Protocol

Title:	**Comparison of Responses to Two Hepatitis A Vaccine Dosing Regimens Among Pediatric Patients with Autoimmune Diseases on Immunosuppressive Therapy: A Pilot Study**
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List of Abbreviations

ACH	Alberta Children's Hospital
AEFI	Adverse event following immunization
BMI	Body mass index, a measure of obesity
CI	Confidence interval
CLS	Calgary Laboratory Services
CRF	Case report form
Ecrf	Electronic case report form
GCP	Good clinical practices
GMT	Geometric mean titer
HAV	Hepatitis A Vaccine
IM	Intramuscular
NACI	National Advisory Committee on Immunization
PHAC	Public Health Agency of Canada
PID #	Personal identification number
PL	Provincial Laboratory of Alberta
CHREB	Calgary Health Research Ethics Board
SP	Sanofi Pasteur Vaccine Company
SAE	Serious adverse event
SID	Secondary identifier
SOP	Standard of practice
SST	Serum separator tube

INVESTIGATOR SUMMARY

The study is a prospective, single centre, double-blinded randomized controlled trial whose **goal** is to compare the immune response of a population of immunosuppressed pediatric patients with autoimmune diseases on immunosuppressive medications to two different doses of Hepatitis A vaccine. The objectives are (a) to confirm that adolescents, like their adult counterparts, have a reduced immune response to hepatitis A vaccine, and (b) to compare the immunogenicity of two different dosing options of vaccine for this age group after one and two doses. A total of 60 adolescents aged 12 – 15 years with confirmed autoimmune disease for which they are being treated with an immunosuppressive therapy will be recruited from the Rheumatology and Gastroenterology Clinic at Alberta Children's Hospital (ACH). Those found to have no immunity to hepatitis A will be enrolled. Informed, written consent will be obtained from the parent or guardian of subject, with assent obtained from the study subjects. Subjects will be randomly assigned to two doses of either Avaxim Pediatric® or Avaxim ® (adult) vaccine (Sanofi Pasteur Canada), six months apart, with hepatitis A titres done at baseline and one month after each dose. Both formulations are licensed for this age group.

1. INTRODUCTION

A variety of childhood autoimmune diseases such as granulomatosis with polyangiitis, juvenile idiopathic arthritis, inflammatory bowel disease or systemic lupus erythematosus are now controlled using immunosuppressive medications. These commonly include biologics such as tumor necrosis factor-, interleukin 1 or 6 - antagonists along with disease-modifying anti-rheumatic drugs (DMARDs) such as methotrexate. Disease control comes at the risk of increased infection. Many important childhood infections can be prevented with effective vaccines, most of which are administered early in life. For vaccines that can be safely given while on immunosuppressive medication, a blunted and/or rapidly waning immune response may occur. This has been demonstrated for several routine vaccines, but there is virtually no such data available for prevention of travel-related diseases in immunocompromised children.

Hepatitis A vaccine is given as 2 doses; in healthy pediatric hosts, 97-100% will have a protective response a month after the first dose, therefore they are protected for an imminent trip without completing the 2-dose series. Similar results are documented for all inactivated hepatitis A vaccines, which can be used interchangeably. The second dose is given in 6-12 months to ensure lasting protection. In adults with drug-induced immunosuppression, there are recent publications reporting significantly reduced responses to hepatitis A vaccine. An open label study in adults with rheumatoid arthritis on tumor necrosis factor-inhibitors and / or methotrexate, only 10% had an adequate immune response one month after the first dose. This rose to 83% a month after the second dose. This confirms the deleterious impact of immunosuppressive medications on response to this vaccine, most dramatically so in those on increasingly suppressive regimens. The increase in protective rates after the second dose suggests that given sufficient exposure to antigens in the vaccine, these individuals could mount a much better response. However it is unrealistic for most people to plan and prepare for their trips so far in advance, therefore it would be ideal to find a way of protecting immunosuppressed persons more rapidly, in other words after the first dose. An increased dose of vaccine has been shown to be successful in eliciting a protective immune response to hepatitis B in immunocompromised hosts, such as those on dialysis. There has been success with use of double-dose hepatitis A and B vaccines in such populations (personal communication – S. Kuhn).

