Diagnosis and management of non-primary infections

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CONGENITAL CMV INFECTION

Non-primary maternal CMV infection leads to congenital CMV infection.

Increasing observations demonstrate the risk for symptomatic infection at birth and sequelae, especially hearing loss, are similar in primary and non-primary maternal CMV infection.

Ahlfors K et al. 1999, Boppana SB et al. 1999, Gaytant MA et al. 2003, Zalel Y et al. 2008, Mussi-Pinhata MM et al. 2009, Yamamoto AY et al. 2011.

Diagnosis of non primary maternal infection

- Clinical diagnosis unreliable

  Clinical CMV symptoms are more common with primary than with nonprimary infections, 60.2 vs 36.6%.
  Rahav G. et al 2007.

- Serological diagnosis ?
- Virological diagnosis

Diagnosis of maternal CMV infection

- Serological diagnosis

Detection of IgG AND IgM

Diagnosis of CMV infection

Serological diagnosis is reliable

The serological reaction employing antibodies linked to a tracer enzymatic or fluorescent or chemiluminescent.

Screening tests

EIA (ELISA), MEIA, CMIA, ELFA ,CLIA

EIA (Enzyme Immuno Assay) o ELISA (Enzyme-Linked-ImmunoSorbent-Assay)
MEIA (Microparticle Enzyme Immunoassay)
CMIA (Chemiluminescent Microparticle Immunoassay)
ELFA (Enzyme-linked fluorescent assay)
CLIA (chemiluminescence immunoassay)

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Diagnosis of maternal CMV infection

- Virological diagnosis

Detection of DNA-CMV in whole blood, saliva and urine

Diagnosis of maternal CMV infection

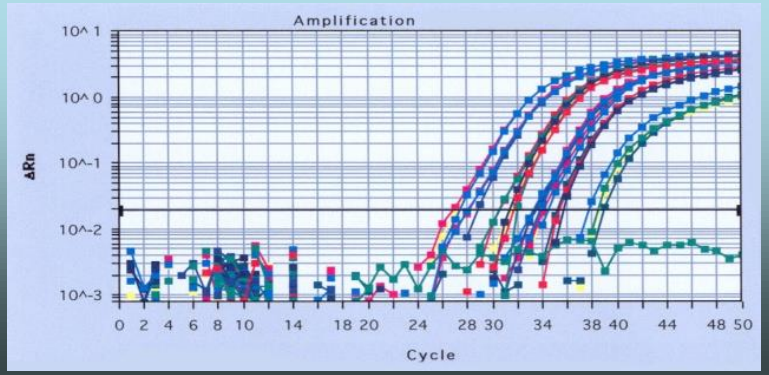
Real-time PCR assays are now the most commonly used diagnostic method for DNA quantification.

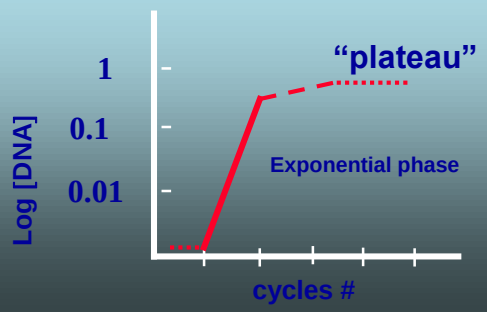
Quantitative molecular test

"Real Time"

"Real Time" quantification:

known*: *"dynamics PCR" or "kinetic quantification".





Virological diagnosis of non primary maternal infection

Pregnant women with non-primary CMV infection

Real Time PCR	n. of patients total	n. of positive patients	sensitivity
Whole blood	27	5	18.6%
saliva	27	13	48.1%
urine	27	12	44.4%

Lazzarotto et al. 2014

When and how are we able to identify non primary infection during pregnancy ?

IgG+ IgM- **RELIABLE**

CMV serostatus known before pregnancy

However

IgG? IgM? **CAN BE UNRELIABLE**

CMV serostatus unknown before pregnancy

Diagnosis of non primary maternal infection

IgG+ IgM- **RELIABLE**

CMV serostatus known before pregnancy

IgG + IgM+ or (IgG+ IgM-)

High avidity IgG-index

Presence of CMV-DNA → WB and/or saliva and/or urine

reliable → Non primary CMV infection

IgG + IgM+ (screening tests) → IB (confermatory tests)

High avidity index IgG

Absence of CMV-DNA → WB and saliva and urine

reliable → Non primary CMV infection

Diagnosis of non primary maternal infection

IgG? IgM? **CAN BE UNRELIABLE**

CMV serostatus unknown before pregnancy

IgG + IgM+ or IgG+ IgM-

High avidity index IgG <12-16 WG

Presence of CMV-DNA → WB and/or saliva and/or urine

reliable → *Non primary CMV infection*

IgG + IgM+ (screening tests) → IB (confermatory tests)

High avidity index IgG <12-16 WG

Absence results of CMV-DNA → WB and/or saliva and/or urine

High risk → *Non primary CMV infection*

Attention !

