ICDDRB: Centre for Health & Population Research RRC APPLICATION FORM

RESEARCH PROTOCOL

2000-015

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FOR OFFICE USE ONLY
Protocol No:2000-15 Date received:
RRCApproval: Yes/ No Date:
ERC Approval: Yes/No Date:
AEEC Approval: Yes/No Date:

Project Title: A Prospective, Randomized, Double-Blind, Placebo-Controlled, Trial to Determine the Safety and Efficacy of Influenza Virus Vaccine, Trivalent, Types A & B, Live Cold-Adapted (CAIV-T) in Healthy Children

Theme and key words: Infectious disease; Inflenza, Vaccine, Immunogenecity, Bangldaesh

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Co-Principal Investigator(s): Dr. Zahid Hasan and Dr. A K Siddique
Co-Investigator(s): Dr. K Zaman, Dr. Shams El Arifeen, Dr. Anwar Hossain, Dr. R. Breiman
Intern Investigator: Dr. K Mizanur Rahman
Collaborating Institute(s) : Kumudini Hospital, Mirzapur, Bangladesh and Johns Hopkins University, USA

Population: Inclusion of special groups (*Check all that apply*):

Gender
- ☐ ✔ Male
- ☐ ✔ Females

Age
- ☐ ✔ 0 – 5 years
- ☐ 5 – 9 years
- ☐ 10 – 19 years
- ☐ 20 +
- ☐ > 65

- ☐ Pregnant Women
- ☐ Fetuses
- ☐ Prisoners
- ☐ Destitutes
- ☐ Service providers
- ☐ Cognitively Impaired
- ☐ CSW
- ☐ Others (specify _____)
- ☐ Animal

Project / study Site (*Check all the apply*):
- ☐ Dhaka Hospital
- ☐ Matlab Hospital
- ☐ Matlab DSS area
- ☐ Matlab non-DSS area
- ☐ ✔ Mirzapur
- ☐ Dhaka Community
- ☐ Chakaria
- ☐ Abhoynagar
- ☐ Mirsarai
- ☐ Patyia
- ☐ Other areas in Bangladesh
- ☐ Outside Bangladesh
  name of country: _____
- ☐ ✔ Multi centre trial
  (Name other countries involved)
  _____

Type of Study (*Check all that apply*):
- ☐ Case Control study
- ☐ ✔ Community based trial / intervention
- ☐ Program Project (Umbrella)
- ☐ Secondary Data Analysis
- ☐ Clinical Trial (Hospital/Clinic)
- ☐ Family follow-up study
- ☐ Cross sectional survey
- ☐ Longitudinal Study (cohort or follow-up)
- ☐ Record Review
- ☐ Prophylactic trial
- ☐ Surveillance / monitoring
- ☐ Others

Targeted Population *(Check all that apply)*:

- ☐ ✔ No ethnic selection (Bangladeshi)
- ☐ Bangalee
- ☐ Tribal groups
- ☐ Expatriates
- ☐ Immigrants
- ☐ Refugee

Consent Process (Check all that apply):

- ☐ ✔ Written
- ☐ Oral
- ☐ None
- ☐ ✔ Bengali language
- ☐ English language

Proposed Sample size: 300 from Bangladesh site, 3,000 from all sites combined

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Determination of Risk: Does the Research Involve *(Check all that apply)*:

- ☐ Human exposure to radioactive agents?
- ☐ Fetal tissue or abortus?
- ☐ Investigational new device?
  (specify_____)
- ☐ ✔ Existing data available from Co-investigator
- ☐ Human exposure to infectious agents?
- ☐ Investigational new drug
- ☐ Existing data available via public archives/source
- ☐ ✔ Pathological or diagnostic clinical specimen only
- ☐ Observation of public behavior
- ☐ New treatment regime

Yes/No

✔☐ ☐ Is the information recorded in such a manner that subjects can be identified from information provided directly or through identifiers linked to the subjects?

☐ ✔☐ Does the research deal with sensitive aspects of the subject's behavior; sexual behavior, alcohol use or illegal conduct such as drug use?

Could the information recorded about the individual if it became known outside of the research:

☐ ✔☐ a. place the subject at risk of criminal or civil liability?

☐ ✔☐ b. damage the subject's financial standing, reputation or employability; social rejection, lead to stigma, divorce etc.

Do you consider this research *(Check one)*:

- ☐ greater than minimal risk
- ☐ no risk
- ☐ ✔ no more than minimal risk
- ☐ only part of the diagnostic test

Minimal Risk is "a risk where the probability and magnitude of harm or discomfort anticipated in the proposed research are not greater in and of themselves than those ordinarily encountered in daily life or during the performance of routine physical, psychological examinations or tests. For example, the risk of drawing a small amount of blood from a healthy individual for research purposes is no greater than the risk of doing so as a part of routine physical examination".

Yes/No

☑ ☐ Is the proposal funded?

If yes, sponsor Name: Wyeth Lederle Vaccines, NY, USA

Is the proposal being submitted for funding ?

☐ ☑ If yes, name of funding agency: _____

Do any of the participating investigators and/or their immediate families have an equity relationship (e.g. stockholder) with the sponsor of the project or manufacturer and/or owner of the test product or device to be studied or serve as a consultant to any of the above?

IF YES, submit a written statement of disclosure to the Director. *Not applicable*

Dates of Proposed Period of Support	Cost Required for the Budget Period ($)
(Day, Month, Year - DD/MM/YY)	*a. 1st Year* *2nd Year* *3rd Year* *Other years*
Beginning date September 1, 2000	150,502 137,564 65,267 -
End date February 28, 2003	*b. Direct Cost:* 282,667 *Total Cost:* 353,334

Approval of the Project by the Division Director of the Applicant

The above-mentioned project has been discussed and reviewed at the Division level as well by the external reviewers. The protocol has been revised according to the reviewer's comments and is approved.

Professor Lars Ake Persson
Name of the Division Director

Signature

5/6/2000
Date of Approval

Certification by the Principal Investigator

I certify that the statements herein are true, complete and accurate to the best of my knowledge. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application.

Malyni
Signature of PI

June 5, 2000
Date:

Name of Contact Person (if applicable)

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Resources, Coordination, Collaboration, and Study Site

Personnel and Responsibilities:

Dr. Abdullah H Baqui: Principal investigator responsible for:

1. Protocol development, modifications, submission to review committees at ICDDR,B, and Bangladesh Drug Adminstration, Government of Bangladesh.
2. Study oversight, including subject recruitment, screening, monitoring; personnel hiring , training and oversight ; submission of timely reports as needed; local budget management; study integrity and quality.
3. Data compilation, analysis, interpretation and security.
4. Manuscript preparation and submission to scientific journals.

Dr. Zahid Hassan and Dr. A.K. Siddique: Co- Principal Investigators responsible for:

1. Ensure implementation of the project and quality collection of data.
2. Submission of timely reports.
3. Data Compilation, analysis, interpretation and security.
4. Manuscript preparation and submission to scientific journals.

Dr. K. Zaman, Dr. Shams El Arifeen, Dr. Anowar Hossain, Dr. R Braiemen, Dr. K M Rahman : Recruitment of subjects, randomization, supervision of field activities, laboratory testing, study monitoring, data compilation and analysis.

Dr. A. N. Alam, Head Training and Education Department will be the independent local safety monitor.

Study site and collaboration:

Field site: Mirzapur sub-district of Bangladesh in collaboration with Kumudini Hospital, Mirzapur, Bangladesh, Weyth Lederle Vaccines, USA and Johns Hopkins University, USA. The proposed PI is a full-time faculty of the Johns Hopkins University and seconded to ICDDR,B to work on a part-time basis. Kumudini Hospital will provide assistance with field implementation of the study.

All subject recruitment and screening will be conducted in Mirzapur. Limited laboratory support and required clinical care to study subjects will be provided by Kumudini Hospital. Blood samples will be taken in Mirzapur, then transported to Dhaka for processing. Volunteers will be recruited from villages around the Kumudini Hospital. Data management support will be provided by the ICDDR,B Child Health Program. Data forms collected in Mirzapur will be transported to Dhaka for entry and analysis.

ICDDR,B facilities in Dhaka, Bangladesh will provide laboratory support in terms of supplies for the field site.

Project Summary

This is a randomized, double-blind, placebo-controlled, multinational, multicenter outpatient study to assess the safety and efficacy of a primary series of two doses of Influenza Virus Vaccine, Trivalent, Types A & B, Live Cold-Adapted (CAIV-T) in children 6-35 months of age. Approximately 3,000 children will be enrolled at multiple study sites in Asia. About 300 children will be enrolled in the study from Mirzapur sub-district of Bangladesh.

Approximately 1,800 (60%) children will receive the two doses of the vaccine and they will be evaluated in comparison to approximately 1200 (40%) recipients of a primary series of two doses of placebo. At the start of the second year, study subjects who completed the two dose primary series will be randomized again to receive either a single dose of CAIV-T or placebo to evaluate the efficacy of the vaccine over a second year period. Healthy children age 6-35 months who meet all the inclusion criteria and none of the exclusion criteria will be eligible to participate in this study. Children with current upper respiratory illness including the common cold or nasal congestion requiring medical intervention, treatment or special clinical management within 72 hours of vaccination will have their participation deferred until the illness resolves.

Liquid CAIV-T consists of three cold-adapted reassortants grown in specific pathogen-free eggs. Placebo consists of physiological saline. The total volume of 0.2 mL of the vaccine or placebo will be administered intranasally with a spray applicator (approximately 0.1 mL into each nostril). Each subject will be required to participate for approximately 24 months. In the first year of the trial, participants will be recruited over approximately a 4 to 6 week period beginning September 1, 2000, and receive two sequential doses of study vaccine or placebo at least one month apart. In the second year, the study subjects will receive a single dose of study vaccine or placebo in a 4 to 6 week period beginning September 1, 2001. Surveillance for influenza illness will be conducted each week by home visits. The criteria for obtaining a nasal swab for viral culture are either one sign, symptom, or condition in Category A, or at least two signs, symptoms, or conditions from Category B (below) during the surveillance period:

- Category A – Fever (≥38°C rectal or oral, or ≥37.5°C axillary), Wheezing, Shortness of breath, Pulmonary congestion, Pneumonia, Ear infection (acute otitis media, suspected or diagnosed).
- Category B – Runny nose or nasal congestion (rhinorrhea), Sore throat (pharyngitis), Cough, Muscle aches, Chills, Headache, Irritability, Decreased activity, Vomiting.

