ToRCH IgG/IgM Assay Development

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ToRCH Overview

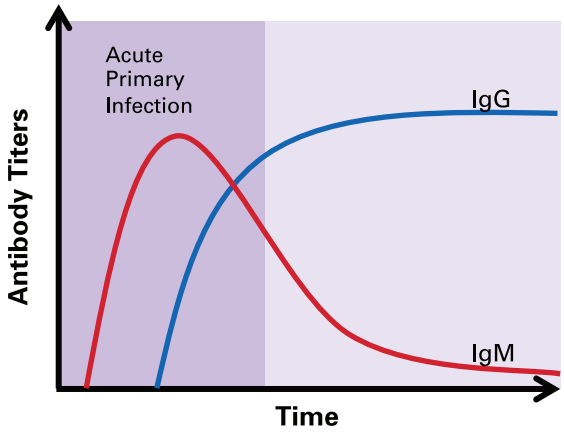
ToRCH is an acronym for a group of infections that can cause significant birth defects and even fetal death. Meridian Life Science offers a complete range of antigens and other reagents for the detection of IgG and IgM antibodies in various assay formats such as EIA, rapid anti-IgM assays and Immunofluorescence (IFA).

The ToRCH test measures the levels of an infant's antibodies against five groups of chronic infections: toxoplasmosis, rubella, cytomegalovirus (CMV), herpes simplex virus (HSV) and other infections. The "other infections" usually include syphilis, hepatitis B, coxsackie virus, Epstein-Barr virus (EBV), varicella-zoster virus (VZV), and human parvovirus.

These infectious diseases are all associated with congenital abnormalities resulting from maternal infection. Although these organisms typically cause only asymptomatic or mild infection in the mother, they can have serious consequences for the fetus.

If the infection occurs during the first three months of pregnancy and if it is a primary infection (newly acquired during pregnancy), the risk of congenital abnormalities is much higher as compared to a secondary or reactivated infection. CMV is the most common cause of congenital infectious disease with a much higher rate of transmission (10% vs. 1%) for mothers with a primary infection compared to a reactivation. Consequently it is a very important part of prenatal care to recognize these infections in the first trimester of pregnancy.

IgM vs IgG SEROLOGY TIMELINE



For most ToRCH organisms, the initial screening test is based on the detection of antibodies to the organism. Subsequent screening, if required, is carried out using a monoclonal antibody-based immunofluorescent assay (IFA). Assays are commercially available for the detection of IgG, IgM, or both IgG/IgM antibodies. In most cases, IgG reactivity in the absence of IgM reactivity is indicative of a past infection, while IgM reactivity in the absence of IgG reactivity indicates a current infection. However, for some ToRCH diseases such as toxoplasmosis and CMV infections, IgG avidity has recently been found to be useful for identifying primary infections. An IgG antibody produced in the first few months following an initial infection has a lower avidity than an IgG antibody produced several months or years later; consequently, low-avidity antibody can be used to specifically identify high-risk mothers with a primary infection. To protect a fetus from ToRCH infection, early diagnosis through first trimester screening is critical.

Meridian ToRCH Antigens & Antibodies

Antigens and Antibodies

Toxoplasma gondii

8200	Native Ag
R01573	Rec. Ag. p30 (SAG1), *E. coli*
R01581	Rec. Ag. p35 (GRA8), *E. coli*
C01523M	MAb to *T. gondii* SAG1 (p30) protein
C01589M	MAb to *T. gondii* (38kDa protein)

Rubella Virus

6075	Native Ag., Grade III, highly pure
6076/6123	Native Ag., Grade IV, highly pure
R01491	Rec. Ag., E1 Mosaic, *E. coli*
EV9525	MAb to Rubella, purified
EV9526	MAb to Rubella, purified

Cytomegalovirus (CMV)

7511	Native Ag., IgM Concentrate
7600	Native Ag, IgG/IgM Concentrate
EV9268	Native Ag., Grade II
EV7509	Native gB Ag.
R18102	Rec. gB Ag., *E. coli*
R01561	Rec. Ag pp52 (UL44), *E. coli*
R01562	Rec. Ag pp65 (UL83), *E. coli*
R01563	Rec. Ag pp150 (UL32), *E. coli*
C86314M	MAb to Early Antigen (65kDa protein)
C8A022M	MAb to Immediate Early Antigen, pp72

