Done**

7. **P**rint worksheet

8. Whilst it is printing, **check** levels of **distilled water**, **wash solution**, **stop solution** are approximately $\frac{3}{4}$ full and **waste bottle** empty. Top up / empty as necessary.

9. Prepare reaction block from worksheet. Place any empty tubes into the block first, then Total IgE Antibody disc tubes (ENSURE DISCS ARE IN THE BOTTOM OF EACH TUBE). **N.B. Calibrator, Controls and Tests all require the same discs.**

10. Make appropriate amount of substrate and tablets (**DO NOT touch PNPP tablets with hands**) described on worksheet and ensure adequate volumes of conjugate and sample diluent. Place into reagent block- **NO LIDS**.
11. Place calibrators into positions in calibrator rack.
12. Place controls, samples into sample rack as per worklist, checking sera volumes & make sure **NO LIDS** are left on and no air bubbles.
13. Place correct number of empty tubes into dilution rack.
14. Place reagent, sample and reaction blocks onto analyser – **NO LIDS**.
15. Click on '**Show liquid levels**'. Click '**Check levels**' Script will change from **red** to **green** if **OK**.
16. Click on Done
17. Start Assay. "*Load system Solutions- Verify solutions have been loaded i.e. wash soln, substrate, stop soln*". Click OK. *Running. Assay preparation* is displayed.
18. **Wait in Hytec 288 vicinity until calibrator and sample pipetting steps are done, as operator intervention may be required (See ALARMS)**.
19. Alarm will sound when all samples calibrators and controls have been dispensed into relevant reaction tubes. At this point in the assay the calibration block, sample diluent and patient samples can be removed from the analyser.
20. **Give substrate bottle a mix, ensuring all tablets are dissolved. Remember to remove the lid.**
21. Once removed, visually check the workstation is OK, close doors, Click 'OK'. Alarm will stop. The first incubation step is now in progress. The current assay status is visible on the VDU throughout the assay.
22. When assay is complete the results will print out.

23. **DO NOT remove reagents or tubes from analyser until assay analysis is complete and validated – nil OD's, CV's and ranges.**

24. **Check all dilution tubes have the correct volume.**

25. **Check that there are discs in all tubes as you remove them from the heating block.**

- Print cumulative positive control graphs. Click Evaluate. Click Controls. Click Selections. Click Assay class- Total IgE. Select C1 and C2. Click Graph. *From Legend Options* choose Mean 1sd etc. calc.

From Display Options choose Points.

- Print All Graphs.
- Click Done.

26. Place HAZ-TAB into waste container and leave overnight before emptying.

SUMMARY

HYTEC pipettes 50µl of calibrators, controls and patient samples into the bottom of the appropriate tubes.

Incubates for 1 hour at 37°C

Washes all tubes with wash solution.

Adds 50µl of specific IgE conjugate to tubes.

Incubates for 30 minutes at 37°C

Repeats wash procedure

Adds 200µl of prepared substrate to all tubes including the assay blanks.

Incubates for 1 hour at 37°C on the heat block.

Pipettes 400µl stop solution to all tubes including the assay blanks.

Measures the absorbance of the colour solution spectrophotometrically at 405nm.

Calculates the assay results automatically from the prepared calibrator curve.

REPORTING RESULTS: -

Using the **INTERFACE**: -

Also see “EXPORT OF RESULTS” SOP

- a) Click on ‘**Evaluate**’
- b) Click on ‘**Assay results**’
- c) Highlight correct assay run
- d) ‘**Export results**’

Wait approximately 5 minutes; check that results are in APEX. PRINTworksheet, check results. Any results >2000 need to be repeated diluted 1/20 or 1/50.

- e) Approve all other checked results.

QUALITY CONTROL AND INTERPRETATION

HYCOR Total IgE Positive and Negative Controls (Cat. No. 73020) are used with each assay. Reference Ranges are provided on the product insert sheet.

The sensitivity of the assay 0.35IU/ml. The HYTEC Total IgE quantitative results are given as IU/ml.

NORMAL RANGE

1 – 12 Months	0 – 11 IU/ml
1 – 2 Years	0 – 29
2 – 3 Years	0 – 42
3 – 5 Years	0 – 52
5 – 7 Years	0 – 56
7 – 10 Years	0 – 63
10 – 12 Years	0 – 45
12 – 15 Years	0 – 70
15 – 99 Years	0 – 100

NOTES / CONSIDERATIONS / PITFALLS

Plasma samples are unsuitable for testing.

Haemolysed, lipaemic and rheumatoid factor do not interfere with this assay, unless in gross amounts.

Bubbles and / or foaming samples / reagents can cause incorrect sampling

Beware of incorrect positioning of calibrators / control in the rack.

Incorrect response to alarm prompts can cause assay to abort.

Incompletely dissolved / unmixed PNPP solution can cause low calibrator absorbance values / high values at latter end of assay run.