Bringing these various considerations together, a pilot study of vaccine responsiveness of adolescents on immunosuppressive medications for autoimmune diseases to 2 different doses of Hepatitis A vaccine

will confirm the suboptimal response to this important travel vaccine in a pediatric immunocompromised population, and explore one means of improving it.

2. OBJECTIVES

The objectives of this study of adolescents aged 12 – 15 years with autoimmune diseases treated with immunosuppressive medication are:

1. To confirm whether this population has a reduced immune response to pediatric Hepatitis A vaccine compared to published data on immunocompetent adolescents; and

2. To compare the immunogenicity of 2 formulations of Hepatitis A Vaccine (pediatric and adult) after the first and second doses

3. STUDY DESIGN

This is a prospective, single centre, randomized, controlled trial comparing immune responses to two doses of a licensed Hepatitis A vaccine in children. Subjects will receive 2 doses (0.5 mL each) of the assigned study vaccine (Avaxim Pediatric® or Avaxim ® (adult) vaccine). One lot of each vaccine formulation will be studied. All subjects will provide safety observations using a diary and sequential blood samples will be obtained to measure serologic/immunologic responses.

4. STUDY PARTICIPANTS

4.1 Population/number of subjects

The study population will include 60 subjects, males and females, in the age range 12 – 15 years. The maximum age at enrollment is 15 years and 5 months, to allow for up to 7 months in which to complete 2 doses of vaccine before age 16 years.

4.2 Study center

This pilot will be conducted at the Alberta Children's Hospital site, with patients recruited through the ACH Rheumatology and Gastroenterology Clinics.

4.3 Inclusion criteria

Subjects must meet all of the following inclusion criteria and none of the exclusion criteria to participate in this study.

Criteria that apply to all subjects include:

- Written informed consent provided for the subject by a parent or legal guardian.

- Written informed assent from the participants themselves.

- Subjects whose parents the investigator believes can and will comply with the requirements of the protocol (i.e. return for follow-up visits, record safety observations, able to converse with study personnel and contactable by telephone).

- Age 12 years to 15 years and 5 months

- Confirmed autoimmune disease

- Maintained on any immunosuppressive medication with the exception of pulse steroids

4.4 Exclusion criteria

The following criteria apply:

- Systemic hypersensitivity to any Hepatitis A vaccine component such as neomycin, 2-phenoxyethanol, formaldehyde, aluminum hydroxide, Medium 199 Hanks, or Polysorbate 80.

- Thrombocytopenia or any bleeding disorder that contraindicates IM injection or blood collection.

- Receipt of blood or any blood-derived products (including IVIG) within the past 3 months.

- Previous laboratory-confirmed infection with Hepatitis A

- Previous vaccination with any Hepatitis A vaccine

The following conditions are **temporary or self-limiting exclusions** so a subject can be vaccinated once the condition has resolved and no other exclusion criteria exist:

- Current febrile illness or oral temperature of ≥ 38.5 °C (or equivalent by other route) or other moderate to severe illness within 24 hours before study vaccine administration

- Receipt of any Hepatitis B vaccine within 28 days of planned study vaccine doses. When Twinrix® has been studied, the response to Hepatitis B is amplified by the presence of Hepatitis A. It is unknown whether the reverse is also true.

- Routine childhood vaccines (except MMR and Varicella) must be separated from a study vaccination by at least 7 days.

4.5 Contraindications to subsequent blood draw

- Receipt of blood or blood-derived products
- New bleeding disorder that begins after Visit 1
- Receipt of non-study Hepatitis A vaccine

4.6 Contraindications to subsequent immunization

- Severe reaction to study Hepatitis A vaccine
- New bleeding disorder that contraindicates IM injection or blood collection.

