



LEIDEN UNIVERSITY MEDICAL CENTER

Quantitative Real-time PCR

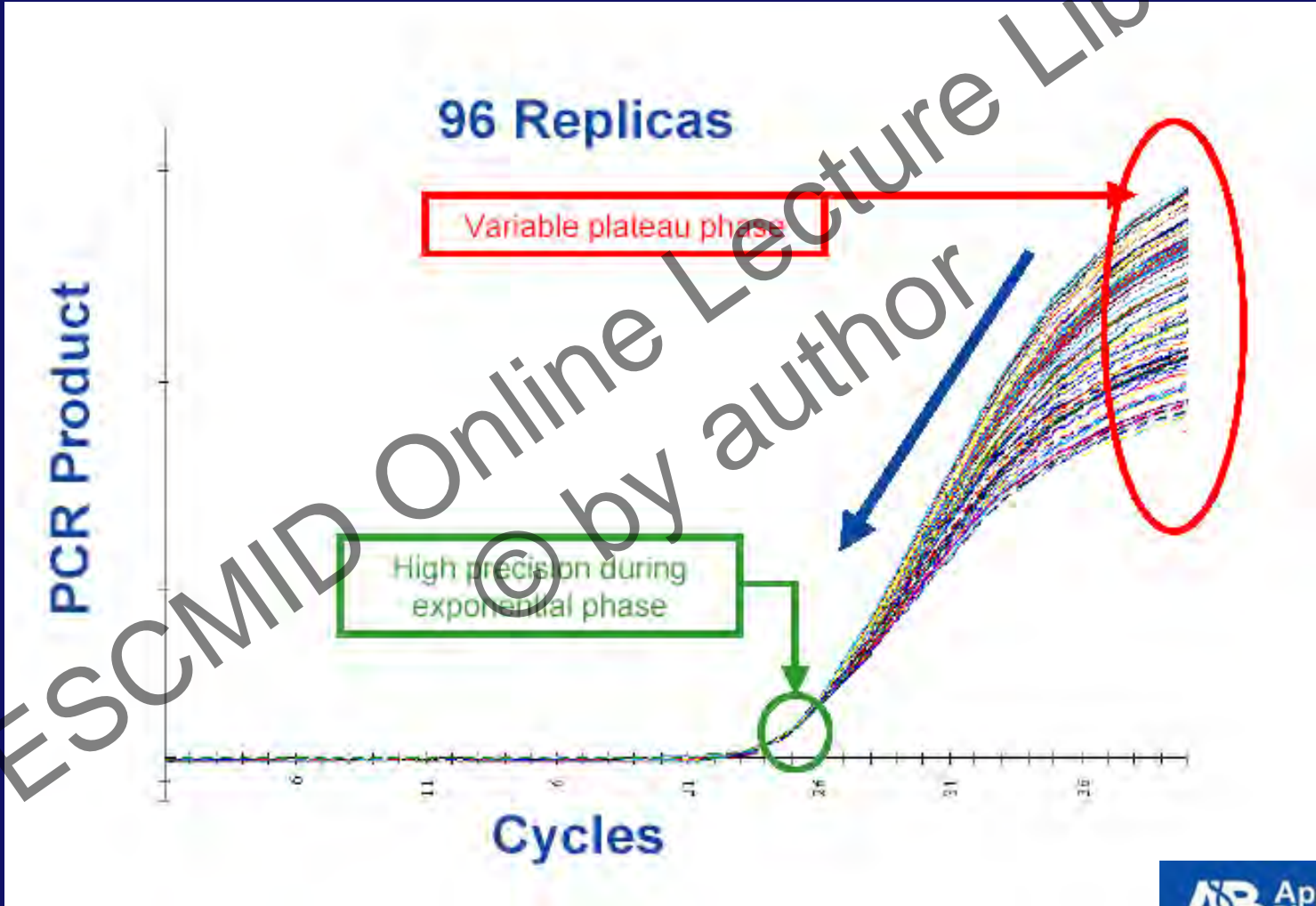
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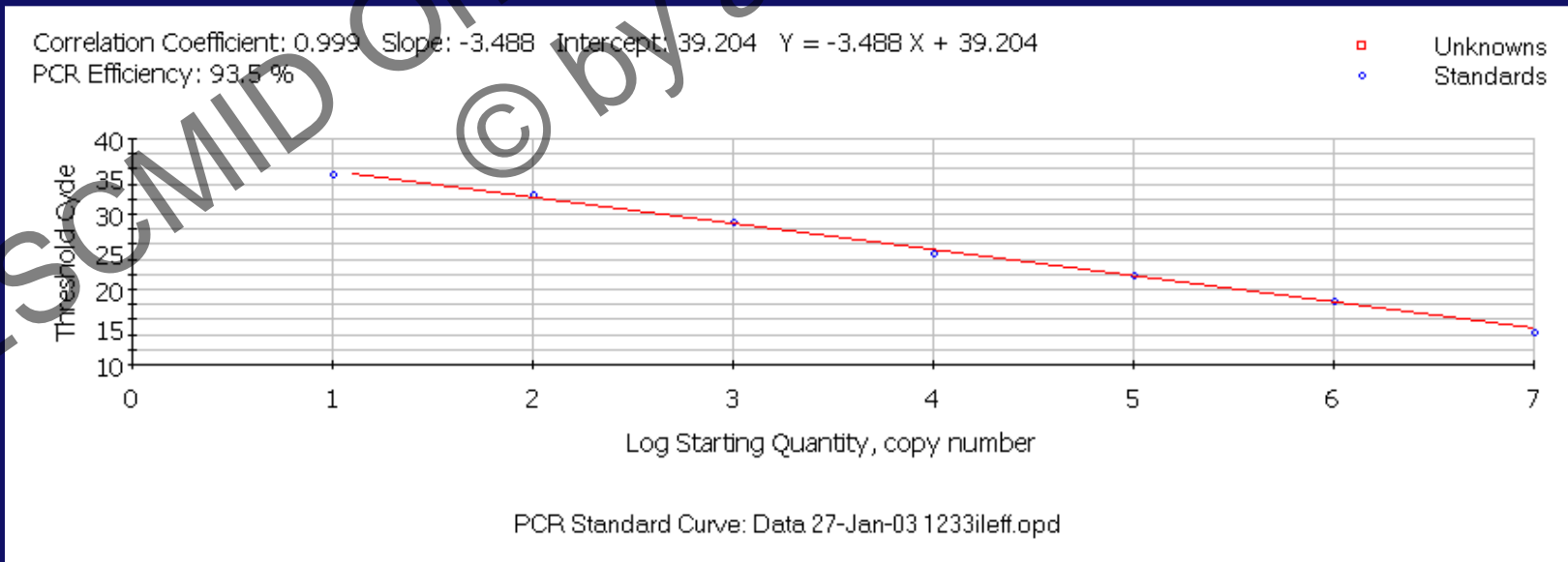
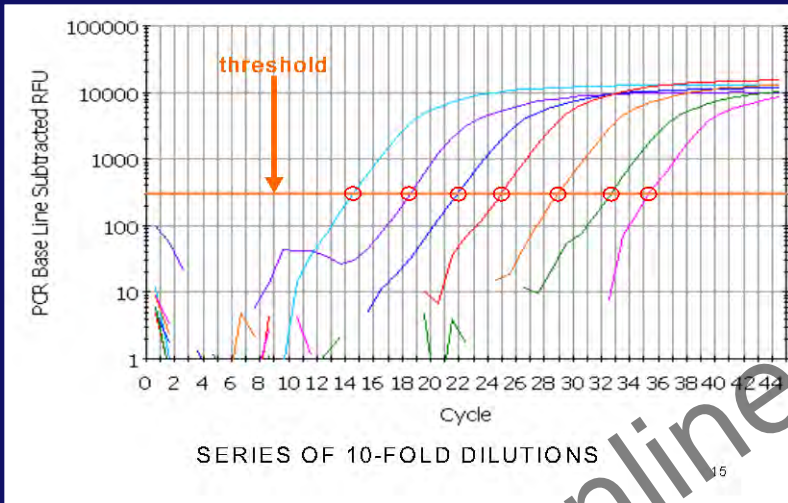
- infectious diseases may have many different causes
- the presentation may be rather similar
 - many infectious disease start as a “flu-like” illness
- while all relevant properties may be different:
 - severity and prognosis
 - infectiveness for others
 - therapeutic options
- a precise diagnosis is therefore required
- General unspecified diagnoses of infections:
 - “it will be some virus, it’s nothing serious”

