are divided into two subfamilies: the *Parvovirinae* and the *Densovirinae*, which infect vertebrate and arthropod hosts, respectively.

Bovine parvovirus 3 (BPV-3) belongs to the subfamily *Parvovirinae*, genus of *Erythroparvovirus* (species of *Ungulate erythroparvovirus 1*). While it is known that BPV-3 infect bovines, its pathogenesis and clinical manifestation remains unclear.

Per the requirements for testing in the Code of Federal Regulations (9 CFR113), the presence or absence of certain viruses in ingredients of animal origin used in production of biologics are required to be evaluated by incubating the animal-derived raw materials on specific indicator cells and which are subsequently observed for virus induced cytopathic effects and tested by hemadsorption or antibody fluorescence. Among the bovine parvoviruses, BPV-1 is capable of replicating in at least one of the selected indicator cell lines and can be detected by 9 CFR 113 test.

However, a permissive cell line to support BPV-3 replication has not been identified to date and, therefore, it cannot be detected by routine 9 CFR 113 testing. Although BPV-3 was detected by next-generation sequencing (NGS) a few years ago, it remains an emerging virus with limited information available. Traditional cell-culture based virus testing is not capable of supporting replication for BPV-3, thus, molecular biology assays are an essential alternative method to detect BPV-3 genomic DNA. The impact of BPV-3 in cell culture-based manufacturing remains unclear. There is a need for a rapid and specific method to detect the presence or absence of BPV-3 genomic DNA in raw materials such as fetal bovine serum (FBS) and/or unprocessed non-GMP bulk harvest samples containing FBS from cell culture-based manufacturing. The invention described herein meets this need.

~~BRIEF~~ SUMMARY ~~OF THE INVENTION~~

In one aspect, the present disclosure provides a composition comprising oligonucleotides having the nucleic acid sequence of SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9; optionally including a second composition comprises oligonucleotides having the sequence of SEQ ID NO: 16, SEQ ID NO:17, and SEQ ID NO:18.

In another aspect, the present disclosure provides a reagent for detecting *Bovine parvovirus* 3 (BPV-3) genomic DNA in the extracted DNA of a test sample consisting of a primer probe combination having the nucleic acid sequences of SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9; optionally SEQ ID NO:9 has a fluorescent reporter dye and/or a non-fluorescent quencher; optionally the fluorescent reporter dye 6-carboxyfluorescein (FAM) at the 5' end and/or either a Minor Groove Binder non-fluorescence quencher (MGB-NFQ) or ZEN-IB and Iowa Black Fluorescence quencher (IBFQ) at the 3' end; optionally the primer probe combination is used with an internal positive control primer combination; and wherein the primer probe combination detects DNA encoding the structural capsid (VP) protein and/or the non-structural (NS) protein of *Bovine parvovirus* 3 in the test sample.

In another aspect, the present disclosure provides a reagent for use as an internal positive control primer probe combination in an assay for detecting *Bovine parvovirus* 3 (BPV-3) genomic DNA in the extracted DNA of a test sample comprising a primer probe combination having the nucleic acid sequences of SEQ ID NO: 16, SEQ ID NO:17, and SEQ ID NO:18; optionally SEQ ID NO: 18 has a fluorescent reporter dye and/or a non-fluorescent quencher; optionally SEQ ID NO: 18 has a fluorescent reporter dye, 2′-chloro-7′phenyl-1,4-dichloro-6-carboxy-fluorescein (VIC) at the 5' end and a Minor Groove Binder non-fluorescent quencher (MGB-NFQ) at the 3' end.

In another aspect, the present disclosure provides a primer probe combination for detecting *Bovine parvovirus* 3 genomic DNA in an extracted DNA of a test sample in combination with an internal positive control primer probe combination for detecting *Bovine parvovirus* 3 (BPV-3) genomic DNA wherein the primer probe combination has the nucleic acid sequences of SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9; optionally SEQ ID NO:9 has a fluorescent reporter dye and/or a non-fluorescent quencher; optionally SEQ ID NO:9 has a fluorescent reporter dye 6-carboxyfluorescein (FAM) at the 5' end and/or either a Minor Groove Binder non-fluorescence quencher (MGB-NFQ) or ZEN-IB and Iowa Black Fluorescence quencher (IBFQ) at the 3' end; optionally the internal positive control primer probe combination has the nucleic acid sequences of SEQ ID NO: 16, SEQ ID NO:17, and SEQ ID NO:18; optionally SEQ ID NO: 18 has a fluorescent reporter dye and/or a non-fluorescent quencher; optionally SEQ ID NO: 18 has a fluorescent reporter dye, 2′-chloro-7′phenyl-1,4-dichloro-6-carboxy-fluorescein (VIC) at the 5' end and a Minor Groove Binder non-fluorescent quencher (MGB-NFQ) at the 3' end, and wherein the primer probe combination detects DNA encoding the structural capsid (VP) protein and/or the non-structural (NS) protein of *Bovine parvovirus* 3 in a test sample.

In another aspect, the present disclosure provides a primer probe combination for detecting *Bovine parvovirus* 3 genomic DNA in the extracted DNA of a test sample comprising SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9 in combination with an internal positive control primer probe combination comprising SEQ ID NO: 16, SEQ ID NO:17, and SEQ ID NO:18, wherein SEQ ID NO: 9 has a fluorescent reporter dye 6-carboxyfluorescein at the 5' end and a Minor Groove Binder non-fluorescent quencher (MGB-NFQ) non-fluorescence quencher at the 3' end and SEQ ID NO:18 has a fluorescent reporter dye, 2′-chloro-7′phenyl-1,4-dichloro-6-carboxy-fluorescein at the 5' end and a Minor Groove Binder non-fluorescent quencher (MGB-NFQ) at the 3' end.

In another aspect, the present disclosure provides a kit for detecting *Bovine parvovirus* 3 (BPV-3) genomic DNA contamination in the extracted DNA of a test sample comprising primer probe combination that detects DNA encoding BPV-3 selected from the group consisting of a primer probe combination having the nucleic acid sequences of SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9; and optionally an internal positive control primer probe combination having the nucleic acid sequences of SEQ ID NO: 16, SEQ ID NO:17, and SEQ ID NO:18.

