



Evaluation of a new protocol for retrospective diagnosis of Congenital Toxoplasmosis: DNA detection by PCR and specific recovery of anti-*Toxoplasma gondii* IgM by Western Blot from dried blood spots in Guthrie cards filter papers



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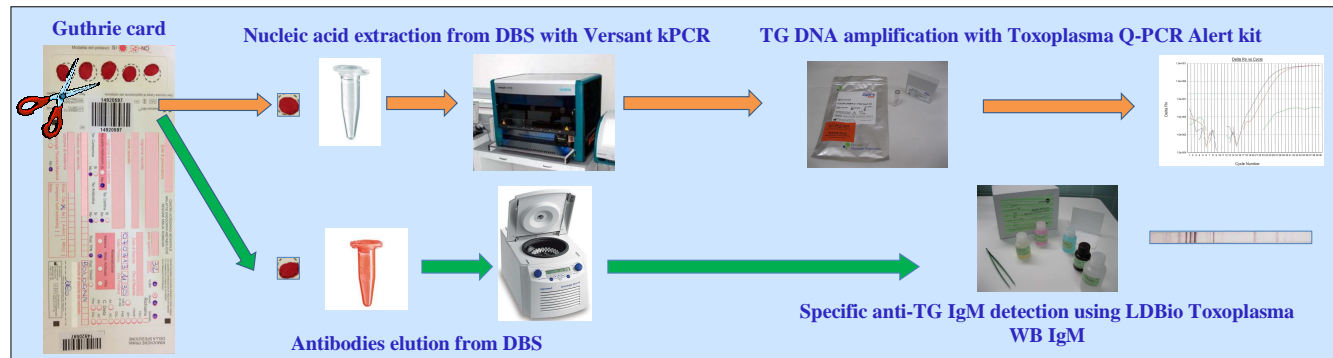
INTRODUCTION AND PURPOSE.

Congenital Toxoplasmosis (CT) in newborns results from primary maternal infection with *Toxoplasma gondii* (TG). Many infected children are asymptomatic at birth but at high risk of neurological sequelae during early childhood. Guthrie Cards are obtained routinely from newborns in many developed countries to screen for metabolic disorders. These specimens also provide a long-term, cost-effective and convenient alternative to freezing blood and have been used for different purposes in epidemiological studies, research areas and clinical applications. Analysis of dried blood samples (DBS) on the Guthrie card has been proposed as a sensitive and specific method to screen for congenital infections, in particular CMV. A National neonatal screening programme for CT was conducted in Denmark from 1999 to 2007 by using DBS obtained from newborns 5-10 days old. These were analysed for TG-specific IgM antibodies by conventional serological methods. This programme ended because it was not cost-effective. Anyway, in case of missed diagnosis at birth, retrospective testing of neonatal Guthrie cards for TG DNA or specific IgM anti-TG detection could help to distinguish congenital from acquired Toxoplasmosis. The aim of this study was to investigate the sensitivity and specificity of IgM testing by Western Blot (WB) and DNA amplification in DBS of infants born from mothers infected by TG during pregnancy.

METHODS.

A retrospective study was performed in 18 infants born from mothers who acquired toxoplasmosis during the second or third trimester of pregnancy. At birth, all mother-child serum pairs were tested by conventional assays (Enzygnost Toxoplasmosis-Siemens; Vidas Toxo-bioMérieux) and by comparative WB (Toxoplasma WB IgG/IgM-LDBio Diagnostics). We collected Guthrie cards of each child (informed written consent was obtained from parents). One DBS spot was used for TG DNA detection and another one for antibodies elution.

Nucleic acids were extracted from DBS with Versant kPCR Sample Preparation system (Siemens) and Toxoplasma Q-PCR Alert Kit (Nanogen) was used for amplification. Specific IgM anti-TG were detected in eluates from DBS by using LDBio Toxoplasma WB IgM.



RESULTS.

At birth CT was diagnosed in 8 of the 18 newborns, because of IgM/IgA positivity and/or different IgG WB pattern in infant's serum compared to the corresponding mother's one. CT was excluded in the remaining 10 children because their sera were IgM/IgA negative and their IgG titres decreased during the follow-up period; at 1 year of age all these 10 babies were IgG negative. In the present study, we were able to confirm CT diagnosis in 4 out of the 8 cases by using Guthrie cards. In particular, TG DNA amplification was successful in one case, while in other 3 cases we found specific IgM anti-TG. Specificity of DBS examination was 100%, since no TG DNA or IgM was found in the group of 10 non-infected babies. Serological testing at birth and Guthrie card results of the 8 CT cases are shown in detail in the table on the right.

| Cases | IgM anti-TG by conventional tests at birth | IgA anti-TG at birth | IgM anti-TG by WB at birth | Comparison of IgG WB patterns between mother and child at birth | TG PCR on Guthrie cards | IgM anti-TG in Guthrie cards |
|-------|--|----------------------|----------------------------|---|-------------------------|------------------------------|
| 1 | - | + | + | Different | - | + |
| 2 | - | - | + | Same | - | + |
| 3 | Border-line | - | + | Different | - | + |
| 4 | - | - | - | Different | - | - |
| 5 | - | + | + | Different | + | - |
| 6 | + | - | + | Same | - | - |
| 7 | - | - | + | Different | - | - |
| 8 | - | + | - | Different | - | - |

CONCLUSIONS.

Although serological evaluation at birth and during the first year remains basic for the laboratory diagnosis of CT, examination of Guthrie cards could be considered a retrospective method to evaluate infants (>1 year of age) with clinical signs suggestive of CT.