Herpesviruses

Tools of diagnosis : what to use and when

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Evolution of the techniques in the virology lab

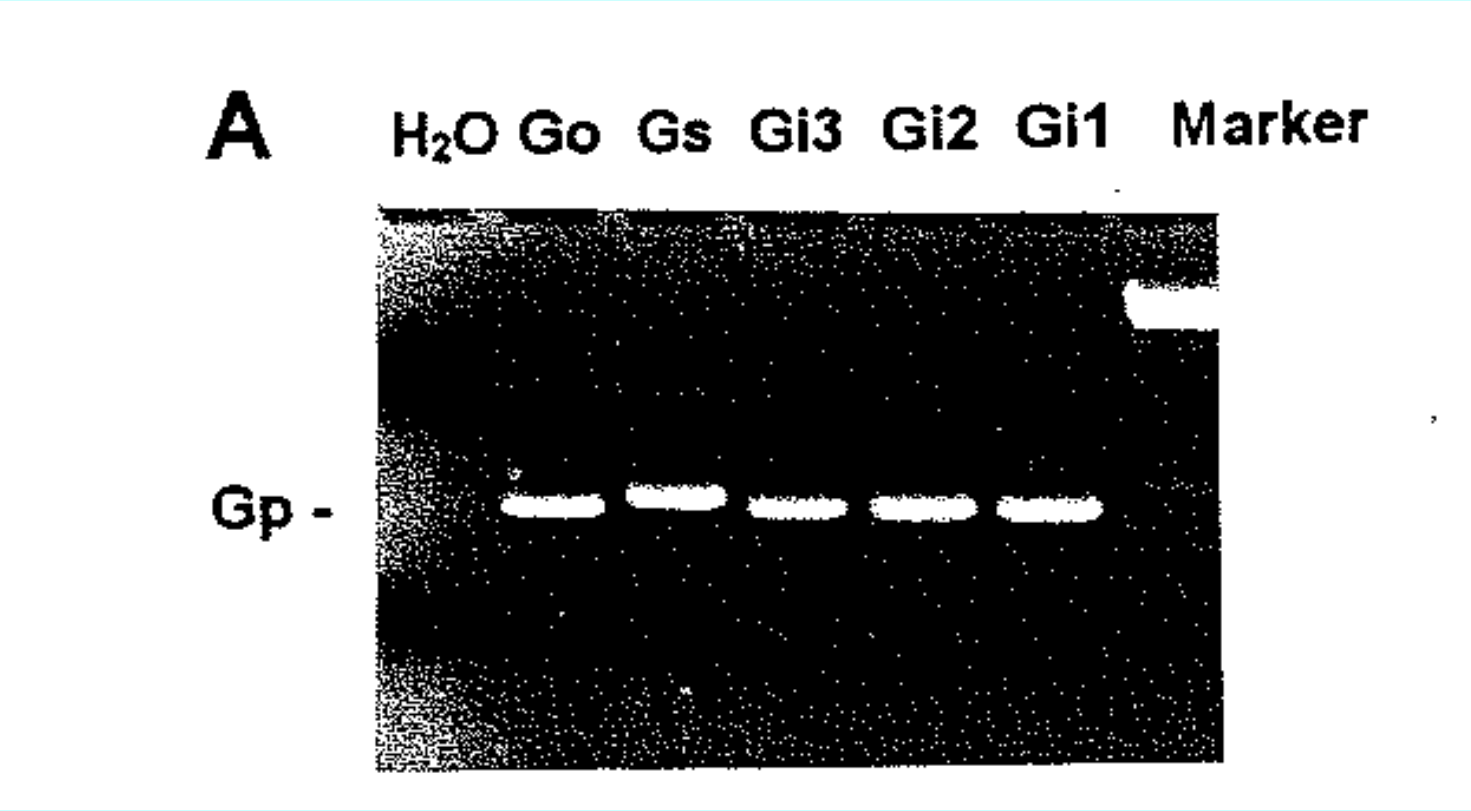
Techniques : "Classic" methods

	Ag detection	Viral culture	Serology
HSV 1-2	direct examination (DE)	+	+, ...
VZV	DE	+/-	+
CMV	Ag pp65 (blood)	+	+
	DE		
EBV	NA	NA	+
HHV6	NA	coculture	+
HHV7	NA	NA	+, but ...
HHV8	NA	NA	+

Techniques : Molecular methods

- Detection and quantification of herpesviruses
- Rapid evolution of molecular diagnosis techniques

PCR end point : <u>detection</u> <u>quantification</u>



Techniques : Molecular methods – quantification

Real time PCR (detection and quantification)

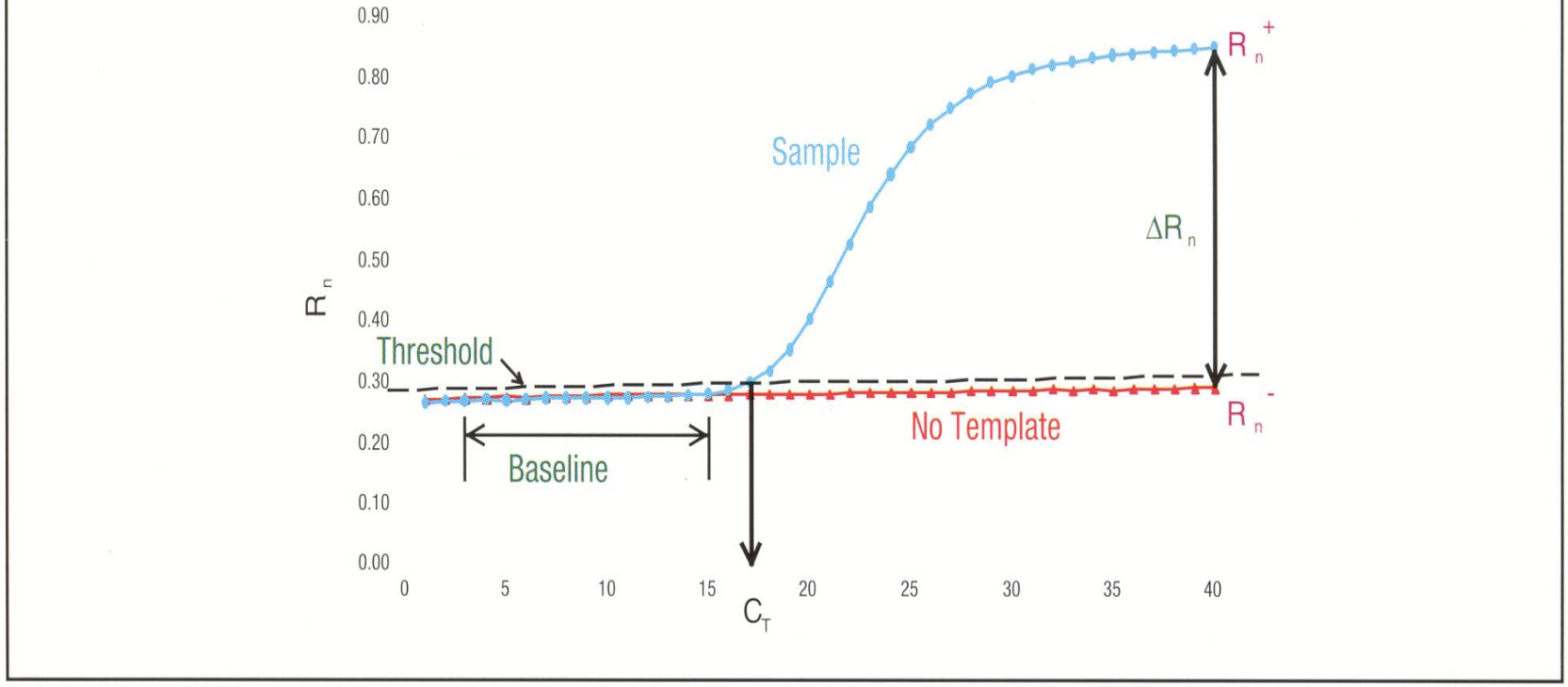


Figure 2. Model of a single amplification plot, showing terms commonly used in real-time quantitative PCR.

