

**Claims**

1. A method for preparing positive serum against a low pathogenic avian influenza virus, characterized by comprising the following steps:
  - performing a primary immunization on an animal using a seed virus containing live low-pathogenic avian influenza virus;
  - performing a secondary immunization using an inactivated vaccine against this low-pathogenic avian influenza after the primary immunization; and the serum is harvested from the animal after the secondary immunization.
2. The preparation method according to claim 1, characterized in that: the viral titer of the seed virus is from  $10^{4.0}$  EID<sub>50</sub>/0.1 ml to  $10^{6.0}$  EID<sub>50</sub>/0.1 ml; and the low pathogenic avian influenza virus comprises H9, H3, or H6 subtype avian influenza virus.
3. The preparation method according to claim 1, characterized in that: the route of administration for the primary immunization is intranasal instillation.
4. The preparation method according to claim 1, characterized in that: the secondary immunization using the inactivated vaccine against low pathogenic avian influenza is performed 13 days after the primary immunization, and serum is harvested 7 days after the secondary immunization; and the route of administration for the secondary immunization comprises intramuscular injection.
5. The preparation method according to claim 1, characterized in that: the preparation method for said inactivated vaccine against low pathogenic avian influenza comprises the following steps:
  - inactivating a seed virus containing live low pathogenic avian influenza virus to obtain an inactivated antigen;
  - mixing the inactivated antigen with Tween-80 to form an aqueous phase; and
  - mixing the aqueous phase with an oil phase to obtain an inactivated water-in-oil

emulsion vaccine.

6. The preparation method according to claim 5, characterized in that: the volume ratio of the inactivated antigen to Tween-80 is 97:3; the volume ratio of the aqueous phase to the oil phase is 2:3; the oil phase comprises white oil and Span-80; and the volume ratio of the white oil to Span-80 is 95:5.
7. The preparation method according to claim 1, characterized in that: the animal comprises an avian animal; the avian animal comprises a chicken; the age of immunized chickens is from 14 days to 21 days at the time of the primary immunization.
8. A positive serum against low-pathogenic avian influenza virus prepared by the preparation method according to any one of claims 1 to 7, characterized in that:
- the low-pathogenic avian influenza virus comprises H9, H3, or H6 subtype avian influenza virus;
- the HI titer of the H9 subtype avian influenza virus positive serum is  $\geq 12\log_2$ , the neutralizing antibody titer is  $\geq 1:4096$ , and the H9 subtype positive serum reacts positively only with H9 subtype avian influenza virus or antigen, and reacts negatively with Newcastle disease virus, egg drop syndrome virus, H3, H5, H6, and H7 avian influenza viruses;
- the HI titer of said H6 subtype avian influenza virus positive serum is  $\geq 12\log_2$ , the neutralizing antibody titer is  $\geq 1:2048$ , and said H6 subtype positive serum reacts positively only with H6 subtype avian influenza virus or antigen, and reacts negatively with Newcastle disease virus, egg drop syndrome virus, H3, H5, H7, and H9 avian influenza viruses;
- the HI titer of said H3 subtype avian influenza virus positive serum is  $\geq 11\log_2$ , the neutralizing antibody titer is  $\geq 1:1024$ , and said H3 subtype positive serum reacts positively only with H3 subtype avian influenza virus or antigen, and reacts negatively with Newcastle disease virus, egg drop syndrome virus, H5, H6, H7, and H9 avian influenza viruses.

9. A use of the low-pathogenic avian influenza virus positive serum according to claim 8, characterized in that the scope of use comprises at least one of the following aspects:

1) preparing a product for detecting, identifying, or diagnosing low pathogenic avian influenza virus;

5 2) preparing a product for using in research on the pathogenesis and transmission mechanisms of low pathogenic avian influenza virus;

3) preparing a product for evaluating or monitoring the quality of live vaccines against low-pathogenic avian influenza virus;

10 4) preparing a reference standard for low pathogenic avian influenza virus positive serum.

10. The use according to claim 9, characterized in that: the serum product is constructed based on at least one of the following detection techniques: indirect immunofluorescence assay, enzyme-linked immunosorbent assay, Western blot, and immune-histochemistry.