Development and Characterization of A Multiplexed RT-PCR Species Specific Assay for Bovine and one for Porcine Foot-and-Mouth Disease Virus Rule-Out

July 23, 2007

UCRL-TR-233661

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This work was performed under the auspices of the U.S. Department of Energy by the University of California, Lawrence Livermore National Laboratory under Contract No. W-7405-Eng-48, with funding through the Department of Homeland Security (DHS). Distribution authorized to US government agencies and their contractors for administrative and operational use.

Executive summary

Lawrence Livermore National Laboratory (LLNL), in collaboration with the Department of Homeland Security (DHS) and the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Services (APHIS) has developed candidate multiplexed assays that may potentially be used within the National Animal Health Laboratory Network (NAHLN), the National Veterinary Services Laboratory (Ames, Iowa) and the Plum Island Animal Disease Center (PIADC). This effort has the ability to improve our nation's capability to discriminate between foreign animal diseases and those that are endemic using a single assay, thereby increasing our ability to protect food and agricultural resources with a diagnostic test which could enhance the nation's capabilities for early detection of a foreign animal disease. In FY2005 with funding from the DHS, LLNL developed the first version (Version 1.0) of a multiplexed (MUX) nucleic-acid-based RT-PCR assay that included signatures for foot-and-mouth disease virus (FMDV) detection with rule-out tests for two other foreign animal diseases (FADs) of swine, Vesicular Exanthema of Swine (VESV) and Swine Vesicular Disease Virus (SVDV), and four other domestic viral diseases Bovine Viral Diarrhea Virus (BVDV), Bovine Herpes Virus 1 (BHV-1), Bluetongue virus (BTV) and Parapox virus complex (which includes Bovine Papular Stomatitis Virus [BPSV], Orf of sheep, and Pseudocowpox). In FY06, LLNL has developed Bovine and Porcine species-specific panel which included existing signatures from Version 1.0 panel as well as new signatures. The MUX RT-PCR porcine assay for detection of FMDV includes the FADs, VESV and SVD in addition to vesicular stomatitis virus (VSV) and porcine reproductive and respiratory syndrome (PRRS). LLNL has also developed a MUX RT-PCR bovine assay for detection of FMDV with rule out tests for the two bovine FADs malignant catarrhal fever (MCF), rinderpest virus (RPV) and the domestic diseases vesicular stomatitis virus (VSV), bovine viral diarrhea virus (BVDV), infectious bovine rhinotracheitus virus (BHV-1), bluetongue virus (BTV), and the Parapox viruses (which are of two bovine types) bovine papular stomatitis virus (BPSV) and psuedocowpox (PCP). A timeline for this development is presented in Table 1. The development of the Version 1.0 panel for FMDV rule-out and the most current efforts aimed to designed species specific panels has spanned over 2 ½ years with multiple collaborative partnerships.

This document provides a summary of the development, testing and performance data at OIE Stage 1 Feasibility into Stage 2 Assay Development and Standardization[1] (see Table 2), gathered as of June 30th, 2007 for the porcine and bovine MUX assay panels. We present an overview of the identification and selection of candidate genetic signatures, the assay development process, and preliminary performance data for each of the individual signatures as characterized in the multiplexed format for the porcine and bovine panels. The Stage 1 Feasibility data of the multiplexed panels is presented in this report also includes relevant data acquired from the Version 1.0 panel as supporting information where appropriate. In contrast to last years' effort, the development of the bovine and porcine panels is pending additional work to complete analytical characterization of FMDV, VESV, SVD, RPV and MCF. The signature screening process and final panel composition impacts this effort. The unique challenge presented this year was having strict predecessor limitations in completing characterization, where efforts at LLNL must precede efforts at PIADC, such challenges were alleviated in the 2006 reporting by having characterization data from the interlaboratory comparison and at Plum Island under AgDDAP project. We will present an addendum at a later date with additional data on the characterization of the porcine and bovine multiplex assays when that data is available. As a summary report, this document does not provide the details of

[1] World Organization for Animal Health. (**Office International des Epizooties**)
http://www.oie.int/vcda/eng/en_download_application_VCDA.htm

signature generation, evaluation, and testing, nor does it provide specific methods and materials used. This information has been provided in the separate 488 page Supplementary Materials document.

TABLE 1. Development timeline for the Version 1.0, Bovine and Porcine FMDV rule-out panels.

Panel	2005				2006				2007	
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2
Porcine										
1. FMDV_TC										
2. FMDV_PIR										
3. PRRS_1807709										
4. PRRS_1810351										
5. PRRS_1807706										
6. PRRS_1810383										
7. PRRS_1810386										
8. SVD_1										
9. SVD_2										
10. SVD_3										
11. VESV_1										
12. VESV_4										
13. VESV_5										
14. VSV_1798943										
15. VSV_1811409										
16. VSV_1798947										
17. VSV_1798949										
18. VSV_1811405										
19. VSV_1811408										
Bovine										
20. BHV_1										
21. BHV_3										
22. BTV_1759932										
23. BTV_1810207										
24. BTV_1810199										
25. BTV_1810205										
26. BVD_1a										
27. BVD_2										
28. FMDV_TC										
29. FMDV_PIR										
30. MCF_1										
31. MCF_2										
32. MCF_3										
33. PPox_1										
34. PPox_2										
35. PPox_4										
36. RPV_1814853										
37. RPV_1814855										
38. RPV_1814856										
39. VSV_1798943										
40. VSV_1798947										
41. VSV_1798949										
42. VSV_1811408										

TaqMan LLNL
TaqMan PIADC
TaqMan USDA ABADRL
TaqMan UM VDL
Multiplex assembly LLNL
Multiplex development PIADC
Multiplex development Canada
Multiplex characterization LLNL&PIADC

TABLE 2. Assay development and validation status according to OIE Chapter I 1.4 for Validation and Quality Control of Polymerase Chain Reaction Methods Used for the Diagnosis of Infectious Diseases for Stage 1 Validation: Feasibility. The goal of this stage of validation is to determine whether or not an assay can determine a wide range of target concentrations.

1.2. Stage 1 Validation: Feasibility	**Status for FY06 Development (Bovine and Porcine panels)**
1.2.1. Calibration	
1.2.1.1.Calibration testing of in-house standards	90% complete: during development, each MUX panel was tested against viral nucleic acid extracts (total nucleic acids, non-titered) from each organism. Preliminary results assessed by low-resolution titration curves, for each step-wise addition of assay to the multiplexed panel.
1.2.1.2.Calibration test of reference standards	90% complete: reference standards were obtained from PIADC and NVSL from isolates that had been identified using "gold standard" test methods. These isolates were cultured and quantified in TCID50 and/or PFU/mL using standard methods.
1.2.2. Repeatability data	
1.2.2.1.Minimum of 3 in-house samples representing activity in linear range of assay	90% complete; For each multiplexed assay addition, each analyte is titrated across the linear dynamic range of each assay against a minimum of 3 strains of virus for each organism. Final high-resolution titration curves using titered reference standards are used to characterize panel at final development stage.
1.2.2.2.Replicates within runs (quadruplicates)	90% complete; For each multiplexed assay addition, each analyte is titrated across the linear dynamic range of each assay to determine if the sensitivity of the assay shifts with each new assay addition. Final high-resolution titration curves using titered reference standards are used to characterize panel at final development stage.
1.2.2.3.Between run tests (minimum set of 20, 2 or more operators, independent runs on separate days).	0% complete: however, Version 1.0 panel testing was completed to determine long-term regent stability (over the course of 90+ days of testing, 20+ replicate runs, using 2 or more operators each day). Serial repeatability will occur after MUX panels are complete.
1.2.2.4.Serial repeatability (ideally 3 reagent batches)	0% complete: pending completion of panel development
1.2.2.5.Data with mean, SD, upper and lower control (UCL and LCL) on unprocessed and processed data.	0% : pending above.
1.2.3. Analytical Specificity Data	
1.2.3.1.Cross-reactivity and near neighbor data	90% complete: real-time RT-PCR screening complete for all analytes to include near-neighbor panels of isolates of similar clinical presentation as well as genetically related organisms. Final near neighbor screening in multiplexed format will conclude testing.
1.2.3.2.Document cross-reactivity (organisms of similar clinical presentation or organisms genetically related).	90% complete: preliminary evaluation of cross-reactivities of multiplexed constituents has been established in the development process.
1.2.3.3.Type/group specificity data	60% complete: With the exception of remaining work at PIADC, each assay has been screened against all available isolates or serotypes from each assay in the final multiplex.
1.2.3.4.Documentation affirming serotype or group specificity.	100% complete: samples sourced from PIADC and NVSL, titered viral extractions previously tested as reliable samples. Identifications recorded.
1.2.4. Analytical Sensitivity Data	
1.2.4.1.Specify standard of comparison	Standard of comparison is derived from dose-response curves using titered virus extracts.
1.2.4.2.Comparisons may be end point titrations; earliest time of detection post-exposure.	60% complete: Dose-response curves are generated for each isolate of each assay panel using titered virus extracts. Occurs after full completion of each multiplexed assay; porcine and bovine panel development has concluded, bovine and porcine panel is currently finishing characterization.
1.2.4.3.Duration of detection post-exposure (if applicable).	N/A

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Author's Notes

I. Only limited information is available for those signatures that were not generated at/by LLNL. In some cases, we have not been able to establish with certainty how the signatures were developed (parameters, etc), and which genome(s) were used for the alignment.
II. Externally developed signatures were not subjected to the same level of wet-screening as internally developed signatures. They have been adapted to multiplexed PCR format based on significant characterization data acquired by others in real-time PCR format.
III. Reported concentration units of nucleic acid extracts vary depending on stage of screening and testing location. Preliminary screening using nucleic acids was conducted with total extracts of untitered material reported in pg/rxn units, where 5uL sample added to each reaction. Limit of detection studies and multiplex PCR analysis were performed using titered nucleic acid extracts from titered virus, with a few exceptions where titered virus was not available. Actual titer of nucleic acids is reported in this report in one of 2 methods (dependant on source and virus type) (1) $TCID_{50}$/rxn or (2) pfu/rxn. Dose-response curves are reported on a double log-scale (response (MFI) vs concentration).
IV. A portion of the assay development work was carried out off-site at several facilities some of which were approved for select work with foreign animal diseases (FADs). LLNL staff conducted assay development work of foot-and-mouth disease virus [FMDV], swine vesicular disease virus [SVD], malignant catarrhal fever [MCF] and vesicular exanthema of swine virus [VESV] at Plum Island Animal Disease Center [PIADC], Plum Island, New York. Some screening work for BTV under 2006 funding was carried out in Dr. William Wilson's lab at the USDA Agricultural Research Service [ARS] Laboratory Arthropod Bourne Animal Disease Laboratory [ABADRL] in Laramie, WY. Part of the screening work in 2006 for PRRS was carried out in Dr. Kay Faaberg's laboratory in the Minnesota State Veterinary Diagnostic Laboratory in Minneapolis, MN. Additional work on the aforementioned viruses was conducted with FY05 funding at National Canadian Foreign Animal Disease [NCFAD] laboratory, in Winnipeg, Canada.
V. LLNL bioinformatics group maintains an internal database of reference information. Some of this information (e.g. TAQSIM analysis reports) is not included here, for brevity, but is included in the Supplemental Materials Document.

INTRODUCTION

Agriculture is a major sector of the U.S. economy, accounting for more than 13% of the gross domestic product, and employing more than 15% of the U.S. population. Cattle and dairy farmers alone earn between $50 billion and $54 billion a year through meat and milk sales, and roughly $50 billion is raised every year through farm-related exports. Overall livestock sales in 2001 were in excess of $108 billion. An intentional or unintentional introduction of a FAD into the U.S. could cause devastating economic consequences, not only for the affected agribusinesses but also for allied industries and services, possibly disrupting food supplies, trade, and tourism. Moreover, because of the structure of American agribusiness (e.g., highly concentrated herds (dairy farms), suboptimal animal tracking systems, minimal farm security/surveillance), agricultural and food industries are vulnerable to such an event. The agricultural community currently views an introduction of foot-and-mouth disease (FMD) into the United States as one of their greatest concerns. FMD is a severe, highly communicable viral disease of cattle, other ruminants, and swine, FMD is enzootic to many countries in the world, and the virus is easy to obtain. Although FMD does not pose a direct threat to human health, it is considered a significant agro threat agent. Recent estimates associated with the 2001 FMD outbreak in the United Kingdom place economic losses at greater than $30 billion (U.S.).

The Animal and Plant Health Inspection Service (APHIS), a branch of the U.S. Department of Agriculture (USDA), is charged with protecting the nation's livestock and poultry from the introduction of foreign animal diseases and for coordinating the response to an agricultural disease outbreak. The current system for detecting a foreign animal disease (FAD) such as FMD generally involves the following components: (1) observations by veterinary practitioners and livestock owners, who likely will be the first to suspect and report a FAD case; (2) investigation of suspect cases and submission of samples to USDA/APHIS at the Plum Island Animal Disease Center (PIADC); and (3) diagnostic work-up at the Plum Island Foreign Animal Diagnostic Disease Laboratory (FADDL). Currently, all testing for FMD is done (by law) at FADDL on Plum Island, which averages about 300 investigations per year. During a major outbreak, demand could rise to 1000s of investigations per week. The number of required diagnostic tests would far exceed current analysis capacity, and authorities would have to resort to subjective clinical observations to determine if herds must be destroyed.

There is a desire on the part of the USDA APHIS and State agriculture departments in combating an outbreak of FMD for rapid, validated diagnostic assays for detection and identification of FMD. This necessity for improved diagnostics and surveillance programs to better enable the United Sates to detect and respond to foreign animal diseases (FADs) has been highlighted in several reports (e.g. National Association of State Departments of Agriculture and Research Foundation, the General Accounting Office, and the National Research Council) as well as Homeland Security Presidential Directive-9 (HSPD-9). Additionally, outbreaks of FMD in South America and the United Kingdom have heightened concerns about the ability of existing US surveillance systems to rapidly detect a FMD incursion early in the course of an outbreak and then provide the required diagnostic surge capacity needed for an outbreak response and the recovery of disease free status. At PIADC, laboratory methods currently used to detect FADs include polymerase chain reaction (PCR), agar gel immunodiffusion assays, enzyme-linked immunosorbent assays (ELISA), serum neutralization assays, virus isolation in tissue culture, direct fluorescent antibody tests, electron microscopy, and animal inoculation. Some of these methods are time-consuming and labor-intensive. Several single-plex polymerase chain reaction (PCR) assays have been developed and are currently used in reference laboratories such as Plum Island, however none are currently distributed into National Animal Health Laboratories (NAHLN) for routine use as part of a national surveillance program. None of these FMD assays are currently validated by the *Office International des Epizooties* (OIE). For the laboratory identification of FMDV, OIE recommends virus isolation, antigen-

ELISA, and RT-PCR with detection by agarose gel electophoresis, real-time TaqMan flurogenic probes or sequencing[2].

A single multiplexed screening test that simultaneously detects and differentiates FMDV from look-alike disease viruses would be desirable. These "look-alike" domestic diseases produce some clinical signs that are similar to FMD: drooling, blisters, or lameness. In the absence of an FMD outbreak, these look-alike diseases may instill a sense of complacency on the part of practitioners and producers to report suspicions of FMD, as they are accustom to seeing such signs associated with domestic diseases and believe these signs are the result of common enzootic diseases. When a practitioner notifies a regulatory agency of a FAD suspect animal, samples are sent to PIADC for FAD diagnosis only, not for diagnosis of look-alike domestic diseases. Consequently, without the ability to offer a timely diagnosis for the look-alike diseases that are indigenous, practitioners and producers might disregard important disease signs or develop a disinterest in becoming involved in an FAD investigation of their animals. Without the availability of rapid diagnostic testing for FMD during an actual FMD outbreak response, dependency on clinical diagnosis will lead to the tendency to err on the side of over-diagnosis. In the 2001 United Kingdom FMD outbreak, field diagnoses based on clinical observations resulted in a large number of false positive diagnoses and unnecessary slaughter of uninfected herds (6 million animals were culled: 4.9 million sheep, 0.7 million cattle and 0.4 million pigs and 4.2 million were slaughtered), with only 2030 declared cases of FMD[3].

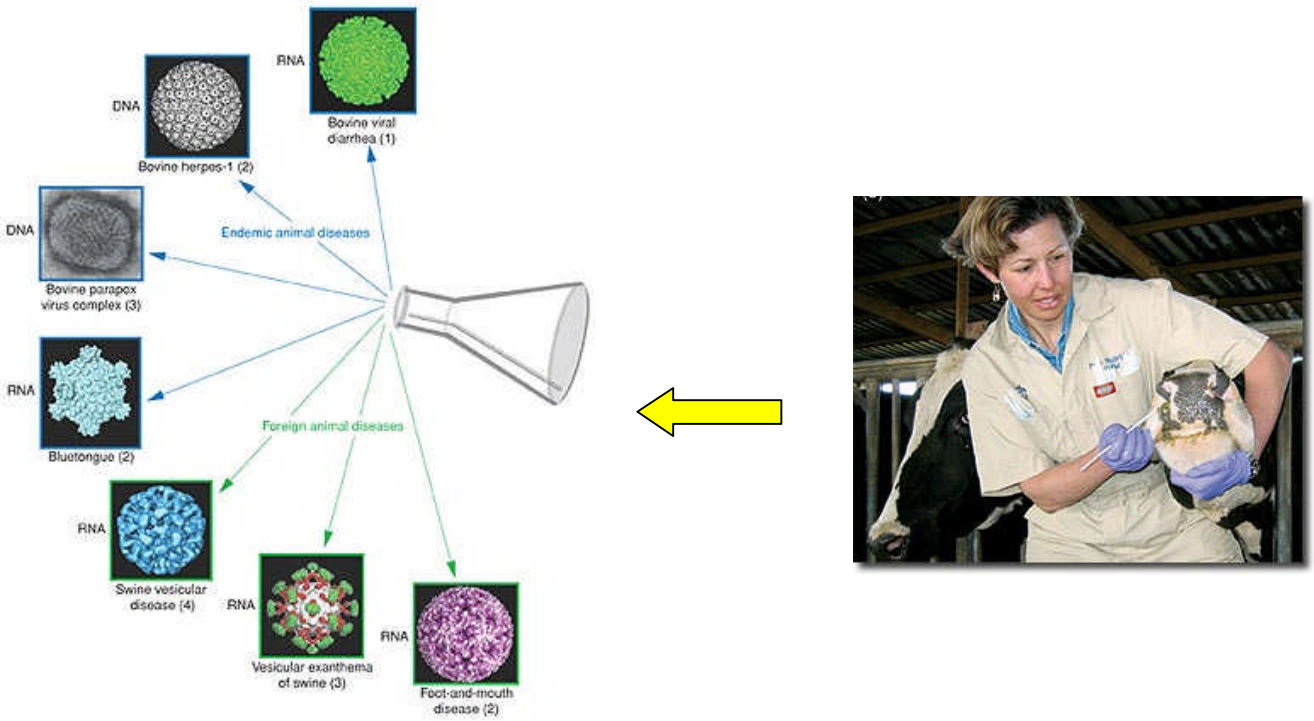


FIG. 1 Figure shows LLNL veterinarian Dr. Pam Hullinger collecting nasal swab samples from a dairy cow in Davis, CA. Samples are processed by performing an extraction procedure to release nucleic acids. The nucleic acids (Version 1 Foot-and-Mouth Disease

[2] Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, vol. Part 2, Section 2.1, Chapter 2.1.1.

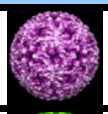
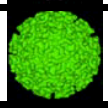
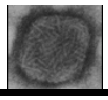
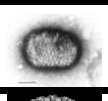
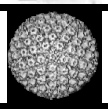
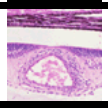
[3] The Royal Society Report - Infectious Diseases in Livestock, 2002; K Department for Environment, Food and Rural Affairs (DEFRA) (formerly the Ministry of Agriculture, Fisheries and Food (MAFF)).

rule-out panel shown) are the subjected to reverse transcriptase-PCR amplification followed by hybridization to beads for the simultaneous detection and identification of all analytes (shown above).

Researchers at Lawrence Livermore National Laboratory, in collaboration with the USDA National Animal Health Laboratory Network (NAHLN) and PIADC developed, under 2005 funding from DHS, a Version 1.0 panel for the detection of FMD and its differentiation from a number of other viruses that cause clinical signs in animals that are indistinguishable from FMD (i.e., "look-alikes"). The "Version 1.0" FMD look-alike rule out diagnostic assay panel contained 17 signatures for 7 diseases (numbers in parentheses indicate the number of signatures per disease), including FMDV (2), BHV-1 (2)), the parapox virus complex (3), BVDV (1), BTV domestic serotypes only (2), SVD (3) and VESV (4). See figure 1 above. The panel also incorporated 4 inbuilt assay controls.

Under 2006 DHS support LLNL has developed two species specific panels, one for bovine and one for porcine diseases, which detect FMD in addition to the look-alike disease rule outs for FMD in each species . The bovine panel has 23 signatures for 9 diseases (numbers in parenthesis indicate number of signatures per diseases), FMDV (2), malignant catarrhal fever (MCF/AlHV-1 (3)), rinderpest virus (RPV (3)), BHV-1 (2), Parapox (3) including BPSV and pseudocowpox PCP, BVDV (2), BTV all 24 foreign and domestic serotypes (4), vesicular stomatitus virus (VSV (4)). See figure below. The bovine and porcine panels also incorporate the same 4 inbuilt assay controls as the V1.0 panel developed in 2005. The porcine panel has 18 signatures for 5 diseases (numbers in parenthesis indicate number of signatures per diseases) FMDV (2), SVD (3), VESV (3), VSV (6), and porcine reproductive and respiratory syndrome (PRRS (5)) virus. See Tables below. This document provides a comprehensive summary of the development, testing and performance of the new bovine and porcine multiplexed assay panels. We present an overview of the identification and selection of candidate genetic signatures, the assay development process, and finally we present performance data for each of the individual signatures as they perform in the full compliment of the multiplexed bovine or porcine panel signature environment.

Table 3. Bovine multiplexed assays. * Indicates both BPSV and PCPV are detected by the assay.

Disease of Bovine	Loci	Nucleic Acid	
1. Foot-and-Mouth disease virus (FMDV)	2	ssRNA	
2. Bovine viral diarrhea (BVD)	2	ssRNA	
3. Bovine Papular Stomatitis Virus (BPSV)	3	dsDNA	
4. Parapox virus (PPOX)	3*	dsDNA	
5. Bovine herpes-1 (BHV, IBR)	2	dsDNA	
6. Malignant catarrhal fever (MCF)	3	dsDNA	

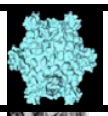
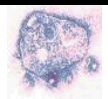
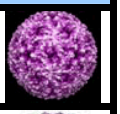
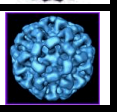
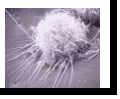
7. Blue tongue virus (BTV)	4	dsRNA	
8. Vesicular stomatitis virus (VSV	4	ssRNA	
9. Rinderpest virus (RPV)	3	ssRNA	

Table 4. Porcine multiplexed assays.

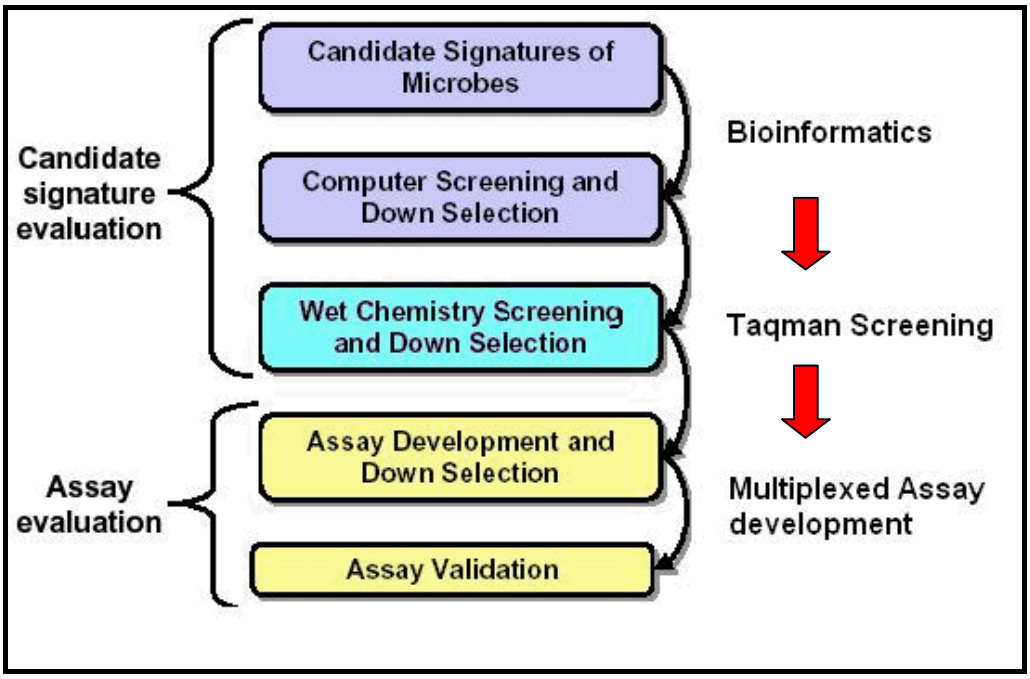
Disease of Swine	Loci	Nucleic Acid	
1. Foot-and-Mouth disease virus (FMDV)	2	ssRNA	
2. Swine vesicular disease SVD)	3	ssRNA	
3. Vesicular exanthema of swine (VESV)	3	ssRNA	
4. Vesicular stomatitis virus (VSV)	6	ssRNA	
5. Porcine reproductive and respiratory syndrome (PRRS)	5	ssRNA	

1. METHODS SUMMARY

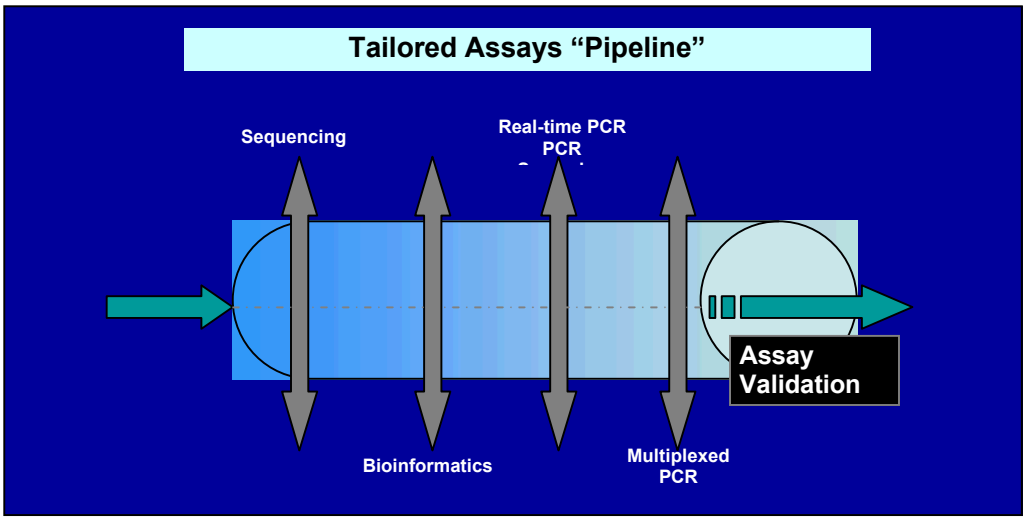
1.1. Pipeline Approach

Traditional approaches to nucleic acid signature development start with the hypothesis that a particular gene is vital to the organism's virulence, host range, or other factors that might be considered "unique". Suitable primers and probes are designed for the detection system of choice, with or without computational screening (via BLAST or equivalent) for uniqueness. The resulting assay is then tested with the available target strain(s) and considered successful if targets are detected, but the assay doesn't cross react with genetic near-neighbors. The whole-genome approach, pioneered at LLNL, constituted a major change in the strategy for assay development. Rather than focus more narrowly on specific genes, the entire genome is computationally examined to identify candidate regions that might be unique to the microorganism of interest. Knowledge about the function of genes is not required, and large numbers of candidate signatures can be rapidly generated. Furthermore, candidate signatures can be selected based on performance criteria for specific detection technologies. Primer/probe sets for the candidate signatures are then screened against strain panels, near neighbor panels and background collections using high-throughput laboratory techniques. The whole-genome approach has demonstrated the ability to generate very high quality assays in a short period (days to weeks).

The LLNL assay development team has developed a robust technical architecture (Figure 2a) for the rapid development of highest-quality nucleic acid assays, tailored to end-users specifications. The process begins with an analysis of all available genomic sequence information, which forms the basis for the development of signatures. A signature is a unique region or set of regions on the genome of a target microorganism. The entire genome is computationally examined to identify candidate regions that might be unique to the property or characteristic of interest and conserved among all available genomes for the pathogen of interest. Candidate signatures can be selected based on performance criteria for specific detection technologies such as TaqMan PCR or multiplex PCR or both. Our nucleic acid assays employ PCR with primer pairs to generate the signature fragment(s) of interest. The candidate signatures are screened *in-silico* to ensure that the primer pairs are amenable to the assay chemistry requirements. This approach provides rapid, low-cost initial screening of signatures. Primers that emerge from computational screening and down-selection are then laboratory tested against an extensive panel of nucleic acids. The wet-chemistry screening consists of a panel of 2,000 to 3,000 samples, representing a wide range of organisms and backgrounds. Each sample is tested in triplicate, comprising more than 7,500 individual challenges to each signature. The bench screening ensures that the primers will detect the strain diversity of the pathogen but will not react with the nucleic acids of other organisms that could be present in a sample. Primer pairs that successfully pass the wet chemistry screening criteria are advanced to assay development. This stage includes the optimization of extraction and detection protocols such that the assays perform consistently to required specifications on the selected instrument platform. The signatures comprising multiple real-time PCR assays targeting multiple organisms are then sent forward for multiplexed assay development. Through a series of step-wise tests, each signature is tested, evaluated, and merged into a multiplexed panel. At this phase of development, assays are re-screened (as a complete panel) against environmental backgrounds as well as target nucleic acids. Performance of each signature is characterized in both simplex and multiplex assay formats.



(a)



(b)

FIG. 2 (a) Assay selection for the generation of multiplexed PCR assays follow a proven technical architecture we refer to as the assay development "pipeline". (b) This "pipeline" describes the sequential process of genome sequencing, bioinformatics analysis, and real-time PCR screening, followed by multiplexed PCR assay development. Each step in the assay development process plays a crucial role in the development of the final product; a highly optimized, multiplexed assay panel.

1.2. Bioinformatics

1.2.1. Bioinformatics approach

The LLNL Bioinformatics team has developed "KPATH", a whole-genome comparative analysis software system that uses available DNA sequence information for a given target organism to generate candidate DNA signature sequences that are conserved and unique. Signatures are primarily used to underlie reliable and specific pathogen detection assays. Evidence of the success of the process is that several of the real-time PCR signatures that are used in the national BioWatch monitoring system were generated by this process, and to date, has resulted in no false positives, even with more than 6 million samples tested.

The general approach is the following: All available complete genomes of different strains of the target species are compared using a multiple genome alignment programs. A *consensus gestalt* is formed from the alignments that contain the sequence conserved amongst all target inputs. (This step is bypassed if only one target sequence is available). To establish that organism-conserved sequence does not occur in any other sequenced microbial genome, the consensus gestalt is compared against an immense, continuously updated database of microbial sequences. A customized algorithm accomplishes this electronic subtraction, and the result is a *uniqueness gestalt* that is mined for potential signature candidates. A final computational screening is done to verify that cross-reactions are not detected.

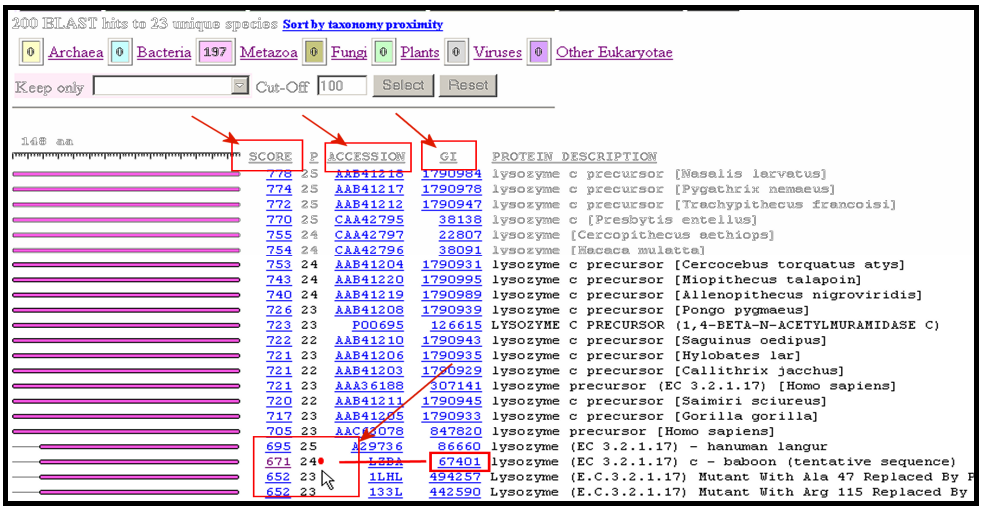


FIG. 3 The screen shot above shows the computational platform by which signature candidates are generated. This is referred to as a signature "BLAST" that identifies homology between like signatures from a database that includes Genbank repositories as well as sequenced genomes that are not publicly available.

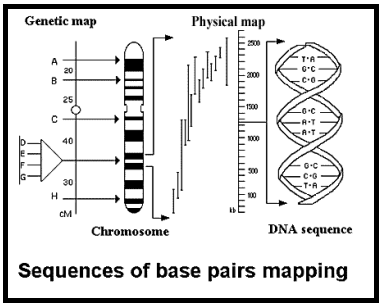


FIG. 4 Diagram showing the mapping of genetic target regions used in signature generation.

KPATH allows the genome to define potential signature candidates. However, rather than selecting candidate signatures randomly (often there are more candidates than is economically feasible to screen in the wet lab), they can be prioritized based on annotation. Annotation allows signatures to be scrutinized in a biological context. Identifying genes responsible for rendering a pathogen virulent is one component of a good diagnostic signature set. Candidate sets are manually selected, associated with genes of interest, and include a random selection of candidates within intergenic regions, for wet lab screening. The random unique intergenic regions are selected as a guard against gene deletion or substitution engineering to evade DNA-based detection. We note that there are few tools focused on viral gene finding, and none known to us that can adequately predict genes in certain RNA virus families.

Because signature candidates are generated using exact matches in the *Vmatch* step described above, additional electronic screening on the signature candidates is performed to catch potential non-exact matches that might result in false positives in the wet lab. We have seen cases where this would predict cross-reactions with near-neighbor species that had not been caught by the exact-matching step (due to as few as 1 or 2 fortuitously-placed mis-matched bases.)

1.2.2. Signature generation input parameters

We used a computational TaqMan simulator program, "TaqSim", to identify all potential targets for each signature from all sequences available in Genbank. TaqSim is a BLAST-based comparison of each signature as

a triplet against all sequences in Genbank to identify the targets that are predicted to produce a TaqMan reaction at 57 °C primer annealing and 67 °C for probe annealing (these temperatures are according to Primer 3 oligo T_m calculations).

TABLE 5. Universal settings used for signature generation. Input parameters allow for standardized signature informatics that allows for universal protocol development and assay compatibility.

Primer3 Parameters ***Note: All sequences are listed in the 5'→ 3' direction.**	
Parameters	**Standard Settings**
PRIMER_OPT_SIZE	**20**
PRIMER_MIN_SIZE	**18**
PRIMER_MAX_SIZE	**27**
PRIMER_PRODUCT_OPT_SIZE	**100**
PRIMER_PRODUCT_SIZE_RANGE	**71-600**
PRIMER_OPT_TM	**62**
PRIMER_MIN_TM	**61**
PRIMER_MAX_TM	**63**
PRIMER_MIN_GC	**20**
PRIMER_MAX_GC	**80**
PRIMER_PICK_INTERNAL_OLIGO	**1**
PRIMER_INTERNAL_OLIGO_OPT_SIZE	**31**
PRIMER_INTERNAL_OLIGO_MIN_SIZE	**18**
PRIMER_INTERNAL_OLIGO_MAX_SIZE	**36**
PRIMER_INTERNAL_OLIGO_OPT_TM	**72**
PRIMER_INTERNAL_OLIGO_MIN_TM	**71**
PRIMER_INTERNAL_OLIGO_MAX_TM	**73**

1.3. Real-time PCR™

Real-time PCR laboratory screening technical approach

To ensure extremely high selectivity and sensitivity, we have established a screening protocol that rigorously screens candidate DNA signatures to select the optimal sets for eventual field use. Candidate signature primer pairs that survive computational screening proceed to wet chemistry screening utilizing standard PCR with agarose gel electrophoresis. This step ensures that the primers will react across strains representative of the diversity of the pathogen (avoid false negative), but will not react with the nucleic acids of other organisms that could be present in a sample (avoid false positives). The resulting signatures are carried forward into Real-time screening where remaining signatures are re-screened against additional background confounders as well as target and near-neighbor isolates. **Constituents of the wet-screening panels can be found in Appendix II. Target and Near-neighbors screened are detailed in Appendix III.** The final signatures are then further screened to determine relative limit of detection for each candidate signature. During this process of down-selection, final signatures are screened against over 2,400 potential confounders (described in detail below).

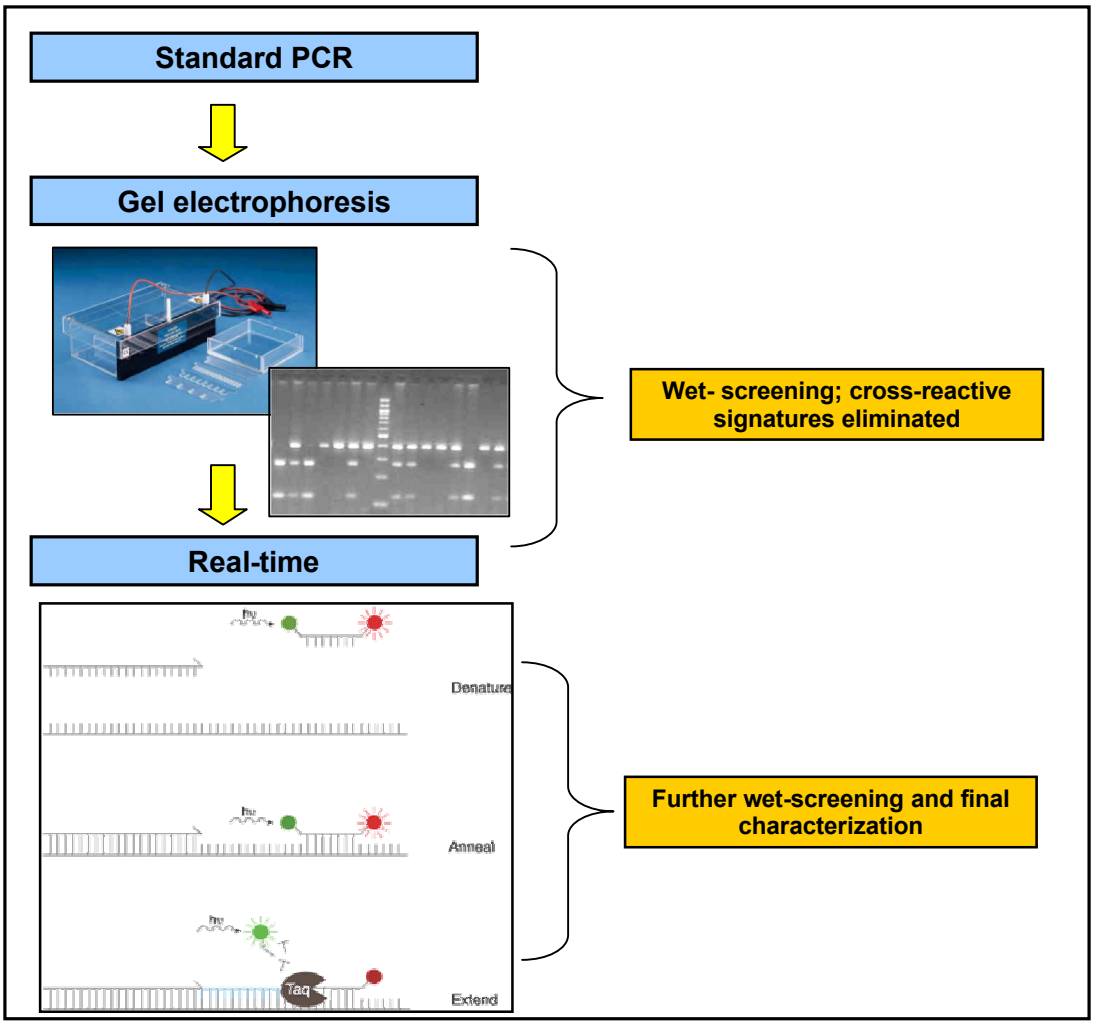


FIG. 5 Illustration depicts the work-flow of wet-screening for signature down-selection. Standard PCR and gel electrophoresis followed by real-time PCR amplification in the presence of potential cross-reactors eliminates non-specific signatures. Real-time PCR on final signatures for further wet-screening and complete characterization of signature be defining relative limits of detection for each.

1.4. Multiplexed PCR

1.4.1. Multiplex Development Overview

The Version 1.0 panel for FMD look-a-like rule out contained 17 signatures for 7 diseases (shown in figure 1). As described previously, 2006 under DHS support it was determined to take these signatures from the first generation panel and combine them with additional signatures to produce two species specific panels (one for bovine FMDV rule out and one for porcine FMDV rule out). While the first generation panel was a 17 plex for detection of 7 mixed bovine, porcine and small ruminant diseases, the bovine panel thus has 23 signatures for 9 bovine diseases (see Table 3). The bovine panel consists of the FMDV, BHV-1, and Parapox (BPSV, Pseudocowpox (PCP)) signatures from the first generation panel combined with one additional plus the original signature for BVDV(2), and one first generation signature for BTV combined with three additional signatures for BTV(4). The bovine panel also has an expanded capability to detect additional bovine viruses vesicular stomatitis virus (VSV (4)), rinderpest virus (RPV(3)), and one cause of malignant catarrhal fever (AlHV-1 (3)) not found in the first

generation panel. The porcine panel consists of the FMDV, SVD and VESV signatures from the first generation panel combined with an expanded capability to detect VSV (6). In order to enable the porcine panel to be used in routine surveillance for prevalent porcine disease, we have added signatures for the detection of porcine respiratory and reproductive syndrome virus (PRRS (5)) to the porcine multiplex. The porcine multiplex panel thus has 19 signatures for the detection of five diseases. Additionally, all panels have been constructed with four in-built assay controls.

1.4.2. Multiplex bead-based assays technical approach

The multiplexed assays (liquid arrays) were developed on a Luminex™ flow cytometer. The liquid arrays (Figure 6a) utilize surface-functionalized polystyrene microbeads, embedded with unique ratios of two fluorophores (red and infrared). When excited by a 635-nm laser, the two internal fluorophores emit light at different wavelengths, 658 and 712 nm, yielding an array of 100 beads, each with a unique spectral address. Because the microbead classes can be distinguished, they can be combined and up to 100 different analytes can be measured simultaneously within the same sample. The versatility of the liquid arrays has been well-demonstrated for detection of antigen, antibodies, and peptides. For nucleic acid-based detection, oligonucleotide probes with sequences that are complimentary to the target nucleic acid sequences are covalently coupled to beads (Figure 6b). Nucleic acids from pathogens (targets) are amplified using standard PCR techniques (Figure 7). After target amplification, the amplicons, half of which contain the biotinylated forward (5'-3') primer are introduced to the beads and allowed to hybridize to their complimentary probes on the appropriate bead. A fluorescent reporter molecule (strepavidin-phycoerythrin) is added, and binds the biotin functional groups within the forward primers. The completed assay product comprises a bead + probe + biotinylated (and fluorescently tagged) amplicon (Figure 6c). Each optically encoded and fluorescently-labeled microbead is then analyzed by the flow cytometer. The 635-nm red laser excites the dyes inside the bead and classifies each bead to its unique bead class, and a green laser (532 nm) quantifies the assay at the bead surface. The flow cytometer is capable of reading several hundred beads each second; analysis can be completed in as little as 15 seconds. Conducting the assay requires multiple steps and significant thermocycling times and the process currently takes approximately 4-5 hours to complete a 96-well plate of samples.

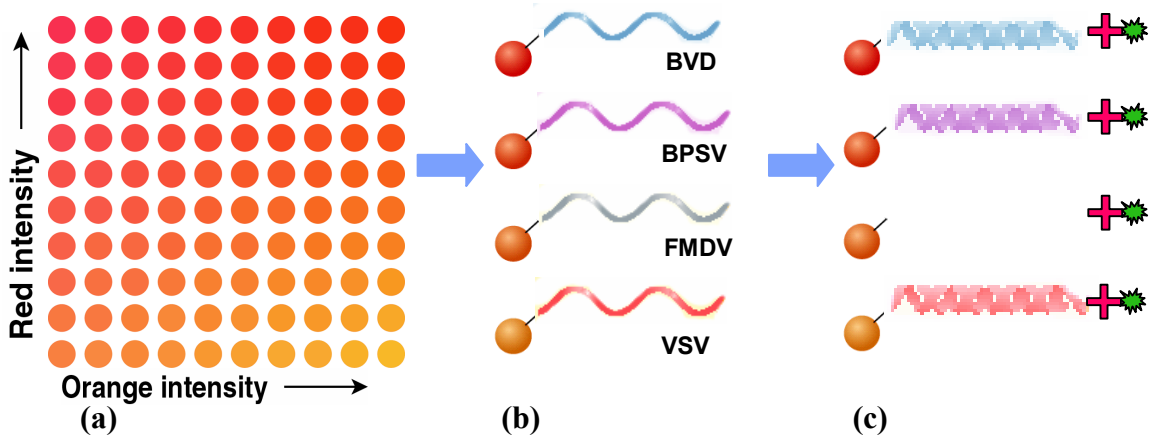


FIG. 6 (a) Liquid array of 100 beads, each with unique spectral address. (b) Capture beads with covalently-coupled oligonucleotide probes complimentary to target DNA. (c) Amplified nucleic acid (PCR product) is hybridized to beads, fluorescently labeled, and analyzed.

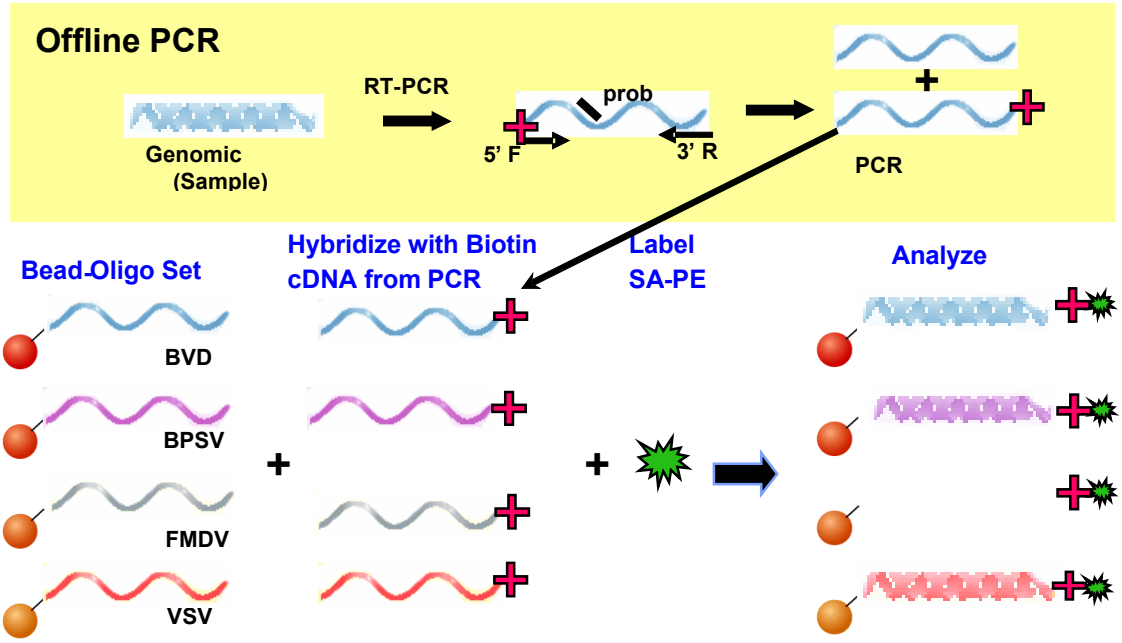


FIG. 7 Individual primer pairs (biotinylated forward and standard reverse) that bracket the target genomic sequence are included in an automated PCR master mix of buffers, Taq polymerase, dntps, etc. After target amplification by PCR, the amplicons are mixed with beads where target amplicons containing the forward biotinylated primer hybridize to the complimentary probe on the appropriate beads. A fluorescent reporter molecule (strepavidin-phycoerythrin) then binds biotin functional groups. Therefore, the completed assay comprises a bead + probe + biotinylated (and fluorescently tagged) amplicon. The sample is then analyzed using the Luminex detector.

1.4.3. Conversion of a Real-time PCR Signature to a Luminex-based Multiplexed PCR Signature

Due to the nature of the assays, inherent chemistry differences exist between Real-time and Luminex PCR. Real-time oligo-modification chemistry (primers and probe) are standardized for every signature set. For Luminex assays the design is sequence specific and contingent upon other factors such as oligonucleotide length. This is generally only affected by the forward primer; the reverse primer, and probe have standardized modifications. The internal biotins placement in the forward primers depends on the location and quantity of the thiamines in the sequence. For the first generation multiplex assay each forward primer had a 5 prime biotin attached and 2 internal biotins, unless with a desired 10-base separation, it is only possible for 1 internal biotin to be placed (see **TABLE 6** below).

TABLE 6. Conversion of a Real-time PCR assay to a Luminex binding-assay is accomplished by the modification of one of the primers and the probe. The modifications are described below.

Oligonucleotide	Modification
Forward Primer:	5' biotin label and 1-2 internal biotins Example: Real-time PCR primer 5'-AATCGGATCAGATCCA-3" becomes 5'-/5Bio/AA/iBiodT/CGGA/iBiodT/CAGATCCA-3' for Luminex assay.
Reverse Primer:	unmodified sequence
Probe:	5'amine and a spacer 18 modification Example: If the real-time PCR probe sequence is 5' FAM-ATCCGCGCATAG-TAM3', the Luminex binding sequence becomes 5'/5AmMC12//iSp18//ATCCGCGCATAG-3'.

