



Toxoplasmosis: Challenges in Diagnosis

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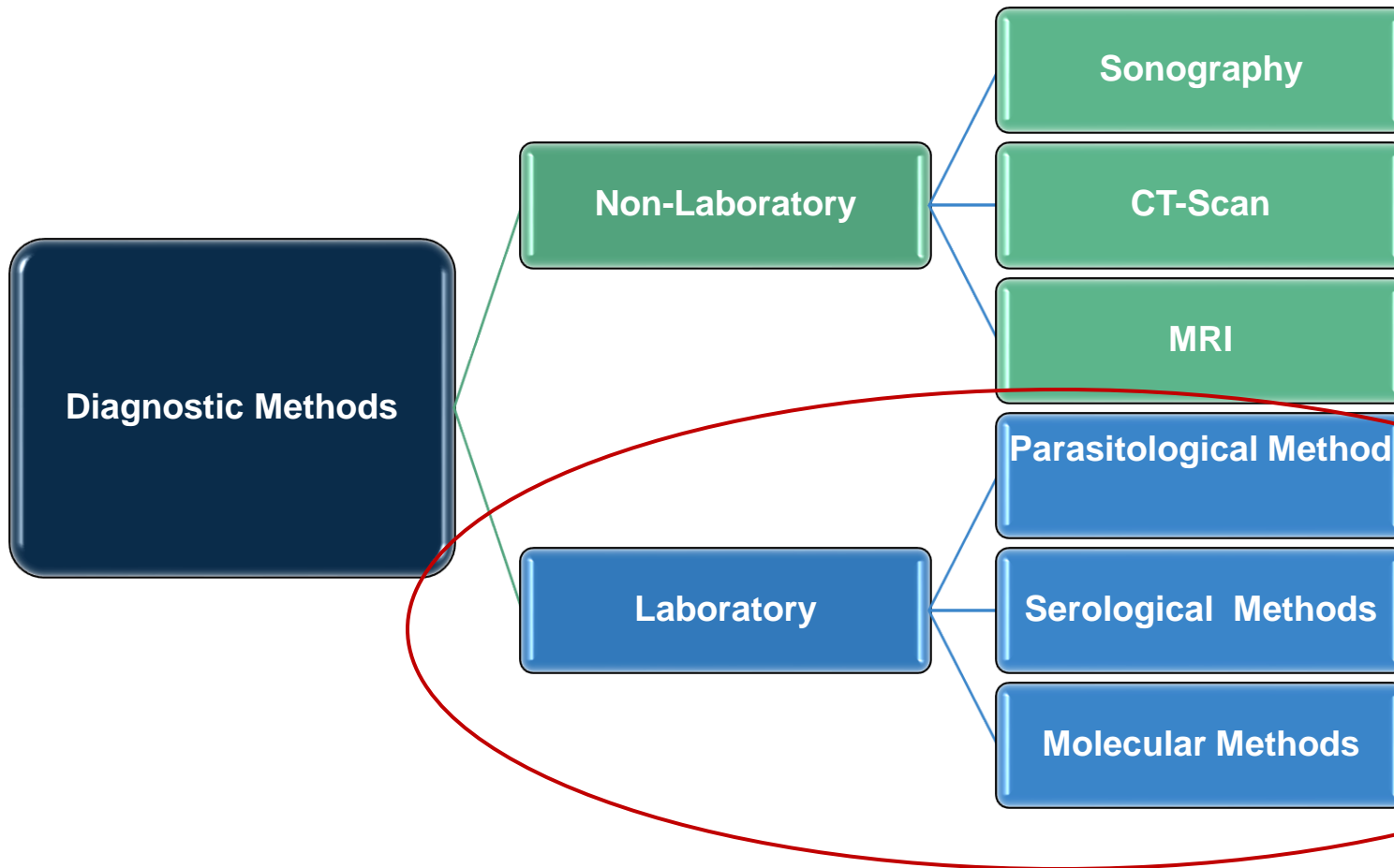


Diagnosis of Congenital Toxoplasmosis



Diagnosis of Ocular Toxoplasmosis

Diagnostic Methods



❖ Parasitological Method

Lymph node aspiration

- i: Giemsa staining**
- ii: Inoculation to a sensitive laboratory animal**

❖ Serological methods:

ELISA

IgG avidity

Indirect immunofluorescence

Indirect hemagglutination

Dye-Test (Sabin-Feld test)

Modified agglutination test (MAT)

Western blot

Immunocytochemistry and Immunohistochemistry

Goldmann - Witmer coefficient (GWC)

