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Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), Swine Influenza Virus type A (SIV), Porcine Circovirus type 2 (PCV2) and *Mycoplasma hyopneumoniae* Antibody Test Kit Microsphere-based multiplex fluorescent immunoassay (MFIA)

Swinecheck MP® PRRSV type 1 and 2, SIV, PCV2, M. hyopneumoniae

Product code: TRM-554

- ✓ **Detect antibodies against 5 pathogens in a single well**
- ✓ **Highly sensitive and specific test**
- ✓ **Reduced handling costs per sample**
- ✓ **Simple and user-friendly protocol**

INTRODUCTION

Porcine Reproductive and Respiratory Syndrome (PRRS) is caused by the PRRS virus (PRRSV) which comprises two major genotypes: type 1 (“European”) and type 2 (“North-American”). The disease appeared in the late ’80 in Europe and North America and is now present in most swine producing countries. PRRS is characterized by reproductive problems such as increased abortion in late gestation, mummification and stillbirth as well as increased mortality rates and pneumonia in young pigs.

Influenza is a highly contagious viral infection in many animal species including swine and poultry. In swine, most of the clinical infections are caused by influenza viruses of type A. Swine influenza virus (SIV) are subdivided in different subtypes based on their hemagglutinin (HA) and neuraminidase (NA) proteins. The subtypes involved in clinical infections in swine are the hemagglutinins H1 or H3.

PCV2 has been associated to various clinical signs in weaned and growing pigs (the so-called porcine circovirus associated diseases, PCVAD), the most important being the post-weaning multisystemic wasting syndrome (PMWS). Other frequent conditions associated with PCV2 include porcine dermatitis and nephropathy syndrome (PDNS), enteritis, myocarditis, and abortions or stillbirths.

Mycoplasma hyopneumoniae belongs to the class Mollicutes and is the etiological agent of Porcine Enzootic Pneumonia. This agent affects mostly the growing and finishing pigs resulting in persistent cough and growth retardation. *M. hyopneumoniae* is also implicated in the pathogenesis of porcine respiratory disease complex (PRDC), a disease involving both bacterial and viral (PRRS, PCV2, ADV and porcine respiratory coronavirus) pathogens.

PRRSV, SIV, PCV2 and *M. hyopneumoniae* diagnosis relies mostly on the demonstration of nucleic acids (PCR) or the detection of antibodies in body fluids (serum, oral fluids, etc). Numerous serological tests have been developed to detect antibodies, but conventional ELISAs do not allow simultaneous detection.

INTENDED USE

The Swinecheck MP® PRRSV type 1 and 2, SIV, PCV2, M. hyopneumoniae assay is a microsphere-based mutiplex fluorescent immunoassay (MFIA) intended for the simultaneous detection of antibodies directed against Porcine Reproductive and Respiratory Syndrome virus (PRRSV) type 1 (EU) and 2 (NA), swine Influenza type A virus (SIV), porcine circovirus type 2 (PCV2) and/or *Mycoplasma hyopneumoniae* in swine serum.

