US CDC Serological Investigations

In response to H7N9 Outbreak

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I. Development of a modified horse RBC HI assay to detect H7N9 sera antibody response with improved sensitivity.
A Modified HI assay Using Horse RBC for Detection of H7N9 Serum Antibodies

- The receptor specificity of influenza virus HA correlates with their ability to agglutinate RBCs from different animal species:
  - Avian viruses: SA α-2, 3
  - Human viruses: SA α-2, 6

- Horse RBCs express a high proportion of SA α-2, 3-gal linkages compared with turkey RBCs.

- With H7 influenza subtype viruses, an improved sensitivity has been observed using horse RBCs for detecting HI antibodies than using turkey RBCs.
A modified sample treatment procedure is developed

Heat inactivation of sera
Test for non-specific agglutinins
If positive, adsorption with horse RBCs
RDE treatment to remove non-specific virus inhibitors
Confirm the absence of non-specific agglutinins
HI assay with horse RBCs

Higher levels of non-specific agglutinins can be detected in sera samples using horse RBCs causing false negative results. The serum must be adsorbed with horse RBC before testing in the HI assay.

However, during adsorption with horse RBCs, non-specific virus inhibitors may be introduced into serum, which will cause a false positive result in HI assay with horse RBC. These non-specific inhibitors can be eliminated by RDE treatment.
HI assay using horse RBCs provides better sensitivity to detect serological responses against several H7 viruses, including wild type H7N9 virus. Ab titers detected by modified horse RBC HI assay are 2-8 folds higher than titers detected using turkey RBCs.

Comparison of horse and turkey RBCs in HI assay using human sera from a H7 vaccine study (Two doses of A/NL/219/2003 live attenuated vaccine prime and One dose of A/Mallard/NL/12/2000 inactivated vaccine boost). Sera were kindly provided by K. Subbarao and J. Treanor.
HI assay using horse RBC yielded generally comparable titers as the microneutralization assay.

Comparison of antibody titers achieved by horse RBC HI assay vs microneutralization (MN) assay using human sera from a H7 vaccine study with A/NL/219/2003 live attenuated vaccine prime and A/Mallard/NL/12/2000 inactivated vaccine boost.
Protocol Shared with Other Labs

- H7N9 Modified horse RBC HI protocol was shared with WHO, China CDC, and CONSISE on July 3rd, 2013.

- Protocol was also shared with Public Health Agency of Canada (PHAC) for confirmation of a H7N9 suspected case.

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Other studies conducted:

II. To assess the level of pre-existing cross-reactive antibodies to H7N9 in the current US population.

III. Evaluation if existing vaccinations (e.g. seasonal vaccines) provide any cross reactive antibodies to H7N9 infection.
Thank you