Modified latex agglutination test for antibodies to *Toxoplasma gondii* in eluates from Guthrie cards

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Abstract

***Aims*: To determine whether the Eiken particle agglutination test could be modified to make it sufficiently sensitive to screen blood samples collected on Guthrie cards for the presence of antibodies to *Toxoplasma gondii*; to evaluate the specificity of the modified system; and to compare seroepidemiological data on the prevalence of *T gondii* in pregnant women.**

***Methods*: Simulated dried blood spots were prepared from sera from pregnant women booking for antenatal care. Eluates from the simulated dried blood spot cards and sera were tested in parallel using the modified test (1 in 5 dilution of latex) and the standard assay (neat latex particles) and endpoints determined. Guthrie card eluates, from neonates in three Thames regions, were then tested using the modified test.**

***Results*: The modified test produced a 4·21-fold increase in antibody titre in 85 sera when tested in parallel with the standard test. Eluates of 168/170 from simulated dried blood spots derived from seropositive patients gave a positive result in the modified test. The two eluates which gave a negative result were derived from patients with an equivocal titre of 1/16 in the standard serum test. Of the eluates derived from serum negative patients all 103 were negative at a dilution of 1 in 4 in the modified test. The seroprevalence of antibodies to *T gondii* in pregnancy was 21·8% using the standard test. A similar value of 20·5% was obtained when dried blood spots from neonates in a similar region of London were tested by the modified test.**

***Conclusions*: The modified Eiken Toxoreagent test is sensitive, simple, and economic for screening large numbers of dried blood spots. The procedure could be easily semiautomated and the technique applied to the mass screening of neonatal blood samples collected on Guthrie cards to determine the seroprevalence of *T gondii* in pregnant women.**

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It has been suggested recently that the true extent of problems associated with congenital toxoplasmosis infection are being overlooked. As a result, the proposed introduction of a programme for screening pregnant women in the United Kingdom has provoked controversy.

The epidemiology of *Toxoplasma gondii* in women has been reviewed,[1-3] and serological studies have shown a wide variation in the prevalence of antibodies in European countries: France 80%[4]; Belgium 50%[5]; Netherlands 40%[6]; Finland 19%[7]; and England 19%.[8] However, seroprevalence has fallen rapidly over the past 20 years in Sweden[9] and in parts of England.[10] Inexpensive and effective methods of surveillance are reported to monitor changes in seroprevalence so that appropriate strategies to prevent infection can be implemented.

Blood collected from neonates on Guthrie cards has been used for many years in the United Kingdom and the United States of America for screening for the presence of metabolic diseases.[11,12] Such samples have been used in the USA for seroepidemiology studies of the prevalence of antibodies to HIV-1 in pregnant women.[13,14] To reduce the cost of neonatal screening in the United Kingdom the Fujirebio anti-HIV-1 particle agglutination assay was modified.[15] Its efficacy has been confirmed for the determination of the prevalence of maternal HIV-1 infection based on unlinked anonymous testing of neonates.[16-18] As a result of our experience with the modified HIV-1 test, we decided to evaluate the use of the Toxoreagent latex agglutination test, which we modified in a similar way.

Methods

SOURCE OF SERA

Positive (n = 170) and negative (n = 103) sera were obtained from pregnant women when they booked in at an antenatal clinic in inner London. These samples had previously been screened for the presence of antibodies to *T gondii*. Sera had been stored at −20°C and frozen and thawed several times during the past 10 years for other studies[8,19,20] but not heat inactivated.

SOURCE OF NEONATAL SAMPLES

Dried blood spot samples were obtained from Guthrie cards which had been collected from 1023 neonates for routine metabolic screening. These samples originated from health districts in the inner London area. Samples were collected during 1991 and stored at 4°C in sealed bags for a period of up to one month, before testing.

