# Developments in understanding transmission risk and efficacy of screening scenarios

Nico Lelie, Director Scientific Affairs

Vilnius, Lithuania, May 19 2010

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# HBV genotype A2 panels and assays in EFS study[1]

| Panels | Assays |
|---|---|
| 5 HBV genotype A seroconversion panels (Zeptometrix 6254, 6289, 6292, 11006 and 11008)[1] | ▪Ultrio (5-18 replicates)<br>▪Ultrio Plus (2-16 replicates)<br>▪TaqScreen (4 replicates)<br>▪bDNA 3.0 assay (single)<br>▪PRISM HBsAg (2 replicates)<br>▪Biorad HBsAg (2 replicates) |
| HBV-DNA genotype A standard dilution panel (PeliCheck, Sanquin-VQC)[1,2] | ▪Ultrio (24 replicates)<br>▪Ultrio Plus (12 replicates)<br>▪TaqScreen (12 replicates) |

[1] EFS study (Assal et al, Transfusion 2009:49:289-300, Assal et al, Transfusion 2009:49:301-310)

[2] HBV standard was recalibrated in copies/ml by multiple bDNA 3.0 assays in DDL Diagnostic Laboratory (Voorburg, the Netherlands)

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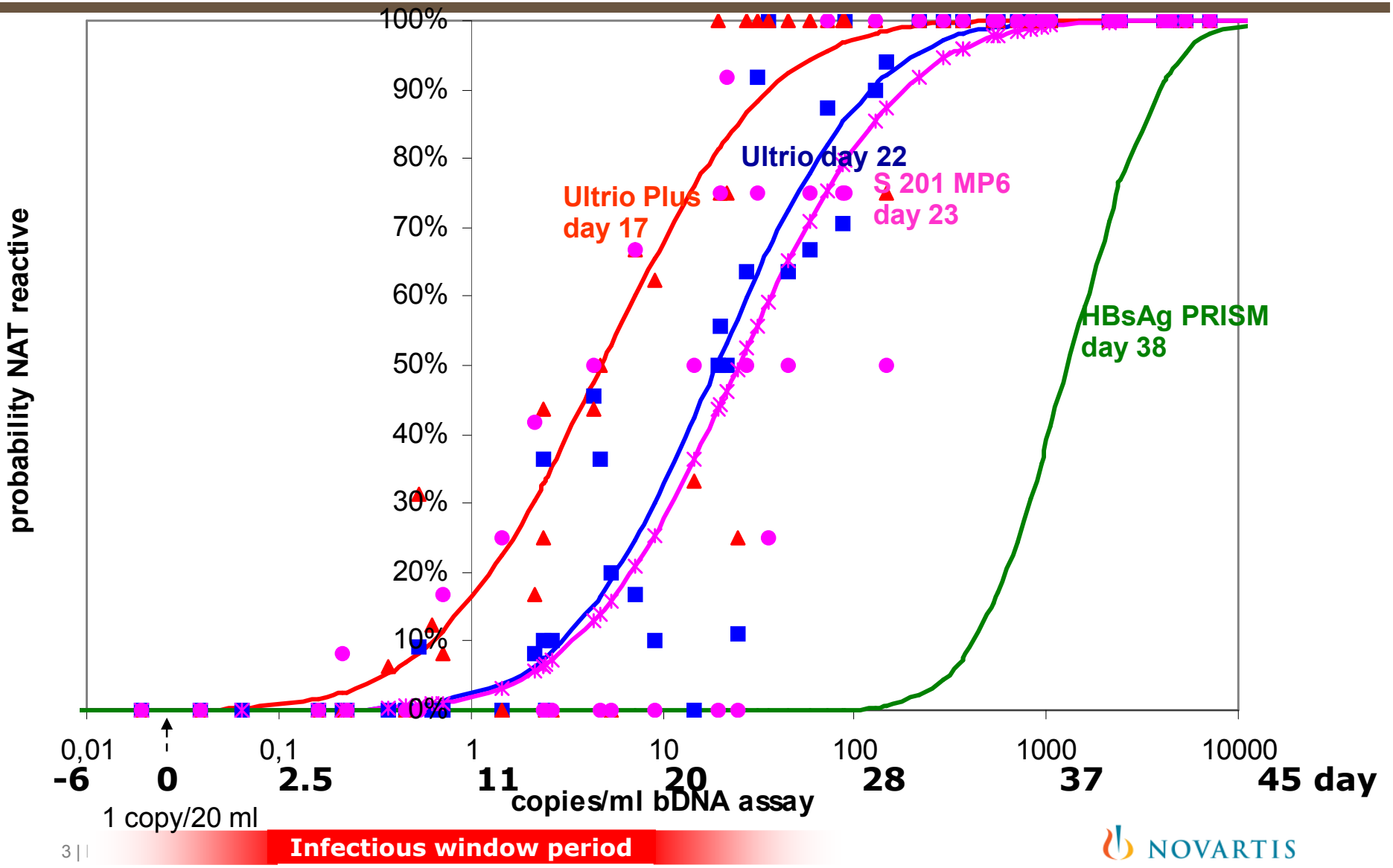
# Probability of detection* of HBV by NAT methods and HBsAg in five seroconversion panels



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Van Drimmelen et al, ISBT, 2010

# Comparison of HBV NAT response on five seroconversion panels and on genotype A standard dilution series by probit analysis

| Assay | panels | Sam-ples | Repli-cates | 50% LOD copies/ml | 95% LOD copies/ml | Potency dilution to SC panels |
|---|---|---|---|---|---|---|
| Ultrio | 5 SC | 69 | 5-18 | 19 (15-26) | 208 (140-347) | 1.04 (0.62-1.74) |
| | dilution | 10 | 24 | 19 (12-29) | 200 (120-375) | |
| Ultrio Plus | 5 SC | 69 | 2-16 | 4.6 (3.4-6.3) | 61 (38-110) | 0.81 (0.41-1.56) |
| | dilution | 10 | 12 | 5.7 (3.2-10) | 75 (39-166) | |
| s 201 MP-6 | 5 SC | 69 | 4 | 32 (20-49) | 381 (206-913) | 1.84 (0.63-8.85) |
| | dilution | 10 | 12 | 17 (9.5-30) | 207 (104-527) | |

Van Drimmelen et al, ISBT, 2010

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# Analytical sensitivity* of NAT systems on seroconversion panels and HBV gt A dilution panel

| assay | 50 % LOD (C.I.) copies/ml | 95% LOD (C.I.) copies/ml |
|---|---|---|
| Ultrio ID | 19 (16-24) | 206 (146-319) |
| Ultrio plus ID | 4.8 (3.7-6.4) | 64 (41-113) |
| s 201 MP-6 | 25 (17-36) | 316 (181-713) |

*50 % and 95 % detection limits determined by probit analysis on seroconversion panels and genotype A standard dilution series taken together

Van Drimmelen et al, ISBT, 2010

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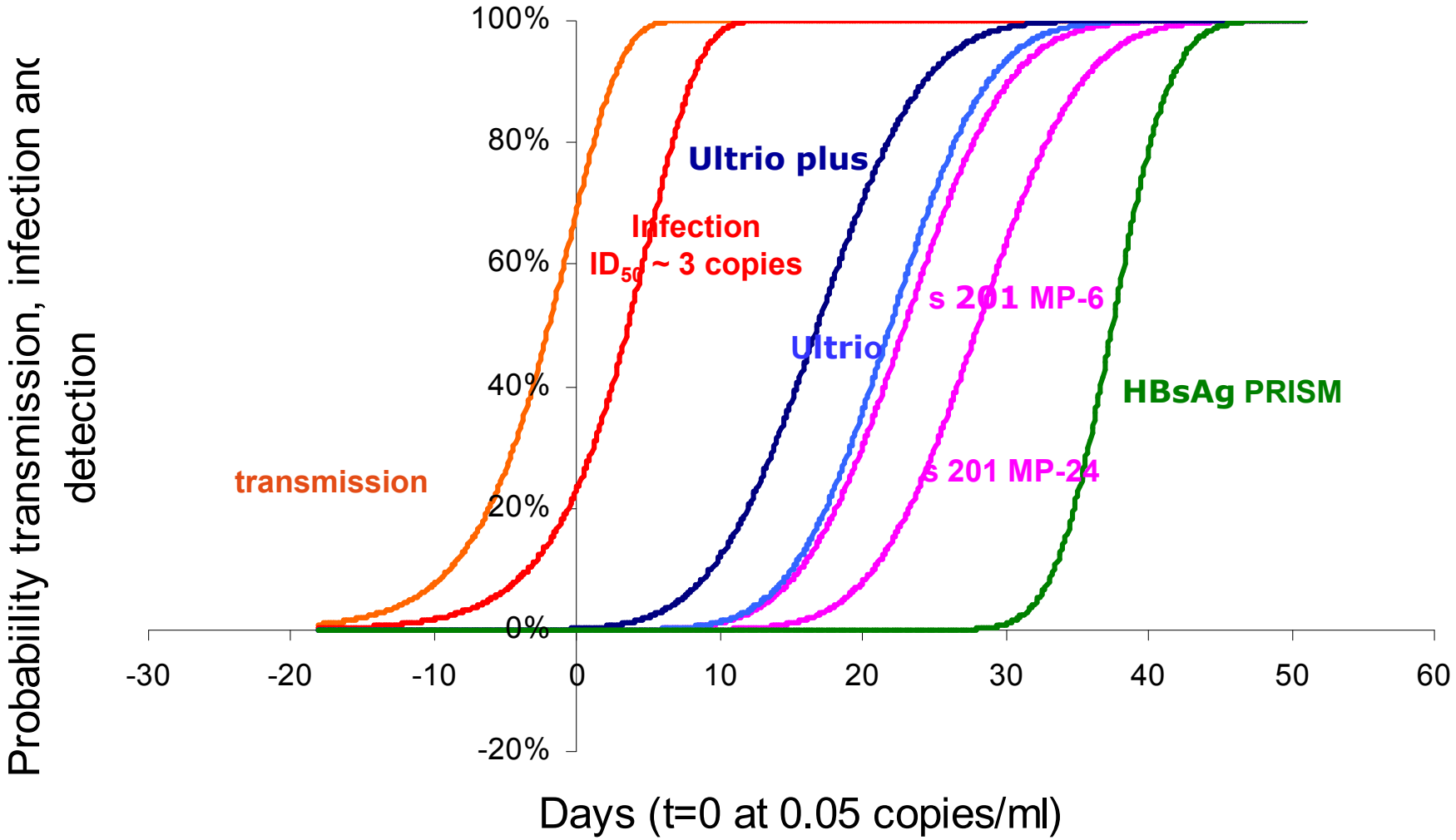
# Probability of transmission, infection and detection of HBV by NAT and HBsAg in infectious WP