Herpes Simplex Virus (HSV) 1

7305	Native Ag.
VTI520	Rec. Ag. glycoprotein G 1 *(S. cerevisiae)*
C05014MA	MAb to Nucleocapsid protein (155kDa)
C66150M	MAb to Glycoprotein G 1

Herpes Simplex Virus (HSV) 2

7705	Native Ag.
VTI530	Rec. Ag. glycoprotein G 2 *(S. cerevisiae)*

Assay Reagents

Anti-human IgM

Z01235M	MAb to IgM
W01258G	Goat PAb (μ chain)
W01259G	Goat PAb (μ chain), low cross-reactivity to IgA & IgG

Anti-human IgG

Z86238M	MAb to IgG (Fc), no cross-reactivity with IgA, IgM or IgE

IgG Absorbent (Required for IgM Assays)

L15406G	Goat anti-human IgG (Fc)
8120	IgM Diluent

Solid Phase Blocking Buffers

J82100B	ELISA blocking buffer
J82300B	Lateral Flow blocking Buffer
J16403D	Coating stabilizer and Blocking Buffer

To order

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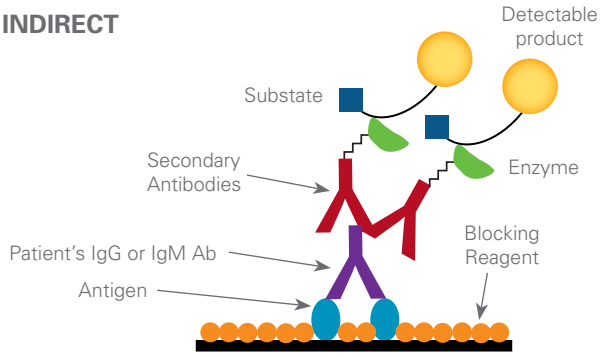
Common Types of ToRCH Assays

ToRCH IgG & IgM Capture Assays

A ToRCH serologic test detects IgM and IgG antibodies to the ToRCH panel of infectious diseases (Toxo, Rubella, CMV and HSV). IgM is the immediate antibody that is produced once a human is exposed to a bacteria, virus or a toxin and disappears within 2-3 weeks. It is then replaced by IgG which lasts for life and provides lasting immunity. Meridian's ToRCH antigens are suitable for IgG and IgM detection. They can be used in a range of immunoassay formats including, but not limited to, ELISA, LF, CLIA, rapid assays, and bead-based assays.

INDIRECT



GENERAL ASSAY PRINCIPLE

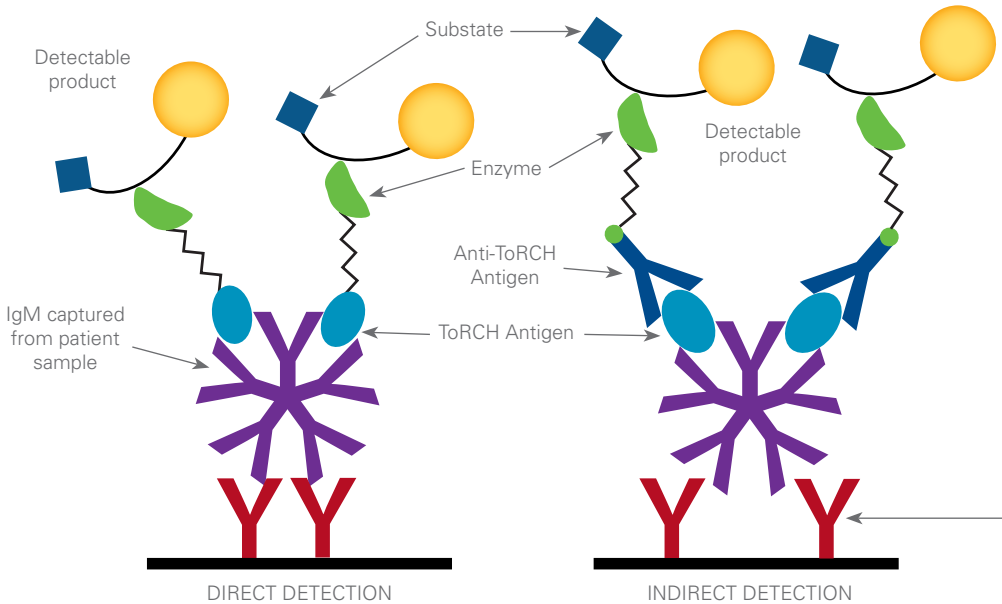
1. Solid-phase (assay plate, beads, etc) is coated with the antigen
2. Blocking buffer is added to block the remaining binding site
3. Sample is added and patient's IgG or IgM antibody binds to the antigen
4. Detection can either be by direct or indirect methods

INDIRECT DETECTION: uses a labeled secondary antibody for detection. The secondary antibody has specificity for human IgG or IgM.

