To
Professor A K M Nurul Anwar
Member, ERC

International Centre for Diarrhoeal Disease Research, Bangladesh
CENTRE FOR HEALTH AND POPULATION RESEARCH
Mail : ICDDR, B, GPO Box 128, Dhaka-1000, Bangladesh
Phone : 880-2-8811751-60, Telex : 642486 ICDD BJ
Fax : 880-2-8823116, 8812530, 8811568, 8826050, 9885657, 8811686, 8812529
Cable : Cholera Dhaka

Please review the modified version of the protocol and provide your comments whether the ERC observations have been addressed or not. Thank you.

6/8/2002

HSID
2002

Memorandum

To: Professor Mahmudur Rahman
Chairman, Ethical Review Committee (ERC)

CC: David Sack, MD, Robert Breiman, MD
Director, Assoc Director HSID

From: W. Abdullah Brooks, MD, MPH
Acting Head, Programme of Infectious Diseases & Vaccine Sciences

Date: 05.08.2002

Re: Protocol 2002-012

Thank you for your further comments from the ERC on our protocol # 2002-012, entitled, "A prospective, randomized, double blind, placebo-controlled, multi-centre trial to assess safety, efficacy, tolerability, and immunogenicity of influenza virus vaccine, trivalent, types A & B, live cold-adapted, liquid formulation (CAIV-T), administered concomitantly with a combination live, attenuated, mumps, measles, and rubella vaccine in healthy children aged 11-24 months". The observations are helpful, and, once again we address them in serial fashion below.

a) *"PI's response in reply to the observation #(a) should be incorporated in the protocol. The PI should clearly state that no initiative will be taken that may encourage deliberate delay in EPI schedule of measles vaccination."*

We have taken the Committee's suggestion and have incorporated the following statement into the text of the protocol (p11):

MMR vaccine is in routine use worldwide. In the United States, for instance MMR vaccine has been licensed since 1971, is recommended for each child, and has been in wide use for over 30 years. The vaccine has been recommended for every child in the United Kingdom since 1988. Well over 100 million children have received this vaccine. While MMR is licensed in Bangladesh, it is not used routinely by EPI because of cost considerations. MMR is more expensive than measles vaccine. MMR provides equivalent protection to measles vaccine against measles; however, in addition it provides protection against mumps and rubella.

The first dose of MMR vaccine is usually given between 9 and 24 months of age. In many countries where vaccine costs are a key issue, like Bangladesh, measles vaccine is given alone (mumps and rubella vaccine are not given). However, the vaccine schedules for

Memorandum

To: Professor Nizamuddar Rahman
Chairman, Ethical Review Committee (ERC)

CC: David Sack, MD, Robert Breiman, MD
Director, Assoc Director HSID

From: W. Abdullah Brooks, MD, MPH
Acting Head, Programme of Infectious Diseases & Vaccine Sciences

Date: 05.08.2002

Re: Protocol 2002-012

Thank you for your further comments from the ERC on our protocol # 2002-012, entitled, "A prospective, randomized, double blind, placebo-controlled, multi-centre trial to assess safety, efficacy, tolerability, and immunogenicity of influenza virus vaccine, trivalent, types A & B, live cold-adapted, liquid formulation (CAIV-T), administered concomitantly with a combination live, attenuated mumps, measles, and rubella vaccine in healthy children aged 11-24 months". The observations are helpful, and, once again we address them in serial fashion below.

a) "PI's response in reply to the observation #1a) should be incorporated in the protocol. The PI should clearly state that no initiative will be taken that may compromise definitive delay in EPI schedule of measles vaccination."

We have taken the Committee's suggestion and have incorporated the following statement into the text of the protocol (p11):

MMR vaccine is in routine use worldwide. In the United States, for instance MMR vaccine has been licensed since 1971, is recommended for each child, and has been in wide use for over 30 years. The vaccine has been recommended for every child in the United Kingdom since 1988. Well over 100 million children have received this vaccine. While MMR is licensed in Bangladesh, it is not used routinely by EPI because of cost considerations. MMR is more expensive than measles vaccine. MMR provides equivalent protection to measles vaccine against measles however, in addition it provides protection against mumps and rubella.

The first dose of MMR vaccine is usually given between 9 and 24 months of age. In many countries where vaccine costs are a key issue, like Bangladesh, measles vaccine is given alone (mumps and rubella vaccine are not given). However, the vaccine schedules for

MMR vaccine or measles vaccine are the same, and the trial will result in no delay, nor will deliberate delay of EPI schedule be tolerated or accepted.

b) *"The Committee viewed insurance coverage as a provision for providing compensation to the study participants in case of permanent disability and/or death attributed due to vaccination; and not for providing treatment...in case of illness. The protocol should clearly and categorically state that study participants will be provided free treatment both at the study site clinic in Kamalapur and if needed in the hospital. The study should also be incorporated in the protocol as well as in the consent forms."*

We fully concur with the Committee, and have added the following language to the protocol (p18) and to the consent form (p 5), respectively.

Any and all study-related illnesses will be treated, free of cost, at the field clinic for the duration of the study, including for those children who voluntarily withdraw or are withdrawn from the study. If a child requires hospitalisation, all costs pertaining to hospitalisation and transportation to hospital will be paid for.

Your child will be treated, free of cost, at our clinic for any and all study-related illnesses throughout the duration of the study. If you withdraw your child from the study before it is completed, your child will still have access to our clinic. If your child requires hospitalisation, all costs pertaining to hospitalisation and transportation to hospital will be paid for.

c) *"The PI should take further efforts for real improvement of the treatment facility at the ICDDR,B Kamalapur field office."*

We appreciate the Committee's concerns, and share them. We are pleased to inform you that we have just concluded negotiations with the landlord for renting the entire building (apart from her living quarters) and shall dedicate the entire ground floor to clinical services, which will increase the dedicated clinic space approximately three-fold. We will occupy these new spaces on 04 September 2002. We will continue to monitor quality of care, train and test our medical staff, to strive for the highest standard of medical practice we can provide on site.

Attachment 1 Date: 05 August 2002

(FACE SHEET) **ETHICAL REVIEW COMMITTEE, ICDDR,B.**

Principal Investigator: Robert F. Breiman, MD

Application No. 2002-012

Title of Study: A Prospective, Randomized, Double Blind, Placebo-Controlled, Multi-Centre Trial to Assess Safety, Efficacy, Tolerability and Immunogenicity of Influenza Virus Vaccine, Trivalent, Types A & B, Live Cold-Adapted, Liquid Formulation (CAIV-T), Administered Concomitantly with a Combination Live, Attenuated, Mumps, Measles, and Rubella Vaccine in Healthy Children Aged 11 – 24 Months.

Trainee Investigator (if any): _____

Supporting Agency (if Non-ICDDR,B) **Wyeth Lederle Vaccines**

Project Status:

[✓] New Study

[ ] Continuation with change

[ ] No change (do not fill out rest of the form)

Circle the appropriate answer to each of the following (If Not Applicable write NA)

1. Source of Population:
   (a) Ill subjects — (Yes) No
   (b) Non-ill subjects — (Yes) No
   (c) Minor or persons under guardianship — (Yes) No

2. Does the Study Involve:
   (a) Physical risk to the subjects — (Yes) No
   (b) Social risk — Yes (No)
   (c) Psychological risks to subjects — Yes (No)
   (d) Discomfort to subjects — (Yes) No
   (e) Invasion of privacy — Yes (No)
   (f) Disclosure of information damaging to subject or others — Yes (No)

3. Does the Study Involve:
   (a) Use of records (hospital, medical, death or other) — Yes (No)
   (b) Use of fetal tissue or abortus — Yes (No)
   (c) Use of organs or body fluids — (Yes) No

4. Are Subjects Clearly Informed About:
   (a) Nature and purposes of the study — (Yes) No
   (b) Procedures to be followed including alternatives used — (Yes) No
   (c) Physical risk — (Yes) No
   (d) Sensitive questions — (Yes) No
   (e) Benefits to be derived — (Yes) No
   (f) Right to refuse to participate or to withdraw from study — (Yes) No
   (g) Confidential handling of data — (Yes) No
   (h) Compensation &/or treatment where there are risks or privacy is involved in any particular procedure — (Yes) No

5. Will Signed Consent Form be Required:
   (a) From subjects — Yes (No)
   (b) From parents or guardian (if subjects are minor) — (Yes) No

6. Will precautions be taken to protect anonymity of subjects — (Yes) No

7. Check documents being submitted herewith to Committee:
   ✓ Umbrella proposal - Initially submit an with overview (all other requirements will be submitted with individual studies
   ✓ Protocol (Required)
   ✓ Abstract Summary (Required)
   ✓ Statement given or read to subjects on nature of study, risks, types of questions to be asked, and right to refuse to participate or withdraw) (Required
   ✓ Informed consent form for subjects
   ✓ Informed consent form for parent or guardian
   ✓ Procedure for maintaining confidentiality
   ✓ Questionnaire or interview schedule*

   * If the final instrument is not completed prior to review, the following information should be included in the abstract summary
   1. A description of the areas to be covered in the questionnaire or interview which could be considered either sensitive or which would Constitute an invasion of privacy
   2. Example of the type of specific questions to be asked in the sensitive areas
   3. An indication as to when the questionnaire will be presented to the Committee for review

We agree to obtain approval of the Ethical Review Committee for any changes involving the rights and welfare of subjects before making such change.

Principal Investigator

Trainee

RESEARCH PROTOCOL

Protocol No. 2002-12

FOR OFFICE USE ONLY

RRC Approval: Yes/ No Date:

ERC Approval: Yes/No Date:

AEEC Approval: Yes/No Date:

Project Title: A Prospective, Randomized, Double Blind, Placebo-Controlled, Multi-Centre Trial to Assess Safety, Efficacy, Tolerability and Immunogenicity of Influenza Virus Vaccine, Trivalent, Types A & B, Live Cold-Adapted, Liquid Formulation (CAIV-T), Administered Concomitantly with a Combination Live, Attenuated, Mumps, Measles, and Rubella Vaccine in Healthy Children Aged 11 – 24 Months.

Theme: *(Check all that apply)*

- ☐ Nutrition
- ☒ Emerging and Re-emerging Infectious Diseases
- ☐ Population Dynamics
- ☐ Reproductive Health
- ☒ Vaccine evaluation
- ☐ Environmental Health
- ☐ Health Services
- ☐ Child Health
- ☐ Clinical Case Management
- ☐ Social and Behavioural Sciences

Key words: Influenza, vaccine, nasal spray, immunogenicity, efficacy

Relevance of the protocol: The findings from this trial will be relevant to use of a nasal spray influenza vaccine to prevent influenza and complications from influenza in Bangladesh.

