Laboratory Investigation of Suspected Congenital Toxoplasma Infection in the Neonate and Infant

In cases where congenital infection has not been confirmed as a result of investigations carried out during pregnancy, laboratory investigation of congenital toxoplasma infection in the neonate or infant may be required. These investigations can be based upon serological methods for the demonstration of an immune response to toxoplasma in the child, and nucleic acid amplification tests (NAAT) for the direct detection of the parasite in infant specimens.

1) Serological investigation
The key challenges facing serological diagnosis in the neonate/infant are:

1. The presence of passively-acquired maternal antibody, particularly IgG that can mask the child's own immune response.

2. Low levels of specific IgM and/or IgA antibody (or the delayed appearance of these) in the child, due to the immaturity of the infant immune system.

IgM/IgA Testing

**False negative findings** may occur due to these antibody classes not appearing in some cases for up to 3 months after birth. Further, levels of IgM/IgA may be below the positive threshold for some commercial assays (such thresholds are often determined based on the typical adult immune response).

**False positive findings** may occur in some cases, due to passive transfer of maternal IgM during parturition. In cases where the mother has significantly raised levels of toxoplasma-specific IgM, and particularly where highly sensitive Toxoplasma IgM assays are employed (e.g. ISAGA), positive findings in neonatal samples taken within the first few days of life may not confirm congenital infection. Repeat testing at 2-4 weeks to confirm the persistence of IgM may be helpful in this context.
IgG
In cases where congenital toxoplasma is suspected the mother will usually have been relatively recently infected with toxoplasma (this may not be the case, however, in HIV/AIDS where reactivation in the mother was suspected) and so will usually have relatively high levels of toxoplasma-specific IgG that will be passed on to the unborn child.

Thus, discrimination of passively-acquired maternal IgG and neonatal IgG is problematic. Significantly raised levels of IgG in the child compared to the mother are strong evidence to support congenital infection. An alternative approach is to determine whether the IgG response in the child includes toxoplasma antigens not recognised by the mother, i.e. evidence of a unique IgG response in the child. One method available commercially that can support this approach is immunoblot (western blot). If any key antigens are recognised by the IgG response (or IgM response if present) in the child but not the mother, this is evidence of the unique production of these immunoglobulins in the child and, hence, of congenital toxoplasma infection.

2) Nucleic Acid Amplification Tests – NAAT (PCR)
The direct detection of toxoplasma in any specimen from the neonate confirms congenital infection. However, a negative NAAT result does not unequivocally exclude congenital toxoplasma infection. For example, in asymptomatic cases in particular, there is commonly no parasite detected in blood.

Confirmation and Exclusion of congenital toxoplasma infection
Confirmation of congenital toxoplasma infection requires either the demonstration of a toxoplasma-specific immune response in the child, or confirmation (using NAAT methods) that the parasite is present in the child.

Exclusion of congenital toxoplasma infection requires demonstration of the complete clearance of any IgG acquired passively from the mother, by approximately 12 months of age. This is required to confirm there is no residual IgG response unique to the child.