Transfusion Transmissible Infections

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South African National Blood Service
Introduction

• The South African National Blood Service (SANBS) collects approximately 750,000 donations per annum.

• The organisation is responsible for all aspects related to the collection, processing, testing, cross matching and issuing of blood and blood products to all areas of SA excluding the Western Cape.

• Approximately 900,000 blood products issued per year.
  ▪ Over 700,000 red cell units
  ▪ 120,000 FFP
  ▪ 57000 platelet concentrates

The safety of the blood supply is a primary concern.
Regulatory requirements

**What SANBS Does**

**Defer**
- Donor deferral
- Regular donors
- Education
- Target low risk communities

**Test**
- Tests as per regulations and standards
- Preparation of products from low-risk donors
- Quality system
- Staff training
- Accreditation

**Issue**
- Appropriate use of blood
- Blood user education
- Lookback programme
- Haemovigilance
- Quality systems
Various steps in the surveillance of blood transfusion safety

Donor

- Good practices related to blood collection
- *medical interview
- *physician's training

- Whole blood

Processing

- Good practices related to blood processing
- Virological screening

- Plasma
- Buffy coat
- RCC

- +4°C max. 42 days

- Blood component qualification

- Good practices related to storage

- Hospital traceable
- Hospital transfusion

Recipient

- Good practices related to distribution
- Recipient follow-up

- *Biological tests
- *Look-back

Donors

- Candidates for donation (% excluded)
- Potential donors
- Actual donors

Donor registry

Haemovigilance

Standards

Transfusion safety
Risks Exist for Blood Transfusion

1. Bacteria
   Introduced during collection

2. Window Period

3. Immunologic reactions
   Eg Haemolytic, Allergic

4. Emerging/Unknown Viruses

5. Known Pathogens
   For which no assay is available

6. Non immunologic reactions
   Eg circulatory overload

7. Leukocytes
   Adverse immune responses
- HIV transmission is often the key focus area at the expense of other risks
- The leading course of death related to blood transfusion is incompatible transfusion as a result of clerical error
What is the Risk of HIV Transmission by Blood?

- Injury at home
- Maternal Mortality
- Risk of G.A.
- Smoker + Oral Contraceptive
- Non Smoker + Oral Contraceptive
- HIV from Blood
- Murder Gunshot
- Acute Haemolytic Reaction
- Rape
- TB
# Pathogens Known to be Transmitted by Blood Transfusion

<table>
<thead>
<tr>
<th>Family</th>
<th>Pathogen</th>
<th>Disease</th>
<th>Routinely Screened</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatitis viruses</td>
<td>HBV, HCV</td>
<td>Hepatitis</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>HEV, HGV</td>
<td>Hepatitis</td>
<td>X</td>
</tr>
<tr>
<td>Retroviruses</td>
<td>HIV-1 &amp; -2</td>
<td>AIDS</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>HTLV-I &amp; -II</td>
<td>Malignant lymphoproliferative disorders, neuropath</td>
<td>X</td>
</tr>
<tr>
<td>Herpes viruses</td>
<td>CMV</td>
<td>CMV retinitis, hepatitis, pneumonia</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>EBV</td>
<td>Epstein-Barr Syndrome</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>HHV-8</td>
<td>Kaposi’s Sarcoma</td>
<td>X</td>
</tr>
<tr>
<td>Paroviruses</td>
<td>B19</td>
<td>Aplastic anemia</td>
<td>X</td>
</tr>
<tr>
<td>Bacteria</td>
<td>Gram-negative, Gram-positive</td>
<td>Sepsis</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td><em>Treponema pallidum</em></td>
<td>Syphilis</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td><em>Borrelia burgdorferi</em></td>
<td>Lyme disease</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td><em>Rickettsia rickettsii</em></td>
<td>Rocky Mountain Spotted Fever</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td><em>Ehrlichia chafeensis</em></td>
<td>Ehrlichiosis</td>
<td>X</td>
</tr>
<tr>
<td>Parasites</td>
<td><em>Trypanosoma cruzi</em></td>
<td>Chagas’ disease</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td><em>Babesia microti</em></td>
<td>Babesiosis</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td><em>Leishmania donovani</em></td>
<td>Leishmaniasis</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td><em>Plasmodium spp.</em></td>
<td>Malaria</td>
<td>X</td>
</tr>
</tbody>
</table>
Blood Safety Procedures