Principal Investigator: Robert F. Breiman, MD **Division: HSID** **Phone: x2500**

Address: ICDDR,B **Email: breiman@icddrb.org**

Co-Principal Investigator(s): W. Abdullah Brooks, MD, MPH

Co-Investigator(s): Doli Goswami, MBBS

Student Investigator/Intern:

Collaborating Institute(s): Wyeth-Lederle Vaccines

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 -- **Vaccine Trial**

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:

Sub-group _____150 in Bangladesh (☒ _____ ☐

Total sample size: 1200 in entire multi-centre trial ___ _____ ☐ _____ ☐

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 behaviour
- ☐ 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 behaviour; sexual behaviour, 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

Yes/No

☐ ☒ Is the proposal being submitted for funding?

If yes, name of funding agency: (1) _____

(2) _____

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.

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: 09/01/02	$105,989			
End date: 08/30/03	*b. Direct Cost :* $84,116	*Total Cost :* $105,989		

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.

Robert Belman _____ 27-6-02

Name of the Division Director | Signature | 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.

Signature of PI _____

Date: 6-27-02

Name of Contact Person (if applicable)

_____

A Prospective, Randomized, Double Blind, Placebo-Controlled, Multi-Centre Trial to Assess Safety, Efficacy, Tolerability and Immunogenicity of Influenza Virus Vaccine, Trivalent, Types A & B, Live Cold-Adapted, Liquid Formulation (CAIV-T), Administered Concomitantly with a Combination Live, Attenuated, Mumps, Measles, and Rubella Vaccine in Healthy Children Aged 11 – 24 Months.

Abstract Summary

In Asia, influenza rates are highest in young children. In community surveillance of children under 5 years with acute lower respiratory infections (ALRI) in Manila and Dhaka, influenza was confirmed by culture as the probable etiology in 22% and 14% cases if ALRI, respectively. Similarly, in Singapore, children under 4 years of age accounted for approximately 22% of all influenza isolations, representing the highest age-specific rates, and among those infants hospitalized for acute respiratory tract infections, influenza was associated with 11%.

Cold adapted influenza virus vaccine (CAIV-T) produced by Aviron Inc. (Mountain View, CA, USA) and Wyeth Lederle Vaccines (Marietta, PA, USA) is a live, trivalent, cold-adapted, temperature sensitive and attenuated influenza vaccine consisting of two influenza strains of type A and one influenza strain of type B. CAIV-T 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. The vaccine is administered via nasal spray. It is well tolerated and a variety of studies have demonstrated high efficacy in prevention of influenza and influenza-related conditions including lower respiratory infections and otitis media. Because of the role of influenza as a direct cause of respiratory illness and because of its role in predisposing patients to secondary bacterial pneumonia, this vaccine may be of substantial use in prevention of morbidity and mortality in developing countries.

The first dose of Measles, Mumps, Rubella Vaccine (MMR) is usually given between 9 and 24 months of age. In many countries where vaccine costs are a key issue, like Bangladesh, measles vaccine is given alone (mumps and rubella vaccine are not given routinely). However, the vaccine schedules for MMR or measles vaccine are the same, and MMR is licensed in Bangladesh. MMR obviously provides additional protection against mumps and rubella, both of which are responsible for illness and sequelae worldwide.

At the present time, no data exist regarding the effect on immune responses to the components of MMR vaccine when co-administered with CAIV-T. For CAIV-T to be used routinely in Bangladesh and other developing countries, it is necessary to show that administration of CAIV-T would not interfere with immune responses to each of the components of MMR (including measles vaccine), which could potentially be given simultaneously. Likewise, it is important to evaluate whether MMR, administered concomitantly, interferes with protective immune responses elicited by the influenza vaccine (CAIV-T). The principal purposes of this clinical trial is to investigate the effect, if any, of the co-administration of CAIV-T and MMR on the immune responses to the

components of the MMR vaccine, and on the efficacy of CAIV-T against influenza infections.

CAIV-T has been shown to be generally safe, well tolerated, efficacious, and effective in both healthy children and healthy adults. In the pediatric protective efficacy trial, children receiving two doses of CAIV-T had 92% overall protection from culture-confirmed influenza compared to placebo recipients for both years of the trial combined. 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. No serious adverse events have been associated with use of this vaccine.

To further minimize risks and a potential that ongoing acute illness might aggravate symptoms associated with receipt of the vaccine, children will not be given the vaccine while they have symptoms of acute illness. All adverse events will be monitored in daily home visits, beginning on the day of vaccination, by field research assistants who will record all symptoms and check for fever with a thermometer. Any child with fever or other complications will be referred to clinic for evaluation and management by the medical officers

Strict confidentiality will be maintained during the study and only research staff that need this information for follow-up and completion of the study will have access to personal identifiers. Records will be kept in a locked cabinet. All personal identifiers will be stripped from the data set at conclusion of the study (including the follow-up period).

This clinical trial will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, and that are consistent with GCP and the applicable regulatory requirements.

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 Institutional Review Board/Ethics Committee (IRB/EC) 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(s)/legal guardian(s) will receive information on the vaccines to be used in the study. The scope of the trial will be explained. By signing the Informed Consent Form, the parent(s)/legal guardian(s) confirms the child's voluntary participation and his/her intention to follow the study protocol and the instructions of the Investigator.

The Investigator is responsible to obtain written informed consent for each subject enrolled. The Informed Consent Form must be signed and dated by the parent(s)/legal guardian(s) prior to study enrollment. Each parent(s)/legal guardian(s) will receive a

copy of the signed Informed Consent Form. The study will not be conducted among sick individuals. The objectives of this study cannot be achieved other than among human subjects (an animal model could not adequately address the study questions).

This vaccine may be of substantial use in prevention of morbidity and mortality in developing countries. Influenza is a global pathogen capable of causing severe epidemics worldwide. Given studies showing excellent immune responses to influenza antigens and protection against influenza following immunization of children with CAIV-T, it is likely that children who receive this vaccine during the trial will experience protection against influenza and influenza-related complications. There is a risk of minor adverse events as stated above. Given the absence of known serious adverse events, we believe that the potential benefits to the individual and to society far outweigh potential risks.

The project will not require use of records, organs, tissues, the fetus or abortus, but it will require the use of body fluids (blood) for evaluating serum antibody levels at baseline and after the first dose of the vaccine to measure immune responses to influenza and MMR vaccines, which is a principal aim of the study.

Table of Contents

☐ Check here if appendix is included

PROJECT SUMMARY: Describe in concise terms, the hypothesis, objectives, and the relevant background of the project. Describe concisely the experimental design and research methods for achieving the objectives. This description will serve as a succinct and precise and accurate description of the proposed research is required. This summary must be understandable and interpretable when removed from the main application. **(TYPE TEXT WITHIN THE SPACE PROVIDED).**

Principal Investigator Robert F. Breiman, M.D.

Project Name: A Prospective, Randomized, Double Blind, Placebo-Controlled, Multi-Centre Trial to Assess Safety, Efficacy, Tolerability and Immunogenicity of Influenza Virus Vaccine, Trivalent, Types A & B, Live Cold-Adapted, Liquid Formulation (CAIV-T), Administered Concomitantly with a Combination Live, Attenuated, Mumps, Measles, and Rubella Vaccine in Healthy Children Aged 11 – 24 Months.

Total Budget: $105,989 Beginning Date: September 1, 2002 Ending Date: August 30, 2003

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. In cool and temperate climates, influenza occurs annually in the autumn and winter months causing widespread infection and morbidity in all age groups. 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. The attack rate is greatest in individuals with minimal prior exposure to influenza, particularly children. Although complete recovery occurs in uncomplicated influenza, pneumonia and other serious complications may occur in the very young, the elderly, the immune compromised and persons with underlying conditions such as chronic pulmonary or heart diseases.

In Asia, influenza rates are highest in young children. In community surveillance of children under 5 years with acute lower respiratory infections (ALRI) in Manila and Dhaka, influenza was confirmed by culture as the probable etiology in 22% and 14% cases if ALRI, respectively. Similarly, in Singapore, children under 4 years of age accounted for approximately 22% of all influenza isolations, representing the highest age-specific rates, and among those infants hospitalized for acute respiratory tract infections, influenza was associated with 11%.

Cold adapted influenza virus vaccine (CAIV-T) produced by Aviron Inc. (Mountain View, CA, USA) and Wyeth Lederle Vaccines (Marietta, PA, USA) is a live, trivalent, cold-adapted, temperature sensitive and attenuated influenza vaccine consisting of two influenza strains of type A and one influenza strain of type B. CAIV-T 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. The vaccine is administered via nasal spray. It is well tolerated and a variety of studies have demonstrated high efficacy in prevention of influenza and influenza-related conditions including lower respiratory infections and otitis media. Because of the role of influenza as a direct cause of respiratory illness and because of its role in predisposing patients to secondary bacterial pneumonia, this vaccine may be of substantial use in prevention of morbidity and mortality in developing countries.

The first dose of Measles, Mumps, Rubella Vaccine (MMR) is usually given between 9 and 24 months of age. In many countries where vaccine costs are a key issue, like Bangladesh, measles vaccine is given alone (mumps and rubella vaccine are not given). However, the vaccine schedules for MMR or measles vaccine are the same. MMR obviously provides additional protection against mumps and rubella.

At the present time, no data exist regarding the effect on immune responses to the components of MMR when co-administered with CAIV-T. For this vaccine to be used routinely in Bangladesh and other developing countries, it is necessary to show that administration of CAIV-T would not interfere with

immune responses to MMR which could potentially be given simultaneously. Likewise, it is important to evaluate whether MMR administered concomitantly interferes with protective immune responses elicited by the influenza vaccine (CAIV-T).

In clinical trials to date, no other live virus vaccine may be administered within 1 month of a dose of CAIV-T. The first dose of MMR is routinely given between the age of 11– 24 months of age. Healthy children within this age range may also benefit from an effective live influenza vaccine, such as CAIV-T. However, use of CAIV-T would be severely restricted should the timing of opportunity for influenza vaccination coincide with the time that the children are due to receive MMR without data to support the concomitant use of CAIV-T and MMR. Therefore, the principal purpose of this clinical trial is to investigate the effect, if any, of the co-administration of CAIV-T and MMR on the immune responses to the components of the MMR vaccine, and on the efficacy of CAIV-T.

KEY PERSONNEL (List names of all investigators including PI and their respective specialties)

Name	Professional Discipline/ Specialty	Role in the Project
1. Robert F. Breiman, MD	Infectious Diseases/Epidemiology	PI
2. W. Abdullah Brooks, MD, MPH	Epidemiology	Co-PI
3. Doli Goswami, MBBS	Medical Doctor/Research Investigator	Co-Investigator

DESCRIPTION OF THE RESEARCH PROJECT

Hypotheses to be tested:

Concisely list in order, in the space provided, the hypothesis to be tested and the Specific Aims of the proposed study. Provide the scientific basis of the hypothesis, critically examining the observations leading to the formulation of the hypothesis.