In 2006 we found no functional difference between the performance of forward primers labeled with a single or multiple biotins. All forward primers for those look-alike diseases added in 2006 to the bovine and porcine panels (BTV, PCP, MCF, RPV, VSV and PRRS) have primers labeled with only a single biotin. This has resulted in lower cost of the primers used in the assay and more reproducible and rapid forward primer synthesis.

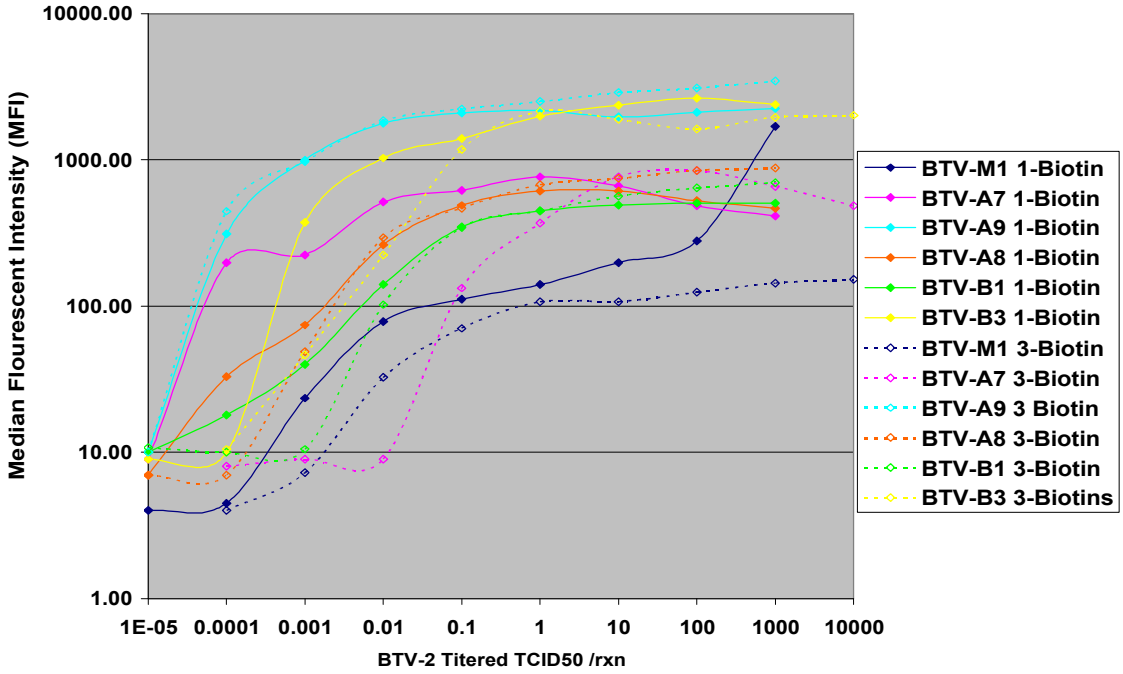


FIG 8. Comparison of BTV assays' forward primers labeled with either 1 or multiple (3) biotins. Six different assays were compared to determine the effects of having one or multiple biotins used for fluorescent molecule detection. Results suggest that all assays either showed no significant change or showed slight enhancement with the reduced number of biotin molecules present.

In addition to differences in oligo modification, there is also a functional difference between probes. In Real-time assays there is no difference between the reverse and forward orientation of the probe relevant to the success of the amplification. For Luminex assays the orientation of the probe can also be either orientation (a result of complex hybridization networks); however there may be instances when one orientation works preferably to another. For the purposes of this testing, all probes were optimized in the FCP orientation for multiplexed assay development.

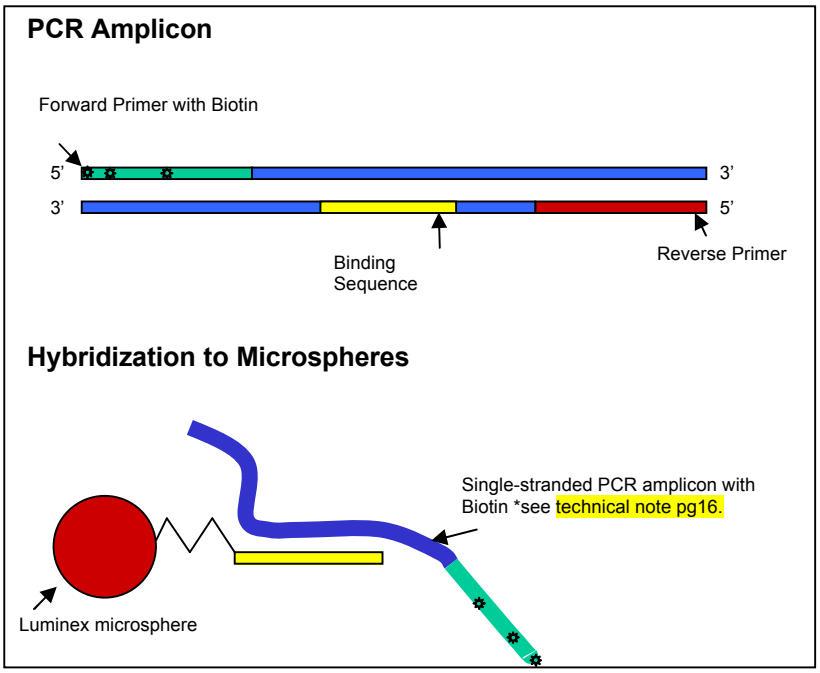


FIG. 9 The forward primer is modified with a 5 prime biotin and 2 additional internal biotins for a total of 3 biotins. During PCR amplification the biotins are integrated into the amplified PCR product. These are the amplicons that are complimentary to the probe sequence. During the hybridization process, the probe binds complimentary amplicon sequence that is labeled with biotins.

1.4.4. Multiplexed assay development

Signatures that pass gel and real-time PCR screening are candidates for multiplexed assays. If the number of candidate signatures is large, then a subset of the available signatures are selected based on measured sensitivity and specificity observed in real-time screening. In preliminary screening the background of individual assays is measured by running "blank" (no sample added) controls and observing assay response. In some cases a primer-to-primer non-specific interference will eliminate candidate signatures. Assays are then added iteratively to each multiplexed panel, titrated, and collectively, all assays are re-titrated to determine if the presence of one assay will interfere with the performance of another. During this process assays are added or subtracted from the multiplexed panel depending on individual outcomes until all desirable multiplex compatible assays have been added.

1.4.5. Multiplexed PCR optimization

Once all of the signature components of the multiplexed assay are determined, all of the different assay variables can then be systematically tested. Examples of these variables is presented below.

Assay optimization (examples):

- PCR: $MgSO_4$ concentration optimization of PCR reaction
- PCR: Optimization of primer concentration. Assays with poor reactivity may be enhanced with a proportionately larger amount of primer.
- PCR: Optimization of thermal cycling conditions (i.e. annealing temperature, time, etc) for PCR.
- PCR: Effect of increasing enzyme content in RT-PCR kit formulation; (2 x enzymes) for enhancing PCR.

- PCR: reagent quality optimization. Evaluation of lot-to-lot differences in materials and standardization of quality requirements for synthesized oligonucleotides to reduce the effect of bi-product interference.
- Luminex Assay: Amount of PCR product added to **hybridization** reaction (in 2006 work changes from adding 1uL PCR product to adding 5 uL to increase reproducability of the assays).

Signatures generated external to LLNL's process present additional optimization challenges. External signatures must be integrated into the multiplexed assay, but such signatures may have very different melt temperatures, relative GC content, amplicon size, or may exhibit significant secondary structure, etc. The challenge of multiplexing is developing a universally robust set of reaction conditions that prevents or reduces the effects of competition or interference that might result from having so many primers in one reaction vessel. This exemplifies why the LLNL bioinformatics process of unique signature generation is key to the successful formulating of a multiplexed assay panel.

1.4.6. Multiplexed PCR assay controls

Controls that convey important diagnostic information regarding assay integrity, including reagent addition, quality and concentration, assay operator performance and instrument stability are incorporated without compromising or limiting the screening capabilities of the assay. Our assays employ a unique set of four rationally-designed controls built into every sample that monitor and report on certain key steps of the assay. Every sample is analyzed in the context of the performance of the controls, thereby increasing confidence in the assay results.

1.4.6.1. Negative contro*l (NC)*

A Luminex bead conjugated to a Maritima (MT-7) oligonucleotide sequence serves as the negative control (NC). MT-7 is a conserved DNA sequence from a maritima organism (a thermal vent microbe) that does not match those of published genomes of terrestrial organisms, and serves as a non-specific binding control in the multiplex PCR assay. In the absence of non-specific binding, the MFI values for the NC MT-7 bead should remain consistently low.

1.4.6.2. Fluorescence control (FC)

A Luminex bead conjugated to a biotinylated MT-7 (b-MT7) oligonucleotide sequence serves as the fluorescence control (FC). The biotin moiety has a high affinity for SA-PE and confirms that the fluorescent labeling step of the assay was performed correctly. MFI values of the b-MT7 bead should consistently high.

1.4.6.3. Instrument control (IC)

A Luminex bead conjugated to a Cy3 labeled MT-7 (MT7/Cy3) oligonucleotide sequence serves as the instrument control (IC). Both Cy3 and SA-PE have similar fluorescence characteristics in terms of excitation and emission wavelengths. The MT7/Cy3 bead is used to confirm that the reporter optics within the Bio-Plex are functioning correctly. Large fluctuations in the MT7/Cy3 may indicate optics failure.

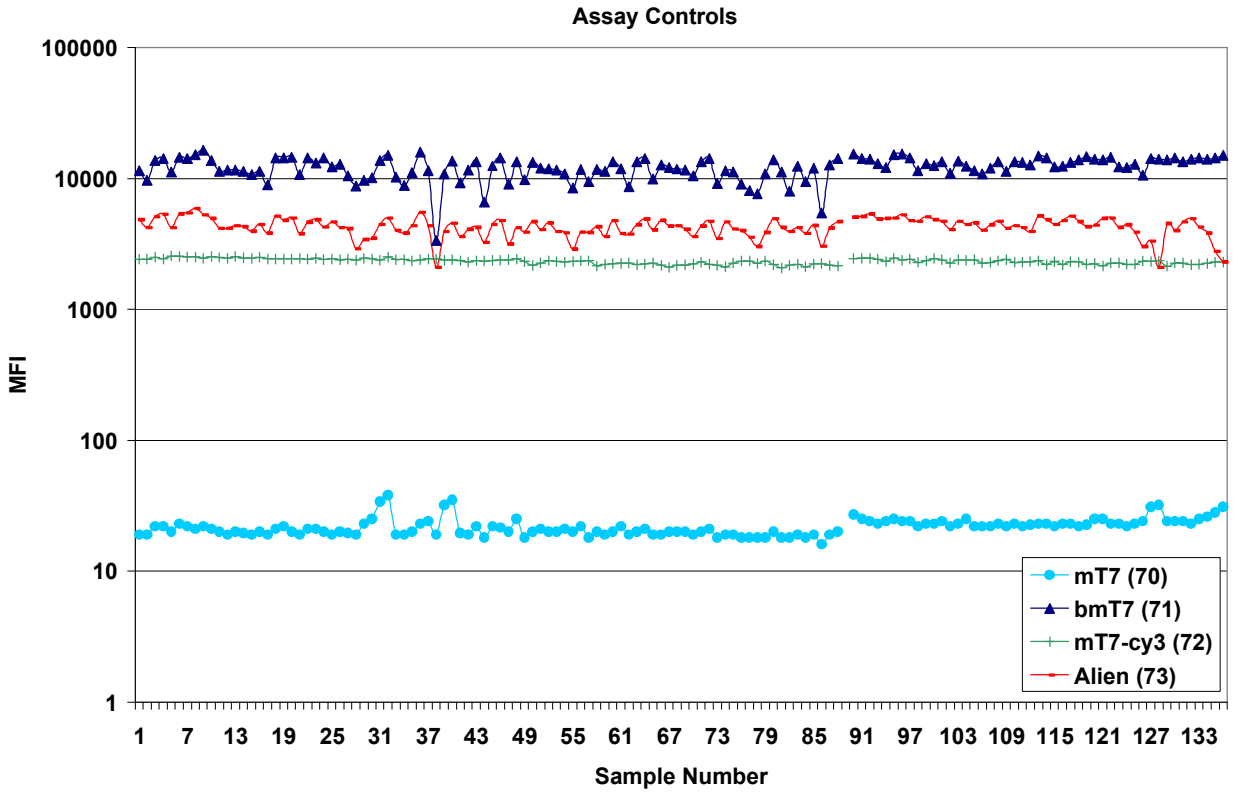


FIG. 10 Example "normal" control data from Luminex multiplexed PCR assay across a microtiter plate (96-wells, 2 plates). The log of the median fluorescence intensity (MFI) is plotted on the Y-axis versus sample number shown on the X-axis. Each sample contains 4 internal controls. Controls should produce data that is constant form one sample to the next; therefore data in plots like this one should exhibit 4 straight lines. Fluctuations in MFI values for any of the 4 controls may indicate a problem with the assay. Additionally, each control is characterized by its inherent variation. Some controls produce data that is much less variable than others. The high deviation from point to point shown on this plot from the positive control Armored RNA is expected and normal.

1.4.6.4. Armored RNA (Internal Positive Control)

Armored RNA (arRNA) is a synthetic RNA sequence, ~1000 nucleotides in length, packaged in an MS2 phage (protein capsid). The sequence has no homology to currently annotated GenBank sequences. Packaging increases the stability of the RNA in clinical sample matrices and more closely mimics the behavior of target virus particles during processing. An internal control signature for the arRNA was incorporated into the multiplex PCR assay using specific primers and probe. ArRNA is used as an end-to-end internal control for virus lysis, nucleic acid purification, reverse transcription, PCR amplification, Luminex microsphere array hybridization and Bio-Plex detection.

The arRNA concentration used was 1000 copies per reaction (see FIG 11 below), which consistently yielded an MFI above the assay detection limit. A low number copy number for the internal control was selected to minimize competition within the PCR reaction with the agent signatures. Low copy number can also better reflect detrimental changes in assay performance that could potentially result in a false negative. MFI values below threshold may indicate incomplete nucleic acid purification (e.g. inhibitors or the PCR reaction were not completely removed during the extraction), failed reverse transcription and PCR amplification, or a failed hybridization reaction.

Armored RNA was prepared as per manufacturer's recommendation. Concentrated stock is stored in a Tris-based buffer, TSM: [10 mM Tris (pH7.0), 100 mM NaCl and 1 mM $MgCl_2$] TSM buffer provided by the vendor. A sub-stock is prepared in TSM buffer and aliquotted for daily use. Armored RNA dilutions were prepared fresh prior to use by diluting in PCR grade water. Prior to adding to the PCR reaction, the armored RNA is heat lysed for 3 min at 75°C.

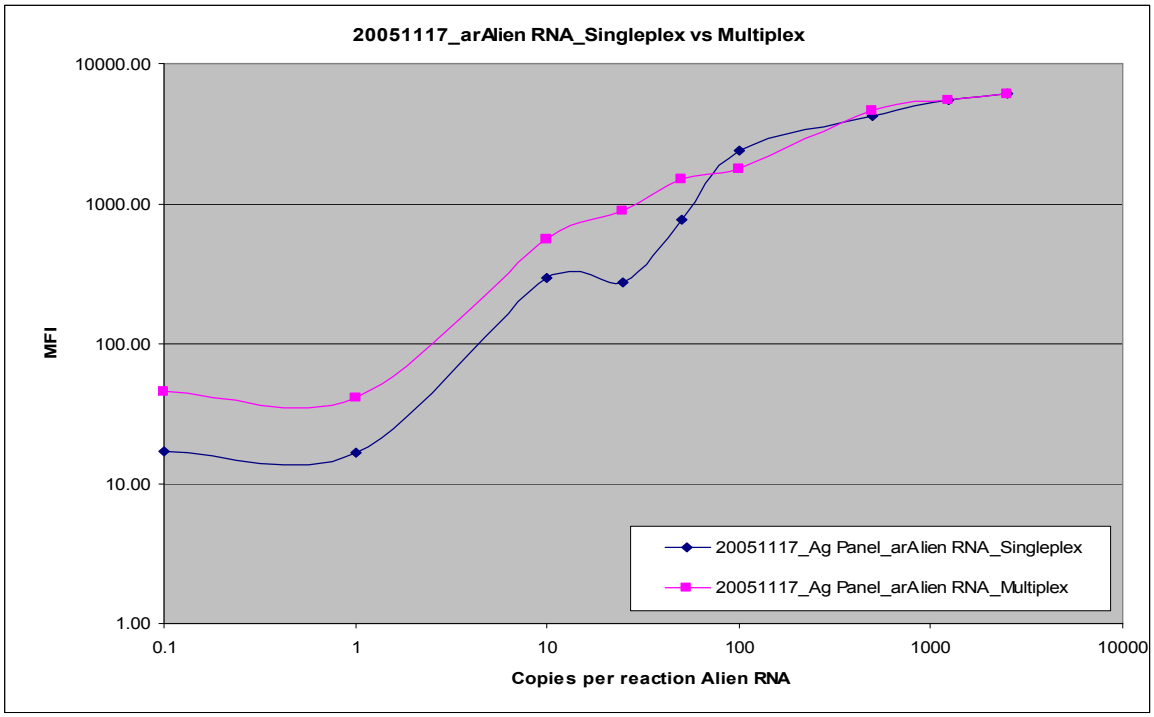


FIG. 11 Singleplex versus multiplex assay characterization of arRNA internal control assay. A dose-response curve plotted by the log of median fluorescence intensity units (MFI) versus the log of concentration in copies of RNA per reaction with 10-fold serial dilutions spanning 8-logs defines assay limit of detection as between 1-10 copies when nucleic acid material is added directly to the PCR reaction. For use as a positive control, each sample is spiked with the equivalent of 100 copies of arRNA per reaction. This shows that there is a negligible difference between the singleplex and multiplexed (when assay is added to Version 1.0 17-plex panel) assay and establishes which concentration is optimal for incorporation into the multiplexed reaction.

1.4.7. Establishment of Thresholds

For each individual assay, in the first generation multiplex assay, developed under 2005 DHS funding, adjustable thresholds were established by testing and evaluating many thousands of samples, both positive and negative in appropriate sample matrices, and then selecting values that provide a specific probability of false positives and false negatives. For the bovine and porcine multiplex assays, developed under 2006 support, these thresholds remain to be set by the AgDDAP project once the final panel's composition is determined. A specific form of a Receiver Operating Characteristic (ROC) curve is used to establish thresholds. Using all negative sample data, a ROC function describing the relationship between threshold values and the rate at which false positives occur was plotted. The threshold is determined from the ROC function by finding the MFI values that would yield a false positive at a given rate. The resolution in false positive rate is determined by the number of samples that

are present. For example to establish a false positive rate of 0.0001, or 1 false positive per 10,000 samples, a minimum of 10,000 samples are required.

TABLE 7: Threshold values used for the assay controls and each signature in the multiplex assay Version 1.0 panel.

Signature	Threshold (MFI)
Fluorescent Control (b-MT7)	>10000
Instrument Control (MT7/Cy3)	>1000
Negative Control (MT7)	<80
Positive Control (Synthetic RNA)	>20

1.4.8. Multiplexed Assay integrity

Assay integrity was verified in the context of inbuilt controls. Assay control results determined whether the results for a given sample were valid. Assay integrity was determined using the following processes. The assay included 4 inbuilt controls (described above). For each sample, the MFI values for the 4 control beads classes were checked against a corresponding threshold. The thresholds used for the Version 1.0 panel are shown in Table 7 If an MFI value for the IC, NC or FC controls were out of range then the results from that sample was deemed invalid and excluded from further analysis.

If the MFI value of the arRNA control was out of range *AND* none of the MFI values for the other 17 agent channels exceeded threshold, then the results from that sample were deemed invalid and excluded from further analysis. If the MFI for the arRNA control was out of range *AND* one or more of the MFI values for the agent channels exceeded threshold, then the results from that sample was deemed valid and included in further analysis. We have observed that agent spikes above certain concentrations can cause a decrease in the arRNA MFI, probably due to competition in the PCR reaction. When the arRNA MFI drops below threshold on a sample considered negative for all signatures, the analysis would be discarded and would need to be repeated. This control reduces the probability of false negatives.

If the MFI values for all four controls were within range, then the bead counts were checked. First, the bead counts for each of the 4 controls were checked. If the bead count minimum (40 beads) for any of the 4 controls was not reached, then the control MFI values were deemed invalid, and all assay results for that sample were excluded from further analysis. It was determined from experimental data acquired at LLNL that a bead count less than 40 generates unreliable results.

The final step was to check the individual bead count for each of the signatures for a given sample (non-control beads) in a particular multiplex panel. If an individual agent bead class (signature) did not reach the bead count minimum (40 beads), that individual assay result was deemed invalid and only that individual result for that signature was removed from the analysis. If the bead counts for any of the agent signatures exceeded the minimum, they were considered valid and included in the analysis.

The flow cytometer counts the number of beads of each type, counting until at least 100 beads of each class are counted. The fluorescent intensity of each bead is also recorded. Each bead class has a distribution of fluorescence values associated with it, summarized by its median value. The data output is provided as median fluorescent intensity (MFI). Validity of the data are determined first by evaluating the MFI values of the internal assay controls, which must fall within pre-determined specifications. Controls outside these values are out of specification and the assay result is invalid. If the data are valid by this test, results of the individual analytes are then analyzed. To interpret the MFI values for each bead class as either positive or negative, MFI values from each bead class in the assay are compared to pre-determined threshold values. For each individual assay, adjustable thresholds are established by testing and evaluating many thousands of samples, both positive and negative in appropriate sample matrices, and then selecting values that provide a specific probability of false positives and false negatives. During the course of an assay, if a bead reports an MFI signal above its

associated threshold value, that assay would be ruled positive, otherwise the assay is ruled negative. Finally, positive assay results are analyzed using a detection algorithm.

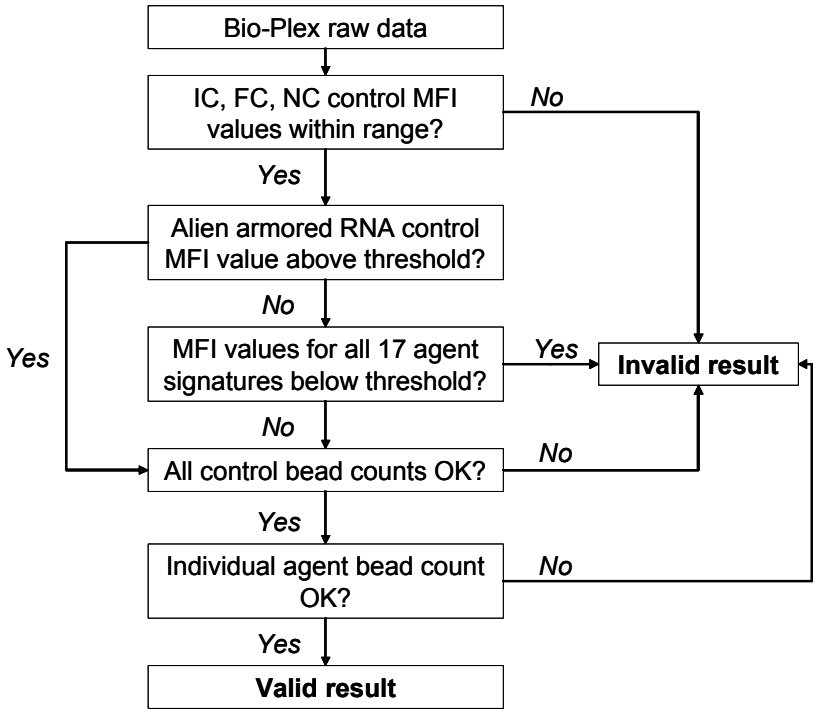


FIG 12 Flow chart showing the decision points used to process Bio-Plex data to determine integrity of an assay result based on the controls data.

The testing of numerous clinical positives and negatives in the AgDDAP testing in Dec FY05 allowed for the development of thresholds, this work remains to be completed for the bovine and porcine panels. Preliminary data has been collected, but not in significant enough volume to be used in the generation of ROC curves (requiring 500+ samples tested from each panel it the final configuration).

1.4.9. Additional criteria for down-selection of multiplexed assays

- **Specificity.** Analytical specificity is defined as the ability of an assay to distinguish the target agent from other infectious agents, determined by analyzing similar pathogens. Diagnostic specificity is the proportion of virus-free samples that are tested negative by the assay. For our purposes, specificity is defined as a measure of an assay's ability to correctly characterize truly negative samples at relevant concentrations in relevant sample matrixes.
- **Sensitivity.** A measure of the ability of the assay to identify true positive samples. When sensitivity is discussed in the context of assay performance, the terms analytical sensitivity or diagnostic sensitivity are typically used. Analytical sensitivity (often called limit of detection) is defined as the smallest amount of an analyte detected by the assay. Diagnostic sensitivity is defined as the proportion of known infected samples (usually tested against a gold standard reference) that tested positive in an assay.
- **Optimal signal to noise ratio.** In the multiplex assay system the potential for cross reactivity is larger than in single reaction testing, and this may cause unwanted PCR reaction products or non-specific cross-reactivity. Assays are preferred to have a high signal to noise ratio, where

desired signal is significantly greater than the background noise of the assays when there is no target nucleic acid in the reaction.

- **Compatibility with other signatures in multiplex.** In some cases a primer sequence may interfere with other primers or probe sequences in the multiplex and there by cause a decrease in sensitivity for one or more assay in the multiplex. For each assay it must be evaluated whether the shift in detection level is significant enough to remove, what could be a very desirable signature, from the panel.

2. Version 1 Panel: Foot-and-Mouth Disease Rule-Out (Historic Data Overview)

This section of the report summarizes work that was completed on the Version 1.0 FMDV rule-out panel as reported in FY06. It was deemed necessary to include summary information from previous work to provide a relevant correlation to the bovine and porcine panel development which includes assays carried over from the Version 1.0 panel. This summary is limited to a brief overview of bioinformatics efforts, real-time PCR screening and final multiplexed PCR summary data.

2.1. Bioinformatics Summary

Signatures were developed de novo using multiple genome alignments targeting organism-conserved sequence for parapox virus (BPSV, Orf, pseudowcowpox), bovine herpes virus, bluetongue virus, swine vesicular disease virus, and vesicular exanthema of swine virus; signatures for FMDV and BVD were provided from external sources and described in Table 8 below.

TABLE 8. Summary of signatures generated and down-selected for the Version 1 assays.

Agent	Initial # of candidate signatures	# of signatures forwarded to Real-time PCR	# of signatures released to multiplex	# of signatures in frozen panel
PARAPOX	**8**	**7**	**4**	**3**
BHV	**177**	**101**	**4**	**2**
BVD[1]	**1**	**1**	**1**	**1**
FMDV[2]	**4**	**4**	**4**	**2**
BTV	**8**	**8**	**4**	**2**
SVD	**4**	**4**	**4**	**3**
VESV	**44**	**20**	**6**	**4**

[1]BVD signature was externally developed (CAHFS, UC Davis)
[2]One of the two FMDV signatures were externally developed (Tetracore[4], Pirbright Laboratories[5])

[4] **Callahan, J. D., F. Brown, F. A. Csorio, J. H. Sur, E. Kramer, G. W. Long, J. Lubroth, S. J. Ellis, K. S. Shoulars, K. L. Gaffney, D. L. Rock, and W. M. Nelson.** 2002. Use of a portable real-time reverse transcriptase-polymerase chain reaction assay for rapid detection of foot-and-mouth disease virus. Journal of the American Veterinary Medical Association **220:**1636-1642.

[5] **Shaw, A. E., S. M. Reid, D. P. King, G. H. Hutchings, and N. R. Ferris.** 2004. Enhanced laboratory diagnosis of foot and mouth disease by real-time polymerase chain reaction. Revue Scientifique Et Technique-Office International Des Epizooties **23:**1003-1009.

2.2. Real-time PCR Summary

2.2.1. Target / near neighbor screening

Candidate signature primer pairs that survived computational screening were forwarded to wet chemistry screening utilizing standard PCR with agarose gel electrophoresis followed by down-selection and additional screening in real-time PCR format. In some cases (external signatures, such as FMDV and BVD) were not subjected to the same level of gel and real-time PCR screening as LLNL-developed signatures. These signatures have been extensively screened externally in real-time format. The signature screening effort was highly collaborative requiring a high level of off-site coordination with external laboratories. For Version 1.0 panel development most of the FAD screening (FMDV, SVD, and VESV) was conducted at PIADC, New York by LLNL staff. Table 8 above summarizes how many signatures survived the gel and real-time PCR screening and the below table summarizes the extent of target and near neighbor screening performed in gel and real-time PCR format, however, detailed gel and real-time data is not included herein but can be a summary can be found in Section 4 of this report under each agent summary and also in the Supplemental Materials Document.

TABLE 9. Summary of target and near-neighbor viruses used in Version 1.0 panel gel and real-time PCR screening. A complete listing of these agents can be found in Appendix III.

Agent	Gel PCR Screening Total # of Virus isolates screened	Real-time PCR Screening Total # of Virus isolates screened
BHV Targets	5	10
BHV Near-Neighbors	5	19
Parapox Targets	0	37
Parapox Near-Neighbors	7	7
BTV Targets	5	5
BTV Near-Neighbors	2	0
BVD Targets	1	1
BVD Near-Neighbors	0	0
FMDV Targets[2]	0	0
FMDV Near-Neighbors[1]	0	0
SVDV Targets[2]	0	7
SVDV Near-Neighbors	0	11
VESV Targets[1]	12	11
VESV Near-Neighbors	0	0

[1]Testing completed at Plum Island Animal Disease Center (PIADC), New York. [2]Screening was conducted at both PIADC and the NCFAD, Winnipeg, CAN.

2.3. Multiplexed PCR Version 1.0 Panel

The first generation FMD look-a-like rule out diagnostic assay panel contains 17 signatures for 7 diseases (numbers in parentheses indicate the number of signatures per disease), including FMDV (2), Bovine Herpes Virus-1 (BHV-1 (2)), Bovine Papular Stomatitis Virus (BPSV (3)), Bovine Viral Diarrhea Virus (1), Bluetongue virus (BTV (domestic, 2)), Swine Vesicular Disease Virus (3) and Vesicular Exanthema of Swine Virus (4). The panel also incorporated 4 inbuilt assay controls. See Table 10 below.

2.3.1. Panel Configuration

TABLE 10. Frozen panel layout for the Version 1.0 multiplexed Foot-and-Mouth disease "rule-out" assay.

Bead #	MUX Internal ID	Source	Forward Primer (5' -3')	Reverse Primer (5' -3')	Luminex Probe (5' -3')	Probe strand[1]	Amplicon length
117	BVH-1	LLNL	GTGCCAGCCGCGTAAAAG	GACGACTCCGGGCTCTTTT	TCCTGGTTCCAGAGCGCTAACATGGAG	FCP	140bp
118	BVH-3	LLNL	TGAGGCCTATGTATGGGCAGTT	GCGCGCCAAACATAAGTAAA	AAATAACACGGTGTGCACTTAAATAAGATTCGCG	FCP	114bp
119	PPOX-1	LLNL	GCAGATGCGCTCCTGGTT	GCACCTCTGCTGCTGCAA	CCGACTCCGACGTGGAGAACGTG	FCP	178bp
120	PPOX-2	LLNL	GATGGCCGTGCAGCTCTT	CGTACAAGATCACGGCCAACT	TGTACGGGCTCATGGGCTTCCG	FCP	95bp
122	PPOX-4	LLNL	GCAGCAGTGCACCACGTAGT	CGCTGAACCCGTACATCCT	GACTTCGAGGCGGACAACAAGCG	FCP	167bp
132	FMDV. TC	Tetracore	ACTGGGTTTTACAAACCTGTGA	GCGAGTCCTGCCACGGA	GTCCCACGGCGTGCAAAGGA	FCP	107bp
133	FMDV.Pir	Pirbright	CACYTYAAGRTGACAYTGRTACTGGTAC	CAGATYCCRAGTGWCICITGTTA	CCTCGGGGTACCTGAAGGGCATCC	FCP	97bp
134	BVD-Sig1	UCD, CAHFS lab	GGTAGTCGTCAGTGGTTCGAC	CATGTGCCATGTACAGCAGAGAT	CCTCGTCCACGTGGCATCTCGAG	FCP	195 bp
135	BTV-9932	LLNL	GCACCCTATATGTTTCCAGACCA	CAGCTAACTCTTCAGCCACACG	CTAACTCGTGGGCCAATCATCATCTTCTGT	FCP	271bp
136	BTV-9933	LLNL	AGAATTCAGGATGGGCAGGA	GCACAATTCCCATCCCCTTA	CCATCACACCATTATACTGTACCCGCGTAGC	FCP	187bp
150	SVD-1	LLNL	CAGGATAATTTCTTCCAAGGGC	ACGTGAACATTTCGAGCTTCC	TGCATTGTGTCTGATGGTACAACTTGTGACG	FCP	349bp
151	SVD-2	LLNL	GACTTGTTGTGGCTGGAGGA	CAGCGCCATGGTGAGGTAG	TGACCGTAATGAGGTCATCGTGATTTCTCAC	FCP	281bp
152	SVD-3	LLNL	GACAAAGTGGCCAAGGGAAA	CACGTAAACCACACTGGGCT	CTGGCGTCATAGCCTGAATAGTCAAACGCTA	FCP	248bp
154	VESV-1	LLNL	GCCTTCTCCCTTCCCAAAA	TGAAGGAATGGTTCCGTCAGT	CATCATCGTTGATAACCTTAGATGTGCAATTTGG	FCP	153bp
156	VESV-3	LLNL	GGGAATGAGGTGTGCATCATT	CACGTCTTGATGTTGGCTTGAC	AAATTGGCATAATCAACCTTGTCAGATGAGTCG	FCP	199bp
157	VESV-4	LLNL	GGTCGCTCTCACTGATGATGAGTA	GGTGTTATCAGCACCCATTGC	GCTCGGTGCCTGAGTTGGAGGAAG	FCP	124bp
158	VESV-5	LLNL	ACCACCTCTGGAAACATCTATGG	TTTGTGCACGTGTCACGAAT	CGGGACGGGCATTTGTCACCA	FCP	200bp
170	NC (MT-7)	LLNL bioinformatics	N/A	N/A	CAAAGTGGGAGACGTCGTTG	N/A	N/A
171	b-MT7 (FC)	LLNL	N/A	N/A	CAAAGTGGGAGACGTCGTTG	N/A	N/A
172	MT7/Cy3 (IC)	LLNL	N/A	N/A	CAAAGTGGGAGACGTCGTTG	N/A	N/A
173	arRNA Control	Ambion	GACATCAAGGCTCAAACTAATTTTACC	CAAAGGCTGCCAACATAAAATG	CAAGCGTAAATGCAGCGTCCA	FCP	N/A

[1] "RCP" refers to Reverse Compliment Probe, and "FCP" refers to Forward Compliment Probe. This information is included to indicate the strand of the PCR product to which the probe is expected to bind. All probes in this panel are designed so that the probe binds (hybridizes) to the labeled PCR strand primed by the labeled forward primer (forward compliment probe orientation FCP).

2.3.2. "Version 1" Multiplexed panel LOD

TABLE 11. "Version 1" FMDV rule-out panel limit of detection chart. Multiplexed PCR data is reported in Median Fluorescent Intensity (MFI) as a mean value of several hundred replicates.

Assay ID	Target DNA	Reference Test Strain	Singleplex LOD	Multiplex LOD	LOD shift (logs)	Bkgrd SP (MFI)	Bkgd Mux (MFI)	Threshold (MFI)[3]
BVH-1	Bovine Herpes Virus	BHV_N/A	100 $TCID_{50}$/uL	100 $TCID_{50}$/uL	0	9	60	>49
BVH-3	Bovine Herpes Virus	BHV_N/A	100 $TCID_{50}$/uL	100 $TCID_{50}$/uL	0	10	40	>43
PPOX-1	Bovine Papular Stomatitis Virus	BPSV_N/A	1000 $TCID_{50}$/uL	100 $TCID_{50}$/uL	-1	10	30	>35
PPOX-2[4]	Bovine Papular Stomatitis Virus	BPSV_N/A	10 $TCID_{50}$/uL	10 $TCID_{50}$/uL	0	10	65	>400
PPOX-4	Bovine Papular Stomatitis Virus	BPSV_N/A	100 $TCID_{50}$/uL	100 $TCID_{50}$/uL	0	10	35	>41
FMDV.TC	Foot and Mouth Virus	01 Korea	0.00065 pfu/mL	0.065 pfu/mL	2	10	10	>42
FMDV.Pir	Foot and Mouth Virus	01 Korea	6.5 pfu/mL	6.5 pfu/mL	0	10	10	>60
BVD_Sig1	Bovine Viral Diarrhea	BVD_N/A	10 $TCID_{50}$/uL	10 $TCID_{50}$/uL	0	7	30	>40
BTV-9932	Bluetongue Virus	BTV-13	1 $TCID_{50}$/uL	1 $TCID_{50}$/uL	0	11	30	>55
BTV-9933	Bluetongue Virus	BTV-13	1 $TCID_{50}$/uL	10 $TCID_{50}$/uL	1	9	20	>31
SVD_1	Swine Vesicular Disease	ITL/1/66	0.4 $TCID_{50}$/uL	4 $TCID_{50}$/uL	1	5	8	>38
SVD_2	Swine Vesicular Disease	ITL/1/66	0.4 $TCID_{50}$/uL	40 $TCID_{50}$/uL	2	10	10	>28
SVD_3	Swine Vesicular Disease	ITL/1/66	4 $TCID_{50}$/uL	4 $TCID_{50}$/uL	0	16	16	>40
VESV_1	Vesicular Exanthema of Swine Virus	E54[1]/A48[2]	2.69 x 10^{-4} $TCID_{50}$/uL	0.27 $TCID_{50}$/uL	N/A	5	5	>24
VESV_3	Vesicular Exanthema of Swine Virus	A48[1]/A48[2]	2.69 x 10^{-4} $TCID_{50}$/uL	2.69 x 10^{-4} $TCID_{50}$/uL	N/A	22	18	>39
VESV_4	Vesicular Exanthema of Swine Virus	E54[1]/A48[2]	2.69 x 10^{-4} $TCID_{50}$/uL	2.69 x 10^{-3} $TCID_{50}$/uL	N/A	25	17	>105
VESV_5	Vesicular Exanthema of Swine Virus	A48[1]/A48[2]	2.69 x 10^{-4} $TCID_{50}$/uL	2.69 x 10^{-4} $TCID_{50}$/uL	N/A	23	20	>56

2.3.3. Multiplexed Assay Data

2.3.3.1. Version 1.0 Panel Backgrounds and Near-neighbor Screening

Backgrounds Screening

Screening was performed in multiplexed PCR format in the finalized version of the panel. The panel was screened against 16 eukaryotes, 44 prokaryotes, 52 soils and 768 aerosols, in triplicate totaling over 2600 PCR reactions. For each test, 200pg per reaction of extracted total nucleic acid is added to each PCR reaction in triplicate. **No cross-reactions were observed for any of the backgrounds tested. Please refer to Appendix II for a complete list of the backgrounds tested.**

Near-Neighbor Screening

In 2006 additional near-neighbor screening was conducted against the Version 1.0 panel to complete the screening necessary for characterization. Additional screening at both LLNL and PIADC was conducted as newly available strains of virus were acquired. Please refer to Appendix III for a list of near-neighbor viruses screened in multiplex format against the Version 1.0 panel.

TABLE 12. Summary of the near-neighbor viruses used in Version 1.0 panel screening. A complete listing of these agents can be found in Appendix III. This screening was conducted at both LLNL and PIADC.

Agent	TOTAL # of NNs screened	Near-neighbor Screening Summary
BTV	17	In Version 1.0, the BTV assays were screened against BTV_9932 did not respond to nucleic acid (2 extracted from three EHD-1 serotypes (isolates Georgia, New Jersey, Santa Barbara) and EHD-Alberta.
Parapox	7	The signatures in multiplex did not generate responses above background when screened against near-neighbors including Rhadinovirus Caprine Herpes-2, V. Pseudorabies, Pseudorabies Shope, Equine Herpesvirus-1, Equine Herpesvirus-2, Feline herpes and Bovine Herpesvirus-5.
BHV	5	The BHV signatures were screened against 17 bovine herpes virus near-neighbors. None of the signatures cross-reacted.
BVD	7	In the Version 1.0 panel, BVD_1a signature responded to Border Disease Virus (BDV) Aveyron and Frijters strains but not 137-7.[6] Also in the Version 1.0 panel, BVD_1a signature responded to Classical Swine Fever Virus (CSFV) Brescia (≥550 $TCID_{50}$/mL) and Paderborn (≥6550 $TCID_{50}$/mL) but not Kanagawa.
FMDV	114[1]	FMD signatures in the Version 1.0 multiplex was examined by assays by screening FMDV assays against numerous (114) isolates of bovine and porcine enterovirus (BEV and PEV), SVD, VSV, VESV, Rinderpest, BHV-1 and BVDV. No response greater than the background was observed for either FMD signature.
SVD	12	Near-neighbor testing of the SVD signatures was recently conducted for Version 1.0, against 12 isolates of Human Entero Virus B. None of the SVD signatures were found to cross react with these viruses.
VESV	2	In Version 1.0 the VESV signatures were screened against San Miguel Sea Lion Virus (SMSV) serotypes 6-13; bovine, cetacean, feline, primate, reptile and skunk caliciviruses. VESV_4 exhibited strong response to Cetacean calicivirus, SMSV serotypes 7, 10 and 13. VESV_4 generated weak responses to SMSV Serotypes 6 and 9, whilst 11 and 12 were not detected.

[1]Total number screened includes all target testing for Version 1.0 panel agents.

FMDV Near-neighbor screening: SVD Near-neighbor screening: VESV Near-neighbor and Target screening:

2.3.3.2. Version 1.0 Panel Target Screening

Multiplexed PCR Assay Titrations

Upon finalization of the assay panel constituents each agent assay is characterized in multiplexed PCR format by running dose-response (titration) curves against titered virus extracted from virus infected cell culture for each available agent strain. Each sample is tested in duplicate and reported as a mean value of the median fluorescence intensity units (MFI) against agent concentration (per reaction volume, 1uL) on a double log plot. Concentration units are reported in TCID50/rxn (assumes 1uL sample volume per reaction) or pfu/rxn depending on virus type and source. In some cases where titered virus was not available data is reported as picogram units (pg). Each assay is evaluated by its ability to detect agent strains and differentiate from near neighbors (specificity) and by its range of sensitivity to each strain

[6] Federal Research Centre of Virus Diseases of Animals, Riems Island, Germany.
Concentration of material is presently unknown; the signature response increased above background for Averyron and Frijters strains at 10,000 and 10-fold dilution of the original samples, respectively.

relative to each signature tested for that agent. Because this method is not aimed at determination of analytical sensitivities (requiring a more extensive sample set tested in clinical sample matrices which is not practical for this stage of development) it does however, provide tentative limits of detection that are used to measure the performance of each assay.

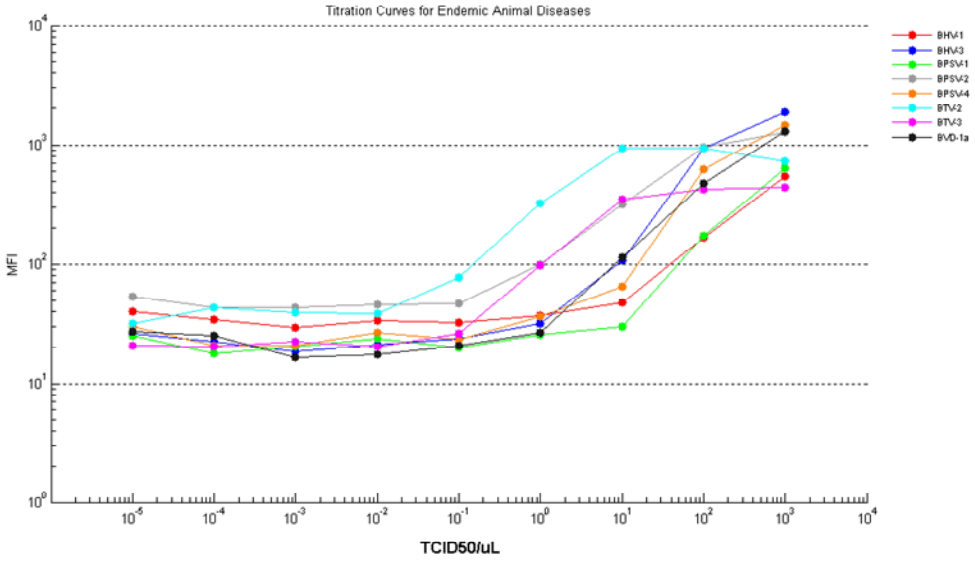


FIG. 12 Dose-response curves for select domestic and foreign animal diseases that constitute the FMDV rule-out panel. The curves combined above represent common viral extraction units () of representative strains for BHV (Colorado vaccine strain), PPOX (BPSV, Texas A&M strain), BTV (BTV serotype 2 NVSL), and BVDV (Type 1 Singer cytopathic). Serial dilution of nucleic acid extracted from titered virus-infected cell culture. Each point represents the mean response (n=2), with the first point being the no template control (NTC).

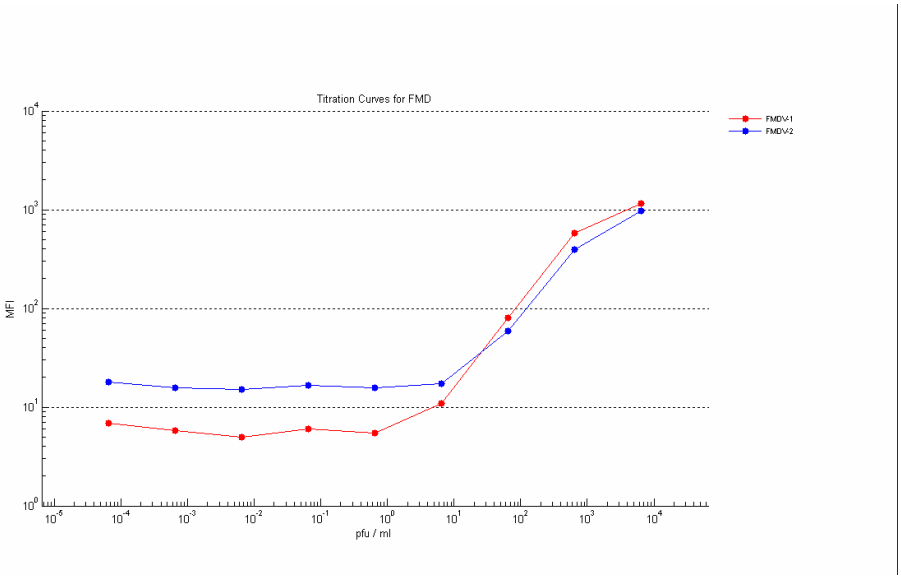


FIG. 13 Dose-response curves for select domestic and foreign animal diseases that constitute the FMDV rule-out panel. The curves combined above represent common viral extraction units (pfu/mL) of representative strain FMDV (Asia 1 serotype).

Serial dilution of nucleic acid extracted from titered virus-infected cell culture. Each point represents the mean response (n=2), with the first point being the NTC.

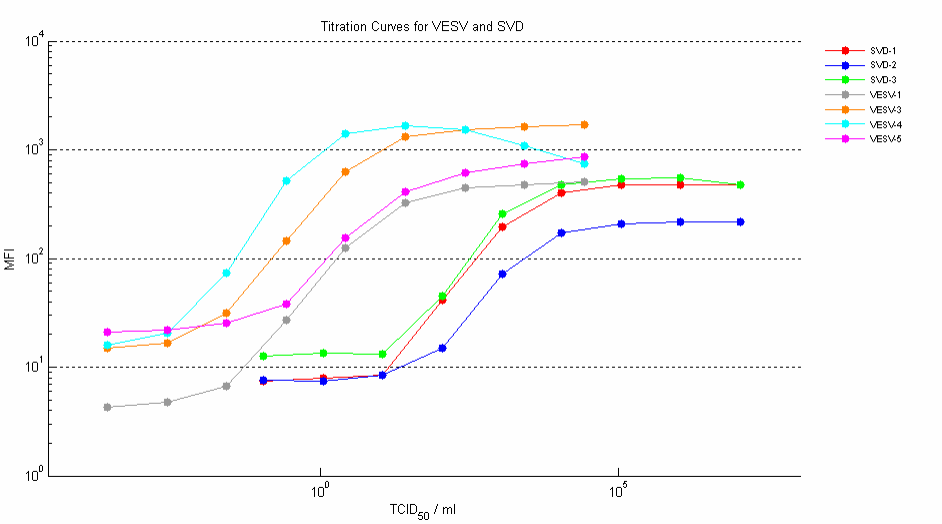


FIG. 14 Dose Dose-response curves for select foreign animal diseases that constitute the FMDV rule-out panel. The curves combined above represent common viral extraction units ($TCID_{50}$/mL) of representative strains for SVD (UKG-72) and VESV (B51). Serial dilution of nucleic acid extracted from titered virus-infected cell culture. Each point represents the mean response (n=2), with the first point being the NTC.

3. Species-Specific (Bovine/Porcine) Panel Development (FY06-07): FMDV Rule-Out Panel

3.1. Bioinformatics Summary

Depending on the genomes available for each pathogen and the inherent divergence of those genomes, assays may be designed against 1 or several genomes by performing an alignment or multiple alignments to determine conserved regions of the genomes and generate candidate signatures that match the LLNL input algorithms. In some cases the genomes may be limited or so divergent that it may be necessary to design signatures that work as a set, or minimal set cluster (MSC) to cover all available genomes such as those for PRRS.

For the species specific panel development assays for BTV, BVD and FMDV were re-visited for the purposes of re-designing or generating anew signatures that would enhance the current detection capabilities of the assays. New BTV signatures were designed against segment 5 and segment 10 of the genome. We teamed with Bill Wilson (USDA/ARS arbovirus research lab Laramie WY) using 21 unpublished sequences available for segment 5 in addition to the 6 sequences in Genbank. For segment 10 Bill Wilson provided 25 sequences combined with the 27 in Genbank to design signatures. This gave us 11 signatures for segment 5 and 16 signatures for segment 10 for PAN BTV detection. For the BVD assay a new signature generation process was initiated to enhance for the detection of BVD-2. The three candidate signatures were modified versions of the BVD assay from the Version 1.0 panel. A re-design of the FMDV assay produced candidate signatures, but they found to be equivalent in capability to the current external signatures, so these signatures were dropped from further screening.

