

Some basic virology for blood bankers

26th IPFA/PEI Workshop on Blood-borne Pathogens
Krakow, May 22th 2019

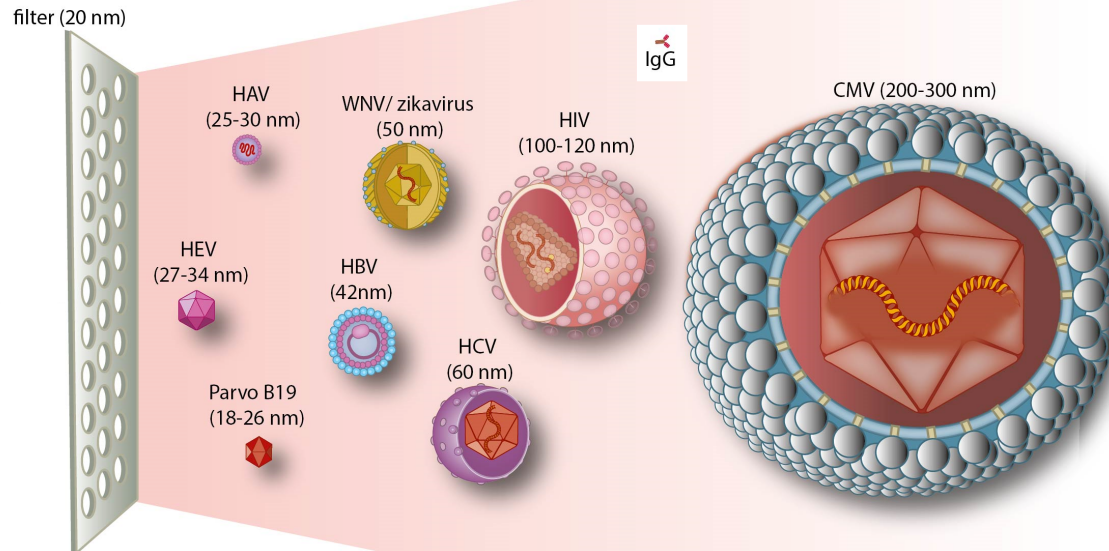
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outline

1. intro: some history
2. pitfalls of NAT (PCR)
3. persistent (latent, occult) viruses; occult hep B.
4. the origin of kissing
5. two kinds of immunity
6. viral load and dose

relevant viruses



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1. Some history of detection of pathogens

Since the 19th century:
Bacteriologists culture bacteria.
Sometimes culture is not feasible.

> Serology!

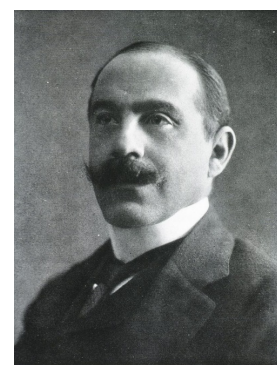
E.g. the von Wassermann test (1906):

Virologists liked to culture too, but:

- viruses are harder to culture:
- insensitive, slow, expensive (labor-intensive).

> Extensive serology for viral infections.

IgG serology reliably detects chronic/latent infections.



PROFESSOR AUGUST VON WASSERMANN

1983:

1983: along route 128 Kary Mullis has a brainwave:



the principle of Polymerase Chain Reaction

2. Some pitfalls of NAT (PCR)

False or irrelevant positive:

- contamination: $\geq$ once a year in each lab.
- primerset not specific, too broad: rhino-entero, WNV-USUV, HIV-lentiviral vector.
- fragments, remnants of viral genomes detected, or non-infectious viruses.
- old-fashioned false positives do occur.

False negative

- Technical error, etc.
- primerset mismatch, too 'narrow'.
- suboptimal biocompartment sampled.

Test results: copies/mL, IU/ml, $\log^{10}$

False negative HIV NAT

False negative HIV serology

2007-2010 in Germany:

5 HIV-infected blood donors: mismatched primerset caused negative PCR.
2/5 early infection (EIA negative), infecting 2 recipients.

> 2e primerset added to HIV NAT.

1990-1994, in Belgium, Germany and France:

Some anti-HIV EIAs negative in AIDS-patients from Cameroon: "HIV-O" *
(Older lysate-based EIAs and Western blot correctly positive).

> additional HIV-O-antigen in anti-HIV EIAs.