1. Intranasally-administered live, cold-adapted influenza virus vaccine, trivalent, types A and B (CAIV-T), liquid formulation, when administered concomitantly to children aged 11 to 24 months will not interfere with immune responses to the components of a combination live, attenuated mumps, measles, and rubella virus (MMR) vaccine administered subcutaneously.

2. Two doses of CAIV-T, administered prior to the anticipated commencement of the influenza season, will be efficacious over one season against culture-confirmed influenza-illness caused by community-acquired subtypes antigenically similar to those contained in the vaccine, in children who are at least 11 months and less than 24 months of age at enrollment, when compared with children in the same age range who receive placebo, each with concomitant administration of MMR vaccine (single dose, given with the first dose of study vaccine).

3. Two doses of intranasally-administered live, cold-adapted influenza virus vaccine, trivalent, types A and B (CAIV-T), liquid formulation will be efficacious against clinically defined acute otitis media, febrile otitis media, and influenza-associated otitis media.

4. Concomitant administration of CAIV-T with MMR vaccines will be safe and tolerable.

Specific Aims:

Describe the specific aims of the proposed study. State the specific parameters, biological functions/ rates/ processes that will be assessed by specific methods (TYPE WITHIN LIMITS).

1. To determine if intranasally-administered influenza virus vaccine, trivalent, types A and B, live, cold-adapted (CAIV-T), when administered concomitantly to children aged 11 to 24 months, interferes with the immune responses to the components of a combination live, attenuated mumps, measles, and rubella virus vaccine administered subcutaneously.

2. To compare the efficacy over one season against culture-confirmed influenza-illness caused by community-acquired subtypes antigenically similar to those contained in the vaccine, as well as against any community-acquired influenza subtypes, in children who are aged at least 11 months and less than 24 months at enrollment, between those who receive two doses of either a liquid formulation of CAIV-T and those who receive placebo, each with concomitant administration of combined measles, mumps and rubella vaccine (single dose) administered prior to the anticipated commencement of the influenza season.

3. To assess the efficacy of CAIV-T vaccine against clinically defined acute otitis media, febrile otitis media, and influenza-associated otitis media.

4. To assess the safety and tolerability of concomitant administration of CAIV-T with a combination live, attenuated, mumps, measles, and rubella vaccine.

Background of the Project including Preliminary Observations

Describe the relevant background of the proposed study. Discuss the previous related works on the subject by citing specific references. Describe logically how the present hypothesis is supported by the relevant background observations including any preliminary results that may be available. Critically analyze available knowledge in the field of the proposed study and discuss the questions and gaps in the knowledge that need to be fulfilled to achieve the proposed goals. Provide scientific validity of the hypothesis on the basis of background information. If there is no sufficient information on the subject, indicate the need to develop new knowledge. Also include the **significance and rationale** of the proposed work by specifically discussing how these accomplishments will bring benefit to human health in relation to biomedical, social, and environmental perspectives. **(DO NOT EXCEED 5 PAGES, USE CONTINUATION SHEETS).**

Cold adapted influenza virus vaccine (CAIV-T) produced by Aviron Inc. (Mountain View, CA, USA) and Wyeth Lederle Vaccines (Marietta, PA, USA) is a live, trivalent, cold-adapted, temperature sensitive and attenuated influenza vaccine consisting of two influenza strains of type A and one influenza strain of type B. CAIV-T 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.[i,ii] 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 segments coding for the hemagglutinin (HA) and neuraminidase (NA) genes from wild type influenza strains A (H1N1 and H3N2) and B. The reassorted cold-adapted strains are called 6:2 reassortments. All three influenza strains are cold-adapted so that the virus is able to replicate in the relatively cool temperatures of the nose and nasopharynx (25°C); temperature sensitive so that they are not able to replicate efficiently in the warm temperature of the lungs (37°C for type B and 39°C for type A) and attenuated so that the virus does not spread and replicate in the lungs.

Frozen CAIV-T

Clinical trials of a frozen formulation of CAIV-T have been conducted by Aviron in over 8,936 children, over 3,774 healthy adults and approximately 2,334 high risk adults (co-administered with the licensed injectable influenza vaccine). Prior to Aviron's trials, cold-adapted influenza vaccines derived from type A or type B cold-adapted master strains[ii] were used since 1976 in clinical trials sponsored by the National Institute of Health (NIH) and from 1991-1993 in clinical trials sponsored by Wyeth-Ayerst Research. Monovalent and bivalent type A, monovalent type B, and trivalent CAIV-T were administered in trials prior to Aviron sponsorship to more than 8,000 subjects whose ages ranged from 2 months to more than 100 years. The frozen CAIV-T formulation is intended for North American distribution only, and requires storage at –20°C or below.

Liquid CAIV-T

The liquid formulation of CAIV-T jointly developed by Aviron, Inc. and Wyeth Lederle Vaccines is derived from the same three attenuated influenza strains that are used to manufacture the frozen formulation. The difference in the production of the liquid formulation from the frozen formulation occurs

after the harvest of allantoic fluid from SPF chicken eggs infected with the three cold-adapted influenza strains. The harvested material for the liquid formulation is concentrated and purified by centrifugation (refer to the Clinical Investigator's Brochure). The liquid CAIV-T formulation is stable at 2 to 8°C and was developed for more convenient storage and simplified administration of the vaccine, enabling distribution outside of North America.

In ferrets, a liquid CAIV-T formulation was found to be comparable to the frozen CAIV-T for immunogenicity, safety and protection against wild-type influenza virus challenge. There did not appear to be a demonstrable difference between the frozen and liquid CAIV formulations for induction of immunogenicity and protective efficacy in the ferret model (Wyeth-Ayerst technical report [TR#10], a confidential internal company report). A comparable safety study between the liquid and frozen CAIV-T formulation was performed in 420 healthy adults from 18 years of age. To date, eight (8) clinical trials of the liquid formulation of CAIV-T are ongoing or complete in 18 countries. Approximately 7,270 additional subjects, ranging in age from $\geq$ 6 months to $\geq$60 years, have been administered the liquid formulation of CAIV-T in clinical trails currently underway. These studies remain blinded, and therefore, the exact number of vaccinees is estimated at this time based on the expected randomization. This includes approximately 5,109 healthy children 6 to 36 months of age, 450 healthy adolescents 6-17 years of age, 40 healthy adults age 18-45 years of age, and 1670 elderly adults $\geq$65 years of age.

Influenza

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. In cool and temperate climates, influenza occurs annually in the autumn and winter months causing widespread infection and morbidity in all age groups.[iii] 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.[iv] The attack rate is greatest in individuals with minimal prior exposure to influenza, particularly children. Although complete recovery occurs in uncomplicated influenza, pneumonia and other serious complications may occur in the very young, the elderly, the immune compromised and persons with underlying conditions such as chronic pulmonary or heart diseases.

The currently approved, parenterally administered, trivalent inactivated influenza vaccines (TIV) are most commonly recommended for the prevention of influenza in adults and children with chronic disorders, residents of chronic care facilities, and healthy persons 65 years of age or older.[iv] Vaccination recommendations vary by region, but some countries recommend vaccination for persons who desire to avoid influenza infection and to reduce the severity of disease or the chance of transmitting influenza to high-risk persons with whom the individual has frequent contact. However, these recommendations do not

include healthy children, who serve as a source of endemic spread of influenza in the community. Variable efficacy of the inactivated vaccines in the youngest and oldest age groups and poor acceptance of annual administration by injection, particularly in children, have delayed widespread use of influenza vaccination outside the high-risk populations and have stimulated research to identify alternative, practical vaccination methods.

Live, cold-adapted influenza virus vaccines that are protective against naturally circulating influenza types A and B viruses could offer certain potential advantages over inactivated influenza vaccines: Intranasal administration requires no injection; local and systemic immune responses are produced to mediate protection against influenza infection; induction of cell-mediated immunity (e.g., cytotoxic T-cell activity) may contribute to recovery from infection; effective immunization of young children may reduce epidemic transmission of wild-type influenza viruses in households and to the elderly and chronically ill who are at increased risk from serious illness. Live, attenuated vaccines are among the most efficacious, durable and cost-effective vaccines available.

Epidemiology of Influenza in Children

In the USA, the rate of influenza infection is 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). 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 influenza infections occurred in infants aged 6 to 11 months (21 per 100 person-years).[v] In recently reported studies, hospitalization rates for influenza in healthy infants aged 1 year or less represented the highest age-specific hospitalization rates.[vi,vii]

Similar patterns of disease caused by influenza have been reported across a variety of European regional settings. A collaborative surveillance program involving national data from Belgium, France, The Netherlands, Portugal, Spain, and the United Kingdom[viii] reported that the highest proportions of clinical cases of influenza occurred in children under 14 years of age (ranging from 15% in Spain to 49% in northern France). Further, data from respiratory viral surveillance of the Rhône-Alpes region in France indicated that influenza accounted for 22% of all viral isolates obtained from children aged 1 to 4 years with an influenza-like illness, and 58% of viral isolates in children aged 5 to 9 years.[ix] Similar reports from Norway, the Czech Republic, and Scotland, support the significant role of influenza in childhood respiratory illness in Europe.[x,xi,xii]

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.[xiii] In community surveillance of children under 5 years with acute lower respiratory infections in Manila, Philippines, and in Dhaka, influenza was confirmed by culture as the probable etiology in 22% and 14%, respectively.[xiv,xv] Similarly, in Singapore, children under 4 years of age accounted for approximately 22% of all influenza isolations, representing the highest

age-specific rates,[xvi] and among those infants hospitalized for acute respiratory tract infections, influenza was associated with 11%.[xvii]

Data are available from some countries in Southern Africa and South America for influenza in children. The published data confirm the very high rates of influenza infection in young children in diverse populations in the Southern Hemisphere, with influenza rates from South Africa,[xviii,xix,xx,xxi] Argentina,[xxii,xxiii] Brazil,[xxiv,xxv,xxvi,xxvii] and Chile[xxviii] also appearing to be highest in young children. These findings are comparable to those observed in the USA.

Use of Measles-Mumps-Rubella (MMR) Vaccine

MMR vaccine is in routine use worldwide. In the United States, for instance MMR vaccine has been licensed since 1971, is recommended for each child, and has been in wide use for over 30 years. The vaccine has been recommended for every child in the United Kingdom since 1988. Well over 100 million children have received this vaccine. While MMR is licensed in Bangladesh, it is not used routinely by EPI because of cost considerations. MMR is more expensive than measles vaccine. MMR provides equivalent protection to measles vaccine against measles; however, in addition it provides protection against mumps and rubella.