- Powerful advancement of basic PCR technique
- Performed in single tube, no Post PCR analysis.
- Fluorescent reporter monitors PCR reaction as it occurs.

Real-time PCR provides quantitative results



Quantify Pathogen Load

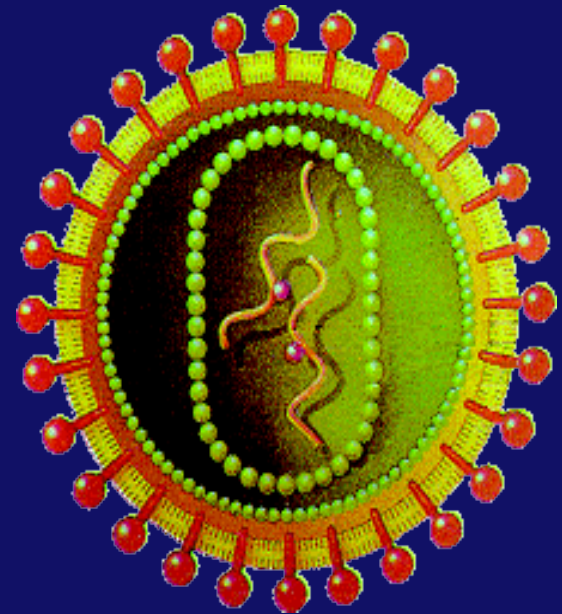


Chronic infections requiring therapy

- Human immunodeficiency virus (HIV)
- Hepatitis B virus (HBV)
- Hepatitis C virus (HCV)

Persistent infections requiring continuous monitoring in the immunocompromised host