• At Donor site prior to donation
  – self exclusion
  – health questionnaire
  – Donor deferral and code registry
  – Confidential Unit Exclusion
  – Interview with nursing sister
The impact of donor selection

- HIV rate antenatal attendees: ~245/1000
- HIV rate population: ~150/1000
- HIV rate all donors: 1.89/1000
- HIV rate LP donors: 0.0062/1000
Screening tests for certain diseases performed on every blood donation

- Hepatitis B surface antigen (HBsAg)
- Anti-hepatitis C antibody (anti-HCV antibody)
- Anti-HIV 1&2 antibody
- Nucleic acid amplification test (NAT) for HIV-1, HCV and HBV
- TPHA for syphilis
Progress in Detection of Transfusion-Transmitted Pathogens

<table>
<thead>
<tr>
<th>Surrogate Marker</th>
<th>Antibody Testing</th>
<th>Viral Antigen Detection</th>
<th>Viral RNA/DNA Detection</th>
<th>Pathogen Inactivation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum ALT</td>
<td>Anti-HIV 1,2</td>
<td>HIV p24 Ag</td>
<td>NAT</td>
<td>Inactivates viral and bacterial RNA/DNA</td>
</tr>
<tr>
<td>T-cell count</td>
<td>Anti-HBc</td>
<td>HBsAg</td>
<td>HIV-1</td>
<td>HBV</td>
</tr>
<tr>
<td></td>
<td>Anti-HCV</td>
<td>HCV Ag</td>
<td>HCV</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anti-HTLV</td>
<td></td>
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</tr>
</tbody>
</table>

NAT is the only direct test for the infectious agent.

*Earlier Viral Detection = Safer Blood Supply*
Individual Donation Nucleic Acid Amplification Testing (ID-NAT) was introduced in the South African National Blood Service on 3 Oct 2005.

Routine Screening for HIV:p24 ag was discontinued.

Serological screening for anti-HIV 1&2, HBsAg, and anti-HCV on Abbott Prism was continued.

Platform chosen for NAT was Procleix Tigris using the Ultrio multiplex assay to screen blood donations for HIV-1, HBV, HCV.
- Further reduction of window period for HIV by 5-6 days
- Detects very low levels of viral RNA or DNA
- Highly sensitive & specific — targets specific viral nucleic acid sequences
- Reduces window period through direct detection of viral nucleic acid sequences
- Provides additional layer of safety to the world’s blood supply
Testing Centres

- Johannesburg (Area 1)
  - 5 instruments
  - Average Number tests
    - 54 000 / month
    - Ave 1800 / day (1600 – 2200)
    - 7 days per week

- Durban (Area 2)
  - 2 instruments
  - Average Number tests
    - 19 600 / month
    - Ave 650 / day (550 – 1000)
    - 7 days per week
Complete Automation: Procleix® TIGRIS® System from Chiron
COMPARING TMA TO PCR

- TMA is a RNA transcription amplification system using two enzymes to drive the reaction: RNA polymerase and reverse transcriptase. (PCR-Taq polymerase)
- TMA is isothermal, i.e. performed at a single temperature (PCR- thermal cycling)
- TMA produces RNA amplicon rather than DNA amplicon. Since RNA is more labile in the lab environment than DNA, this helps to reduce the possibility of carryover contamination.
- TMA produces 100-1000 copies per cycle in contrast to PCR that produces only 2 copies per cycle. This results in a 10 billion fold increase of copies within about 15-30 minutes.
• The Procleix Ultrio Assay is an in vitro nucleic acid amplification test for the qualitative detection of HIV-1 RNA, HCV RNA, and HBV DNA, all done simultaneously.

• The discriminatory probe reagents can then be used to discriminate between these viruses in the case of a reactive Ultrio assay.

• The system is designed as a batch analyzer with the capacity to perform 500 tests in less than 9 hours, taking about 4 hours for the first results, and 125 sample results each subsequent hour.
WHY USE NAT FOR BLOOD SCREENING in SA?