The first dose of MMR vaccine is usually given between 9 and 24 months of age. In many countries where vaccine costs are a key issue, like Bangladesh, measles vaccine is given alone (mumps and rubella vaccine are not given). However, the vaccine schedules for MMR vaccine or measles vaccine are the same, and the trial will result in no delay, nor will deliberate delay of EPI schedule be tolerated or accepted.

MMR is safe and obviously provides additional protection against mumps and rubella. An additional dose of MMR (or measles vaccine alone) is recommended later in childhood in many countries.

Following a single dose of MMR vaccine in the second year of life, the immune response to each of the 3 components of the vaccine is as follows:

Antigen	Response Criterion	Proportion Responders (%; 95% CI)	Data Source
Mumps virus	See note a.	80 (69, 88)	WLV CSR Protocol D124P2
		82 (63, 94)	Rennels[xxix] et al. 2001
Measles virus	See note a.	96 (89, 99)	WLV CSR Protocol D124P2
		100 (88, 100)	Rennels[xxix] et al. 2001

Rubella virus	See note a.	95 (86, 99)	WLV CSR Protocol D124P2
		89 (72, 98)	Rennels[xxix] et al. 2001
a. seroconversion is defined as a change from seronegative to seropositive as determined by serum IgG specific antibody as measured by ELISA, or a four-fold increase in specific IgG antibody titer if the subject was seropositive prior to vaccination. Subjects with inconclusive results prevaccination are considered as seronegative.			

Purpose of study

At the present time, no data exist regarding the effect on immune responses to the components of MMR when co-administered with CAIV-T. In clinical trials to date, no other live virus vaccine may be administered within 1 month of a dose of CAIV-T.

The first dose of MMR is routinely given between the age of 11– 24 months of age. Healthy children within this age range may also benefit from an effective live influenza vaccine, such as CAIV-T. However, use of CAIV-T would be severely restricted should the timing of opportunity for influenza vaccination coincide with the time that the children are due to receive MMR without data to support the concomitant use of CAIV-T and MMR.

Therefore, the principal purpose of this clinical trial is to investigate the effect, if any, of the co-administration of CAIV-T and MMR on the immune responses to the components of the MMR vaccine, and on the efficacy of CAIV-T.

Risks and Benefits

The frozen formulation of CAIV-T, manufactured by Aviron, Inc. has been evaluated in over 8,936 healthy children and 3,774 healthy adults. The results of completed pivotal studies are summarized in Table 1.

Table 1
CAIV-T Efficacy and Effectiveness Studies

STUDY	POPULATION	OBJECTIVE	RESULTS
AV006	Children	Assess the efficacy of CAIV-T 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 in prevention of culture-confirmed influenza in children following the first annual revaccination	• 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 against viral shedding of H1N1 vaccine virus	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	• 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	• 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

The frozen formulation of CAIV-T has been shown to be generally safe, well tolerated, efficacious, and effective in both healthy children[xxx] and healthy adults.[xxxi] In the pediatric protective efficacy trial, children receiving two doses of CAIV-T had 92% overall protection from culture-confirmed influenza compared to placebo recipients for both years of the trial combined.[xxx]

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 are additional 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. or Wyeth Lederle Vaccines.

- 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 vaccination with CAIV-T is a contraindication to further use of this product.

Clinical Investigator Brochure

The Clinical Investigator Brochure (CIB) for the liquid formulation of the Intranasal Influenza Virus Vaccine, Trivalent, Types A and B, Live, Attenuated Cold Adapted, provides additional background.

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 and regulatory requirements for clinical trials and within the laws of Bangladesh.

Research Design and Methods

Describe in detail the methods and procedures that will be used to accomplish the objectives and specific aims of the project. Discuss the alternative methods that are available and justify the use of the method proposed in the study. Justify the scientific validity of the methodological approach (biomedical, social, or environmental) as an investigation tool to achieve the specific aims. Discuss the limitations and difficulties of the proposed procedures and sufficiently justify the use of them. Discuss the ethical issues related to biomedical and social research for employing special procedures, such as invasive procedures in sick children, use of isotopes or any other hazardous materials, or social questionnaires relating to individual privacy. Point out safety procedures to be observed for protection of individuals during any situations or materials that may be injurious to human health. The methodology section should be sufficiently descriptive to allow the reviewers to make valid and unambiguous assessment of the project. **(DO NOT EXCEED TEN PAGES, USE CONTINUATION SHEETS).**

This is a phase III, randomized, double blind, placebo-controlled, multi-centre out-patient study to assess the safety, tolerability, and immunogenicity of a combination live attenuated measles, mumps, and rubella (MMR) vaccine when given concomitantly with Influenza Virus Vaccine, Trivalent, Types A & B, Live Cold-Adapted (CAIV-T), administered intranasally. The safety, tolerability, and efficacy of CAIV-T administered concomitantly with MMR vaccine will also be assessed. The total number of children who will be evaluated in this multi-centre trial will be 1200 healthy children aged 11 to less than 24 months. It is anticipated that this will include 150 children from Bangladesh. Other sites likely to participate in this trial are located in: Singapore, Hong Kong, Manila Philippines, Kuala Lumpur Malaysia, Seramban Malaysia, Bangkok Thailand, Seoul South Korea, and Puebla Mexico.

Subjects will be prospectively randomized to one of two study groups in a 2:1 ratio as detailed below:

Table 2

Vaccines to be Administered (by Study Visit)

Study Group	No. of Subjects	Visit 1	Visit 2
1	800	CAIV-T MMR*	CAIV-T
2	400	Placebo** MMR*	Placebo**

*MMR is administered subcutaneously

** Placebo consisting of physiological-normal saline administered intranasally

The use of placebo as a control is necessary:

- to allow a valid comparison with a control to provide a quantitative assessment of vaccine effect.
- because no proven prophylactic, method of treatment exists for this indication.
- to provide reliable scientific evidence of efficacy and safety to ensure a reliable evaluation of the balance of benefits and risks.

Informed consent will be sought from all parent(s)/legal guardian(s) of children before any study-related procedures are performed.

In order to be considered for the trial, the child must be due to receive their first dose of measles vaccine.

Children with current respiratory illness including the common cold or nasal congestion requiring medical intervention, treatment or special clinical management within 72 hours prior to vaccination will have their participation deferred until the illness resolves. Resolution of upper respiratory illness will be determined by clinical judgment. All subjects will have an initial physical examination (including axillary or rectal temperature (according to local practice)) and medical history review (including current medication usage and vaccination history) prior to vaccination at enrollment (study visit 1).

Each dose of intranasal CAIV-T or placebo will consist of approximately 0.2 mL in total volume administered as an intranasal spray using a spray applicator. Approximately 0.1 mL will be sprayed into each nostril. The combination, live, attenuated, trivalent mumps, measles, and rubella virus vaccine used in the study will be licensed in Bangladesh. MMR will be administered subcutaneously according to the manufacturer's instructions. Immediately following the vaccination, each subject will be observed for at least 30 minutes for signs and symptoms of anaphylaxis and local reactions (e.g., nasal congestion/runny nose).

The time interval between the assigned first dose and the assigned second dose will be 35 +/- 7 days. Prior to the second vaccination the subject will be assessed for continued eligibility.

A blood specimen of approximately 5 milliliters will be collected from subjects on two occasions. The first sample will be assayed using a serum hemagglutination inhibition assay for influenza virus types A/H1N1, A/H3N2 and B. In addition, measles, mumps and rubella virus antibody responses will be determined by serum IgG as measured by ELISA. The second specimen will be assayed for measles, mumps and rubella virus antibody responses by serum IgG as measured by ELISA. These blood specimens will be collected prior to the first dose (study visit 1), and prior to the second dose (study visit 2). Specimen collection and processing guidelines will be provided separately from this document.

Following vaccination, the parent(s)/legal guardian(s) will be instructed to complete a diary card for 11 consecutive days including the day of immunization, Day 0 to Day 10. The following information will be collected in the diary card: axillary or rectal temperature (according to local practice), runny nose/nasal congestion, cough, sore throat, vomiting, decreases in appetite and activity level, abdominal pain, irritability, local reactions at the injection site. Study staff will provide parent(s)/legal guardian(s) with digital thermometers supplied by the Sponsor. The parent(s)/legal guardian(s) will also be instructed to record any other concomitant medications (excluding fluoride supplements and vitamins) in the subjects' diary card for 11 days after each vaccination. A field research assistant will visit the household on each

day for the first 11 days following immunization and assist in the recording of all information required and the measuring and recording of temperature. The diary card will be reviewed at the next study visit. In addition, any unscheduled physicians visits will also be recorded for 28 days after immunization (day 0 to 27).

For each subject, surveillance for influenza illness will be initiated on Day 10 following the first dose of CAIV-T/placebo and MMR and will continue for the duration of the study. Surveillance consists of weekly contacts with the parent(s)/ legal guardian(s) to assess whether the subject meets the criteria for influenza illness. Where a subject is considered to meet the criteria for influenza illness, the study staff will arrange a clinic or home visit to complete an illness worksheet and obtain a viral culture (nasal swabs) to confirm influenza. The criteria for an illness visit and viral culture are provided below. Parent(s)/ legal guardian(s) will also be instructed to inform the study staff should the subject develop any clinically significant event or illness. Where this event or illness is deemed by the study staff to meet the criteria of influenza, then an illness visit as described above will be performed. Resolution of the illness will be determined at subsequent surveillance contacts.

Surveillance contacts may be reduced in frequency towards the end of the study if it is deemed that the influenza season in any one region / study site has ended. Any decision to reduce the frequency of surveillance contacts will be taken by the Sponsor and confirmed to the Principal Investigator of the study site in writing.

Adverse events will be recorded for 11 days following receipt of each dose of study vaccine. Adverse events that require unscheduled healthcare provider visits/consultations from Day 11 to Day 27 following receipt of each dose of study vaccine will also be recorded. Adverse events after Day 27 may also be recorded if they result in a subject being withdrawn from the study or are judged clinically significant by the investigator.

Information on adverse events for all subjects will be collected by the field research assistants during home visits. In addition, parents or legal guardians will be asked to contact the study site should the subject develop any clinically significant illness/event, including, but not limited to those requiring the following that the parent(s) / legal guardian(s) has concerns about:

- Physician intervention (visit or consultation) within 28 days (Days 0 to 27) after immunization;
- Prescription or non-prescription medication use within 11 days (Days 0 to 10) after immunization.

Serious adverse events, including hospitalizations, will be monitored for the duration of the study. Details of the event, including concomitant medication(s) and treatment will be recorded on the SAE form provided.