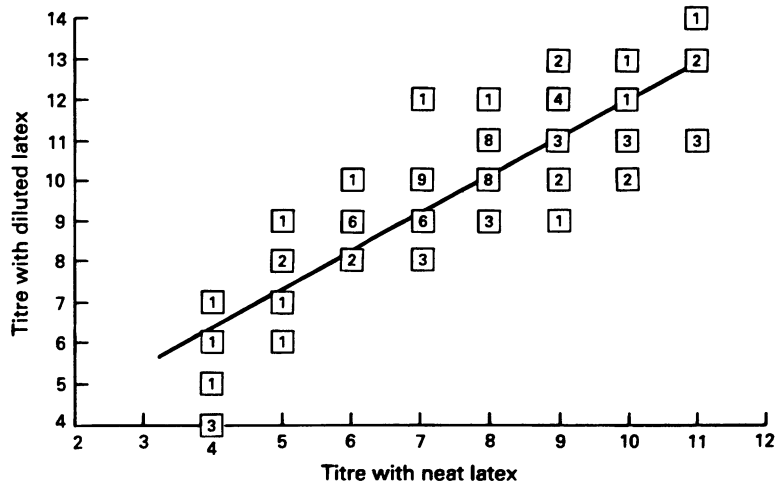

Figure 1 Comparison of 85 antibody titres in sera using neat or diluted (1 in 5) latex. Data expressed in log base 2.

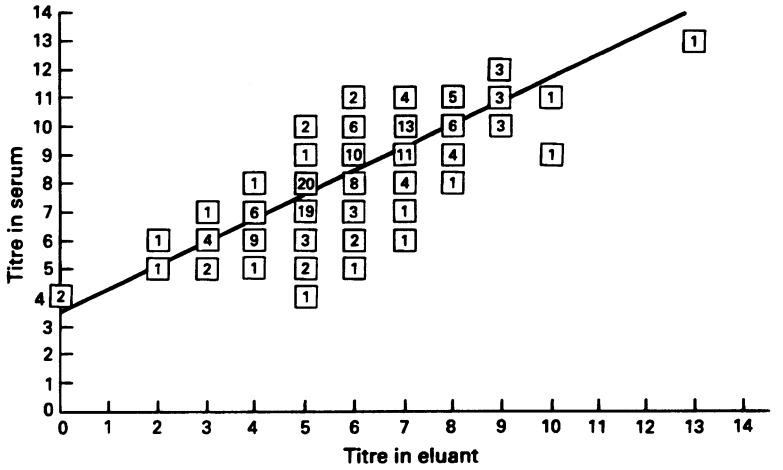

Figure 2 Distribution of titres obtained in 339 antenatal sera and 338 Guthrie cord eluates.

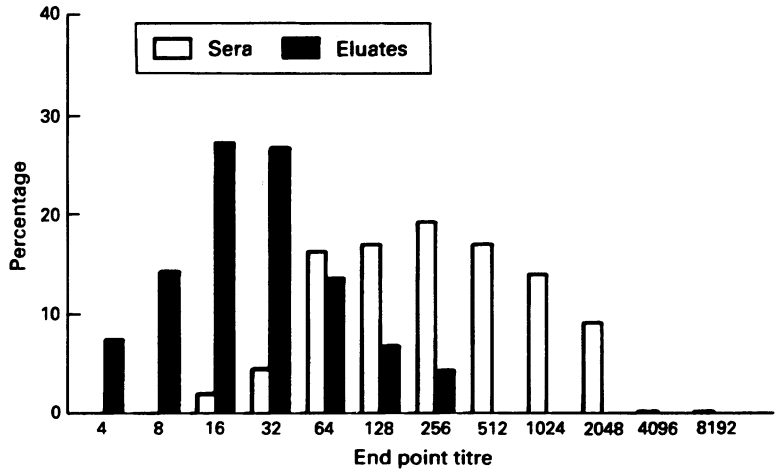

Figure 3 Comparison of 170 antibody titres in sera and Guthrie card eluates. Data expressed in log base 2.

PREPARATION OF SIMULATED DRIED BLOOD SPOTS

Fresh whole blood was screened to ensure the absence of antibodies to *T gondii* and used to make a 1 in 2 dilution of each of the 273 antenatal serum samples provided. A 110 μl aliquot of each dilution was spotted on to a 15–20 mm diameter circle on a Guthrie card and allowed to dry at room temperature for 24 hours, then stored at 4°C in sealed bags.

ELUTION

A 5·5–6·0 mm diameter blood spot was punched out of each Guthrie card circle and placed in the well of a flat bottomed microtitre plate. The dried blood was then eluted in 100 μl of buffer (phosphate buffered saline, pH 7·2, containing 0·05% Tween 20, 0·005% sodium azide). Plates were shaken for 30 minutes, covered, and then left to eluate overnight at 4°C. Plates were shaken again for three minutes before the eluate was removed for testing.

The Eiken test was used according to the manufacturer's instructions for testing sera.