• SAFER BLOOD
• REDUCED RISK OF TRANSFUSION TRANSMITTED INFECTIONS
• MOST SENSITIVE TEST AVAILABLE
• SOUTH AFRICA HAS ONE OF THE HIGHEST HIV and HBV PREVALENCE RATES
## Confirmed HIV NAT Yields – Index Donation p24 Ag Positive (7)

<table>
<thead>
<tr>
<th>Month</th>
<th>First Rep</th>
<th>Unit No</th>
<th>Ultrio S/CO</th>
<th>dHIV S/CO</th>
<th>Ultra Bag</th>
<th>dHIV Bag</th>
<th>p24 S/CO</th>
<th>Viral load</th>
<th>Follow-up</th>
<th>Anti-HIV S/CO</th>
<th>Conf</th>
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<tbody>
<tr>
<td>Oct 06</td>
<td>L</td>
<td>19252094</td>
<td>11.3, 11.11</td>
<td>18.9</td>
<td>13.5</td>
<td>n.t</td>
<td>21.06</td>
<td>320667</td>
<td>10.9, 10.10</td>
<td>NT</td>
<td>25.9, 21.8</td>
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<tr>
<td>Aug 06</td>
<td>F</td>
<td>2759058</td>
<td>17.1, 16.3, 16.1</td>
<td>27.86</td>
<td>18.3</td>
<td>25.5</td>
<td>8.47</td>
<td>305460</td>
<td>18.32</td>
<td>26.84</td>
<td>101.92</td>
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<tr>
<td>Sept 06</td>
<td>R</td>
<td>19738501</td>
<td>18.4, 3.1, 16.5</td>
<td>22.01</td>
<td>18.26</td>
<td>n.t</td>
<td>7.59</td>
<td>151635</td>
<td>18.1, 17.5</td>
<td>23.9</td>
<td>0.46</td>
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<tr>
<td>Oct 05</td>
<td>R</td>
<td>19157834</td>
<td>16.5, 18.5, 18.0</td>
<td>17.95</td>
<td>0.05</td>
<td>21.67</td>
<td>6.04</td>
<td>127309</td>
<td>16.8</td>
<td>22.32</td>
<td>20.7</td>
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<tr>
<td>Mar 06</td>
<td>R</td>
<td>19367877</td>
<td>19.6, 19.1, 19.3</td>
<td>32.11</td>
<td>19.44</td>
<td>24.13</td>
<td>13.9</td>
<td>126187</td>
<td></td>
<td></td>
<td>No follow-up</td>
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<tr>
<td>Apr 06</td>
<td>R</td>
<td>2693348</td>
<td>17.4, 20.0, 19.6</td>
<td>26.3</td>
<td>No pack</td>
<td>No pack</td>
<td>22, 21.6</td>
<td>No samples</td>
<td>16.5</td>
<td>23.6</td>
<td>73.11</td>
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<td>Oct 07</td>
<td>R</td>
<td>18814602</td>
<td>12.6, 12.8, 12.2</td>
<td>16.23</td>
<td>12.93</td>
<td>18.4</td>
<td>1</td>
<td>16123</td>
<td>9.8, 9.9</td>
<td>7.95</td>
<td>11.85</td>
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### Confirmed HIV NAT Yields – Index Donation p24 Negative (7)

<table>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
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<th></th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>18814602</td>
<td>12.6, 12.8, 12.2</td>
<td>16.23</td>
<td>12.93</td>
<td>18.4</td>
<td>Neg</td>
<td>1</td>
<td>16123</td>
<td>Rea</td>
<td>Rea</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>19707346</td>
<td>15.9, 15.7, 15.4</td>
<td>17.39</td>
<td>18.09</td>
<td>23.67</td>
<td>Neg</td>
<td>0.4</td>
<td>7943</td>
<td>Rea</td>
<td>Rea</td>
<td>Pos</td>
<td>Pos</td>
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<tr>
<td>5401633</td>
<td>17.6, 14.6, 15.3</td>
<td>29.7</td>
<td>n.t</td>
<td>30.1</td>
<td>Neg</td>
<td>0.54</td>
<td>5467</td>
<td>Rea</td>
<td>Rea</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>2593697</td>
<td>15.9, 14.6, 14.6</td>
<td>17.9</td>
<td>16.34</td>
<td>n.t</td>
<td>Neg</td>
<td>0.75</td>
<td>2920</td>
<td>Rea</td>
<td>Rea</td>
<td>Pos</td>
<td>Pos</td>
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<tr>
<td>2647509</td>
<td>13.3, 13.7, 13.6</td>
<td>22.9</td>
<td>11.62</td>
<td>ND</td>
<td>Neg</td>
<td>0.5</td>
<td>216</td>
<td>Rea</td>
<td>Rea</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>2640514</td>
<td>4.01, 8.66, 3.56</td>
<td>5.5</td>
<td>2.16</td>
<td>ND</td>
<td>Neg</td>
<td>0.54</td>
<td>62</td>
<td>Rea</td>
<td>Rea</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>19441512</td>
<td>13.75, 13, 12.9</td>
<td>22.5</td>
<td>11.98</td>
<td>13.5</td>
<td>Neg</td>
<td>0.53</td>
<td>Neg</td>
<td>Rea</td>
<td>Rea</td>
<td>Pos</td>
<td>Pos</td>
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<tr>
<td>19036358</td>
<td>11.3, 9.02, 4.3</td>
<td>6.5</td>
<td>n.t</td>
<td>n.t</td>
<td>Neg</td>
<td>n.t</td>
<td>n.t</td>
<td>Rea</td>
<td>Rea</td>
<td>Pos</td>
<td>Pos</td>
</tr>
</tbody>
</table>
Prevalence of HIV NAT Yields (Window Phase Donations) in First, Lapsed and Repeat Donors

