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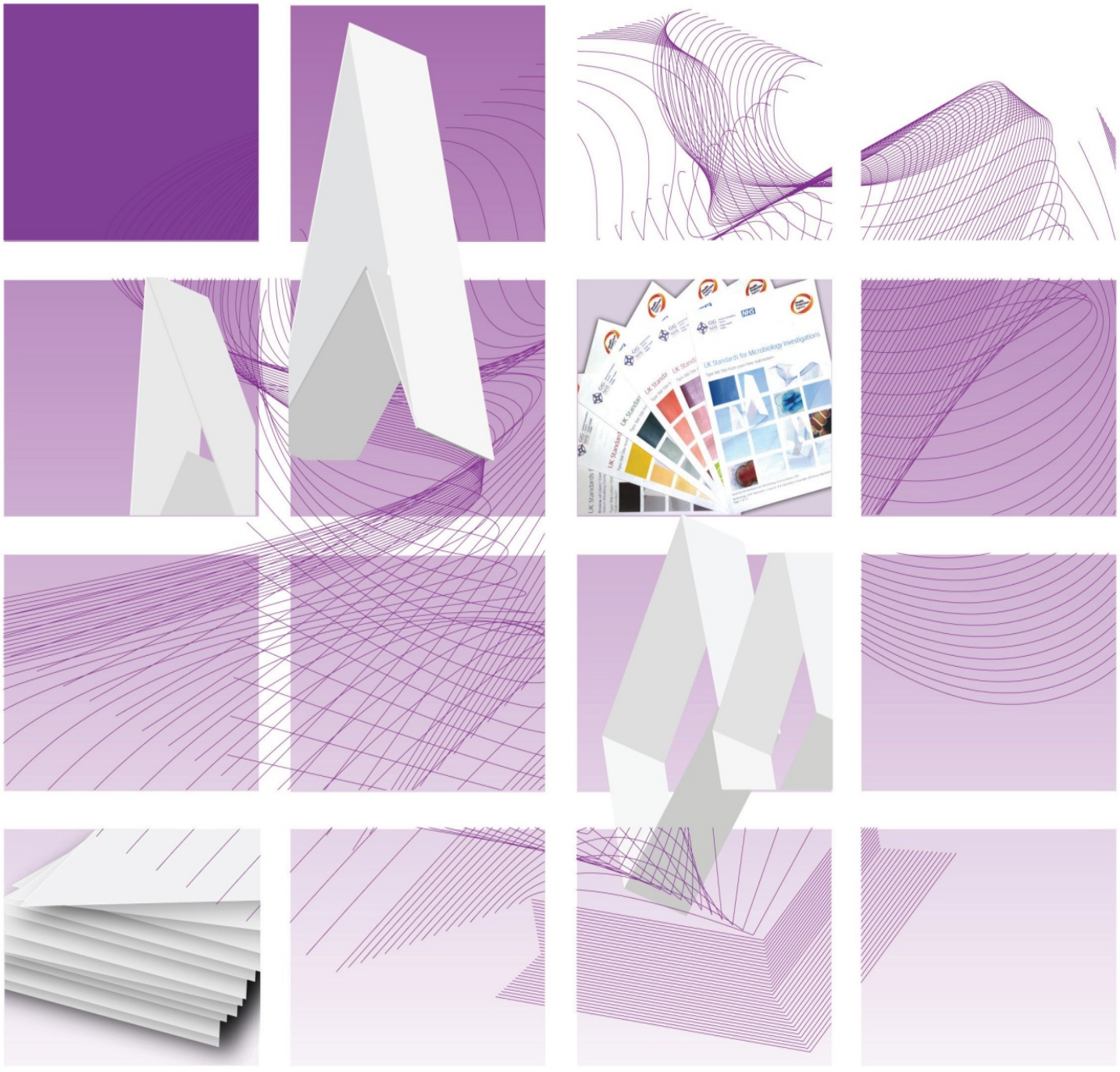
Health Protection Agency

UK Standards for Microbiology Investigations

Investigation of Toxoplasma Infection in Pregnancy



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Acknowledgments

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The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the Medical Editors for editing the medical content.

