Evaluation of a Field-deployable Real-Time PCR Platform for Rapid and Accurate Detection of Significant Avian and Equine Pathogens

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ABSTRACT

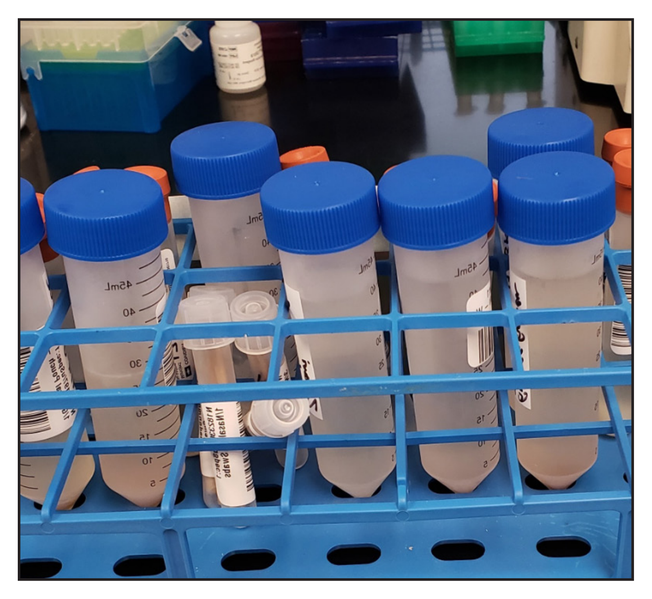
Early detection of veterinary diseases play a crucial role in containing and preventing outbreaks, which can imperil food security, human health, and cause significant economic loss. Current work-flows often take more than 24 hours from sample collection to results, potentially increasing the risk of disease spread. Disease surveillance efforts would benefit greatly from tools that enable rapid, point of need detection. Biomeme has developed a portable, hand-held real-time PCR (rtPCR) machine that, when used in conjunction with their rapid ~one minute M1 Sample Prep™ method and ambient temperature Go-Strip™ reagents, allows the user to carry all necessary reagents and equipment in a backpack and go from sample collection to results in one hour. Here, we assess the feasibility of applying Biomeme's rapid tools for nucleic acid isolation and detection in avian and equine commonly used but technically challenging sample types and matrices. This platform has the potential to serve as a field-deployable tool for rapid, specific, and sensitive pathogen detection.

INTRODUCTION

In this study, respiratory panels for both avian and equine pathogens were developed, validated, and compared to rtPCR tests routinely used by the Pennsylvania Animal Diagnostic Laboratory system (PADLS). Samples with known positive or negative status for the avian pathogens Mycoplasma gallisepticum (MG) and Mycoplasma synoviae (MS) from either tracheal/oropharyngeal or eye conjunctiva swab samples were used to compare the efficacy of the Biomeme platform against the lab's validated protocol, which uses MagMAX-96 DNA extraction followed by IDEXX's RealPCR™ (MG/MS duplex) platform. Detection of equine pathogens Equine Herpes Virus-1 (EHV-1) and Streptococcus equi subs. equi (Strangles) in nasal swabs using the Biomeme platform was also compared to our diagnostic lab's PrepMan Ultra or Qiagen extraction and in-house validated assays.