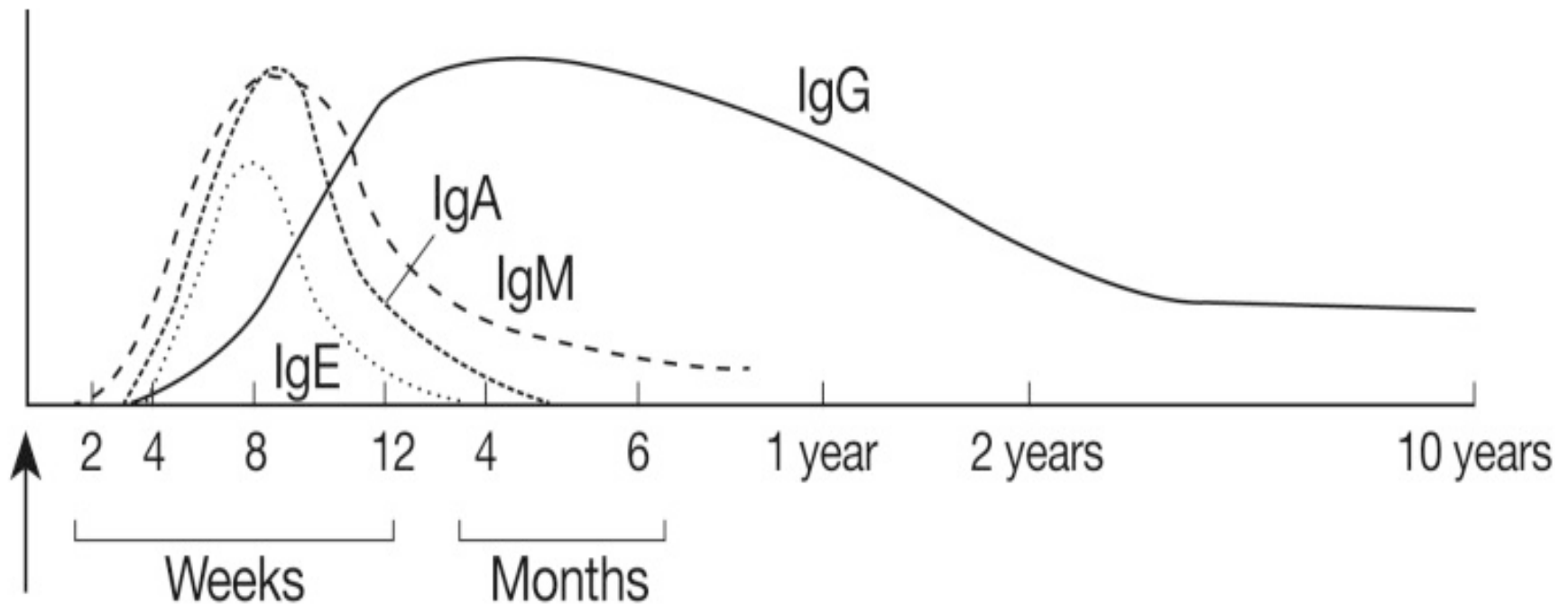
Diagnosis of Toxoplasmosis

❖ Molecular Methods

- **Nested PCR**
- **Q PCR (Real time PCR)**
- **LAMP (Loop-mediated isothermal Amplification)**
- **HAD (Helicase-Dependent)**
- **HRM (High-Resolution Melting)**

Diagnosis of Toxoplasmosis

Serological methods



Diagnosis of Toxoplasmosis

❖ Immunoglobulin M (IgM)

- IgM antibodies are **the first** to be produced by the body in response to a Toxoplasma infection.
- IgM antibody titers rise starting on **day 5** and reach the **maximum** level at **1 to 2 months**
- The most commonly used methods are the **IFAT, ELISA, and ISAGA**
- In patients with recently acquired primary infection, these specific IgM antibodies are detected initially and in most cases, these titers **become negative within a few months**

Diagnosis of Toxoplasmosis

- Among serological tests, the **IgM ISAGA**
- Because of its overall higher sensitivity compared to the ELISA (81.1% versus 64.8%)
- It is considered to be the method of choice for the detection of Toxoplasma IgM in **infants younger than 6 months of age** [8].