Once the criteria are met, a viral culture will be obtained and an illness worksheet completed. However, a viral culture may also be obtained if, in the opinion of the Investigator, the symptom complex so warrants. Nasal swab specimens will be obtained and shipped to Weyth Lederle Vaccines (WLV) for culture. Sera will be obtained from a subset of approximately 20 children on 5 occasions over the two years as follows: prior to the first dose, 1 month following completion of the second dose of the primary vaccination series, prior to the second year's dose, 1 month following the second year's dose, and at the completion of the second year. The samples will be shipped to WLV for assaying for vaccine-type influenza serum haemagglutination inhibition (HAI) antibody. Immuno-genicity will be evaluated by comparison of pre- and post-vaccination strain-specific titers of serum antibody by HAI. Seroconversion will be defined as a four-fold or greater rise in titer. Nasal wash fluid will be

obtained from the same subset of subjects 4 times over the two year study period for determination of specific IgA antibody.

Following each immunization, the child will be visited daily by a health worker who will collect information regarding axillary temperature, runny nose/nasal congestion, cough, vomiting, decreased appetite, activity level, and irritability for 11 days including the day of immunization. Parents/caregivers will be asked to contact the study site should the subject develop any clinically significant illness/event. Information on serious adverse events, including hospitalizations, will be collected and monitored for the entire duration of the study. Incidence rates of adverse events and reactogenicity adverse events will be compared between treatment groups by Fisher's Exact test.

The primary efficacy endpoint is the first episode during the first year in a study child of a culture-confirmed influenza illness, caused by community-acquired subtypes antigenically similar to those contained in the vaccine, following receipt of the second dose of study vaccine or placebo. The secondary efficacy endpoint is the first episode during the second year in a study child of a culture-confirmed influenza illness, caused by community-acquired subtypes antigenically similar to those contained in the vaccine, following receipt of a primary series of two doses of vaccine or placebo and one dose during second year. Point and interval estimates of efficacy will be reported for each efficacy comparison of the study objectives. The superiority test of CAIV-T versus placebo will be based on the point and interval estimate of the efficacy ($e=1- I_v/I_p$, where I_v and I_p represent the incidence rates in the vaccinated and control groups respectively). If the lower limit of the 95% confidence interval is greater than zero then the vaccine is significantly superior to placebo.

Description of the Research Project

1. STUDY OBJECTIVES AND PURPOSE

1.1 Primary Objective

- To compare the efficacy over one year against culture-confirmed influenza-illness caused by community-acquired subtypes antigenically similar to those contained in the vaccine, in children aged 6 months to less than 36 months at enrollment, of a primary series consisting of two doses of a liquid formulation of influenza virus vaccine, trivalent, types A and B, live, cold-adapted (CAIV-T) with placebo administered at the beginning of the first year (Study group 1.1 vs. Study group 1.2).

1.2 Secondary Objectives

- To compare the efficacy over a second year against culture-confirmed influenza-illness caused by community-acquired subtypes antigenically similar to those contained in the vaccine, in children given placebo at the beginning of the second year, and who were aged 6 months to less than 36 months at enrollment and received at the beginning of the first year a primary series of two doses of either CAIV-T or placebo (Study group 2.2 vs. Study group 2.4);
- To compare the efficacy over a second year against culture-confirmed influenza-illness caused by community-acquired subtypes antigenically similar to those contained in the vaccine, in children given either CAIV-T or placebo at the beginning of the second year, and who were aged 6 months to less than 36 months at enrollment and received at the beginning of the first year a primary series of two doses of CAIV-T (Study group 2.1 vs. Study group 2.2);
- To compare the efficacy over a second year against culture-confirmed influenza-illness caused by community-acquired subtypes antigenically similar to those contained in the vaccine, in children given CAIV-T at the beginning of the second year, and who were aged 6 months to less than 36 months at enrollment and received at the beginning of the first year a primary series of two doses of CAIV-T, or given placebo at the beginning of the second year, and who were aged 6 months to less than 36 months at enrollment and received at the beginning of the first year a primary series of two doses of placebo (Study group 2.1 vs. Study group 2.4);
- To compare the efficacy over a second year against culture-confirmed influenza-illness caused by community-acquired subtypes antigenically similar to those contained in the vaccine, in children given CAIV-T at the beginning of the second year, and who were aged 6 months to less than 36 months at enrollment and received at the beginning of the first year a primary series of two doses of either CAIV-T or placebo (Study group 2.1 vs. Study group 2.3);
- To compare the efficacy over two consecutive seasons against culture-confirmed influenza-illness of any subtype, in children aged 6 months to less than 36 months at enrollment (above Objectives but for culture-confirmed influenza-illness associated with any influenza virus strain or subtype) (Study group 2.1 vs. Study group 2.4);
- To assess the efficacy of CAIV-T vaccine against clinically-defined acute otitis media, febrile otitis media, and influenza-associated otitis media (first year comparison: Study

group 1.1 vs. Study group 1.2; second year comparison: Study group 2.1 vs. Study group 2.4).

1.3 Safety Objective

To assess the safety and tolerability of CAIV-T in healthy children.

2. BACKGROUND OF THE PROJECT

2.1 Name and Description of the Investigational Material

Influenza virus vaccine, trivalent, Types A & B, live cold-adapted (CAIV-T) co-produced by Aviron (Mountain View, CA, USA) and Wyeth Lederle Vaccines (WLV) (Marietta, PA, USA) is based on two master donor strains, A/Ann Arbor/6/60 and B/Ann Arbor/1/66, that were developed three decades ago by H.F. Maassab (1). CAIV-T contains three components: two attenuated influenza A strains, H1N1 and H3N2, based on the influenza A master donor strain and one attenuated influenza B strain based on the influenza B master donor strain. Two of the eight gene segments of the master donor are replaced by gene segment coding for the hemagglutinin (HA) and neuraminidase (NA) genes from wild type influenza selected by the United States Food and Drug Administration (FDA) that are expected to circulate in the community during a particular season. The reassorted cold-adapted strains are called 6:2 gene reassortants. The attenuated viruses replicate efficiently at 25°C, the temperature in the upper respiratory tract (nasal passages) that restricts replication of wild-type viruses. Two additional phenotypes are recognized for the cold-adapted reassortants: temperature sensitivity, which limits replication at higher temperatures (37°C for type B and 39°C for type A) such as those found in the lower respiratory tract, and attenuation, which indicates that the virus will not replicate in the lungs of seronegative ferrets.

The liquid formulation of CAIV-T is produced by harvesting the allantoic fluid from SPF eggs infected with the three cold-adapted influenza strains. The harvested material is then concentrated and purified by centrifugation (refer to the Clinical Investigator's Brochure). The final formulation of each dose consists of the three influenza strains and is stored at 2 – 8°C. The volume for administration is 0.2 mL (0.1 mL in each nostril).

2.2 Background and Rationale

Epidemiology of Influenza in Children

Influenza is an acute viral respiratory illness characterized by abrupt onset of fever, myalgia, non-productive cough, headache, sore throat, nasal congestion, and malaise. The infection is spread primarily as an aerosol by transfer of virus-containing droplets from an infected to a susceptible person. Influenza respiratory infections occur annually in the fall and winter months causing widespread infection and morbidity in all age groups (2). The infection is frequently epidemic with an acute community onset and rapid spread. During epidemic periods, it may be responsible for 25% of all respiratory illnesses (3). The attack rate and morbidity are greatest among individuals with immune deficiencies and those with minimal prior exposure to influenza, with the highest attack rates occurring in children (4).

In the USA, the rate of influenza infection was found to be highest in children aged 6 to 11 years (48 per 100 person-years), and in children aged 2 to 5 years (45 per 100 person-years). Hospitalizations for influenza occurs most commonly in children aged 6 months to 11

months of age, and that children under 2 years of age have the highest influenza disease rate on any age groups (23, 24).

The risk of lower respiratory tract infection associated with influenza was found to be highest in children under 2 years (7.8 per 100 person-years) while in children under 2 years of age, two-thirds of influenzal infections occurred in infants aged 6 to 11 months (21 per 100 person-years).

In Asia, influenza rates also appear to be highest in young children. For example, in residential kindergartens in Beijing, China, 19% of acute respiratory infections of children aged 6 months to 7 years were confirmed by culture to be attributed to influenza (5). In community surveillance of children under 5 years with acute lower respiratory infections in Manila, Philippines, influenza was confirmed by culture as the probable etiology in 22% (6).

A study was conducted among 601 hospitalized children with ALRI in Bangladesh. Among the viral infections detected, influenza A and B viruses accounted for 14.4% (7). Among the viruses, about 10% influenza viruses A and B were isolated from a study conducted among 401 children admitted with ALRI and diarrhoea in Dhaka, Bangladesh (21). These publications reported that influenza was the second most common viral pathogen identified in children hospitalized with diarrhea and ALRI in Dhaka, Bangladesh.

A recently conducted study in Mirzapur area examined the selected etiological agents in a cohort of 252 newborns from birth to 24 months of age. This study detected about 4% influenza viruses from pneumonia cases (22).

Similarly, in Singapore, children under 4 years of age accounted for approximately 22% of all influenza isolations, representing the highest age-specific rates (8), and among those infants hospitalized for acute respiratory tract infections, influenza was associated with 11%. (9).

While published data confirm the very high rates of influenza infection in young children in diverse populations in Asia, rates comparable or higher to those in the USA, regional differences in seasonality of infection are apparent between tropical and temperate climatic regions (9).

CAIV-T and Heterotypic Protection Against Influenza

Previous studies have documented heterotypic efficacy of cold-adapted influenza vaccines (CAIV) against antigenic drift strains of influenza (10).

Serological studies examining the capacity of anti-influenza A/H3N2 HAI antibodies in sera obtained from children immunized with CAIV-T, in which the vaccine contained A/Wuhan/W3N2, to cross-react with heterotypic A/H3N2 strains, demonstrated that following a single dose of CAIV-T, ≥84% of children had serum HAI antibodies that cross-reacted to the Thessalonika, Nanchang, Wuhan, and Sydney A/H3N2 strains, and 36% cross-reacted to the Russian and Johannesburg A/H3N2 strains. After a second dose of CAIV-T, ≥96% of children seroconverted to Thessalonika, Nanchang, Wuhan, and Sydney A/H3N2 strains, 78% seroconverted to Russia and 52% to Johannesburg A/H3N2 strains.

