“Hepatitis C”: general update on NAT vs serology

IPFA, Bilbao 2026

Dr Marion Vermeulen

I have no conflict of interest to declare

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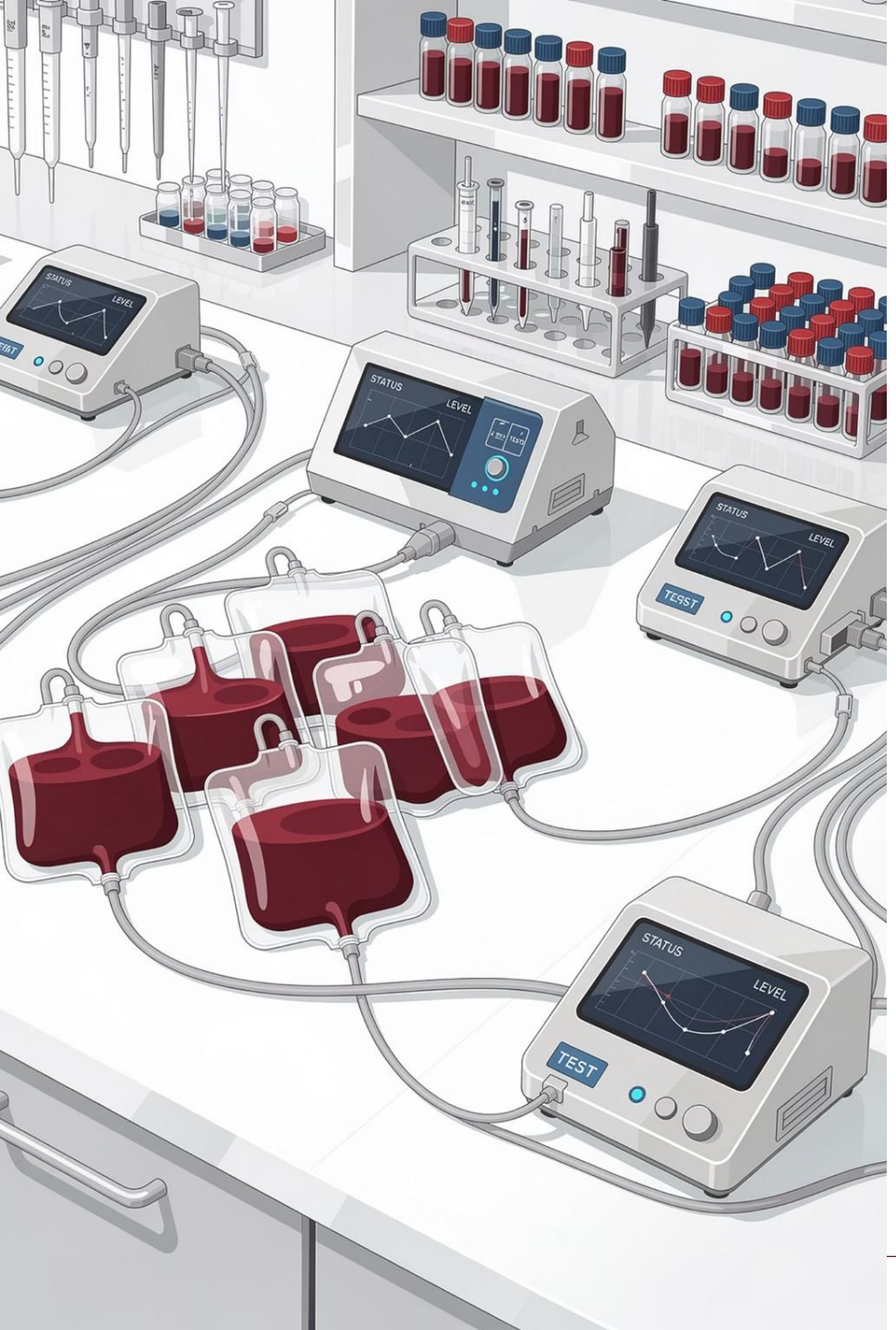


Is Dual Testing for Hepatitis C Necessary?

- El Ekiaby et al. — Transfusion 2015;55:1186–1194
- Roy Choudhury A, Hoad VC, Seed C, Bentley P — Vox Sanguinis, 2023;118:480–7
- Cheng Q, Hoad VC, Roy Choudhury A, Seed CR, Bentley P, Shih STF, Kwon JA, Gray RT, Wiseman V - Vox Sanguinis, 2023; 118:471–479

Background and Context

Current Practice

Parallel testing of blood donations for HCV antibody **and** HCV RNA by nucleic acid testing (NAT) has been standard since 2000.

What Has Changed?

- NAT technologies have significantly improved
- HCV is now a **curable disease** with direct-acting antivirals (DAAs)
- Risk and clinical consequences of HCV transmission through transfusion have substantially reduced

Study Aim

Estimate the residual risk (RR) under various testing options to determine the **optimal testing strategy**.

Viremia levels in hepatitis C infection among Egyptian blood donors and implications for transmission risk with different screening scenarios

Magdy El Ekiaby,[1] Faten Moftah,[2] Heidi Goubran,[2] Harry van Drimmelen,[3] Syria LaPerche,[4] Steve Kleinman,[5] Michael Busch,[6] and Nico Lelie[7]

Egypt has the world's highest HCV prevalence (~15% general population), largely attributed to inadequately sterilized syringes during historical antischistosomal treatment campaigns. HCV seroprevalence among blood donors ranged from 5–28% by region.