TABLE 13. Summary of signatures generated and down-selected for the Bovine-Specific Panel. Agents shown in red were developed in the "Version 1" panel (described in section 2 of this report).

Bovine-Specific Panel for Foot-and-Mouth Disease Rule-Out

Agent	Genomes used for Alignment or Minimal Set Cluster (MSC)	Initial # of candidate signatures	# of signatures forwarded to Gel/Real-time PCR screening	# of signatures released to multiplex PCR screening	# of signatures in frozen Bovine panel
Parapox (PPOX)	4	8	7/4	4	3
BHV	1	177	101/4	4	2
FMDV[1]	N/A	N/A	N/A	2	2
BVD[2]	N/A	1	1/1	1	1
LLNL BVD	48	3	3/3	3	1
BTV	4, 4, 5[3]	8	8/5	4	2
MCF	1	1108	40/5	5	3
"Pan"[5] BTV	MSC	27	27/14	5	3
VSV	4	14	14/14	8	4
RPV	5, 5, 6[4]	12	9/5	5	3

[1]The two FMDV signatures were externally developed (Tetracore, Pirbright Laboratories). [2]BVD signature was externally developed (UC Davis).[3]For BTV three separate Kpath runs were performed using 4,4 and 5 genomes in each attempt.[4]For RPV three separate Kpath runs were performed using 5, 5 and 6 genomes in each attempt. [5] "Pan" refers to signature development designed to detect all serotypes.

TABLE 14. Summary of signatures generated and down-selected for the Porcine-Specific Panel. Agents shown in red were developed in the "Version 1" panel (described in section 2 of this report).

Porcine-Specific Panel for Foot-and-Mouth Disease Rule-Out					
Agent	Genomes used for Alignment or Minimal Set Cluster (MSC)	Initial # of candidate signatures	# of signatures forwarded to Gel/Real-time PCR screening	# of signatures released to multiplex PCR screening	# of signatures in frozen Porcine panel
FMDV[1]	N/A	N/A	N/A	2	2
SVD	5	4	4/4	4	3
VESV	1	44	20/6	6	4
PRRS	8, 14, 3[2]	62	62/30	27	5
VSV	4	14	14/14	8	6

[1]The two FMDV signatures were externally developed (Tetracore, Pirbright Laboratories).
[2]Multiple (3) Kpath runs were performed to generate signatures.

3.2. Real-time PCR Summary

3.2.1. Target / near neighbor screening

For the Bovine and Porcine panel development effort additional signatures were developed for BTV, and BVD, and new signatures for MCF, RPV, VSV, and PRRS. These new or added candidate signatures were screened in gel and real-time PCR format using the same methodology employed in the development of the Version 1.0 panel. In summary, candidate signature primer pairs that survived computational screening were forwarded to wet chemistry screening utilizing standard PCR with agarose gel electrophoresis followed by down-selection and additional screening in real-time PCR format to characterize tentative limits of detection in real-time PCR format. Table 14 above summarizes how many signatures survived the gel and real-time PCR screening.

The signature screening effort was highly collaborative requiring several coordination efforts with several external laboratories. In addition to the work conducted at LLNL, extensive real-time testing of the BTV signatures was conducted at Dr. William Wilson's lab at the USDA Agricultural Research Service [ARS] Laboratory Arthropod Bourne Animal Disease Laboratory [ABADRL] in Laramie, WY against a library of 24 serotypes of BTV as well as near-neighbor EHD isolates. Part of the PRRS real-time screening was carried out in Dr. Kay Faaberg's laboratory in the Minnesota State Veterinary Diagnostic Laboratory in Minneapolis, MN. Several other assays (SVD, VESV, VSV, PRRS, MCF, and RPV) were screened against target and near-neighbors at PIADC, NY. These important collaborations allowed for access to critical agent libraries that have been essential in completing this work

TABLE 15. Summary of target and near-neighbor viruses used in **2006** Bovine and Porcine panels gel and real-time PCR screening. A complete listing of these agents can be found in Appendix III. N/A agent not found in panel.

	Bovine Panel		**Porcine Panel**	
Agent	**Gel PCR Screening**	**Real-time PCR Screening**	**Gel PCR Screening**	**Real-time PCR Screening**
BHV Targets	0	0	N/A	N/A
BHV Near-Neighbors	0	0	N/A	N/A
Parapox Targets	0	0	N/A	N/A
Parapox Near-Neighbors	0	0	N/A	N/A
BTV Targets	7	23	N/A	N/A
BTV Near-Neighbors	0	10	N/A	N/A
BVD Targets	0	8	N/A	N/A
BVD Near-Neighbors	0	1	N/A	N/A
MCF Targets	10	3	N/A	N/A
MCF Near-Neighbors	7	7	N/A	N/A
RPV Targets	0	9	N/A	N/A
RPV Near-Neighbors	0	3	N/A	N/A
VSV Targets	2	16	2	16
VSV Near-Neighbors	0	0	0	0
FMDV Targets	0	7	0	7
FMDV Near-Neighbors	24	57	3	18
SVDV Targets	N/A	N/A	0	0
SVDV Near-Neighbors	N/A	N/A	0	0
VESV Targets	N/A	N/A	0	0
VESV Near-Neighbors	N/A	N/A	0	0
PRRS Targets	N/A	N/A	9	9
PRRS Near-Neighbors	N/A	N/A	0	0

3.3. Multiplexed PCR Assay Development Summary

In developing the multiplexed assay panels the general approach was to take the Version 1.0 panel and reduce it to the existing signatures that would be carried over into the new panel configurations. Newly developed assays were systematically added in a step-wise fashion and each individual assay was re-screened during each configuration change to ensure that the assays retained their original detection capabilities. Upon completing the step-wise addition process, the assay panels are then "frozen" and each assay undergoes a characterization process to define sensitivity ranges of the assays in their complete panel configuration. After the panels have been frozen, each panel is also screened against backgrounds (nucleic

acid extracts of soils, prokaryotes, eukaryotes, and near-neighbors) to test for cross-reactivity. The below section summarizes these assay development efforts.

3.3.1. Assay Down-Selection Summary

Multiplexed assay down-selection criteria: Preliminary screening can be broken down into several test methods. In the absence of target testing, or concurrently, the assays are preliminarily screened by first assessing how the assays will perform in the presence of other assay constituents in multiplex and whether or not the addition of those assays will adversely effect the other assays, those methods are described as "assay baseline screening" and "multiplex addition screening" respectively. Assay baseline screening indicates the performance of the assay in a no-sample reaction (blank), a "passing" score is indicated by a low MFI. All signatures passed the assay baseline screening. In the multiplex addition screening the primers are added one-by –one to the other panel constituents to determine if there would be any cross-reactions between primer pairs in the panel.

TABLE 16. Bovine Panel assay screening and down-selection. Each assay is rigorously screened to ensure its performance in the multiplexed PCR panel. Results are displayed as pass or fail with explanation for a signature failing.

Bovine Panel Signature	Mux Screening: Assay Down Selection				
	Assay baseline screening	Multiplex addition screening	Target screening	Near-neighbor screening	Limit of detection screening
MCF-1 (emcf_94975.F)	Pass	Pass	Pending additional screening	Pass	Pending additional screening
MCF-2 (emcf_95059.F)	Pass	Pass	Pending additional screening	Pass	Pending additional screening
MCF-3 (emcf_95155.F)	Pass	Pass	Pending additional screening	Pass	Pending additional screening
MCF-4 (emcf_95416.F)	Pass	Fail (12-29-06): Cross reacts with BTV10_1810199 (A7) assay	No further testing	No further testing	No further testing
MCF-5 (emcf_95476.F)	Pass	Fail (8-29-06): Cross reacts with MCF-4 (95416) assay	No further testing	No further testing	No further testing
BTV_9932 (2)	Pass	Pass	Pass	Pass	Pass
BTV_9933 (3)	Pass	Fail (4-30-07): Cross-reaction with PPOX-2 verified. Also it is overall about the worst BTV assay in Mux and TaqMan	No further testing	No further testing	No further testing
BTV10_0199 (A7)	Pass	Pass	Pass	Pass	Pass
BTV10_0200 (A8)	Pass	Fail (12-19-06): High backgrounds in multiplex	No further testing	No further testing	No further testing
BTV10_0201 (A9)	Pass	Fail (12-29-06): Cross reacts with BVD-1a assay	No further testing	No further testing	No further testing
BTV10_0205 (B1)	Pass	Pass	Pass	Pass	Pass
BTV10_0207 (B3)	Pass	Pass	Pass	Pass	Pass
PPOX-1 (BPSV_95719.F)	Pass	Pass	Pass	Pass	Pass
PPOX-2 (BPSV_95722.F)	Pass	Pass (12-28-06): Stepwise addition of BTV assays show historical background issues may be caused by BTV_1759933 (3) assay	Pass	Pass	Pass
PPOX-4 (BPSV_95731.F)	Pass	Pass	Pass	Pass	Pass
BHV-1	Pass	Pass	Pass	Pass	Pass

(BHV__94666.F)					
BHV-3 (BVH__94738.F)	Pass	Pass	Pass	Pass	Pass
FMD_TC	Pass	Pass	Pass	Pass	Pass
FMD_PIR	Pass	Pass	Pass	Pass	Pass
BVD-Sig1	Pass	Pass	Pass	Pass	Pass
BVD-Sig2 (BVD_1821165.F)	Pass	Pass	Pass	Pass	Pass
VSV_8941	Fail: Based on testing in the Porcine this assay not tested here	No further testing	No further testing	No further testing	No further testing
VSV_8943	Pass	Pass	Pending additional screening	Pass	Pending additional screening
VSV_8947	Pass	Pass	Pending additional screening	Pass	Pending additional screening
VSV_8949	Pass	Pass	Pending additional screening	Pass	Pending additional screening
VSV_1405	Pass	Fail (4-25-07): Cross reacts with BVD-1a,2 and BTV_1759932	No further testing	No further testing	No further testing
VSV_1406	Fail: Based on testing in the Porcine this assay not tested here	No further testing	No further testing	No further testing	No further testing
VSV_1408	Pass	Pass	Pending additional screening	Pass	Pending additional screening
VSV_1409	Pass	Fail (5-1-07): Cross reacts with BVD-1a,2	No further testing	No further testing	No further testing
RPV_1628	Pass	Fail	No further testing	No further testing	No further testing
RPV_4853	Pass	Pass	Pending additional screening	Pass	Pending additional screening
RPV_4855	Pass	Pass	Pending additional screening	Pass	Pending additional screening
RPV_4856	Pass	Pass	Pending additional screening	Pass	Pending additional screening
RPV_4893	Pass	Fail	No further testing	No further testing	No further testing

TABLE 17. Porcine Panel assay screening and down-selection. Each assay is rigorously screened to ensure its performance in the multiplexed PCR panel. Results are displayed as pass or fail with explanation for a signature failing.

Porcine Panel Signature	**Mux Screening: Assay Down Selection**				
	Assay baseline screening	**Multiplex addition screening**	**Target screening**	**Near-neighbor screening**	**Limit of detection screening**
FMD-TC	Pass	Pass	Pass	Pass	Pass
FMD-PIR	Pass	Pass	Pass	Pass	Pass
SVD-1 (SVD_172049.F)	Pass	Pass	Pending additional Screening	Pass	Pending additional Screening
SVD-2 (SVD_172050.F)	Pass	Pass	Pending additional Screening	Pass	Pending additional Screening
SVD-3 (SVD_172051.F)	Pass	Pass	Pending additional Screening	Pass	Pending additional Screening
VESV-1 (vesv_95653.F)	Pass	Pass	Pending additional Screening	Pass	Pending additional Screening
VESV-3 (vesv_95680.F)	Pass	Fail (4-16-07): Cross reacts with VSV-1811409	No further testing	No further testing	No further testing
VESV-4 (vesv_95686.F)	Pass	Pass	Pending additional Screening	Pass	Pending additional Screening
VESV-5 (vesv_95692.F)	Pass	Pass	Pending additional Screening	Pass	Pending additional Screening
PRRS_7658	Pass	Fail (3-1-07): Cross reacts with PRRS_1807706	No further testing	No further testing	No further testing
PRRS_7706	Pass	Pass	Pending additional	Pass	Pending additional

			Screening		Screening
PRRS_7709	Pass	Pass (4-13-07): Some cross-reaction seen with other PRRS assays	Pending additional Screening	Pass	Pending additional Screening
PRRS_0351	Pass	Pass	Pending additional Screening	Pass	Pending additional Screening
PRRS_0368	Pass	Fail (2-13-07): High backgrounds in multiplex also appears to cross-react with SVD-1 based on subsequent tests	No further testing	No further testing	No further testing
PRRS_0374	Pass	Pass	Fail (2-13-07): Does not react with any templates	No further testing	No further testing
PRRS_0383	Pass	Pass	Pending additional Screening	Pass	Pending additional Screening
PRRS_0386	Pass	Pass	Pending additional Screening	Pass	Pending additional Screening
VSV_8941	Pass	Fail (4-4-07): Cross reacts with VESV-3 and VSV_1811409	No further testing	No further testing	No further testing
VSV_8943	Pass	Pass	Pending additional Screening	Pass	Pending additional Screening
VSV_8947	Pass	Pass	Pending additional Screening	Pass	Pending additional Screening
VSV_8949	Pass	Pass	Pending additional Screening	Pass	Pending additional Screening
VSV_1405	Pass	Pass	Pending additional Screening	Pass	Pending additional Screening
VSV_1406	Pass	Fail (4-11-07) Cross reacts with VSV_1811406	No further testing	No further testing	No further testing
VSV_1408	Pass	Pass	Pending additional Screening	Pass	Pending additional Screening
VSV_1409	Pass	Pass	Pending additional Screening	Pass	Pending additional Screening

3.3.2. Multiplex panel layouts (Bovine and Porcine –Specific Panels)

TABLE 18. Panel layout for the 23-plex (plus controls) **Bovine-Specific Panel** for Foot-and-Mouth disease "rule-out". Assay controls are shown in red. Signatures that carried over from the Version1 panel are shown in blue.

Bead ID	Assay/ Target Name	Mux Group ID	Organism	Source	Forward Primer	Reverse Primer	Luminex Probe (-strand, FCP[1])
113	emcf_94975.F, emcf_94976.R, emcf_94977.P	MCF_1	Malignant Catarrhal Fever	LLNL	ATGCCAGTCACTGGCTCTCA	GGGTGTTGTAGAATCCTGAAATGG	GTTGATCACGGTGGCACCCTGG
114	emcf_95059.F, emcf_95060.R, emcf_95061.P	MCF_2	Malignant Catarrhal Fever	LLNL	GTTCTGGAAACTGACCAAACAGTGT	AGTGGCACTTGAGTGTAACTTTTATTG	GCACTCTGGCAGGCATAAGGGAAATACA
115	emcf_95155.F, emcf_95156R, emcf_95157.P	MCF_3	Malignant Catarrhal Fever	LLNL	CCCTGGAAGCTGTCATACAAAA	AAACATTGGCATATCTTGCAAGGT	CAGTAGAGTCCAGGGCTGCAGTTGTCTCA
117	BVH__94666.F, BVH_94667.R, BVH_94668.P	BHV__1	Bovine Herpes Virus	LLNL	GTGCCAGCCGCGTAAAAG	GACGACTCCGGGCTCTTTT	TCCTGGTTCCAGAGCGCTAACATGGAG
118	BVH__94738.F, BVH_94739.R, BVH_94740.P	BHV__3	Bovine Herpes Virus	LLNL	TGAGGCCTATGTATGGGCAGTT	GCGCGCCAAACATAAGTAAA	AAATAACACGGTGTGCACTTAAATAAGATTCGCG
119	BPSV_95719.F, BPSV_95720.R, BPSV_95721.P	PPOX_1	Bovine Papular Stomatitis Virus	LLNL	GCAGATGCGCTCCTGGTT	GCACCTCTGCTGCTGCAA	CCGACTCCGACGTGGAGAACGTG
120	BPSV_95722.F, BPSV_95723.R, BPSV_95724.P	PPOX_2	Bovine Papular Stomatitis Virus	LLNL	GATGGCCGTGCAGCTCTT	CGTACAAGATCACGGCCAACT	TGTACGGGCTCATGGGCTTCCG
122	BPSV_95731.F, BPSV_95732.R, BPSV_95733.P	PPOX_4	Bovine Papular Stomatitis Virus	LLNL	GCAGCAGTGCACCACGTAGT	CGCTGAACCCGTACATCCT	GACTTCGAGGCGGACAACAAGCG
132	FMDV.TC	FMDV.TC	Foot and Mouth Virus	Tetracore	ACTGGGTTTTACAAACCTGTGA	GCGAGTCCTGCCACGGA	GTCCCACGGCGTGCAAAGGA

133	FMDV.Pir	FMDV.Pir	Foot and Mouth Virus	Pirbright	CACYTYAAGRTGACAYTGRTACTGGTAC	CAGATYCCRAGTGWCICITGTTA	CCTCGGGGTACCTGAAGGGCATCC
134	BVD_1a	BVD_Sig1	Bovine Viral Diarrhea	UCD, CAHFS lab	GGTAGTCGTCAGTGGTTCGAC	CATGTGCCATGTACAGCAGAGAT	CCTCGTCCACGTGGCATCTCGAG
138	BVD_1821165.F, BVD_1821164.R, BVD_1821166.P	BVD_Sig2	Bovine Viral Diarrhea	LLNL	GGGAGTCGTCAATGGTTCGAC	TCCATGTGCCATGTACAGCAGAG	CCTCGTCCACITGGCATCTCGAG
135	BTV_1759932	BTV-9932	Bluetongue Virus	LLNL	GCACCCTATATGTTTCCAGACCA	CAGCTAACTCTTCAGCCACACG	CTAACTCGTGGGCCAATCATCATCTTCTGT
136	BTV10_1810207	BTV-0207	Bluetongue Virus	LLNL	CAAACACAAAAGGCGGAGAAG	GGCGTTTAATCTGTCTTAGTCTTACGT	GAAACGCTTCTGCGTACGATGCGA
137	BTV10_1810199	BTV-0199	Bluetongue Virus	LLNL	CACATGTCGCTTAATTTGTCTTAACC	GCGGAGAAGGCTGCATT	ACGAAACGCTTCCGCGTACGATG
139	BTV10_1810205	BTV-0205	Bluetongue Virus	LLNL	TCAATTTTGGTAGAATTTGTTCATTCA	GCGGAGAAGGCTGCATTC	ACGAAACGCTTCCGCGTACGATG
163	VSV_1798943	VSV-8943	Vesicular Stomatitis Virus	LLNL	CGCCACAAGGCAGAGATGT	TGTCAAATTCTGACTTAGCATACTTGC	GCATACTGCATCATATCAGGAGTCGGTTTTCTG
166	VSV_1798947	VSV-8947	Vesicular Stomatitis Virus	LLNL	CCCAATCAATGCCATGATACA	CTCCAATGGAAGGGTCCAAA	TTTGAAAGTAGAACTGTGCAAGCCCGGTATC
167	VSV_1798949	VSV-8949	Vesicular Stomatitis Virus	LLNL	GGCGCTCATTATAAAATTCGGA	ACATTTTCTCGTAGTAATGCAGCAG	GAAGTCCCTGTAATGGATTCCCATTCCATGT
169	VSV_1811408	VSV-1408	Vesicular Stomatitis Virus	PIADC	CTCACAACATGGGTCCTGAA	TTCTTGACCTGGATACATCAT	GGCATAGYTCGTCTGCRACTTCCCT
160	RPV_1814853	RPV-4853	Rinderpest Virus	LLNL	GGATCGCTGAAATGATCTGTGA	GGAGCCAGTTCACCCATTTG	CTGGCCAACCCTGCCTCCACTATGTA
161	RPV_1814855	RPV_1814855	Rinderpest Virus	LLNL	TGCATCTTATGTGACTTTGGTTCA	GGCTATCCGCACAGCTGAC	CAGTCCTCTCATCTGTTGTCGATCCGATGTA
162	RPV_1814856	RPV-4856	Rinderpest Virus	LLNL	AACTCCTGACCTCATTCCTTGC	GGCTCTATAATCCCACTATGCCA	TGGCTCAGTGCATTCACAAAGACCTTGAATA
170	N/A	NC (MT-7)	Maritima	LLNL	N/A[2]	N/A[2]	CAAAGTGGGAGACGTCGTTG
171	N/A	b-MT7 (FC)	N/A	LLNL	N/A[2]	N/A[2]	CAAAGTGGGAGACGTCGTTG
172	N/A	MT7/Cy3 (IC)	N/A	LLNL	N/A[2]	N/A[2]	CAAAGTGGGAGACGTCGTTG
173	Armored RNA	arRNA Control	None	Ambion	GACATCAAGGCTCAAACTAATTTTACC	CAAAGGCTGCCAACATAAAATG	CAAGCGTAAATGCAGCGTCCA

[1] "RCP" refers to Reverse Compliment Probe, and "FCP" refers to Forward Compliment Probe. This information is included to indicate the strand of the PCR product to which the probe is expected to bind. All probes in this panel are designed so that the probe binds (hybridizes) to the labeled PCR strand primed by the labeled forward primer (forward compliment probe orientation FCP). [2]Control assays that consist of probe-only binding, no primers used.

TABLE 19. Panel layout for the 19-plex (plus controls) **Porcine-Specific Panel** for Foot-and-Mouth disease "rule-out". Assay controls are shown here in red. Signatures that carried over from the Version1 panel are shown here in blue.

Bead ID	Assay/ Target Name	Mux Group ID	Organism	Source	Forward Primer	Reverse Primer	Luminex Probe (-strand, FCP[1])
132	FMDV.TC	FMDV.TC	Foot and Mouth Virus	Tetracore	ACTGGGTTTTACAAACCTGTGA	GCGAGTCCTGCCACGGA	GTCCCACGGCGTGCAAAGGA
133	FMDV.Pir	FMDV.Pir	Foot and Mouth Virus	Pirbright	CACYTYAAGRTGACAYTGRTACTGGTAC	CAGATYCCRAGTGWCICITGTTA	CCTCGGGGTACCTGAAGGGCATCC
142	PRRS_1807709	PRRS-7709	Porcine Reproductive and Respiratory Syndrome	LLNL	GAGCGGCAATTGTGTCTGTC	GCTGAGGGTGATGCTGTGAC	CGCACAGTATGATGCGTAGGCAAACTAAACTC
144	PRRS_1810351	PRRS-0351	Porcine Reproductive and Respiratory Syndrome	LLNL	TTCTTGTGACCACGATTCGC	GACCCACCGAGTAACTTGCC	GCTCAAGAGCCAAAAGCTCAGCATGACA
145	PRRS_1807706	PRRS-7706	Porcine Reproductive and Respiratory Syndrome	LLNL	ATTGGTTTGCTCCGCGATAC	AAATGAGCCACCACATCCAA	CGGTACATTCGACGCGACACCATTTC
148	PRRS_1810383	PRRS-0383	Porcine Reproductive and Respiratory Syndrome	LLNL	CAGTGTGCACGCTTCCATTT	CTCGAATGATGTGTTGCCGT	AAACATAGCGTAGAGCTGGAATTCGAAGCCA
149	PRRS_1810386	PRRS-0386	Porcine Reproductive and Respiratory Syndrome	LLNL	GCTTTCTGCGTGCCTTTTCT	ACAACGCCAGAGACATTCCC	TGACTTTGAAGCCTTTCTCGCTCATTTCTGA
150	SVD_1727049	SVD_1	Swine Vesicular	LLNL	CAGGATAATTTCTTCCAAGGGC	ACGTGAACATTTCGAGCTTCC	TGCATTGTGTCTGATGGTACAACTTGTGACG

			Disease				
151	SVD_1727050	SVD_2	Swine Vesicular Disease	LLNL	GACTTGTTGTGGCTGGAGGA	CAGCGCCATGGTGAGGTAG	TGACCGTAATGAGGTCATCGTGATTTCTCAC
152	SVD_1727051	SVD_3	Swine Vesicular Disease	LLNL	GACAAAGTGGCCAAGGGAAA	CACGTAAACCACACTGGGCT	CTGGCGTCATAGCCTGAATAGTCAAACGCTA
154	VESV_95653.F, VESV_95654.R, VESV_95655.P	VESV_1	Vesicular Exanthema of Swine Virus	LLNL	GCCTTCTCCCTTCCCAAAA	TGAAGGAATGGTTCCGTCAGT	CATCATCGTTGATAACCTTAGATGTGCAATTTGG
157	VESV_95686.F, VESV_95687.R, VESV_95688.P	VESV_4	Vesicular Exanthema of Swine Virus	LLNL	GGTCGCTCTCACTGATGATGAGTA	GGTGTTATCAGCACCCATTGC	GCTCGGTGCCTGAGTTGGAGGAAG
158	VESV_95692.F, VESV_95693.R, VESV_95694.P	VESV_5	Vesicular Exanthema of Swine Virus	LLNL	ACCACCTCTGGAAACATCTATGG	TTTGTGCACGTGTCACGAAT	CGGGACGGGCATTTGTCACCA
163	VSV_1798943	VSV-8943	Vesicular Stomatitis Virus	LLNL	CGCCACAAGGCAGAGATGT	TGTCAAATTCTGACTTAGCATACTTGC	GCATACTGCATCATATCAGGAGTCGGTTTTCTG
164	VSV_1811409	VSV-1409	Vesicular Stomatitis Virus	PIADC	CTCACAACATGGGTCCTGAA	TTCTTGCCCCGGATACATCAT	GGCACAGCTCATCTGCGACTTCCCT
166	VSV_1798947	VSV-8947	Vesicular Stomatitis Virus	LLNL	CCCAATCAATGCCATGATACA	CTCCAATGGAAGGGTCCAAA	TTTGAAAGTAGAACTGTGCAAGCCCGGTATC
167	VSV_1798949	VSV-8949	Vesicular Stomatitis Virus	LLNL	GGCGCTCATTATAAAATTCGGA	ACATTTTCTCGTAGTAATGCAGCAG	GAAGTCCCTGTAATGGATTCCCATTCCATGT
168	VSV_1811405	VSV-1405	Vesicular Stomatitis Virus	PIADC	AAGAGATGGTCACGAGTGAC	GAGCATTTGTGGAAACCGAGC	TGGGTATTTGGTCATTGGTGACACA
169	VSV_1811408	VSV-1408	Vesicular Stomatitis Virus	PIADC	CTCACAACATGGGTCCTGAA	TTCTTGACCTGGATACATCAT	GGCATAGYTCGTCTGCRACTTCCCT
170	N/A	NC (MT-7)	Maritima	LLNL	N/A	N/A	CAAAGTGGGAGACGTCGTTG
171	N/A	b-MT7 (FC)	N/A	LLNL	N/A	N/A	CAAAGTGGGAGACGTCGTTG
172	N/A	MT7/Cy 3 (IC)	N/A	LLNL	N/A	N/A	CAAAGTGGGAGACGTCGTTG
173	Armored RNA	arRNA Control	None	Ambion	GACATCAAGGCTCAAACTAATTTTACC	CAAAGGCTGCCAACATAAAATG	CAAGCGTAAATGCAGCGTCCA

[1] "RCP" refers to Reverse Compliment Probe, and "FCP" refers to Forward Compliment Probe. This information is included to indicate the strand of the PCR product to which the probe is expected to bind. All probes in this panel are designed so that the probe binds (hybridizes) to the labeled PCR strand primed by the labeled forward primer (forward compliment probe orientation FCP).

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3.3.3. Multiplexed PCR Assay Development Data

3.3.3.1. Background and Near-neighbor Screening

Screening was performed in multiplexed PCR format in the finalized version of each of the two panels. The Bovine and Porcine panels were screened against identical backgrounds that included: 16 eukaryotes, 44 prokaryotes, 52 soils, and 768 aerosols, all tested in triplicate totaling over 2700 PCR reactions. For each test, 200pg per reaction of extracted total nucleic acid is added to each PCR reaction in triplicate. **Please refer to Appendix II for a complete list of the backgrounds tested.**

TABLE 20. Summary of background and standard screening panels. Additional near-neighbors from panel constituents were screened as a "standardized panel" of target and near-neighbors, even though clearly, some of these are not near-neighbors of the Porcine panel agents this is reported in Appendix II.

Standardized Screening Panels (See Appendix II)	**Bovine Panel Results Summary**	**Porcine Panel Results Summary**
Eukaryotes (16)	No Cross-reactions observed	No Cross-reactions observed
Prokaryotes (44)	RPV-4853 and VSV-1408 had several low-level cross-reactions.	No Cross-reactions observed
Soils (52)	No Cross-reactions observed	No Cross-reactions observed
Aerosols (768)	No Cross-reactions observed	No Cross-reactions observed

Standardized Near-neighbor panel (45)	Several BVD cross-reactions were observed[1]	No Cross-reactions observed

[1]BVDV is a known contaminate of serum used to propagate cells for virus stocks and is likely to be responsible for the cross reactions seen with the BVDV signatures.

Please refer to Appendix III for a complete lists of near-neighbor viruses screened in multiplex format against the Bovine and Porcine Panels. The lists are separated by species-specific panels, however, a large volume of testing against targets and near-neighbors is in process at PIADC these are reported as "pending" in the data tables referenced in Appendix III.

Bovine Panel Near-Neighbor Screening Summary

TABLE 21. Summary of the near-neighbor viruses used in Version 1.0 panel screening. A complete listing of these agents can be found in Appendix III. This screening was conducted at both LLNL and PIADC. Additional screening that is in process at PIADC is represented by the numbers in parentheses.

Agent	TOTAL # of NNs screened[1]	Near-neighbor Screening Summary
BTV	4	The BTV assays were screened against 4 isolates of Epizootic Hemorrhagic Disease virus (EH Georgia, New Jersey, Santa Barbara and EHD-2 Alberta). None of the BTV assays cross-reacted v the four EHD isolates.
Parapox	18 (2)	The Parapox signatures were screened against 18 near-neighbor viruses including Rhadinovirus Caprine Herpes-2, V. Pseudorabies, Pseudorabies Shope, Equine Herpesvirus-1, Equine Herpesvirus-2, Feline herpes, Fowl pox and Bovine Herpesvirus-5. None of the Parapox signatures cross-reacted with any of the near-neighbors screened.
BHV	20 (3)	The BHV signatures were screened against 20 near-neighbor viruses including Rhadinovirus Caprine Herpes-2, V.Pseudorabies, Pseudorabies Shope, Equine Herpesvirus-1, Equine Herpesvirus-2, Feline herpes, Fowl pox and Bovine Herpesvirus-5. Either BHV signature cross-reacted with any of the near-neighbors screened.
BVD	1(7)	The two BVD signatures were screened at LLNL against border disease virus (Coos Bay) and did not cross-react. Additional screening is in process at PIADC against 3 additional isolates of border disease virus (Frijters, 134/7, and Aveyron) and 4 isolates of classical swine fever (including Brescia, Kanagawa, and Paderborn).
MCF	31	In multiplex near-neighbor testing was repeated using several (31) Herpes virus isolates and three V.Pseudorabies isolates. None of these near-neighbors cross-reacted with the MCF signatures.
RPV	3(7)	This work has not yet been conducted; testing of 7 isolates of Peste de Petits Ruminants virus (PPRV) currently in process at PIADC.
VSV	(8)	This work has not yet been conducted; it is in process at PIADC.
FMDV	(7)	FMD signatures were examined by running titrations against SVD (UKG-72), VSV (NJ 95366), VESV (A48), Rinderpest (Nig Buffalo), BHV-1 and BVDV (NY 1). No response greater than the background was observed for either FMD signature. Additional screening of several bovine enteroviruses is in process at PIADC.

[1]Screening is in process at PIADC for several target and near-neighbors, a list of these agents pending screening can be found in Appendix III.

Porcine Panel Near-Neighbor Screening Summary

TABLE 22. Summary of the near-neighbor viruses used in Version 1.0 panel screening. A complete listing of these agents can be found in Appendix III. This screening was conducted at both LLNL and PIADC. Additional screening that is in process at PIADC is represented by the numbers in parentheses.

Agent	TOTAL # of NNs screened[1]	Near-neighbor Screening Summary

VSV	(8)	This work has not yet been conducted; it is in process at PIADC.
FMDV	(8)	FMD signatures were examined by running titrations against SVD (UKG-72), VSV (NJ 95366), VESV (A48), Rinderpest (Nig Buffalo), BHV-1 and BVDV (NY 1). No response greater than the background was observed for either FMD signature. Additional screening of several porcine enteroviruses is in process at PIADC.
SVD	(12)	The SVD assays were screened against human enterovirus B (HEV-B) Coxsackie B5 (Faulkner) and no cross-reactions were observed. Additional screening against several other HEV-B isolates is in process at PIADC.
VESV	2 (8)	The VESV assays were screened against 2 isolates of San Miguel Sea Lion Virus (SMSV, types 2 and 12). No cross reactions were observed with any of the signatures. Additional near-neighbor screening is in process at PIADC.
PRRS	2	The PRRS signatures in the current porcine panel were screened against porcine respiratory coronavirus and transmissible gasteroenteritis of swine, none of the signatures cross-reacted with those near neighbors when screened.

[1]Screening is in process at PIADC for several target and near-neighbors, a list of these agents pending screening can be found in Appendix III.

3.3.4. Multiplexed PCR Target Screening

Each assay is characterized in the multiplexed panels by various stringencies for desired assay sensitivity, specificity, and overall reliability. For both the Bovine and Porcine panels all agents are screened against several target isolates by running dose-response (titration) curves against titered virus (when available) extracted from virus infected cell culture for each available agent strain. Because this method is not aimed at determination of analytical sensitivities (requiring a more extensive sample set tested in clinical sample matrices which is not practical for this stage of development) it does however, provide tentative limits of detection that are used to measure the performance of each assay. In the table below this is reported as a range of sensitivity that is defined by results acquired from testing one or more isolates. The results of this screening is described below in the tables describing each panels' attributes and in the limit of detection summary section below.

Multiplexed PCR Panel Attributes

TABLE 23. Bovine Panel attributes.

Signature	Detection Range	Noted cross-reactions	Designated Specificity	Detected all serotypes/ strains tested? (Y/N)
MCF_1	Pending	None	AlHV MCF	Y
MCF_2	Pending	None	AlHV MCF	N
MCF_3	Pending	None	AlHV MCF	Y
BHV-4668	$1x10^{-1}$ - $5x10^{3}$ $TCID_{50}$/rxn	None	BHV-1	N
BHV-4740	$5x10^{-2}$ - $1x10^{2}$ $TCID_{50}$/rxn	None	BHV-1	Y
PPOX-1	10^7 $TCID_{50}$/0.1mL	None	BPSV and Pseudocowpox	N
PPOX-2	10^7 $TCID_{50}$/mL	PPOX-2 probe cross reacts with dropped BTV-9933 primers	BPSV and Pseudocowpox	N
PPOX-4	10^7	None	BPSV and Pseudocowpox	Y

	$TCID_{50}$/mL			
FMDV.TC	Pending	None	FMDV, all serotypes	Y
FMDV.Pir	Pending	None	FMDV, all serotypes	N
BVD-Sig1	$5x10^{-3}$ - $1x10^{1}$ $TCID_{50}$/rxn	Cross-reacts with several NNs; due to BVD contamination in cell lines	BVD, all strains	Y
BVD-Sig-2	$5x10^{-3}$ - $1x10^{1}$ $TCID_{50}$/rxn	Cross-reacts with several NNs; due to BVD contamination in cell lines	BVD, all strains	Y
BTV-9932	$5x10^{-3}$ - $5x10^{1}$ $TCID_{50}$/rxn	None	Pan BTV: detects all domestic and 17/19 exotic serotypes	N
BTV-0207	$1x10^{0}$ - $1x10^{3}$ $TCID_{50}$/rxn	None	BTV, all serotypes	Y
BTV-0199	$1x10^{0}$ - $1x10^{3}$ $TCID_{50}$/rxn	None	BTV, all serotypes	Y
BTV-0205	$1x10^{0}$ - $1x10^{3}$ $TCID_{50}$/rxn	None	BTV, all serotypes	Y
VSV-8943	$1x10^{-2}$ $TCID_{50}$/rxn	None	VSV Indiana-1	Y
VSV-8947	$1x10^{-2}$ $TCID_{50}$/rxn	None	VSV Indiana-1	Y
VSV-8949	$1x10^{-2}$ $TCID_{50}$/rxn	None	VSV Indiana-1	Y
VSV-1408	$1x10^{-3}$ $TCID_{50}$/rxn	Low cross-reactions with zoological isolates: cat, monkey, tick	VSV New Jersey	Y
RPV-4853	Pending	Low cross-reactions with zoological isolates: bovine, cat, chicken, dog, equine, monkey, mouse, sheep, tick	RPV, all strains	Y
RPV-4855	Pending	None	RPV, all strains	Y
RPV-4856	Pending	None	RPV, all strains	Y

TABLE 24. Porcine Panel attributes.

Signature	Detection Range	Noted cross-reactions	Designated Specificity	Detects all serotypes/ strains tested? (Y/N)
FMDV.TC	Pending	None	FMDV, all serotypes	Y
FMDV.Pir	Pending	None	FMDV, all serotypes	N
PRRS-7709	Pending	None	PRRS: domestic	Y
PRRS-0351	Pending	None	PRRS: European	Y
PRRS-7706	Pending	None	PRRS: domestic	Y
PRRS-0383	Pending	None	PRRS: European	Y
PRRS-0386	Pending	None	PRRS: European	Y
SVD_1	Pending	None	SVD, all strains	Y
SVD_2	Pending	None	SVD, all strains	N
SVD_3	Pending	None	SVD, all strains	Y
VESV_1	Pending	None	VESV, all strains	N
VESV_4	Pending	None	VESV, all strains	Y
VESV_5	Pending	None	VESV, all strains	N
VSV-8943	$1x10^{-2}$	None	VSV Indiana-1	Y

	$TCID_{50}$/rxn			
VSV-1409	$1x10^{-2}$ $TCID_{50}$/rxn	None	VSV New Jersey	Y
VSV-8947	$1x10^{-2}$ $TCID_{50}$/rxn	None	VSV Indiana-1	Y
VSV-8949	$5x10^{0}$ $TCID_{50}$/rxn	None	VSV Indiana-1	Y
VSV-1405	$1x10^{-3}$ $TCID_{50}$/rxn	None	VSV Indiana-1	Y
VSV-1408	$1x10^{-1}$ $TCID_{50}$/rxn	None	VSV New Jersey	Y

Limit of Detection for Multiplexed Detection Assays

TABLE 25. Bovine panel limit of detection summary. Extracted nucleic acid from titered virus is added to each PCR reaction in a 10-fold dilution series. Reactions are performed in triplicate and what is reported below is the detection limit at which the signal for a positive exceeds the signal for the assay in the absence of virus by a factor of greater than three standard deviations above the mean. The values set for the limits reported herein lack sufficient threshold data to analytically characterize actual limit of detection. What is reported herein is a relative sensitivity of each assay in the panel. The specific virus isolates referenced below are further detailed in Appendix III. **Data for FMDV, SVD, VESV, VSV, RPV, and MCF is still being collected at PIADC at the time of this report. Orf is not a bovine target virus.**

Virus	BTV_9932 (TCID50/rxn)	BTV_0199 (TCID50/rxn)	BTV_0205 (TCID50/rxn)	BTV_0207 (TCID50/rxn)
BTV-2(NVSL)	1×10^{-3}	1×10^{0}	1×10^{0}	1×10^{0}
BTV-10(NVSL)	5×10^{1}	1×10^{3}	1×10^{3}	1×10^{3}
BTV-11(NVSL)	5×10^{1}	5×10^{1}	5×10^{1}	5×10^{1}
BTV-13(NVSL)	1×10^{-3}	1×10^{-3}	1×10^{-3}	1×10^{-3}

Virus	BHV-4668 (TCID50/rxn)	BHV-4740 (TCID50/rxn)
Colorado	1×10^{-1}	5×10^{-2}
ATCC VR-188	1×10^{2}	1×10^{1}
TX 20072	5×10^{3}	1×10^{2}

Virus	PPOX-1 (TCID50/rxn)	PPOX-2 (TCID50/rxn)	PPOX-4 (TCID50/rxn)
BPSV(NVSL)	5×10^{-1}	5×10^{-1}	5×10^{-2}
Orf vaccine	5×10^{0}	1×10^{0}	1×10^{1}
PCPV(NVSL)	1×10^{0}	1×10^{0}	1×10^{-2}

Virus	BVD-SIG1 (TCID50/rxn)	BVD-SIG2 (TCID50/rxn)
BVD-1b NY-1	1×10^{-1}	1×10^{-1}
BVD-2b FSB	1×10^{-2}	1×10^{-2}
BVD-1a Singer	1×10^{1}	1×10^{1}
BVD-1b TGAN	5×10^{0}	5×10^{0}
BVD-2a 5085	1×10^{-1}	1×10^{-1}
BVD-1a NADL	1×10^{-2}	1×10^{-2}
BVD-2 165	5×10^{-3}	5×10^{-3}
BVD-2b AU501	1×10^{-1}	1×10^{-1}

Virus	VSV-8943 (TCID50/rxn)	VSV-8947 (TCID50/rxn)	VSV-8949 (TCID50/rxn)	VSV-1408 (TCID50/rxn)
VSVIN-1 (NVSL)	1×10^{-2}	1×10^{-2}	1×10^{-2}	X
VSVNJ (NVSL)	X	X	X	1×10^{-3}

TABLE 26 Porcine panel limit of detection summary. Extracted nucleic acid from titered virus is added to each PCR reaction in a 10-fold dilution series. Reactions are performed in triplicate and what is reported below is the detection limit at which the signal for a positive exceeds the signal for the assay in the absence of virus by a factor of greater than three standard deviations above the mean. The values set for the limits reported herein lack sufficient threshold data to analytically characterize actual limit of detection. What is reported herein is a relative sensitivity of each assay in the panel**.** The specific virus isolates referenced below are further detailed in Appendix III. **Data for FMDV, SVD, VESV and PRRS is still in process at PIADC.**

Virus	VSV-8943 (TCID50/rxn)	VSV-8947 (TCID50/rxn)	VSV-8949 (TCID50/rxn)	VSV-1408 (TCID50/rxn)	VSV-1409 (TCID50/rxn)	VSV-1405 (TCID50/rxn)
VSVIN-1 (NVSL)	1×10^{-2}	1×10^{-2}	1×10^{-2}	X	X	5×10^{0}
VSVNJ (NVSL)	X	X	X	1×10^{-3}	1×10^{-1}	X

Virus	PRRS-7706 (TCID50/rxn)	PRRS-7709 (TCID50/rxn)	PRRS-0351 (TCID50/rxn)	PRRS-0383 (TCID50/rxn)	PRRS-0386 (TCID50/rxn)
North American (NVSL)	1×10^{-1}	1×10^{-2}	X	X	X

4. Assay Development Summaries

This section is organized by agent, where each agent section reports sequential assay development step in the development process. The results presented in this section are in a format that closely mirrors those presented in a recent executive summary of multiplex assay development presented to DHS and USDA on June 11 of 2007. This report section contains not only new development efforts for FY06, but also previous years development as it is applicable to the development of species-specific assay panels (Parapox, BHV, BVD, BTV, FMDV, VESV, and SVD). The order of the agents in this document are respective to their panel membership. The first 8 agents are bovine-panel agents, and the last 5 are porcine panel agents, with overlap of FMDV and VSV which belong to both assay panels. Each sections begins with an objective statement, followed by background information on the agent, then the bioinformatics and computations summary which provides detailed information on signature generation and attributes, followed by the gel and real-time PCR screening report, and lastly a section covering the multiplexed PCR assay development effort.

4.1. Section overview and general comments

Bioinformatics

For some viral agents, sequence information is limited or not publicly available, in such cases LLNL acquired sequence information through private collections or collaborative efforts. Externally developed signatures that do not follow the LLNL signature generation process were screened in silico to verify that they were, indeed, organism conserved sequences.

Gel and Real-Time PCR Assay Screening

As a conservative approach, candidate signatures developed with in silico screening proceed to wet chemistry screening utilizing standard PCR with agarose gel electrophoresis this ensures that the primers will react across strains representative of the diversity of the pathogen, but will not react with the nucleic acids of other organisms that could be present in a sample. For gel screening a subset of the vast backgrounds (soils, aerosols, prokaryotes and eukaryotes) are screened. Candidate signatures that pass gel screening are forwarded to real-time TaqMan-based PCR screening. Further testing against a large set of nucleic acid extracts from backgrounds (over 2000 samples in total), in addition to available target and near-neighbors isolates is conducted and the signatures that prove successful are characterized by dose-response titrations to determine tentative sensitivities in real-time PCR format.

Multiplexed PCR Assay Development

Signatures that pass gel and real-time PCR screening are candidates for multiplexed assays. If the number of candidate signatures is large than a subset of the available signatures are selected based on measured sensitivity and specificity observed in real-time screening. In preliminary screening the background of individual signatures is measured by running "blank" (no sample added) controls and observing signature response. In some cases a primer-to-primer non-specific interference will eliminate candidate signatures. Signatures are then added iteratively to each multiplexed panel, titrated, and with each signature addition, collectively, all signatures are re-titrated to determine if the presence of one signature will interfere with the performance of another. During this process signatures are added or subtracted from the multiplexed panel depending on individual outcomes until all desirable signatures have been added.

Upon finalization of the assay panel constituents each agent assay is characterized in multiplexed PCR format by running dose-response (titration) curves against titered virus extracted from virus infected cell culture for each available agent strain. Each sample is tested in duplicate and reported as a mean value of the median fluorescence intensity units (MFI) against agent concentration (per reaction volume, 5uL) on a double log plot. Concentration units are reported in TCID50/rxn (assumes 5uL sample volume per reaction) or pfu/rxn depending on virus type and source. In some cases where titered virus was not available data is reported as picogram units (pg). Each assay is evaluated by its ability to detect agent strains and differentiate from near neighbors (specificity) and by its range of sensitivity to each strain relative to each signature tested for that agent. Because this method is not aimed at determination of analytical sensitivities (requiring a more extensive sample set tested in clinical sample matrices which is not practical for this stage of development) it does however, provide tentative limits of detection that are used to measure the performance of each assay.

4.2. Bluetongue Virus (BTV) –Bovine Panel

Purpose: Bluetongue is an infectious, non-contagious, vector borne disease affecting cattle, sheep, and other domestic and wild ruminants. Bluetongue is considered an FMD look-alike disease. BTV has 24 known serotypes, five of which are endemic to the US (2, 10, 11, 13, and 17). Incorporating BTV signatures in a

bovine panel could allow for routine BTV diagnostic testing for US strains, surveillance for exotic strains, and embedded FAD surveillance. The BTV signatures (in combination) are intended to provide pan-serotype detection of all 24 BTV serotypes. The signatures should also differentiate BTV from FMDV. The signatures are specific for BTV and not respond to the BTV look-alike Epizootic hemorrhagic disease virus (EHDV). The BTV signature set is not intended to provide serogroup or serotype identification. The primary sample matrixes for BTV detection is bovine blood or tissue.[7]

Signature candidates: One signature, BTV_9932, was adopted from the Version 1.0 panel without change. In 2006 we re-designed additional signatures to detect the diversity of BTV. These 5 candidate assays were screened in the Bovine panel, and of these five, three were found suitable. The three new BTV signatures for consideration are BTV_0199, BTV_0205 and BTV_0207.

Signature origin: BTV_1759932 was developed by LLNL was adopted without change from the Version 1.0 panel. BTV_1810199, BTV_1810205 and BTV_1810207 were developed in 2006 as part of a collaborative effort between LLNL and USDA ARS ABADRL using published (Genbank) and unpublished (ABADRL) sequence information for segments 5 and 10. Signature screening and characterization was also performed in partnership between LLNL and USDA ABADRL.

BTV Taqman

Near-neighbor screening: Signatures were screened at USDA ABADRL against eleven EHDV isolates from all eight EHDV serotypes. Samples were dsRNA purified by LiCl precipitation of RNA extracted from virus-infected cell culture. No signature cross-reaction was observed at 200 pg of EHDV dsRNA.

Target screening: All signatures were screened at USDA ABADRL against purified dsDNA from BTV strains representing twenty-four serotypes. Samples were dsRNA purified by LiCl precipitations of RNA extracted from virus-infected cell culture. The new signatures BTV_0199, BTV_0205 and BTV_0207 responded to 24/24 serotypes tested. BTV_9932 responded to 20/24 serotypes tested; no response was observed for BTV_9932 for serotypes 16, 20, 21 and 23. Additional Taqman screening was conducted at LLNL and USDA ABARDL to determine qualitative limits of detection for each signature.

[7] Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, 5th edition, 2004, Part 2 Section 2.1., Chapter 2.1.9. (http://www.oie.int/eng/normes/MMANUAL/A_00032.htm)

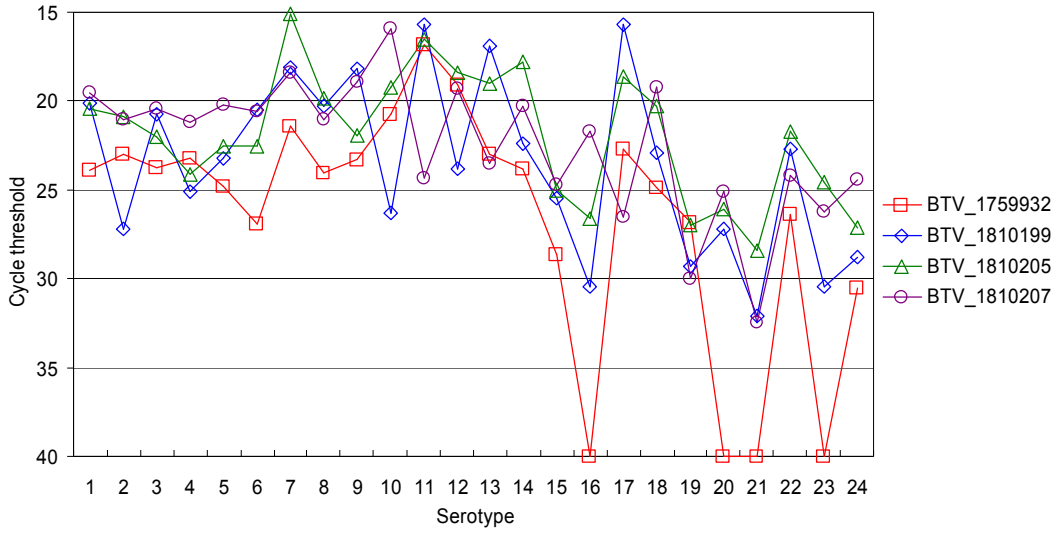

FIG. 16 Taqman screening of BTV signatures against isolates representing twenty-four BTV serotypes. Testing was conducted at UDSA ABARDL (University of Wyoming). Samples were dsRNA purified by LiCl precipitations of RNA extracted (Pure Script, Gentra Systems) from virus-infected cell culture. Each reaction was spiked with 200 pg of template. Each point represents the mean (n=3) cycle threshold. If no cycle threshold was reached (i.e. infinite cycle threshold), a cycle threshold of 40 was assigned in this plot.