The cross-reactivity between the A/H3N2 Wuhan and Sydney strains was important, as genetic sequence and antigenic analyses demonstrated significant differences between the two

strains (11, 12). During a season (1997/1998) in which an A/Wuhan/ H3N2-containing inactivated parenterally-administered vaccine failed to afford protection against A/Sydney associated disease in a placebo-controlled trial in healthy adults in the USA (13), an A/Wuhan/H3N2-containing CAIV-T conferred 86% efficacy (95% CI: 75-92%) on children aged 15 to 71 months against culture-confirmed influenza-illness caused by A/Sydney/H3N2 in the same season.

Therefore, although immune responses to other influenzal proteins may be contributory to heterotypic protection, the serum HAI antibody cross-reactivity demonstrated across influenza virus A/H3N2 strains were supported by subsequently demonstrated heterotypic efficacy against an antigenic drift strain. In addition this efficacy was demonstrated during a season when the commercially-available inactivated vaccine did not confer significant protection.

CAIV-T and its components formulated as either bivalent (CAIV-B) or monovalent (CAIV-M) has been evaluated clinically in trials involving children and infants under 12 months of age (14-18). In these trials, CAIV-T, CAIV-B and CAIV-M have been demonstrated to be immunogenic, very safe and generally well-tolerated in 692 infants as young as 2 months of age as well as being efficacious, immunogenic, very safe and generally well tolerated in infants and children aged 6 to 18 months of age. No serious adverse events have been reported from these trials.

Purpose of Study

The purpose of this study is to determine the efficacy of CAIV-T in a diverse Asian population aged from 6 months to less than 36 months, in tropical and temperate climates, against culture-confirmed influenza illness.

In addition, the trial provides the opportunity to investigate the efficacy of CAIV-T over multiple influenza seasons, and to investigate the effect of CAIV-T on acute otitis media.

2.3 Risks and Benefits

A frozen formulation of CAIV-T manufactured by Aviron, Inc. (Mountain View, CA, USA) has been evaluated in over 6,500 healthy children and 3,700 healthy adults. The results of completed pivotal studies are summarized in Table 1.

CAIV-T has been shown to be generally safe, well tolerated, efficacious, and effective in both healthy children and healthy adults. In the first year of the pediatric protective efficacy trial, children receiving two doses of CAIV-T had 93% overall protection from culture-confirmed influenza compared to placebo recipients (19).

The ease of administration of CAIV-T by intranasal sprayer could lead to increased compliance and better public health control of influenza and therefore, a reduction in the impact of this disease.

As with any vaccine, CAIV-T may not protect 100% of individuals given the vaccine. The following risks that may be associated with CAIV-T:

- Common Post-vaccination Reactions

  CAIV-T was generally well tolerated in clinical trials. In children in the 10 days following Dose One, the most common events were fever (5% over placebo), runny

nose/nasal congestion (11% over placebo), and vomiting, muscle aches, headaches and decreased activity (each approximately 2% over placebo). However, following Dose Two and after annual re-vaccination, there were no significant differences between the vaccine and placebo group. Currently, there have been no serious adverse experiences attributed to CAIV-T in the completed clinical trials performed by Aviron, Inc. (Mountain View, CA, USA).

- Hypersensitivity

CAIV-T is contraindicated in individuals hypersensitive to any component of the vaccine. Intranasal influenza virus vaccine is propagated in eggs. Therefore, CAIV-T should not be administered to anyone with a history of hypersensitivity to eggs. The occurrence of a hypersensitivity reaction to the vaccine following immunization with CAIV-T is a contraindication to further use of this product.

Table 1
CAIV-T Efficacy and Effectiveness Studies

STUDY	POPULATION	OBJECTIVE	RESULTS
AV006	Children	Assess the efficacy of CAIV-T(containing three components: two attenuated influenza A strains, H1N1 and H3N2, based on the influenza A master donor strain and one attenuated influenza B strain based on the influenza B master donor strain) in prevention of culture-confirmed influenza in children. 15-71 months of age	• Vaccine efficacy rate of 93% [95%CI: 88,96] against culture-confirmed influenza was observed. CAIV-T was efficacious against both strains of influenza circulating in 1996-97: A/H3N2 and B. • One dose (89% efficacy) and two doses (93% efficacy) were efficacious • CAIV-T was 98% efficacious against influenza-associated otitis media
AV006 Year Two	Children	Assess the efficacy of CAIV-T (containing three components: two attenuated influenza A strains, H1N1 and H3N2 and one attenuated influenza B strain –same as above) in prevention of culture-confirmed influenza in children following the first annual revaccination. 27-83 months of age	• Vaccine efficacy of 87% [95%CI:78,93] against culture-confirmed influenza was observed • Vaccine efficacy rate of 86% [95%CI: 75,92] against culture-confirmed influenza due to a variant H3N2 strain A/Sydney • CAIV-T was 94% efficacious against influenza-associated otitis media
AV011	Children	Assess the efficacy of CAIV-T (three components of Influenza strain – same as above) against viral shedding of H1N1 vaccine virus. 39-95 months of age (children from AV006 trial)	Reduction in viral shedding of A/H1N1 vaccine virus after intranasal challenge (efficacy 83%, 95% [CI: 60.2, 92.7])
AV003	Adults	Assess the efficacy of CAIV-T and the inactivated influenza vaccine compared to placebo in protecting adults from a challenge dose of H1N1, H3N2, or B wild-type virus 18-40 years of age	• Reduction in laboratory-documented influenza compared to placebo was statistically significant for the CAIV-T [$p<0.001$, efficacy 85%, (95%CI: 28,100)]
AV009	Adults	Assess the effectiveness of CAIV-T against influenza-like illness, worker absenteeism and health resource use. 18-64 years of age	• Reduction in the numbers of severe febrile illnesses (18.8% reduction, 95% [CI: 7, 29]) and of febrile upper respiratory illness (23.6% reduction, [95% CI: 13,33]) and the associated days of work lost, of healthy care provider visits, use of prescription antibiotics and use of over the counter medication for these events during the peak influenza period

2.4 Good Clinical Practice and Regulatory Compliance

The study will be conducted in compliance with the procedures outlined in this protocol, Good Clinical Practice (GCP) and according to applicable guidelines [International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH)] and regulatory requirements for clinical trials.

3. STUDY DESIGN

3.1 Study Endpoints

3.1.1 Case Definition

A culture-confirmed case of influenza-illness is one that occurs at least 15 days after the receipt of a dose of vaccine or placebo and that is defined by a positive viral culture of a wild-type virus subtype.

3.1.2 Primary Endpoint

The primary endpoint for efficacy is the first episode during the first year in a study child of a culture-confirmed influenza-illness, caused by community-acquired subtypes antigenically similar to those contained in the vaccine, at least 15 days following receipt of the second dose of study vaccine or placebo.

3.1.3 Secondary Endpoint

The secondary endpoint for efficacy is the first episode during the second year in a study child of a culture-confirmed influenza-illness, caused by community-acquired subtypes antigenically similar to those contained in the vaccine, following receipt of two doses administered in the prior season. All isolates will be analyzed in the subtype specific efficacy.

3.1.4 Overview of the Trial Design

This is a prospective, randomized, double-blind, placebo-controlled, trial designed to determine the safety and efficacy of CAIV-T. Approximately 3,000 healthy children ≥ 6 months and < 36 months of age will be enrolled from 10 Asian countries. Approximately 300 will be enrolled from Mirzapur, a rural sub-district of Bangladesh. About 60% of the study participants will receive two doses of the study vaccine and the remaining 40% will receive two doses of placebo at the beginning of year 1. The safety and efficacy of a primary series of two doses of CAIV-T vaccine administered to children will be evaluated in comparison to recipients of a primary series of two doses of placebo. At the start of the second season, study subjects who completed the two dose primary series will be randomized again to receive either a single dose of CAIV-T or placebo. The distribution of study participants at the Bangladesh site to vaccine or placebo will be as follows:

		First Year		Second Year		
Study Group	No. of Subjects	Dose 1	Dose 2	Group	# of Subjects	Dose
1.1	180	CAIV-T	CAIV-T	2.1	90	CAIV-T_2
				2.2	90	Placebo
1.2	120	Placebo	Placebo	2.3	60	CAIV-T_2
				2.4	60	Placebo

Reactogenicity and other Adverse Events (AEs) will be evaluated in all study recipients. Each subject will be included in the safety and efficacy surveillance for approximately 2 years.

3.2 Trial Design Diagram

Tables 2a through 2d illustrate the study procedures for all subjects participating in the study:

Table 2a

SCHEDULE OF PROCEDURES – First Year

	Primary Vaccination Series – First Year						
Study Visits/Contacts (Days post-vaccination)	**1** (0)	**Day 4** (4 to 6)	**Day 10** (10 to 12)	**2** (28 to 56)	**Day 4** (4 to 6)	**Day 10** (10 to 12)	**End of First Year***
Informed Consent	X						
Medical History	X						
Interim Medical History				X			
Review of Subject Eligibility	X			X			
Physical Exam	X			X			
Immunization	X			X			
Post-Immunization Contact By daily home visit for first 11 days		X	X		X	X	
Serious Adverse Event Reporting	X	X	X	X	X	X	X
Surveillance for Influenza Illness		Beginning on the 11th day after receipt of the first dose of study vaccine, parent/guardian are contacted weekly by home visit.					

Day 0 is defined as day of first vaccination.

* First year ends on the day of third study vaccination, approximately one year from subject enrollment.

Table 2b

SCHEDULE OF PROCEDURES – First Year (Immunogenicity Cohort Only)

In addition to the procedures outlined in Table 2a, subjects involved in the immunogenicity cohort will also complete the following procedures:

		Primary Vaccination Series - First Year	
Study Visits/Contacts (Days post-vaccination)	1 (0)	2 (28 to 56)	3 (28 to 35 post second dose)
Obtain Nasal Wash Sample*	X		X
Obtain Blood Sample *	X		X
Immunization **	X	X	

* Serum and nasal wash samples will be obtained from a subset of subjects (approximately 20 children)

**As described in Table 2a.

Table 2c

SCHEDULE OF PROCEDURES – Second Year

	Second Year①			
Study Visits/Contacts (Days post-vaccination)	**1** (0)	**Day 4** (4 to 6)	**Day 10** (10 to 12)	**End of Second Year③**
Interim Medical History	X			
Review of Subject Eligibility	X			
Physical Exam	X			
Immunization	X			
Post-Immunization daily Contacts by home visit		X	X	
Serious Adverse Event Reporting	X	X	X	X
Study Termination				X
Surveillance for Influenza Illness				②

① Second Year begins on the day of third vaccination, approximately one year from subject enrollment

Day 0 is defined as day of vaccination.

② Beginning on the 11th day after receipt of this dose of study vaccine, parent/guardian are contacted weekly by home visit until the end of the study.

