Protocol for l_antibod_ex05_1_0582d

This is the protocol for l_antibod_ex05_1_0582. This information was taken from documents found years after the tests were run, and were not able to be verified by the lab that ran these tests. These documents include two from the lab that ran the tests, giving cut-off information and outlining the tests, and 2 pages from a research proposal.

Key to result interpretation
Framingham Study

Test	Result in Excel file	Actual result
C. pneumoniae IgG and IgA	15	<1:16
"	16	1:16
"	32	1:32
"	64	1:64
"	128	1:128
"	256	> or = 1:256
H. pylori IgG ELISA	1.7	<1.8
"	6.5	> 6.4
CMV IgG ELISA	0.90	< or = 0.90

For H. pylori, values between 1.7 and 6.5 (non-inclusive) are the actual values.

For CMV IgG, the upper cutoff (for example, >=4.46) varies with each run. We therefore cannot eliminate >= values for this test. Values between 0.90 and the upper cutoff are the actual values.

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Procedure summaries – Framingham Study
February 3, 1998
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H. pylori IgG by ELISA (Enteric Products International kit):
Sera are diluted 1:101 in sample buffer, and 0.1 mL is added to individual microtiter wells. After 20 minutes at room temperature (RT), plates are washed three times, and 0.1 mL of horseradish peroxidase-conjugated goat anti-human IgG is added. After 20 minutes at RT, the plates are washed as before. TMB is added, and after 10 minutes, 1N sulfuric acid is added to stop the reaction. Absorbance is read at 450 nm. Absorbance values are converted to ELISA values (EV) using a 3-point standard curve. <1.8 EV is considered negative, 1.8-2.2 EV is considered Equivocal, and >2.2 EV is considered positive.

CMV IgG by ELISA (Zeus Scientific kit):
Sera are diluted 1:21 in sample buffer, and 0.1 mL is added to individual microtiter wells. After 20 minutes at RT, plates are washed three times, and 0.1 mL of horseradish peroxidase-conjugated goat anti-human IgG is added. After 20 minutes at RT, the plates are washed as before. TMB is added, and after 10 minutes, 1N sulfuric acid is added to stop the reaction. Absorbance is read at 450 nm. An index value is calculated by dividing the patient absorbance value by the cutoff absorbance value (determined by multiplying the low positive control absorbance value by a kit-specific conversion factor). Index values < or = 0.90 are considered negative, 0.91-1.09 equivocal, and > or = 1.10 positive.

C. pneumoniae IgG and IgA by IFA (slides purchased from MRL Diagnostics):
Slides contain C. pneumoniae elementary bodies attached to the glass; there are 12 wells per slide. Sera are diluted 1:16, 1:64, and 1:256 in sample buffer. For IgG measurements, 0.025 mL of each dilution is added to slide wells. For IgA measurements, only the 1:16 dilution is added to a well. After 1 hour at 37C, the slides are washed and dried, and then receive FITC-conjugated goat anti-human IgG or FITC-conjugated goat anti-human IgA. After 30 minutes at 37C, slides are washed and dried as before. Glycerol mounting medium is added, along with a coverslip. Slides are examined using a fluorescent microscope at 400X. The presence of antibody is indicated by fluorescence of elementary bodies. If the IgA is positive, then all 3 dilutions are tested to determine the titer. For both IgG and IgA, titers of 1:16 or greater are considered positive.

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Assays for Chlamydia antibodies

Chronic *Chlamydia pneumoniae* infection will be indicated by elevated antibody titers against Chlamydia pneumoniae, presence of Chlamydial lipopolysaccharide-containing immune complexes, or both. Antibody and immune complex determinations will be done in a blinded fashion. Immunoglobulin A (IgA) and G (IgG) antibodies to Chlamydia pneumoniae will be measured using the microimmunofluorescence method (University of Washington Research Foundation, Seattle, Washington). Lipopolysaccharide-containing immune complexes will be measured using antigen-specific enzyme immunoassays, the lipopolysaccharide-capture and immunoglobulin M (IgM)-capture methods.[38]

Assays for CMV antibodies

Tests for anti-CMV IgG antibiodies will performed with an enzyme-linked immunosorbent assay (ELISA) kit (Cytomegelisa II, BioWhittaker, Walkersville, MD), according to the manufacturer's directions. Antibody titers will be determined on the basis of a standard curve. The threshold value will be determined prospectively; an ELISA value of less than 0.25 unit may be considered a negative result, and a value of 0.25 units or higher will be considered a positive result, indicating prior exposure to *CMV*. Tests for anti-CMV IgM antibodies will be performed with an enzyme-linked antibody-capture assay kit (CMV CAP-M, BioWhittaker), according to the manufacturer's directions. An index value of less than 0.9 will be interpreted as a negative result, and a value of more than 1.1 will be interpreted as a positive result; values between 0.9 and 1.1 will be considered equivocal results.

Assays for Helicobacter pylori

Blood samples will be evaluated for the presence of *Helicobacter pylori* antibodies by an ELISA test (Enteric Products Inc.). If the antibodies are detected in the blood sample, the subjects will be considered to have an infection with *Helicobacter pylori.* If the antibodies are not detected, the subjects will be considered as not infected.