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The reader is informed that all taxonomy in this document was correct at time of issue.

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## AMENDMENT PROCEDURE

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<th>Amendment Number/Date</th>
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Each National Standard Method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@hpa.org.uk.

On issue of revised or new pages each controlled document should be updated by the copyholder in the laboratory.
INVESTIGATION OF TOXOPLASMA INFECTION IN PREGNANCY

Types of specimen: Serum
Plasma
Whole blood
Amniotic Fluid

INTRODUCTION

Toxoplasma gondii is an extremely successful protozoan parasite that can infect all mammalian and bird species throughout the world. Following the acute active phase of infection the parasite will persist for many years (probably lifelong) in the form of latent cysts located throughout the body, especially in cardiac and skeletal muscle and central nervous system tissues. Individuals who acquire toxoplasma infection either notice no significant symptoms or experience a self-limiting, mild to moderate, 'flu' or glandular fever-like illness. In the immunocompetent, the latent tissue cyst form of the parasite presents no further risk to health.

However, where toxoplasma infection is acquired by a mother during pregnancy, the parasite presents a significant risk of adverse outcome to the foetus. The risk of transmission from mother to foetus is lower when maternal infection is acquired in the early stages of pregnancy but the outcome in such cases can be severe or life-threatening to the foetus. Conversely, while maternal infection acquired later in pregnancy confers a higher risk of transmission to the foetus, the clinical outcome is characteristically less severe, or the child may even be born asymptomatic. Infection acquired in the 2–3 months prior to conception can very rarely present a risk of damage to the foetus. The cumulative incidence of congenital toxoplasmosis for England and Wales was estimated at 3.4/100,000 live births in 2002-04, with the most common symptoms among live births being retinochoroiditis and/or intracranial abnormalities (with or without developmental delay).

This guidance note will summarise current knowledge regarding risk of transmission and clinical outcome and will provide a summary of strategies for the investigation and management of suspected or confirmed toxoplasma infection. Toxoplasma screening is not part of routine screening for women in the UK. The principal aim is to address cases where toxoplasma infection acquired by the mother during or immediately prior to pregnancy is suspected or confirmed. Therefore, the issue of prenatal screening for toxoplasma infection will not be considered.
1 OVERVIEW OF INVESTIGATION STRATEGY

Laboratory investigation for toxoplasma infection in pregnancy aims to provide critical information to support appropriate and timely clinical management. Therefore, in determining the most appropriate laboratory investigation strategy, it is also essential to consider what management options are available.

Essentially, there are three separate patient groups that need to be considered in the investigation of toxoplasma infection in pregnancy: the mother, the foetus, and the neonate.

The key information sought is summarised in the table below:

<table>
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<tr>
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<th>Aim of laboratory investigation</th>
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<tbody>
<tr>
<td>Mother</td>
<td>Confirm or exclude risk to pregnancy by determining whether maternal infection was acquired before conception</td>
</tr>
<tr>
<td>Foetus</td>
<td>If risk to pregnancy is confirmed, determine whether foetal infection can be confirmed</td>
</tr>
<tr>
<td>Neonate</td>
<td>If foetal infection is not confirmed, confirm or exclude congenital toxoplasma infection in the neonate</td>
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Mother – If laboratory investigation can exclude risk to the pregnancy by confirming that infection took place before conception, then further investigation is unnecessary. However, if risk to the pregnancy cannot be excluded, management options include further clinical and laboratory investigation of the foetus. Concurrent antitoxoplasma treatment (spiramycin) aimed at reducing the risk of transmission from mother to foetus can also be considered. The precise choice of management option will depend upon a range of factors including stage of pregnancy and parental choice, which, in some cases, might be elective termination.

Foetus – where foetal infection is either strongly suspected or confirmed, management options include intervention with reportedly more effective, but potentially more toxic, antitoxoplasma therapy, or a reconsideration of elective termination.

Neonate – where unequivocal exclusion or confirmation of congenital toxoplasma infection was not possible during prenatal investigation, postnatal exclusion can preclude the need for treatment and further clinical investigation, while confirmation of infection in the neonate can ensure appropriate treatment and clinical follow-up.