- Herpesviruses (CMV, EBV)
- Adenovirus
- BK virus

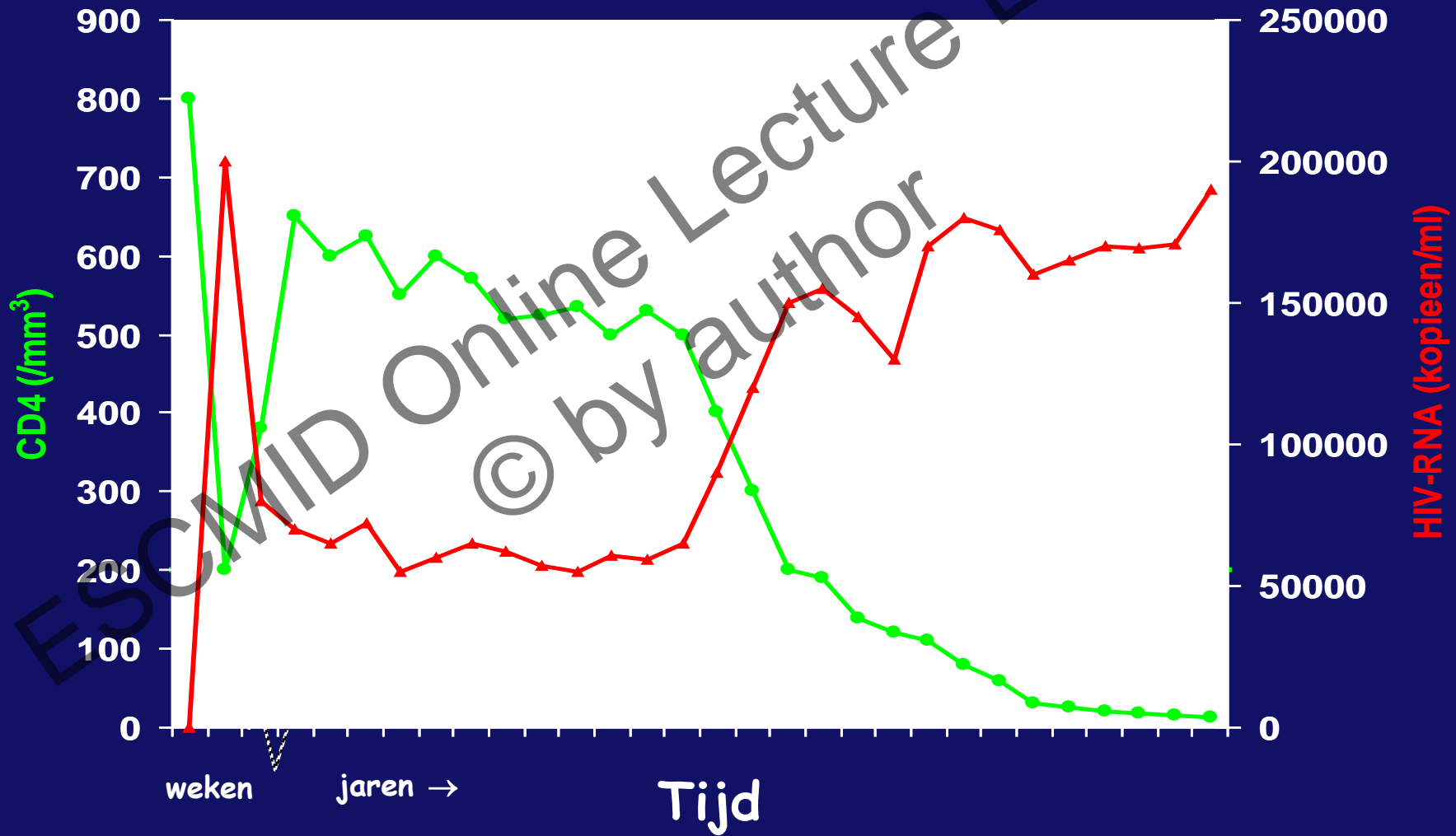


Adults and children estimated to be living with HIV | 2009

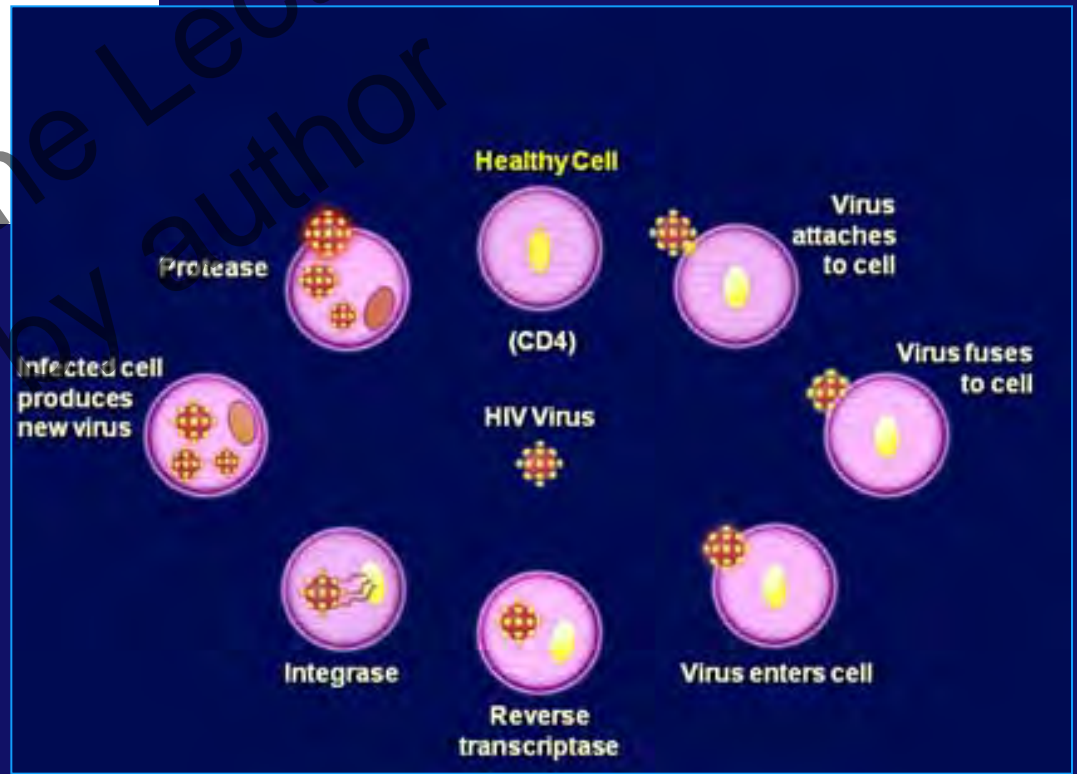
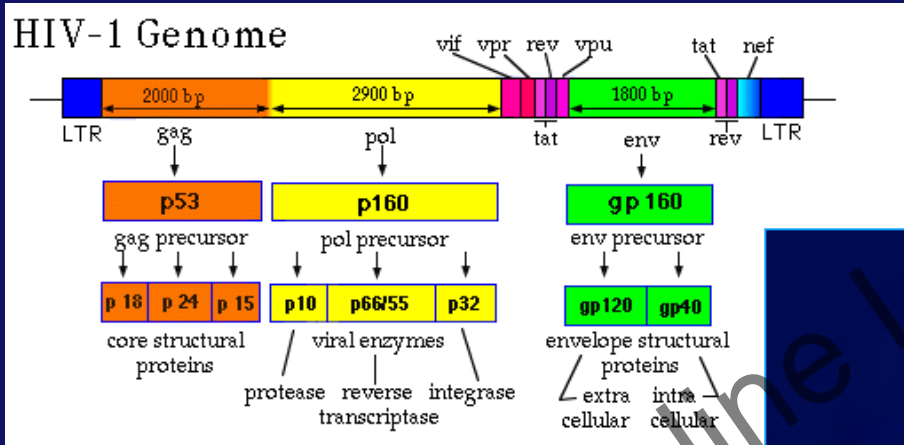


