



## Evaluation of the kit **LDBIOTOXO II IgG** for the confirmation of IgG results obtained by toxoplasmosis serology screening tests.



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### INTRODUCTION

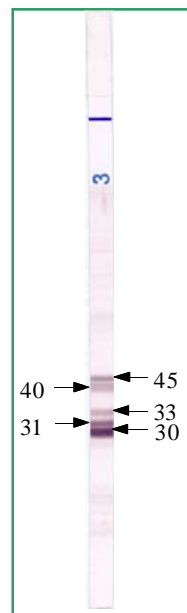
The aim of the study was to compare on 529 serum samples the results obtained by using **LDBIOTOXO II IgG**, *Ldbio Diagnostics* (LDBIO IgG), the **Dye test of Sabin and Feldman** (Dye test) and 2 screening tests: **TOXO IgG EIA II COBAS CORE**, *Roche* (COBAS IgG) and **VIDAS TOXO IgG II**, *bioMérieux* (VIDAS IgG). All patients were characterised by clinical and biological data. The Dye test was performed in the *Toxoplasmosis Laboratory of Docteur Thulliez, Institut de Puériculture de Paris, France*. COBAS IgG and VIDAS IgG were performed in the *Laboratory of Parasitology, CHU La Timone, Marseille, France*, following the instructions of the manufacturers and by using their corresponding analysers. LDBIO IgG was performed in the *Laboratory of Parasitology, CHU La Timone* following the instructions of the manufacturer.

### MATERIALS & METHODS

#### **The test LDBIOTOXO II IgG:**

It is a qualitative immunoblot. Strips and liquid reagents are supplied ready for use in the kit. Incubation times (60', 60', 30') are standardized. Reading is standardised by matching the revealed bands on the patient's strip with the standard profile (between 30 and 45 kDa) obtained by using the positive control included in the kit. The test is positive by the presence on the strip of 3 bands among the 5 bands of the specific profile (fig.1).

Additionally, for this study, we made a semi quantitative reading by using an index from 1 to 5 corresponding to the intensity of the coloration of the bands.



**fig.1 - LDBIOTOXO II IgG specific profile of 5 bands (MW in kDa)**

Other bands may be present on the strip. They are not used for reading the test.



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### Cutoff value of the tests:

	negative	équivocal	positive
DYE TEST (UI/ml)	<2	-	>=2
VIDAS IgG (UI/ml)	<4	4-8	>=8
COBAS IgG (UI/ml)	<6	-	>=6
LDBIO IgG (index)	0	-	>=1

### Statistics:

We calculated the values of specificity, sensitivity, positive predictive value (PPV) and negative predictive value (NPV) when data made it possible. The statistical correlations between results from different tests were made by using CHI-square paired test of Mac Nemar.

### Patients:

All sera were stored frozen at  $-20^{\circ}\text{C}$  before analysis.  
Patient's samples are from 5 groups.

#### **Group I – Dye test**

This study was performed on 200 sera from systematic screening of pregnant women for toxoplasmosis. These samples were supplied by the *Institut de Puériculture de Paris*. All sera were first tested by Dye test.

The sub-group “positive” corresponded to 98 sera from chronically infected women and positive in Dye test: the anti-*T.gondii* IgG titre was especially chosen between 2 and 32 UI/ml for testing the sensitivity of LDBIO IgG versus both screening tests.

The sub-group “negative” corresponded to 102 sera from pregnant women who were not immunized against *T.gondii* and negative in Dye test.

The 200 samples have been tested in parallel by using the 3 kits: LDBIO IgG, COBAS IgG et VIDAS IgG.

#### **Group II – Seroconversions**

It was a retrospective study on sequential samples from 17 pregnant women (101 samples in total) who acquired a *T.gondii* infection during their pregnancy. These patients were followed in the *Laboratory of Parasitology, CHU la Timone, Marseille* (diagnostic of acute toxoplasmosis by using COBAS IgG, VIDAS IgM and ISAGA IgM, *bioMérieux*). Each sequence included the last negative sample followed by 3 - 5 sequential sera showing the appearance of specific IgM and then specific IgG.

#### **Group III – follow up of non infected newborns**

It was a retrospective study on sequential samples from serological follow-up of 20 newborns (74 samples in total). All the mothers of the newborns acquired a *T.gondii* infection during their pregnancy. These patients were followed in the *Laboratory of Parasitology, CHU la Timone, Marseille*. (diagnostic of acute toxoplasmosis by using COBAS IgG, VIDAS IgM and ISAGA IgM, *bioMérieux*).