Van Drimmelen et al, ISBT, 2010

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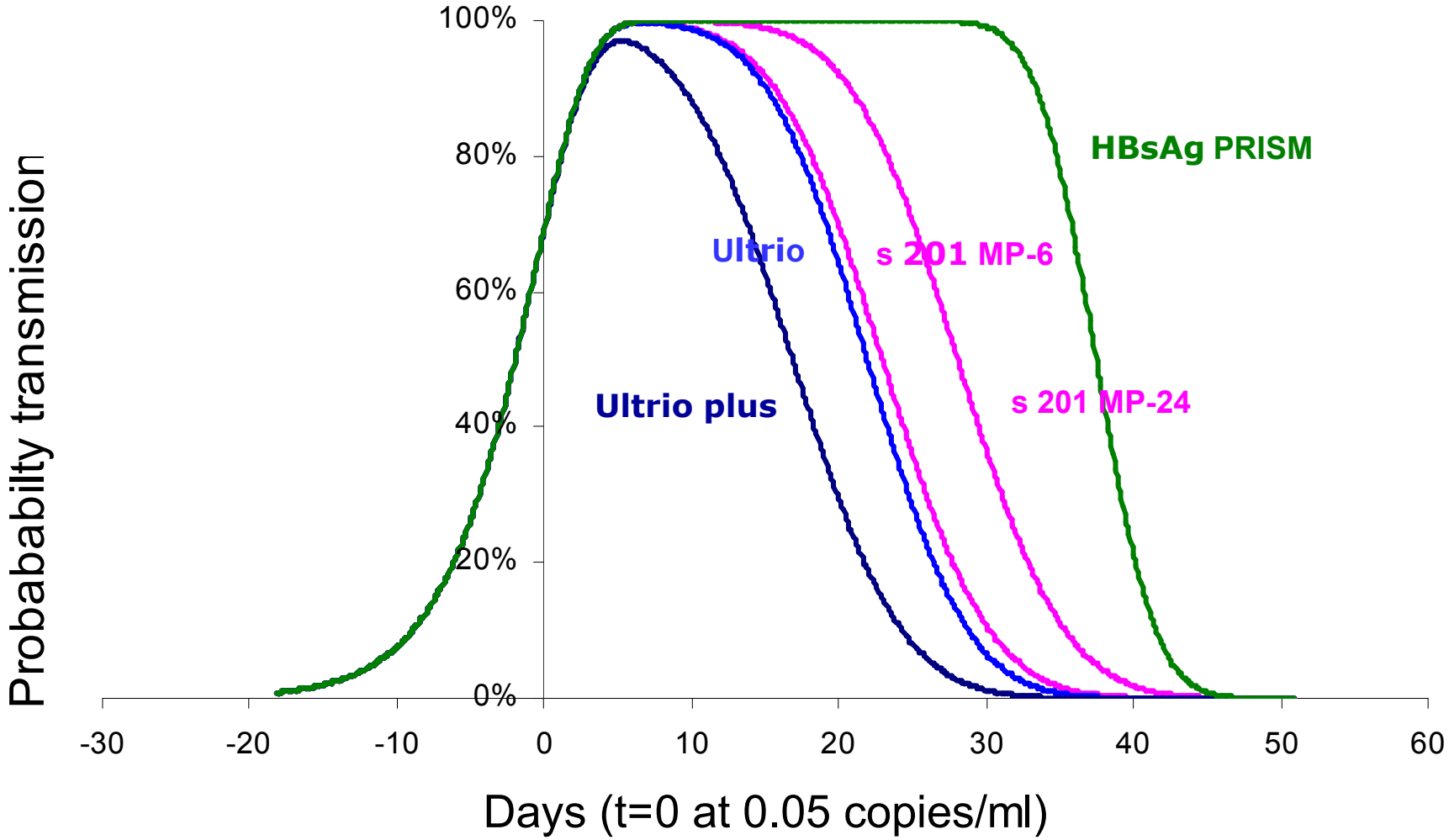
# Risk of transmitting virions* with HBV-NAT and HBsAg testing




* Weusten et al. Transfusion, in press



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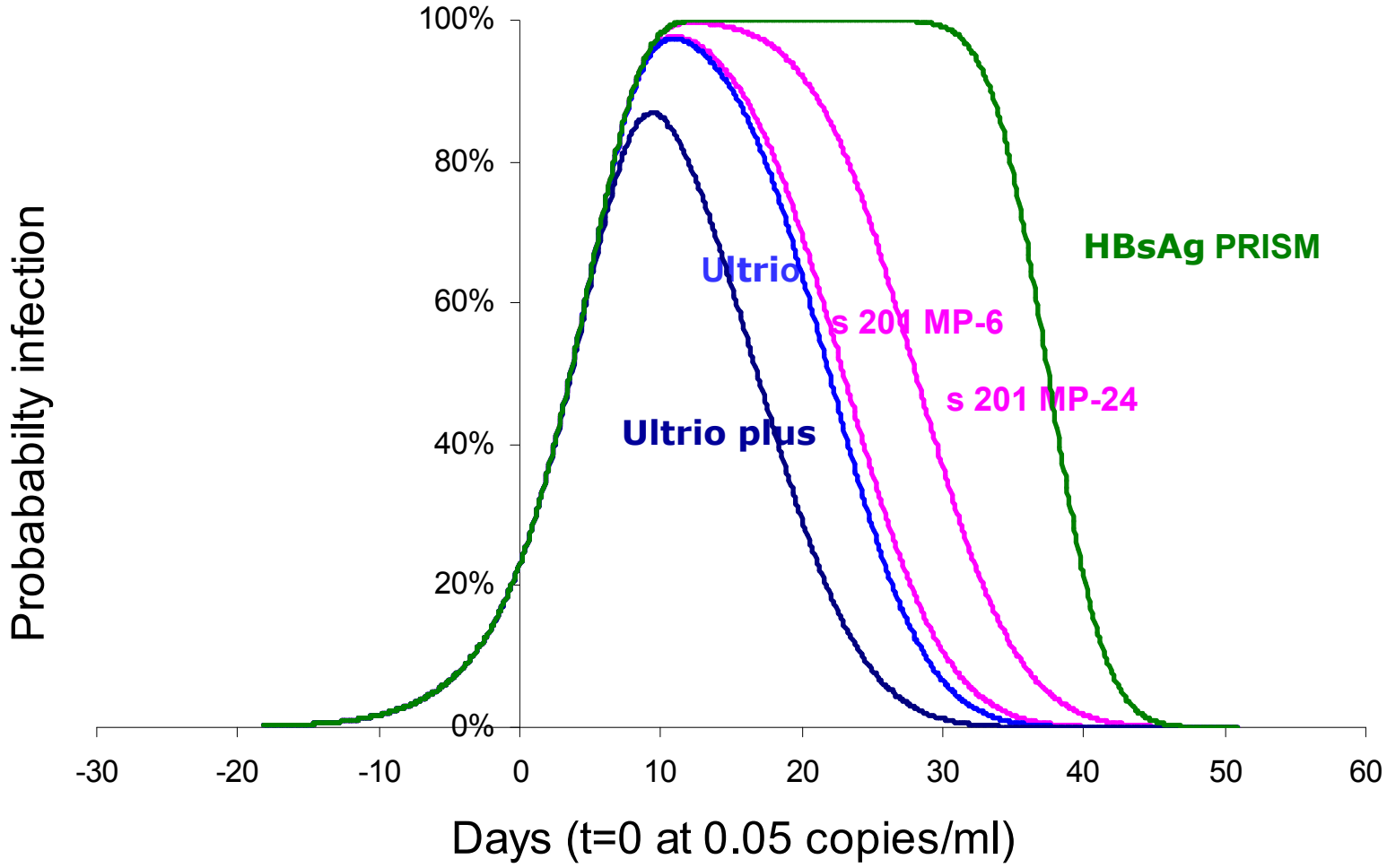
# Risk of HBV infection* with HBV-NAT and HBsAg testing ($ID_{50}$ ~ 3 copies)




* Weusten et al. Transfusion, in press

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# WP transmission risk day equivalents* with HBV-NAT and contribution of HBsAg testing

| | $ID_{50}$ = 1 copy (virion) | | | $ID_{50}$ = 3 copies (virions) | | |
|---|---|---|---|---|---|---|
| Assay | risk days | WP reduction by NAT | Extra WP reduction HBsAg | risk days | WP reduction by NAT | Extra WP reduction HBsAg |
| Ultrio Plus ID | 19.6 | 20.6 | 0.0017 | 14.1 | 20.6 | 0.0017 |
| Ultrio ID | 24.7 | 15.5 | 0.013 | 19.1 | 15.6 | 0.013 |
| s 201 MP-6 | 25.7 | 14.5 | 0.030 | 20.1 | 14.6 | 0.030 |
| s 201 MP-24 | 30.8 | 9.4 | 0.22 | 25.3 | 9.4 | 0.22 |
| PRISM | 40.2 | | | 34.7 | | |