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PRINCIPLE OF THE TEST

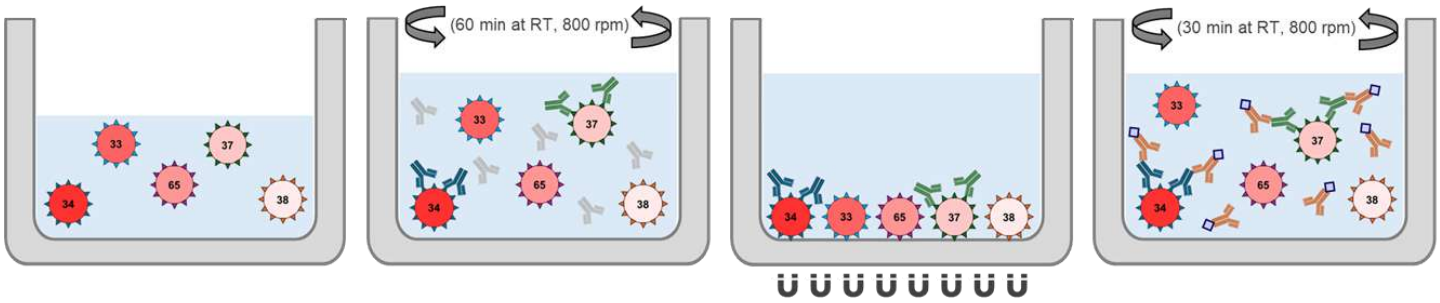
a. Assay description

1. Five distinct microsphere sets (Region) coated with different antigens are added to the wells.

2. Diluted serum samples are added to the wells and the plate is incubated under agitation.

3. After the incubation, the plate is moved on a magnetic separator and wells are washed.

4. The plate is then removed from the magnetic separator and the detection antibody is added, followed by an incubation.

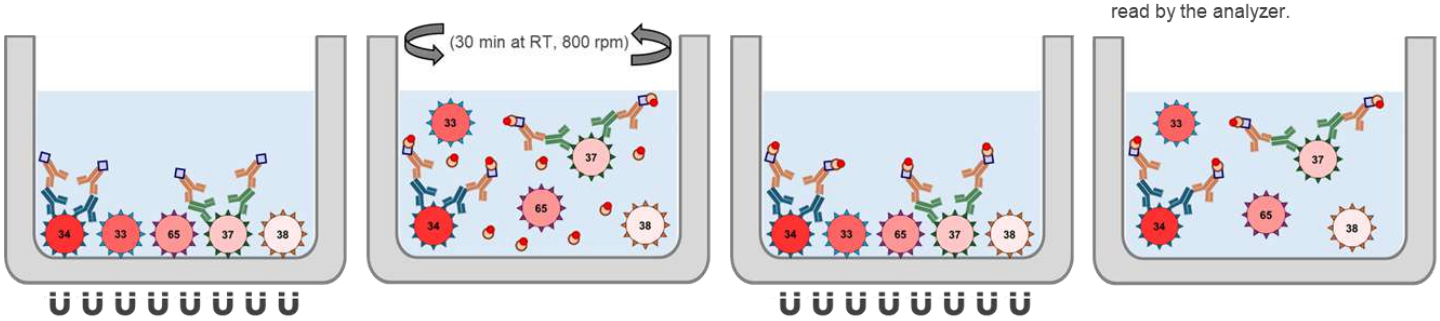


5. After the incubation, the plate is moved on a magnetic separator and wells are washed.

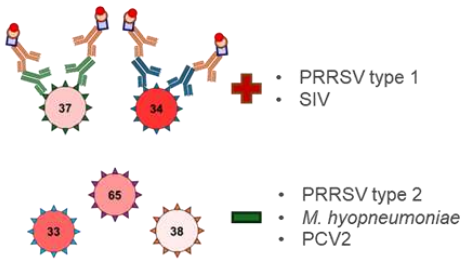
6. The plate is then removed from the magnetic separator and the SA-PE Reporter is added, followed by an incubation.

7. After the incubation, the plate is moved on a magnetic separator and wells are washed.

8. The plate is then removed from the magnetic separator and microspheres are resuspended in the wash buffer. The plate is then read by the analyzer.



9. The analyzer will read the SA-PE signal separately for each set (region) of microspheres.



Legend:

- 34 — Microsphere Region 34 coated with PRRSV type 1 (EU) Ag
- 33 — Microsphere Region 33 coated with PRRSV type 2 (NA) Ag
- 65 — Microsphere Region 65 coated with *Mycoplasma hyopneumoniae* Ag
- 37 — Microsphere Region 37 coated with Swine Influenza Virus Ag
- 38 — Microsphere Region 38 coated with Porcine Circovirus type 2 Ag
- Positive PRRSV1 antibody
- Positive SIV antibody
- Negative antibody
- Detection antibody
- SA-PE Reporter
- Magnet

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b. Results interpretation

The analyzer will read the wells and give the Median Fluorescence Intensity (MFI) signal for each set of microsphere/antigen. Subtract the MFI value obtained for the negative control from those of the samples or the positive control to obtain the corrected MFI. If the negative control is run in duplicate, use the mean MFI for subtraction.