The absence of congenital infection was proved by the absence of specific IgM / IgA



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(ISAGA IgM / IgA, *bioMérieux*) in the infant's serum, the negativity of the Comparative Immuno-Profiles Western Blot at birth (TOXOPLASMA WB IgG/IgM, *Ldbio Diagnostics*) and the decrease and disappearance of specific IgG in the infant's serum during the serological follow-up (COBAS IgG). Each sequence included 2 - 6 samples showing the decrease of the specific IgG titres until negativity by using COBAS IgG (5 - 13 months).

### Group IV – follow up of infected new born

It was a retrospective study on sequential samples from serological follow-up of 30 newborns (85 samples in total) who acquired a *T.gondii* congenital infection.

The diagnostic of congenital toxoplasmosis was antenatal for 7 cases (positive PCR on amniotic fluid), neonatal for 20 cases and postnatal for 3 cases.

Neonatal and postnatal diagnostic methods:

- At birth: presence of specific IgM / IgA in the infant's serum (ISAGA IgM / IgA *bioMérieux*) ; positivity (IgG and/or IgM) of the Comparative Immuno-Profile Western Blot (TOXOPLASMA WB IgG/ IgM, *Ldbio Diagnostics*).
- During the infant's follow-up: underlining of the synthesis of specific anti-*T.gondii* IgG by the infant (COBAS IgG).

### Group V – sensitivity and specificity (viral infections and malaria)

It was a retrospective study on 69 sera from patients infected by viruses or malaria (table 1). These samples were first tested by COBAS IgG and VIDAS IgM. All VIDAS IgM results were negative. All COBAS IgG negative samples and all discordant results COBAS IgG / LDBIO IgG were tested by Dye test.

infect. agent	COBAS IgG POS	COBAS IgG NEG	TOTAL
EBV	0	5	5
VZV	2	1	3
CMV	2	3	5
HBV	8	1	9
HAV	0	2	2
HCV	8	2	10
HIV	6	4	10
<i>P.falciparum</i>	18	7	25
			69

**Table 1** : 69 sera from viral infections or malaria



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## RESULTS & DISCUSSION

### Group I : Dye test

	DYE TEST	LDBIO IgG	VIDAS IgG	COBAS IgG
POS	98	97	61	93
NEG	102	103	114	107
EQUIVOCAL	-	-	25	-

**Table 2** Positive and negative results by using each test. Remark: only the test VIDAS IgG is showing equivocal values (4 - 8 UI/ml)

REF = DYE TEST (200 sera)		
	COBAS IgG	LDBIO IgG
sensitivity	94.90%	98.98%
specificity	100.00%	100.00%
PPV	100.00%	100.00%
NPV	95.33%	99.03%

**Table 3** Performances of COBAS IgG versus LDBIO IgG

REF = DYE TEST (175 sera)		
	VIDAS IgG	LDBIO IgG
sensitivity	83.56%	98.63%
specificity	100.00%	100.00%
PPV	100.00%	100.00%
NPV	90.48%	99.13%

**Table 4** Performances of VIDAS IgG versus LDBIO IgG

<b>25 VIDAS IgG equivocal results (4 - 8 UI/ml)</b>
24 samples are positive with LDBIO IgG and Dye test
1 sample (7UI/ml) is negative with LDBIO IgG and Dye test

**tables 3 et 4 - RESULTS:** sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for the 3 tests LDBIO IgG, COBAS IgG and VIDAS IgG. Dye test was the reference for statistical calculations. The 25 equivocal results of VIDAS IgG were not used for the calculation of the performances of this test.



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### **Comparison LDBIO IgG / DYE TEST:**

The correlation LDBIO IgG with the reference Dye test is very good. One sample is false negative (band P30 isolated) among 98 positive samples (table 2): the sensitivity of LDBIO IgG is 98,98%. The specificity of LDBIO IgG is 100%.

### **Comparison LDBIO IgG / COBAS IgG (table 3):**

- 5 samples are negative by using COBAS IgG (3 - 5 UI) but positive with LDBIO IgG and Dye test,
- 1 sample is negative by using LDBIO IgG but positive with COBAS IgG and Dye test.

