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Reproducibility of serology assays for pandemic influenza H1N1: Collaborative study to evaluate a candidate WHO International Standard☆

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ABSTRACT

Haemagglutination–inhibition (HI) and virus neutralisation (VN) assays are used to evaluate immunogenicity of pandemic H1N1 vaccines; however these bioassays are poorly standardised leading to inter-laboratory variation. A candidate International Standard (IS) for antibody to H1N1pdm virus (09/194) was prepared from pooled sera of subjects who had either recovered from H1N1pdm infection or who had been immunised with an adjuvanted subunit vaccine prepared from reassortant virus NYMC X-179A (derived from A/California/7/2009 virus). Ten laboratories from seven countries tested the candidate IS, 09/194 and a panel of human sera by HI and VN using the A/California/7/2009 virus (six laboratories) and/or the reassortant virus NYMC X-179A (ten laboratories). As expected, the inter-laboratory variability for HI and VN assay results was high. For results of antibody tests to NYMC X-179A, the % geometric coefficient of variation (%GCV) for 09/194 between laboratories was 83% for HI and 192% for VN. For tests of all sera, the median %GCV ranged from 95 to 345% for HI (80-fold variation) and 204 to 383% for VN (109-fold variation), but for the titres relative to 09/194 the median %GCV was much reduced (HI 34–231%; VN 44–214%). For tests of antibody to the A/California/7/2009 wild type virus there were similar reductions in %GCV when 09/194 was used.

These results suggest that 09/194 will be of use to standardise assays of antibody to A/California/7/2009 vaccine and 09/194 has now been established by WHO as an IS for antibody to A/California/7/2009 with an assigned potency of 1300 IU per ml.

1. Introduction

In August 2010, the WHO listed over 40 clinical trials of candidate pandemic H1N1 vaccines that had either already occurred or were taking place [1]. In most of these trials the vaccines were assessed by their ability to stimulate serum antibody to influenza H1N1pdm haemagglutinin (HA). In addition serum surveys of antibody to pandemic H1N1 virus have been conducted in order to estimate either the susceptibility of populations before the pandemic or exposure to virus after the pandemic [2–4]. Most of these serology assays rely on either haemagglutination–inhibition (HI) [5], where agglutination of erythrocytes by influenza virus is blocked by strain-specific antibody binding to receptors on the HA; or virus neutralisation (VN) where antibody to HA inhibits virus infection of mammalian cells. As serum HI titres have been shown to correlate with protection [6], HI is widely used to evaluate the immunogenicity of influenza vaccines [7–9]. However, there are some concerns about the relevance of HI for pandemic influenza because the information on correlates of protective immunity was generated many years ago, primarily in adult populations, and with seasonal influenza viruses. Following the emergence of H5N1 viruses and associated problems with HI assays of antibody to H5N1, there has been increased use of VN assays although there is no general agreement on VN levels associated with protection.

Poor reproducibility of serological assays has been observed in several studies and is partly attributed to differences in assay protocols, reagents and expression of endpoints [10–12]. This limits comparison of candidate H1N1pdm vaccines in different clinical

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trials, and poses challenges to licensing authorities, particularly if specific seroprotective titres are required as an endpoint [7–9]. It may also limit comparison of serum surveys performed by different laboratories. The use of standards in bioassays to improve inter-laboratory agreement is well recognized [13,14] and an International Standard for antibody to H5N1 clade 1 virus has already been established by the WHO [15]. It is likely that a standard for H1N1pdm antibody would also be beneficial.

A study was organised to assess the inter-laboratory agreement of HI and VN for the detection of H1N1pdm antibody, and to evaluate the suitability of a freeze-dried human serum pool to act as a standard for detection of antibody to H1N1pdm antigens.

2. Methods

2.1. Candidate International Standard code 09/194

The candidate standard 09/194 was a freeze-dried pool of serum obtained from 22 subjects who had received inactivated MF59-adjuvanted subunit NYMC X-179A (X-179A) (derived from A/California/7/2009) vaccine (clinical trial at the Leicester Royal Infirmary, UK under the direction of Dr. Iain Stephenson) and 9 subjects from NIBSC who had recently recovered from pandemic influenza (not virologically confirmed). The serum pool was aliquoted, freeze-dried and backfilled with nitrogen at NIBSC following standard procedures [16] to produce 0.5 g weight and stored at −20 °C. Stability studies by HI and VN found no significant change after storage for 8 months at −20 °C, +4 °C or +20 °C when compared to −70 °C samples. There were 1135 vials of 09/194 prepared, of which only 40 now remain. Consequently 9000 vials of a replacement preparation were made (10/202) and this has now been evaluated by a collaborative study to assign unitage based on 09/194. Both 09/194 and 10/202 are available from NIBSC at http://www.nibsc.ac.uk/products/biological_reference_materials.aspx.

