Network di Microbiologia e Virologia del Nord Est Incontro di Aggiornamento
INTERFERON GAMMA RELEASE ASSAYS (IGRAs) NELLA DIAGNOSI E MONITORAGGIO DELLE MALATTIE INFETTIVE
19 aprile 2013
Sala Ferrari Incontri - Cantine Ferrari
Trento

IGRA nella diagnosi dell' infezione da *Toxoplasma gondii*

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SC di Virologia e Microbiologia
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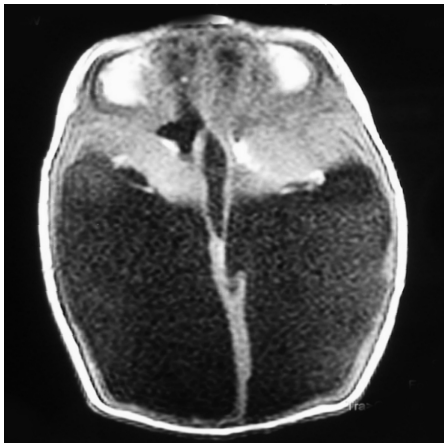
Pavia

Outcome dei neonati con Toxoplasmosi congenita

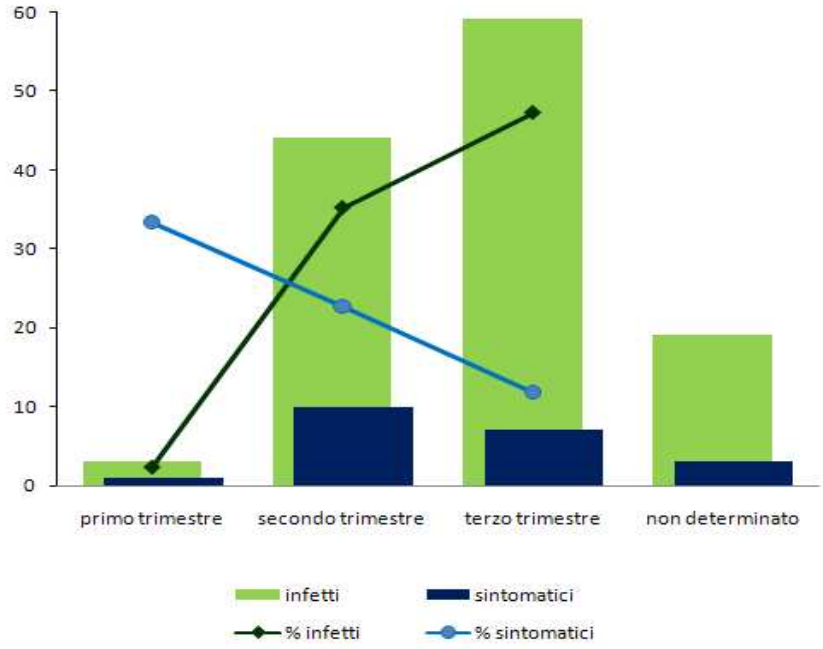
Montoja Liesenfeld Lancet 2004

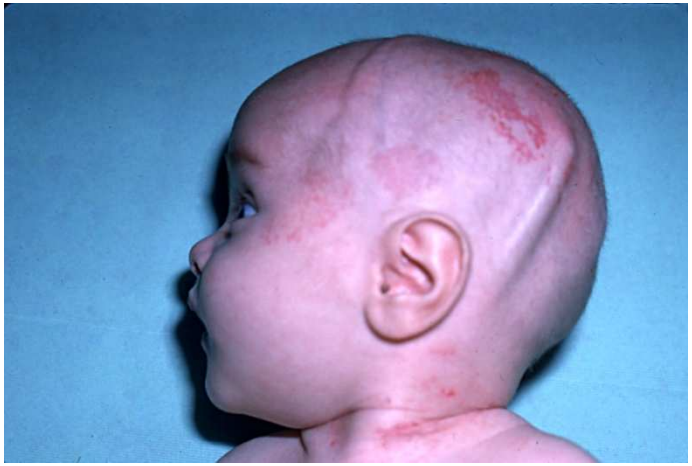
N 542	Infection acquired		
Outcome in the offspring	I Trimester	II Trimester	III Trimester
Congenital toxoplasmosis	9%	27%	59%
Subclinical	22,2%	74,4%	89,8%
Clinicaly apparent	77,8%	15,6%	10,2%
Perinatal death or stillbirth	5%	2%	0%