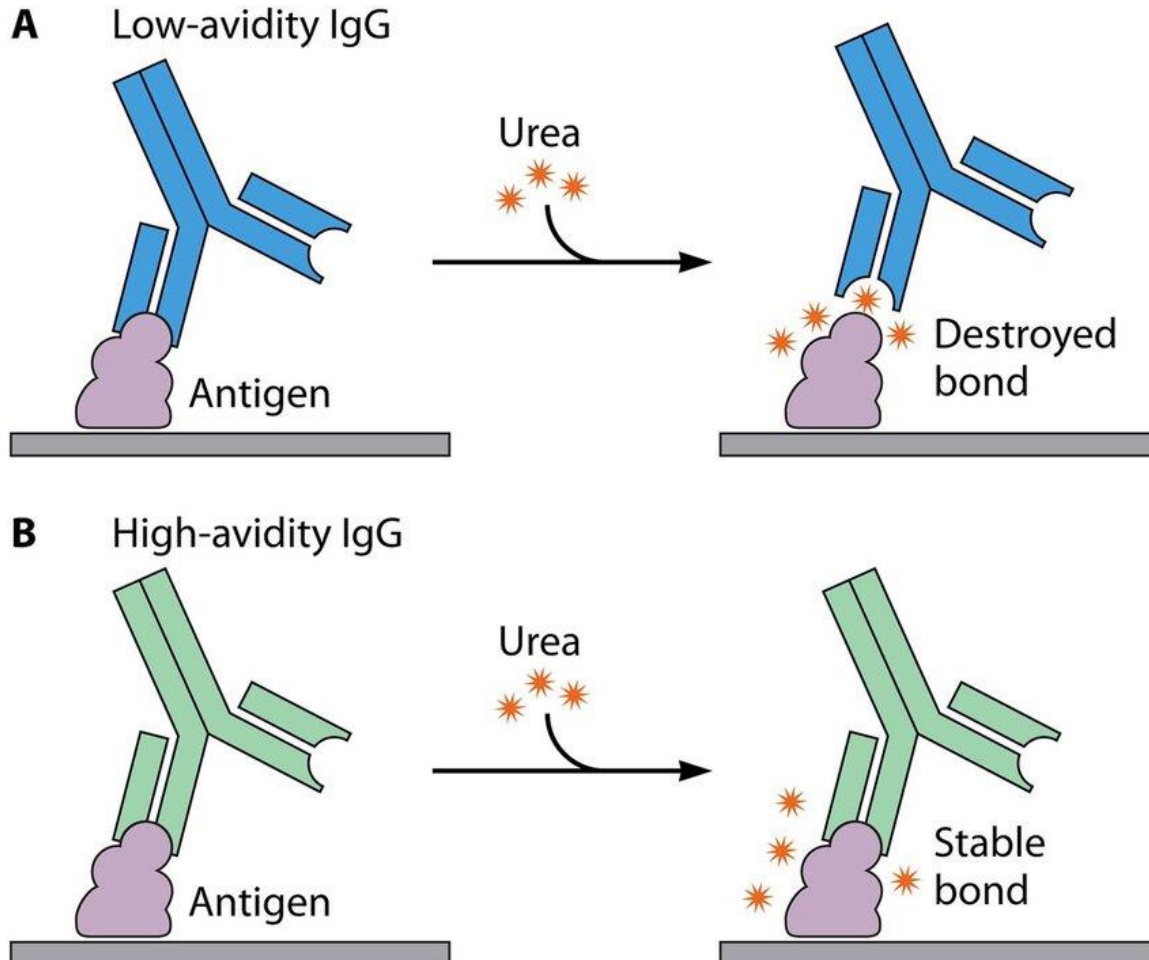
General Considerations

❖ Immunoglobulin G (IgG)

- IgG antibodies usually appear within **1 to 2 weeks** of acquisition of the infection.
- These antibodies **peak within 2 to 4 months, decline at various rates, and usually persist for life**
- The most commonly applied tests are the **DT, ELISA, IFAT, and MAT**
- More specific tests, like **IgG antibody avidity test**, are used to more accurately determine the likelihood of a recently acquired infection

Diagnosis of Toxoplasmosis

IgG avidity



Diagnosis of Toxoplasmosis

❖ Immunoglobulin A (IgA)

- IgA antibodies may persist for **many months or more than a year**.
- These antibodies may be detected in **sera** of acutely infected adults and congenitally infected infants.
- The most commonly used methods are the **ELISA** and **ISAGA**.
- In a number of neonates with congenital toxoplasmosis and negative IgM antibodies, the **serologic diagnosis** has been established by **the presence of IgA and IgG antibodies**

Diagnosis of Toxoplasmosis

❖ Immunoglobulin E (IgE)

- IgE antibodies persist **briefer** than IgM and IgA antibodies.
- These antibodies are detectable in **sera** of acutely infected adults, congenitally infected infants, and children with congenital toxoplasmic chorioretinitis
- The most commonly used method is the **ELISA** Since the duration of IgE seropositivity is **relatively short**,
- these antibodies can be considered to identify **recently acquired infections**

Diagnosis of Toxoplasmosis

❖ Molecular Methods (PCR)

Gene targets	Samples	Methods	fold	Sequence
RE	Blood, Amniotic fluid, Placenta, Urine, CSF....	RT-PCR, Real-time PCR, Nested PCR	200 to 300	529 bp
B1	Blood, Amniotic fluid, Placenta, Urine, CSF....	RT-PCR, Real-time PCR, Nested PCR, Q-PCR, LAMP	35	
18 S rDNA	Amniotic fluid, Placenta, Cord blood	PCR	10	
P30 (SAG1)	Amniotic fluid	PCR	1	1500

