Molecular diagnosis of toxoplasmosis by Q-LAMP method

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BACKGROUND

Molecular assays have been of paramount importance for the diagnosis of toxoplasmosis in pregnant women and immunocompromised patients. Today detection of *Toxoplasma gondii* DNA in amniotic fluid (AF) , aqueous humor (AH), cerebrospinal fluid (CSF) and whole blood using real-time PCR assays is a standard approach. The DiaSorin Q-LAMP (loop mediated Isothermal amplification - DiaSorin SpA, Saluggia, Italy) offers all the benefits of isothermal LAMP technology with the addition of a real time fluorescent detection and multiplexed amplification. Furthermore extraction and Q-LAMP assays are automatized and performed on the Liaison IAM platform (Diasorin SpA, Saluggia, Italy). Aim of the study was to evaluate the performance of the new Q-LAMP Iam TOXO assay on clinical samples (AF, AH, CSF, blood) and European quality controls for molecular diagnosis (QCMD – Glasgow United Kingdom)

MATERIAL/METHODS

47 amniotic fluids from pregnant women referred to the Infectious Diseases outpatient clinic for a suspected or confirmed Toxoplasma infection, 2 CSF, 1 AH sample and 24 blood samples from immunocompromised patients with suspected toxoplasmic infection/reactivation were stored frozen at – 80° C until use. Ten samples of CQMD 2014 were also tested. All these lyophilized samples were reconstituted and stored according to the manufacture's instructions.
From all the samples Toxoplasma DNA was extracted with NucliSENS easyMAG (BioMerieux Marcy l'Etoile France). Real time PCR was run in triplicate using the commercial kit TOXOPLASMA g ELITe MGB Kit (Elitech group SpA, Turin, Italy) on a 7300 Real-Time PCR System (Applied Biosystems, Foster City, USA). For all the samples, DNA was also extracted with Liaison IXT platform (Diasorin SpA, Saluggia, Italy) after addiction of an internal control (Fig 1) . Internal control was used to check extraction and amplification steps.
Q-LAMP assay was run using Iam Toxo Kit on Liaison IAM instrument (Fig 2). Each test was performed according to the manufacturer's instructions.

RESULTS

All the 47 amniotic fluid gave negative results with the Q-LAMP assay as was recorded with real time PCR (Tab 1). No newborn born from the mothers who underwent amniocentesis resulted infected at the end of one year follow up. The 2 CSF from immunocompromised patients that were positive with real time PCR gave positive results also with Q-LAMP (Tab 2). The patients improved after therapy with Pyrimethamine sulphadiazine. The AH sample was negative also with Q-LAMP and the patients was diagnosed a Cytomegalovirus chorioretinis. Sixteen of the 24 blood samples were negative and 8 positive in accordance with EliTe MGB results. All the CQMD samples gave the same results with real time PCR and Q-LAMP: 4 of the 5 amniotic fluid samples were positive and 1 negative, 2 of the 5 plasma samples scored negative and 3 positive, according with the CQMD expected results (Tab 3) .

Fig 1 EXTRACTION

Fig 2 AMPLIFICATION

	Real-time PCR (Elitech group)	
Samples	Positive	Negative
Amniotic fluid		47
Cerebrospinal fluid	2	
Aqueous humor		1
Blood	8	16

Tab 1

	Q-LAMP (DiaSorin)	
Samples	Positive	Negative
Amniotic fluid		47
Cerebrospinal fluid	2	
Aqueous humor		1
Blood	8	16

Tab 2

CQMD	Real-time PCR (Elitech group)		Q-LAMP (DiaSorin)	
Samples	Positive	Negative	Positive	Negative
Amniotic fluid	4	1	4	1
Plasma	3	2	3	2

Tab 3

CONCLUSIONS

These preliminary data show the good diagnostic accuracy of the Q-LAMP that seems to be a valid alternative to the classic real time PCR tests. In particular, Q-LAMP is fully automated , less time consuming , less expensive and also useful in routine practice with small sample numbers.

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