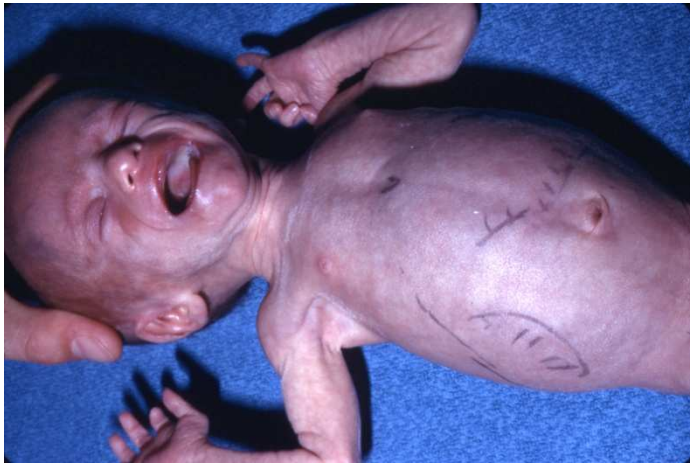


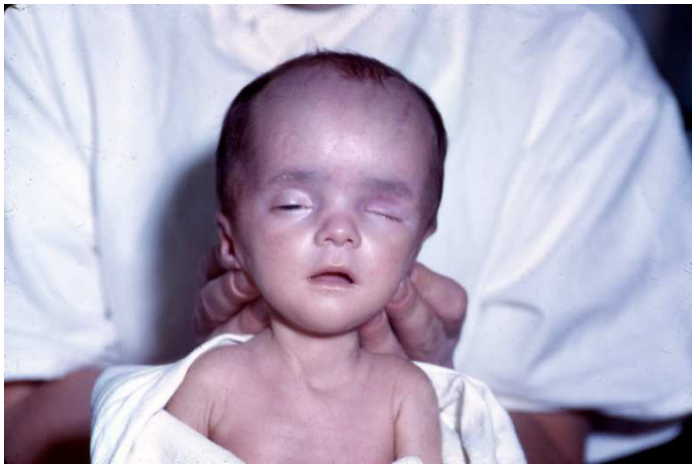
Meroni 2009

N 128	Infection acquired		
Outcome in the offspring	I Trimester	II Trimester	III Trimester
Congenital toxoplasmosis	2%	34%	48%
Subclinical	65%	88%	89%
Clinicaly apparent	33%	22%	11%
Perinatal death or stillbirth	2%	0%	0%









Nei neonati trattati prima e dopo la nascita

- **I casi gravi sono estremamente rari**
- **la toxoplasmosi congenita è una malattia oculare cronica**
- **che ha globalmente una prognosi buona**
- **ma può durare tutta la vita**

Come escludere un'infezione congenita?