* Weusten et al. Transfusion, in press

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# WP transmission risk day equivalents* with HBV-NAT and HBsAg testing, effect of $ID_{50}$

| | risk days | | | | |
|---|---|---|---|---|---|
| Assay | $ID_{50}$ =1 copy | *$ID_{50}$ =3 copies* | $ID_{50}$ =10 copies | $ID_{50}$ =30 copies | $ID_{50}$ =100 copies |
| Ultrio Plus ID | 19.6 | *14.1* | 9.6 | 6.1 | 3.3 |
| Ultrio ID | 24.7 | *19.1* | 14.4 | 10.4 | 6.5 |
| s 201 MP-6 | 25.7 | *20.1* | 15.4 | 11.4 | 7.4 |
| s 201 MP-24 | 30.8 | *25.3* | 20.5 | 16.4 | 12.0 |
| PRISM | 40.2 | *34.7* | 29.9 | 25.8 | 21.4 |

* Weusten et al. Transfusion, in press

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# New study protocol

- Multi-center analysis of the efficacy and cost effectiveness of HBV, HCV and HIV blood screening scenarios based on observed yield with serologic assays and individual donation nucleic acid amplification testing (ID NAT)
  - Sponsored by Novartis Diagnostics
  - Lead investigators: Steven Kleinman, Brian Custer, Michael Busch, Nico Lelie
  - Other investigators from multiple participating laboratories worldwide that use Ultrio ID NAT and chemiluminescent (or reasonably equivalent) serology assays

# Primary study objectives

- Compare the efficacy of different possible NAT and serology screening scenarios in first time and repeat donors for HIV, HCV, and HBV infection
  - Calculate modelled residual risk based on observed ID NAT and serology yield in many countries
  - Efficacy is defined as percentage of transmission risk that is removed by a given testing strategy
- Compare the cost effectiveness of these scenarios
- Examine if different screening scenarios could be selected in low and high incidence/prevalence countries

# Secondary study objectives

- Classify HIV, HCV, and HBV infections into various phases of infection and analyze these data by geographic region
  - South Africa, Egypt, Mediterranean, Eastern Europe, Nordic (North European), Southeast Asia, NZ
- Compare the ratio of cases detected by serology alone, ID NAT alone, and serology plus NAT in regions with differing infection incidence
- Extend the analysis to antigen/antibody combo assays and MP-NAT of various sizes and separately to lapsed donors

# Input data required from each participating lab

- Number of donations tested:
  - classified by donor status: first time, repeat, and lapsed (> 1 year)
- Test yield data:
  - NAT only, serology only, and concordant NAT/serology classified by donor status
- More detailed data on positive donations
  - Pre-seroconversion or pre-NAT conversion interdonation interval
  - Data to validate true positivity and the phase of infection

# Additional data for positive donations

- Differentiate true positives from false positives
  - Serology confirmatory testing procedures and detailed data (e.g., RIBA or Western blot band patterns)
  - Donor follow-up testing data
  - NAT data on an alternative sample (e.g., plasma bag)
  - Additional HBV assay results
- Classify stage of infection
  - Follow-up testing data
  - HIV: p24 Ag status, Western blot results if using combined Ag-Ab EIA
  - HBV: vaccination history, anti-HBc status, anti-HBc IgM status, anti-HBs titer in mIU/mL

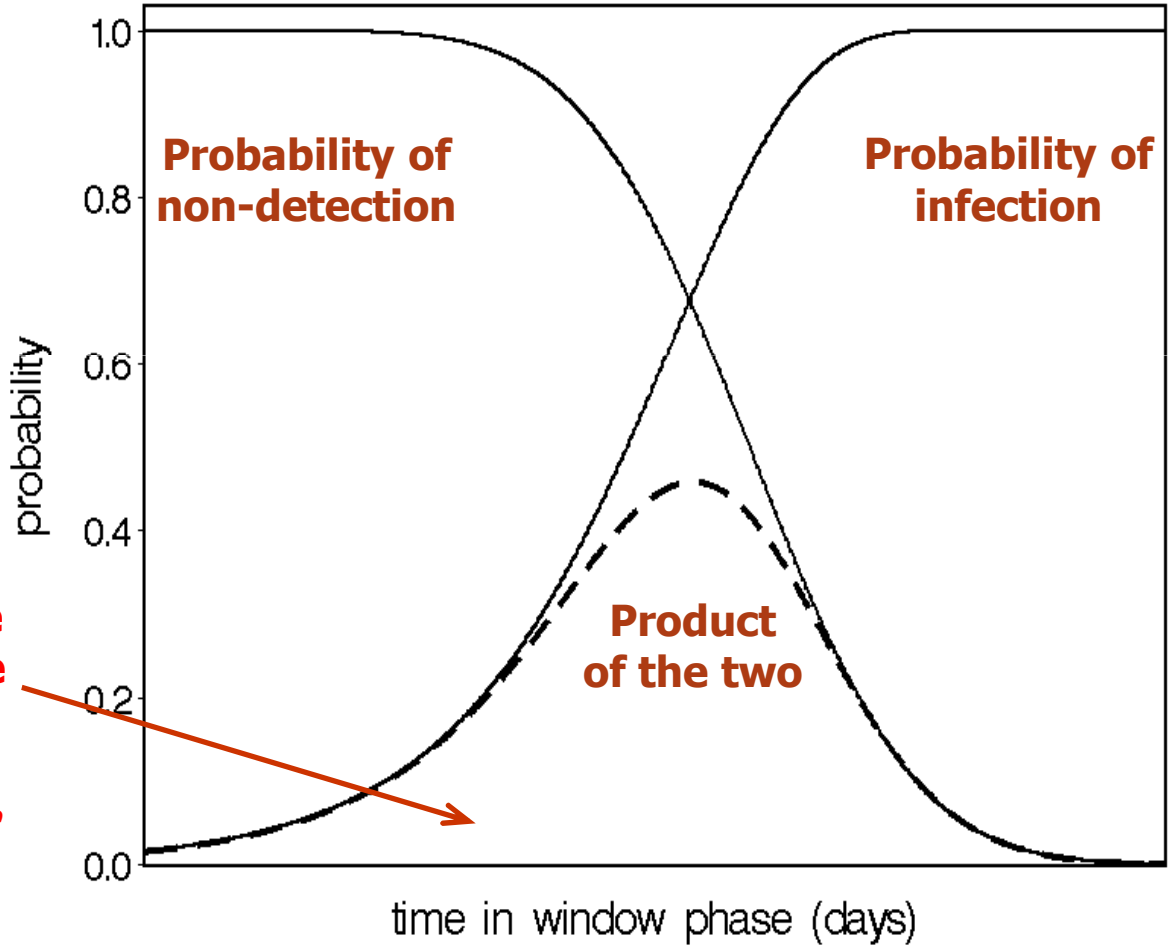
# Data standardization issues

- Determining when data from different countries can/should be combined
- Definition of a “lapsed” donation
  - proposed to be a repeat donation with an interdonation interval of > one year
- Missing data
  - particularly impacts classifying the phases of HBV infection

# Model for residual risk

- Calculate residual risk for packed RBC transfusion
- Use the statistical risk day equivalent model (Weusten et al) to calculate risk in repeat donors
  - Baseline scenario is ID NAT plus serology: this gives minimal residual risk
  - Evaluate residual risk in other testing scenarios: ID NAT only, serology only; MP NAT at various pool sizes
- Calculate risk in first time donors by using the ratio of ID NAT yield rate in first time to repeat donors as a conversion factor

# Probability of infectivity during the window period
# Weusten et al, Transfusion, in press)




**Area under the curve gives the overall risk in days** ***("Window phase risk days equivalents")***



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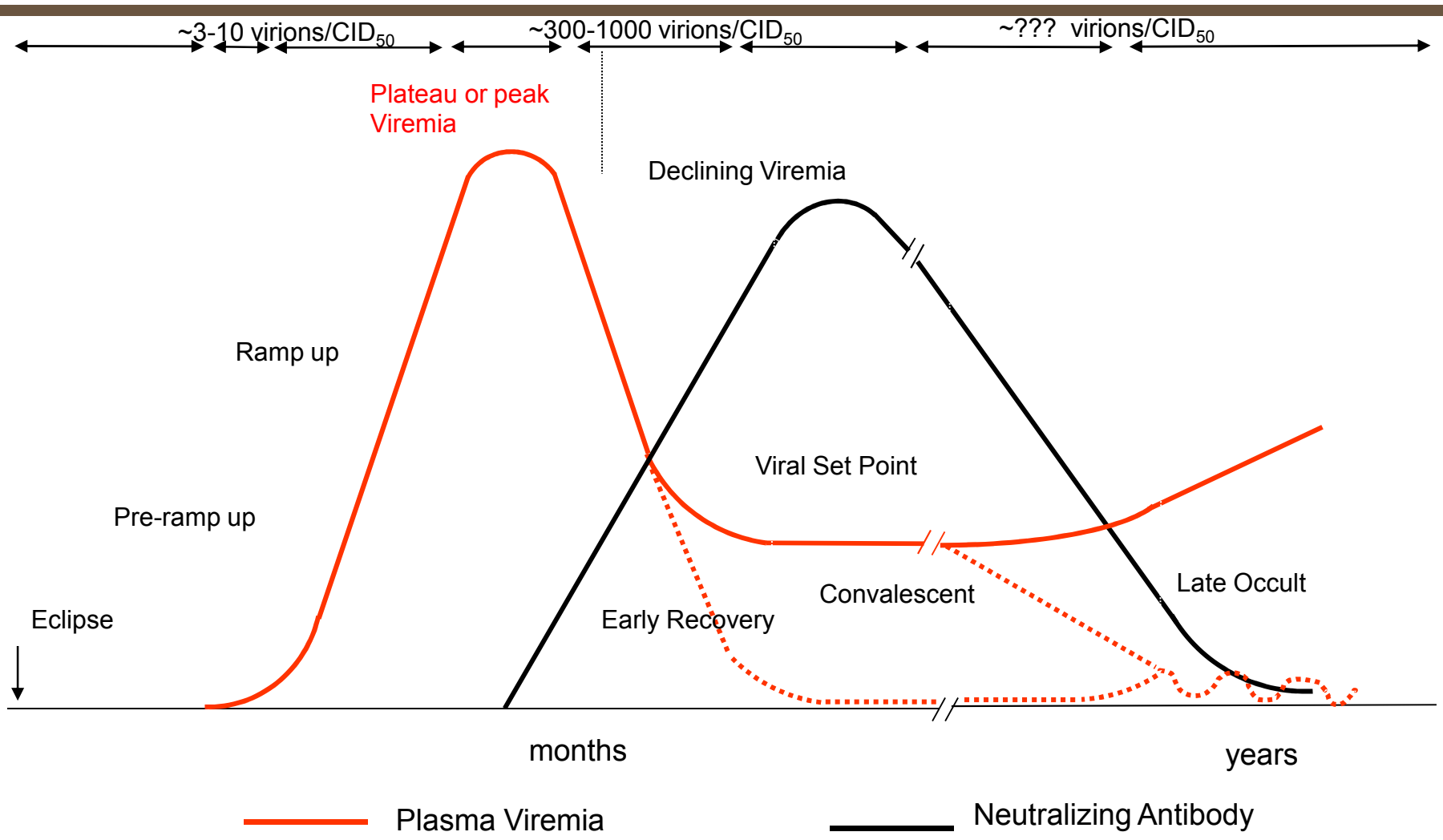
# Standardization and input parameters for modelling

