Validation of a Point-of-Care Polymerase Chain Reaction Assay for the Detection of *Streptococcus equi* subspecies *equi*, Agent of Strangles

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INTRODUCTION

Strangles is a bacterial infection of the upper respiratory tract of equids, caused by *Streptococcus equi* subspecies *equi* (*S. equi*).

The current workflow for PCR detection requires access to a laboratory for testing, often with turnaround times between 24-72 hours from sample collection.

The objective of this study was to validate a stall-side PCR assay for the detection of *S. equi* in respiratory secretions from horses with strangles and to compare results against the molecular gold standard of quantitative PCR (qPCR).

METHODS

Design: Prospective, randomized, blinded laboratory evaluation.

Samples: 232 individual frozen banked clinical nasal secretions.

Testing: Respiratory secretions were collected via rayon-tipped swabs that were immersed in one milliliter (mL) of phosphate buffer solution (PBS). For the qPCR analyzer, nucleic acid was extracted and subsequently analyzed for the presence of *S. equi* and *S. zooepidemicus* on a Biosystems 7900 HTA. An aliquot of these samples was available for the testing using the point-of-care (POC) PCR platform (Fluxergy). For each test the nasal secretions were mixed with a rehydration buffer and pipetted into a microfluidic test card targeting the eqbE gene of S. equi. The test card was inserted into the device and the *S. equi* PCR test was initiated. Total test time for each test was 55 minutes.

RESULTS

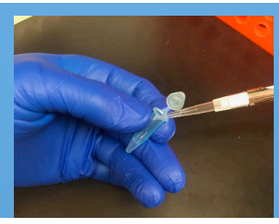
Sample Group	n	*S. equi* qPCR CT Value*	Antigen Load (target gene/µL)	Median (Range)	Agreement
S. equi (-) *S. zooepidemicus* (-)	42	>40	-	-	100%
S. equi (-) *S. zooepidemicus* (+)	40	>40	-	-	100%
S. equi Strong (+)	42	<32	>828	8,087 (897-32,083)	100%
S. equi Moderate (+)	49	32-35	92-828	346 (128-828)	100%
S. equi Weak (+)	59	>35	<92	15 (5-91)	71%

*Number of heat cycles required for the fluorescent signal to cross a threshold to confirm the presence of the target gene

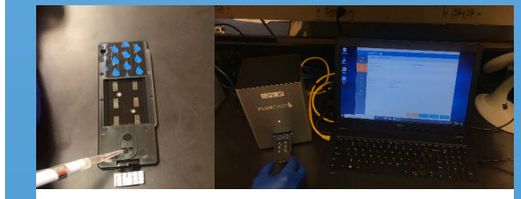
	qPCR		
POC PCR	S. equi (+)	S. equi (-)	Total
S. equi (+)	126	0	126
S. equi (-)	16	73	89
Total	142	73	215
Sensitivity	88.7% (95%, [CI] 85.4%, 96%)		
Specificity	100% (95% [CI] 95.1%, 100%)		

FIGURE 1: POC PCR PROCEDURE

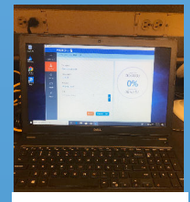
Nasal Secretions + 1 mL PBS



Mixing Sample with PCR Buffer



Loading Microfluidic Test Card



Result

CONCLUSIONS

The POC PCR device showed strong agreement with the qPCR analyzer and detected *S. equi* in the majority of the study samples. The reduced sensitivity of the POC PCR is a result of the reduced detection rate in samples with a CT value >35. Strong agreement and short turn-around-time make the POC PCR device the first molecular diagnostic platform allowing detection of *S. equi* stall-side. The availability of an accurate POC for the detection of *S. equi* will enhance the diagnostic capability of equine veterinarians to timely support a diagnosis of strangles and institute proper biosecurity protocols.

CONFLICT OF INTEREST DISCLOSURE

The authors have no conflicts of interest to disclose.

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