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New Technologies for Virus Detection: Where Donor Screening is Going

Thomas R. Kreil
Global Pathogen Safety

September 26, 2013 – WFH Global Forum, Montreal
Testing: From the Past …

- Addition of tests over time has greatly increased safety margins
  - 100 to 10,000-fold, depending on the virus

- Limitations
  - Sensitivity: non-reactive ≠ negative
  - Range of agents tested

- Methods
  - Indirect: antibody
  - Direct: antigen / NAT
  - Surrogate: unspecific
• **Indirect**
  Depends on antibody formation by infected individual delayed → prolonged „window period“

• **Direct**
  Reduction of window period
Window Period Reduction

- Virus **Nucleic Acid Testing** (NAT)
  - Shorter window period
  - NAT window period donation: minimal virus load
West Nile Virus: a learning …

• 1930-ies: discovered blood of a febrile woman …

• 1999: first in the US

• 2002: unprecedented
  – ~ 3,500 cases
  – ~ 240 deaths

• Blood transfusion → transmission
Safety Tripod: Selection & Testing

- Donor **Selection**
  - 20% or \( 0.3 \log_{10} \) risk reduction
  - \( \sim 81\% \) viremic donors \( \rightarrow \) asymptomatic (654 of 818)

- Donation **Testing**
  - 93% or \( 1 \log_{10} \) risk reduction, by mini-pool PCR
  - only 1/2 to 2/3 of viremic donations identified
  - only 8-15% of viremic donations are infectious \( \rightarrow \) high WNV load, w/o IgM

Safety Tripod: **Reduction**

- **Inactivation**
  
  → investigated using a WNV infectivity assay (BSL-3):
  
  NY zoo snowy owl isolate / Vero
  
  - Solvent detergent, IVIG & F VIII
  
  - Vapor heating, FEIBA
  
  - Low pH incubation, IVIG
  
  - Pasteurization, HSA
  
  → all effectively inactivate **WNV**
Present: **Safety Tripod**

- **Selection** of donors
- **Testing** of donations
- **Reduction**
  Virus inactivation / removal during manufacturing processes

Pathogen Safety

- Selection
- Testing
- Reduction
• Primarily based on **Reduction (!)** products enjoy significant safety margins, even without testing plasma for some viruses, e.g. WNV

• PLUS: Virus reduction is fairly generic, i.e. similar viruses → similar inactivation
Reduction → **Other Flaviviruses**, too

- **SLEV**
  - 1975 US epidemic, similar in size to WNV
- **DEN**
- **JEV**
- **YFV**
- **TBEV**

→ Effective reduction, instead of an **unending series of tests** …
... to the Future: **Testing**

- Can we do better?
- New technologies available?

- Options
  - Multiplex PCR
  - Arrays, protein / nucleic acids
  - Next Generation / Deep / Massively Parallel Sequencing
• Design of primers / conditions optimized for target, **exponential** amplification → **sensitivity**

• Use of several different fluorescent dyes in parallel → **several analytes** in one test

• Increase of fluorescent signal is directly proportional to the analyte concentration; plus: standard for comparison → **quantitative**
Multiplex PCR

- Concept currently used for plasma testing
- Delicate sensitivity
- Quantification of several analytes in one test
- Specific for certain viruses, not all
- Current standard NAT program
  - HIV
  - HBV
  - HCV
  - B19V
  - HAV
Arrays

- Probes “printed” onto a chip: >100,000 / cm² (!)
  - many analytes on one chip
  - only those included in the chip design
- Hybridisation of a sample (NA or antibody) to several probes for one target
- Quantification not possible
• Technologies: Illumina, Ion Torrent, Roche
  – Longer reads: more easy construction of full genome
  – More reads: better sensitivity
• Challenge: Bio-Informatics (!)
Next Generation / Deep / Massively Parallel Sequencing

• Successes: **Virus Discovery**
  A selection form one group* within 2 years …
  – A member of a new Picornaviridae genus is shed in pig feces.  
  – Human skin microbiota: high diversity of DNA viruses identified on the human skin by high throughput sequencing.  
  – Identification of the first human gyroivirus, a virus related to chicken anemia virus.  
  – Identification of a novel neuropathogenic Theiler's murine encephalomyelitis virus.  

• Others, too: EL Delwart, MP Busch, WI Lipkin …

*Marc Eloit & team, PathoQuest / Institut Pasteur
• Successes: **Adventitious Virus** in a Live-Attenuated Vaccine *
  – Porcine Circovirus in two rotavirus vaccines

• Future Application (?): **Biotech Donor** Qualification
  – Analysis of (less-well known) cell substrates: TBD
New Technologies → Which, for What?

- **Multiplex PCR**
  - Rapid, sensitive; limited number of targets

- **Arrays**
  - More targets, less sensitive

- **NGS**
  - All targets, sensitivity (?); significantly more effort (!)

A STAGED STRATEGY FOR PATHOGEN DETECTION

Clinical specimen

- Specific candidate list <30 agents
- Long candidate list >30 agents

Refine:
MassTag primers
GreeneChip probes

MassTag PCR
~6 hr, $

GreeneChip Array
~15 hr, $$

Sequencing

Surveillance assays
- Quantitative real time PCR
- Serology
- IHC/ISH
- Koch's Postulates

High Throughput Sequencing
72 hr, $$$$
• **CBER/OBRR Research Priority**

• **Blood** products for transfusion, without manufacturing processes → detection of a virus contaminant is critical

• **Plasma** product manufacturing processes incorporate virus inactivation / removal → reduction is a critical component of safety margins
  – New viruses of concern & reduction ? → verification studies

• NAT, Nucleic Acid Testing
  – 1983: Polymerase Chain Reaction (PCR)
  – 1993: Kary Mullis – Nobel prize for Chemistry
  – 1994: HIV, HBV, HCV testing of plasma pools
  – 1998: EU submission
  – 1999: EMEA requirement for HCV
  – 2000: Five viruses

• Invention to industry: only one decade!

• My perspective:
The pace of innovation has not gone slower …
New Technologies → First Successes

• PCV in Rotavirus Vaccines
  – FDA / EMA: March 22, ’10:
    „extraneous virus detected in (live !) Rotarix,
  – DNA from Porcine Circovirus 1 (PCV1),
  – no evidence at this time that there is a safety concern.“
• PCV in Rotarix: Infectivity (!)

• Philip R. Krause, M.D. FDA/CBER/OVRR
  – Incubation of Rotarix with ST cells
  – Direct extraction of supernatant, or capsid preparation by ultracentrifugation
  – Virus detection: qPCR

New Technologies ➔ New Questions

• Presence of Signal vs. Infectivity vs. Pathogenicity
  ➔ *Is it relevant for safety?*
  – SENV

• Reliability, Validation, Licensing
  ➔ *Is it real?*
  – Xenotropic murine leukemia virus-related virus in the pathogenesis of prostate cancer and/or chronic fatigue syndrome

• “Classical” vs. “New” methods: which role for what?
  – Routine Testing vs. Outbreak Investigation vs. Virus Discovery

➔ Answers needed in less than a decade … (!)
Summary

• **Current testing strategies**
  – Have reached a very mature status
  – Are, by far, not the only contribution to safety (*Safety Tripod*)

• **New technologies**
  – Carry potential
  – Specific / reasonable applications to be evaluated