- Calibration of NAT detection limits and minimum infectious dose in virions or nucleic acid copies using bDNA 3.0 assay as reference method[1,2]
- Determination of seroconversion point in antigen and combo assays during ramp up phase in copies/ml (bDNA assay)[3,4,5]
- Random appearance of donors in WP[3]
- Half life of nucleic acid and antigen in clearance phase[6]
- Minimum infectious dose in different stages of infection[7,8,9,10]

1). Van Drimmelen A.A.J. Vox Sang 96 Suppl1 ISBT abstract P075. 2). Weusten J et al, Transfusion, in press 3). Vermeulen M et al, Vox Sang ISBT abstract ( in press). 4). LaPerche et al, to be published. 5). Van Drimmelen et al. Vox Sang ISBT abstracxt (in press) 6). Yoshikawa A et al, Vox Sang 2005:88:77-86 7) Komiya K et al. Transfusion 2008;48:286 8). Katayama K et al, Intervirology, 2004, 47, 57 9). Tabuchi A et al. J Med Virol 2008;80:2064-8 10) Ma ZM et al, J of Virology, 2009, 83:3288-97

# Schematic of phases of infection



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# Assumptions for minimum infectious dose ($ID_{50}$) in different infection stages

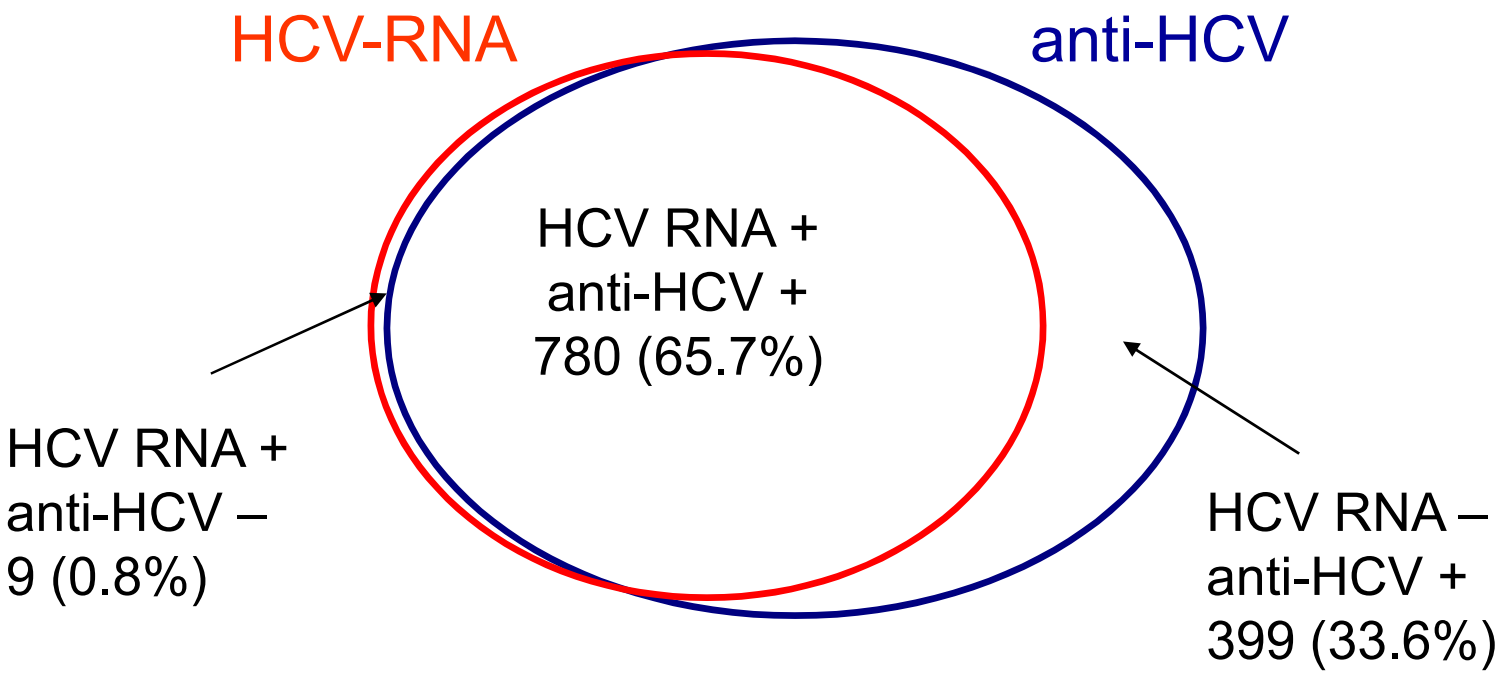
- in pre-seroconversion phase: 3 (1-10 virions)[1,2,3,4]
- in HB vaccine breakthrough infection (?)
- in clearance or early recovery phase (?)
- in chronic viremia phase: 300 (100-1000) virions[1,5,6,7]
- in chronic occult HBV infection phase: (?)
- in HBsAg positive low viral load carriers: (?)
- in HIV elite controllers: (?)
- in HCV low viral load (occult) carriers: (?)

**(?)** proposed to assume $ID_{50}$ of 300 (100-1000) virions in plasma in modelling

1) Ma ZM et al, J of Virology, 2009, 83:3288-97 2) Komiya K et al. Transfusion 2008;48:286 3). Katayama K et al, Intervirology, 2004, 47, 57 4) Kleinman S et al Vox Sang 96, Suppl1, ISBT abstract 5). Tabuchi A et al. J Med Virol 2008;80:2064-8 6) Hijikata J. Virol 1993 67:1953 7) Alter H et al. J. Viral Hep 1995, 2:121;

# Preliminary HCV and HIV data

# HCV screening results of **first time** donations in Egypt




1188 HCV infections in 27,655 first time donors
(prevalence 4.3%)

**El Ekiaby M et al. and Goubran et al. Vox Sang 96, Suppl1, ISBT abstracts, Cairo, March, 2009**



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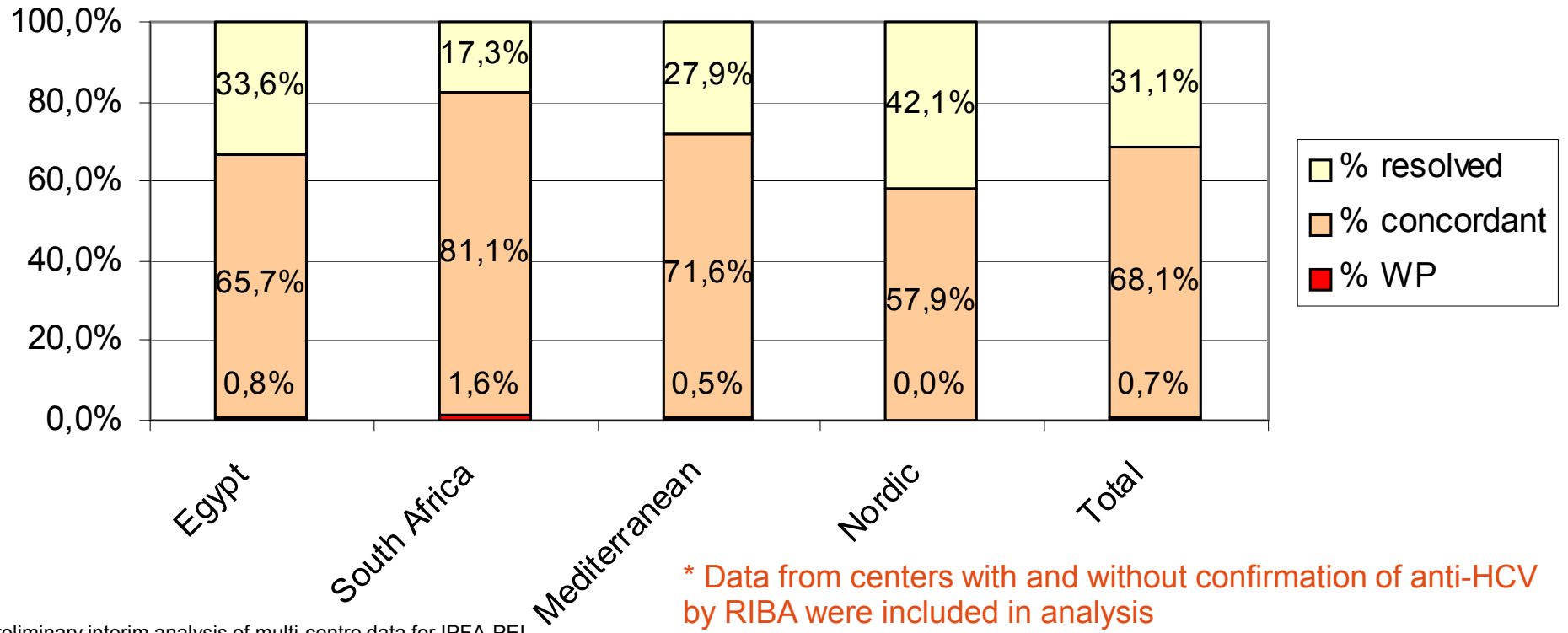
# Proportion HCV-RNA and antibody positives* in HCV infected **first time** donors from 4 regions

| | Egypt | South-Africa | Mediterranean | Nordic | Total |
|---|---|---|---|---|---|
| First time donors | 27,655 | 327,968 | 231,221 | 43,058 | 629,902 |
| Infections (prevalence) | 1188 (4.3%) | 148 (0.05%) | 419 (0.18%) | 19 (0.04%) | 1753 (0.28%) |
| WP NAT yield (rate) | 9 (1:3070) | 2 (1:164,000) | 2 (1:116,000) | 0 (0.00%) | 13 (1:48,454) |