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Users of SMIs

Three groups of users have been identified for whom SMIs are especially relevant:

- SMIs are primarily intended as a general resource for practising professionals in the field operating in the field of laboratory medicine in the UK. Specialist advice should be obtained where necessary.
- SMIs provide clinicians with information about the standard of laboratory services they should expect for the investigation of infection in their patients and the documents provide information that aids the electronic ordering of appropriate tests from hospital wards.
- SMIs also provide commissioners of healthcare services with the standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population.

Background to SMIs

SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post analytical (result interpretation and reporting) stages.

Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe essential laboratory methodologies which underpin quality, for example assay validation, quality assurance, and understanding uncertainty of measurement.

Standardisation of the diagnostic process through the application of SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health interventions, surveillance, and research and development activities. SMIs align advice on testing strategies with the UK diagnostic and public health agendas.

Involvement of Professional Organisations

The development of SMIs is undertaken within the HPA in partnership with the NHS, Public Health Wales and with professional organisations.

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SMIs are developed, reviewed and updated through a wide consultation process. The resulting documents reflect the majority view of contributors. SMIs are freely available to view at http://www.hpa.org.uk/SMI as controlled documents in Adobe PDF format.

[#] UK Standards for Microbiology Investigations were formerly known as National Standard Methods.

Microbiology is used as a generic term to include the two GMC-recognised specialties of Medical Microbiology (which includes Bacteriology, Mycology and Parasitology) and Medical Virology.

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NHS Evidence has accredited the process used by the HPA to produce SMIs. Accreditation is valid for three years from July 2011. The accreditation is applicable to all guidance produced since October 2009 using the processes described in the HPA's Standard Operating Procedure SW3026 (2009) version 6.

SMIs represent a good standard of practice to which all clinical and public health microbiology laboratories in the UK are expected to work. SMIs are well referenced and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. SMIs also provide a reference point for method development. SMIs should be used in conjunction with other SMIs.

UK microbiology laboratories that do not use SMIs should be able to demonstrate at least equivalence in their testing methodologies.

The performance of SMIs depends on well trained staff and the quality of reagents and equipment used. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

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Microbial taxonomy is up to date at the time of full review.

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Contents

Amendment Table

Each SMI 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.

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment No/Date.	3/11.09.12
Issue no. discarded.	2.1
Insert Issue no.	2.2
Section(s) involved.	**Amendment.**
Contents page.	NICE logo removed.

Amendment No/Date.	2/03.05.12
Issue no. discarded.	2
Insert Issue no.	2.1
Section(s) involved.	**Amendment.**
Whole document.	P 5 formally QSOP 59. Document presented in new format.
References.	Some references updated.

Scope of Document

This SMI provides a summary of strategies for the investigation and management of suspected or confirmed toxoplasma infection.

This SMI should be used in conjunction with other SMIs.

Introduction

Toxoplasma gondii is an extremely successful protozoan parasite that can infect all mammalian and bird species throughout the world[1]. 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[2].

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[3]. 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)[4].

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[5,6]

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:

	Aim of laboratory investigation
Mother	Confirm or exclude risk to pregnancy by determining whether maternal infection was acquired before conception
Foetus	If risk to pregnancy is confirmed, determine whether foetal infection can be confirmed
Neonate	If foetal infection is not confirmed, confirm or exclude congenital toxoplasma infection in the neonate

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[7]. 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.

Toxoplasma Test	Pregnant Women	Foetus		Neonate	
	Blood	Amniotic fluid	Blood	Cord Blood/Neonatal Blood	Amniotic Fluid
IgG	√	x	√	√	x
IgM/IgA EIA	√	x	√	√	x
IgM/IgA ISAGA	x	x	√	√	x
IgG Avidity	√	x	x	x	x
IgG Immunoblot	√[a]	x	x	√[b]	x
PCR	x	√	√	√	√

[a] as matched sample with neonatal blood.

[a] and [b] must be tested as paired samples for comparison.

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[8]. 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[9,10].

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 woman it is essential that the parents should be counselled regarding the risk to the foetus and management options.

3 Pregnant Women Who Are Immuno-Compromised

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, Hodgkins' 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[11,12]. 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 of infection 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[13].

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[14]. 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.

