BIOVET
A DIVISION OF ANTECH®

PRRSV and *Mycoplasma hyopneumoniae* Antibody Test Kit
Microsphere-based multiplex fluorescent immunoassay (MFIA)

Swinecheck MP® PRRSV type 1 (EU) and 2 (NA), M. hyopneumoniae

Product code: TRM-601

- ✓ **Detect antibodies against 3 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.

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 and/or *M*. *hyopneumoniae* diagnosis relies mostly on the demonstration of nucleic acids (PCR) or the detection of antibodies in body fluids (serum, meat juice, oral fluids). 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 (EU) and 2 (NA), 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 (PRRSV) type 1 (EU) and 2 (NA) or *Mycoplasma hyopneumoniae* in swine serum.

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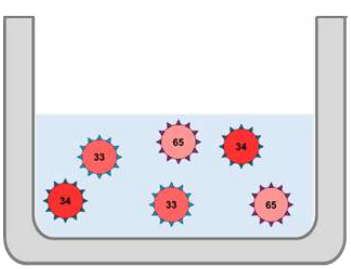
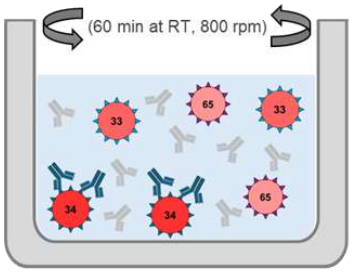
PRINCIPLE OF THE TEST

a. Assay description

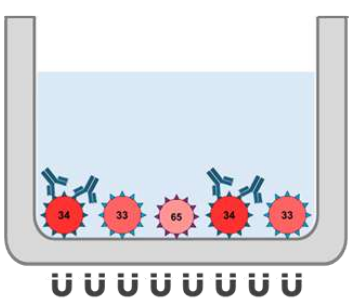
1. Three distinct microsphere sets of different "Region" coated with different antigens are added to the wells.



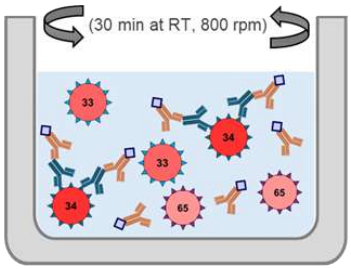
2. Diluted serum samples are added to the wells and the plate is incubated under agitation.



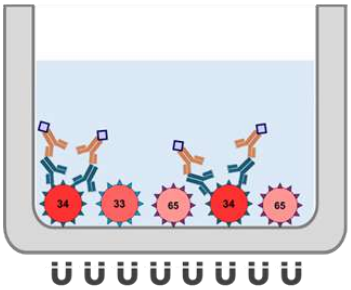
3. After the incubation, the plate is moved on a magnetic separator and wells are washed.



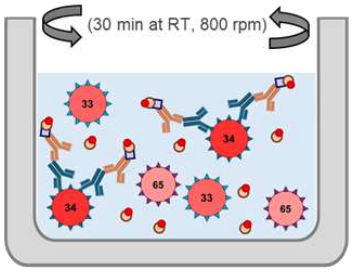
4. The plate is then removed from the magnetic separator and the detection antibody is added, followed by an incubation.



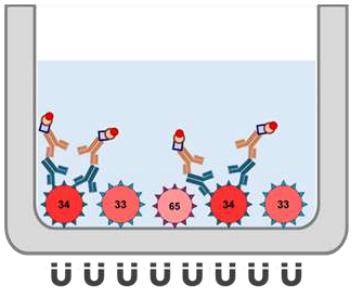
5. After the incubation, the plate is moved on a magnetic separator and wells are washed.



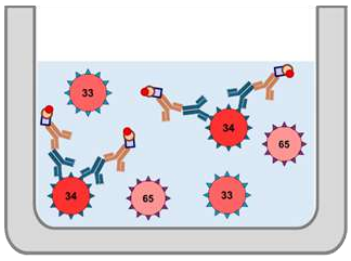
6. The plate is then removed from the magnetic separator and the SA-PE Reporter is added, followed by an incubation.



