Pseudomonas aeruginosa IgG ELISA

Quantitative test for serum Ps. Aeruginosa IgG antibodies

For Research Use Only. Not For Use In Diagnostic Procedures.

Updates from previous versions: 021098 – ALPCO 08/2001 are non-critical to the assay procedure.

Catalog Number: 27-GD07
Size: 96 Wells
Version: 061103 - ALPCO 10/04/04

American Laboratory Products Company
PO Box 451 • Windham, NH 03087
Tel.(800)592-5726 • Fax.(603)898-6854
www.alpco.com

This protocol is for reference purposes only. DO NOT use this copy to run your assay; use the protocol included with the kit ONLY.
Intended use
The Ps. aeruginosa kit is a rapid ELISA method for the detection of serum antibodies to Ps. aeruginosa in dilute human sera. In patients with cystic fibrosis and burn injuries, the kit may be used to monitor immune response to infection. Results are unaffected by antibiotics. The components of the kit are for research use only.

Introduction
Many hospital patients, particularly those with cystic fibrosis and severe burns are prone to Ps. aeruginosa infection. Pseudomonas aeruginosa may be difficult to eradicate and can be particularly virulent in CF patients. Regular monitoring of a patient’s immune status can be helpful in dealing with early onset of infection.

Levels of serum antibodies to Ps. aeruginosa are low in the general population, but rise rapidly during infection. The Ps. aeruginosa ELISA kit allows quantitative results to be obtained allowing longitudinal studies of a patients response to infection and therapy.

Principle of the test
Diluted serum samples are incubated with partially purified Ps. Aeruginosa lipopolysaccharides immobilized on microtiter wells. After washing away unbound serum components, rabbit anti-human IgG conjugated to horseradish peroxidase is added to the wells and this binds to surface bound antibodies in the second incubation. Unbound conjugate is removed by washing, and a solution containing 3,3’, 5,5’-tetramethylbenzidine (TMB) and enzyme substrate is added to trace specific antibody binding. Addition of Stop Solution terminates the reaction and provides the appropriate pH for color development. The optical densities of the standards, control and samples are measured using a microplate reader at 450nm. Optical density is directly proportional to antibody activity in the sample.

Materials included in the Kit
• Microplate 96 wells in 12 X 8 “break-apart” strips, pre-coated with Ps. aeruginosa (Serotype 10)
• Reagent 1 Sample Diluent (15 x) 15ml, Blue.
• Reagent 2 Wash Buffer (15 x) 75 ml
• Reagent 3 Enzyme Conjugate (POD-Anti-IgG Solution), 12 ml, Red, ready to use
• Reagent 4 Enzyme Substrate (TMB), 12 ml, ready to use
• Reagent 5 Stopping Solution, 12 ml, ready to use.
• Pre-calibrated standards 0, 6.25 12.5, 25, 50, 100 U/ml, 1ml Liquid, ready to use.
• Positive Control, 1ml Ready to use.
• Instructions for use

Other equipment required
10 X 60mm tubes for dilution, pipettes 10µl, 100µl, repeating dispenser 100µl, microplate reader with 450/630nm filter, a microplate washing device. distilled or R/O water, general laboratory apparatus.

Safety Precautions
On arrival, store the kit at 2 - 8°C. Once opened the kit is stable for three months (or until its expiration date if less than three months) It is important to protect the unused wells from excess moisture. Do not use kits beyond their expiration date.

The Ps aeruginosa used in the kit is not infectious. The assay calibrators are manufactured from dilute human serum which has tested negative for HIV and HbsAg. Normal clinical laboratory safety procedures should be maintained at all times. Operators should wear gloves and protective clothing when handling any patient sera or serum based products.

The stopping solution contains 0.25 sulphuric acid.

This protocol is for reference purposes only. DO NOT use this copy to run your assay; use the protocol included with the kit ONLY.
Technical Precautions

1. Strips and solutions should not be used if the foil bag is damaged or liquids have leaked.
2. Allow all reagents and the microplate to reach room temperature before use. Ensure that the microplate foil bad is containing any unused strips is well sealed and contains the desiccant to avoid moisture. Store at 2-8 °C after use.
3. The sample diluent x15 concentrate contains 0.09% sodium azide as preservative. Prepare sufficient working strength diluent for assay run. However, if the working strength diluent is to be stored for more than 1 week, add sodium azide (0.9g/L). Store unused sample diluent concentrate and dilute sample diluent at 2-8 °C.
4. Include the Positive Control in every test run to monitor for reagent stability and correct assay performance.
5. Strictly observe the indicated incubation times and temperature.
6. When automating, consider excess volumes required for setting up the instrument and dead volume of robot pipette.
7. Ensure that no cross-contamination occurs between wells. Keep all pipettes and other equipment used for Conjugate completely separate from the TMB substrate reagent.
8. When pipetting Conjugate or TMB Substrate, aliquots for the required numbers of wells should be taken to avoid multiple entry of pipette tips into the reagent bottles. Never pour unused reagents back into the original bottles.
9. Do not allow microwells to dry between incubation steps.
10. Strictly follow the described wash procedure. Insufficient washing may cause high background signal.
11. Avoid direct sunlight and exposure to heat sources during all incubation steps.
12. Replace color-coded caps on their correct vials to avoid cross-contamination.
13. It is important to dispense all samples and the positive control in the wells without delay. Therefore ensure that all samples are ready to dispense.

