

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Stephenson I, Nicholson KG, Hoschler K, et al. Antigenically distinct MF59-adjuvanted vaccine to boost immunity to H5N1. *N Engl J Med* 2008;359:1631-3.

Supplementary online material and methods

Subjects Women of childbearing potential were required to have a negative urine pregnancy test on the day of any immunization and agree to use adequate contraception for the study period. Subjects were excluded from enrollment if they had known anaphylaxis or allergy to eggs, antibiotics or any vaccine; disorders of immune function as a result of disease or treatment including oral or parenteral steroids, high-dose inhaled steroids or immunosuppressive drugs; any active neoplastic process; any significant acute or chronic medical condition that would interfere with evaluation of responses to vaccine (this includes but is not limited to: chronic liver disease, significant renal disease, unstable diabetes mellitus, significant cardiopulmonary disorders); pregnancy or refusal of women to use reliable contraception during the study; receipt of blood products or immunoglobulins in the preceding 3 months; any other vaccination or experimental drug use during the previous 4 weeks; acute respiratory disease or use of antibiotics or antivirals within previous 7 days; fever of more than 38°C in the previous 3 days; and inability to give informed consent.

Regulatory approval The trial was registered with the ClinicalTrials.gov, number NCT00478816. The study was designed by the investigators. The UK Medicines and Human products Regulatory Agency, independent ethics committee, and University Hospitals Leicester approved the study. Dr Stephenson served as principal investigator and assumed responsibility for preparation of the manuscript and vouches for its accuracy and completeness.

Vaccine The vaccine strain was a reassortant H5N1 virus (A/Vietnam/1194/2004/NIBRG-14) supplied by the UK National Institute for Biological Standards and Controls. The vaccine was inactivated MF59-adjuvanted surface-antigen H5N1 vaccine produced by Novartis (Siena, Italy) by standard processes used for interpandemic vaccines, and formulated as 0.5ml in pre-filled monodose syringes (lot W52P07H1B). Each dose contained 7.5µg of H5 hemagglutinin, 9.75mg squalene, 1.175mg polysorbate 80, 1.175mg sorbitan trioleate, sodium citrate dehydrate

and citric acid monohydrate. Hemagglutinin content was determined by single-radial-immunodiffusion.