Blood screening nucleic acid amplification tests for human immunodeficiency virus Type 1 may require two different amplification targets

Chudy ea.
Transfusion 2012; 52; 431-439

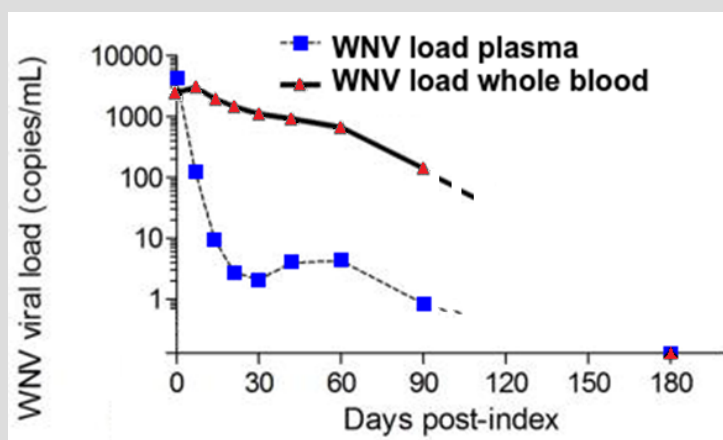
Isolation and partial characterization of an unusual human immunodeficiency retrovirus from two persons of west-central African origin.

De Leys ea.
J.Virol. 1990; 64: 1207-1216

* Later more outliers in patients from Cameroon: HIV-N, HIV-P.
Hence active monitoring of HIV variants in Cameroon.

WNV sticks to erythrocytes

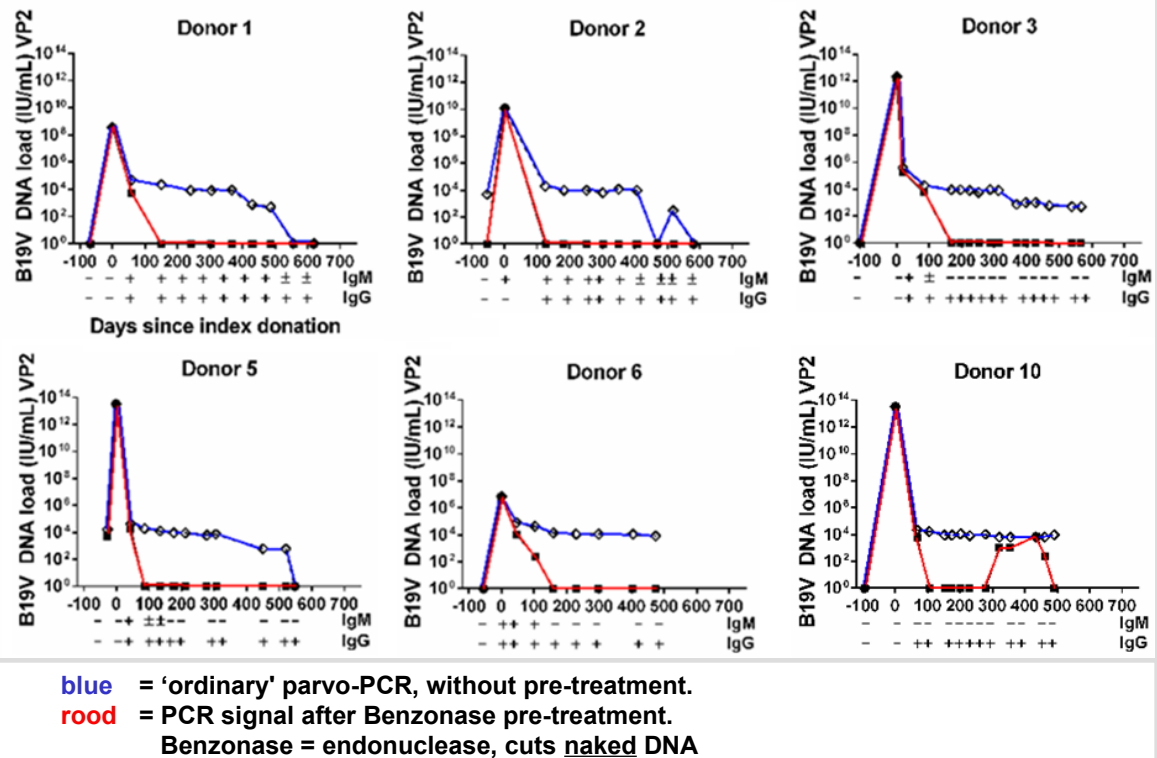
follow-up of 54 persons with WNV infection:
WNV RNA rapidly clears from plasma,
but 'sticks' to RBCs up to 3 months:



adapted from: Lanteri ea. Transfusion 2014; 54: 3232-3241
(life span of erythrocytes: 100-132 days)