Rapid viral culture / Real time PCR (711 specimens)

- throat, cutaneous, genital swabs (% positive)*

	HSV1	HSV2	VZV	CMV
Culture	19	10.8	4.6	14
RT-PCR	21.4	14.5	7.4	19.8

* most of them associated with lesions

TABLE 5. Ct values of real-time PCR according to virus and culture-immunofluorescence result

Virus and culture result	No. of specimens[a]	Ct value		Difference between sample means (95% confidence interval)	*P*
		Mean	SD		
CMV					
Positive	10	33.1	6.25	−2.14 (−8.39–4.12)	NS[b]
Negative	6	36.3	4.35		
VZV					
Positive	15	19.5	4.75	−4.57 (−9.00–1.29)	>0.01, <0.05
Negative	11	24.1	5.95		
HSV-1					
Positive	125	27.0	6.33	−7.77 (−10.7–−4.79)	<0.01
Negative	19	34.8	4.25		
HSV-2					
Positive	72	23.2	4.35	−10.5 (−12.6–−8.39)	<0.01
Negative	25	33.7	5.16		

[a] Some specimens are missing Ct data.
[b] NS, not significant.

Real time PCR / nested PCR comparison in patients suspected of HSV2 meningitis

	N pts	RT	nested
HSV2 meningitis total	65	52 (80%)	47 (72%)
primary	38	33 (87%)	33 (87%)
recurrent	27	19 (70%)	14 (52%)
Aseptic meningitis*	45	1	0

* 2 VZV +

(- for borrelia, TBE, HSV1)

Franzen-Röhl, 2007

Prevalence of viruses detected in the CSF by PCR (1995-2001)

Real time PCR

Virus	N of PCR + in CSF samples (576)
EV	409 (71%)
HSV1	54 (9,4%)
CMV	41 (7,1%)
VZV	29 (5%)
HSV2	13 (2,3%)
JC virus	12 (2,1%)
EBV	11 (1,9%)
HHV6	5 (0,9%)
Parvovirus	2 (0,3%)

26% = Herpesviruses

↗ diagnosis by testing for different viruses

↗ in VZV diagnosis

Aberle, 2003

***Table 1** Etiology of aseptic meningitis*

Etiology	Confirmed	Probable	Possible	Total, no. (%)
Enterovirus	33	5		38 (26)
HSV-2	22	2		24 (17)
VZV	8	4		12 (8)
TBEV	2	6		8 (6)
HSV-1*	3			3 (2)
Other defined agents†	4	4	2	10 (7)
Undefined agents				49 (34)

* Both HSV-1 and VZV etiologies in one patient.
† EBV (n = 2), *M pneumoniae* (n = 2), *B burgdorferii* (n = 2), adenovirus (n = 1), parainfluenzavirus (n = 1), *T gondii* (n = 1), and trimethoprim (n = 1).

HSV = herpes simplex virus; VZV = varicella zoster virus; TBEV = tick-borne encephalitis virus.

***Table 4** Diagnostic findings for entero- and herpesviruses from CSF samples*

	PCR		Culture		AB-1		AB-2	
Virus	%	n	%	n	%	n	%	n
Aseptic meningitis, n = 144								
Enterovirus	23	(30/133)	14	(17/121)	3	(2/78)	0	(0/34)
HSV-1	2	(3*/136)	0	(0/121)	0	(0/87)	0	(0/41)
HSV-2	16	(22/136)	0	(0/121)	0	(0/87)	2	(1/41)
VZV	7	(8*/121)	0	(0/121)	3	(2/79)	3	(1/34)
Encephalitis, n = 42								
Enterovirus	0	(0/35)	0	(0/37)	0	(0/13)	0	(0/7)
HSV-1	10	(4†/42)	0	(0/37)	0	(0/33)	9	(2†/22)
HSV-2	0	(0/42)	0	(0/37)	0	(0/33)	0	(0/22)
VZV	11	(4†/35)	0	(0/37)	3	(1/33)	9	(2†/22)

* In one patient, CSF PCR tests were positive for HSV-1 and VZV.

† In one patient, CSF PCR tests were positive for HSV-1 and VZV and intrathecal antibody production developed for both of these viruses.

AB-1 and AB-2 = CSF antibodies in early (1) and in follow-up (2) CSF (only intrathecal antibody production is considered); HSV = herpes simplex virus; VZV = varicella zoster virus.

Etiology of meningitis in Finland (2006)

- PCR
- Antibodies to virus, mycop., chlam., borrelia
- CSF, throat and fecal swabs for viral culture

Aseptic meningitis : 66% etiology

EV : 26% > HSV2 : 17% (25% women) > VZV: 8%

Encephalitis : 36% etiology

VZV : 12% > HSV1 : 9%, TBE : 9%

PCR : + in 45% of aseptic meningitis

+ in 17% of encephalitis

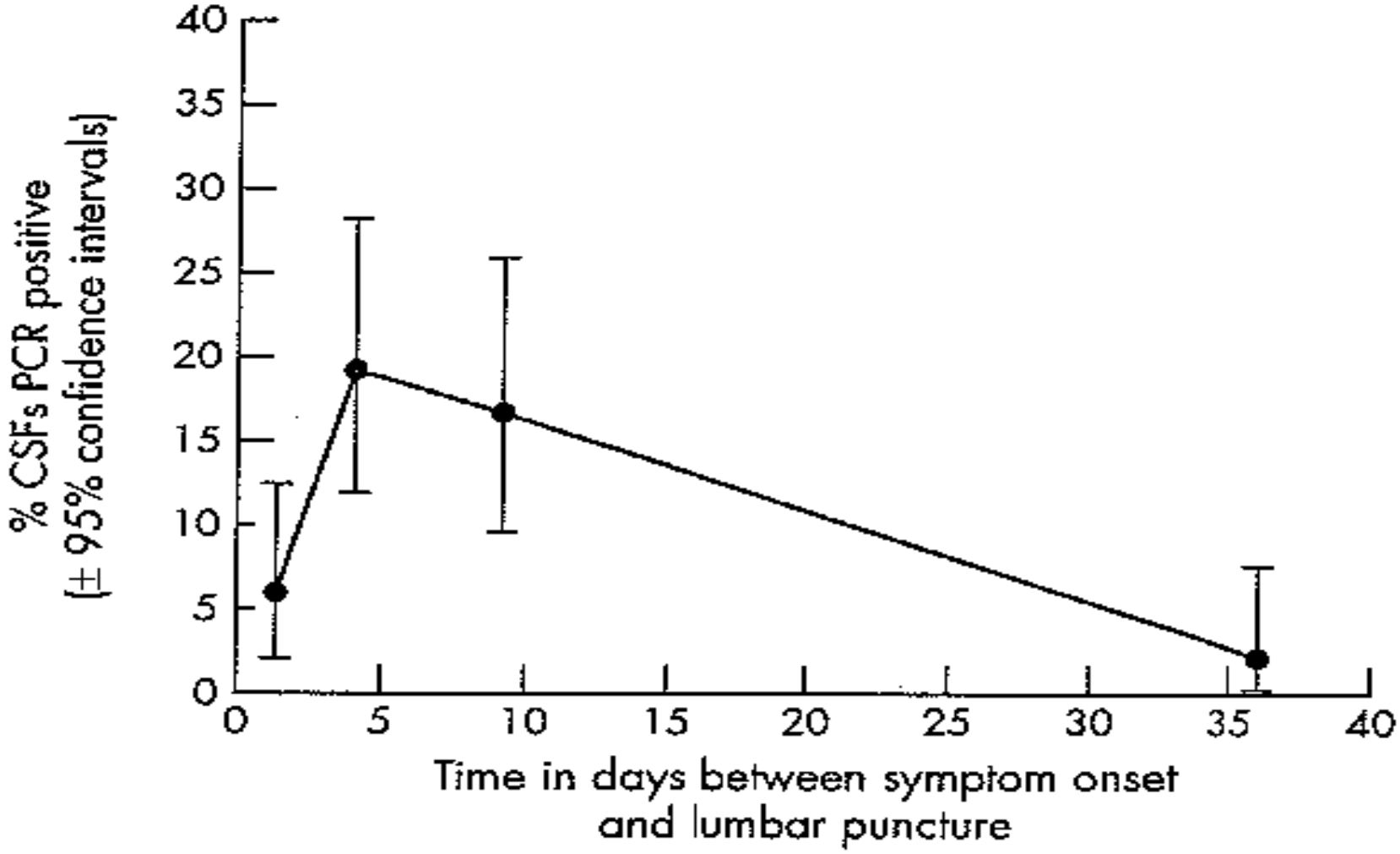
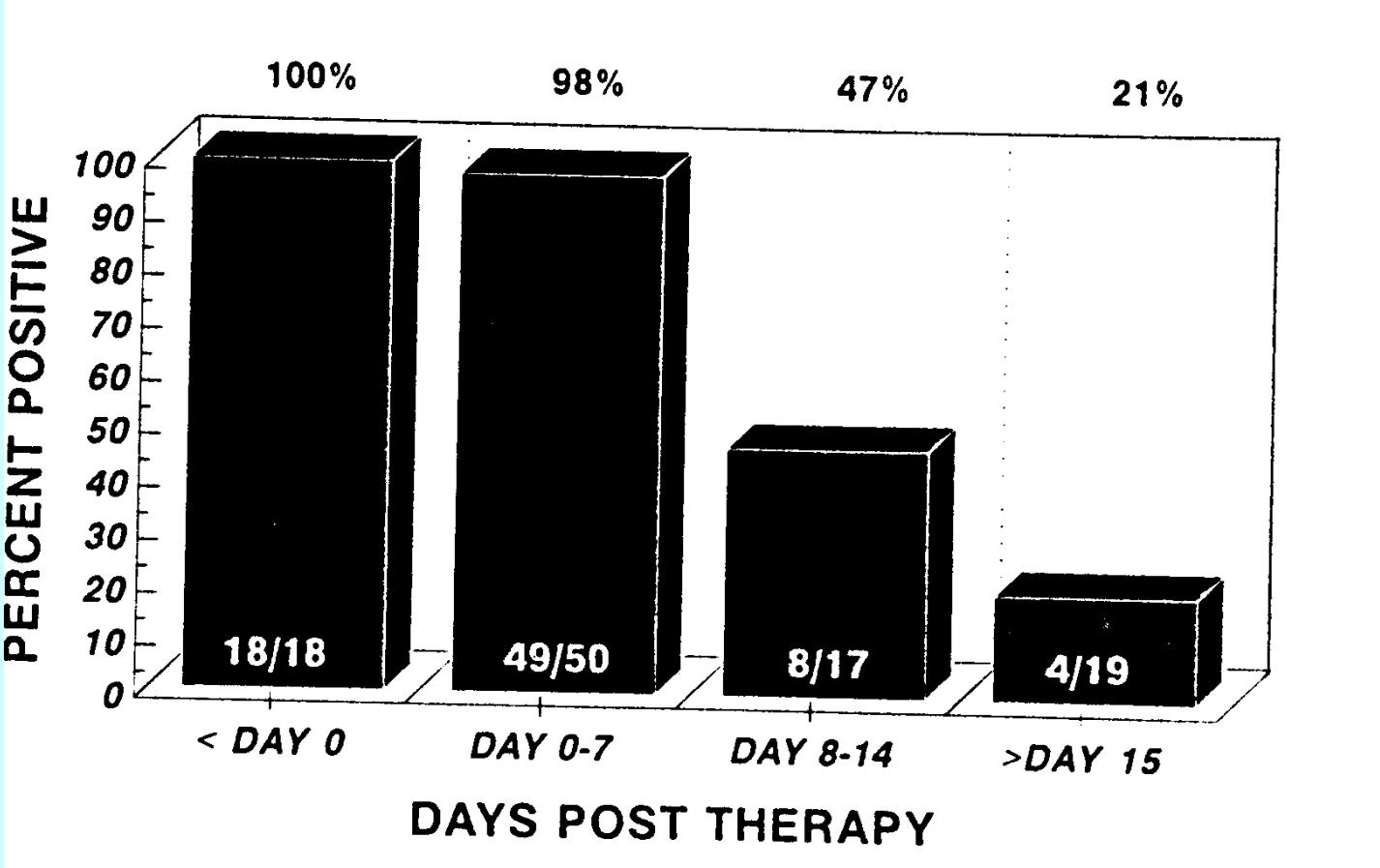


