



Evaluation of the effectiveness of the hemagglutination inhibition (hi) test in the diagnosis of avian influenza

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SUBMITTED 21 March 2025

ACCEPTED 17 April 2025

PUBLISHED 19 May 2025

VOLUME Vol.07 Issue03 2025

I.B. Butaeva

LLC "Afrosiab Parranda", Samarkand, Uzbekistan

CITATION

I.B. Butaeva. (2025). Evaluation of the effectiveness of the hemagglutination inhibition (hi) test in the diagnosis of avian influenza. The American Journal of Veterinary Sciences and Wildlife Discovery, 7(03), 6-13. <https://doi.org/10.37547/tajvswd/Volume07Issue03-02>

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Abstract: Avian influenza remains one of the most dangerous viral infections affecting both wild and domestic birds. Effective diagnostics are essential to prevent outbreaks and virus dissemination. This article discusses the use of the hemagglutination inhibition test (HI test) as a standard serological method for identifying antibodies against the avian influenza virus. The methodology for performing the HI test, interpretation of results, and its significance in the veterinary control system in the Republic of Uzbekistan are presented.

Keywords: Avian influenza, HI test, diagnostics, serology, H5N1 virus, infection control.

Introduction: Avian influenza is a highly pathogenic viral disease caused by various subtypes of influenza A virus, particularly H5 and H7. The disease poses a serious threat both to poultry farming and human health. Among various diagnostic methods, the hemagglutination inhibition (HI) test holds a prominent place due to its utility in virus serotyping and determining antibody levels in serum from infected or vaccinated birds.

METHODS

Study subjects: Serum samples were collected from chickens raised at the poultry farm LLC "Afrosiab Parranda" (Samarkand region) following vaccination against avian influenza (strain H5).

Antigens and erythrocytes: Inactivated H5N1 subtype avian influenza virus was used as the antigen. Chicken erythrocytes were used at a concentration of 1% in

Alsever's solution.

HI test procedure:

- Serum samples were heat-inactivated at 56°C for 30 minutes.
- Serial twofold dilutions of sera were prepared in 96-well plates.
- Virus antigen was added, followed by incubation for 30 minutes at room temperature.
- Chicken erythrocytes were then added, and the plates were incubated for an additional 30-45 minutes.
- Results were assessed based on the presence or absence of hemagglutination.

RESULTS

Blood samples for HI testing were collected between 21–28 days post-vaccination, aligning with the optimal period for the development of post-vaccination immunity. The overall immunity rate was found to be 85%, which is a satisfactory level of protection according to veterinary norms.

According to the vaccine manufacturer's instructions, immunity duration post-vaccination is up to 6 months, provided that vaccine administration protocols are strictly followed and no immunosuppressive factors are present.

The analysis included two age categories: young birds (up to 6 months old) and adults. A total of 120 serum samples were examined, of which 70 were from adult chickens and 50 from young birds. Positive antibody detection in adults was observed in 64 cases (91.4%), whereas among young birds, antibodies were detected in 38 cases (76%). Thus, it can be concluded that adult chickens developed reliable immunity, while younger birds demonstrated a lower immunity level, indicating the need for additional monitoring and potential revaccination.

Among the 120 samples studied, the average antibody titer was 1:64, confirming the vaccine's effectiveness.

DISCUSSION

The HI test has proven to be a reliable serological diagnostic method for avian influenza, demonstrating high sensitivity, ease of execution, and accessibility. However, the method requires stringent adherence to protocol conditions, particularly concerning antigen and erythrocyte quality. Given the limited laboratory resources available in Uzbekistan, the HI test remains the primary method for post-vaccination immunity monitoring.

Furthermore, the HI test complies with international WOAH (OIE) standards and is widely used in serological

monitoring for export purposes.

CONCLUSION

The hemagglutination inhibition test is a reliable diagnostic tool for avian influenza. At the poultry farm LLC "Afrosiab Parranda," the method showed high informativeness and reproducibility. The obtained results can be utilized to adjust vaccination protocols and enhance biosecurity measures.

Conducting the HI test allowed for the assessment of post-vaccination immunity against avian influenza virus. An overall immunity rate of 85% confirmed the effectiveness of the vaccination program. Nevertheless, younger birds showed a slightly reduced seropositivity rate (76%), indicating a necessity for ongoing monitoring and, if needed, adjustment of the vaccination scheme.

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