

# The serum plate agglutination test (SPA)\*

*\*also known as the Rapid Plate agglutination test*

*The Intervet serum plate agglutination antigens have provided the poultry industry with a cheap, rapid and effective flock screening test. The test is mainly used for the fast screening of poultry flocks for infections with *Salmonella pullorum*, *Mycoplasma gallisepticum*, *Mycoplasma synoviae* and *Mycoplasma meliagridis*.*

*Basically the test allows the macroscopic recognition (called agglutination), resulting from the union of specific antibodies in the tested sera samples and the antigen. Usually the antigen consists of inactivated colored bacteria, in order to make the reaction better visible.*

*It is very important to perform the SPA test according to the manufacture's instructions. The interpretation of the results should be considered in the context of a flock screening. If agglutination reactions are observed further testing (HI test, ELISA, PCR, and/or isolation of the disease agent) are needed to confirm if the flock is indeed "positive".*

## Procedure

The SPA test is conducted by mixing a drop (0.05ml) of test serum with a drop (0.05ml) of antigen on a clean glass plate at room temperature (25°C). The glass plate should be illuminated from below as to facilitate observing the reaction, avoiding excessive heat from the light source. The standard procedure is:

- the antigen should be stored in the dark at 4<sup>o</sup>-8<sup>o</sup>C
- only fresh sera (not older than 1 day) should be tested
- it is not recommended to test frozen sera as this could contribute to aspecific agglutination reactions.

Allow antigen and sera to warm up to room temperature (20<sup>o</sup>-25<sup>o</sup>C). Testing at other temperatures might reduce specificity and sensitivity.

Check for agglutination before starting the test. A too high temperature may cause auto agglutination.

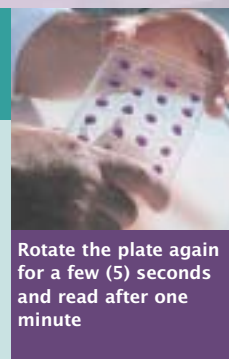
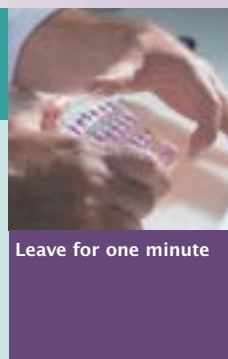
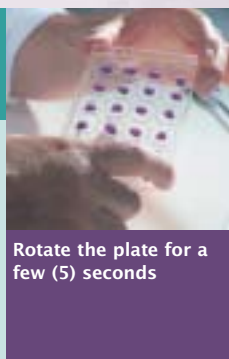
Dispense test sera (rinse between samples!) in amounts of 0.05ml to ±4cm squares on a ruled glass plate. The serum should not dry out before being mixed with the antigen.

Limit the number of samples (usually 25) according to the speed of the technician.

Shake antigen prior to use.

Dispense antigen in amounts of 0.05ml on the tests sera and mix until a homogenous distribution is achieved.

Rotate the plate for a few (5) seconds and leave for one minute, then rotate the plate again for a few (5) seconds and read after one minute.