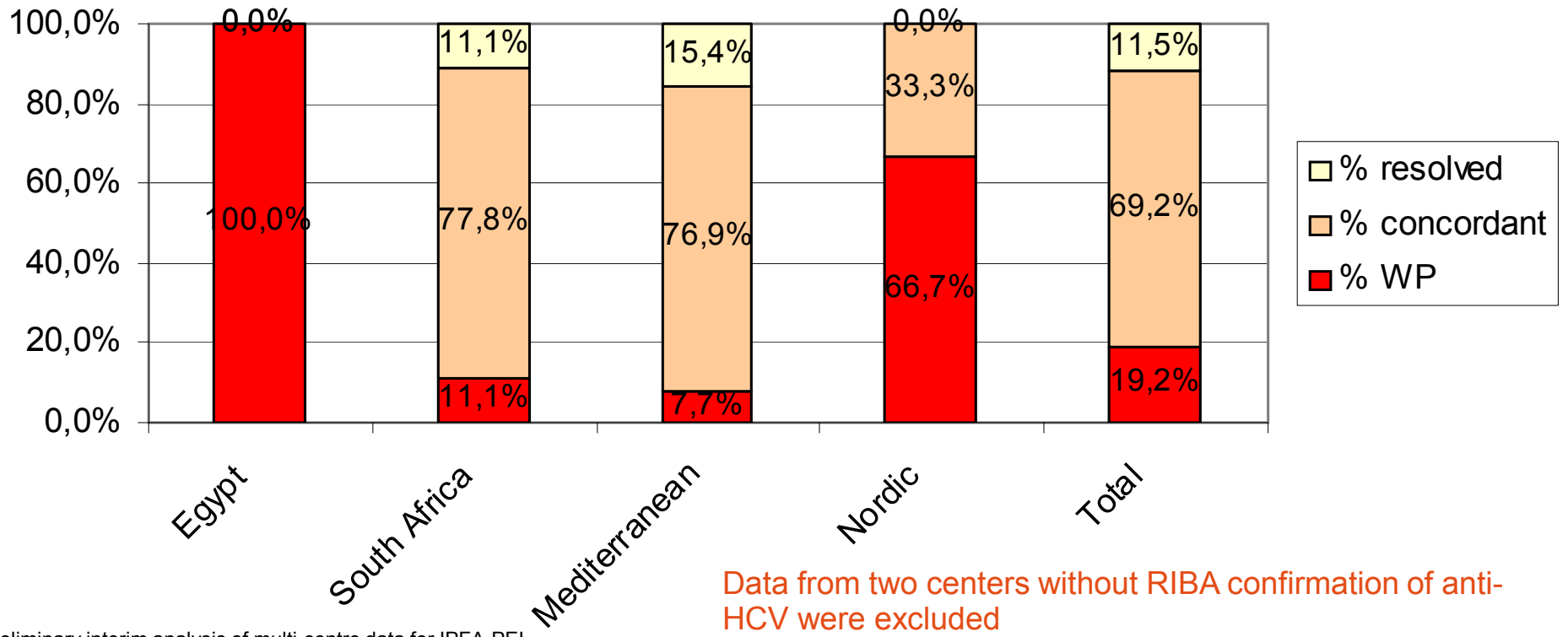

* Data from centers with and without confirmation of anti-HCV by RIBA were included in analysis

Preliminary interim analysis of multi-centre data for IPFA-PEI meeting in Zagreb by Kleinman et al

# Proportion HCV-RNA and antibody positives in acutely infected **repeat** donors from 4 regions

| | Egypt | South-Africa | Mediterranean | Nordic | Total |
|---|---|---|---|---|---|
| **Repeat donors** | **12,644** | **1,769,441** | **1,431,726** | **507,750** | **3,721,561** |
| **infections (rate)** | **1 (1:12,644)** | **9 (1:196,605)** | **13 (1:110,133)** | **3 (1:169.250)** | **26 (1:143,137)** |
| **WP NAT yield (rate)** | **1 (1:12,644)** | **1 (1:1,769,441** | **1 (1:1,431,726** | **2 (1:253,875)** | **5 (1:744,312)** |

HCV infections in lapsed donations (interval >365 days) excluded

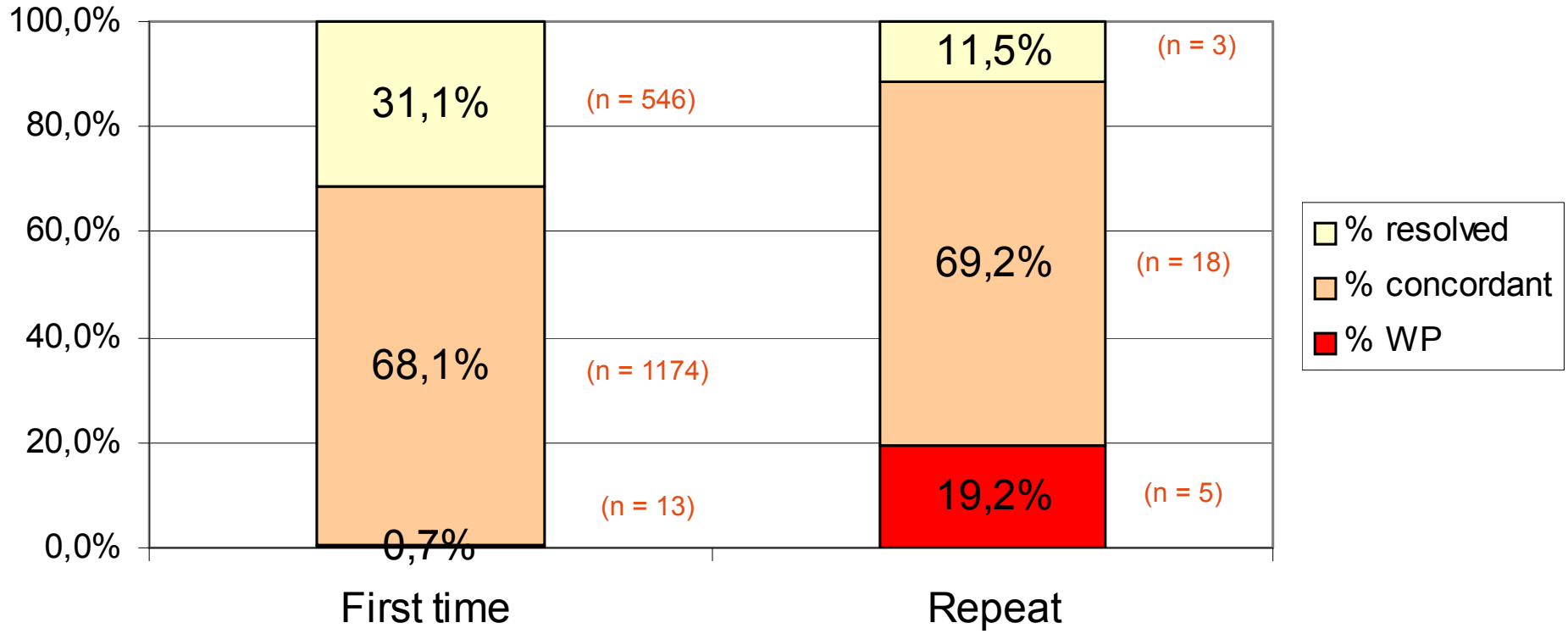



Data from two centers without RIBA confirmation of anti-HCV were excluded

Preliminary interim analysis of multi-centre data for IPFA-PEI meeting in Zagreb by Kleinman et al

# Proportion HCV-RNA and antibody positives in first time and repeat donations (interval <365 days)

| | First time | Repeat |
|---|---|---|
| donations | 629,902 | 3,721,561 |
| HCV infections | 1774 | 26 |




Data from two centers were excluded from repeat donation data set because anti-HCV reactivity was not confirmed by RIBA. Also infections in lapsed donations with intervals>365 days were excluded from repeat donation data.