In another aspect, the present disclosure provides a method for determining the presence or absence of *Bovine parvovirus* 3 genomic DNA in the extracted DNA of a test sample comprising 1) a reaction mixture comprising a test sample, a positive control, a BPV-3 IPC positive control plasmid DNA, nucleic acid amplification reagents, an internal positive control primer probe combination having the nucleic acid sequences of SEQ ID NO: 16, SEQ ID NO:17, and SEQ ID NO:18, wherein SEQ ID NO:18 has a fluorescent reporter dye at the 5' end and a non-fluorescence quencher at the 3' end, and a primer probe combination selective for a DNA sequence of *Bovine parvovirus* 3 having a primer probe combination having the nucleic acid sequences of SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9, wherein SEQ ID NO:9 has a fluorescent reporter dye at the 5' end and a non-fluorescence quencher at the 3' end, optionally the fluorescent reporter dye is 6-carboxyfluorescein (FAM) and the non-fluorescence quencher is a Minor Groove Binder non-fluorescent

quencher (MGB-NFQ) or ZEN-IB and Iowa Black Fluorescence quencher (IBFQ); optionally further comprising one or more negative extraction control, no template control, positive extraction control, positive control, and/or inhibition control; optionally the primer probe combination selective for a DNA sequence of *Bovine parvovirus* 3 detects DNA encoding the non-structural (NS) protein and/or structural capsid (VP) protein of *Bovine parvovirus* 3; 2) subjecting the reaction mixture to a quantitative PCR technique to obtain copies of the target sequence, 3) measuring any increase in fluorescence signal, wherein an increase in fluorescence signal indicates the presence of *Bovine parvovirus* 3 genomic DNA in the test sample.

In another aspect, the present disclosure provides a method for the quantification of 1E3 to 1E8 genome copies of *Bovine parvovirus* 3 genomic DNA in a PCR reaction comprising 1) a reaction mixture comprising the extracted DNA of a test sample, a positive control, a BPV-3_IPC positive control plasmid DNA, nucleic acid amplification reagents, an internal positive control primer probe combination having the nucleic acid sequences of SEQ ID NO: 16, SEQ ID NO:17, and SEQ ID NO:18, wherein SEQ ID NO:18 has a fluorescent reporter dye at the 5' end and a non-fluorescence quencher at the 3' end, and a primer probe combination having the nucleic acid sequences of SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9, wherein SEQ ID NO:9 has a fluorescent reporter dye at the 5' end and a non-fluorescence quencher at the 3' end; optionally the method includes one or more of a no template control, a positive control, a negative extraction control, a positive extraction control, an inhibition control, an internal positive control, and a standard; 2) subjecting the reaction mixture to a quantitative PCR technique to obtain copies of the target sequence, and 3) measuring any increase in fluorescence signal; optionally the limit of detection (LOD95%) of the method is 27 genome copies of *Bovine parvovirus* 3 genomic DNA per reaction with a 95% confidence interval of 22 and 34 genome copies per reaction; optionally the linearity of the method has a correlation coefficient (R2) $\geq$ 0.98 and a PCR amplification efficiency within 90-110%; optionally the method has a repeatability value that is a %CV of quantity equal or less than 25%; optionally the method has an intermediate precision value that is %CV of quantity equal or less than 30%; optionally the method has an accuracy value within ±30% of the accepted reference value (ST) across the whole dynamic range of the assay; optionally the method has a limit of quantitation that is the %CV of quantity for repeatability at $\leq$ 25%, intermediate precision at $\leq$ 30% and acceptance criterion for the accuracy within ±30% of the expected standard reference value; and optionally the method has a robustness that has a percent CV of quantity for repeatability of $\leq$ 25%, an intermediate precision of $\leq$ 30%, and an accuracy of the mean of quantity of the combination matrix condition tested of ±30% of the mean of quantity of the optimized condition.

The ~~invention~~disclosure provides a composition comprising oligonucleotides selected from the groups consisting of a) oligonucleotides having the nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3; b) oligonucleotides having the nucleic acid sequence of SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6; c) oligonucleotides having the nucleic acid sequence of SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9; d) oligonucleotides having the nucleic acid sequence of SEQ ID NO:10, SEQ ID NO:11, and SEQ ID NO:12; and e) oligonucleotides having the nucleic acid sequence of SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15; f) oligonucleotides having the nucleic acid sequence of SEQ ID NO:20, SEQ ID NO:21, and SEQ ID NO:22; g) oligonucleotides having the nucleic acid sequence of SEQ ID NO:23, SEQ ID NO:24, and SEQ ID NO:25; and h) oligonucleotides having the nucleic acid sequence of SEQ ID NO:26, SEQ ID NO:27, and SEQ ID NO:28. In one ~~embodiment~~example the composition optionally includes a second composition comprising oligonucleotides having the sequence of SEQ ID NO: 16, SEQ ID NO:17, and SEQ ID NO:18. In one ~~embodiment~~example the

composition comprises oligonucleotides having the nucleic acid sequence of SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9. In one ~~embodiment~~example the composition comprises a first composition comprises oligonucleotides having the nucleic acid sequence of SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9 and a second composition comprises oligonucleotides having the sequence of SEQ ID NO: 16, SEQ ID NO:17, and SEQ ID NO:18.

The ~~invention~~disclosure provides a reagent for detecting DNA encoding *Bovine parvovirus* 3 (BPV-3) genomic DNA in the extracted DNA of a test sample selected from the group consisting of a) a primer probe combination having the nucleic acid sequences of SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3; b) a primer probe combination having the nucleic acid sequences of SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6; c) a primer probe combination having the nucleic acid sequences of SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9; d) a primer probe combination having the nucleic acid sequences of SEQ ID NO:10, SEQ ID NO:11, and SEQ ID NO:12; and e) a primer probe combination having the nucleic acid sequences of SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15; f) a primer probe combination having the nucleic acid sequences of SEQ ID NO:20, SEQ ID NO:21, and SEQ ID NO:22; g) a primer probe combination having the nucleic acid sequences of SEQ ID NO:23, SEQ ID NO:24, and SEQ ID NO:25; and h) a primer probe combination having the nucleic acid sequences of SEQ ID NO:26, SEQ ID NO:27, and SEQ ID NO:28.

In one ~~embodiment~~example SEQ ID NO: 3, SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:12, SEQ ID NO:15, SEQ ID NO:22, SEQ ID NO:25; or SEQ ID NO:28 have a fluorescent reporter dye and/or a non-fluorescent quencher. In a related ~~embodiment~~example one or more of SEQ ID NO: 3, SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:12, SEQ ID NO:15, SEQ ID NO:22, SEQ ID NO:25; or SEQ ID NO:28 have a fluorescent reporter dye 6-carboxyfluorescein (FAM) at the 5' end and/or either a Minor Groove Binder non-fluorescence quencher (MGB-NFQ) or ZEN-IB and Iowa Black Fluorescence quencher (IBFQ) at the 3' end. In one ~~embodiment~~example the primer probe combination detects DNA encoding the structural capsid (VP) protein and/or the non-structural (NS) protein of *Bovine parvovirus* 3 in a test sample. In one ~~embodiment~~example the reagent in combination with an internal positive control primer combination. In one ~~embodiment~~example the reagent comprises SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9.

