NETSCC, HTA

23 October 2009

PROTOCOL

A randomised, partially observer-blind, multi-centre, head-to-head comparison of a two dose regimen of Baxter and GSK H1N1 pandemic vaccines, administered 21 days apart.'

Short title: Head to head study of influenza H1N1 vaccines in adults

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STUDY SYNOPSIS

TITLE OF STUDY:

A randomised, partially observer-blind, multi-centre, head-to-head comparison of a two dose regimen of Baxter and GSK H1N1 pandemic vaccines, administered 21 days apart.

OBJECTIVES:

Primary:

1. To evaluate the immunogenicity of Baxter cell-culture, non-adjuvanted, whole virus H1N1 vaccine, and GSK AS03-adjuvanted, split H1N1 vaccine with respect to Committee of Human Medicinal Products (CHMP) and FDA licensing criteria.

Secondary:

1. To identify whether one or two doses of vaccine are required to satisfy the licensing criteria,
2. To examine the short term reactogenicity of the vaccines,
3. To examine the kinetics of the antibody responses to vaccination,
4. To examine persistence of antibody at 6 months,
   AND if appropriate (i.e., an antigenic drift variant emerges prior to the 2010-2011 influenza season),
5. To evaluate the breadth of the antibody response to the antigenic variant.

DESIGN:

An observer-blind, multi-centre study in which 6 groups of 60 male and female adults stratified by age (18-44, 45-64, and 65 years and older) will be randomly allocated to receive two 7.5µg haemagglutinin doses of cell culture plain (i.e., non-adjuvanted) whole virus A/California/2009 (H1N1) vaccine, or two doses of AS03-adjuvanted influenza A/California/2009 (H1N1) split virus vaccine containing 3.75µg haemagglutinin by intramuscular injection. A second dose of the same vaccine containing the same quantity of antigen as in the first dose will be administered 21 days later. Subjects will be observed for local and systemic reactions for 30 minutes after each immunisation and will be monitored for any reactions and other adverse events for 7 days after each immunisation.

Blood for immunogenicity studies will be obtained at day 0 (pre-immunisation), day 7 ($\pm$1 day), day 14 ($\pm$2 days), day 21 ($\pm$2 days), day 28 ($\pm$2 days), day 35 ($\pm$3 days), day 42 ($\pm$3 days) and day 180 ($\pm$10 days).

Immunogenicity to influenza viruses will be evaluated by haemagglutination-inhibition (HI), virus neutralization (MN), and possibly single radial haemolysis (SRH) responses.

DURATION:

Approximately 6 months per subject. Subjects will screened to ensure entry criteria are met and then vaccinated. After a second dose of vaccine on day 21, subjects will be followed-up for an additional 159 days.

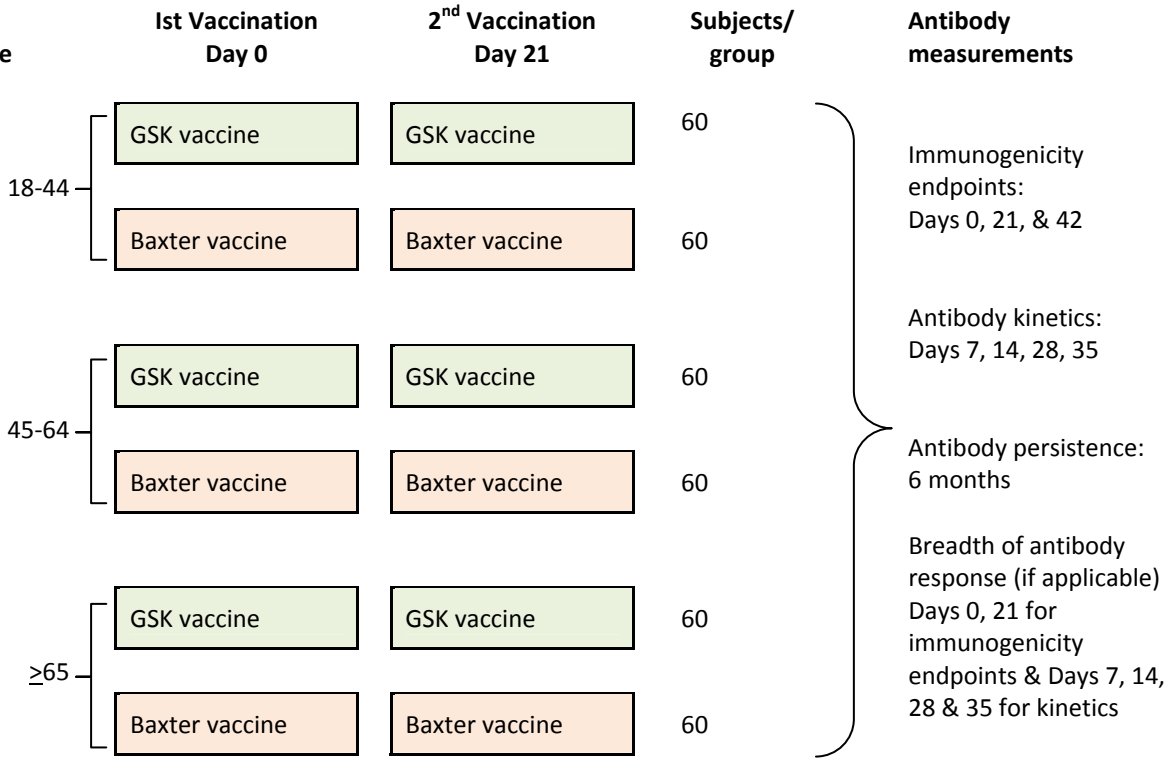
START DATE:

The study is planned to commence early September 2009.

SETTING:

This multicentre study will be conducted in University Hospitals of Leicester, Nottingham, and Sheffield, and possibly in GP surgeries in Leicestershire, Nottinghamshire, Yorkshire and Derbyshire.

STUDY SCHEDULE: FLOW DIAGRAM



TIME AND EVENTS TABLE

Study visit	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
	Days after the first vaccination							
Window (days)	**0**	**(±1)**	**(±2)**	**(±2)**	**(±2)**	**(±3)**	**(±3)**	**(±10)**
Study day	**0**	**7**	**14**	**21**	**28**	**35**	**42**	**180**
Informed consent	X							
Inclusion/exclusion criteria	X			X				
Medical/medication history	X							
Pregnancy test	X			X				
Blood sample – antibody studies	X	X	X	X	X	X	X	X
Vaccination	X			X				
Thermometer/Diary card	X			X				
Diary card training	X							
Diary card returned/review		X			X			
Reminder re unsolicited events	X	X	X	X	X	X	X	
Adverse events monitoring		X	X	X	X	X	X	X
Termination of study								X

NUMBER OF SUBJECTS:

		Vaccine dose (µg)		
Vaccine	**Age**	**3.75**	**7.5**	**Total**
GSK	18-44	60	-	60
	45-64	60	-	60
	≥65	60	-	60
Baxter	18-44	-	60	60
	45-64	-	60	60
	≥65	-	60	60
Total		**180**	**180**	**360**

INCLUSION CRITERIA:

1. Mentally competent adults, who have signed an informed consent form after having received a detailed explanation of the study protocol.
2. Clinically healthy, male or female volunteers aged 18 years of age and older, including the over 65's, and those with **stable** high-risk medical conditions. (NOTE: 'Stable' is defined as having no medical consultations for an exacerbation or worsening of any chronic medical condition during the preceding 8 weeks, AND have been maintained on a stable drug regimen for at least 2 weeks prior to study entry as assessed by the medical history).
3. Are able to understand and comply with all study procedures and to complete study diaries,
4. Individuals who can be contacted and are available for all study visits.
5. Females should either be using secure contraceptive precautions including a) the oral contraceptive pill, b) condom/barrier contraception c) partner has had a vasectomy, d) be surgically sterilised, or e) post-menopausal (defined as at least two years since the last menstrual period).

EXCLUSION CRITERIA:

1 Subjects who are unable to lead an independent life either physically or mentally;

2 Women should not be pregnant or lactating;

3 Women who refuse to use a reliable contraceptive method Days 0 to 42 of the study;

4 Confirmed H1N1 infection, as determined by laboratory tests;

5 Have received Oseltamivir or Zanamivir for influenza-like illness since May 2009;

6 Have a household member who had confirmed H1N1 infection, as determined by laboratory tests, and/or received Oseltamivir or Zanamivir for influenza-like illness since May 2009;

7 Receipt of another investigational agent (vaccine or medicinal product) in the preceding 4 weeks;

8 Unwilling to refuse participation in another study during Days 0 to 42 of the study;

9 Any clinically significant concurrent illness or unstable medical condition including: malignant tumours, acute or progressive renal or hepatic pathology, chronic obstructive pulmonary disease requiring oxygen therapy, and any active neurological disorder;

10 Individuals who have had acute respiratory pathology or infections requiring systemic antibiotic or antiviral therapy during the preceding 7 days (chronic antibiotic therapy for prevention of urinary tract infections is acceptable);

11 Subjects who had a temperature $\geq 38^{o}C$ within 3 days of vaccination;

12 Any acute illness at the time of vaccination. Note: minor infections without fever or systemic upset are not contraindications/exclusion criteria;

13 Subjects with known or suspected impairment/alteration of immune function, including:

- receipt of oral immunosuppressive drugs or other drugs listed in section 8 of the British National Formulary (BNF) or chloroquine, gold or penicillamine or other drugs listed in section 10.1.3 of the BNF to suppress a chronic disease process (Note: long-term, inhaled steroids for asthma management is acceptable),
- receipt of immunostimulants or interferon,
- receipt of an immunoglobulin preparation, blood products, and/or plasma derivatives within 3 months of the study;
- Anyone at high risk of developing immunocompromising condition;
- Received radiotherapy or chemotherapy during the 6 months preceding the study;

14 Subjects for whom surgery is planned during Days 0 to 42 of the study;

15 Regularly drink more than 40 units of alcohol weekly;

16 Known or suspected drug abuse (recreational or prescribed);

17 Individuals who, in the opinion of the investigator, have conditions that might complicate interpretation of the study results;

18 Subjects with a history of anaphylaxis or serious reactions to vaccines; known hypersensitivity (other than anaphylactic reaction) to influenza viral protein, to any component of the study vaccines, to products containing mercury and to residues (egg and chicken protein, ovalbumin, formaldehyde, gentamicin sulphate, sodium deoxycholate, and benzonase).

19 Subjects with a history of any neurological symptoms and signs, or anaphylactic shock following administration of any vaccine;

20 Actual or planned receipt of another vaccine, excluding seasonal influenza vaccine, during the period 3 weeks before to 3 weeks after vaccination on Days 0 and 21.

TEST VACCINES, ANTIGEN CONTENT, DOSAGE REGIMEN, ROUTE OF ADMINISTRATION

All subjects will be allocated either two doses of Baxter vaccine (i.e., cell-culture non-adjuvanted, whole virus influenza A/California/2009 (H1N1) vaccine, containing 7.5µg of haemagglutinin), or two doses of GSK vaccine (i.e., egg-grown, AS03-adjuvanted, split virus influenza A/California/2009 (H1N1) vaccine, containing 3.75µg of haemagglutinin), administered 21 days apart, by intramuscular injection into the deltoid muscle, preferably of the non-dominant arm.

CONCOMITANT VACCINES/MEDICATIONS

There are no concomitant vaccines or medication.

ASSESSMENTS

Study entry:

1. Inclusion/exclusion criteria,
2. Medical history,
3. Demography.

During the study

1. Exclusion criteria,
2. Solicited and unsolicited events (local and systemic symptoms),
3. Immune responses to influenza virus

ANALYSIS

1. Baseline demographic data, including age, sex, ethnicity, previous influenza vaccination (past three seasons), influenza-like illness (since May 2009), and pre-vaccination antibody to the vaccine strain,
2. Solicited and unsolicited events, including local and systemic symptoms, relief medication, and absence from work due to any adverse events,
3. Measures of immunogenicity (see below).

MEASURES OF IMMUNOGENICITY

Immunogenicity will be measured by haemagglutination inhibition (HI), neutralizing antibody (MN) and possibly single radial haemolysis (SRH) antibody responses to influenza H1N1 at each visit.

The principal objective of the study is to evaluate the immunogenicity of each dose of Baxter cell-culture, non-adjuvanted, whole virus H1N1 vaccine, and GSK AS03-adjuvanted, split H1N1 vaccine with respect to Committee of Human Medicinal Products (CHMP) and FDA licensing criteria, i.e., 21 days after the first and second doses.