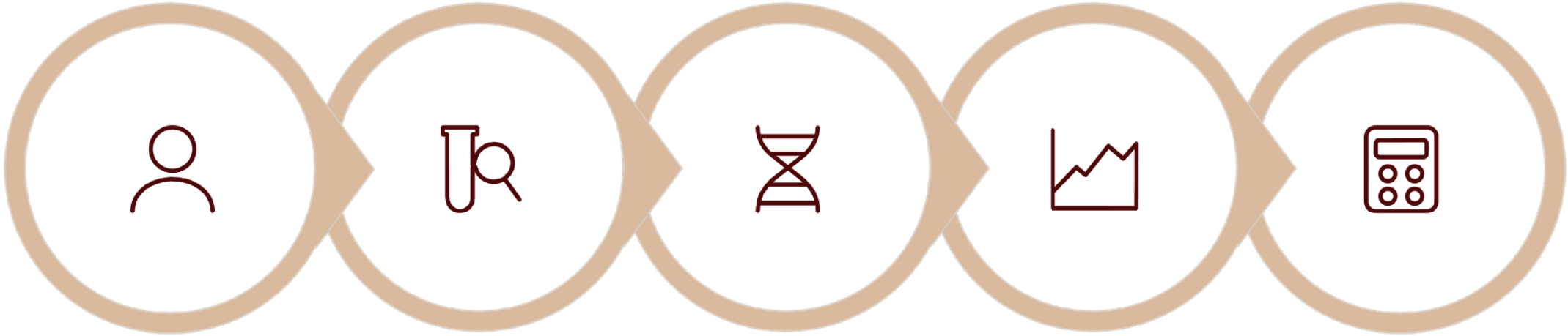
The Core Question

How do viral load (VL) distributions across HCV infection stages compare the efficacy of serologic screening vs. nucleic acid testing (NAT) in preventing transfusion transmission?

Study Setting

Two Cairo blood centers — Shabrawishi Hospital (SH, first Egyptian center to introduce ID-NAT in 2007) and the National Blood Transfusion Centre (NBTC, 2008) — screened 119,756 donors simultaneously with ID-NAT and anti-HCV testing.