- Receipt of blood or any blood-derived products.
- Receipt of non-study Hepatitis A vaccine

4.7 Deviations

Subjects may be retained in the study in the following circumstances but will be flagged as having deviated from the protocol:

- Serial blood samples provided but one or more beyond the accepted 7 day window
- Blood sample not obtained
- Vaccine administration outside protocol parameters (wrong formulation, dosage etc)
- Vaccine or blood samples stored outside cold-chain parameters

5. CONDUCT OF STUDY

5.1 Ethics and regulatory

5.1.1 Informed consent

Each authorized representative of a child subject must participate in the informed consent process and sign and date the informed consent form before any procedures specified in this protocol are performed. Given that all subjects are adolescents, informed assent will also be required.

The informed consent discussion, the informed consent and assent forms, and letter of information will include explanations of the following:

a) That the study involves research

b) The purpose of the study

c) The study treatment(s)

d) The study procedures to be followed, including all invasive procedures

e) The subject's responsibilities (or those of the subject's representative in the case of children)

f) The aspects of the study, that are experimental

g) The reasonably foreseeable risks or inconveniences to the subject

h) The reasonably expected benefits. When there is no intended clinical benefit to the subject, the subject should be made aware of this

i) The alternative procedure(s) or course(s) of treatment that may be available to the

subject, and their important potential benefits and risks

j) The compensation and/or treatment available to the subject in the event of trial-related injury

k) The anticipated pro-rated payment, if any, to the subject for participating in the trial

l) The anticipated expenses, if any, to the subject for participating in the trial

m) That the subject's participation in the trial is voluntary and that the subject may refuse to participate or withdraw from the trial at any time, without penalty or loss of benefits to which the subject is otherwise entitled

n) That any REB will be granted direct access to the subject's original medical (study) records for verification of clinical trial procedures and/or data, without violating the confidentiality of the subject, to the extent permitted by applicable laws and regulations and that, by signing a written informed consent form, the subject or the subject's legally acceptable representative is authorizing such access

o) That records identifying the subject will be kept confidential and, to the extent permitted by applicable laws and/or regulations, will not be made publicly available. If the results of the trial are published, the subject's identity will remain confidential

p) That the subject or the subject's legally acceptable representative will be informed in a timely manner if information becomes available that may be relevant to the subject's willingness to continue participation in the trial

q) The person(s) to contact for further information regarding the trial and the rights of trial subjects, and whom to contact in the event of trial-related injury

r) The foreseeable circumstances and/or reasons under which the subject's participation in the trial may be terminated

s) The expected duration of the subject's participation in the trial

t) The approximate number of subjects involved in the trial

5.1.2 **Regulatory considerations**

Both vaccine formulations used in this trial has been licensed for use in Canada in the age range of the subjects being studied.

5.1.3 **Monitoring**

Investigators will be made aware of any concerns with respect to vaccine reactions or changes to the stability of the underlying disease condition of the subject(s).

5.2 Outline of study procedures

5.2.1 Study outline

		Vaccine Dose #1		Vaccine Dose #2	
Activity	Screening (Visit 0)	Day 0 (Visit 1)	Day 28 (Visit 2)	Day 0 (Visit 3)	Day 28 (Visit 4)
Window			+7 days		+7 days
Recruitment	X				
Informed consent	X	X (can be done in advance)			
Eligibility review		X	X	X	X
Health assessment		X	X	X	X
Blood Sample	X	X	X	X	X
Randomization, Group A or B		X			
Vaccination		X		X	
Safety review			X		X
Safety monitoring instruction		X	X	X	X

5.2.2 Randomization Process

Subject group assignment will be determined following enrolment and eligibility confirmation by sealed envelope to be opened by the unblinded vaccination nurse. A randomly-generated list will assign study vaccine group by subject study number, and maintained by an unblinded Study Staff member. Given the narrow age range of subjects, they will not be stratified by age.

