Actinobacillus pleuropneumoniae Antibody Test Kit

Swinecheck MP® APP 1-9-11, 2, 3-6-8-15, 4-7, and 5

A new kit for detecting and differentiating antibodies to multiple APP serogroups in a single assay

MAJOR ADVANTAGES

- Allows testing for multiple serotypes **in a single well assay**
- **Saves time, labour and materials** ($$$)
- **Requires minimal sample volume** (50 µL of 1/100 serum dilution)
- The entire assay is performed **at room temperature**
- **93 samples may be tested at once (one plate)**
- Test results are available **within 2 ½ hours**
- The assay may be **partially or fully automated**
- The test is **sensitive, specific and reproducible**

Components Supplied in the Kit

Component	Quantity	Storage
Sample Diluent	150 mL	2 to 7°C
APP1-9-11 Positive Control (ready to use)	2 mL	2 to 7°C
APP2 Positive Control (ready to use)	2 mL	2 to 7°C
APP3-6-8-15 Positive Control (ready to use)	2 mL	2 to 7°C
APP4-7 Positive Control (ready to use)	2 mL	2 to 7°C
APP5 Positive Control (ready to use)	2 mL	2 to 7°C
APP Negative Control (ready to use)	3 mL	2 to 7°C
Microsphere Mix (antigens)	28 mL	2 to 7°C
Wash Buffer (10X)	500 mL	2 to 7°C
Detection Antibody	52 mL	2 to 7°C
SA-PE Conjugate	52 mL	2 to 7°C
Microtiter Plate	5	20 to 25°C

Equipment and Consumables Required But Not Provided

- Purified water
- Table-top bath sonicator
- Vortex
- Plate shaker
- Magnetic microsphere separator
- Plate analyzer (e.g., LX200, Magpix)
- Data analysis software (e.g., xPONENT)
- Aluminum foil
- Bottles, tubes or plates for dilution
- Micropipettes and tips
- Microcentrifuge
- Plate washer (optional)

The materials provided are sufficient for testing up to 445 samples.

IMPORTANT:

- Requires a permit to release veterinary biologics from CFIA in Canada. This product is not licensed by CFIA and any claims made have not been substantiated by CFIA.
- Requires a Research and Evaluation import permit from the USDA in the US. This product is not licensed by the USDA and any claims made have not been substantiated by the USDA.

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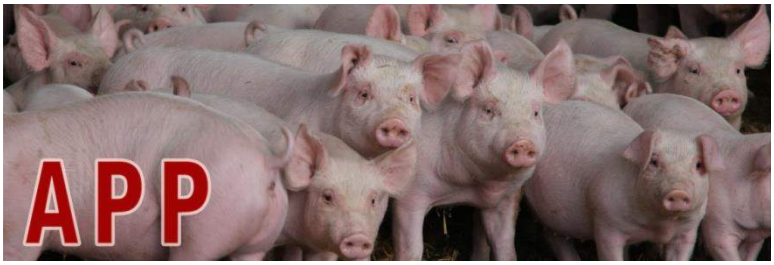

Detecting and differentiating antibodies to multiple APP serogroups in a single assay

Actinobacillus pleuropneumoniae (APP)

APP is still an important swine respiratory pathogen in many countries worldwide. Fifteen APP serotypes corresponding to nine serogroups have been identified so far (APP1-9-11, APP2, APP3-6-8-15, APP4-7, APP5, APP10, APP12, APP13, and APP14). The prevalence of the various serotypes and their virulence greatly vary depending on the country.

The surveillance of APP infections mostly relies on the detection of serotype or serogroup specific antibodies in serum samples. Various serological assays have been developed for that purpose. The most sensitive and specific are ELISA, using highly purified long chain lipopolysaccharides (LC-LPS) as antigen.

