Development and Validation of the VetMAX™-Gold SIV Subtyping Kit

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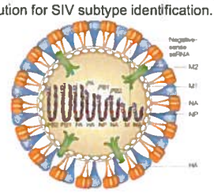
ABSTRACT

Swine influenza virus (SIV) is a highly contagious viral infection of pigs, resulting in significant economic losses to the swine industry. We have validated an SIV subtyping workflow consisting of high throughput nucleic acid purification, SIV detection, and SIV subtyping from porcine nasal swab samples. The VetMAX™-Gold SIV Subtyping Kit is a pair of real-time RT-PCR assays to detect and differentiate the H1, H3, N1 and N2 alleles. When performed in conjunction with the VetMAX™-Gold SIV Detection Kit to screen samples for SIV as well as monitor sample isolation and inhibition, the VetMAX™-Gold SIV Subtyping Kit provides a robust method for subtyping the predominant SIV subtypes in swine.

The SIV subtyping workflow was evaluated with 169 SIV-positive and 150 SIV-negative porcine nasal swab field samples and virus isolates originating from diverse geographic regions in the US. The SIV status and subtype of each sample was confirmed prior to the start of the study. The characterized positive samples processed in this study consisted of the H1N1, H3N2, H1N2, and H2N3 genotypes.

Collaborator laboratories purified the viral nucleic acid using the MagMAX™-96 Viral RNA Isolation Kit (AM1836) and MagMAX™ Express instruments. Samples were first tested with the VetMAX™-Gold SIV Detection Kit prior to testing with the H1/H3 and N1/N2 SIV subtyping reactions on the AB 7500-Fast Real-Time PCR System.

The VetMAX™-Gold SIV Subtyping Kit assays resulted in >97% sensitivity and specificity for identifying the SIV subtype from nasal swab samples. This study indicates that RNA isolated from diagnostic porcine nasal swab samples, tested with the VetMAX™-Gold SIV Subtyping Kit in conjunction with the VetMAX™-Gold SIV Detection Kit, provides an economical and rapid solution for SIV subtype identification.



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INTRODUCTION

Body text - 10 pt. Arial (the blank space above, between subtitle and body text, is 7 pt.) Swine influenza virus (SIV) subtypes are defined by the surface glycoproteins: hemagglutinin and neuraminidase, with H1N1, H3N2, and H1N2 representing the predominant subtypes in swine.

We have validated an SIV subtyping workflow consisting of high throughput nucleic acid purification, SIV detection, and SIV subtyping from porcine nasal swab samples. The VetMAX™-Gold SIV Subtyping Kit is a pair of single-well real-time RT-PCR assays to detect and differentiate the H1, H3, N1 and N2 alleles. The H1 and H3 assays are multiplexed into a single reaction with the H3 probe labeled with FAM™ and the H1 probe labeled with VIC®. The N1 and N2 assays are multiplexed into a single reaction with the N2 probe labeled with FAM™ and the N1 probe labeled with VIC®. All fluorescent probes in the kit are modified with a 3' non-fluorescent quencher, Eclipse™ Q. When performed in conjunction with the VetMAX™-Gold SIV Detection Kit to screen samples for SIV as well as monitor sample isolation and inhibition, the VetMAX™-Gold SIV Subtyping Kit provides a robust method for subtyping the predominant SIV subtypes in swine (Figure 1).

Figure 1. SIV Subtyping Workflow

MATERIALS AND METHODS

The sensitivity and specificity of the SIV subtyping workflow was evaluated with 169 SIV-positive and 150 SIV-negative porcine nasal swab field samples and virus isolates originating from diverse geographic regions in the US. The SIV status and subtype of each sample was confirmed prior to the start of the study with virus isolation (VI) as well as with whole-genome sequencing or qRT-PCR. The characterized positive samples processed in this study consisted of the H1N1, H3N2, H1N2, and H3N1 genotypes. Of the 169 characterized positive samples processed in this study 42% were of the H1N1 genotype, 47% were of the H3N2 genotype, 10% were of the H1N2 genotype, and 0.5% were of the H3N1 genotype. These values roughly correspond to the typical distribution of genotype prevalence described in the literature (1,2), with the majority of samples representing strains circulating within the swine population in the last five years (Table 1).

Table 1: SIV Samples used in Sensitivity and Specificity study

Subtype	Source	Year	Number of Samples (n)
H1N1	IL	2009	11
	IL	2010	10
	MN	2010	10
	OH	2007	40
H3N2	CO	1999	13
	IA	2012	1
	IN	2011	7
	IN	2012	6
	NY	2011	19
	PA	2010	14
	TX	1998	20
H1N2	IA	2010	1
	IL	2010	1
	TX	2008	15
H3N1	NC	2013	1

The collaborator laboratory purified the viral nucleic acid from 50µL porcine nasal swab supernatant using the MagMAX™-96 Pathogen RNA/DNA Kit (Life Tech #4462359) and MagMAX™ Express-96 Deep Well Magnetic Particle Processor. 20,000 copies of Xeno™ RNA were spiked into each nucleic acid isolation to serve as an extraction control and to monitor for the presence of PCR inhibitors. Two negative extraction controls (PBS) per extraction plate were processed through the testing workflow to monitor for contamination of the sample preparation reagents. 8µL of extracted RNA was tested with the VetMAX™-Gold SIV Subtyping Kit to classify the hemagglutinin and neuraminidase alleles. Samples were tested in the Applied Biosystems 7500 Fast Real-Time PCR System. The sensitivity, specificity, and positive/negative predictive values were calculated for the SIV subtyping workflow.

