PARVOVIRUS B19 MOLECULAR BEACON ASSAY

INTENDED PURPOSE

The assay was intended for “in process” testing only and may be used with both whole blood and source donation plasma samples.

Super pool samples are created using a Hamilton Star pipetting instrument which transfers an aliquot from an appropriate tube to a specific well in a deep well plate. Then the Hamilton Star transfers an aliquot from each well in the deep well plate to a reservoir creating a Primary Pool. Super pools contain aliquots from 3 separate Primary Pools and contain a total of 288 individual samples. A Proteinase K digestion is performed on an aliquot of the superpool and then the isolation is performed using the Magnasil Blood Genomic kit from Promega and a Biomek FX from Beckman Coulter. It is an automated system which generates a 96 well plate containing each individual isolated sample.

A mastermix is prepared which contains the molecular beacons and primers for both the internal control and Parvovirus B19. Single stranded beacons are used as indicators of specific amplified products. Their primary nucleic acid sequence is complementary to the amplified portion of the amplicons for B19 and Bovine Viral Diarrhea Virus (BVDV) (internal control). The mastermix also contains other components necessary for the amplification process. The tubes are placed in a GeneAmp PCR System 9700 thermocycler from Applied Biosystems for the amplification process.

Following amplification the samples are transferred to a fluorometric plate and read using a FLUOstar fluorometer from BMG LABTECH. The plate is read at the appropriate excitation and emission filter sets for each beacon. Parvo B19 is read at 485 and 520 nm. BVDV is read at 584 and 620 nm. The fluorescence of each sample is determined by calculating the ratio of the fluorescent reading of the sample over the fluorescence of the beacon when hybridized with the complementary target sequence oligonucleotide (maximum fluorescence).

Use of this device for other purposes or in a manner not in accordance with this work instruction must be validated.

SPECIMEN COLLECTION, STORAGE AND HANDLING

A. Blood specimens collected in plastic tubes may be used.

B. Plasma collected in K_2EDTA, K_3EDTA, ACD-A or 4% Sodium Citrate is acceptable.

C. Whole blood specimens must be centrifuged within 72 hours.

D. Samples can be stored at temperatures ≤25ºC for up to eight days, followed by an additional twelve days at 2 - 8ºC. Plasma may be stored at ≤-18ºC for up to one year.

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E. No adverse affect on assay performance was observed when plasma was subjected to three freeze-thaw cycles.

F. The user must validate other collection and storage conditions.

REAGENTS

A. Room temperature is defined as 15° to 30°C.

B. Do not use reagents after the expiration date.

C. Do not refreeze the Kit Controls and External Quality Controls after the initial thaw.

D. Consult the following table for storage information.

Reagents	Unopened Storage	Opened Storage
Premix (Beacon/Primer Premix)	Store at ≤-20°C until the expiration date	Store at ≤-20°C for 30 days, maximum of 4 freeze - thaw cycles
2-plex comp	Store at ≤-20°C until the expiration date	Store at ≤-20°C until the expiration date
Internal Control Pellet	Store at ≤-20°C until the expiration date	Store at RT for 30 days once it has been added to the Magnasil Lysis Buffer*
Kit Controls	Store at ≤-20°C until the expiration date	Store at 2° to 8°C up to 24 hours
External Quality Controls (EQC)	Store at ≤-20°C until the expiration date	Store at 2° to 8°C up to 24 hours

*RT = Room Temperature

E. The premix contains the molecular beacons and primers for both the internal control and Parvovirus B19.

F. Internal control primer sequences were developed from a region of the Bovine Viral Diarrhea Virus (BVDV) genome. The primer sequences are proprietary information.

G. The internal control molecular beacon nucleotide sequence was developed from a region of the BVDV genome. The fluorescent tag on the 5' end is Tx Red and the quencher on the 3' end is Blackhole Quencher 2. The beacon sequence is proprietary information.

H. The Parvovirus B19 primer sequences were developed from a highly conserved region of the VP1 gene. The primer sequences are proprietary information.

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I. The Parvovirus B19 beacon nucleotide sequence was developed from a highly conserved region of the VP1 gene. The fluorescent tag on the 5' end is FAM and the quencher on the 3' end is Blackhole Quencher 1. The beacon sequence is proprietary information.

QUALITY CONTROL PROCEDURES / INTERPRETATION OF RESULTS

A. Contents of each well are read 5 times for detection of Parvovirus B19 (B19), and 5 times for detection of the Internal Control (IC). Readings are reported in Relative Light Units (RLUs).

B. B19 Raw Averages of the 5 B19 RLU values are computed for each well.

C. IC Raw Averages of the 5 IC RLU values are computed for each well.

D. A B19 Blanks Average is calculated from the three blank wells:

   a. If a B19 Raw Average for a blank well is less than or equal to 10,440, it is considered qualified.

   b. If there are fewer than two qualified B19 Raw Averages, all of the results on the plate will be disqualified.

   c. The B19 Blanks Average is calculated by averaging the two or more qualified B19 Raw Averages and rounding to two decimal places.