Negativizzazione delle IgG specifiche

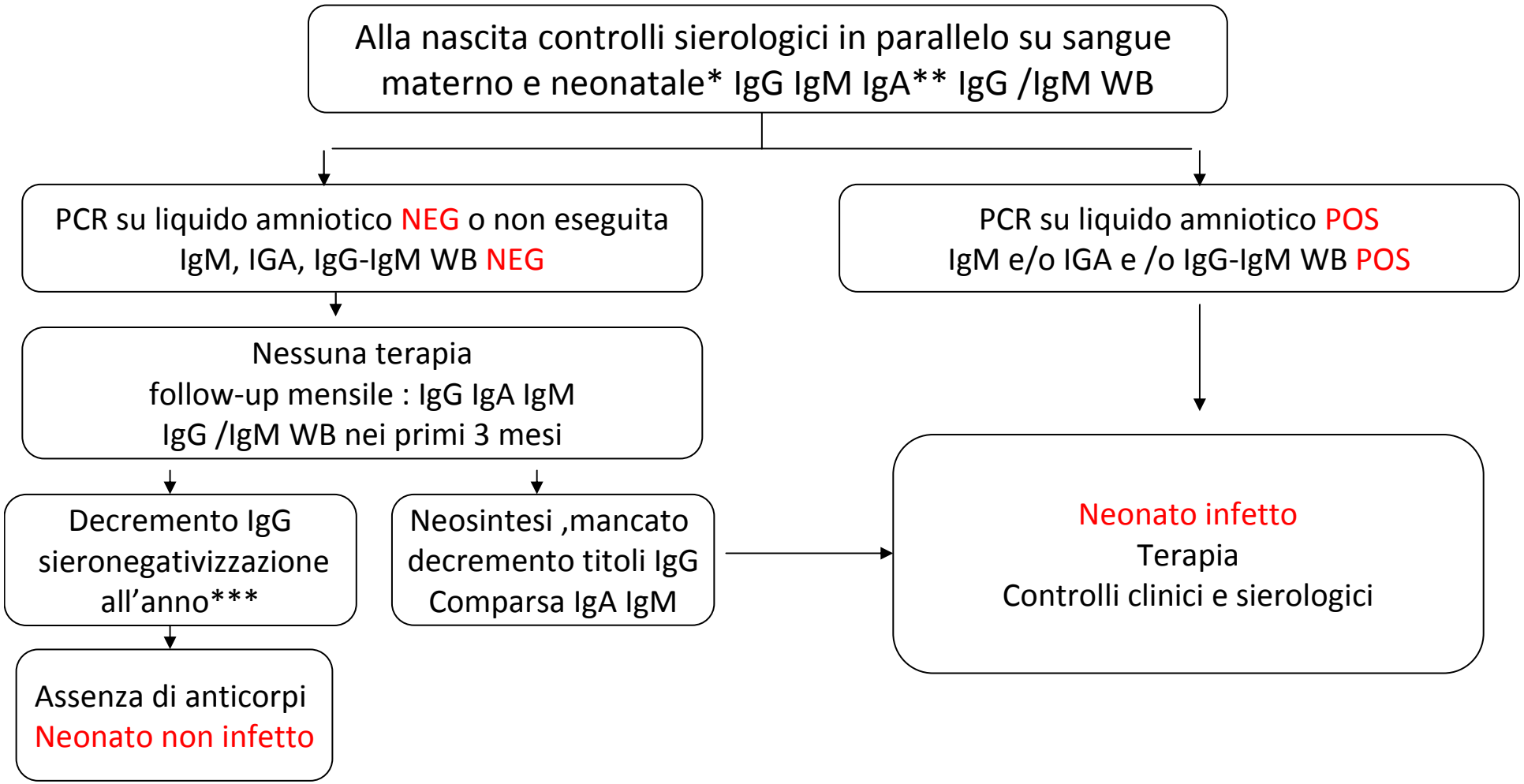
IgG materne
In utero

IgG alla nascita

Decremento

Negativizzazione all'anno

Diagnosi sierologica della toxoplasmosi connatale



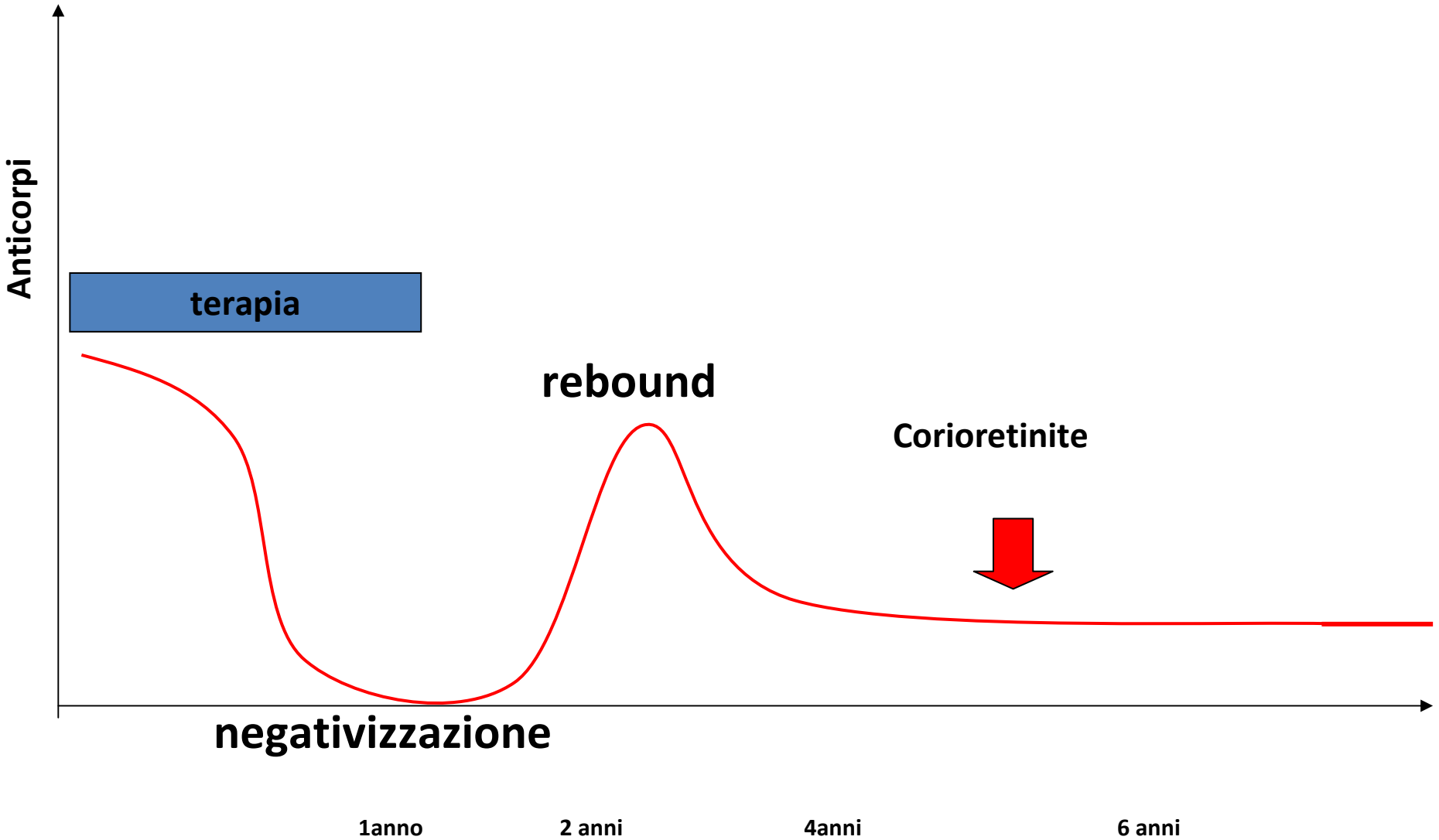
*è preferibile usare il sangue periferico del neonato

** i test più sensibili sono IgM e IgA ISAGA

*** tutti i test vanno eseguiti nello stesso laboratorio e i sieri conservati congelati per un anno

E' possibile osservare la sieronegativizzazione dei titoli anticorpali in corso di terapia

Cinetica della sierologia



Western Blot comparativo eseguito nei primi 3 mesi di vita

IgG-IgM WESTERN BLOT			
	POS	NEG	TOT
POS	38	6	44
NEG	2	178	180
TOT	40	184	224

Sens 95,0 (IC 95% : 83,1-99,4)*
Spec 96,7 (IC 95% : 93,0-98,8)

IgM ISAGA + IgA ELISA			
	POS	NEG	TOT
POS	30	2	32
NEG	10	182	192
TOT	40	184	224

Sens 75,0 (IC 95% : 58,8-87,3)*
Spec 98,9 (IC 95% : 96,1-99,3)

Early and Accurate Diagnosis of Congenital Toxoplasmosis

Laura Ciardelli, PhD, Valeria Meroni, PhD,† Maria Antonietta Avanzini, PhD,* Lina Bollani, MD,‡ Carmine Tinelli, MD,§ Francesca Garofoli, PhD,* Antonella Gasparoni, MD,‖ and Mauro Stronati, MD‡*

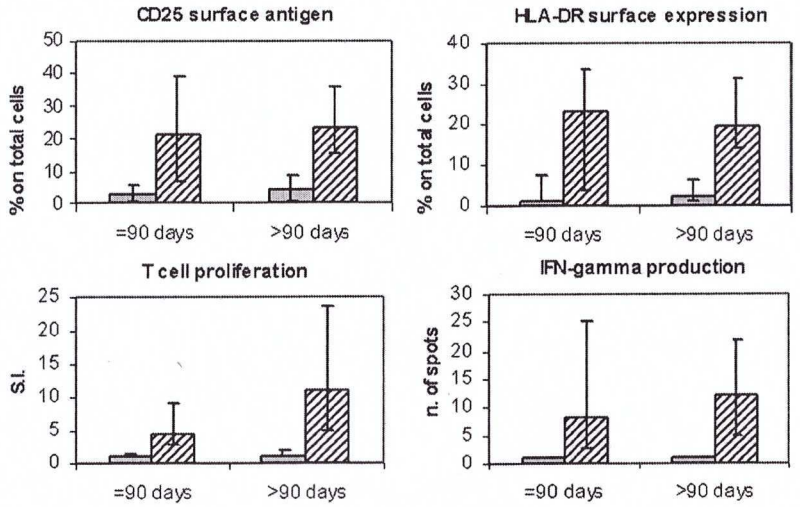


FIGURE 1. Activation antigen expression, T cell proliferation and IFN-γ production in infected and uninfected newborns ≤90 and >90 days of age. Activation antigen CD25 at ≤90 and at >90 days of age, $P < 0.001$ and $P < 0.00001$; activation antigen HLA-DR at ≤90 and at >90 days of age, $P < 0.01$ and $P < 0.00001$; T cell proliferation at ≤90 and at >90 days of age, $P < 0.0001$ and $P < 0.00001$; IFN-γ production, at ≤90 and at >90 days of age, $P < 0.001$ and $P < 0.00001$. Filled bars: uninfected newborns. Striped bars: infected newborns.