Figure 1 The relationship of virus detection by PCR with time delay between onset of neurological symptoms and lumbar puncture. *Numbers of PCR positive and negative CSF samples in each group are found in table 3.

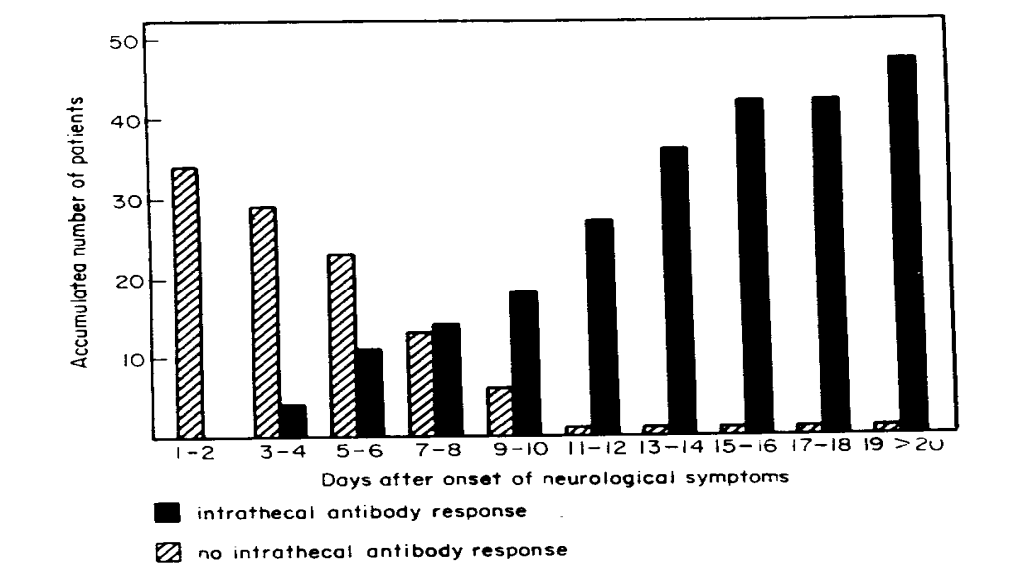
Herpes simplex encephalitis:

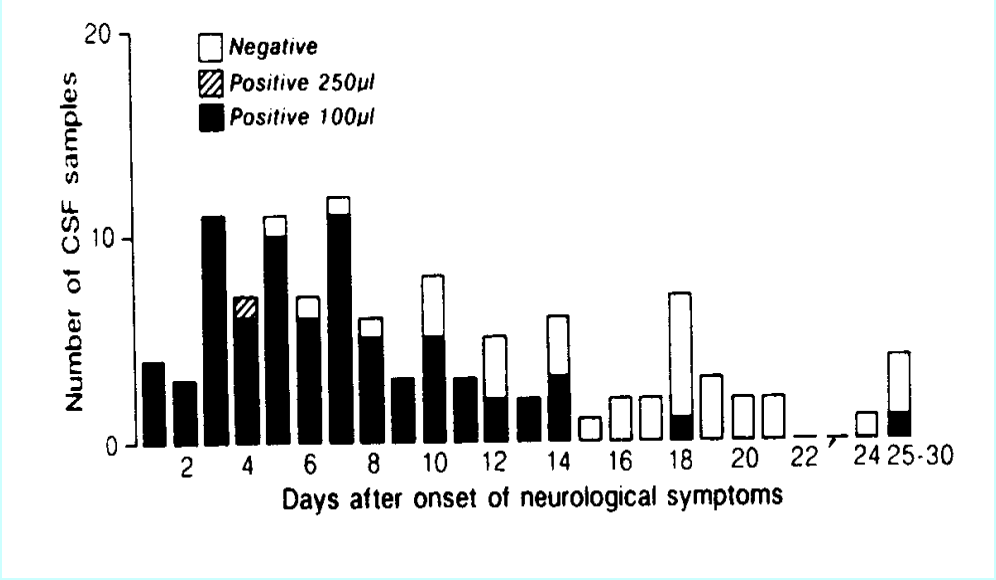
PCR results according to days of therapy



Herpes simplex encephalitis:

Intrathecal antibody production and PCR results





Diagnosis of viral meningitis and encephalitis

What and When to order ?