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Management of non primary maternal infection

Prenatal diagnosis

Invasive prenatal diagnosis	+	Non invasive prenatal diagnosis	+	COUNSELLING
Amniocentesis		**Ultrasound examination**		
discussion		***YES***		***YES***

Since 1994 pregnant women at risk of transmitting CMV are referred to our CMV reference center in Bologna

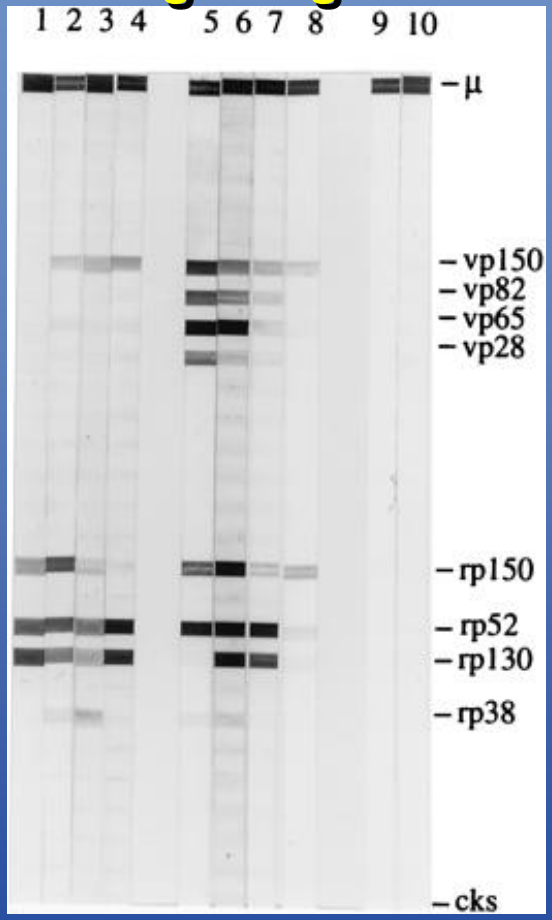
Our clinical experience carried out in 2015 for a 12 months period involving 327 pregnant women that underwent screening tests and were identified as being at risk of transmitting the virus to their fetus.

Serological diagnosis

SCREENING tests

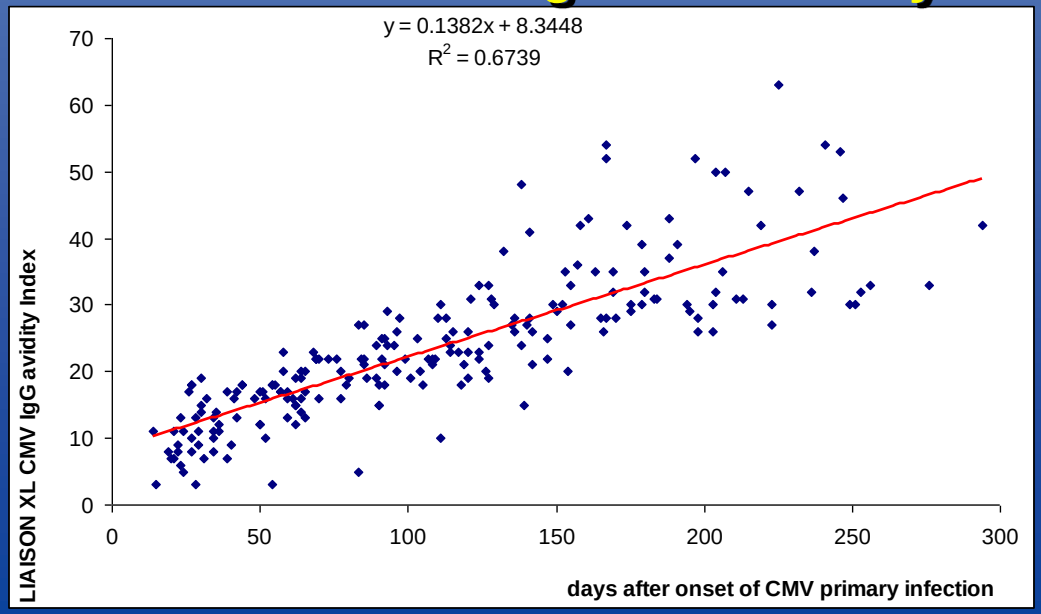
The serological reaction employing antibodies linked to a tracer chemiluminescent.