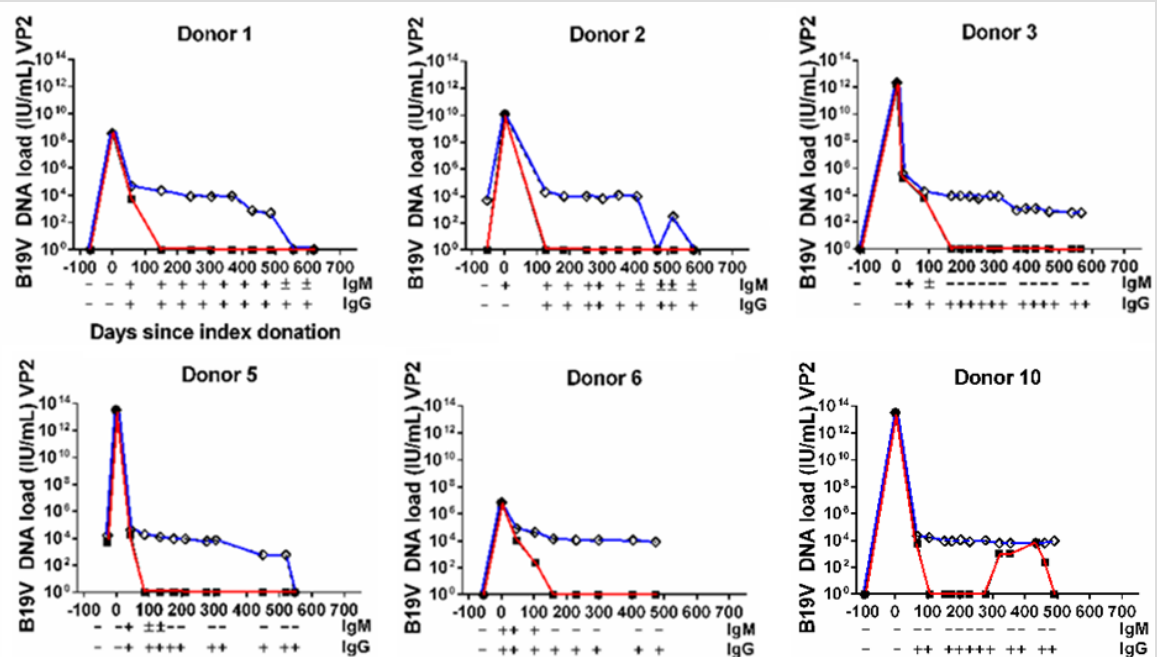
Parvovirus 'viremia' in Dutch blood donors.

Molenaar-de Backer ea, J.Clin.Vir. 2016;84:19-23



Parvovirus 'viremia' in Dutch blood donors.

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Earlier concept: parvo viremia $<10^5$ IU/mL is not infectious, due to neutralizing antibodies.

Alternative: parvo viremia $<10^5$ IU/mL concerns naked fragments of parvo DNA.

Quantitative PCR results

- sometimes in IU/mL. (1 IU often is a few viruses)
- sometimes in copies/mL (HIV).

FDA and WHO are in charge? They decide *per virus*.

500.000 IU/mL = 5,0 E5 IU/mL = $5,0 \times 10^5$ IU/mL = 5,7 $\log^{10}$ IU/mL
Two viral loads differ when at least 2-fold difference on linear scale.

Dear colleagues virologists:

Please no longer send us quantitative PCR results in logs of 10. ☹

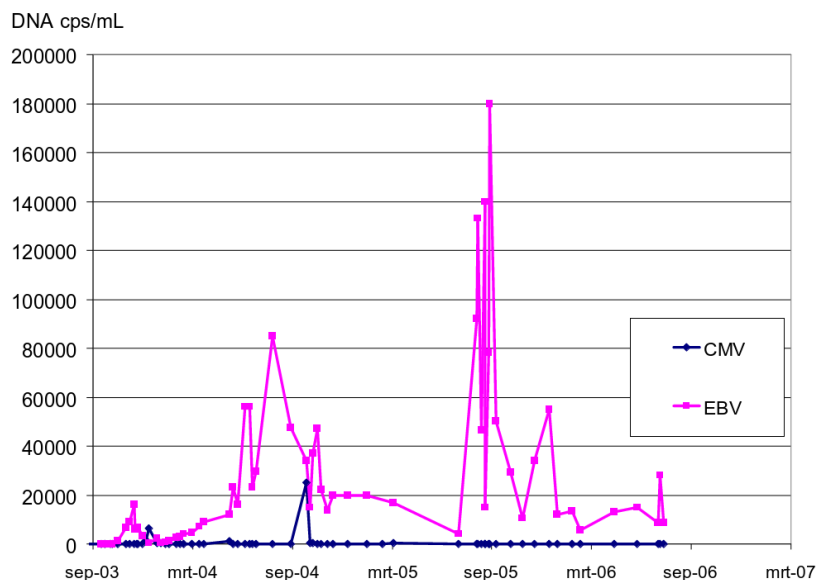
Why 5,65 $\log^{10}$ instead of $4,5 \times 10^5$ or 450,000 IU/mL HBV DNA ?

Nobody understands. It even is dangerous:

Is 4,25 $\log^{10}$ IU/mL HBV DNA more or less than 20.000 IU/mL HBV DNA ?

Next: Comparing quantitative viral loads...

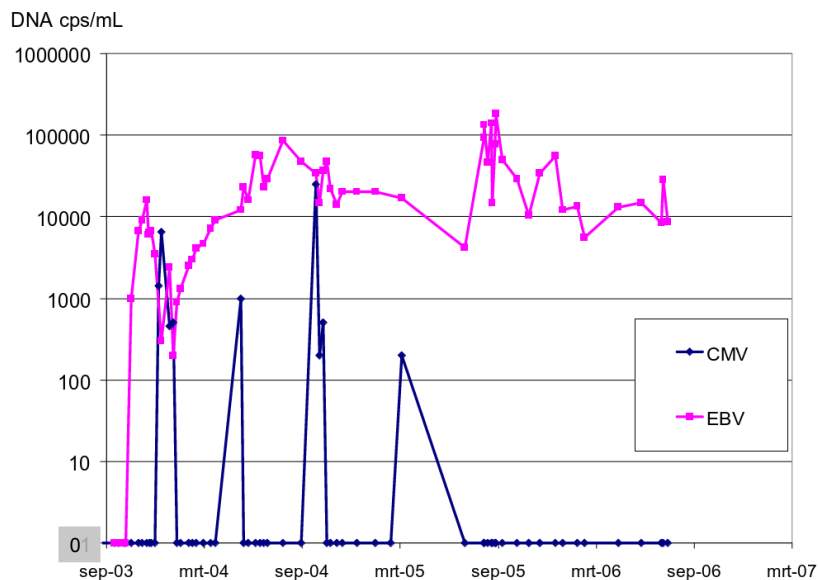
Course of CMV and EBV loads, in a boy after kidney Tx



This boy has:

1. no CMV and no EBV problem
2. EBV problem, no CMV problem
3. EBV and CMV problem

Course of CMV and EBV loads, in a boy after kidney Tx



Tip: depicting viral loads:
in Excell force Y-axis to logarithmic,
and edit all loads of zero ('neg') into 1.



3. Which viruses persist in our donors?

- 1) All human herpes viruses (HSV-1/2, CMV, EBV, VZV, HHV-6/7/8)
- 2) HIV, HTLV
- 3) Some HBV, but not all
- 4) Polyoma viruses. (5,4% of 1016 Dutch donors PyV-DNA pos; JC-virus, MCPyV, HPyV9, etc).

Which viruses are gone after spontaneous clearance or after cured infection?

- 1) HCV (but no immunity)
- 2) HAV
- 3) arboviruses (but cave some bio-compartments)
- 4) Some HBV, but not all
- 5) parvo-B19 (some non-believers)
- 6) HEV (some non-believers)

'Occult hep B': confusing for newbies.

We now know:

After resolved HBV infection (HBsAg=neg, anti-HBcore=pos, anti-HBs=pos/neg) HBV lingers in the liver of some or all (healthy!) persons, for shorter or longer time.

> reactivation & recurrence of hep B by chemo, transplantation, nibs, mabs.

> some (low) infectious blood donors.