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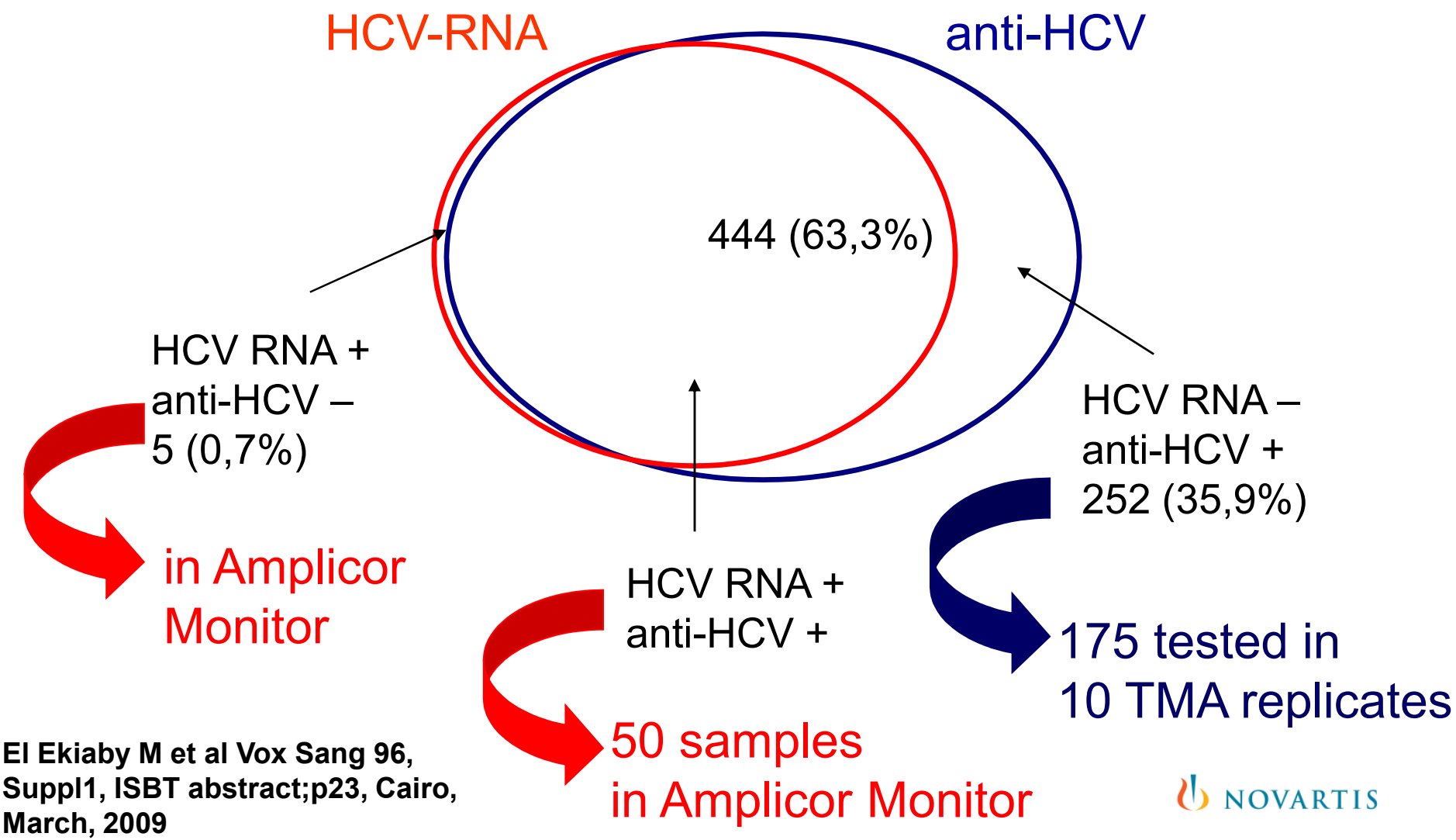
# Viral load analysis




**El Ekiaby M et al Vox Sang 96, Suppl1, ISBT abstract;p23, Cairo, March, 2009**

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# Replicate TMA results in subset of anti-HCV+/RNA- samples

- Random subset of 175 out of 252 anti-HCV+/RNA- samples were tested in 10 replicates
- 173/175 (98,8%) were non reactive in 10 replicates
- One donation was reactive in 3/25 (12%) of replicates and contained ~0.5 copies/ml plasma*
- Another donation was reactive in 10/25 (40%) of replicates and contained ~1.8 copies/ml plasma*

*determined by probit analysis against replicate TMA results on HCV genotype 1 and 4 standard dilutions

**El Ekiaby M et al. Vox Sang 96, Suppl1, ISBT abstract;p23, Cairo, March, 2009**

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# Viral load in 5 HCV window period samples in Egypt (NAT yield rate 1:3,100)

| donor | HCV-RNA copies/ml |
|---|---|
| 1 | 1.7E+07 |
| 2 | 2.0E+07 |
| 3 | 3.2E+06 |
| 4 | 9.1E+05 |
| 5 | 82* |

| dilution | TMA reactive* |
|---|---|
| 1:1 | 24/24 (100%) |
| 1:4 | 22/24 (92%) |
| 1:8 | 14/24 (58%) |
| 1:16 | 12/24 (50%) |
| 1:32 | 9/24 (38%) |
| 1:64 | 1/24 (4%) |

* determined by probit analysis against replicate TMA results on HCV genotype 1 and 4 standard dilutions

**El Ekiaby M et al. Vox Sang 96, Suppl1, ISBT abstract;p23, Cairo, March, 2009**

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# Modeling residual risk of HCV transmission by RBCs from Egyptian first time donors with different screening scenarios

| | WP risk by transfusion of RBCs[1,2] | |
|---|---|---|
| Screening with: | risk day equivalents | Risk |
| Anti-HCV only | 65 | 1:3100 |
| HCV Ag/Ab Combo only | 39[3] | 1:5200[1] |
| ID NAT only | 1.5[4] | 1:135,700 |
| Serology + ID NAT | 1.3 | 1:151,900 |
| Serology + MP 16 NAT | 3.1 | 1:65,600 |

1. adapted from WP ratio model (Busch MP et al. Transfusion 2005;45:254-264)
2. Transmission risk model (Weusten J et al,Transfusion 2002;42:537-548).
3. 60% of WP samples missed by Biorad combo ELISA
4. assumed both ramp up and recovery WPs

**El Ekiaby M et al. Vox Sang 96, Suppl1, ISBT abstract;p23, Cairo, March, 2009**

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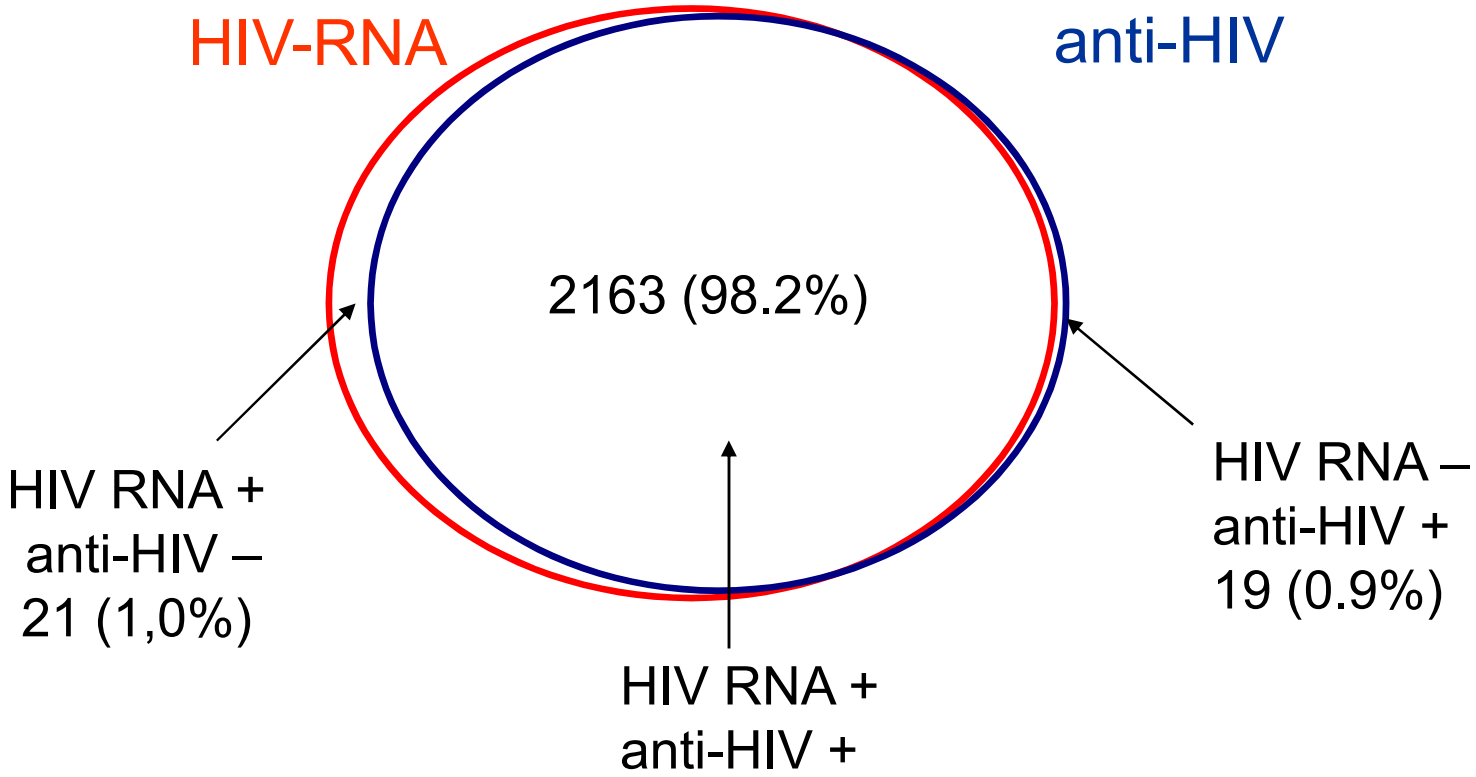
# HIV infections in **first time** donors during three years of ID-NAT screening in South Africa (SANBS, WPBTS)




2203 confirmed HIV infections in 327,928 first time donations

Preliminary interim analysis of multi-center data for IPFA-PEI meeting in Zagreb by Kleinman et al

