

are added to the wells and antibodies recognizing the antigen bind during the incubation. After washing the wells to remove unbound proteins, a peroxidase labelled conjugate is added. The bound conjugate is visualized with a TMB substrate which gives a blue colour change whose intensity is proportional to the concentration of antibody in the sample.

References

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Helen Sandy
Laboratory Manager
0121 507 4606
Email: helen.Sandy@nhs.net

Dr Sadia Noorani
Consultant Immunologist
Tel: 0121 507 5157
Email: sadia.noorani@nhs.net

Immunology Laboratory
Tel: 0121 507 4258

Mailing Address
Immunology Department
City Hospital
Dudley Road
Birmingham
B18 7QH
Fax: 0121 507 4567

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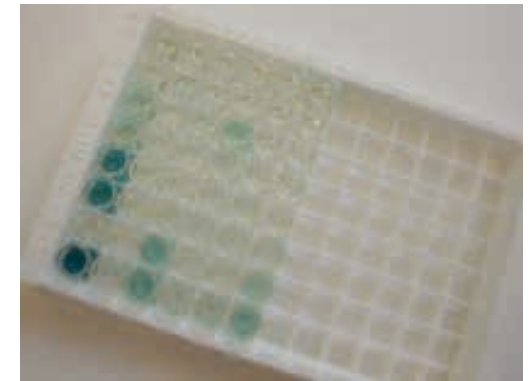
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Functional Antibody Testing

Immunology
Version No. 1.00



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Specimen Requirement:

- 10ml of clotted blood or 1-2mls of separated serum
- Samples are stable left at 4°C
- Haemolysed samples are unsuitable for analysis
- Please send samples by first class post at ambient temperature to the address on the back of this leaflet.

Reference Ranges:

Pneumococcal antibody assay:

- >20 U/ml- normal adult value

Haemophilus assay:

- <0.15 mg/L –immunodeficiency
- 0.15-1.0 mg/L-minimum protective level
- >1.0mg/L-optimum level

Tetanus assay:

- <0.01 IU/ml- susceptible
- 0.01-0.09IU/ml- basic level of protection
- 0.1-1.0IU/ml- full protective level
- >1.0IU/ml-long term protection

Clinical use of Functional Antibody Testing

The functional antibody assays include Pneumococcal, Haemophilus and Tetanus and are routinely performed in the Immunology department.

Streptococcus pneumonia is a major human pathogen causing pneumonia, sepsis, meningitis and otitis media. It causes infections mostly in children and the elderly because their immune systems are not able to respond effectively to pneumonia. Measurement of pneumococcal antibodies may be indicated when patients have increased frequency of infections or for pre and post vaccination levels. The laboratory measures Total IgG pneumococcal antibody levels in addition to subclasses IgG1 and IgG2.

Haemophilus influenza is a small gram negative coccobacillus. H. Influenzae type B (HIB) is the most virulent strain accounting for 95% of invasive diseases in children and adults including bacteraemia, meningitis and pneumonia. The antibody to the HIB PRP capsule plays the primary role in conferring immunity. Newborns have a low risk of infection, because of acquired maternal antibodies. When these transplacental antibodies to the PRP antigen wane, infants are at high risk of developing invasive H. influenzae disease, and their immune responses are low even after the disease. Therefore, they are at high risk of repeat infections since prior episodes of H influenzae do not confer immunity. By age 5 years, most children have naturally acquired

antibodies.

The HIB conjugate vaccine induces protection by inducing antibodies against the PRP capsule.

Clostridium tetani is an anaerobic gram negative bacillus which causes tetanus. Measurement of antibody titre for tetanus provides an indication of immunity and possible need for vaccination.

Method

For Pneumococcal antibody testing a solution of protein antigen is bound to plastic microtitre plate. A dilution of the patient serum is then added and any antibodies specific for the antigen will bind. A monoclonal antibody step takes place to differentiate between the total IgG, IgG1 and IgG2 against the pneumococcal antigen. Anti-human antibody labelled with an enzyme is then added which binds any human Immunoglobulin bound to the antigen. A substrate which undergoes a colour change when split by the enzyme is added. The resultant colour change is proportional to the antibody bound i.e. antibody present in the patient's serum. The HIB and Tetanus methods use microwells which are pre-coated with the appropriate antigen. The calibrators, controls and diluted patient samples