Immunogenicity will be assessed in terms of the ***'magnitude'*** and ***'kinetics'*** of the antibody response, and, when appropriate, the ***'breadth'*** of the antibody response:

- MAGNITUDE: i.e., measurement of the antibody titres (to the vaccine strain) to one and two 0.5mL intramuscular (IM) doses of Baxter and GSK vaccines (i.e., by comparing (i) mean geometric increases [ratio of day 21 GMT/day 0 GMT and ratio of day 42 GMT/day 0 GMT, by age group and all age groups combined]; (ii) the seroconversion rate, or significant increases in titre; and (iii) the seroprotection rate;
- KINETICS: i.e., application of the above immunogenicity criteria 7 and 14 days after each dose, after each vaccine type, in each age group, and in all age groups combined;

  and
- BREADTH: i.e., application of the above immunogenicity criteria 21 days after each dose, after each vaccine type, in each age group, and in all age groups combined, to any antigenic drift variant that emerges prior to the 2010-2011 influenza season.

SEROLOGY

Serum samples will be assessed by means of HI, MN, and possibly SRH tests. HI and MN assays will be performed at the Health Protection Agency Centre for Infections, Enteric, Respiratory & Neurological Virus Laboratory, London, UK. SRH tests may be done at the National Institute for Biological Standards and Control.

STATISTICAL HYPOTHESIS

The aim of the trial is to establish whether GSK and Baxter vaccines satisfy all three CPMP criteria, and if so compare them in terms of immunogenicity (for each vaccine/age group and each vaccine type). The sample/group size is in line with standard practice. The protocols for seasonal EU vaccine clinical trials and the criteria for assessment have been standardised within the EU. They stipulate that trials should be done with groups of at least 50 subjects. We will recruit 60 per group, allowing for up to 17% dropout.

INTERIM/PRELIMINARY ANALYSES

To provide DH with information as rapidly as possible with the goal of informing DH vaccination strategy, the following interim analyses of data from this study are planned:

1. Solicited/unsolicited events (local and systemic symptoms, relief medication, and absence from work due to any adverse events) during:
   Days 0-6 (1st vaccination)
   Days 0-21 (1st vaccination)
   Days 21-27 (2nd vaccination)
   Days 21-41 (2nd vaccination)
2. HI and MN antibody titres on days:
   Days 0, 7, 14, 21 (1st tranche of sera measuring antibodies before and after 1st injection)
   Days 21, 28, 35, 42 (2nd tranche of sera measuring antibodies before and after 2nd injection).

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1.0 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

1.1 Abbreviations:

AE	Adverse Event
AP	(Statistical) analysis plan
CCA	Chick cell agglutination
CPMP	Committee for Proprietary Medicinal Products
CI	Confidence interval
CRF	Case report form
EC	Ethics committee
EMEA	European Agency for the Evaluation of Medicinal Products
GCP	Good clinical practice
GMA	Geometric mean area
GMR	Geometric mean ratio
GMT	Geometric mean titre
HA	Haemagglutinin
HI	Haemagglutination inhibition
ICH	International Conference on Harmonization
ICF	Informed consent form
IM	Intramuscular
ITT	Intention to treat
IUD	Intra-uterine device
LSLV	Last subject last visit
MHRA	Medicines and Healthcare Products Regulatory Agency
MRC CDVIP	Medical Research Council Committee for the Development of Vaccines and Immunisation Procedures
MedDRA	Medical Dictionary for Regulatory Activities
MN	Microneutralisation
NA	Neuraminidase
SA	Surface antigen
SAE	Serious adverse event
SOP	Standard operating procedure
SP	Split product
SRH	Single radial haemolysis
SUSAR	Suspected Unexpected Serious Adverse Reaction
UHL	University Hospitals of Leicester
WHO	World Health Organisation
WV	Whole virion

1.2 Definition of terms

Adverse Event: An Adverse Event (AE) is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product at any dose that does not necessarily have to have a causal relationship with this treatment. An AE can, therefore be any unfavourable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of an investigational product, whether or not considered related

to the investigational product. This definition includes intercurrent illnesses or injuries and exacerbations of pre-existing conditions.

Concomitant medication: All prescription medication, being taken by the subjects on entry to the study and all prescription medication given in addition to the study vaccine during 21 days after each vaccination are to be regarded as concomitant medication

End of Trial: The End of Trial corresponds to the last visit of the last subject undergoing the trial (LSLV, Last Subject Last Visit).

Local and Systemic Reactions: Selected local and systemic AEs are routinely monitored in vaccine clinical trials as indicators of vaccine reactogenicity. It is recognized that each of these events, and particularly those of a systemic nature, may under some circumstances, in any individual subject, have a cause that is unrelated to the study vaccine. However, as a matter of convenience and in accordance with common clinical practice, all such events occurring within 6 days after immunization are herein termed 'local and systemic reactions'.

Month, Day: Study months are based upon 30-day cycles. The study day refers to the number of days after enrollment, with the day of first vaccination being designated day 0.

Serious Adverse Event: Any experience or reaction that suggests a significant hazard, contraindication, side effect, or precaution. These events include any experience that is fatal or life-threatening, requires or prolongs inpatient hospitalization, is permanently disabling, leads to congenital abnormality, requires intervention to prevent permanent impairment or damage, or is important and significant medical event that, based upon appropriate medical judgment, may jeopardize the subject.

Stable medical condition: Is defined as having no medical consultations for an exacerbation or worsening of any chronic medical condition during the preceding 8 weeks, AND have been maintained on a stable drug regimen for at least 2 weeks prior to study entry as assessed by the medical history).

Study Monitor: The study monitor is the sponsor's designated representative responsible for managing, supervising and monitoring the overall conduct of the trial.

2.0 ETHICS

2.1 Approval of study protocol

This protocol and any accompanying material provided to the patient (such as patient information sheets or descriptions of the study used to obtain informed consent), will be submitted for expedited IRAS ethical approval for projects on pandemic influenza by the principal investigator. Approval will be obtained before starting the study, and will be documented in a letter to the investigator specifying the date on which the committee met and granted approval for the study and the protocol identification (title, version, date).

The EC should also be asked for a written statement regarding the composition of the committee and should comply with GCP and the applicable regulatory requirement(s). The trial will not be initiated until appropriate EC approval of the protocol and informed consent document. In addition,

all documents will be submitted to other authorities (e.g., MHRA) in compliance with local jurisdictions.

Prior to enrolment, the sponsor and the investigator must exchange written confirmation that their ethical and legal responsibilities have been observed. The EC and, if applicable, other authorities, must be informed of protocol amendments in accordance with local legal requirements. Appropriate reports on the progress of the study will be made to the EC and the sponsor by the investigator in accordance with applicable governmental regulations and in agreement with policy established by the sponsor.

Any modifications made to the protocol after receipt of the Ethics Committee approval must be submitted by the investigator to the Committee in accordance with local procedures.

2.2 Ethical conduct and good clinical practice

This trial will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, and that are consistent with Good Clinical Practices (GCPs) and the applicable regulatory requirement(s) for the country in which the trial is conducted, Good Clinical Practice (GCP) according to International Conference on Harmonisation (ICH) guidelines, and applicable Standard Operating Procedures (SOPs). Specifically, this trial is based on adequately performed laboratory procedures; the trial will be conducted under a protocol reviewed and approved by an EC, the trial will be conducted by scientifically and medically qualified persons; the benefits of the study are in proportion to the risks; the rights and welfare of the subjects will be respected; the physicians conducting the trial do not find the hazards to outweigh the potential benefits; each subject, or where applicable, each subject's legally acceptable representative(s) will give his or her written informed consent before any protocol-driven tests or evaluations are performed. A copy of the ICH GCP guidelines and of the Declaration of Helsinki (version 1996) will be included in the investigator's study file.

2.3 Informed consent of subject and confidentiality

2.3.1. Informed consent

The investigator is responsible to obtain informed consent in adherence to GCP and according to applicable regulations prior to entering the subject into the trial.

The information about the trial must be given orally and in an understandable form. Written information about the trial will also be provided. In addition to the explanation of the trial and the subject's legal rights the information should comprise that access to original medical records and processing of coded personal information must be authorized. The informed consent discussion must be conducted by a person who is qualified according to applicable local regulations. The subject should have the opportunity to inquire about details of the trial and to consider participation.

The informed consent form (ICF) must be signed and dated by the subject and must be countersigned by the person who conducted the informed consent discussion (according to local laws and GCP).

If a person is unable to read or write, oral consent in the presence of an impartial witness is possible, if this is permitted by local legislation. In this case, the witness is to be present during the meeting in which the significance of the informed consent will be orally explained. After the informed consent discussion and after the subject has orally consented to participate in the clinical trial the witness should sign and personally date the consent form to attest that information concerning the clinical trial and the subject's rights was accurately explained to, and apparently understood by the subject and that informed consent was freely given.

The investigator will provide a copy of the signed informed consent to the subject, and will maintain the original in the investigator's study file.

The written informed consent form and any other written information to be provided to subjects should be revised whenever important new information becomes available that may be relevant to the subject's consent. Any revised written informed consent form, and written information should receive EC's approval before use.

The subject should be informed in a timely manner if new information becomes available that may affect the decision to participate in the clinical trial. The communication of this information should be documented.

2.3.2 Subject confidentiality

Subject names will not be supplied to the sponsor (UHL Trust). Only the subject numbers and subject identification codes will be recorded in the Case Report Form (CRF), and if a subject's name appears on any other document, it will be obliterated before a copy of the document is supplied to the sponsor. Study findings stored on a computer will be subject to local data protection laws. The subject, or where applicable, the subject's legally acceptable representative, will be informed that representatives of the sponsor, EC, or regulatory authorities may inspect their medical records to verify the information collected, and that all personal information made available for inspection will be handled in strictest confidence.

The investigator or designee will maintain a personal list of subject numbers and subject identification codes to enable records to be found at a later date.

2.4 Indemnity

This study is being undertaken in response to pandemic H1N1 influenza following a call for scientific proposals to help inform national strategy/policy. This study is being done with vaccine purchased by the Department of Health to help protect the population from pandemic influenza. The investigators and the Sponsor (University Hospitals of Leicester NHS Trust) and others who facilitate the study will be indemnified by the Department of Health of England and Wales against non-negligent harm (in accordance with applicable laws and regulations, against financial loss resulting from personal injury and/or other damages), which may arise as a consequence of the administration of Baxter and GSK H1N1 vaccines used in this study. This indemnity is applicable to subjects vaccinated in this study in health-care settings (University Hospitals of Leicester, Nottingham, and Sheffield, and general practice facilities) in Leicestershire, Nottinghamshire, Yorkshire, and Derbyshire.

3.0 INVESTIGATORS

The trial will be administered and monitored by employees or representatives of the University Hospitals of Leicester NHS Trust, University Hospitals of Nottingham NHS Trust, and University Hospitals of Sheffield NHS Trust. The Principal Investigators in Leicester (Dr Iain Stephenson), Nottingham (Dr Weishen Lim), and Sheffield (Professor Robert Read), together with the Chief Investigator (Professor Karl Nicholson), will be responsible for the timely reporting of serious adverse events.

The Investigators and Study Nurses undertaking the trial either hold, or will hold (when appointed) appointments, or honorary appointments, with the University Hospitals of Leicester Trust, University Hospitals of Nottingham NHS Trust, or University Hospitals of Sheffield NHS Trust.

Study Monitors will monitor the sites on a periodic basis and perform verification of source documentation for volunteers. The principal investigator at each study site will be readily available to provide appropriate medical expertise on trial related medical questions. The sponsors and investigators responsibilities as regards reporting SAEs and SUSARs will be in accordance with the European Directive 2001/20/EC.

4.0 BACKGROUND AND RATIONALE

Influenza virus diversity Influenza A viruses are antigenically distinguished and classified by subtypes of haemagglutinin (HA) and neuraminidase (NA) with 16 HA and 9 NA subtypes identified within the natural reservoir of aquatic birds. The HA and NA surface antigens of influenza A virus are responsible for virus attachment and release from host cell receptors and are targeted by host antibodies. The HA is the major component of influenza vaccines and is subject to mutations resulting in antigenic drift that enables the virus to escape immune recognition.

In the northern hemisphere influenza is characterised by the occurrence of annual outbreaks during winter and worldwide pandemics, which have occurred at 11-52 year intervals during the past 300 years.[1] Pandemics inflict huge socio-economic costs. The 1918-19 pandemic caused an estimated 40-100 million deaths globally. Pandemic influenza results from the emergence of an influenza A virus possessing a 'new' HA (antigenic 'shift') to which the population possesses little or no immunity and which is capable of spreading with a high attack rate in all parts of the world. Despite this viral diversity, only three HAs and two NAs have established human lineages during the last 100 yrs. In 1918, (Spanish flu: A/H1N1), 1957 (Asian flu: A/H2N2), 1968 (Hong Kong flu: A/H3N2) and 1977 (A/H1N1) strains emerged to cause widespread human infections.