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Score --



Score --



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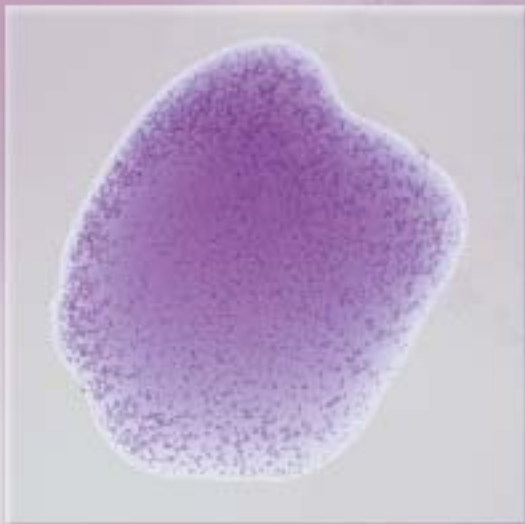
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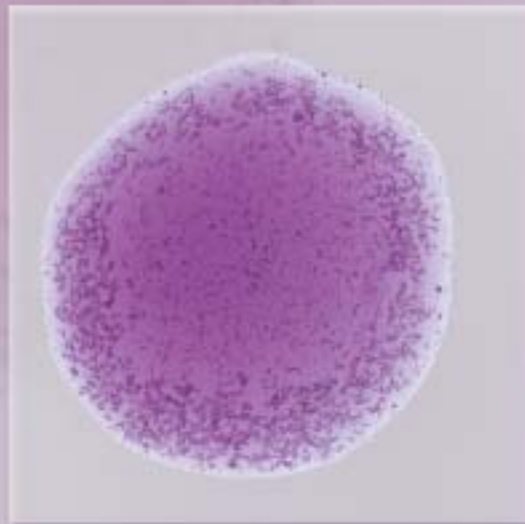
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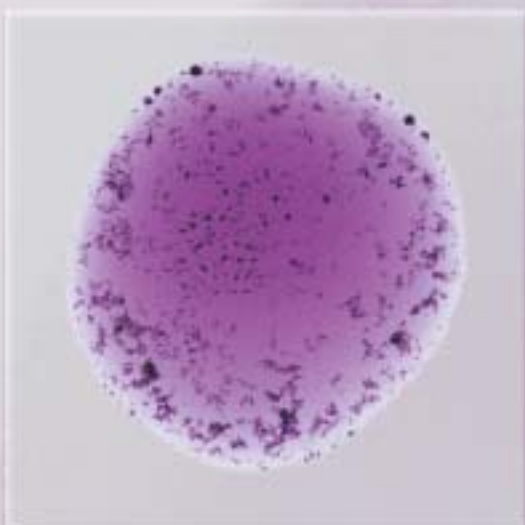
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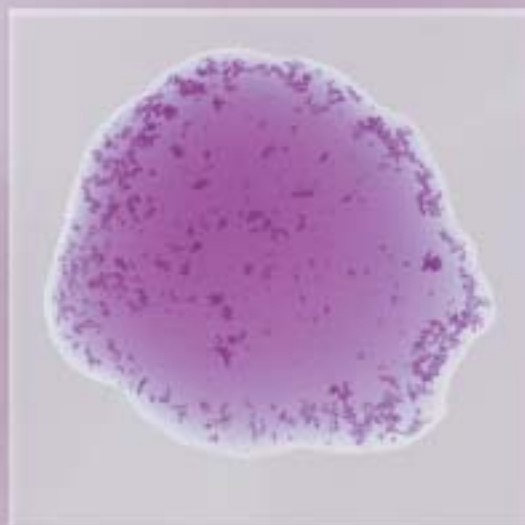
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**Score +++**



**Score ++++**



**Score ++++**

# The serum plate agglutination test (SPA)

## Positive reaction

Is characterized by the formation of definitive clumps within 2 minutes after mixing the test sera with the antigen. The clumps usually start and may concentrate at the periphery of the mixture. It is possible to measure the strength of the agglutination reaction according to the following scheme:

- no clumps, no background clearing
- + small clumps, no background clearing
- ++ medium sized clumps, almost complete background clearing
- +++ large clumps, almost complete background clearing
- ++++ very large clumps, mostly in the periphery, complete background clearing

## Negative reaction

If no agglutination is observed the reaction to the test is considered as negative. Care should be taken that the natural granulation of the antigen (due to the presence of whole cells) is not taken for a positive reaction.

## Controls

Whenever samples are tested, the antigen should be tested against known positive and negative sera. Only then the results to the test may be considered reliable.

## Confirmatory testing

Thermic inactivation of sera:

Heating serum samples during 30-60 minutes at 56°C can limit the number of aspecific reactions.

## SPA titer determination

Dilution of the test sera may indicate whether a positive reaction should be considered as false positive. Real positive reactions will be encountered even if the test sera are diluted > 1:8.

### *Specificity of the SPA antigen*

*Under normal circumstances, the rate of unspecific reactions is low. However, unspecific reactions may occasionally be high for a variety of reasons. Some of the causes are:*

*Bacterial contamination of sera, prolonged storage of sera at 4°C, freezing of sera before testing, anti-gamma-globulin activity of sera, presence of swine serum in the antigen, isohemagglutinating antibody, staphylococcal infection, exposure of the flock to vaccination a short time before testing, use of inactivated vaccine, cross-reactions between *Mycoplasma gallisepticum* antigens and *Mycoplasma synoviae* antibodies*

## Retesting after 2-4 weeks

In flocks recently contaminated, the number and intensity of reacting sera will increase.

## Other tests

- Haemagglutination Inhibition test
- ELISA
- PCR
- Isolation of the disease agent

## Interpretation of the results

In general with an Mg or Ms infection the percentage of positive samples will increase rapidly within a few weeks. At the same time the strength of the agglutination reaction (and HI titers or Elisa positives) will increase and will stay at a high level thereafter. It has to be taken into account that with a *Mycoplasma* infection SPA agglutinins (among which IgM) can be detected 2-3 weeks earlier than other types of antibodies (mainly IgG). Repeated investigation with a few weeks interval will give a clear answer in the majority of the cases.

According to the regulations of the European Union (to be found under: [www.europa.eu.int/eur-lex](http://www.europa.eu.int/eur-lex)) poultry flocks should be tested as follows:

- 60 serum samples should be taken per house
- a flock is considered positive when >15% of the undiluted samples are positive in the SPA
- >3% of the samples show a SPA titer of >1:8
- and >3% of the samples show a HI titer of >1:16

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