If a subject terminates from the study due to an adverse event, the Investigator will report this to the Sponsor's pharmacovigilance group or designee, within 24 hours of learning of termination, by faxing the completed, signed and dated Subject Outcome page of the Case Report Form (CRF).

Any and all study-related illnesses will be treated, free of cost, at the field clinic for the duration of the study, including for those children who voluntarily withdraw or are withdrawn from the study. If a child requires hospitalisation, all costs pertaining to hospitalisation and transportation to hospital will be paid for.

Table 3 summarizes the study procedures for all subjects participating in the study. In order that study visits are scheduled within the correct timeframes, the day of study vaccination visit should be considered as Day 0:

Table 3

Study Visits	**1**		**2**		**3**	**4****
Visit Window (Days After Previous Visit)	(0)	(2-4)	(35 ± 7)	(2-4)	(35 ± 7)	7 – 9 Months Post Enrollment
Informed Consent	X					
Subject Eligibility	X		X			
Demography	X					
Medical History	X					
Physical Examination	X					
Blood Sample	X		X			
Vaccination	X		X			
Assess Acute Reactions – 15 Minutes Post Vaccination	X		X			
Provision of Diary Card	X		X			
Post Vaccination Contact Including Review of Diary Card		X		X		
Surveillance for Influenza Illness (Weekly Post-Vaccination Contact)			Beginning on the 11th day (Day 10) after receipt of the first dose of study vaccine, all parent(s)/legal guardian(s) must be contacted to assess if the subject qualifies for an illness visit and viral sample collection.			
Collection and Review of Diary Cards			X		X	
Concomitant Medication Collection	X		X		X	
*Adverse Event Collection	X	X	X	X	X	X
Serious Adverse Event Reporting	X	X	X	X	X	X

* All AEs will be recorded up to 11 days following receipt of study vaccine. Only AEs that require healthcare provider visit and /or healthcare provider consultation will be recorded from Day 11 to Day 27 (28 days following receipt of study vaccine). AEs after day 27 may also be recorded outside of this time period if they directly result in a subject being withdrawn from the study or if they are judged clinically significant by the investigator

** Visit 4 is the final surveillance contact / visit conducted at the end of the surveillance period (provisionally 31 May 2003). Visit 4 may be clinic or home visit.

Randomization

A randomization schedule will be prepared and provided by the Sponsor, WLV or by their designee.

Randomization Procedures

Eligible subjects will be assigned to receive the study vaccines according to a randomization schedule generated by the Sponsor's statistician. Study personnel at each site responsible for preparing study vaccine for administration will be informed of the treatment allocation.

Study participants will be prospectively randomized in a 2:1 proportion among Study Group 1, and Study Group 2 respectively (see table 2). Once a subject number and/or treatment is assigned to a particular subject, it will not be reused, even if the subjects drops out of the study prior to administration of the first study vaccination.

Unblinding of the Randomization Code

A single representative of the Sponsor's Regulatory Affairs Department will have access to the subject numbers and treatment assignments in the event that unblinding is necessary. This information will be stored in a secure location. A written log documenting instances of unblinding will be maintained and available for review at study completion.

Blinding

To minimize bias in the reporting of post-vaccination reactions and other study events, neither the study subjects, their parent(s)/legal guardian(s) nor the clinical personnel will know whether intranasal CAIV-T or placebo is being administered.

Responsibilities of the Vaccine Dispenser

All vaccines will be dispensed according to applicable local procedures, laws and regulations. Dispensing procedures will be provided to the Principal Investigator at each site and filed with all study-related documents. In addition, individuals with the responsibility of dispensing vaccines in this study will be listed on the Authorized Signature Record/Investigator Delegation Log.

The dispenser will:

- Identify the correct treatment for the particular subject based on the randomization 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.
- 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.

Study Vaccine

Liquid CAIV-T consists of three cold-adapted influenza virus reassortants that have been concentrated and purified by centrifugation from the allantoic fluid of specific pathogen-free (SPF) chicken eggs. The formulation contains sucrose-phosphate-glutamate, acid-hydrolyzed porcine gelatin and arginine as stabilizers. The total volume of 0.2 mL is administered intranasally with a spray applicator (approximately 0.1 mL into each nostril). Each dose of CAIV-T used in this study will contain approximately $10^{7\ \pm 0.5}$ FFU (equivalent to $10^{7\ \pm 0.5}$ $TCID_{50}$) of each of the three influenza virus strains.

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

The concomitant vaccine, given at the first vaccination only, will be a combination, live, attenuated, MMR vaccine. MMR will be administered subcutaneously according to the manufacturer's instructions.

Packaging and Labeling of Study Vaccine

Each dose of CAIV-T or placebo contained in the spray applicator will be supplied as identically packaged, single-dose units. Study vaccine sprayers and outer packaging boxes will be labeled as investigational products in accordance with applicable local, legal and regulatory requirements. All supporting regulatory documentation, as required by local regulatory agencies and the Sponsor, will be in place and verified prior to shipment of vaccine to the clinical study centres.

Vaccine Storage

Liquid CAIV-T and placebo will be shipped to each study centre upon request of the Sponsor. Receiving departments should be notified that rapid handling of the shipment is required. Upon receipt at study site, the liquid CAIV-T and placebo should be immediately transferred to a 2°C to 8°C refrigerator. Once the vaccine is stored in refrigerated conditions it should not be frozen. MMR will be stored according to the manufactures instructions.

The refrigerator must be secure and with limited access. The investigator will monitor refrigerator temperature and maintain daily temperature logs during the working week. Temperature monitors that record minimum/maximum temperatures will be provided, if required.

Receipt and storage of all study treatments will be documented on the Vaccine Accountability Record (or equivalent) provided by the Sponsor. Study vaccine will not be used after the specified expiry date.

Duration of Subject Participation

Approximately 1200 subjects will be recruited in multiple centers over a period of approximately 6 weeks beginning in approximately September 2002. It is anticipated that 150 subjects will be recruited at the Bangladesh site. The study duration for each participant will be until the end of the influenza season, defined for this study as May 31, 2003 (approximately 8 months).

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, or clinically significant adverse events which constitute contraindications to further vaccine administration (see below).

Vaccine Accountability

Exact written records of receipt and storage of the study vaccine, including date received, lot/LPCS 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. The Investigator will not use the vaccine in any other manner than that provided for in the protocol.

Source Data

It is the policy of Wyeth Lederle Vaccines that study data must be verifiable from the source data, which necessitates access to all original recordings, laboratory reports, and subjects' records. Source data will be made available for all study data. The parent(s)/legal guardian(s will be informed of this and will be signing their agreement when giving informed consent. The subject diary card is considered to be a source document and relevant information regarding adverse events and concomitant medications from this document will be recorded onto the Case Report Form (CRF).

Inclusion and Exclusion criteria

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

Subject Inclusion Criteria

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

- Be at least 11 months of age and less than 24 months of age at the time of first vaccination
- Due to receive their first dose of measles vaccine (or MMR vaccine).
- Who are in good health as determined by medical history, physical examination and clinical judgment
- Whose parent(s)/legal guardian(s) have provided written informed consent after the nature of the study has been explained
- Who, along with their parent(s)/legal guardian(s), will be available for duration of the trial (approximately 8 months)
- Whose parent(s)/legal guardian(s), can be reached by study staff for the post-vaccination contact [clinic or home visit]

Subject Exclusion Criteria

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

- Parent(s)/legal guardian(s) are perceived to be unavailable or difficult to contact for evaluation or study visits during the study period
- Any serious chronic disease (e.g., with signs of cardiac or renal failure or severe malnutrition), including progressive neurological disease
- Down's syndrome or other known cytogenetic disorders
- Known or suspected disease of the immune system or those receiving immunosuppressive therapy, including systemic corticosteriods. (NB: Subjects receiving high doses of systemic corticosteriods given daily or on alternate days, for 14 days or more, should be excluded from vaccination until corticosteriod therapy has been discontinued for at least 1 month. High doses are defined as 2mg/kg/day or more of Prednisolone or its equivalent, or 20mg or more daily if they weigh more than 10kg.)[xxxii]
- Received, or are anticipated to receive, any blood products, including immunoglobulin, in the period from six months prior to vaccination through to the conclusion of the study

- There is intent to administer any other investigational vaccine or agent from one month prior to enrollment through to the conclusion of the study
- There is an immunosuppressed or a immunocompromised 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)
- At any time prior to entry into this study, received a dose of MMR vaccine or any of the individual components of the MMR vaccine (commercial or investigational)
- Anticipated to receive a subsequent does of MMR or measles vaccine within 1 month after receiving the second dose of CAIV-T or placebo.
- Documented history of hypersensitivity to egg or egg protein or any other components of CAIV-T or MMR.
- Received aspirin (acetylsalicylic acid) or aspirin-containing products in the two weeks prior to vaccination or for which use is anticipated during the study
- Any medical conditions that in the opinion of the Investigator might interfere with interpretation of the study results

If any of these criteria are met following enrollment, the subject will be excluded from subsequent vaccine dosing

Note: Pregnancy in a household member or any person who has regular contact with the subject is not a contraindication to the enrollment or ongoing participation of the subject in the study.

Criteria for Temporarily Delaying Vaccine Administration

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

- A febrile illness (≥ 38°C rectal temperature, or axillary temperature ≥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 (e.g. 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 at the time of vaccination for prophylaxis against a non-respiratory related illness, the subject may vaccinated.
- Administration of any live virus vaccine (other than assigned study vaccine) within one month prior to enrollment or expected to receive another live virus vaccine (other than assigned study vaccine) within one month of vaccination in this study.
- Receipt of a dose of any influenza treatment (commercial or investigational) in the two weeks prior to vaccination.
- A clinically confirmed respiratory illness with wheezing within two weeks prior to vaccination.

All subjects must receive the first dose of CAIV-T or placebo and MMR within the defined recruitment period and the second dose of CAIV-T or placebo no later than 42 days following the first dose of study vaccines.

Subject Withdrawal Criteria

Participating subjects will be contraindicated to subsequent doses of study vaccine if the subject 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.

An Investigator and/or Sponsor can withdraw a subject from the study if deemed appropriate. Criteria for withdrawal of a subject from the study may include, but are not limited to: request for withdrawal; subject lost to follow-up; or, clinically significant adverse events. (Refer to Adverse Event Section of the protocol for details).

Following enrollment, development of any condition specified in the exclusion criteria will constitute a contraindication to subsequent dosing and may result in the subject:

a) continuing in the trial, completing all study surveillance for illness visits and safety follow-up as per protocol; or,

b) continuing in the trial, completing the safety follow-up as per protocol, but not completing study surveillance for illness visits; or,

c) withdraw from all study surveillance for illness visits and safety follow-up.

The determination as to which of the above options are applicable is a decision that may be made either by the investigator, subjects parent(s) / legal guardian(s), or both. A subjects parent / legal guardian(s) may for any reason elect to one of the above three options for any reason without further elaboration.

