Reviewer's report

Title: Development of a sensitive novel diagnostic kit for the highly pathogenic avian influenza A (H5N1) virus

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Reviewer: Fang He

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Major Compulsory Revisions

The manuscript by Yasuko etc. describes a diagnostic method for H5N1 influenza virus. 19 clinical specimens from 13 patients in Vietnam infected with clade 1.1 or clade 2.3.4 H5N1 HPAI virus was tested in this luminescence-linked enzyme immunoassay developed. Approximately 80% of clinical specimens were H5-positive using the POCube system. The author concluded that this novel H5/A kit using POCube is a rapid and sensitive screening test for H5N1 HPAI virus infection in humans. The kit is still equipment-dependent. The study may promote H5N1 diagnosis in humans. However, the current presentation of results is not complete. The missing or unclear information includes:

Specificity study: Sequence variance in H5 leads to different clades (clade 0-9) of H5N1 and new H5N1s are emerging every year. In this study, only four H5 strains were tested. It is not sufficient. No results were shown about tests with non-H5 AIVs, such as H1N1 and H3N2.

Sensitivity study: For a clear understanding to readers, the sensitivity of the test should be compared to any conventional methods, such as Hemagglutination and TCID50. Table 1 is confusing. The results of different methods should be presented in the same table with details like sample volume used.

Cut-off value: Is this cut-off value fixed for all samples or adjusted before every batch of tests? If it is fixed, more negative samples in different conditions should be included to generate this cut-off value, such as bloody samples, non-H5 AIVs and other type of microorganism. If it is adjusted every time, this test may not be claimed as rapid test with 5-15 mins.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:
'I declare that I have no competing interests'