Calculate the S/P ratio for each set of microsphere/antigen as follow:

$$\text{S/P ratio} = \frac{\text{(Sample MFI – Negative Control MFI)}}{\text{(Positive Control MFI – Negative Control MFI)}} = \frac{\text{Sample corrected MFI}}{\text{Positive Control corrected MFI}}$$

The following cut off values were chosen:

Result	Negative	Positive
PRRSV type 2 (NA)*	S/P < 0.30	S/P ≥ 0.30
PRRSV type 1 (EU)*	S/P < 0.30	S/P ≥ 0.30
M. hyopneumoniae	S/P < 0.30	S/P ≥ 0.30
SIV	S/P < 0.15	S/P ≥ 0.15
PCV2	S/P < 0.30	S/P ≥ 0.30

* PRRSV type1 and 2 antigens share common epitopes. PRRSV positive antibody samples usually cross-react with both antigens. The PRRSV type involved corresponds to the antigen with the highest S/P.

The following criteria must be met in order to validate the test:

- Each sample requires the analysis of at least 50 microspheres per antigen for valid data. Rerun any sample with a lower microsphere count for any antigen.
- The mean MFI signal for the Negative Control must be < 1 500 for PRRSV NA (type 2), PRRSV EU (type 1), SIV and *M. hyopneumoniae*, and < 2 500 for PCV2. Higher Negative Control signals can indicate a systematic error for the assay plate and require repeating the assay plate.
- The “corrected MFI” signal for the Positive Controls must be ≥ 5 000 for all controls. Lower Positive Control signals can indicate a systematic error for the assay plate and require repeating the assay plate.

KIT COMPOSITION

Components	Quantity	Storage
Ready-to-use Sample Diluent	2 x 75 mL	2 – 8°C
Ready-to-use PRRSV type 2 (NA) Positive Control	2 mL	2 – 8°C
Ready-to-use PRRSV type 1 (EU) Positive Control	2 mL	2 – 8°C
Ready-to-use *M. hyopneumoniae* Positive Control	2 mL	2 – 8°C
Ready-to-use SIV Positive Control	2 mL	2 – 8°C
Ready-to-use PCV2 Positive Control	2 mL	2 – 8°C
Ready-to-use Negative Control	3 mL	2 – 8°C
Ready-to-use Microsphere Mix	28 mL	2 – 8°C
Wash Buffer (10X)	2 x 100 mL	2 – 8°C
Ready-to-use Detection Antibody	55 mL	2 – 8°C
Ready-to-use SA-PE Conjugate	55 mL	2 – 8°C
Microtiter Plate	5	2 – 25°C

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TECHNICAL DATA

a. Sensitivity & Specificity

A panel of 519 serum samples was used for evaluating the test sensitivity & specificity for *M. hyopneumoniae* (**A**). The serum samples originated from various Canadian herds either free of *M. hyopneumoniae* (169 samples) or naturally infected (350 samples). The M. hyopneumoniae Ac ELISA kit (Dako/Oxoid) was used as a reference test. A different panel of 500 serum samples was used for evaluating the test sensitivity & specificity for PRRSV (**B**). The Idexx PRRS X3 Ab Test was used as a reference test. This test did not differentiate between type 1 and type 2 strains. Positive results for one of the two types, as tested with the Biovet kit, were grouped together. A panel of 349 samples was examined for PCV2 (**C**). The reference test was the Ingezym Circo IgG ELISA kit (Ingenasa, Spain). Finally, 209 samples were examined for SIV (**D**) using the Influenza A Ab ELISA (Idexx, USA). Testing was performed according to the instructions of the manufacturers.