BTV Multiplex

Near-neighbor screening: In Version 1.0, BTV_9932 did not respond to nucleic acid (200 pg/rxn) extracted from three EHD-1 serotypes (isolates Georgia, New Jersey, Santa Barbara) and EHD-2 serotype Alberta. In the Bovine panel the BTV assays were screened against the same 4 isolates of EHD and did not cross-react.

Target screening: The four signatures have consistently low background response in the absence of template in the current Bovine panel. The Bovine panel was screened against five US strains of BTV (2, 10, 11, 13 and 17). The signatures responded over a wide concentration. BTV_9932 generated a stronger response to BTV serotypes 2 and 10, compared to the three newly developed signatures. Testing conducted at USDA ABARDL was done using BTV strains different to those used for at LLNL.

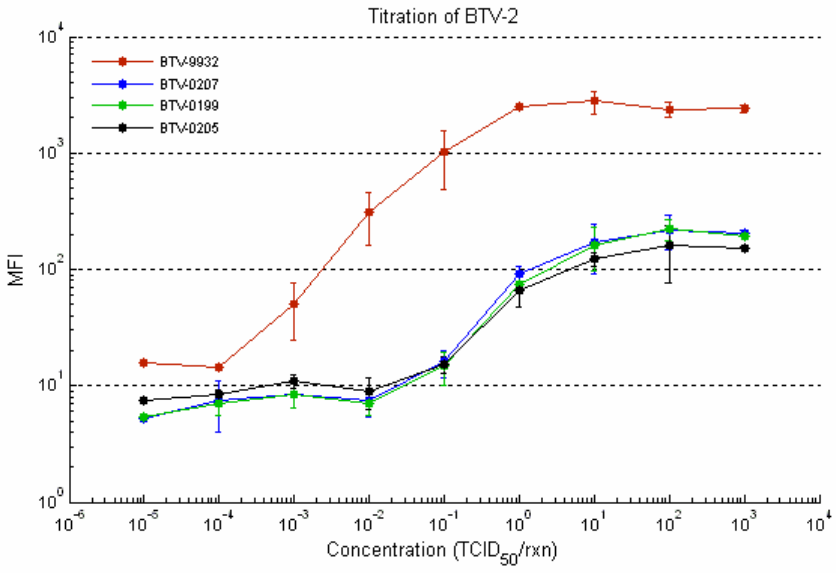

FIG. 17 Multiplex screening data for the four BTV signatures against extracted nucleic acids from isolate BTV-2. Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture. Each point represents the mean response (n=2). Error bars indicate ±1 σ of the mean.

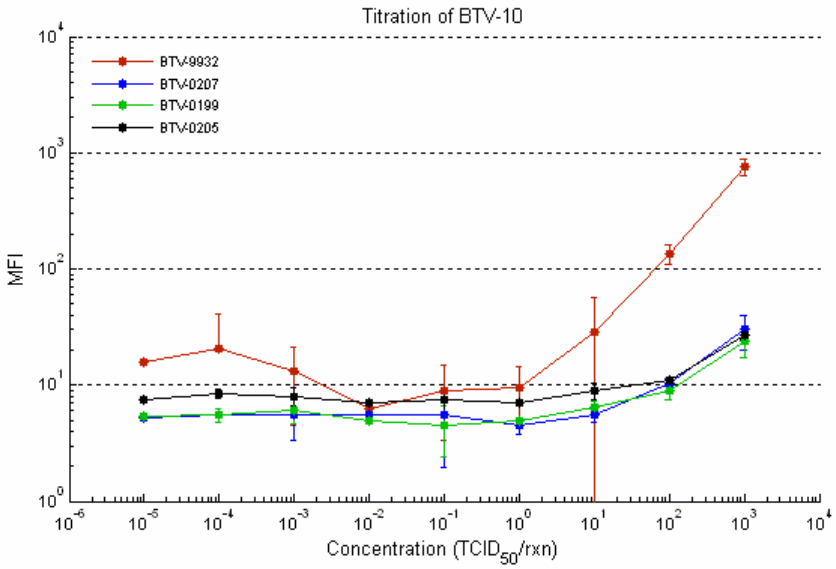

FIG. 18 Multiplex screening data for the four BTV signatures against extracted nucleic acids from isolate BTV-10. Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture. Each point represents the mean response (n=2). Error bars indicate ±1 σ of the mean.

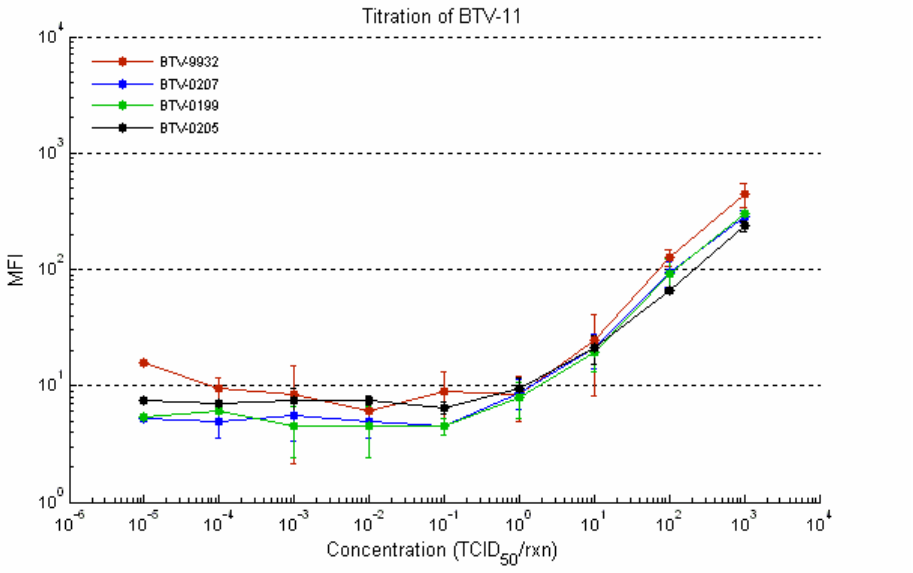

FIG. 19 Multiplex screening data for the four BTV signatures against extracted nucleic acids from isolate BTV-11. Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture. Each point represents the mean response (n=2). Error bars indicate ±1 σ of the mean.

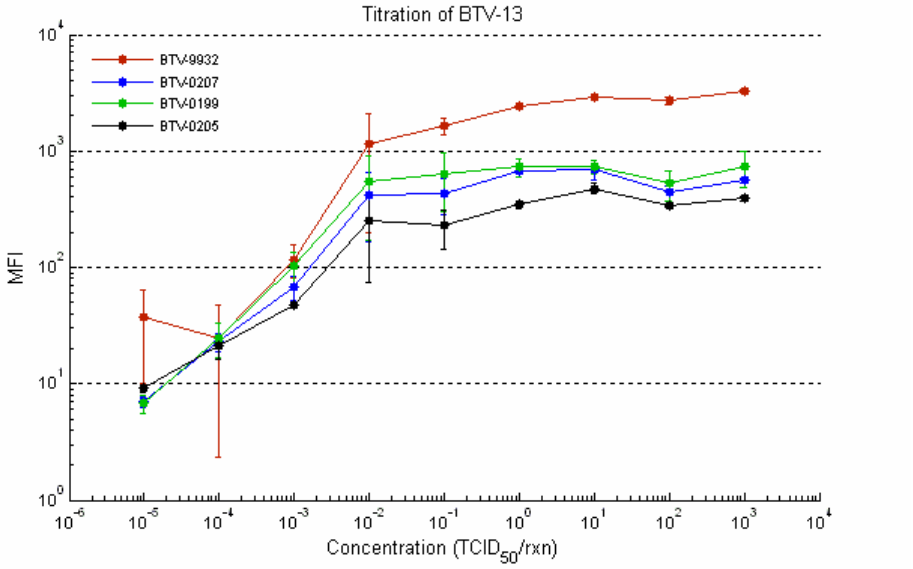

FIG. 20 Multiplex screening data for the four BTV signatures against extracted nucleic acids from isolate BTV-13. Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture. Each point represents the mean response (n=2). Error bars indicate ±1 σ of the mean.

Summary: The BTV signatures together demonstrated promising performance in both Taqman and multiplex formats. The four signatures have the potential to provide pan-serotype detection capability for BTV and potentially laboratory differentiation from foot-and-mouth disease within a multiplex panel. Further screening of the multiplex panel against a reference panel of diverse BTV isolates is required. The continuation, and possible expansion, of the collaborative effort that led to BTV signature generation and preliminary

characterization could accelerate their evaluation against a comprehensive set of clinical samples in the Bovine multiplex panel.

4.3. Bovine Herpes Virus (BHV) – Bovine Panel

Purpose: Bovine herpesvirus-1 (BHV-1) is associated with infectious bovine rhinotracheitis (IBR) which is considered and FMD look-alike disease in cattle. Incorporating BHV signatures in a Bovine panel could potentially allow for embedded FAD surveillance whilst testing for an endemic disease of cattle. The BHV signatures should detect and differentiate BHV from FMDV in bovine samples including oral swabs.[8]

Signature candidates: The two signatures available for consideration are BHV_1 and BHV_3. These two signatures were adopted from the Version 1.0 panel without change.

Signature origin: Signatures were designed at LLNL using one complete genome[9] for the BHV-1.1 subtype (respiratory subtype). The two signatures target two different genes[10] for glycoproteins. The BHV-1 signature targets the glycoprotein C gene and the BHV-3 signature targets the glycoprotein B gene.

BHV Taqman

Near-neighbor screening: The signatures did not respond to twenty near-neighbors including Rhadinovirus Caprine Herpes-2, V. Pseudorabies, Psuedorabies Shope, Equine Herpesvirus-1, Equine Herpesvirus-2, Feline herpes and Bovine Herpesvirus-5.[11]

Target screening: Ten BHV- isolates[12] were screened and both signatures responded to 10/10 isolates and did not cross-react with any of the above near neighbors.

[8] Following infection, nasal viral shedding can be detected for 10-14 days, with peak titres of 10^8-10^{10} $TCID_{50}$/ml of nasal secretion. Source: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, 5th edition, 2004, Part 2 Section 2.3, Chapter 2.3.5.
[9] GI Number: 9629818/NC_001847.1
[10] Gene ID: UL43/1487394 (BHV-1); UL43/1487382 (BHV-3)
[11] Materials obtained from the California Animal Health and Food Safety (CAHFS) Laboratory, Davis, CA and ATCC as nucleic acids extracted from virus-infected cell culture.
[12] Isolates were from ATCC, NVSL and the California Animal Health and Food Safety (CAHFS) Laboratory, Davis, CA.

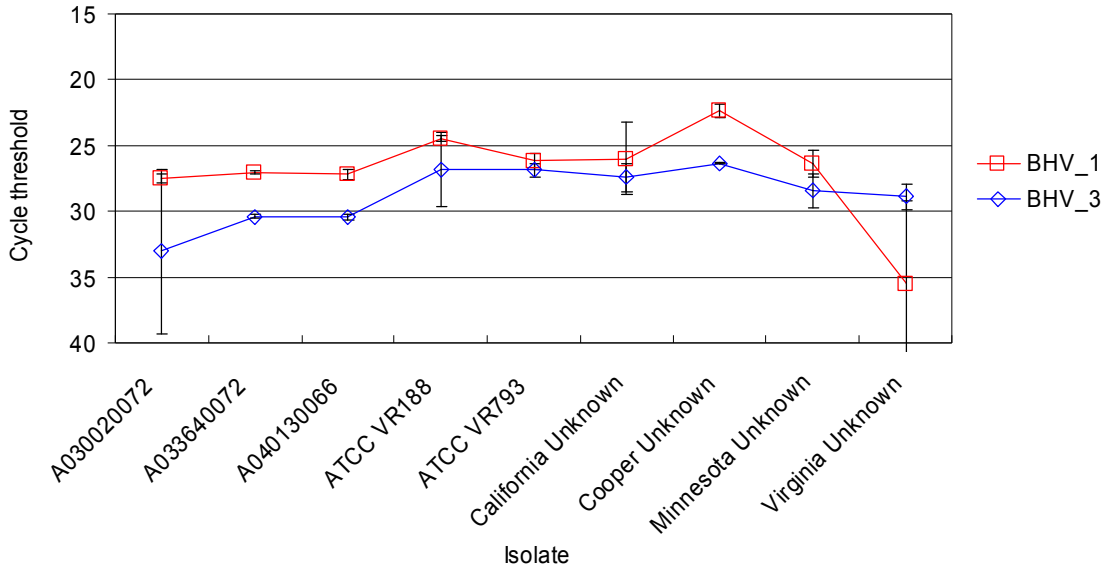

FIG. 21 Taqman screening of BHV signatures against ten isolates of BHV-1 subtype. DNA extracted from virus-infected cell culture media was used as a template. Each point represent the mean (n=3) cycle threshold. Error bars indicate ±1σ of the mean.

BHV Multiplex

Near-neighbor screening: The signatures in multiplex (Version 1.0 panel and Bovine panel) did not generate responses above background when screened against near-neighbors including Rhadinovirus Caprine Herpes-2, V. Pseudorabies, Pseudorabies Shope, Equine Herpesvirus-1, Equine Herpesvirus-2, Feline herpes and Bovine Herpesvirus-5. These results agree with those obtained during Taqman screening.

Target screening: Both signatures were added to the current Bovine panel where they generate consistently low background response in the absence of template. These results compare to results from the Version 1.0 panel.

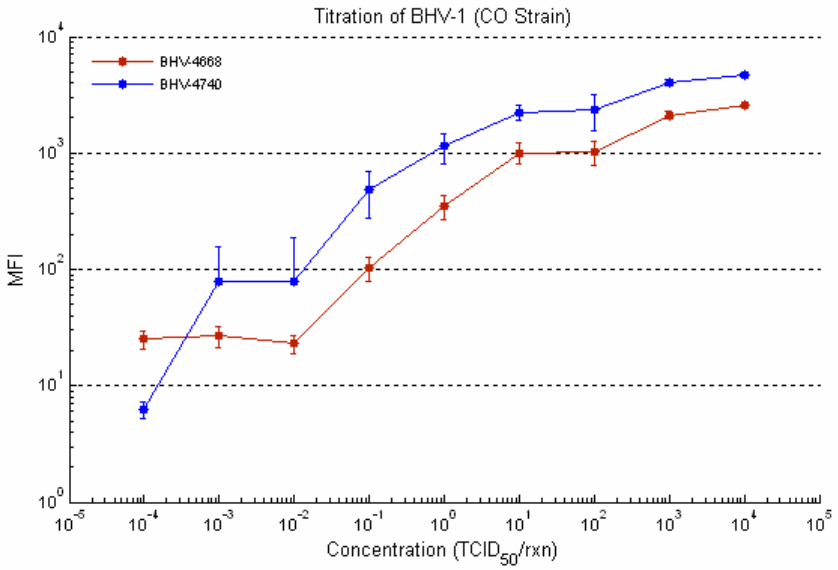

FIG. 22 Multiplex screening data for the two BHV signatures against extracted nucleic acids from isolate BHV-Colorado vaccine strain. Serial dilution of nucleic acid extracted with phenol chloroform virus-infected cell culture media then used as template. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1σ of the mean.

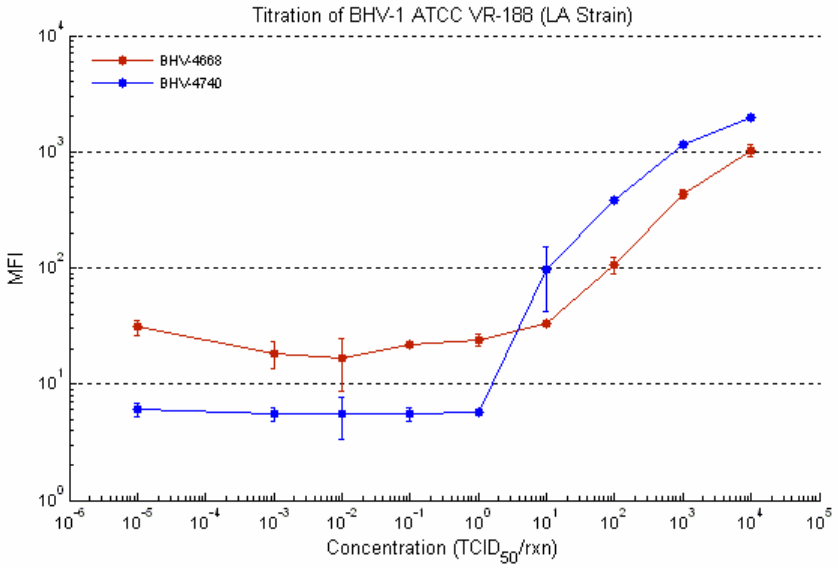

FIG. 23 Multiplex screening data for the two BHV signatures against extracted nucleic acids from isolate BHV-1 ATCC VR188 (LA strain) Serial dilution of nucleic acid extracted with phenol chloroform virus-infected cell culture media then used as template. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1σ of the mean.

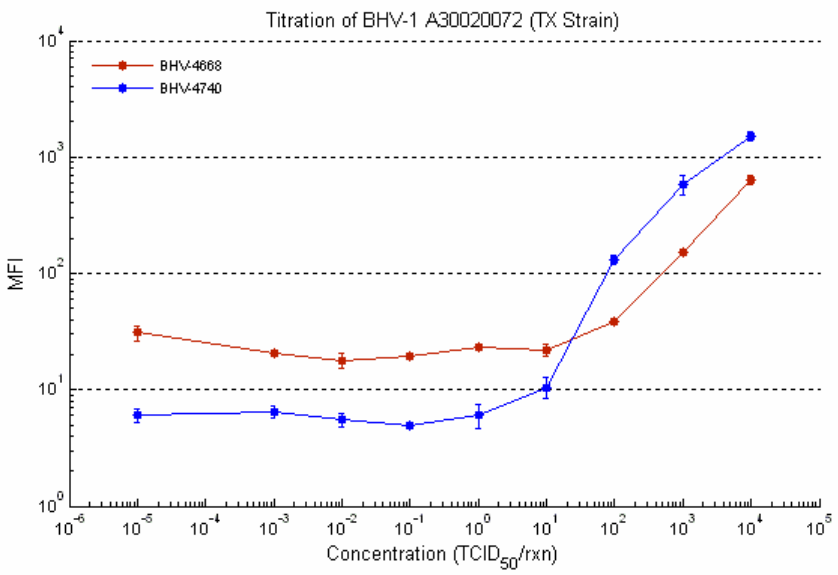

FIG. 24 Multiplex screening data for the two BHV signatures against extracted nucleic acids from isolate BHV-1 Texas strain A30020072. Serial dilution of nucleic acid extracted with phenol chloroform virus-infected cell culture media then used as template. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1σ of the mean.

Summary: The two Version 1.0 panel signatures were re-screened for the bovine panel. The two BHV signatures BHV_1 and BHV_3 respond to the target when incorporated into the current Bovine panel. The near-neighbor testing conducted to date indicates that both signatures do not respond to genetic near-neighbors. The signature response indicates that the signatures should be capable of detecting BHV-1 virus in clinical samples and potentially laboratory differentiation from foot-and-mouth disease within a multiplex panel.

4.4. Parapox (PPOX) Virus –Bovine Panel

Purpose: Incorporating signatures for parapox viruses, including pseudocowpox virus, and bovine papular Stomatitis virus, in a Bovine panel could potentially allow for embedded FAD surveillance whilst conducting routine diagnostic testing for an endemic domestic disease. These signatures should detect and differentiate parapoxviruses from FMDV in bovine samples including oral/nasal/ swab in viral transport medium, vesicular fluid, and epithelial tissue.

Signature candidates: Three signatures were adopted from the Version 1.0 panel without change, including PPOX_1, PPOX_4 and PPOX_5.

Signature origin: Signatures were designed at LLNL using a four genome alignment[13] each varying in size from 134,000-137,000bp. The PPOX_1 signature targets the ORF108 DNA packaging protein ATPase gene with similarities to Vaccinia strain Copenhagen A32L and Molluscum contagiosum virus MC140L. The PPOX_2 signature targets the ORF025 DNA polymerase. The PPOX_4 signature targets the ORF083 DNA early transcription factor VETFL gene.

[13] GI Numbers for alignment: 32167392, 40019122, 40019123, 40019124.

Parapox Taqman

Near-neighbor screening: Seven near-neighbor isolates of goatpox (V717 Pendik, Held LT5, Nigerian G165), sheeppox (Kenyan LT1, Vaccine 0-180 15LK V2164 and X783 6LT) were screened found to not cross react with any of the signatures.

Target screening: Thirty-seven parapoxvirus isolates[14] were screened. Signatures PPOX-1 and PPOX-4 detected 36/37 isolates, while PPOX_2 detected 33/37.

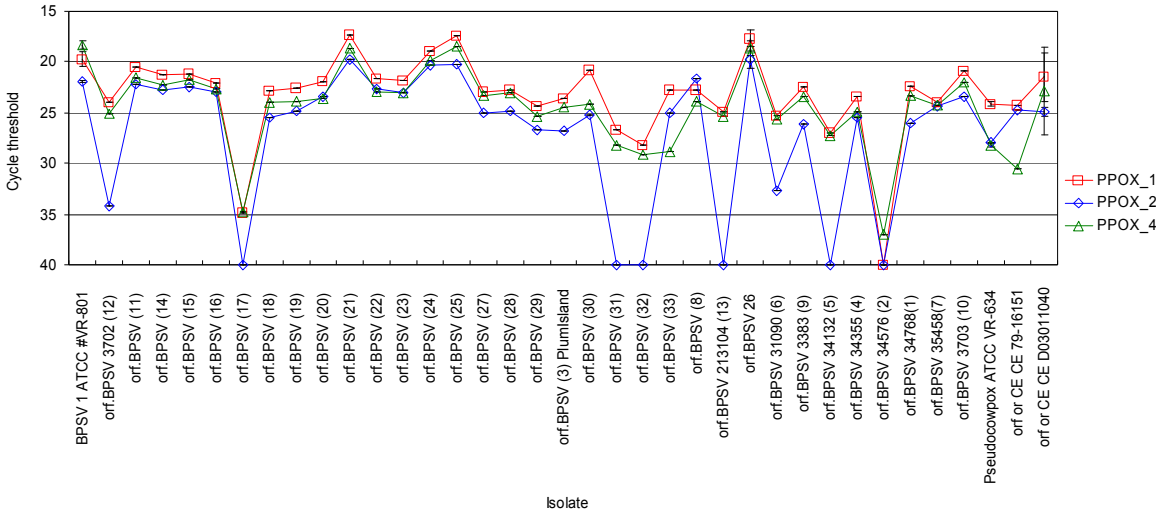

FIG. 25 Taqman screening of Parapox signatures against thirty-seven isolates of parapoxvirus. Purified nucleic acid was used as a template. Each reaction was spike with 200 pg of DNA. Each point represents the mean (n=3) cycle threshold. If no cycle threshold was reached (i.e. infinite cycle threshold), a cycle threshold of 40 was assigned in this plot.

Parapox Multiplex

Near-neighbor screening: The Parapox signatures were screened against 18 near-neighbor viruses including Rhadinovirus Caprine Herpes-2, V. Pseudorabies, Pseudorabies Shope, Equine Herpesvirus-1, Equine Herpesvirus-2, Feline herpes, Fowl pox and Bovine Herpesvirus-5. None of the Parapox signatures cross-reacted with any of the near-neighbors screened.

Target screening: During testing of the diagnostic accuracy testing of the FMD signatures in the Version 1.0 panel at the Institute for Animal Health, Pirbright, parapox virus was detected in suspect FMDV clinical samples submissions (confirmed negative for FMDV).

[14] Isolates were from the Foreign Animal Disease Diagnostic Laboratory at the Plum Island Animal Disease Center (USDA APHIS).

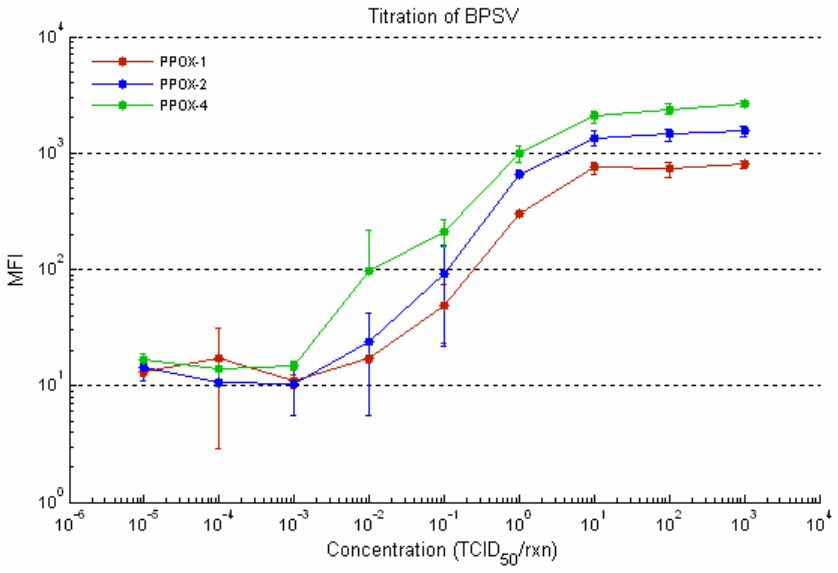

FIG. 26 Multiplex screening data for the three PPOX signatures against extracted nucleic acids from isolate PPOX-Texas A&M strain. Serial dilution of nucleic acid extracted with phenol chloroform virus-infected cell culture media then used as template. nucleic acid was extracted with phenol chloroform virus-infected cell culture media then used as template. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1σ of the mean.

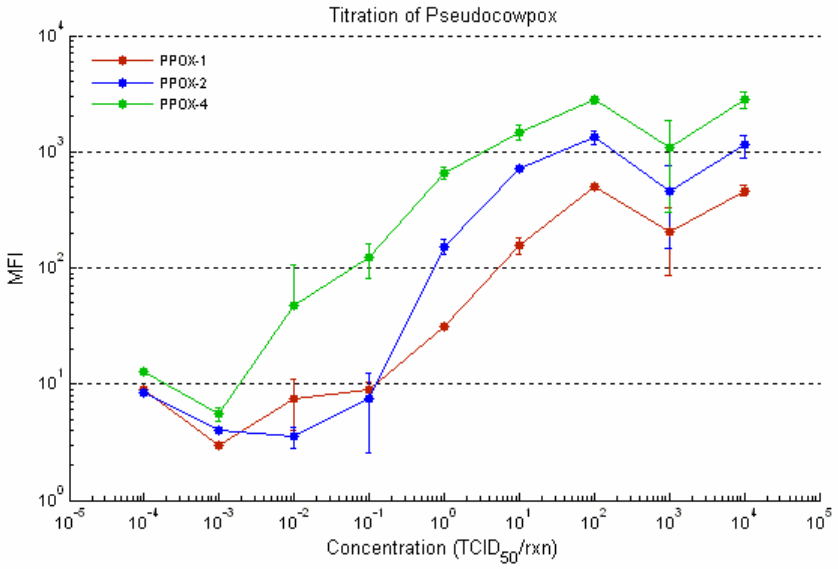

FIG. 27 Multiplex screening data for the three PPOX signatures against extracted nucleic acids from isolate Pseudocowpox (NVSL). Serial dilution of nucleic acid extracted with phenol chloroform virus-infected cell culture media then used as template. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1σ of the mean.

Summary: The three Version 1.0 panel signatures were screened in the bovine panel and results indicate that these signatures will work well with the new constituents that comprise the Bovine panel. Additional screening of the PPOX signatures against the parapoxvirus pseudowcowpox provided further information to characterize the performance of these assays. The three Parapox signatures performed well, responding equivalently to target

material over a wide range of concentrations when incorporated into the current Bovine multiplex panel. Together the signatures provide detection capability for Bovine Papular Stomatitis, and Pseudocowpox and potentially laboratory differentiation from foot-and-mouth disease within a multiplex panel.

4.5. Bovine Viral Diarrhea (BVD) Virus –Bovine Panel

Purpose: BVDV causes disease in cattle of all ages and is closely related to classical swine fever and ovine Border disease viruses. Incorporating BVDV signatures in a bovine panel could potentially allow for embedded FAD surveillance whilst testing for and endemic disease of cattle. These signatures should detect BVDV genotypes 1 and 2 and differentiate BVDV from FMDV in bovine samples. Candidate sample matrices for the detection of BVDV RNA include a nasal swab in viral transport medium, epithelial tissue, buffy coat cells, whole blood, washed leukocytes or serum and ear notches.

Signature candidates: Two signatures, BVD_1a and BVD_2, are available for consideration. BVD_1a was adopted from the Version 1.0 panel without change. BVD_2 is a redesigned version of BVD_1a. The BVD_2 forward primer includes 2 nucleotide substitutions, reverse primer was shifted 2 nucleotides, and the probe uses the same sequence except for 1 inosine substitution.

Signature origin: BVD primers designed by Ridpath et al.[15] were adapted to Taqman format by Faaberg et al.,[16] and subsequently modified by scientists at the California Animal Health and Food Safety Laboratories.[17] The CAHFS version of the Taqman signature was adapted to the Luminex multiplex format.

BVDV Taqman

Target screening: No TaqMan screening was run on the BVDV signatures as the location of the probe is not ameneable to TaqMan assay testing. The Taqman signature published by Faaberg et al. detected BVDV Type I and II, and border disease virus.

BVDV Multiplex

Near-neighbor screening: In the Version 1.0 panel, BVD_1a signature responded to high concentrations of Border Disease Virus (BDV) Aveyron and Frijters strains but not 137-7.[18] Also in the Version 1.0 panel, BVD_1a signature responded to Classical Swine Fever Virus (CSFV) Brescia ($\geq$550 $TCID_{50}$/mL) and Paderborn ($\geq$6550 $TCID_{50}$/mL) but not Kanagawa. In the Bovine panal the same near-neighbors are pending screening at PIADC. One isolate of Border Disease virus has been screened at LLNL against the BVD signatures and no cross-reactions were seen.

Target screening: Titration curves were acquired for the BVD_1a signatures in the Version 1.0 panel (data not shown). Each titration series was constructed from serially diluted samples of titered virus-infected cell culture

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[15] Rev. Sci. Tech. Off. Int. Epizoot., 1998, 17, 733-742
[16] J. Vet. Diagn. Invest., 2002, I, 120-125
[17] Developed by Dr Hietala and colleagues at CAHFS and shared with LLNL as part of this collaborative effort.
[18] Federal Research Centre of Virus Diseases of Animals, Riems Island, Germany. Concentration of material is presently unknown; the signature response increased above background for Averyron and Frijters strains at 10,000 and 10-fold dilution of the original samples, respectively.

for both BVDV genotypes[19] that were subsequently extracted (Ambion MagMax 96). In general, the BVD_1a signature response was stronger for lower concentrations of BVDV Type 1 compared to Type 2. To improve the response of a candidate Bovine multiplex panel to BVD Type 2 strains, the BVD_1a signature was modified leading to the generation of an additional signature BVD_2. Signature responses were compared using three developmental Bovine panels that included either BVD-1a only, BVD-2 only, or BVD_1a and BVD_2 in combination. The data shows that the BVD-1a LOD for BVD Type 2 strains improved when BVD_2 was present in the multiplex. However, the new BVD_2 signature alone performed as well as, or better than the BVD_1a signature alone or in combination. No signature combination responded to the near-neighbor BDV (Coos Bay #4, 4-6-92).

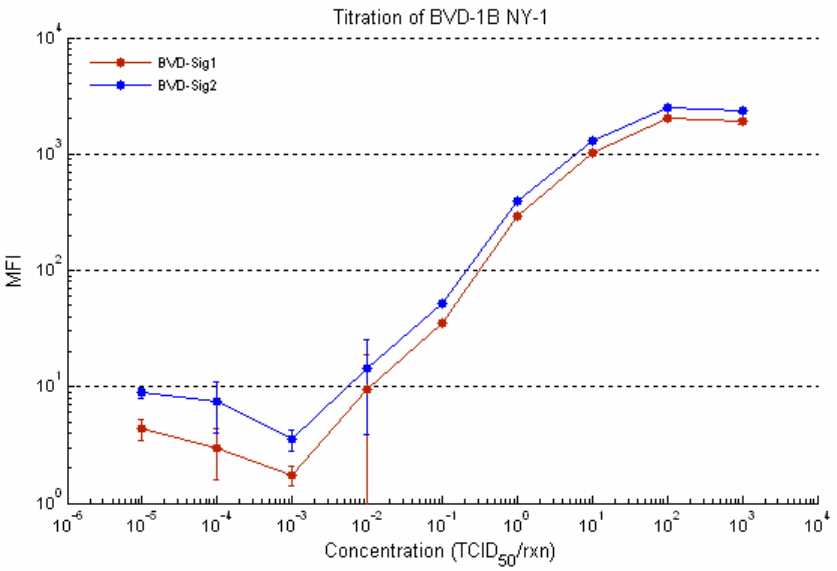


FIG. 28 Multiplex screening data for the two BVDV signatures against extracted nucleic acids from isolate BVD-1B NY-1. Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1σ of the mean.

[19] Testing was conducted at LLNL using a panel of blinded samples kindly provided by National Animal Disease Center (USDA ARS, Ames IA).

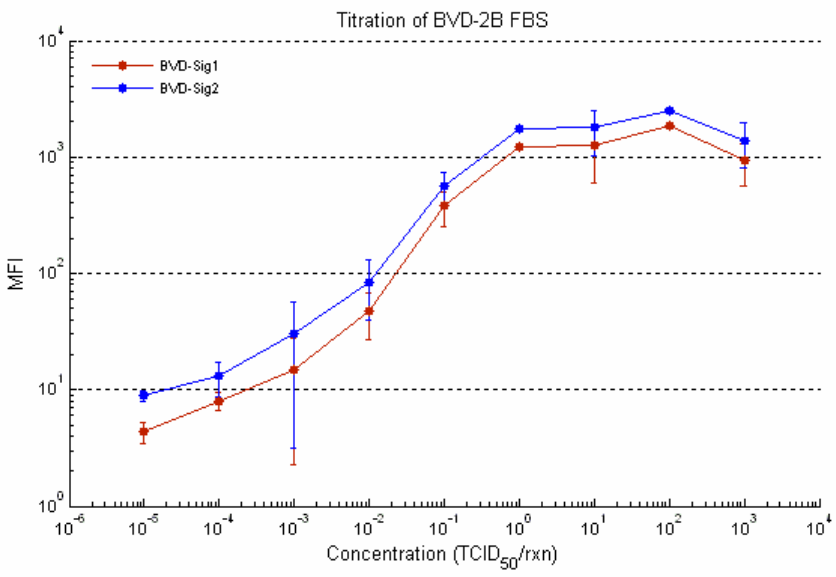


FIG. 29 Multiplex screening data for the two BVDV signatures against extracted nucleic acids from isolate BVD-2B FBS. Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1σ of the mean.

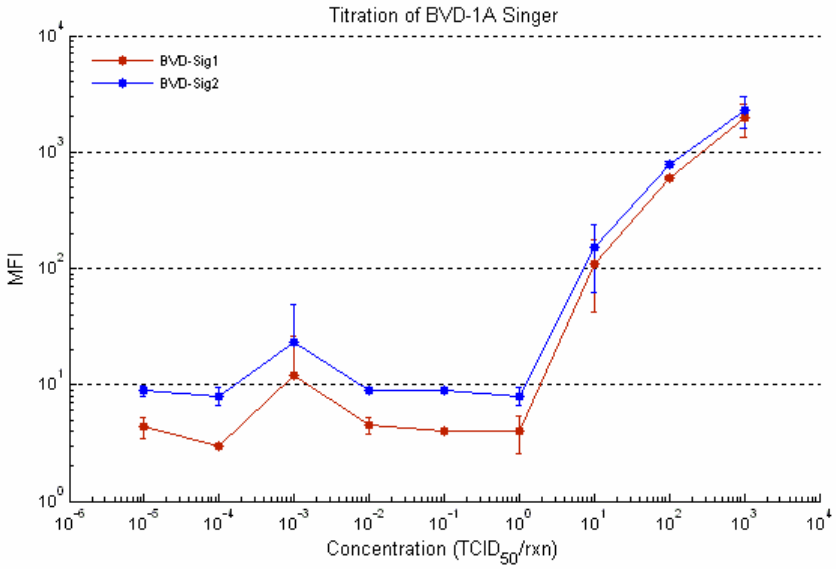


FIG. 30 Multiplex screening data for the two BVDV signatures against extracted nucleic acids from isolate BVD-1A Singer. Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1σ of the mean.

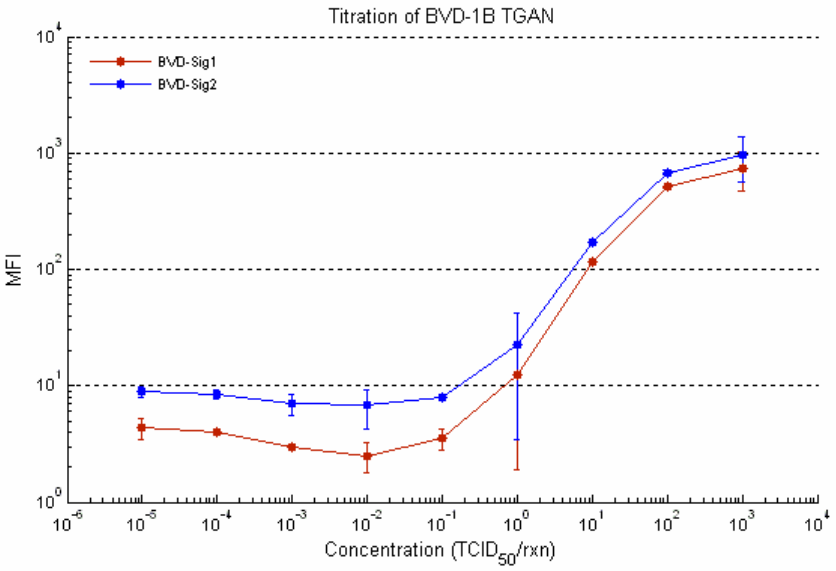


FIG. 31 Multiplex screening data for the two BVDV signatures against extracted nucleic acids from isolate BVD-1B TGAN. Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1σ of the mean.

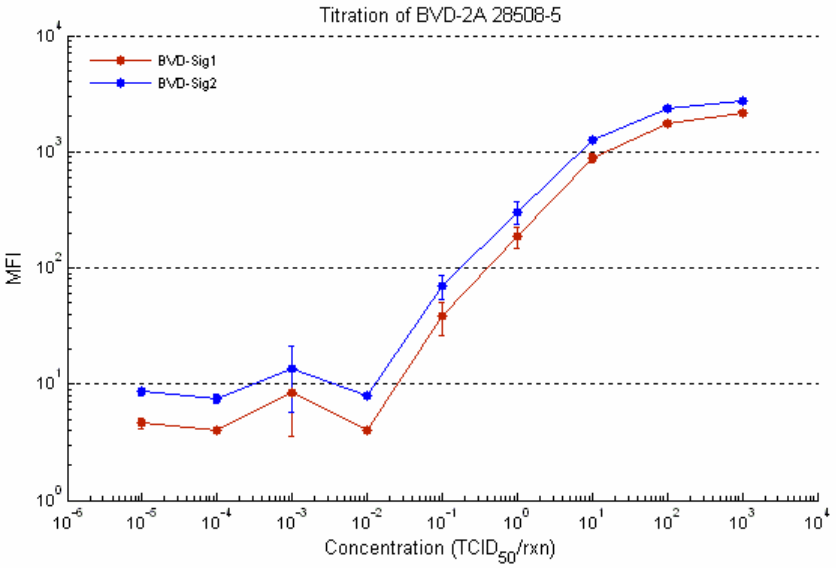


FIG. 32 Multiplex screening data for the two BVDV signatures against extracted nucleic acids from isolate BVD-2A 28508-5. Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1σ of the mean.

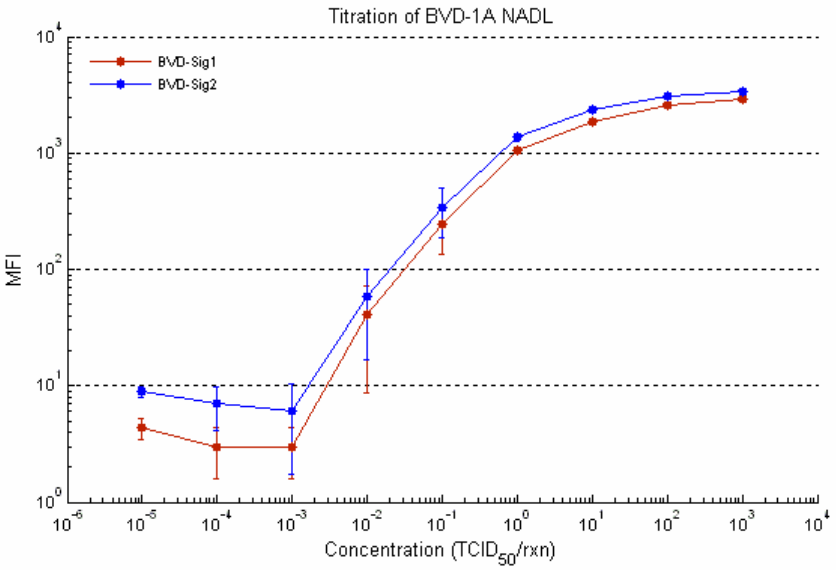


FIG. 33 Multiplex screening data for the two BVDV signatures against extracted nucleic acids from isolate BVD-1A NADL. Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture.. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1σ of the mean.

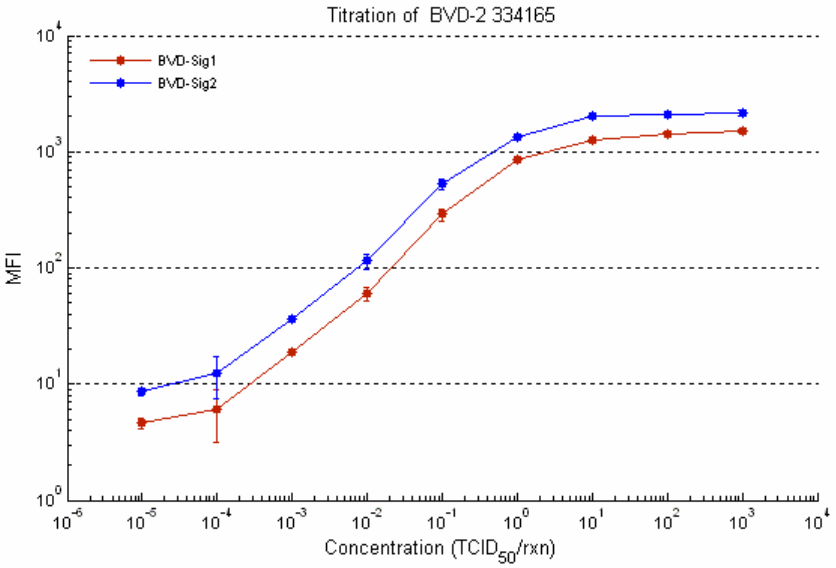


FIG. 34 Multiplex screening data for the two BVDV signatures against extracted nucleic acids from isolate BVD-2 334165. Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1σ of the mean.

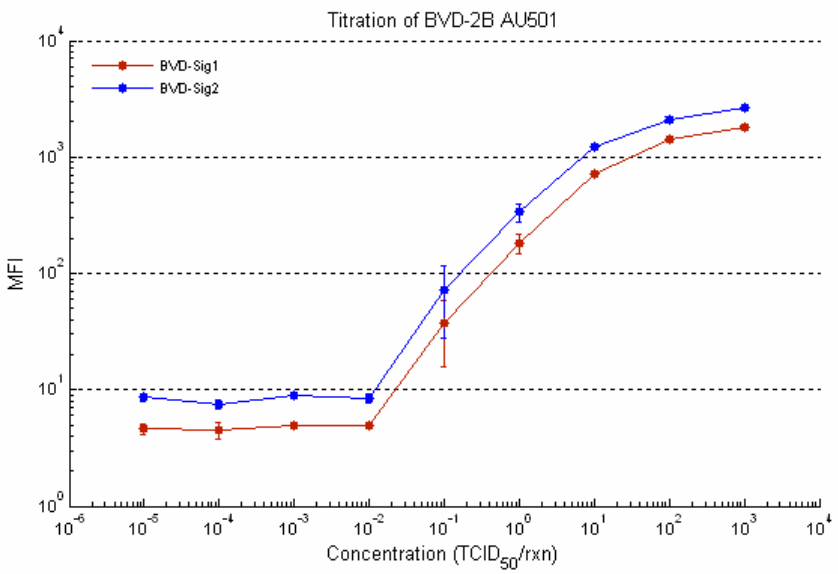


FIG. 35 Multiplex screening data for the two BVDV signatures against extracted nucleic acids from isolate BVD-2B AU501. Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1σ of the mean.

Summary: To improve the response of a candidate Bovine multiplex panel to BVD Type 2 strains, the BVD_1a signature was modified leading to the generation of an additional signature BVD_2. The new BVD_2 signature alone or in combination with BVD _1a when added to the Bovine panel provides better coverage for BVDV Type 2. Additional target and near-neighbor screening of the current Bovine panel is underway. This testing includes seven titered genotypes or subgenotypes of target (BVDV 1a, two strains of BVDV 1b, BVDV 2, BVDV 2a and two strains of BVDV 2b) and two near-neighbors (CSF, BDV).

4.6. Malignant Catarrhal Fever Virus (MCF) – Bovine Panel

Purpose: Malignant catarrhal fever (MCF) is viral disease of ruminants and rarely swine presenting as a variable complex of lesions making it an FMD look-alike disease. Incorporating MCF signatures in a Bovine panel could potentially allow for embedded FAD surveillance. The disease can be caused by two gammaherpesviruses. Wildebeest are the natural hosts of alcelaphine herpesvirus-1 (AlHV-1) whereas ovine herpesvirus-2 (OvHV-2) is prevalent in sheep and goats. These signatures are intended to detect AlHV-1 DNA to confirm clinical disease and simultaneously differentiate AlHV-1 associated MCF from FMD in bovine samples including cell suspensions of either of peripheral blood leukocytes, lymph nodes or other affected tissues and nasal swabs.

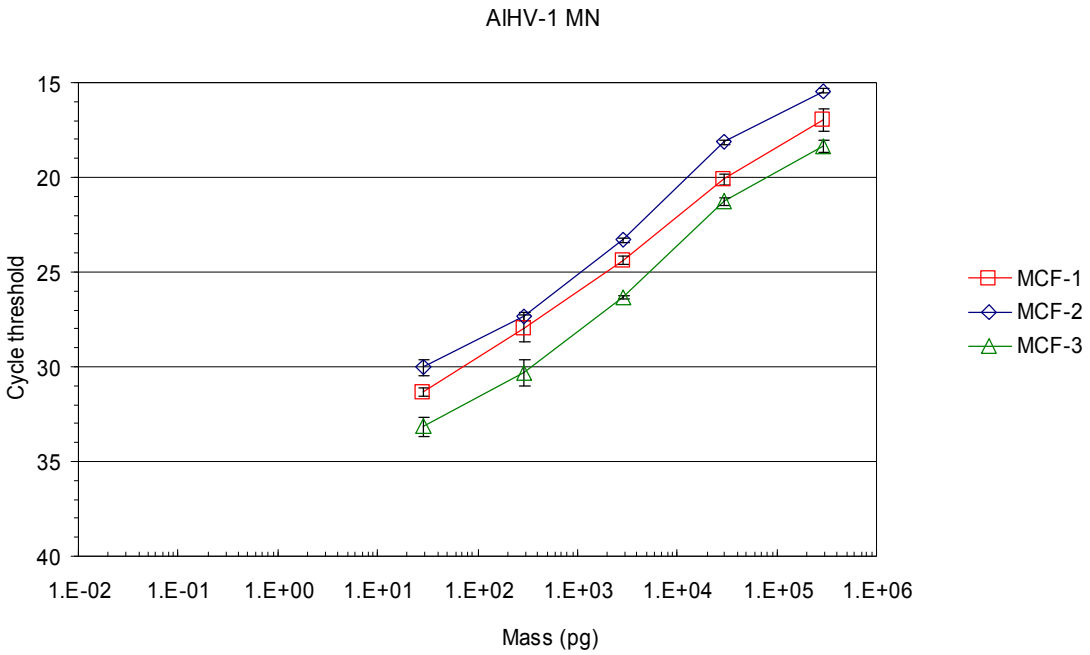
Signature candidates: The bioinformatics group generated 1108 candidate signatures; 200 of these went to bench screening, however this was down-selected to 40 that were screened in gel-PCR format. Of this 40, 5 were moved forward into Real-time PCR assay format and all of these 5 were forwarded to multiplexed assay development. These signatures were further down-selected in multiplexed and the remaining 3 were tested in multiplexed PCR format. Three new signatures are available for consideration, including MCF-1, MCF-2 and MCF-3.

Signature origin: Signatures were designed at LLNL using one complete genome.[20] All three signatures target different genes. MCF-1 targets the DNA polymerase gene ORF09. MCF-2 was not found to be associated with a known gene region. MCF-3 targets the putative major envelope glycoprotein gene ORF68.[21]

MCF Taqman

Near-neighbor screening: MCF was screened against 15 near neighbor viruses; several different Herpes viruses and a few isolates of pseudorabies virus (refer to TABLE 8). No cross-reactions were observed with any of the MCF signatures and the near-neighbors tested (data not shown).

Target screening: The three signatures were screened against AlHV-1(MN), AlHV-1 (WC11, an attenuated isolate) and OvHV-2[22] over a wide concentration range. All signatures responded well to AlHV-1 as shown below. The signatures did not respond to the OvHV-2 strain. At the time the signatures were generated, no complete genome sequence information was available for OvHV-2.