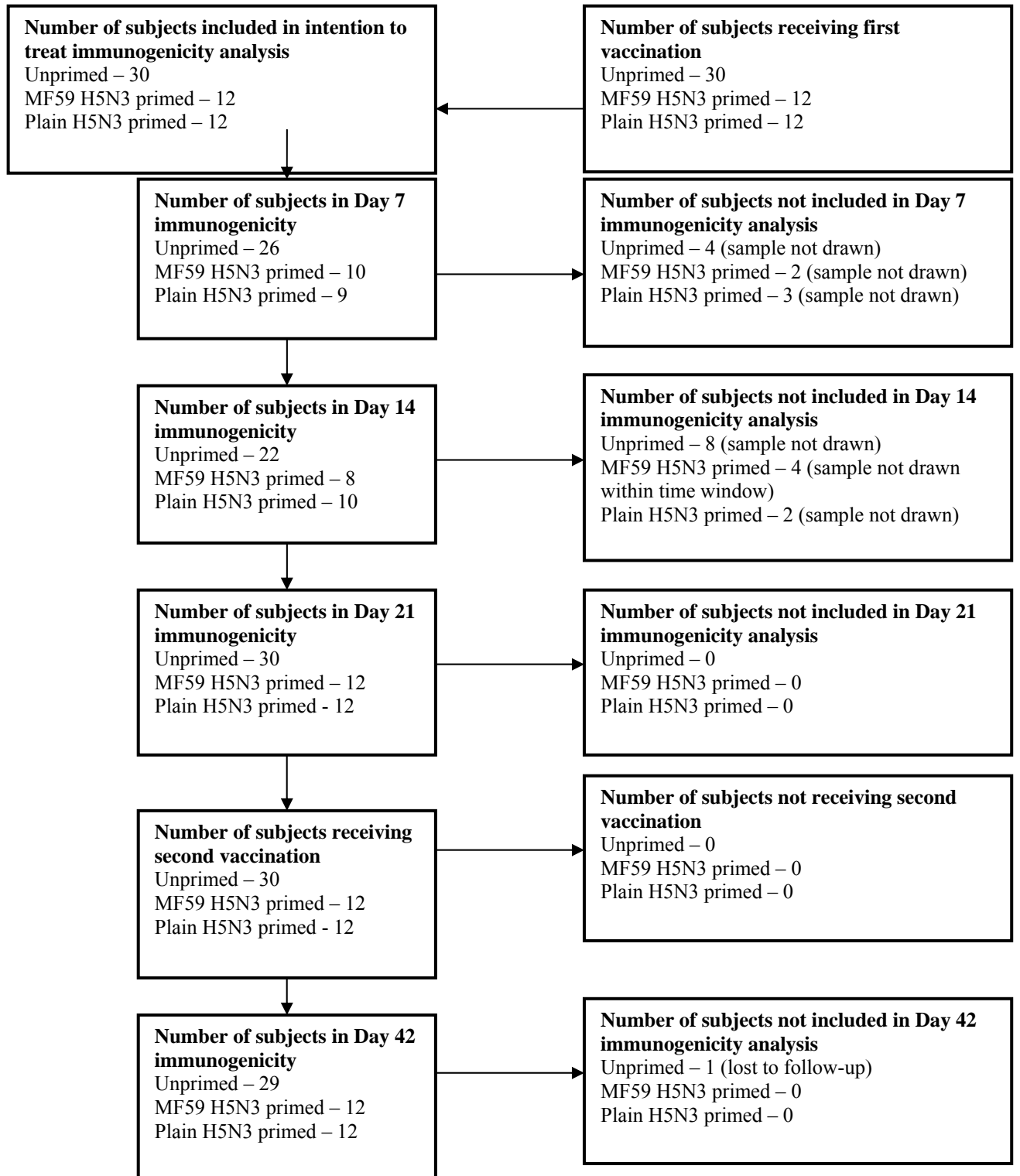
Laboratory assays Antibody responses by microneutralization (MN) assay and hemagglutination-inhibition (HAI) with horse erythrocytes by standard methods were performed at Health Protection Agency, UK with A/Vietnam/1194/2004/NIBRG-14 and A/turkey/Turkey/1/2005/NIBRG-23 viruses. For MN, sera were tested at an initial dilution of 1:20, and those that were negative were assigned a titer of 1:10. For HAI, sera were tested at an initial dilution of 1:8 and those that were negative were assigned a titer of 1:4. Antibody responses were measured by HAI using horse erythrocytes at Influenza Division, Centers for Disease Control and Prevention, Atlanta, US with wild-type A/Vietnam/1194/2004, A/Indonesia/5/2005, A/Turkey/15/2006, and A/Anhui/1/2005 (H5N1) viruses. Sera were tested at an initial dilution of 1:10 and those that were negative were assigned a titer of 1:5. All sera were tested separately and in duplicate and the geometric mean value used for analysis.

Statistical analysis Group sizes were not based on sample or power calculations. Descriptive statistics were used to summarize demographic, immunogenicity and safety variables. Geometric mean titers of antibody and 95% CI were calculated by taking the exponential of the least square means and of the upper and lower limits of associated 95% CI of log₁₀ transformed titers, which were obtained from an analysis of variance with one factor for the priming vaccination group. P-values for between priming group comparisons were also obtained from an ANOVA with one factor for the priming vaccination group. In a supportive robustness analysis, Wilcoxon rank sum test was used to analyze differences between priming groups. For each priming vaccination group, 95% CI for seroprotection or seroconversion were calculated according to Clopper-Pearson. Generally confidence intervals and p-values were not adjusted for multiplicity. P-values ≤ 0.05 were regarded as significant.