If an intervening illness does not resolve, the subject may be discontinued from the study at the discretion of the Investigator.

Where possible, the tests and evaluations listed for the next scheduled study visit should be performed. Information must be collected on any new/continuing adverse events and concurrent medications since the last study visit. Any serious adverse events that are continuing at the time of withdrawal from the study must be followed to resolution.

An effort must be made to determine why a subject fails to return for the necessary visits or discontinued from the study. Information detailing the circumstances leading to the withdrawal of a subject from the study, as well as the date of withdrawal, will be recorded on the Study Outcome Case Report Form.

Additional subjects will not be enrolled to replace discontinued/withdrawn subjects.

Study Screening Procedures

Prior to enrolment, the Investigator will review the subject's medical history to ensure that the subject meets all of the inclusion criteria and none of the exclusion criteria.

Informed Consent

- The parent(s)/legal guardian(s) of subjects who fulfill all the study eligibility criteria will be informed of the purpose and conduct of the study. The information leaflet and consent form will be read to the parent(s)/legal guardian(s) in its entirety. Voluntary written study-specific informed consent will be obtained from the parent(s)/legal guardian(s) before any study-related procedures are performed. The parent(s)/legal guardian(s) must sign and date the consent form or provide a witness documented thumb print.
- The Investigator (or authorized designee listed on the Authorized Signature Record/Investigator Delegation Log) is responsible for obtaining written informed consent for each subject enrolled. Each signature on the informed consent form should only be personally dated by the signatory.
- A copy of the signed and dated Informed Consent Form is given to the subject's parent(s)/ legal guardian(s). The subject's source documentation must reflect that informed consent was obtained prior to participation in the study.

Study Visit Procedures

Study Visit One, (Day 0)

- Prior to performing any study-related activities, obtain informed consent (see above).
- Obtain and record demographics and medical history, including current medication usage and vaccination history.
- Perform physical examination, including axillary or rectal temperature (according to local practice), to determine that no acute illness is present.

If the subject continues to meet all of the inclusion criteria and none of the exclusion criteria or temporary delay criteria assign the next sequentially-available subject identification number.

- Collect approximately 5 mL of blood (before vaccination) for immunogenicity testing.
- Administer a single dose of assigned study treatment and MMR. The appropriate study personnel must update the vaccine accountability records.

Note: The assigned intranasal study treatment should be administered first, with the MMR vaccine administered no more than 30 minutes later.

- After vaccination, observe each subject for a minimum of 30 minutes. Emergency management supplies (AMBU bag, adrenaline [epinephrine], and antihistamines) must remain available for initial treatment of an allergic reaction if needed. Record any local reactions or systemic events on the adverse event pages in the Case Report Form (CRF).
- Inform the parent(s)/legal guardian(s) that a Field Research Assistant will visit each home daily (beginning that day) with a digital thermometer and diary card and will assist with recording of adverse event data. Beginning that evening and for the following 10 days, daily axillary or rectal temperature (according to local practice) and reactogenicity events will be captured on a diary card by the field research assistants. Also recorded will be any other adverse events, unscheduled physician visits, concomitant prescription and non-prescription medication used.
- The parent(s)/ legal guardian(s) are asked to contact the Investigator immediately if any significant illness or hospitalization occurs during the study period.
- Serious adverse events (SAEs), regardless of causality, will be collected up to study completion.
- Arrange to contact the parent(s)/legal guardian(s) for a surveillance assessment and confirm that these contacts will continue on a weekly basis until study completion.
- The subject and parent(s)/legal guardian(s) will be given an appointment to return for their next study visit.
- Record all information in the appropriate source documents, and complete the Case Report Form (CRF).

First Dose Post-Vaccination Contact

- Contact the parent(s)/legal guardian(s) on Day 0 after the first vaccination and continuing daily through Day 11 post vaccination.
- The contact will be by clinic or home visit.
- Discuss the diary card completion with the parent(s) / legal guardian(s) ensuring correct diary card completion and answering any questions that the parent(s) / legal guardian(s) may have. Ask the parent(s) / legal guardian(s) about specific local and systemic reactions, occurrence of AEs and/or medical problems, the use of prescription / non-prescription medications.

Record all information in the appropriate source documents and Case Report Form.

Surveillance Contact

- Start surveillance procedures on Day 10 after receipt of the first dose of CAIV-T or placebo and MMR and continue on a weekly basis throughout the trial. (See below).
- Contacts will be by clinic or home visit.

Record all information in the appropriate source document

Study Visit Two

- This visit occurs 35 (+/- 7) days after the first dose of assigned study vaccines.
- Review diary card with the parent(s) / legal guardian(s). Any missing data or clarifications required should be discussed with the parent(s) / legal guardian(s). Omitted data should not be entered retrospectively.
- Obtain and record medical history since visit 1, including all adverse events and concomitant medications for 11 consecutive days, including the day of vaccination and any unscheduled physicians visits for 28 days after immunization (day 0 to 27).
- Assess subject to ensure continued study eligibility. Record axillary or rectal temperature (if a vaccine delay criteria is met the temperature must be re-assessed immediately prior to vaccination).

If the subject continues to meet the study eligibility criteria:

- Collect approximately 5 mL of blood for immunogenicity testing.
- Administer a single dose of assigned study treatment (see below). The appropriate study personnel must update the vaccine accountability records.
- After vaccination, observe each subject for a minimum of 30 minutes. Emergency management supplies (AMBU bag, adrenaline [epinephrine], and antihistamines) must remain available for initial treatment of an allergic reaction if needed. Record any local reactions or systemic events on the adverse event pages in the Case Report Form (CRF).

- Remind the parent(s)/ legal guardian(s) that a field research assistant will be coming to the house daily to assist completion of the diary card, beginning that evening and for the following 10 days, and to record any other adverse events, unscheduled physician visits, and concomitant medication used (see section 6.2).
- The parent(s)/legal guardian(s) are asked to contact the Field Research Assistant, Field Clinic, and/or the Investigator immediately if any significant illness or hospitalization occurs during the study period.
- Confirm with the parent(s)/legal guardian(s) that surveillance contacts will continue on a weekly basis until study completion.
- The subject and parent(s)/legal guardian(s) will be given an appointment to return for their next study visit.
- Serious adverse events (SAEs), regardless of causality, will be collected up to study completion.
- Record all information in the appropriate source documents, and complete the Case Report Form (CRF).

Second Dose Post-Vaccination Contact

- Contact the parent(s)/legal guardian(s) beginning on Day 0 after the second vaccination and continuing daily through day 11.
- The contact will be via clinic or home visit.
- Discuss diary card completion with the parent(s) / legal guardian(s) ensuring correct diary card completion and answering any questions that the parent(s) / legal guardian(s) may have. Ask the parent(s) / legal guardian(s) about specific local and systemic reactions, occurrence of AEs and/or medical problems, the use of prescription / non-prescription medications.

Record all information in the appropriate source documents and Case Report Form.

Study Visit Three

- This visit occurs 35 (+/- 7) days after the second dose of assigned study vaccine.
- Review subject diary card from visit 2 with the parent(s) / legal guardian(s). Any missing data or clarifications required should be discussed with the parent(s) / legal guardian(s). Omitted data should not be entered retrospectively.
- Obtain and record medical history since the last study visit (visit two), including all adverse events and concomitant medications for 11 consecutive days (including the day of vaccination) following the second dose of assigned study treatment and any unscheduled physicians visits for 28 days after immunization (day 0 to 27).

- The parent(s)/legal guardian(s) are asked to contact the Clinic and/or Investigator immediately if any significant illness or hospitalization occurs during the study period.
- Serious adverse events (SAEs), regardless of causality, will be collected up to study completion.
- Confirm with the parent(s)/legal guardian(s) that the surveillance contacts will continue on a weekly basis until study completion.
- The subject and parent(s)/legal guardian(s) will be given an appointment to return for their next study visit.
- Record all information in the appropriate source documents, and complete the Case Report Form (CRF).

Study Completion Study Visit Four

- Study Visit 4 is the final surveillance contact / visit conducted at the end of the surveillance period (provisionally 31 May 2003). This may be a clinic or home visit.
- Collect all serious adverse events (SAEs), regardless of causality that occurred since the last contact or study visit (as appropriate).
- Record all information in the appropriate source documents, and complete the Case Report Form.

Study Surveillance Procedures

- Start surveillance procedures on the 11th day (Day 10) after receipt of the first dose of CAIV-T/placebo and MMR.
- Continue this surveillance contact on a weekly basis until completion of the surveillance phase (provisionally May 31, 2003). Contacts do not need to be 7 days apart. One successful contact should occur in each 7-day period.
- A contact may be a clinic or home visit. If the parent(s) / legal guardian(s) contact the study staff, this may also be defined as a surveillance contact provided an assessment for illness visit criteria is made.
- During the surveillance contact assess whether the subject meets the requirements for an illness visit (see below), and complete the surveillance contact Case Report Form.
- The weekly surveillance contact may be reduced to a contact every two weeks by the Sponsor. The Sponsor will make this decision based on local surveillance reports.
- The Sponsor will advise the study centres in writing of changes to the frequency of the surveillance contact with parent(s)/legal guardian(s) or if a decision is made by the Sponsor to complete the surveillance phase prior to May 31, 2003.

Criteria for Obtaining a Culture for Influenza Virus During an Illness Visit

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

Category A – fever (≥ 38°C rectal temperature or ≥ 37.5°C axillary temperature), shortness of breath, pulmonary congestion, pneumonia, ear infection (acute otitis media, suspected or diagnosed), wheezing.

Category B* – runny nose or nasal congestion (rhinorrhea), sore throat (pharyngitis), cough, muscle aches, chills, headache, irritability, decreased activity, vomiting.

*Some symptoms may not be identifiable in children at the lower end of the age group

A viral culture may also be obtained if, in the opinion of the Investigator, the symptom complex so warrants.

Conduct of the Illness Visit

- When illness criteria are met the subject will be seen in the clinic or home within four days of the onset of the illness. If an illness visit cannot be arranged within four days of the onset of the illness, a visit should still be performed as soon as possible.
- Collect a nasal swab specimen from the subject for viral culture, (refer to the Influenza Study Specific Manual for methodology). Details of the nasal swab must be recorded in the source data and the appropriate Case Report Form. A Nasal Swab Requisition Form must also be completed and will accompany the swab to the laboratory, this should include date and time of swab collection.
- If an otitis media assessment is made the results must be recorded in the source data and the appropriate Case Report Form. For the purposes of the study, otitis media assessments must be performed by a medically-qualified Investigator (see otitis media assessment section below). If an initial otitis media assessment is performed by a non-medically qualified member of the study team, for instance the study nurse, and otitis media is suspected, this should be verified by the Investigator using the definition below (otitis media assessment section). The otitis media assessment must be made at the first available opportunity after initiation of the illness visit.
- Resolution of an illness must be confirmed at subsequent surveillance contacts.
- Record all information in the appropriate source documents, and complete the Case Report Forms (CRF).
- All participants within the trial will be provided with medical care for influenza, ear infection, or any other condition that may occur during the conduct of the trial (including the lengthy surveillance period).