[20] GI Number: 10140926/NC_002531.1, Sequence length 130608 base pairs, K-path ID 13760 S Segment.
[21] Gene ID: AlHVIgp10/911748 (MCF-1); no gene associated (MCF-2); AlHVIgp66/911771 (MCF-3).
[22] AlHV-1 MN and OvHV-2 isolates were provided by Hong Li at Washington State University. The WC11 isolate was from the Foreign Animal Disease Diagnostic Laboratory (FADDL) at PIADC.

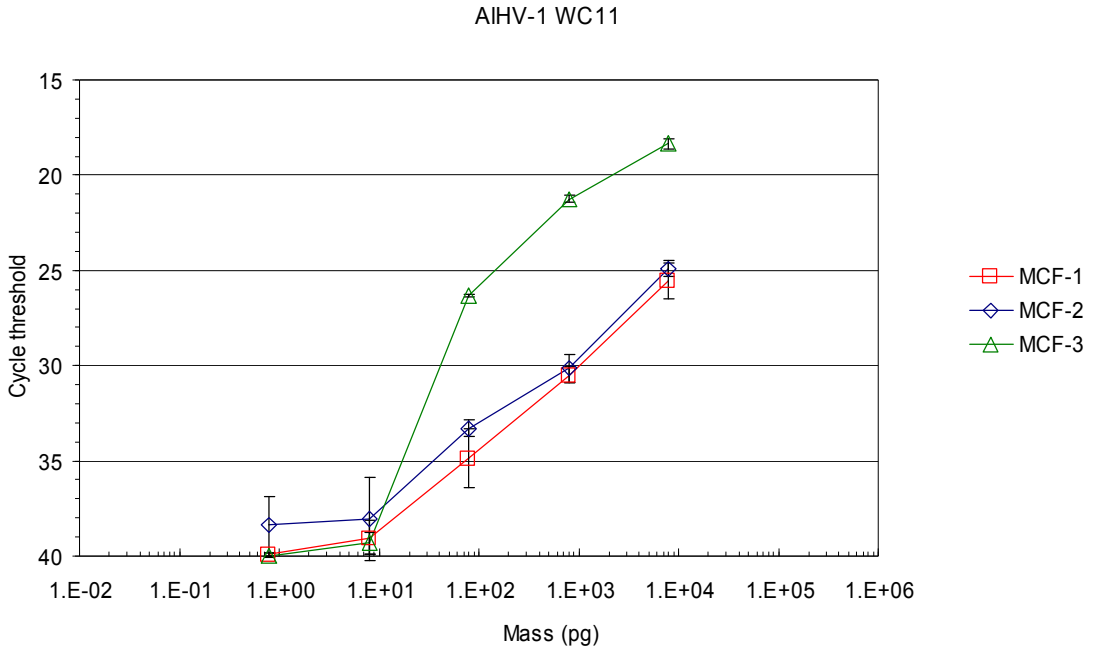


FIG. 36 Taqman screening of MCF signatures against AlHV-1 (MN, WC11). Total nucleic acid extracted (Ambion MagMax) from virus-infected cell culture media was used as template. Each point represents the mean response (n=3). Error bars indicate ±1σ of the mean. If no cycle threshold was reached (i.e. infinite cycle threshold), a cycle threshold of 40 was assigned in this plot.

MCF Multiplex

Near-neighbor screening: In the Bovine panel near neighbor screening was conducted against several herpes virus isolates and three pseudorabies isolates, totaling over 30 different virus (see appendix III). None of the near-neighbors cross-reacted with the MCF signatures.

Target screening: All three signatures were added to current Bovine panel where they show low background response in the absence of template. The same samples used during the TaqMan screening phase were used to conduct multiplex screening using a **developmental version** of the Bovine panel[23]. Testing of MCF signatures in the final bovine multiplex is in process at PIADC. The multiplex titration results show that all three signatures responded similarly to both AlHV-1 strains. The results obtained in Taqman and multiplex formats agreed over the concentration ranges tested. Likewise, the three signatures did not respond to OvHV-2 DNA (6.4 ng) in multiplex format (data not shown).

[23] Signatures in the developmental panel included MCF-1,2,3,4; BHV-1,3; PPOX-1,2,4; FMDV-TC,-Pir; BVD-1a; BTV-2,3

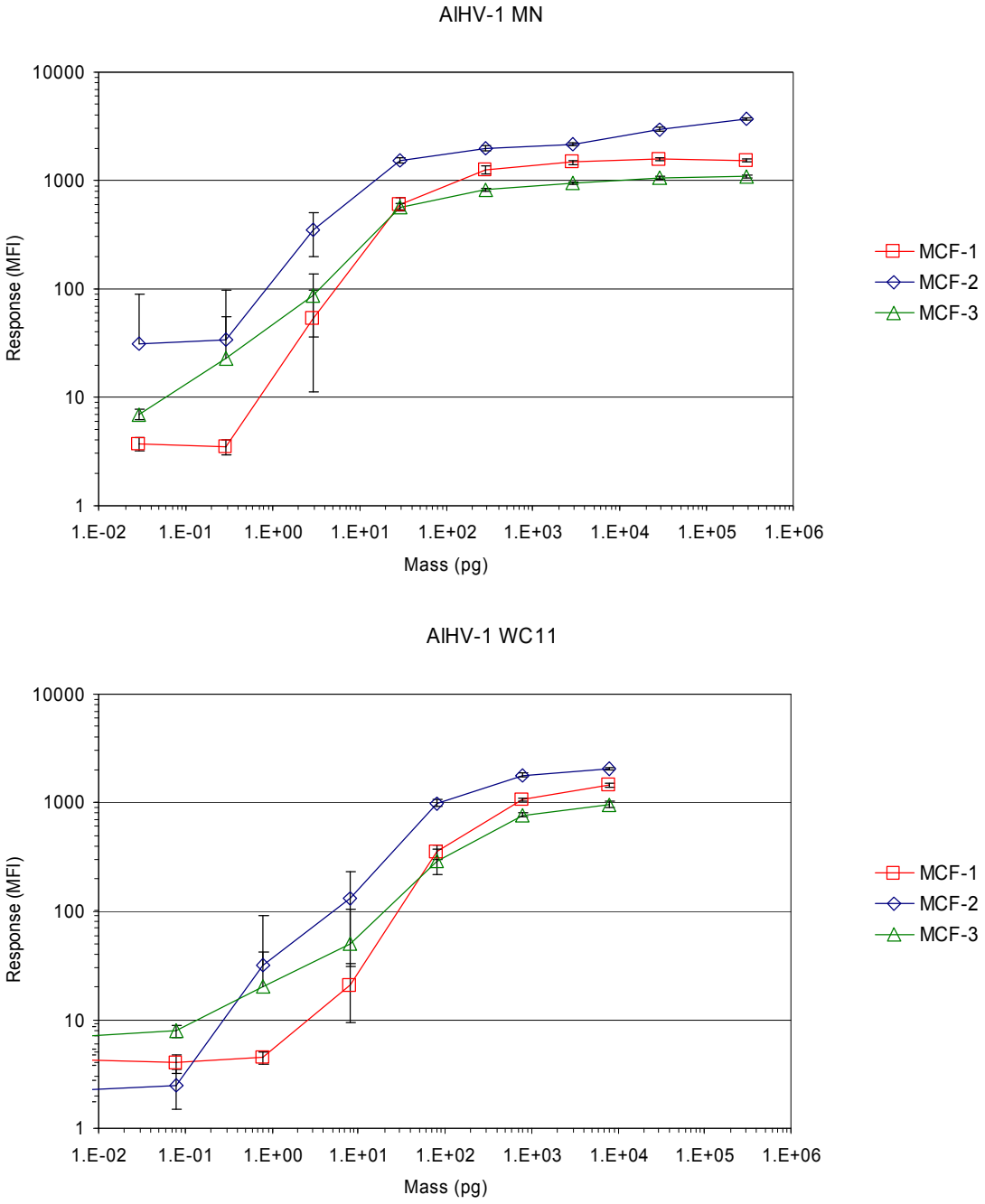


FIG. 37 Multiplex screening data for the three MCF signatures against AIHV-1 (MN, WC11) using a **developmental version** of the Bovine Panel. Serial dilution of nucleic acid extracted (Ambion MagMax 96) from virus-infected cell culture. Each point represents the mean response (n=4). Error bars indicate ±1σ of the mean.

Summary: All three signatures perform equivalently against the limited strains of material available during the development phase. The data indicates that all three MCF signatures could be incorporated into a Bovine panel. If detection of OvHV-2 is desired, the data presented suggests that additional signature development would be required. Further screening is in process at PIADC to characterize the MCF assays.

4.7. Rinderpest Virus (RPV) –Bovine Panel

Purpose: Rinderpest is a viral foreign animal disease of cattle and other species including wildlife and is considered an FMD-look-alike disease. Rinderpest is also clinically indistinguishable from BVD and MCF. In the multiplex panel the RPV signatures should detect RPV for laboratory diagnosis and differentiation from FMDV, BVD and MCF in bovine whole blood, but potentially other tissue samples.[24]

Signature candidates: The bioinformatics group generated 12 candidate signatures; 9 of these went to bench screening, and 3 were down-selected in multiplexed screening. Three new RPV signature candidates are available for consideration. RPV signatures were not included in the Version 1.0 panel. The signatures are designated RPV_4853, RPV_4855 and RPV_4856.

Signature origin: Signatures developed by LLNL using one published genome[25] and five unpublished genomes.[26] RPV_4853 targets the nucleocapsid (N) protein[27] RPV_4855 and RPV_4856 both target the polymerase (L protein) gene[28].

RPV Taqman

Near-neighbor screening: Signatures were screened at PIADC against five Peste de Petits Ruminants virus (PPRV) strains (RCA, Burkina Faso, Egypt 87, Dorcas and Ghana 76/1) at 200 pg (n=3) using extracted RNA template from virus-infected cell culture (Qiagen RNeasy Mini kit). No cross-reactivity was observed.

Target screening: All signatures were screened against nine RPV strains[29] strains; three signatures responded well to 9/9 strains tested.

[24] Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, 5th edition, 2004, Part 2 Section 2.1.4, Chapter 2.1.1. (http://www.oie.int/eng/normes/MMANUAL/A_00027.htm)
[25] GI Number: 56410431
[26] Kindly provided by our USDA ARS collaborator.
[27] Gene ID: RPVgp1/3021777
[28] Gene ID: RPVgp7/3021780
[29] Testing conducted at the Plum Island Animal Disease Center (USDA APHIS).

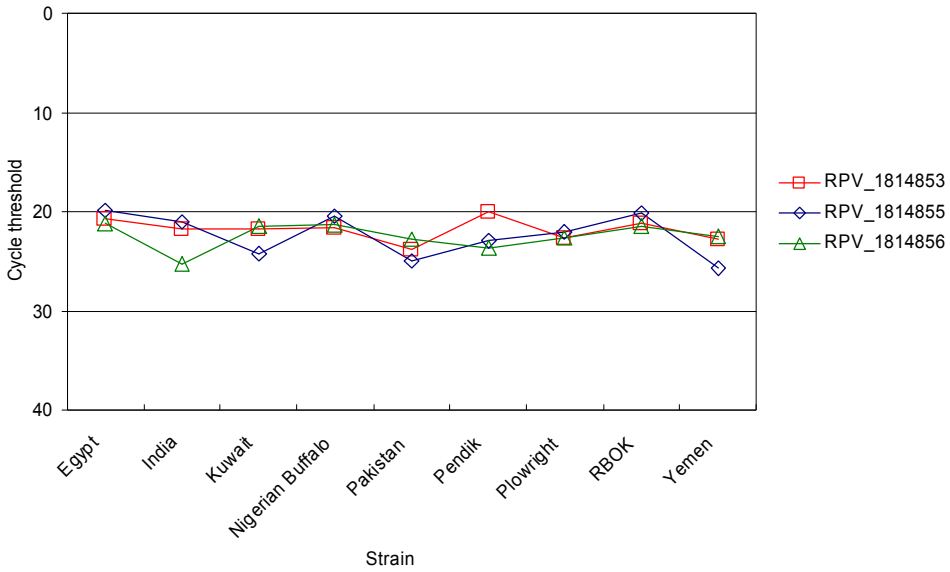
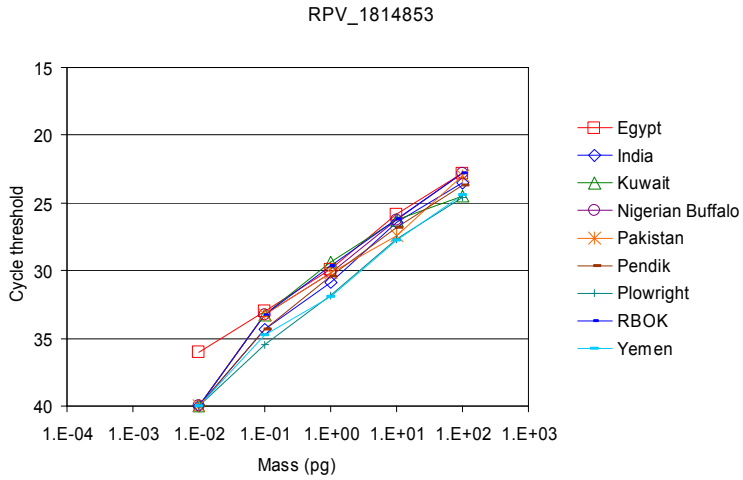


FIG. 38 Taqman screening of RPV signatures against nine RPV strains (PIADC). Samples were total RNA extracted (Qiagen RNeasy Mini kit) from virus-infected cell culture (Qiagen RNeasy Mini kit). Each reaction was spiked with 200pg of template. Each point represents the mean (n=3) cycle threshold. If no cycle threshold was reached (i.e. infinite cycle threshold), a cycle threshold of 40 was assigned in this plot.

Additional Taqman screening was conducted at PIADC using serial dilutions of purified RNA of the same nine strains. RPV_4853 signature showed the most consistent results over all concentrations for all strains.



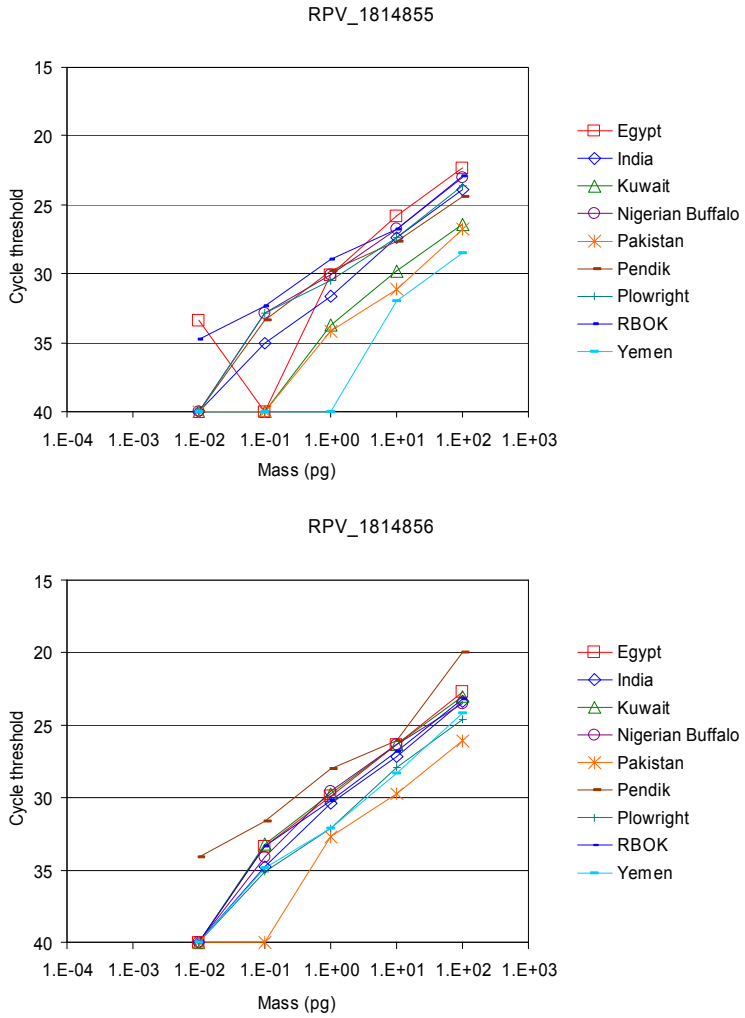


FIG. 39 Taqman response of RPV signatures (RPV_4853, RPV_4855 and RPV_4856) against nine RPV strains over four orders of magnitude in template concentration. Samples were serial dilutions of total RNA extracted (Qiagen RNeasy Mini kit) from virus-infected cell culture (Qiagen RNeasy Mini kit). Each point represents the mean (n=3) cycle threshold. If no cycle threshold was reached (i.e. infinite cycle threshold), a cycle threshold of 40 was assigned in this plot.

RPV Multiplex

Near-neighbor screening: This work has only been conducted using human parainfluenza virus; additional NN testing is currently in process at PIADC.

Target screening: All three RPV signatures have been added to the current Bovine panel where they show consistently low background response in the absence of template. RPV target testing information should be acquired at PIADC in July 2007 based on our current testing plan.

Summary: The RPV signatures together demonstrate promising performance in Taqman formats. Multiplex screening against target and near-neighbor material is underway.

4.8. Vesicular Stomatitis Virus (VSV) –Bovine/Porcine Panels

Purpose: Vesicular Stomatitis is a vesicular disease of swine and cattle is a FMD look-alike disease. Vesicular Stomatitis is also clinically indistinguishable from SVD and VES. These signatures should detect and differentiate VSV Indiana (IND) and New Jersey (NJ) species from FMDV in samples including vesicle fluid, epithelium covering unruptured vesicles, epithelial flaps of freshly ruptured vesicles, swabs of ruptured vesicles, or throat swabs.[30]

Signature candidates: Six new VSV signature candidates are available for consideration for the Porcine panel. VSV signatures were not included in the Version 1.0 panel. Four signatures intended to detect VSV IND are designated VSV_3943, VSV_8947, VSV_8949 and VSV_1405. Two signatures intended to detect VSV NJ are designated VSV_1408 and VSV_1409. Four of these signatures including VSV signature candidates are candidates for the Bovine panel. VSV_3943, VSV_8947, VSV-8949 and VSV_1409.

Signature origin: Signatures developed by others[31] were modified to match LLNL's assay design criteria to increase compatibility in the multiplex format. VSV_1405[32] targets the polymerase (L protein) gene[33] whilst VSV_1408 and VSV_1409[34] both target the nucleocapsid (N) protein gene. The three signatures VSV_3943, VSV_8947, VSV_8949 were designed at LLNL using four complete VSV IND genomes.[35] VSV_3943 targets the nucleocapsid (N) protein gene[36] whilst VSV_8947, VSV_8949 both target the polymerase (L protein) gene.[37]

VSV Taqman

Near-neighbor screening: Signatures were screened at PIADC against eight vesiculovirus near-neighbors (as determined by serological analysis, not sequence) at 200 pg (n=3) of extracted RNA template from virus-infected cell culture (Qiagen RNeasy Mini kit). These included BeAr, Hilo CT AN, Calchaqui, Klamath, NM-85-488, Jurona, Piry, Chandipura isolates. No cross-reactivity was observed. Additional near-neighbor testing of these signatures at PIADC is underway.

Target screening: All signatures were screened against four IND-1 and four NJ[38] strains. These results indicate selectivity between signature sets in that IND signatures responded to IND-1 but not NJ strains, whereas NJ

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[30] Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, 5th edition, 2004, Part 2 Section 2.1., Chapter 2.1.2. (www.oie.int/eng/normes/MMANUAL/A_00025.htm)
[31] External collaborators: Plum Island Animal Disease Center, USDA ARS; NCFAD, Winnipeg, Canada.
[32] Original signature (NCFAD) had a very long amplicon. LLNL selected additional oligos around the appropriate loci that split the original signature into 2 signatures, resulting in 1811405 and 1811406 (eliminated).
[33] Gene ID: VSIVgp5/1489835
[34] Original signature (USDA ARS) was modified to meet LLNL's standard TaqMan criteria and to match LLNL's VSV genome alignments resulting in VSV_1811408 and VSV_1811409.
[35] GI Number: 23305074, 23305068, 23305062, 9627229. Sequence length 8284 base pairs, K-path ID 13753
[36] Gene ID:VSIVgp1/1489831
[37] Gene ID: VSIVgp5/1489835
[38] Isolates were from the Rodriguez lab at the Plum Island Animal Disease Center (USDA APHIS).

signatures responded to NJ but not IND-1 strains. NJ strain NJ118HDB generated a weak response on VSV_1811408 and VSV_1811409 signatures. In concert, the two signature sets responded to 7/8 strains tested and worked well to provide complementary coverage.

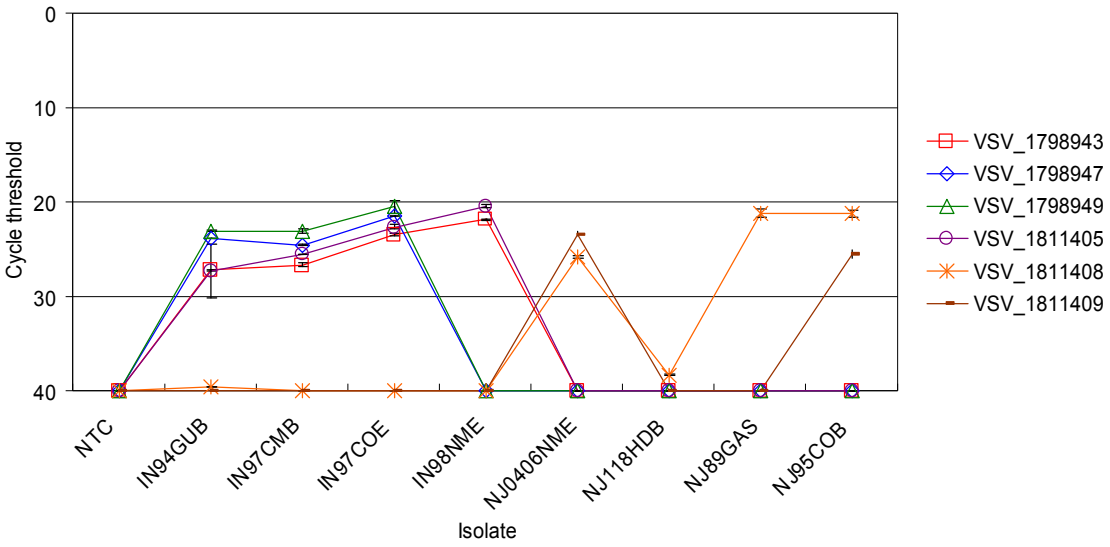

FIG. 40 Taqman screening of VSV signatures against eight VSV isolates conducted at PIADC. Samples were serial dilutions of total nucleic acid Trizol extracted from virus-infected cell culture. Each reaction was spiked with 200pg of template. Each point represents the mean (n=3) cycle threshold. If no cycle threshold was reached (i.e. infinite cycle threshold), a cycle threshold of 40 was assigned in this plot.

Additional Taqman screening was conducted at LLNL using one IND and one NJ strain acquired from the NVSL.[39] The results show selectivity between signature sets. As expected, the cycle threshold decreased as the mass of template per reaction increased. VSV_1408 showed a stronger response than VSV_1409 against this single NJ strain.

[39] Isolated obtained from the NVSL Bovine & Porcine Viruses Section, Diagnostic Virology Laboratory. NJ VSV strain used for this testing was originally isolated from horse epithelial tissue (Colorado 1982). VSV IND was originally isolated from bovine vesicular fluid (New Mexico, 1965) and is the Chimayo strain.

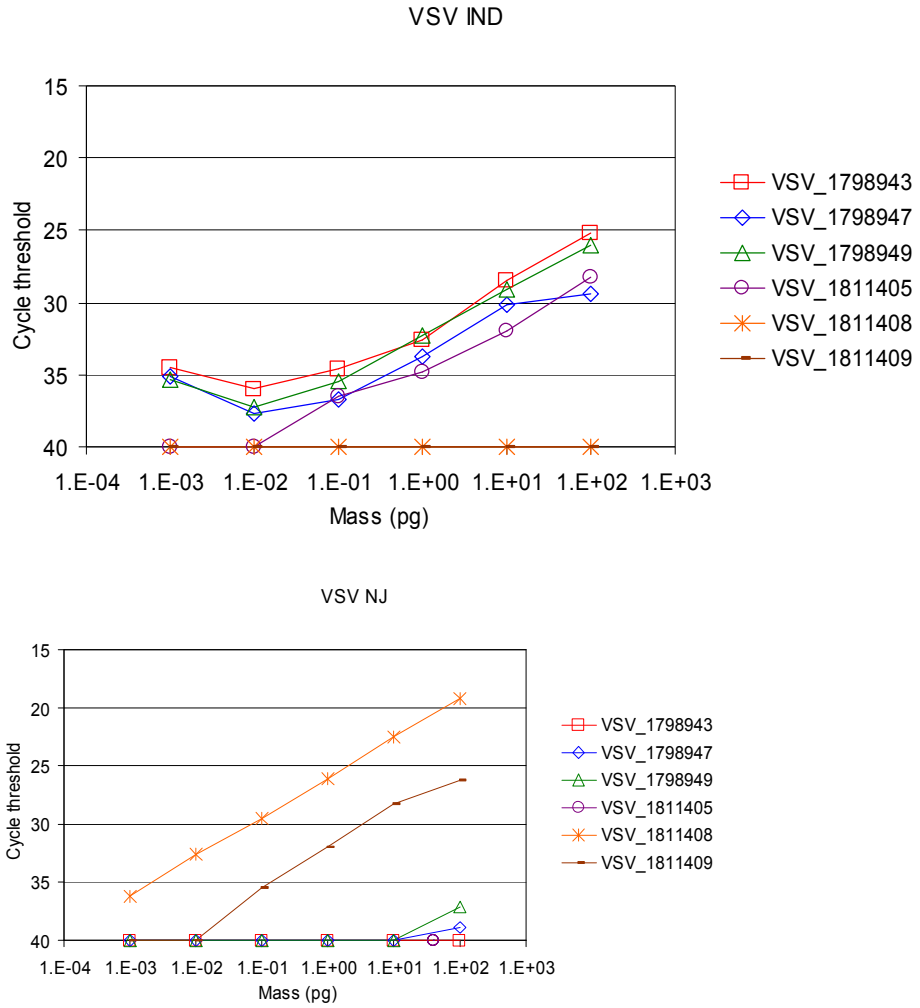

FIG. 41 Taqman screening of six VSV signatures against one VSV IND and one NJ strain showing response over a wide range of template concentrations. Samples were serial dilutions of total RNA Trizol extracted from virus-infected cell culture. Each reaction was spiked with 200pg of template. Each point represents the mean (n=3) cycle threshold. If no cycle threshold was reached (i.e. infinite cycle threshold), a cycle threshold of 40 was assigned in this plot.

VSV Multiplex

Near-neighbor screening (Porcine and Bovine panels): This work has not yet been conducted; it is in process at PIADC.

Target screening (Porcine Panel): All six VSV signatures have been added to the current porcine multiplex. Five of the signatures show consistently low background response in the absence of template. VSV_1409 in the current panel configuration exhibits an elevated background response (MFI values ranging from 50-200) in the reaction mixture.
Multiplex screening results were in excellent agreement with the Taqman results presented above. In multiplex, the VSV_1408 also showed a stronger response than VSV_1811409 against the NJ species. Additional calibration data has been acquired at LLNL using titered material for the two NVSL VSV isolates.

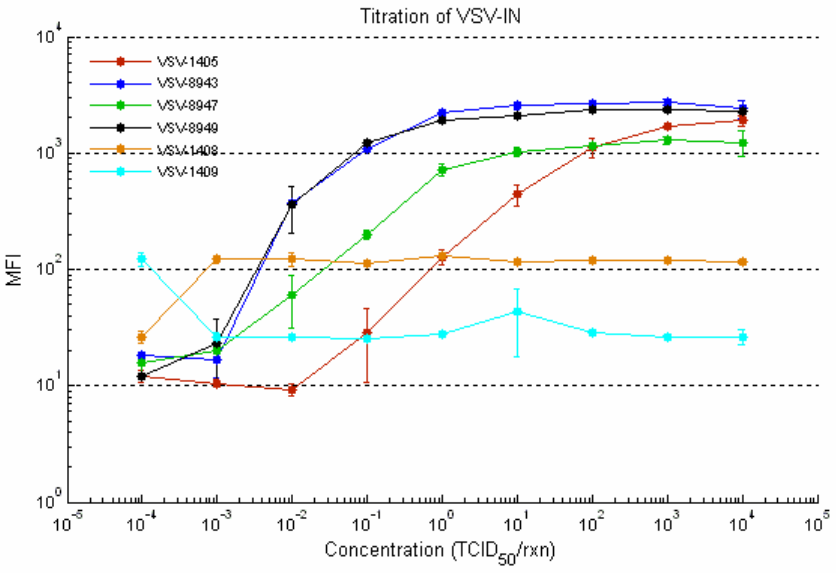

FIG. 42 Porcine multiplex screening data for the six VSV signatures against extracted nucleic acids from isolate VSV-IN-1(NVSL). Signatures VSV-1408 and VSV-1409 have design specificity for the New Jersey Strain, so they are not expected to react with the Indiana strain as shown here. Serial dilution of nucleic acid Trizol extracted from titered virus-infected cell culture. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1σ of the mean.

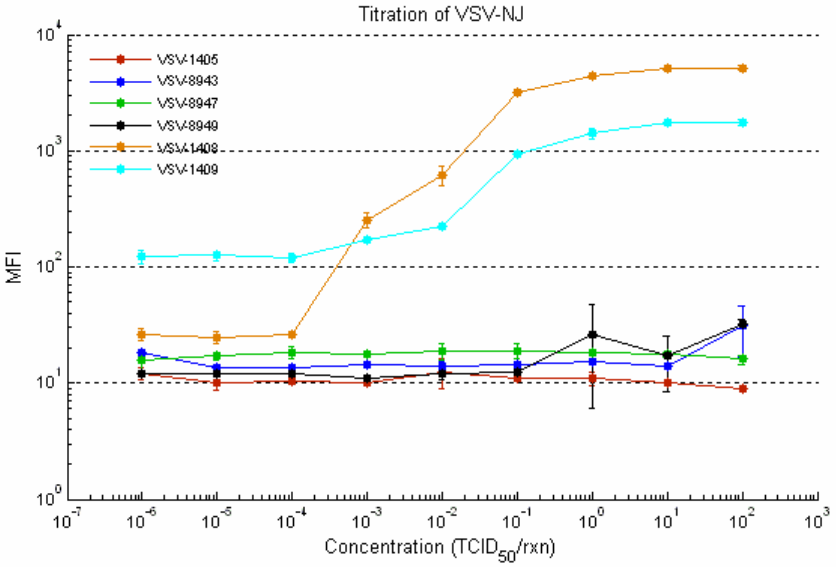

FIG. 43 Porcine multiplex screening data for the six VSV signatures against extracted nucleic acids from isolate VSV-NJ(NVSL). Signatures VSV-1408 and VSV-1409 have design specificity for the New Jersey Strain, so they are the only signature of the six that is expected to react with the New Jersey strain as shown here. Serial dilution of nucleic acid Trizol extracted from titered virus-infected cell culture. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1σ of the mean.

Target screening (Bovine Panel): Four VSV signatures have been added to the current Bovine multiplex. The Bovine panel has two less VSV signatures than the Porcine panel, as two signatures were found to be incompatible during attempts to add them to multiplex. All four signatures show consistently low background

response in the absence of template. Multiplex screening results were also acquired using total RNA Trizol extracted from titered virus-infected cell culture. Good agreement was obtained between the performance of these signatures in the current Bovine and Porcine panels.

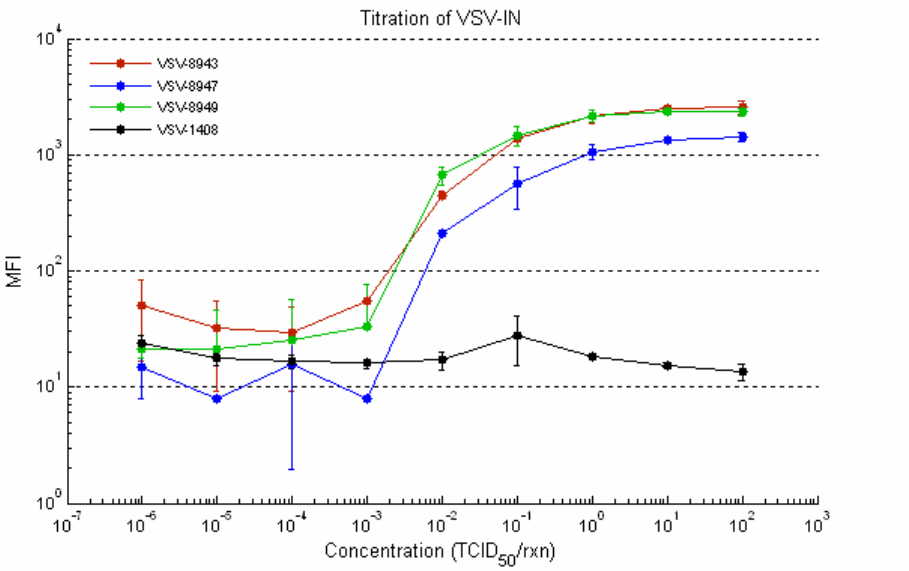

FIG. 44 Multiplex screening data for the four VSV signatures against extracted nucleic acids from isolate VSV-IN. Signature VSV-1408 has design specificity for the New Jersey Strain, so it is not expected to react with the Indiana strain as shown here. Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1σ of the mean.

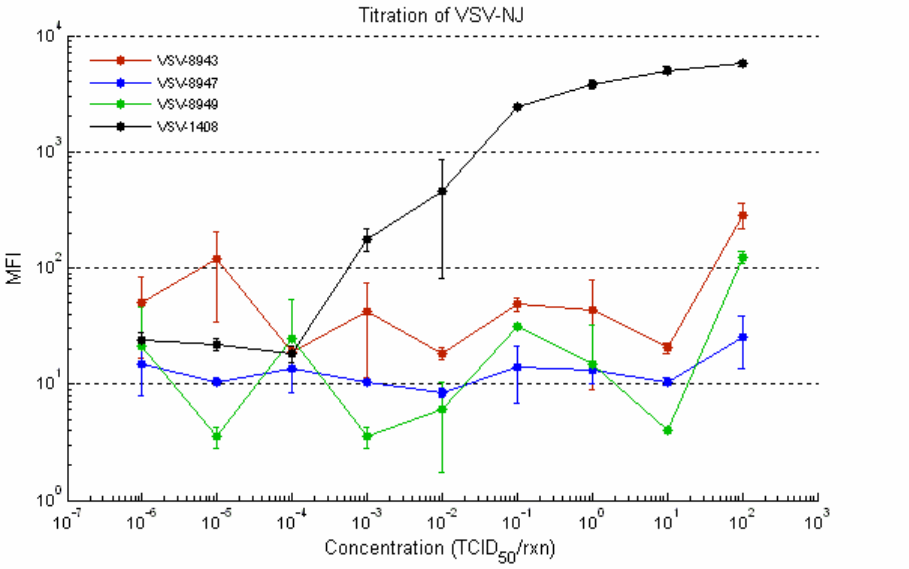

FIG. 45 Multiplex screening data for the four VSV signatures against extracted nucleic acids from isolate VSV-NJ. Signature VSV-1408 has design specificity for the New Jersey Strain, so it is the only signature of the four that is expected to react with the New Jersey strain as shown here. Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture. Total nucleic acid was extracted with Ambion MagMax 96 from non-infected (no template control, NTC) and virus-infected cell culture media then used as template. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1σ of the mean.

Summary: The VSV signatures together demonstrate promising performance in both Taqman and multiplex (porcine and bovine) formats. Together, the signatures are capable of detecting VSV NJ and IND-1 and with apparent species selectivity. Further screening against target and near-neighbor material is underway at Plum island.

4.9. Foot-and-Mouth Disease Virus (FMDV) – Bovine/Porcine Panels

Purpose: Foot-and-mouth disease (FMD) is a highly infectious and contagious vesicular disease affecting domestic and wild ruminants and swine caused by a single stranded positive sense RNA virus having seven distinct serotypes. Clinical diagnosis of FMD can be confounded by species that present little or no clinical symptoms and other vesicular diseases, termed "look-alikes". The purpose of these signatures is to provide pan-serotype detection of FMDV RNA in samples taken from suspect clinical animals. The Bovine panel will provide differential laboratory diagnosis of FMDV from look-alikes including malignant catarrhal fever, bovine herpes-1 (IBR), parapox, bovine viral diarrhea, bluetongue, vesicular stomatitis and rinderpest. The Porcine panel will provide differential laboratory diagnosis of FMDV from swine vesicular disease, vesicular exanthema of swine and vesicular stomatitis.

Signature candidates: FMDV-TC (USDA ARS/Tetracore) and FMDV-Pir (Institute for Animal Health, Pirbright). Both signatures were adopted from published assays and the Version 1.0 panel without change.

Signature origin: Two independent real-time reverse transcriptase polymerase chain reaction (rRT-PCR) assays targeting conserved regions of the internal ribosomal entry site of 5´untranslated region (5´UTR)[40] and the viral RNA polymerase (3D)[41] on the highly variable FMD genome. An in-depth comparative evaluation of diagnostic sensitivity (D-SN)[42] was reported in which samples of epithelial suspension from tissue collected from suspect FMD cases were analyzed. In combination, these singleplex Taqman assays reported higher D-SN compare to virus isolation and antigen-ELISA. The 5´UTR (FMD_PIR) and 3D (FMD_TC) Taqman signatures were adapted directly to the multiplex format.

FMDV Taqman

Target screening: No Taqman screening was conducted by LLNL on the FMDV signatures as a large body of validation data had already been acquired by others.

FMD Multiplex

Near-neighbor screening: FMD signatures in the Version 1.0, Bovine and Porcine multiplex was examined by running titrations against panel targets such as SVD, VESV, BHV, and BVD. No response greater than the

[40] Reid, S. M.; Ferris, N. P.; Hutchings, G. H.; Zhang, Z. D.; Belsham, G. J.; Alexandersen, S. Journal of Virological Methods 2002, 105, 67-80.
[41] Callahan, J. D.; Brown, F.; Csorio, F. A.; Sur, J. H.; Kramer, E.; Long, G. W.; Lubroth, J.; Ellis, S. J.; Shoulars, K. S.; Gaffney, K. L.; Rock, D. L.; Nelson, W. M. Journal of the American Veterinary Medical Association 2002, 220, 1636-1642.
[42] King, D. P.; Ferris, N. P.; Shaw, A. E.; Reid, S. M.; Hutchings, G. H.; Giuffre, A. C.; Robida, J. M.; Callahan, J. D.; Nelson, W. M.; Beckham, T. R. Journal of Veterinary Diagnostic Investigation 2006, 18, 93-97.

background was observed for either FMD signature against any panel targets. Additional near-neighbor work is in process at PIADC to screen a number of porcine and bovine enteroviruses.

Target screening: Titration curves were acquired for the FMDV signatures in the Version 1.0 panel. The samples were a titration series constructed from titered virus-infected cell culture for each FMDV serotype. The FMD_TC signature had a qualitative limit of detection typically 1-2 orders of magnitude higher. The FMD_PIR signature did not detect SAT1/6 SWA or SAT3/3 Bech 1Nov05 samples at any concentration tested.

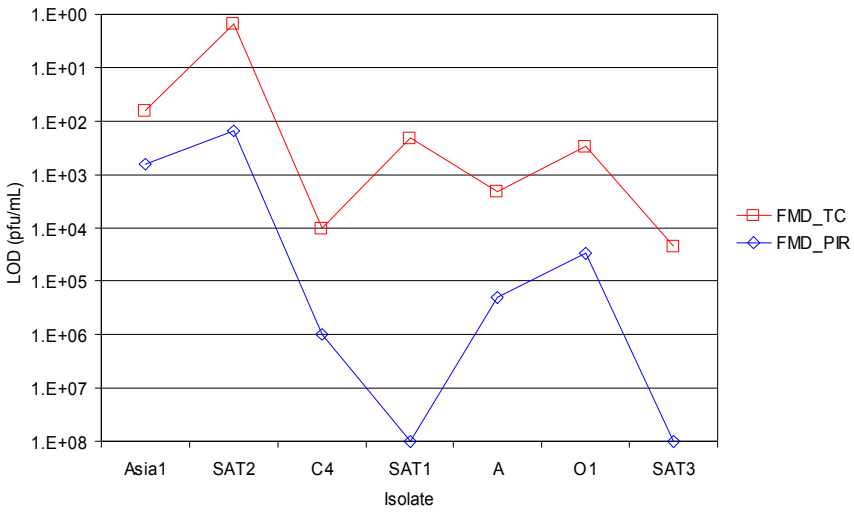

FIG. 46 The FMD_TC signature in the Version 1.0 panel detected lower concentrations of virus than FMD_Pir for all seven serotypes. The LOD (pfu/ml) represents the lowest sample concentration tested that generated response above threshold. The threshold values for FMD_PIR and FMD_TC were 42 and 60 MFI units, respectively. Samples were A Argentina 2001, O1 South Korea, C4 Tierra Del Fuego, Asia1, SAT 1/6 SWA, SAT 2 Zim and SAT 3 /3 Bech 1Nov05 (PIADC).

Bovine Panel screening

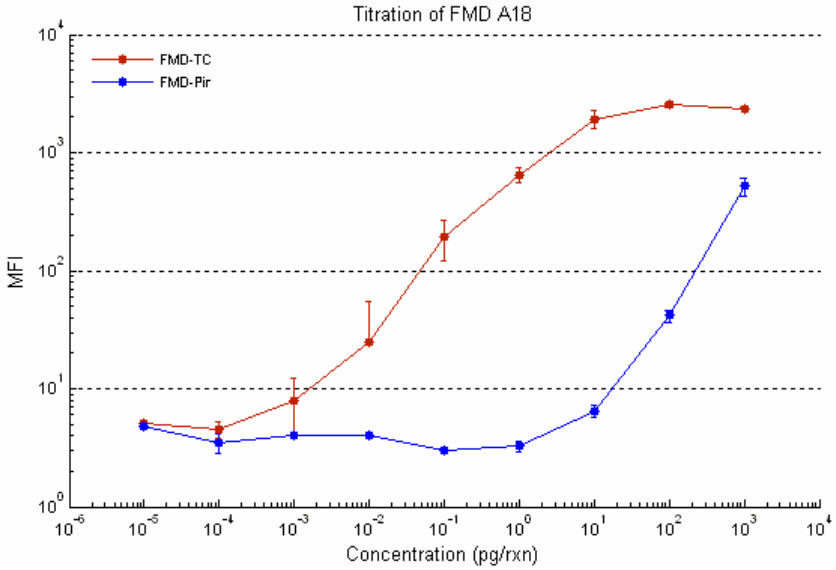

FIG. 47. LLNL preliminary bovine multiplex screening data for the two FMDV signatures against extracted nucleic acids from isolate FMDV serotype A18. Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture. Total nucleic acid was extracted with Ambion MagMax 96 from non-infected (no template control, NTC) and virus-infected cell culture media then used as template. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1σ of the mean.

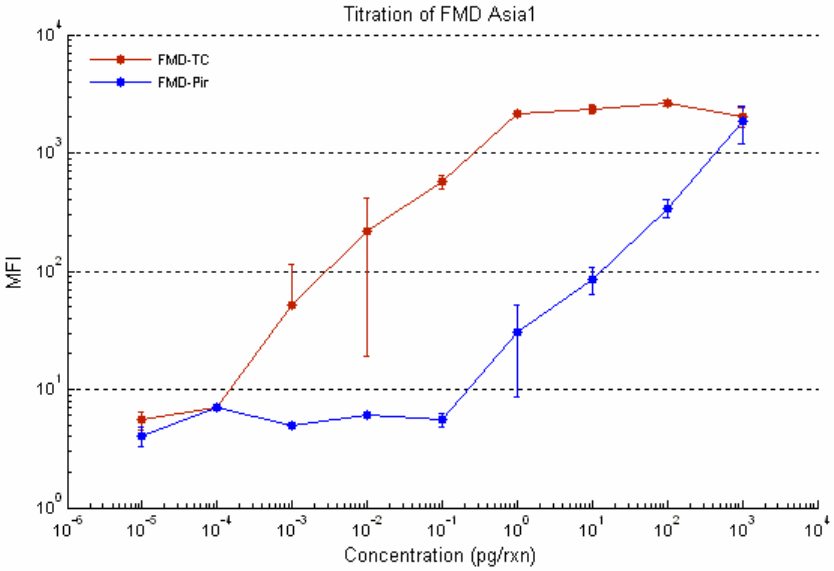

FIG. 48. LLNL preliminary multiplex screening data for the two FMDV signatures against extracted nucleic acids from isolate FMDV serotype Asia1. Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture. Total nucleic acid was extracted with Ambion MagMax 96 from non-infected (no template control, NTC) and virus-infected cell culture media then used as template. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1σ of the mean.

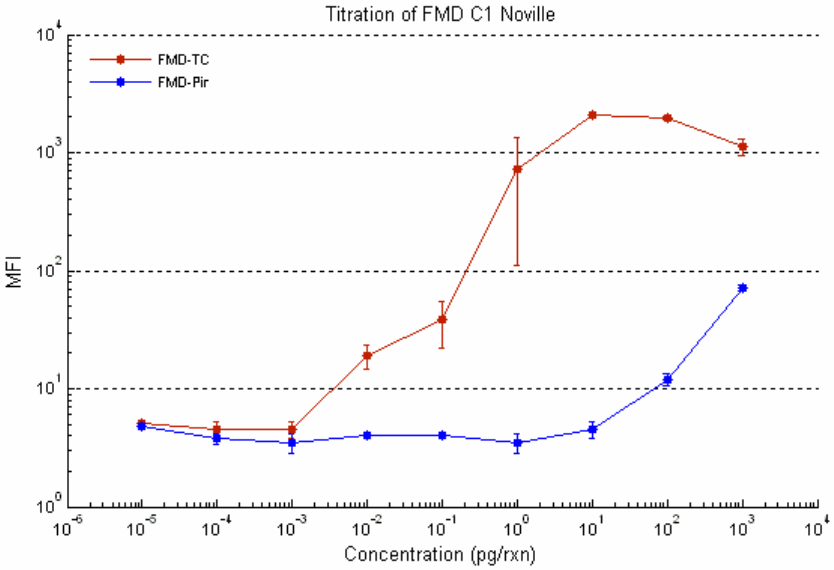

FIG. 49. LLNL preliminary bovine multiplex screening data for the two FMDV signatures against extracted nucleic acids from isolate FMDV serotype C1 Noville. Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture. Total nucleic acid was extracted with Ambion MagMax 96 from non-infected (no template control, NTC) and virus-infected cell culture media then used as template. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1σ of the mean.

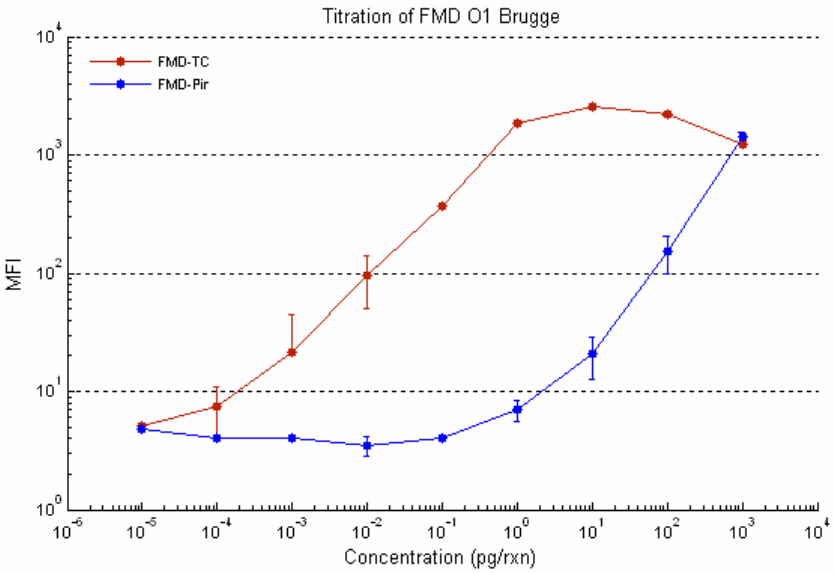

FIG. 50. LLNL preliminary multiplex screening data for the two FMDV signatures against extracted nucleic acids from isolate FMDV serotype O1 Brugge. Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture. Total nucleic acid was extracted with Ambion MagMax 96 from non-infected (no template control, NTC) and virus-infected cell culture media then used as template. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1σ of the mean.

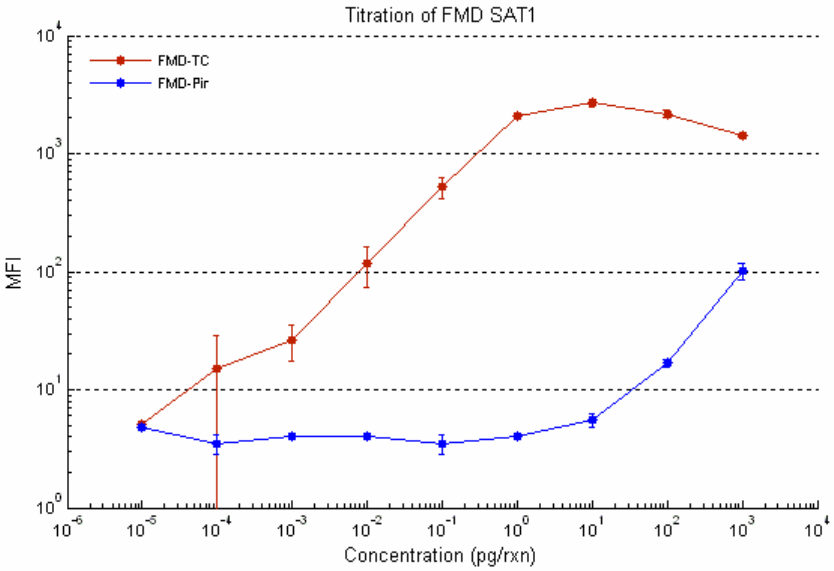

FIG. 51. LLNL preliminary multiplex screening data for the two FMDV signatures against extracted nucleic acids from isolate FMDV serotype SAT1. Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture. Total nucleic acid was extracted with Ambion MagMax 96 from non-infected (no template control, NTC) and virus-infected cell culture media then used as template. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1σ of the mean.