E. An IC Blanks Average is calculated from the three blank wells:

   a. If an IC Raw Average for a blank well is less than or equal to 11,518, it is considered qualified.

   b. If there are fewer than two qualified IC Raw Averages, all of the results on the plate will be disqualified.

   c. The IC Blanks Average is calculated by averaging the two or more qualified IC Raw Averages and rounding to two decimal places.

F. If the B19 Raw Average for the Fmax well is greater than or equal to 54,000, the B19 Raw Average is considered qualified.

G. If the IC Raw Average for the Fmax well is greater than or equal to 51,000, the IC Raw Average is considered qualified.

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H. If either the B19 Raw Average or the IC Raw Average fails to qualify, all of the results on the plate will be disqualified.

I. Normalized B19 for each well, including the Fmax, is calculated by subtracting B19 Blanks Average from the B19 Raw Average.

J. B19 Percentage of Fmax for each sample and control well is calculated by dividing the Normalized B19 by the Normalized B19 Fmax, multiplied by 100 and rounded to a whole number.

K. IC Percentage of Fmax for each sample and control well is calculated by dividing the Normalized IC by the Normalized IC Fmax, multiplied by 100 and rounded to a whole number.

L. A Positive Control is considered qualified if the B19 Percentage of Fmax for the well is greater than or equal to 5.

M. If any of the Positive Controls are not qualified, then all of the results on the plate will be disqualified.

N. A Negative Control is considered qualified if the B19 Percentage of Fmax for the well is less than 5 and the IC Percentage of Fmax is greater than or equal to 10.

O. If any of the Negative Controls are not qualified, then all of the results on the plate will be disqualified.

P. Interpret sample and External Control results:

   a. A sample is considered positive if the B19 Percentage of Fmax is greater than or equal to 5.

   b. A sample is considered Negative if the B19 Percentage of Fmax is less than 5 and the IC Percentage of Fmax is greater than or equal to 10.

Q. Evaluate performance of External Controls:

   a. If the Negative External Control is missing or is not negative, the control is considered invalid, the plate is considered Invalid and Negative sample results are disqualified, while Positive sample results are still considered Positive.

   b. If the Positive External Control is missing or is not Positive, the control is considered invalid, the plate is considered Invalid. Negative sample results are disqualified, while Positive sample results are still considered Positive.

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c. If both Positive and Negative External Controls are considered valid, all sample results are considered qualified.

R. All calculations are performed as described, with standard rounding rules applied only to the products of four calculations:

a. The internal control blanks average and the Parvovirus B19 blanks average, which are both rounded to 2 decimal places after calculation.

b. The internal control percentage of internal control Fmax and the Parvovirus B19 percentage of Parvovirus B19 Fmax, which are both rounded to whole numbers before comparison with cutoff values and interpretation.

S. Additional assay parameters and acceptance criteria must be validated by the user.

PERFORMANCE CHARACTERISTICS

A. Sensitivity: The assay has been validated to detect equal to or greater than 347 IU/ml per 288 member superpool and equal to or greater than 100,000 IU/ml per individual sample.

B. Pool Resolution: Positive pools are resolved to a single sample. The three plates of 96 samples that comprise the super pool of 288 samples are each tested separately to determine the positive plate(s). Each plate of 96 samples is in a 8 x 12 grid with eight rows and 12 columns. Each row from each positive plate is made into a pool containing 12 samples and then tested. The twelve samples in each positive row are then tested separately to determine the positive sample(s).

C. Potentially Interfering Substances: The assay demonstrated the ability to detect Parvovirus B19 DNA in the presence of potentially interfering substances including anticoagulants (K_2EDTA, K_3EDTA, ACD, Sodium Citrate), and other viruses (HCV, BVDV, HBV, HAV and HIV-1). The assay also detected Parvovirus B19 DNA in lipemic and hemolytic plasma samples.

D. Repeatability and Reproducibility (Intra and Inter Run Validity): The repeatability and reproducibility of the assay was determined by using three different lots of reagents and two different technicians. Each technician tested a panel of samples using each lot five different times.

E. Genotypes: The assay has been shown to detect all known genotypes (1, 2 & 3). The primer and probe sequences have 100% homology to published nucleotide sequences from genotype 1 & 3 and 97% homology to published nucleotide sequences from genotype 2.

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QUALTEX LABORATORIES

PARVOVIRUS B19 MOLECULAR BEACON ASSAY

APPROVAL:

Name	Authority	Signature	Date
Amanda Munoz	Director, Laboratory Services, Infectious Diseases	**Pdf**	**12/03/2012**
Kim Ratliff	Director, QualTex Quality Assurance	**Pdf**	**11/14/2012**
N. D. Kalmin, MD	Medical Director, QualTex Laboratories	**Pdf**	**11/14/2012**

DISTRIBUTION:

Distribution	Distribution
Genetic Testing	
Research and Specialty Laboratory	
Quality Assurance/Batch Release	
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