A	Reference +	Reference -	Total
Biovet +	160	16	**176**
Biovet -	9	334	**343**
Total	**169**	**350**	**519**

Statistic	Value	95% CI
Relative sensitivity	94.67%	90.13% to 97.54%
Relative specificity	95.43%	92.68% to 97.36%
Kappa	**0.891**	

B	Reference +	Reference -	Total
Biovet +	199	1	**200**
Biovet -	1	299	**300**
Total	**200**	**300**	**500**

Statistic	Value	95% CI
Relative sensitivity	99.50%	97.25% to 99.99%
Relative specificity	99.67%	98.16% to 99.99%
Kappa	**0.992**	

C	Reference +	Reference -	Total
Biovet +	142	33	**175**
Biovet -	46	128	**174**
Total	**188**	**161**	**349**

Statistic	Value	95% CI
Relative sensitivity	75.53%	68.75% to 81.50%
Relative specificity	79.50%	72.44% to 85.45%
Kappa	**0.547**	

D	Reference +	Reference -	Total
Biovet +	122	2	**124**
Biovet -	9	76	**85**
Total	**131**	**78**	**209**

Statistic	Value	95% CI
Relative sensitivity	93.13%	87.36% to 96.81%
Relative specificity	97.44%	91.04% to 99.69%
Kappa	**0.890**	

The MFIA test showed an excellent agreement with the ELISAs tests for *M. hyopneumoniae*, PRRSV and SIV samples. On the contrary the agreement was poor for PCV2 samples. The lack of correlation between PCV2 antibody tests (various IFA and ELISA) has been observed previously (data not shown). This may be partially explained by the use of different antigens and the origin of the samples examined (infected or vaccinated pigs).

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b. Repeatability

A panel of 7 samples was selected, including positive and negative samples for each target pathogen. Samples were tested in 4 distinct serials to assess lot to lot repeatability. MFI were measured and used to calculate the S/P ratio. The coefficient of variation (%) was calculated for each sample according to the target pathogen (microsphere region), using results obtained in the 4 serials. Positive results are presented in bold.

PRRSV2 (NA)

Sample ID	Serial A	Serial B	Serial C	Serial D	CV (%)
CQ # 718	**0,35**	**0,38**	**0,40**	**0,41**	*6,4%*
USDA # 87	**1,00**	**0,90**	**0,92**	**0,97**	*4,9%*
USDA # 92	**0,65**	**0,63**	**0,64**	**0,61**	*2,6%*
USDA # 93	**0,66**	**0,63**	**0,65**	**0,64**	*2,3%*
USDA # 98	**0,39**	**0,42**	**0,37**	**0,39**	*5,3%*
754304-1	0,02	0,03	0,02	0,03	*23,1%*
775778-13	0,08	0,10	0,08	0,09	*9,2%*

PRRSV1 (eu)

Sample ID	Serial A	Serial B	Serial C	Serial D	CV (%)
CQ # 718	**0,64**	**0,63**	**0,62**	**0,65**	*1,8%*
USDA # 87	**0,69**	**0,66**	**0,61**	**0,66**	*5,6%*
USDA # 92	**0,18**	**0,18**	**0,17**	**0,18**	*2,8%*
USDA # 93	**0,29**	**0,29**	**0,28**	**0,30**	*3,3%*
USDA # 98	0,07	0,07	0,05	0,06	*15,2%*
754304-1	0,02	0,01	0,00	0,01	*65,5%*
775778-13	0,00	0,00	0,00	0,00	*100,7%*

SIV

Sample ID	Serial A	Serial B	Serial C	Serial D	CV (%)
CQ # 718	0,01	0,01	0,01	0,01	*18,5%*
USDA # 87	**1,26**	**1,10**	**1,09**	**1,10**	*6,9%*
USDA # 92	**0,33**	**0,30**	**0,29**	**0,24**	*13,0%*
USDA # 93	0,05	0,05	0,05	0,04	*12,2%*
USDA # 98	**0,25**	**0,26**	**0,21**	**0,19**	*14,5%*
754304-1	**1,15**	**1,13**	**1,08**	**1,10**	*2,7%*
775778-13	**1,26**	**1,04**	**1,11**	**1,15**	*7,9%*