Total: 33.3 million [31.4 million – 35.3 million]

Course of CD4 cells and HIV-RNA during infection



Targets for HIV medication



Antiretroviral drugs: different classes



Reverse Transcriptase Inhibitors						Protease inhibitors		
NRTI			NNRTI					
Zidovudine	AZT	Retrovir	Delavirdine	DLV	Rescriptor	Amprenavir	APV	Agenerase
Zalcitabine	ddC	Hivid	Efavirenz	EFV	Stocrin	Atazanavir	ATV	Reyataz
Didanosine	ddl	Videx	Etravirine	ETV	Intencele	Darunavir	DRV	Prezista
Emtricitabine	FTC	Emtriva	Nevirapine	NVP	Viramune	Indinavir	IDV	Crixivan
Lamivudine	3TC	Epivir				Lopinavir	LPV	Kaletra
Abacavir	ABC	Ziagen				Nelfinavir	NFV	Viracept
Stavudine	d4T	Zerit				Ritonavir	RTV	Norvir
Tenofovir	TDF	Viread				Saquinavir	SQV	Fortovase
						Tipranavir	TPV	Aptivus



Fusie remmers			CCR5 antagonist (entry inhibition)			Integrase remmers		
Enfuvirtide	ENF	Fuzeon	Maraviroc	MVC	Selzentry	Raltegravir	RAL	Isentress
			Vicriviroc	VVC	phIII	Elvitegravir	EVG	phIII



- Compliance
- Side effects
- Interaction other medication
- Antiviral Resistance
 - Primary
 - Acquired

- HCV
 - Genotype and viral load (real-time PCR) determines the duration of treatment

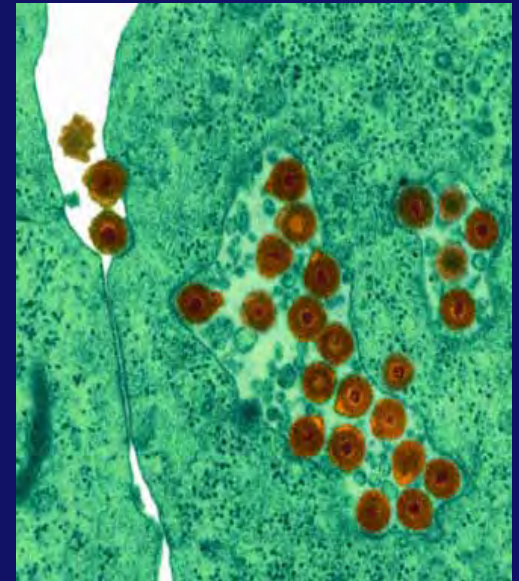
- HBV
 - Viral load analyses (real-time PCR) to monitor successful treatment

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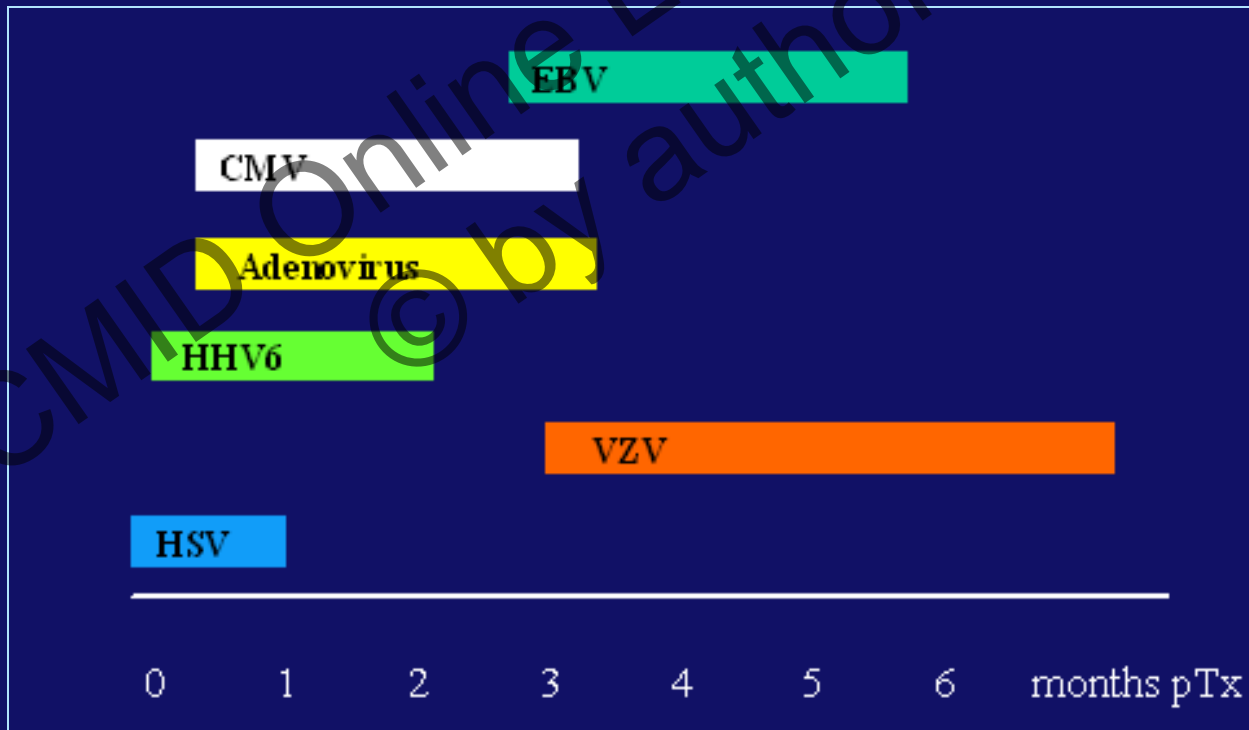