Reasons for decline and emergence of dominant subtypes is unclear, although it seems likely that during interpandemic intervals, population immunity broadens to a point where the prevalent strain loses its capacity for further drift capable of eluding host defences.

Swine influenza in humans Influenza as a disease of pigs was first described during 1918 when outbreaks of respiratory disease occurred simultaneously in humans and swine-herds living and working in close proximity. Pigs are thought to have an important role in inter-species transmission as they possess receptors in their respiratory tract capable of binding both avian and human influenza. Consequently they have been proposed as a possible mixing vessel in which novel reassortant viruses of pandemic potential may be generated. Occasional isolation of swine influenza viruses from humans with respiratory illness has confirmed that sporadic human infection can

occur.[2] Generally, cases have been limited to laboratory workers or those with occupational swine exposure. However, a pandemic alert was raised in 1976 when swine H1N1 caused an outbreak of respiratory illness with one fatality among 13 soldiers at a military base in Fort Dix, New Jersey, USA.[3] No exposure to pigs was found and sero-epidemiological investigation identified up to 230 further soldiers had been infected suggesting human-to-human transmission. Mass vaccination of the US public was initiated and halted amid reports of adverse vaccine reactions, media scepticism and the lack of pandemic activity.[4]

Pandemic A/H1N1 emergence in 2009 In April 2009, near the end of the usual influenza season in the Northern Hemisphere, the first two cases of swine origin H1N1 influenza virus were identified in the United States.[5] The CDC confirmed that these cases were caused by a genetically similar swine virus that had not been previously identified in the United States. Genetic analysis of the strains showed that they were derived from a new reassortment of six gene segments from the known triple reassortant swine virus, and two gene segments (NA and matrix protein) from the Eurasian influenza A (H1N1) swine virus lineage.[6] Effectively all isolates are susceptible to neuraminidase inhibitors, but resistant to M2 inhibitors. The World Health Organization raised its pandemic alert level to Level 6 on June 11 2009,[7] reflecting community level outbreaks of novel H1N1 infection in at least two different WHO regions and the onset of a new pandemic. H1N1 virus has reached over 170 countries and territories worldwide as of August 6 2009, causing at least 177457 cases and 1462 deaths.[8] In the UK, the first pandemic wave is waning, but a substantial increase in cases of novel H1N1 infection is expected following the re-opening of schools in September.

Burden of H1N1 disease, hospitalisations and admissions to ITU As of August 7, analysis of the distribution by age of 8974 individual case reports of influenza A(H1N1)v infection in 27 EU/EEA countries reveal that the age-distribution of non-hospitalised cases of H1N1 influenza is highest in the 10-19 year age group, followed by 0-9 and 20-29 year age groups. The age-distribution of hospitalised cases is significantly higher in the 20-29 year age group than in the under 20's.[9] Estimates of transmissibility (R_0 1.4-1.6) are significantly higher than observed in seasonal influenza and comparable to previous pandemics.[10] In England, admission rates have been highest in the under 5's, but life-threatening complications requiring admissions to ITU increase with age and presence of 'high-risk' co-morbidity. It is unclear whether the higher prevalence of infection in the under 30's is related primarily to social mixing, cross-reacting antibodies that are more prevalent with increasing age, or both.

Preliminary studies in the United States have shown that cross-reactive microneutralisation (MN) antibody titres of ≥160 to A/California/2009 H1N1 were detected in 6% of adults aged 18 to 40 years, 9% of adults 18 to 64 years, and 33% of adults aged 60 years and older.[11] After vaccination with seasonal vaccine, 7% of adults aged 18 to 40 years, 25% of adults aged 18 to 64 years, and 43% of adults aged 60 years and older had post-vaccination titres of ≥160.

Infection with the current influenza H1 pandemic virus is mostly mild, with no increase in mortality above threshold national statistics the United States, despite widespread infection. The observed age distribution is unusual and different from seasonal influenza, being skewed towards younger age groups, resulting in deaths and ITU admissions in people who are normally affected less frequently.

Currently it is difficult to be certain about the impact of novel H1N1 infection on hospitalisation rates. A rate of 11% has been observed in the United States, but this is likely to be an overestimate due to the mild nature of the disease in many cases, and differences in clinical practice. An overall rate for Europe is around 5%-6%, but this too may be an overestimate due to the practice in some countries of admitting patients for isolation to control spread, rather than for severity of illness. In the UK, the observed rate has been around 1-2%.

Current seasonal influenza vaccines Inactivated influenza virus vaccines represent the mainstay of efforts to prevent influenza and its complications. Current licenced vaccines are produced from virus grown in eggs or cell culture systems and consist of either whole-virus, detergent-treated 'split-product', or purified HA and NA (subunit) surface antigen formulations.

Vaccine efficacy of 70-95% in healthy adults is obtained when there is a good match between the vaccine and the circulating strains.[12] They display reduced efficacy against antigenically drifted viruses and are considered ineffective against unrelated subtypes.

The use of mammalian cell lines, notably Vero and MDCK cells, to grow influenza virus are approved substrates for production of licenced trivalent seasonal vaccines which may allow for increased vaccine production at short notice to meet unexpected demand.

As vaccine responses are generally lower in elderly subjects, efforts to improve immunogenicity have been investigated. The addition of MF59, a squalene-containing oil-in-water emulsion adjuvant, has been shown to increase post vaccination antibody titres and seroconversion rates in elderly and immunocompromised subjects.[13] MF59-adjuvanted seasonal influenza vaccines have been licenced for clinical use since 1997. More recently, two other squalene-containing oil-in-water adjuvants have been developed by GSK and Sanofi. The GSK AS03 oil-in-water adjuvant has been extensively evaluated in association with H5N1 antigens.

Global manufacturing capacity for pandemic influenza vaccine Seasonal influenza vaccines are given at doses of 15µg HA per virus strain. The global human population is estimated at 6.77 billion.[14] The annual global vaccine manufacturing capacity for trivalent seasonal influenza vaccines was 852 million doses in May 2009.[15] Assuming that the yield of H1N1(v) antigen is comparable to that for seasonal virus strains, the present manufacturing capacity equates to 2.56 billion doses of monovalent H1N1(v) vaccine containing 15µg HA per dose. This would be enough for only 2.56 billion people if two doses containing 7.5µg HA were immunogenic, but could protect more people if one dose was sufficient in older people. Pandemic H1N1(v) vaccines will be supplied over a period of 6 months or more, so it is essential that dose sparing formulations and regimens are identified and deployed rapidly to protect as many vulnerable people as possible.

Experience of pandemic and mock pandemic vaccines since the 1970's Historically, influenza vaccines were first developed as 'whole virus' formulations. During the late 1970's whole virus vaccines were replaced by 'split' , and highly purified 'surface antigen' formulations that caused fewer local and systemic reactions than whole virus vaccine, but are equally immunogenic when given to primed individuals as 'seasonal' or 'interpandemic' vaccine.

Experience with H1N1 vaccines during the 1970's Experience in unprimed individuals with vaccines produced from Hsw1N1 viruses (A/New Jersey/8/76) or H1N1 viruses (A/USSR/90/77) indicated that

high concentrations of antigen (>50µg HA) were needed in a single vaccine dose to generate HI titres that met the current European licensing criteria. In a two-dose schedule, HI titres ≥ 40 could be achieved with two doses containing 5µg HA. Overall, whole virus vaccines were more immunogenic than split or subunit vaccines. The split and surface antigen vaccine formulations were notably less immunogenic than whole virus vaccine when given to children, both during 1976 when influenza A/New Jersey/76 (H1N1) posed a pandemic threat,[16] and during 1977 when A/USSR/77 (H1N1) virus re-emerged.[17]

European licensing criteria During the late 1970's, influenza vaccines were poorly standardised. Subsequently, improved methods of measuring vaccine potency and ensuring vaccine standardisation were introduced, and in Europe, by criteria for licensure of seasonal,[18] and latterly pandemic vaccines.[19]

As specified in the Committee for Medicinal Products for Human Use (CHMP) 'Guidelines on dossier structure and content for pandemic influenza vaccine marketing authorisation application',[19] it is anticipated that a pandemic candidate vaccine should at least be able to elicit sufficient immunological responses to meet and preferably exceed all three of the current standards set for existing vaccines in unprimed adults or elderly subjects as specified for seasonal vaccines.[18]

These include assessments of the mean geometric increase in antibody titre (the seroconversion factor), the number of seroconversions or significant increases in antibody, and the seroprotection rate (i.e., the proportion attaining 'protective' levels of antibody). The criteria are based on the HI assay or single radial haemolysis. Both assays have been established as surrogates for protection.

The CHMP guidelines stipulate that vaccines should be tested in adults (18-60 yrs) and elderly (>60 yrs), in groups of >50 subjects, and attain the following:

Adults (18-60 yrs):

1. Seroconversions/ or significant rises (i.e., a 4-fold increase in post-vaccination titre) by > 40%
2. Mean fold increase in GMT post-vaccination >2.5
3. Significant levels of antibody (i.e., having post-vaccination HI titres >1:40) in >70%.

Elderly (>60 yrs):

1. Seroconversions/ or significant rises (i.e., a 4-fold increase in post-vaccination titre) by > 30%
2. Mean fold increase in GMT >2
3. Significant levels of antibody (i.e., having post-vaccination HI titres >1:40) in >60%.

Immunogenicity of plain (i.e., non-adjuvanted split and subunit avian influenza vaccines As outlined below, neither split nor subunit vaccine formulations of H5, H7, and H9 avian influenza satisfy all three CHMP licensing criteria when given at doses of up to 90µg HA.

Treanor *et al.* showed that neither two 90µg doses of plain (i.e., non-adjuvanted) recombinant, baculovirus-expressed, H5 HA[20] nor two 90µg doses of egg-grown, plain, inactivated, subvirion influenza A/Vietnam/1203/2004 (H5N1) vaccine satisfied the CHMP regulatory criteria.[21] Nicholson *et al.* showed that two doses of all three 7.5-30µg formulations of plain A/Duck/Singapore/97 (H5N3) surface antigen vaccine failed to meet the CHMP criteria.[22]

Bresson *et al.* showed that two doses of all three 7.5-30µg HA formulations of plain, split virus, A/Vietnam/1194/2004 (H5N1) vaccine satisfied the CHMP criterion for a greater than 2.5-fold increase in antibody titre, but 47% vaccinees failed to achieve protective levels of antibody after a second dose.[23] Nolan *et al.* evaluated two doses of split A/Vietnam/1194/2004 (H5N1) vaccine containing 7.5-45µg HA with and without alum adjuvant.[24] All formulations met the CPMP criterion for a greater than 2.5-fold increase in HI antibody titres after the second dose, but not the criterion for greater than 70% of participants achieving sero-protection.

Keitel *et al.*[25] evaluated subvirion inactivated influenza A/H5N1 vaccine containing 3.75, 7.5, 15, or 45 µg of HA. Dose-related increases in antibody responses were noted after both vaccinations, but no formulation attained the CHMP criteria.[25]

Stephenson *et al.* evaluated two 7.5, 15 and 30µg doses of plain, subunit, influenza A/Hong Kong/1073/99 (H9N2) vaccines in people before and after their 32nd birthday.[26] The CHMP criterion for a greater than 2.5-fold increase in HI antibody titres was met after the second dose, but 86% vaccinees failed to attain protective levels of antibody. Cox *et al* evaluated two doses of split H7N1 virus vaccine containing 12µg or 24µg HA. Neither formulation fulfilled the CHMP licensing criteria.[27]

Immunogenicity of whole virus vaccines and vaccines adjuvanted with oil-in-water emulsions
Whole virus vaccines and vaccines adjuvanted with oil-in-water emulsion are more immunogenic in man than split and subunit vaccines.[22,26,28-35]

Lin *et al*[36]*.* showed that a two-dose regimen of an aluminium hydroxide adjuvanted whole virus A/Vietnam/1194/2004 (H5N1) vaccine containing 10µg HA met all CHMP regulatory requirements for annual licensing of seasonal influenza vaccine. Ehrlich *et al.*[37] evaluated whole virus A/Vietnam/1203/2004 (H5N1) vaccine (manufacturer by Baxter Healthcare) at doses of 3.75µg, 7.5µg, 15µg or 30µg HA with alum adjuvant, and 7.5µg or 15µg without adjuvant. Maximum responses to the vaccine strain were obtained with formulations without alum adjuvant. When assessed by single radial haemolysis, the 7.5µg dose met all three CHMP licensing criteria. Two criteria were met when antibodies were measured by haemagglutination inhibition.[37] The vaccine also induced a neutralizing immune response against clade 2 and 3 strains and results without alum adjuvant elicited significantly higher immune responses than those with alum.

