Blood Donor Screening 2009 - Current Tests & Risks

Dr Patrick Coghlan
National Transplantation Services
Australian Red Cross Blood Services
Melbourne
Source and Type of Regulated Blood Products

- Source Plasma
  - Plasmapheresis
  - ~450 tonne

- Whole Blood Donations
  - Recovered Plasma
    - Manufacture of Plasma Derivatives
      - And in-vitro reagents
    - Cellular components & Plasma
      - ~2,500,000 units

- Transfusion
  - ~1,000,000 units
  - ~500,000 donors
Ref: SHOT Annual Report, 2007
**Donor Derived Viral Infections**

- HBV, HCV: hepatitis, cirrhosis, HCC
- HIV: AIDS
- HTLV: Adult T-Cell Leukaemia, TSP/HAM
- CMV: CMV disease
- EBV: Infectious Mononucleosis, Lymphoma PTLPD
- HHV-8: Kaposi Sarcoma (immunocompromised)
- Erythrovirus: RBC aplasia
- Dengue: Dengue Fever
- CHIKV: Chikungunya Fever
- WNV: WNME
- Lyssavirus: Rabies
- Arenavirus: Lymphocytic Chorio-Menigitis
- Plasmodium: Malaria
- Babesia: Babesiosis
- Trypanosoma: Chaga’s disease
- Treponema: Syphilis
- Borrelia: Lyme Disease
- Mycobacteria: TB
- TSEs: CJD, vCJD,
Blood is much safer now, but is it safe enough?

Clinically significant viral infections by blood transfusion have been virtually eliminated by:

- Increasingly stringent donor eligibility criteria
- Increasingly sensitive/additional serological screening tests
- Nucleic acid amplification testing (NAT)

Sources of Residual Risk

- Window period (>90% of risk)
  - time between exposure to an agent and detection with screening tests
- Viral variants (strains, subtypes) not detected by current tests
- Infectious chronic antibody neg carriers
- Testing errors

Pat’s Take Home: Current estimates of risk

Minuscule Risk

Massive Risk

Neg Min VL

One in 1 Million = Effective Zero

The Paling Perspective Scale © John Paling

Blajchman - 2002
Calman 1995
Dzik 2003
‘Blood borne’ viral transmissibility

- Three primary criteria that generally need to be met for a virus to be transmitted by blood:
  - It gives rise to asymptomatic infection in the donor
  - It is present in the bloodstream
  - It is able to survive during subsequent storage/processing
Safeguarding the blood supply

‘Safety tripod’ *

1. Donor selection
2. Testing
3. Pathogen reduction
4. [Haemovigilance]

* Farrugia A. Vox Sang 2004 86: 1-7
HIV prevalence in persons aged 15 - 49 years in selected countries

- Papua New Guinea
- Thailand
- Myanmar
- Cambodia
- Vietnam
- France
- Italy
- Malaysia
- Australia
- Indonesia
- China
- New Zealand
- United Kingdom
- India
- Spain
- United States
- United Kingdom
- France
- Vietnam
- Malaysia
- Italy
- Australia
- Indonesia
- China
- New Zealand

HIV prevalence per 100,000

National Centre in HIV Epidemiology and Clinical Research: 2007
Diagnoses of HIV infection and AIDS in Australia

1. AIDS diagnoses adjusted for reporting delays.

Source: State and Territory health authorities
Donor Selection

Select from ‘low’ risk donors

- ARCBS – voluntary, non remunerated
- Donor education – ‘risk’ focused
- Comprehensive medical history
  - Infectious diseases - asymptomatic deferral period > usually 2x incubation period (e.g. WNV 28 days)
  - Minimum deferral period after cessation of symptoms
  - Additional deferrals for high risk behaviour, immunisations, medications, travel, transfusion, carcinoma etc.
How effective is donor selection?