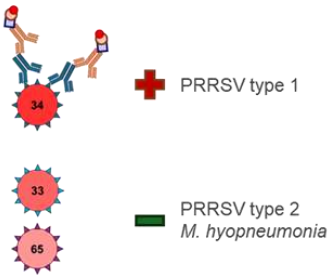
7. After the incubation, the plate is moved on a magnetic separator and wells are washed.

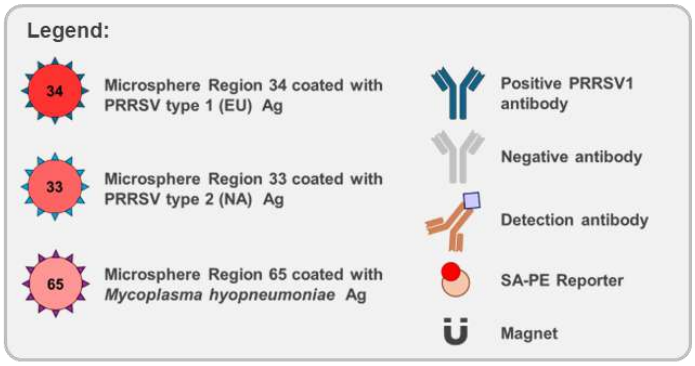


8. The plate is then removed from the magnetic separator and microspheres are resuspended in the wash buffer. The plate is then read by the analyzer.



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





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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

* 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. 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 ≥ 4,000 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 125 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 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. Testing was performed according to the instructions of the manufacturers.

A	Reference +	Reference -	Total
Biovet +	160	16	**176**
Biovet -	9	334	**143**
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	**0.894**	

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	**0.996**	

The study, with both *M. hyopneumoniae* and PRRSV samples, showed an excellent agreement between Biovet kit and the reference tests.

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b. Stability

A panel of 8 samples was tested in 3 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	
		0 mo.	19 mo.	0 mo.	19 mo.	0 mo.	19 mo.
Weak Pos.	667508-1	**0,52**	**0,37**	**0,46**	**0,41**	**0,52**	**0,43**
Positive	USDA 103	**1,13**	**1,25**	**1,18**	**1,36**	**1,24**	**1,37**
Negative	USDA 105	0,23	0,15	0,21	0,14	0,23	0,16
Negative*	CQ 717	**0,45**	**0,33**	**0,39**	**0,52**	**0,44**	**0,38**
Negative	CQ-537	0,05	0,02	0,06	0,02	0,05	0,02
Negative	PIG 21 1/6	0,11	0,06	0,10	0,06	0,10	0,06
Negative	775778-13	0,11	0,07	0,09	0,07	0,12	0,10
Weak Pos.	775778-15	**0,48**	**0,47**	**0,49**	**0,39**	**0,59**	**0,46**

*Sample was 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	
		0 mo.	19 mo.	0 mo.	19 mo.	0 mo.	19 mo.
Negative	667508-1	0,08	0,06	0,09	0,07	0,08	0,07
Negative*	USDA 103	**0,99**	**0,99**	**1,01**	**1,03**	**0,97**	**0,95**
Negative	USDA 105	0,12	0,08	0,14	0,08	0,11	0,09
Positive	CQ 717	**0,94**	**0,88**	**0,91**	**0,97**	**0,88**	**0,85**
Negative	CQ-537	0,02	0,02	0,04	0,02	0,03	0,02
Weak Pos.	PIG 21 1/6	**0,48**	**0,44**	**0,51**	**0,46**	**0,46**	**0,42**
Negative	775778-13	0,00	0,00	0,00	0,00	-0,01	-0,01
Negative	775778-15	0,26	0,28	0,29	0,22	0,29	0,24