A phase I randomised trial of subunit and whole virus A/Hong Kong/1073/99 (H9N2) vaccine, given in two doses containing doses of 7·5µg, 15µg, or 30µg HA, revealed the presence of cross-reacting antibodies in participants born before 1969 who were older than 32 years[38] – this finding is comparable to the recent observation of an age-related presence of cross-reacting antibodies to A/California/2009 (H1N1v) in the United States. In participants older than 32 years, one dose of whole virus or subunit vaccine evoked antibody responses associated with protection. However, in people aged 32 years or younger, whole virus vaccine produced a significantly higher probability of seroconversion compared with subunit virus for this age group.[38]

Nicholson *et al.*[22] evaluated two doses of subunit A/Duck/Singapore/97 (H5N3) vaccine containing 3.75µg, 7.5µg, and 15µg HA with and without MF59 oil-in-water adjuvant. In this phase I randomised trial, the geometric mean titres of antibody, and seroconversion rates, were significantly higher with MF59 adjuvanted vaccine. After the second injection, all MF59-adjuvanted vaccine

doses met all three CHMP licensing criteria. Further studies showed improved antibody persistence with MF59 containing vaccine, improved immune responses to other clades of H5 virus, and significantly higher antibody responses on boosting.[29-32]

Leroux-Roels *et al.* evaluated A/Vietnam/1194/2004 (H5N1) vaccine manufactured by GlaxoSmithKline at doses of 3.8µg, 7.5µg, 15µg and 30µg HA with and without its proprietary AS03 adjuvant. The adjuvanted formulations were significantly more immunogenic than the non-adjuvanted formulations at all antigen doses. At the lowest antigenic dose, immune responses for the adjuvanted vaccine against the vaccine strain met or exceeded all US Food and Drug administration and EU CHMP licensure criteria.[33] Further research showed broad cross-clade immune responses at the lowest antigen dose (3.8µg) with adjuvant, but no cross-clade response in the non-adjuvanted group.[34,35]

Reproducibility of the serology assays for influenza For research purposes and vaccine licensure, influenza vaccines are evaluated by clinical trials that assess immunogenicity by the presence of serum antibody. Collaborative studies have shown that the serology assays are highly variable between laboratories – with variability between laboratories for HI assays varying by up to 32-fold.[39-41] This leads to difficulties in interpreting results from different manufacturers. At the 5th WHO Meeting on Evaluation of Pandemic Influenza Prototype Vaccines in Clinical Trials, 12-13 February 2009, WHO highlighted the need for standardised assays and internationally accepted antiserum standards.[42]

The H1N1v vaccines purchased by the Government The UK Departments of Health have purchased pandemic vaccines from Baxter Healthcare and GlaxoSmithKline. The Baxter vaccine (trade name, Celvapan) is a plain (i.e., non-adjuvanted), whole virus, vero-cell-grown, influenza A (H1N1)v pandemic formulation, containing 7.5µg HA per 0.5mL dose. The GlaxoSmithKline vaccine (trade name, Pandemrix) is an AS03-adjuvanted, split-product, egg-grown, influenza A (H1N1)v formulation, containing 3.75µg HA and oil-in-water AS03 adjuvant composed of squalene, DL-α-tocopherol and polysorbate 80. The expectation is that the initial limited supplies of both vaccines will be prioritised for those deemed to a greatest risk, but eventually everyone will have access to either vaccine. To ensure that protection is provided as rapidly as possible, it is imperative that vaccine is used efficiently.

Conclusions The available evidence indicates that whole virus vaccines, and split and subunit vaccines that contain oil-in-water adjuvant, are more immunogenic for avian H5, H7, and H9 HAs, and A/New Jersey/76 (HSw1N1) and A/USSR/77 (H1N1) than split and surface antigen vaccines without adjuvant. They also offer the potential for broadened antibody responses that could be critical in the event of antigenic drift during the course of the pandemic. Vaccine is likely to be in short supply (vaccine production takes time and is subject to various rate-limiting factors) and demand will be high worldwide. The available antigen will therefore need to be given optimally with consideration being given to the logistics of vaccine administration, immune responses, and the frequency and nature of adverse clinical reactions.

There have been no head-to-head comparisons of avian vaccines manufactured by Baxter Healthcare and GSK. Due to variability of serological assays, there are uncertainties as to whether

one preparation offers advantages over the other in terms of dose sparing in 'older' people, and significantly more 'seroconversions' in the young.

5.0 STUDY OBJECTIVES:

Primary:

1. To evaluate the immunogenicity of Baxter cell-culture, non-adjuvanted, whole virus H1N1 vaccine, and GSK AS03-adjuvanted, split H1N1 vaccine with respect to Committee of Human Medicinal Products (CHMP) and FDA licensing criteria.

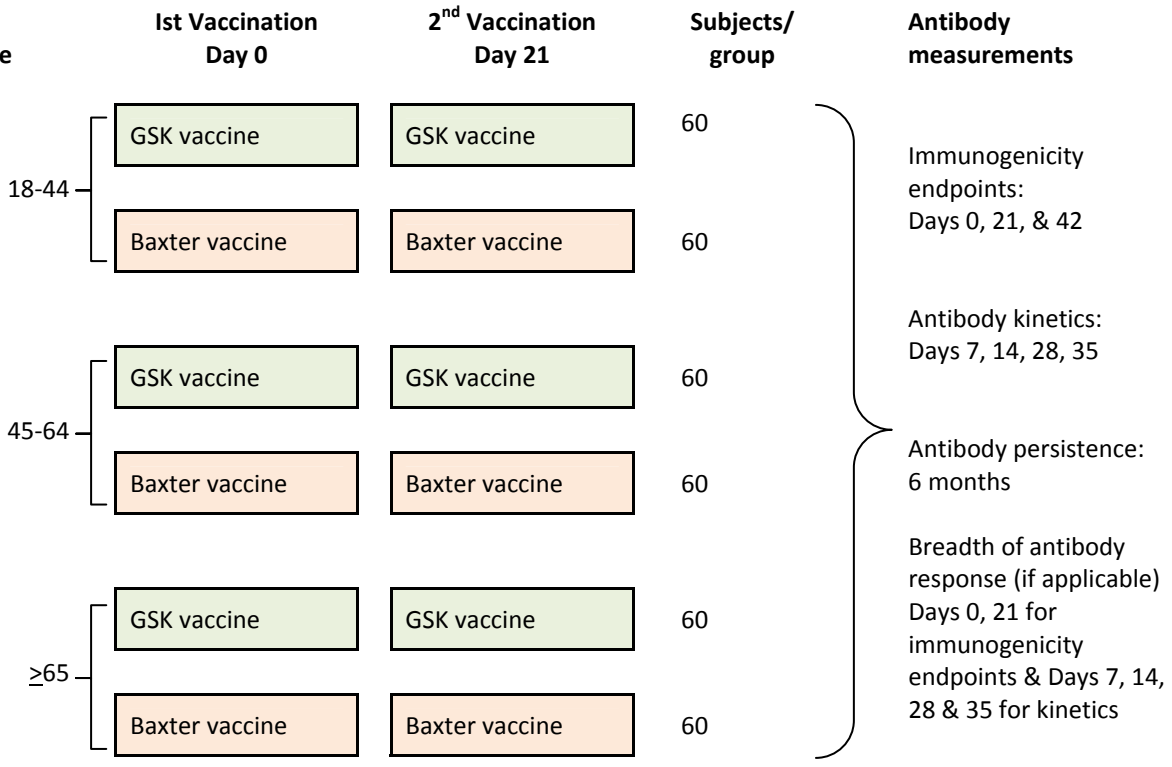
Secondary:

2. To identify whether one or two doses of vaccine are required to satisfy the licensing criteria,
3. To examine the short term reactogenicity of the vaccines,
4. To examine the kinetics of the antibody responses to vaccination,
5. To examine persistence of antibody at 6 months,
   AND if appropriate (i.e., an antigenic drift variant emerges prior to the 2010-2011 influenza season),
6. To evaluate the breadth of the antibody response to the antigenic variant.

6.0 OVERALL STUDY DESIGN

6.1 Overall study design

This observer-blind, multi-centre study will be performed at three study sites in England (Leicester, Nottingham, and Sheffield) in a study population of healthy male and female adults, or adults with stable chronic medical conditions. Six groups of 60 male and female adults will be stratified by age (18-44, 45-64, and 65 years and older):



At least 360 subjects will be randomly allocated to receive two 7.5µg HA doses of cell culture plain (i.e., non-adjuvanted) whole virus A/California/2009 (H1N1) vaccine, or two doses of AS03-adjuvanted influenza A/California/2009 (H1N1) split virus vaccine containing 3.75µg HA by IM injection. A second dose of the same vaccine containing the same quantity of antigen as in the first dose will be administered by the same route 21 days later. Subjects will be observed for local and systemic reactions for 30 minutes after each immunisation and will be monitored for any reactions and other adverse events for 7 days after each immunisation.

Blood for immunogenicity studies will be obtained at day 0 (pre-immunisation), day 7 (±1 day), day 14 (±2 days), day 21 (±2 days), day 28 (±2 days), day 35 (±3 days), day 42 (±3 days) and day 180 (±10 days).

TIME AND EVENTS TABLE

Study visit	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
				Days after the first vaccination				
Window (days)	**0**	**(±1)**	**(±2)**	**(±2)**	**(±2)**	**(±3)**	**(±3)**	**(±10)**
Study day	**0**	**7**	**14**	**21**	**28**	**35**	**42**	**180**
Informed consent	X							
Inclusion/exclusion criteria	X			X				
Medical/medication history	X							
Pregnancy test	X			X				
Blood sample – antibody studies	X	X	X	X	X	X	X	X
Vaccination	X			X				
Thermometer/Diary card	X			X				
Diary card training	X							
Diary card returned/review		X			X			
Reminder re unsolicited events	X	X	X	X	X	X	X	
Adverse events monitoring		X	X	X	X	X	X	X
Termination of study								X

Immunogenicity to influenza viruses will be evaluated by HI, MN, and possibly SRH responses.

6.2 Planned duration of the study

Expected enrolment interval	Approximately 2 weeks
Duration of individual subject's participation	6 months
Total duration of study	Approximately 7 months
End of trial:	Corresponds to the last visit of the last subject undergoing the trial (LSLV)

Subjects will be screened and consented to ensure entry criteria are met and then vaccinated. After a second dose of vaccine on 21 days later, subjects will be followed-up for an additional 159 days.

6.3 Premature discontinuation of the Study

The sponsor (UHL Trust), or the Chief Investigator (following consultation with the Department of Health/NIHR – the funder) has the right to discontinue this study at any time. If the clinical study is prematurely terminated, the Chief Investigator is to promptly inform the study subjects and should assure appropriate follow-up for the subjects. If the study is prematurely terminated, all procedures and requirements pertaining to archiving of documents will be observed.

6.4 Discussion of overall study design

This study was designed to evaluate immunogenicity of Baxter cell-culture, non-adjuvanted, whole virus H1N1 vaccine, and GSK AS03-adjuvanted, split H1N1 vaccine with respect to Committee of Human Medicinal Products (CHMP) and FDA licensing criteria, and occurrence of local and systemic symptoms and signs following vaccine administration in adult and elderly people who have never previously been vaccinated with pandemic H1N1 influenza vaccine. The exclusion criteria reduce the likelihood of prior pandemic H1N1 infection among vaccinees, but the timing of the study cannot avoid this possibility. Moreover it is possible that a further outbreak of pandemic H1N1infection may occur during the first 42 days of the study. Nonetheless, this study design was considered best to evaluate the pandemic H1N1 vaccines purchased by the Government. The need for the study was discussed by advisors to the Department of Health. The study proposal was reviewed anonymously as part of the NIHR funding process.

6.5 Start date

The study is planned to commence early September 2009.

6.6 Study setting

This multicentre study will be conducted in University Hospitals of Leicester, Nottingham, and Sheffield, and possibly in GP surgeries in Leicestershire, Nottinghamshire, Yorkshire and Derbyshire.

6.7 Study population

6.7.1 Initial approach

We will approach staff and healthcare workers in university and healthcare settings in Leicester, Nottingham, and Sheffield either directly (i.e., personal contact), or through written information by post, email, or poster advertising. We may also request GPs to contact potentially suitable patients, either in writing or verbally, asking whether they would be prepared to learn more about the study with a view to participation. We may advertise the study through news items or advertisements on the local radio or in local newspapers. Respondents will be enrolled after they receive a detailed explanation of the study protocol and providing they meet inclusion and exclusion criteria and give signed informed consent. We will approach staff in frontline areas (acute admissions unit, acute wards, intensive care, bone marrow units) before non-health care workers are enrolled.