CSF

early(first days of symptoms)

- **Virus detection by nucleic acid amplification tests**
  Enterovirus (80% sensitivity)
  HSV (95% sensitivity)
- **Culture :**
  Enterovirus (60% sensitivity)
  HSV (4% sensitivity)

late (2-3 weeks)

- **Virus specific antibody intrathecal synthesis**
  HSV (80% sensitivity)
  Enterovirus (serology not available)
  Do not forget a blood sample !

but also : stool sample
throat swab
urine

for viral culture : enterovirus
mumps

Intrathecal production of specific antibodies

1. $\frac{\text{Albumine in CSF}}{\text{Albumine in blood}}$ has to be < 0,009

2. $\frac{\frac{\text{specific antiviral antibodies (quant) in CSF}}{\text{Albumine in CSF}}}{\frac{\text{specific antibodies (quant) in blood}}{\text{Albumine in blood}}}$ >1.91

= Intrathecal production of specific antibodies

CMV pp65 Antigenemia

- phosphoprotein of the viral tegument in the nucleus of PMN in blood

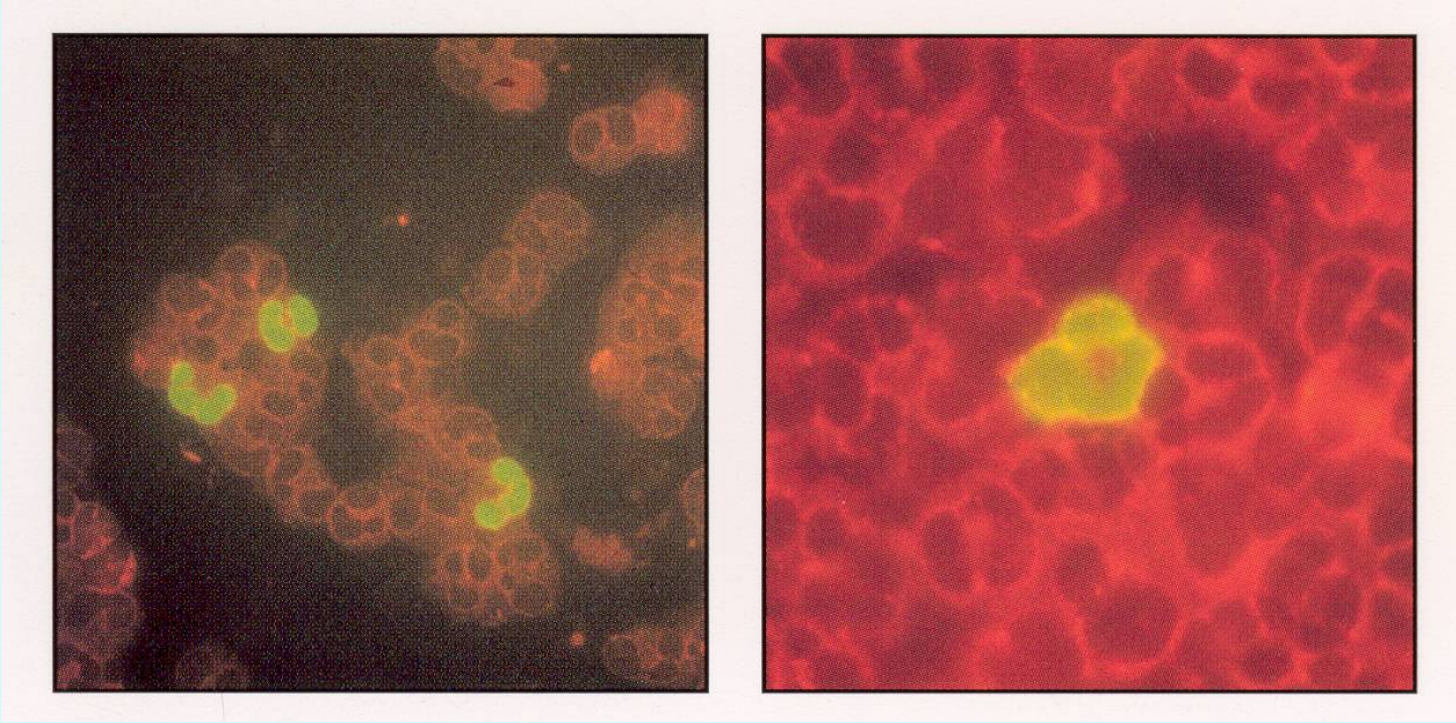
  = active CMV infection

  N. of positive cells/100.000 cells (PMN)

  ± good correlation with viral load as measured by molecular methods

- neutropenia : loss of sensitivity

CMV pp65 antigenemia



Quantification of viral load by Real time PCR

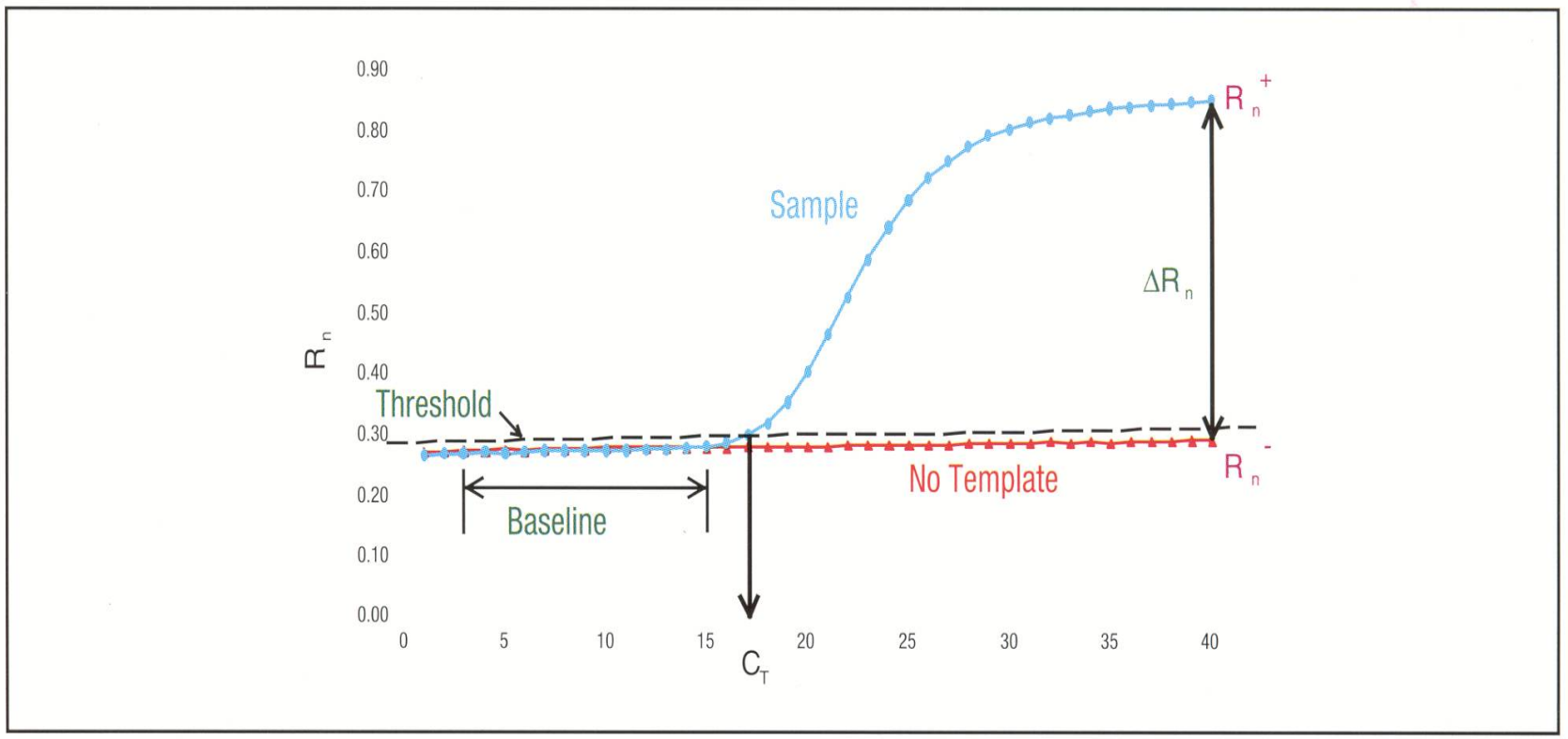
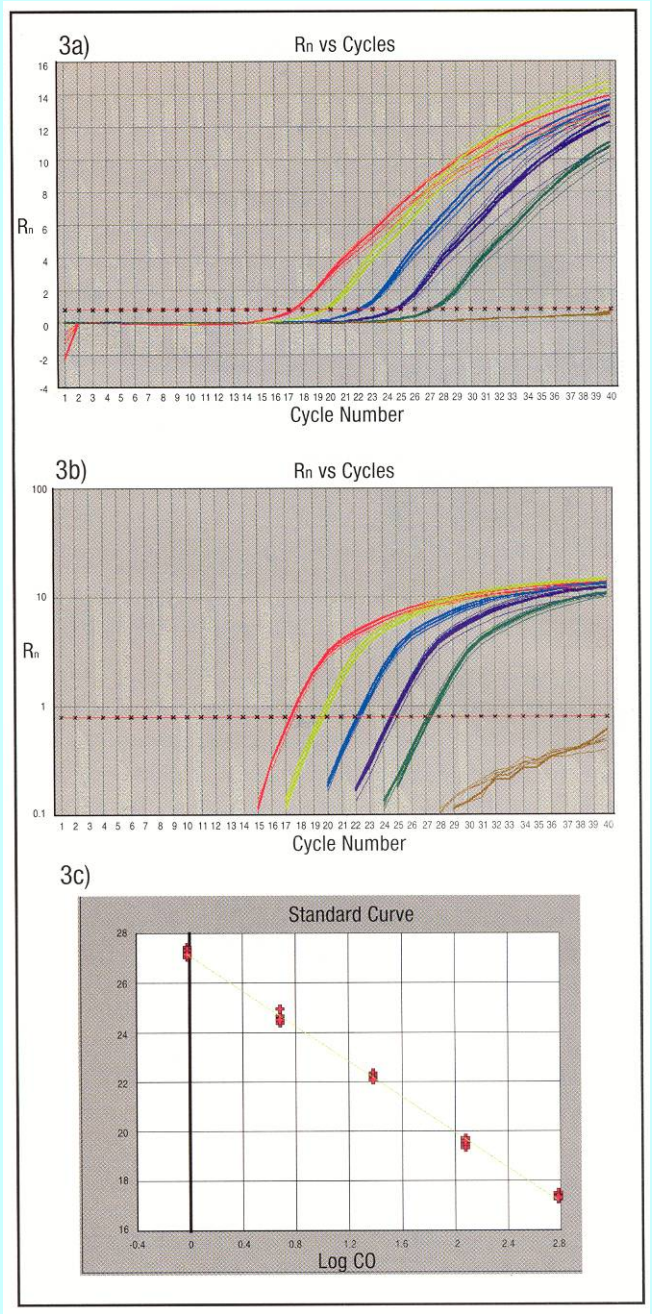
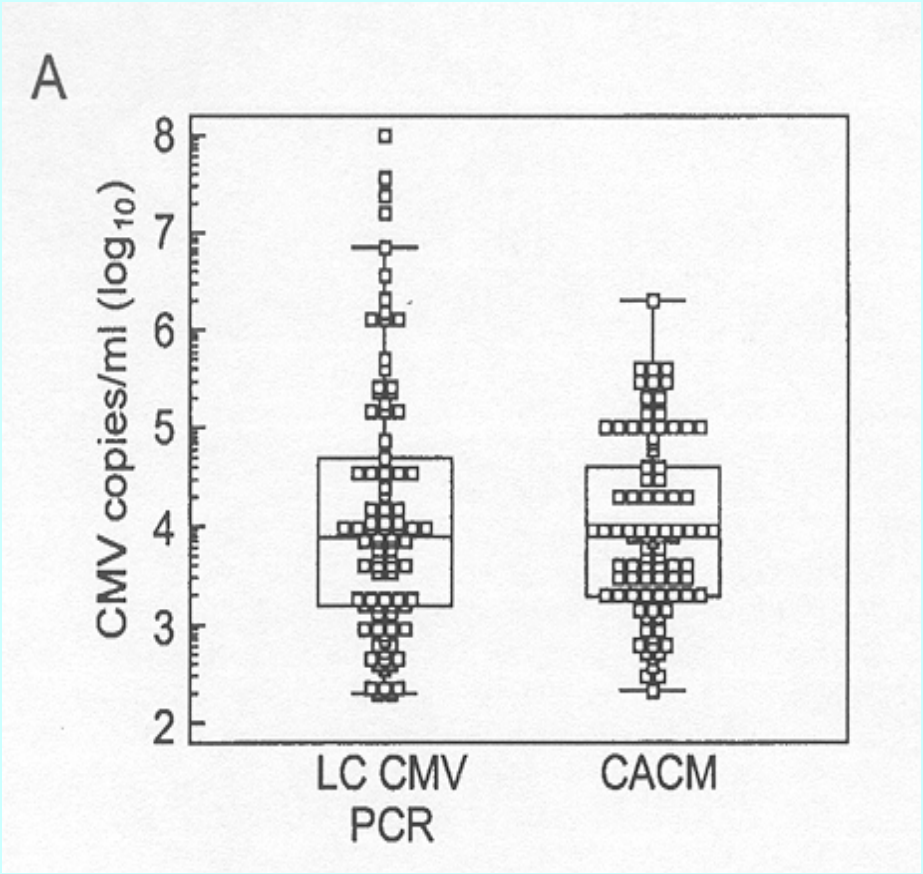


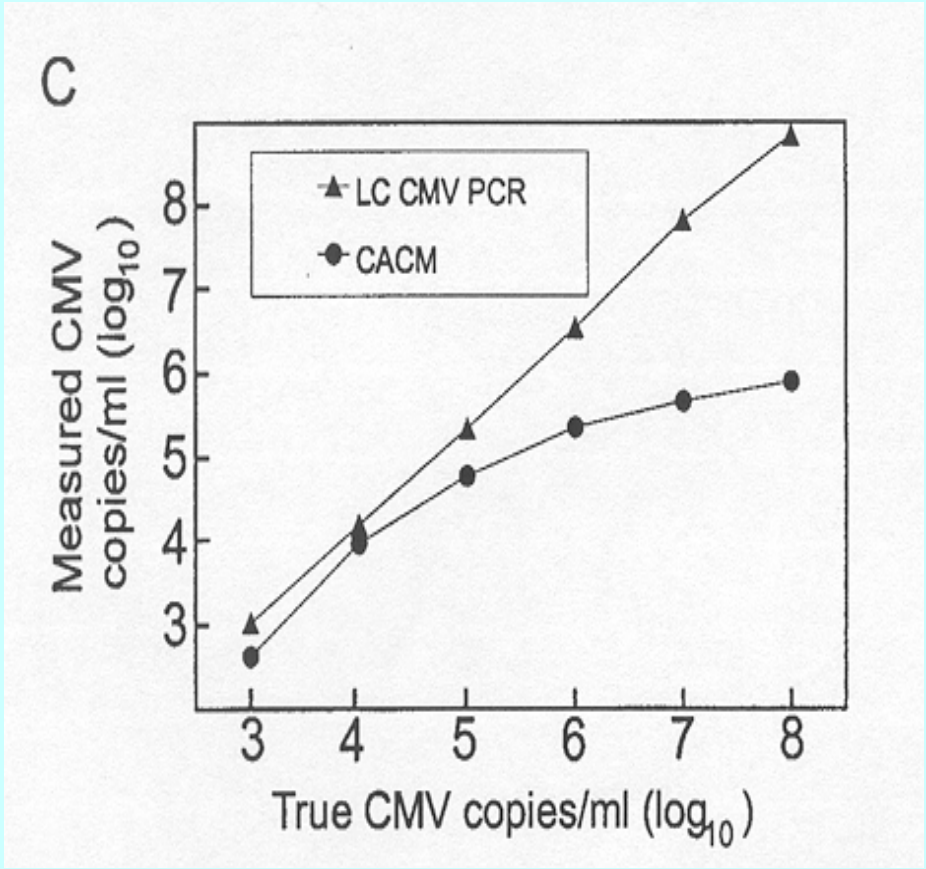
Figure 2. *Model of a single amplification plot, showing terms commonly used in real-time quantitative PCR.*


3a)
Rn vs Cycles
Rn
Cycle Number
3b)
Rn vs Cycles
Rn
Cycle Number
3c)
Standard Curve
Log CO

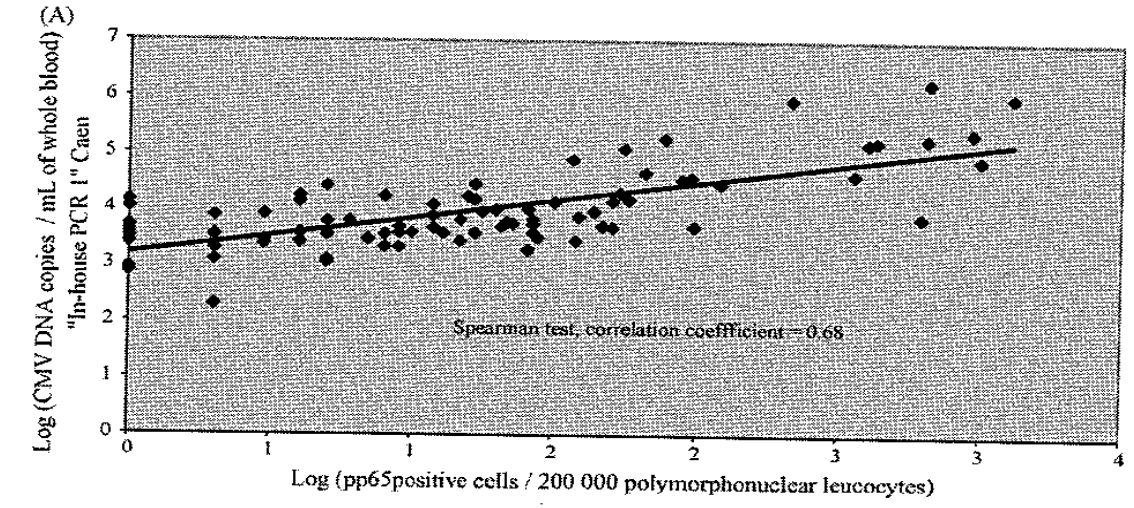
Comparison of real-time quantitative PCR and Cobas amplicor CMV monitor.

