

**A RANDOMIZED CONTROLLED TRIAL TO COMPARE THE IMMUNOGENICITY OF SELF-  
ADMINISTERED AND NURSE-ADMINISTERED INTRADERMAL INFLUENZA VACCINE**

**CLINICAL TRIAL PROTOCOL**

**Clinical Trials Research Center**

**Canadian Center for Vaccinology, Halifax**

**Dalhousie University**

**IWK Health Centre**

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## 1. Background and Rationale

### 1.1 Introduction

Influenza is a highly contagious viral respiratory tract infection that affects the nose, throat, and lungs. The disease is caused by the influenza virus types A or B, which are highly infectious and spread primarily when an infected carrier coughs or sneezes. Influenza typically starts with a headache, chills and cough, followed rapidly by fever, loss of appetite, muscle aches, fatigue, running nose, sneezing, watery eyes and throat irritation. Nausea, vomiting and diarrhea may also occur, especially in children. Most people will recover from influenza within a week or ten days, but some including those over 65 and adults and children with chronic conditions, such as diabetes and cancer are at greater risk of more severe complications, such as pneumonia [1].

According to the World Health Organization (WHO), approximately 5-15% of the population is affected with upper respiratory tract infections during annual influenza epidemics. During these annual epidemics, roughly three to five million cases of influenza result in severe sickness, and 250,000 and 500,000 deaths occur. Most deaths associated with influenza occur among those over 65 years of age. Vaccination programs greatly reduce the incidence of the disease as well as the economic costs in treating influenza. Among healthy adults, influenza vaccine prevent 70% to 90% of influenza-specific illness. Among the elderly, the vaccine reduces severe illnesses and complications by up to 60%, and deaths by 80%[2].

The most commonly used route for delivery of inactivated influenza vaccines is intramuscular injection; however, other routes such as intradermal delivery of vaccine have shown equal or superior results in healthy persons between the ages of 18 and 49 years [3, 4]. Intradermal vaccination also has the advantage of using a lower dose per person than that required for intramuscular vaccine: in situations of vaccine shortages (eg. pandemics), this means that more persons can be vaccinated with the same vaccine supply.

In a randomized control trial, Kenney et al. demonstrated that intradermal administration of one fifth the standardized intramuscular dose of an inactivated trivalent influenza vaccine resulted in similar or superior immunogenicity as intramuscular injection. Subjects 18-40 years of age received an intradermal injection of one fifth the standard dose (3 µg of hemagglutinin per strain) and the increase in geometric mean hemagglutination-inhibition (HAI) titre on day 21 was compared to the standard intramuscular (15 µg of hemagglutinin per strain) vaccination group. Participants who received an intradermal vaccination of one fifth the dose had increases in geometric mean HAI titre by a factor of 15.2 for the H1N1 strain, 19.0 for H3N2 strain, and 12.4 for the B strain. In contrast, the intramuscular vaccination group had respective increases by a factor of 14.9, 7.1, and 15.3. The seroprotection and seroconversion rates against each of the three strains were not statistically significantly different in the two vaccination groups [4]. Similarly, Holland et al. demonstrated that intradermal vaccination elicited superior immune responses when compared with the intramuscular route in older adults [8]. Reduced dose intradermal influenza vaccination in children as young as 3 years of age (3 µg of hemagglutinin per strain) and in patients with solid tumours (7.5 µg of hemagglutinin per strain) elicited immune responses comparable to those elicited by a full dose of intramuscular vaccine [9, 10].

The efficiency of intradermal immunization is due to the direct and effective access of antigen to the immune system. The dermal skin layer has an extensive vascularized capillary and lymphatic vessel network along with a large number of dendritic cells, macrophages, and lymphocytes. Once antigen is administered to the dermis, it can activate T and B cell responses via 3 methods:

- (1) dendritic cells in the dermis capture the antigen, migrate to lymph nodes, and activate T and B cell responses
- (2) vaccine may diffuse into the epidermis through the basement membrane and be captured by epidermal Langerhan cells; and or
- (3) antigen can drain directly into lymph nodes where resident dendritic cells can capture and present antigen to T and B cells [5].