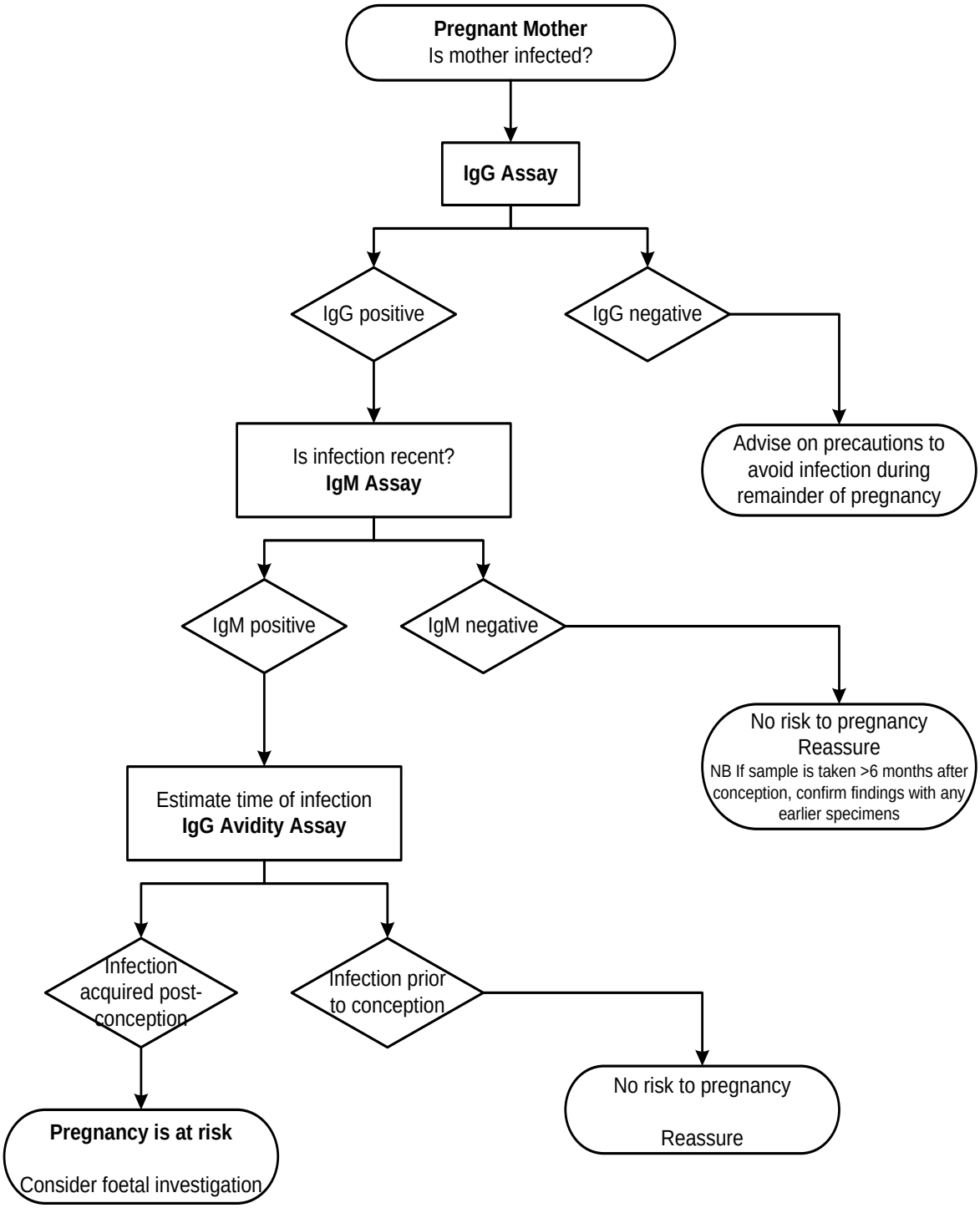
Direct detection of the parasite is attempted by culture and PCR of the amniotic fluid and cord blood. Investigation for placental infection is considered less helpful since detection of parasite in the placenta alone, while providing strong supporting evidence, can not be considered as unequivocal confirmation 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.