2.2. Test serum samples

A total of 6 human sera (coded A–F) including serum from a convalescent subject (sample A) and 2 post-vaccination sera selected for medium and high antibody titres (samples B and F) from subjects who had received inactivated MF59-adjuvanted subunit A/California/7/2009 (X-179A reassortant) vaccine were used. Samples B and E were duplicates, sample C was from a pool of human serum used to prepare 09/194 and sample D was from a subject known to be negative for antibody to H1N1pdm.

2.3. Ethical approval

The clinical trial of MF59-adjuvanted vaccine was approved by the ethical committee of the Leicester Royal Infirmary and was performed in compliance with the 'Declaration of Helsinki'. All human subjects gave informed consent for the study.

The human subjects at NIBSC gave informed consent for their blood donations and the study was approved by the ethical committee of NIBSC.

2.4. Virus reagents

Egg-grown A/California/7/2009 and reassortant X-179A (derived from A/California/7/2009) H1N1pdm viruses were used in serology assays and were either supplied by NIBSC or were from laboratory stocks of participating laboratories.

2.5. Design of study

Fifteen participants were sent reagents on solid CO_2 and asked to store sera at −20 °C and viruses at −70 °C. Laboratories were instructed to reconstitute 09/194 with 0.5 ml distilled water and test it and the serum panel for antibody to the two H1N1pdm viruses on at least three separate occasions by HI and VN. Participants could use their in-house assays and were asked to return details of their assays.

The fifteen participants were assigned a code number 1–15. Ten of the participating laboratories from 7 countries returned results for inclusion in the analysis covered in this report

2.6. Statistical analysis

Submitted HI and VN data consisted of replicate 'absolute' titres expressed as the reciprocal of serum dilution. A serum was considered negative if below the minimum detectable titre of the assay, and positive if any titre was detectable. For calculation, negative titres were assigned the value of half the minimum detectable titre, and titres reported as greater than the final dilution were assigned a value twice the largest titre (e.g., titre <10 assigned a value of 5; titre >1280 assigned a value of 2560). Overall titres were calculated as the geometric mean (GMT) of the individual laboratory replicates. Variation between laboratories was expressed as the percentage geometric coefficient of variation (%GCV). To assess within-laboratory reproducibility, a comparison was made of replicate assays and calculating the percentage of tests giving >2-fold or >4-fold variation for all sera in each laboratory for each virus, giving 14 sets of replicate titres (7 sera and 2 viruses). To assess the ability of the serum standard to improve inter-laboratory agreement, titres were expressed relative to 09/194 by taking the ratio of the GMT for a sample to the GMT for 09/194 obtained by a laboratory, and multiplying by an assigned value for 09/194: the assigned value was the overall GMT by HI and VN. The impact on inter-laboratory agreement, and the %GCV, is independent of the value chosen. The relationship between HI and VN test results was assessed by plotting the log HI titre against the log VN titre across the study samples for each individual laboratory and by calculating the correlation coefficient.

3. Results

3.1. Assays and analysis

Nine participants returned data from both assays and one laboratory only returned HI data. All laboratories returned data from X-179A virus and 6 laboratories also supplied data using A/California/7/2009 (A/California) virus. Laboratory 10 provided two sets of VN assay results, described as using viruses from different sources and following different protocols. There were differences of approximately 1.5–2-fold between VN assays of laboratory 10 and they were combined to give a single VN GMT for subsequent analysis. Negative serum D was excluded from analysis, as all titres were negative. For inter-laboratory comparisons, the GMT for each serum, virus and assay was calculated.

3.2. Reproducibility within laboratories: comparison of identical samples B and E

Comparing titres of samples B and E within individual assays, only one of the laboratories (laboratory 12) had HI or VN titres that differed by >2-fold. For the VN assays, laboratories 5, 6, 8, 11 and 12 each had a single assay where titres differed by more than 2-fold. There were no assays where titres differed by more than 4-fold. Laboratory 12 had a consistent difference in HI results