Shortened protocol for PrioCHECK FMDV NS ELISA

Introduction

Foot-and-mouth disease (FMD) is an extremely contagious and severe vesicular disease, caused by foot-and-mouth disease virus (FMDV), that affects cloven-hoofed animals. The virus is a non-enveloped, single-stranded, positive-sense RNA virus of the *Picornaviridae* family. There are 7 distinct serotypes—O, A, C, Asia1, and SAT 1, 2, and 3. The viral capsid consists of 4 structural proteins (VP1–4), and antibodies against these proteins are serotype-specific. The nonstructural proteins (NSPs)—3ABC and 3D—are highly conserved and nonspecific. FMDV particles are chemically inactivated and purified during vaccine preparation. Generally, best-in-class vaccine formulations do not contain NSP; vaccinated livestock have antibodies against the structural proteins only. Therefore, NSP antibody assays can differentiate infected from vaccinated animals (DIVA).

Controlling FMD requires extreme measures to prevent rapid spread among susceptible livestock and is a major concern in countries with meat and dairy industries. Early and quick detection via laboratory diagnosis is essential for FMD control. Therefore, it is important to verify that the FMDV diagnostic assays used by German veterinary diagnostic laboratories are fit for this purpose. In 2015, Friedrich-Loeffler-Institut (FLI, German national reference laboratory for FMD) conducted a trial of the Applied Biosystems™ PrioCHECK™ FMDV NS Antibody ELISA Kit to confirm diagnostic specificity, and verified a shorter protocol for testing [1].

In 2017 the Pirbright Institute, the world reference laboratory for FMD, performed a verification study on a statistically sufficient number of samples (n = 500) to verify the observations from the FLI study [2].

ThermoFisher SCIENTIFIC

Verification of a shortened protocol for PrioCHECK FMDV NS ELISA

The protocol for PrioCHECK FMDV NS ELISA recommends an overnight incubation. However, FLI evaluated shorter incubation times (1 or 2 hr) at 37°C with larger sample volumes (up to 50 µL), with the shortened protocol allowing testing to be completed in ~4 hr (Table 1). A total of three dilutions of positive reference serum containing FMDV-specific antibodies and one negative-control sample were used for the comparisons.

Pirbright verification trial

For the Pirbright verification trial, 250 characterized serum samples obtained from experimental studies and 250 serum samples from an FMDV-free country without vaccination were tested in parallel by both the PrioCHECK FMDV NS ELISA overnight protocol and the shortened (single-day) incubation protocol [2]. These samples were from vaccinated and/or infected animals. The majority of samples were bovine (n = 194), with some samples porcine (n = 12) and ovine (n = 44). The samples represented sera of O, A, Asia 1, and SAT2 FMDV serotypes (Table 2). Of these samples, 209 were NSP-positive and 291 were NSP-negative using the reference PrioCHECK FMDV NS ELISA overnight protocol.

Table 1. PrioCHECK FMDV NS kit—original and shortened protocols. Differences are highlighted in bold.

Original protocol [3-5]	Shortened protocol [2]
Day 1: Incubation with test serum	
1. Mix 80 µL buffer with **20 µL control or test sample**.	1. Mix 80 µL buffer with **50 µL control or test sample**.
2. Seal and shake gently.	2. Seal and shake gently.
3. **Incubate overnight (16–18 hr) at room temperature (RT) (22 ±3°C)**.	3. **Incubate for 2 hr at RT (22 ±3°C)**.
Day 2: Incubation with conjugate	
1. Empty plate.	
2. Wash 6x with 200–300 µL washing solution.	
3. Add 100 µL of diluted conjugate.	
4. Incubate for 1 hr at RT.	
5. Empty plate.	
6. Wash 6x with 200–300 µL washing solution.	
7. Add 100 µL of TMB substrate.	
8. Incubate for 20 min at RT.	
9. Add 100 µL of stop solution and mix gently.	
10. Measure at 450 nm.	

Note: RT = room temperature, TMB = trimethylbenzidine.

Table 2. Samples used for the comparison study.

Source of serum		Bovine	Ovine	Porcine	Caprine	Total
NSP-positive	Serotype O	68	39	11	0	118
	Serotype A	64	0	1	0	65
	Serotype Asia 1	48	5	0	0	53
	Serotype SAT 2	14	0	0	0	14
NSP-negative	NA	150	25	50	25	250
Total		**344**	**69**	**62**	**25**	**500**

Results

An increased sample volume allowed a shortened protocol for PrioCHECK FMDV NS ELISA. Shortening the sample incubation time for the PrioCHECK FMDV NS kit without increasing the sample volume reduced the sensitivity independently of the incubation temperature (Figure 1), but increasing the sample volume to 50 µL restored the original sensitivity, even with incubation for 2 hr at RT (Figure 2). With the increased sample volume, the kit controls and reference samples showed good agreement between the long and short protocols (Figure 3).

