Viral Inactivation of Blood Products

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Human and animal
Blood derived medicinal products &
related *in vitro* diagnostic devices

**Human blood derived products**
- Blood components (red cells, platelets, plasma)
- Blood Coagulation Factors
- Immunoglobulins
  - Anti-hepatitis B
  - Anti-rabies
  - Anti-tetanus
  - Anti-rhesus (anti-D)
- Fibrin sealant
- Albumin

**Animal-derived sera**
- Anti-rabies
- Anti-venoms (snake/scorpion)
- Anti-tetanus toxins
- Anti-diphtheria toxins
- Anti-botulism toxins

**Other related products**
- Anticoagulant & fibrinolysis biological therapeutic products

**In vitro diagnostic devices** for the control of quality and safety of blood derived medicinal products including *in vitro* diagnostic devices
The main Concern:

encouragement of voluntary & unpaid blood donations
Quality and Safety: Biological Products

Starting material
- Blood collection & Plasma quality & safety

Production Process
- Fractionation Technology/ Viral inactivation /Removal Procedures

Final product consistency
- Product characteristics: Bulk & Formulated Product
Transfusion Transmissible Diseases

- Hepatitis B
- Hepatitis C
- HIV 1&2
- HTLV 1&11
- Syphilis
- CMV
- Bacterial contamination
- vCJD
- West Nile Virus
- Parasites
## Residual risks of major TTIs*

<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>WP  days  Serology</td>
<td>WP  days  NAT</td>
</tr>
<tr>
<td>HIV</td>
<td>22  1: 3 000 000</td>
<td>11  1: 5 000 000</td>
</tr>
<tr>
<td>HCV</td>
<td>70  1: 500 000</td>
<td>10  1: 5 000 000</td>
</tr>
<tr>
<td>HBV</td>
<td>68  1: 135 000</td>
<td>45  1: 250 000**</td>
</tr>
</tbody>
</table>

* Schreiber et al NEJM 1996 ;334:1685-90

** serology
Transfusion Safety

- Careful selection of donors
- Testing
- Viral inactivation /recombinant products
- Appropriate blood use
- Haemovigilance
Why Viral Inactivation

- The growing list of blood-borne pathogens is outpacing ability to devise and implement new screening tests
Blood component vs. plasma product

- blood components
  - red blood cells
  - platelets (thrombocytes)
  - fresh frozen plasma
  - → patient is exposed to one donor only

- plasma products
  - gammaglobulin, coagulation factor products
  → plasma from several donors is pooled
    - industry
    - viral inactivation methods

- FFP → pooled plasma in 2007 (Octaplas®)
Plasma
Platelets & leucocytes
Red Cells
Platelets - four donors

Red blood cells - one donor
Platelet Production

2\textsuperscript{nd} separation in an extractor

Leucoreduction by filtration
Principle of Collection and Manufacturing Process

Donor Screening
Donor Testing
Viral removal/ inactivation
Quarantine
Product release
Plasma Testing

- Plasma tested for
  - HBsAg
  - Anti-HCV
  - Anti-HIV
  - ALT
  - PCR for HCV, HBV, HIV, Parvovirus B19

- Plasma pools tested for
  - HBsAg
  - Anti-HCV
  - Anti-HIV
  - PCR for HCV, HBV, HIV, Parvovirus B19
Approaches to Control Potential Viral Contamination of Biologicals

Three principal complementary approaches can be adopted to control potential viral contamination of biologicals:

- selecting and testing source material for the absence of viruses,
- testing the capacity of the production processes to remove or inactivate viruses,
- and testing the product at appropriate stages of production for freedom from contaminating viruses.
Nucleic Amplification Testing (NAT)

- HCV NAT SNBTS Nov 99
- HIV NAT Sept 01
- Approx 950 000 screened
- No HCV RNA, anti-HCV neg detected
- No HIV RNA, anti-HIV neg detected

- UK 3: 3,500,000 HCV RNA pos
### HCV NAT in Plasma Pools

**Pools before HCV NAT:**

<table>
<thead>
<tr>
<th>Initial anti-HCV screening test</th>
<th>Anti-HCV positive pools (anti-HCV 2nd)</th>
<th>No. of plasma pools tested</th>
<th>No. of HCV-PCR positive plasma pools</th>
</tr>
</thead>
<tbody>
<tr>
<td>none</td>
<td>+++</td>
<td>8</td>
<td>8 (100%)</td>
</tr>
<tr>
<td>anti-HCV 1st</td>
<td>+/-</td>
<td>85</td>
<td>65 (76%)</td>
</tr>
<tr>
<td>anti-HCV 2nd</td>
<td>-</td>
<td>123</td>
<td>49 (39%)</td>
</tr>
</tbody>
</table>

After introduction of HCV NAT, the HCV burden in all plasma pools used in the EC is below the detection limit, ensuring a high safety margin for the virus inactivation steps.
NAT: Appropriate Stage for Testing for Freedom from Contaminating Viruses