PCV2

Sample ID	Serial A	Serial B	Serial C	Serial D	CV (%)
CQ # 718	**1,52**	**1,49**	**1,29**	**1,22**	*10,7%*
USDA # 87	**0,55**	**0,64**	**0,74**	**0,69**	*12,5%*
USDA # 92	**1,19**	**1,14**	**1,16**	**1,01**	*6,9%*
USDA # 93	**1,86**	**1,77**	**1,34**	**1,26**	*19,4%*
USDA # 98	**1,05**	**1,09**	**1,04**	**0,96**	*5,3%*
754304-1	0,01	0,02	0,02	0,02	*47,0%*
775778-13	0,07	0,10	0,18	0,17	*40,1%*

M. hyopneumoniae

Sample ID	Serial A	Serial B	Serial C	Serial D	CV (%)
CQ # 718	0,01	0,01	0,01	0,03	*61,5%*
USDA # 87	**1,91**	**2,05**	**2,01**	**2,00**	*2,9%*
USDA # 92	**2,00**	**2,28**	**2,19**	**2,04**	*6,1%*
USDA # 93	**0,84**	**0,80**	**0,86**	**0,76**	*5,0%*
USDA # 98	**1,56**	**1,82**	**1,62**	**1,54**	*7,8%*
754304-1	0,06	0,08	0,07	0,07	*13,2%*
775778-13	**0,42**	**0,55**	**0,43**	**0,41**	*14,7%*

The results demonstrate the kit's high repeatability. Only one positive sample had a coefficient of variation greater than 15% (USDA#93 for PCV2).

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c. Stability

A panel of 10 was selected and tested, in 5 distinct serials, at final QC and 19 months post-manufacture. The results presented are the S/P ratios of the selected samples for each targeted pathogen (microsphere region). The kits used for the stability tests were stored at 2-8°C until final testing.

A. PRRSV type 2 (NA) – Microspheres region 34

Reactivity	Sample ID	Serial A		Serial B		Serial C		Serial D		Serial E	
		0 mo.	19 mo.	0 mo.	19 mo.	0 mo.	19 mo.	0 mo.	19 mo.	0 mo.	19 mo.
Positive	CQ # 429	**0,82**	**0,76**	**0,79**	**0,76**	**0,82**	**0,77**	**0,89**	**0,82**	**0,82**	**0,78**
Positive	CQ # 723	**1,14**	**1,23**	**0,97**	**1,03**	**1,10**	**1,11**	**1,23**	**1,20**	**1,11**	**1,20**
Positive	USDA # 103	**1,24**	**1,37**	**1,04**	**1,19**	**1,19**	**1,25**	**1,21**	**1,28**	**1,22**	**1,27**
Negative	USDA # 105	0,16	0,15	0,16	0,17	0,16	0,14	0,20	0,18	0,16	0,11
Weak pos.	USDA # 108	**0,43**	**0,43**	**0,43**	**0,44**	**0,42**	**0,38**	**0,50**	**0,46**	**0,42**	**0,37**
Negative	USDA # 127	0,30	0,24	0,28	0,26	0,24	0,23	0,30	0,27	0,25	0,23
Negative*	CQ # 717	0,29	0,27	**0,35**	**0,34**	**0,36**	**0,32**	**0,54**	**0,37**	**0,40**	**0,31**
Negative*	CQ # 718	**0,35**	**0,34**	**0,38**	**0,37**	**0,40**	**0,33**	**0,52**	**0,52**	**0,41**	**0,33**
Negative	453172-3	0,03	0,03	0,04	0,02	0,03	0,02	0,04	0,02	0,03	0,02
Negative	773217-1	0,00	0,01	0,01	0,00	0,00	0,01	0,00	0,01	0,01	0,01

*Samples were considered negative because the signal with PRRSV1 (Eu) was stronger.