Campioni alla nascita

Sierologia

- Sangue periferico materno

Sierologia e immunologia

- 1ml sangue periferico del neonato in eparina

Materiali e Metodi

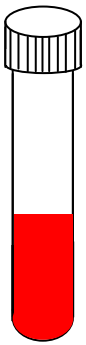
622 campioni da 1ml di sangue intero in Litio Eparina provenienti da 283 bambini (120 femmine e 163 maschi) nati da madre con infezione certa o probabile da *Toxoplasma* in gravidanza.

546 da 251 neonati non infetti e 76 da 32 neonati con infezione congenita accertata alla nascita e/o al termine dell'anno di follow-up.

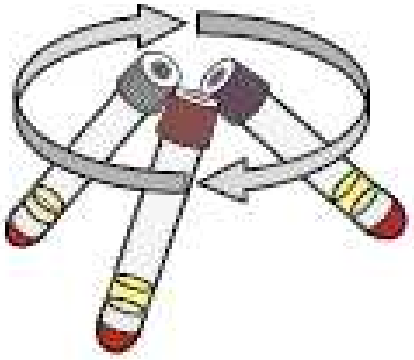
Di questi, 113 sono stati seguiti dalla nascita, 143 entro i primi tre mesi di vita e comunque tutti sono stati controllati con i test sierologici in uso presso il laboratorio fino all'anno di età per escludere eventuali infezioni

1. ESECUZIONE DEL TEST

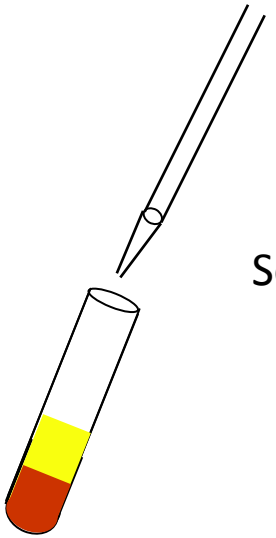
QuantiFERON Cellestis® (ADA)



1 ml di sangue in
Litio Eparina

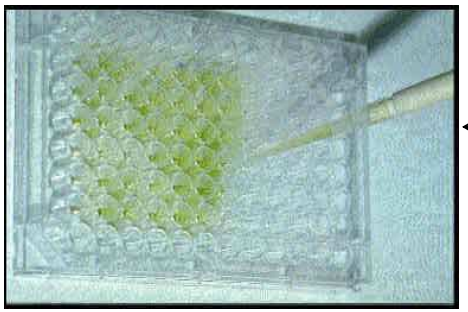


Centrifugato a bassa velocità
per 10 min

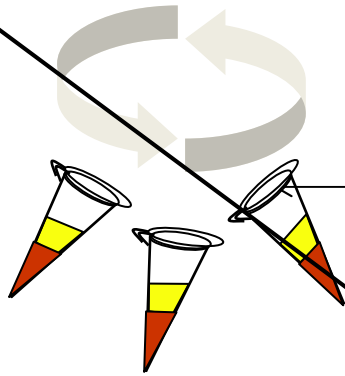


PLASMA UTILIZZATO PER I TEST SIEROLOGICI

Sostuire il plasma
con uno stesso
volume di
terreno RPMI

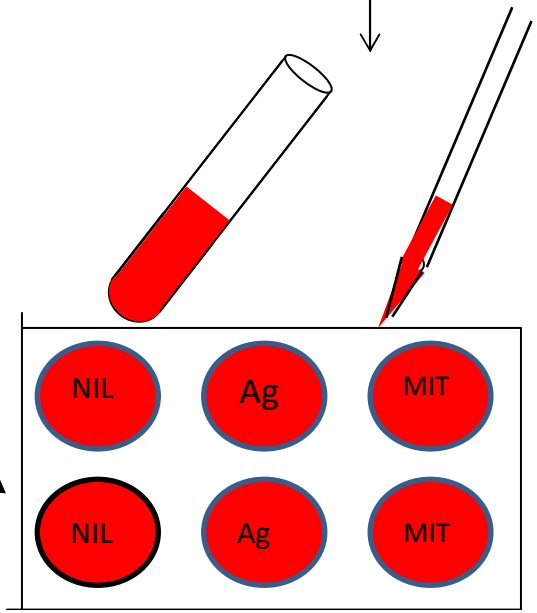


Conservare il plasma a -
80°C fino all'esecuzione del
test ELISA

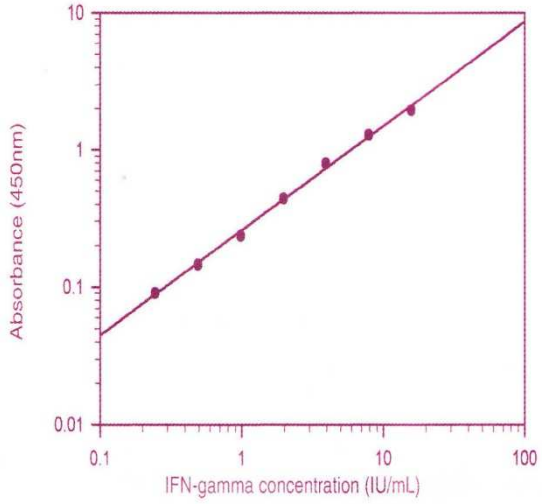


Raccogliere il surnatante
di ogni pozzetto

Incubare a
37°C + CO_2
per 24h



Il dosaggio quantitativo dell'INF-γ viene eseguito mediante un test ELISA (QuantiFERON CMI Cellestis®) e calcolato grazie ad un apposito software per l'analisi dei dati grezzi, espresso poi in UI/ml