Demographics of enrolled subjects

| Characteristic | Number of subjects (%) in each vaccine group | | |
|-------------------------------|--|------------------------|-------------------------|
| | Unprimed n=30 | MF59 H5 primed n=12 | Plain H5 primed n=12 |
| Number of previous H5N3 doses | | | |
| 0 | 30 (100%) | 0 (0%) | 0 (0%) |
| 2 | 0 (0%) | 5 (42%) | 7 (58%) |
| 3 | 0 (0%) | 7 (58%) | 5 (42%) |
| Ethnicity | | | |
| White | 20 (67%) | 11 (92%) | 12 (100%) |
| Asian | 10 (33%) | 1 (8%) | 0 (0%) |
| Female | 17 (57%) | 7 (58%) | 9 (75%) |
| Age (years, median, range) | 36.5 (23-60) | 33 (28-47) | 36 (26-47) |
| Weight (kgs, median, range) | 72 (49-105) | 72 (56-106) | 71 (50-122) |

Trial profile



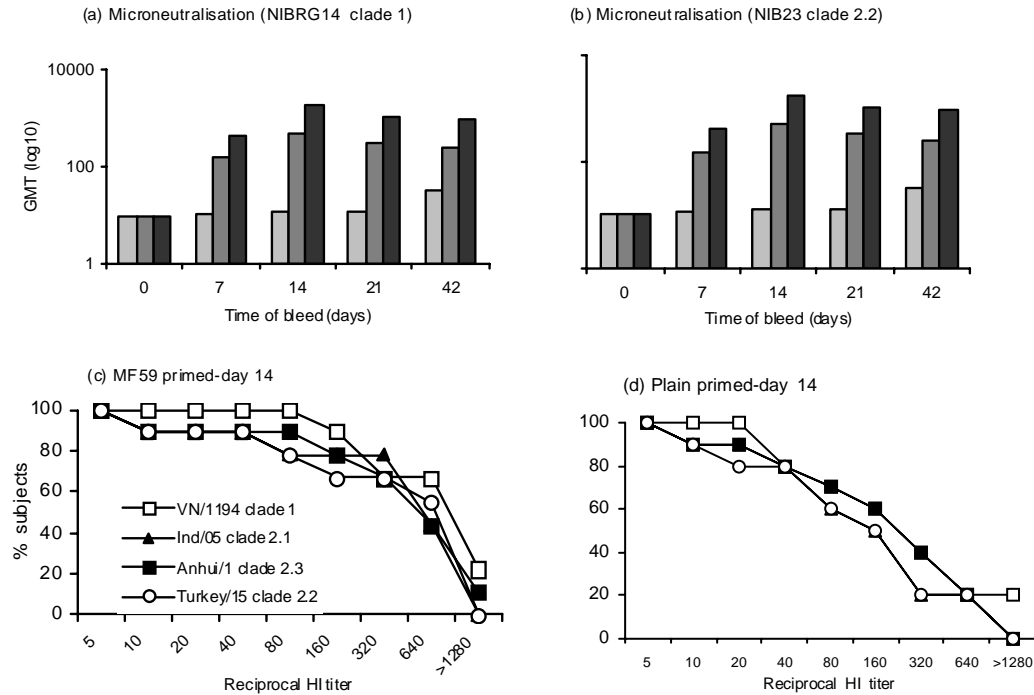


Figure: Geometric mean (log₁₀) antibody titers by neutralization to (a) A/Vietnam/1194/2004/NIBRG-14 and (b) A/turkey/Turkey/1/2005/NIBRG-23 in unprimed (n=30), plain-H5 (n=12) and MF59-H5 primed (n=12) subjects after one and two doses of vaccine. Reverse cumulative distribution curves at 14 after one dose of vaccine in (c) MF59-H5 primed and (d) plain H5 primed groups. The percentage of recipients achieving HI titer is based on the total number of samples available. Antibody titers are shown are from HI testing using wild-type H5 viruses: A/Vietnam/1194/2004, A/Indonesia/5/2005, A/Turkey/15/2006 and A/Anhui/1/2005