The diversity of pathways by which dendritic cells can acquire, process, and present antigen to T and B cells provides insight behind the mechanism of the high immunogenicity observed in intradermal vaccine administration. Dendritic cells are the most powerful antigen presenting cells and there is evidence that they induce cell-mediated immune responses via CD4+ and CD8+ T-cell activation [6]. In addition, dendritic cells have also been shown to be effective in enhancing antibody production by B cells indirectly via CD4+ T-cell activation and directly by binding surface integrin proteins on B cells [7]. Activation of both the humoral and cell mediated immune responses by dendritic cells makes intradermal vaccination a promising candidate for influenza vaccination programs.

The first intradermal delivery technique was performed by Mantoux in 1908 and it was used to deliver tuberculin intradermally to perform a tuberculosis diagnostic skin test [11]. The technique developed by Mantoux is currently used as the standard intradermal administration technique. The procedure involves using a 26 gauge, 3/8 inch, short bevel hypodermic needle attached to a 1 ml syringe. The need is inserted approximately parallel to the skin in order to gain entry into the dermal skin layer prior to injection of the active substance [12]. As previously stated, intradermal vaccine delivery provides immunological and economical advantages; however, the Mantoux technique is not commonly used for vaccination due to lack of ease, reliability, consistency, and efficiency of performing the technique in clinical practice. Effective vaccination using the Mantoux technique requires training and practice to correctly place the needle in the dermal layer [13]. Furthermore, the dose provided shows a great deal of variation due to inconsistency of syringe filling and air bubble purging. Finally, the Mantoux technique results in wasting of vaccine stores due to the vaccine left in the vial and syringe after injection, referred to as dead volume [14]. Due to the disadvantages associated with Mantoux technique, interests in developing intradermal vaccines by pharmaceutical companies have been scarce. The only vaccines currently delivered via intradermal route using the Mantoux technique are BCG and rabies [11].

Recently, BD (Becton-Dickinson, USA) has developed a new intradermal delivery technique. This microinjection technique uses a 1.5 mm, 30 gauge needle inserted perpendicular to the skin surface. Antigen can be delivered accurately, consistently, and easily into the dermis. The new microinjection technique is also associated with reduced pain: using a visual analogue scale for pain perception, the pain experienced by BD microinjection system was 10.2 as compared to 27.6 for the standard Mantoux technique 27.6 [11]. Studies of the BD Microinjection device conducted in large adult populations representing various ethnic groups have evaluated the consistency of depth and thickness of the dermal tissue at various anatomic sites appropriate for intradermal injection including the deltoid region, suprascapular region, waist and thigh. Mean 95% CI values of skin thickness in deltoid, suprascapular, and waist body sites are greater than the BD micro needle length (1.5mm) regardless of age, gender, ethnic origin, or BMI, thus ensuring intradermal delivery [12].

Recent studies using the BD microinjection system for influenza vaccine have demonstrated that using this system with a 0.1ml dose with 9ug of each influenza antigen in

persons aged 18-59 years of age achieves antibody levels that are non-inferior to those achieved using standard intramuscular influenza vaccines with the same antigens (15). In older adults in this age range, lower doses of antigen were associated with a reduced immunogenicity.

One other potential advantage of the new microinjection system is that vaccines can potentially be self-administered. Studies to date have shown that nurses without training can use this system successfully, and the device is simple enough to use that there is every reason to believe that it can be used to self-administer the vaccine. If this is true, self-administration may permit significant reductions in the cost of delivering vaccine (at a minimum to healthcare workers), as well as facilitating vaccination for shift workers, and reducing the need for training in currently organized peer-to-peer vaccination programs.

This study will therefore compare the immunogenicity and safety of nurse administration and self administration of the approved 2010/11 seasonal trivalent split –virus influenza intradermal vaccine Intanza in healthy adults aged 18-59 years of age.

There is growing evidence that stress, behaviour and social determinants of health are important mediators of immunity and risk for infectious diseases (16-20). Studies have shown that low socio-economic status is associated with increased risk of many infectious diseases (16), and others have shown that life stresses impact on immunogenicity to adult vaccinations (17-21). One recent study has demonstrated that short sleep duration was associated with increased susceptibility to experimental rhinovirus infection (22). Despite this evidence, studies of response to vaccination have not traditionally included any assessment of these risk putative risk factors for poor response. This is, in part, because easy to use measures do not exist. We will therefore use the opportunity of this study to ask if recent average sleep duration, stress as measured by the primary stress question on the Canadian Community Health survey, and socio-economic status (using postal code to impute the local deprivation index ([http://www.msss.gouv.qc.ca/statistiques/atlas/atlas/index.php?id\\_carte=331#da](http://www.msss.gouv.qc.ca/statistiques/atlas/atlas/index.php?id_carte=331#da)) are associated with the antibody reponse to influenza vaccination in healthy adults.