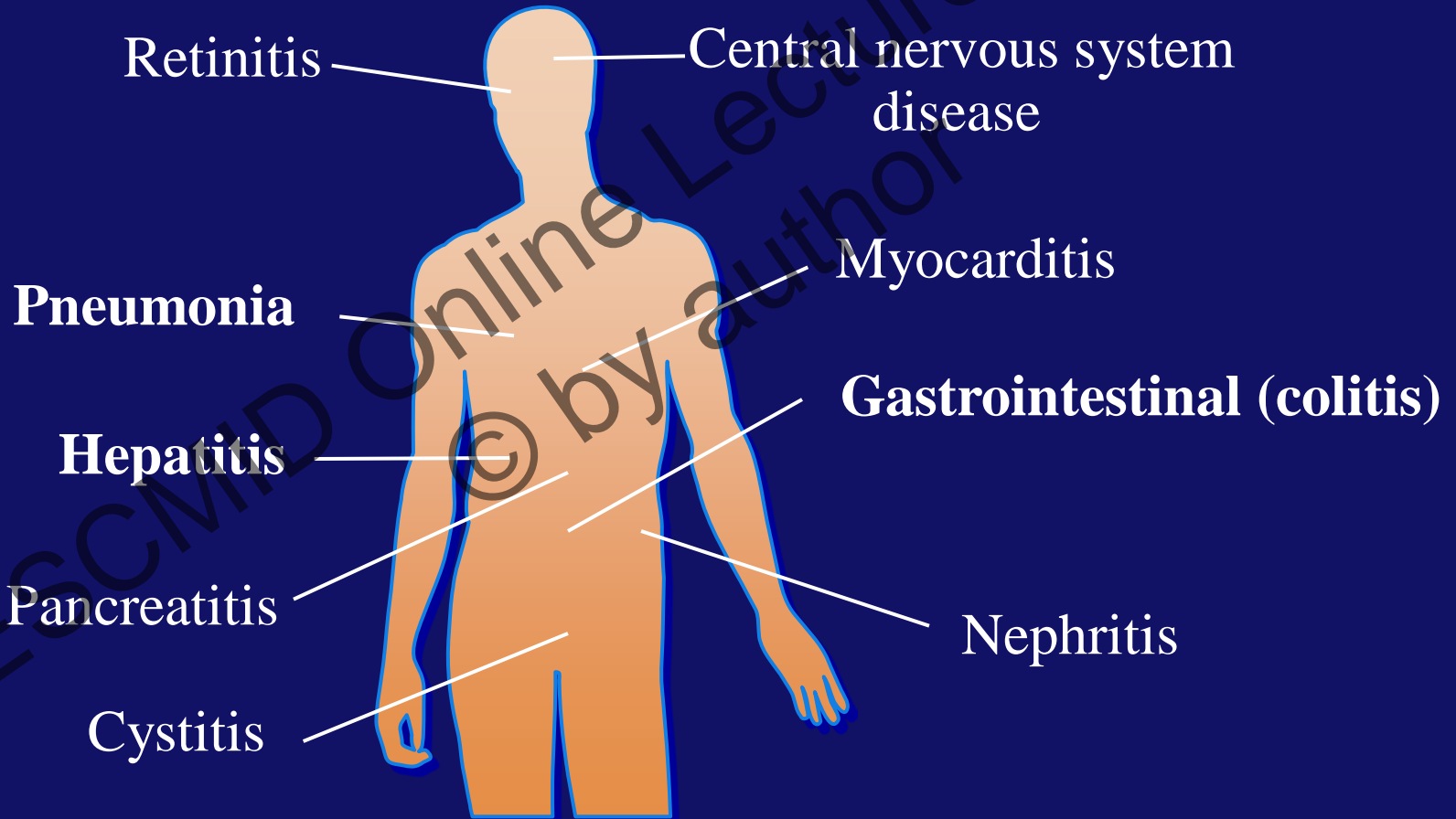
Quantitative analysis

- Chronic infections: HCV, HBV, HIV
- Reactivating infections: CMV, EBV, ADV
 - Immunocompromised patients