### **Comparison LDBIO IgG / VIDAS IgG (table 4):**

- 11 samples are negative by using VIDAS IgG (1 - 3 UI) but positive with LDBIO IgG and Dye test.
- 24 samples are equivocal by using VIDAS IgG (4 - 8 UI) and positive with LDBIO IgG and Dye test.
- 1 sample is equivocal by using VIDAS IgG (7 UI) and negative with LDBIO IgG and Dye test.

**LDBIOTOXO II IgG could confirm the immune status of the patients showing borderline or equivocal results by using IgG screening tests.**

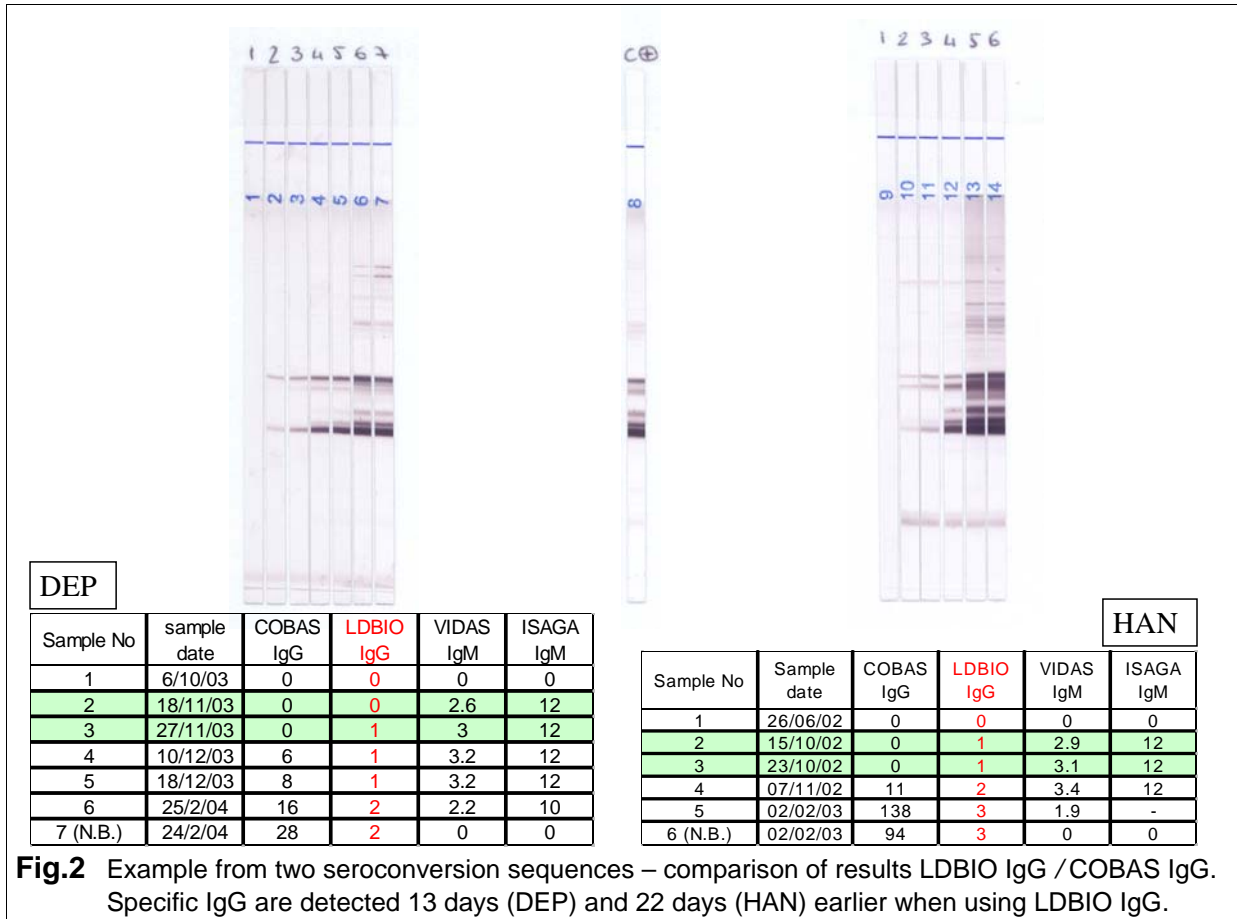


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## Group II : Seroconversion



**Fig.2** Example from two seroconversion sequences – comparison of results LDBIO IgG / COBAS IgG. Specific IgG are detected 13 days (DEP) and 22 days (HAN) earlier when using LDBIO IgG.