The ~~invention~~disclosure provides a reagent for use as an internal positive control primer probe combination in an assay for detecting *Bovine parvovirus* 3 (BPV-3) genomic DNA in the extracted DNA of a test sample comprising a primer probe combination. In a related ~~embodiment~~example the internal positive control primer probe combination has the nucleic acid sequences of SEQ ID NO: 16, SEQ ID NO:17, and SEQ ID NO:18. In one ~~embodiment~~example SEQ ID NO: 18 has a fluorescent reporter dye and/or a non-fluorescent quencher. In a related ~~embodiment~~example SEQ ID NO: 18 has a fluorescent reporter dye, 2′-chloro-7′phenyl-1,4-dichloro-6-carboxy-fluorescein (VIC) at the 5' end and a Minor Groove Binder non-fluorescent quencher (MGB-NFQ) at the 3' end. In one ~~embodiment~~example the reagent comprises SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9.

The ~~invention~~disclosure provides a primer probe combination for detecting *Bovine parvovirus* 3 genomic DNA in the extracted DNA of a test sample in combination with an internal positive control primer probe combination for detecting *Bovine parvovirus* 3 (BPV-3) genomic DNA. In one ~~embodiment~~example the primer probe combination is selected from the groups consisting of a) a primer probe combination having the nucleic acid sequences of SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3; b) a primer probe combination having the nucleic acid sequences of SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6; c) a primer probe combination having the nucleic acid sequences of SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9; d) a primer probe combination having the nucleic acid sequences of SEQ ID NO:10, SEQ ID NO:11, and SEQ ID NO:12; e) a primer probe combination having the nucleic acid sequences of SEQ ID NO:13, SEQ ID NO:14, and SEQ ID NO:15, f) a primer probe combination having the nucleic acid sequences of SEQ ID NO:20, SEQ ID NO:21, and SEQ ID NO:22; g) a primer probe combination having the nucleic acid sequences of SEQ ID NO:23, SEQ ID NO:24, and SEQ ID NO:25; and h) a primer probe combination having the nucleic acid sequences of SEQ ID NO:26, SEQ ID NO:27, and SEQ ID NO:28. In one ~~embodiment~~example SEQ ID NO: 3, SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:12, or SEQ ID NO:15 have a fluorescent reporter dye and/or a non-fluorescent quencher. In one ~~embodiment~~example one or more of SEQ ID NO: 3, SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:12, SEQ ID NO:15, SEQ ID NO:22, SEQ ID NO:25; or SEQ ID NO:28 have a fluorescent reporter dye 6-carboxyfluorescein (FAM) at the 5' end and/or either a Minor Groove Binder non-fluorescence quencher (MGB-NFQ) or ZEN-IB and Iowa Black Fluorescence quencher (IBFQ) at the 3' end. In one ~~embodiment~~example the primer probe combination detects DNA encoding the structural capsid (VP) protein and/or the non-structural (NS) protein of *Bovine parvovirus* 3 in a test sample. In one ~~embodiment~~example an internal positive control primer probe combination having the nucleic acid sequences of SEQ ID NO: 16, SEQ ID NO:17, and SEQ ID NO:18 is used in combination with a primer probe combination used for detecting *Bovine parvovirus* 3 (BPV-3) genomic DNA in a test sample as described above. In a related ~~embodiment~~example SEQ ID NO: 20 has a fluorescent reporter dye and/or a non-fluorescent quencher. In a related ~~embodiment~~example SEQ ID NO: 18 has a fluorescent reporter dye, 2′-chloro-7′phenyl-1,4-dichloro-6-carboxy-fluorescein (VIC) at the 5' end and a Minor Groove Binder non-fluorescent quencher (MGB-NFQ) at the 3' end.

The ~~invention~~disclosure provides a primer probe combination for detecting DNA encoding *Bovine parvovirus* 3 genomic DNA in the extracted DNA of a test sample comprising SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9 in combination with an internal positive control primer probe combination comprising SEQ ID NO: 16, SEQ ID NO:17, and SEQ ID NO:18, wherein SEQ ID NO: 9 has a fluorescent reporter dye 6-carboxyfluorescein at the 5' end and a Minor Groove Binder non-fluorescent quencher (MGB-NFQ) non-fluorescence quencher at the 3' end and SEQ ID NO:18 has a fluorescent reporter dye, 2′-chloro-7′phenyl-1,4-dichloro-6-carboxy-fluorescein at the 5' end and a Minor Groove Binder non-fluorescent quencher (MGB-NFQ) at the 3' end.

The ~~invention~~disclosure provides a kit for detecting *Bovine parvovirus* 3 (BPV-3) genomic DNA contamination in the extracted DNA of a test sample comprising primer probe combination that detects DNA encoding BPV-3 selected from the group consisting of a) a primer probe combination having the nucleic acid sequences of SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3; b) a primer probe combination having the nucleic acid sequences of SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6; c) a primer probe combination having the nucleic acid sequences of SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9; d) a primer probe combination having the nucleic acid sequences of SEQ ID NO:10, SEQ ID NO:11, and SEQ ID NO:12; and e) a primer probe combination having the nucleic acid sequences of SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, f) a primer probe combination having the nucleic acid sequences of SEQ ID NO:20, SEQ ID NO:21, and SEQ ID NO:22; g) a primer probe combination having the nucleic acid sequences of SEQ ID NO:23, SEQ ID NO:24, and SEQ ID NO:25; and h) a primer probe combination having the nucleic acid sequences of SEQ ID NO:26, SEQ ID NO:27, and SEQ ID NO:28; and optionally an internal positive control primer probe combination having the nucleic acid sequences of SEQ ID NO: 16, SEQ ID NO:17, and SEQ ID NO:18. In one ~~embodiment~~example the kit comprises a primer probe combination that detects DNA encoding BPV-3 selected from the group consisting of a) a primer probe combination having the nucleic acid sequences of SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3; b) a primer probe combination having the nucleic acid sequences of SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6; c) a primer probe combination having the nucleic acid sequences of SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9; d) a primer probe combination having the nucleic acid sequences of SEQ ID NO:10, SEQ ID NO:11, and SEQ ID NO:12; and e) a primer probe combination having the nucleic acid sequences of SEQ ID NO:13, SEQ ID NO:14, and SEQ ID NO:15, f) a primer probe combination having the nucleic acid sequences of SEQ ID NO:20, SEQ ID NO:21, and SEQ ID NO:22; g) a primer probe combination having the nucleic acid sequences of SEQ ID NO:23, SEQ ID NO:24, and SEQ ID NO:25; and h) a primer probe combination having the nucleic acid sequences of SEQ ID NO:26, SEQ ID NO:27, and SEQ ID NO:28; and an internal positive control primer probe combination having the nucleic acid sequences of SEQ ID NO: 16, SEQ ID NO:17, and SEQ ID NO:18.