The table below summarises the range of laboratory investigations that are helpful in assessing risk to a pregnancy from toxoplasma, and confirming or excluding congenital toxoplasma infection in the foetus and neonate;
2 MATERNAL INFECTION

The prevalence and incidence of toxoplasma infection among pregnant women varies significantly between countries, probably reflecting differing risk factors and modes of transmission\(^9\). In the majority of cases the infection is either asymptomatic or symptoms may pass unnoticed. If symptoms are present they are usually mild to moderate and non-specific, for example fatigue, malaise, myalgia, sore throat, low-grade fever and lymphadenopathy (often involving the posterior cervical region). Symptoms can last from a few weeks to several months.

There have been very few reports of women who are immunocompetent acquiring toxoplasma infection before becoming pregnant and transmitting the organism to the foetus. It is therefore generally accepted that when infection occurs before conception there is no significant risk of congenital infection in the foetus.

It is therefore important to consider the following questions when trying to determine whether or not a pregnancy is at risk from toxoplasma infection:

- Is there evidence of maternal toxoplasma infection? Use IgG assays
- Is the infection recent? Use IgM, IgA assays
- When did infection occur in relation to conception? Use IgG avidity assays

As the clinical features of acute toxoplasma infection are non-specific, diagnosis relies primarily upon serological tests. The demonstration of seroconversion is seldom possible in UK practice as neither serial sampling during pregnancy nor preconception testing are performed routinely. Therefore, current standard practice is often serological testing by the local laboratory of a single maternal sample, using an appropriate toxoplasma screening assay. However, if possible, it is always useful to compare results in an antenatal booking sample (and any preconception samples collected for other purposes, if available) with the sample collected at first presentation. Where acute toxoplasma infection is suspected, it is recommended that investigation be undertaken utilising separate IgG and IgM assays. It should be emphasised that the more sensitive IgM immunosorbent agglutination assay (IgM-ISAGA) is not helpful in this particular situation as it can detect IgM persisting for longer than one year after infection has been acquired, and therefore may detect IgM in a significant proportion of women whose foetuses are not at risk from toxoplasma infection. If the serum is found to be positive for IgM using an appropriate assay, it is recommended that further specialist investigation be considered, including IgG avidity testing and comparison of both IgG and IgM levels in sequential samples in order to gain insight into the likely duration of infection\(^{10,11}\).

\(\text{a}\) as matched sample with neonatal blood

\(\text{a}\) and \(\text{b}\) must be tested as paired samples for comparison
Samples can be sent to the Toxoplasma Reference Unit (TRU) where initial investigation will be undertaken using the Sabin-Feldman Dye test (DT) and IgM enzyme immunoassay (EIA). The DT is the international ‘gold standard’ reference test for toxoplasma, detecting both IgG and IgM. The DT can confirm whether the pregnant woman has become infected at any time previously with toxoplasma and the detection of IgM can identify infections probably (but not invariably) acquired within the past 6-9 months.

If the mother is confirmed as being negative for IgG (eg DT negative), advice should be given on precautions aimed to reducing the risk of infection for the remainder of the pregnancy. When IgG is positive and IgM is not detected, the patient can be reassured that the pregnancy should not be at risk. However, when IgG and IgM are confirmed as being positive, further laboratory testing is required in order to provide a more precise estimate of the duration of infection. Measurement of IgG avidity can be particularly helpful for this purpose since this method can discriminate between infection acquired recently and those acquired several months or more earlier. Several commercial IgG avidity assays are available and the precise range of discrimination between ‘early’ and ‘later’ infection will depend upon each manufacturer’s specifications. For example, the IgG avidity assay provided by TRU currently discriminates infections of less than three months versus greater than six months duration. In addition detailed questioning of the pregnant women can be helpful in revealing clinical features which may help in timing the onset of infection, and archives should be checked for any stored serum samples collected prior to conception.

Following identification of a pregnancy potentially at risk from toxoplasma, it is very important to request a second serum sample immediately in order both to confirm the original result and to allow comparison for possible changes in titre. When an acute toxoplasma infection is confirmed in a pregnant women it is essential that the parents should be counselled regarding the risk to the foetus and management options.