Presently, in order to detect antibodies to the nine serogroups, the same number of ELISA tests have to be performed. Testing for several serogroups at the same time in a single assay (multiplex testing) could save a lot of labour, time, and cost. Unfortunately ELISA using combinations of multiple antigens (mix-ELISA) have been difficult to develop and they do not allow to differentiate the reacting serogroups.

A new technology (xMAP) allowing the development of multiplex immunoassays has been recently introduced in veterinary medicine. We have used this new particular platform to develop a kit allowing for the detection and differentiation of antibodies to five major APP serogroups (APP1-9-11, APP2, APP3-6-8-15, APP4-7, and APP5).

Multiplexed Fluorometric Immunoassays (MFIA)

Multiplexed Fluorometric Immunoassays (MFIA) use suspensions of microspheres (beads) with unique internal fluorescent dyes. The beads are coupled to their surface with unique antigens (e.g., APP LC-LPS) (one antigen per bead type).

Bead sets and samples are added to microtitre plates. Antigen-antibody complexes formed during the incubation of the beads and diluted sample incubation are detected through successive incubations with biotinylated anti-swine IgG followed by streptavidin coupled to R-phycoerythrin (SA-PE). Incubations are followed by wash steps to remove unbound sample constituents and reagents.

MFIA plates are finally read and analyzed using a microtiter plate fluorescence analyzer. The intensity of the R-phycoerythrin fluorescence is reported as a Median Fluorescence Index (MFI) which denotes how much reporter fluorescence of a given microsphere set carries.

An S/P ratio of the SA-PE on the antigen-coated microspheres above a specific threshold indicates that antibodies to the corresponding antigen are present in the sample.

MFIA kit for APP 1-9-11, 2, 3-6-8-15, 4-7, and 5

We have developed a 5-plex MFIA to detect and differentiate antibodies to APP1-9-11, APP2, APP3-6-8-15, APP4-7, and APP5 LC-LPS in swine serum samples. LC-LPS corresponding to these serogroups have been coupled to five sets of magnetic beads (Magplex®, Luminex). Antigen-antibody complexes formed during incubation are detected through successive incubations with biotinylated anti-swine IgG followed by streptavidin coupled to R-phycoerythrin (SA-PE). Incubation steps are followed by serial washings which can be easily performed manually or with an automatic plate washer and a magnetic bead separator. MFIA plates are finally read and analyzed using a microtiter plate fluorescence analyzer (eg Magpix® or LX200®, Luminex). The diagnostic sensitivity and specificity of the assay are similar to those of the corresponding individual serogroup specific ELISA.

References

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2. **Christopher-Hennings J, Araujo KP, Souza CJ, Fang Y, Lawson S, Nelson EA, Clement T, Dunn M, Lunney JK.** Opportunities for bead-based multiplex assays in veterinary diagnostic laboratories. J Vet Diagn Invest. 2013 Nov;25(6):671-91
3. **Giménez-Lirola LG, Jiang YH, Sun D, Hoang H, Yoon KJ, Halbur PG, Opriessnig T.** Simultaneous detection of antibodies against Apx toxins ApxI, ApxII, ApxIII, and ApxIV in pigs with known and unknown *Actinobacillus pleuropneumoniae* exposure using a multiplexing liquid array platform. Clin Vaccine Immunol. 2014 Jan;21(1):85-95.
4. **Gottschalk M.** *Actinobacillus pleuropneumoniae*: an old but still relevant swine pathogen in the XXI century. Proc. 22nd International Pig Veterinary Society Congress, 2012, Jeju, Korea, p.26-31
5. **Opriessnig T, Hemann M, Johnson JK, Heinen S, Giménez-Lirola LG, O'Neill KC, Hoang H, Yoon KJ, Gottschalk M, Halbur PG.** Evaluation of diagnostic assays for the serological detection of *Actinobacillus pleuropneumoniae* on samples of known or unknown exposure. J Vet Diagn Invest. 2013 Jan;25(1):61-71.

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