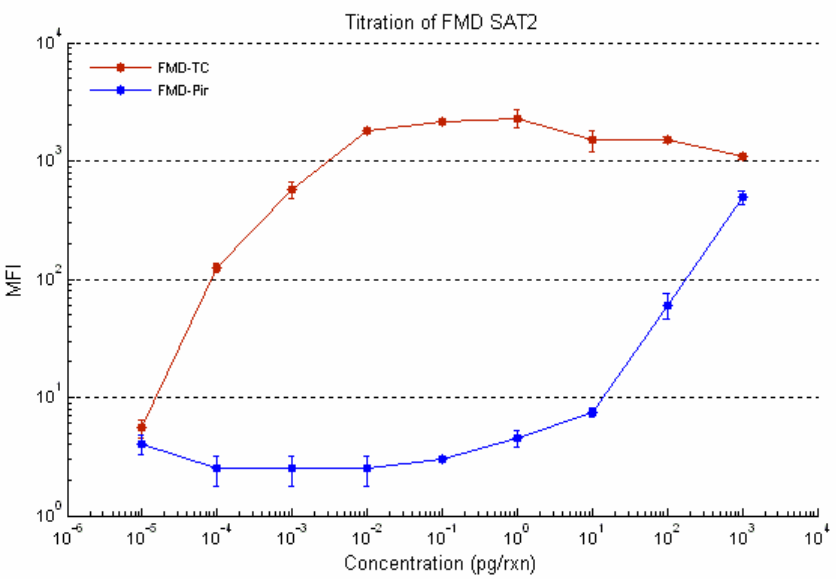

FIG. 52. LLNL preliminary multiplex screening data for the two FMDV signatures against extracted nucleic acids from isolate FMDV serotype SAT2. Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture. Total nucleic acid was extracted with Ambion MagMax 96 from non-infected (no template control, NTC) and virus-infected cell culture media then used as template. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1σ of the mean.

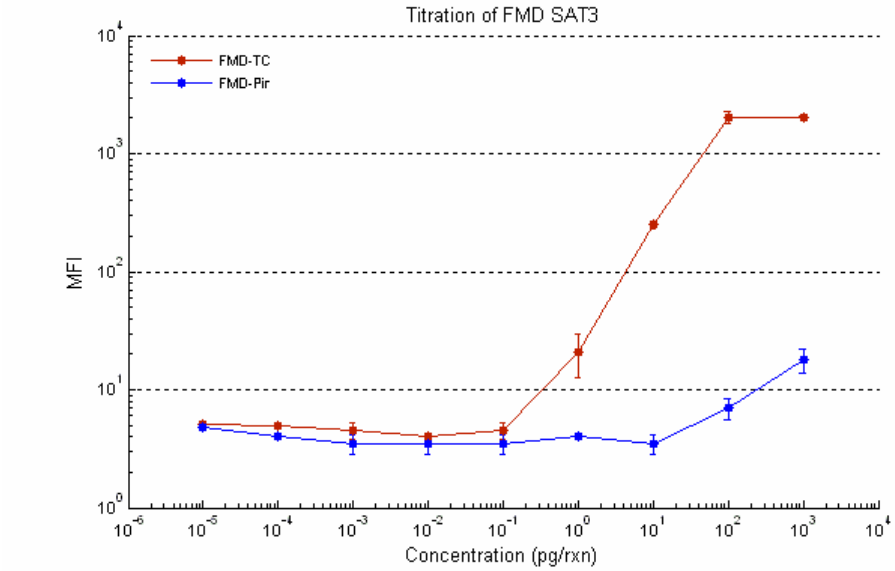

FIG. 53. LLNL preliminary bovine multiplex screening data for the two FMDV signatures against extracted nucleic acids from isolate FMDV serotype SAT3. Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture. Total nucleic acid was extracted with Ambion MagMax 96 from non-infected (no template control, NTC) and virus-infected cell culture media then used as template. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1σ of the mean.

Porcine Panel screening

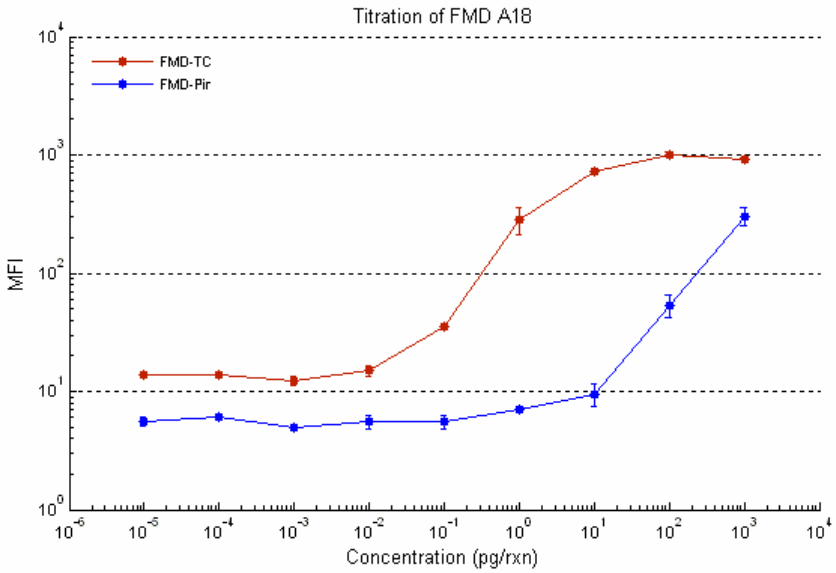

FIG. 54. LLNL preliminary porcine multiplex screening data for the two FMDV signatures against extracted nucleic acids from isolate FMDV serotype A18. Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture. Total nucleic acid was extracted with Ambion MagMax 96 from non-infected (no template control, NTC) and virus-infected cell culture media then used as template. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1σ of the mean.

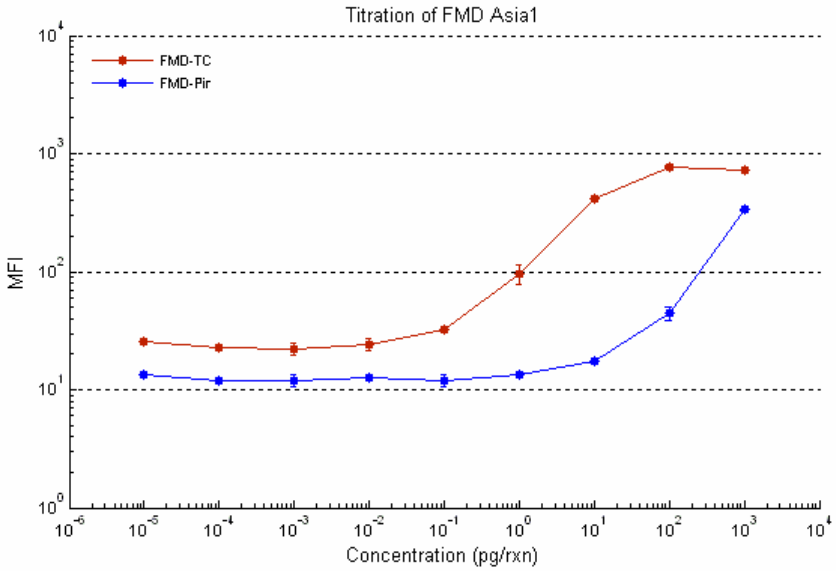

FIG. 55. LLNL preliminary multiplex screening data for the two FMDV signatures against extracted nucleic acids from isolate FMDV serotype Asia1. Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture. Total nucleic acid was extracted with Ambion MagMax 96 from non-infected (no template control, NTC) and virus-infected cell culture media then used as template. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1σ of the mean.

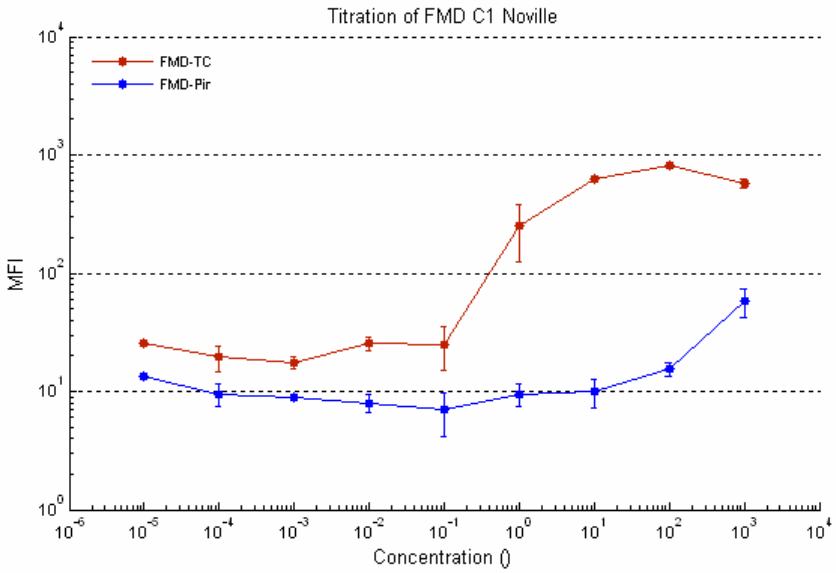

FIG. 56. LLNL preliminary multiplex screening data for the two FMDV signatures against extracted nucleic acids from isolate FMDV serotype C1 Noville. Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture. Total nucleic acid was extracted with Ambion MagMax 96 from non-infected (no template control, NTC) and virus-infected cell culture media then used as template. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1σ of the mean.

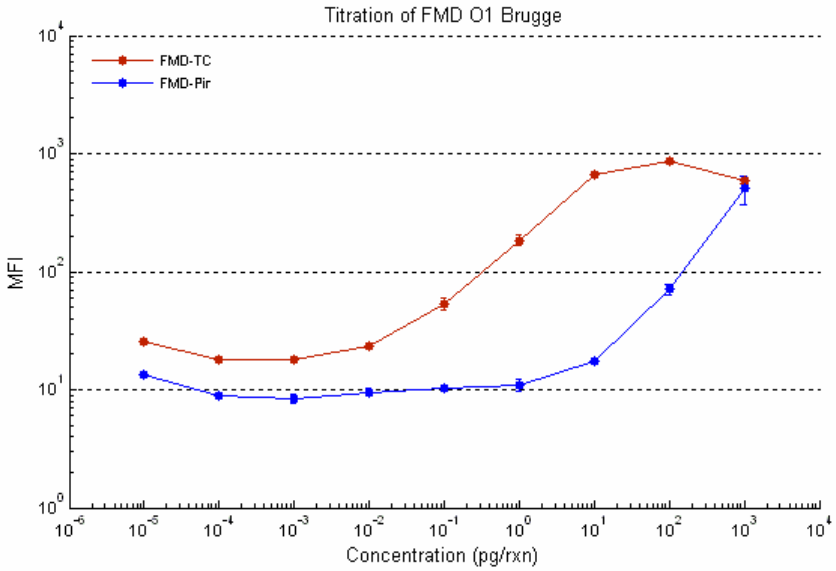

FIG. 57. LLNL preliminary multiplex screening data for the two FMDV signatures against extracted nucleic acids from isolate FMDV serotype O1 Brugge. Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture. Total nucleic acid was extracted with Ambion MagMax 96 from non-infected (no template control, NTC) and virus-infected cell culture media then used as template. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1σ of the mean.

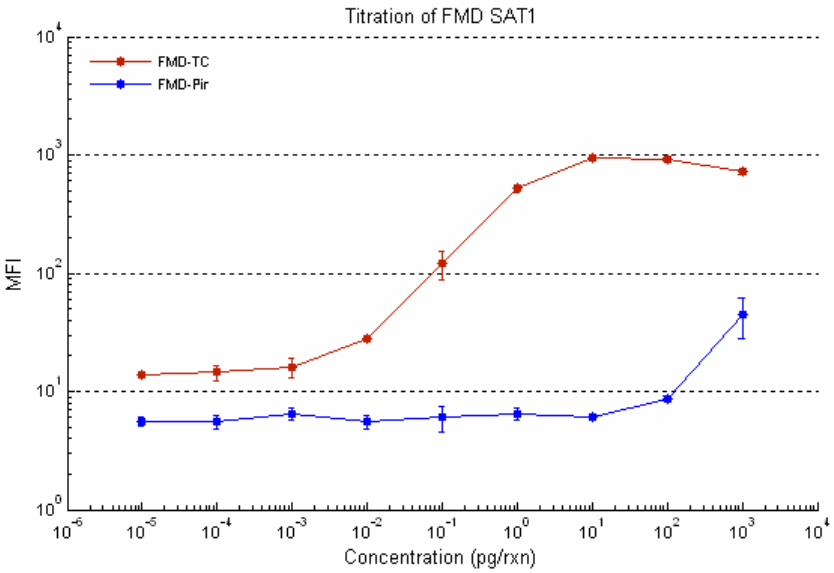

FIG. 58. LLNL preliminary multiplex screening data for the two FMDV signatures against extracted nucleic acids from isolate FMDV serotype SAT1. Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture. Total nucleic acid was extracted with Ambion MagMax 96 from non-infected (no template control, NTC) and virus-infected cell culture media then used as template. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1σ of the mean.

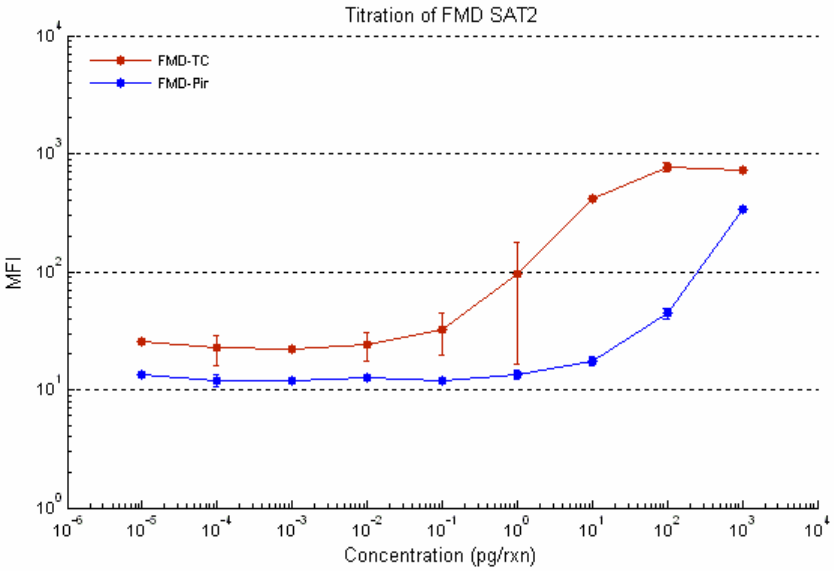

FIG. 59. LLNL preliminary multiplex screening data for the two FMDV signatures against extracted nucleic acids from isolate FMDV serotype SAT2. Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture. Total nucleic acid was extracted with Ambion MagMax 96 from non-infected (no template control, NTC) and virus-infected cell culture media then used as template. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1σ of the mean.

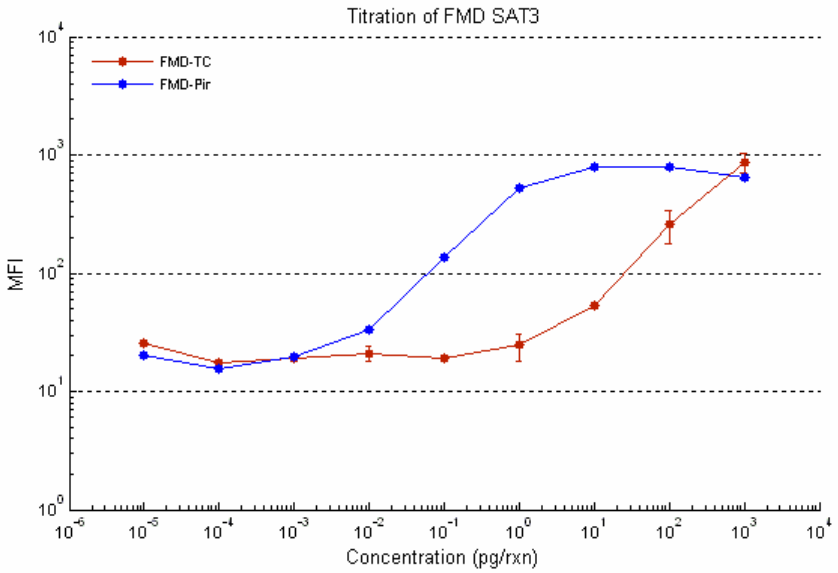

FIG. 60. LLNL preliminary porcine multiplex screening data for the two FMDV signatures against extracted nucleic acids from isolate FMDV serotype SAT3. Serial dilution of nucleic acid Trizol extracted from virus-infected cell culture. Total nucleic acid was extracted with Ambion MagMax 96 from non-infected (no template control, NTC) and virus-infected cell culture media then used as template. Each point represents the mean response (n=2), with the first point being the NTC. Error bars indicate ±1σ of the mean.

Summary: The LOD of both FMD signatures in the multiplex assay is higher than the singleplex Taqman assays, which in effect causes a reduction of diagnostic sensitivity. Using the signatures in combination does increase the overall diagnostic sensitivity of the multiplex assay. The combination of signatures in a finalized panel can cause LOD for any signature within the panel to change. Once a panel's composition has been finalized, further work could be conducted to determine the analytical sensitivity of all signatures including FMDV and undertake further optimization if required. Additional comprehensive screening is in process for both the porcine and bovine panels at PIADC.

4.10. Swine Vesicular Disease (SVD) –Porcine Panel

Purpose: Swine Vesicular Disease (SVD) is a foreign animal disease and an FMD look-alike disease. Incorporating SVDV signatures within a Porcine panel could potentially allow for the simultaneous screening for multiple vesicular FADs in swine. These signatures should detect and differentiate SVDV from FMDV in Porcine samples including oral/nasal swab in viral transport medium, vesicular fluid, and epithelial tissue. The signatures should provide pan-serotype detection of SVDV.

Signature candidates: SVD_1, SVD_2, SVD_3. These signatures were adopted from the Version 1.0 panel without change.

Signature origin: Signatures were designed at LLNL using five complete genomes.[43] SVD_1, SVD_2 and SVD_3 signatures target ID protein (coat protein), membrane permeability enhancement and RdRp genes, respectively

SVDV Taqman

[43] GI Numbers 8896132, 61167, 37993797, 402536, 1228947, K-path ID 61307

Near-neighbor screening: SVD-1 and SVD-2 did not cross-react with 10 human enterovirus B strains (Cox A9, B1, B4, B5, B6, Echo 11, Echo 9 H11 and Echo24). Taqman near-neighbor screening for SVD-3 was not conducted.

Target screening: The three SVD signatures were screened against eleven isolates of SVDV at PIADC. SVD-1 detected 7/7 isolates. SVD-2 detected 4/7 isolates, missing HKN/12/8, RON-1-87, TAW/119/97. Taqman target screening for the SVD-3 signature is lacking data for five isolates and may need to be screened further. Of the two isolates screened to date, one generated a weak response (ITL/1/88) and the other no response (ITL/1/99).

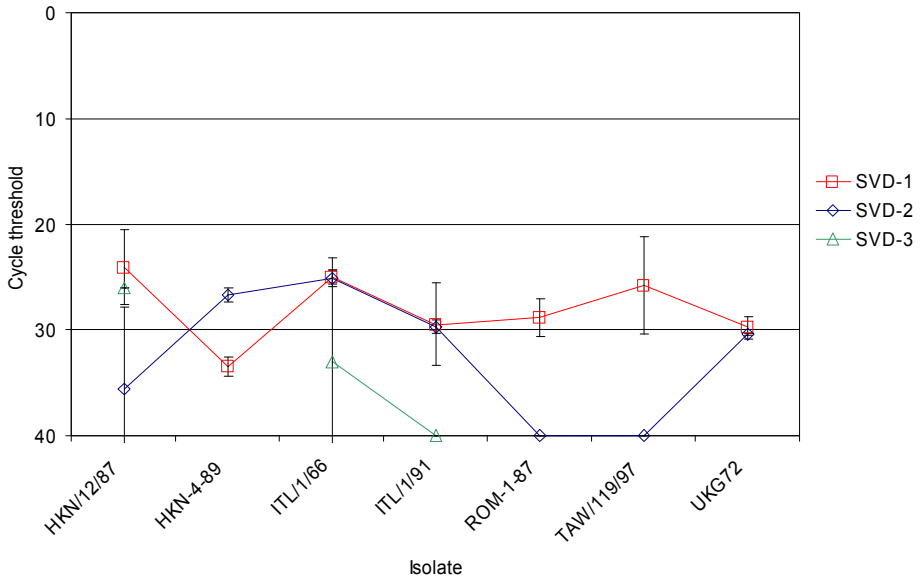

FIG. 61 Taqman screening of SVD signatures against seven isolates of SVDV (PIADC). Virus-infected cell culture media was used as template. Each point represents the mean (n=3) cycle threshold. If no cycle threshold was reached (i.e. infinite cycle threshold), a cycle threshold of 40 was assigned in this plot.

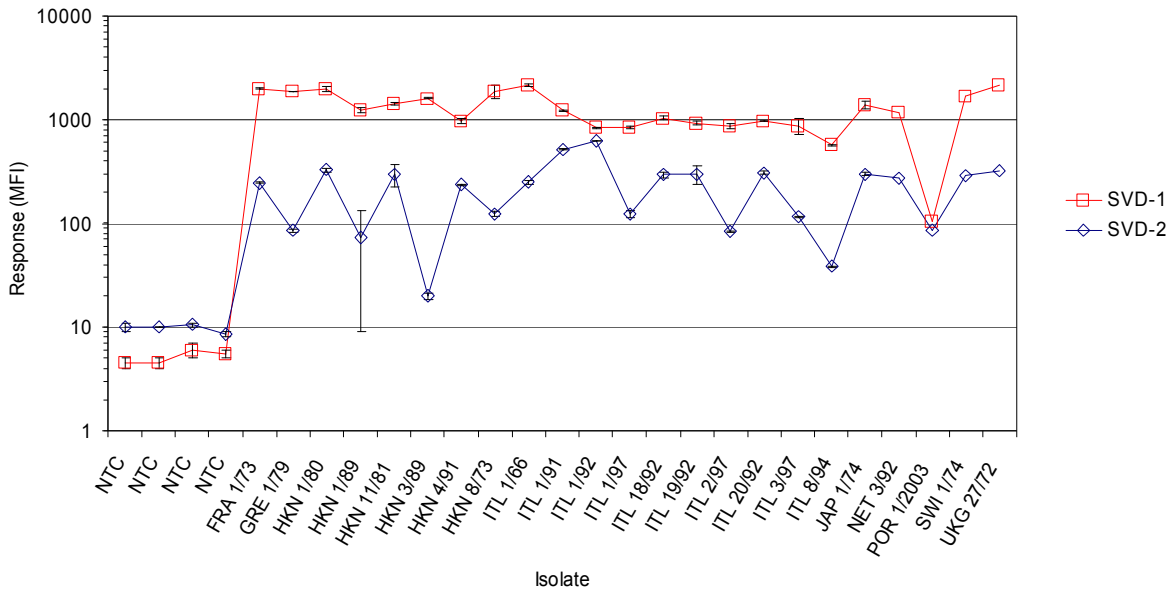

FIG. 62 SVDV signature screening in singleplex format against twenty-three isolates of SVDV (Canada). Samples comprised of a non-infected culture media (no template control, NTC) and virus-infected culture media spiked into each PCR reaction. Each point represents the mean response (n=2). Error bars indicate ±1σ of the mean.

Further testing of the three signatures was undertaken in singleplex format during testing at NCFAD (Winnipeg, Canada). These serial dilutions of untitered virus indicate that all three signatures responded over seven orders of magnitude of template concentration.

SVDV Multiplex

Near-neighbor screening: Near-neighbor testing of the SVD signatures in the porcine panel is underway at plum island.

Target screening: The three signatures were added to the Porcine panel and are currently being screened against target at Plum island. Characterization data captured for these signatures in the Version 1.0 panel is supporting information. The data indicates that three SVD signatures offer complementary coverage of SVD isolates in a multiplex assay.

Summary: All three SVD signatures are suitable candidates for inclusion in the Porcine panel additional screening is in process at PIADC.

4.11. Vesicular Exanthema of Swine Virus (VESV) - Porcine Panel

These four VESV signatures were added to the Porcine panel and tested against various background confounders. Target and near-neighbor is in process at PIADC and reporting will be amended when that data becomes available.

Purpose: VESV is a vesicular disease of swine and is an FMD look-alike disease. These signatures should detect and differentiate VESV from FMDV in Porcine samples including oral/nasal swab in viral transport medium, vesicular fluid, and epithelial tissue.

Signature candidates: Three of the four signatures from the Version 1.0 panel were adopted without change: VESV_1, VESV_4, VESV_5. When the new constituents were added to the Porcine panel a cross-reaction with VESV-3 was and one of the VSV assays was observed. As a result of the cross-reaction, the VESV-3 signature was dropped from the Porcine panel and the other two VESV signatures were retained.

Signature origin: Signatures were designed at LLNL using one complete genome.[44] All three signatures target different regions of a large polyprotein gene[45] that spans over 5000 base pairs.

VESV Taqman

Target screening: VESV isolates 1934B, A_{48}, B_{51}, C_{52}, D_{53}, E_{54}, F_{55}, G_{55}, H_{54}, I_{55}, J_{56} and K_{54}[46] were screened. VESV_4 detected 8/11 strains tested. VESV_5 only detected 1/11 strains.

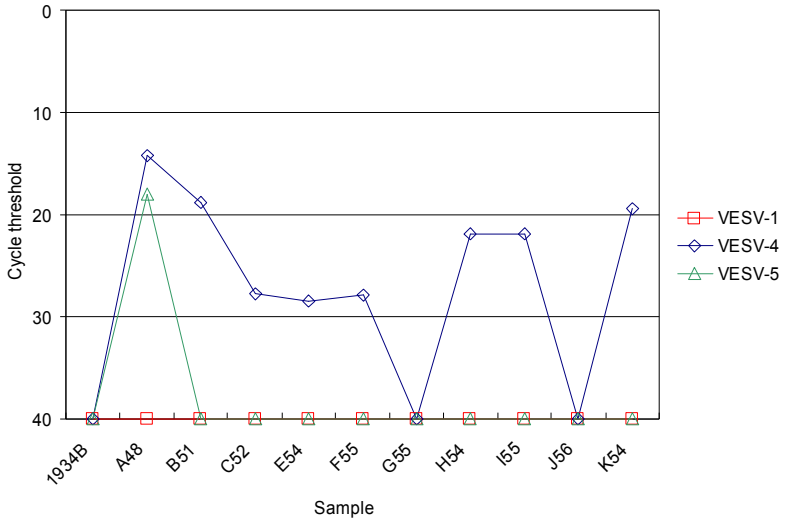

FIG. 63 Taqman screening of VESV signatures against eleven isolates of VESV. Virus-infected cell culture media was used as a template. Each point represent the mean (n=3) cycle threshold. If no cycle threshold was reached (i.e. infinite cycle threshold), a cycle threshold of 40 was assigned in this plot. Each point represents the mean response (n=3).

VESV Multiplex

Near-neighbor and Target screening: In Version 1.0, VESV_4 was the best performing signature against the samples available for testing. VESV_1 had a qualitative limit of detection approximately 100 to 1000 times higher than VESV_4. During characterization of the Version 1.0 panel at the Institute for Animal Health, Pirbright, target screening was conducted against the following nucleic acids extracted from virus-infected cell culture; VESV serotypes B1-34 (1934B), B51, and H54m; San Miguel Sea Lion Virus (SMSV) serotypes 6-13; bovine, cetacean, feline, primate, reptile and skunk caliciviruses. VESV_4 exhibited strong response to Cetacean calicivirus, SMSV serotypes 7, 10 and 13. VESV_4 generated weak responses to SMSV Serotypes 6 and 9, whilst 11 and 12 were not detected. VESV_4 generated strong responses to VESV serotypes B51 and

[44] GI Number: 10314005/NC_002551.1, Sequence length 8284 base pairs, K-path ID 13753
[45] Gene ID: VESVgp1/911834
[46] Isolates were from the Foreign Animal Disease Diagnostic Laboratory at the Plum Island Animal Disease Center (USDA APHIS).

H54 but did not response to B1-34 (1934B) which is consistent with Taqman target screening results. VESV_1 and VESV_4 signatures did not response to any of these isolates. In the Porcine panel near-neighbor screening is in process at PIADC.

Summary: VESV_4 was the best performing signature in multiplex Version 1.0. The three VESV signatures have been currently added the Porcine panel but have not been screened against targets; this work is in process at PIADC.

4.12. Porcine Respiratory and Reproductive Sydrome (PRRS) –Porcine Panel

Purpose: Although Porcine Respiratory Reproductive Syndrome (PRRS) is not a vesicular look-alike disease, incorporating these signatures in the Porcine panel could potentially allow for embedded FAD surveillance whilst testing for a prevalent domestic disease of swine. The signatures were designed for detection of North American and European strains of PRRS in Porcine samples including serum, and potentially oral/nasal swab in viral transport medium or vesicular fluid.

Signature candidates: Five new signatures are available. Two signatures designed to detect North America (domestic) PRRSV include PRRS_7706 and PRRS_7709. Three signatures designed to detect European PPRSV include PRRS_0351, PRRS_0383 and PRRS_0386.

Signature origin: Signatures were designed at LLNL in two main sets North American or domestic and European. For the domestic signatures PRRS genomes cluster into two sets identified by Dr. Kay Faaberg (our collaborator) as VR-2332/Minn and those identified as belonging to the RFLP 142 group. We used 8 complete genomes of the 142 group and 14 genomes for the VR-2332/Minn group which contained two unpublished complete genomic sequences of genomes from the latter group. For the European signatures we used a set of three complete genomes suggested by Dr. Faaberg that constitute that group.[47] North American signatures PRRS_7706 and PRRS_7709 target the envelope protein GP2 and the nucleocapsid protein N genes, respectively. The European signatures PRRS_0351, PRRS_0383 and PRRS_0386 target the replicase polyprotein 1A, GP2, and GP3-GP4 genes, respectively.

PRRS Taqman

Near-neighbor screening: No near neighbor viruses were available at the time screening was done. However no cross reaction was seen even between signatures reactive for either North American and Euro strains of PRRS. Near neighbors were tested in multiplex screening below.

Target screening: Taqman screening of PRRS signatures was conducted at the University of Minnesota using seven North American (RFLP112, 124, 134, 184, 251 and 262) and two European (EU-8, EU-13) isolates[48] shown in the data below. As intended, PRRS_7706 and PRRS_7709 signatures responded to North American

[47] GI Numbers: 14250956, 12744849, 7650192, 20271246, 40646796, 45360239, 31747018, 25361009, 12240324, 11192298, 9931316, 27549163, 22658020, 46519708, 9630807, 66735372, 66735498, 51980219, 92090664, 38385769, 2 sequences were unpublished genomes from University of Minnesota, Sequence lengths were between 15072 and 15520 base pairs, K-path IDs Domestic: 99484, Domestic: 99485 and European: 99872

[48] Kindly provided by the University of Minnesota Veterinary Diagnostic Laboratory

isolates, although the response to RFLP112 was weak for both signatures. The signatures PRRS_0351, PRRS_0383 and PRRS_0386 only responded to European isolates.

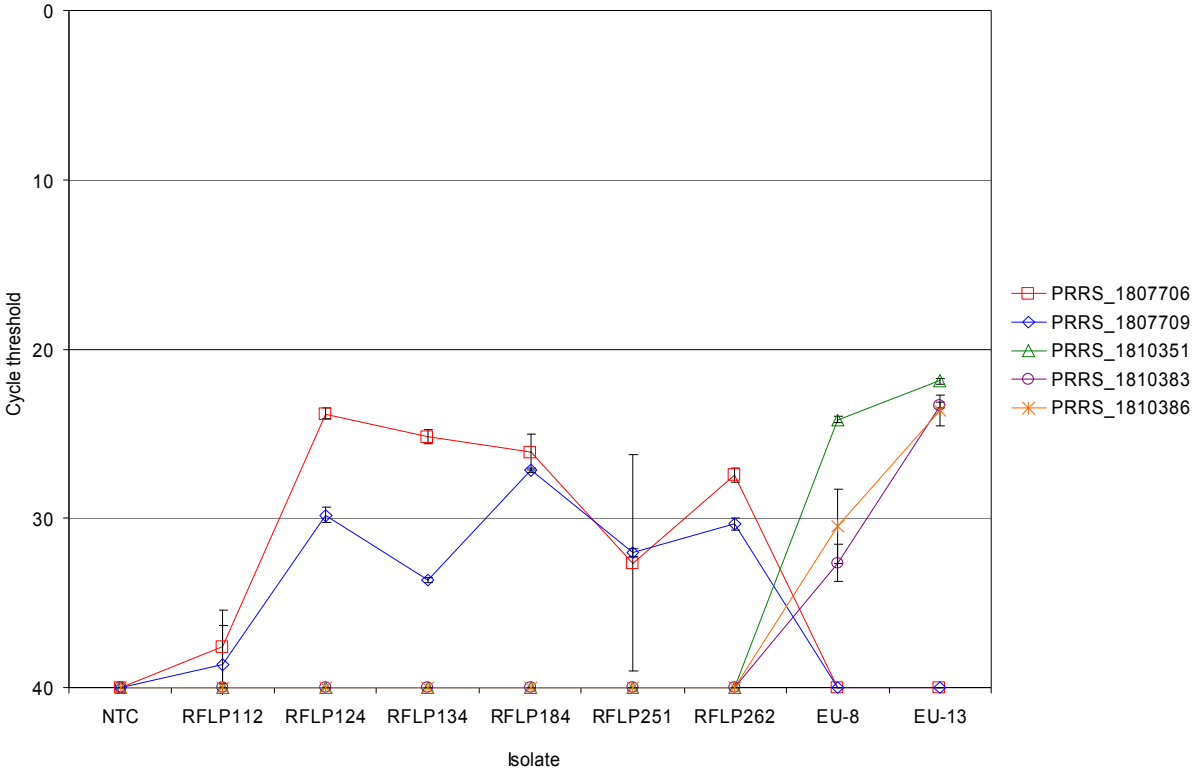

FIG.64 Taqman screening of PRRS signatures against six North American and two European isolates. Total RNA (Trizol extracted) from non-infected (no template control, NTC) and virus-infected cell culture media wad used as a template. Each reaction was spiked with 200pg. Each point represents the mean (n=3) cycle threshold. If no cycle threshold was reached (i.e. infinite cycle threshold), a cycle threshold of 40 was assigned in this plot. Error bars indicate ±1σ of the mean.

PRRS Multiplex

Near-neighbor screening: The PRRS signatures in the current porcine panel was screened against porcine respiratory coronavirus and transmissible gasteroenteritis of swine, none of the signatures cross-reacted with those near neighbors when screened (data not shown).

Target screening: All five signatures were added to the Porcine panel. The PRRS_1807709 exhibits an elevated background response (~50 to 200 MFI) in the Porcine panel. One possible cause of this behavior is a non-specific interaction between the PRRS_1807709 signature with another primer in the reaction mixture.
Different samples of the same isolates used during the Taqman screening phase (U. Minnesota) were used to conduct multiplex screening at LLNL. The multiplex spot-test results show that PRRS_1807706 and PRRS_1807709 signatures responded only to North American isolates, although their response to RFLP112 was weak. The signatures PRRS_1810351, PRRS_1810383 and PRRS_1810386 designed to respond to European PRRS strains only responded to European isolates. Taqman and multiplex target screening results agree.

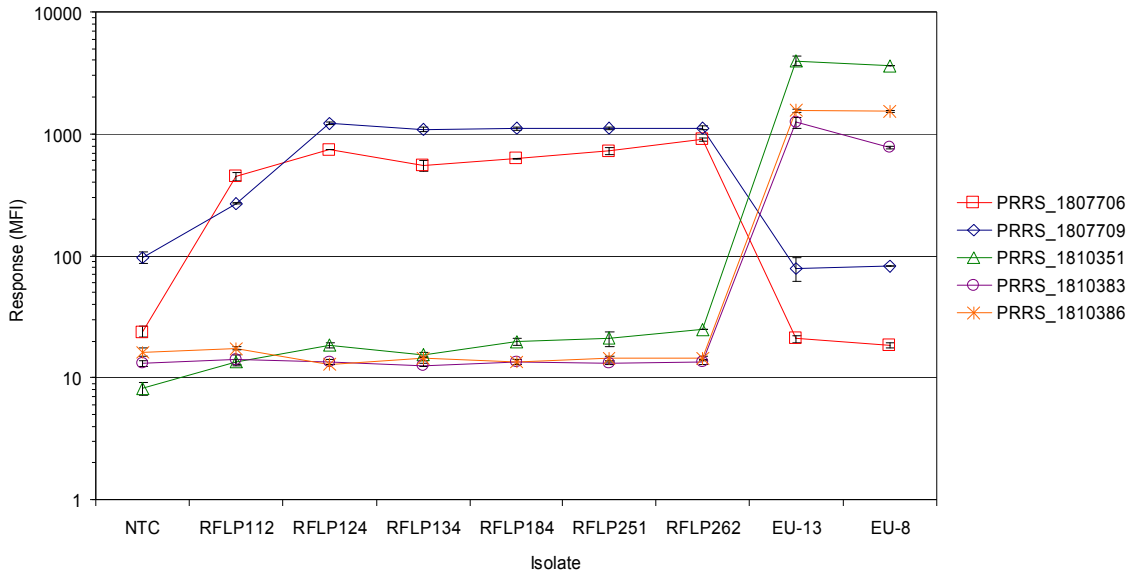

FIG. 65 Multiplex screening data for five PRRS signatures against six North American and two European isolates. Total nucleic acid was Trizol extracted from non-infected (no template control, NTC) and virus-infected cell culture media then used as a template. Each reaction was spiked with 200pg. Each point represents the mean response (n=2). Error bars indicate ±1σ of the mean.

Target screening using a North American strain (NVSL 89) was conducted at LLNL. Data shows that both signatures PRRS_1807706 and PRRS_1807709 responded as expected to the North American strain over four orders of magnitude in concentration. The signatures PRRS_1810351, PRRS_1810383 and PRRS_1810386 designed to detect European strains did not respond to the North American strain.

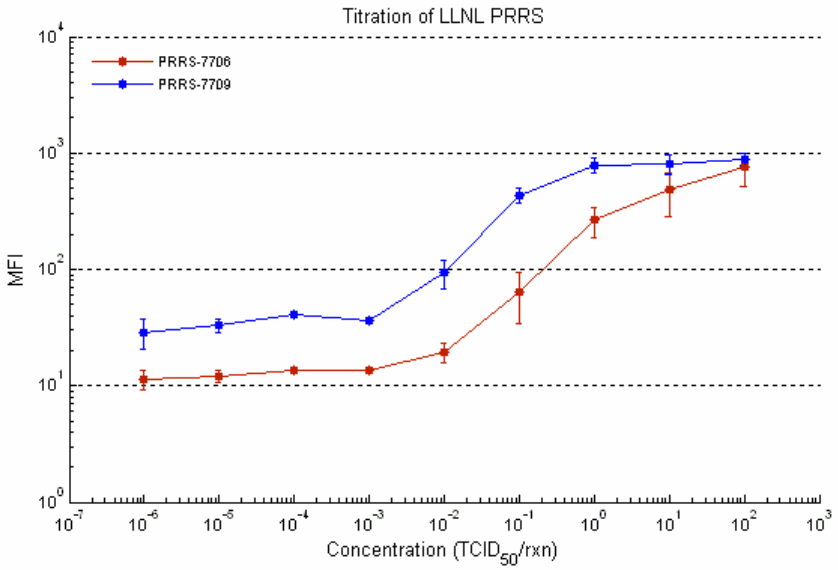

FIG. 66 Multiplex screening data for the 2 North American PRRS signatures against the North American Strain (NVSL, 1989). Serial dilution of total nucleic acid Trizol extracted from virus-infected cell culture. Total nucleic acid was Trizol extracted from non-infected (no template control, NTC) and virus-infected cell culture media then used as template. Each point represents the mean response (n=2). Error bars indicate ±1σ of the mean.

Summary: Taqman and multiplex target screening results are supportive of the advancement of these signatures to subsequent phases. Additional near-neighbor testing in process at PIADC.

Concluding Remarks

There work herein provides an overview of the development of the Version 1.0 multiplexed panel for foot-and-mouth disease rule and the subsequent work to develop species-specific panels for bovine and porcine FMD detection or rule out. As defined by the OIE describing the validation and certification of diagnostic assays, the bovine and porcine panels have demonstrated success at Stage I Feasibility; additional screening is required to further the validation and certification of these panels for use as a diagnostic.

Additional screening, not included in this report, is in process at PIADC and will provided as an addendum to this report when the data becomes available.

APPENDICES I: DEFINITIONS

Armored RNA: Positive control for RT-PCR. An armored RNA (RNA that is packaged in a protein coat for added stability). This material is added to the RT-PCR reaction and verifies that both RT and PCR have occurred in the reaction.

Amine modified probe: Probe that is modified with a 5 prime amine and carbon spacer for the attachment to Luminex carboxylated microspheres.

Amplicon: The sequence that results from PCR or RT-PCR amplification that incorporates the primers and replicated sequence.

Annealing: Describes the step in nucleic acid amplification where the reaction is heated for the attachment of the primers to their compliment regions.

Background screening: describes the process of assay down-selection where signatures are screened against various environmental confounders for assay interference or cross-reactivity.

Bead number/ID: indicates the bead type used. More specifically, corresponds to a specific bead in the set of 100 in the Luminex xMAP, based on fluorescent properties.

Biotinylated primer: Forward primer that is modified with a 5-prime biotin and internal biotins for multiplexed bead-based assays.

Bt: *Bacillus thuringiensis***,** bacterial DNA used as positive control in real-time assays.

DAD: Domestic Animal Disease, endemic diseases. In this report this refers to the following viruses: Bovine herpes virus [BHV], Bovine Viral Diarrhea [BVD], Bovine Papular Stomatitis Virus [BPSV], and Blue Tongue Virus [BTV].

Denaturation: Describes the step in nucleic acid amplification where the reaction is heated to "denature" or separate the double stranded nucleic acid in preparation for the amplification process. May also be used to destroy or remove unwanted reagent components at a given step in the process.

Extension: Describes the step in nucleic acid amplification where the primers "extend" along the template sequence to replicate.

FAD: Foreign Animal Disease. In this report refers to the following: foot-and-mouth disease virus [FMDV], swine vesicular disease virus [SVD] and vesicular exanthema of swine virus [VESV].

FCP: Forward compliment probe. Describes the orientation of the probe sequence that is used for the reaction hybridization. Forward compliment indicates that the sequence is the compliment to the forward primer strand.

Fluorescent control: Control designed to detect stability of the fluorescent label (steptavidin phycoerythrin) or to verify that it was added to the reaction.

Hybridization: Describes process by which PCR product is introduced to microspheres that have oligonucleotide probes attached and heated slightly for hybridization to occur.

Internal controls: Describes the in-built assays that are incorporated into the reaction process to verify that particular steps of the reaction occurred properly, without failures.

Kpathrun ID: LLNL-internal identification number that references internal database of signature generation information. LLNL has been granted access via collaborators to several unpublished genomes that have been used in the work described here. The LLNL-internal identification numbers are the only unambiguous identifiers for such genomes.

Labeled amplicon: The sequence that results from PCR or RT-PCR amplification that incorporates the primers and replicated sequence; includes the forward primer that is labeled with a biotin. Synonymous with "PCR product"; plural reference.

Limit of detection: described as the point at which the signal crosses the pre-established threshold for that assay.

Luminex: The Luminex instrument is a flow cytometer with in-built optics used to detect the fluorescent signal from both the microspheres and the fluorescently labeled reaction that is hybridized to the beads. Software allows detection measurements to be semi-quantifiable. (Quantifiable means that the assay result produces a numeric result that is traceable to a standard. Semi-quantitative means that the assay produces a positive or negative result and provides information such that a positive result can be associated with a numeric result. Our assays are semi-quantitative. Instrument is also called "Bioplex" (Biorad), "Liquiplex" (Qiagen), etc.

MFI: Median fluorescence intensity. Describes fluorescence intensity reported on the Luminex/Bioplex systems that indicate the amount of reporter label is on the bead.

Microsphere: Luminex polystyrene beads (5.6 microns in diameter), optically encoded with precise ratios of two fluorescent dyes designed for use in the Luminex Xmap technology. Surface modification of beads allow for covalent attachment to substrate. Synonymous with "beads".

MSC: MSC=Minimal Set Cluster. A computational technique developed by the LLNL bioinformatics team to cope with the high sequence variation of RNA viral agents. The available genomes are clustered so that at least one TaqMan PCR signature can be found for each cluster that is predicted to identify all members of that cluster. It is usually the case that multiple signatures will be required to cover all known (sequenced) isolates of RNA viral agents.

Multiplex: Indicates that the PCR reaction contains multiple primer sets including the internal control assay(s).

MUX: Abbreviation used for Multiplexed bead-based PCR.

Negative control: Designed such that under "normal" assay conditions there will be no signal detected.

Oligonucleotides: Synthesized sequences of nucleic acids that are used for PCR reactions (primers, probes).
PCR: Polymerase Chain Reaction, technique for amplifying nucleic acids in a thermal cycler. Involves use of forward and reverse primer pairs that start off the reaction. End yield is many orders of magnitude more DNA of the target sequence than one started with.
PCR product: Describes the resulting product of several cycles of PCR/RT-PCR amplification. The sequence that results from PCR or RT-PCR amplification that incorporates the primers and replicated sequence. Synonymous with "amplicon"; singular reference).
PFU: Plaque forming unit.

Pg: Picogram = 10^{-12} gram
PIADC: Acronym for Plum Island Animal Disease Center

Positive control: Control designed to verify that each step in the reaction process occurred correctly. Verifies RT-PCR, PCR or both.
Probe: Oligonucleotide sequence that is found internal of the target gene and is used to detect the entire sequence. In multiplex PCR this sequence is covalently coupled to the microsphere and directly hybridizes to the amplified sequence that is complimentary to the probe sequence.
RCP: Reverse compliment probe. Describes the orientation of the probe sequence that is used for the reaction hybridization. Reverse compliment indicates that the sequence is the compliment to the reverse primer strand.

Real-time PCR: Taqman-based PCR utilizing a fluorescent probe and quencher for real-time reporting.
RT-PCR: Reverse transcriptase polymerase chain reaction. Process of nucleic acid amplification of RNAs that requires a reverse transcriptase step to convert mRNA into cDNA and subsequently to be amplified by polymerase chain reaction.
Singleplex: Indicates that the PCR reaction contains only one primer set with an added primer set for the internal control assay.

Spot check or "pre-screen": Describes process by which multiplex assay candidates are rapidly screened in singleplex format to determine relative reactivities across all signatures. This process is called also called "spot checking" because the assays are subjected to a single concentration of target DNA, rather than in a dilution series.
Streptavidin phycoerithrin: Fluorophore used in labeling that binds with high affinity to biotin.
Real-time PCR: Synonymous to Taqman polymerase chain reaction (PCR). PCR utilizing a fluorescent probe that is quenched until it is cleaved during PCR amplification, at which time an optical system can detect the amount of cleaved probe present in the reaction and report this value in relative fluorescence.
Target/Near-Neighbor (NN) screening: describes the process of assay down-selection where signatures are screened against target nucleic acids and nucleic acids from organism that are phylogenically nearest to the target organism.
$TCID_{50}$: Tissue Culture Infectious Dose at which half (50%) of recipients are infected. The endpoint of the infectious dose is that dilution of virus, 1 ml of which will infect 50% of the cell cultures to which it is added.

Wash assay: Describes process of taking beads with hybridized PCR product attached and washing with buffer in a 96-well filter plate format using a vacuum manifold station. In this process the beads are washed several times to remove unbound nucleic acid and labeled with streptavidin phycoerythrin before they are transferred to the Luminex instrument for processing.

APPENDICES II: BACKGROUND SCREENING PANELS

Standardized Background Screening Panels for: Version 1.0 Panel, Bovine and Porcine Panels

Aerosols: Nucleic acid extractions from aerosol samples collected by environmental sampling systems using a BioWatch air collector and filtration system (data is restricted release). Screened 2304 samples (3 aerosol plates, each well = 8 pooled samples)

Soils: Nucleic acids extracted from soil collections distributed over geographically diverse locations. Screened 54 soils from various locations

D 000402 # 53	D 000542 - 6	S 254	S 274	S 286	S 297
D 000109 # 50	D 000533 - 17 -1	S 255	S 275	S 287	S 298
D 000107-49	D 000561 - 8 - 6	S 256	S 276	S 288	S 299
D 000500 - 26 - 1	D 000562 - 30 - 5	S 257	S 277	S 289	S 300
D 000505 - 11 - 4	D 000501-14-1	S 259	S 279	S 290	S 301
D 000521 - 23	D 000550 - 20	S 260	S 280	S 291	S 303
D 000551 - 5	S 251	S 271	S 282	S 292	S 304
D 000527 - 3	S 252	S 272	S 283	S 295	S 305
D 000531 - 21	S 253	S 273	S 284	S 296	S 307

Prokaryotes (45):

Actinobacillus suis	Erwina herbicola	Porphyrobacter sanguineus
Aneurinbacillus migulanus	Escherichia coli	Proteus mirabillis
Bacillus cereus	Geobacillus caldoxylosilyticus	Pseudomonas aeruginosae
Bacillus globigii	Halomonas halmophila	Pseudomonas oleovorans
Bacillus subtilis	Heamophilus influenza	Rhizobium leguminosarum
Bacillus thuringiensis	Herbaspirillium seropedicae	Rhodococcus rhodochrous
Bifidobacterium denticum	Lactobacillus garvieae	Salmonella typhimurium
Borrellia burgdorferi	Lactobacillus gasseri	Simonsiella muelleri
Burkholderia capacia	Listeria monocytogenes	Sphingomonas sp. (Alcaligenes sp)
Caulobacter vibriodes	Listeria seeligeri	Staphylococcus aureus
Clavibacter michganensis	Micrococcus luteus	Streptococcus pneumoniae
Clostridium butyricum	Moraxella lacunatica	Streptomyces scabiei
Corynebacterium pseudodipthericum	Oceanospirillium ssp. Maris	Tatlockia maceachernii
Cytophaga marinoflava	Paenibacillus napthalaenovorans	Vibrio paraheamolyticus
Erwina amylovora	Paracoccus dentrificans	Xanthomonas translucens

Eukaryotes (16):

Bovine	Drosophila Melanogaster	Monkey	Rabbit
Cat	Equine	Mosquito	Rat
Chicken	Flea	Mouse	Sheep
Dog	Human	Porcine	Tick

APPENDICES III: VIRUS SCREENING RECORDS

SUMMARY OF GEL, REAL-TIME AND MULTIPLEXED PCR SCREENING OF TARGETS AND NEAR-NEIGHBORS FOR EACH MULTIPLEX PANEL (VERSION 1.0, BOVINE AND PORCINE).
***Detailed virus information and history is provided in the next section "Virus Information and History"**

Version 1.0 Panel List of Viral Targets and Near-Neighbors Screened

Below summarizes the agents used in screening the Version 1 assays to determine assay analytic specificity for each candidate signature. Results from this screening are detailed in the accompanied Supplemental Material Document. Data that is not available or unknown is indicated by a ~ sign.