③ Second Year ends approximately September 1, 2002, or two years from subject enrollment.

Table 2d

SCHEDULE OF PROCEDURES – Second Year (Immunogenicity Cohort Only)

In addition to the procedures outlined in Table 2c, subjects involved in the immunogenicity cohort will also complete the following procedures:

	Second Year①		
Study Visits/Contacts (Days post-vaccination)	**1** (0)	**2** (28 - 35)	**3②**
Obtain Nasal Wash Sample*	X	X	
Obtain Blood Sample *	X	X	X
Immunization**	X		

① Second Year begins on the day of third vaccination, approximately one year from subject enrollment.

Day 0 is defined as day of first vaccination.

② Second Year ends approximately September 1, 2002, or two years from subject enrollment.

*Serum and nasal wash samples will be obtained from the same subset of subjects (approximately 20 children)

** As described in Table 2c.

3.3 Randomization and Blinding

The Investigator and the study staff will remain blinded for each immunization. The randomization schedule will be prepared by WLV, or their designate. A double-blind study design can be implemented because the study products appear identical.

3.3.1 Randomization

A randomization schedule will be provided for each study clinic by the Sponsor, WLV.

3.3.2 Randomization Procedures

The subjects will be randomized according to a schedule provided by WLV separately for the first and second years. Subjects will remain blinded and use the same study number throughout the entire study period (from day of study entry to approximately September 1, 2002, or two years from subject enrollment).

3.3.3 Unblinding of the Randomization Code

A representative of the Sponsor's Regulatory Affairs Department and a regional designee will have a masked randomization code-breaker list of corresponding treatment assignments for each vaccine administration. The code-breaker list will be stored in a secure location. The person who holds the code-breaker list will maintain a written log of the indications for all instances of unblinding. This log will be made available for review at study completion. Unblinding may only occur with the permission of WLV.

3.3.4 Blinding

All vaccines will be dispensed and administered only by individuals qualified to perform that function under applicable local laws and regulations for the specific study site. To minimize bias in the reporting of post-immunization reactions and other study events, neither the study subjects, their parents/guardians nor the clinical personnel will know which vaccine is being administered. A double-blind study design is possible because the vaccine and placebo preparations appear identical.

3.3.5 Responsibilities of the Vaccine Dispenser

The dispenser will sign a statement that assures WLV and the regulatory authorities that he/she understands his/her obligations and responsibilities as specified in the protocol relative to the dispensing of the study vaccine and the manner in which it is to be administered. The original signed statement will be returned to the study monitor. Copies of this statement will be retained by the dispenser, the Investigator, and the WLV monitors.

The vaccine dispenser will be responsible for all clinical material provided by WLV, such as the study vaccine, randomization schedules, vaccine accountability forms, and any other supplies.

The vaccine dispenser will:

- Identify the correct treatment for the particular patient based on the randomization table generated by WLV and prepare the doses according to the instructions provided.
- Adhere to good dispensing practices (i.e., clear an appropriate space, minimize distractions, open only one container at a time).
- Dispense study vaccine as indicated.
- Safely and efficiently deliver dispensed vaccine directly to the Investigator or his/her designee administrator.
- Complete all dispensing information on the Vaccine Accountability Form, including quantities that are wasted or discarded.
- Return unused clinical material including the vaccine, Vaccine Accountability Forms and any other supplies to WLV according to the shipping instructions.

3.4 Study Vaccine

3.4.1 Dosage Form of Study Vaccine

CAIV-T consists of three cold-adapted reassortants grown in specific pathogen-free eggs. The allantoic fluid containing virus is harvested, concentrated and purified. The total volume of 0.2 mL is administered intranasally with a spray applicator (approximately 0.1 mL into each nostril).

The placebo is comprised of physiological saline. The total volume of 0.2 mL is administered intranasally with a spray applicator (approximately 0.1 mL into each nostril).

3.4.2 Packaging and Labeling of Study Vaccine

Each dose of CAIV-T and placebo contained in the spray applicator will be supplied as single-dose units.

3.4.3 Vaccine Storage

Vaccine receipt and storage will be documented on the Investigational Vaccine Accountability Record (or equivalent).

All supplies are to be kept in a secure, limited access storage area. Refrigerator temperatures must be monitored regularly and regularly documented in the study records.

3.5 Duration of Subject Participation

The study duration for each participant will be approximately 24 months.

3.6 Termination of a Subject from the Study

An Investigator and/or Sponsor may drop a subject from the study if deemed appropriate. Criteria for termination of a subject from the study may include, but are not limited to, request for withdrawal, subject lost to follow-up, and/or clinically significant AEs which constitute contraindications to further vaccine administration (see Section 4.4.1 of this protocol).

3.7 Vaccine Accountability

Exact written records of receipt and storage of the vaccine, including date received, lot number, quantity received, and each administered dose with the identification of the subject, must be present. Any known discrepancies in the accountability of the vaccine must be adequately documented. At the end of the trial, the unused vaccine will be returned to the Sponsor (or designee) by the Investigator (or designee). The Investigator will not use the vaccine in any other manner than that provided for in the protocol.

3.8 Source Data

It is the policy of WLV that study data must be verifiable to the source data, which necessitates access to all original recordings, laboratory reports, and subjects' records. The Investigator must therefore agree to allow access to subjects' records, and source data must be made available for all study data. The subjects [or their legal representatives] must also allow access to the subjects' medical records, and they will be informed of this and will be signing their agreement when giving informed consent.

4. SELECTION AND WITHDRAWAL OF SUBJECTS

The following selection criteria must be met in order for the subject to participate in the study.

4.1 Subject Inclusion Criteria

Subject must fulfill all of the following conditions or characteristics in order to be considered for study enrollment or participation:

- who are $\geq$ 6 months and less than 36 months of age at the time of enrollment, and in good health as determined by medical history, physical examination, and clinical judgment;
- whose parent or legal guardian has provided written informed consent after the nature of the study has been explained;

- who, along with their parent or legal guardian, will be available for the duration of the study (24 months);
- whose parent/legal guardian can be reached by study staff for the post-immunization contacts by home visits.

4.2 Subject Exclusion Criteria

Subjects with any of the following conditions or characteristics will be excluded from study enrollment or continued participation:

- whose parents or caregiver are perceived to be unreliable or difficult to contact for evaluation or study visits during the study period;
- with any serious chronic disease (e.g., with signs of cardiac or renal failure) including progressive neurological disease;
- Severely malnourished children;
- with Down's syndrome or other known cytogenetic disorders;
- with a known or suspected disease of the immune system or those receiving immunosuppressive therapy, including systemic corticosteroids;
- who received any blood products, including immunoglobulin, in the period from six months prior to vaccination through to the conclusion of the study;
- for whom there is intent to administer any other investigational vaccine or agent from one month prior to enrollment through to the conclusion of the study;
- with an immunosuppressed or compromised individual living in the same household;
- who, at any time prior to entry into this study, received a dose of any influenza vaccine (commercial or investigational);
- who, in the two weeks prior to entry into this study, received a dose of any influenza treatment (commercial or investigational);
- who were administered any live virus vaccine within one month prior to study vaccination or expected receipt of another live virus within one month of vaccination in this study;
- with a documented history of hypersensitivity to egg or egg protein, or any other component of the study vaccines or placebo;
- with a respiratory illness with wheezing within two weeks prior to enrollment;
- who received aspirin (acetylsalicylic acid) or aspirin-containing products in the two weeks prior to enrollment or for which use is anticipated during the study;
- with any medical conditions that in the opinion of the Investigator might interfere with interpretation of the study results;

Note: A pregnant household member or day care provider is not considered a contraindication to enrollment.

4.3 Criteria for Temporarily Delaying Vaccination Administration

In general, the subject must be in good health (by physical examination and history) at the time of each study vaccination.

The following conditions are temporary or self-limiting and a subject may receive study vaccine once the condition(s) has/have resolved and no other exclusion criteria are met:

- A febrile illness (axillary temperature $\geq$ 37.5°C) or other acute illness within 36 hours prior to study vaccine administration.
- A respiratory illness in the subject and/or household member within 72 hours prior to study vaccine administration. Study vaccination of the subject may occur if a subject is experiencing mild symptoms of the common cold (eg. slight cough and a runny nose) which is not going to be medically treated and if the investigator feels that the subject's condition is not a respiratory illness and does not require any special clinical management. If the symptoms require medical intervention, it should be considered a Respiratory Illness and the study vaccination should be delayed until the illness has resolved. Vaccination should be delayed if a subject is taking medication (other than an antibiotic—see below) to prevent a particular respiratory related symptom (e.g., runny nose). In addition, if nasal blockage exists, it must be minimal enough to not reduce the likelihood of successful administration of the vaccine.
- Treatment of the subject with an antibiotic within 72 hours prior to study vaccine administration. Vaccination should be delayed if the subject requires any medical intervention with an antibiotic at the time of vaccination. If a subject is currently taking medicine (other than an antibiotic) at the time of vaccination for prophylaxis against a non-respiratory related illness, the subject may vaccinated.

4.4 Subject Withdrawal Criteria

4.4.1 Events Which Warrant Withdrawal from Further Doses of Study Vaccine

Participating subjects will be contraindicated to subsequent doses of study vaccine if the child has a related Serious Adverse Event (SAE) or any other related alarming or unusual event, including anaphylaxis. Any AE which is not considered vaccine related, but that is of concern will be discussed with the sponsor prior to the administration of the subsequent dose.

4.4.2 Subject Withdrawal from Study

Following enrollment, development of any condition specified in the initial exclusion criteria cited in Section 4.2 (Subject Exclusion Criteria) may not constitute a criterion for withdrawal. If an intervening illness does not resolve, the subject may be discontinued from the study at the discretion of the Investigator.

4.4.3 Subject Replacement

Additional subjects will not be enrolled to replace discontinued subjects.

5. CONDUCT OF THE STUDY

The schedule of procedures for individual study participants is presented in Section 3.2 (Tables 2a through 2d).

5.1 Study Screening Procedures

5.1.1 Informed Consent

- Prior to participation in the study, the parent or legal guardian of a prospective subject will be informed of the purpose and conduct of the study.
- The appropriate informed consent form is used to obtain parent/legal guardian signature. The parent/legal guardian must date their signature.

5.1.2 Physical Exam and Medical History

- A physical examination (including temperature), medical history (includes current non-prescription and prescription medication use, and immunization history), weight, gender, and ethnic origin must be obtained on the day of study entry (when a subject receives the dose of study vaccine).
- The subject must meet all of the inclusion criteria and none of the exclusion criteria to be eligible to be enrolled in the study.