MODIFIED TEST FOR GUTHRIE CARD BLOOD SPOTS

The Eiken Toxoreagent latex agglutination test was modified by diluting the latex 1 in 5 in AMP buffer, 0·2 M 2-amino-2-methyl-1-propanol (HCl) (Sigma Ltd, UK).

Tests were performed according to the manufacturer's instructions but in V well microtitre plates. Following overnight incubation at room temperature the plates were placed on a light box sloped at an angle of 70° and the results read after 10 minutes. Positive tests were distinguished by a tight discrete agglutination pattern; negative samples formed a teardrop pattern. Samples found to be positive in the initial screen were titrated in a series of doubling dilutions from 1 in 4 in AMP buffer to 1 in 8192 to obtain an endpoint.

Results

COMPARISON OF TITRES IN SERA OBTAINED USING NEAT LATEX AND A 1 IN 5 DILUTION OF LATEX PARTICLES

The results of tests on 85 sera are shown in fig 1. The mean value of the endpoints obtained using a 1 in 5 dilution of latex was 4·21 times greater than with neat latex. The agglutination patterns were easy to read and there was no evidence of false negative results when diluted latex was used.

COMPARISON OF ANTIBODY TITRES IN SERA TESTED BY THE STANDARD TECHNIQUE AND ELUATES FROM GUTHRIE CARDS USING LATEX AT A 1 IN 5 DILUTION

The results of tests on 170 seropositive antenatal sera and corresponding eluates from simulated blood spots are shown in fig 2. The mean value of titres in sera using the standard technique was 4·13 times greater than in the eluates from simulated blood spots.

Using the modified test 168 out of 170 (98·8%) had detectable antibody titres ($\geq$4 (>2 $\log_2$)). The two eluates in which antibody was not detected originated from patients that had equivocal titres of 1/16 by the standard test.

A further 103 eluates from simulated antibody negative blood spots gave a negative result (<4(<2 $\log_2$)) using the modified test.

Figure 3 compares the distribution of antibody titres in sera using the standard technique and in eluates from Guthrie cards using the modified assay. The results show a similar

antibody distribution curve in pregnant women and in a comparable sized sample of unrelated neonates. However, the endpoints of the eluates were four-to eight-fold lower, presumably due to the dilution factor involved. Using the standard test, 334 of 339 of the antibody positive sera had titres of $\geqslant 1/32$. The other five sera had low titres of 1/16 which represents the cutoff value.

SEROPREVALENCE OF ANTIBODIES TO *T gondii* IN PREGNANT WOMEN BY THE NEONATAL SCREENING OF GUTHRIE CARDS

The overall seroprevalence of antibodies to *T gondii* when screening neonates using the modified technique on eluates from Guthrie cards was 20·5% (214 out of 1043). A similar value would be expected with the mothers, as neonatal blood contains maternal antibody.

Discussion

The Eiken Toxoreagent test has been evaluated and found to be both sensitive and specific.[8 21–23] It is widely used in the United Kingdom for screening sera, but has not been evaluated for screening samples collected on Guthrie cards.

The modified test detected 98·8% of all positive sera, giving 100·0% specificity when compared with the standard test. This is a sensitive and simple method for screening large numbers of samples. The prevalence of maternal antibody found in the present study of 1043 eluates from Guthrie cards was 20·5%. This is similar to the figure of 21·8% obtained when sera were screened from 1000 pregnant women attending an antenatal clinic in London using the standard test.[8]

Using the standard Eiken test, sera with a titre of 1/16 are regarded as weakly positive and testing a follow up sample is recommended. If a screening procedure was introduced, all negative samples would need to be followed up two to three times during pregnancy. Although the modified latex test failed to detect two simulated samples in which the sera had a titre of 1/16, the outcome would be identical, as both these patients would have been retested. The standard technique was not sufficiently sensitive to detect low titres of antibody in eluates from Guthrie cards, but this problem was overcome by modifying the assay as described.

The advantages of the Eiken latex agglutination test are that it detects total antibody, is simple to perform, and requires no expensive capital equipment. The modified test retains these advantages and the cost is reduced from about 10 pence to 2 pence per well, between 50 and 100 times cheaper than many commercially available enzyme immunoassays.

The use of blood samples collected on Guthrie cards from both adults and babies also reduces the problems of transport and storage. This makes the test ideal for epidemiological surveillance in the United Kingdom or third world countries.

The method described can be used to screen rapidly large numbers of samples and the procedures can easily be semiautomated. The modified assay is ideally suited to link in with other neonatal screening programmes which rely on the use of blood collected on Guthrie cards.

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