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

C. *M. hyopneumoniae* – Microspheres region 65

Reactivity	Sample ID	Serial A		Serial B		Serial C	
		0 mo.	19 mo.	0 mo.	19 mo.	0 mo.	19 mo.
Positive	667508-1	**2,60**	**2,68**	**2,60**	**2,96**	**2,42**	**2,49**
Positive	USDA 103	**0,77**	**0,75**	**0,76**	**0,75**	**0,92**	**0,77**
Positive	USDA 105	**0,95**	**0,84**	**0,89**	**0,84**	**0,94**	**0,87**
Negative	CQ 717	0,01	0,02	0,06	0,13	0,11	0,14
Negative	CQ-537	0,07	0,05	0,08	0,07	0,09	0,06
Negative	PIG 21 1/6	0,01	0,01	0,01	0,02	0,03	0,03
Weak Pos.	775778-13	**0,48**	**0,36**	**0,46**	**0,38**	**0,46**	**0,42**
Negative	775778-15	0,14	0,16	0,15	0,12	0,22	0,14

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

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c. Repeatability

A panel of 7 samples, including negatives, weak positives and strong positives, was 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.

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

Reactivity	Sample ID	Serial A	Serial B	Serial C	Serial D	CV (%)
Weak pos.	667508-2	**0,49**	**0,51**	**0,49**	**0,53**	*3,5%*
Positive	667508-8	**0,95**	**0,98**	**0,93**	**1,03**	*4,2%*
Negative*	CQ 718	**0,39**	**0,46**	**0,40**	**0,42**	*7,0%*
Negative	CQ-536	0,03	0,04	0,03	0,03	*12,6%*
Negative	PIG 25 1/6	0,08	0,12	0,11	0,11	*17,1%*
Negative	400071-7	0,03	0,04	0,04	0,05	*12,7%*
Weak pos.	775771-1	**0,34**	**0,35**	**0,32**	**0,41**	*11,5%*

*Sample was 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	CV (%)
Negative	667508-2	0,23	0,26	0,27	0,22	*9,6%*
Negative*	667508-8	**0,51**	**0,58**	**0,56**	**0,55**	*5,3%*
Positive	CQ 718	**0,67**	**0,70**	**0,68**	**0,64**	*3,7%*
Negative	CQ-536	0,00	0,00	0,01	0,00	*258,2%*
Weak pos.	PIG 25 1/6	**0,35**	**0,39**	**0,40**	**0,39**	*5,4%*
Negative	400071-7	0,01	0,01	0,03	0,02	*48,9%*
Negative	775771-1	0,11	0,14	0,14	0,13	*10,3%*

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

C. *M. hyopneumoniae* – Microspheres region 65

Reactivity	Sample ID	Serial A	Serial B	Serial C	Serial D	CV (%)
Positive	667508-2	**1,46**	**1,51**	**1,54**	**1,49**	*2,4%*
Positive	667508-8	**2,45**	**2,59**	**2,64**	**2,47**	*3,5%*
Negative	CQ 718	0,05	0,02	0,05	0,09	*62,0%*
Negative	CQ-536	0,03	0,04	0,04	0,04	*11,3%*
Negative	PIG 25 1/6	0,01	0,01	0,02	0,03	*53,5%*
Weak pos.	400071-7	**0,37**	**0,42**	**0,40**	**0,41**	*4,8%*
Positive	775771-1	**1,67**	**1,69**	**1,64**	**1,77**	*3,4%*

The results demonstrate the Swinecheck MP® PRRSV type 1 (EU) and 2 (NA), M. hyopneumoniae kit's high repeatability. None of the positive samples had a CV greater than 15% and negative samples with high variability had a S/P ratio close to zero.

CONCLUSION

The Swinecheck MP® PRRSV type 1 (EU) and 2 (NA), M. hyopneumoniae 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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