ARCBS study

Reducing the risk of transfusion transmissible viral infection through blood donor selection: The Australian experience 2000-2006


- 6.2 million allogeneic blood donations collected by ARCBS between July 2000 and June 2006
- Tested for hepatitis C, hepatitis B, HIV, and HTLV I/II
- Donors with positive test results contacted for reassessment of risk factors and repeat testing
Prevalence Reduction

<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>Donor Prevalence</th>
<th>Population Prevalence*</th>
<th>Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatitis C</td>
<td>818</td>
<td>13 in 1x10^5</td>
<td>1-2 in 10^2</td>
<td>75-150</td>
</tr>
<tr>
<td>Hepatitis B</td>
<td>605</td>
<td>9.6 in 1x10^5</td>
<td>5-10 in 10^3</td>
<td>50-100</td>
</tr>
<tr>
<td>HIV</td>
<td>18</td>
<td>2.9 in 1x10^6</td>
<td>1 in 10^3</td>
<td>350</td>
</tr>
<tr>
<td>HTLV</td>
<td>20</td>
<td>3.2 in 1x10^6</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Reflects combined impact of education and selection

*Sourced from National Surveillance of Notifiable Infectious Diseases Reporting

(Source: Polizzotto et al)
## Comparing Requirements for Blood Testing

<table>
<thead>
<tr>
<th></th>
<th>Western</th>
<th>Developing</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Epidemiology</strong></td>
<td><strong>Low endemicity</strong></td>
<td><strong>High endemicity</strong></td>
</tr>
<tr>
<td>HBV</td>
<td>&lt; 0.1%</td>
<td>&gt; 5-10</td>
</tr>
<tr>
<td>HIV</td>
<td>&lt; 0.1</td>
<td>&gt; 2-15</td>
</tr>
<tr>
<td>HCV</td>
<td>&lt; 0.1</td>
<td>&gt; 0.5-12</td>
</tr>
<tr>
<td>CMV</td>
<td>± 50</td>
<td>&gt; 90</td>
</tr>
<tr>
<td><strong>Blood Bank size</strong></td>
<td>&gt;10,000/y</td>
<td>&lt; 10,000/y</td>
</tr>
<tr>
<td><strong>Test emphasis</strong></td>
<td>Specificity</td>
<td>Sensitivity</td>
</tr>
<tr>
<td><strong>Testing strategy</strong></td>
<td>Automated</td>
<td>Manual</td>
</tr>
<tr>
<td></td>
<td>EIA/NAT</td>
<td>Rapid tests/EIA</td>
</tr>
<tr>
<td><strong>Who pays</strong></td>
<td>Govt/Insurance</td>
<td>Patient/family</td>
</tr>
<tr>
<td><strong>Cost per unit</strong></td>
<td>$200</td>
<td>$10-40</td>
</tr>
</tbody>
</table>

*Source: J-P Allain*
Characterising Assays

<table>
<thead>
<tr>
<th>Assay Result</th>
<th>Presence of Disease</th>
<th>+</th>
<th>-</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>True Positive (TP)</td>
<td>+</td>
<td>Predictive value $\frac{TP}{(TP+FP)}$</td>
</tr>
<tr>
<td>-</td>
<td>False Negative (FN)</td>
<td></td>
<td>- Predictive value $\frac{TN}{(TN+FN)}$</td>
</tr>
<tr>
<td></td>
<td>False Positive (FP)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>True Negative (TN)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **Sensitivity**: $\frac{TP}{(TP+FN)}$
- **Specificity**: $\frac{TN}{(TN+FP)}$

*PJC 2000*

**Probability of a reactive sample being confirmed as positive.**

**Probability that test is negative in the absence of disease.**
**Universal Viral Screening Markers (ARCBS, 2007)**

<table>
<thead>
<tr>
<th>Serological markers:</th>
<th>NAT:</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBsAg</td>
<td>HIV-1 RNA</td>
</tr>
<tr>
<td>Anti-HIV-1/2</td>
<td>HCV RNA</td>
</tr>
<tr>
<td>Anti-HCV</td>
<td>(Chiron/Genprobe Procleix TMA multiplex)</td>
</tr>
<tr>
<td>Anti-HTLV-I/II</td>
<td></td>
</tr>
<tr>
<td>(Abbott PRISM ChLIA)</td>
<td></td>
</tr>
<tr>
<td>O&amp;T donations:</td>
<td></td>
</tr>
<tr>
<td>[Anti- HBc/Anti-HBs]</td>
<td></td>
</tr>
</tbody>
</table>

**NAT:**
- HIV-1 RNA
- HCV RNA

Chiron/Genprobe Procleix TMA multiplex
Initial Reactive IA-1 → Repeat Reactive IA-1 (anti-HIV, anti-HCV, anti-HTLV) → Product discard

Test on IA-2 (NAT non-reactive)

NEG on IA-2: biological false reactive (BFR)

Reactive on IA-2

Immunoblot
Selection

Screening

Donors-----Donation

Test System Optimisation
• Registration
• Supplier accreditation
• Supplier audit
• Assay evaluation
• Batch release certification
• Pre-acceptance testing
• Automation
• Monitoring SPC
• EQAS

Viral filtration

Blood Safety Strategies

Ab & Ag ChLIA (HIV & HCV Antigen) (US$20m/QALY)

NAT (US$~2M/QALY)

Patients

Viral Inactivation*

Fractionation

The transient increase in initial reactive rate (IR) and repeat reactive rate (RR) of a problematic HIV antibody assay.

