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August 25-26, 2002 from 17:00 to 19:00
ISBT 27th Annual Congress, 2002
Poster Presentation

Performance Characteristics of PCR Based Parvovirus B19 Nucleic Acid Testing Confirmatory Assay for the Procleix Parvovirus B19/Hepatitis A Virus Assay

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Background:

Parvovirus B19 is a blood borne, single stranded DNA, nonenveloped virus which is not inactivated easily by solvent and/or detergent treatment of plasma. The Procleix Parvo B19/Hepatitis A Virus (HAV) assay, a transcription-mediated amplification (TMA) test for screening blood donations for Parvovirus B19 and HAV B19 nucleic acid is being developed by Gen-Probe. An alternative confirmatory Parvovirus nucleic acid test (NAT) is needed to test TMA positive samples. The assay will also be used as a quantitative test needed to prepare calibrators and proficiency panels for Parvovirus B19 diagnosis. Here we describe the development of an alternative confirmatory and quantitative NAT for Parvovirus B19 DNA.

Methods:

A magnetic bead based protocol was used for the isolation of Parvovirus B19 DNA. The isolated target on the beads was amplified by Taqman technology, with amplification primers corresponding to VP1 region. A single stranded Internal Control DNA of 680 nt corresponding to the VP1 region with altered probe sequence was added to the lysis buffer. It serves as a control for both target capture and amplification for each sample. The analytical sensitivity panel was built from CBER sample (106 IU/ml) diluted to 8,000-30 IU/ml. Clinical sensitivity was determined by its ability to detect all Parvovirus B19 IgM positive samples from European Red Cross blood bank. The specificity of the assay was determined in the presence of hepatitis B virus, which is also a blood borne DNA virus. Normal donor population specificity was examined with fifty samples.`

Results:

The Parvovirus B19 NAT confirmatory assay had 100% detection at 60 IU/ml. Use of HBV DNA positive plasma did not interfere or affect the detection of Parvovirus B19 by the alternate test. Of the six clinical plasma samples, four had high titer of >106 IU/ml, supporting the observations that it is a high copy virus. No positive specimens were detected in the fifty normal donor samples.

Conclusion:

Using a combination of magnetic bead based target capture and Taqman assay, a rapid, sensitive, user-friendly Parvovirus NAT confirmatory and quantitative assay has been developed to test samples reactive in the Procleix Parvovirus B19/Hepatitis A Virus (HAV) assay and to prepare calibrators and proficiency panels.