B. PRRSV type 1 (Eu) – Microspheres region 33

Reactivity	Sample ID	Serial A		Serial B		Serial C		Serial D		Serial E	
		0 mo.	19 mo.	0 mo.	19 mo.	0 mo.	19 mo.	0 mo.	19 mo.	0 mo.	19 mo.
Negative*	CQ # 429	**0,65**	**0,65**	**0,67**	**0,59**	**0,62**	**0,62**	**0,81**	**0,75**	**0,65**	**0,67**
Negative*	CQ # 723	**0,42**	**0,41**	**0,39**	**0,38**	**0,38**	**0,34**	**0,54**	**0,55**	**0,44**	**0,47**
Negative*	USDA # 103	**1,02**	**1,04**	**0,99**	**0,96**	**0,98**	**1,01**	**1,02**	**0,99**	**1,00**	**0,96**
Negative	USDA # 105	0,09	0,09	0,07	0,08	0,07	0,08	0,15	0,13	0,08	0,07
Negative	USDA # 108	0,07	0,07	0,07	0,05	0,06	0,05	0,14	0,10	0,07	0,06
Negative	USDA # 127	0,15	0,13	0,13	0,11	0,09	0,09	0,20	0,17	0,11	0,13
Positive	CQ # 717	**0,87**	**0,88**	**0,83**	**0,82**	**0,86**	**0,88**	**0,96**	**0,89**	**0,90**	**0,87**
Positive	CQ # 718	**0,64**	**0,67**	**0,63**	**0,62**	**0,62**	**0,61**	**0,78**	**0,79**	**0,65**	**0,66**
Negative	453172-3	0,02	0,02	0,01	0,00	0,00	0,00	0,02	0,00	0,01	0,00
Negative	773217-1	0,00	0,00	-0,01	0,00	0,00	0,00	-0,01	-0,01	0,00	0,00

*Samples were considered negative because the signal with PRRSV2 (NA) was stronger.

C. Swine Influenza Virus – Microspheres region 37

Reactivity	Sample ID	Serial A		Serial B		Serial C		Serial D		Serial E	
		0 mo.	19 mo.	0 mo.	19 mo.	0 mo.	19 mo.	0 mo.	19 mo.	0 mo.	19 mo.
Positive	CQ # 429	**0,99**	**0,88**	**0,90**	**0,80**	**0,82**	**0,81**	**0,81**	**0,84**	**0,82**	**0,88**
Positive	CQ # 723	**0,90**	**0,78**	**0,77**	**0,75**	**0,79**	**0,76**	**0,74**	**0,79**	**0,77**	**0,81**
Positive	USDA # 103	**0,67**	**0,57**	**0,56**	**0,56**	**0,52**	**0,55**	**0,57**	**0,52**	**0,54**	**0,54**
Positive	USDA # 105	**1,02**	**0,91**	**0,84**	**0,87**	**0,87**	**0,84**	**0,89**	**0,93**	**0,83**	**0,84**
Positive	USDA # 108	**0,61**	**0,53**	**0,56**	**0,51**	**0,55**	**0,48**	**0,53**	**0,53**	**0,48**	**0,48**
Positive	USDA # 127	**1,29**	**1,21**	**1,09**	**1,14**	**1,14**	**1,13**	**1,08**	**1,17**	**1,18**	**1,24**
Negative	CQ # 717	0,01	0,01	0,01	0,01	0,01	0,01	0,01	0,01	0,01	0,01
Negative	CQ # 718	0,01	0,01	0,01	0,01	0,01	0,01	0,01	0,02	0,01	0,01
Negative	453172-3	0,01	0,01	0,01	0,01	0,01	0,01	0,01	0,01	0,01	0,01
Weak pos.	773217-1	**0,30**	**0,23**	**0,30**	**0,21**	**0,25**	**0,19**	**0,25**	**0,31**	**0,23**	**0,19**

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D. Porcine Circovirus type 2 – Microspheres region 38