3 PREGNANT WOMEN WHO ARE IMMUNOCOMPROMISED

Reactivation of latent infection and consequent transmission to the foetus has been reported in women with a cell-mediated immune deficiency. This includes patients with systemic lupus erythematosus treated with corticosteroids, Hodgkin’s lymphoma and HIV infection. Pregnant women in the last category require careful monitoring of both their immune status and toxoplasma infection. Where required, specialist advice should be sought in the management of such patients. Such advice is available from the Toxoplasma Reference Unit.

4 FOETAL INFECTION

Infection of the foetus is not an inevitable outcome of every maternal toxoplasma infection. The risk of transmission from mother to foetus increases depending in which trimester maternal infection is acquired. Based on a range of reported studies, the mean risk of transmission in the first trimester is estimated to be 10-15%, rising to 70-80% in the third trimester. However, although foetal infection in the first trimester is less likely, the outcome is generally more severe (eg gross abnormality or spontaneous termination) compared to infection acquired in the third trimester which may result in more subtle neurological, ocular or systemic signs or may be sub-clinical, with the child born apparently normal. Transmission of toxoplasma to the foetus typically occurs after the placenta has become infected. This transmission from placenta to foetus may take place almost immediately or may be delayed for several weeks. Probably a major factor influencing the risk of transmission is the development of placental blood flow; this may well explain the increased rate of transmission later in pregnancy.

The diagnosis of foetal infection is based upon the detection of the parasite and/or specific antibody responses in the foetus. Ultrasound alone can support, but not confirm, the diagnosis. Typical abnormalities found in an infected foetus by this technique are cerebral ventricular dilation and intracranial densities.
Cordocentesis affords the opportunity to demonstrate non-specific biochemical and haematological abnormalities, detection of both the parasite and specific anti-toxoplasma IgM/IgA. However, negative serological findings are not reliable in excluding congenital infection; one study found positive IgM results in only 12% of infected foetuses aged 22-24 weeks, 39% at 25-30 weeks and 59% after 30 weeks. No positive results were reported before 22 weeks of gestation. The presence of IgA also confirms congenital infection but, like IgM, is frequently not found. However, because the risk of contamination of foetal blood by maternal blood is difficult to exclude, the diagnostic significance of these tests is reduced where maternal IgM/IgA can also be demonstrated.

Direct detection of the parasite from foetal blood or amniotic fluid using the polymerase chain reaction (PCR) provides unequivocal evidence of infection. Detection in amniotic fluid has been found to have as good a level of detection as the methods involved in cordocentesis and has fewer risks to the foetus. PCR allows for earlier diagnosis of toxoplasma infection and allows therapy to be introduced sooner and amniocentesis is now the recommended sample for investigation of toxoplasma infection. However, although PCR is a highly specific and sensitive technique it still has potential limitations and PCR results should not be interpreted in isolation from other tests. For example, few commercial nucleic acid amplification tests are yet available for the detection of toxoplasma, and European interlaboratory quality assurance studies have suggested that performance of PCR can be highly variable between centres. Laboratories offering toxoplasma PCR testing for diagnostic purposes should subscribe to external quality control/quality assurance schemes as these become available.

5 NEONATAL INFECTION

While the classical triad of congenital infection (hydrocephalus, cerebral calcification, chorioretinitis) strongly indicates congenital toxoplasmosis, many children are born either with more subtle signs or are born apparently normal. In the latter, clinical features can present in the first weeks or months of life but may not be apparent for several years or even decades. The range of presentations that may occur months or years after birth include:

- Chorioretinitis
- Hydrocephalus
- Cerebral calcification
- Seizures
- Hepatosplenomegaly
- Jaundice
- Rash
- Mental retardation
- Deafness
- Spasticity
- Cataracts, strabismus
- Blindness

Early treatment of congenital toxoplasmosis appears to decrease the frequency of chorioretinitis and be associated with the disappearance of cerebral opacities.