'Occult HBV infection' =

a long term state of HBsAg=neg and HBV-DNA=pos. (not during acute infection).

However;

Low levels of HBV DNA vary (blip?) over time, and may or may not be picked up.

'HBV-DNA=pos' depends on sensitivity of NAT, and hence on chance.

A frequent Dutch donor infected some recipients, with 'clean' interval of 14 yrs.

Eg. in Holland: anti-HBcore positive donors only suitable if anti-HBs $\geq$ 200 IU/L.

Summary:

In some persons HBV is a latent virus, like the human herpes viruses; and HBV-DNA may (= 'occult') or may not be detected or detectable.

5. Two kinds of immunity.

Increasingly donors have been vaccinated against hep B.

Hurrah: less acute hep B in our donors! ☺ **or ?**

2 forms of 'immunity' exist. Focussing on hep B:

A) 'sterilizing immunity', by high levels of anti-HBs:

HBVs that enter the body are neutralised, before any cell can be infected. No virus replication, no viremia, no booster effect.

B) 'abortive immunity', when anti-HBs has waned (as it does) :

Invading HBVs infect hepatocytes and replication starts.

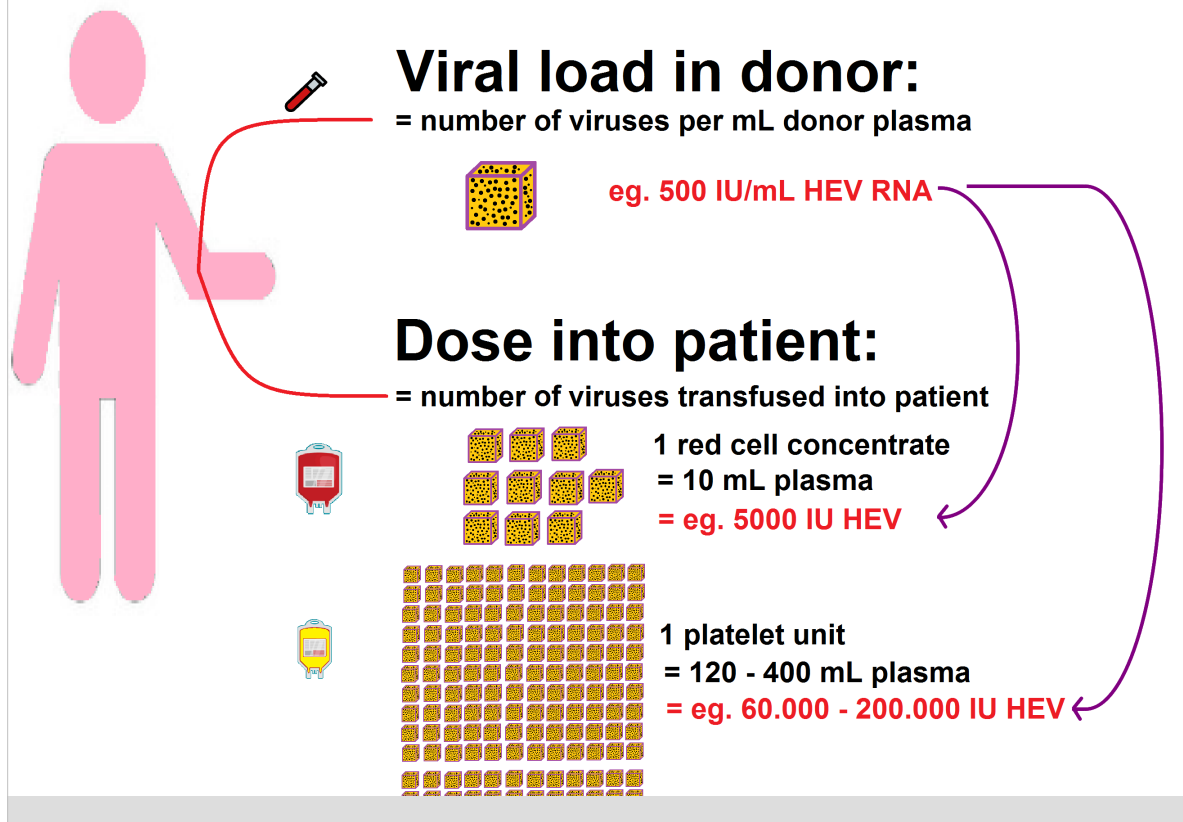
The immune system awakes and aborts infection:

no disease and no 'societal' HBV infectivity or transmission.