## **2. Objectives and Hypotheses**

### **2.1 Primary Hypotheses**

That the immunogenicity of self-administered intradermal 2010/11 seasonal influenza vaccine (Intanza) is non-inferior to that of nurse administered intradermal 2010/11 seasonal influenza vaccine. Specifically, that ratio of increase in geometric mean titres between day 0 and day 21 post-vaccination are greater than 2/3 (i.e.  $\text{GMT (IDS)}/\text{GMT (IMN)} > 0.65$ ).

### **2.2 Secondary Hypotheses**

- (i) that the reactogenicity of self-administered intradermal 2010/11 seasonal influenza vaccine (Intanza) is not significantly greater than that of nurse administered intradermal 2010/11 seasonal influenza vaccine.
- (ii) that 85% or more of healthy adults can self-administer Intanza successfully; that is, they are willing to self-administer vaccine and successfully administer it, as judged by observation by a trained nurse.
- (iii) that stress (as measured with a single question from the Canadian community health survey), socioeconomic status (as imputed from postal code of residence) and self-reported sleep duration of  $\leq 6$  hours per night is associated with reduced immunogenicity.

## **3. Trial Design**

### **3.1 Description and Justification of the Trial Design**

This will be an open randomized controlled trial. Nurses administering/observing vaccine will not be blinded. Staff following up with adverse events, and those measuring antibody levels will be blinded to subject allocation.

### **3.2 Randomization/Treatment Allocation Procedure/Stratification**

Randomization will be performed by randomize.net, with randomization balanced in blocks of four. Participants will be randomly assigned in a ratio of 1:1 to self-administered intradermal versus routine, nurse-administered intradermal groups.

### **3.3 Blinding and Code-Breaking Procedures**

The study nurse will use randomize.net to assign study codes. All other study staff (those responsible for assessment of adverse events and laboratory evaluation of immune response will be blinded to participant group assignment). In the event a code break is required, a paper copy of the randomization code will be stored in a locked filing cabinet which is accessible to the medical principal investigator or designate.

### **3.4 Trial Centers**

The trial will be conducted at the Clinical Trials Research Center of the Canadian Center for Vaccinology at Dalhousie University and the IWK Health Centre in Halifax, and the Infectious Disease Epidemiology Research Group at the Mount Sinai Hospital, University of Toronto. .

## **4. Trial Population**

### **4.1 Inclusion Criteria**

- Medically stable persons between age of 18-50 years of age (inclusive)
- Available during the trial period and for follow-up
- Able to read, understand, and sign informed consent
- Able to be contacted by telephone for follow-up of adverse events

### **4.2 Exclusion Criteria**

- Use of experimental vaccines within the month prior to study entry, or expected use of experimental or licensed vaccines or blood/blood products during the duration of the study.
- Receipt of immunoglobulin or other blood product within 3 months prior to enrollment
- Receipt of other licensed vaccines within the preceding 4 weeks
- History of a severe reaction following influenza vaccination
- Use of cytotoxic therapy or biologic modifiers in the previous 2 years.
- Plans to receive cytotoxic therapy during the study period.
- Concurrent acute moderate to severe illness. (Vaccination will be deferred until recovery. Subjects with mild illnesses with fever  $\leq 37.8^{\circ}\text{C}$  orally may be enrolled).
- History of medical disorder associated with immunosuppression (eg. HIV-infected individuals, transplant recipients)
- History of chronic lung, cardiac, renal or liver disease, which has required hospitalization within 1 the last year.
- Receipt of any high-dose daily systemic corticosteroids (inhaled steroids are acceptable) within two weeks of study entry. High dose is defined as a dose of 20 mg of prednisone daily or its equivalent. Topical steroids are allowed.
- Failure to give written, informed consent

- History of febrile illness (>37.8°C orally) within the past 72 hours (immunization may be deferred).
- Known allergy to eggs or other components of vaccine (i.e., thimerosal)
- History of Guillain-Barré Syndrome (GBS)

#### **4.3 Prior and Concomitant Therapy**

Prescription and nonprescription medications used 7 days prior to the vaccination visit will be recorded. Any concomitant medications used to treat any adverse events during the 7 day diary period will be recorded.