ToRCH Rapid Anti-IgM Assays

Rapid anti-IgM assays are particularly sensitive in demonstrating IgM responses early in the illness. These assays work by binding IgM-specific antibodies in the patient's specimen to a solid phase coated with an anti-IgM capture antibody. Soluble antigen is added in excess allowing the specific IgM antibody-antigen reaction to occur in the absence of competing immunoglobulin isotypes. Finally, a labelled detection antibody is added which has specific reactivity against the antigen. Assay sensitivity can be highly dependent on the purity of the antigen used.

ELISA capture for IgM determination minimizes interference of rheumatoid factor.



DIRECT DETECTION: uses a labeled antigen that reacts directly with the antibody.

INDIRECT DETECTION: uses a labeled secondary antibody for detection.

GENERAL ASSAY PRINCIPLE

1. Solid-phase is coated with anti-human IgM (MAb or PAb Blocking buffer is added to block the remaining binding site)
2. IgM-specific antibodies in the patient's sample bind to the anti-human IgM
3. Antigen (e.g. Rubella, toxo) is added in excess and a antibody-antigen-antibody complex forms
4. Detection can either be by direct or indirect methods

Anti-human IgM antibody

Recommended:
Cat# Z01235M
Cat# W01258G
Cat# W01259G

How to Increase Assay Sensitivity

Use a solid phase blocking buffer

Solid phase blocking buffers are designed to efficiently prevent non-specific binding, reduce background noise, and stabilize coated proteins to enable more sensitive immunoassays. They work by blocking unoccupied spaces on the surface to prevent non-specific binding to this surface by other proteins or biomolecules.

Recommended Reagents:

J82100B	Blocking Buffer for ELISA
J82300B	Blocking Buffer for Lateral Flow, PBS Based
J16430D	Coating Stabilizer and Blocking Buffer

Use IgG absorbant to remove IgG and RF

The sensitivity and specificity of IgM detection can be compromised by the presence of IgG in the patient sample. There are two major mechanisms by which IgG can interfere with assays for IgM and cause a false result:

- by competing with specific IgM for substrate binding sites
- by forming immune complexes with Rheumatoid Factor (RF) which can compete with specific IgM for substrate binding sites

Removal of IgG and RF-IgM can be accomplished by pre-treating the patient specimen with goat anti-human IgG.

Recommended Reagents:

L15406G	**Goat anti-human IgG FC (GAHG)** Dilute prior to adding to patient sample. Recommend diluting 1:10 in PBS. Add in a ratio of 1:10 to patient sample and allow to incubate 5-30 minutes.
8120	**IgM Diluent** In a separate tube, dilute the patient serum sample in the IgM Assay diluent at a 1:21 dilution or greater (mix well). The diluent must be standardized with the other assay components.

How to use Blocking Buffer and GAH IgG

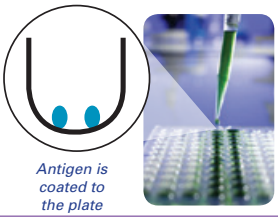
General Protocol

STEP 1 Optimize the plate/nitrocellulose-coating conditions for the antigen

A. Coat the plate with antigen: 2-10 µg/mL solution of protein dissolved in an alkaline buffer such as phosphate-buffered saline (pH 7.4) or carbonate-bicarbonate buffer (pH 9.4)

B. Incubate plate for several hours to overnight at 4-37°C

C. Remove coating solution, perform wash steps



Antigen is coated to the plate

STEP 2 Add blocking buffer

A. Add the blocking solution directly to the wells, blotting membrane or nitrocellulose membrane depending on the assay type being used.
Use at 1X concentration or with further dilution