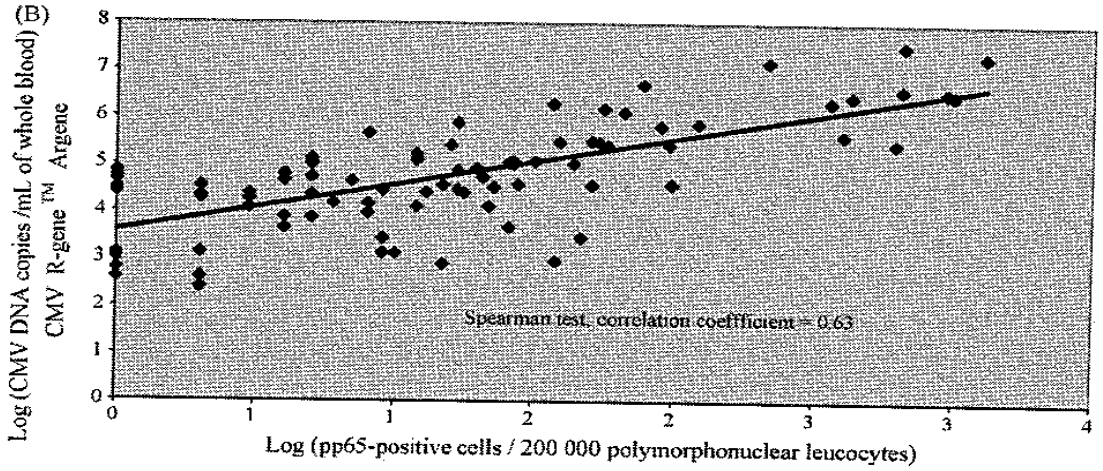
Schaade et al, 2000





CMV viral load : pp65 Ag or real-time PCR (whole blood) in renal transplant patients





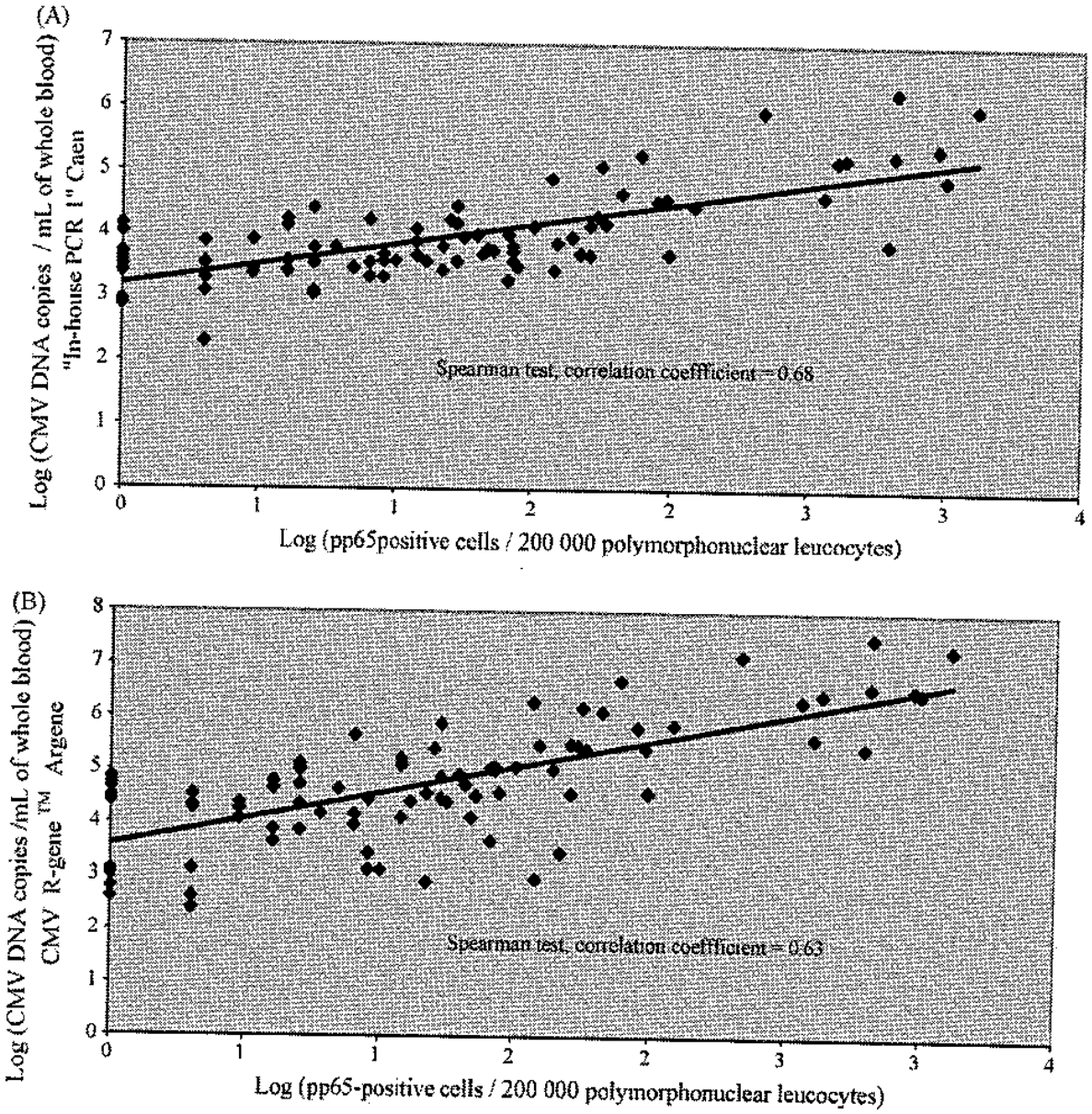


Fig. 1. Correlation between copy number of CMV DNA in whole blood and pp65-positive cells count in polymorphonuclear leukocytes. One hundred and seventeen samples from renal transplant patients of laboratory 1 were analysed using both real-time PCR and pp65 antigenemia assay. The copy numbers of CMV DNA were plotted on a logarithmic graph against the pp65-positive cell numbers obtained by the antigenemia assay. (A) Correlation between the CMV DNA viral load obtained by the "in-house 1" PCR and the pp65-positive cell number (Spearman's rank test $r = 0.68$, $p < 0.0001$). (B) Correlation between the CMV DNA viral load obtained by the CMV R-gene™ test and the pp65-positive cell number (Spearman's rank test $r = 0.63$, $p < 0.0001$).