MATERIALS & METHODS

CURRENT METHODS IN LAB



Clinical Samples
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Extraction
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Primers/Probes Master Mix
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Amplification
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Result Interpretation
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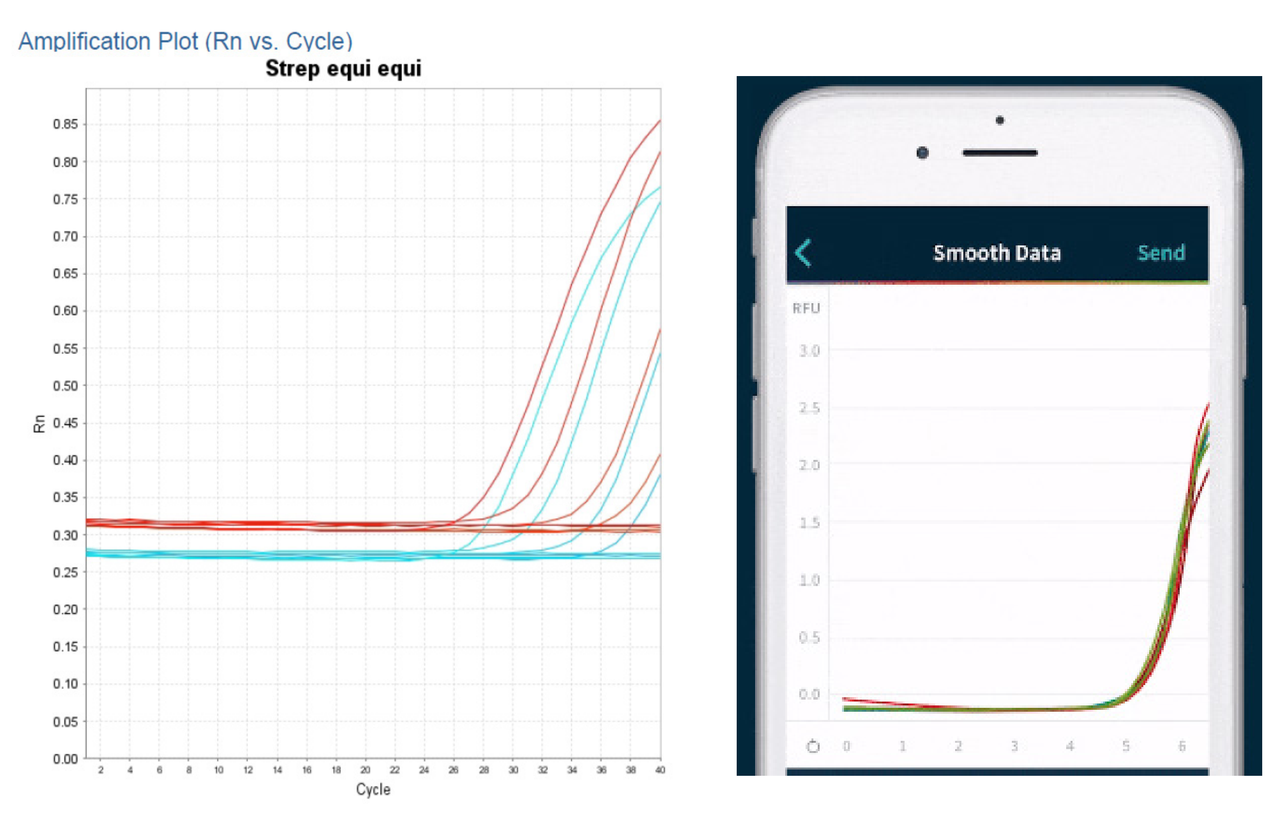
BIOMEME METHOD

Samples
Can be obtained from anywhere: field, environment, stall-side, pen-side or in the lab.

Sample Prep
Pre-aliquoted, disposable buffers in color-coded tubes require no refrigeration or special lab equipment. Up to 9 samples can easily be processed in under 15 minutes.

Go-Strips
Provides lyophilized Hot-Start Taq polymerase, stabilizers and dUTPs that are shelf stable for up to two years. Primers, probes, and 20μl from sample prep final step are added before running on the three9.

Amplification
The three9 real-time thermocycler can detect up to 3 targets in each of 9 wells simultaneously and weighs under 2lbs. Total sample to result can be achieved in under 60 minutes.



Result Interpretation

Figure 1
Sample results from Strep. equi standard curve with IPC run using Biomeme reagents on 7500 fast (A) and example of data screen for the Biomeme three(9) (B).

PRELIMINARY RESULTS

Banked diagnostic samples of equine nasal swabs and chicken tracheal/oropharyngeal/ conjunctiva swabs with known pathogen status were tested for Strep equi or MG/MS, respectively, using standard protocols and the Biomeme platform. The results for Strep equi are summarized in **Table 1**. Samples deemed suspect (SUS) by the laboratory's protocol were further analyzed via culture and MALDI-TOF. Suspect samples that tested positive on the Biomeme platform were culture-confirmed positive, whereas suspect samples that were negative on the Biomeme platform were both culture- and MALDI-TOF negative. The results for MG/MS are shown in **Table 2**. The sensitivity and specificity of the Biomeme methods compared to standard methods are summarized in **Table 3**.

Table 1. *Strep equi*

Sample type, *Strep*		Standard	Biomeme
POS		27.95	24.58
POS		30.81	28.48
POS		34.97	30.11
POS		28.85	26.89
POS		28.85	25.42
POS		29.66	26.19
POS		36.99	26.56
POS		36.95	26.67
POS		38.09	21.70
POS		33.91	30.22
POS		35.68	30.81
SUS	+	37.09	34.19
SUS	+	36.99	32.41
SUS	+	38.31	36.50
SUS	+	36.97	36.03
SUS	-	37.08	0
SUS	-	37.08	0
SUS	-	36.99	0
SUS	-	37.01	0
SUS	-	36.95	0
SUS	-	38.09	0
SUS	-	37.01	0
NEG		0	0
NEG		0	0
NEG		0	0
NEG		0	0
NEG		0	0
NEG		0	0
NEG		0	0
NEG		0	0
PEC		21.76	18.44
PEC		21.85	18.89
PEC		21.62	18.15
PAC		29.00	25.23
PAC		27.56	24.64
PAC		28.18	24.40
PAC		28.99	25.02
PAC		28.95	25.06
NTC		0	0
NTC		0	0
NTC		0	0
NTC		0	0
NTC		0	0