5.2 Study Conduct, First Year

5.2.1 Safety Monitoring

- Initially 30 children will be vaccinated for safety monitoring of the vaccine. These children will be followed for 10 days and an independent local safety monitor will monitor the safety. After reviewing the safety rest of the children will be vaccinated.

5.2.2 Visit 1, First Immunization, Day 0

- After eligibility is confirmed and the informed consent procedure is completed, the participant will be randomized. The subject number is maintained throughout the entire study.
- A qualified member of the study staff will administer a single dose of either CAIV-T or placebo. Vaccine accountability records will be updated.
- After vaccination, the subject will be observed for a minimum of 15 minutes by the study staff. Emergency supplies, (for example: AMBU bag, adrenaline (epinephrine), and antihistamines) will remain available for initial treatment of an allergic reaction if needed.

5.2.3 First Dose Post-Immunization Contact, Day 0-11

- Beginning the evening of vaccination and for the following 10 days, a health worker will visit the children daily in their home and will record axillary temperatures and reactogenicity events on a case report form. The parent/guardian will be asked to report any occurrence of AEs (including unscheduled physician visits), and non-prescription and prescription medication use.
- Serious Adverse Events (SAEs), regardless of causality, will be collected for the duration of the study.
- Information will be recorded in the appropriate source documentation record(s) and CRF(s).

5.2.4 Start of First Year Study Surveillance Period

- Beginning on the 11th day after receipt of the first dose of CAIV-T or placebo, parents/guardians will be contacted weekly by home visit.
- See Section 5.4.2 for surveillance definitions and procedures.
- First year study surveillance periods ends on the day of third study vaccination, approximately one year from subject enrollment.

5.2.5 Visit 2, Second Immunization, Day 0

- This visit occurs within 28 to 56 days after the first immunization.
- A physical examination and interim medical history must be obtained on the day of immunization.
- The subject must meet appropriate inclusion/exclusion criteria to continue the study.
- A final review of the first post-immunization CRFs is conducted including occurrence of AEs and/or medical problems, and use of non-prescription and prescription medication.
- A qualified member of the study staff will administer a single dose of either CAIV-T or placebo. Vaccine accountability records will be updated.
- After vaccination, the subject will be observed for a minimum of 15 minutes by the study staff. Emergency supplies, (for example: AMBU bag, adrenaline (epinephrine), and antihistamines) will remain available for initial treatment of an allergic reaction if needed.

5.2.6 Second Dose Post-Immunization Contact, Day 0-11

- Beginning the evening of vaccination and for the following 10 days, a health worker will visit the children daily in their home and will record axillary temperatures and reactogenicity events on a case report form. The parent/guardian will be asked to report any occurrence of AEs (including unscheduled physician visits), and non-prescription and prescription medication use.
- Serious Adverse Events (SAEs), regardless of causality, will be collected for the duration of the study.
- Information will be recorded in the appropriate source documentation record(s) and CRFs.

5.3 Study Conduct, Second Year

5.3.1 Visit 1, Third Immunization, Day 0

- A physical examination and interim medical history must be obtained on the day of immunization.
- The subject must meet appropriate inclusion/exclusion criteria to continue the study.
- A qualified member of the study staff will administer a single dose of either CAIV-T or placebo. Vaccine accountability records will be updated.

- After vaccination, the subject will be observed for a minimum of 15 minutes by the study staff. Emergency supplies, (for example: AMBU bag, adrenaline (epinephrine), and antihistamines) will remain available for initial treatment of an allergic reaction if needed.

5.3.1 Third Dose Post-Immunization Contact, Day 0-11

- Beginning the evening of vaccination and for the following 10 days, a health worker will visit the children daily in their home and will record axillary temperatures and reactogenicity events on a case report form. The parent/guardian will be asked to report any occurrence of AEs (including unscheduled physician visits), and non-prescription and prescription medication use.
- Serious Adverse Events (SAEs), regardless of causality, will be collected for the duration of the study.
- Information will be recorded in the appropriate source documentation record(s) and CRFs.

5.3.2 Start of Second Year Study Surveillance Period

- Beginning on the 11th day after receipt of the third dose of CAIV-T or placebo, parents/guardians will be contacted weekly by home visit.
- See Section 5.4.2 for surveillance definitions and procedures.

5.3.3 End of Study Surveillance Period, Study Termination

- A study staff member will interview by home visit the parent/guardian to determine if any SAEs have occurred since the last study visit.
- Information will be recorded in the appropriate source documentation record(s) and CRF(s).

5.4 Study Surveillance Procedures

5.4.1 Start of Surveillance

Beginning on the 11th day after receipt of the first dose (first year) and third dose (second year) of CAIV-T or placebo, parents/guardians will be contacted weekly by home visit to ensure specific medical conditions (see section 5.4.2.1) are promptly being reported to the study center.

5.4.2 Surveillance Procedures

5.4.2.1 Illness Visit Criteria

The field workers should have at least 12 th grades of education. They will be given 2 weeks training at Mirzapur hospital. The field workers will be trained on interviewing techniques, counting of a child's respiratory rate, recording of temperature and to recognise cases of chest indrawing.On the day of visit the field workers will check temperature, count respiratory

rates, observe presence of chest indrawing and ear pain/discharge to all children. Children having hurried respiration (> 50 in cases of children aged <12 months and > 40 in cases of >12 months), chest indrawing, ear discharge will be referred to the study center for examination by the project physician.

The criteria for obtaining a culture are either one sign, symptom, or condition in Category A, or at least two signs, symptoms, or conditions from Category B (below) during the surveillance period:

- Category A – Fever (≥ 38°C rectal or oral, or ≥ 37.5°C axillary), Wheezing, Shortness of breath, Pulmonary congestion, Pneumonia, Ear infection (acute otitis media, suspected or diagnosed).
- Category B – Runny nose or nasal congestion (rhinorrhea), Sore throat (pharyngitis), Cough, Muscle aches, Chills, Headache, Irritability, Decreased activity, Vomiting.

5.4.2.2 Conduct of the Illness Visit

- Illness visit criteria are met. The subject is seen in the clinic or home within four days of the onset of the illness.
- Information regarding the details of the illness, which may include symptom(s)/ diagnosis, duration, treatment, and medication use, are collected from the parent/guardian.
- A nasal swab is collected for viral culture from the subject. However, a viral culture may also be obtained if, in the opinion of the Investigator, the symptom complex so warrants.
- If otitis media is suspected, an ear examination should be performed.
- The illness visit will be followed by home visit to confirm resolution of the illness.
- Information will be recorded in the appropriate source documentation record(s) and CRF(s).

5.4.3 Duration of Study Surveillance for Influenza Illness

We plan to do weekly surveillance to be consistent with all other study areas. A pilot study is actually not necessary. The weekly contacts are not surveillance contacts only. They are also reminder contacts to the parents or guardians that should their child develop any of the per protocol symptoms that require culture, that they should contact the study site. Parents of study children will not wait for the weekly contact, but they must contact study site/health workers immediately if their child has any of the symptoms listed.

There is no doubt that there may be some missed cases, but the protocol analysis for the primary objective is the first episode only, so a missed opportunity in any individual subject will not affect the power of the trial to meet its statistical objectives

All subjects will be followed for illness for the duration of the study. The first year surveillance period will begin on the 11th day after the first dose and continue up to the day of the third dose (second year). The second year surveillance period will begin on the 11th day after the third dose (second year) and continue until approximately September 1, 2002, or two years from subject enrollment.

5.4.4 Viral Cultures for Influenza

An attempt will be made to collect viral culture specimens from symptomatic subjects within four days of onset of any illness that prompted a study center or home visit. Nasal swabs will be obtained on all subjects. Cultures that cannot be obtained within four days of illness onset should still be performed as soon as possible. Site and laboratory processing of all samples will be detailed in a separate Manual of Laboratory Procedures.

5.4.5 Differentiation Between Vaccine and Viral Strain

We will obtain a nasal swab for every respiratory illness that is listed in the protocol, which also includes pneumonia at any time of the trial. Since the vaccine is only shed for 24 to 36 hours after a vaccine dose is administered there is very little likelihood of it being associated with pneumonia. However, we are able to easily distinguish between wild-type and the vaccine type based on phenotypic characteristics, and so could do this for serious events such as pneumonia occurring shortly after vaccination strain is causing pneumonia.

6. VACCINE ADMINSTRATION

6.1 Vaccine to be Administered

- At the time of study entry (first year), each randomized subject will receive two doses of either CAIV-T or placebo approximately one month apart (as per Table 2a).
- At the start of the second year, each subject will receive a single dose of either CAIV-T or placebo (as per Table 2c).

6.1.1 Handling of CAIV-T and Placebo

- CAIV-T and placebo should be allowed to come to room temperature for 5 minutes just prior to use.

6.1.2 Administration of CAIV-T and Placebo

- A single administration comprises delivery of approximately 0.1 ml CAIV-T or placebo into each nostril.
- Each spray applicator of CAIV-T or placebo has an adapter that allows delivery of approximately half the contents to one nostril.
- Removal of the adapter allows delivery of the remaining vaccine to the other nostril.
- CAIV-T or placebo will be sprayed as a fine mist into each nostril while the child is in an upright position (preferably sitting/held on the parent/guardian's lap).
- Significant precautions will be taken to minimize any chance of accidental mix-up of study sprayers.
- Accidental use of used study sprayers after administration must be prevented by placement into locked containers immediately after use.

- After vaccination, all participants will be observed for a minimum of 15 minutes by the study staff. Emergency supplies (for example: AMBU bag, epinephrine and antihistamine) will remain available for initial treatment of an allergic reaction if needed.

6.1.3 Accountability and Disposal of Used/Unused Study Vaccines

- Used CAIV-T and placebo spray applicators should not be discarded until instructed to do so by a WLV representative. Instructions will be provided as to the correct disposal of applicators.
- Used spray applicators will be placed in locked containers immediately after use and destroyed at the clinical site by autoclaving or other appropriate means according to the site's Standard Operating Procedures (SOP) only after review by a WLV representative. Documentation of the destruction method will be placed in the Investigator's study binder and signed and dated by the Investigator.
- Unused CAIV-T and placebo will be maintained at 2 - 8°C in a refrigerator and monitored regularly until return of supplies are arranged per instructions provided.

6.2 Concurrent Vaccinations and Other Treatments

Information about medication(s) use will be obtained from the parent/guardian.