The ~~invention~~disclosure provides a method for determining the presence or absence of *Bovine parvovirus* 3 genomic DNA in the extracted DNA of a test sample comprising 1) a reaction mixture comprising a test sample, a positive control, a BPV-3_IPC positive control plasmid DNA, nucleic acid amplification reagents, an internal positive control primer probe combination having the nucleic acid sequences of SEQ ID NO: 16, SEQ ID NO:17, and SEQ ID NO:18, wherein SEQ ID NO:18 has a fluorescent reporter dye at the 5' end and a non-fluorescence quencher at the 3' end, and a primer probe combination selective for a DNA sequence of *Bovine parvovirus* 3, selected from the group consisting of a) a primer probe combination having the nucleic acid sequences of SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3, wherein SEQ ID NO:3 has a fluorescent reporter dye at the 5' end and a non-fluorescence quencher at the 3' end; b) a primer probe combination having the nucleic acid sequences of SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6, wherein SEQ ID NO:6 has a

fluorescent reporter dye at the 5' end and a non-fluorescence quencher at the 3' end; c) a primer probe combination having the nucleic acid sequences of SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9, wherein SEQ ID NO:9 has a fluorescent reporter dye at the 5' end and a non-fluorescence quencher at the 3' end; d) a primer probe combination having the nucleic acid sequences of SEQ ID NO:10, SEQ ID NO:11, and SEQ ID NO:12, wherein SEQ ID NO:12 has a fluorescent reporter dye at the 5' end and a non-fluorescence quencher at the 3' end; e) a primer probe combination having the nucleic acid sequences of SEQ ID NO:13, SEQ ID NO:14, and SEQ ID NO:15, wherein SEQ ID NO:15 has a fluorescent reporter dye at the 5' end and a non-fluorescence quencher at the 3' end; g) a primer probe combination having the nucleic acid sequences of SEQ ID NO:23, SEQ ID NO:24, and SEQ ID NO:25, wherein SEQ ID NO:25 has a fluorescent reporter dye at the 5' end and a non-fluorescence quencher at the 3' end; and h) a primer probe combination having the nucleic acid sequences of SEQ ID NO:26, SEQ ID NO:27, and SEQ ID NO:28, wherein SEQ ID NO:28 has a fluorescent reporter dye at the 5' end and a non-fluorescence quencher at the 3' end; 2) subjecting the reaction mixture to a quantitative PCR technique to obtain copies of the target sequence, 2) subjecting the reaction mixture to a quantitative PCR technique to obtain copies of the target sequence, 3) measuring any increase in fluorescence signal, wherein an increase in fluorescence signal indicates the presence of *Bovine parvovirus* 3 genomic DNA in the test sample. In one ~~embodiment~~example the fluorescent reporter dye is 6-carboxyfluorescein (FAM) and the non-fluorescence quencher is a Minor Groove Binder non-fluorescent quencher (MGB-NFQ) or ZEN-IB and Iowa Black Fluorescence quencher (IBFQ). In one ~~embodiment~~example the ~~invention~~disclosure further comprises one or more negative extraction control, no template control, positive extraction control, positive control, and/or inhibition control. In one ~~embodiment~~example the sensitivity or analytical limit of detection is 22 genome copies per reaction. In one ~~embodiment~~example the sample limit of detection is 25 genome copies per reaction. In one ~~embodiment~~example the primer probe combination selective for a DNA sequence of *Bovine parvovirus* 3 detects genomic DNA encoding the non-structural (NS) protein and/or structural capsid (VP) protein of *Bovine parvovirus* 3. In one ~~embodiment~~example the primer probe combination selective for a DNA sequence of *Bovine parvovirus* 3 amplifies a 144 bp fragment. In one ~~embodiment~~example the primer probe combination selective for a DNA sequence of *Bovine parvovirus* 3 comprises the combination of SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9, wherein SEQ ID NO:9 has a 6-carboxyfluorescein (FAM) at the 5' end and a Minor Groove Binder non-fluorescent quencher (MGB-NFQ) at the 3' end. In one ~~embodiment~~example the method further comprises an internal positive control primer probe combination. In a related ~~embodiment~~example the primer probe combination has the nucleic acid sequences of SEQ ID NO: 16, SEQ ID NO:17, and SEQ ID NO:18. In a related ~~embodiment~~example SEQ ID NO: 18 has a fluorescent reporter dye and/or a non-fluorescent quencher. In a related ~~embodiment~~example SEQ ID NO: 18 has a fluorescent reporter dye, 2′-chloro-7′phenyl-1,4-dichloro-6-carboxy-fluorescein (VIC) at the 5' end and a Minor Groove Binder non-fluorescent quencher (MGB-NFQ) at the 3' end.

The ~~invention~~disclosure provides a method for the quantification of 1E3 to 1E8 genome copies of *Bovine parvovirus* 3 genomic DNA in a PCR reaction comprising 1) a reaction mixture comprising the extracted DNA