5.2.3 Study visit description

Visit 1

- Obtain written informed consent for study participation for each subject
- Obtain written informed assent for study participation for each subject
- Check inclusion and exclusion criteria
- Complete a health assessment and confirm / document an autoimunedisease and the immunosuppressive medication being used, as well as any other chronic health conditions.
- Collect blood sample (10-30 mL) from all subjects.
- Determine the randomized study vaccine assignment (unblinded vaccinating RN)

- Administer the assigned study vaccine, without divulging the formulation to the parent

- Observe subject for 15 minutes, with appropriate medical treatment readily available in case of an anaphylactic reaction

- Instruct the subject's parent/guardian to contact the investigator promptly to report any serious adverse event (SAE) occurring during the subsequent 6 days using a memory aid / diary supplied for that purpose

- Instruct the subject's parent/guardian to record daily any symptoms or signs of illness that occur during days 0 to 6 after vaccination using the diary.

- Advise the parent regarding appropriate medications and dosages to use if the adolescent requires treatment for discomfort or fever.

Visit 2 (Day 28 after immunization; window to Day 35)

- Confirm parent's ongoing consent / subject's ongoing assent to subject's participation

- Review parent's safety and health observations for subject for days 1-28 following vaccination, including the diary. If the safety review is delayed beyond day 28 - 35, it should remain focused on observations for days 7 to 28.

- Report any AEFI provincially as required, with a de-identified copy to VSS at PHAC and to the PCIRN Manager.

- Review contraindications to subsequent immunization and blood sample collection

- Collect blood sample (10-30 mL) from all subjects.

- Administer dose 2 of Hepatitis A vaccine.

- Observe subject for 15 minutes with appropriate medical treatment readily available in case of emergency

- Instruct the parent to contact the investigator promptly to report any serious adverse event (SAE) occurring during the subsequent 28 days

- Instruct the parent to record daily any symptoms or signs of illness that occur during days 0 to 6 after vaccination using the diary.

Visit 3 (Day 0 for Vaccine Dose #2; minimum 6 months after Dose #1 with 1 month window)

- Confirm parent's ongoing consent / subject's ongoing assent to subject's participation

- Review contraindications to subsequent immunization and blood sample collection

- Collect blood sample (10-30 mL) from all subjects.

- Administer dose 2 of Hepatitis A vaccine (same type as dose #1).

- Observe subject for 15 minutes with appropriate medical treatment readily available in case of emergency

- Instruct the parent to contact the investigator promptly to report any serious adverse event (SAE) occurring during the subsequent 28 days

- Instruct the parent to record daily any symptoms or signs of illness in a provided diary.

Visit 4 (28-35 days after Visit 3)

- Confirm parent's ongoing consent to subject's participation

- Review parent's safety and health observations for subject for days 1-28 following vaccination, including the diary. If the safety review is delayed beyond day 28, it should remain focused on observations for days 7 to 28.

- Report any AEFI provincially as required, with a de-identified copy to VSS at PHAC and to the PCIRN Manager. If the AEFI is an SAE ensure that it is provided to PHAC within 24 hours of being reported

- Review contraindications to subsequent blood sample collection

- Collect blood sample (10-30 mL) from all subjects.

- Obtain consent from subject's parent/guardian to participate in future trials eg to assess duration of protection after vaccination.

5.2.4 **Non-study vaccines**

Hepatitis B vaccinations should not be given while subjects are participating in the study as they could interfere with evaluation of the study vaccines. Subjects can receive any other routine childhood vaccines during the study if essential, provided those vaccinations are separated from a study vaccination by at least 7 days. Non-study Hepatitis A vaccines cannot be given.

5.2.5 **Health assessments**

A health history will be reviewed at Visit 1 to confirm an autoimmunedisease for which the subject is receiving immunosuppressive medications (except for pulse steroids); additional pre-existing conditions including whether or not any prescription medications are being taken for the condition will be noted (no medication details will be collected). The health assessment will be sufficiently detailed to confirm eligibility to participate (subject has no medical exclusions) and to identify a baseline for appropriate assessment and management of any reportable AEFIs. Height and weight measurements will be noted at enrolment.