6.7.2 Inclusion criteria:

1. Mentally competent adults, who have signed an informed consent form after having received a detailed explanation of the study protocol.
2. Clinically healthy, male or female volunteers aged 18 years of age and older, including the over 65's, and those with **stable** high-risk medical conditions. (NOTE: 'Stable' is defined as having no medical consultations for an exacerbation or worsening of any chronic medical condition during the preceding 8 weeks, AND have been maintained on a stable drug regimen for at least 2 weeks prior to study entry as assessed by the medical history).
3. Are able to understand and comply with all study procedures and to complete study diaries,
4. Individuals who can be contacted and are available for all study visits.
5. Females should either be using secure contraceptive precautions including a) the oral contraceptive pill, b) condom/barrier contraception c) partner has had a vasectomy, d) be surgically sterilised, or e) post-menopausal (defined as at least two years since the last menstrual period).

6.7.3 Exclusion criteria:

1 Subjects who are unable to lead an independent life either physically or mentally;

2 Women should not be pregnant or lactating;

3 Women who refuse to use a reliable contraceptive method Days 0 to 42 of the study;

4 Confirmed H1N1 infection, as determined by laboratory tests;

5 Have received Oseltamivir or Zanamivir for influenza-like illness since May 2009;

6 Have a household member who had confirmed H1N1 infection, as determined by laboratory tests, and/or received Oseltamivir or Zanamivir for influenza-like illness since May 2009;

7 Receipt of another investigational agent (vaccine or medicinal product) in the preceding 4 weeks;

8 Unwilling to refuse participation in another study during Days 0 to 42 of the study;

9 Any clinically significant concurrent illness or unstable medical condition including: malignant tumours, acute or progressive renal or hepatic pathology, chronic obstructive pulmonary disease requiring oxygen therapy, and any active neurological disorder;

10 Individuals who have had acute respiratory pathology or infections requiring systemic antibiotic or antiviral therapy during the preceding 7 days (chronic antibiotic therapy for prevention of urinary tract infections is acceptable);

11 Subjects who had a temperature $\geq 38^{o}C$ within 3 days of vaccination;

12 Any acute illness at the time of vaccination. Note: minor infections without fever or systemic upset are not contraindications/exclusion criteria;

13 Subjects with known or suspected impairment/alteration of immune function, including:

- receipt of oral immunosuppressive drugs or other drugs listed in section 8 of the British National Formulary (BNF) or chloroquine, gold or penicillamine or other drugs listed in section 10.1.3 of the BNF to suppress a chronic disease process (Note: long-term, inhaled steroids for asthma management is acceptable),
- receipt of immunostimulants or interferon,
- receipt of an immunoglobulin preparation, blood products, and/or plasma derivatives within 3 months of the study;
- Anyone at high risk of developing immunocompromising condition;
- Received radiotherapy or chemotherapy during the 6 months preceding the study;

14 Subjects for whom surgery is planned during Days 0 to 42 of the study;

15 Regularly drink more than 40 units of alcohol weekly;

16 Known or suspected drug abuse (recreational or prescribed);

17 Individuals who, in the opinion of the investigator, have conditions that might complicate interpretation of the study results;

18 Subjects with a history of anaphylaxis or serious reactions to vaccines; known hypersensitivity (other than anaphylactic reaction) to influenza viral protein, to any

component of the study vaccines, to products containing mercury and to residues (egg and chicken protein, ovalbumin, formaldehyde, gentamicin sulphate, sodium deoxycholate, and benzonase).

19 Subjects with a history of any neurological symptoms and signs, or anaphylactic shock following administration of any vaccine;

20 Actual or planned receipt of another vaccine, excluding seasonal influenza vaccine, during the period 3 weeks before to 3 weeks after vaccination on Days 0 and 21.

6.7.4 Prior and concomitant treatment

During this trial medication prescribed to the subject prior to the start of the study will not be collected. All prescription medication (except minerals and vitamins), including non-study vaccines, being taken by the subjects on entry to the study and all prescription medication given in addition to the study vaccine during this clinical trial are to be regarded as concomitant medication and must be documented on the Concomitant Medications CRF.

In consideration of the overlapping Northern Hemisphere influenza vaccination campaign, the use of seasonal flu vaccines during the period 3 weeks before to 3 weeks after vaccination on Days 0 and 21 is an exclusion criterion.

All subjects may continue therapy for chronic medical conditions provided that they are not listed in section 8 of the British National Formulary (BNF) or include chloroquine, gold or penicillamine or other drugs listed in section 10.1.3 of the BNF to suppress a chronic disease process. Subjects with chronic medical conditions must be maintained on a stable regimen for at least 2 weeks prior to study entry as assessed by the medical history.

Use of other medication including over-the-counter products should be discouraged during the study. Investigational drugs are prohibited during the course of the study.

The following concomitant treatments are discouraged and, if used, might lead to a major protocol violation and result in withdrawal of the subject from the study according to the medical judgment of the lead physician (See exclusion criteria, 6.7.3; and Removal of subjects from therapy or assessments, 6.7.5):

- Systemic steroids,
- Other immunosuppressive agents,
- Blood or plasma derivates, including immunoglobulin,
- Non-study vaccines (with the exception of post-exposure vaccinations in a medical emergency, e.g., hepatitis, rabies, tetanus) within 2 weeks.

6.7.5 Removal of subjects from therapy or assessments

The subject, or where applicable, the subject's legally acceptable representative(s) can withdraw consent for participation in the study at any time without prejudice. The investigator can withdraw a subject if, in his or her clinical judgment, it is in the best interest of the subject or if the subject cannot comply with the protocol.

In addition, a subject may not be eligible for subsequent immunization or may be discontinued from the study following occurrence of:

- Convulsions or any other neurological disturbances after vaccination,
- Hypersensitivity to the investigational vaccine,
- Other suspected side effects that could compromise the subject's wellbeing.

Any subject who, despite the requirement for adequate contraception, becomes pregnant during the trial will not receive further immunization. The site should maintain contact with the pregnant subject, and obtain pregnancy outcome information. It should be noted that pregnant women are at substantially increased risk from pandemic H1N1 influenza. WHO and ACIP recommend the administration of pandemic H1N1 vaccine to pregnant women. It is likely that the Department of Health will also recommend that pregnant women should receive pandemic H1N1 vaccine.

The subject will be followed up after withdrawal, the cause of which will be recorded in detail on the Study Termination CRF, and where appropriate, on the Adverse Events and/or Concomitant Medications CRF. Where the withdrawal of a subject resulted from an adverse event, this will be documented in accordance with the procedures in section 8.6.

Whenever possible, the tests and evaluations listed for the termination visit will be carried out.

Withdrawn subjects will not be replaced.

All subjects who have received investigational vaccines should be included in clinical events assessments, and all who provided pre-immunisation and post-immunisation blood samples at the scheduled times should be included in the immunogenicity assessments.

6.7.6 Stopping/pausing rule

There are no predetermined stopping rules other than those described above in 6.7.5.

7.0 VACCINES

7.1 Vaccines

All subjects in this study will be randomized to receive two doses of the two pandemic influenza vaccines purchased by the Government to confront the current H1N1 pandemic. Specifically the vaccine are:

(a) Baxter, plain (i.e., non-adjuvanted), whole virus, vero-cell-grown, influenza A/California/2009 (H1N1) pandemic vaccine, containing 7.5µg of viral HA per 0.5mL dose (given the trade name, Celvapan);

(b) GlaxoSmithKline, AS03-adjuvanted, split-product, egg-grown, influenza A/California/2009 (H1N1) pandemic vaccine, containing 3.75µg of viral HA and AS03 adjuvant composed of squalene, DL-α-tocopherol and polysorbate 80 (given the trade name, Pandemrix).

The vaccines will be supplied by the Department of Health as part of its initial consignment of vaccine from each manufacturer. It will be labelled, packaged, and supplied with a package information leaflet exactly as procured from the manufacturers for the Government's national pandemic influenza vaccine administration programme.

7.2 Vaccine labelling, storage, and packaging

All vaccine supplies must be stored between +2°C to +8°C, protected from light. The vaccine **must not** be frozen. Vaccines that have not been stored according to manufacturer's instructions **must not** be used. In the event that the vaccine cannot be used, the vaccine must be replaced with fresh stock. The Department of Health will supply the investigational H1N1 vaccine. The investigator (or pharmacist) will make an inventory and acknowledge receipt of all shipments of study vaccine.

7.3 Vaccine administration

The Principal Investigators at each study site will be responsible for the administration of the vaccine to subjects enrolled into the study according to the procedures stipulated in this study protocol. All vaccines will only be administered by personnel who are qualified to perform that function under applicable local laws and regulations for the specific study site.

The vaccine should be allowed to reach room temperature before use. The vaccine must be **gently shaken** and visually inspected before use. The vaccination site should be disinfected with a skin disinfectant (e.g., 70% alcohol). Before vaccination, the skin must be dry. **DO NOT inject intravascularly or subcutaneously.**

PRECAUTIONS TO BE OBSERVED IN ADMINISTERING STUDY VACCINE:

Study vaccines should not be administered to individuals with known hypersensitivity to any component of the vaccine.

An axillary temperature ≥38°C or serious active infection (with fever and systemic symptoms) are reasons for delaying vaccination.

Standard immunization practices should be observed and care should be taken to administer the injection intramuscularly. As with all injectable vaccines, appropriate medical treatment and supervision should be readily available in case of rare anaphylactic reactions following administration of the study vaccine. Epinephrine 1:1000 and Chlorphenamine (or equivalent adrenalin and antihistamine agents) should be available in case of any anaphylactic reactions. Care must be taken to ensure the vaccine is not injected into a blood vessel.

7.3.1 Administration of GSK Pandemrix vaccine

Nature and contents of container:

Pandemrix is supplied in multidose vials (type I glass) containing 2.5 ml vaccine (antigen) suspension (10 x 0.25 ml doses) with a stopper (butyl rubber), AND vials (type I glass) of 2.5 ml adjuvant (emulsion) (10 x 0.25 ml doses) with a stopper(butyl rubber).

Prior to administration, the two components should be mixed. The volume after mixing 1 vial of suspension (2.5 ml) with 1 vial of emulsion (2.5 ml) corresponds to 10 doses of vaccine (5 ml).

Instructions for mixing and administration of the vaccine:

1. Before mixing the two components, the emulsion and suspension should be allowed to reach room temperature, shaken and inspected visually for any foreign particulate matter and/or abnormal physical appearance. In the event of either being observed, discard the vaccine.
2. The vaccine is mixed by withdrawing the contents of the vial containing the emulsion (Vial B) by means of a syringe and by adding it to the vial containing the suspension (Vial A).

3. After the addition of the emulsion to the suspension, the mixture should be well shaken. The mixed vaccine is a whitish emulsion. In the event of other variation being observed, discard the vaccine.
4. The volume of Pandemrix (5 ml) after mixing corresponds to 10 doses of vaccine.
5. The vial should be shaken prior to each administration.
6. Each vaccine dose of 0.5 ml is withdrawn into a syringe for injection.
7. The needle used for withdrawal must be replaced by a needle suitable for intramuscular injection.

7.3.2 Administration of Baxter Celvapan vaccine

Celavapan is supplied in multidose vials (type I glass) of 5 ml suspension (10 x 0.5 ml doses) with a stopper (bromobutyl rubber). The vaccine should be allowed to reach room temperature before use. Shake before use. Each vaccine dose of 0.5 ml is withdrawn into a syringe for injection.

7.4 Method of assigning subjects to Baxter and GSK vaccine groups

It would be desirable if vaccinees could be assigned Baxter and GSK vaccines at the same time, i.e., both vaccines arrived in each centre before regulatory approval (ethics and MHRA) is obtained. This may not occur, so procedures will be put in place to meet the following scenarios:

A. Both vaccines arrive at each centre prior to regulatory approval:
B. Only one vaccine arrives at each centre prior to regulatory approval:

A. BOTH VACCINES ARRIVE AT EACH CENTRE PRIOR TO REGULATORY APPROVAL

We will use a block randomisation scheme to ensure that balance between vaccines is maintained and all volunteers are randomly allocated to groups. Subjects who meet the study admission criteria will be enrolled into the study and will be assigned a **5 digit subject number**:

The first digit identifies the study site (1 for Leicester; 2 for Nottingham; 3 for Sheffield).
The second digit reflects the age of the subjects on Day 0 (1 for 18-44; 5 for 45-64; and 9 for 65 years and older).
The following three digits identify the subject within the site and will be assigned sequentially, starting with 001 corresponding to the first subject enrolled within each age band.
Thus each centre will have three randomisation lists, each list corresponding to each age band.
Each centre will continue recruiting volunteers within an age band until the tally for the centres reaches a total of 120 subjects for that age band.
Volunteers in each age band will be randomised by a computer generated randomization code (1:1 proportions in block size(s) that will be determined by the statistician to ensure balance across groups). The randomisation code for each age band will be stored in individual, sequentially-numbered envelopes, specific for each centre, and will be opened by the nurse with responsibility for vaccine administration. The type of vaccine for administration will be printed on an adhesive label. The label will be peeled from its backing and entered into an appropriate space in the 'Vaccine Log', ensuring that the instruction and vaccine that is administered actually agree (See below).