		COBAS IgG	
		POS	NEG
LDBIO IgG	POS	70	10
	NEG	0	21

**Table 5** Correlation LDBIO IgG / COBAS IgG on 91 samples from 17 seroconversions. Mac Nemar paired test **p=0.0016**

The results by using sequential sera from 17 seroconversion sequences show 10 discrepancies: negative results with COBAS IgG but positive with LDBIO IgG (Table 5). All 10 sera presented high titres of specific anti-*T.gondii* IgM in accordance with a recent infection. The presence of specific IgG in further samples confirmed the *Toxoplasma* seroconversion for all the patients.

The detection of specific IgG is earlier when using LDBIO IgG versus COBAS IgG at the beginning of seroconversions (fig.2). In 8 cases among 17 toxoplasmosis seroconversions (47%) the anti *T.gondii* IgG are detected 2 – 3 weeks earlier by using LDBIO IgG (Mac Nemar paired test p=0.0016).

**LDBIOTOXO II IgG could confirm a seroconversion earlier.**

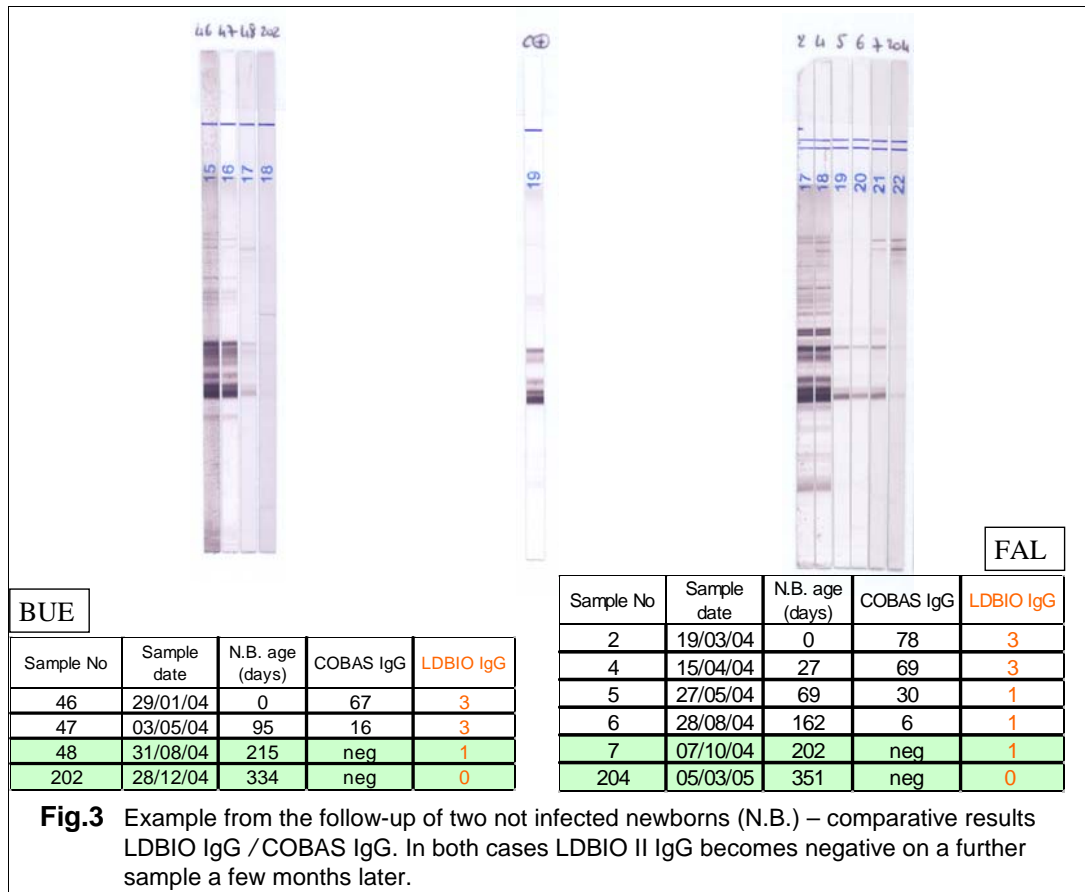


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## Groups III et IV: follow-up of newborns



**Fig.3** Example from the follow-up of two not infected newborns (N.B.) – comparative results LDBIO IgG / COBAS IgG. In both cases LDBIO II IgG becomes negative on a further sample a few months later.

