Laboratory 17 –Cryptosporidium (17kDA), Cryptosporidium (27kDA), Toxoplasma (IgG), Toxoplasma (IgM),Toxoplasma (Dye),Toxoplasma Differential Agglutination,Toxoplasma Differential Agglutination Interpretation, Toxoplasma (Avidity),and Toxoplasma (Avidity) IgG Interpretation

Description

Toxoplasma

Serologic tests are available to determine who has become infected with toxoplasmosis. Toxoplasma-specific IgG antibodies are detectable 1-3 weeks after infection and remain detectable for the life of the individual. Toxoplasma-specific IgM antibodies are also detectable 1-3 weeks after infection but generally decline to nil by one year after infection. The presence of both IgG and IgM is evidence for infection within the last year. The presence of IgG antibody without IgM is considered indicative of past infection. All eligible participants were tested for serum IgG and positive sera were tested for the presence of IgM. These data will be used to estimate the prevalence of toxoplasmosis in the U.S. and to determine if the rates are changing over time. Toxoplasma IgG antibody was measured in NHANES III with an overall prevalence of 22.5%.

Eligible Sample and Exclusion Criteria

Participants aged 6 to 49 years of age

Data Collection Methods

Blood specimens are processed, stored and shipped to the Division of Parasitic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention for analysis.

Examination Protocol

Detailed specimen collection and processing instructions are discussed in the NHANES Laboratory/Medical Technologists Procedures Manual (LPM). Vials were stored under appropriate frozen (-20 degrees Centigrade)conditions until they were shipped to Division of Parasitic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention for testing. The analytical methods are described in the Analytic methodology section.

Analytic Methodology

• Cryptosporidium

Enzyme-linked immunosorbent assay.

Antigens diluted in 0.1 M $NaHCO_3$ buffer (pH 9.6) were used to sensitize 96-well

plates overnight at 4 ^{o}C (Immulon 2 flat-bottom microtiter immunoassay plates, Dynatech Industries, Inc.,McLean, Va.). Each well contained 50 µl of either the recombinant 27-kDa antigen (0.2 µg/ml)(BCA protein microassay , Pierce Biotech. Company, Rockford, Ill.).The plates were washed in 0.05%Tween-20 PBS and blocked with 0.3%Tween-20 PBS for 1 hour at 4 ^{o}C. After a series of three washes (subsequent washes were all with 0.05%Tween-20 PBS),50 µl aliquots of serum-diluted 1:50 with wash buffer were added to each well. All serum samples were tested in duplicate. A two-fold serial dilution (1:50 to 1:6400) of a strong positive control was used to generate a standard curve on each individual plate. Two buffer blanks, three confirmed positive sera, and a battery of four serum samples known by Western blot assay to be negative for C. parvum antibodies were also included on each plate. Plates were incubated for 2 hours at room temperature, and then washed four times with wash buffer. A biotinylated mouse monoclonal antibody against human IgG (clone HP6017, Zymed Laboratories,50 µ l of a 1:1000 dilution in wash buffer)was added to each well and incubated for 1 hour at room temperature. Following four washes, the wells were filled with alkaline phosphatase-labeled streptavidin (Life Technologies, 50 µl of a 1:500 dilution in wash buffer) and incubated an additional hour at room temperature. After four washes (the final wash for 10 minutes at room temperature), p-nitrophenylphosphate substrate was added in 3 mM $MgCl_2$ and 10%diethanolamine at pH 10,and the color was allowed to develop until the 1:50 positive control wells had reached an absorbance of about 1.5 at 405 nm. Absorbances were measured using a Molecular Devices UVmax kinetic microplate reader. Antibody levels of the unknown samples were assigned a unit value based on the 8-point positive control standard curve with a four-parameter curve fit. The 1:50 dilution of the positive control was arbitrarily assigned a value of 6400 units. Unknown samples with absorbance values above the standard curve were diluted further and reassayed. Arbitrary unit values were expressed per microliter of serum.

• Toxoplasma

Toxoplasma (IgG)

The presence and quantity of IgG antibodies to *Toxoplasma gondii* in the test sample were determined by performing an EIA test with *Toxoplasma* antigen. A standard curve was constructed using optical density readings from positive control sera wells; these readings were calibrated to WHO Toxo 60 serum and read as International Units (IU/mL). Those test samples with results below 7 IU/mL indicated a non-significant level of antibody; thus, they were considered to be negative, indicating no infection. Those test samples with results greater than 6 IU/mL were considered to be positive, indicating *Toxoplasma* infection at some undetermined time.

Toxoplasma (IgM)

The presence and quantity of IgM antibodies to *Toxoplasma gondii* in the test sample were determined by performing an IgM capture EIA test with *Toxoplasma* antigen. Results are obtained by dividing the optical density of the test sample

well by the optical density of the positive standard well and multiplying the result by 100. Those test samples exhibiting ratios below 2.0 indicated a non-significant level of IgM antibody according to this technique; thus, they were considered to be negative for IgM antibodies. Those test samples with ratios greater than 2.0 were considered to be IgM positive, indicating either *Toxoplasma* infection within the last 2 years or a false-positive reaction.

Toxoplasma (Dye)
The presence and quantity of antibodies to *Toxoplasma gondii* in the test sample were determined by performing the Sabin-Feldman Dye Test with live *Toxoplasma* organisms. Test samples are diluted and mixed with dye and live organisms. If the sample dilution contains antibodies to Toxoplasma, the organisms are lysed and unable to take up the dye. The titer reported is that dilution of serum at which half of the organisms are not killed (stained) and the other half are killed (unstained). Those test samples with results less than 1:16 indicated a non-significant level of antibody; thus, they were considered to be negative, indicating no infection. Those test samples with results equal to or greater than 1:16 were considered to be positive, indicating *Toxoplasma* infection at some undetermined time.

Toxoplasma Differential Agglutination and Toxoplasma Differential Agglutination Interpretation
The presence and quantity of antibodies to *Toxoplasma gondii* in the test sample were determined by performing the Differential Agglutination Test with *Toxoplasma* organisms. Test samples are diluted; one aliquot is mixed with formalin-fixed organisms and another aliquot is mixed with methanol-fixed organisms. Agglutination titers are reported for both types of fixed organisms and the combined results are interpreted by comparison of titers.

Test samples are classified as having a nonacute pattern, an equivocal pattern, or an acute pattern. The results may be useful in determining whether the patient has an acute infection or not.

Toxoplasma (Avidity) and Toxoplasma (Avidity)IgG Interpretation
The avidity of IgG antibodies to *Toxoplasma gondii* in the test sample was determined by performing the IgG Avidity Assay with *Toxoplasma* organisms. Optical density results of the test sample well are compared with and without dissociation treatment. The index is the percentage of antibodies that resist dissociation. Test samples are classified as having low, equivocal, or high avidity. The results may be useful in determining whether the patient has an acute infection or not.

Analytic Notes

Toxoplasma :

LBXT01
This test is performed on all examinees aged 6-49 years.

LBXT02
This test is performed only if LBXT01 is greater than or equal to 8.

LBXT03
This test is performed only if LBXTO2 is greater than or equal to 2.0.

LBXT04 AND LBXT04IN
These tests are performed only if LBXTO3 is greater than or equal to 16.

LBXT05 AND LBXT05IN
These tests are performed only if LBXTO4 is >25 and >50 because it is a serial dilution test .