Study Design & Methods



Screen Donors

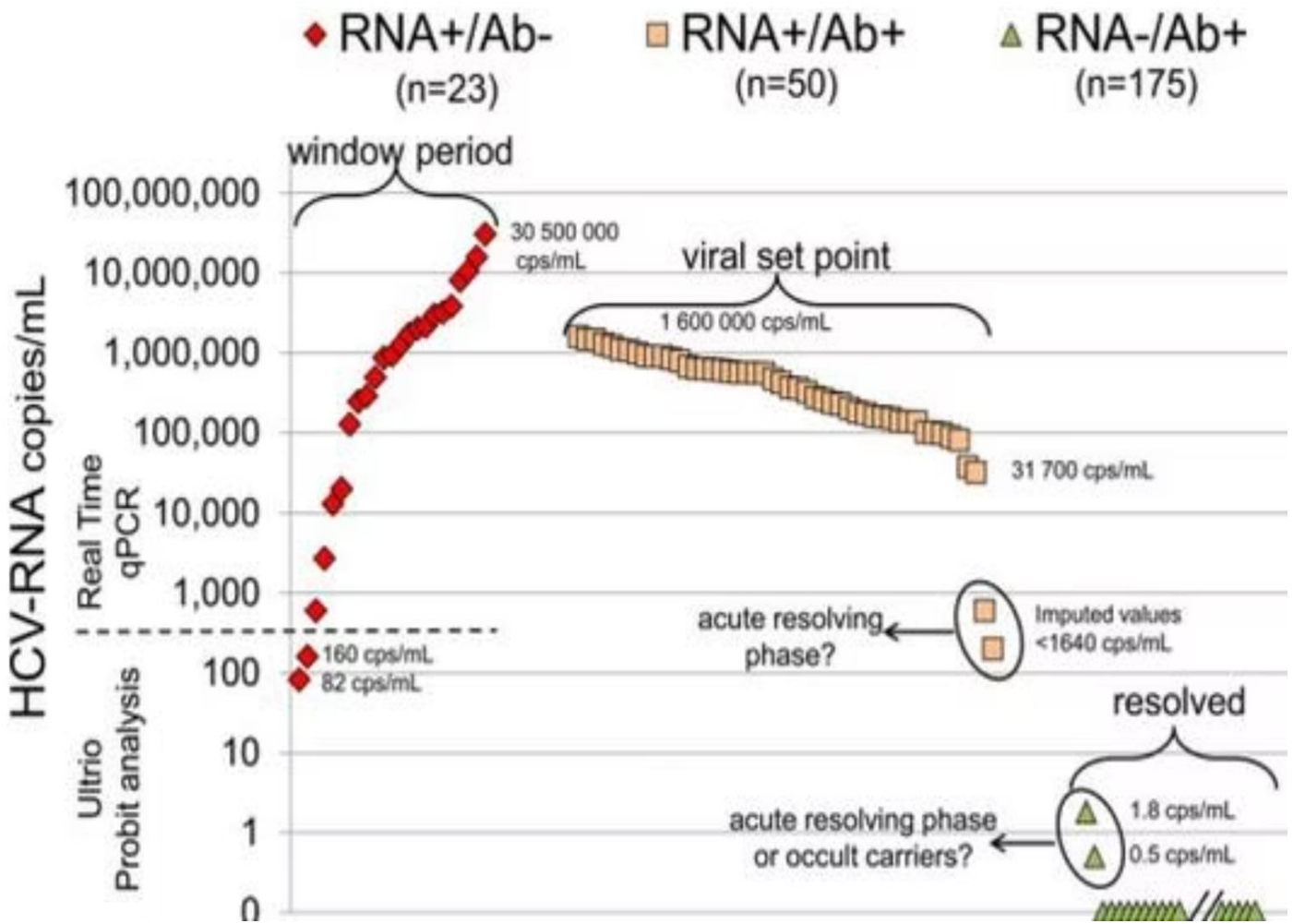
ID-NAT + anti-HCV

qPCR Quantification

Low Viremia Probit

Estimate Risk (Poisson)

Viral Load Distribution Across Infection Stages



Window Period (n=23)

VL: 82 – 3×10^7 copies/mL. Rapid ramp-up; nearly 100% infectious.

Concordant RNA+/Ab+ (n=50)

VL: 31,700 – 1.6×10^6 copies/mL. Chronic viral set point; ~98% infectious.

Resolved RNA−/Ab+ (n=175)

Only 2/175 (1.1%) had detectable RNA at 0.5 and 1.8 copies/mL.

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El Ekiaby et al. — TRANSFUSION 2015;55:1186–1194

Transmission Risk Estimation

1,1% & 3,9%

transmission risk in a RBC

0,5 and 1,8 copies/ml detectable HCV-RNA (2 of 175)

10,4% & 32,6%

transmission risk in a FFP

Donors with probable resolved infection showing detectable HCV-RNA (2 of 175)

0,028%

RBC Transmission Risk

Probability of HCV transmission via RBC transfusion (20 mL plasma) from ID-NAT-negative, anti-HCV-positive donors

0,246%

FFP Transmission Risk

Probability of HCV transmission via FFP transfusion (200 mL plasma) from the same donor group

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El Ekiaby et al. — TRANSFUSION 2015;55:1186–1194

Conclusions & Implications for Blood Safety Policy

99% Viral Clearance

Almost 99% of anti-HCV-reactive, ID-NAT-nonreactive donors had eradicated HCV; the remaining ~1% had extremely low VLs unlikely to be infectious.

NAT Is the Core Safety Layer

This study provides evidence that ID-NAT, not serology, should be considered the primary blood safety measure — reversing conventional WHO guidance.

Policy Consideration

Where infrastructure permits, dropping anti-HCV screening in favor of ID-NAT alone could reduce costs and increase blood availability without meaningfully increasing transmission risk.

Study Limitations

Immunoblot confirmation was not performed; some anti-HCV reactivity may be false positive. Larger studies in immunoblot-confirmed donors are needed to validate the 99% clearance rate.

"This study provides evidence that it may be the other way around" — challenging the WHO position that serology is the core of blood safety and NAT a secondary measure.

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El Ekiaby et al. — TRANSFUSION 2015;55:1186–1194

ORIGINAL ARTICLE

Vox Sanguinis ISBT

Is dual testing for hepatitis C necessary? Modelling the risk of removing hepatitis C antibody testing for Australian blood donations

Avijoy Roy Choudhury[1] | Veronica C. Hoad[2] | Clive Seed[2] | Peter Bentley[1,2]

ORIGINAL ARTICLE

Vox Sanguinis ISBT

Removing hepatitis C antibody testing for Australian blood donations: A cost-effectiveness analysis

Qinglu Cheng[1] | Veronica C. Hoad[2] | Avijoy Roy Choudhury[3] | Clive R. Seed[2] | Peter Bentley[2,3] | Sophy T. F. Shih[1] | Jisoo A. Kwon[1] | Richard T. Gray[1] | Virginia Wiseman[1,4]

1 **Universal Dual Testing**

Anti-HCV + NAT for all donations **(status quo)**

2 **Risk-Targeted Testing**

Anti-HCV + NAT for first-time donors; NAT only for repeat donors

3 **Component-Targeted Testing**

Anti-HCV + ID-NAT for transfusible components; MP16-NAT only for plasma for fractionation

4 **Universal NAT Only**

NAT for all donations; no anti-HCV testing

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Screening Data: 2016–2020

Over the 6-year period, **7,107,210 donations** were included in the analysis. The table below shows the breakdown of positive results by testing method and donor category.

Donation Category	Anti-HCV+ & NAT+	Anti-HCV+ NAT non-reactive	NAT yield only
First-time (n=501,450)	101	107	0
Repeat (n=6,605,800)	17	41	1
Transfusible components (n=3,845,771)	105	133	1
Plasma for manufacture (n=3,261,479)	13	15	0
Total	**118**	**148**	**1**

Notably, **148 donations** were anti-HCV positive but NAT non-reactive — the population central to assessing the marginal value of antibody testing.

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Roy Choudhury A, Hoad VC, Seed C, Bentley P — Vox Sanguinis, 2023;118:480–7

Residual Risk Results

All four strategies produced residual risk estimates **considerably less than 1 in 1 million** — Lifeblood's defined tolerable risk threshold for HCV transmission.