1. Single donation
2. Testing pools
3. Minipools
4. Production pools
5. Intermediate products
6. Final products
Appropriate Stage for Testing for Freedom from Contaminating Viruses

It is due to statistics (Poisson distribution) that testing of final products for the presence of viruses (antigen tests, NAT) cannot ensure freedom from contaminating agents.
Viral Safety of Blood Transfusions after Introduction of NAT

- The selection of healthy donors and highly developed testing methods have reduced the risk drastically.
- The “residual risk“ of contracting a virus infection through a blood transfusion is extremely low and can only be assessed very roughly:
  - For HIV and HCV it is markedly below 1 : 3,000,000
  - For HBV, NAT is difficult to perform and is not obligatory; in spite of this, only isolated transmissions HBV occur; testing for anti-HBc is currently introduced.
- Experience will show whether new developments in the pathogen inactivation of blood components will bring about further progress.
Spontaneous Reports of probable Transmissions of Hepatitis C Virus via Transfusions 1990-2005 (n = 60)

Introduction of NAT
Viral reduction methods for fractionated blood products

- **Donor methods**
  - donor selection
  - donor screening
  - inventory hold and donor re-screening

- **Viral inactivation (effective against HBV, HCV, HIV)**
  - heat treatment (not effective against parvovirus B19)
  - solvent / detergent (not effective against HAV or parvovirus B19)

- **Viral purification**
  - nanofiltration (potential to eliminate HAV, parvovirus B19, prions)
Product Safety

- Full Traceability From Donor To Final Product
- Notification System For Advising Of Post Donation Infections
- Validated Virus Elimination Step(s)
- Control Of Process To Prevent Re-contamination After Virus Elimination Step
- Clinical Trial Data
- Post Marketing Surveillance
Viral inactivation techniques
Lipid enveloped viruses and emerging non-lipid enveloped viruses and prions

Eliminated with current inactivation technology

- Examples of known lipid enveloped viruses
  - HCV
  - HIV
  - HBV
  - West Nile Virus
  - monkeypox
  - SARS

Resistant to current inactivation technology

- Examples of non-lipid enveloped viruses
  - parvovirus B19
  - canine / feline parvovirus
  - enteroviruses
  - chicken anaemia virus (CAV)
  - porcine circovirus type 2 (PCV-2)

- Prions
  - vCJD
Viral Safety

- Research into effective methods for inactivation or removal of viruses from blood products

- Chemical Inactivation
- Inactivation by heat
- Removal by virus filter (nanofiltration)
- Removal by alcohol precipitation
Virus Inactivation and Removal

- Devise methods that will selectively inactivate and/or remove viruses without undue product damage/loss.
- Study relevant test viruses in scaled-down process.
- Ensure that method is capable of giving the degree of virus inactivation/removal required.
# Common Virus Clearance Methods

<table>
<thead>
<tr>
<th>Virus inactivation:</th>
<th>Virus removal</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chemical</strong>: organic solvents; pH extremes; solvent detergent; alcohol</td>
<td><strong>Precipitation</strong>: ammonium sulfate etc.</td>
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<tr>
<td><strong>Physical</strong>: Heat treatment (dry heat or pasteurization)</td>
<td><strong>Chromatography</strong>: ion exchange; gel filtration; affinity; reverse phase</td>
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<td></td>
<td><strong>Membrane filtration</strong>: Omega, Planova, DV50</td>
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Criteria for An Effective Virus Clearance Step

- Significant viral kill
- Reproducible and controllable at process scale and model-able at the laboratory scale
- Should have minimal impact on product yield and activity
- Not generate neo-antigens or leave toxic residues
Virus Safety

- Cold ethanol fractionation
- pH4/pepsin virus inactivation

- effective against
  - enveloped viruses e.g. HIV, Hep B and C
  - and non-enveloped viruses e.g. Hep A
Red Blood Cells

- Hb has phootoabsorptive effects.
- Light – independent compounds have been devised.
- S- 303, PEN110 have no adverse effects.
Fresh Frozen Plasma

- Psoralen
- Riboflavin
- Solvent – detergent plasma
  - Large pools: 250,000 donors/batch
  - Thrombotic complications
Platelets

- No approved method of viral inactivation of platelets
- S-59 is effective but less effective clinically than untreated platelets.
Pooled screened human plasma

Cold ethanol fractionation to yield fraction II

Remove process ethanol

pH4/pepsin VI step

Product returned to a neutral pH

Sterilisation, aseptic dispensing and freeze drying

SNBTS Intravenous Immunoglobulin
Conclusion

- Manufacturing processes for blood derived products should contain two effective steps for removal/inactivation of viruses.

- At least one step should be effective against non-enveloped viruses.
At least one stage in a production process must inactivate rather than remove viruses.

A single step having a large effect gives more assurance of viral safety than several steps having the same overall effect.