CMV IgG e IgM IB



Lazzarotto et al
J Clin Microbiol 1998

LIAISON ® XL CMV IgG-CLIA avidity



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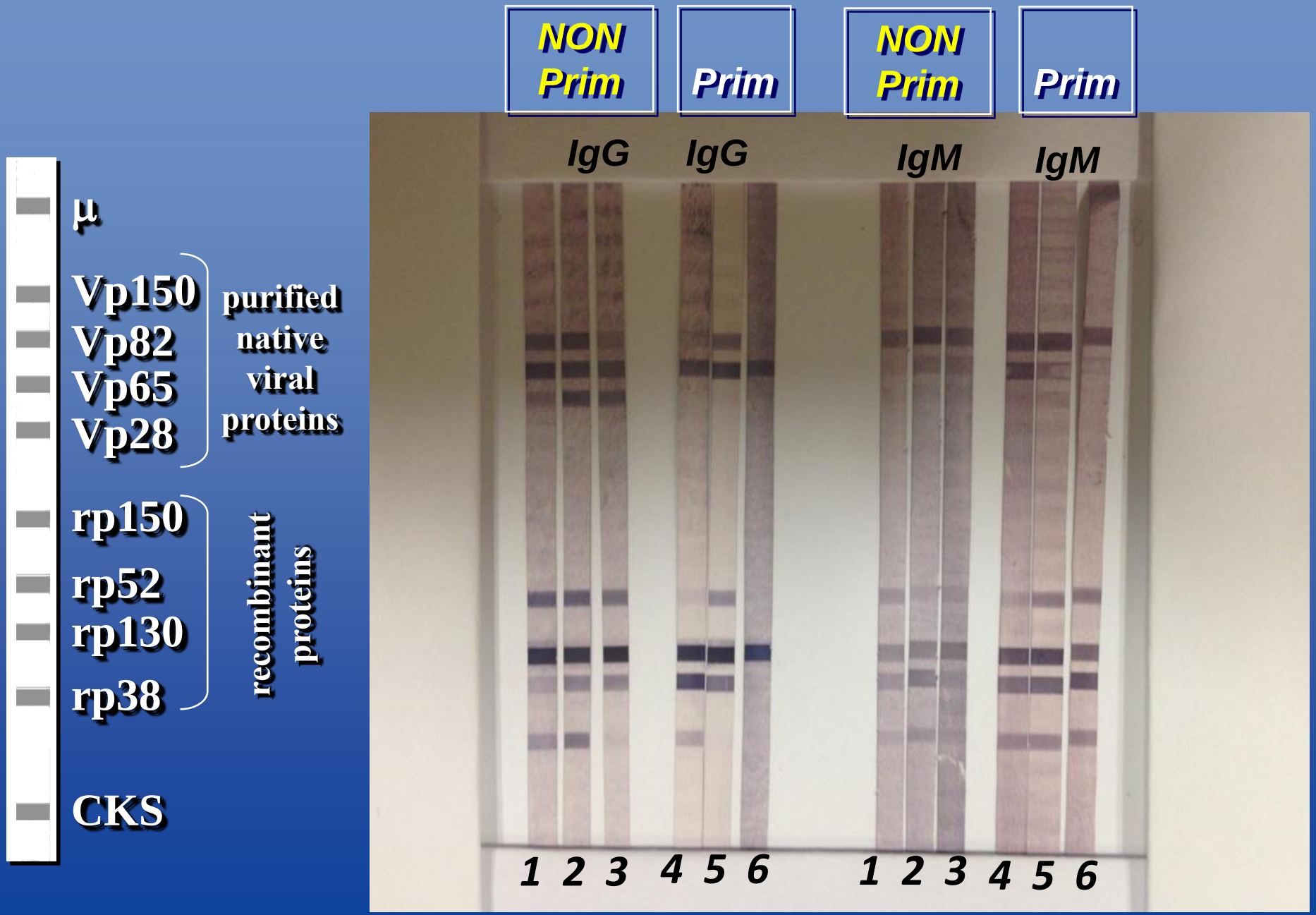
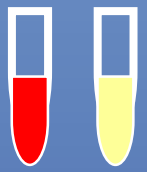
CMV IgG-IgM confirmation by blot