5.2.6 **Data collection**

Data will be collected using source documentation and will be transferred as applicable to the electronic equivalent of a case report form. A secure, PC-based database program will be used to enter data.

The following data elements will be collected (along with safety data described in section 7.0):

Demographics: date of birth, gender.

Autoimmune condition: autoimmune disease, disease activity status, immunosuppressive medication.

History of Hepatitis A vaccination(s): lack of prior vaccination will be confirmed.

Height and weight, obtained at Visit 1. These measurements will be used to calculate BMI.

Protocol compliance: consent date and time, visit dates; immunization details (protocol adherence regarding administration and cold chain, product, date, time); blood sample details, vaccine cold chain); completion of safety reviews and dates.

5.3 **Laboratory**

5.3.1 **Sample collection and handling of specimens**

Blood (10-30 ml) as a source of serum/plasma will be taken by standard venipuncture and processed according to a standardized operating protocol. All serum/plasma specimens collected will be catalogued using Study Number, processed, aliquoted to limit repeated freeze-thaw cycles and then kept in storage at -80°C at a local lab at the University of Calgary until required for assessment.

5.3.2 **Serology testing**

Hepatitis A antibodies will be analyzed quantitative in serum using the commercially available immunoassay Elecsys Anti-HAV (Cobas®) according to the manufacturer's protocol (Roche Diagnostics GmbH, Mannheim, Germany). This testing will be performed by Dr. Andonov, Public Health Agency of Canada.

5.3.3 **Other tests**

Additional testing of the humoral and T-cell immune response in serum and/or plasma will be performed including cytokine profiles and autoantibodies (cross-reactions).

Serum samples will be tested for a spectrum of autoantibodies (Dr. Fritzler's lab, Calgary). These assays include a screening indirect immunofluorescence assay on HEp-2 substrate (Inova Diagnostics Inc., San Diego, CA); autoantigen arrays covering a spectrum of over 25 different well-established targets by ALBIA (TheraDiag, Paris, France), line immunoassays (Euroimmun, Lubeck, Germany) and BioFlash CIA (Inova).

Plasma cytokine/chemokine levels will be assessed using customized multiplexed laser bead assays provided by Eve Technologies (Calgary, AB), according to the manufacturer's instructions (For a

complete list of the cytokines/chemokines in the 64-plex array see:
https://www.evetechnologies.com/discoveryAssayListHuman.php).

In patients for whom consent is obtained, samples will be also stored for future immunology studies related to immunization responses.

6. VACCINES AND ADMINISTRATION

6.1 Hepatitis A study vaccines

The HAV vaccines to be used in this study will be produced by Sanofi Pasteur (Toronto, ON). Supplies will be obtained from single , following strict cold chain procedures. Each 0.5 mL dose of Avaxim Pediatric® contains 80 antigen units, while Avaxim® (adult) contains double this amount (160 antigen units). Both vaccines contain the following: excipients 2-phenoxyethanol, formaldehyde, aluminum hydroxide (expressed as aluminum to which HAV is adsorbed), Medium 199 Hanks, and polysorbate 80. Manufacturing process residuals include neomycin.

6.2. Dosage and administration of pandemic vaccines

The dosage to be evaluated is 0.5 mL of either formulation. Two doses will be given, separated by 6 – 7 months.

The procedure for administering AVAXIM® Pediatric or AVAXIM® is as follows: Shake the pre-filled syringe well until a uniform, cloudy suspension results. AVAXIM® Pediatric may be packaged with a choice of two needles. Select a needle of appropriate length to ensure that the vaccine will be delivered intramuscularly; in most adolescents this will be 25mm. Remove the tip cap from the syringe, take the chosen needle from the blister pack and fix to the tip of the pre-filled syringe. If a syringe with attached needle is present, the vaccine is ready to administer.