<table>
<thead>
<tr>
<th>Donor category</th>
<th>NAT Yield Cases</th>
<th>Number donations</th>
<th>NAT Yield Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>First time</td>
<td>3</td>
<td>73293</td>
<td>1:24,431</td>
</tr>
<tr>
<td>Lapsed</td>
<td>1</td>
<td>55393</td>
<td>1:55,393</td>
</tr>
<tr>
<td>Repeat</td>
<td>10</td>
<td>603564</td>
<td>1:60,356</td>
</tr>
</tbody>
</table>
HIV Seropositive (SP) and ID-NAT Window Phase (WP) Yield Rates in 3 Regions (test sites) of South Africa

Preliminary analysis, Sykes et al, Cable et al, ISBT 2007
Increase of virus concentration (C) in window doubling time. Busch MP et al

\[ C = C_0 \cdot 2^{t/\lambda} \]

- HIV-RNA
- HCV-RNA
- HBV-DNA

λ = 0.74 days
λ = 0.90 days
λ = 2.5 days
Closing the infectious window with Ultrio on Procleix Tigris System

**eclipse phase**

- **viral load**
  - geq/ml
  - $10^3$
  - $10^6$

- **days**
  - day 3
  - day 6
  - day 15
  - day 21
  - day 24
  - day 65

- **below infectivity threshold?**
- **infectious**

- **RNA**
- **HIV-Ag**
- **Anti-HIV**
- **HCV-RNA**
- **Anti-HCV**
- **HBV-DNA**
- **HBsAg**

- **1 geq/20 ml**
- **10 geq/20 ml**

- **days**
  - 0
  - 5
  - 10
  - 15
  - 20
  - 25
  - 30
  - 35
  - 40
  - 60
  - 65
  - 70
## Reduction of Window Period
(claimed by Chiron, based on European studies)

<table>
<thead>
<tr>
<th></th>
<th>Days of Infection to Procleix Detection</th>
<th>Days of Infection to Ab or Ag Detection</th>
<th>Reduction of Window by NAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1</td>
<td>11</td>
<td>22</td>
<td>50%</td>
</tr>
<tr>
<td>HCV</td>
<td>23</td>
<td>82</td>
<td>72%</td>
</tr>
<tr>
<td>HBV</td>
<td>34</td>
<td>59</td>
<td>42%</td>
</tr>
</tbody>
</table>

![Chart showing reduction of window periods for HIV-1, HCV, and HBV](chart.png)
Probability of transmission of HIV during the window phase

- Probability that virus infection in donor is not detected by NAT
- Probability that virus infection develops in patient receiving the blood product

Graph showing the probability over time after infection for 100 copies and 1 copy.
Risk of HIV transmission post NAT

- Based on current data calculated risk on average between 1/80,000 - 1/200,000 per unit
- Multiple transfusions increase risk
- No reported cases since NAT implemented
- Data from lookback studies not yet available
NAT yield for Hepatitis B

- 9 yield cases i.e HBsAG negative
- 2 serology yields- NAT negative
- Discordance between HBsAG and NAT
- Yield cases confirmed by follow up samples and additional testing i.e anti HBc and anti HBs
- HCV – no yield cases. HCV low prevalence in S.A.
NAT & Serology Testing Algorithm

- **Confirmed Viral Positive**
  - Ultrio RR/dHXV Reactive and PRISM RR & 2nd ELISA / Confirmation Positive.
  - Donor deferred and notified. No further testing.