Testing Strategy	Low Estimate	Mid Estimate	High Estimate
Strategy 1: Universal dual testing	1 in 151M	1 in 151M	1 in 151M
Strategy 2: Targeted (first-time donors)	1 in 148M	1 in 111M	1 in 89M
Strategy 3: Targeted (transfusible components)	1 in 151M	1 in 151M	1 in 151M
Strategy 4: Universal NAT only	1 in 146M	1 in 66M	1 in 43M

Based on Egypt prevalence

Even the worst-case estimate for universal NAT only (1 in 43 million) remains far below the 1 in 1 million tolerable risk threshold.

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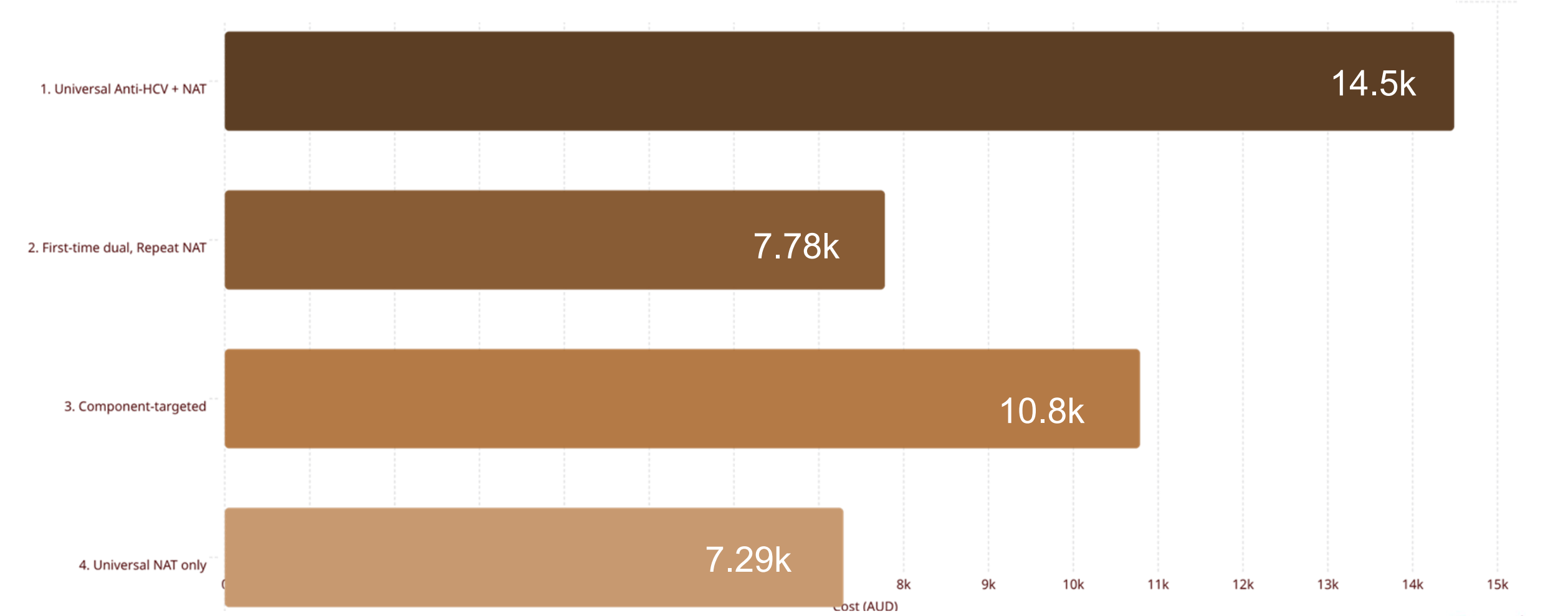
Baseline Results: Costs & QALYs per 1,000 Donations

Strategy 4 (Universal NAT only) is the most cost-effective, with the lowest cost per 1,000 donations across all three age groups (0–35, 36–65, 66+). QALYs were identical: 22,817 (0–35 yrs), 9,432 (36–65 yrs), and 4,634 (66+ yrs).



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Cheng Q, Hoad VC, Roy Choudhury A, Seed CR, et al *Vox Sanguinis*, 2023; 118:471–479

Conclusions

Not Cost-Effective

Current dual testing (anti-HCV + NAT) is not cost-effective compared with targeted or NAT-only strategies.

No Safety Compromise

Partial or total removal of anti-HCV testing would bring significant cost savings *without* compromising blood recipient safety.