of a test sample, a positive control, a BPV-3_IPC positive control plasmid DNA, nucleic acid amplification reagents, an internal positive control primer probe combination having the nucleic acid sequences of SEQ ID NO: 16, SEQ ID NO:17, and SEQ ID NO:18, wherein SEQ ID NO:18 has a fluorescent reporter dye at the 5' end and a non-fluorescence quencher at the 3' end, and a primer probe combination selective for a DNA sequence of *Bovine parvovirus* 3, selected from the group consisting of a) a primer probe combination having the nucleic acid sequences of SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3, wherein SEQ ID NO:3 has a fluorescent reporter dye at the 5' end and a non-fluorescence quencher at the 3' end; b) a primer probe combination having the nucleic acid sequences of SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6, wherein SEQ ID NO:6 has a fluorescent reporter dye at the 5' end and a non-fluorescence quencher at the 3' end; c) a primer probe combination having the nucleic acid sequences of SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9, wherein SEQ ID NO:9 has a fluorescent reporter dye at the 5' end and a non-fluorescence quencher at the 3' end; d) a primer probe combination having the nucleic acid sequences of SEQ ID NO:10, SEQ ID NO:11, and SEQ ID NO:12, wherein SEQ ID NO:12 has a fluorescent reporter dye at the 5' end and a non-fluorescence quencher at the 3' end; e) a primer probe combination having the nucleic acid sequences of SEQ ID NO:13, SEQ ID NO:14, and SEQ ID NO:15, wherein SEQ ID NO:15 has a fluorescent reporter dye at the 5' end and a non-fluorescence quencher at the 3' end; f) a primer probe combination having the nucleic acid sequences of SEQ ID NO:20, SEQ ID NO:21, and SEQ ID NO:22, wherein SEQ ID NO:22 has a fluorescent reporter dye at the 5' end and a non-fluorescence quencher at the 3' end; g) a primer probe combination having the nucleic acid sequences of SEQ ID NO:23, SEQ ID NO:24, and SEQ ID NO:25, wherein SEQ ID NO:25 has a fluorescent reporter dye at the 5' end and a non-fluorescence quencher at the 3' end; and h) a primer probe combination having the nucleic acid sequences of SEQ ID NO:26, SEQ ID NO:27, and SEQ ID NO:28, wherein SEQ ID NO:28 has a fluorescent reporter dye at the 5' end and a non-fluorescence quencher at the 3' end; 2) subjecting the reaction mixture to a quantitative PCR technique to obtain copies of the target sequence, and 3) measuring any increase in fluorescence signal. In one ~~embodiment~~example the limit of detection (LOD95%) of the method is 27 genome copies of *Bovine parvovirus* 3 genomic DNA per reaction with a 95% confidence interval of 22 and 34 genome copies per reaction. In one ~~embodiment~~example the linearity of the method has a correlation coefficient (R2) $\geq$ 0.98 and a PCR amplification efficiency within 90-110%. In one ~~embodiment~~example the method has a repeatability value that is a %CV of quantity equal or less than 25%. In one ~~embodiment~~example the method has an intermediate precision value that is %CV of quantity equal or less than 30%. In one ~~embodiment~~example the method has an accuracy value within ±30% of the accepted reference value (ST) across the whole dynamic range of the assay. In one ~~embodiment~~example the method has a limit of quantitation that is the %CV of quantity for repeatability at $\leq$ 25%, intermediate precision at $\leq$ 30% and acceptance criterion for the accuracy within ±30% of the expected standard reference value. In another ~~embodiment~~example the method has a robustness that has a percent CV of quantity for repeatability of $\leq$ 25%, an intermediate precision of $\leq$ 30%, and an accuracy of the mean of quantity of the combination matrix condition tested of ±30% of the mean of quantity of the optimized

condition. In another ~~embodiment~~example the method includes one or more of a no template control, a positive control, a negative extraction control, a positive extraction control, an inhibition control, an internal positive control, and a standard.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. The Probit analysis used for determination of ALOD in BPV-3 qPCR with 95% of probability (LOD95%).

Figure 2. POD graph and LOD95% analysis results.

DETAILED DESCRIPTION OF THE INVENTION

As described herein, a qualitative real-time PRC method has been developed to detect potential *Bovine parvovirus 3* (BPV-3) genomic DNA contamination in the extracted of test samples. Qualification parameters, including specificity, limit of detection (LOD), robustness and repeatability were used to test and confirm the performance of the assay.

The assay makes use of optimized primer probe combinations for detecting *Bovine parvovirus* 3 (BPV-3) genomic DNA in the extracted DNA of a test sample. The BPV-3 PCR primers amplify conserved regions in the genes encoding the BPV-3 non-structural (NS) protein and/or structural capsid (VP) protein in the BPV-3 genome. "Method Validation of U.S. Environmental Protection Agency (EPA) Microbiological Methods of Analysis", REVISION: December 21, 2016, was consulted and considered in the design and development of the assay.

The invention provides oligonucleotide primer probe combinations that can be used in detection methods, such as in quantitative polymerase chain reaction (qPCR) techniques, to detect DNA encoding *Bovine parvovirus* 3 genomic (BPV-3) and for use in methods for determining the presence or absence of BPV-3 in the extracted DNA of a test sample.

As used herein, "oligonucleotides" are short single stranded synthetic DNA or RNA molecules, less than 200 nucleotides in length, typically in the range of 13-25 nucleotides in length. Oligonucleotides bind to their complement oligonucleotide to form a duplex. As a result, oligonucleotides are commonly used in a variety of applications where detection of the presence or absence of specific DNA or RNA sequences is desired. In particular, oligonucleotides are useful as primers for use in polymerase chain reactions (PCR). Methods to synthesize oligonucleotides are known in the art, equipment to produce oligonucleotides are commercially available and there are service providers who will make custom oligonucleotides on demand. Oligonucleotides can also be obtained from the breakdown of larger nucleic acid molecules, or naturally occurring oligonucleotides, such as micro RNA.

What is claimed is:

1. A composition comprising ~~oligonucleotides selected from the groups consisting of~~

~~a) oligonucleotides having the nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3;~~

~~b) oligonucleotides having the nucleic acid sequence of SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6;~~

~~c)~~ oligonucleotides having the nucleic acid sequence of SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9;

~~d) oligonucleotides having the nucleic acid sequence of SEQ ID NO:10, SEQ ID NO:11, and SEQ ID NO:12;~~

~~e) oligonucleotides having the nucleic acid sequence of SEQ ID NO:13, SEQ ID NO:14, and SEQ ID NO:15;~~

~~f) oligonucleotides having the nucleic acid sequence of SEQ ID NO:20, SEQ ID NO:21, and SEQ ID NO:22;~~

~~g) oligonucleotides having the nucleic acid sequence of SEQ ID NO:23, SEQ ID NO:24, and SEQ ID NO:25; and~~

~~h) oligonucleotides having the nucleic acid sequence of SEQ ID NO:26, SEQ ID NO:27, and SEQ ID NO:28.~~

~~2. A composition according to claim 1, wherein the composition~~ optionally ~~includes~~ including a second composition comprises oligonucleotides having the sequence of SEQ ID NO: 16, SEQ ID NO:17, and SEQ ID NO:18.

