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Abstract (poster session)

Evaluation of a new protocol for retrospective diagnosis of congenital toxoplasmosis: DNA detection by polymerase chain reaction (PCR) and specific recovery of anti-Toxoplasma gondii IgM by Western Blot from dried blood spots in Guthrie cards filter paper

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Objectives: 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. 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 dried blood samples (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 every child (informed consensus was obtained from parents); one DBS 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 confirmed CT in 4 out of the 8 CT cases. In particular, we were able to amplify TG DNA from one of the cards, 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 test at birth and Guthrie card results of the 8 CT cases are shown in detail in table 1. **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.

Table 1. Serological test results at birth and Guthrie cards analysis (TG DNA amplification and recovery of IgM anti-TG) in newborns with Congenital Toxoplasmosis.

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

- = negative; + = positive; +/- = border line