

Specifications Sheet

Fluxergy EHV-1, EHV-4, EIV, and *S. equi* subsp. *equi* (ERP) Assay

Specifications		CAT#
Name:	Fluxergy EHV-1, EHV-4, EIV, and <i>S. equi</i> subsp. <i>equi</i> (ERP) Assay	
Convenient Pack:	10 Units	12646
Test Card Modality	Multi	
Modality Type(s):	Real Time PCR Multiplex	
Targets (Assays):	<i>Streptococcus equi</i> subsp. <i>equi</i> (SeM / eqbE) Equine herpesvirus 1 (gB / gD) Equine herpesvirus 4 (ORF17 / gB) Equine influenza virus (MP / Nucleoprotein)	
User Type:	Research Use Only (RUO)	
Sample Type:	Equine NS Sample in 3mL UTM	
Time to Result:	60 minutes	
Result Output:	Qualitative*	
Collection Method:	Nasal Swab (NS)	12256
	Transport Media (TM)	7994
Preparation:	Respiratory Specimen Collector	12628
	ERP Test Card	12311
Hardware:	Fluxergy Analyzer PC** Barcode Scanner**	5506-CE
Software:	Fluxergy Works	Download
Storage:	Fluxergy ERP test card: 15°C to 25°C (room temperature)	

Workflow

Please see Instructions for Use for the Fluxergy Analyzer.

Required Components

- ERP Test Card
- Respiratory Specimen Collector



Important Notes:

* CT Values and additional data analytics available.

** Part not included. Refer to Instructions for Use for suitable parts.



Handling:

Do not open individual kit packaging until you are ready to test. Use card immediately after opening.

Reference Materials:

Instructions for use, SDS, and support documents can be found at: fluxergy.com/downloads

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WHITE PAPER

A New POC Multiplex PCR Assay for the Rapid Detection of Common Equine Respiratory Targets (EHV-1, EHV-4, EIV and *S. equi* subsp. *equi*)

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Background

Respiratory infections in horses remain a significant concern for equine practitioners. Accurate and rapid identification of causative pathogens is essential—not only to guide effective treatment but also to reduce the spread of disease within barns, veterinary practices, and equine events [1-2].

Over the past few decades, molecular diagnostics have transformed equine medicine, with polymerase chain reaction (PCR) emerging as the gold standard for detecting key infectious agents [1-4]. This

white paper describes a Research Use Only (RUO) point-of-care (POC) multiplex real-time polymerase chain reaction (RT-PCR) assay for the detection of four common equine respiratory targets: equine herpesvirus 1 (EHV-1), equine herpesvirus 4 (EHV-4), equine influenza virus (EIV) and *Streptococcus equi* subsp. *equi* (*S. equi* subsp. *equi*).

Principle of the Assay

This RUO POC Multiplex RT-PCR assay is developed for use with the Fluxergy Multiplex PCR test card and a compatible specimen collector on the Fluxergy Analyzer platform.

The multiplex PCR card is a disposable card with on-chip lyophilized PCR reagent beads featuring a sample loading port and six reaction wells—two dedicated per target. The assay amplifies two highly conserved gene regions for each of the four targets (see **Table 1**) for redundancy in detection.

Table 1 - Target Gene Regions

Target	
Equine herpesvirus 1 (EHV-1)	gB / gD
Equine herpesvirus 4 (EHV-4)	ORF17/ gB
Equine influenza virus (EIV)	Matrix protein / Nucleoprotein
<i>Streptococcus equi</i> subsp. <i>equi</i>	SeM / eqbE

Workflow

Approximately 300 µL of sample (nasal secretions in transport media) is mixed with a compatible buffer at a 1:5 ratio. Five drops of the sample/buffer mixture are dispensed into the loading port of the multiplex PCR card, which can then be inserted into the Fluxergy Analyzer to initiate the test.

Upon initiation, the Fluxergy Analyzer interacts and enables the multiplex PCR card to move sample/buffer mixture to the on-chip PCR rehydration chambers for reconstitution with lyophilized reagent beads. Each reconstituted and homogeneous sample/reagent mixture is then moved to a corresponding on-chip PCR reaction chamber to complete PCR reactions and generate a qualitative result in approximately 60 minutes.