~$11M Annual Savings

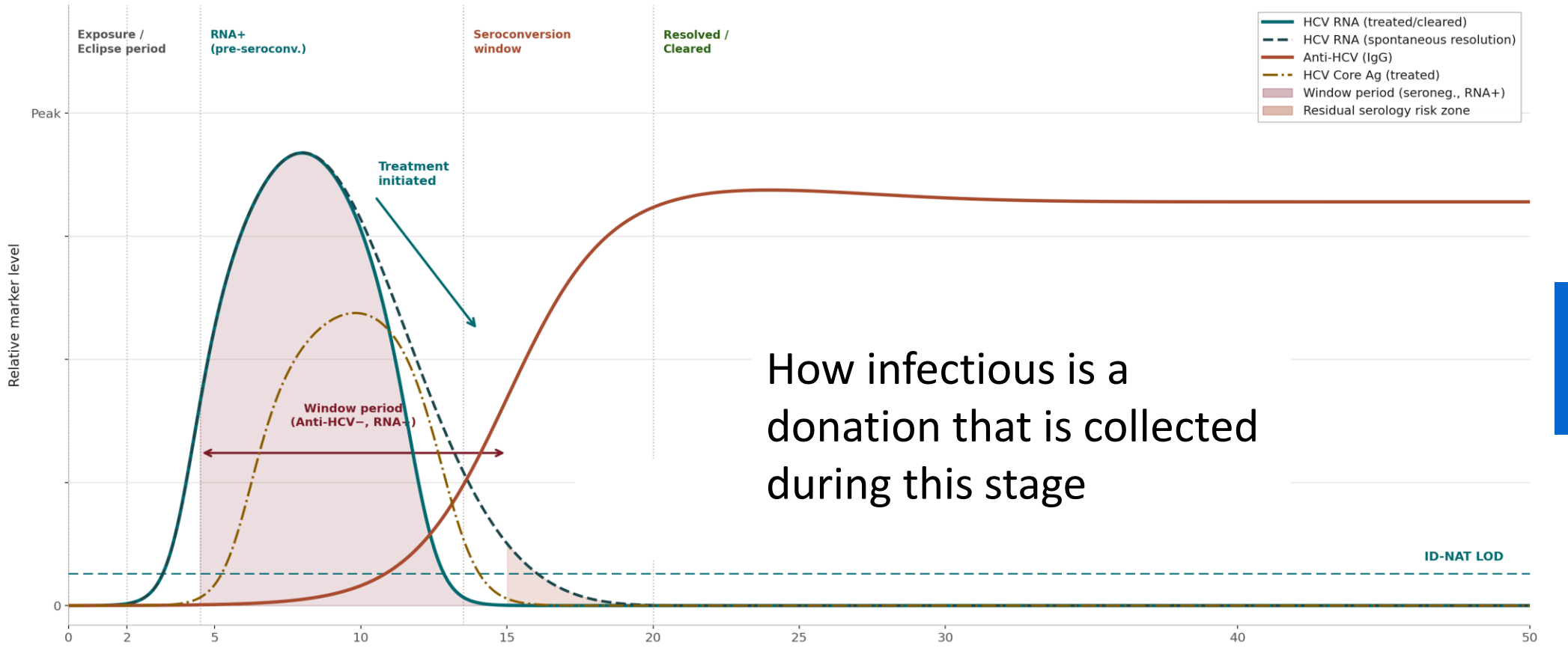
Switching to universal NAT-only could halve HCV testing costs, saving approximately A$11 million per year.

Threshold finding: For the current dual-testing strategy to be cost-effective, the residual risk of alternative strategies would need to be at least **1 HCV infection per 2,424 donations** — over **60,000 times** the baseline residual risk of 1 in 151 million.

Cheng Q, Hoad VC, Roy Choudhury A, Seed CR, et al *Vox Sanguinis*, 2023; 118:471–479

Efficacy of anti-HCV assays in parallel with ID-NAT in interdicting infectious donations

Josephine Mitchel · Laura Tonnetti · Jamel Groves · Kacie Grimm · Nico Lelie · Silvia Sauleda · Magdy Elkiaby · Jose Eduardo Levi · Grace Chin · Marion Vermeulen



How infectious is a donation that is collected during this stage

KEY QUESTION:

In donors with prior/resolved HCV (RNA−, anti-HCV+), does anti-HCV screening add interdiction value beyond ID-NAT? Anti-HCV detects past infection (spontaneous clearance or successfully treated) — if ID-NAT already removes all RNA+ (infectious) units, the residual anti-HCV yield comprises RNA-undetectable donations, raising the question: is transfusion transmission risk still measurable in this population, and does parallel serology meaningfully reduce it?

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Background & Research Question

The Core Uncertainty

The probability of HCV transmission from **antibody-positive but ID NAT nonreactive (NR)** blood donations remains uncertain. While NAT screening is highly sensitive, occasional low-level viremia may escape detection.

Phase 1 Study Objective

This cross-sectional study assesses whether donations from donors with **spontaneously resolved HCV infections** have low or undetectable viremia — and the risk posed if serology testing were removed in settings where ID NAT is performed.

Phase 2

To still be completed. A study performed in Egypt testing donors who have been successfully treated for HCV for a period of one year.

The infectiousness of low-level viremia with accompanying antibodies is currently unknown.

Phase 1: spontaneously resolved

500 Frozen Plasma Samples

Anti-HCV positive but ID NAT-NR donors across three countries.

United States

370 samples

South Africa

100 samples

Spain

30 samples

Phase 2: Successfully treated

Egypt

400 samples successfully treated

Egypt

100 samples spontaneously resolved

100 anti-HCV negative / ID NAT-NR plasma samples were also tested to rule out false reactivity.

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Methods: Replicate NAT Testing Protocol

Initial Replicate Testing

Each sample tested in **10 replicates** using Ultrio Elite (UE) dHCV and **12 replicates** using the cobas HCV assay (note: cobas MPX was not used — a stated study limitation).

Extended Replicates

Samples with at least one reactive replicate were further tested to reach a total of **20 or 24 replicates**.

Viral Load Estimation

Estimated by **probit analysis** on replicate UE, UltrioPlex E, and cobas MPX tests using S0009 HCV-RNA genotype 1 standard dilution panels (BioQControl, Netherlands; 2.73 copies/IU).

Transmission Risk Modeling

Calculated using the **Weusten infectivity-risk model**, assuming an ID50 (range) of 316 (100–1,000) virions.