The vaccine is injected intramuscularly. The deltoid muscle is the preferred site. The non-dominant arm can be selected if preferred. Vaccinators can alternate between sides if desired. Vaccines must not be injected intravascularly. The full recommended dose of vaccine must be used.

6.3 Vaccine Formulation Blinding

Parents/guardians will not be told which study vaccine formulation is administered at the vaccination visits. This information will be provided when the study results are conveyed to parents.

6.4 Storage

Vaccines and adjuvant multi-dose vials should be stored refrigerated at 2° to 8°C, avoiding freezing (ref). Vaccine storage temperature should be monitored and recorded daily.

6.5 Allocation

Vaccine allocation is from the open supply of study vaccines. Documentation of wasted and replaced vaccine is not necessary.

7. VACCINE SAFETY

7.1 Immediate post-vaccination observation for anaphylaxis

Subjects should be observed for at least 15 minutes after the vaccination for any signs or symptoms of anaphylaxis. Research personnel should be equipped to assess such changes and administer appropriate initial medication.

7.2 Observations during days 0-6 post-vaccination

Parents will be asked to make daily notes of any illness symptoms in subjects during the week after vaccination, using a diary supplied for this purpose.

a) **injection site:** presence or absence of **local and armpit pain or tenderness**, and if present, the maximal severity on that day based on the most appropriate descriptive term as follows:

- mild: present but doesn't interfere with daily activities or limb motion
- moderate: interferes with daily activities or limb motion
- severe: prevents usual daily activities or limb motion

b) **injection site:** maximum diameter that day of any **redness** or **swelling, induration (lump)** based on daily measurements using a transparent device (supplied) with concentric circles 1, 2, 3, 4 and 5 cm diameter.

c) **fever:** daily (maximum) temperature to be measured and recorded each day using an electronic thermometer to be provided. Values ≥ 38.5 °C (oral or equivalent) will be defined as fever in the analysis. Measurements should be made each evening or whenever subjects feel feverish, with the highest value to be recorded. Either rectal or axillary measurements will be made, depending upon the age and cooperativeness of the child. Measurement site to be noted.

d) **general symptoms:** shivering, sweating, irritability, drowsiness, sleep disturbance decreased appetite to be noted and rated each day for severity as above.

e) **other (unsolicited) symptoms that interfered with daily activities** will be recorded, with start/stop dates and maximum severity.

f) **health impact:** The health impact of post-immunization symptoms will be described using assessment indicators such as requiring a doctor visit, ER visit, hospitalization, newly prescribed medication or change in baseline prescription medications or missed days of school or parent employment resulting from illness of the adolescent. The specific (most bothersome) type of symptom (local or specific systemic complaint) that compelled the parent to seek medical advice or the adolescent to miss school will be identified.

7.3 Observations during days 7-28 post-vaccination

Parents will be asked to record only significant health events affecting subjects (see 7.2 a-f) defined as events that required medical attention or interfered with daily activities. Adverse events that begin on day 7 or later and are still present on day 28 will be clinically followed to the final resolution date.

7.4 Reporting of Serious Adverse Events

Parents will be asked to advise the investigator as soon as possible of any Serious Adverse Event (SAE, defined below) that occurs while subjects are enrolled in the study Details of the SAE are to be explored in sufficient detail to enable the investigator to complete the provincial AEFI form and to assess the likelihood that it was caused by the vaccination. SAEs will be identified on the adverse event section of the e-CRF. Since the vaccine will have been approved for distribution, it will not be a requirement to report SAEs that are clearly unrelated to the study vaccination, such as respiratory virus infections.

7.5 Collecting safety observations from subjects

At Visits 2 and 4 parents will have a debriefing for post-vaccination days 1-28 by personal interview. While the window for post-vaccination visits is 28-35 days, the safety observations shall conclude on day 28.