M J Nightingale et al. Transfusion Medicine, 17, 404-412, 2007

Initial reactive rate (IR) and repeat reactive rate (RR) during the ‘settling in’ of an HIV Ab assay

M J Nightingale et al. Transfusion Medicine, 17, 404-412, 2007

CMV Neg
Leucodepletion
Appropriate Use
How good are current tests?

- Current serological tests for HIV, HCV, HBV, HTLV, CMV are capable of detecting >99.9% of infectious donations.
- ‘Window period’ risk
Comparative timing of first detection of HBsAg after primary HBV infection (mean value).

John Barbara, Steve Ramskill, Keith Perry, John Parry, and Mark Nightingale

Transfusion Medicine Reviews, Vol 21, No 2 (April), 2007: pp 147-158
Comparative timing of detection of primary HCV seroconversion (mean value).

This figure is based on data generated by testing 19 seroconversion panels in each of the HCV screening tests shown.

- Combined Ag/Ab assay
- Antigen capture assay
- Antibody capture assay
- PCR

John Barbara, Steve Ramskill, Keith Perry, John Parry, and Mark Nightingale

Transfusion Medicine Reviews, Vol 21, No 2 (April), 2007: pp 147-158
## PRISM Assay Performance

<table>
<thead>
<tr>
<th>Assay</th>
<th>donations tested</th>
<th>negative</th>
<th>repeatedly reactive (RR)</th>
<th>confirmed positive</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBsAg</td>
<td>1,153,645</td>
<td>1,153,544</td>
<td>207</td>
<td>101</td>
<td>99.99</td>
<td>48.8</td>
</tr>
<tr>
<td>Anti-HIV</td>
<td>1,153,645</td>
<td>1,153,642</td>
<td>1752</td>
<td>3</td>
<td>99.85</td>
<td>0.17</td>
</tr>
<tr>
<td>Anti-HCV</td>
<td>1,153,645</td>
<td>1,153,538</td>
<td>924</td>
<td>107</td>
<td>99.93</td>
<td>11.6</td>
</tr>
<tr>
<td>Anti-HTLV</td>
<td>1,153,645</td>
<td>1,153,643</td>
<td>674</td>
<td>2</td>
<td>99.94</td>
<td>0.30</td>
</tr>
</tbody>
</table>

Source: ARCBS 2006
Evolution of Approaches to Estimate Transfusion Risks

Risk per unit

Modeled Risk: I – WP Model

Measured Risk:
- Prescreening donor prevalence
- PCR/culture studies
- Recipient SC studies

Retrospective Cohorts:
- TTVS
- NIH
- TSS

Source: Mike Busch
Viral transmission: Measuring risk

- Classical approaches to measure risk (i.e. follow-up studies/missed infections in screened donors) - too few events

- Risk estimates now use mathematical modelling yielding theoretical risk levels based on:
  - Frequency of marker-negative, window period donations
  - Rare transmission events (variants)
  - Antibody negative carriers
  - Procedural testing errors
Incidence-Window Period Model

- Assumes that Window Period transmissions represent the major component of the residual risk.
- Probably holds true for HIV and HCV, but less so for HBV where chronic infection can be marked by transient HBsAg detection.
- \[ P = \lambda \times WP \]
  where \( P \) = probability donor gave infectious unit during window period,
  \( \lambda \) = the incidence and
  \( WP \) = window period

Source: Seed et al ARCBS 2005
Incidence-Window Period Model

\[ P = \lambda \times WP \]

where \( P \) = probability donor gave infectious unit during window period,
\( \lambda \) = the incidence and
\( WP \) = window period

For HIV NAT

\[ [\lambda = 6 \times 10^{-7} ; WP = 9 \text{ days} (9/365) = 0.02465] \]

\[ P = 6 \times 10^{-7} \times 0.02465 \]

\[ = 1.479 \times 10^{-7} \]

or 1 in 6,759,259

Source: Seed et al ARCBS 2005
Transfusion Transmitted Infections: Residual Risk Estimates for Periods 2005-06 and 2006-07