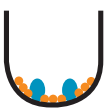
ELISA Blocking Buffer: Cat# J82100B

Lateral Flow Blocking Buffer: Cat# J82300B

Coating Stabilizer & Blocking Buffer: Cat# J16430D

B. Incubate at room temperature for 30 minutes to 2 hours

C. Continue with your process and reagents according to the assay protocol

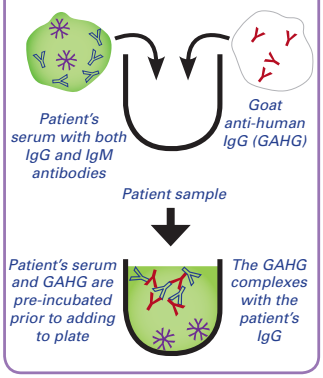


STEP 3 Pre-incubate GAHG with the patient's serum

A. Dilute GAH IgG 1:10 in PBS

B. Add diluted GAH IgG to patient sample 1:10 and mix

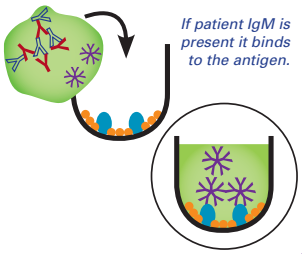
C. Incubate for 3-5 min and proceed with sample testing



STEP 4 Add patient sample mixture to the reaction well

A. Incubate patient sample with the antigen

B. Perform wash steps: remove non-bound reagents



STEP 5 Detection by direct or indirect methods

Commercial Assays & Recommended Products

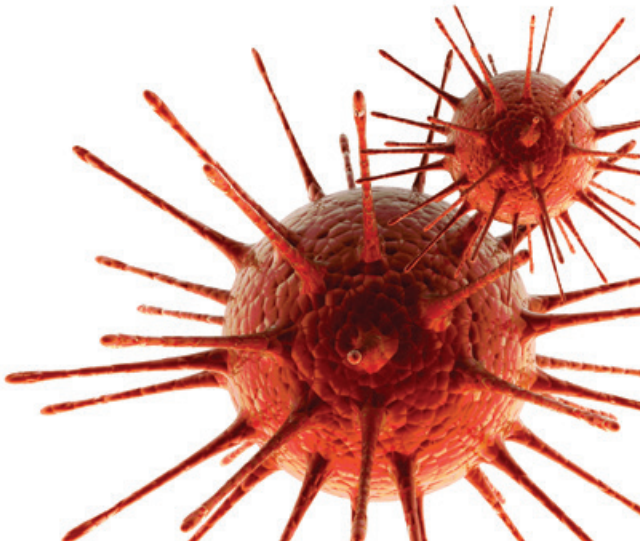
Toxoplasma IgM

	Assay Type	Solid Phase	Antigen	Detection System	Recommended Products
Abbott ARCHITECT	CMIA IgM-capture sandwich method	Paramagnetic microparticles	Mouse anti-human IgM, F(ab')2 to Toxo & Rec. p30 antigen	Direct, CL	Z01235M, C01523M and R01573
Abbott AxSYM	MEIA IgM-capture sandwich method	Microparticles	Mouse anti-human IgM, MAb to Toxo p30 & native lysate	Indirect, MUB	Z01235M, C01523M and 8200
Siemens IMMULITE	CLIA IgM-capture sandwich method	Paramagnetic particles	Mouse anti-human IgM, MAb to Toxo & p30 antigen	Direct, CL	Z01235M, C01523M and 8200
DiaSorin LIAISON	CLIA IgM-capture sandwich method	Paramagnetic particles	Mouse anti-human IgM, MAb to Toxo & native antigen	Indirect, CL	Z01235M, C01523M and 8200
BioMérieux VIDAS	ELFA IgM-capture sandwich method	Solid phase receptacles	Goat anti-human IgM, MAb to Toxo p30 & native antigen (RH Sabin strain)	Direct, MUB	W01259G, C01523M and 8200