~~3. A composition according to claim 1, wherein the composition comprises oligonucleotides having the nucleic acid sequence of SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9.~~

~~4. A composition according to claim 1, wherein the composition comprises a first composition comprises oligonucleotides having the nucleic acid sequence of SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9 and a second composition comprises oligonucleotides having the sequence of SEQ ID NO: 16, SEQ ID NO:17, and SEQ ID NO:18.~~

~~5.~~2 A reagent for detecting *Bovine parvovirus* 3 (BPV-3) genomic DNA in the extracted DNA of a test sample ~~selected from the group~~ consisting of

~~a) a primer probe combination having the nucleic acid sequences of SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3;~~

~~b) a primer probe combination having the nucleic acid sequences of SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6;~~

~~c)~~ a primer probe combination having the nucleic acid sequences of SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9;

~~d) a primer probe combination having the nucleic acid sequences of SEQ ID NO:10, SEQ ID NO:11, and SEQ ID NO:12;~~

~~e) a primer probe combination having the nucleic acid sequences of SEQ ID NO:13, SEQ ID NO:14, and SEQ ID NO:15;~~

~~f) a primer probe combination having the nucleic acid sequences of SEQ ID NO:20, SEQ ID NO:21, and SEQ ID NO:22;~~

~~g) a primer probe combination having the nucleic acid sequences of SEQ ID NO:23, SEQ ID NO:24, and SEQ ID NO:25; and~~

~~h) a primer probe combination having the nucleic acid sequences of SEQ ID NO:26, SEQ ID NO:27, and SEQ ID NO:28.~~

~~6. The reagent according to claim 5, wherein SEQ ID NO: 3, SEQ ID NO:6,~~ optionally SEQ ID NO:9, ~~SEQ ID NO:12, SEQ ID NO:15, SEQ ID NO:22, SEQ ID NO:25; or SEQ ID NO:28 have~~ has a fluorescent reporter dye and/or a non-fluorescent quencher~~.~~

~~7. The reagent according to claim 5, wherein one or more of SEQ ID NO: 3, SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:12, SEQ ID NO:15, SEQ ID NO:22, SEQ ID NO:25; or SEQ ID NO:28~~ ; optionally ~~have a~~ the fluorescent reporter dye 6-carboxyfluorescein (FAM) at the 5' end and/or either a Minor Groove Binder non-fluorescence quencher (MGB-NFQ) or ZEN-IB and Iowa Black Fluorescence quencher (IBFQ) at the 3' end~~.~~;

optionally the primer probe combination is used with an internal positive control primer combination; and

~~8. The reagent according to claim 5,~~ wherein the primer probe combination detects DNA encoding the structural capsid (VP) protein and/or the non-structural (NS) protein of *Bovine parvovirus* 3 in the test sample.

~~9. The reagent according to claim 5 in combination with an internal positive control primer combination.~~

~~10. The reagent according to claim 5, comprising SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9.~~

~~11~~3. A reagent for use as an internal positive control primer probe combination in an assay for detecting *Bovine parvovirus* 3 (BPV-3) genomic DNA in the extracted DNA of a test sample comprising a primer probe combination~~.~~ having

~~12. The reagent according to claim 11, wherein the primer probe combination has~~ the nucleic acid sequences of SEQ ID NO: 16, SEQ ID NO:17, and SEQ ID NO:18~~.~~;

~~13. The reagent according to claim 11, wherein~~ optionally SEQ ID NO: 18 has a fluorescent reporter dye and/or a non-fluorescent quencher~~.~~;

~~14. The reagent according to claim 13, wherein~~ optionally SEQ ID NO: 18 has a fluorescent reporter dye, 2′-chloro-7′phenyl-1,4-dichloro-6-carboxy-fluorescein (VIC) at the 5’ end and a Minor Groove Binder non-fluorescent quencher (MGB-NFQ) at the 3’ end.

~~15~~4. A primer probe combination for detecting *Bovine parvovirus* 3 genomic DNA in ~~the~~ an extracted DNA of a test sample in combination with an internal positive control primer probe combination for detecting *Bovine parvovirus* 3 (BPV-3) genomic DNA wherein ~~.~~

~~16. A primer probe combination for detecting *Bovine parvovirus* 3 genomic DNA according to claim 15 wherein the primer probe combination is selected from the groups consisting of~~

~~a) a primer probe combination having the nucleic acid sequences of SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3;~~

~~b) a primer probe combination having the nucleic acid sequences of SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6;~~

~~c) a~~ the primer probe combination ~~having~~ has the nucleic acid sequences of SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9;

~~d) a primer probe combination having the nucleic acid sequences of SEQ ID NO:10, SEQ ID NO:11, and SEQ ID NO:12;~~

~~e) a primer probe combination having the nucleic acid sequences of SEQ ID NO:13, SEQ ID NO:14, and SEQ ID NO:15;~~

~~f) a primer probe combination having the nucleic acid sequences of SEQ ID NO:20, SEQ ID NO:21, and SEQ ID NO:22;~~

~~g) a primer probe combination having the nucleic acid sequences of SEQ ID NO:23, SEQ ID NO:24, and SEQ ID NO:25; and~~

~~h) a primer probe combination having the nucleic acid sequences of SEQ ID NO:26, SEQ ID NO:27, and SEQ ID NO:28.~~

~~17. The primer probe combination according to claim 16, wherein SEQ ID NO: 3, SEQ ID NO:6,~~ optionally SEQ ID NO:9~~, SEQ ID NO:12, SEQ ID NO:15 have~~ has a fluorescent reporter dye and/or a non-fluorescent quencher~~.~~;

~~18. The primer probe combination according to claim 16, wherein one or more of SEQ ID NO: 3, SEQ ID NO:6,~~ optionally SEQ ID NO:9 ~~, SEQ ID NO:12, SEQ ID NO:15, SEQ ID NO:22, SEQ ID NO:25; or SEQ ID NO:28 have~~has a fluorescent reporter dye 6-carboxyfluorescein (FAM) at the 5' end and/or either a Minor Groove Binder non-fluorescence quencher (MGB-NFQ) or ZEN-IB and Iowa Black Fluorescence quencher (IBFQ) at the 3' end~~.~~;

optionally the internal positive control primer probe combination has the nucleic acid sequences of SEQ ID NO: 16, SEQ ID NO:17, and SEQ ID NO:18;

optionally SEQ ID NO: 18 has a fluorescent reporter dye and/or a non-fluorescent quencher;

optionally SEQ ID NO: 18 has a fluorescent reporter dye, 2′-chloro-7′phenyl-1,4-dichloro-6-carboxy-fluorescein (VIC) at the 5' end and a Minor Groove Binder non-fluorescent quencher (MGB-NFQ) at the 3' end, and wherein

~~19. The primer probe combination according to claim 15, wherein~~ the primer probe combination detects DNA encoding the structural capsid (VP) protein and/or the non-structural (NS) protein of *Bovine parvovirus* 3 in a test sample.