Samples

Serum or plasma samples may be used and should be stored at -20°C for long term storage. Repeated freezing and thawing can affect results. Frozen samples must be mixed well after thawing and prior to testing. Microbially contaminated, heat-treated or specimens containing particulate matter should not be used. Grossly haemolyzed, icteric or lipaemic specimens should be avoided.

Method

Ensure that all materials are at room temperature before beginning the procedure.

Dilute the Wash Buffer (Reagent 2), 1 to 14 in distilled water to make sufficient buffer for the assay run. The diluted wash buffer is stable for two months at 2 - 8 °C. Concentrated wash buffer is stable until the expiry date of the kit at 2 - 8 °C.

Assemble the number of strips required for the assay. It is recommended that the calibrators and blanks are run in duplicate. Samples may be run singly or in duplicate.

Prepare the working strength Sample Diluent (Reagent 1) by diluting it 1 to 14 in distilled water. The Sample Diluent is colour coded “Blue” and is stable for three months after dilution.

Samples must be diluted 1:101 (e.g. 10µl serum plus 1 ml diluent) in buffer before assay.

It is important to dispense all samples and standards into the wells without any delays. Ensure that all samples are ready to dispense. Slow pipetting or breaks in the flow of pipetting can cause drift. If it is necessary to stop the flow of dispensing, for any reason, a second set of calibrators should be dispensed.

Dispense 100 µl of each calibrator or diluted sample into the wells. Use sample diluent 100µl, as zero standard. Incubate for 30 minutes at room temperature, avoid direct sunlight.
and close proximity of any heat sources. For example, a laboratory cupboard is convenient for this purpose.

After 30 minutes, discard or aspirate the well contents and wash the wells thoroughly using a microplate washer three times. Careful washing is the key to good results. Gently tap the inverted microplate dry on absorbent paper before proceeding to the next step. **Do not allow the wells to dry out. Add conjugate immediately.**

**Manual Wash Procedure**

Empty the wells by inversion. Using a multi-channel pipette, dispense 350 µl of wash buffer per well. Empty the wells by inversion and dry the microplate on absorbent paper. Repeat the above wash process two more times.

Dispense 100µl of Enzyme Conjugate (**Reagent 3**) into each well. This reagent is colour coded “Red”.

Incubate the wells for 30 minutes in the incubation bag at room temperature. After 30 minutes, discard the well contents and carefully wash the wells using a microplate washer or manually four times. Dry the microplate on absorbent paper to remove final drops of wash fluid.

Do not allow the wells to dry out. Using a clean repeating dispenser, rapidly dispense 100µl of Enzyme Substrate (**Reagent 4**) into each well.

Incubate the plate for 10 minutes then add 100µl of Stopping Solution (**Reagent 5**) to each well. In order to allow each well the same development time, the Stopping Solution should be added to the wells in the same order sequence as the Enzyme Substrate.

Read the optical density at 450 nm (or 450/620nm dual) in a microplate photometer within 10 minutes. **For quantitative results** it is essential to ensure that each well is allowed to react for the same length of time.

**Quality Control**

Quality Control data supplied on the lot-specific QC certificate included in the kit.

The positive control is intended to monitor for substantial reagent failure.

Any well positive by spectrophotometer but without visible color should be cleaned on the underside and re-read. If OD values below zero are observed, the wavelengths used should be verified, the reader re-blanked to air and the measurements repeated.

**Results**

Plot the Optical Density of each calibrator against its concentration and draw the best fit curve through the points. Read the unknowns off this curve. We have established that 95% of normal blood donors have values less than 6 U/ml.

Each laboratory is advised to establish its own normal range data. Values above 100 U/ml should be re-assayed at a higher dilution.
Performance characteristics

Precision

Within Assay

\[
\begin{array}{cccc}
\text{Mean U/ml} & \text{SD} & n & \text{CV}\% \\
22.0 & 1.4 & 12 & 6.3 \\
78.4 & 6.6 & 12 & 8.4 \\
\end{array}
\]

Between Assay Imprecision <12%

Assay Sensitivity

Calculated from the variance of the zero standard = 0.75 U/ml

Method Summary

• Dilute sera 1:101 with sample diluent (Reagent 1)
• Dispense 100µl of each standard, control, and diluted sample into microplate wells
• Incubate at room temperature for 30 minutes.
• Wash the wells three times
• Dispense 100µl of Enzyme Conjugate (Reagent 3) into each well
• Incubate at room temperature for 30 minutes.
• Wash the wells four times
• Add 100µl of Enzyme Substrate (Reagent 4) to each well
• Incubate at room temperature for 10 minutes
• Add 100µl Stopping Solution (Reagent 5) to each well
• Read OD at 450nm within 10 minutes

Further reading

Brett, MM et al (1990) Serum IgA against Ps aeruginosa in cystic fibrosis Arch Dis Child 65 (3) : 259-263
Schaad, UB et al (1990) Serotype specific IgG antibodies to LPS of Ps aeruginosa in cystic fibrosis: Correlation to disease, sub-class distribution and experimental protective capacity. Paed Res 27(5) : 508 513
Pressler, T et al (1990) IgG subclass antibodies to Ps aeruginosa in sera from patients with chronic Ps A infection investigated by ELISA. Clin Exp Immunol 81(3) 428-434
Johansen, HK and Holby, N (1992) Local IgA and IgG response to intr-tracheal Immunisation with Ps aeruginosa antigens. APMIS 100(1) : 87 - 90
Appropriate coating methods and other conditions for ELISA of smooth, rough and neutral LPS of Pseuderomas Aeruginosa.