- **Potential NAT Yield**
  - Ultrio RR and/or dHXV Reactive, Serology Negative

- **Potential Serology Yield**
  - PRISM RR, Confirmation / Second ELISA Pos, Ultrio NR / NRR

- **Confirmation of Potential Yields**
  - Testing plasma bag – repeat Ultrio and dHXV, p24 Ag, Immunoblot, anti-HBc (IgM & IgG), anti-HBs titre, Q-PCR, bDNA – depending on marker
  - Follow-up samples

- **Ultrio NRR**
  - Ultrio IR, Repeat and dHXV NR, Serology Negative
  - Donation discarded but donor eligible to donate again
Bacterial Contamination

Causes.

Units become contaminated during collection –most common
or processing.-uncommon- closed system
Donor may have transient bacteremia at time of donation.
Characteristic Growth of Bacteria in Platelets and Red Cells

ARC (unpublished data)
Prevention

• Venepuncture site is appropriately prepared prior to venesection
• Use of diversion pouch
  – 1st 30 to 50 ml blood is diverted into a pouch
  – Skin plug is diverted into this pouch and not into main collection bag
    • Further minimise contamination risk
• All product preparation performed using a closed system to prevent introducing external contaminants in the Processing centre
• Expiry date of product and storage conditions strictly adhered to
• In US and UK systems in place to culture platelet prods.
Syphilis

- TPHA screening test
- No confirmatory test
- May remain pos long after cure
- Large number of false pos
- Used as surrogate marker
- Syphilis may be transmitted by blood
Other viruses

• Hepatitis A – not routinely tested for. Screening of plasma pools for fractionation by NAT
• Parvovirus B19 – not screened for. NAT for plasma pools
• West Nile Virus – not a problem here
• HTLV1&2 – not screened for
CJD

• nvCJD may be transmitted by blood
• Classical CJD-no evidence
• No test available
• Plasma from donors who have spent more than year cumulative in UK not used for fractionation.
Malaria

- Endemic areas in South Africa
- No immunity
- Visitors to malaria area deferred from donating for 1 month
- Malaria risk warning tag from 1-3 months after return. Advised to avoid in splenectomised patients
- Not used to make paed packs, for obstetric use
- Residents in malaria area are accepted-tagged. Used in area if possible
- 2 cases of TT malaria in Gauteng
Prospective Pathogen Inactivation

1. Inactivates bacteria
   - The highest risk of contamination
2. Has the potential to inactivate unknown or emerging pathogens
3. Reduces many of the risks still inherent in blood transfusions
   - Window period/false negative risk
   - Donor selection risk
4. Inactivates viruses and parasites
   - Including many of those that are not screened for
5. Inactivates contaminating donor leukocytes
   - Important for prevention of TA GvHD
Psoralens
Mechanism of Action

Psoralen

UVA Illumination

DNA or RNA of pathogen

Docking

Permanent Crosslinking
Riboflavin binds to DNA by intercalation. Photolysis of the complex induces guanine oxidation, single strand breaks, and the formation of covalent adducts.

Ennever et. al., Pediatric Res 1983; 17: 234
Ito et. al., J Biol Chem 1993; 268: 13221
Additional safety measures

- Components such as platelets only prepared from donations given by regular donors (at least 4 times a year)
- Red cells from repeat donors used routinely
- Red cells from 1st time donors are only used during times of shortage.
- Donor retest FFP
- Bioplasma S/D treated-inactivates lipid enveloped viruses i.e HIV, Hep B, not Hep A, Parvo
Responsibility of SANBS

• To ensure a low-risk blood supply by:
  – Stringent selection of donors
  – Adopting state of the art systems for testing of blood
  – Ensuring good laboratory practice in collection, processing, testing and issue of blood
Responsibility of the prescriber

- Appropriate usage
- Consider alternatives
  - Autologous-pre-deposit, cell saving
  - Optimise Hb
  - Haemostasis
- Obtain informed consent for transfusion.