Adverse events meeting the provincial criteria for reporting will be reported to the province in which they occur and to PHAC (VSS), using the prescribed federal-provincial reporting form.

8. SUBJECT COMPLETION AND WITHDRAWAL

Study staff will attempt to contact parents/guardians of subjects who do not return for a scheduled visit, making at least 4 attempts by various means of communication. Each attempt should be documented in the source document/study file.

A subject completing the last scheduled visit will be considered to have completed the study.

Withdrawals will not be replaced. Withdrawals will be documented in the study essential documents file, including the date of withdrawal and reason if known. All data on hand before withdrawal may be included in the study analysis.

9. STATISTICAL ANALYSIS

9.1 Choice of outcomes

The **serologic endpoints** will be based on evaluating antibody responses to HAV vaccine. Seroconversion is defined as change from a negative baseline result to a positive test (≥ 20 mIU/mL). A quantitative result will be provided for each positive result.

1. Primary Outcomes: Seroconversion after the first dose of vaccine.

2. Secondary Outcomes: After the second dose of vaccine
   a) Seroconversion rates
   b) Titre of seroconvertors

9.2 Data analysis plan

For comparison of proportions between study groups such as hepatitis A seroconversion the Chi-square test or Fisher's exact test will be used. For comparisons of quantitative variables including serologic titres, Student's t-test will be used. A P value of 0.05 will be considered statistically significant. Multivariate

logistic regression will be used if appropriate to adjust for gender, autoimmune disease, immunosuppressive medication, and presence of autoantibodies with respect to seroconversion status and final titre.

9.2.1 **Safety observations**

The 2 vaccines in this study are both licensed for use in the age group of the study population. Reactions will be documented in order to identify potential differences in the rates of local reactions and/or significant health events between groups.

9.2.2 **Immune responses**

The manufacturer's criteria for positive test result for HAV (≥ 20 mIU/mL) will be applied in the analysis for each formulation. Quantitative titres will be provided for every positive test result.

9.2.3 **Study completion timeline**

Field work should be completed within ~16 months of commencing vaccinations and analysis should be completed during the following 1 month. Time required to test sera will likely be 1 month, with data assembly and analysis taking a further 1-2 months. Thus the projected timeline from initial vaccination to full report completion is ~ 18 months.

9.3 **Sample size**

Assuming seroconversion rates of 35% (midway between 2 adult studies) to standard dose hepatitis A vaccine and 85% to double dose (response after 2 standard doses in adults), a minimum sample size of 36 (18 in each group; alpha 0.05, power 0.80) is required. Assuming that 20% of the population may be immune due to immigration (25% of the Calgary population are immigrants) from an endemic country, and a drop-out rate of 20%, a total of 60 patients will be enrolled.

9.4 **Data management**

A case report form (source document for local use) will be completed by study personnel at the time of enrolment and each subsequent visit or contact, documenting compliance with the protocol. A study identification number (SID) will be assigned to each subject and used to label the case report form and blood samples. Study personnel will transcribe case information as soon as possible into a secure PC-based electronic case report form (eCRF). Data edits will be checked by a data manager before individual subject files are locked for analysis. Back-up copies of the database will be made whenever new data is entered. No personal identifiers will be included in the database, access to which will be limited by pass code. Data will be viewable by authorized study personnel.

10. **OTHER**

10.1 **Protocol amendment procedures**

Any modification of this protocol will be filed and approved by with CHREB. Any administrative changes or amendments to this protocol will apply to all subjects.

10.2 **Records retention**

Data and study documents at all centres will be stored securely for 25 years beyond completion of the study. Destruction will occur in keeping with privacy and confidentiality regulations and guidelines in Canada and/or at the site and will be the responsibility of the site.

All personal information pertaining to subjects (such as mailing information) will be securely stored in a locked area and/or password protected AHS systems, under the supervision of the local study coordinator(s) and accessible only to local study staff. The storage location of study files must be clearly documented.

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