Only one study nurse/doctor will be responsible for vaccine administration in each centre. This study nurse/doctor will be un-blinded with respect to the type of vaccine administered; he/she will play no

other role in the study. To maintain blinding, volunteers will be told to look away, both during preparation and administration of the vaccine. It is essential that volunteers do not see the syringe that is used or whether the vaccine is translucent or cloudy. The 'Vaccine' study nurse/doctor will document in the CRF: (a) the time of vaccine administration; and (b) the site of vaccine administration (LEFT or RIGHT – vaccine will normally be given into the non-dominant arm). The 'Vaccine' study nurse/doctor will record the volunteer's trial number, together with the vaccine that was administered (the adhesive label in the envelope) in the 'Vaccine Log'. The study nurse will check that the instruction in the envelope corresponds with the type of vaccine that is administered before and after each injection. He/she must inform the Principal Investigator immediately if the wrong vaccine was administered.

B. ONLY ONE VACCINE ARRIVES AT EACH CENTRE PRIOR TO REGULATORY APPROVAL:
This scenario is the one most likely to occur. It will be implemented if all regulatory approval is in place, AND only one of the two trial vaccines is available in each centre, AND the second vaccine will not arrive within five days of arrival of the first vaccine. Subjects who meet the study admission criteria will be enrolled into the study and will be assigned a **5 digit subject number**:

As before the statistician would generate three 'randomisation' lists for each centre, with a list for each age band.

As before, the first digit identifies the study site (1 for Leicester; 2 for Nottingham; 3 for Sheffield);
As before, the second digit reflects the age of the subjects on Day 0 (1 for 18-44; 5 for 45-64; and 9 for 65 years and older);

As before the next three digits identify the subject within the site.
HOWEVER, the distribution of the numbers will be randomised, with the first 60 numbers corresponding to the first vaccine to arrive (Vaccine 1), and the second 60 numbers corresponding to the second vaccine (Vaccine 2).

As an example, the first person to be vaccinated in Leicester could have the number 10054, i.e., with '1' corresponding to Leicester, ' 0' reflecting that he/she is aged 18-44 years, and '054' being the volunteer's unique trial number.

Each centre will continue recruiting volunteers within an age band until the tally for the centres reaches a total of 60 subjects for that age band for Vaccine 1. The same process will then be carried out with Vaccine 2, when it arrives.

Depending on the interval between arrival of GSK and Baxter vaccines, we will endeavour to ship sera from some volunteers who received Vaccine 2 in the first tranche of sera that was sent for analysis. The laboratory staff will not know when Vaccine 2 arrived and was first given, so will not know whether sera were collected from recipients of GSK or Baxter vaccine.

Vaccinees will not be told which vaccine they receive. They will be instructed to look away, both during preparation and administration of the vaccine. It is essential that volunteers do not see the syringe that is used or whether the vaccine is translucent or cloudy.

As before, only one study nurse/doctor will be responsible for vaccine administration in each centre. This study nurse/doctor will be un-blinded with respect to the type of vaccine administered; he/she will play no other role in the study.

The 'Vaccine' study nurse/doctor will record the volunteer's trial number, together with the vaccine that was administered (the adhesive label in the envelope) in the 'Vaccine Log'.

7.5 Adherence to randomisation

Vaccine will be given according to the randomization list. Subjects will not be able to choose between GSK or Baxter vaccines.

7.6 Code break

Should both vaccines arrive at each centre prior to regulatory approval and GSK and Baxter vaccines be allocated randomly, the Principal Investigators will be provided with a sealed envelope containing individual code-break envelopes for each subject. These would be opened in a medical emergency only should a serious adverse reaction (SAE) occur, defined as: requiring medical intervention; frank myonecrosis; ulceration, superinfection or phlebitis at the injection site; extreme pain or tenderness with complete limitation of use of arm; or severe intractable headache requiring repeated narcotic treatment. In the event of such reactions, the investigators will notify the Sponsor immediately and document the event in the Case Report Form (CRF).

7.7 Vaccination compliance

The site Principal Investigator will be responsible for adequate and accurate accounting of vaccine usage. The investigator or designee will administer the study vaccines only to individuals included in this study following the procedures set out in this study protocol. The date and time of vaccinations will be recorded. The investigator or delegate will track vaccines received, used and wasted and will retain all unused or expired products until it has been established that all accountability records are correct. Thereafter, all unused vaccines will be returned to the Department of Health or destroyed at the investigational site. An overall summary of vaccines supplied, received, wasted, used, and returned, re-assayed, or destroyed will be prepared at the conclusion of the study.

8.0 STUDY PROCEDURES

8.1 Clinical procedures

Informed consent must be obtained from the subject, or where applicable, the subject's legally acceptable representative(s) prior to the performance of any trial specific tests or evaluations, i.e., any unusual or non-routine procedures that involve risk, however trivial, to the subject.

The following procedures will be done during Visits 1 to 8:

Visit 1: Day 0: Screening and first vaccination

1. Subjects will be enrolled providing they meet inclusion and exclusion criteria and give fully informed, written consent.
2. For females of childbearing potential, perform a urine pregnancy test.

3. If the subject meets all the inclusion criteria and none of the exclusion criteria, assign a study subject number relevant to the subject's age and study centre (for further information please refer to paragraph 7.4.).
4. Obtain basic demography, including age, sex, ethnicity, previous influenza vaccination (past three seasons), and influenza-like illness (since May 2009).
5. Obtain and record significant medical history.
6. Record any current medications taken. Verify if the subject has taken an analgesic/antipyretic medication on Day 0 prior to study vaccination. Document this information on the subject's appropriate CRF.
7. Collect 10ml clotted blood pre-immunisation for baseline antibodies. Process blood and store serum for serology assays as described in section 8.2.
8. Record oral temperature.
9. The vaccine administrator will give vaccine according to the randomisation list (if GSK and Baxter vaccines are both available) by IM injection in the deltoid muscle of the upper non-dominant arm.
10. Examine the site of injection of the vaccine for local reactions at the end of 30 minutes. These findings and any systemic reactions will be recorded on the appropriate CRF page.
11. Instruct each subject in the evaluation of local and systemic reactions (e.g. how to measure the maximum diameter of induration and erythema in millimetres at the injection site and how to record temperature with a thermometer). As a guide for subsequent evaluations, enter the findings from the 30-minute post-injection evaluation onto the subject diary card.
12. Give the subject diary card and thermometer for immunisation reactions to subjects and instructions for its completion. Tell subjects to:
    - Complete the diary at approximately the same time each day;
    - Notify study personnel immediately if the subject experiences a SAE. Serious adverse events are defined in Sections 1.2 and 8.5
    - Return the diary card to the site on the Day 7 visit.
13. Schedule the Day 7 visit.

Visit 2: Day 7 (±1): Serology and Diary Card review

The following activities will be carried out on day 7 and recorded in the CRF:

1. Collect and review the 1st Immunisation Diary Card, including new medication/analgesia/ antipyretics taken during the preceding week (concomitant medication).
2. Remind subject to notify study personnel immediately if the subject experiences a SAE.
3. Collect 10ml clotted blood for serology. Process blood and store serum.
4. Schedule the Day 14 visit.

Visit 3: Day 14 (±2): Serology

The following activities will be carried out on day 14 and recorded in the CRF:

1. Collect 10ml clotted blood for serology. Process blood and store serum.
2. Remind subject to notify study personnel immediately if the subject experiences a SAE.
3. Schedule the Day 21 visit.

Visit 4: Day 21 (±2): Second vaccination

The following activities will be carried out on day 21 and recorded in the CRF:

1. Collect 10ml clotted blood for serology. Process blood and store serum.
2. For females of childbearing potential, perform a urine pregnancy test.
3. Check if the subject has taken an analgesic/antipyretic medication on Day 21 prior to study vaccination. Document this information on the subject's appropriate CRF.
4. Record oral temperature.
5. The vaccine administrator will give the same type of vaccine as before by IM injection in the deltoid muscle of the upper non-dominant arm.
6. Examine the site of injection of the vaccine for local reactions at the end of 30 minutes. These findings and any systemic reactions will be recorded on the appropriate CRF page.
7. Remind each subject in the evaluation of local and systemic reactions (e.g. how to measure the maximum diameter of induration and erythema in millimetres at the injection site and how to record temperature with a thermometer).
8. Give the second diary card. Tell subjects to:
    - Complete the diary at approximately the same time each day and to return it at the next visit;
    - Notify study personnel immediately if the subject experiences a SAE. Serious adverse events are defined in Sections 1.2 and 8.5.
    - Return the diary card to the site on the Day 28 visit.
9. Schedule the Day 28 visit.

Visit 5: Day 28 (±2): Serology and Diary Card review

The following activities will be carried out on day 28 and recorded in the CRF:

1. Collect and review the 2nd Immunisation Diary Card, including new medication/analgesia/ antipyretics taken during the preceding week (concomitant medication).
2. Remind subject to notify study personnel immediately if the subject experiences a SAE.
3. Collect 10ml clotted blood for serology. Process blood and store serum.
4. Schedule the Day 35 visit.

Visit 6: Day 35 (±3): Serology

The following activities will be carried out on day 35 and recorded in the CRF:

1. Collect 10ml clotted blood for serology. Process blood and store serum.
2. Remind subject to notify study personnel immediately if the subject experiences a SAE.
3. Schedule the Day 42 visit.

Visit 7: Day 42 (±3): Serology

The following activities will be carried out on day 42 and recorded in the CRF:

1. Collect 10ml clotted blood for serology. Process blood and store serum.
2. Remind subject to notify study personnel immediately if the subject experiences a SAE.

3. Schedule the Day 180 visit.

Visit 8: Day 180 (±10): Study termination

The following activities will be carried out on day 180 and recorded in the CRF:

1. Collect 10ml clotted blood for serology. Process blood and store serum.
2. Ensure Completion of all study termination CRFs.

8.2 Processing of samples for serology

At least 10 mL **serum** should be available for immunogenicity assays (HI, MN, and possibly SRH). On each of the scheduled days requiring serum samples, the clotted blood will be stored at 2°C to 8°C and centrifuged within 24 hours. Sera will be stored in triplicate in cryovials, with all cryovials labelled with the volunteer's trial code number, and day of collection. Samples should be stored frozen (below -14°C) until shipment.

8.3 Diary cards

Diary cards will be issued to all subjects instructing them to record their temperature and local and systemic symptoms at 6 and 24 hours after vaccination, and then daily for a total of 7 days, or longer should symptoms persist.

Information will be sought concerning the presence or absence of: redness at the injection site; local itching; local swelling; ulceration at the injection site; local pain; warm feeling at the injection site; tenderness to touch at the injection site; limitation of the use of the arm; headache; nausea; dizziness; diminished appetite; breathlessness; cough; coryza; wheeze; skin rash; generalised itching; fatigue; 'other' symptoms, use of antipyretic/relief medication and any changes in medication.

Subjects will score the severity of symptoms and effect on daily activities ranging from 0 (symptom absent), 1 (symptom occurred but not often or severe enough to cause inconvenience), 2 (symptom occurred often and severe enough to interfere with daily activities and requires no medical intervention), 3 (symptom occurred often, severe enough to markedly interfere with daily activities; requires medical intervention).

Subjects will also be asked to document any unsolicited symptoms.

Subjects will be instructed that in the event of any serious adverse event, he/she must notify the investigator immediately and be reviewed clinically. Serious adverse events are defined in (see Definitions (Sections 1.2 and 8.5).

8.4 Local and systemic reactions

The occurrence of selected indicators of reactogenicity (listed below), which by definition, can only occur up to 6 days post-vaccination, will be recorded on the Local and Systemic Reactions CRF rather than the Adverse Events CRF:

- ***Local Reactions:*** Ecchymosis, erythema, induration, swelling and pain at injection site;

- ***Systemic Reactions:*** Chills, malaise, myalgia, arthralgia, nausea, headache, sweating and fatigue;

- ***Other indicators of reactogenicity:*** Stayed at home due to reactions, oral temperature and use of analgesic/antipyretic medication.

8.5 Adverse events, SUSARS, and serious adverse events

An ***adverse event*** (AE) is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product at any dose that does not necessarily have to have a causal relationship with this treatment. An AE can, therefore be any unfavourable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of an investigational product, whether or not considered related to the investigational product. This definition includes intercurrent illnesses or injuries and exacerbation of pre-existing conditions.