### **5. Trial Plan**

#### **5.1 Trial Timelines**

This is a randomized, open label controlled clinical trial. During October and November 2010, healthy adults aged 18 to 59 years (inclusive) who have not received an influenza vaccine within 6 months will be randomized in a 1:1 ratio to receive an inactivated split virion influenza vaccine formulated according to the WHO strain recommendations for the 2009 Southern Hemisphere influenza season. Group one will receive one 0.1ml dose of 9 µg /strain of the intradermal vaccine (Intanza) by self-administration in the non-dominant deltoid by the BD Microinjection system; Group 2 will receive one 0.1 ml dose of 9 µg/strain of the intradermal vaccine (Intanza) in the non-dominant deltoid, administered by a study nurse.

Participants will record solicited and unsolicited adverse events following immunization in a daily diary. Solicited local and systemic reactions will be collected for days 0 to 7, and serious adverse events will be recorded for days 8-21 at the final study visit on day 21.

**Day 0** Subjects will be screened for eligibility and informed consent obtained (eligible subjects who require additional time to review the information and decide on study participation may return at a later date). Ten millilitres of serum will be drawn for antibody testing against influenza. The subject will then be randomized. Those randomized to nurse-administered vaccine will receive their intradermal vaccine as usual. Those randomized to self-administered intradermal vaccine will be given a package with instructions for self-administration (Appendix A), and the vaccine. They will be permitted as long as necessary to read and understand the instructions.

The study nurse will observe the vaccine self-administration. S/he will record the time each subject required to read and understand the instructions, and the time required to administer the vaccine (subjects will be asked to tell the study nurse when they are ready to administer vaccine). If the subject decides that they cannot administer the vaccine by themselves, they will be withdrawn from the study, and offered vaccine. If the subject decides that they need the answers to one or two questions before proceeding, these questions will be recorded; the study nurse will answer them. Subjects who elect to try vaccinating themselves will be watched by the nurse. If the nurse identifies an error that is unsafe (finger touches needle prior to injection) or an obvious failure (vaccine is released from the syringe before the system is applied to the arm) then s/he will stop the procedure and the subject will be withdrawn and offered vaccination. If the subject makes such an error, recognizes it, and stops, they may try with a second dose of vaccine. The requirement for a second attempt will be recorded. If the subject continues the attempt, and is either successful or not definitely a failure (eg. nurse thinks that angle of syringe is too far from perpendicular, but fluid appears to have been injected

intradermally), the subject will continue in the trial. When they return for their day 21 blood, they will be told if the nurse was uncertain about the vaccination, and will then be offered a nurse administered dose of intradermal or intramuscular vaccine.

Post-immunization, subjects will be shown how to use the study diary; 15 minutes after vaccination they will record symptoms for the first time.

**Day 1-6** – Subjects will record symptoms in their daily diary each evening, and take and record their temperature. They will be asked to record any additional adverse events, any new medications (prescription or non-prescription) taken on that day, and any medical visits.

**Day 8 (day 7-9)** – On day 8, a telephone call will be used (may be a study visit if the subject prefers) to ensure that daily diaries have been completed, and to ask about any serious adverse events.

**Day 21 (± 3 days)** – At the final study visit, the subject will have 10 milliliters of blood drawn for serum antibodies, and an interview to identify any adverse events requiring medical care or missed work/school. Subjects in the self-administered Intanza group will be told their score in self-administration, and will be offered routine IM vaccination.

## 6. Adverse Events

|  | Day 0<br>Visit | Day 8<br>Telephone contact    | Day 21<br>Visit                      |
|--|----------------|-------------------------------|--------------------------------------|
|  | Enrolment      | 7-9 days post<br>immunization | Day 21 ± 3 days<br>post immunization |
| Informed consent                         | X              |                               |                                      |
| Review of inclusion/exclusion            | X              |                               |                                      |
| Medical history                          | X              |                               |                                      |
| Baseline Questionnaire                   | X              |                               |                                      |
| History-directed physical exam           | X              |                               |                                      |
| Informed consent                         | X              |                               |                                      |
| Obtain serology                          | X              |                               | X                                    |
| Vaccine administration                   | X              |                               |                                      |
| Record pain 10-15 minutes post-infection | X              |                               |                                      |
| Distribute daily diary                   | X              |                               |                                      |
| Review diary records                     |                | X                             |                                      |
| Collect daily diary                      |                |                               | X (or before)                        |
| Review adverse events                    |                | X                             | X                                    |

Solicited adverse events including systemic and injection-site reactions (redness, tenderness, and swelling) will be collected for 7 days following the vaccination. Any adverse events that resulted in a doctor or emergency room visit or resulted in missed work or school, and all serious adverse events for the participant will be collected for the duration of the study. What constitutes a medically significant event is at the discretion of the medical principal investigator.

