

PolyCheck Allergie NF Veterinary-Panel (Horse)

Test principle

This new *in vitro* test for the determination of allergen-specific IgE in serum is based on an immunoassay principle, using coated allergens and **monoclonal antibodies** directed against horse-IgE. During the first incubation steps with serum the allergen-specific IgE molecules bind to the corresponding allergens. Subsequent to a washing step, the biotinylated antibodies detect the bound IgE and react in a further incubation step with streptavidin-alkaline-phosphatase conjugates. After washing and addition of 5-bromo-4-chloro-3-indolyl-1-phosphate/nitro blue tetrazolium (BCIP/NBT) the enzymes develop a coloured precipitate. The amount of precipitate is directly proportional to the concentration of specific IgE in the applied serum, which results in a more or less intense colouring of each individual allergen.

Benefits of this procedure are determination of 20 allergens in only one assay-run. Only 0.25 ml is necessary. Individual standard-curve, high sensitivity because of the high concentration of allergens coated in form of thin lines on a membrane.

Reagents in test kit

In case of correct storage (2-8°C) all not opened reagents are usable till exp. date.

- *PolyCheck Allergy*-Strips (24 Strips):
Each membrane is coated with 20 allergens and 5 standards.
- Starter (1 vial, 7,5 ml, ready to use)
buffered protein solution
- 1. Detection antibody (1 vial, 7,5 ml, ready to use):
Monoclonal antibody against horse-IgE in a protein solution.
- 2. Detection antibody (1 vial, 7,5 ml, ready to use):
Antibody against mice-IgE in a protein solution
- Streptavidin conjugate (1 vial, 7,5 ml, ready to use):
Streptavidin conjugated with alkaline phosphatase.
- BCIP/NBT substrate solution (1 vial, 7,5 ml, ready to use):
5-Bromo-4-Chloro-3-Indolyl-1-phosphate/nitro blue tetrazolium substrate for alkaline phosphatase.
- Wash buffer (1 package):
Dried powder out of buffer + additives to wash the membrane.

Materials not provided in the kit

- Pipette (200 - 1000 µl)
- Shaker and special tools
- Computer with scanner and special software

Precautionary measures

- Reagent of different lots mustn't be mixed.
- Do not pipette solutions with mouth.
- All recommended measures for handling of potential infectious material should be followed.

Test-Procedure

Common advice:

- Bring all reagents to room temperature.
 - Proceed without interruption.
 - Avoid drying of the membranes.
 - Pipette reagents onto the gap not covered by the membranes.
1. Strip shortly moisten with wash buffer and pat out on paper.
 2. Coat membrane first with **250 µl starter solution** and incubate **5 minutes**. Pat out carefully on paper.
 3. Add directly after moistening **250 µl serum** in the gap beside the membrane. Incubate **60 minutes** at room temperature under constant shaking at 1000 rpm; 300 rpm (tilting shaker). Tip the serum and *wash the membranes three times with 1 ml wash solution (about 1 ml Wash buffer is a filled strip-gap)*; pat out on paper.
 4. Add **250 µl wash buffer** in the gap beside the membrane. Incubate **5 minutes** at room temperature under constant shaking at 1000 rpm. *Wash membrane as described in step 2.*
 5. Add **250 µl 1st detection antibody** in the gap beside the membrane. Incubate **90 minutes** at room temperature under constant shaking at 1000 rpm. *Wash membrane as described in step 2.*
 6. Add **250 µl 2nd detection antibody** in the gap beside the membrane. Incubate **60 minutes** at room temperature under constant shaking at 1000 rpm. *Wash membrane as described in step 2.*
 7. Add **250 µl Streptavidin conjugate** in the gap beside the membrane. Incubate **20 minutes** at room temperature under constant shaking at 1000 rpm. *Wash membrane as described in step 2.*
 8. Add **250 µl substrate solution (BCIP/NBT)** in the gap beside the membrane. Incubate **in the dark** (i.e. cover with aluminium foil or opaque cover) **20 minutes** at room temperature, under constant shaking 1000 rpm. *Wash membrane as described in step 2.*
(Time over all 195 minutes)
 9. **Let membrane dry sufficiently** and analyse strip as described below.

Shaker: ELISA Vibration-Shaker with small amplitude or tilting shaker (300 rpm)

Calculation of results

A computer with scanner and special software serves to calculate the results:

Placed on top of a flatbed scanner the strips are read out and documented. A special software programme analyzes and calculates the image data. In comparison to the standard curve the amount of allergen-specific IgE for each allergen is given as relative kilo units per litre (kU/l) (see Table 1)

Tab. 1 The 5 levels of allergic reactions

Concentration of IgE	level
< 0.5	0
0.5 – 2.0	1
2.0 – 20	2
> 20	3,4

Allergen Panel

Polycheck-Veterinary-Panel contains 15 individual allergens and five mixtures:

- Pollen
- Insects
- House dust mites
- Supply mites
- Fungus



Standard 1 – 5

D. farinae
D. pteronyssinus
Lepidoglyphus destructor
Asp. fumigatus / Pen. notatum
Alt. tenuis / Clad. herbarum
Birch / Alder / Hazel
Platane / Willow / Poplar
Rye pollen
6-Grassmix
Plantain
Mugwort
Sheep Sorrel
Horse fly (Tabanus)
Biting midges (Culicoides nub.)
Mosquito
Black fly (Simulium eq.)
Stable fly (Stomoxys cal.)
Acarus siro
Tyrophagus