The Pirbright study compared the standard protocol with the modified short protocol for 500 fully characterized serum samples. The results achieved with the overnight incubation protocol have been compared against the short incubation protocol; whereas the shortened incubation protocol was the test under investigation, the overnight protocol determines the status of the sample.

There was an overall agreement of 96% between the overnight and the shortened incubation protocols. The shortened incubation protocol resulted in 98% sensitivity and 95% specificity when compared with the overnight incubation protocol (Table 3). In comparison with the actual predetermined sample status of the samples, 13 of the 14 false-positive samples were in fact true positives. Specificity against the true sample status is therefore 99%.

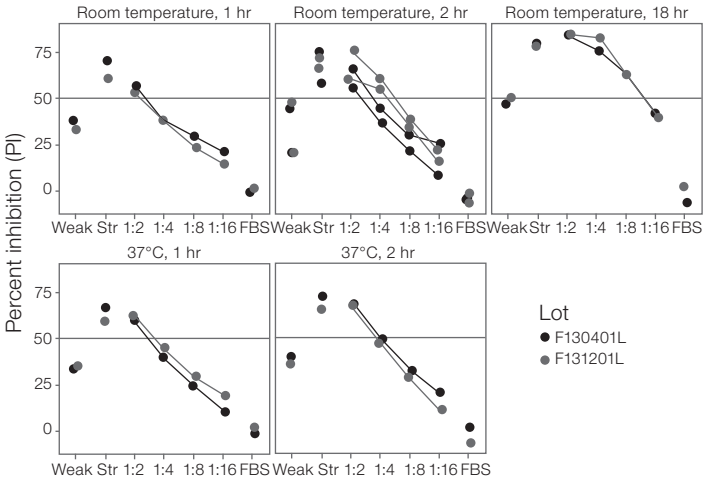

Figure 1. PrioCHECK FMDV NS ELISA with modified incubation time and temperature. Kit controls and known positive sample dilutions were tested using 20 µL samples for the two kit lots. Positive cut-off (50% inhibition) is marked with a horizontal line. Str: strong.

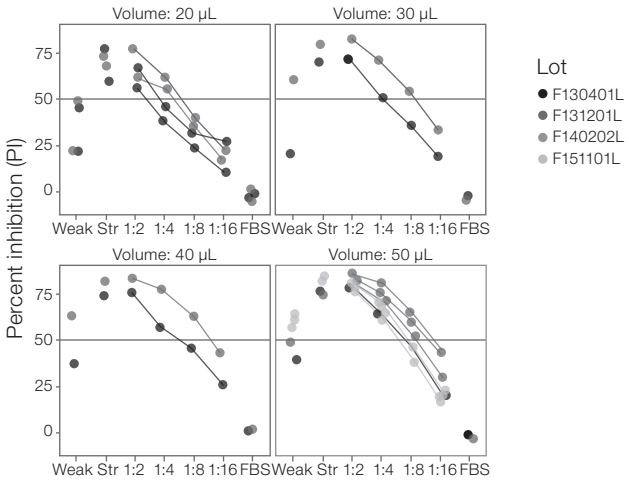

Figure 2. PrioCHECK FMDV NS ELISA with shortened incubation time and increased sample volume. Known positive samples and controls were incubated for 2 hr at RT. Four kit lots were used for the tests. Str: strong.

Table 3. Sensitivity and specificity calculations.

		PrioCHECK FMDV NS ELISA (overnight protocol)*	
		Positive result	Negative result
PrioCHECK FMDV NS ELISA (shortened incubation protocol)	Positive result	True positive (TP) = 205	False positive (FP) = 14
	Negative result	False negative (FN) = 4	True negative (TN) = 277
Agreement: (TP + TN)/total number of samples = 96%		Sensitivity: TP/(TP + FN) = 98%	Specificity: TN/(FP + TN) = 95%

* Note: The PrioCHECK FMDV NS ELISA overnight incubation protocol was used as the reference test.