All AEs will be monitored until resolution or, if the AE becomes chronic, a cause identified. If an AE is unresolved at the conclusion of the study, a clinical assessment will be made by the investigator and Medical Monitor whether continued follow-up of the AE is warranted

The severity of events reported on the Adverse Events CRF will be determined by the investigator as:

- **Mild:** Transient effect with no limitation in normal daily activity.
- **Moderate:** Some limitation in normal daily activity, but no medical attention needed.
- **Severe:** Unable to perform normal daily activity and requiring medical attention.

The relationship of the study treatment to an AE will be determined by the investigator based on the following definitions:

- **Not Related:** The AE is not related if exposure to the investigational vaccine has not occurred, **or** the occurrence of the AE is not reasonably related in time, **or** the AE is considered unlikely to be related to use of the investigational vaccine, i.e. there are no facts (evidence) or arguments to suggest a causal relationship.
- **Possibly Related:** The administration of the investigational vaccine and AE are considered reasonably related in time **and** the AE could be explained by causes other than exposure to the investigational vaccine
- **Probably Related:** Exposure to the investigational vaccine and AE are reasonably related in time **and** the investigational vaccine is more likely than other causes to be responsible for the AE, **or** is the most likely cause of the AE.

The relationship of the study treatment to an AE will be determined by the Principal Investigator.

A ***suspected unexpected serious adverse event*** (SUSAR) is one that is not listed in the current Summary of Product Characteristics or the Investigator's Brochure or an event that is by nature more specific or more severe than a listed event.

A ***serious adverse event*** (SAE) is defined as any untoward medical occurrence that at any dose:

- Results in death;

- Is life-threatening (i.e., the subject was, in the opinion of the investigator, at immediate risk of death from the event as it occurred); it does not refer to an event which hypothetically might have caused death if it were more severe;
- Requires or prolongs inpatient hospitalization;
- Results in persistent or significant disability/incapacity (i.e., the event causes a substantial disruption of a person's ability to conduct normal life functions);
- Results in a congenital anomaly/birth defect;
- Requires intervention to prevent permanent impairment or damage;
- Is an important and significant medical event that may not be immediately life threatening or resulting in death or hospitalization but, based upon appropriate medical judgment, may jeopardize the patient/subject or may require intervention to prevent one of the other outcomes listed above.

Of note: a "possible vaccine failure" should be reported as a serious AE only if it resulted in an infectious disease which should have been prevented by the vaccine implied.

Adverse events which do not fall into these categories are defined as **non-serious**. It should be noted that a severe adverse event need not be serious in nature and that a serious adverse event need not, by definition, be severe.

In addition, a pre-existing event or condition that results in hospitalization should be recorded on the medical history CRF. If the onset of an event occurred before the subject entered the trial (e.g., any pre-planned hospitalization for conditions like cosmetic treatments or for non-emergency routine visits for a pre-existing condition), the hospitalization would not lead to an AE being classified as serious unless, in the view of the investigator, hospitalization was prolonged as a result of participation in the clinical trial or was necessary due to a worsening of the pre-existing condition

8.6 Documentation/reporting of adverse and other clinical events

All study subjects will be observed for at least 30 minutes after a vaccination for evidence of immediate reactions in general and in particular for symptoms of allergic phenomena (such as rashes, itching, or other allergic manifestations). Each subject, or where applicable, the subject's legally acceptable representative(s) will be instructed to complete a diary card for 7 days following each administration, to describe local and systemic reactions and other selected indicators of reactogenicity. If a local and systemic reaction or fever (derived from measured oral temperatures $\geq$38.0°C) continues beyond the 7-day period after a vaccination, it will also be recorded on the Adverse Events CRF. If the subject recovers on the last day, then this fact will be recorded on the Local and Systemic Reaction CRF. All AEs must be reported and documented. The period of observation for adverse events extends from the time the subject receives vaccination through until 3 weeks after vaccination.

All AEs necessitating a physician's visit or consultation and/or leading to premature study discontinuation and all SAEs will be collected throughout the entire study and data will be reconciled at study termination.

All adverse events, regardless of severity, will be monitored by the investigator until resolution. All subjects experiencing adverse events - whether considered associated with the use of the study

vaccine or not - must be monitored until symptoms subside and any abnormal laboratory values have returned to baseline, or until there is a satisfactory explanation for the changes observed, or until death, in which case a full pathologist's report should be supplied, if possible. All findings must be reported on an Adverse Events CRF and on the "Serious Adverse Event" form, if necessary, which is part of the investigator's study file. All findings in subjects experiencing adverse events must be reported also in the subject's medical records.

In addition, any event resulting in a subject's withdrawal from subsequent vaccinations or from follow-up should be reported according to the protocol instructions.

All SAEs which occur during the course of the trial, whether considered to be associated with the study vaccination or not, have to be reported **within 24 hours** by telephone or fax to either of the following:

Study Sponsor: Mrs Carolyn Maloney, Research and Development, Leicester General Hospital, Gwendolen Road, Leicester LE4 5PW. Phone +44 116 258 4109; Fax; +44 116 258 4226.

Chief Investigator: Professor Karl Nicholson, Infectious Diseases Unit, Leicester Royal Infirmary, Leicester. Mob: +44 (0) 7880746939.

For trial related **emergencies out of office hours** please contact the Principal Investigators at each site
Principal Investigator, Leicester: Dr Iain Stephenson, Infectious Diseases Unit, Leicester Royal Infirmary, Leicester. Tel +44 116 258 6528; Fax +44 116 258 5067

Principal Investigator, Nottingham: Professor Jonathan Nguyen-Van-Tam, Room A40d Clinical Sciences Building, City Hospital, Nottingham NG5 1PB. Phone: +44 (0) 115 823 0276; Fax

Principal Investigator, Sheffield: Professor Robert Read, Royal Hallamshire Hospital, Sheffield.
Tel +44 (0) 114 272 4072

As far as possible, all points raised on the "Serious Adverse Event" form need to be addressed and faxed immediately to the Study Monitor. The original must be retained by the investigator. The event must also be documented on the Adverse Events CRF. After receipt of the initial report, the Study Monitor/Sponsor will review the information and contact the investigator if it is necessary to obtain further information for assessment of the event.

Any medication or other therapeutic measures used to treat the event will be recorded on the appropriate CRF(s) in addition to the outcome of the AE. Any serious adverse reaction must be reported to the EC in a timely manner, according to local regulations. Adequate documentation will be provided to the sponsor showing that the EC has been properly notified. The sponsor must also comply with the applicable regulatory requirement(s) relating to the reporting of unexpected serious and non-serious adverse drug reactions to the regulatory authority(ies) and the EC.

Post-Study Events

Any AE occurring at any time outside the observation period or after the end of the study and considered to be caused by the study vaccine - and therefore a possible adverse drug reaction - must be reported to the sponsor.

Halting criteria

Any serious adverse reaction will be reported to the sponsor and EC in accordance with the European Directive 2001/20/EC. If any serious adverse event is considered as probably related to the study vaccine, this will be considered by the Chief Investigator, study co-applicants, sponsor, and DH representatives who will make a collective decision to continue or halt the study.

8.7 Study monitoring/auditing

Investigators and/or their study staff will be trained at the latest during the initiation meeting. Monitoring and auditing procedures will be followed in order to comply with GCP guidelines and to ensure validity of the study data. During each monitoring visit source data verification will be performed by qualified staff.

8.7.1 Monitoring

The clinical study sites will be monitored by regular site visits and telephone calls to the investigator by qualified staff representing the Sponsor. By frequent communication, the site monitor will ensure that the study is conducted according to the protocol. CRFs and all original data collected at the site should be available for review during monitoring visits. During these visits, the site monitor should review drug accountability records and might review document retention including the Investigator's Study File. Additionally, the site monitor should check that clinical study procedures are observed and discuss any problems with the investigators.

8.7.2 Source data verification

Inspection and examination of CRFs and source documents (all original recordings, medical records) - giving due consideration to data protection and medical confidentiality - will be undertaken by representatives of the Sponsor.

All data not recorded directly on the CRFs as defined in section 8.7.3 of this study protocol will be verified by checking CRF entries against source documents in order to ensure that the data have been completely and accurately reported as required by the study protocol.

Source data verification will be performed and recorded following the sponsor's SOP. The subject or the subject's legally acceptable representative must also allow access to the subject's medical records, if required. Each subject, or the subject's legally acceptable representative, will be informed of this prior to the start of the study.

During or after the clinical study, the regulatory authorities, the EC and/or representatives of the sponsor may request access to all source documents, CRFs and other study documentation for on-site audit or inspection.

8.7.3 Documentation of study findings

All study data must be entered into the CRFs by the investigator who will sign and date the entries. If the investigator authorizes other persons in his/her staff to make entries on the CRF, the names, positions, signatures, and initials must be supplied to the sponsor.

The following data may be reported directly on the CRFs and are considered to be source data:

- Medical history,
- Vaccination time, Concomitant medication,
- Study termination, and
- Comments.

CRFs must be completed during/after each study visit.

A reasonable explanation must be given by the investigator for all missing data.

If corrections are made to entries in the CRF by the investigator or designates, the words or figures must be crossed through, leaving the initial entry legible. The correction must then be dated and initialed. Incorrect entries must not be covered with correcting fluid, obliterated, or made illegible in any way. If further corrections are made after review and signature by the investigator, he/she must confirm and endorse the changes by signing and dating the study termination CRF again.

As part of the conduct of the trial, the sponsor may have questions about the CRF data. These questions will be documented using Data Clarification Forms (DCFs). The investigator will file each of the DCFs for the trial.

8.8 Record retention

Investigators must retain all study records according to applicable regulations in a secure and safe facility. The investigator must notify the sponsor of any change in the location, disposition, or custody of the study files.

Upon completion of the study, all study documents will be collated and stored by the Chief Investigator.

The Committee for Human Medicinal Products (CHMP) requires retention for the maximum period of time permitted by the institution, but not less than 15 years. It is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained.

8.9 Data protection

The sponsor respects the subjects' rights to privacy and will ensure the confidentiality of their medical information in accordance with all applicable laws and regulations. The sponsor as Data Controller according to the European Directive on the protection of individuals with regard to the processing of personal data and on the free movement of such data [95/46/EC] confirms herewith compliance to Directive 95/46/EC in all stages of Data Management.

8.10 Changes in the conduct of the study or planned analysis

Planned changes in the conduct of the study will be described in protocol amendments; changes in the planned analysis will be described in the clinical study report. An amendment is a written description of change(s) to, or formal clarification of, a study protocol. The EC must be informed of all amendments and if necessary prior review and documented approval/favourable opinion must be sought for ethical aspects. Approval must also be obtained from the authorities, if necessary. Such amendment will be agreed upon by the sponsor, the investigator, the EC and authorities, if necessary, prior to implementation.

9.0 ANALYSIS

9.1 Blood sampling windows

Blood samples taken in the following time windows will be evaluable:

- Pre-immunization: Day 0
- Post-immunization: Day 7 (window: Day 6–Day 8)
  Day 14 (window: Day 12–Day 16)
  Day 21 (window: Day 19–Day 23)
  Day 28 (window; Day 26–Day 30)
  Day 35 (window: Day 32–Day 38)
  Day 42 (window: Day 39–Day 45)
  Day 180 (window: Day 170–Day 190)

9.2 Statistical methods, sample size, and analyses

9.2.1 Sample size

The aim of the trial is to establish whether GSK and Baxter vaccines satisfy all three CPMP criteria, and if so compare them in terms of immunogenicity (for each vaccine/age group and each vaccine type). The sample size is in line with standard practice. The protocols for seasonal EU vaccine clinical trials and the criteria for assessment have been standardised within the EU. They stipulate that trials should be done with groups of at least 50 subjects. We will recruit 60 per group, allowing for up to 17% dropout. This will enable for example the trial to detect a 10% to 20% difference in seroprotection and seroconversion rates at the 5% significance level with 80% power.

9.2.2 Definition of populations to be analysed

Definition of populations to be analyzed:

(a) All enrolled population

- All subjects in the enrolled population

(b) Full analysis set, Immunogenicity

- All subjects in the enrolled population who:
  - actually receive at least one dose of study vaccination, and
  - provide at least one evaluable serum sample both before and after baseline

(c) Per protocol (PP) population, Immunogenicity

- All subjects in the enrolled population who:

- receive all the relevant doses of vaccine correctly, and
- provide evaluable serum samples at the relevant time points, and
- have no major protocol violation as defined prior to statistical analysis

A major deviation is defined as a protocol deviation that is considered to have a significant impact on the immunogenicity result of the subject.