Virus	Type	Strain, Serotype, or Subtype	Isolate or Identifier	Source	Titered (Y/N)	sample type	Gel screened (Y/N)	Real-time screened (Y/N)	MUX Screened (Y/N)
Bovine Herpes Virus	BHV NN	BHV 2	BHMv	PIADC	N	extracted NA	N	N	Y
Bovine Herpes Virus	BHV NN	BHV 5	A032540006	CAHFS	N	extracted NA	N	Y	Y
Bovine Herpes Virus	BHV NN	BHV 5	A040150085	CAHFS	N	extracted NA	N	Y	Y
Bovine Herpes Virus	BHV NN	BHV 5	D9402133	CAHFS	N	extracted NA	N	Y	Y
Equine Herpes 1	BHV NN	Equine Herpes 1	ATCC VR2003	ATCC	N	extracted NA	N	Y	Y
Equine Herpes 1	BHV NN	Equine Herpes 1	A011120004	CAHFS	N	extracted NA	N	Y	Y
Equine Herpes 1	BHV NN	Equine Herpes 1	A99043047	CAHFS	N	extracted NA	N	Y	Y
Equine Herpes 2	BHV NN	Equine Herpes 2	ATCC VR701	ATCC	N	extracted NA	N	Y	Y
Equine Herpes 2	BHV NN	Equine Herpes 2	D990	CAHFS	N	extracted NA	N	Y	Y
Equine Herpes 2	BHV NN	Equine Herpes 2	NVSL 002	NVSL	N	extracted NA	N	Y	Y
Feline Herpes	BHV NN	~	ATCC VR636	ATCC	N	extracted NA	N	Y	Y
Porcine Herpes Pseudorabies Shope	BHV NN	~	RA 180	CAHFS	N	DNA extract	N	Y	Y
Rhadinovirus Caprine Herpes 2	BHV NN	~	ATCC VR462	ATCC	N	extracted NA	N	Y	Y
Rhadinovirus Caprine Herpes 2	BHV NN	~	D0201157	CAHFS	N	extracted NA	N	Y	Y
Rhadinovirus Caprine Herpes 2	BHV NN	~	S0201998	CAHFS	N	extracted NA	Y	Y	Y
V. Pseudorabies	BHV NN	~	92-12013	CAHFS	N	DNA extract	N	Y	Y
V. Pseudorabies	BHV NN	~	93-11745	CAHFS	N	DNA extract	Y	Y	Y
Bovine Herpes Virus	BHV Target	BHV1	Infectious Bovine Rhinotracheitis	PIADC	N	VI media	N	N	Y
Bovine Herpes Virus	BHV Target	BHV2	Bovine Herpes Mammalitis virus v03173	PIADC	N	VI media	N	N	Y

Bovine Herpes Virus-1	BHV Target	BHV1	Colorado vaccine strain	NVSL	Y	extracted NA	N	N	Y
Bovine Herpes Virus	BHV Target, MCF NN	BHV1	ATCC VR188	ATCC	N	extracted NA	N	Y	Y
Bovine Herpes Virus	BHV Target, MCF NN	BHV1	ATCC VR793	ATCC	N	extracted NA	N	Y	Y
Bovine Herpes Virus	BHV Target, MCF NN	BHV1	Texas A030020072	CAHFS	N	extracted NA	N	Y	Y
Bovine Herpes Virus	BHV Target, MCF NN	BHV1	A033640072	CAHFS	N	extracted NA	N	Y	Y
Bovine Herpes Virus	BHV Target, MCF NN	BHV1	A040130066	CAHFS	N	extracted NA	N	Y	Y
Bovine Herpes Virus	BHV Target, MCF NN	BHV1	Cooper	CAHFS	N	extracted NA	N	Y	Y
Bovine Herpes Virus	BHV Target, MCF NN	BHV1	California	CAHFS	N	extracted NA	Y	Y	Y
Bovine Herpes Virus	BHV Target, MCF NN	BHV1	MSVDL	CAHFS	N	extracted NA	Y	Y	Y
Bovine Herpes Virus	BHV Target, MCF NN	BHV1	Virginia	CAHFS	N	extracted NA	Y	Y	Y
GoatpoxVirus	BPSV(Ppox) NN	Held LT5 05-05-1975	v00824	PIADC	N	VI media	Y	N	N
GoatpoxVirus	BPSV(Ppox) NN	V717 Pendik 26-11-1976	~	PIADC	N	VI media	Y	N	N
GoatpoxVirus	BPSV(Ppox) NN	Pellor	pathogenic field isolate	PIADC	N	DNA extract	N	N	Y
Sheeppox	BPSV(Ppox) NN	~	Romania	PIADC	N	~	N	N	Y
Sheeppox	BPSV(Ppox) NN	Kenyan O 180 vaccine	v02164	PIADC	N	VI media	N	N	Y
Sheeppox	BPSV(Ppox) NN	Nishki attenuated Khazak vaccine	SN	PIADC	N	DNA extract	N	N	Y
Sheeppox	BPSV(Ppox) NN	Strain A Khazakistan -	virulent field isolate	PIADC	N	DNA extract	N	N	Y
Sheeppox	BPSV(Ppox) NN	Turkey, virulent	~	PIADC	N	DNA extract	N	N	Y
Sheeppox	BPSV(Ppox) NN	HELD LT6	V02990	PIADC	N	extracted NA	Y	N	Y
Bovine Papular Stomatitis Virus (BPSV)	BPSV(Ppox) target	#15	California	PIADC	N	extracted NA	N	N	Y
Bovine Papular Stomatitis Virus (BPSV)	BPSV(Ppox) target	#8	Kansas	PIADC	N	extracted NA	N	N	Y
Bovine Papular	BPSV(Ppox) target	~	Illinois 721 ATCC VR801	ATCC, PIADC	N	extracted NA	Y	Y	Y

Stomatitis Virus (BPSV)									
Bovine Papular Stomatitis Virus (BPSV)-1	BPSV(Ppox) target	Texas A&M Strain	~	NVSL	Y	extracted NA	N	N	Y
Orf	BPSV(Ppox) target	#1 Oregon	~	PIADC	N	extracted NA	N	N	Y
Orf	BPSV(Ppox) target	#21 Indiana	~	PIADC	N	extracted NA	N	N	Y
Orf	BPSV(Ppox) target	#4 West Virginia	~	PIADC	N	extracted NA	N	N	Y
Parapox (Orf/BPSV)	BPSV(Ppox) target	~	CE 79-16151	CAHFS	N	extracted NA	N	Y	Y
Parapox (Orf/BPSV)	BPSV(Ppox) target	~	CE D03011040	CAHFS	N	extracted NA	N	Y	Y
Parapoxvirus Pseudocowpox	BPSV(Ppox) target	~	VR634		Y	extracted NA	N	N	Y
Epizootic Hemorrhagic Disease virus	BTV NN	EHD-2	ATCC Alberta, Canada 1962 Deer-1;	USDA/ARS (Wy)	N	extracted dsRNA	N	Y	N
Epizootic Hemorrhagic Disease virus	BTV NN	2	ATCC Alberta, Canada 1962 Deer-1;	USDA/ARS (Wy)	N	extracted dsRNA	N	Y	Y
Epizootic Hemorrhagic Disease virus	BTV NN	EHD-1	GA	USDA/ARS (Wy)	N	extracted dsRNA	N	Y	Y
Epizootic Hemorrhagic Disease virus	BTV NN	EHD-1	NJ	USDA/ARS (Wy)	N	extracted dsRNA	N	Y	Y
Epizootic Hemorrhagic Disease virus	BTV NN	EHD-1	SB	USDA/ARS (Wy)	N	extracted dsRNA	N	Y	Y
Bluetongue Virus	BTV Target	BTV-10	lot #001 ODV 0001 Dec 5, 2000	NVSL	Y	extracted NA	Y	Y	Y
Bluetongue Virus	BTV Target	BTV-11	lot#002 ODV0101 Nov 30, 2001	NVSL	Y	extracted NA	Y	Y	Y
Bluetongue Virus	BTV Target	BTV-13	no lot or strain given	NVSL	Y	extracted NA	Y	Y	Y
Bluetongue Virus	BTV Target	BTV-17	lot#004 ODV 0201 Nov 28, 2002	NVSL	Y	extracted NA	Y	Y	Y
Bluetongue Virus	BTV Target	BTV-2	no lot given	NVSL	Y	extracted NA	Y	Y	Y
Boorder Disease Virus	BVD NN	Frijters	~	PIADC	N	extracted NA	N	N	Y
Boorder Disease Virus	BVD NN	134 / 7	~	PIADC	N	extracted NA	N	N	Y
Boorder Disease Virus	BVD NN	Aveyron	~	PIADC	N	extracted NA	N	N	Y
Classical Swine Fever Virus	BVD NN	~	~	PIADC	~	extracted NA	N	N	Y
Classical	BVD NN	Brescia	~	PIADC	Y	extracte	N	N	Y

Swine Fever Virus						d NA			
Classical Swine Fever Virus	BVD NN	Kanagawa	~	PIADC	Y	extracte d NA	N	N	Y
Classical Swine Fever Virus	BVD NN	Paderborn	~	PIADC	Y	extracte d NA	N	N	Y
Bovine Viral Diarrhea	BVD Target	genotype 1 cytopathic	Singer	NVSL	Y	extracte d NA	Y	Y	Y
Bovine enterovirus (BEV)	FMDV NN	Type 1	~	PIADC	N	VI Media	N	N	Y
Bovine enterovirus (BEV)	FMDV NN	Type 2	~	PIADC	N	VI Media	N	N	Y
Bovine enterovirus (BEV)	FMDV NN	Type 3	~	PIADC	N	VI Media	N	N	Y
Bovine enterovirus (BEV)	FMDV NN	Type 4	~	PIADC	N	VI Media	N	N	Y
Bovine enterovirus (BEV)	FMDV NN	Type 5	~	PIADC	N	VI Media	N	N	Y
Bovine enterovirus (BEV)	FMDV NN	Type 6	~	PIADC	N	VI Media	N	N	Y
Bovine enterovirus (BEV)	FMDV NN	Type 7	~	PIADC	N	VI Media	N	N	Y
Porcine enterovirus (PEV)	FMDV NN	Type 1	~	PIADC	N	VI Media	N	N	Y
Porcine enterovirus (PEV)	FMDV NN	Type 2	~	PIADC	N	VI Media	N	N	Y
Porcine enterovirus (PEV)	FMDV NN	Type 3	~	PIADC	N	VI Media	N	N	Y
Porcine enterovirus (PEV)	FMDV NN	Type 4	~	PIADC	N	VI Media	N	N	Y
Porcine enterovirus (PEV)	FMDV NN	Type 5	~	PIADC	N	VI Media	N	N	Y
Porcine enterovirus (PEV)	FMDV NN	Type 6	~	PIADC	N	VI Media	N	N	Y

Porcine enterovirus (PEV)	FMDV NN	Type 7	~	PIADC	N	VI Media	N	N	Y
Porcine enterovirus (PEV)	FMDV NN	Type 8	~	PIADC	N	VI Media	N	N	Y
Foot and mouth Disease Virus	FMDV target	O	O1 Korea	PIADC	N	extracted NA	N	N	Y
Foot and mouth Disease Virus	FMDV target	Sat 3	~	PIADC	N	extracted NA	N	N	Y
Foot and mouth Disease Virus	FMDV target	A	22 Iran/98	NCFAD, CAN	Y	extracted NA	N	N	Y
Foot and mouth Disease Virus	FMDV target	A	22 Iraq 24/64	NCFAD, CAN	Y	extracted NA	N	N	Y
Foot and mouth Disease Virus	FMDV target	A	24 Cruzeiro	NCFAD, CAN	Y	extracted NA	N	N	Y
Foot and mouth Disease Virus	FMDV target	A	ARG 2/2001	NCFAD, CAN	Y	extracted NA	N	N	Y
Foot and mouth Disease Virus	FMDV target	A	ARG/87	NCFAD, CAN	Y	extracted NA	N	N	Y
Foot and mouth Disease Virus	FMDV target	A	COL/85	NCFAD, CAN	Y	extracted NA	N	N	Y
Foot and mouth Disease Virus	FMDV target	A	Irn 1/96	NCFAD, CAN	Y	extracted NA	N	N	Y
Foot and mouth Disease Virus	FMDV target	Asia 1	Pak 1/54	NCFAD, CAN	Y	extracted NA	N	N	Y
Foot and mouth Disease Virus	FMDV target	Asia 1	Shamir	NCFAD, CAN	Y	extracted NA	N	N	Y
Foot and mouth Disease Virus	FMDV target	C1	Noville	NCFAD, CAN	Y	extracted NA	N	N	Y
Foot and mouth Disease Virus	FMDV target	C3	Resende	NCFAD, CAN	Y	extracted NA	N	N	Y
Foot and mouth Disease Virus	FMDV target	O	TAW 10/97	NCFAD, CAN	Y	extracted NA	N	N	Y
Foot and mouth Disease Virus	FMDV target	O	UKG 1/2001	NCFAD, CAN	Y	extracted NA	N	N	Y
Foot and mouth Disease Virus	FMDV target	O	O1 BFS 1860	NCFAD, CAN	Y	extracted NA	N	N	Y
Foot and mouth Disease Virus	FMDV target	O	O1 manisa	NCFAD, CAN	Y	extracted NA	N	N	Y

Foot and mouth Disease Virus	FMDV target	Sat 1	Ken 4/98	NCFAD, CAN	Y	extracted NA	N	N	Y
Foot and mouth Disease Virus	FMDV target	SAT1	Bot 1/68	NCFAD, CAN	Y	extracted NA	N	N	Y
Foot and mouth Disease Virus	FMDV target	SAT2	Sau 1/2000	NCFAD, CAN	Y	extracted NA	N	N	Y
Foot and mouth Disease Virus	FMDV target	SAT2	SWA 1/69	NCFAD, CAN	Y	extracted NA	N	N	Y
Foot and mouth Disease Virus	FMDV target	SAT2	Zim 10/91	NCFAD, CAN	Y	extracted NA	N	N	Y
Foot and mouth Disease Virus	FMDV target	SAT2	Zim 5/81	NCFAD, CAN	Y	extracted NA	N	N	Y
Foot and mouth Disease Virus	FMDV target	SAT3	Bec 1/65	NCFAD, CAN	Y	extracted NA	N	N	Y
Foot and mouth Disease Virus	FMDV target	SAT3	Zim 4/81	NCFAD, CAN	Y	extracted NA	N	N	Y
Foot and mouth Disease Virus	FMDV target	A18	~	PIADC	N	extracted NA	N	N	Y
Foot and mouth Disease Virus	FMDV target	C1	~	PIADC	N	extracted NA	N	N	Y
Foot and mouth Disease Virus	FMDV target	Sat 2	~	PIADC	N	extracted NA	N	N	Y
Foot and mouth Disease Virus	FMDV target	A	Argentina 2001 V02764	PIADC	Y	extracted NA	N	N	Y
Foot and mouth Disease Virus	FMDV target	Asia 1	V02594	PIADC	Y	extracted NA	N	N	Y
Foot and mouth Disease Virus	FMDV target	C	C4 Tierra Del FuegoV02367	PIADC	Y	extracted NA	N	N	Y
Foot and mouth Disease Virus	FMDV target	O	O1 South Korea V02722	PIADC	Y	extracted NA	N	N	Y
Foot and mouth Disease Virus	FMDV target	Sat 1	Sat1 /6V02412	PIADC	Y	extracted NA	N	N	Y
Foot and mouth Disease Virus	FMDV target	Sat 2	Zim VV02403	PIADC	Y	extracted NA	N	N	Y
Foot and mouth Disease Virus	FMDV target	Sat 3	Sat3 /3 Bech 1Nov05 V02376	PIADC	Y	extracted NA	N	N	Y
Varicellovirus Pseudorabies	MCF NN	~	NVSL 92_12013	NVSL	N	extracted NA	Y	Y	Pending
Varicellovirus Pseudorabies	MCF NN	~	NVSL 93_11745	NVSL	N	extracted NA	Y	Y	Pending
Varicellovirus Pseudorabies Shope	MCF NN	~	RA180	Not available	N	extracted NA	Y	Y	Pending

Malignant Catarrhal Fever	MCF target, BHV NN	~	AIHV-1 (MN)	PIADC: Hong Li in Washington State	N	extracted NA	Y	Y	Y
Malignant Catarrhal Fever	MCF target, BHV NN	~	OvHV-2	PIADC: Hong Li in Washington State	N	extracted NA	Y	Y	Y
Malignant Catarrhal Fever	MCF target, BHV NN	~	AvHV-1 (WC11 BTH50 6D)	PIADC	Pending	VI Media	Y	Y	Y
Human Entero Virus B	SVD NN	Coxsackie B5	Faulkner TC 583 A 890	PIADC	N	VI Media	N	N	Y
Human Entero Virus B	SVD NN	Coxsackie A9	~	CAHFS	N	extracted NA	N	Y	N
Human Entero Virus B	SVD NN	Coxsackie B3	~	CAHFS	N	extracted NA	N	Y	N
Human Entero Virus B	SVD NN	Coxsackie B1	~	CAHFS	N	extracted NA	N	Y	N
Human Entero Virus B	SVD NN	Coxsackie B2	~	CAHFS	N	extracted NA	N	Y	N
Human Entero Virus B	SVD NN	Coxsackie B4	~	CAHFS	N	extracted NA	N	Y	N
Human Entero Virus B	SVD NN	Coxsackie B5	~	CAHFS	N	extracted NA	N	Y	N
Human Entero Virus B	SVD NN	Coxsackie B6	~	CAHFS	N	extracted NA	N	Y	N
Human Entero Virus B	SVD NN	Echovirus 11	Echo 11	CAHFS	N	extracted NA	N	Y	N
Human Entero Virus B	SVD NN	Echovirus 12	Echo 12	CAHFS	N	extracted NA	N	Y	N
Human Entero Virus B	SVD NN	Echovirus 24	Echo 24	CAHFS	N	extracted NA	N	Y	N
Human Entero Virus B	SVD NN	Echovirus 9	Echo 9	CAHFS	N	extracted NA	N	Y	N
Swine Vesicular Disease	SVD target	~	FRA 1/73	NCFAD, CAN	N	extracted NA	N	N	Y
Swine Vesicular Disease	SVD target	~	GRE 1/79	NCFAD, CAN	N	extracted NA	N	N	Y
Swine Vesicular Disease	SVD target	~	HKN 1/80	NCFAD, CAN	N	extracted NA	N	N	Y
Swine Vesicular Disease	SVD target	~	HKN 1/89	NCFAD, CAN	N	extracted NA	N	N	Y
Swine Vesicular Disease	SVD target	~	HKN 11/81	NCFAD, CAN	N	extracted NA	N	N	Y
Swine Vesicular Disease	SVD target	~	HKN 3/89	NCFAD, CAN	N	extracted NA	N	N	Y
Swine Vesicular Disease	SVD target	~	ITL 1/92	NCFAD, CAN	N	extracted NA	N	N	Y

Swine Vesicular Disease	SVD target	~	ITL 1/927	NCFAD, CAN	N	extracted NA	N	N	Y
Swine Vesicular Disease	SVD target	~	ITL 18/92	NCFAD, CAN	N	extracted NA	N	N	Y
Swine Vesicular Disease	SVD target	~	ITL 19/92	NCFAD, CAN	N	extracted NA	N	N	Y
Swine Vesicular Disease	SVD target	~	ITL 2/97	NCFAD, CAN	N	extracted NA	N	N	Y
Swine Vesicular Disease	SVD target	~	ITL 20/92	NCFAD, CAN	N	extracted NA	N	N	Y
Swine Vesicular Disease	SVD target	~	JAP 1/74	NCFAD, CAN	N	extracted NA	N	N	Y
Swine Vesicular Disease	SVD target	~	NET 3/92	NCFAD, CAN	N	extracted NA	N	N	Y
Swine Vesicular Disease	SVD target	~	PORT 1/2003	NCFAD, CAN	N	extracted NA	N	N	Y
Swine Vesicular Disease	SVD target	~	SWI 1/74	NCFAD, CAN	N	extracted NA	N	N	Y
Swine Vesicular Disease	SVD target	~	HKN/12/87	NCFAD, CAN	N	extracted NA	N	N	Y
Swine Vesicular Disease	SVD target	~	ITL/1/91	NCFAD, CAN	N	extracted NA	N	N	Y
Swine Vesicular Disease	SVD target	~	ROM-1-87	NCFAD, CAN	N	extracted NA	N	N	Y
Swine Vesicular Disease	SVD target	~	SVD ITL/1/66	NCFAD, CAN	N	extracted NA	N	N	Y
Swine Vesicular Disease	SVD target	~	TAW/119/97	NCFAD, CAN	N	extracted NA	N	N	Y
Swine Vesicular Disease	SVD target	~	UKG72	NCFAD, CAN	N	extracted NA	N	N	Y
Swine Vesicular Disease	SVD target	~	ROM-1-87	PIADC	Y	extracted NA	N	Y	Y
Swine Vesicular Disease	SVD target	~	HKN-4-89	PIADC	Y	extracted NA	N	Y	Y
Swine Vesicular Disease	SVD target	~	HKN/12/87	PIADC	Y	extracted NA	N	Y	Y
Swine Vesicular Disease	SVD target	~	ITL/1/91	PIADC	Y	extracted NA	N	Y	Y

Swine Vesicular Disease	SVD target	~	SVD ITL/1/66	PIADC	Y	extracted NA	N	Y	Y
Swine Vesicular Disease	SVD target	~	TAW/119/97	PIADC	Y	extracted NA	N	Y	Y
Swine Vesicular Disease	SVD target	~	UKG72	PIADC	Y	extracted NA	N	Y	Y
Swine Vesicular Disease	SVD target	~	HKN 4/91	NCFAD, CAN	N	extracted NA	N	N	Y
Swine Vesicular Disease	SVD target	~	HKN 8/73	NCFAD, CAN	N	extracted NA	N	N	Y
Swine Vesicular Disease	SVD target	~	ITL 3/97	NCFAD, CAN	N	extracted NA	N	N	Y
Swine Vesicular Disease	SVD target	~	ITL 8/94	NCFAD, CAN	N	extracted NA	N	N	Y
Swine Vesicular Disease	SVD target	~	HKN-4-89	NCFAD, CAN	N	extracted NA	N	N	Y
Swine Vesicular Disease	SVD target	~	ROM-1-87	PIADC	Y	extracted NA	N	N	Y
Swine Vesicular Disease	SVD target	~	HKN-4-89	PIADC	Y	extracted NA	N	Y	Y
San Miguel Sea Lion Virus (SMSV)	VESV NN	Type 12	V02040	PIADC	N	VI Media	N	N	Y
San Miguel Sea Lion Virus (SMSV)	VESV NN	Type 2	V02056	PIADC	N	VI Media	N	N	Y
Vesicular Exanthema of Swine Virus (VESV)	VESV Target	V02029	1934B	PIADC	Y	extracted NA	Y	Y	Y
Vesicular Exanthema of Swine Virus (VESV)	VESV Target	V02031	C52	PIADC	Y	extracted NA	Y	Y	Y
Vesicular Exanthema of Swine Virus (VESV)	VESV Target	V02035	H54	PIADC	Y	extracted NA	Y	Y	Y
Vesicular Exanthema of Swine Virus (VESV)	VESV Target	K54	V02038	PIADC	Y	extracted NA	Y	Y	Y
Vesicular Exanthema of Swine Virus (VESV)	VESV Target	B51	V00758	PIADC	Y	extracted NA	Y	Y	Y

Vesicular Exanthema of Swine Virus (VESV)	VESV Target	A48	V02028	PIADC	Y	extracted NA	Y	Y	Y
Vesicular Exanthema of Swine Virus (VESV)	VESV Target	D53	V02032	PIADC	Y	extracted NA	Y	Y	Y
Vesicular Exanthema of Swine Virus (VESV)	VESV Target	E54	V02033	PIADC	Y	extracted NA	Y	Y	Y
Vesicular Exanthema of Swine Virus (VESV)	VESV Target	G55	V02034	PIADC	Y	extracted NA	Y	Y	Y
Vesicular Exanthema of Swine Virus (VESV)	VESV Target	I55	V02036	PIADC	Y	extracted NA	Y	Y	Y
Vesicular Exanthema of Swine Virus (VESV)	VESV Target	J56	V02037	PIADC	Y	extracted NA	Y	Y	Y

[1]Sources for viruses: California Animal Health and Food Safety (CAHFS), Davis, CA.; Plum Island Animal Disease Center (PIADC), Plum Island, NY; National Veterinary Service Laboratory (NVSL), Ames, IA; National Center for Foreign Animal Disease (NCFAD), Winnepeg, CAN; American Type Culture Collection (ATCC), Manassas, VA; Minnesota State Veterinary Diagnostic Laboratory (MSVDL) in Minneapolis, MN; USDA ARS National Animal Disease Center, IA; USDA ARS, Wy.

Bovine Panel List of Viral Targets and Near-Neighbors

Below summarizes the agents used in screening the Bovine Panel assays to determine assay analytic specificity for each candidate signature. Results from this screening are detailed in the accompanied Supplemental Material Document. Data that is not available is indicated by a ~ sign.

Virus	Type	Strain, Serotype, or Subtype	Isolate or Identifier	Source	Titered (Y/N)	sample type	Gel screened (Y/N)	Real-time screened (Y/N)	MUX Screened (Y/N)
Bovine Herpes Virus	BHV Target	BHV1	ATCC VR188	ATCC	Y	extracted NA	N	N	Y
Bovine Herpes Virus	BHV Target	BHV1	A030020072	CAHFS	Y	extracted NA	N	N	Y
Bovine Herpes Virus-1	BHV Target	BHV1	Colorado vaccine strain	NVSL	Y	extracted NA	N	N	Y
Bovine Papular Stomatitis Virus (BPSV)-1	BPSV(Ppox) target	Texas A&M Strain	~	NVSL	Y	extracted NA	N	N	Y
Orf	BPSV(Ppox) target	#1 Oregon	~	PIADC	N	extracted NA	N	N	Y
Orf	BPSV(Ppox) target	#21 Indiana	~	PIADC	N	extracted NA	N	N	Y
Orf	BPSV(Ppox) target	#4 West Virginia	~	PIADC	N	extracted NA	N	N	Y
Parapoxvirus Pseudocowpox	BPSV(Ppox) target	~	VR634		Y	extracted NA	N	N	Y
Epizootic Hemorrhagic Disease virus	BTV NN	EHDV-1	GA	USDA/ARS (Wy)	N	extracted dsRNA	N	Y	Y

Epizootic Hemorrhagic Disease virus	BTV NN	EHDV-1	SB	USDA/ARS (Wy)	N	extracted dsRNA	N	Y	Y
Epizootic Hemorrhagic Disease virus	BTV NN	EHDV-1 (SV123)	New Jersey (3TD) Deer, NJ 1955	USDA/ARS (Wy)	N	extracted dsRNA	N	Y	Y
Epizootic Hemorrhagic Disease virus	BTV NN	EHDV-2 (SV124)	Alberta (59) Deer-1;	USDA/ARS (Wy)	N	extracted dsRNA	N	Y	N
Epizootic Hemorrhagic Disease virus	BTV NN	EHDV-3	NVSL 047 ODV 0001 Nigeria	USDA/ARS (Wy)	N	extracted dsRNA	N	Y	N
Epizootic Hemorrhagic Disease virus	BTV NN	EHDV-4	NVSL 046 ODV 9201 Nigeria	USDA/ARS (Wy)	N	extracted dsRNA	N	Y	N
Epizootic Hemorrhagic Disease virus	BTV NN	EHDV-5	NVSL CSIRO 157	USDA/ARS (Wy)	N	extracted dsRNA	N	Y	N
Epizootic Hemorrhagic Disease virus	BTV NN	EHDV-6	NVSL C6753	USDA/ARS (Wy)	N	extracted dsRNA	N	Y	N
Epizootic Hemorrhagic Disease virus	BTV NN	EHDV-7	NVSL CS775	USDA/ARS (Wy)	N	extracted dsRNA	N	Y	N
Epizootic Hemorrhagic Disease virus	BTV NN	EHDV-8	NVSL DPP059	USDA/ARS (Wy)	N	extracted dsRNA	N	Y	N
Bluetongue Virus	BTV Target	BTV-24	NVSL 024 ODV 9101	USDA/ ARS (Wy)	N	extracted dsRNA	N	Y	N
Bluetongue Virus	BTV Target	BTV-3	600565	USDA/ ARS (Wy)	N	extracted dsRNA	N	Y	N
Bluetongue Virus	BTV Target	BTV-4	600566	USDA/ ARS (Wy)	N	extracted dsRNA	N	Y	N
Bluetongue Virus	BTV Target	BTV-5	600567	USDA/ ARS (Wy)	N	extracted dsRNA	N	Y	N
Bluetongue Virus	BTV Target	BTV-6	600568	USDA/ ARS (Wy)	N	extracted dsRNA	N	Y	N
Bluetongue Virus	BTV Target	BTV-7	600561	USDA/ ARS (Wy)	N	extracted dsRNA	N	Y	N
Bluetongue Virus	BTV Target	BTV-8	600570	USDA/ ARS (Wy)	N	extracted dsRNA	N	Y	N
Bluetongue Virus	BTV Target	BTV-9	600571	USDA/ ARS (Wy)	N	extracted dsRNA	N	Y	N
Bluetongue Virus	BTV Target	BTV-10	lot #001 ODV 0001 Dec 5, 2000	NVSL	Y	extracted NA	Y	Y	Y
Bluetongue Virus	BTV Target	BTV-11	lot#002 ODV0101 Nov 30, 2001	NVSL	Y	extracted NA	Y	Y	Y
Bluetongue Virus	BTV Target	BTV-24	NVSL 024 ODV 9101	USDA/ ARS (Wy)	N	extracted dsRNA	N	Y	N
Bluetongue Virus	BTV Target	BTV-3	600565	USDA/ ARS (Wy)	N	extracted dsRNA	N	Y	N
Bluetongue Virus	BTV Target	BTV-4	600566	USDA/ ARS (Wy)	N	extracted dsRNA	N	Y	N
Bluetongue Virus	BTV Target	BTV-5	600567	USDA/ ARS (Wy)	N	extracted dsRNA	N	Y	N

Bluetongue Virus	BTV Target	BTV-6	600568	USDA/ ARS (Wy)	N	extracted dsRNA	N	Y	N
Bluetongue Virus	BTV Target	BTV-7	600561	USDA/ ARS (Wy)	N	extracted dsRNA	N	Y	N
Bluetongue Virus	BTV Target	BTV-8	600570	USDA/ ARS (Wy)	N	extracted dsRNA	N	Y	N
Bluetongue Virus	BTV Target	BTV-9	600571	USDA/ ARS (Wy)	N	extracted dsRNA	N	Y	N
Bluetongue Virus	BTV Target	BTV-10	lot #001 ODV 0001 Dec 5, 2000	NVSL	Y	extracted NA	Y	Y	Y
Bluetongue Virus	BTV Target	BTV-11	lot#002 ODV0101 Nov 30, 2001	NVSL	Y	extracted NA	Y	Y	Y
Bluetongue Virus	BTV Target	BTV-13	no lot or strain given	NVSL	Y	extracted NA	Y	Y	Y
Bluetongue Virus	BTV Target	BTV-17	lot#004 ODV 0201 Nov 28, 2002	NVSL	Y	extracted NA	Y	Y	Y
Bluetongue Virus	BTV Target	BTV-2	no lot given	NVSL	Y	extracted NA	Y	Y	Y
Boorder Disease Virus	BVD NN	Frijters	~	PIADC	N	extracte d NA	N	N	Y
Boorder Disease Virus	BVD NN	134 / 7	~	PIADC	N	extracte d NA	N	N	Y
Boorder Disease Virus	BVD NN	Aveyron	~	PIADC	N	extracte d NA	N	N	Y
Border Disease Virus	BVD NN	~	Coos Bay 4-6-92	USDA/A RS (IA)	Y	extracte d NA	N	Y	Y
Classical Swine Fever Virus	BVD NN	~	~	PIADC	~	extracted NA	N	N	Pending
Classical Swine Fever Virus	BVD NN	Brescia	~	PIADC	Y	extracted NA	N	N	Y
Classical Swine Fever Virus	BVD NN	Kanagawa	~	PIADC	Y	extracted NA	N	N	Y
Classical Swine Fever Virus	BVD NN	Paderborn	~	PIADC	Y	extracted NA	N	N	Y
Bovine Viral Diarrhea	BVD Target	genotype 1a	NADL	USDA/AR S (IA)	Y	extracted NA	N	Y	Y
Bovine Viral Diarrhea	BVD Target	genotype 1b	NY-1 5-18-02	USDA/AR S (IA)	Y	extracted NA	N	Y	Y
Bovine Viral Diarrhea	BVD Target	genotype 1b	TGAN	USDA/AR S (IA)	Y	extracted NA	N	Y	Y
Bovine Viral Diarrhea	BVD Target	genotype 2	334165+BVD 2	USDA/AR S (IA)	Y	extracted NA	N	Y	Y
Bovine Viral Diarrhea	BVD Target	genotype 2a	28508-5	USDA/AR S (IA)	Y	extracted NA	N	Y	Y

Bovine Viral Diarrhea	BVD Target	genotype 2b	AU 501 1-6-2006	USDA/ARS (IA)	Y	extracted NA	N	Y	Y
Bovine Viral Diarrhea	BVD Target	genotype 2b	BVD-FBS B69519	USDA/ARS (IA)	Y	extracted NA	N	Y	Y
Bovine Viral Diarrhea	BVD Target	genotype 1 cytopathic	Singer	NVSL	Y	extracted NA	Y	Y	Y
Foot and mouth Disease Virus	FMDV target	A18	~	PIADC	N	extracted NA	N	N	Y
Foot and mouth Disease Virus	FMDV target	C1	~	PIADC	N	extracted NA	N	N	Y
Foot and mouth Disease Virus	FMDV target	Sat 2	~	PIADC	N	extracted NA	N	N	Y
Foot and mouth Disease Virus	FMDV target	A	Argentina 2001 V02764	PIADC	Y	extracted NA	N	N	Y
Foot and mouth Disease Virus	FMDV target	Asia 1	V02594	PIADC	Y	extracted NA	N	N	Y
Foot and mouth Disease Virus	FMDV target	C	C4 Tierra Del FuegoV02367	PIADC	Y	extracted NA	N	N	Y
Foot and mouth Disease Virus	FMDV target	O	O1 South Korea V02722	PIADC	Y	extracted NA	N	N	Y
Foot and mouth Disease Virus	FMDV target	Sat 1	Sat1 /6V02412	PIADC	Y	extracted NA	N	N	Y
Foot and mouth Disease Virus	FMDV target	Sat 2	Zim VV02403	PIADC	Y	extracted NA	N	N	Y
Foot and mouth Disease Virus	FMDV target	Sat 3	Sat3 /3 Bech 1Nov05 V02376	PIADC	Y	extracted NA	N	N	Y
Varicellovirus Equine Herpes 2	MCF NN	Type 2	D990	Not available	N	extracted NA	Y	Y	Pending
Varicellovirus Equine Herpes 2	MCF NN	Type 2	NVSL 0002	NVSL	N	extracted NA	Y	Y	Pending
Varicellovirus Pseudorabies	MCF NN	~	96-10866	Not available	N	extracted NA	Y	Y	Pending
Varicellovirus Pseudorabies	MCF NN	~	NVSL 93_21246	NVSL	N	extracted NA	Y	Y	Pending
Varicellovirus Pseudorabies	MCF NN	~	NVSL 93_27020	NVSL	N	extracted NA	Y	Y	Pending
Varicellovirus Pseudorabies	MCF NN	~	NVSL 92_12013	NVSL	N	extracted NA	Y	Y	Pending
Varicellovirus Pseudorabies	MCF NN	~	NVSL 93_11745	NVSL	N	extracted NA	Y	Y	Pending
Varicellovirus Pseudorabies	MCF NN	~	NVSL 93_27020	Not available	N	extracted NA	Y	Y	Pending

Varicellovirus Pseudorabies	MCF NN	~	NVSL 92_12013	NVSL	N	extracted NA	Y	Y	Pending
Varicellovirus Pseudorabies Shope	MCF NN	~	RA180	Not available	N	extracted NA	Y	Y	Pending
Malignant Catarrhal Fever	MCF target, BHV NN	~	V1918 San Diego ZooSteer117	PIADC	N	~	Y	N	Pending
Malignant Catarrhal Fever	MCF target, BHV NN	~	V2201 Oklahoma BCET91B006 Serial9109	PIADC	N	~	Y	N	Pending
Malignant Catarrhal Fever	MCF target, BHV NN	~	ET4499	PIADC	Y	extracted NA	Y	N	Pending
Malignant Catarrhal Fever	MCF target, BHV NN	~	V1912 X648	PIADC	Y	extracted NA	Y	N	Pending
Malignant Catarrhal Fever	MCF target, BHV NN	~	AIHV-1 (MN)	PIADC: Hong Li in Washington State	N	extracted NA	Y	Y	Y
Malignant Catarrhal Fever	MCF target, BHV NN	~	OvHV-2	PIADC: Hong Li in Washington State	N	extracted NA	Y	Y	Y
Malignant Catarrhal Fever	MCF target, BHV NN	~	AvHV-1 (WC11 BTH50 6D)	PIADC	Pending	VI Media	Y	Y	Y
Peste de Petits Ruminants virus (PPRV)	RPV NN	~	Egypt	PIADC	N	extracted NA	N	Y	Pending
Peste de Petits Ruminants virus (PPRV)	RPV NN	~	Ghana	PIADC	N	extracted NA	N	Y	Pending
Peste de Petits Ruminants virus (PPRV)	RPV NN	~	RCA	PIADC	N	extracted NA	N	Y	Pending
Rinderpest virus	RPV Target	~	Egypt	PIADC	Y	extracted NA	N	Y	Pending
Rinderpest virus	RPV Target	~	India	PIADC	Y	extracted NA	N	Y	Pending
Rinderpest virus	RPV Target	~	Kuwait	PIADC	Y	extracted NA	N	Y	Pending
Rinderpest virus	RPV Target	~	Nigerian Buffalo	PIADC	Y	extracted NA	N	Y	Pending
Rinderpest virus	RPV Target	~	Pakistan	PIADC	Y	extracted NA	N	Y	Pending
Rinderpest virus	RPV Target	~	Plowright	PIADC	Y	extracted NA	N	Y	Pending
Rinderpest virus	RPV Target	~	RBOK	PIADC	Y	extracted NA	N	Y	Pending
Rinderpest virus	RPV Target	~	Pendik	PIADC	Y	extracted NA	N	Y	Pending
Rinderpest virus	RPV Target	~	Yemen	PIADC	Y	extracted NA	N	Y	Pending
Vesicular Stomatitis	VSV Target	Indiana	Indiana	NVSL	Y	extracted NA	Y	Y	Y

Virus									
Vesicular Stomatitis Virus	VSV Target	New Jersey	New Jersey	NVSL	Y	extracted NA	Y	Y	Y
Vesicular Stomatitis Virus	VSV Target	Indiana	IN94GUB	PIADC: Luis Rodriguez	Pending	VI Media	N	Y	Pending
Vesicular Stomatitis Virus	VSV Target	Indiana	IN97CMB	PIADC: Luis Rodriguez	Pending	VI Media	N	Y	Pending
Vesicular Stomatitis Virus	VSV Target	Indiana	IN97COE	PIADC: Luis Rodriguez	Pending	VI Media	N	Y	Pending
Vesicular Stomatitis Virus	VSV Target	Indiana	IN98NME	PIADC: Luis Rodriguez	Pending	VI Media	N	Y	Pending
Vesicular Stomatitis Virus	VSV Target	New Jersey	NJ0604NME	PIADC: Luis Rodriguez	Pending	VI Media	N	Y	Pending
Vesicular Stomatitis Virus	VSV Target	New Jersey	NJ1184HDB	PIADC: Luis Rodriguez	Pending	VI Media	N	Y	Pending
Vesicular Stomatitis Virus	VSV Target	New Jersey	NJ89GAS	PIADC: Luis Rodriguez	Pending	VI Media	N	Y	Pending
Vesicular Stomatitis Virus	VSV Target	New Jersey	NJ95COB	PIADC: Luis Rodriguez	Pending	VI Media	N	Y	Pending
Vesicular Stomatitis Virus	VSV Target	Indiana	2	PIADC	N	extracted NA	N	Y	Pending
Vesicular Stomatitis Virus	VSV Target	Indiana	2: Cocal	PIADC	N	extracted NA	N	Y	Pending
Vesicular Stomatitis Virus	VSV Target	Indiana	2: Maipu	PIADC	N	extracted NA	N	Y	Pending
Vesicular Stomatitis Virus	VSV Target	Indiana	2: Parana (118)	PIADC	N	extracted NA	N	Y	Pending
Vesicular Stomatitis Virus	VSV Target	Indiana	3: Alagoas (146)	PIADC	N	extracted NA	N	Y	Pending
Vesicular Stomatitis Virus	VSV Target	Indiana	3: Alagoas (174)	PIADC	N	extracted NA	N	Y	Pending

[1]Sources for viruses: California Animal Health and Food Safety (CAHFS), Davis, CA.; Plum Island Animal Disease Center (PIADC), Plum Island, NY; National Veterinary Service Laboratory (NVSL), Ames, IA; National Center for Foreign Animal Disease (NCFAD), Winnepeg, CAN; American Type Culture Collection (ATCC), Manassas, VA; Minnesota State Veterinary Diagnostic Laboratory (MSVDL) in Minneapolis, MN; USDA ARS National Animal Disease Center, IA; USDA ARS, Wy.

Porcine Panel List of Viral Targets and Near-Neighbors

Below summarizes the agents used in screening the Porcine panel assays to determine assay analytic specificity for each candidate signature. Results from this screening are detailed in the accompanied Supplemental Material Document. Data that is not available is indicated by a ~ sign.

Virus	Type	Strain, Serotype, or Subtype	Isolate or Identifier	Source	Titered (Y/N)	sample type	Gel screened (Y/N)	Real-time screened (Y/N)	MUX Screened (Y/N)

Foot and mouth Disease Virus	FMDV target	A18	~	PIADC	N	extracted NA	N	N	Y
Foot and mouth Disease Virus	FMDV target	C1	~	PIADC	N	extracted NA	N	N	Y
Foot and mouth Disease Virus	FMDV target	Sat 2	~	PIADC	N	extracted NA	N	N	Y
Foot and mouth Disease Virus	FMDV target	A	Argentina 2001 V02764	PIADC	Y	extracted NA	N	N	Y
Foot and mouth Disease Virus	FMDV target	Asia 1	V02594	PIADC	Y	extracted NA	N	N	Y
Foot and mouth Disease Virus	FMDV target	C	C4 Tierra Del FuegoV02367	PIADC	Y	extracted NA	N	N	Y
Foot and mouth Disease Virus	FMDV target	O	O1 South Korea V02722	PIADC	Y	extracted NA	N	N	Y
Foot and mouth Disease Virus	FMDV target	Sat 1	Sat1 /6V02412	PIADC	Y	extracted NA	N	N	Y
Foot and mouth Disease Virus	FMDV target	Sat 2	Zim VV02403	PIADC	Y	extracted NA	N	N	Y
Foot and mouth Disease Virus	FMDV target	Sat 3	Sat3 /3 Bech 1Nov05 V02376	PIADC	Y	extracted NA	N	N	Y
Porcine Respiratory Coronavirus	PRRS NN	~		~	N	extracted NA	N	N	Y
Transmissible gasteroenteritis of Swine	PRRS NN	~	~	~	N	extracted NA	N	N	Y
Porcine Reproductive and Respiratory Sydrome	PRRS Target	~	1807706	LLNL	N	extracted NA	N	N	Y
Porcine Reproductive and Respiratory Sydrome	PRRS Target	~	1810351	MSVDL	N	extracted NA	N	N	Y
Porcine Reproductive and Respiratory Sydrome	PRRS Target	~	1810383	MSVDL	N	extracted NA	N	N	Y
Porcine Reproductive and Respiratory Sydrome	PRRS Target	~	1810386	MSVDL	N	extracted NA	N	N	Y
Porcine Reproductive and Respiratory Sydrome	PRRS Target	~	DOM 1807709	MSVDL	N	extracted NA	N	N	Y
Porcine Reproductive and Respiratory Sydrome	PRRS Target	~	North American	NVSL	N	extracted NA	Y	Y	Y

Vesicular Stomatitis Virus	VSV Target	Indiana	Indiana	NVSL	Y	extracted NA	Y	Y	Y
Vesicular Stomatitis Virus	VSV Target	New Jersey	New Jersey	NVSL	Y	extracted NA	Y	Y	Y

[1]Sources for viruses: California Animal Health and Food Safety (CAHFS), Davis, CA.; Plum Island Animal Disease Center (PIADC), Plum Island, NY; National Veterinary Service Laboratory (NVSL), Ames, IA; National Center for Foreign Animal Disease (NCFAD), Winnepeg, CAN; American Type Culture Collection (ATCC), Manassas, VA; Minnesota State Veterinary Diagnostic Laboratory (MSVDL) in Minneapolis, MN; USDA ARS National Animal Disease Center, IA; USDA ARS, Wy.

List of Pending Targets and Near-Neighbors for Bovine and Porcine Panel Testing at PIADC

Below summarizes the agents used in screening the Porcine panel assays to determine assay analytic specificity for each candidate signature. Results from this screening are detailed in the accompanied Supplemental Material Document. Data that is not available is indicated by a ~ sign.