		COBAS IgG	
		POS	NEG
LDBIO IgG	POS	130	18
	NEG	0	11

**Table 6** Correlation LDBIO IgG / COBAS IgG on 159 samples from 50 follow-ups of newborns. Mac Nemar paired test  $p < 0.0001$

The serological follow-up of 50 newborns (20 non-infected children and 30 congenital toxoplasmosis) underlines the greater sensitivity of LDBIO IgG when compared to COBAS IgG on 18 samples (from 13 different postnatal follow-ups -Table 6). Non-infected children: 13 samples (from 10 different postnatal follow-ups) are negative by using COBAS IgG but remain positive with LDBIO IgG that detects transmitted maternal antibodies when COBAS IgG does not detect any more specific IgG (fig.3). Congenital toxoplasmosis: 5 samples (from 3 different postnatal follow-ups) are discordant. One follow-up presents a temporary negative serology by using COBAS IgG when it remains positive by using LDBIO IgG which confirms the congenital toxoplasmosis. For both other children LDBIO IgG detects specific IgG earlier than COBAS IgG. However, it is impossible to prove the neo-synthesis of anti-*T.gondii* IgG by using LDBIO IgG. This test cannot distinguish between maternal transmitted antibodies and neo-synthesized antibodies of the child. The sensitivity of LDBIO IgG is underlined by Mac Nemar paired test  $p < 0.0001$ . **LDBIOTOXO II IgG could confirm the serological status of the child during the post-natal biological follow-up.**



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### Group V: Sensitivity and Specificity (viral infections and malaria)

		COBAS IgG	
		POS	NEG
LDBIO IgG & DYE TEST	POS	42	2
	NEG	2	23

**Table 7** Correlation LDBIO IgG / DYE TEST/COBAS IgG on 69 sera from viral infections and malaria.

**The correlation between LDBIO IgG and Dye test is excellent (100%): these results confirm the sensitivity and the specificity of LDBIOTOXO II IgG.**

The study shows a discrepancy concerning four results of COBAS IgG, **2 false negatives** (4 and 5 UI/ml, one HIV and one *P.falciparum*) and **2 false positives** (7 and 9 UI/ml, two *P.falciparum*). These data underline the usefulness of a confirmatory test for checking all samples with borderline results (table 7).

Remark: the false negative result concerning the HIV patient could underline the usefulness of a confirmatory test for the control of samples from immunocompromised patients. However a larger prospective study is required to confirm these results.



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### CONCLUSION

The kit LDBIOTOXO II IgG is standardised. The reading of the strips is easy. The specific profile (showed by the positive control in the kit) includes 5 bands at 30, 31, 33, 40 and 45 kDa. The test is positive by the presence of 3 bands among the 5 specific bands.

#### **Group I** (Dye test):

the correlation LDBIO IgG / DYE TEST is very good (sensitivity 99%, specificity 100%). LDBIOTOXO II IgG could confirm the immune status of the patients with borderline or equivocal IgG screening results.

#### **Group II** (seroconversions):

LDBIO IgG is more sensitive than COBAS IgG ( $p=0.0016$ ). LDBIOTOXO II IgG could confirm a seroconversion earlier than COBAS IgG.

#### **Groups III et IV** (follow-up of newborns):

LDBIO IgG is more sensitive than COBAS IgG ( $p<0.0001$ ). LDBIOTOXO II IgG could be used for confirming the serological status of the child during the postnatal follow-up. However LDBIOTOXO II IgG cannot distinguish between maternal transmitted antibodies and neo-synthesized antibodies of the child.

#### **Group V** (viral infections and malaria):

the correlation LDBIO IgG / DYE TEST is excellent (sensitivity 100%, specificity 100%). These data underline the usefulness of a confirmatory test for checking all samples with borderline results.

The good performances of the kit LDBIOTOXO II IgG justify its purpose for confirmatory testing. It should be useful for checking equivocal, borderline or non interpretable results after using anti-*T.gondii* IgG serological screening tests.

*Acknowledgements: We would like to thank Dr Ph. Thulliez for having performed all the Dye tests present in this study and for his assistance in the correction of this work.*