Toxoplasma IgG

	Assay Type	Solid Phase	Antigen	Detection System	Recommended Antigens
Abbott ARCHITECT	CMIA	Paramagnetic microparticles	Rec. antigens to 30 and p35 (GRA8), anti-human IgG	Indirect, CL	R01573, R01581 and Z86238M
ARCHITECT Avidity	CMIA 2 assays with and without blocking buffer to dissociate low-avidity antibodies	Paramagnetic microparticles	Rec. antigens to 30 and p35 (GRA8), anti-human IgG	Indirect, CL	R01573, R01581 and Z86238M
Siemens AVIDA Centaur	CLIA	Paramagnetic particles	Native p30 antigen	Direct, CL	8200
Siemens IMMULITE 2000	CLIA	Paramagnetic particles	Partially purified antigen, mouse anti-human IgG	Indirect, CL	8200
DiaSorin LIAISON	CLIA	Paramagnetic microparticles	Native antigen, anti-human IgG	Indirect, CL	8200 and Z86238M
DiaSorin LIAISON Avidity	CLIA 2 assays with and without a 6M urea elution step to dissociate low-avidity antibodies	Paramagnetic microparticles	Native antigen, anti-human IgG	Indirect, CL	8200 and Z86238M
BioMérieux VIDAS	ELFA	Solid phase receptacles	Native antigen	Direct, MUB	8200
BioMérieux VIDAS Avidity	ELFA 2 assays (reference and test) with and without a disassociation wash step to remove low-avidity	Solid phase receptacles	Native antigen (RH Sabin strain), anti-human IgG	Direct, CL	8200 and Z86238M

ELFA: Enzyme-linked fluorescence assay
CLIA: Chemiluminescent Immunoassay
CMIA: Chemiluminescent Microparticle Immunoassay
MUB: Methyl-umbellifery
CL: Chemiluminescence
MEIA: Microparticle Enzyme Immunoassay
ECLIA: Electrochemiluminescence Immunoassay



Rubella IgM

	Assay Type	Solid Phase	Antigen	Detection System	Recommended Products
Abbott ARCHITECT i2000SR	CMIA	Paramagnetic microparticles	Viral lysate (strain HPV77) and mouse anti-human IgM MAb	Indirect, CL	6076/6123
Roche ELECSYS	CLIA IgM-capture sandwich method	Magnetic beads	Recombinant rubella like particles and rubella-specific antibodies	Indirect, CL	R01491 and EV9525 or EV9526
Siemens IMMULITE 2000	CLIA IgM-capture sandwich method	Polystyrene beads	Viral lysate (strain HPV77)	Indirect, CL	6076/6123
Siemens AVIDA Centaur XP	CLIA IgM-capture sandwich method	Paramagnetic particles	Viral lysate and rubella specific antibody	Indirect, CL	6076/6123 and EV9525 or EV9526

Rubella IgG

	Assay Type	Solid Phase	Antigen	Detection System	Recommended Products
Abbott ARCHITECT i200SR	CMIA	Paramagnetic microparticles	Partially purified viral lysate (strain HPV77), mouse anti-human IgG MAb	Indirect, CL	6075 and Z86238M
Abbott AxSYM	MEIA	Microparticles	Purified viral lysate and anti-human IgG	Indirect, MUB	6075 and Z86238M
Siemens AVIDA Centaur XP	CLIA moderate complexity	Paramagnetic particles	Purified viral lysate (strain HPV77) and mouse anti-human IgG FC	Indirect, CL	6075 and Z86238M
Siemens IMMULITE 2000	CLIA moderate complexity	Paramagnetic particles	Viral lysate	Indirect, CL	6075
Beckman ACCESS 2	EIA	Paramagnetic particles	Viral lysate	Indirect, CL	6075
Roche ELECSYS	CLIA, double antigen sandwich with an IgG-capture method	Magnetic beads	Recombinant E1 antigen, rubella like particles and rubella specific antibodies	Indirect, CL	R01491 and EV9525 or EV9526
BioMérieux VIDAS	ELFA, sandwich immunoassay method	Solid phase receptacles	Viral lysate (strain MR383) and anti-human IgG	Indirect, MUB	6075 and Z86238M
Ortho VITROS Eci	CLIA high complexity	Plastic wells	Viral lysate and mouse-anti-human IgG	Indirect, Luminescence	6075 and Z86238M