Reactivity	Sample ID	Serial A		Serial B		Serial C		Serial D		Serial E	
		0 mo.	19 mo.	0 mo.	19 mo.	0 mo.	19 mo.	0 mo.	19 mo.	0 mo.	19 mo.
Weak pos.	CQ # 429	**0,37**	**0,39**	**0,45**	**0,36**	**0,64**	**0,57**	**0,55**	**0,53**	**0,55**	**0,56**
Negative	CQ # 723	0,08	0,08	0,09	0,07	0,13	0,08	0,10	0,10	0,13	0,10
Positive	USDA # 103	**1,70**	**1,98**	**1,57**	**1,76**	**1,29**	**1,48**	**1,18**	**1,41**	**1,18**	**1,57**
Positive	USDA # 105	**1,02**	**1,10**	**0,98**	**1,03**	**1,01**	**1,12**	**0,95**	**1,08**	**0,94**	**1,05**
Positive	USDA # 108	**1,28**	**1,50**	**1,26**	**1,34**	**1,19**	**1,28**	**1,07**	**1,26**	**1,06**	**1,29**
Positive	USDA # 127	**1,51**	**1,76**	**1,36**	**1,58**	**1,25**	**1,51**	**1,10**	**1,41**	**1,16**	**1,54**
Weak pos.	CQ # 717	**0,42**	**0,42**	**0,46**	**0,42**	**0,71**	**0,53**	**0,68**	**0,61**	**0,69**	**0,57**
Positive	CQ # 718	**1,52**	**1,81**	**1,49**	**1,61**	**1,29**	**1,53**	**1,27**	**1,50**	**1,22**	**1,46**
Negative	453172-3	0,03	0,04	0,05	0,03	0,09	0,06	0,11	0,07	0,09	0,07
Negative	773217-1	0,01	0,02	0,03	0,01	0,03	0,03	0,02	0,04	0,04	0,03

E. *M. hyopneumoniae* – Microspheres region 65

Reactivity	Sample ID	Serial A		Serial B		Serial C		Serial D		Serial E	
		0 mo.	19 mo.	0 mo.	19 mo.	0 mo.	19 mo.	0 mo.	19 mo.	0 mo.	19 mo.
Negative	CQ # 429	0,14	0,14	0,15	0,20	0,12	0,14	0,14	0,14	0,14	0,14
Negative	CQ # 723	0,14	0,10	0,16	0,15	0,11	0,12	0,13	0,13	0,12	0,11
Positive	USDA # 103	**0,67**	**0,67**	**0,79**	**0,75**	**0,67**	**0,75**	**0,66**	**0,61**	**0,63**	**0,67**
Positive	USDA # 105	**0,83**	**0,87**	**0,93**	**0,85**	**0,79**	**0,90**	**0,76**	**0,85**	**0,73**	**0,78**
Positive	USDA # 108	**0,89**	**0,89**	**1,02**	**1,04**	**0,95**	**0,97**	**0,82**	**0,84**	**0,83**	**0,85**
Positive	USDA # 127	**1,12**	**1,25**	**1,30**	**1,24**	**1,12**	**1,24**	**1,05**	**1,17**	**1,10**	**1,26**
Negative	CQ # 717	0,01	0,02	0,00	0,01	0,01	0,02	0,04	0,04	0,03	0,04
Negative	CQ # 718	0,01	0,02	0,01	0,02	0,01	0,02	0,03	0,06	0,03	0,03
Weak pos.	453172-3	**0,51**	**0,48**	**0,64**	**0,62**	**0,52**	**0,49**	**0,49**	**0,47**	**0,45**	**0,50**
Positive	773217-1	**0,76**	**0,68**	**0,89**	**0,95**	**0,72**	**0,68**	**0,65**	**0,78**	**0,69**	**0,63**

The S/P ratio of each sample remained stable over time for all five serials. No different results in terms of positivity were observed throughout the study, for any of the targeted pathogens.

CONCLUSION

The Swinecheck MP® PRRSV type 1 and 2, SIV, PCV2, M. hyopneumoniae assay has demonstrated excellent performances in terms of stability, repeatability, relative sensitivity, relative specificity and agreement when compared to the reference test.

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