Virological diagnosis

DNA purification by automated instrument

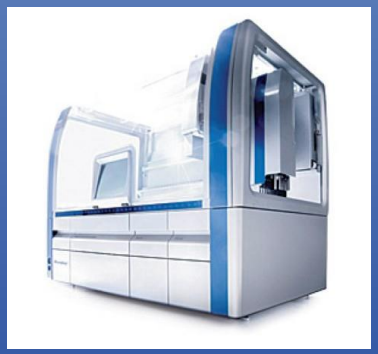
QIAsymphony SP/AS (Qiagen)

Whole Blood  200 µl → 90 µl

Urine 500 µl → 115 µl

EasyMag (BioMerieux)

Saliva 500 µl → 55 µl 



20 µl of purified DNA

amplification using

CMV ELITe MGB® Kit ELITechGroup
Combined with ABI 7300 Applied Biosystems instrument (Life Technologies)

Real Time PCR: n. of copies of CMV-DNA/mL

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(January – December 2015) → 327 pregnant women

288 → first or second trimester - 39 → third trimester

IgG and IgM screening tests ⟶ **IgG pos and IgM bl/pos**

	Maternal CMV Infection	Total (%)	number of fetuses/newborns infected (%)	number of amniocentesis (%)
1	non immune	31 (9.5)	0	0
2	Past	99 (30.3)	0	0
3	Primary	102 (31.2)	33 (32.4)	32 (31.4)
4	Non primary	73 (22.3)	1 (1.4)	6 (8.2)
5	Undefined	22 (6.7)	1 (4.5)	1 (4.5)

73 pregnant women with non primary CMV infection (6-22 WG)

IgG and IgM screening tests ⟶ IgG pos and IgM bl/pos

n. of PW (%)	
42 (57.5)	CMV serostatus unknown before pregnancy
28 (38.4)	CMV serostatus known before pregnancy and NO symptoms
3 (4.1)	CMV serostatus known before pregnancy and YES symptoms

Results of samples from 73 pregnant women with non primary CMV infection (6-22 WG)

IgG and IgM screening tests ⟶ IgG pos and IgM bl/pos

n. of PW (%)	CMIA test	IB test	IgG Avidity CLIA Test (range %)	Real Time PCR WB, saliva, urine	outcome
28 (38.4)	IgG + IgM+	IgG + IgM+	High (40-60%)	Pos	neg
23 (31.5)	IgG + IgM+	IgG + IgM+	High (30-95%)	Neg	neg
15 (20.5)	IgG + IgM bl	IgG + IgM+	High (44-72%)	Pos	1 pos
7 (9.6)	IgG + IgM neg	IgG + IgM neg	High (31-95%)	Pos	neg

219 samples from 73 pregnant women with non primary CMV infection (6-22 WG)

Real Time PCR	n. of samples total	n. of positive samples	sensitivity
Whole blood <500 cps/ml	73	9	12.3%
Saliva <500 – 37.630 cps/mL	73	38	52.0%
Urine <500 – 4.968 cps/mL	73	25	34.2%

1 pos body fluid	2 pos body fluids	3 pos body fluids	N. PW
31 (62%)	15 (30%)	4 (8%)	50

31 serum samples from pregnant women with non primary CMV infection

SAMPLES	whole blood	saliva	urine
2	+ both values <500 cps/mL	-	-
19	-	+ Median values 1.155 cps/mL	-
10	-	-	+ Median values 500 cps/mL

Our findings confirm that the saliva sample is the biological sample that contains the highest viral load and for a long time, this is in agreement with most recent literature.

CLINICAL CASE

2016 - a 35-year-old woman in her first pregnancy
2009 IgG 6.4 UI/mL (+) IgM <8.0 (-)

Screening tests

16 weeks of gestation	CMV-IgG *>250 AU/mL* *Positive*	Neg < 6.0 Equivocal 6≤x<15 Pos ≥ 15	CMV-IgM *1.00 Index* *Weak Positive*	Neg < 0.85 Equivocal 0.85≤x<1 Pos ≥ 1

Advanced diagnosis

19 weeks of gestation	CMV-IgG *>250 AU/mL*	Neg < 12 Equivocal 12≤x<14 Pos ≥ 14	CMV-IgM *0.69 Index*	Neg < 18 Equivocal 18≤x<22 Pos ≥ 22
	> 250 IU/mL *Positive*		*6.87 AU/mL* *Negative*	
	CMV-IgG *IB* *Positive*	CMV-IgM *IB* *Weak Positive*	*CMV IgG avidity* *55% (high AI)*	*CMV-DNA* *Real Time PCR* *urine* *<500 copies/mL* *WB & saliva NEG*

DIAGNOSIS: NON primary infection during pregnancy.