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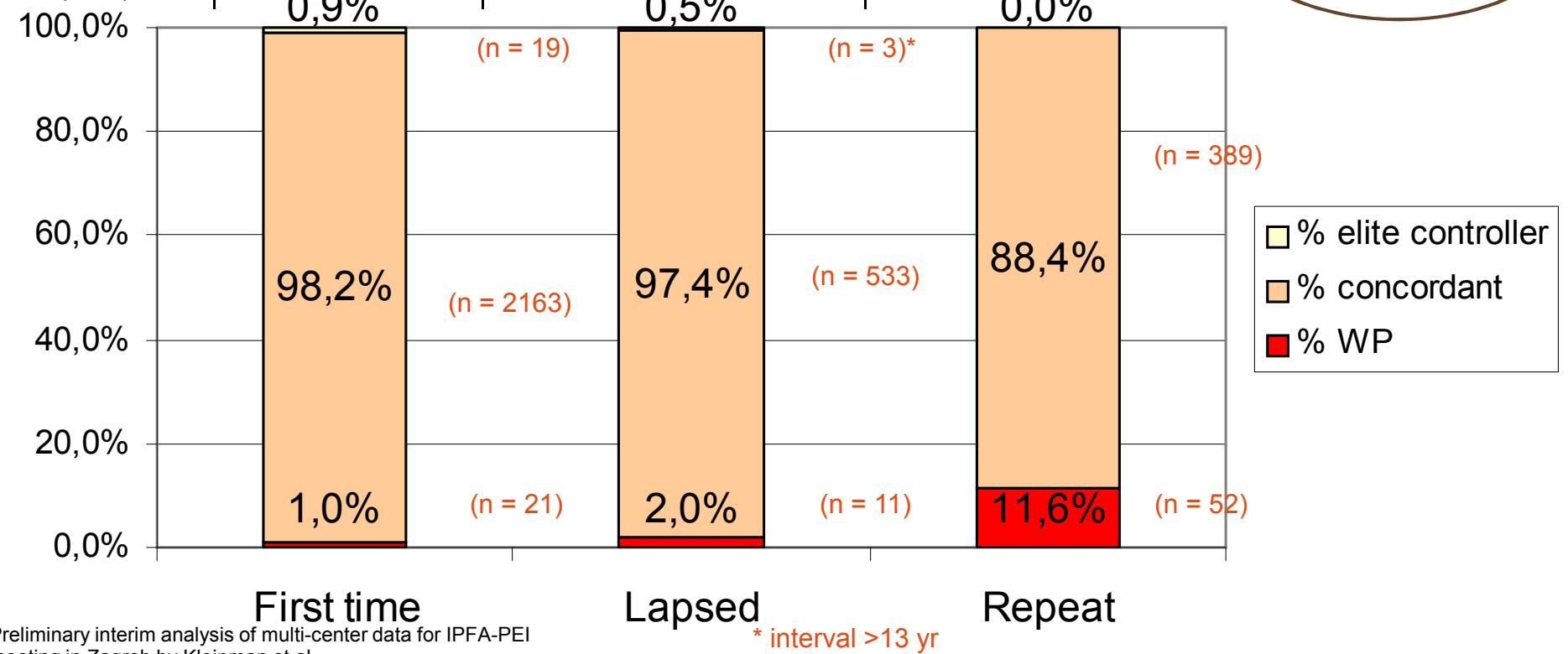
# Proportion detectable HIV-RNA in first time, lapsed, and repeat donors in South Africa (SANBS, WPBTS)

| | First time | Lapsed | Repeat |
|---|---|---|---|
| donations | 327,968 | 125,609 | 2,087,402 |
| infections (prevalence) | 2203 (0.67%) | 547 (0.44%) | 450 (0.02%) |
| NAT WP yield (rate) | 21 (1:15,618) | 11 (1:11,419) | 52 (1:40,142) |

2.51 and 3.51 fold lower yield rate than in first time and lapsed donors




Preliminary interim analysis of multi-center data for IPFA-PEI meeting in Zagreb by Kleinman et al

* interval >13 yr

# HIV infections in **first time** donations from three regions

| Region | **South-Africa** | **Mediterranean** | **Nordic** |
|---|---|---|---|
| donations | 327,968 | 231,261 | 43,058 |
| WP yield | 21 | 1 | 0 |
| All infections | 2203 | 51 | 1 |
| prevalence | 1:149 | 1:4535 | 1:43,058 |
| NAT yield rate | 1:15,618 | 1:231,261 | |
| % WP | 1.0% | 2.0% | 0% |
| % concordant | 98.2% | 98.0% | 100% |
| % elite controller | 0.9% | 0% | 0% |

Preliminary interim analysis of multi-center data for IPFA-PEI meeting in Zagreb by Kleinman et al

# HIV infections in **repeat** donations from three regions

| Region | **South-Africa** | **Mediterranean** | **Nordic** |
|---|---|---|---|
| donations | 2,087,402 | 1,851,220 | 507,750 |
| WP yield | 52 | 4 | 1 |
| All infections | 450 | 71 | 2 |
| Seroconv. rate | 1:4639 | 1:26,074 | 1:253,875 |
| NAT yield rate | 1:40,142 | 1:462,805 | 1:507,750 |
| % WP | 11.6% | 5.6% | 50% |
| % concordant | 88.4% | 94.4% | 50% |
| % elite controller | 0% | 0% | 0% |

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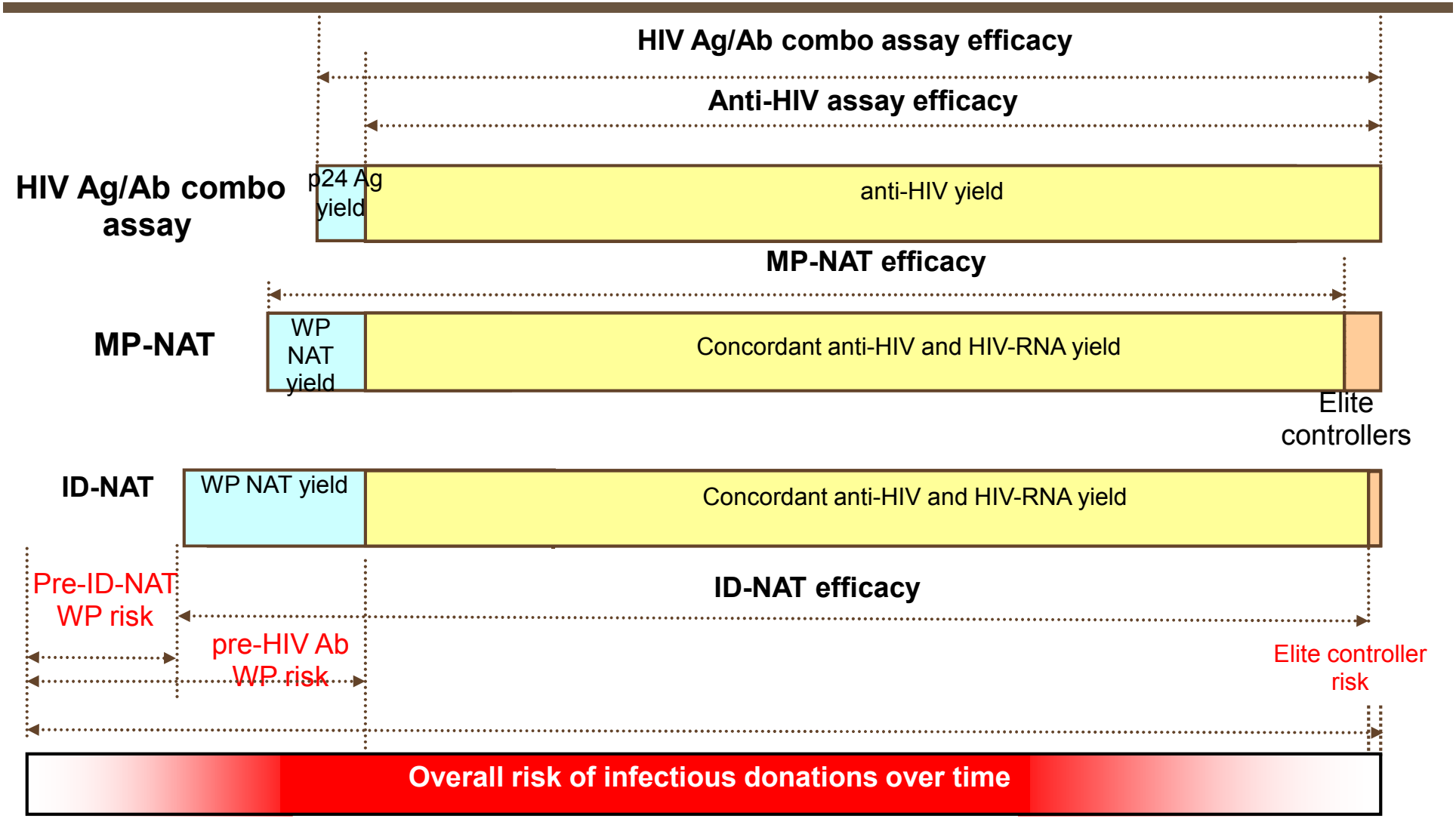
# Estimated viral load in 21 HIV elite controllers*(SANBS, 3 year)

| Year 1 | | | | | Year 2 | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **unit nr** | **pos** | **N test** | **% pos** | **cps/ml** | **unit nr** | **pos** | **N test** | **% pos** | **cps/ml** |
| 1 | 6 | 30 | 20% | 0.3 | *3* | destroyed | | | |
| 2 | 9 | 30 | 30% | 0.4 | 4 | 8 | 20 | 40% | 0.6 |
| **Year 3** | | | | | 5 | 12 | 25 | 48% | 0.8 |
| 14 | destroyed | | | | 6 | 2 | 20 | 10% | 0.2 |
| 15 | 4 | 10 | 40% | 0.6 | 7 | 0 | 31 | 0% | <0.1 |
| 16 | 6 | 6 | 100% | 2.7 | 8 | 20 | 23 | 87% | 2.9 |
| 17 | 0 | 20 | 0% | <0.1 | 9 | 2 | 30 | 7% | 0.1 |
| 18 | 12 | 20 | 60% | 1.1 | 10 | 4 | 25 | 16% | 0.3 |
| 19 | 1 | 29 | 3% | 0.1 | 11 | 0 | 10 | 0% | <0.2 |
| 20 | 2 | 20 | 10% | 0.2 | 12 | 0 | 33 | 0% | <0.1 |
| 21 | 4 | 25 | 16% | 0.3 | 13 | 5 | 25 | 20% | 0.3 |