Table 2. MG and MS

Sample type,	Mg standard	Mg Biomeme	Ms standard	Ms Biomeme
POS/POS	30.7	30.27	22.55	22.51
POS/POS	35.54	38.40	23.48	23.28
POS/POS	29.04	29.88	25.31	25.14
POS/POS	27.02	32.11	25.59	25.99
POS/POS	39.57	38.25	26.46	25.83
POS/POS	33.87	34.56	27.40	27.43
POS/POS	35.79	36.70	27.73	26.59
POS/POS	27.12	27.12	27.99	27.13
POS/POS	30.56	29.35	28.59	27.51
POS/POS	28.08	27.05	28.69	28.50
POS/POS	36.55	0	29.02	28.21
POS/POS	29.56	29.22	29.24	29.52
POS/POS	34.87	34.03	30.59	30.04
POS/POS	32.72	33.14	32.70	33.24
POS/POS	30.84	31.92	34.95	36.17
POS/NEG	30.02	34.15	0	0
NEG/POS	0	38	23.56	23.50
NEG/POS	0	0	24.92	25.37
NEG/POS	0	36.01	25.89	26.93
NEG/POS	0	0	26.16	26.77
NEG/POS	0	0	26.34	26.34
NEG/POS	0	0	26.64	25.56
NEG/POS	0	0	26.67	25.12
NEG/POS	0	0	26.68	27.95
NEG/POS	0	0	26.71	26.56
NEG/POS	0	0	27.04	27.93
NEG/POS	0	0	27.27	27.61
NEG/POS	0	0	27.41	27.25
NEG/POS	0	0	27.86	27.27
NEG/POS	0	0	27.95	27.73
NEG/POS	0	0	28.88	28.57
NEG/POS	0	0	29.00	31.06
NEG/POS	0	0	29.08	29.17
NEG/POS	0	0	30.01	30.18
NEG/POS	0	0	31.65	32.44
NEG/POS	0	0	34.25	36.86
NEG/POS	0	0	37.81	0
NEG/NEG	0	0	0	0
NEG/NEG	0	0	0	0
NEG/NEG	0	0	0	0
NEG/NEG	0	0	0	0
NEG/NEG	0	0	0	0
NTC	0	0	0	0
NEG/NEG	NEG	0	NEG	0
NEG/NEG	NEG	0	NEG	0
NEG/NEG	NEG	0	NEG	0
NEG/NEG	NEG	0	NEG	0
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NEG/NEG	NEG	0	NEG	0
NEG/NEG	NEG	0	NEG	0
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NEG/NEG	NEG	0	NEG	0
NEG/NEG	NEG	0	NEG	0
NEG/NEG	NEG	0	NEG	0
NEG/NEG	NEG	0	NEG	0

Table 1. Comparison between Strep equi PCR Ct values determined via standard protocol (Prepman Ultra nucleic acid extraction, XX primers, probe, and master mix and 7500 FAST thermocycler) and Biomeme protocol (M1 Sample Prep, Go-Strips, in-house validated primers and probes, and three9 thermocycler). Samples were known positive (POS), suspect (SUS) or negative (NEG). The culture status (+ or -) of SUS samples is indicated. All runs included positive extraction controls (PEC), positive amplification controls (PAC) and no template controls (NTC).

Table 2. Comparison between MG/MS PCR Ct values determined via standard protocol (MagMAX-96 DNA extraction, IDEXX RealPCR MG/MS duplex assay, and XX thermocycler) and Biomeme protocol (M1 Sample Prep, Biomeme Master Mix, multiplexed in-house validated primers and probes, and CFX96 thermocycler). Samples were determined positive (POS), or negative (NEG) for MG and MS via the standard protocol. An additional 21 NEG/NEG samples were found to have Ct values of 0 for both MG and MS using the Biomeme protocol (data not shown).

Table 3. Sensitivity and specificity

Sample type/Pathogen	Sensitivity	Specificity
Equine nasal swabs/*Strep equi*	100%	100%
Chicken tracheal/MG	93.8%	95.7%
Chicken tracheal/MS	97.2%	100%

Table 3. Summary of sensitivity and specificity of the Biomeme method. The results of standard laboratory methods were used to determine true positives, false positives, false negatives, and true negatives. Suspect (SUS) samples were excluded from analysis in the Equine nasal swabs/Strep equi.

CONCLUSIONS

Assays on the Biomeme platform showed both high sensitivity and specificity for all pathogens tested to date, with further optimization in development. We anticipate from this proof of concept study that the Biomeme platform could enable more rapid and efficient surveillance and diagnosis in a field deployable system to support animal health management and monitoring programs, ultimately improving industry preparedness and disease response.

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ACKNOWLEDGEMENTS

This research is financially supported by a grant from the Pennsylvania Animal Department, NIH-Merial scholarship, and in conjunction with Biomeme, Inc. The Authors would like to thank all practitioners that have submitted samples to the PADLS-New Bolton Center lab to aid in this research.