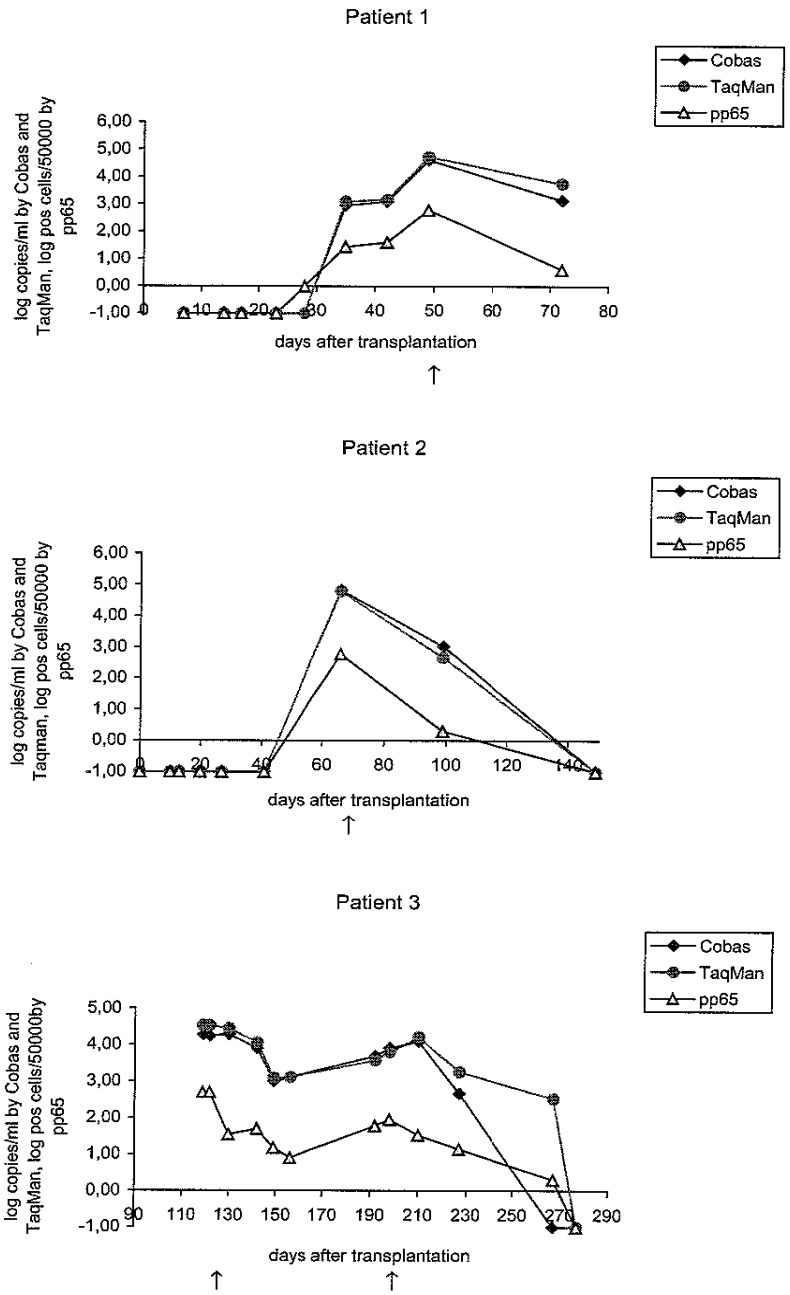


Fig. 2. Results of monitoring of CMV viral load in three individual patients after transplantation measured by Cobas Monitor, TaqMan and pp65 antigenemia. Patient 1 (monitored until discharged to another hospital) and patient 2 are liver transplant patients and patient 3 a kidney transplant recipient. The values below zero on the y axis indicate copy numbers below the detection limits of the PCR assays (Cobas <400 cps/ml and TaqMan <250 cps/ml) or negative antigenemia results. Ganciclovir treatments are marked with arrows.

Ocular Herpetic diseases

Acute retinal necrosis (ARN) syndrome :

- necrotizing retinitis
- retinal arteritis
- inflammatory reaction in vitreous and anterior chamber
- VZV 66%, HSV 22%, EBV 17% (+ VZ)
- diagnosis : PCR and intraocular antibodies

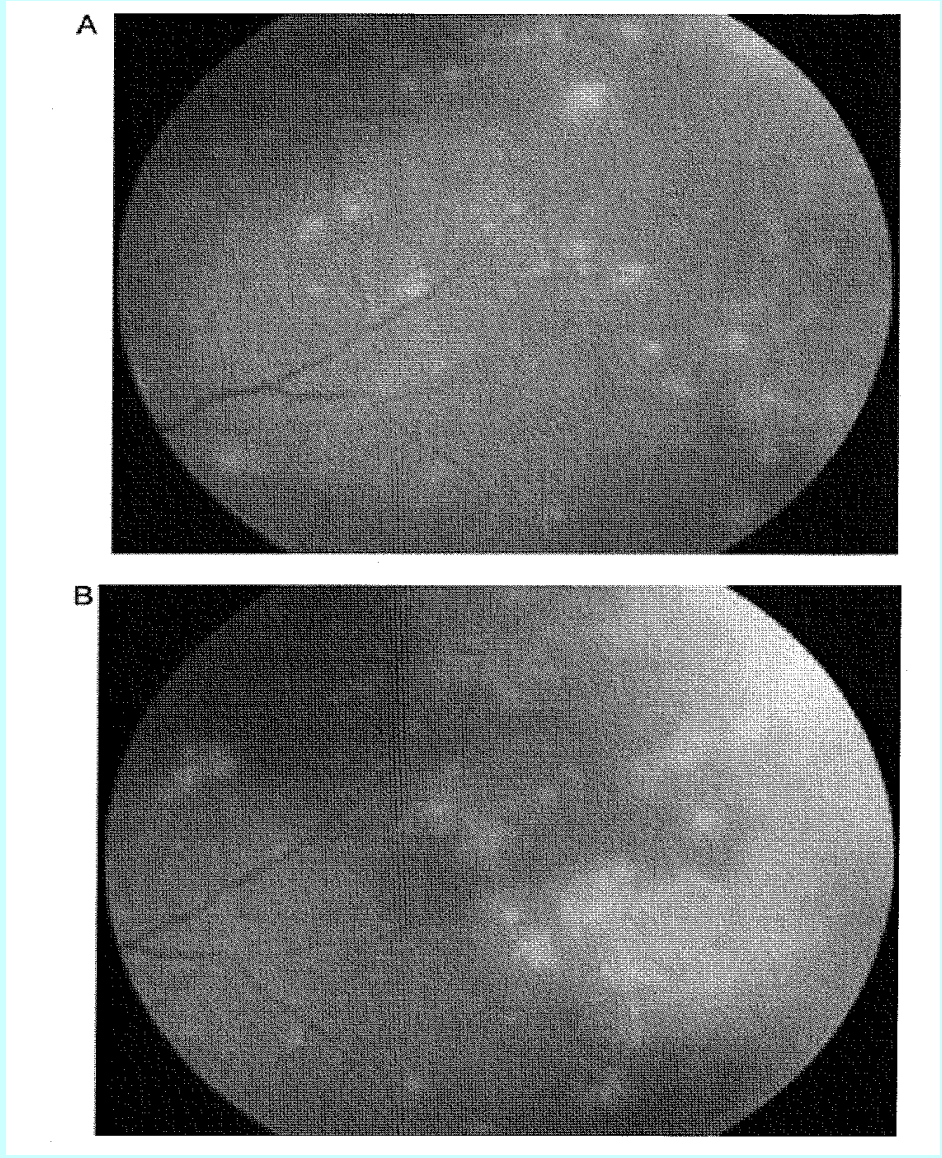
Ocular Herpetic diseases

ARN due to HSV2 (11 cases) :

- mean 22,6 years (25 days – 56)
- 30% bilateral involvement
- immunocompetent individuals

associated with herpes neonate (1)

- corticoïds (3)
- trauma (1)
- chorioretinal scars (3)
- previous episodes of ARN (3)


A
B

Ocular Herpetic diseases

Complications : cataract, epiretinal membrane, retinal detachment, optic nerve atrophy

frequence : 1 cas / 2.10^6 / an

Ocular Herpetic diseases

Diagnosis

- PCR on aqueous and/or vitreous humor
- PCR could help monitoring the treatment
- Japan : ARN due to HSV2 frequent : no preexistence of HSV1 antibodies ?