If clinical benefit can be achieved by appropriate treatment, then the diagnosis of neonatal infection becomes crucial. This can be straightforward in a child with characteristic clinical and serological findings that are confirmed by parasite detection. Unfortunately in the majority of cases the diagnosis is less straightforward. Thorough clinical and ophthalmological examinations of the neonate must be performed together with an ultrasound of the brain. However, frequently it is not possible to detect these changes and diagnosis has to be based solely on laboratory findings.

Direct detection of the parasite is attempted by culture and PCR of the amniotic fluid and cord blood. Investigation for placentation infection is considered less helpful since detection of
parasite in the placenta alone, while providing strong supporting evidence, can not be considered as unequivocal confirmation of foetal infection. Further, if placental testing is being considered, multi-site sampling of the placenta is recommended as the distribution of the parasite may be localised into discrete foci of infection within placental tissues.

Cord blood and a matched maternal sample are subjected to serological testing. Since maternal IgG is transferred passively to the foetus in utero, detection of IgG in the neonate is of limited value unless levels are significantly elevated compared to maternal titres. However, comparison of maternal and neonatal IgG by immunoblot may be helpful since detection of a neonatal immune response to any antigens not recognised by the maternal immune response would imply this IgG is unique to the neonate.

Detection of neonatal IgM and IgA by EIA and/or ISAGA are regarded as being diagnostic for neonatal infection, but the possibility of contamination by maternal blood should be excluded if IgM and/or IgA are present in the mother at the time of birth.

It is important to note that IgM and IgA may only be present in 50-60% of congenitally infected children in the first month of life but may appear subsequently. It is therefore essential to monitor the child serologically throughout the first year of life by which time any passively-acquired maternal IgG antibodies will decline and disappear. The disappearance of IgG within the first year of life excludes congenital infection. Persistence of positive DT after 12 months confirms infection. Treatment of an infected neonate may initially result in a reduction in antibody levels or even a complete disappearance. In such cases antibodies reappear when therapy is stopped.

6 RELEVANT NATIONAL STANDARD METHODS

For additional details on specific areas of diagnosis refer to the relevant NSMs available through the Department for Evaluations, Standards and Training web page (www.hpa-standardmethods.org.uk).
7 ACKNOWLEDGEMENTS AND CONTACTS

This National Standard Method has been developed, reviewed and revised by Dr Guy of the Toxoplasma Reference Unit and Dr Ho-Yen of the Scottish Toxoplasma Reference Unit for the Virology Working Group on Standards and Quality (http://www.hpa-standardmethods.org.uk/wg_virology.asp). The contributions of many individuals in clinical virology laboratories and specialist organisations who have provided information and comment during the development of this document, and final editing by the Medical Editor are acknowledged.

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APPENDIX 1: PREGNANT MOTHER - GUIDANCE

1. **Pregnant Mother**
   - **Is mother infected?**
     - **IgG Assay**
       - **IgG positive**
         - **Is infection recent?**
           - **IgM Assay**
             - **IgM positive**
               - Estimate time of infection
                 - **IgG Avidity Assay**
                   - Infection acquired post-conception
                     - Pregnancy is at risk
                       - Consider foetal investigation
                   - Infection prior to conception
                     - No risk to pregnancy
                       - Reassure
             - **IgM negative**
               - No risk to pregnancy
                 - Reassure
       - **IgG negative**
         - Advise on precautions to avoid infection during remainder of pregnancy

This NSM should be used in conjunction with the series of NSMs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk
APPENDIX 2: PREGNANCY CONFIRMED TO BE AT RISK - GUIDANCE

Pregnancy confirmed to be At Risk
(For additional advice contact the reference unit)

Foetal samples available?

Amniotic Fluid or Foetal Blood

Yes

Evidence of foetal infection?
(Management decisions will also rely upon any clinical evidence of foetal infection)

PCR

No

Consider Spiramycin for duration of pregnancy
(Management decisions will also rely upon any clinical evidence of foetal infection)

Yes

Commence Pyrimethamine/Sulphadiazine/Folinic Acid treatment or Consider Termination

No

DELIVERY

Neonatal Investigation
REFERENCES