RESULTS

Primary testing of 169 characterized positive samples with the VetMAX™-Gold SIV Subtyping Kit produced 161 initial positive test results for both hemagglutinin and neuraminidase. 67 samples were called positive for H1N1, 16 samples were positive for H1N2, 77 samples were positive for H3N2, and 1 sample was positive for H3N1. 6 positive samples produced a suspect result for at least one genotype during initial testing. 4 out of the 6 suspect samples were correctly identified after completion of the suspect workflow. One sample could not identify the correct hemagglutinin allele even after the suspect workflow, resulting in a false-negative call. Another sample resulted in presumptive positive final calls for both the hemagglutinin and neuraminidase alleles. The instructions for use direct that this sample should be confirmed with a secondary test method. Since a final diagnostic call could not be made with the VetMAX™-Gold SIV Subtyping Kit alone, this sample was omitted from sensitivity and specificity calculations. 2 samples produced a result that was discordant with the result reported from initial sample characterization. Both were negative by the VetMAX™-Gold SIV Subtyping Kit and the VetMAX™-Gold SV Detection Kit but positive by initial sample characterization (VI and sequencing). These samples were classified as false-negative (Table 2).

Table 2: Results of SIV-positive samples sensitivity testing

Call	Sample #
Final Call: True Positive	166
Final Call: False Negative	3
Diagnostic Sensitivity: 98.2%	

Primary testing of 150 characterized negative samples with the VetMAX™-Gold SIV Subtyping Kit produced 142 initial negative test results for both hemagglutinin and neuraminidase. Eight (8) samples produced a suspect result for at least one genotype during initial testing. There were no samples that produced a discrepant call in the initial testing. Of the 8 suspect samples, 5 samples were suspect for the N2 genotype, 1 sample was suspect for H1, 1 sample was suspect for H3, and 1 sample was suspect for both H3 and N2. Suspect samples were retested according to the suspect workflow provided in the instructions for use. All 8 suspect samples produced the correct final call when processed through the suspect workflow (Table 3).

Table 3: Results of SIV-negative samples specificity testing

Call	Sample
Final Call: True Negative	150
Final Call: False Positive	0
Diagnostic Specificity: 100%	

The VetMAX™-Gold SIV Subtyping Kit produced 98.2% sensitivity and 100% specificity for identifying the SIV subtype from porcine nasal swab samples. This study indicates that RNA isolated from diagnostic porcine nasal swab samples, tested with the VetMAX™-Gold SIV Subtyping Kit in conjunction with the VetMAX™-Gold SIV Detection Kit, provides an economical and rapid solution for SIV subtype identification.

To test assay robustness, a panel of 20 porcine nasal swab samples (n=16 SIV positive; n=4 SIV negative) were tested in duplicate by three collaborator laboratories. Collaborators extracted the nucleic acid as described above and tested the RNA with the VetMAX™-Gold SIV Detection Kit followed by the VetMAX™-Gold SIV Subtyping Kit. Each sample was tested by each lab on two different days with two independent kit lots to determine the amount of variation in diagnostic calls between the all variables (Figure 2). There was 100% agreement amongst all labs and test conditions for the H1H3 assay. There was >99% agreement in diagnostic call amongst all N1 samples tested and >98% agreement between labs testing the N2 samples (Tables 4 and 5).

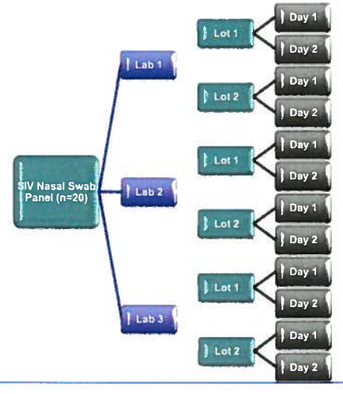

Table 4. Repeatability and Reproducibility SIV-negative sample results

SIV Subtype	True Negative Samples	Test Negative Result	% Agreement
H1	240	240	100%
H3	336	336	100%
N1	336	333	99.1%
N2	240	236	98.3%

Table 5. Repeatability and Reproducibility SIV-positive sample results

SIV Subtype	True Positive Samples	Test Positive Result	% Agreement
H1	240	240	100%
H3	144	144	100%
N1	144	145	99.3%
N2	240	241	99.6%

CONCLUSIONS

The VetMAX™-Gold SIV Subtyping Kit demonstrated excellent diagnostic sensitivity and specificity, 98.2% and 100% respectively. The VetMAX™-Gold SIV Subtyping Kit showed highly repeatable and reproducible results across multiple labs, kit lots, and testing days. Results of the field study have been submitted to the USDA-CVB in support of a license for the VetMAX™-Gold SIV Subtyping Kit.

REFERENCES

1. Choi et al, Arch Virol, Volume 147, Issue 6. June 2002: 1209-20.
2. Pascua et al, Virus Res., Volume 138, Issue 1-2. December 2008: 43-9.

ACKNOWLEDGEMENTS

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TRADEMARKS/LICENSING

Disclaimer: At the time of writing this paper, the VetMAX™-Gold SIV Subtyping kit had not been licensed by the USDA.