Duration of Study Surveillance for Influenza Illness

All subjects will be followed for influenza illness for the duration of the surveillance phase. Surveillance will begin 11 days (Day 10) following the first dose of study vaccine and will provisionally continue up to May 31, 2003 for each subject.

In the event that there is no further evidence of influenza virus activity prior to May 31, 2003, the Sponsor may determine that it is no longer necessary to continue influenza illness surveillance in study participants. The Sponsor will then advise study sites in writing of the revised completion date for the study surveillance phase.

Viral Cultures for Influenza

Study sites will attempt to collect viral culture specimens (nasal swabs) from symptomatic subjects within four days of onset of any illness that prompted a clinic or home visit. Specimens that cannot be obtained within four days of illness onset should still be collected as soon as possible.

The appropriate Nasal Swab Requisition Form must accompany all nasal swab samples sent to the laboratory for analysis. Processing of all specimens will be detailed in the Influenza Study Specific Manual.

Otitis Media Assessment

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 ≥37.5°C axillary), ear ache, irritability, diarrhea, vomiting, acute otorrhea not caused by external otitis, or other symptoms of respiratory infection[xxxiii].

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 ≥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.

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

Vaccine Administration

Vaccine to be Administered

At study Visit 1, each enrolled subject will receive either intranasal CAIV-T, or physiological-normal saline placebo, as assigned. In addition each subject will also concomitantly receive MMR. At Study Visit

2, the subject will receive the same intranasal study treatment which he/she received at Visit 1. The time interval between Dose 1 and Dose 2 will be 35 (+/- 7) days. MMR is not given drug Study Visit 2.

Adrenaline (Epinephrine) and resuscitative equipment should be available in the event of an anaphylactic reaction.

Observe standard immunization practices.

Handling of CAIV-T and MMR

CAIV-T should be allowed to come to room temperature for 5 minutes just prior to use. MMR will be handled according to the manufacturer's instructions.

Administration of CAIV-T or 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 into one nostril.
- Removal of the adapter allows delivery of the remaining vaccine into 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.
- Care 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 or sealed bags immediately after use.
- After vaccination, all participants will be observed for a minimum of 30 minutes by the study staff. Emergency management supplies (for example: AMBU bag, adrenaline [epinephrine] and antihistamine) will remain available for initial treatment of an allergic reaction if needed. Local reactions or systemic events must be recorded in the Case Report Form.

Administration of MMR

- MMR will be administered according to the manufacturer's instruction.
- A single administration of MMR will be delivered by subcutaneous injection.
- After vaccination, all participants will be observed for a minimum of 30 minutes by the study staff. Emergency management supplies (for example: AMBU bag, adrenaline [epinephrine] and antihistamine) will remain available for initial treatment of an allergic reaction if needed. Local reactions or systemic events must be recorded in the Case Report Form.

Accountability and Disposal of Used/Unused CAIV-T/placebo and MMR

- Used CAIV-T/placebo and MMR applicators/vials will be placed in locked containers or sealed bags immediately after use. Applicators/vials should not be discarded until instructed to do so by the Sponsor (or designee). Instructions will be provided as to the correct disposal of vials/applicators.
- Unused CAIV-T/placebo and MMR will be maintained at 2 to 8°C in a refrigerator and monitored daily until return or destruction of supplies are arranged. The Sponsor (or designee) will provide written instruction for shipment conditions and procedures.

Concurrent Vaccinations and Other Treatments

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

- All routine childhood vaccinations administered from one month prior to administration of CAIV-T/placebo and MMR to the final study visit will be recorded. Live virus vaccines (excluding study vaccines) must not be administered within one month prior to study vaccination to one month following receipt of the final study vaccination.
- Medication(s) the child is taking at the time of enrolment will be obtained from the parent(s)/ legal guardian(s) in order to understand any risk of reactions.

During the Day 0 to Day 10 post-vaccination period, use of any non-prescription and prescription medications (including antipyretics, decongestant/antihistamines, analgesic preparations and antibiotics) will be reported (exceptions: vitamins, iron, fluoride supplements, and topical medications). This includes any prescription or non-prescription medications or treatments given as countermeasure therapy during an Adverse Event.

Mandatory Concurrent Vaccines

None

Allowable Concurrent Vaccines

CAIV-T may be administered concomitantly with routinely scheduled vaccines (excluding live viral vaccines). Any concomitantly administered vaccines must be recorded.

Restricted Use of Other Vaccines

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

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) will not be given to study participants prophylactically during the trial as these may interfere with assessment of true illness incidence.

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/vial inventory will be tracked using a Vaccine Accountability Form.

Assessment of Efficacy

Efficacy Parameters

Nasal swab specimens will be cultured and typed for influenza at central or regional laboratories selected by the Sponsor.

Analysis of Efficacy Parameters

Positive specimens will be shipped to sponsor (or designee) for official identification. Efficacy against virologically confirmed influenza illness will be calculated by comparing the proportion of children with virologically-confirmed influenza illness receiving CAIV-T with those children receiving placebo (1-Relative Risk; see statistical section below).

Immunogenicity Parameters

In order to evaluate immunogenicity, blood will be drawn from each subject on two occasions during the course of the subject's participation in the study: pre-vaccination and at one month (35 +/- 7 days) after the first vaccination.

The primary measures of immunogenicity will be:

- Each antigen component of MMR as determined by serum IgG specific antibody as measured by ELISA

In addition:

- Influenza baseline serology for A/H3N2, A/H1N1, and B will be performed on the pre-dose 1 vaccination serum sample by hemagglutination inhibition assay (HAI) to determine the influenza serostatus of the subject at entry into the study.

Blood sample collection and processing will be done in accordance with specimen handling guidelines provided by the Sponsor.

Analysis of Immunogenicity Parameters

The samples will be shipped to the Sponsor or Sponsor-designated laboratory for assaying for vaccine-type influenza HAI antibodies, and MMR antibodies.

Assessment of Safety

Safety Parameters

Reactogenicity events are predefined AEs that could occur after vaccine administration. They include but are not limited to the following:

- Fever (temperature $\geq$ 37.5°C axillary or $\geq$ 38°C rectally)
- Cough
- Sore throat
- Runny nose or nasal congestion
- Irritability
- Vomiting
- Decreased activity
- Decreased appetite
- Abdominal pain
- Local reactions at injection site

Reactogenicity events will be collected on diary cards by the parent(s)/legal guardian(s). Reported reactogenicity events collected on diary cards should not be recorded as adverse events unless they are associated with prescription/non-prescription medication use or unscheduled physician visits. All other AEs and medication (prescription or non-prescription) with onset within the 11-day post-vaccination period will be recorded on the Case Report Form (CRF). In addition any unscheduled physicians visits for 28 days after immunization (day 0 to 27) will also be recorded.

Analysis of Safety Parameters

All subjects who receive at least one dose of study vaccine will be evaluated for safety. All adverse events will be summarized based on severity during the follow-up period. The distributions of the maximum severity will be presented.

Adverse Event Reporting

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 8.3.1).

For this study, an AE is any clinically significant event, including but not limited to those events: 1) requiring prescription or non-prescription medication within 11 days of vaccination; 2) requiring an unscheduled health care provider visit within 28 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 prescription or non-prescription medication or a medical visit within 11 days, or events occurring beyond 28 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.

All AEs must be recorded on the AE page of the Case Report Form.

Serious Adverse Event (SAE) Definition

A Serious AE is an AE occurring following any vaccination related to this study 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 offspring of vaccine recipient, where applicable);

Additionally, **important medical events** that may not result in death, be life-threatening, or require hospitalization may be considered SAEs 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 only as an overnight admission. Hospitalization or prolongation of a hospitalization constitutes criteria for an AE to be serious. In the absence of an AE, there should not be any reporting for a hospitalization or prolongation of a hospitalization by the participating investigator through a Serious Adverse Event Report Form (WLV/CR002). Any elective hospitalization for a preexisting condition that has **not** worsened does **not** constitute an SAE: (e.g., surgical repair of hypospadias).

Disability is defined as a substantial disruption in a person's ability to conduct normal life functions.

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.

SAEs will be collected for the duration of the study regardless of causality.

Unexpected Adverse Event

An unexpected adverse event is defined as 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).

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 immediate risk of death from the event as it occurred.

Causality Assessment

Relationship to Product/Investigational Product

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

The following parameters should be considered when assessing the possible causal relationship between an investigational product and an adverse event:

- the temporal relationship between administration of the investigational product and the event,
- the biological plausibility of a relationship,
- the subject's underlying clinical state or concomitant agents or therapies,
- any known response patterns to the investigational product or related compounds [de-challenge and re-challenge, where applicable].

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

Definite:	event can be fully explained by administration of the investigational product
Probable:	event is most likely to be explained by administration of the investigational product, rather than the subject's clinical state or other agents/therapies
Possible:	event may be explained by administration of the investigational product, or by the subject's clinical state or other agents/therapies
Probably Not:	event is most likely to be explained by the subject's clinical state or other agents/ therapies, rather than the investigational product
Not Related:	event can be fully explained by the subject's clinical state or other agents or therapies

The adverse event will be determined to be vaccine-related by the Wyeth
Medical Monitor. The following documentation must be provided to Wyeth through Quintiles East Asia:

- Medical records for the event
- Detailed description of all procedures conducted & length of stay in the hospital
- All invoices & receipts for expenses incurred
- Copy of the signed informed consent form

All documentation will be sent to the Wyeth claims department for legal review. Once the claim has been approved, a set of release documents will be sent to the investigator / site. The parent / legal guardian of the subject will be required to sign the
release documents and return to Wyeth. Upon receipt of the signed release documents by Wyeth, the payment will be released directly to the parent.

Relationship to Protocol

SAEs 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 (i.e., 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. These events should be reported to WLV following the same procedure for reporting product-related events.

Relationship to Other Causes

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

Reporting Adverse Events

Adverse Events may be observed by the Investigator, elicited from the parent/legal guardian(s) or subject, reported on subject diary cards, or volunteered by the parent/legal guardian(s) 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 investigational product, will be followed until resolution.

Reporting Serious Adverse Events (SAEs)

For Serious Adverse Events only: a Serious Adverse Event Report Form (WLV/CR002) must be completed by the Investigator in addition to the Adverse Event page of the Case Report Form.