### 6.1 Definitions

The following definitions are from the ICH E2A Guideline for Clinical Safety Data Management:

Definitions and Standards for Expedited Reporting:

Adverse event (AE)

An AE is any untoward medical occurrence in a patient or clinical investigation participant administered a pharmaceutical product and 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 a medicinal product, whether or not considered related to the medicinal product. The worsening of an existing sign or symptom is also considered as an AE. Therefore an AE may be

- a new illness;
- the worsening of a concomitant illness;
- an effect of vaccination, whether with the investigative or comparator product; or
- a combination of the above.

Surgical procedures are not adverse events; they are the action taken to treat a medical condition. It is the condition leading to the action taken that is the adverse event (if it occurs during the trial period). Surgery performed as a result of medical conditions that started prior to the trial, but did not worsen during the trial, are not to be reported as adverse events.

#### Serious adverse event (SAE)

*Serious* and *severe* are not synonymous. The term *severe* is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This is not the same as *serious*, which is based on patient or event outcome or action criteria usually associated with events that pose a threat to a patient's life or functioning. Seriousness, not severity, serves as a guide for defining regulatory reporting obligations.

An SAE is any untoward medical occurrence that at any dose (including overdose).

- results in death of participant;
- is life-threatening;  
*The term "life-threatening" refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event, which hypothetically might have caused death if it were more severe.*
- requires in-patient hospitalization or prolongation of existing hospitalization;  
*All medical events leading to hospitalizations will be recorded and reported as Serious Adverse Events, with the exception of the following: hospitalization planned before inclusion into the trial or outpatient hospitalization with no overnight hospitalization.*
- results in persistent or significant disability or incapacity;  
*"Persistent or significant disability or incapacity" means that there is a substantial disruption of a person's ability to carry out normal life functions.*
- is an important medical event.  
*Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious. Examples of such events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse, new onset diabetes, or autoimmune disease.*



Additionally, the following important medical events are to be considered as SAEs and reported according to the procedure described in section 6.3:

*Adverse drug reaction (ADR) or its synonym Adverse reaction:*

All noxious and unintended responses to a medicinal product related to any dose should be considered adverse drug reactions. (The phrase “responses to a medicinal product” means that a causal relationship between a medicinal product and an adverse event is at least a reasonable possibility.)

*Unexpected adverse drug reaction:*

An adverse reaction, the nature or severity of which is not consistent with the applicable product information.

## **6.2 Recording of Adverse Events**

At each study visit staff will record on site source documentation any medically significant adverse event that required a doctor or emergency room visit for the participants.

### **6.2.1 Relatedness of Adverse Events**

The medical principal investigator will evaluate the relationship of the adverse events based on the following definitions:

**Definite/Certain (yes)** are terms applied to an adverse events which have a timely relationship to the study vaccine and no alternative etiology is present. The adverse event must have occurred within a reasonable temporal sequence of the vaccine administration, must not be reasonably explained, and must follow a known pattern of response.

**Probable (yes)** means that the adverse event has a timely relation to the study vaccine and a potential alternative etiology is not apparent (i.e., fever or malaise when no other symptoms suggestive of an illness are present).

**Possible (yes)** means that the adverse event has a timely relation to the study vaccine; however, a potential alternative etiology exists, which may be responsible for the symptom (i.e., fever or malaise when other symptoms are present that suggest another etiology such as upper respiratory infection).

**Unrelated/Unlikely (no)** means that the adverse event is applied to those adverse events for which evidence exists that the symptom is definitely related to an etiology other than the study vaccine (i.e., auto accident, or a symptom suggestive of another illness that is not accepted to have a possible relatedness to the vaccine).

**Unknown** is noted when causality is unknown.

## **6.3 Reporting of Serious Adverse Events**

### **6.3.1 Reporting Procedure**

Participants will be asked to notify study staff immediately of any serious adverse event that occurs during the study period. All information will be recorded on the source document. The principal investigator will be notified immediately of any SAE. Any serious adverse event will be reported to the Biologics and Genetic Therapies Directorate (BGTD) of Health Canada and the Research Ethics Board directly by the sponsor (Canadian Center for Vaccinology) with copies of the reports sent to sanofi pasteur.