Replicate NAT Results in 15 Reactive Donor Samples

Reactivity rates ranged from 4.2% to 60% across replicates, reflecting a wide range of ultra-low viral loads.

Sample ID	Country	Ultrio Elite reactive	cobas MPX reactive
ARC199823*	USA		1/24 (4,2%)
ARC134007*	USA		1/24 (4,2%)
ARC170196*	USA	1/20 (5%)	
ARC132934*	USA		1/24 (4,2%)
ARC164552*	USA		1/24 (4,2%)
ARC094340*	USA		1/24 (4,2%)
30229263	South Africa	12/20 (60%)	3/24 (12,5%)
32202448*	South Africa	5/20 (25%)	
30236865*	South Africa		1/24 (4,2%)
35045366*	South Africa	1/20 (5%)	
31481608*	South Africa		2/24 (8,3%)
33207239	South Africa	2/20 (10%)	2/24 (8,3%)
S501123297237O0*	South Africa		1/24 (4,2%)
33004747	South Africa	2/20 (10%)	1/24 (4,2%)
HCV 0207*	Spain		2/24 (8,3%)

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Analytical Sensitivity: Probit-Derived LODs

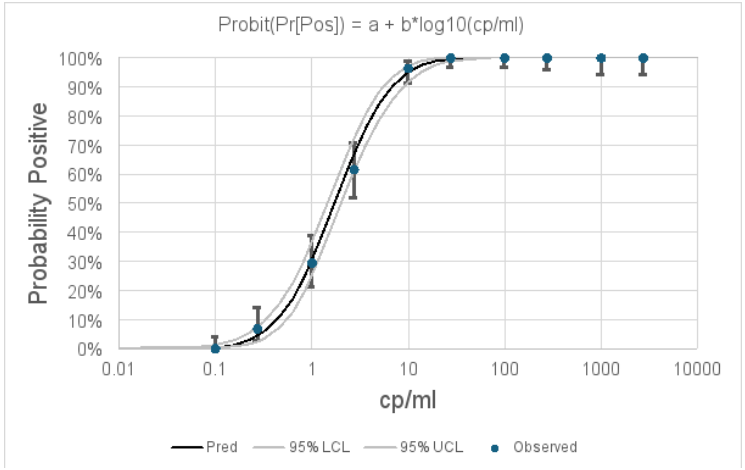
HCV-RNA concentrations were estimated using probit curves derived from the **S0009 HCV-RNA genotype 1 standard** (Sanquin–BioQControl), calibrated in copies/mL and traceable to the first WHO standard (conversion factor: **2.73 copies/IU**).

cat no stand - panel	test combi	n	50% LOD (CI) copies/mL	95% LOD (CI) copies/mL	Dataset used for analysis
IBTS study 2022					
S0009 - P0288	Ultrio Elite	24	2.52 (1.74 - 3.69)	20.3 (11.7 - 47.5)	
S0009 - P0288	UltrioPlex E	24	2.09 (1.04 - 4.56)	15.6 (6.44 - 140)	
IBTS study 2024					
S0009 - P0288	UltrioPlex E	24	2.63 (1.94 - 3.59)	10.5 (6.82 - 22.4)	
S0009 - P0288	UltrioPlex E dHCV	24	1.95 (1.35 - 2.83)	15.1 (8.79 - 35.4)	
S0009 - P0288	UltrioPlex E + dHCV	48	2.25 (1.77 - 2.87)	13.4 (9.23 - 22.5)	
IBTS studies combined					
S0009 - P0288	UltrioPlex E	48	2.32 (1.50 - 3.67)	13.5 (7.38 - 41.7)	yes
S0009 - P0288	UltrioPlex E + dHCV	72	2.19 (1.51 - 3.23)	14.1 (8.19 - 35.1)	
Old studies combined					
S0009 - P0019	Ultrio Plus	48	1.76 (1.35 - 2.29)	15.1 (9.93 - 26.7)	
S0009 - P0019	Ultrio Elite	112	1.73 (1.48 - 2.03)	9.97 (7.73 -- 13.8)	yes
S0009 - P0019	Ultrio Plus + Elite	160	1.73 (1.51 - 1.99)	11.5 (9.17 - 15.1)	
S0009 - P0019	Cobas MPX	60	2.86 (2.27 - 3.59)	20.4 (14.3 - 33.2)	
S0009 - P0272	Cobas MPX	166	3.28 (2.95 - 3.63)	16.3 (13.7 - 20.0)	yes

Probit Curves: Visualizing Assay Detection Probability

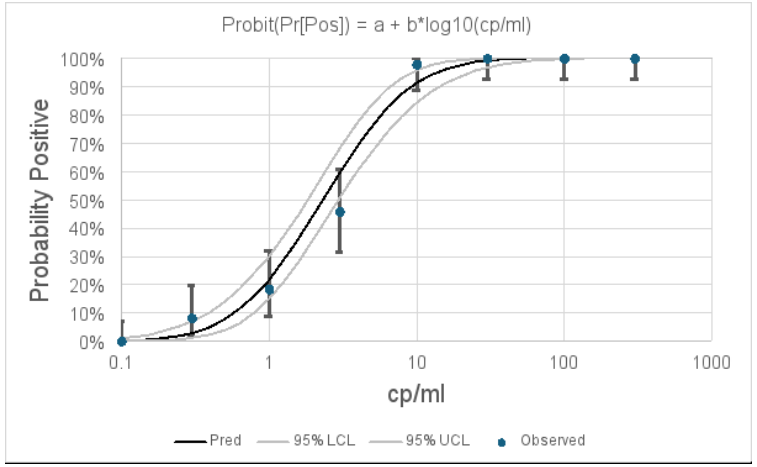
UltrioPlex E (P0288)