Sovrapponibilità dei dati

Su 122 campioni è stato possibile effettuare il dosaggio sia in S (sangue intero) che in R (plasma sostituito con RPMI)

RISULTATI SOVRAPPONIBILI ⟶ Solo R

```
. kap  risul_r risul_s, t

           |            risul_s
   risul_r |         N         ND          P |     Total
-----------+---------------------------------+----------
         N |        39         13          2 |        54
        ND |         5         36          1 |        42
         P |         2          2         18 |        22
-----------+---------------------------------+----------
     Total |        46         51         21 |       118

             Expected
Agreement    Agreement      Kappa    Std. Err.          Z      Prob>Z
------------------------------------------------------------------------
  78.81%       36.54%     0.6661       0.0672       9.92      0.0000
```

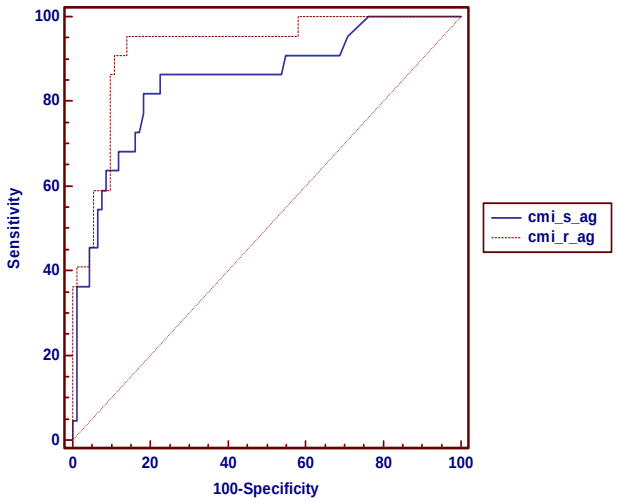
Sensibilità, Specificità, Cut-Off

SENSIBILITA' ⟶ **S: 81,58% (IC 95%)**
R: 93% (IC95%)

SPECIFICITA' ⟶ **S: 83,48% (IC 95%)**
R: 89,7% (IC95%)

CUT OFF ⟶ **S: 0,151 (IC 95%)**
R: 0,156 (IC95%)

P=0.076



CONCLUSIONI

Nessuno dei neonati con infezione congenita è risultato negativo a tutti i prelievi effettuati, in qualche caso si è verificata un'assenza di risposta nella produzione di INF-γ nei primi prelievi

Minor capacità di risposta del neonato

Precoce somministrazione della terapia

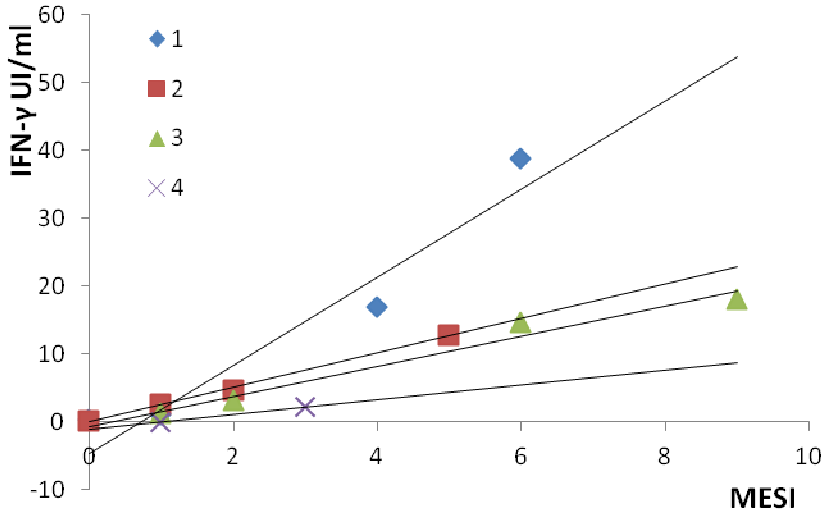
Il numero di test non determinati è stato del 16%