Virus	Type	Strain, Serotype, or Subtype	Isolate or Identifier	Source	Titered (Y/N)	sample type	Gel screened (Y/N)	Real-time screened (Y/N)	MUX Screened (Y/N)	Panel(s) Screened
Sheeppox	BPSV(Ppox) NN	~	Romania	PIADC	N	~	N	N	Y	Version 1.0, Pending for bovine NN
Sheeppox	BPSV(Ppox) NN	Kenyan O 180 vaccine	v02164	PIADC	N	VI media	N	N	Y	Version 1.0, Pending for bovine NN
Sheeppox	BPSV(Ppox) NN	Nishki attenuated Khazak vaccine	SN	PIADC	N	DNA extract	N	N	Y	Version 1.0, Pending for bovine NN
Sheeppox	BPSV(Ppox) NN	Strain A Khazakistan -	virulent field isolate	PIADC	N	DNA extract	N	N	Y	Version 1.0, Pending for bovine NN
Sheeppox	BPSV(Ppox) NN	Turkey, virulent	~	PIADC	N	DNA extract	N	N	Y	Version 1.0, Pending for bovine NN
Sheeppox	BPSV(Ppox) NN	HELD LT6	V02990	PIADC	N	extracted NA	Y	N	Y	Version 1.0, Pending for bovine NN
Classical Swine Fever Virus	BVD NN	~	~	PIADC	~	extracted NA	N	N	Pending	Version 1.0, Bovine
Porcine enterovirus (PEV)	FMDV NN	Type 1	~	PIADC	N	VI Media	N	N	Y	Version 1.0, Pending for Porcine Pnael screening
Porcine enterovirus (PEV)	FMDV NN	Type 2	~	PIADC	N	VI Media	N	N	Y	Version 1.0, Pending for Porcine Pnael screening
Porcine enterovirus (PEV)	FMDV NN	Type 3	~	PIADC	N	VI Media	N	N	Y	Version 1.0, Pending for Porcine Pnael screening
Porcine enterovirus (PEV)	FMDV NN	Type 4	~	PIADC	N	VI Media	N	N	Y	Version 1.0, Pending for Porcine Pnael screening
Porcine enterovirus (PEV)	FMDV NN	Type 5	~	PIADC	N	VI Media	N	N	Y	Version 1.0, Pending for Porcine Pnael

										screening
Porcine enterovirus (PEV)	FMDV NN	Type 6	~	PIADC	N	VI Media	N	N	Y	Version 1.0, Pending for Porcine Pnael screening
Porcine enterovirus (PEV)	FMDV NN	Type 7	~	PIADC	N	VI Media	N	N	Y	Version 1.0, Pending for Porcine Pnael screening
Porcine enterovirus (PEV)	FMDV NN	Type 8	~	PIADC	N	VI Media	N	N	Y	Version 1.0, Pending for Porcine Pnael screening
Caprine Herpes 2	MCF NN	~	(ATCC VR462)	ATCC	N	extracted NA	Y	Y	Pending	TBD
Equine Herpes 1	MCF NN	~	(ATCC VR2003)	ATCC	N	extracted NA	Y	Y	Pending	TBD
Equine Herpes 2	MCF NN	~	(ATCC VR701)	ATCC	N	extracted NA	Y	Y	Pending	TBD
Equine Herpes 2	MCF NN	~	(NVSL 0002)	NVSL	N	extracted NA	Y	Y	Pending	TBD
Feline Herpes	MCF NN	~	(ATCC VR636)	ATCC	N	extracted NA	Y	Y	Pending	TBD
Varicellovirus Equine Herpes 2	MCF NN	Type 2	NVSL 0002	NVSL	N	extracted NA	Y	Y	Pending	TBD
Varicellovirus Pseudorabies	MCF NN	~	NVSL 93_21246	NVSL	N	extracted NA	Y	Y	Pending	TBD
Varicellovirus Pseudorabies	MCF NN	~	NVSL 93_27020	NVSL	N	extracted NA	Y	Y	Pending	TBD
Varicellovirus Pseudorabies	MCF NN	~	NVSL 92_12013	NVSL	N	extracted NA	Y	Y	Pending	Version 1.0
Varicellovirus Pseudorabies	MCF NN	~	NVSL 93_11745	NVSL	N	extracted NA	Y	Y	Pending	Version 1.0
Varicellovirus Pseudorabies Shope	MCF NN	~	RA180	Not available	N	extracted NA	Y	Y	Pending	Version 1.0
Malignant Catarrhal Fever	MCF target, BHV NN	~	V1918 San Diego ZooSteer117	PIADC	N	~	Y	N	Pending	TBD
Malignant Catarrhal Fever	MCF target, BHV NN	~	V2201 Oklahoma BCET91B006 Serial9109	PIADC	N	~	Y	N	Pending	TBD
Malignant Catarrhal Fever	MCF target, BHV NN	~	ET4499	PIADC	Y	extracted NA	Y	N	Pending	TBD
Malignant Catarrhal Fever	MCF target, BHV NN	~	V1912 X648	PIADC	Y	extracted NA	Y	N	Pending	TBD
Porcine Reproductive and Respiratory Sydrome	PRRS Target	~	Domestic 112	MSVDL	N	extracted NA	Y	Y	Pending	TBD
Porcine Reproductive and Respiratory Sydrome	PRRS Target	~	Domestic 124	MSVDL	N	extracted NA	Y	Y	Pending	TBD

Porcine Reproductive and Respiratory Sydrome	PRRS Target	~	Domestic 134	MSVDL	N	extracted NA	Y	Y	Pending	TBD
Porcine Reproductive and Respiratory Sydrome	PRRS Target	~	Domestic 184	MSVDL	N	extracted NA	Y	Y	Pending	TBD
Porcine Reproductive and Respiratory Sydrome	PRRS Target	~	Domestic 251	MSVDL	N	extracted NA	Y	Y	Pending	TBD
Porcine Reproductive and Respiratory Sydrome	PRRS Target	~	Domestic 262	MSVDL	N	extracted NA	Y	Y	Pending	TBD
Porcine Reproductive and Respiratory Sydrome	PRRS Target	~	European 13	MSVDL	N	extracted NA	Y	Y	Pending	TBD
Porcine Reproductive and Respiratory Sydrome	PRRS Target	~	European 8	MSVDL	N	extracted NA	Y	Y	Pending	TBD
Peste de Petits Ruminants virus (PPRV)	RPV NN	~	Burkina Faso	PIADC	N	extracted NA	N	Y	Pending	Pending for bovine NN
Peste de Petits Ruminants virus (PPRV)	RPV NN	~	Dorcas	PIADC	N	extracted NA	N	Y	Pending	Pending for bovine NN
Peste de Petits Ruminants virus (PPRV)	RPV NN	~	Egypt	PIADC	N	extracted NA	N	Y	Pending	Pending for bovine NN
Peste de Petits Ruminants virus (PPRV)	RPV NN	~	Ghana	PIADC	N	extracted NA	N	Y	Pending	Pending for bovine NN
Peste de Petits Ruminants virus (PPRV)	RPV NN	~	RCA	PIADC	N	extracted NA	N	Y	Pending	Pending for bovine NN
Rinderpest virus	RPV Target	~	Egypt	PIADC	Y	extracted NA	N	Y	Pending	Pending for bovine Target
Rinderpest virus	RPV Target	~	India	PIADC	Y	extracted NA	N	Y	Pending	Pending for bovine Target
Rinderpest virus	RPV Target	~	Kuwait	PIADC	Y	extracted NA	N	Y	Pending	Pending for bovine Target
Rinderpest virus	RPV Target	~	Nigerian Buffalo	PIADC	Y	extracted NA	N	Y	Pending	Pending for bovine Target
Rinderpest virus	RPV Target	~	Pakistan	PIADC	Y	extracted NA	N	Y	Pending	Pending for bovine Target
Rinderpest	RPV Target	~	Plowright	PIADC	Y	extracted	N	Y	Pending	Pending for

virus						NA				bovine Target
Rinderpest virus	RPV Target	~	RBOK	PIADC	Y	extracted NA	N	Y	Pending	Pending for bovine Target
Rinderpest virus	RPV Target	~	Pendik	PIADC	Y	extracted NA	N	Y	Pending	Pending for bovine Target
Rinderpest virus	RPV Target	~	Yemen	PIADC	Y	extracted NA	N	Y	Pending	Pending for bovine Target
Swine Vesicular Disease	SVD target	~	ROM-1-87	PIADC	Y	extracted NA	N	Y	Y	Version 1.0, Pending Porcine Target screening
Swine Vesicular Disease	SVD target	~	HKN-4-89	PIADC	Y	extracted NA	N	Y	Y	Version 1.0, Pending Porcine Target screening
Swine Vesicular Disease	SVD target	~	HKN/12/87	PIADC	Y	extracted NA	N	Y	Y	Version 1.0, Pending Porcine Target screening
Swine Vesicular Disease	SVD target	~	ITL/1/91	PIADC	Y	extracted NA	N	Y	Y	Version 1.0, Pending Porcine Target screening
Swine Vesicular Disease	SVD target	~	SVD ITL/1/66	PIADC	Y	extracted NA	N	Y	Y	Version 1.0, Pending Porcine Target screening
Swine Vesicular Disease	SVD target	~	TAW/119/97	PIADC	Y	extracted NA	N	Y	Y	Version 1.0, Pending Porcine Target screening
Swine Vesicular Disease	SVD target	~	UKG72	PIADC	Y	extracted NA	N	Y	Y	Version 1.0, Pending Porcine Target screening
San Miguel Sea Lion Virus (SMSV)	VESV NN	Type 12	V02040	PIADC	N	VI Media	N	N	Y	Version 1.0, Pending for Porcine NN
San Miguel Sea Lion Virus (SMSV)	VESV NN	Type 2	V02056	PIADC	N	VI Media	N	N	Y	Version 1.0, Pending for Porcine NN
Vesiculovirus	VESV NN	BeAr	~	PIADC	Pending	extracted NA	N	N	Pending	Pending for Porcine NN
Vesiculovirus	VESV NN	Calchaqui	~	PIADC	Pending	extracted NA	N	N	Pending	Pending for Porcine NN
Vesiculovirus	VESV NN	Chandipura	~	PIADC	Pending	extracted NA	N	N	Pending	Pending for Porcine NN
Vesiculovirus	VESV NN	Hilo CT AN	~	PIADC	Pending	extracted NA	N	N	Pending	Pending for Porcine NN
Vesiculovirus	VESV NN	Jurona	~	PIADC	Pending	extracted NA	N	N	Pending	Pending for Porcine NN
Vesiculovirus	VESV NN	Klamath	~	PIADC	Pending	extracted NA	N	N	Pending	Pending for Porcine NN
Vesiculovirus	VESV NN	NM 85-488	~	PIADC	Pending	extracted NA	N	N	Pending	Pending for Porcine NN
Vesiculovirus	VESV NN	Piry	~	PIADC	Pending	extracted NA	N	N	Pending	Pending for Porcine NN
Vesicular Exanthema of	VESV Target	B51	V00758	PIADC	Y	extracted NA	Y	Y	Y	Version 1.0, Pending

Swine Virus (VESV)										Porcine target
Vesicular Exanthema of Swine Virus (VESV)	VESV Target	A48	V02028	PIADC	Y	extracted NA	Y	Y	Y	Version 1.0, Pending Porcine target
Vesicular Exanthema of Swine Virus (VESV)	VESV Target	D53	V02032	PIADC	Y	extracted NA	Y	Y	Y	Version 1.0, Pending Porcine target
Vesicular Exanthema of Swine Virus (VESV)	VESV Target	E54	V02033	PIADC	Y	extracted NA	Y	Y	Y	Version 1.0, Pending Porcine target
Vesicular Exanthema of Swine Virus (VESV)	VESV Target	G55	V02034	PIADC	Y	extracted NA	Y	Y	Y	Version 1.0, Pending Porcine target
Vesicular Exanthema of Swine Virus (VESV)	VESV Target	I55	V02036	PIADC	Y	extracted NA	Y	Y	Y	Version 1.0, Pending Porcine target
Vesicular Exanthema of Swine Virus (VESV)	VESV Target	J56	V02037	PIADC	Y	extracted NA	Y	Y	Y	Version 1.0, Pending Porcine target
Vesicular Stomatitis Virus	VSV Target	Indiana	IN94GUB	PIADC: Luis Rodriguez	Pending	VI Media	N	Y	Pending	Pending for bovine/porcine target
Vesicular Stomatitis Virus	VSV Target	Indiana	IN97CMB	PIADC: Luis Rodriguez	Pending	VI Media	N	Y	Pending	Pending for bovine/porcine target
Vesicular Stomatitis Virus	VSV Target	Indiana	IN97COE	PIADC: Luis Rodriguez	Pending	VI Media	N	Y	Pending	Pending for bovine/porcine target
Vesicular Stomatitis Virus	VSV Target	Indiana	IN98NME	PIADC: Luis Rodriguez	Pending	VI Media	N	Y	Pending	Pending for bovine/porcine target
Vesicular Stomatitis Virus	VSV Target	New Jersey	NJ0604NME	PIADC: Luis Rodriguez	Pending	VI Media	N	Y	Pending	Pending for bovine/porcine target
Vesicular Stomatitis Virus	VSV Target	New Jersey	NJ1184HDB	PIADC: Luis Rodriguez	Pending	VI Media	N	Y	Pending	Pending for bovine/porcine target
Vesicular Stomatitis Virus	VSV Target	New Jersey	NJ89GAS	PIADC: Luis Rodriguez	Pending	VI Media	N	Y	Pending	Pending for bovine/porcine target
Vesicular Stomatitis Virus	VSV Target	New Jersey	NJ95COB	PIADC: Luis Rodriguez	Pending	VI Media	N	Y	Pending	Pending for bovine/porcine target

[1]Sources for viruses: California Animal Health and Food Safety (CAHFS), Davis, CA.; Plum Island Animal Disease Center (PIADC), Plum Island, NY; National Veterinary Service Laboratory (NVSL), Ames, IA; National Center for Foreign Animal Disease (NCFAD), Winnepeg, CAN; American Type Culture Collection (ATCC), Manassas, VA; Minnesota State Veterinary Diagnostic Laboratory (MSVDL) in Minneapolis, MN; USDA ARS National Animal Disease Center, IA; USDA ARS, Wy.

VIRUS INFORMATION AND HISTORY

Summary List of for all Targets and Near-neighbors screened in the Bovine and Porcine panels

Data is organized alphabetically by virus. The ~ sign indicates data that is not applicable or unknown. Viruses shown in red are pending screening at PIADC.

Target Agent	Type	Virus	Serotype, Strain or Isolate	Unique ID	Source[1]	Original Titer	Passage History	Extraction date/ID	Extraction Method	NA conc. used or stock titer	Titer Method
BTV	Target	bluetongue virus	BTV-10	lot #001 ODV 0001 Dec 5, 2000	NVSL	~	(NVSL)BHK?(LLNL)BHK1	12/14/2006	Trizol	$6.32x10^5$ $TCID_{50}$/0.1mL	Reed-Meunch
BTV	Target	bluetongue virus	BTV-11	lot#002 ODV0101 Nov 30, 2001	NVSL	~	(NVSL)BHK?(LLNL)BHK1	12/14/2006	Trizol	$6.32x10^5$ $TCID_{50}$/0.1mL	Reed-Meunch
BTV	Target	bluetongue virus	BTV-13	no lot or strain given	NVSL	~	~	12/14/2006	Trizol	$3.56x10^3$ $TCID_{50}$/0.1mL	Reed-Meunch
BTV	Target	bluetongue virus	BTV-17	lot#004 ODV 0201 Nov 28, 2002	NVSL	~	(NVSL)BHK?(LLNL)BHK1	12/14/2006	Trizol	$1.12x10^5$ $TCID_{50}$/0.1mL	Reed-Meunch
BTV	Target	bluetongue virus	BTV-2	no lot given	NVSL	~	(NVSL)BHK?(LLNL)BHK2	12/14/2006	Trizol	$6.32x10^5$ $TCID_{50}$/0.1mL	Reed-Meunch
BVD	NN	border disease virus	Coos Bay	4-6-92	USDA ARS, IA	$1.5x10^8$ $TCID_{50}$/mL	~	12/14/2006	Trizol	1.18 x 10^6 TCID50/mL	Reed & Muench
BVD	NN	border disease virus	134 / 7		PIADC	~	~	~	~	~	~
BVD	NN	border disease virus	Aveyron		PIADC	~	~	~	~	~	~
BVD	NN	border disease virus	Coos Bay 4-6-92		USDA/ ARS (IA)	~	~	~	~	~	~
BVD	NN	border disease virus	Frijters		PIADC	~	~	~	~	~	~
MCF	NN	bovine herpes virus -5	BHV 5	D9403153	CAHFS	~	~	~	~	40 pg/uL	~
BHV	NN	bovine herpes virus -5	BHV 5	D9403153	CAHFS	~	~	~	~	40 pg/uL	~
BHV	NN	bovine herpes virus -5	D9402133	A040150085	CAFHS	~	CAHFS Lab (MDBK), (1flasks) 11/19/2003	11/19/2003	Phenol/Chloroform	40 pg/uL	~
BHV	NN	bovine herpes virus -5	DN-599	R. Mock, A032540006	CAFHS	~	2BFK2 CAHFS Lab 4/30/2004	6/3/2004	Phenol/Chloroform	40 pg/uL	~
BHV	NN	bovine herpes virus -5	DN-599	R. Mock, #2 Lung A040150085	CAFHS	~	CAHFS Lab 5BFK5 4/23/2004	7/7/2004	Phenol/Chloroform	40 pg/uL	~
BHV	Target	bovine herpes virus-1	California	NVSL 200032	NVSL, CAHFS	~	MDBK CAHFS	11/19/2003	Phenol/ Chloroform	40 pg/uL	~
MCF	NN	bovine herpes virus-1	California	NVSL 200032	NVSL, CAHFS	~	MDBK CAHFS	11/19/2003	Phenol/ Chloroform	40 pg/uL	~
BHV	Target	bovine herpes virus-1	California	NVSL 20032 111903	CAHFS	~	~	~	Phenol/ Chloroform	40 pg/uL	~
MCF	NN	bovine herpes	California	NVSL 20032	CAHFS	~	~	~	Phenol/ Chloroform	40 pg/uL	~

		virus-1		111903							
BHV	Target	bovine herpes virus-1	California	NVSL 51619	NVSL, CAHFS	~	MDBK CAHFS	11/12/2003	Phenol/ Chloroform	40 pg/uL	~
MCF	NN	bovine herpes virus-1	California	NVSL 51619	NVSL, CAHFS	~	MDBK CAHFS	11/12/2003	Phenol/ Chloroform	40 pg/uL	~
BHV	Target	bovine herpes virus-1	Colorado Vaccine	~	NVSL	10^8 $TCID_{50}$/0.1 ml	~	Dec-05	Phenol/Chloroform	7.63 x 10^6/0.1mL $TCID_{50}$	Reed & Muench
BHV	Target	bovine herpes virus-1	Cooper	NVSL 86741 111903	CAHFS	~	~	~	Phenol/ Chloroform	40 pg/uL	~
MCF	NN	bovine herpes virus-1	Cooper	NVSL 86741 111903	CAHFS	~	~	~	Phenol/ Chloroform	40 pg/uL	~
BHV	Target	bovine herpes virus-1	Cooper	NVSL-Cooper RA 309	NVSL, CAHFS	~	MDBK CAHFS	~	Phenol/ Chloroform	40 pg/uL	~
MCF	NN	bovine herpes virus-1	Cooper	NVSL-Cooper RA 309	NVSL, CAHFS	~	MDBK CAHFS	~	Phenol/ Chloroform	40 pg/uL	~
BHV	Target	bovine herpes virus-1	IBR	R. Mock, #5 Swab A033640072	CAHFS	~	1BDFK1,	4/23/2004	Phenol/ Chloroform	40 pg/uL	~
MCF	NN	bovine herpes virus-1	IBR	R. Mock, #5 Swab A033640072	CAHFS	~	1BDFK1,	4/23/2004	Phenol/ Chloroform	40 pg/uL	~
BHV	Target	bovine herpes virus-1	IBR	R. Mock, #4 Lung A040130066	CAHFS,	~	1BFK1	4/23/2004	Phenol/ Chloroform	40 pg/uL	~
MCF	NN	bovine herpes virus-1	IBR	R. Mock, #4 Lung A040130066	CAHFS,	~	1BFK1	4/23/2004	Phenol/ Chloroform	40 pg/uL	~
BHV	Target	bovine herpes virus-1	IBR	Texas R. Mock, #80 Swab A030020072	CAHFS,	~	1BDFK1	4/23/2004	Phenol/ Chloroform	40 pg/uL	~
MCF	NN	bovine herpes virus-1	IBR	Texas R. Mock, #80 Swab A030020072	CAHFS,	~	1BDFK1	4/23/2004	Phenol/ Chloroform	40 pg/uL	~
BHV	Target	bovine herpes virus-1	Los Angeles	Los Angeles ATCC VR188	ATCC (CAHFS)	1 x 10^5 TCID50/mL	(CAHFS)MDBK2(LLNL)MDBK1	5/17/07	Phenol/ Chloroform	40 pg/uL	~
MCF	NN	bovine herpes virus-1	Los Angeles	Los Angeles ATCC VR188	ATCC (CAHFS)	1 x 10^5 TCID50/mL	(CAHFS)MDBK2(LLNL)MDBK1	5/17/07	Phenol/ Chloroform	40 pg/uL	~
BHV	Target	bovine herpes virus-1	Minnesota	NVSL 86741	NVSL, CAHFS	~	MDBK CAHFS	11/19/2003	Phenol/ Chloroform	40 pg/uL	~
MCF	NN	bovine herpes virus-1	Minnesota	NVSL 86741	NVSL, CAHFS	~	MDBK CAHFS	11/19/2003	Phenol/ Chloroform	40 pg/uL	~
BHV	Target	bovine herpes virus-1	Minnesota	NVSL 97-10720	NVSL, CAHFS	~	MDBK CAHFS	11/12/2003	Phenol/ Chloroform	40 pg/uL	~
MCF	NN	bovine herpes virus-1	Minnesota	NVSL 97-10720	NVSL, CAHFS	~	MDBK CAHFS	11/12/2003	Phenol/ Chloroform	40 pg/uL	~
BHV	Target	bovine	RLB-106	ATCC	ATCC	~	~	~	Phenol/	40 pg/uL	~

		herpes virus-1		VR793	(CAHFS)				Chloroform		
MCF	NN	bovine herpes virus-1	RLB-106	ATCC VR793	ATCC (CAHFS)	~	~	~	Phenol/ Chloroform	40 pg/uL	~
BHV	Target	bovine herpes virus-1	Virginia	NVSL 231221	NVSL, CAHFS	~	MDBK CAHFS	11/12/2003	Phenol/ Chloroform	40 pg/uL	~
MCF	NN	bovine herpes virus-1	Virginia	NVSL 231221	NVSL, CAHFS	~	MDBK CAHFS	11/12/2003	Phenol/ Chloroform	40 pg/uL	~
MCF	NN	bovine herpes virus-5	D9402133	Sonoma Co. 3/94 D9402133	CAHFS	~	CAHFS Lab (MDBK), (1flasks) 11/19/2003	11/19/2003	Phenol/Chloroform	40 pg/uL	~
MCF	NN	bovine herpes virus 5	DN-599	R. Mock A032540006	CAHFS	~	2BFK2 4/30/2004	6/3/2004	Phenol/Chloroform	40 pg/uL	~
MCF	NN	bovine herpes virus 5	DN-599	R. Mock A040150085	CAHFS	~	CAHFS Lab 5BFK5	7/7/2004	Phenol/Chloroform	40 pg/uL	~
Parapox	Target	bovine papular stomatitis virus	~	Illinois 721	ATCC	~	~	~	~	~ total NA	~ (pg/uL)
Parapox	Target	bovine papular stomatitis virus	~	Texas A&M	NVSL	1 x 10^7 $TCID_{50}$/0.1 mL	~	Dec-05	Phenol/Chloroform	3.49x10^6 $TCID_{50}$/0.1mL	Reed-Meunch
BVD	Target	bovine viral diarrhea	genotype 1a	NADL-BVDV	USADARS (IA)	6.8 x 10^6 $TCID_{50}$/mL	~	12/14/2006	Trizol	2.9 x 10^4 $TCID_{50}$/mL	Reed & Muench
BVD	Target	bovine viral diarrhea	genotype 1 cytopathic	Singer	NVSL	$10^7$$TCID_{50}$/ 0.1mL	~	Dec-05	Trizol	1.98 x 10^6 $TCID_{50}$/0.1mL	Reed & Muench
BVD	Target	bovine viral diarrhea	genotype 1b	NY-(5/18/02) BVDV	USADARS (IA)	6.8 x 10^7 $TCID_{50}$/mL	~	12/14/2006	Trizol	8.38 x 10^5 $TCID_{50}$/mL	Reed & Muench
BVD	Target	bovine viral diarrhea	genotype 1b	TGAN-BVDV	USADARS (IA)	1.5 x 10^9 $TCID_{50}$/mL	~	12/14/2006	Trizol	1.32 x 10^7 $TCID_{50}$/mL	Reed & Muench
BVD	Target	bovine viral diarrhea	genotype 2	334165 = BVD2-BVD2	USADARS (IA)	2.4 x 10^5 $TCID_{50}$/mL	~	12/14/2006	Trizol	1.1 x 10^3 $TCID_{50}$/mL	Reed & Muench
BVD	Target	bovine viral diarrhea	genotype 2a	2850805 - BVDV	USADARS (IA)	4.2 x 10^6 $TCID_{50}$/mL	~	12/14/2006	Trizol	3.36 x 10^4 $TCID_{50}$/mL	Reed & Muench
BVD	Target	bovine viral diarrhea	genotype 2b	AU-501 (1-6-2006) BVDV	USADARS (IA)	1.47 x 10^7 $TCID_{50}$/mL	~	12/14/2006	Trizol	8.64 x 10^4 $TCID_{50}$/mL	Reed & Muench
BVD	Target	bovine viral diarrhea	genotype 2b	BVD-FBS B69519 - BVDV	USADARS (IA)	2.4 x 10^5 $TCID_{50}$/mL	~	12/14/2006	Trizol	1.7 x 10^3 $TCID_{50}$/mL	Reed & Muench
BHV	NN	caprine herpes virus	S0201998	San Diego Co. 2/02 S0201998	CAHFS	~	CAHFS Lab (MDBK)	11/19/2003	Phenol/ Chloroform	40 pg/uL	~
BPSV	NN	caprine herpes virus	S0201998	San Diego Co. 2/02 S0201998	CAHFS	~	CAHFS Lab (MDBK)	11/19/2003	Phenol/ Chloroform	40 pg/uL	~
MCF	NN	caprine herpes virus	S0201998	San Diego Co. 2/02 S0201998	CAHFS	~	CAHFS Lab (MDBK)	11/19/2003	Phenol/ Chloroform	40 pg/uL	~
BHV	NN	caprine herpes virus	~	VR462	ATCC	~	~	~	Phenol/ Chloroform	40 pg/uL	~
BPSV	NN	caprine herpes virus	~	VR462	ATCC	~	~	~	Phenol/ Chloroform	40 pg/uL	~
MCF	NN	caprine	~	VR462	ATCC	~	~	~	Phenol/	40 pg/uL	~

		herpes virus							Chloroform		
BPSV	NN	caprine herpes virus	~	San Joaquin Co. 2/02 D0201157	CAHFS	~	CAHFS Lab (MDBK)	~	Phenol/ Chloroform	40 pg/uL	~
MCF	NN	caprine herpes virus	~	San Joaquin Co. 2/02 D0201157	CAHFS	~	CAHFS Lab (MDBK)	~	Phenol/ Chloroform	40 pg/uL	~
BHV	NN	caprine herpes virus		San Joaquin Co. 2/02 D0201157	CAHFS	~	CAHFS Lab (MDBK)	~	Phenol/ Chloroform	40 pg/uL	~
BVD	NN	classical swine fever virus	~		PIADC	~	~	~	~	~	~
BVD	NN	classical swine fever virus	Brescia		PIADC	~	~	~	~	~	~
BVD	NN	classical swine fever virus	Kanagawa		PIADC	~	~	~	~	~	~
BVD	NN	classical swine fever virus	Paderborn		PIADC	~	~	~	~	~	~
BHV	NN	equine herpes 1	Equine Herpes 1	A9904309	CAHFS	~	CAHFS, ~	6/3/04	Phenol/ Chloroform	40 pg/uL	~
BPSV	NN	equine herpes 1	Equine Herpes 1	A9904309	CAHFS	~	CAHFS, ~	6/3/04	Phenol/ Chloroform	40 pg/uL	~
MCF	NN	equine herpes 1	Equine Herpes 1	A9904309	CAHFS	~	CAHFS, ~	6/3/04	Phenol/ Chloroform	40 pg/uL	~
BPSV	NN	equine herpes 1	Bovine 1247	Bovine 1247 ATCC VR2003	ATCC	~	CAHFS, ~	~	Phenol/ Chloroform	40 pg/uL	~
MCF	NN	equine herpes 1	Bovine 1247	Bovine 1247 ATCC VR2003	ATCC	~	CAHFS, ~	~	Phenol/ Chloroform	40 pg/uL	~
BHV	NN	equine herpes 1	Bovine 1247	Bovine 1247 VR2003	ATCC	~	CAHFS, ~	~	Phenol/ Chloroform	40 pg/uL	~
BHV	NN	equine herpes 1	Equine Herpes 1	A01112000 4	CAHFS	~	CAHFS, 2BFK2	6/3/04	Phenol/ Chloroform	40 pg/uL	~
BPSV	NN	equine herpes 1	Equine Herpes 1	A01112000 4	CAHFS	~	CAHFS, 2BFK2	6/3/04	Phenol/ Chloroform	40 pg/uL	~
MCF	NN	equine herpes 1	Equine Herpes 1	A01112000 4	CAHFS	~	CAHFS, 2BFK2	6/3/04	Phenol/ Chloroform	40 pg/uL	~
BHV	NN	equine herpes 1	Equine Herpes 1	A99043047	CAHFS	~	CAHFS, 1BFK1	6/3/04	Phenol/ Chloroform	40 pg/uL	~
BPSV	NN	equine herpes 1	Equine Herpes 1	A99043047	CAHFS	~	CAHFS, 1BFK1	6/3/04	Phenol/ Chloroform	40 pg/uL	~
MCF	NN	equine herpes 1	Equine Herpes 1	A99043047	CAHFS	~	CAHFS, 1BFK1	6/3/04	Phenol/ Chloroform	40 pg/uL	~
BHV	NN	equine herpes 2	D990	D990	CAHFS	~	CAHFS, ~	1/29/03	Phenol/ Chloroform	40 pg/uL	~
BPSV	NN	equine herpes 2	D990	D990	CAHFS	~	CAHFS, ~	1/29/03	Phenol/ Chloroform	40 pg/uL	~
MCF	NN	equine herpes 2	D990	D990	CAHFS	~	CAHFS, ~	1/29/03	Phenol/ Chloroform	40 pg/uL	~
BHV	NN	equine herpes 2	LK	LK ATCC VR701	ATCC	~	CAHFS, ~	~	Phenol/ Chloroform	40 pg/uL	~
BPSV	NN	equine herpes 2	LK	LK ATCC VR701	ATCC	~	CAHFS, ~	~	Phenol/ Chloroform	40 pg/uL	~
MCF	NN	equine herpes 2	LK	LK ATCC VR701	ATCC	~	CAHFS, ~	~	Phenol/ Chloroform	40 pg/uL	~

BHV	NN	equine herpes 2	NVSL 002	NVSL 002 1/28/04	NVSL	~	CAHFS, ~	1/29/03	Phenol/ Chloroform	40 pg/uL	~
BPSV	NN	equine herpes 2	NVSL 002	NVSL 002 1/28/04	NVSL	~	CAHFS, ~	1/29/03	Phenol/ Chloroform	40 pg/uL	~
MCF	NN	equine herpes 2	NVSL 002	NVSL 002 1/28/04	NVSL	~	CAHFS, ~	1/29/03	Phenol/ Chloroform	40 pg/uL	~
BHV	NN	feline herpes	C-27	ATCC VR636	ATCC	~	CAHFS, ~	~	~	40 pg/uL	~
BPSV	NN	feline herpes	C-27	ATCC VR636	ATCC	~	CAHFS, ~	~	~	40 pg/uL	~
MCF	NN	feline herpes	C-27	ATCC VR636	ATCC	~	CAHFS, ~	~	~	40 pg/uL	~
FMDV	Target	*foot-and-mouth disease virus*	A	A18	PIADC	~	BHK P56	Feb-06	Trizol	ng/uL	~
FMDV	Target	*foot-and-mouth disease virus*	A	Argentina 2001 V02764	PIADC	~	BHK P56	~	Ambion MagMax 96	2.05 x 10^7 $TCID_{50}$/mL	Spearman-Karber
FMDV	Target	*foot-and-mouth disease virus*	Asia 1	~	PIADC	~	2 BOV, 1 BHK	Feb-06	Trizol	ng/uL	~
FMDV	Target	*foot-and-mouth disease virus*	Asia 1	LEB '83 V02594	PIADC	~	2 BOV, 1 BHK	~	Ambion MagMax 96	6.5 x 10^4 $TCID_{50}$/mL	Spearman-Karber
FMDV	Target	*foot-and-mouth disease virus*	C	C1 Noville	PIADC	~	1 LK, 1 BHK	Feb-06	Trizol	ng/uL	~
FMDV	Target	*foot-and-mouth disease virus*	C	C4, Tierra Del Fuego V02367	PIADC	~	1 LK, 1 BHK	~	Ambion MagMax 96	1 x 10^7 $TCID_{50}$/mL	Spearman-Karber
FMDV	Target	*foot-and-mouth disease virus*	O	O1 Brugge	PIADC	~	1 LK, 1 BHK	Feb-06	Trizol	ng/uL	~
FMDV	Target	*foot-and-mouth disease virus*	O	O1, South Korea V02722	PIADC	~	1 LK, 1 BHK	~	Ambion MagMax 96	3 x 10^7 $TCID_{50}$/mL	Spearman-Karber
FMDV	Target	*foot-and-mouth disease virus*	SAT 1	~	PIADC	~	1 LK, 1 BHK	Feb-06	Trizol	ng/uL	~
FMDV	Target	*foot-and-mouth disease virus*	SAT 1	Sat 1/6 SWA 40/61 V02412	PIADC	~	1 LK, 1 BHK	~	Ambion MagMax 96	2 x 10^7 $TCID_{50}$/mL	Spearman-Karber
FMDV	Target	*foot-and-mouth disease virus*	SAT 2	~	PIADC	~	3 BTY, 2LK, 7BHK,	Feb-06	Trizol	ng/uL	~
FMDV	Target	*foot-and-mouth disease virus*	SAT 2	Sat 2 Zim 5/81	PIADC	~	3 BTY, 2LK, 7BHK,	~	Ambion MagMax 96	5 x 10^5 $TCID_{50}$/mL	Spearman-Karber
FMDV	Target	*foot-and-mouth disease virus*	SAT 3	~	PIADC	~	3 LK, 1 BHK	Feb-06	Trizol	ng/uL	~
FMDV	Target	*foot-and-mouth disease virus*	SAT 3	Sat 3/3 Bech 1Nov05 V02376	PIADC	~	3 LK, 1 BHK	~	Ambion MagMax 96	2.25 x 10^7 $TCID_{50}$/mL	Spearman-Karber
BPSV	NN	fowl pox	Vaccine	Poxine™	NVSL	~	ECE	5/17/07	Phenol/	40 pg/uL	~

		virus	strain	Jeffers Livestock					Chloroform		
SVD	NN	human entero virus B	Coxsackie A9	~	PIADC	~	~	~	~	~	~
SVD	NN	human entero virus B	Coxsackie B1	~	PIADC	~	~	~	~	~	~
SVD	NN	human entero virus B	Coxsackie B2	~	PIADC	~	~	~	~	~	~
SVD	NN	human entero virus B	Coxsackie B3	~	PIADC	~	~	~	~	~	~
SVD	NN	human entero virus B	Coxsackie B4	~	PIADC	~	~	~	~	~	~
SVD	NN	human entero virus B	Coxsackie B5	~	PIADC	~	~	~	~	~	~
SVD	NN	human entero virus B	Coxsackie B5	Faulkner TC 583 A 890	PIADC	~	~	~	~	~	~
SVD	NN	human entero virus B	Coxsackie B6	~	PIADC	~	~	~	~	~	~
SVD	NN	human entero virus B	Echovirus 11	Echo 11	PIADC	~	~	~	~	~	~
SVD	NN	human entero virus B	Echovirus 12	Echo 12	PIADC	~	~	~	~	~	~
SVD	NN	human entero virus B	Echovirus 24	Echo 24	PIADC	~	~	~	~	~	~
SVD	NN	human entero virus B	Echovirus 9	Echo 9	PIADC	~	~	~	~	~	~
MCF	Target	malignant catarrhal fever virus	AIHV-1 (MN)	~	PIADC: Hong Li in Washington State	~	Sheep	~	Ambion MagMax 96	~	~
MCF	Target	malignant catarrhal fever virus	AvHV-1 (WC11 BTH50 6D)	~	PIADC	~	MDBK	~	Ambion MagMax 96	~	~
MCF	Target	malignant catarrhal fever virus	OvHV-2	~	PIADC: Hong Li in Washington State	~	IMP 7/1/73, 50BTH, 6 EBL	~	Ambion MagMax 96	~	~
Parapox	Target	orf	~	vaccine strain	NVSL	~	~	~	~	~	~
RPV	NN	parainfluenza virus type 3	C-243	TC-81335	DHS-VRDL	10^5 TCID50/0.1 mL	(VL)MK14(LLNL)H292-1	5/17/2007	Trizol	40 pg/uL	Reed and Muench
RPV	NN	peste de petits ruminants virus (PPRV)	Burkina Faso	V02299	PIADC	~	1 LS, 10 VERO	~	Qiagen Rneasy Mini kit	7.94 x 10^3 $TCID_{50}$/mL	Spearman-Karber
RPV	NN	peste de petits ruminants virus (PPRV)	Dorcas	V02322	PIADC	~	8 VERO	~	Qiagen Rneasy Mini kit	5.01 x 10^5 $TCID_{50}$/mL	Spearman-Karber
RPV	NN	peste de petits	Egypt	V02306	PIADC	~	10 VERO	~	Qiagen Rneasy	2.51 x 10^6 $TCID_{50}$/mL	Spearman-Karber

		ruminants virus (PPRV)							Mini kit		
RPV	NN	peste de petits ruminants virus (PPRV)	Ghana	V02329	PIADC	~	7 LK, 6 VERO	~	Qiagen Rneasy Mini kit	5.01 x 10^4 $TCID_{50}$/mL	Spearman-Karber
RPV	NN	peste de petits ruminants virus (PPRV)	RCA	V02297	PIADC	~	2 LK, 6 VERO	~	Qiagen Rneasy Mini kit	6.31 x 10^4 $TCID_{50}$/mL	Spearman-Karber
BHV	NN	porcine herpes pseudorabies	Shope	RA 180	CAHFS	10^5 TCID50/mL	~	5/17/07	Trizol	40 pg/uL	~
BPSV	NN	porcine herpes pseudorabies	Shope	RA 180	CAHFS	10^5 TCID50/mL	~	5/17/07	Trizol	40 pg/uL	~
PRRS	Target	porcine respiratory and reprductive virus	Domestic 112	~	NVSL	10^6 $TCID_{50}$/1.0 mL	Marc-145	2006	TRIZOL	No titer	~
PRRS	Target	porcine respiratory and reprductive virus	Domestic 112	Texas	MSVDL	~	PAM	2006	TRIZOL	No titer	~
PRRS	Target	porcine respiratory and reprductive virus	Domestic 124	Oklahoma	MSVDL	~	PAM	2006	TRIZOL	No titer	~
PRRS	Target	porcine respiratory and reprductive virus	Domestic 134	Nebraska	MSVDL	~	PAM	2006	TRIZOL	No titer	~
PRRS	Target	porcine respiratory and reprductive virus	Domestic 184	Minnesota	MSVDL	~	PAM	2006	TRIZOL	No titer	~
PRRS	Target	porcine respiratory and reprductive virus	Domestic 251	Missouri	MSVDL	~	Marc-145	2006	TRIZOL	No titer	~
PRRS	Target	porcine respiratory and reprductive virus	Domestic 262	Missouri	MSVDL	~	PAM	2006	TRIZOL	No titer	~
PRRS	Target	porcine respiratory and reprductive virus	European 13	North Carolina	MSVDL	~	PAM	2006	TRIZOL	No titer	~
PRRS	Target	porcine respiratory and reprductive virus	European 8	Missouri	MSVDL	~	PAM	2006	TRIZOL	No titer	~
PRRS	Target	porcine respiratory and	North American	field isolate	NVSL	10^6 $TCID_{50}$/1.0 mL	~	9/5/2006	TRIZOL	9.06x10^4 $TCID_{50}$/mL	Reed & Muench

		reprductiv e virus									
PRRS	NN	porcine respiratory coronaviru s	PRRS NN	~	NVSL	~	~	~	~	~	~
Parapo x	Target	pseudoco wpox	~	VR634	NVSL	~	~	~	~	~	~
BHV	NN	pseudorabi es	Shope	~	NVSL	~	~	~	Phenol/ Chloroform	~	Reed & Muench
BPSV	NN	pseudorabi es	Shope	~	NVSL	~	~	~	Phenol/ Chloroform	~	Reed & Muench
MCF	NN	pseudorabi es	Shope	~	NVSL	~	~	~	Phenol/ Chloroform	~	Reed & Muench
BHV	NN	pseudorabi es	Shope	RA180	NVSL	~	CAHFS	12/3/03	Phenol/ Chloroform	40 pg/uL	~
BPSV	NN	pseudorabi es	Shope	RA180	NVSL	~	CAHFS	12/3/03	Phenol/ Chloroform	40 pg/uL	~
MCF	NN	pseudorabi es	Shope	RA180	NVSL	~	CAHFS	12/3/03	Phenol/ Chloroform	40 pg/uL	~
BHV	NN	pseudorabi es	~	92-12013	NVSL	~	CAHFS	12/2/2003	Phenol/ Chloroform	40 pg/uL	~
BPSV	NN	pseudorabi es	~	92-12013	NVSL	~	CAHFS	12/3/03	Phenol/ Chloroform	40 pg/uL	~
MCF	NN	pseudorabi es	~	92-12013	NVSL ,	~	CAHFS	12/3/2003	Phenol/ Chloroform	40 pg/uL	~
BHV	NN	pseudorabi es	~	93-11745	NVSL	~	CAHFS	12/3/03	Phenol/ Chloroform	40 pg/uL	~
BPSV	NN	pseudorabi es	~	93-11745	NVSL	~	CAHFS	12/3/03	Phenol/ Chloroform	40 pg/uL	~
MCF	NN	pseudorabi es	~	93-11745	NVSL	~	CAHFS	12/3/03	Phenol/ Chloroform	40 pg/uL	~
RPV	NN	respiratory syncytial virus	Group A Long	TC-3857	DHS-VRDL	~	(VL)KB6L2K B2HFDL2A549 - 1(LLNL)HeLa3	5/17/2007		40 pg/uL	~
BPSV	NN	respiratory syncytial virus	Long	~	LLNL	~	(VL)KB6L2K B2HFDL2A549 - 1(LLNL)HeLa3	5/17/07	Trizol	40 pg/uL	~
RPV	Target	rinderpest virus	Egypt	V02285	PIADC	~	1 VERO	~	Qiagen Rneasy Mini kit	3.98 x 10^5 $TCID_{50}$/mL	Spearma n-karber
RPV	Target	rinderpest virus	India	V02280	PIADC	~	2 VERO	~	Qiagen Rneasy Mini kit	3.16 x 10^6 $TCID_{50}$/mL	Spearma n-karber
RPV	Target	rinderpest virus	Kuwait	V02278	PIADC	~	2 VERO	~	Qiagen Rneasy Mini kit	3.98 x 10^6 $TCID_{50}$/mL	Spearma n-karber
RPV	Target	rinderpest virus	Nigeria buffalo	V02279	PIADC	~	2 VERO	~	Qiagen Rneasy Mini kit	3.16 x 10^6 $TCID_{50}$/mL	Spearma n-karber
RPV	Target	rinderpest virus	Pakistan	V02284	PIADC	~	1 VERO	~	Qiagen Rneasy Mini kit	5.01 x 10^5 $TCID_{50}$/mL	Spearma n-karber
RPV	Target	rinderpest virus	Plowright Vaccine Master seed, Kabete O-strain	V02057	PIADC	~	92BK, 2VERO, Master seed	~	Qiagen Rneasy Mini kit	3.98 x 10^6 $TCID_{50}$/mL	Spearma n-karber
RPV	Target	rinderpest virus	RBOK vaccine	V02325	PIADC	~	~	~	Qiagen Rneasy Mini kit	1 x 10^7 $TCID_{50}$/mL	Spearma n-karber
VESV	NN	San Miguel Sea Lion Virus (SMSV)	Type 12	V02040	PIADC	~	~	~	~	~	~

VESV	NN	San Miguel Sea Lion Virus (SMSV)	Type 2	V02056	PIADC	~	~	~	~	~	~
SVD	Target	swine vesicular disease virus	~	HKN/12/87	PIADC	~	Pending (SK6)	~	Ambion MagMax96	9.11±0.13 Log10 TCID50	Spearman-karber
SVD	Target	swine vesicular disease virus	~	HKN-4-89	PIADC	~	Pending (SK6)	~	Ambion MagMax96	8.3 ± 0.14 Log10 TCID50	Spearman-Karber
SVD	Target	swine vesicular disease virus	~	ITL/1/66	PIADC	~	Pending (SK6)	~	Ambion MagMax96	8.61±0.15 Log10 TCID50	Spearman-Karber
SVD	Target	swine vesicular disease virus	~	ITL/1/91	PIADC	~	Pending (SK6)	~	Ambion MagMax96	8.49±0.14 Log10 TCID50	Spearman-Karber
SVD	Target	swine vesicular disease virus	~	ROM-1-87	PIADC	~	Pending (SK6)	~	Ambion MagMax96	7.49± 0.14 Log10 TCID50	Spearman-Karber
SVD	Target	swine vesicular disease virus	~	TAW/119/97	PIADC	~	Pending (SK6)	~	Ambion MagMax96	7.43±0.17 Log10 TCID50	Spearman-Karber
SVD	Target	swine vesicular disease virus	~	UKG72	PIADC	~	Pending (SK6)	~	Ambion MagMax96	8.05±0.12 Log10 TCID50	Spearman-Karber
PRRS	NN	transmissible gastroenteritis virus of hogs	Perdue	NVSL 9801	LLNL	$10^{4.75}$ TCID50/0.1 ml	ST 1	~	Trizol	40 pg/ul	Reed and Muench
BTV	NN	transmissible gastroenteritis virus of hogs	Perdue	NVSL 9801	LLNL	$10^{4.75}$ TCID50/0.1 ml	ST 1	~	Trizol	40 pg/ul	Reed and Muench
VESV	Target	vesicular exanthema of swine virus	1934B	V02029	PIADC	~	10PPK, 2ST, 1 Vero	~	Ambion MagMax 96	1.51 x 10^3 $TCID_{50}$/mL	Spearmann-Karber
VESV	Target	vesicular exanthema of swine virus	A48	V02028	PIADC	~	12 PPK, 2 MVPK, 6 Vero	~	Ambion MagMax 96	5.43 ± 0.16 Log10 TCID50	Spearmann-Karber
VESV	Target	vesicular exanthema of swine virus	B51	V00758	PIADC	~	4 Vero	~	Ambion MagMax 96	3.11 ± 0.15 Log10 TCID50	Spearmann-Karber
VESV	Target	vesicular exanthema of swine virus	C52	V02031	PIADC	~	12PPK, 2MVPK, 6 Vero	~	Ambion MagMax 96	1.29 x 10^5 $TCID_{50}$/mL	Spearmann-Karber
VESV	Target	vesicular exanthema of swine virus	D53	V02032	PIADC	~	12 PPK, 2 MVPK, 7 Vero	~	Ambion MagMax 96	5.86 ± 0.16 Log10 TCID50	Spearmann-Karber
VESV	Target	vesicular exanthema of swine virus	E54	V02033	PIADC	~	3 ST, 2 Vero	~	Ambion MagMax 96	5.99 ± 0.1 Log10 TCID50	Spearmann-Karber
VESV	Target	vesicular exanthema	F55	V02027	PIADC	~	2 ST, 3PK, 1SW, 3PK,	~	Ambion MagMax		Spearmann-

		of swine virus					2ST, 1 Vero		96		Karber
VESV	Target	vesicular exanthema of swine virus	G55	V02034	PIADC	~	21 PPK, 2 MVPK, 7 Vero	~	Ambion MagMax 96	3.8 ± 0.12 Log10 TCID50	Spearmann-Karber
VESV	Target	vesicular exanthema of swine virus	H54	V02035	PIADC	~	12PPK, 2MVPK, 5 Vero	~	Ambion MagMax 96	2.69 x 10^3 $TCID_{50}$/mL	Spearmann-Karber
VESV	Target	vesicular exanthema of swine virus	I55	V02036	PIADC	~	12 PPK, 2 MVPK, 6 Vero	~	Ambion MagMax 96	2.18 ± 0.14 Log10 TCID50	Spearmann-Karber
VESV	Target	vesicular exanthema of swine virus	J56	V02037	PIADC	~	17 PPK, 3 ST, 2 Vero	~	Ambion MagMax 96	2.99 ± 0.1 Log10 TCID50	Spearmann-Karber
VESV	Target	vesicular exanthema of swine virus	K54	V02038	PIADC	~	12PPK, 2MVPK, 5 Vero	~	Ambion MagMax 96	9.77 x 10^3 $TCID_{50}$/mL	Spearmann-Karber
VSV	Target	vesicular stomatitis virus	Indiana - 2	Cocal	PIADC	~	~	~	Qiagen Rneasy Mini Kit	~	~
VSV	Target	vesicular stomatitis virus	Indiana - 2	Maipu	PIADC	~	~	~	Qiagen Rneasy Mini Kit	~	~
VSV	Target	vesicular stomatitis virus	Indiana - 2	Parana (118)	PIADC	~	~	~	Qiagen Rneasy Mini Kit	~	~
VSV	Target	vesicular stomatitis virus	Indiana - 3	Alagoas (146)	PIADC	~	~	~	Qiagen Rneasy Mini Kit	~	~
VSV	Target	vesicular stomatitis virus	Indiana - 3	Alagoas (174)	PIADC	~	~	~	Qiagen Rneasy Mini Kit	~	~
VSV	Target	vesicular stomatitis virus	Indiana-1	~	NVSL	10^8 $TCID_{50}$/mL	~	9/14/2006	Trizol	10^6 $TCID_{50}$/mL	Reed & Muench
VSV	Target	vesicular stomatitis virus	Indiana-1	IN94GUB	PIADC: Luis Rodriguez	$10^{9.59}$ TCID50/mL	~	~	Qiagen Rneasy Mini Kit	1 x $10^{9.6}$ $TCID_{50}$/mL	Spearman-Karber
VSV	Target	vesicular stomatitis virus	Indiana-1	IN97CMB	PIADC: Luis Rodriguez	$10^{7.5}$ TCID50/mL	~	~	Qiagen Rneasy Mini Kit	1 x $10^{7.5}$ $TCID_{50}$/mL	Spearman-Karber
VSV	Target	vesicular stomatitis virus	Indiana-1	IN97COE	PIADC: Luis Rodriguez	~	~	~	Qiagen Rneasy Mini Kit	~	~
VSV	Target	vesicular stomatitis virus	Indiana-1	IN98NME	PIADC: Luis Rodriguez	~	~	~	Qiagen Rneasy Mini Kit	~	~
VSV	Target	vesicular stomatitis virus	Indiana-2	Salt	PIADC	~	~	~	Qiagen Rneasy Mini Kit	~	~
VSV	Target	vesicular stomatitis virus	New Jersey	~	NVSL	10^7 $TCID_{50}$/mL	~	9/14/2006	Trizol	10^7 $TCID_{50}$/mL	Reed & Muench
VSV	Target	vesicular stomatitis virus	New Jersey	NJ0604NME	PIADC: Luis Rodriguez	$10^{7.75}$ TCID50/mL	~	~	Qiagen Rneasy Mini Kit	1 x $10^{7.8}$ $TCID_{50}$/mL	Spearman-Karber
VSV	Target	vesicular stomatitis virus	New Jersey	NJ1184HDB	PIADC: Luis Rodrigu	$10^{7.5}$ TCID50/mL	~	~	Qiagen Rneasy Mini Kit	1 x $10^{7.5}$ $TCID_{50}$/mL	Spearman-Karber

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VSV	Target	vesicular stomatitis virus	New Jersey	NJ89GAS	PIADC: Luis Rodriguez	$10^{9.25}$ TCID50/mL	~	~	Qiagen Rneasy Mini Kit	1 x $10^{9.3}$ $TCID_{50}$/mL	Spearman-Karber
VSV	Target	vesicular stomatitis virus	New Jersey	NJ95COB	PIADC: Luis Rodriguez	10^{9} TCID50/mL	~	~	Qiagen Rneasy Mini Kit	1 x 10^{9} $TCID_{50}$/mL	Spearman-Karber
VESV	NN	vesiculovirus	BeAr	~	PIADC	~	~	~	~	~	~
VESV	NN	vesiculovirus	Calchaqui	~	PIADC	~	~	~	~	~	~
VESV	NN	vesiculovirus	Chandipura	~	PIADC	~	~	~	~	~	~
VESV	NN	vesiculovirus	Hilo CTAN	~	PIADC	~	~	~	~	~	~
VESV	NN	vesiculovirus	Jurona	~	PIADC	~	~	~	~	~	~
VESV	NN	vesiculovirus	Klamath	~	PIADC	~	~	~	~	~	~
VESV	NN	vesiculovirus	NM 85-488	~	PIADC	~	~	~	~	~	~
VESV	NN	vesiculovirus	Piry	~	PIADC	~	~	~	~	~	~

[1]Sources for viruses: California Animal Health and Food Safety (CAHFS), Davis, CA.; Plum Island Animal Disease Center (PIADC), Plum Island, NY; National Veterinary Service Laboratory (NVSL), Ames, IA; National Center for Foreign Animal Disease (NCFAD), Winnepeg, CAN; American Type Culture Collection (ATCC), Manassas, VA; Minnesota State Veterinary Diagnostic Laboratory (MSVDL) in Minneapolis, MN; USDA ARS National Animal Disease Center, IA; USDA ARS, Wy.