- Routine childhood vaccinations (excluding live viral vaccines) administered at the time of CAIV-T or placebo administration will be recorded.
- Medication(s) the child is taking at the time of enrollment will be obtained from the parent/guardian in order to understand any risk of reactions.
- During the Day 0 – Day 10 post-immunization period, use of any non-prescription and prescription medications (including antipyretics, decongestant/antihistamines, analgesic preparations, antibiotics, and herbal or traditional remedies) will be reported (exceptions: vitamins, iron, and fluoride supplements). This includes any prescription or non-prescription medications or treatments given as countermeasure therapy during an Adverse Event.

6.2.1 Mandatory Concurrent Vaccines

None.

6.2.2 Allowable Concurrent Vaccines

CAIV-T and placebo may be administered concomitantly with routinely scheduled childhood vaccines (excluding live viral vaccines). Any concomitantly administered vaccines must be recorded as per Section 6.2.

6.2.3 Restricted Use of Other Vaccines

A live virus vaccine must not be administered within one month prior to study vaccination or 1 month following receipt of study vaccination.

6.2.4 Other Allowable and Restricted Treatments

- Aspirin (acetylsalicylic acid) and aspirin-containing products must not be given to study participants during the trial as the use of salicylates in children with concurrent influenza illness has been associated with Reye's syndrome.
- Anti-influenza antiviral therapy (e.g., amantidine, rimantidine) must not be given to study participants prophylactically during the trial as these may interfere with assessment of true illness incidence.

6.3 Procedure for Monitoring Subject Compliance

To ensure subject compliance, the study vaccine will be administered to the subject by the Investigator or a qualified member of the Investigator's staff. Vaccine administration details (i.e., date of administration, vial label identifier, volume administered) will be recorded. Spray applicator inventory will be tracked using a Vaccine Accountability Form (or equivalent).

7. ASSESSMENT OF EFFICACY IMMUNOGENICITY

7.1 Efficacy Assessment

Nasal swab specimen will be shipped to Wyeth Lederle Vaccines (USA) for identification.

7.2 Immunological Parameters

7.2.1 Serum Hemagglutination Inhibition Antibodies (HAI)

Immunogenicity will be evaluated by comparison of pre and post-vaccination strain-specific titers of serum antibody by HAI. Seroconversion will be defined as a four-fold or greater rise in titer.

Serum will be obtained from a subset of subjects for determination of serum HAI antibodies. This cohort, consisting of approximately 20 children will have 3-5 mL of blood (1-1.5 mL serum) collected on 5 occasions over the two years as follows: prior to the first dose, 1 month following completion of the second dose of the primary vaccination series, prior to the second year's dose, 1 month following the second year's dose, and at the completion of the second year.

The samples will be shipped to WLV for assaying for vaccine-type influenza HAI antibody.

7.2.2 Nasal Wash Antibody

Nasal wash specimens will be collected from the same subset of subjects on approximately 4 occasions over the two years as follows: prior to the first dose, 1 month following completion of the second dose of the primary vaccination series, prior to the second year's dose, and 1 month following the second year's dose. These specimens will be shipped to

WLV for determination of specific IgA antibody (as determined by ELISA) to the influenza A/H1N1, A/H3N2, and B vaccine strains.

8. ASSESSMENT OF SAFETY

8.1 Safety Parameters

Reactogenicity events are predefined AEs that could occur after vaccine administration. They are as follows:

- Fever (temperature ≥37.5°C axillary)
- Cough
- Runny nose or nasal congestion
- Irritability
- Vomiting
- Decreased activity (lethargy)
- Decreased appetite

Reactogenicity events will be collected on diary worksheets by the parent/caregiver. All reported reactogenicity events and other AEs (including unscheduled physician visits or any medication use) with onset within the 11-day post-immunization period will be recorded on the case report form (CRF).

8.2 Analysis of Safety Parameters

All subjects will be evaluated for safety following any dose. All adverse events will be summarized based on severity during the follow-up period. The distribution of the maximum severity will be presented.

8.3 Adverse Event Reporting

8.3.1 Adverse Event Definition

An Adverse Event (or Adverse Experience, AE) is any untoward, undesired or unexpected clinical event in the form of signs, symptoms, disease or laboratory or physiological observations occurring (in a human being) in a temporal relationship to the use of a WLV product, regardless of causal relationship. This also includes: a) any clinically significant worsening of a pre-existing condition, b) any episode of overdose or abuse of a WLV product, c) any AE occurring associated with the discontinuation of a WLV product and d) all serious adverse events (as defined in Section 8.3.2).

For this study, an AE is any clinically significant event, including but not limited to those events: 1) requiring any prescription or non-prescription medication within 11 days of vaccination, 2) requiring an unscheduled health care provider visit and/or health care provider consultation within 11 days of vaccination, 3) resulting in study termination; or 4) any other clinically significant event occurring at any time during the course of the study.

Note that these examples represent the minimum set of what must be reported in the Case Report Form, and these must all be reported regardless of causality.

In addition, there may be other events that do not meet the above criteria (e.g., events not requiring medication or a medical visit/consultation, or events occurring beyond 11 days after vaccine) which should be reported nonetheless. It is particularly important that all events which lack a clear alternative explanation (e.g., systemic rash, arthralgia/arthritis, idiopathic thrombocytopenic purpura), be reported. The Investigator's clinical judgment is critical in insuring appropriate reporting of events.

8.3.2 Serious Adverse Event (SAE) Definition

A Serious AE is an AE occurring at any dose that:

- results in death regardless of cause;
- is life-threatening (see below);
- requires inpatient hospitalization or prolongs an existing hospitalization (see following definition);
- results in a persistent or significant disability or incapacity (see following definition);
- results in cancer;
- results in a congenital anomaly or birth defect (in the offspring of a vaccine recipient, where applicable);

Additionally, **important medical events** that may not result in death, be life-threatening, or require hospitalization may be considered serious AEs when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home with resolution of symptoms, blood dyscrasias or convulsions that do not result in hospitalization, or development of dependency or abuse.

Life-threatening refers to **immediate** risk of death from the event as it occurred. A life-threatening event does not include an experience that, had it occurred in a more severe form, might have caused death but as it actually occurred did not create an immediate risk of death. For example, hepatitis that resolved without evidence of hepatic failure would not be considered life-threatening even though drug-induced hepatitis of a more severe nature can be fatal. Similarly, an allergic reaction resulting in angioedema of the face would not be life-threatening even though angioedema of the larynx, allergic bronchospasm, or anaphylaxis can be fatal.

Hospitalization is to be considered a serious adverse event only in the event of an overnight admission. Any elective hospitalization for a preexisting condition that has **not** worsened does **not** constitute an SAE (eg, elective hospitalization for repair of hypospadias).

The following events must also be reported according to the same procedures specified for the reporting of Serious Adverse Events:

- events which suggest a significant hazard, contraindication, side effect, or precaution associated with the use of a study vaccine, that are unexpected (of a nature, severity, specificity or frequency not indicated in the Clinical Investigator Brochure).

The Sponsor requires immediate notification if any subject experiences an overdose or accidental exposure, or develops cancer during the study.

8.3.3 Unexpected Adverse Event

An adverse event, of which the nature, severity, specificity or frequency is not consistent with the applicable product information (i.e., Clinical Investigator Brochure or Commercial Package Insert).

8.3.4 Severity Assessment

Mild	Awareness of sign or symptom, but easily tolerated;
Moderate	Discomfort enough to cause interference with usual activity;
Severe	Incapacitating, with inability to do usual activity;
Life-Threatening	Subject was at risk of death as the event occurred.

8.3.5 Causality Assessment

8.3.5.1 Relationship to Product

A product is defined as any drug, vaccine, biological product, or device under clinical investigation. This includes blinded study drug, placebo, or control agent.

The assessment of causality requires the balanced application of clinical judgment and knowledge of the investigational product and its effects. Additionally, an association between the product and an adverse event should be plausible in terms of what is known of the investigational compound and the biological basis of the adverse response.

Factors that should be considered in assessing the possible causal relationship between an investigational product and an adverse event includes the temporal relationship between treatment and the event; the subject's clinical state; coexisting therapies; and any known response patterns to the study drug or related compounds. For study compounds administered on an ongoing basis, response to withdrawal of the investigational compound, and response to re-challenge (if clinically and ethically feasible) should also be considered.

The following categories are to be used when assessing causality of the investigational product:

Definite	There is a direct cause and effect relationship between the administration of the (WLV) product and the Adverse Event;
Probable	The Adverse Event follows a reasonable temporal sequence from product administration and is likely to be due to the product, rather than another cause;

Possible	The Adverse Event follows a reasonable temporal sequence from product administration but could have been produced by either the subject's clinical state or by product administration.
Remote	The temporal association between the Adverse Event and the product is not likely to have had any reasonable association with the observed illness/event (cause and effect relationship improbable but not impossible).
Not Related	The Adverse Event is completely independent of product administration.

8.3.5.2 Relationship to Protocol

Serious AEs from clinical studies that are not product-related may nevertheless be considered to be related to the conduct of the protocol or conditions of the research (ie, related to the fact that a subject is participating in the study). The expression "conditions of the research" includes all restrictions derived from or imposed by the protocol including all concomitantly administered vaccines. For example, a protocol-related serious AE may be an event related to a procedure required by the protocol independent of administration of the investigational product.

8.3.5.3 Relationship to Other

Serious AEs that are neither product or protocol related may nevertheless be considered to be more likely related to yet another independent cause. (eg, underlying disease, trauma sustained in a motor vehicle accident). These events should be reported following the same procedure, with suspected alternative cause specified.

8.3.6 Reporting Adverse Events

Adverse Events may be observed by the Investigator, elicited from the parent/guardian or subject, reported on subject diary cards, or volunteered by the parent/guardian or subject.

Medical follow-up (such as history, physical examination, and laboratory testing and/or treatment) may be necessary if a subject experiences an Adverse Event.

Details of all Adverse Events must be properly recorded on the Adverse Event Case Report Form. The following information should be provided in the case report form for each Adverse Event:

- Dates of symptom onset and resolution. Note: for hospitalizations, it is important to record not only the date of hospitalization but also the date that the illness leading to hospitalization began.
- Physician evaluation of severity and of the relationship between product administration and the adverse event.
- Action(s) taken
- Subject outcome

All Adverse Events, regardless of relationship to study vaccine, should be followed until resolution.

8.3.7 Reporting Serious Adverse Events (SAEs)

For Serious Adverse Events only: the Investigator must complete a Serious Adverse Event Report Form (WLV/CR002 or equivalent). Additionally, the Adverse Event Case Report Form page may need to be completed.