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CLINICAL CASE

2016 - a 44-year-old woman in her first pregnancy

2009 IgG 6.4 UI/mL (+) IgM <8.0 (-)

Screening tests

16 weeks of gestation	CMV-IgG *>250 AU/mL* *Positive*	Neg < 6.0 Equivocal 6≤x<15 Pos ≥ 15	CMV-IgM *1.00 Index* *Weak Positive*	Neg < 0.85 Equivocal 0.85≤x<1 Pos ≥ 1

Maternal diagnosis: NON primary infection during pregnancy.

Ultrasound findings 29WG	borderline ventriculomegaly
MRI 32WG	bilateral ventriculomegaly, with cystic ectasia of the terminal portion of the temporal horns. Presence of bilateral intraventricular cysts.

Fetus with severe cerebral brain damage compatible with CMV infection.

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Conclusion – diagnosis of maternal non primary CMV infection

Serological diagnosis

Antibodies

IgG and IgM with screening tests <12 WG

Avidity IgG test <16 WG

RELIABLE

Virological diagnosis

DNA

Whole blood - Real Time PCR

Saliva – Real Time PCR

Urine - Real Time PCR

RELIABLE

VIROLOGICAL DIAGNOSIS

- ✓ Plays a role in the diagnosis of non primary CMV infection
- ✓ All three biological samples have to be analyzed
- ✓ We must always take a cautious approach when interpreting the overall results

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January 2000 – December 2014 → 1170 pregnant women

	Non primary maternal infection n. 205	Primary maternal infection n. 965
% of transmission	7 (3.4%)	219 (23%)
% of fetuses/ newborns symptomatic	3 (42.9%)	39 (18%)

Simonazzi G, Cervi F, Guerra B et al. 2015

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January 2005 – December 2015 → 63 newborns

Newborns	Non primary maternal infection n. 10	Primary maternal infection n. 48	Unknown maternal infection n. 5
symptomatic at birth (%)	6 (60%) 3 very severe 1 death	10 (20.8%) 4 very severe	2 (40%) 2 mild
asymptomatic at birth (%)	4 (40%)	38 (79.2%)	3 (60%)

Capretti MG, Lanari M. et al. 2015

Frequency and virulence of congenital CMV infection in relation to maternal immune status

Primary maternal infections have a much greater clinical impact on the fetus than non primary infection

however

the risk for symptomatic infection at birth and sequelae are similar in primary and non-primary maternal CMV infection.

Finally

we must be very careful when we have to perform a diagnosis of non-primary CMV infection during pregnancy.

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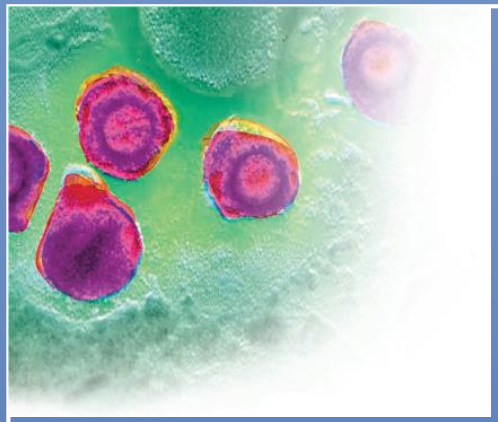
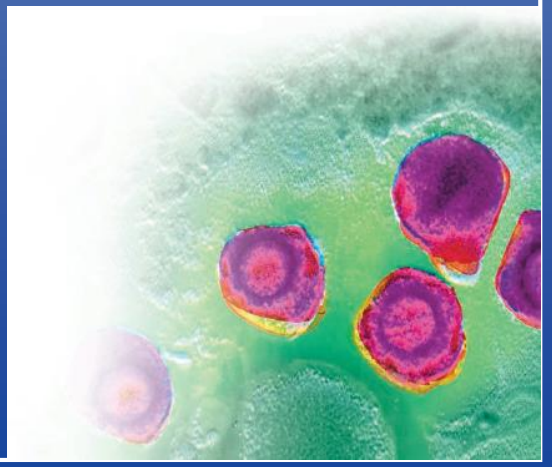


thank you for your attention !



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