### **6.3.2 Trial Relationship to Vaccination**

The scale to assess the causal relationship between SAE and a product will be either related (yes) or unrelated (no):

No = no relationship

Yes = at least possibly related (i.e., possible, probable, or definite causal relationship)

### **6.3.3 Regulatory Requirements**

A report of a serious adverse drug experience will be followed by a written report to the REB, including a full description of the event and any consequences. All deaths during a participant's involvement in the study, whether considered vaccine-related or not, will be reported immediately to the REB. The principal investigator will report all serious adverse experiences/events associated with vaccine administration to the government agencies, adhering to timelines for reporting outlined in the Therapeutic Products Program Directorate Guidelines (ICH/GCP) and Guidelines for Reporting Adverse Events Associated with Vaccine Products (Canada Communicable Disease Report, Feb. 2000, Vol. 26S1).

## **7. Assessment**

### **7.1 Safety Assessment Methods**

#### **7.1.1 Safety Parameters Assessed During the Trial**

The safety parameters assessed during the trial are as follows:

1. Participants will be observed for 15 minutes for immediate reactions to the vaccination.
2. Solicited adverse events will be collected for 7 days following the immunization and recorded on a diary card (appendix B).
3. Participant perception of pain following vaccination using standardized visual analogue scale and verbal pain description scale.
4. Any medically significant adverse event that required a doctor or an emergency room visit during the study period for the participants and associated concomitant medications will be recorded and assessed.
5. Any serious adverse event that occurred for the participants during the study period and associated concomitant medications will be recorded and assessed.

##### **7.1.1.1 Immediate Reactogenicity**

Immediate reactions occurring within the first 15 minutes after administration of the study vaccine and treatment of the reactions will be reported to the principal medical investigator (i.e., hives, difficulty breathing, anaphylaxis and other severe reactions) and observations and treatment recorded.

##### **7.1.1.2 Unsolicited Medically Attended Adverse Events and Serious Adverse Events**

Throughout the study at each visit, study staff will record any medically significant adverse event for the participant that required a doctor or emergency room visit and any serious event that occurs during the study period. The participant will be provided a memory aid to record this information and will be asked to contact study staff if there is any serious adverse event. Serious adverse events will be followed until resolution or unless they develop into a chronic condition as determined by the medical principal investigator.

##### **7.1.1.3 Severity of Adverse Events**

The participant will rate the severity of local and systemic reactions using the following rating scale:

None = symptom was not present

Mild = aware of symptom but did not interfere with usual activities

Moderate = caused interference with usual activities

Severe = unable to go to work or do usual activities

### **7.2 Immunogenicity Assessment Methods**

Serum immunoglobulin G (IgG) antibodies against hemagglutinin for the three influenza strains will be measured at day 0 before vaccination and at 21 days after vaccination by the

haemagglutination-inhibition (HI) assay; titres will be reported in International Units per mL (IU/mL).

## **8. Data Analyses**

### **8.1 Responsibility for the Analysis**

The statistical analysis will be performed by the Clinical Trials Research Center in Halifax. The analysis will be under the direction of the Program Leader (Dr. Shelly McNeil) and the study statistician (Dr. Bruce Smith).

### **8.2 Hypotheses**

#### **8.1 Primary Hypotheses**

That the immunogenicity of self-administered intradermal 2010/11 seasonal influenza vaccine (Intanza) is non-inferior to that of nurse administered intradermal 2010/11 seasonal influenza vaccine (Intanza). Specifically, that ratio of increase in geometric mean titres between day 0 and day 21 post-vaccination are greater than 2/3 (i.e. GMT (IDS)/GMT (IMN) > 0.65).

#### **8.2 Secondary Hypotheses**

(i) that the reactogenicity of self-administered intradermal 2010/11 seasonal influenza vaccine (Intanza) is not significantly greater than that of nurse administered intradermal 2010/11 seasonal influenza vaccine.

(ii) that 85% or more of healthy adults can self-administer Intanza successfully; that is, they are willing to self-administer vaccine and successfully administer it, as judged by observation by a trained nurse.

#### **8.3 Variables and Timepoints of Measurement**

At day 0, baseline serum specimens will be collected and participants will then be randomized to receive one dose of self administered or nurse administered Intanza via deltoid route. At day 21, serum specimens will be collected and analyzed from both study groups.

Success in self-administration will be assessed by study nurses, using direct observation.