- The Investigator (or designee) must report all Serious Adverse Events, whether or not considered study vaccine related, to the Sponsor (via facsimile to + 1-914-732-4042) on the form provided, within 24 hours of learning of the event.
- Additional information concerning the event, including diagnostic information that clarifies the etiology of the event, should be communicated to the Sponsor as soon as it is available.
- It may be necessary for the Investigator to provide the Sponsor with additional relevant information, such as hospital or outpatient records, copies of case report forms, and autopsy reports/death certificates.
- The Investigator will comply with the applicable regulatory requirement(s) relating to Serious Adverse Event reporting to the regulatory authority and the Institutional Review Board/Ethics Committee.
- For specific instructions on the Adverse Event collection period, please refer to the Trial Design section of this protocol.
- In addition, the Medical Monitor can be reached at the following number(s):

Bruce Forrest, MD
Director, Clinical Research
Wyeth Lederle Vaccines
401 N. Middletown Road
Pearl River, NY 10965-1299
USA
+1-914-732-5404

If there is a need to speak with a Medical Monitor concerning Adverse Events during off-business hours, weekends and holidays, the following telephone numbers may be used as well: +1 914-732-2222. This is a voice-mail system from which messages are retrieved periodically by a Medical Monitor.

8.3.8 Other Information Reportable to WLV

Other information reportable to WLV includes the following: any overdose, product abuse, and inadvertent or accidental exposure to product with or without an AE. These events are to be recorded by the Investigator on the Immediately Reportable Event Form (WLV/CR041). These events are handled in the same manner as SAEs, and are subject to the same reporting time frames.

8.3.9 Removal of a Subject

The removal of a subject from the study because of an Adverse Event, whether by the Investigator or by the parent/guardian's or subject's volition, should be reported to the Sponsor within 24 hours of learning of the termination. The Investigator must complete, sign, and date the Subject Outcome page of the Case Report Form and submit it via facsimile

to +1 914-732-4042. If the termination is due to a serious adverse event, the procedure for reporting serious adverse events must be followed as well (refer to Section 8.3.7).

9. STATISTICS AND DATA EVALUATION

Statistical analyses will be the responsibility of WLV; any additional or supplemental data analyses performed independently by the investigator should be submitted to WLV while in preparation for publication or presentation.

9.1 Efficacy Evaluation

Efficacy of CAIV-T with respect to placebo will be evaluated.

9.1.1 Primary Variables and Comparison of Interest

CAIV-T, when given as a primary series of two doses in the first year, protects children aged 6 months to 36 months at enrollment, from culture-confirmed influenza illness caused by community-acquired subtypes antigenically similar to those contained in the vaccine, compared with placebo. Treatment group comparisons will be reported for all subtypes combined and by individual subtype for those subtypes that are antigenically similar to those contained in the vaccine.

Comparisons of Interest: The test of superiority for this hypothesis will be based on a 95% confidence interval about the efficacy endpoint compared between Group 1.1 versus Group 1.2 after two doses of vaccine in the first year. The lower bound greater than zero demonstrates statistically significant superiority for CAIV-T compared to placebo. The lower bound of the 95% confidence interval will be reported as the estimated lower limit on CAIV-T efficacy.

9.1.2 Secondary Variables and Comparisons of Interest

1. CAIV-T, when given as a primary series of two doses in the first year, protects children aged 6 months to 36 months at enrollment, from culture-confirmed influenza illness caused by community-acquired subtypes antigenically similar to those contained in the vaccine in the second year of surveillance compared with those children who received a primary series of the two doses of placebo in the first year.

Comparisons of Interest: Point estimate and 95% confidence interval will be provided for efficacy parameter based on comparison Group 2.2 with Group 2.4 after one dose of placebo in the second year for combined subtypes that are antigenically similar to those contained in the vaccine.

2. CAIV-T, when given as a single dose in the second year to children aged 6 months to 36 months at the time of enrollment and who received a primary series of two doses in the first year, protects against culture-confirmed influenza illness caused by community-acquired subtypes antigenically similar to those contained in the vaccine compared with placebo.

Comparisons of Interest: Point estimate and 95% confidence interval will be provided for efficacy parameter based on comparison Group 2.1 with Group 2.2 after a single dose of CATV-T or placebo in the second year for combined subtypes that are antigenically similar to those contained in the vaccine.

3. CAIV-T, when given as a single-dose in the second year to children aged 6 months to 36 months at enrollment and who received a primary series of two doses of CAIV-T in the first year, protects children from culture-confirmed influenza illness caused by community-acquired subtypes antigenically similar to those contained in the vaccine, compared with children who received a single dose of placebo in the second year following a primary series of two doses of placebo in the first year.

Comparisons of Interest: Point estimate and 95% confidence interval will be provided for efficacy parameter based on comparison Group 2.1 with Group 2.4 after one dose of CAIV-T or placebo in the second year for combined subtypes that are antigenically similar to those contained in the vaccine.

4. CAIV-T, when given as a single-dose in the second year to children aged 6 months to 36 months at enrollment and who received a primary series of two doses of either CAIV-T or placebo in the first year, protects children from culture-confirmed influenza illness caused by community-acquired subtypes antigenically similar to those contained in the vaccine.

Comparisons of Interest: Point estimate and 95% confidence interval will be provided for efficacy parameter based on comparison Group 2.1 with Group 2.3 after one dose of CAIV-T in the second year for combined subtypes that are antigenically similar to those contained in the vaccine.

5. Compared over two consecutive years, CAIV-T, when given as a single dose of liquid formulation in the second year after two-doses of a liquid formulation in the first year versus a single-dose of placebo in the second year after two doses of placebo in the first year, protects children aged 6 months to 36 months at enrollment from culture-confirmed influenza illness associated with any subtype.

Comparisons of Interest: Point estimate and 95% confidence interval will be provided for efficacy parameter based on comparison Group 2.1 with Group 2.4 over two consecutive years for any influenza virus strain or subtype antigenically not similar to those contained in the vaccine.

6. CAIV-T, when given as a primary series of two doses in the first year to children aged 6 months to 36 months at enrollment, reduces the occurrence of protocol defined acute otitis media, febrile otitis media, and influenza associated otitis media in the first and second year of the study, compared with placebo.

Comparisons of Interest: Comparison Group 1.1 versus Group 1.2 in the first year; and, Group 2.1 versus Group 2.4 in the second year for the number of episodes for acute otitis media, febrile otitis media, and influenza associated otitis media will be provided.

9.1.3 Statistical Methods for Efficacy Evaluation

Primary Analysis will be conducted after completing the first year study based on group unblinding.

9.1.3.1 Case Definition for Culture-Confirmed Episode

A culture-confirmed episode of influenza illness is defined by a positive viral culture of a wildtype virus subtype antigenically similar to one contained in the vaccine. Subjects completing the primary vaccination series in the first year less than 15 days prior to the time of development of culture-confirmed illness will not be evaluable for primary efficacy analysis but will be evaluable for intent-to-treat analysis. Subjects completing the vaccination

in the second year less than 15 days prior to the time of development of culture-confirmed illness will not be evaluable for secondary efficacy analysis but will be evaluable for intent-to-treat analysis.

9.1.3.2 Efficacy Endpoints for Culture-Confirmed Episode

The primary efficacy endpoint is the first episode during the first year in a study child of a culture-confirmed influenza illness, caused by community-acquired subtypes antigenically similar to those contained in the vaccine, following receipt of the second dose of study vaccine or placebo (see Case Definition in 9.1.3.1).

The secondary efficacy endpoint is the first episode during the second year in a study child of a culture-confirmed influenza illness, caused by community-acquired subtypes antigenically similar to those contained in the vaccine, following receipt of two doses administered in the first year and one dose during the second year (see Case Definition in 9.1.3.1).

All episodes during both years in a study child of a culture-confirmed influenza illness, caused by community-acquired subtypes antigenically similar to those contained in the vaccine, following receipt of a primary series of two doses of vaccine or placebo.

9.1.3.3 Primary, Secondary and Intent-to-Treat Analysis for Culture-Confirmed Episode

The Primary Analysis will include all subjects receiving placebo or study vaccine. It will be based on the primary endpoint (see 9.1.3.2) of all episodes of culture-confirmed influenza-illness occurring at least 15 days after the receipt of the second dose of study vaccine or placebo (see 9.1.3.1) in the first year of the study.

The Secondary Analysis will include all subjects receiving placebo or study vaccine. It will be based on secondary endpoint (see 9.1.3.2) of all episodes for culture-confirmed influenza-illness occurring at least 15 days after the receipt of a dose of study vaccine or placebo (see 9.1.3.1) in the second year of the study.

The Intent-to-Treat Analysis will include all subjects. It will be based in the first year, on all episodes of culture-confirmed influenza-illness occurring at least 15 days after the first dose of study vaccine or placebo.

The Intent-to-Treat Analysis of protection against all culture-confirmed influenza illness due to subtypes antigenically similar to those contained in the vaccine as well as to unrelated strains will be performed.

9.1.3.4 Efficacy Evaluation of CAIV Vaccine for Culture-Confirmed Episode

The superiority test of CAIV-T_1 versus placebo will be based on the point estimate of the efficacy ($e=1- I_v/I_p$, where I_v and I_p represent the incidence rates in the vaccinated and control groups respectively). A StatXact™ calculation for the exact method confidence interval about the rate ratio (I_v/I_p) will be used to estimate the interval about e. If the lower limit of the confidence interval is greater than zero then the vaccine is significantly superior to placebo. Furthermore, the true efficacy is greater than the estimated lower confidence limit with 97.5% probability.

9.1.3.5 Additional Analysis for the Core Efficacy Study for Culture-Confirmed Episode

- An additional analysis will be performed to compare the two-dose regimen versus the one-dose regimen for placebo;
- An additional analysis will be performed using the Intent-to-Treat analysis (see 9.1.3.3).

9.1.3.6 Secondary Analysis for Culture-Confirmed Episode

The Secondary Analysis will be analogous to that for the Core Efficacy Study (see 9.1.3.4), with the exception that this analysis will estimate efficacy in the study year following a second year's single vaccination. In addition, for children vaccinated in two successive study years, efficacy will be evaluated by:

- Comparing vaccine and placebo groups with respect to the number of children who had at least one culture documented case of influenza in the two study years;
- Comparing vaccine and placebo groups with respect to the distribution of children who had culture documented influenza in neither study years, in the first study year, or in the second study year;
- Comparing vaccine and placebo groups with respect to the total number of culture documented influenza cases in the two study years.