Secrezione spontanea di INF-γ nei controlli

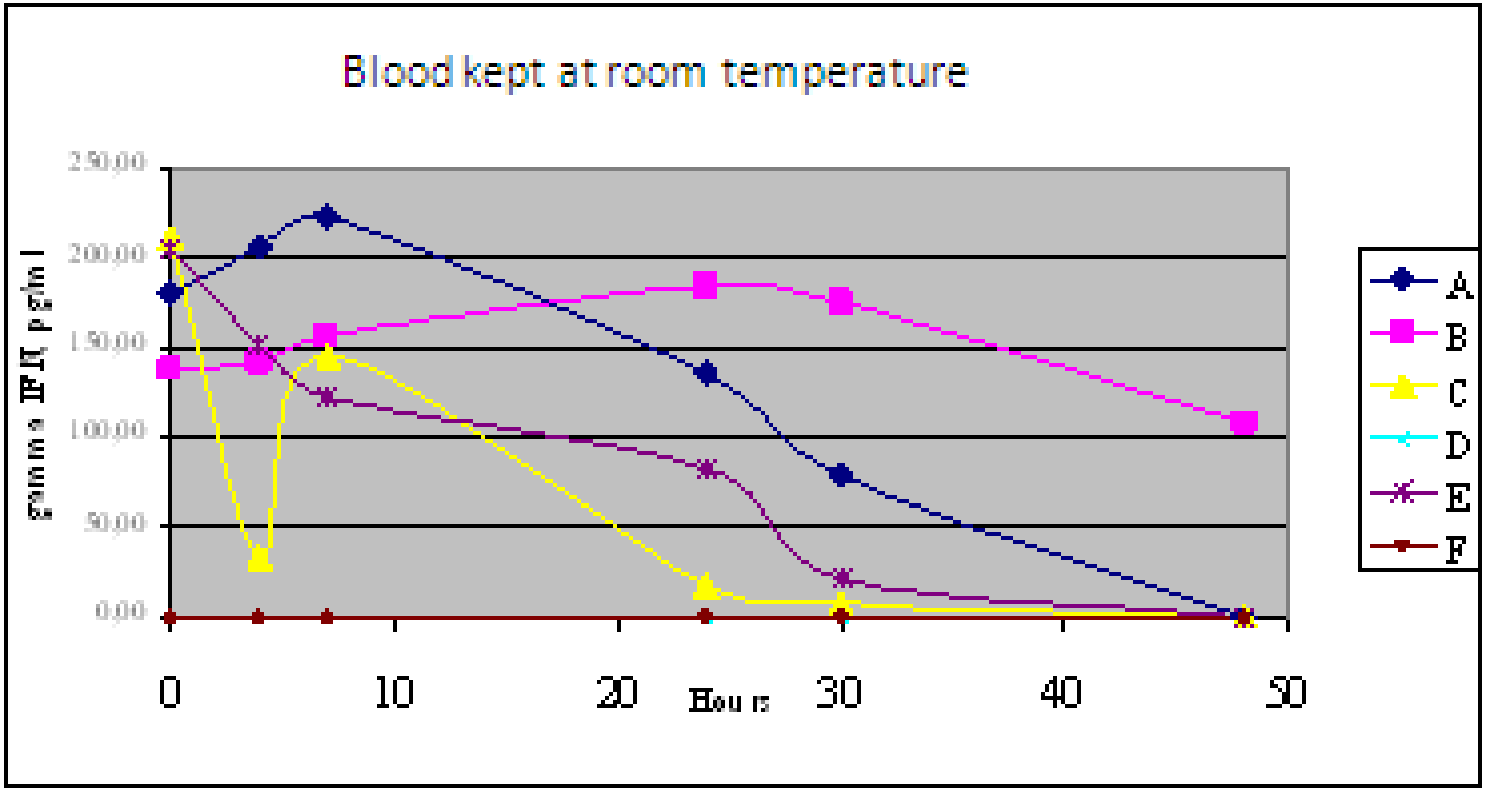
Mancanza di risposta al mitogeno

La ripetizione dell'esame il mese successivo ha portato ad una definizione del risultato del test

Aumento del release di INF-γ al progredire dell'età



Intervallo tra momento del prelievo e esecuzione del test



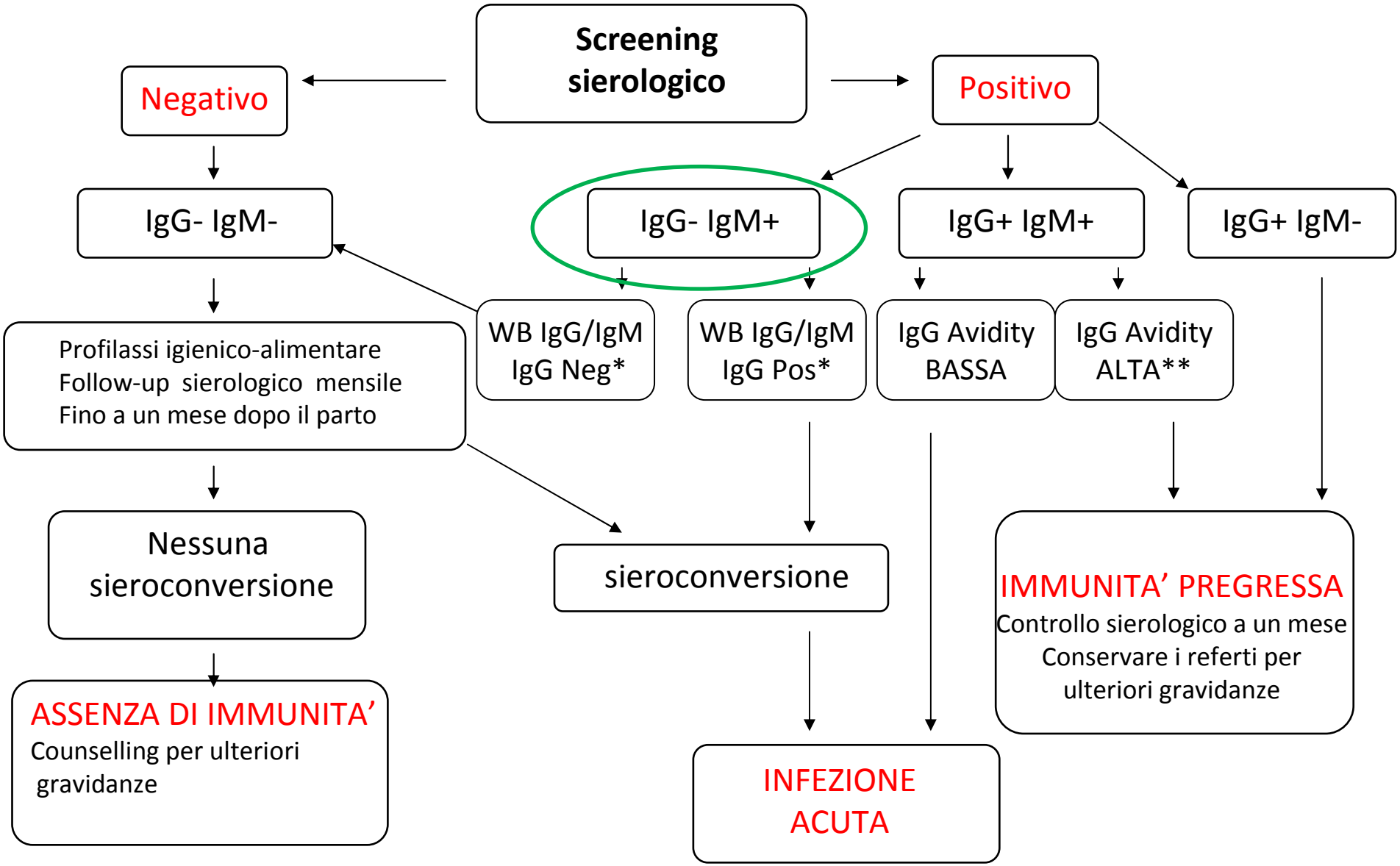
- Decremento importante della secrezione di IFN-γ dopo 24vore