CMV IgM

	Assay Type	Solid Phase	Antigen	Detection System	Recommended Products
Abbott ARCHITECT	CMIA	Paramagnetic microparticles	Viral lysate (strain AD169), rec. antigen pp150 and pp52, and anti-human IgM	Indirect, CL	R01561, R01563 and W01259G
Abbott AxSYM	MEIA	Microparticles	Rec. antigen CMV pp150, pp52, pp65, pp38, and anti-human IgM	Indirect, MUB	R01561, R01562, R01563, R01567 and W01259G
Siemens IMMULITE 2000	CLIA 3 step assay, IgM-capture sandwich method	Paramagnetic particles	Purified antigen (strain AD169), Goat anti-human IgG, goat anti-human IgM	Direct, CL	7600 or 7511 and W01259G and L15406G
Roche ELECSYS	ECLIA IgM-capture sandwich method	Paramagnetic microparticles	Rec. antigens CMV pp150 and pp52, and anti-human IgM	Direct, CL	R01561, R01563 and Z01235M
BioMérieux VIDAS	ELFA IgM-capture sandwich method	Solid phase receptacles	Viral lysate (strain AD169), mouse anti-human IgM	Indirect, MUB	7600 or 7511 and Z01235M

Commercial Assays & Recommended Products *continued*

CMV IgG

	Assay Type	Solid Phase	Antigen	Detection System	Recommended Antigens
Abbott ARCHITECT i2000SR	CMIA	Paramagnetic microparticles	Viral lysate	Indirect, CL	7600 or 7511
Abbott ARCHITECT Avidity	CMIA, two assays with and without liquid CMV antigen to neutralize high-avidity CMV antibodies	Paramagnetic microparticles	Viral lysate, anti-human IgG	Indirect, CL	7600 or 7511 and Z86238M
Abbott AxSYM	MEIA	Microparticles	Viral lysate	Indirect, MUB	7600 or 7511
Siemens IMMULITE 2000	CLIA	Paramagnetic particles	Purified Antigen	Indirect, CL	7600 or 7511
Roche ELECSYS	ECLIA, one step double sandwich method	Paramagnetic microparticles	Rec. antigens CMV pp150, pp52, pp28, pp38	Direct, CL	R01561 or R01563
Roche ELECSYS Avidity	ECLIA, two assays with and without chaotropic conditions to dissociate low-avidity antibodies	Paramagnetic microparticles	Rec. antigens CMV pp150, pp52, pp28, pp38	Direct, CL	R01561 or R01563
BioMérieux VIDAS	ELFA two step sandwich method	Solid phase receptacles	Viral lysate, anti-human IgG	Direct, MUB	7600 or 7511 and Z86238M
BioMérieux VIDAS Avidity	ELFA two assays with and without 6 M urea to dissociate low-avidity antibodies	Solid phase receptacles	Viral lysate, anti-human IgG	Direct, MUB	7600 or 7511 and Z86238M

HSV-1 & HSV-2 IgG/IgM

	Assay Type	Solid Phase	Antigen	Detection System	Recommended Antigens
DiaSorin LIAISON HSV-1 Type Specific	CLIA, two steps	Magnetic particles	Rec. HSV-1 gG 1 and mouse anti-human IgG	Indirect, CL	VTI520, Z86238M
DiaSorin LIAISON HSV-2 Type Specific	CLIA, two steps	Magnetic particles	Rec. HSV-2 gG 1 and mouse anti-human IgG	Indirect, CL	VTI530, Z86238M
Focus PLEXUS HerpesSelect® 1 and 2 IgG	EIA multiplex flow three steps	Beads	Rec. HSV-1 gG 1, HSV-2 gG 1 and goat anti-human IgG	Indirect, Fluorescent	VTI520, VTI530 and L15406G
Biorad BIOPLEX 2200 HSV-1 & HSV-2 IgG	EIA multiplex flow three steps	Carboxy-coated dyed beads	Rec. gG1 and synthetic peptide p8C:BSA derived from gG2 sequence (patented) and mouse anti-human IgG and mouse anti-human FXIII	Direct, Fluorescent	VTI520, VTI530, and Z86238M
Roche ELECSYS HSV-1 IgG	ECLIA, two step antigen sandwich	Paramagnetic microparticles	Rec. HSV-1, HSV-1 viral lysate	Direct, CL	VTI520 and 7305
Siemens Immunlite 2000 Herpes 1/2 IgG	CLIA	Paramagnetic particles	HSV-1 (strain MacIntyre), HSV-2 (strain G) viral lysate and anti-human IgG	Indirect, CL	7305, 7705 and Z86238M

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