50% LOD: **2.32 cp/mL**95% LOD: **13.5 cp/mL**



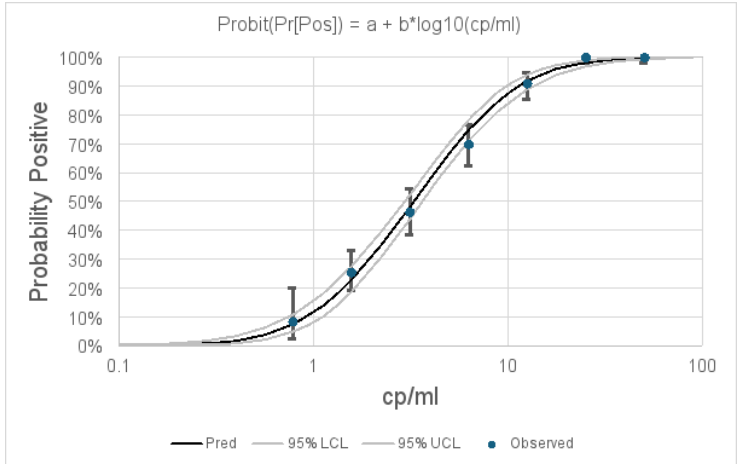
Ultrio Elite (P0019)

50% LOD: **1.73 cp/mL**95% LOD: **9.97 cp/mL**



cobas MPX (P0272)

50% LOD: **3.28 cp/mL**95% LOD: **16.3 cp/mL**



ⓘ The UltrioPlex E probit curve (IBTS combined dataset) was selected for worst-case risk analysis, as a more recent Ultrio Elite dataset showed slightly higher LOD estimates.

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Weusten, J et al *Transfusion*, 2011; 51:203-215

Estimated HCV-RNA Concentrations in 15 Samples

Probit function parameters were used to back-calculate HCV-RNA concentration (copies/mL) from the proportion of reactive replicates in each assay. Concentrations are extremely low — mostly **below 1 copy/mL**.

Sample ID	Ultrio Elite (cp/mL)	UltrioPlex E (cp/mL)	cobas MPX (cp/mL)	Country
ARC199823 / ARC134007 / ARC132934 / ARC164552 / ARC094340/30236865 S50112329723700	—	—	0.61	USA / SA
ARC170196 / 35045366	0.30	0.40	—	USA / SA
30229263	2.27	3.04	1.07	South Africa
32202448	0.85	1.13	—	South Africa
33207239 / 33004747	0.44	0.59	0.85 / 0.61	South Africa
HCV 0207 / 31481608	—	—	0.85	Spain / SA

Per-Sample HCV Transmission Probabilities

Transmission risk was calculated for each of the 15 samples using ID50 = 316 virions (range 100–1000), for both RBC (20 mL) and FFP (200 mL) transfusions.

Sample ID	Ultrio–RBC	MPX–RBC	Ultrio–FFP	MPX–FFP
ARC199823 / ARC134007 / ARC132934 / ARC164552 / ARC094340 / 30236865 S50112329723700	2.64% (0.84–8.1%)	—	23.5% (8.1–57.1%)	—
ARC170196 / 35045366	1.74% (0.55–5.4%)	—	16.1% (5.4–42.6%)	—
30229263	12.49% (4.1–34.4%)	4.59% (1.5–13.8%)	73.7% (34.4–98.5%)	37.5% (13.8–77.3%)
32202448	4.82% (1.6–14.4%)	—	39.0% (14.4–79.0%)	—
HCV 0207 / 31481608	3.66% (1.2–11.1%)	—	31.1% (11.1–69.2%)	—

⚠ FFP transfusions carry substantially higher transmission risk than RBC due to the 10× greater plasma volume (200 mL vs. 20 mL).

Overall Average Transmission Risk Across 500 Donors

Individual sample probabilities were summed and averaged across all 500 anti-HCV positive/NAT nonreactive donors.

	Probability of HCV transmission with ID_{50} = 316 (100 – 1000) virions	
	RBC (20 mL plasma)	FFP (200 mL plasma)
sum probabilities of infectivity in 15 cases	0,52 (0,17 - 1,54)	4,17 (1,54 - 9,12)
n all a-HCV positive/ NAT nonreactive donors	500	500
Average probability of HCV transmission	0,10 (0,03 - 0,31) %	0,83 (0,31 - 1,82) %

Transmission Probability: Per-Donation Risk

Probit Analysis Findings

Estimated viral loads ranged between **0.40 – 3.04 copies/mL**, corresponding to:

8.0 – 60.8 virions in Red Blood Cell (RBC) units

80 – 608 virions in 200 mL Fresh Frozen Plasma (FFP) units

These extremely low viral loads are detectable only through replicate NAT testing — not standard single-replicate screening.

Low Viremic Plasmas (15 samples) — RBC

Transmission probability ranged from **1.74% (0.55–5.4%)** to **12.49% (4.13–43.4%)** per RBC transfusion.

Low Viremic Plasmas (15 samples) — FFP

Transmission probability ranged from **16.1% (5.4–42.6%)** to **73.7% (34.4–98.5%)** per FFP transfusion.