(d) Adverse events population

- All subjects who received at least one dose of the study vaccine who:
  - Provide post-baseline adverse events data

9.2.3 Analysis of demographic and baseline characteristics

Descriptive statistics (mean, standard deviation, median, minimum, and maximum) for age, together with distributions of subjects by sex and ethnic origin, previous influenza vaccination (during the past three seasons), recent influenza-like illness in the patient (since May 2009), and presence of pre-vaccination antibody to the vaccine strain will be summarized overall, for each vaccine group, and by age group.

9.2.4 Analysis of immunogenicity criteria

Blood samples for immunogenicity assays will be collected on Days 0 (prevaccination), 7, 14, 21, 28, 35, 42, and 180.

Antibody response will be evaluated by haemagglutination inhibition (HI), neutralization (MN), and possibly single-radial haemolysis (SRH) in all subjects.

HI and/or MN antibody titres need to be assessed rapidly and so will be analysed in three tranches:
1st tranche of sera measuring antibodies before and after 1st injection: Days 0, 7, 14, and 21
2nd tranche of sera measuring antibodies before and after 2nd injection: Days 21, 28, 35, and 42.
3rd tranche of sera measuring persistence of antibodies: Days 0, 42 and 180.

HI and MN assays will be performed at the Health Protection Agency Centre for Infections, Enteric, Respiratory & Neurological Virus Laboratory, London, UK. SRH tests may be done at the National Institute for Biological Standards and Control. Antibody titrations will be done in duplicate. Pre- and post-vaccination samples from each person will be titrated simultaneously, as specified in the CHMP guidance.[5] The titre assigned will be the geometric mean of two independent determinations.

9.2.5 Immunogenicity objectives

9.2.5.1 Primary immunogenicity objective

The primary immunogenicity objective is to evaluate the immunogenicity of Baxter cell-culture, non-adjuvanted, whole virus H1N1 vaccine, and GSK AS03-adjuvanted, split H1N1 vaccine with respect to Committee of Human Medicinal Products (CHMP) and FDA licensing criteria.

These criteria for adults aged between 18 and 60 years are, for sera collected 'approximately 3 weeks after vaccination':

- Number of seroconversions or significant increase in antihaemagglutinin antibody titres >40%
- Mean geometric increase >2.5
- The proportion of subjects achieving an HI titre of ≥40 or SRH titre of 25 mm^2 should be >70%

For adults aged over 60 years these criteria are:

- Number of seroconversions or significant increase in antihaemagglutinin antibody titres >30%
- Mean geometric increase >2.0
- The proportion of subjects achieving an HI titre of ≥40 or SRH titre of 25 mm^2 should be >60%

The measures of immunogenicity will be calculated as:

Geometric Mean Titre or Geometric Mean Area: For each vaccine/age group and each vaccine type, least squares GMTs for HI and MN data (GMAs for SRH data), associated 95% confidence interval and median, minimal, and maximal titre value will be determined for each visit, i.e., days 0, 7, 14, 21, 28, 35,42, and 180.

Geometric Mean Ratio (increase): For each vaccine/age group and each vaccine type, the least squares GMRs will be calculated for the HI, MN and SRH results for the following time points of the study: day 7/day 0, day 14/day 0, day 21/day 0, day 28/day 0, day 35/day 0, day 42/day 0, as well as the associated 95% confidence intervals and the median, minimal, and maximal n-fold increase. Statistical methods used to analyze GMRs will be identical to those described above for GMTs (GMAs).

Seroconversions: *Percentages of subjects with seroconversion (or significant increase in HI titre).* The number and proportion of subjects achieving seroconversion or significant increase in HI titres or SRH area from pre-immunization to each visit after first immunization will be tabulated for each vaccine/age group and each age group.

- Seroconversion is defined as negative pre-vaccination serum (<10 for HI, <4 for SRH) / positive post-vaccination titre (≥ 40 for HI, area ≥ 25 mm^2 for SRH).
- Significant increase in antibody titre/area is defined as at least a fourfold increase in HI or a 50% increase in area from non-negative pre-vaccination serum (≥ 10 for HI, ≥ 4 for SRH).

Seroprotection: *Percentages of subjects achieving each of the following thresholds: inverse HI titre ≥40, SRH area ≥25 mm^2:* The number and proportion of subjects achieving each threshold at each visit will be tabulated for each vaccine/age group and each vaccine type.
All statistical analyses for HI and SRH will be performed on the logarithmically (base 10) transformed values. Titres below the limit of detection for assays will be set to 1 in 5 for HI and 4mm^2 for SRH for the purposes of analysis. Original values will be presented in all listings

The above immunogenicity criteria will be applied to sera collected 21 days after completion of vaccination, i.e., 21 days after the second vaccine dose. For pandemic vaccines, all three criteria should be met.

Should both GSK and Baxter vaccines satisfy all three CPMP criteria, we will compare the immunogenicity of the two vaccines (for each vaccine/age group and each vaccine type) in terms of:

a. Geometric mean titres 21 days after each vaccination;
b. Geometric mean ratio increases 21 days after each vaccination;
c. Seroprotection rates 21 days after each vaccination;
d. Seroconversion rates 21 days after each vaccination.

Comparisons will be made using parametric or non-parametric tests as appropriate for continuous outcomes, and Pearson's chi-square test or Fisher's exact test where appropriate for categorical outcomes. Further analyses using (generalised) linear models will explore the effect of baseline covariates on the outcomes. For populations (a) and (b), analyses using multiple imputation to allow for missing data will also be undertaken as a sensitivity analysis. No formal adjustment will be made for multiple testing but associated P-values will be interpreted cautiously.

9.2.5.2 Secondary immunogenicity measures

The secondary immunogenicity measures are:

a. *To identify whether one or two doses of vaccine are required to satisfy the licensing criteria.* We will assess whether either GSK or Baxter vaccine is able to meet all three CPMP criteria 21 days after the first vaccine dose. This will be assessed for each vaccine/age group, and each vaccine type;
b. *To examine the kinetics of the antibody responses to vaccination.* This will be measured in terms of the ability of GSK or Baxter vaccine to meet any one, or all three, of the CPMP criteria 7 and 14 days after the first and second doses – for each vaccine/age group, and each vaccine type;
c. *To examine persistence of antibody at 6 months.* This will be assessed in terms of the proportion of vaccinees who have inverse HI antibody titres of $\geq$40, SRH areas of $\geq$25mm^2, and inverse MN antibody titres of $\geq$40 and $\geq$80. Antibody persistence will be assessed for each vaccine/age group, and each vaccine type;
d. *To evaluate the breadth of the antibody response to the antigenic variant* (if appropriate, i.e., an antigenic drift variant emerges prior to the 2010-2011 influenza season). This will be done using the new antigenic variant as test antigen in HI, MN, and possibly by SRH tests by comparing the above primary immunogenicity measures on days 21 and 42 for each vaccine, by age group, and all age groups combined.

As with the primary immunogenicity outcomes comparisons will be made using parametric or non-parametric tests as appropriate for continuous outcomes, and Pearson's chi-square test or Fisher's exact test where appropriate for categorical outcomes. Further analyses using (generalised) linear models will explore the effect of baseline covariates on the outcomes. For populations (a) and (b), analyses using multiple imputation to allow for missing data will also be undertaken as a sensitivity analysis. No formal adjustment will be made for multiple testing but associated P-values will be interpreted cautiously.

9.2.5.3 Exploratory analyses

The possible effect of previous seasonal influenza vaccination, age, presence of detectable levels of antibody before vaccination, as well as vaccine type – and any interactions will be explored using (generalised) linear models.

If one vaccine is available significantly before the other, then the trial will adopt a sequential allocation procedure switching to randomisation when the second one becomes available. If such a situation arises then the comparisons outlined in 9.2.5.1 will be non-randomised ones and potentially subject to bias (for example due to temporal effects). In this situation the use of date (time) of vaccination will also be used as an explanatory covariate in (generalised) linear models in order to explore the potential impact of this on the trial outcomes.

9.2.6 Analysis of local and systemic symptoms and adverse events

For the purpose of data partitioning and analysis, the study will be partitioned into the following broad time intervals to compare short term reactogenicity to vaccination:

- Vaccination to 48 hours;
- 48 hours to 7 days and
- Beyond 7 days.

Analysis will focus on comparisons of the incidence and severity of local and systemic reactions as indicated by the presence of specific symptoms, symptom scores, and temperature elevations.

9.2.6.1 Local and systemic symptoms

The incidences of local and systemic reactions following vaccination will be summarized by vaccine group.

The occurrence of selected indicators of reactogenicity (listed below), which by definition, can only occur up to 6 days post-vaccination, will be recorded on the Local and Systemic Reactions Diary/CRF rather than the Adverse Events CRF.

- *Local reactions:* Ecchymosis, erythema, induration, swelling and pain at injection site.
- *Systemic reactions:* Chills, malaise, myalgia, arthralgia, nausea, headache, sweating and fatigue
- *Other indicators of reactogenicity:* Stayed at home due to reactions, oral temperature, and use of analgesic/antipyretic medication.

If a reaction occurs more than once for a subject, the reaction will be classified according to the highest occurring severity.

All study subjects will be observed for at least 30 minutes after a vaccination for evidence of immediate reactions in general and in particular for symptoms of allergic phenomena (such as rashes, itching, or other allergic manifestations).

Each subject will be instructed to complete a diary card for 7 days following each vaccination, to describe local and systemic reactions and other selected indicators of reactogenicity. If a local and systemic reaction or fever (derived from measured oral temperatures ≥38.0°C) continues beyond the 7-day period after a vaccination, it will also be recorded on the Adverse Events CRF. If the subject recovers on the last day, then this fact will be recorded on the Local and Systemic Reaction CRF.

We will summarise the occurrence of solicited local and systemic symptoms (point estimates and 95%CI) by vaccine type in terms of incidence, intensity, and relation to vaccination. We will use the two-sided Fisher's exact tests to compare groups where appropriate.

Frequencies and percentages (together with 95% CIs) of subjects experiencing each reaction will be presented for each symptom severity. Summary tables showing the occurrence of any local or systemic reaction overall and at each time point will also be presented.

The severity of local reactions, including injection-site ecchymosis, erythema, swelling, and induration, will be categorized as none, 1 to ≤25 mm, 26 to ≤50 mm and >50 mm.

The severity of pain and systemic reactions will be categorized as none, mild (transient with no limitation in normal daily activity), moderate (some limitation in normal daily activity), and severe (unable to perform normal daily activity).

Distribution of body temperature, staying at home due to vaccine reaction and the use of analgesic/antipyretic medication occurring during 7 days after each vaccination will be tabulated. Fever will be defined as a temperature of ≥38.0 ^{o}C, and severe fever as ≥40.0^{o}C. Oral temperature will be categorized as < 38°C, 38°C to < 39°C, 39°C to < 40°C, and ≥ 40°C.

All post-vaccination reactions (local and systemic), use of relief medication and absence from work due to any adverse events will be summarized as none *versus* any.

For the local and systemic reaction safety variables, differences among the groups after vaccination will be analyzed by using Pearson's chi-square test or Fisher's exact test where appropriate.

9.2.6.2 Adverse events

The reporting and documentation of unsolicited events throughout the study is described in Sections 8.5 and 8.6.

The original verbatim terms used by investigators to identify adverse events in the CRFs will be mapped to preferred terms using the MedDRA dictionary. The adverse events will then be grouped by MedDRA preferred terms into frequency tables according to system organ class. All reported adverse events, as well as adverse events at least possibly related to study vaccine, will be summarized according to system organ class and preferred term within system organ class. These summaries will be presented by vaccination group. When an adverse event occurs more than once for a subject, the maximal severity and strongest relationship to the vaccine group will be counted.

Additionally, three separate summaries will be produced: (i) serious adverse events, (ii) adverse events that are possibly or probably related to vaccine, and (iii) adverse events that are unrelated to

vaccine. Data listings of all adverse events will be provided by subject. In addition, a listing of subjects withdrawn from the study because of an adverse event will be presented.

9.2.7 Interim/preliminary analyses

To provide DH with information as rapidly as possible with the goal of informing DH vaccination strategy, the following interim analyses of data from this study are planned:

- Solicited/unsolicited events (local and systemic symptoms, relief medication, and absence from work due to any adverse events) during:
  Days 0-6 (1st vaccination)
  Days 0-21 (1st vaccination)
  Days 21-27 (2nd vaccination)
  Days 21-41 (2nd vaccination)
- HI and MN antibody titres on days:
  Days 0, 7, 14, 21 (1st tranche of sera measuring antibodies before and after 1st injection)
  Days 21, 28, 35, 42 (2nd tranche of sera measuring antibodies before and after 2nd injection).

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