Keeping blood transfusions safe: the challenge of emerging infectious diseases

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What is the **blood supply** made of?

- 500,000 RBC
- 120,000 FFP
- 60,000 PLT

WB
Biologics: need to **reduce** transfusion-transmission of infectious diseases

Donors (persons at risk)  
Recipients (PLT => immunosuppressed patients)
Emergent (& re-emergent) **pathogens** in blood transfusion?

- **Virus**: HIV, HCV, HBV, SARS, HEV gt3, …  
  DENV, WNV [US-Canada], CHIKV, JEV, ZIKV, …

- **Bacteria spirochaete**: Treponema (Syphilis)  
  **Proteobacteria**: Coxiella burnetii (Q fever)

- **Protozoa**: Babesia [US], Plasmodium (malaria), Trypanosoma (Chagas disease [T. cruzi]), Leishmania (Oriental sore, Kala-azar)

- **Helminths**: microfilaria (filariasis)

- **Prions**: vCJD, *cf. mad cow disease* [UK, France]

CMV, ParvoB19, HTLV, bacteria, …
Presence of **asymptomatic** blood phase!

**HIV**

Symptoms *after* peak viremia

**WNV**
Donor *exposures*, separation, fractionation, *cold* chain storage, …

a) **Blood components** = unstable, *1 or few donors*
   - Red blood cells (RBC): 1 donor; +4 °C, up to 35 (42) days
   - Plasma (FFP): 1 donor; **below - 18 (- 30) °C**, up to 3 (12) months
   - Platelets (PLT): 1 to 6 donors; +22 °C, up to 5 (7) days
   - … granulocytes, cryoprecipitate, cryo-depleted plasma, … whole blood …

b) **Blood products** = stable plasma proteins, *hundreds/thousands donors* (plasma pools)
   - Albumin solutions (ALB)
   - Immunoglobulins (IVIG)
   - Coagulation factors (FVIII)
   - …
Residual plasma, centrifugation, ...

<table>
<thead>
<tr>
<th>Blood component</th>
<th>Residual plasma volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red blood cells</td>
<td>15 mL</td>
</tr>
<tr>
<td>Fresh frozen plasma</td>
<td>240 mL</td>
</tr>
<tr>
<td>Platelet concentrate</td>
<td>120 – 133 mL</td>
</tr>
</tbody>
</table>

vs. RBC adherence (WNV, ZIKV, …)

<= total amount of HEV gt3

first RBC ever, not PLT => treated, centrifugation?

First Documented Transmission of Trypanosoma cruzi Infection through Blood Transfusion in a Child with Sickle-Cell Disease in Belgium

Sophie Blumental¹, Micheline Lambermont², Catherine Heijmans³, Marie-Pierre Rodenbach², Hanane El Kenz², Daniel Sondag², Emmanuel Bottleau⁴, Carine Truyens⁵

Friday, 19 May 2017
Closed circuit, leukoreduction, irradiation, first 30 mL discarded, ...

leukodepletion filter

needle ‘hole’ = bit of skin (bacteria)

storage solution: promotes growth!
Plasma for fractionation: pathogen inactivation/elimination, heat, filtration, quarantine hold, ...

plasma = **acellular** => solvent-detergent (removes lipid envelopes) … filtration: **nano**filtration

<table>
<thead>
<tr>
<th>VIRUS</th>
<th>Enveloped (E)</th>
<th>Size (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avian Flu</td>
<td>E</td>
<td>80-120</td>
</tr>
<tr>
<td>SARS</td>
<td>E</td>
<td>80-90</td>
</tr>
<tr>
<td>Chikungunya</td>
<td>E</td>
<td>60-70</td>
</tr>
<tr>
<td>Dengue</td>
<td>E</td>
<td>40-45</td>
</tr>
<tr>
<td>West Nile</td>
<td>E</td>
<td>40-45</td>
</tr>
<tr>
<td>MERS-coronavirus</td>
<td>E</td>
<td>80-90</td>
</tr>
<tr>
<td>Ebola</td>
<td>E</td>
<td>120</td>
</tr>
<tr>
<td>Hepatitis E</td>
<td>NE</td>
<td>27-34</td>
</tr>
</tbody>
</table>

**S/D** indicates solvent-detergent. **nano-filtration** is used for removing viruses.
Strategies to safeguard the blood supply?

a) **Selection of low risk donors**
   - **advantages**: fast implementation, broad coverage, avoids donors in undetectable phase, avoids vCJD, surrogate role
   - **limitations**: incomplete, non-compliance (!), loss of donors, validation?

b) **Donor testing**
   - **advantages**: targeted coverage (public health value), limits waste, validation
   - **limitations**: slow implementation, restricted coverage (no test for vCJD), undetectable marker phase, mutations, errors, false positives, dependence on high tech

c) **Pathogen reduction technologies (PRT)**
   - **advantages**: faster implementation, broad coverage (emerging diseases), limits bacterial proliferation in PLTs, can replace irradiation, validation
   - **limitations**: toxic for cells, damages to cells (shelf-life?), incomplete (high viral load, bacterial spores, prions, intracellular pathogens, …), not (yet) for RBCs, high tech
Limitations of screening technology: window period, mutations (strains), seronegative chronic infection, errors

Mutations: Schmidt et al. 2009; Chudy et al., 2012

Quarantine release error: Sobata et al. 2014

Window period donation:

= detected if came back to donate!
**Bacterial detection (PLTs)**

- Transfusion-transmitted bacterial infection: 1/2,000 – 1/3,000
  
  **fatal sepsis**: 1/100,000

  vs. HIV/HCV/HBV residual risk 1/1,000,000

- Incidence of contamination unknown (underdetection, underreporting)

**Bacterial culture**

- differential growth kinetics: aerobic + anaerobic, production of CO₂, 24 h after collection vs. time of issue, ...