Average Across All 500 Donations — RBC

Average transmission probability: **0.10% (0.03–0.31%)** per RBC transfusion.

Average Across All 500 Donations — FFP

Average transmission probability: **0.83% (0.31–1.82%)** per FFP transfusion.

Determine country specific risk

Country-Specific Policy Matters

The next steps are extrapolating the risk to specific countries. This is dependent on the prevalence of anti-HCV positive, RNA negative donations in the countries blood supply.

The study performed in Australia highlights the difference in residual risk due to different prevalence of spontaneously resolved infections

Study Limitation

The cobas MPX assay was not used; the cobas HCV assay was substituted — acknowledged as a limitation of this study.

Broader Significance

This analysis supports evidence-based decisions about whether serology testing remains necessary alongside ID NAT, particularly in resource-varied settings or densely treated settings.

This study was conducted across the **United States, South Africa, and Spain**, providing a multinational perspective on HCV transfusion safety.

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Conclusions

Extremely Low-Level Viremia Exists

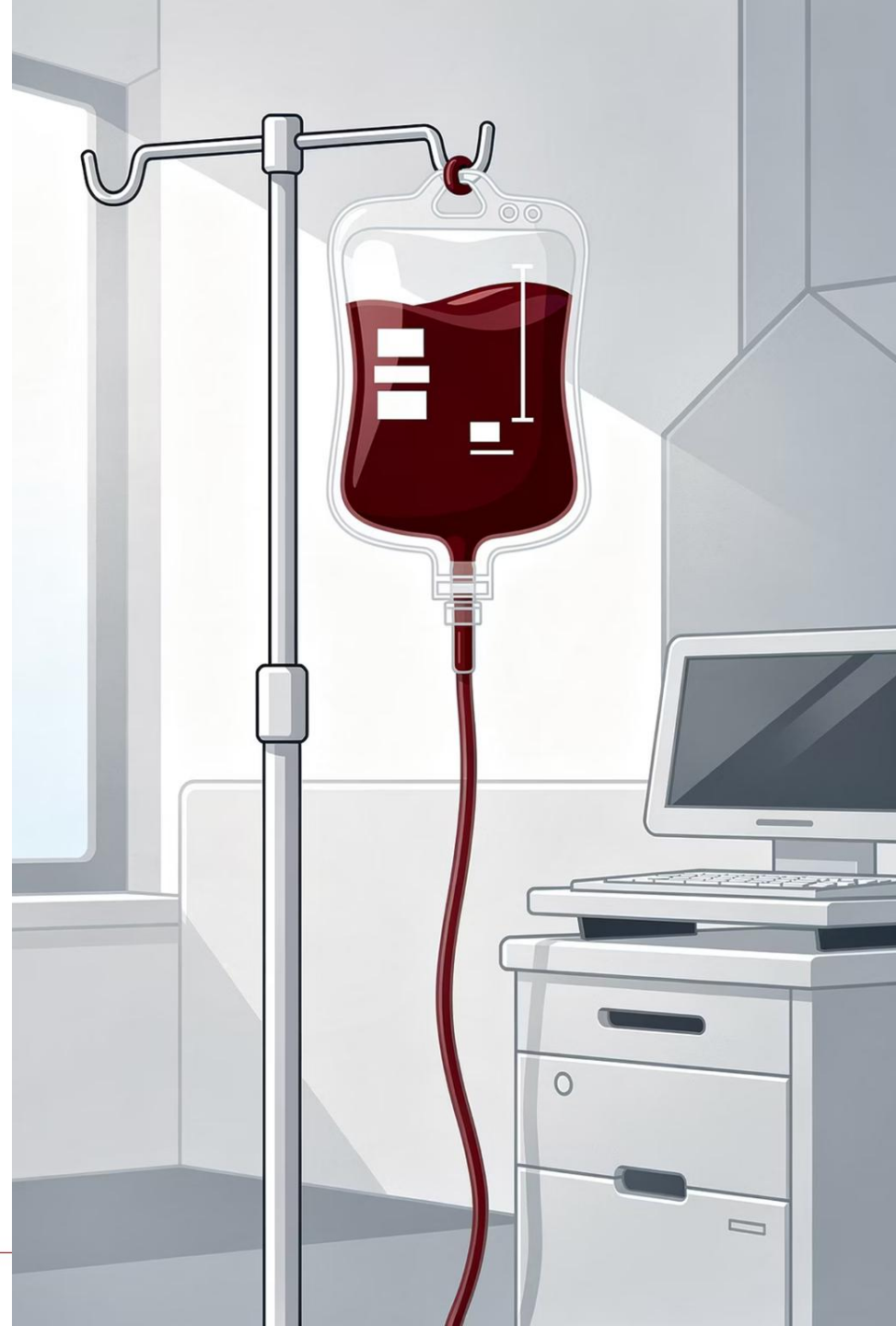
A small proportion (3%) of anti-HCV-positive/NAT-NR donors exhibit viremia detectable only through replicate NAT — not standard screening.

Overall Risk Is Exceedingly Low

Anti-HCV-positive/ID NAT-NR donations are **99.9% safe for RBC** and **99.17% safe for FFP** (assuming 100-fold reduced infectivity by anti-HCV neutralization).

Pathogen Inactivation Eliminates FFP Risk

In countries using pathogen inactivation on top of ID NAT, the associated FFP risk for HCV is likely eliminated entirely.



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