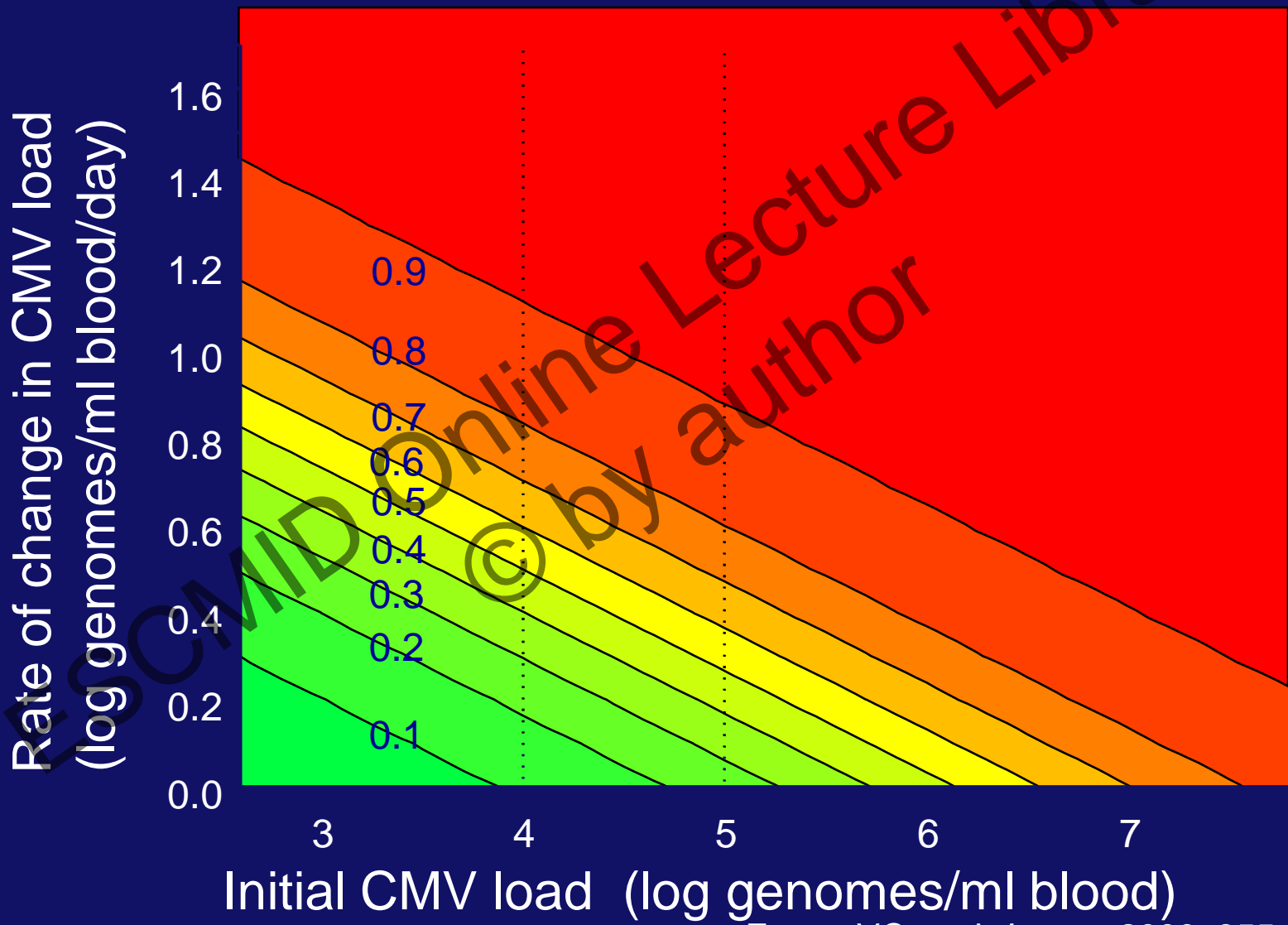
Reactiverende infecties: Immuunsuppressie

- door onderdrukking of afwezigheid (BMT) van immuunrespons worden latent aanwezige virussen gereactiveerd.





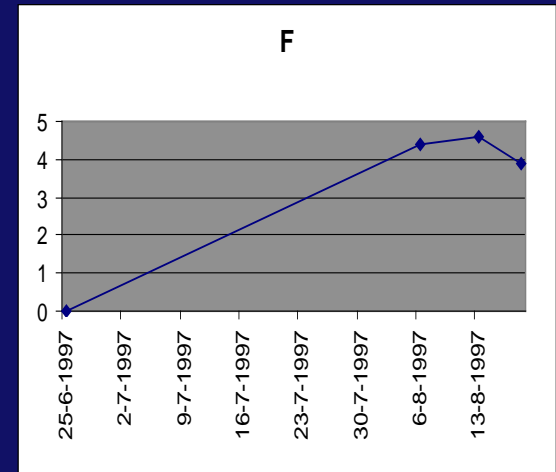
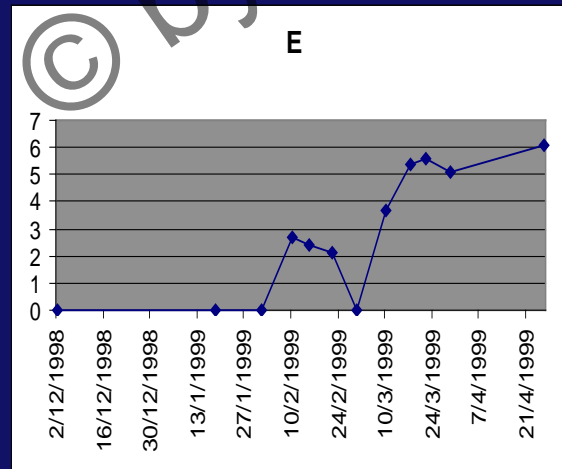
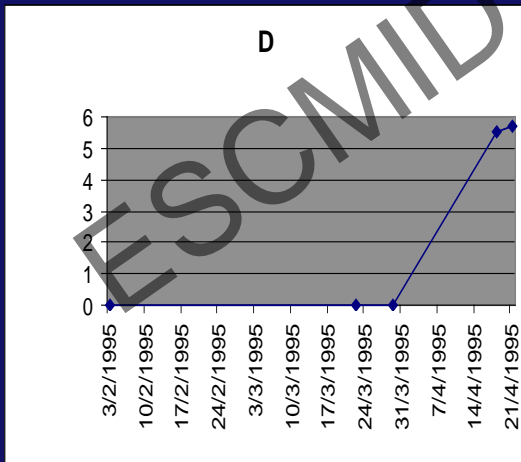
CMV DNA load value *and* rate of change both determine risk of CMV related disease