~~20. An internal positive control primer probe combination for detecting *Bovine parvovirus* 3 (BPV 3) genomic DNA in a test sample according to claim 15, wherein the primer probe combination has the nucleic acid sequences of SEQ ID NO: 16, SEQ ID NO:17, and SEQ ID NO:18.~~

~~21. The internal positive control primer probe combination according to claim 20, wherein SEQ ID NO: 18 has a fluorescent reporter dye and/or a non-fluorescent quencher.~~

~~22. The primer probe combination according to claim 20, wherein SEQ ID NO: 18 has a fluorescent reporter dye, 2′ chloro-7′phenyl-1,4-dichloro-6-carboxy fluorescein (VIC) at the 5' end and a Minor Groove Binder non-fluorescent quencher (MGB-NFQ) at the 3' end.~~

~~23~~5. A primer probe combination for detecting *Bovine parvovirus* 3 genomic DNA in the extracted DNA of a test sample comprising SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9 in combination with an internal positive control primer probe combination comprising SEQ ID NO: 16, SEQ ID NO:17, and SEQ ID NO:18, wherein SEQ ID NO: 9 has a fluorescent reporter dye 6-carboxyfluorescein at the 5' end and a Minor Groove

Binder non-fluorescent quencher (MGB-NFQ) non-fluorescence quencher at the 3' end and SEQ ID NO:18 has a fluorescent reporter dye, 2′-chloro-7′phenyl-1,4-dichloro-6-carboxy-fluorescein at the 5' end and a Minor Groove Binder non-fluorescent quencher (MGB-NFQ) at the 3' end.

~~24~~6. A kit for detecting *Bovine parvovirus* 3 (BPV-3) genomic DNA contamination in the extracted DNA of a test sample comprising

primer probe combination that detects DNA encoding BPV-3 selected from the group consisting of

~~a) a primer probe combination having the nucleic acid sequences of SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3;~~

~~b) a primer probe combination having the nucleic acid sequences of SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6;~~

~~c)~~ a primer probe combination having the nucleic acid sequences of SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9; and

~~d) a primer probe combination having the nucleic acid sequences of SEQ ID NO:10, SEQ ID NO:11, and SEQ ID NO:12;~~

~~e) a primer probe combination having the nucleic acid sequences of SEQ ID NO:13, SEQ ID NO:14, and SEQ ID NO:15;~~

~~f) a primer probe combination having the nucleic acid sequences of SEQ ID NO:20, SEQ ID NO:21, and SEQ ID NO:22;~~

~~g) a primer probe combination having the nucleic acid sequences of SEQ ID NO:23, SEQ ID NO:24, and SEQ ID NO:25; and~~

~~h) a primer probe combination having the nucleic acid sequences of SEQ ID NO:26, SEQ ID NO:27, and SEQ ID NO:28; and~~

optionally an internal positive control primer probe combination having the nucleic acid sequences of SEQ ID NO: 16, SEQ ID NO:17, and SEQ ID NO:18.

~~25~~7. A method for determining the presence or absence of *Bovine parvovirus* 3 genomic DNA in the extracted DNA of a test sample comprising

1) a reaction mixture comprising a test sample, a positive control, a BPV-3_IPC positive control plasmid DNA, nucleic acid amplification reagents, an internal positive control primer probe combination having the nucleic acid sequences of SEQ ID NO: 16, SEQ ID NO:17, and SEQ ID NO:18, wherein SEQ ID NO:18 has a fluorescent reporter dye at the 5' end and a non-fluorescence quencher at the 3' end, and a primer

probe combination selective for a DNA sequence of *Bovine parvovirus* 3~~, selected from the group consisting of~~

~~a) a primer probe combination having the nucleic acid sequences of SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3, wherein SEQ ID NO:3 has a fluorescent reporter dye at the 5' end and a non-fluorescence quencher at the 3' end;~~

~~b) a primer probe combination having the nucleic acid sequences of SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6, wherein SEQ ID NO:6 has a fluorescent reporter dye at the 5' end and a non-fluorescence quencher at the 3' end;~~ having

~~c)~~ a primer probe combination having the nucleic acid sequences of SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9, wherein SEQ ID NO:9 has a fluorescent reporter dye at the 5' end and a non-fluorescence quencher at the 3' end~~;~~

optionally the fluorescent reporter dye is 6-carboxyfluorescein (FAM) and the non-fluorescence quencher is a Minor Groove Binder non-fluorescent quencher (MGB-NFQ) or ZEN-IB and Iowa Black Fluorescence quencher (IBFQ);

optionally further comprising one or more negative extraction control, no template control, positive extraction control, positive control, and/or inhibition control;

optionally the primer probe combination selective for a DNA sequence of *Bovine parvovirus* 3 detects DNA encoding the non-structural (NS) protein and/or structural capsid (VP) protein of *Bovine parvovirus* 3;

~~d) a primer probe combination having the nucleic acid sequences of SEQ ID NO:10, SEQ ID NO:11, and SEQ ID NO:12, wherein SEQ ID NO:12 has a fluorescent reporter dye at the 5' end and a non-fluorescence quencher at the 3' end;~~

~~e) a primer probe combination having the nucleic acid sequences of SEQ ID NO:13, SEQ ID NO:14, and SEQ ID NO:15, wherein SEQ ID NO:15 has a fluorescent reporter dye at the 5' end and a non-fluorescence quencher at the 3' end;~~

~~f) a primer probe combination having the nucleic acid sequences of SEQ ID NO:20, SEQ ID NO:21, and SEQ ID NO:22, wherein SEQ ID NO:22 has a fluorescent reporter dye at the 5' end and a non-fluorescence quencher at the 3' end;~~

~~g) a primer probe combination having the nucleic acid sequences of SEQ ID NO:23, SEQ ID NO:24, and SEQ ID NO:25, wherein SEQ ID NO:25 has a fluorescent reporter dye at the 5' end and a non-fluorescence quencher at the 3' end; and~~

~~h) a primer probe combination having the nucleic acid sequences of SEQ ID NO:26, SEQ ID NO:27, and SEQ ID NO:28, wherein SEQ ID NO:28 has a fluorescent reporter dye at the 5' end and a non-fluorescence quencher at the 3' end~~;

2) subjecting the reaction mixture to a quantitative PCR technique to obtain copies of the target sequence,

3) measuring any increase in fluorescence signal,

wherein an increase in fluorescence signal indicates the presence of *Bovine parvovirus* 3 genomic DNA in the test sample.