*determined by probit analysis against HIV subtype C standard calibrated in bDNA copies/ml (Vermeulen et al, to be published)

# Efficacy of HIV screening assays in repeat or lapsed donations




*



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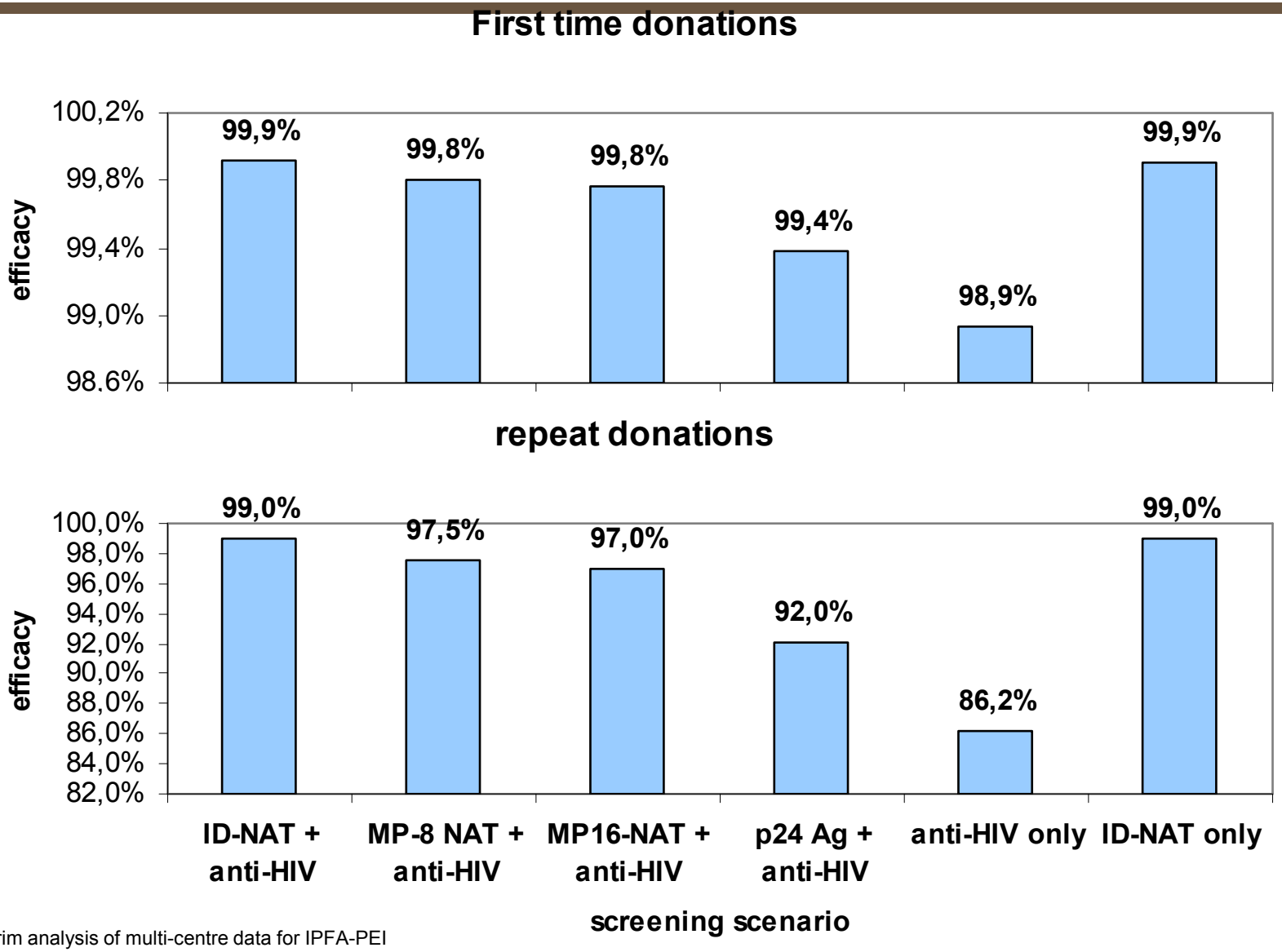
# HIV transmission risk* per million donations in different screening scenarios (SANBS 3 years)

| screening scenario | risk per million | | | |
|---|---|---|---|---|
| | First time | Lapsed | Repeat | All |
| ID-NAT + anti-HIV | 5,63 | 9,72 | 2,26 | 3,02 |
| MP-8 NAT + anti-HIV | 13,50 | 23,31 | 5,42 | 7,24 |
| MP16-NAT + anti-HIV | 16,12 | 27,83 | 6,47 | 8,64 |
| p24 Ag + anti-HIV | 43,10 | 74,41 | 17,30 | 23,10 |
| anti-HIV only | 74,62 | 129,03 | 29,95 | 39,99 |
| ID-NAT only | 6,22 | 9,72 | 2,26 | 3,10 |

*assuming $ID_{50}$ of 3.2 virions for HIV-NAT WP and 320 virions in elite controllers

Preliminary interim analysis of multi-centre data for IPFA-PEI meeting in Zagreb by Kleinman et al

# HIV Screening Efficacy (SANBS 3 years)

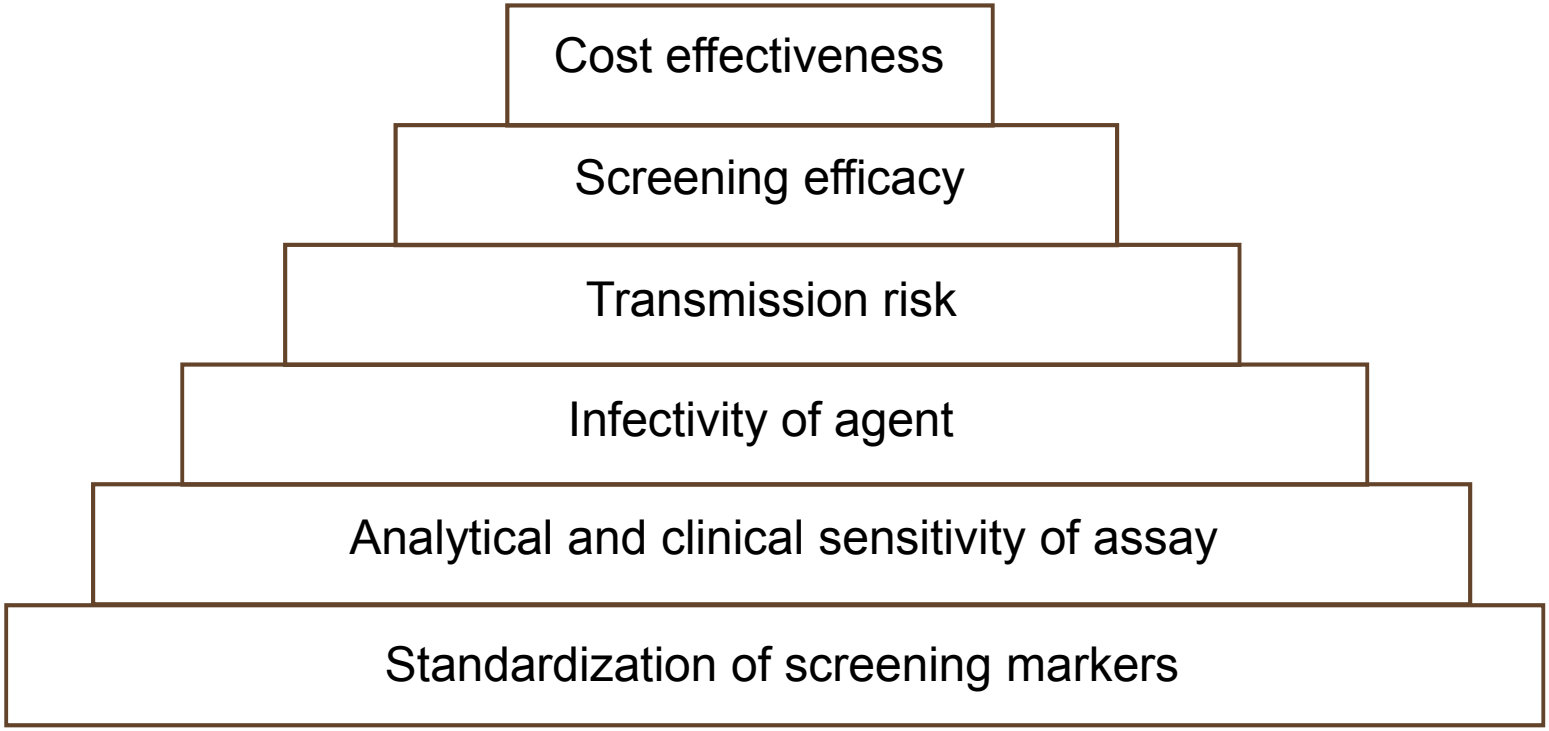



Preliminary interim analysis of multi-centre data for IPFA-PEI meeting in Zagreb by Kleinman et al

# Next steps

- Obtain data from additional countries and use these to extend the HIV and HCV risk analyses
- Perform similar analyses for HBV
- Possibly extend the protocol to include data collection from countries using other NAT testing:
  - MP NAT with Ultrio
  - MP NAT with MPX/s201 or Ultrio Plus
  - ID NAT with Ultrio Plus
- Conduct cost-effectiveness analyses

# Foundations for a robust analysis of screening efficacy and cost-effectiveness



# Co-investigators and laboratories who have supplied data

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