Abstract

Background: Early diagnosis of congenital Toxoplasma infection is essential to avoid deleterious results. Although toxoplasmosis is considered harmless for a non-pregnant woman, it is potentially harmful during pregnancy, especially at first trimester. Toxoplasmosis when diagnosed on time and treated properly can lead to healthy offspring. The laboratory diagnosis of toxoplasmosis is based on detection of antibodies and T. gondii DNA using polymerase chain reaction (PCR).

Even if PCR is now currently applied to the diagnosis of toxoplasmosis, and has a recognized high diagnostic value in the acute disease, many ‘in-house’ PCR assays, suffer from lack of standardization. Methods: This study summarizes the diagnostic performances of a novel ready to use molecular biology test based on the highly repetitive RE gene as an infection marker.

Results: We used as a reference the European quality control for Molecular Biology panel QCMD 2008 Toxoplasma gondii (TGNA008) EQA Programme. The EQA panel for the detection of Toxoplasma gondii consisted of eight samples containing serial concentrations of T. gondii and two negative samples (seven amniotic fluids and plasma). All the negatives were confirmed, as well as the strong-positives. Only the weak positive, corresponding to a concentration of 5 parasites/ml, gave a positive result in the 50% of the ten replications of the intra assay.

Conclusion: The present study, even if preliminary, demonstrates the specificity and sensitivity of the test compared to the gold standards on different biological samples.

Objectives

The aim of the present study is to introduce a new PCR based assays for Toxoplasma detection able to identify a possible mother-to-child transmission, or an infection derived from a positive organ transplantation, or again from an infected blood transfusion with a sensitivity and a specificity as high as possible, and to perform the gold standard serological and direct observation tests.

Toxoplasma gondii detection is performed on electrophoretic agarose gel.

The system contains an internal control to assess the functionality of the test, and to exclude PCR inhibition.

Introduction

The protozoan parasite Toxoplasma gondii is the infective agent responsible for Toxoplasmosis. Whilst in the United States it is estimated that 22.5% of the population 12 years and over have been infected, in other places in the world it has been shown that up to 95% of the population have been in contact with Toxoplasma.

The diffusion of the parasite is more represented in high and humid areas with no high altitudes.

The way of infection is mainly through microscopic cysts included in food that can be transmitted by undercooked, contaminated meat or handling contaminated meat and contact with not washed hands, or, by contact with infected meat. The most important role in the parasitic transmission is played by cats. Cats usually eat rodents, birds, or other small infected animals.

The parasites are then passed in the cat’s feces in a cat’s fecal form. The passage from person to person was never evidenced, except in mother-to-child, and blood transfusion or organ transplantation. The transplantation of an organ from a Toxoplasma positive donor can cause the infection of the recipient, and, in some cases, lead to a severe prognosis.

The most diffuse surveillance for Toxoplasma infection is in mother-to-child (congenital) transmission. A woman who becomes positive to Toxoplasma during pregnancy can pass the infection to the fetus.

The mother may not have symptoms, but severe consequences, such as miscarriage or diseases to the nervous system or to the eyes of the unborn child, could arise during pregnancy or later in the infant.

If the woman has a previous Toxoplasma infection before becoming pregnant, the unborn child will be protected, but she can pass the infection if the contact with the parasite is during or immediately before pregnancy.

The earlier the infection is, the more severe is the damage to the unborn child. The diagnosis of Toxoplasmosis is typically made by serologic testing, but the molecular techniques that are able to detect the Toxoplasma DNA in the amniotic fluid, or in the peripheral blood of the mother, can be very useful in cases of possible mother-to-child (congenital) transmission.

Design and Methods

The assay is a ready-to-use test that includes all the required elements for the amplification of nucleic acid targets.

Amniotic fluid, whole blood, CSF, or tissue samples, have been extracted with a convenient protocol able to give a good quality of DNA.

For this study we used a silica based system developed internally.

The extraction protocol starts with a washing step balanced on the different biological material.

Then, a solution containing a chaotropic agent, and a suspension of silica for binding the DNA, is added to the washed sample. The suspension is left 3-5 minutes at room temperature.

A further washing step with ethanol completes the procedure.

The pellet is left to dry for five minutes at room temperature, and then dissolved in 100-200 µl of sterile water.

The DNA was immediately used for the PCR protocol and then stored at -20°C.

The set of primers for the amplification of Toxoplasma gondii was designed in the RE region (GenBank accession AF146527), a repetitive sequence of 200-300 nucleotides in the Toxoplasma genome, giving an amplified fragment of 134 bp.

In each test tube an internal control was also included.

The primers for the internal control were designed in the human Beta Globin gene.

The choice for a housekeeping gene was done in reason to introduce both a control of the extraction system and a control for the efficiency of the amplification.

The amplified fragment corresponds to a band of 268 bp.

The two sets of primers for the specific region of Toxoplasma and for the internal control were designed to have the same thermal profile and to be amplified simultaneously in a multiplex format.

Each test was performed using two Units of Hot Start DNA Polymerase.

The thermal profile is described in Table 1.

Results

The panel of samples considered in the present study includes strong positives as well as weak positives and negatives.

All the samples were tested in repetitive runs for the intra-assay and inter-assay evaluation.

Qualitative results obtained using RE sequence were compared with the expected results of the panel.

The results showed a perfect agreement in ten out of the ten samples of the panel.

Even in presence of very low levels of parasitic infection (5 parasites/ml in some samples) the developed test was able to detect the presence of Toxoplasma gondii in the sample.

In the two lower concentration samples (5 parasites/ml in some samples) the developed test was able to detect the presence of Toxoplasma gondii in the sample. In the two lower concentration samples (5 parasites/ml in some samples) the developed test was able to detect the presence of Toxoplasma gondii in the sample.

Figure 1 shows the electrophoretic profile of a PCR run.

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The results showed a perfect agreement in ten out of the ten samples of the panel.

The results were presented preparing from Toxoplasma cultures, diluted and resuspended in a matrix. The matrix was clinical amniotic fluid or plasma, each of them tested serologically and by molecular techniques (PCR) to ensure negativity of T. gondii.

In order to evaluate the specificity of the test we also used positive samples for other parasitic infection such as Leishmania and Malaria. No false positives were observed in all the checked samples.

The specificity of the test was equal to 100% (figure 2).

Table 2: Panel composition. The samples from TG08-01 to TG08-07 were dissolved in amniotic fluid. The samples from TG08-08 to TG08-10 were dissolved in plasma.

Table 3: PCR Protocol. The thermal profile and the timing of the amplification protocol are described.

The assay was tested on Toxoplasma gondii QCMD (Quality Control for Molecular Diagnostics) 2008 panel in collaboration with the laboratory of Parasitology of the Foundation IRCCS Policlinico San Matteo (Pavia – Italy).