Multiplex Detection of Transfusion-Transmissible Agents and Blood Cell Antigens in Blood Donations: FDA Workshop Summary

Sanjai Kumar, Ph.D.
Chief, Laboratory of Emerging Pathogens
DETTD, OBRR, CBER, FDA

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Current Status of Blood Safety by Donor Testing for Infectious Agents

- ~18 million units were transfused in 2011
- Risk of transmission through transfusion has significantly reduced with the introduction of tests
- Currently approved tests (NAT or serological):
  - HIV-1/2/O, HTLV-I/II, HCV, HBV, WNV, *T. cruzi*, *Syphilis*, CMV
- Currently being tested under Investigational New Drug Applications (INDs)
  - Babesia, Dengue virus

Nature of Challenges to Further Improve Blood Safety and Availability

- **Emerging pathogens and NTDs**
  - **Dengue virus, CHIKV, TSE agents, Parasitic agents, Biodefense agents**
- **Novel variants of existing pathogens**
  - **Recombinants, antigenic variants, escape mutant and drug resistant forms of existing pathogens such as HIV, HBV and HCV**
  - **Ability of existing tests to identify such variants**
- **Significant donor loss based on risk based deferrals (e.g., malaria, *Leishmania*)**
  - **No screening assays are available**

Nature of Challenges (cont.)

- The current paradigm of donor screening
  - One test per agent
  - Blood volume
  - Implementation of new tests is difficult
- Some intracellular pathogens are difficult to detect by NAT (e.g. malaria, *Babesia*, *Leishmania*)
- Association between antibody and infectivity in blood is not clear
- Reentry of false positives by screening tests
  - Lack of supplemental assays

Technological Advances

- Next Generation Sequencing (NGS)
  - First human genome sequence: $ 3.8 billion
  - Current cost: $6,000
- Microarray platforms
- Protein arrays: >2,000 ORF per pathogen
- Genotyping blood cell antigens
- Multiplex real-time PCR

Workshop Objectives

- **Discuss recent advances in nucleic acid and protein based pathogen detection and blood cell antigen typing**
- **Review the technological status of multiplex platforms**
- **Explore ways to leverage with stakeholders to bring multiplex platforms to market**

Workshop Agenda I

- **Blood safety and Infectious agents: Present and the Future**
- **Advances in Blood-borne Pathogen Detection**
  - **Panel discussion**
- **Molecular DNA-based Typing of Blood Cell Antigens**
  - **Panel discussion**

Workshop Agenda II

- **Highly Multiplex Technologies for Blood Donor Screening**
- **Bioinformatics, Data Analysis and Management**
  - **Panel discussion**
- **Perspectives in Developing Multiplex Devices for Donor Screening**
  - **Panel discussion**
- **Conclusions and Future Steps**
  - **Panel discussion**

Blood Safety from Infectious Agents: Present and the Future

- Advanced technologies are being developed for detection of novel circulating viruses and in surveillance studies in endemic areas
- Advance technologies have application in both diagnosis and donor screening for infectious agents in multiplex format
- Notable among these advanced technologies are next generation sequencing (NGS), and microarrays (DNA and RNA) and protein arrays.
- Scalable Computational databases are being developed to handle the vast amount of data sets generated from NGS and microarray platforms.

Advances in Blood-borne Pathogen Detection

- Mass Tag PCR for emerging agent detection
- Deep sequencing for blood borne pathogen surveillance and discovery
- Microarrays with pre-amplification to achieve sensitivity
- Protein microarrays have been developed to detect antibodies to18 infectious agents
- Nanoparticle based genomic microarrays have potential to achieve multiplex detection

Molecular DNA-based Typing of Blood Cell Antigens

- Overview: Red cell antigens
  - Approximately 30 red cell antigens are significant for alloimmunization of frequently transfused patients. Only 18 serology-based tests are available
  - 10 years experience with lab developed tests for antigen genotypes—automation
  - Automated genotyping will improve patient care and cost effectiveness

Molecular DNA-based Typing of Blood Cell Antigens

- Overview: HLA typing
  - Technique with promise: reverse sequence-specific oligo probe hybridization—multiplexed Luminex assay widely used
  - Antibody detection with solid phase assays
    - Needs optimization and standardization

