Development of a Parvovirus B19 DNA Assay and Systems Software for Plasma Screening

Parvovirus B19 Overview

- First identified in 1975
- Small non-enveloped single-stranded DNA virus
- Present in high titers during acute infection (10^{11} IU/mL)
- Absence of a lipid envelope, virus resists inactivation by solvent-detergent process
- Creates potential for transmission of B19 by plasma derivatives, which can cause disease in specific at-risk patient populations: early pregnancy (fetus) and immunosuppressed patients (red cell aplasia)
- A high throughput semi-automated in-process test for Parvo B19 screening was developed.

Background

- Recently the FDA asked manufacturers of derivatives to include "in-process" screening of recovered plasma for high titer Human Parvovirus B19 (B19) DNA as part of their manufacturing process (a quality assessment of raw material, not a donor screening assay).
- Final manufactured pool: Parvo B19 infectivity level must be $<10^4$ IU/mL.
- Suppliers of recovered plasma for fractionation are required to provide B19 high titer ($>10^5$) negative units.

FDA Defined In-Process Testing

- No donor deferral or notification
- Risk is not significant enough to warrant donor notification or look back
- Recipients of Parvo positive components will not be notified
- Quarantine and destroy in-date units when possible

Laboratory Requirements

- Parvo B19 testing is delayed (>42 days) post-collection to ensure labile components are no longer in-date.
- Parvo B19 test sensitivity is "detuned" by producing a 280-member super-pool.
- For Positive super-pools: refer to Figure 1
  1. 4-minipools containing 70 samples each are tested.
  2. With further testing of seven resolution pools, each containing 10-samples.
  3. Final resolution tests 10-individual samples.

Sample processing: see Figure 2

The sample source is the residual EDTA NAT tube.

1. The plasma is transferred into an archive plate for frozen storage.
2. From this archive plate, samples are pooled into 280-members, known as "Super-pools" (SP).

Figure 2

Assay Method: see Figures 3-4

1. DNA extraction occurs using the Promega kit on the Biomek instrument (Figure 3).
2. The PCR based assay uses molecular beacon specific for B19 (Figure 4).
3. Sensitivity:
   - Detects > 2.5 X 10^3 IU/mL per SP sample
   - Detects 7 X 10^5 IU/mL per individual sample in random blood donors.
4. The assay detects all three B19 genotypes (1,2, 3) in human plasma.

Figure 3

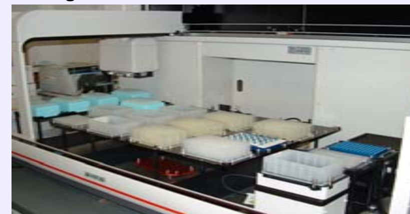
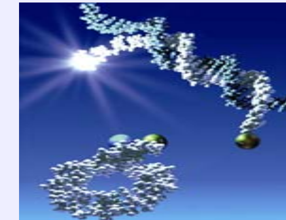
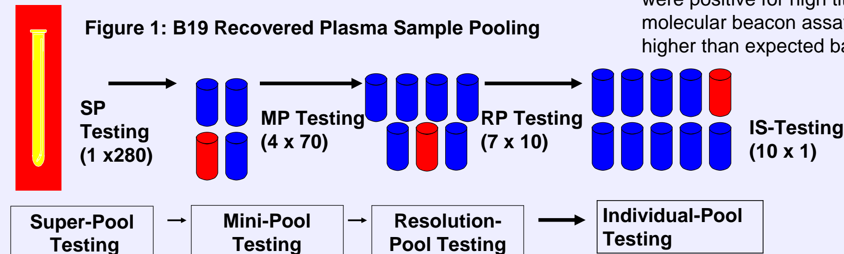


Figure 4



Results

- A random sampling of 18,929 whole blood donors were screened for high titer B19 using this molecular beacon assay with 5-IS positives. The rate of high titer B19 was 0.03% (1:3,786).

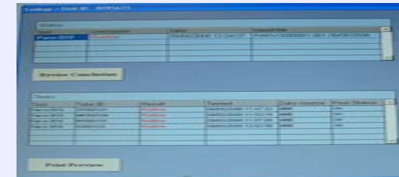


The red tube represents a positive sample.

Systems software functions: see figure 5

1. Pooled sample and test result management is electronically controlled with continuous sample number tracking, from archive, pooling and through assay testing. There is automated interpretation of assay test results.
2. For identification of the positive unit(s) in a positive SP and negative results, the Pool Result Manager de-convolutes sample pools and electronically releases B19 results.
3. Positive B19 donations are reported to the Fractionators and blood centers for product retrieval.

Figure 5



Conclusion

Blood centers are required to identify donations marked for recovered plasma. A collaboration between two high volume testing laboratories allowed for development and implementation of Parvovirus B19 in-process donor screening of both whole blood and source plasma samples. Preliminary data shows 1 in 3,786 donations were positive for high titers of B19 DNA using this molecular beacon assay, a prevalence rate somewhat higher than expected based on published reports.

Session I

TTID1: Testing Issues (Virology)

Abstract: SP161

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