Emery VC et al. *Lancet* 2000; 355: 2032-6

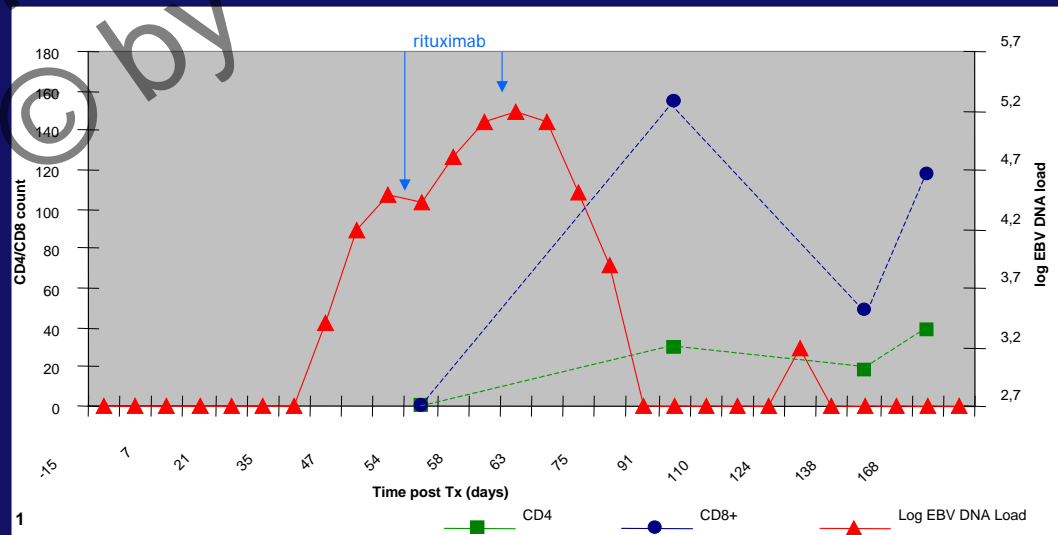
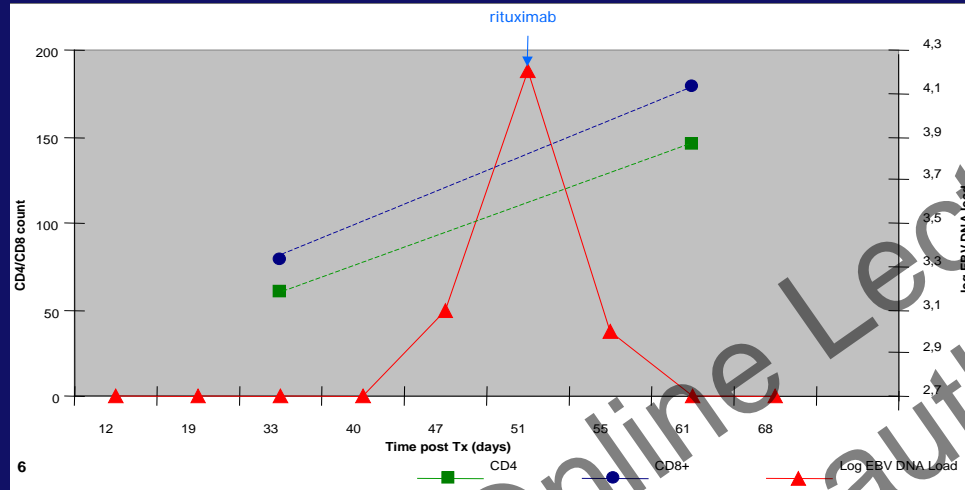
Epstein Barr Virus

- Infectious mononucleosis
- High seroprevalence (70-80%) in the population
- 15% BMT patients: EBV-LPD, majority lethal



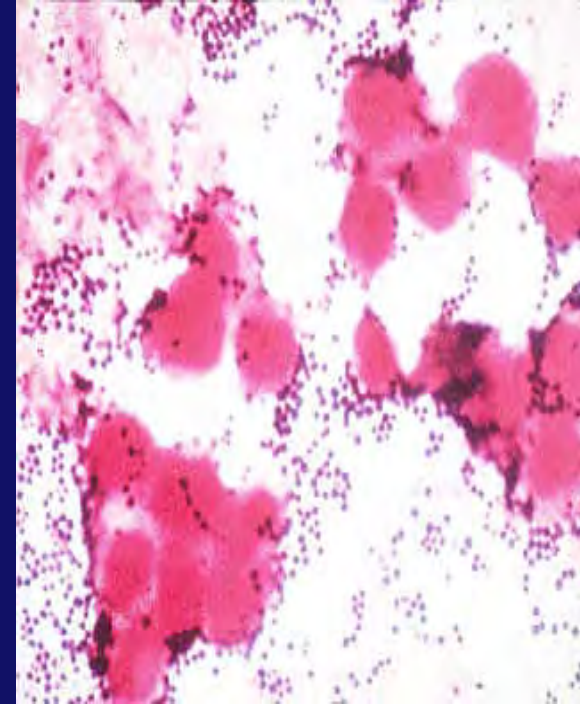
- Post Tx weekly monitoring of plasma using a quantitative EBV assay
- Load > 1000 C/ml: start therapy
 - rituximab
 - reduced immune suppression
 - DLI

EBV treatment prevents EBV-LPD.



Kwantitatieve analyse?

- Chronic infections: HCV, HBV, HIV
- Reactivating infections: CMV, EBV, ADV
- Colonisation vs Infectie
 eg: *S. pneumoniae*
 Interpretation based on Ct value?