J Clin Microbiol. 2010 Jan;48(1):41-5.
Diagnosis of congenital toxoplasmosis by using a whole-blood gamma interferon release assay.
Chapey E, WallonM, Debize G, Rabillaud M, Peyron F

Questo test, associato alla sierologia, ci ha permesso di fare diagnosi di infezione congenita in TUTTI I NEONATI INFETTI ENTRO I PRIMI 3 MESI DI VITA, potendo così sottoporli ad un tempestivo e corretto trattamento farmacologico

A TUTTI I NEONATI risultati NON INFETTI, non è stata somministrata nessuna terapia, ed hanno confermato l'assenza di infezione con la completa sieronegatività all' anno di vita

Diagnosi della toxoplasmosi in gravidanza



* Nei successivi controlli settimanali in assenza di terapia

** Se il test viene eseguito nel primo trimestre di gravidanza

Effectiveness of prenatal treatment for congenital toxoplasmosis: a meta-analysis of individual patients' data

UA1

The SYROCOT (Systematic Review on Congenital Toxoplasmosis) study group*

Summary

Background Despite three decades of prenatal screening for congenital toxoplasmosis in some European countries, uncertainty remains about the effectiveness of prenatal treatment.

Methods We did a systematic review of cohort studies based on universal screening for congenital toxoplasmosis. We did a meta-analysis using individual patients' data to assess the effect of timing and type of prenatal treatment on mother-to-child transmission of infection and clinical manifestations before age 1 year. Analyses were adjusted for gestational age at maternal seroconversion and other covariates.

Findings We included 26 cohorts in the review. In 1438 treated mothers identified by prenatal screening, we found weak evidence that treatment started within 3 weeks of seroconversion reduced mother-to-child transmission compared with treatment started after 8 or more weeks (adjusted odds ratio [OR] 0·48, 95% CI 0·28–0·80; p=0·05). In 550 infected liveborn infants identified by prenatal or neonatal screening, we found no evidence that prenatal treatment significantly reduced the risk of clinical manifestations (adjusted OR for treated vs not treated 1·11, 95% CI 0·61–2·02). Increasing gestational age at seroconversion was strongly associated with increased risk of mother-to-child transmission (OR 1·15, 95% CI 1·12–1·17) and decreased risk of intracranial lesions (0·91, 0·87–0·95), but not with eye lesions (0·97, 0·93–1·00).

Interpretation We found weak evidence for an association between early treatment and reduced risk of congenital toxoplasmosis. Further evidence from observational studies is unlikely to change these results and would not distinguish whether the association is due to treatment or to biases caused by confounding. Only a large randomised controlled clinical trial would provide clinicians and patients with solid evidence of the potential benefit of prenatal

Lancet 2007; 369: 115–22

*Members listed at end of report

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PLoS MEDICINE

Prenatal Treatment for Serious Neurological Sequelae of Congenital Toxoplasmosis: An Observational Prospective Cohort Study

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Abstract

Background: The effectiveness of prenatal treatment to prevent serious neurological sequelae (SNSD) of congenital toxoplasmosis is not known.

Methods and Findings: Congenital toxoplasmosis was prospectively identified by universal prenatal or neonatal screening in 14 European centres and children were followed for a median of 4 years. We evaluated determinants of postnatal death or SNSD defined by one or more of functional neurological abnormalities, severe bilateral visual impairment, or pregnancy termination for confirmed congenital toxoplasmosis. Two-thirds of the cohort received prenatal treatment (189/293; 65%). 23/293 (8%) fetuses developed SNSD of which nine were pregnancy terminations. Prenatal treatment reduced the risk of SNSD. The odds ratio for prenatal treatment, adjusted for gestational age at maternal seroconversion, was 0.24 (95% Bayesian credible intervals 0.07–0.71). This effect was robust to most sensitivity analyses. The number of infected fetuses needed to be treated to prevent one case of SNSD was three (95% Bayesian credible intervals 2–15) after maternal seroconversion at 10 weeks, and 18 (9–75) at 30 weeks of gestation. Pyrimethamine-sulphonamide treatment did not reduce SNSD compared with spiramycin alone (adjusted odds ratio 0.78, 0.21–2.95). The proportion of live-born infants with intracranial lesions detected postnatally who developed SNSD was 31.0% (17.0%–38.1%).