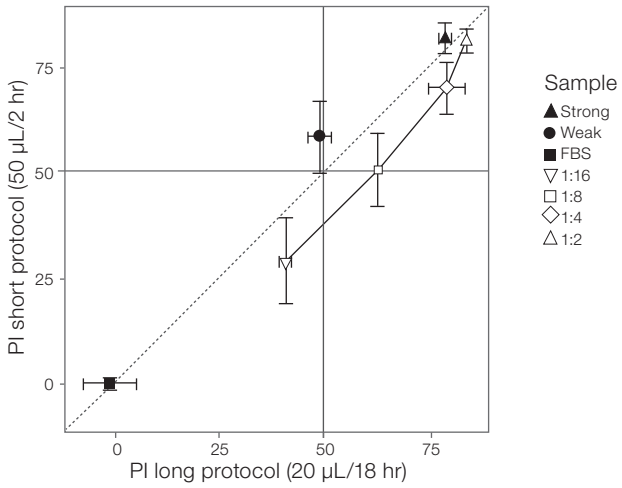

Figure 3. Comparison of ELISA results between short and long protocols using the PrioCHECK FMDV NS kit.

applied biosystems

Percent inhibition (PI) was compared between the two test protocols. The green line represents the cut-offs used in each test. For both assays, a PI over 50% is considered positive. The data include results for 500 serum samples (Figure 4).

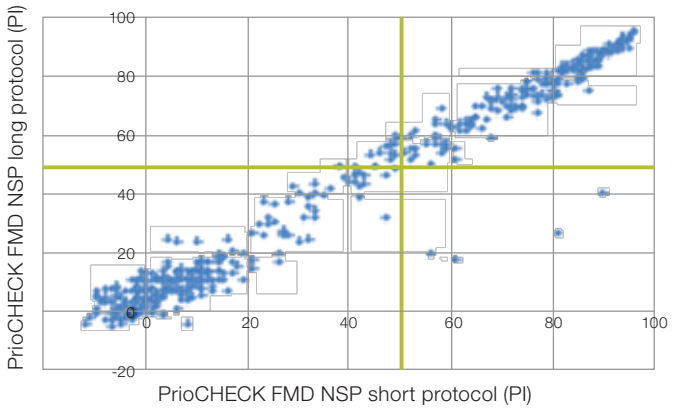

Figure 4. Agreement between the PrioCHECK FMDV NS ELISA overnight and short protocols.

Summary

A shortened protocol for the PrioCHECK FMDV NS ELISA Kit was validated by FLI using 50 µL test samples and 2 hr incubation. The positive controls and serial dilutions of reference sera were reliably detected. The shortened protocol is faster and allowed results to be obtained in ~4 hr.

The Pirbright Institute validation trial revealed an overall 96% agreement between the overnight and the short incubation protocols. The short incubation protocol has sensitivity of 98% and specificity of 95% in direct comparison with the overnight incubation protocol. When compared to the actual sample status, the sensitivity is 99%.

Ordering information

Product	Quantity	Cat. No.
PrioCHECK FMDV NS Antibody ELISA Kit	5-plate kit (450 tests)	7610440
PrioCHECK FMDV NS Antibody ELISA Kit	10-plate kit (900 tests)	7610760
PrioCHECK FMDV NS Antibody ELISA Kit	5-strip plate (450 tests)	7610770
Related products		
PrioCHECK FMDV Type O Antibody ELISA Kit, strip	5-plate kit (450 tests)	7610420
PrioCHECK FMDV Type A Antibody ELISA Kit, strip	5-plate kit (450 tests)	7610850
PrioCHECK FMDV Type Asia 1 Antibody ELISA Kit, strip	5-plate kit (450 tests)	7610870

References

1. Dill V, Eschbaumer M, Beer M et al. (2017) Inter-laboratory validation of foot-and-mouth disease. *Vet Microbiol* 203:62–67.
2. The Pirbright Institute, PrioCHECK NSP Single Day Validation, Stage 2 Validation Study Report Number: PrioCHECK s2-1/18.
3. Package insert of "PrioCHECK FMDV NS ELISA for *in vitro* detection of antibodies against Foot and Mouth Disease Virus in serum of cattle, sheep, goats and pigs".
4. Pirbright Institute, QA-SOP-12 Management and Review of Quality Audits.
5. Pirbright Institute, SAU-SOP-10 Detection of Antibodies against the Non Structural Protein of foot-and-mouth disease virus (FMDV) using PrioCHECK FMDV-NS (Ceditest™ FMDV-NS) kits.

Find out more at **thermofisher.com/animalhealth**

ThermoFisher SCIENTIFIC