Molecular DNA-based Typing of Blood Cell Antigens

- New technologies for red cell and HLA antigen typing
  - BeadChip: probe elongation-35 antigens/chip
  - Next generation sequencing
  - Luminex: probe hybridization, flow cytometry
  - OpenArray: spatially multiplexed real-time PCR assays-32 SNPs, 90 donors per chip

Molecular DNA-based Typing of Blood Cell Antigens: Challenges

- New targets (alleles) need to be added
- Need “black box” automation
- How to deal with single target failure in multiplexing
- Control and Test validation Materials
- Population variations admixture/migration
- Concept of confirmatory testing/re-entry for NTD’s (no type determined)

Highly Multiplexed Technologies for Blood Donor Screening

- **Microarrays are deemed the most suitable for blood-borne pathogen detection although PCR is more specific**
  - **Lawrence Livermore Microbial Detection Array**
  - **ViroChip**
  - **GreenChip**
  - **x-MAP: multiplex PCR/Luminex hybridization/flow reader**
  - **TessArae resequencing platform**
  - **Life Technologies OpenArray Platform**
  - **Gen-Probe Transcription-mediated amplification testing**
- **Different Microarray multiplexing formats**
    - **Individual analyte vs pooled analyte detection**
    - **Need Validation in both formats**

Highly Multiplexed Technologies for Blood Donor Screening

- The use of microarrays in routine donor screening would require extensive standardization
- bioinformatics tools for data analysis
- Turnaround time and high throughput
  - pooled format of samples possible solution
- Sensitivity and specificity equal to currently licensed tests
- Challenges of regulatory approval for multiplexed tests
- Development would be facilitated by access to repositories and pedigreed panels

Bioinformatics, Data Analysis and Management

- **Next Generation Sequencing can find everything; including things that may or may not be real.**
  - **Blood centers must specify relevant levels of concern.**
  - **Thresholding to prevent false-positives**
- **Host contamination: physical subtraction in sample prep, bioinformatic filtering of the host genome.**

Bioinformatics, Data Analysis and Management

- **Bioinformatic components of any platform are highly dependent on the quality of Genomic Databases**
  - **Current public databases (Genbank etc.) are not sufficiently curated. Sequences submitted with large polyA stretches, for example, result in spurious pathogen hits**
  - **Miss-identified pathogens also present**
- **Need for a "clinically curated" database**
- **Bioinformatics must function within the blood bank appropriate turnaround time (~24hrs)**

Perspectives in Developing Multiplex Devices for Donor Screening

- **The blood centers and industry want clarification on the number of pathogens required for testing in a multiplex donor screening platform**
- **FDA flexibility: alternative approaches to analytical validation for multiplex devices-**
  - **panel approach**
  - **in silico testing**
  - **modified clinical approach (prospective, archived, retrospective and mock samples).**
- **Validation for addition or removal of an analyte**

Perspectives in Developing Multiplex Devices for Donor Screening

- **Can the CRDH guidance and experience clearing multiplex diagnostic devices apply to donor screening devices?**
- **Changes in the blood bank computer systems are needed to accommodate the test results, donor notification, deferral and reentry with the implementation of multiplex testing systems**
- **Public funding would be required for the pre-clinical development and clinical testing of multiplex platforms**
  - **NIH (NHLBI), DOD (BARDA, DARPA)**
  - **SBIR**
  - **cost recovery during discovery phase**

Conclusions

- Sensitivity should be equal to or superior to licensed tests
- Highly specific to prevent notifications and loss of donors based on false-positive or difficult-to-interpret test results
- Major challenges identified:
  - Sample size in clinical studies for sensitivity and specificities determination for each pathogen
  - Sample size in clinical studies for sensitivity and specificity determination for variant forms or rare pathogens
  - Studies to determine that alterations made in an individual test would not influence the performance of the other tests
  - Sample repositories for community use
  - Funding to support test development and clinical studies
- Continued dialogue between the industry, blood centers, funding agencies and FDA to facilitate the development and licensure of multiplex tests for infectious agents and red cell antigen typing.

Information Resources

- **Workshop summary document in preparation**
- **Slides for most presentations available through a Freedom of Information request**
- **Workshop transcripts available**
  - **http://www.fda.gov/downloads/BiologicsBloodVaccines/NewsEvents/WorkshopsMeetingsConferences/UCM350572.pdf**
  - **http://www.fda.gov/downloads/BiologicsBloodVaccines/NewsEvents/WorkshopsMeetingsConferences/UCM350571.pdf**

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