Reactogenicity will be assessed by comparing the rates of any local pain vs. none, local pain rated as moderate or severe on at least one day vs. pain consistently rated as mild or none, and as a total pain score (sum of visual analogue score over 7 days) in the two groups.

#### **8.4 Population to be Analysed**

The intent-to-treat population (ITT) is defined as all participants who are enrolled and randomized, and who are vaccinated successfully (i.e. excluding participants unable to attempt self-administered intradermal vaccine). The per-protocol population (PP) is defined as all participants who received the vaccine and had all samples taken according to the protocol schedule. All immunogenicity analyses will be performed in the ITT and PP population.

### **8.5 Statistical Methods and Determination of Sample Size**

#### **8.5.1 Immunogenicity Analysis**

Comparisons of immunogenicity will be made between the following groups:

1. Group 1: Patients immunized with Intanza by self administration (IDs)
2. Group 2: Patients immunized with Intanza by nurse administration (IDn)

For each group, the primary endpoints that will be measured will be strain specific geometric mean titres (GMTs) of anti-hemagglutinin antibodies for each of the three influenza strains 21 days after vaccination. Serum IgG antibody titres against hemagglutinin (HA) will be measured at day 0 before vaccination and at 21 days after vaccination by the haemagglutination-inhibition (HI) assay.

Immunogenicity will be further assessed by calculating the following for each strain in each group and their 95% confidence intervals:

- 1) Geometric mean titre ratio (GMTR) of post-vaccination titre to pre-vaccination titre ( $D_{21}/D_0$ )
- 2) Seroconversion rate: post-vaccination titre  $\geq 40$  IU/mL in subjects with a pre-vaccination titre  $< 10$  IU/mL
- 3) Significant titre increase rate:  $\geq 4$ -fold increase in titre after vaccination in subjects with a pre-vaccination titre  $\geq 10$  IU/mL
- 4) Seroprotection rate: percentage of subjects with a post-vaccination HI titre  $\geq 40$  IU/mL

Using the CHMP note for guidance, the proportion of participants in each group meeting the European CHMP criteria for immunogenicity will be compared. The EMEA/CHMP criteria for the annual relicensure of influenza vaccines are that at least one of the following criteria should be met for each strain:

- seroprotection rate  $> 70\%$
- seroconversion or significant increase rate  $> 40\%$
- GMTR  $\geq 2.5$

### **8.5.2 Safety Analysis**

Additional safety analysis includes the analysis of all solicited injection-site reaction and solicited systemic reaction at vaccination date (day 0) to 7 days post-vaccination; unsolicited adverse events (AEs) and serious adverse events will be analyzed from days 0 to 21. The proportion of subjects reporting local and/or systemic reactions, AEs, and SAEs will be summarized, together with confidence intervals. For AEs and SAEs, the number of events and their relationship to vaccination will be summarized. Solicited injection site reactions consist of induration, redness, tenderness, swelling, pain with arm movement, ecchymosis, and itching. Solicited systemic reactions consist of fever ( $\geq 38.5^\circ\text{C}$ ), malaise, shivering, myalgia, nausea, vomiting, and headache.

### **8.5.3 Statistical Methods**

The primary outcome of interest will be tested using a non-inferiority approach. The immunogenicity of self-administered intradermal vaccination (IDs) will be considered non-inferior to that of nurse-administered ID vaccine (IDn) if, for all three strains, the 95% confidence interval (CI) of the difference of the  $\log_{10}$  transformation of post-vaccination GMTs between IDs and IMN groups was greater than  $-0.176$ . An alternative method that IDs vaccination will be considered non-inferior to IDn if the ratio of the IDs to IDn 21-day geometric mean titre 95% CI is  $> 0.67$  for each strain.

**H<sub>0</sub>:**  $\log(\text{GMT IDs}) - \log(\text{GMT IDn}) \leq -0.176$  OR  $\text{GTM IDs} / \text{GTM IDn} \leq 0.67$

**H<sub>1</sub>:**  $\log(\text{GMT IDs}) - \log(\text{GMT IDn}) > -0.176$  OR  $\text{GMT IDs} / \text{GMT IDn} > 0.67$

For the analyses of continuous titre and fold-increase outcomes, a logarithmic scale will be used so that the distribution would be roughly Gaussian. Subsequently, this permits the use of regression models for the immunogenicity data and allows comparison of adjusted mean log outcomes among the groups.

The analysis of continuous variables will consist of point estimates and interval estimates for means, and differences between groups will be assessed using t-tests and analysis of variance. Logistic regression will be used to adjust for age, sex, BMI, and baseline titre.