Diagnosis of Toxoplasmosis

❖ Molecular Methods (PCR)

Gene	Samples	Sensitivity		Specificity		PPV			NPV			
		L	%	H	L	%	H	L	%	H		
RE	Placenta, Amniotic fluid, Blood	35.9		71	97		100	91		100	78.3	90
B1	Blood, Amniotic fluid, Placenta, Cord blood, Urine, Tissues	21.2	98.3		86.3		100	67		100	70.9	97.6
18 S rDNA	Placenta, Amniotic fluid, Cord blood		25			99			93			95
P30 (SAG1)	Amniotic fluid		83			98.3			---			---

Diagnosis of Toxoplasmosis

❖ Acquired by Immunocompetent Patients (IPs)

Table 1: Overview of strategies for the diagnosis of toxoplasmosis in IPs

Clinical and Epidemiological Situation		Sample	Technique	Diagnostic Criteria
Toxoplasmosis in IPs	Determination of immune status	Serum	Serology	IgG ⁻ , IgM ⁻ : no infection IgG ⁺ , IgM ⁻ or IgG ⁺ , IgM ⁺ , high avidity: Chronic infection
	Primary infection			IgG ⁻ /IgM ⁺ or IgG ⁺ /IgM ⁺ or low avidity: Acute infection

Diagnosis of Toxoplasmosis

Table 2: Overview of strategies for the diagnosis of toxoplasmosis during pregnancy and CT

Clinical and Epidemiological Situation		Sample	Technique	Diagnostic Criteria
CT Diagnosis and Monitoring	Pregnant women	Serum	Serology	IgG ⁻ / IgM ⁺ or IgG ⁺ / IgM ⁺ with low avidity: Acute infection
	Prenatal diagnosis	Amniotic fluid	qPCR	qPCR ⁺
	Diagnosis at delivery	Placenta and/or cord blood	Mouse inoculation	Seropositive mice
	Postnatal follow-up	Sera (cord blood and/or newborn + mother)	Serology	IgM ⁺ and/or IgA ⁺ in newborn serum: biological CT
			Comparative WB	Neosynthesized IgG and/or IgM by the newborn
		If no evidence for CT: serological follow-up	Serology	Confirmation of the absence of CT by total disappearance of immunoglobulin
				Detection of serological rebound during follow-up of immunoglobulin levels
If evidence for CT: monitor child sera until the end of the treatment				

Diagnosis of Toxoplasmosis

❖ Prenatal diagnosis of congenital toxoplasmosis

- 1- Examination of **IgM** class specific antibodies in fetal blood.
- 2- Fetal blood or **amniotic fluid** culture in **Vero cell or mice**
- 3- **Ultrasonography** and observation of the **enlargement of the fetal brain ventricles** in the obtained images
- 4- Using the **PCR** method to identification of *Toxoplasma* DNA in **amniotic fluid**

Diagnosis of Toxoplasmosis

Table 3: Overview of strategies for the diagnosis of toxoplasmosis in ICs

Clinical and Epidemiological Situation		Sample	Technique	Diagnostic Criteria
Toxoplasmosis in ICs	Monitoring (primary infection, reactivation)	Serum	Serology	Seroconversion: primary infection Increase of IgG and/or IgM: Toxoplasmosis reactivation
		Blood	Molecular assay	qPCR ⁺
	Disseminated infection	Blood CSF BAL/AB biopsy	Molecular assay Histology	qPCR ⁺ <i>Toxoplasma gondii</i> on histology

❖ Ocular Toxoplasmosis (OT)

- The diagnosis of OT is primarily based on the **clinical examination** when typical lesions are found in a patient seropositive for *T. gondii*
- Laboratory tests, including the Goldmann-Witmer coefficient (**GWC**), **comparative WB**, and **PCR analysis**, are helpful when atypical lesions are observed or anti-toxoplasmic treatment is not effective

Diagnosis of Toxoplasmosis

- Abnormal amounts of *anti-T. gondii* antibodies in aqueous humor (AH) can be evidenced by the calculation of the Goldmann-Witmer coefficient (GWC) with a sensitivity of up to **81%**.
- The **GWC** is defined as follows:

$$\frac{[\text{anti-Toxoplasma IgG (IU/ml) in AH} / \text{anti-Toxoplasma IgG (IU/ml) in serum}] \times [\text{serum total IgG (g/liter)} / \text{AH total IgG (g/liter)}]}$$

Table 4: Overview of strategies for the diagnosis of toxoplasmosis in patients with **OT**

Clinical and Epidemiological Situation	Sample	Technique	Diagnostic Criteria
OT	Serum + AH	Comparative WB	Intraocular production of immunoglobulin
	AH Vitreous fluid	GWC	qPCR ⁺

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Thank you for your time and attention.