<table>
<thead>
<tr>
<th>Virus</th>
<th>Screening Technology</th>
<th>WP (95 CI)</th>
<th>Median (Point estimate)</th>
<th>Median (Point estimate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV</td>
<td>Procleix NAT</td>
<td>9 (8.0-10.3)</td>
<td>1 in 69,560,000 (8,319,000– 86,473,000)</td>
<td>1 in 35,256,000 (8,971,000– 41,558,000)</td>
</tr>
<tr>
<td>HCV</td>
<td>Procleix NAT</td>
<td>5.4 (4.9- 6.0)</td>
<td>1 in 12,215,000 (3,565,000– 47,783,000)</td>
<td>1 in 3,211,000 (2,855,000– 5,023,000)</td>
</tr>
<tr>
<td>HBV</td>
<td>Prism HBsAg</td>
<td>38.3 (33- 43.7)</td>
<td>1 in 669,000 (447,000– 2,657,000)</td>
<td>1 in 1,927,000 (678,000– 11,002,000)</td>
</tr>
<tr>
<td>HTLV</td>
<td>Prism HTLV 1/2</td>
<td>51 (36-72)</td>
<td>1 in 10,549,000 (2,664,000– 18,578,000)</td>
<td>1 in 14,728,000 (3,728,000– 25,059,000)</td>
</tr>
</tbody>
</table>

Seed et al: ARCBS 2008
### How does Australia benchmark internationally?
#### Residual risk per unit transfused

<table>
<thead>
<tr>
<th></th>
<th>HIV</th>
<th>HCV</th>
<th>HBV</th>
<th>HTLV</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Australia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(ARCBS 2008)</td>
<td>1 in 35 million</td>
<td>1 in 3.2 million</td>
<td>1 in 1.9 million</td>
<td>1 in 14.7 million</td>
</tr>
<tr>
<td><strong>USA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Dodd et al.2002)</td>
<td>1 in 2 million</td>
<td>1 in 2 million</td>
<td>1 in 270,000</td>
<td>1 in 3 million</td>
</tr>
<tr>
<td><strong>Canada</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(O’Brien et al.2007)</td>
<td>1 in 7.8 million</td>
<td>1 in 2.3 million</td>
<td>1 in 153,000</td>
<td>1 in 4.3 million</td>
</tr>
<tr>
<td><strong>UK</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Soldan et al.2005)</td>
<td>1 in 4.5 million</td>
<td>1 in 20 million</td>
<td>1 in 450,000</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>France</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Pillonel et al.2005)</td>
<td>1 in 3.1 million</td>
<td>1 in 10 million</td>
<td>1 in 640,000</td>
<td>N/A</td>
</tr>
</tbody>
</table>
## Documented NAT Breakthrough Infections

<table>
<thead>
<tr>
<th>Region</th>
<th>Country / Details</th>
<th>HIV</th>
<th>HCV</th>
<th>HBV</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AFRICA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rep. So Africa</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>ASIA - PACIFIC</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Australia</td>
<td></td>
<td>0</td>
<td>0</td>
<td>N/A</td>
</tr>
<tr>
<td>Japan</td>
<td></td>
<td>1</td>
<td>2</td>
<td>70</td>
</tr>
<tr>
<td><strong>EUROPE</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>France</td>
<td></td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Germany</td>
<td></td>
<td>2</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>Greece</td>
<td>(&quot;covering 75% of the total blood supply&quot;)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Italy</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Poland</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spain</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UK: (England, Scotland, Wales, No Ireland) + Eire</td>
<td></td>
<td>0</td>
<td>0</td>
<td>1 (since 2002), no NAT</td>
</tr>
<tr>
<td><strong>NORTH AMERICA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canada: (excl Quebec)</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Canada: (Quebec only)</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>USA</td>
<td></td>
<td>4</td>
<td>1</td>
<td>4 (since 2000); no NAT</td>
</tr>
</tbody>
</table>

M. Busch
HBV: To NAT or Not?

- Current screening test is HBsAg
- Should additional test(s) be introduced for detection of Hepatitis B?
  - Anti HBc
  - HBV DNA
Natural Course of Acute HBV Infection

Characteristics of the pre-acute phase of HBV infection

Window/incubation period of
- HBV DNA (individual NAT)
- HBV DNA (20 mini-pool NAT)
- HBsAg (CLIA method)
Hepatitis B NAT virus-positive blood donors in the early and late stages of HBV infection: analyses of the window period and kinetics of HBV DNA

Yoshikawa et al. Vox Sanguinis 88 (2), 77-86
Roche and Chiron have new automated assays that include hepatitis B NAT.