For the analysis of proportions, binomial point estimates and exact binomial confidence intervals will be calculated for each group, and differences between groups will be compared using Fisher's exact tests. Logistic regression will be used to adjust for age, sex, BMI, and baseline titre.

Baseline comparability of treatment groups will be assessed using binomial estimates and Fisher's exact tests for binary variables; and t-tests and confidence intervals for continuous variables.

#### **8.5.4 Determination of Sample Size**

The sample size determination is for the primary immunogenicity analysis.

The alternative hypothesis is that the 21 day post-immunization geometric mean anti-haemagglutinin antibody titre using IDs administration is non-inferior to the titre using IDn vaccination. Non-inferiority is defined as the ratio of geometric mean titres being greater than 2/3 ( $\text{GMT (IDS)/GMT (IMN)} > 2/3$ ). Assuming a common standard deviation of log titres of 1.2 (Holland et al, 2008), a sample size of 111 per group provides power of 0.8 when testing at level 0.05. Allowing for a 10% non-evaluable rate, and for 10% of subjects in the Intanza arm to be unable/unwilling to attempt self-administration, the required sample size is 139 per group.

A second immunogenicity outcome concerns the proportion of subjects achieving seroprotective levels. Where  $p_{\text{IDs}}$  and  $p_{\text{IDn}}$  denote the probabilities that subjects in the self administered ID and nurse-administered ID will achieve a seroprotective level, and assuming equal seroprotective rates of at least 90%, the sample size of 111 evaluable subjects per group provides power of 80% to reject the inferiority hypothesis  $H_0: p_{\text{IDs}} + \delta \leq p_{\text{IDn}}$ , where the margin of non-inferiority is  $\delta=1$ .

With a combined sample of 222 evaluable subjects, the probability of detecting an adverse event occurring with a true frequency of at most 1% is greater than .89, and the probability of detecting an event with a frequency of at most 2% is greater than .98.

#### **8.6 Data Management**

Data management will be the responsibility of the Data Center of the Clinical Trials Research Center at the Canadian Center for Vaccinology, IWK Health Centre and Dalhousie University in Halifax. SAS<sup>®</sup> v.8 software on CTTC's IBM server will be used for data management. Integrity of the data will be assured by limiting access through passwords and account control. The IBM workstation is located in an environment that is protected from fire and water. Weekly archives of the data are stored off-site and daily incremental backups are performed.

Participant case report forms (CRFs) will be double data entered by two data entry staff working independently. The resulting SAS<sup>®</sup> datasets will be compared and corrected by the data manager or designate. Validity checks will be run and data clarifications forms will be issued as required for data corrections.

Laboratory test data received from the Canadian Center for Vaccinology lab will be translated to SAS<sup>®</sup> datasets and transferred to the IBM workstation for analysis.

### **9 Ethics**

#### **9.1 Ethical Conduct of the Trial**

The protocol, including the informed consent document and all recruiting materials, will be submitted to the Research Ethics Board for review and approval. No changes will be made to the protocol without REB approval, except where necessary to eliminate apparent immediate hazards

to participants. Participants will be able to withdraw their consents to participate at any time without prejudice. Additionally, the medical principal investigator may withdraw a participant if, in the investigator's clinical judgement, it is in the best interest of the participant or if the participant is unable to comply with study requirements.

The trial will be conducted in accordance with the latest version of the Declaration of Helsinki, GCP, ICH regulatory guidelines, and requirements regarding ethical committee review, informed consent, and other statutes and regulations regarding the protection of the rights and welfare participants participating in the study.

## **9.2 Benefits/Potential Risks**

A complete description of the potential risks and benefits can be found in the informed consent document and vaccine monographs.

## **9.3 Informed Consent Process and Documentation**

The principal investigator or designate will be responsible for presenting a full description of the research project including risks/benefits and how personal health information may be used and disclosed in research. A written informed consent/authorization will then be obtained from the participant prior to the screening procedures and vaccination. The principal investigator or designate will also be responsible for maintaining up-to-date records of the consent forms and providing a copy to the participant. See appendix 13.4 for a sample of the informed consent to be used in this trial.

Participants will be encouraged and will have ample opportunity to have their questions answered before and after consenting to participate. They are free to withdraw from the study at any time without giving a reason.

## **9.4 Stipends for Participation**

Participants will be provided a stipend of \$25 per visit to compensate for their time and travel required for each visit to the study site.

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