You will encounter: HBV-DNA = pos & HBsAg = neg; and variable Ab response.

Are such donors temporarily infectious via blood components?

HBV vaccination protects against disease and 'societal' transmission, not against silent infection and short-time viremia (in donations).



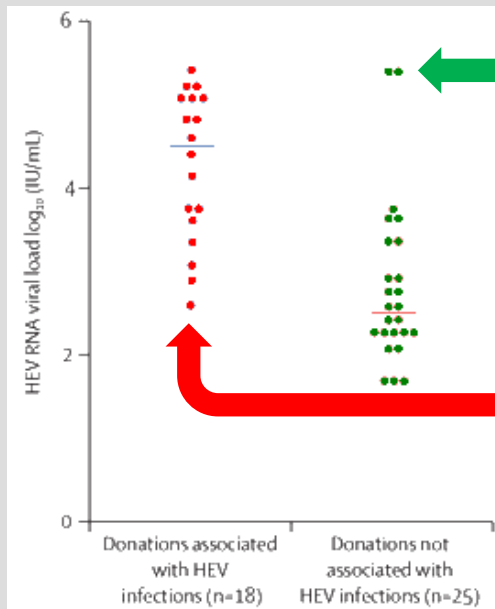
the plasma content of some blood components in Holland

blood component	approx. plasma content
1 packed cells ('transfusion')	10 ml
pooled platelets concentrate in plasma	322 ml
pooled platelets concentrate in PAS II	124 ml
apheresis platelets in plasma	150-400 ml
apheresis platelets in PAS II	160 ml
quarantine plasma ('FFP')	310 ml
SD plasma, Omniplasma	200 ml

HEV loads in UK donors, and transmission

Hepatitis E virus in blood components: a prevalence and transmission study in southeast England

Patricia E Hewitt,¹ Samreen Ijaz,¹ Su R Brailsford,¹ Rachel Brett,¹ Steven Dicks,¹ Becky Haywood,¹ Iain T R Kennedy,¹ Alan Kitchen,¹ Poorvi Patel,¹ John Poth,¹ Katherine Russell,¹ Kate I Tettmar,¹ Joanne Tossell,¹ Ines Ushiro-Lumb,¹ Richard S Tedder¹



Hewitt ea, Lancet 2014

possibly only few mL of highly viremic plasma, via red blood cells; no transmission.

possibly 300 mL of low viremic plasma, via platelets; transmission !

HEV dose and transmission

Hepatitis E virus in blood components: a prevalence and transmission study in southeast England

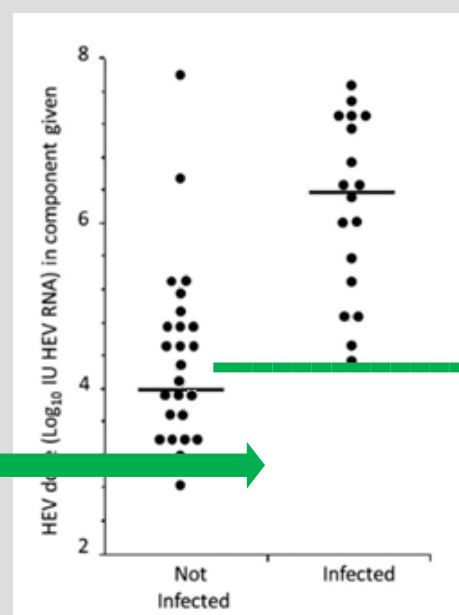
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Hewitt ea, Lancet 2014

Hepatitis E risks: pigs or blood—that is the question

Richard S. Tedder,^{1,2,3} Samreen Ijaz,¹ Alan Kitchen,² Ines Ushiro-Lumb,² Kate I. Tettmar,² Patricia Hewitt,² and Nick Andrews⁴



Tedder ea., Transfusion 2017

summary

Pitfalls of NAT (PCR):

NAT also detects DNA fragments and neutralised viruses.

Primers may be too broad or too narrow.

Persistent (latent, occult) viruses; "occult hep B":

after resolved hep B HBV may linger on, HBV NAT = pos or neg.

Two kinds of immunity:

with or without temporary silent (viremic) infection after exposure.

Viral load and dose:

load = viruses per mL of plasma in a donor.

nn IU/mL

dose = total number of viruses transfused into a patient.

nn IU

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