Need to be performed in single donor (Chiron Ultrio) or very small pools (Roche S201).

ARCBS participated in a trial to evaluate the instrumentation.

Accurate statistics for hepatitis B transmission are unavailable. In the absence of a haemovigilance system transmission rates are estimated by modelling.
### Analytical Sensitivity
- 95% Limit of Detection of WHO standards (IU/mL)

<table>
<thead>
<tr>
<th>WHO Reference Standard</th>
<th>Procleix ULTRIO IDT</th>
<th>Cobas MPX IDT</th>
<th>Significance (P&lt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1 (B) (97/650)</td>
<td>42.2 (24.8 - 99.3)</td>
<td>50.5 (29.6 – 118.2)</td>
<td>No</td>
</tr>
<tr>
<td>HCV (1) (96/798)</td>
<td>2.0 (1.4 - 7.4)</td>
<td>6.0 (3.9 – 12.5)</td>
<td>Yes</td>
</tr>
<tr>
<td>HBV (A) (97/746)</td>
<td>12.2 (7.3 - 29.2)</td>
<td>8.4 (5.0 – 21.7)</td>
<td>No</td>
</tr>
</tbody>
</table>

**The case of WNV**
- 2002 first death – blood donation
- FDA challenge – summer 2003
- 2003 WNV NAT
- 2007 – automated NAT

Margaritis et al. Transfusion 2007; 47:1783-1793
# Prevalence & Incidence of HIV, HCV, HBV and HTLV among Musculoskeletal Tissue Donors and First Time Blood Donors


<table>
<thead>
<tr>
<th>NAT: residual risk* reduction</th>
<th>Sero.</th>
<th>NAT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HIV</strong></td>
<td>WP</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>RR</td>
<td>1:161,000</td>
</tr>
<tr>
<td><strong>HCV</strong></td>
<td>WP</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>RR</td>
<td>1:55,000</td>
</tr>
<tr>
<td><strong>HBV</strong></td>
<td>WP</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>RR</td>
<td>1:172,000</td>
</tr>
</tbody>
</table>

**DDI Risk:** Reg BD << FTBD << O&TD

* Estimated probability of viraemia in donor

**TT - Cytomegalovirus**

- CMV Abs in 30 - 80% blood donors
- 2-12% Ab+ capable of transmitting virus
- WP 42 – 56 days
- Residual Risk of seronegative donation
  - 1:66,000 (1:42,000 – 165,000)
- 2 - 3 cases per year
- Strategies to eliminate TT-CMV:
  - CMV Ab negative blood
  - Leucocyte depletion
  - CMV immune globulin/intravenous immunoglobulin
  - Prophylactic acyclovir

*Source: Seed 2008*
Sample 5,050 random blood donors and 13 seroconversion panels.

Applying a modified cutoff of 9AU/mL for the AxSYM IgG MEIA

Seed et al Transfusion 2008; in press
Blood Testing in Africa

- Most blood centres screen for anti-HIV by ELISA or rapid tests
- 80% screen for HBsAg with rapid tests
- < 20% screen for anti-HCV
- Few countries have a QA system in place
- Kits and equipment are often donated, supply is not sustained

Source: J-P Allain
Simple/Rapid (S/R) Tests

Properties
- Simple
- Instrument-free
- Electricity-free

Type
- Agglutination
- Membrane
  - Immunofiltration (flow through)
  - Immunochromatographic (lateral flow)

Sensitivity/Specificity
- HIV 100% / 98.6%
- HBV 99% / 99.6%
- HCV 100% / 99.4%
Real World Testing – The Need for Simple Rapid Tests:

- Rudimentary lab facilities
- Poorly maintained equipment
- Interruptions to power supply
- Unreliable refrigeration
- Interruption in supply of tests
- Poor training and performance
Global Distribution of Major Human Flaviviruses
Emerging technologies

- 1990: Ag / Ab
- 2000: NAT
- 2010: Microarrays

serology  molecular biology  genomics  nanobiotechnologies
Acknowledgements

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- Clive Seed
- Angelo Margaritis
- Phil Kiely
- Dr Tony Keller