Ocular Herpetic diseases

- Necrotizing retinitis

Diagnosis : Goldmann – Witmer coefficient

$$\frac{\frac{\text{specific antibodies (eye)}}{\text{IgG (eye)}}}{\frac{\text{specific antibodies (serum)}}{\text{IgG (serum)}}} > 3 \text{ or } 4$$

Herpesviruses and serology

IgG and IgM (commercial kit) :

- enzyme immuno assay
- chemiluminescence assay
- immunofluorescence assay
- immunoblot assay (confirmation)

Indications :

Diagnosis of acute infection : EBV, CMV, HHV6

- presence of IgM
- seroconversion of IgG (2 sérums)
  - negative → positive
  - "significant" increase in IgG

"Immunity" or past contact with the virus

- presence of IgG
  - ex : before organ transplantation (EBV, CMV, VZV)

Indirect diagnosis : HHV8

- Kaposi's sarcoma

Frequent cross-reactivity of EBV IgM and CMV IGM in serologic assays

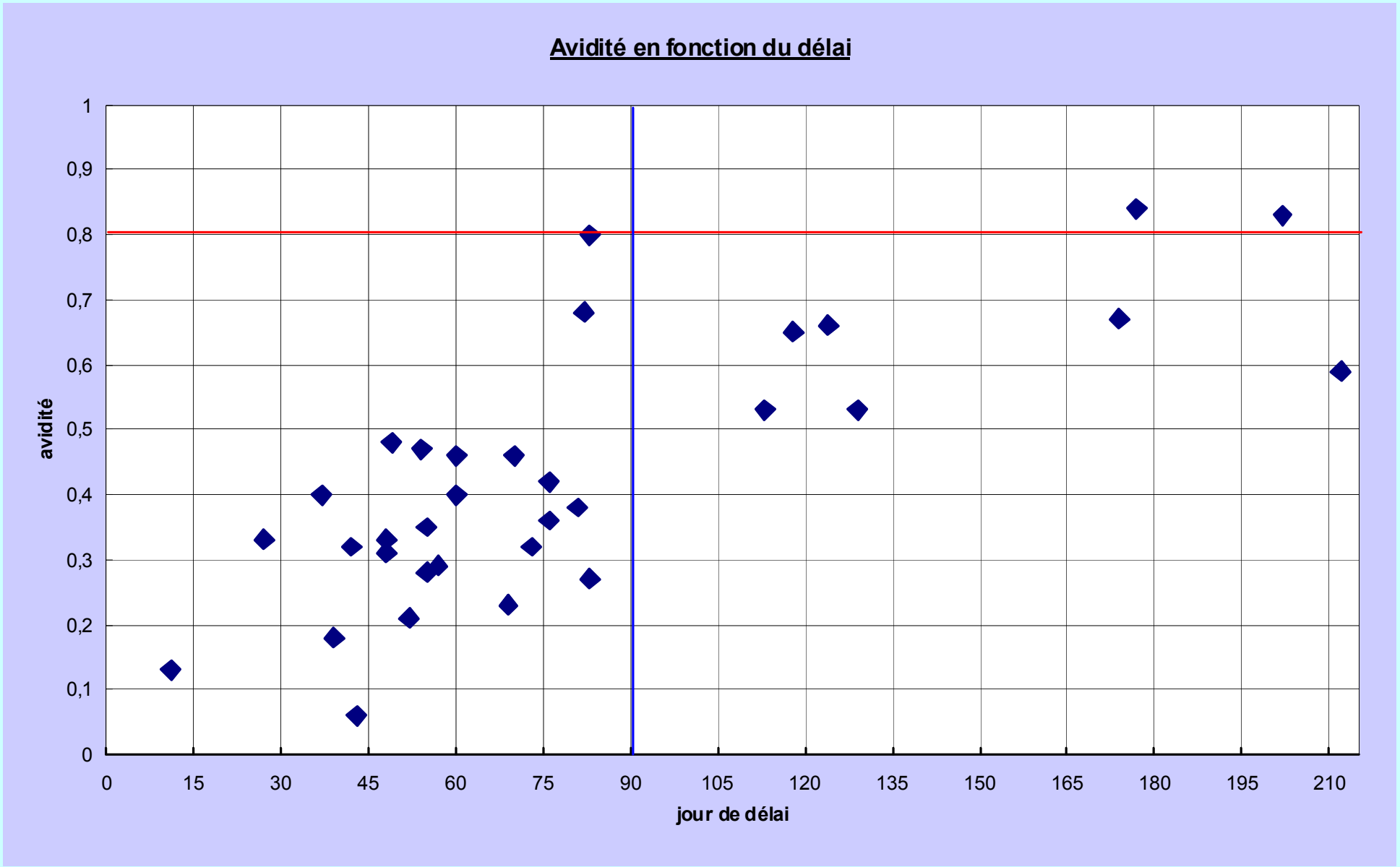
- conventionnal or recombinant Ag

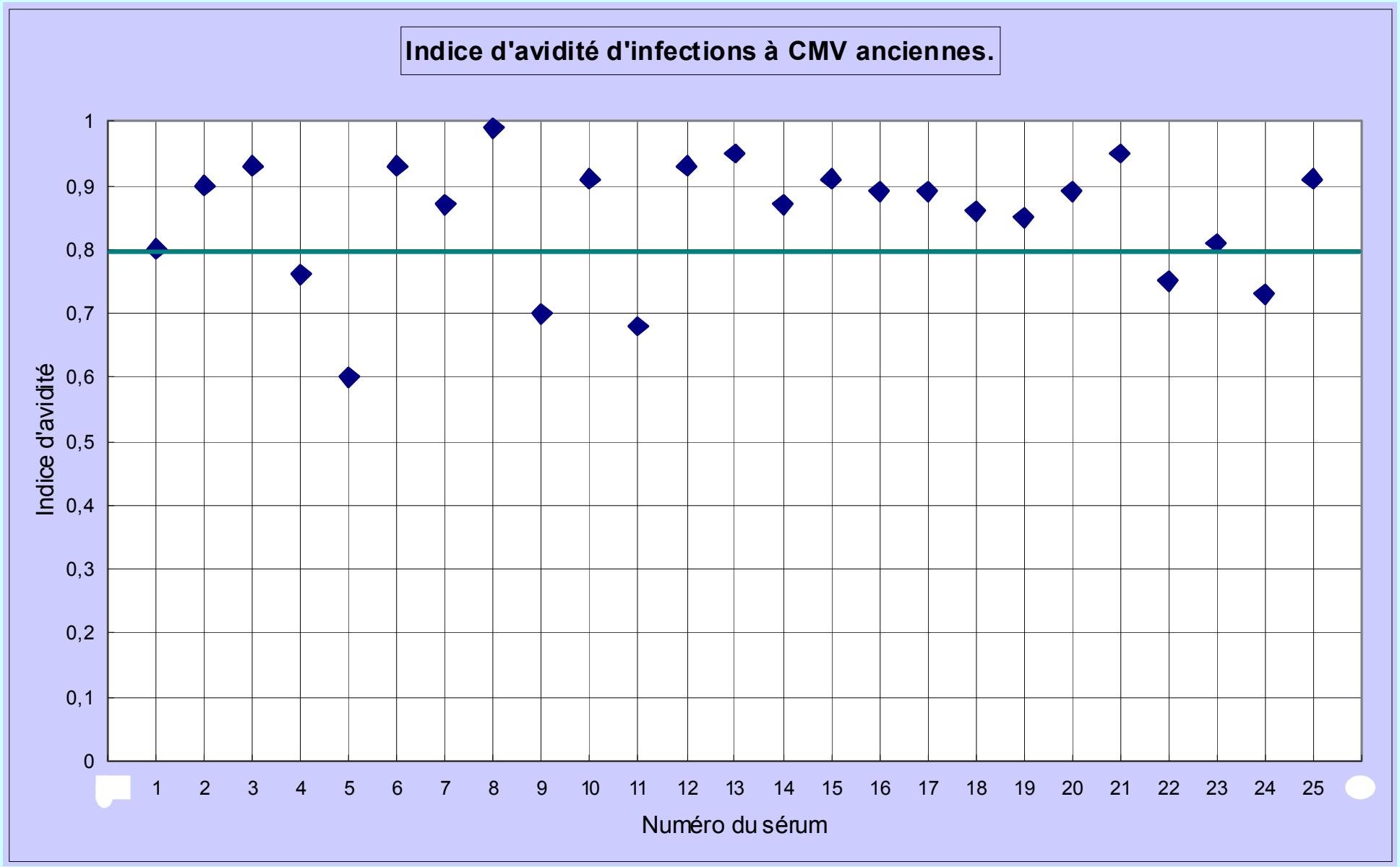
>< - short glycine-rich motifs in pUL44 and pUL57 CMV (major antigenic domain for IgM Ab during CMV)

Primary EBV induces IgM antibodies that bind to widely used diagnostic antigens in CMV IgM tests

IgG avidity

- IgG high avidity : past infection
- IgG low avidity : recent **and** sometimes past infection


Avidité en fonction du délai
avidité
1
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
0
15
30
45
60
75
90
105
120
135
150
165
180
195
210
jour de délai


Indice d'avidité d'infections à CMV anciennes.
Indice d'avidité
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0,8
0,7
0,6
0,5
0,4
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0,1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
Numéro du sérum