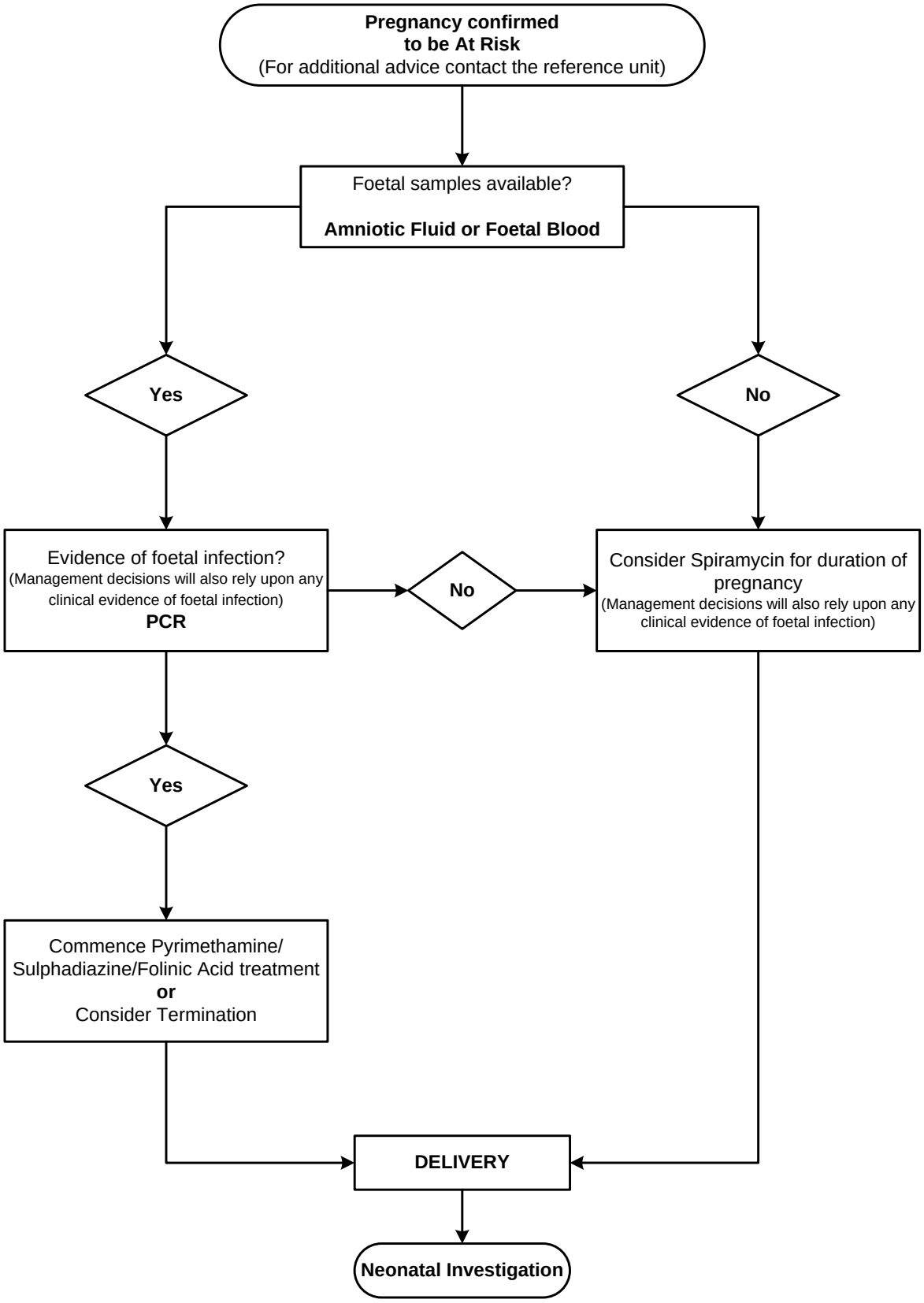
Detection of neonatal IgM and IgA by EIA and/or ISAGA are regarded as being diagnostic for neonatal infection, but the possibility of contamination by maternal blood should be excluded if IgM and/or IgA are present in the mother at the time of birth.

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.

Appendix 1: Pregnant Mother - Guidance



Appendix 2: Pregnancy Confirmed to be at Risk - Guidance



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