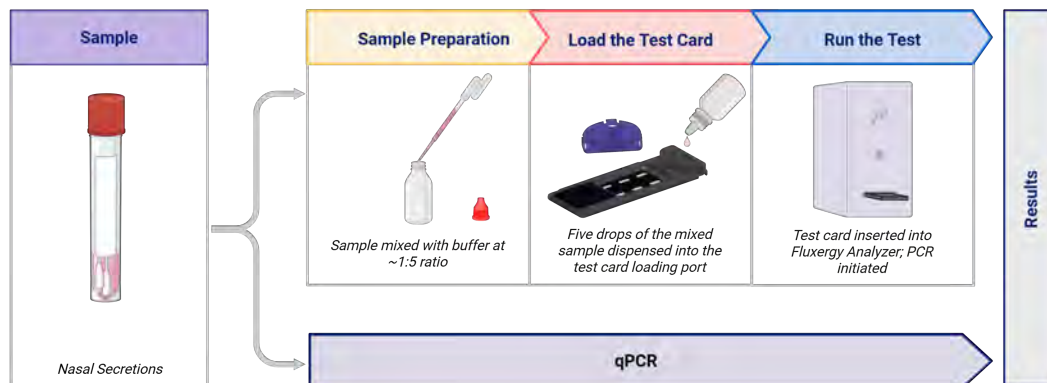


Figure 1 - The Multiplex PCR Assay Workflow

Materials and Methods

A total of 194 equine nasal samples—comprising banked, fresh, and contrived material—were tested either in-house or at the UC Davis School of Veterinary Medicine. All samples were randomized and tested using the RUO POC Multiplex RT-PCR assay. Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) were established relative to the expected outcomes based on a composite reference of contrived plus reference qPCR results.

Results

When comparing the RUO POC Multiplex RT-PCR Assay results for nasal equine samples to the gold standard of qPCR, the overall agreement, PPA, and NPA for each target were listed in **Table 2** here:

Table 2 - PPA, NPA and Overall Agreement with qPCR

Target	PPA	NPA	Overall Agreement
<i>S. equi</i>	94.44% (51/54)	97.86% (137/140)	96.91% (188/194)
EHV-4	94.23% (49/52)	97.18% (138/142)	96.39% (187/194)
EIV	91.30% (42/46)	98.65% (146/148)	96.91% (188/194)
EHV-1	100.00% (39/39)	99.35% (154/155)	99.48% (193/194)

† This evaluation excludes samples that did not pass internal quality control (n=4) with indeterminate results, which may occur due to sample quality or assay inhibition.

Conclusion

The rapid identification of equine respiratory targets—especially from nasal samples—within one hour of sampling enables timely, actionable results and supports early-stage isolation of infected horses. This underscores the value of point-of-care (POC) PCR testing as a supplement to traditional qPCR methods in outbreak scenarios.

Most discrepant samples in this evaluation had comparator qPCR Ct values >36, indicating target levels near or below the detection limit of the RUO POC multiplex RT-PCR assay. Remaining discrepancies may be attributed to differences in sample processing or quality; comparator qPCR was performed on extracted input, while the RUO assay was run directly on raw samples.

While rapid molecular results can inform clinical decision-making, all test findings must be interpreted in context—considering patient history, clinical signs, and other diagnostics findings.

Disclosure

The POC multiplex RT-PCR assay evaluated in this study is for research use only (RUO). It has not been reviewed or approved by the USDA Center for Veterinary Biologics (CVB) for veterinary diagnostic use. This assay is intended solely for use by qualified laboratory personnel who are developing and validating their own non-clinical assays. It is not intended for use in animal disease diagnosis, treatment decisions, or other clinical applications. Use of this assay should be in accordance with applicable local and state regulations by licensed veterinary professionals.

References

1. Newton JR, Wood JL. "Equine respiratory infections: Diagnosis, treatment, and control." *Vet Clin North Am Equine Pract.* 2003;19(2):357-369.
2. Goehring LS, Cook RF. "Equine herpesvirus infections." *Vet Clin North Am Equine Pract.* 2017;33(3):561-574.
3. Pusterla N, et al. "Equine Influenza: A Comprehensive Review." *Vet J.* 2017;221:44-52.
4. Henninger RW, et al. "Advances in the molecular diagnosis of equine respiratory pathogens." *Equine Vet J.* 2019;51(6):735-743.