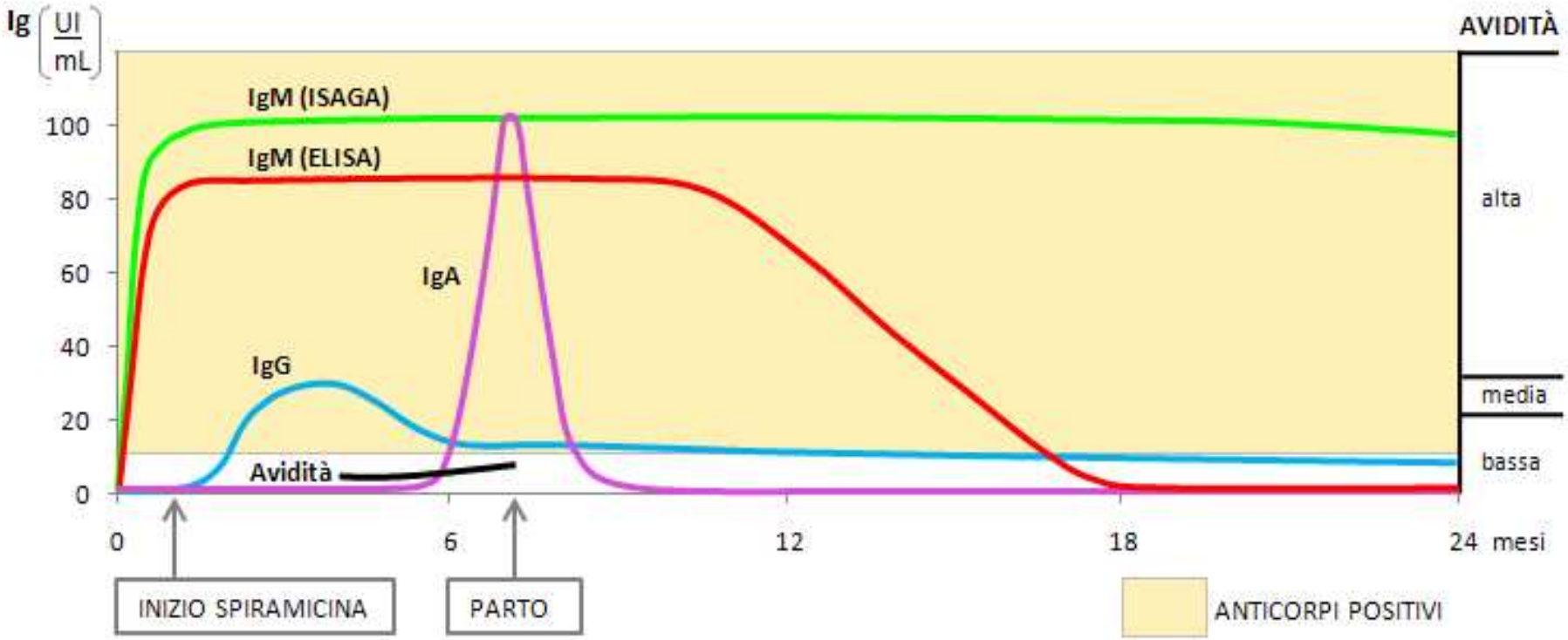
Conclusion: The finding that prenatal treatment reduced the risk of SNSD in infected fetuses should be interpreted with caution because of the low number of SNSD cases and uncertainty about the timing of maternal seroconversion. As these are observational data, policy decisions about screening require further evidence from a randomized trial of prenatal screening and from cost-effectiveness analyses that take into account the incidence and prevalence of maternal infection.

Please see later in the article for the Editors' Summary.

Diapositiva 21

UA1 Utente Autorizzato; 09/05/2007

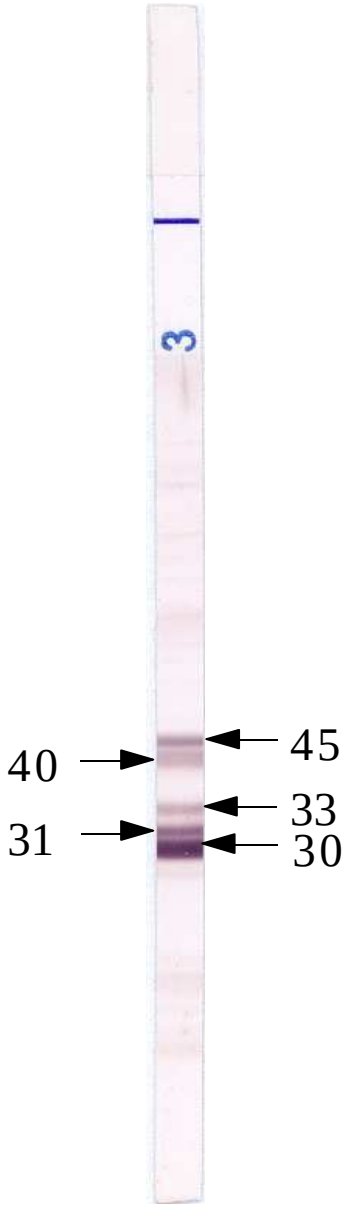
Cinetica anticorpale in una gravida trattata



Cinetica anticorpale in una gravida trattata con spiramicina

TEST	02/11/2010	10/11/2010	30/11/2010	23/12/2010	04/01/2011
IgG CLIA	NEG	NEG	8	NEG	25
IgG ELFA	NEG	NEG	ND	4	24
IgM CLIA	POS	POS	POS	POS	POS
IgM ISAGA	12+	12+	12+	12+	12+
IgA ELISA	200	250	200	150	150
WB IgG/IgM	NEG/POS		POS/POS	POS/POS	
IgG AVIDITY					0,114

Terapia con Spiramicina dal 10/11 al 15/12 2010



LDBIOTOXO II

- (immunoblot)
- Pronto all'uso
- standardizzato(60'- 60'- 30')
- positive control For IgG included

5 bande specifiche:
30 – 31 – 33 – 40 – 45 kDa

IgG positivo: devono essere presenti almeno 3 bande specifiche

IgM positivo: devono essere presenti almeno 2 bande specifiche, inclusa la banda di 30 kDa

41 gravide IgG-/IgM+ trattate con spiramicina

10	IgM ISAGA NEG	IgG WB NEG	IgM WB NEG	IFN-γ NEG	No seroconversion
14	IgM ISAGA POS	IgG WB NEG	IgM WB NEG	IFN-γ NEG	No seroconversion
13	IgM ISAGA POS	IgG WB POS	IgM WB POS	IFN-γ POS	Seroconversion
4	IgM ISAGA POS	IgG WB NEG	IgM WB POS	IFN-γ NEG	No seroconversion

Cinetica anticorpale in un paziente sottoposto a trapianto di rene

TEST	27/09/2010	18/10/2010	19/11/2010	23/12/2010
IgG CLIA	NEG	8	NEG	NEG
IgG ELFA	NEG	6	NEG	NEG
IgM CLIA	NEG	NEG	NEG	NEG
IgM ISAGA	NEG	NEG	NEG	NEG
IgA ELISA	NEG	NEG	NEG	NEG
WB IgG/IgM		POS/NEG		NEG
IFN-γ		NEG		

GRAZIE PER L’ ATTENZIONE