- short shelf-life: ordered fast => automation

- **early sampling** of bag when low seed!

- **high sample volume**: false positives (cells produce CO₂)
Bacterial detection (continued)

‘Rapid’ methods

- **NAT**: real-time 16s or 23s rDNA (3-4 hrs); high background signals
- **Flow cytometry**: labelling of nucleic acids (thiazole orange); very fast, easy; background signals
  - automates: 96 well plates, fluorescent dyes; 15 min – 3 hrs
  - non-fluorescent fluorochrome passes the cell membrane (cells with intact membrane integrity and enzymatic activity) and is cleaved by intracellular esterase; 1 h
- **Enzyme-based colorimetric assays** (peptidoglycan); 1 h
- **Lateral flow immunoassay** (lipoteichoic acid, LPS antigens); 30 min bedside
- ...

**small sample volume**: false positives (1 mL of storage solution)
**release**: up to two days after testing (bacteria continue to proliferate)
Pathogen reduction technology

a) photochemical

► methylene blue + illumination: FFP
► psoralen derivate + UV light: PLT, FFP … RBC
► riboflavin (vit B) + UV light: PLT … WB

b) light

► UVC … plasma proteins protective
► UV illumination (no methylene blue) + heavy shaking: PLT
► pulsed waves

Continuing debates: penetration (amotosalene design), formation of novel surface antigens, shadow (low bacterial spike), in vivo recovery & survival, unexpected long term toxicity (polytransfused patients), …
Limitations of PRT:
high viral load, bacterial breakthroughs

e.g. CHIKV $10^9$

PLT PRT validated with 100 CFU/mL => 100 CFU/bag!

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>High titer test average reduction factor (log)</th>
<th>Low spike titer bacterial reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N = 6</td>
<td>Initial spike titer CFU/product N = 10</td>
</tr>
<tr>
<td><em>S. epidermidis</em>, ATCC# 12228</td>
<td>$\geq 4.15$ (6 of 6 to LOD*)</td>
<td>15,500</td>
</tr>
<tr>
<td><em>S. aureus</em>, ATCC# 25923</td>
<td>$3.56 \pm 0.35^b$ (2 of 6 to LOD*)</td>
<td>100</td>
</tr>
<tr>
<td><em>S. aureus</em>, ATCC# 700787</td>
<td>$4.8 \pm 0.8$</td>
<td>—</td>
</tr>
<tr>
<td><em>B. cereus</em>, ATCC# 7064</td>
<td>$1.9 \pm 0.3$</td>
<td>—</td>
</tr>
<tr>
<td><em>B. cereus</em>, Blood isolate</td>
<td>$2.7 \pm 0.6$</td>
<td>150</td>
</tr>
<tr>
<td><em>E. coli</em>, ATCC# 25922</td>
<td>$\geq 4.38$ (6 of 6 to LOD*)</td>
<td>15,800</td>
</tr>
<tr>
<td><em>P. aeruginosa</em>, ATCC# 43088</td>
<td>$\geq 4.48$ (6 of 6 to LOD*)</td>
<td>21,400</td>
</tr>
<tr>
<td><em>S. marcescens</em>, ATCC# 43862</td>
<td>$4.0 \pm 0.5$</td>
<td>—</td>
</tr>
</tbody>
</table>
Progress with NAT screening?
- real-time RT-PCR, TMA e.g. ZIKV: 45 people, 2 weeks (Jan 2016)
- dual targets, sample concentration
- minipool => individual, multiplexing

Alternative screening technologies: proof-of-concepts
- gene chip based detection assays
- nanoparticle assays
- aptamer assays, …
Microarray detection

4 species of *Plasmodium*

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**Multiplex detection and identification of viral, bacterial, and protozoan pathogens in human blood and plasma using a high-density resequencing pathogen microarray platform**

*Moussa Kourout,¹ Carolyn Fisher,¹ Anjan Purkayastha,² Clark Tibbetts,² Valerie Winkelman,³ Phillip Williamson,³ Hira L. Nakhasi,¹ and Robert Duncan⁷*

Jay Epstein, IABS Cambridge, June 2009
**Nanoparticle-based whole genome array assay**

**versus** **RT-PCR**

<table>
<thead>
<tr>
<th>AG1292 (9.2 fM)</th>
<th>WNV RNA copies</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 x 10^6</td>
<td>15,000</td>
</tr>
<tr>
<td>3 x 10^5</td>
<td>1,500</td>
</tr>
<tr>
<td>3 x 10^4</td>
<td>150</td>
</tr>
<tr>
<td>3,000</td>
<td>15</td>
</tr>
<tr>
<td>300</td>
<td>15</td>
</tr>
<tr>
<td>30</td>
<td>15</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
</tr>
</tbody>
</table>

**NB.** no amplification involved in nanoparticle assay
Aptamer detection

Short strands of oligonucleotides, assembled into RNA: molecular recognition of target protein

Blood sample (~10 mL)

bead capture device

DNA extraction

PCR for pathogen DNA

Pathogen specific ligand

RNA Aptamer
Introducing/removing methods?

Highly regulated environment =

difficult to obtain reliable data for policy changes

- Tendency to remove older techniques?
- Efficiency of NAT vs. serology => remove NAT?
- PRT & MP348-NAT complementary?
- PRT & serology sufficient?
Whether the glass appears half full or half empty depends on the background.