9.1.4 Efficacy Analysis for Acute Otitis Media

9.1.4.1 Case Definition for Acute Otitis Media

Acute otitis media (AOM) is defined by the demonstration of a visually abnormal tympanic membrane (in regard to color, position, and/or mobility) suggesting effusion in the middle ear cavity, concomitantly with at least one of the following signs and/or symptoms of acute infection: fever (≥38°C rectal or oral, or ≥37.5°C axillary), ear ache, irritability, diarrhea, vomiting, acute otorrhea not caused by external otitis, or other symptoms of respiratory infection (20).

An episode of febrile otitis media is defined as an episode of acute otitis media in a child with a documented fever (≥38°C rectal or oral, or ≥37.5°C axillary).

An episode of influenza-associated otitis media is defined as an episode of acute otitis media in a child with a positive culture for influenza virus.

An episode of AOM in a study participant will be included in the efficacy estimations, if it complies with the definition of AOM above, and occurs at least 15 days after receipt of the first dose of vaccine or placebo.

9.1.4.2 Efficacy Endpoints for Acute Otitis Media

For the purposes of analysis of efficacy against otitis media, a new episode of AOM is defined as being when at least 30 days have elapsed since the previous AOM irrespective of etiology.

- *Primary AOM Efficacy Endpoint*

The primary endpoint for efficacy against acute otitis media is all episodes of acute otitis media associated with a positive culture for influenza virus antigenically similar to those contained in the vaccine, occurring during either year in a study child.

- *Secondary AOM Efficacy Endpoint*

The secondary endpoint for efficacy against AOM is first episodes of acute otitis media associated with a positive culture for influenza virus antigenically similar to those contained in the vaccine, occurring during either year in a study child.

In addition, the following will be investigated: efficacy against all episodes of AOM during either year in a study child; and all and first episodes of acute otitis media associated with fever (≥38°C rectal or oral, or ≥37.5°C axillary) during either year in a study child.

9.1.5 Characterization of Study Groups

Demographic characteristics, baseline history, physical exam and laboratory data will be compared between treatment groups for descriptive purposes.

9.1.6 Sample Size Calculation

The sample size of this study is designed to demonstrate at least 45% efficacy of CAIV-T compared to Placebo with at least 90% power at the 5% significance level.

Sample size calculation is based on the following assumptions:

- the true event rate in the CAIV-T group after the first year will be 3%;
- the true event rate in the placebo group after the first year will be 12%;
- the predicted drop-out rate over 2 years will be no more than 60%.

Due to the relatively low influenza incidence rate expected in the vaccine group, optimal number of subjects for a given study power can be achieved by including more subjects in the vaccine group than in the control group. This approach leads to a total sample size of 1,100 with 3:2 ratio (i.e., 660 subjects in vaccine group and 440 subjects in placebo group).

9.1.7 Analysis of Safety

- **Reactogenicity:** Subjects in the treatment groups will be compared with respect to the proportion with fever (temperature ≥ 37.5°C axillary), cough, runny nose or nasal congestion, irritability, vomiting, decreased appetite, and decreased activity (lethargy), using Fisher's Exact test.
- **Adverse Events:** Adverse Events listed by body system, intermediate terms, and severity and causality assessments will be compared between treatment groups using Fisher's Exact test.

The planned sample size for this study (1800 subjects for group 1.1 and 1200 for Group 2.1) should provide at least 80% statistical power to detect from 4.3% to 8.2% increases, depending on the frequency of incidences of the Placebo group (Table 4).

Table 4

Power Analysis for Safety Evaluation

Smallest Detectable Difference in Frequency of Systemic and Local Reactions with 600 Completed and Evaluable Subjects per Group (Power ≥ 80%)	
Assumed Reaction Frequency in the Placebo Group	Smallest Detectable Increase in the Vaccine Group
5%	4.3%
10%	5.6%
15%	6.4%
20%	7.0%
30%	7.8%
40%	8.2%

9.1.8 Interim Analysis

There will be no interim analyses performed.

10. DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

The Investigator agrees to permit trial-related monitoring, audits, ERC review, and regulatory inspection(s), and to provide direct access to source data/documents.

11. QUALITY CONTROL AND QUALITY ASSURANCE

11.1 Monitoring

All aspects of the study will be monitored by WLV or by authorized representatives, with respect to current Good Clinical Practices and Standard Operating Procedures for compliance with applicable government regulations.

Prior to the beginning of the study, the Investigator will be informed of the frequency of monitoring visits. The Investigator will receive reasonable notification prior to each monitoring visit during the course of the study. During the monitoring visit, the monitor will check the availability of the signed Informed Consent Forms and verify the prompt reporting of any SAEs that have occurred. Case Report Forms (CRFs) will be compared with source data for accuracy and completeness.

The sponsor will ensure that appropriate corrections, additions, or deletions that are made to the CRFs are dated, explained (if necessary), and initialed by the Investigator (or designee who is authorized to initial CRF changes for the Investigator). Study documents must also be available for review during the course of the study.

During the monitoring visit, the Investigator (and/or designee) and the study personnel should be available to discuss the study progress.

11.2 Quality Assurance

Studies that are critical for registration may have to undergo Quality Assurance Verification. It is possible that government authorities and/or Sponsor may perform inspections of Investigator compliance with all relevant guidelines and regulations for clinical

trials. Sponsor audits will be conducted by personnel that are independent from the group conducting the clinical trial.

12. DATA HANDLING AND RECORD KEEPING

12.1 Case Report Forms

Case Report Forms (CRFs) and diary cards will be maintained for recording data for each subject enrolled in the study. The Investigator is responsible to ensure the accuracy, completeness, legibility, and timeliness of the data reported to the Sponsor in the CRF. Data reported in the CRF, derived from source documents, should be consistent with the source documents or the discrepancies should be explained.

The Sponsor will provide guidance to Investigators on making corrections to the CRF.

12.2 Source Documentation

Each subject requires complete and adequate source documentation (hospital or medical records, lab reports, test results) for the complete time period of the study. These records must be available to the Sponsor and regulatory authorities upon request for review.

12.3 Study Documentation

As a minimum, the Investigator must retain the following study documents on file:

- Clinical Investigator Brochure, and any updated safety reports or brochures
- Final Protocol with completed Investigator's signature page
- Amendment(s) to the Protocol with completed Investigator's signature page
- Sample of Case Report Forms
- ERC approval of the final Protocol and Protocol Amendment(s)
- ERC approval of the Informed Consent Form and any modifications
- Copy of the ERC approved Informed Consent Form
- ERC approval of advertisements and other written information to be provided to the subject
- ERC annual study review
- Correspondence with ERC
- Study Initiation Monitoring Report
- CV's of Principal Investigator and co-investigators
- Form FDA 1572 (or equivalent Statement of Investigator)
- Correspondence with the Sponsor
- SAE Reports

- Clinical Supply Records
- Shipping records for investigational product(s) and trial-related material
- Vaccine Accountability Records, which include dispensing records of used vaccine during the study and accountability of unused vaccine at study completion (including destruction certification, if applicable)
- Decoding Procedures (for blinded trials only)
- Financial agreement and insurance statement, and financial disclosure statements.
- Copies of Sample Control Form (if applicable)
- Final report by Investigator to ERC
- Clinical Study Report (if applicable)
- Subject identification list, subject screening log, and subject enrollment log
- Monitoring Visit Log
- Authorized Signature Record
- Copies of completed CRFs and corrections to the CRFs

12.4 Retention of Records

The Investigator will retain trial-related documents as required by the applicable regulatory requirements(s) or by an agreement with the Sponsor. The Investigator should take measure to prevent accidental or premature destruction of these documents.

Essential documents should be retained for:

- A period of two years after the last approval of a marketing application in an ICH region (USA, European Community or Japan) and until there are no pending or contemplated marketing applications in an ICH region.

  OR

- A period of two years have elapsed since the formal discontinuation of a clinical development of the investigational product.

The essential documents could be retained for a longer period, however, if required by the applicable regulatory requirements or by an agreement with the Sponsor.

It is the responsibility of the Sponsor to inform the Investigator as to when these documents no longer need to be retained.

12.5 Data Handling

Once monitoring of the Case Report Forms is completed at the study site, the forms are sent for data entry, validation, and analysis. Data queries will be resolved through interaction with the data and site personnel.

12.6 Protocol Revisions

With the exception of emergency situations, no changes or deviations in this protocol will be permitted without the documented approval of a representative of the Sponsor and the ERC that granted original approval for the study. This stipulation does not apply to those changes made to reduce discomfort or overt risk to study subjects. In the event of any medical emergency, the Investigator shall institute any medical procedures that he/she deems appropriate. However, all such events and procedures must be promptly reported to the Sponsor.

13. ETHICAL ASSURANCE FOR PROTECTION OF HUMAN RIGHTS

13.1 Ethical Review Committee (ERC)

The Investigator will be responsible for ERC approval for the study.

Before the study commencement, the appropriate documents (which may include the Protocol, Clinical Investigator Brochure, Informed Consent Form, information sheets, and advertisements) will be submitted to the ERC by the Investigator. A copy of the study appr`oval, including informed consent approval, is to be kept in the Investigator's study document binder and a copy supplied to the Sponsor.

During the study, the Investigator is responsible for providing the ERC with all documents subject to review (i.e., Protocol Amendments, Informed Consent Form updates, advertisements, and other written information that may be provided to the subject).

Appropriate reports on the progress and termination of the study will be made to the ERC by the Investigator in accordance with ERC guidelines and government regulations.

13.2 Declaration of Helsinki

The clinical trial will be performed in accordance with the principles of the Declaration of Helsinki/Somerset West, Republic of South Africa, October 1996.

13.3 Informed Consent

In obtaining and documenting informed consent, the Investigator must comply with the applicable regulatory requirement(s), Good Clinical Practice (GCP), and ethical principles. The written Informed Consent Form must be approved by an ERC prior to its use.

The Informed Consent Form describes that participation is voluntary and can be terminated at any time without reason. In addition, the parent/guardian will receive information on the study vaccine(s) to be used in the study. The scope of the trial will be explained. By signing the Informed Consent Form, the parent/guardian confirms the child's voluntary participation and their intention to follow the study protocol and the instructions of the Investigator.

The Investigator is responsible to obtain written informed consent from the parents/guardians for each subject enrolled. The Informed Consent Form must be signed and dated by the parent/guardian prior to study enrollment.

14. PUBLICATION POLICY

The Investigator may publish/present the results of scientific investigations involving this clinical study, provided that confidential information of the Sponsor is not disclosed and that the Sponsor is provided a copy of the manuscript sixty (60) days prior to presentation or submission for publication for review and approval, to insure that confidential information is not disclosed.

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