Mycoplasma pneumoniae
 Bordetella (para)pertussis
 Legionella sp. - pneum
 Chlamydia pneum & psittaci
 Pneumocystic jiroveci
 Bocavirus/ Adenovirus
 Influenzavirus A, B (incl H5N1)
 RSV
 PIV 1-4
 HCOV 229E, OC43, NL63, HKU1
 Mycobacterium tuberculose
 Mycobacterium genus (avium/haemophilum)
 Clostridium difficile (O27)
 Campylobacter sp.
 Salmonella sp.
 Shigella sp.
 Entamoeba histolytica/dispar
 Giardia lamblia
 Cryptosporidium parvum
 Strongyloides stercoralis
 Dientamoeba fragilis
 Norovirus GI and GII
 Rotavirus
 Astrovirus
 Sapovirus
 Adenovirus F

CMV qual and quant
 EBV qual and quant
 Adenovirus A-F qual and quant
 HHV6 qual and quant
 BK virus qual and quant
 Parvovirus B19 qual and quant
 HBV qual and quant
 HIV quant
 HCV qual and quant
 HEV
 HSV 1/2
 VZV
 JC Virus
 Enterovirus/ Parechovirus
 Toxoplasma gondii
 Neisseria meningitidis
 Listeria monocytogenes
 Stertococcus pneumoniae
 Borrelia sensu latu
 MRSA en MecA/Nuc
 Treponema pallidum
 Chlamydia trachomatis
 LGV
 Neisseria gonorrhoea
 HIV
 HSV 1/2

What does a positive PCR mean?

No. (%) of subjects, by presentation

Pathogen	ILI (n = 166)		ARTI (n = 376)		Control subjects (n = 541)	
	Crude prevalence	Adjusted prevalence ^a	Crude prevalence	Adjusted prevalence ^a	Crude prevalence	Adjusted prevalence ^a
Adenovirus	0 (0)	0 (0)	7 (1.9)	12 (3.2)	0 (0)	0 (0)
Coronavirus	6 (3.6)	5 (3.0)	29 (7.7)	30 (8.0)	21 (3.9)	30 (5.5)
Enterovirus	2 (1.2)	2 (1.2)	13 (3.5)	12 (3.2)	7 (1.3)	12 (2.2)
hMPV	5 (3.0)	4 (2.4)	6 (1.6)	6 (1.6)	0 (0)	0 (0)
Influenza virus						
Type A	49 (29.5)	69 (41.6)	18 (4.8)	17 (4.5)	3 (0.6)	3 (0.6)
Type B	15 (9.0)	14 (8.4)	10 (2.7)	13 (3.5)	0 (0)	0 (0)
Parainfluenza virus	0 (0)	0 (0)	14 (3.7)	17 (4.5)	0 (0)	0 (0)
Rhinovirus	30 (18.1)	50 (30.1)	89 (23.7)	93 (24.7)	60 (11.1)	90 (16.6)
Respiratory syncytial virus	3 (1.8)	3 (1.8)	12 (3.2)	18 (4.8)	3 (0.6)	3 (0.6)
<i>M. pneumoniae</i>	5 (3.0)	4 (2.4)	9 (2.4)	11 (2.9)	3 (0.6)	2 (0.4)
<i>C. pneumoniae/Chlamydophila psittaci</i>	2 (1.2)	2 (1.2)	4 (1.1)	3 (0.8)	10 (1.8)	25 (4.6)
No virus	57 (34.3)	46 (27.7)	191 (50.8)	176 (46.8)	438 (81.0)	385 (71.2)

Clinical relevance of DNA/RNA load?

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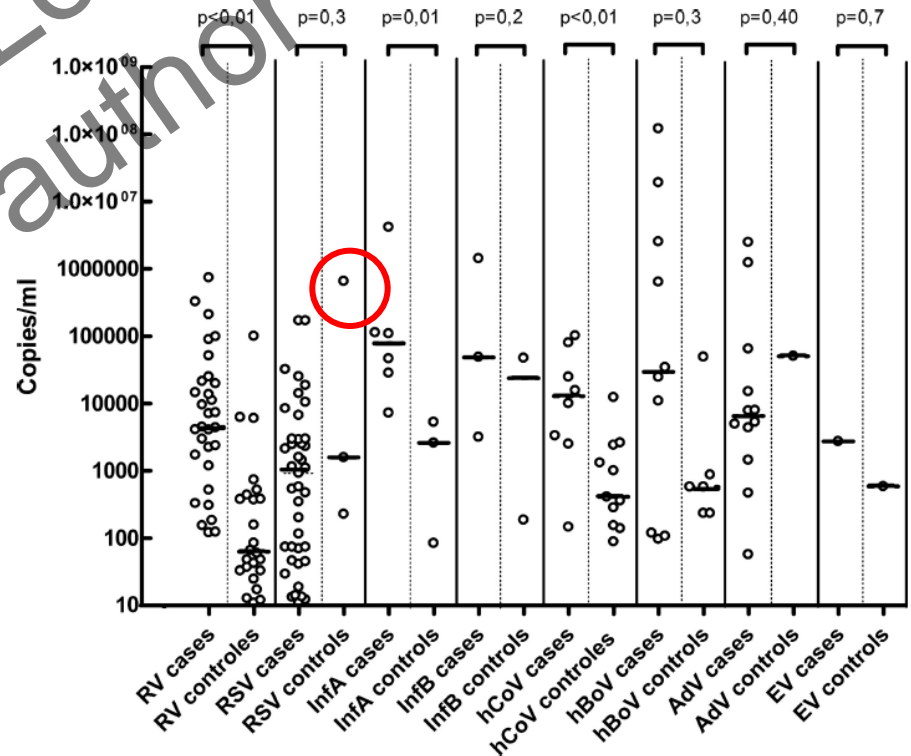
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Frequent Detection of Respiratory Viruses without Symptoms: Toward Defining Clinically Relevant Cutoff Values

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- Luminex system
 - RVP
 - GPP
- Respifinder (Pathofinder)
 - Linker differentiation on length (15 plex)
- Pneumovir (17-plex)
- GenMark e-sensor
- Biofire (Idaho)

- Post PCR processing required
- No quantitative information

Clinical relevance