~~26. The method according to claim 25, wherein the fluorescent reporter dye is 6-carboxyfluorescein (FAM) and the non-fluorescence quencher is a Minor Groove Binder non-fluorescent quencher (MGB-NFQ) or ZEN-IB and Iowa Black Fluorescence quencher (IBFQ).~~

~~27. The method according to claim 25, further comprising one or more negative extraction control, no template control, positive extraction control, positive control, and/or inhibition control.~~

~~28~~8. The method according to claim ~~25~~7, wherein the sensitivity or analytical limit of detection is 22 genome copies per reaction~~.~~;

~~29. The method according to claim 25,~~ optionally wherein the sample limit of detection is 25 genome copies per reaction.

~~30. The method according to claim 25, wherein the primer probe combination selective for a DNA sequence of *Bovine parvovirus* 3 detects DNA encoding the non-structural (NS) protein and/or structural capsid (VP) protein of *Bovine parvovirus* 3.~~

~~31~~9. The method according to claim ~~25~~7, wherein the primer probe combination selective for a DNA sequence of *Bovine parvovirus* 3 amplifies a 144 bp fragment.

~~32. The method according to claim 25, wherein the primer probe combination selective for a DNA sequence of *Bovine parvovirus* 3 comprises the combination of SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9, wherein SEQ ID NO:9 has a 6-carboxyfluorescein (FAM) at the 5' end and a Minor Groove Binder (MGB-NFQ)at the 3' end.~~

~~33. The method according to claim 25 further comprising an internal positive control primer probe combination.~~

~~34. The method according to claim 33, wherein the primer probe combination has the nucleic acid sequences of SEQ ID NO: 16, SEQ ID NO:17, and SEQ ID NO:18.~~

~~35. The method according to claim 34, wherein SEQ ID NO: 18 has a fluorescent reporter dye and/or a non-fluorescent quencher.~~

~~36. The method according to claim 34, wherein SEQ ID NO: 18 has a fluorescent reporter dye, 2′ chloro-7′phenyl-1,4-dichloro-6-carboxy-fluorescein (VIC) at the 5' end and a Minor Groove Binder non-fluorescent quencher (MGB-NFQ) at the 3' end.~~

~~37~~10. A method for the quantification of 1E3 to 1E8 genome copies of *Bovine parvovirus* 3 genomic DNA in a PCR reaction comprising

1) a reaction mixture comprising the extracted DNA of a test sample, a positive control, a BPV-3_IPC positive control plasmid DNA, nucleic acid amplification reagents, an internal positive control primer probe combination having the nucleic acid sequences of SEQ ID NO: 16, SEQ ID NO:17, and SEQ ID NO:18, wherein SEQ ID NO:18 has a fluorescent reporter dye at the 5' end and a non-fluorescence quencher at the 3' end, and a primer probe combination ~~selective for a DNA sequence of *Bovine parvovirus* 3, selected from the group consisting of~~

~~a) a primer probe combination having the nucleic acid sequences of SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3, wherein SEQ ID NO:3 has a fluorescent reporter dye at the 5' end and a non-fluorescence quencher at the 3' end;~~

~~b) a primer probe combination having the nucleic acid sequences of SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6, wherein SEQ ID NO:6 has a fluorescent reporter dye at the 5' end and a non-fluorescence quencher at the 3' end;~~

~~c) a primer probe combination~~ having the nucleic acid sequences of SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9, wherein SEQ ID NO:9 has a fluorescent reporter dye at the 5' end and a non-fluorescence quencher at the 3' end;

~~d) a primer probe combination having the nucleic acid sequences of SEQ ID NO:10, SEQ ID NO:11, and SEQ ID NO:12, wherein SEQ ID NO:12 has a fluorescent reporter dye at the 5' end and a non-fluorescence quencher at the 3' end;~~

~~e) a primer probe combination having the nucleic acid sequences of SEQ ID NO:13, SEQ ID NO:14, and SEQ ID NO:15, wherein SEQ ID NO:15 has a fluorescent reporter dye at the 5' end and a non-fluorescence quencher at the 3' end; f) a primer probe combination having the nucleic acid sequences of SEQ ID NO:20, SEQ ID NO:21, and SEQ ID NO:22, wherein SEQ ID NO:22 has a fluorescent reporter dye at the 5' end and a non-fluorescence quencher at the 3' end;~~

~~g) a primer probe combination having the nucleic acid sequences of SEQ ID NO:23, SEQ ID NO:24, and SEQ ID NO:25, wherein SEQ ID NO:25 has a fluorescent reporter dye at the 5' end and a non-fluorescence quencher at the 3' end; and~~

~~h) a primer probe combination having the nucleic acid sequences of SEQ ID NO:26, SEQ ID NO:27, and SEQ ID NO:28, wherein SEQ ID NO:28 has a fluorescent reporter dye at the 5' end and a non-fluorescence quencher at the 3' end~~;

optionally the method includes one or more of a no template control, a positive control, a negative extraction control, a positive extraction control, an inhibition control, an internal positive control, and a standard;

2) subjecting the reaction mixture to a quantitative PCR technique to obtain copies of the target sequence, and

3) measuring any increase in fluorescence signal~~.~~;

~~38. The method according to claim 37, wherein~~ optionally the limit of detection (LOD95%) of the method is 27 genome copies of *Bovine parvovirus* 3 genomic DNA per reaction with a 95% confidence interval of 22 and 34 genome copies per reaction~~.~~;

~~39. The method according to claim 37, wherein~~ optionally the linearity of the method has a correlation coefficient (R2) ≥ 0.98 and a PCR amplification efficiency within 90-110~~%.~~ %;

~~40. The method according to claim 37, wherein~~optionally the method has a repeatability value that is a %CV of quantity equal or less than 25~~%.~~ %;

~~41. The method according to claim 37, wherein~~optionally the method has an intermediate precision value that is %CV of quantity equal or less than 30~~%.~~ %;

~~42. The method according to claim 37, wherein~~optionally the method has an accuracy value within ±30% of the accepted reference value (ST) across the whole dynamic range of the assay~~.~~;

~~43. The method according to claim 37, wherein~~ optionally the method has a limit of quantitation that is the %CV of quantity for repeatability at ≤ 25%, intermediate precision at ≤ 30% and acceptance criterion for the accuracy within ±30% of the expected standard reference value~~.~~; and

~~44. The method according to claim 37, wherein~~ optionally the method has a robustness that has a percent CV of quantity for repeatability of ≤ 25%, an intermediate precision of ≤ 30%, and an accuracy of the mean of quantity of the combination matrix condition tested of ±30% of the mean of quantity of the optimized condition.

~~45. The method according to claim 37, wherein the method includes one or more of a no template control, a positive control, a negative extraction control, a positive extraction control, an inhibition control, an internal positive control, and a standard.~~

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