- The Investigator (or designee) must report all SAEs, whether or not considered study vaccine related, to the Sponsor's Pharmacovigilance Group or designee (via facsimile) on the form provided, within 24 hours of learning of the event. (Details of the fax numbers will be provided in the Influenza Study Specific Manual).
- 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.
- At the Sponsor's request, 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 SAE 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):

Dr. Beate Schmöle-Thoma
Director Medical Affairs Vaccines Europe
Fraunhoferstr. 15
82152 Martinsried b.
München
+ (49) 89 / 8565 1541 phone
+ (49) 89 / 8565 1543 fax

If there is a need to contact a Medical Monitor concerning Adverse Events during weekends and holidays, the following telephone number may be used as well: +1 845-602-2222. This is a voice-mail system from which messages are retrieved periodically by a Medical Monitor.

Other Information Reportable to WLV

Other information reportable to WLV includes the following: overdose, product abuse, inadvertent or accidental exposure to product with or without an AE. These events are to be recorded by the Investigator on the appropriate form as detailed in the Influenza Study Specific Manual. These events are handled in the same manner as SAEs, and are subject to the same reporting time frames.

Removal of a Subject

The removal of a subject from the study due to an Adverse Event, whether by the Investigator or by the parent(s) /legal guardian(s), should be reported to the Sponsor on the appropriate Case Report Form page. If the termination is due to a SAE, the procedure for reporting SAEs must be followed as well .

Statistics and Data Evaluation

See Data Analysis Section below.

Direct Access to Source/Data Documents

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

Quality Control and Quality Assurance

Monitoring

All aspects of the study will be monitored by Wyeth Lederle Vaccines as the study Sponsor or by authorized representatives, with respect to current Good Clinical Practices and Standard Operating Procedures for compliance with applicable government regulations. The Principal Investigator (and/or designee) and the study personnel will cooperate fully with the Sponsor, provide all appropriate documentation, and be available to discuss the study progress during any monitoring visits.

Prior to the beginning of the study, the Principal Investigator will be informed of the frequency of monitoring visits. The Principal 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 Serious Adverse Events which 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 Case Report Forms (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.

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.

Institutional Review Board/Ethics Review Committee

The Investigator will be responsible for obtaining Institutional Review Boards/Ethics Review Committee (IRB/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 approval, 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 which 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 (if applicable). The ERC may appoint a Data Safety Monitoring Board (DSMB) to monitor progress, SAEs and adverse events related to the trial.

For other ethical issues, including Declaration of Helsinki and informed consent, see Ethical Assurance of Protection of Human Rights section below.

Data Handling and Record Keeping

Case Report Forms

Case Report Forms (CRFs) and diary cards will be maintained for recording data for each subject enrolled in the study. The Principal 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.

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.

Study Documentation

As a minimum, the Principal Investigator will 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 Form
- IRB/EC approval of the final Protocol and Protocol Amendment(s)
- IRB/EC approval of the Informed Consent Form and any modifications
- Copy of the IRB/EC approved Informed Consent Form

- IRB/EC approval of advertisements and other written information to be provided to the subject
- IRB/EC annual study review
- Correspondence with IRB/EC
- Study Initiation Monitoring Report of Principal Investigator and Sub-Investigators
- Form FDA 1572 (or equivalent Statement of Investigator)
- Correspondence with the Sponsor
- Serious Adverse Event Reports
- Clinical Supply Records
- Shipping records for investigational product(s) and trial-related material
- Vaccine Accountability Records, which include dispensing records of 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 Forms (if applicable)
- Final report by Investigator to IRB/EC
- Clinical Study Report (if applicable)
- Subject identification list, subject screening log, and subject enrollment log
- Monitoring Visit Log
- Authorized Signature Record
- Copies of completed Case Report Form (CRF)s and corrections to the CRFs

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 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.

The Sponsor could arrange for storage of the documents if the Investigator is unable to arrange for appropriate storage. This would be agreed to prior to the start of the study and documented.

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

Data Handling

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

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 Ethics Review Committee which 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 which he/she deems appropriate. However, all such events and procedures must be promptly reported to the Sponsor and ERC.

Clinical Investigator Brochure

Each Investigator will receive from the Sponsor or designate, the current version of the Clinical Investigator Brochure, which comprehensively describes the pre-clinical and clinical experience available to date. If any relevant new findings are made during the course of the study, the Investigator will receive an updated Clinical Investigator Brochure or an amendment to the currently available version.

Facilities Available

Describe the availability of physical facilities at the place where the study will be carried out. For clinical and laboratory-based studies, indicate the provision of hospital and other types of patient's care facilities and adequate laboratory support. Point out the laboratory facilities and major equipments that will be required for the study. For field studies, describe the field area including its size, population, and means of communications. **(TYPE WITHIN THE PROVIDED SPACE).**

Features of the Study Site Where the Visit Will Occur

Kamalapur is an urban slum located in the southeastern sector of the Capitol city, Dhaka approximately 30 – 40 minutes drive from ICDDR,B. The intervention area is spread over 4.0 sq Km (1.5 sq mile or 988 acres)

There are 32,339 households in the community, all of which have been entered into an electronic database maintained at ICDDR,B. The total population as of 30 November 2000 was 118,654. The outmigration rate averages < 2%, however the population of the community has doubled since 1997, largely as a result of immigration from rural areas. The population is relatively young. In our 1998 census, the mean age of mean was 32.8 years, of women was 24.2 years, while 11.2% of the population was less than five years old.

There is a mixture of squatter settlements and formal permanent structures. The mean household size 4.0 but ranges from 1 – 20 persons per household. Population density is 29,663 per square kilometre (191 persons per acre or 77,048/sq mile). Educational levels in the community among adults are low, however the majority of children less than 12 years attend school. Mean level of schooling for men above 18 years old is 4.5 years, and for women is 3.1 years. Unemployment is relatively low. Most men less than 60 years are employed at least part time, resulting in total unemployment of only 1.6%. Among occupations, the most common for men are merchant (24.2%), office worker (21.6%), rickshaw puller (17.0%), and skilled labourers (12.3%). Day labourers and unskilled workers comprise only 7.7%. (See Figure 1 below.) The mean family income is TK 3,000 or about US $60/month.

Vaccine coverage for the community is inconsistent and varies by age and type of vaccine. Measles coverage among children 9 – 12 months old = 54%.

Surveillance System

- Active surveillance system, i.e. we actively collect data using a standardised calendar questionnaire.
- Once weekly home visits by a field research assistant (FRA) to every household for data collection.
- The questionnaire asks about specific signs associated with disease for the past seven days since the last visit.
- Those with illness are referred to the field clinic.
- A field research officer (FRO) supervises the FRAs.
- The FRO sends daily field reports to our office as indicator of the system's performance. Quality assurance also maintained by repeat visits of a sub-sample of the home interviews and weekly staff meetings.

The Clinic

- Staff: 3 medical officers (MO), 3 nurses and 6 health workers.
- MO conducts a history and physical exam on all visitors using a standardised questionnaire to protect the uniformity of case detection.
- Perform appropriate lab tests on all patients meeting our standardised case definitions for diseases under study.
- Treat illnesses using standardised protocols. Follow-up all patients by 48 hours of starting therapy.
- Refer anyone with severe to ICDDR,B hospital or to one of our partner hospitals.

Research Activities

1) Pneumonia surveillance in children less than 2 years old: just completed. Study was designed to determine disease burden (incidence and prevalence) and provide primary disease prevention using pharmacological dose of zinc to reduce to the attack rate (lower the incidence). Results being analysed.

2) Dengue Emergency Response Surveillance: To determine disease burden in the community and conduct a nested case control analysis to determine risk factors dengue and dengue haemorrhagic fever for this population.

3) *Shigella* Burden of Disease Study: To define population-based incidence of diarrhea and complications from *Shigella*.
4) Evaluation of immunogenicity of CAIV-T and OPV when given simultaneously among children 6-36 months of age.

Data Analysis

Describe plans for data analysis. Indicate whether data will be analyzed by the investigators themselves or by other professionals. Specify what statistical softwares packages will be used and if the study is blinded, when the code will be opened. For clinical trials, indicate if interim data analysis will be required to monitor further progress of the study. **(TYPE WITHIN THE PROVIDED SPACE).**

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

Comparisons Of Interest

The multidimensional comparison of primary interest is to evaluate non-inferiority of the serum antibody response to MMR, co-administered with CAIV-T, relative to the response to MMR, co-administered with intranasal placebo. Serum IgG specific antibody responses to the various antigenic components of MMR are measured by ELISA. The response criterion for each antigen is seroconversion, defined as a change from seronegative to seropositive or a four-fold increase in specific IgG antibody titer if the subject was seropositive prior to vaccination. The primary analysis endpoint for serum antibody response will be achieved if the lower bound of each 95% two-sided confidence interval, for the difference (Group 1 – Group 2) in immunogenicity responses to the mumps, measles, and rubella antigens, is greater than -10%.

Secondary study objectives evaluate superiority of CAIV-T treatment efficacy relative to placebo based on incidence of culture-confirmed influenza illness and AOM occurrence frequencies. Interval estimates of vaccine efficacy (1 – relative risk) will be presented for each influenza illness and AOM endpoint. Interval estimates for incidence rate relative risk will be derived from exact method interval estimates for the proportion of all cases that were in Group 1 (MMR co-administered with CAIV-T). Interval estimates for AOM occurrence frequency relative risk will be derived from the generalized Cox regression. The efficacy of CAIV-T co-administered with MMR will be found statistically significant ($p<0.05$ for the two-sided test) if the lower bound of the two-sided 95% confidence interval for vaccine efficacy is greater than 0.

Sample Size

It is expected that approximately 1200 subjects aged 11 to 24 months will be enrolled at multiple study sites. Sample size was based on the following assumptions:

- The proportions of subjects that respond to individual components of the MMR vaccine is 80% for mumps, 95% measles and 89% for rubella;
- The primary study objective, to demonstrate non-inferior immunogenicity response for MMR co-administered with CAIV-T relative to MMR co-administered with placebo, will be achieved if each lower 95% two-sided confidence interval, for the difference (Group 1 – Group 2) in immunogenicity response to the mumps, measles, and rubella antigens, is greater than -10%.
- The drop-out rate is no greater than 25%.

Based on these assumptions, a study with at least 580 subjects completed in group 1 and at least 290 subjects completed in group 2 provides greater than 90% power to achieve the primary study objective.

For the secondary study objective, assuming that:

- The influenza attack rate in the placebo group is 12%
- The influenza attack rate in the CAIV-T group is 3%
- The drop-out rate is no greater than 25%.

The plan to enroll 1200 subjects provides 90% power to demonstrate statistically significant vaccine efficacy of CAIV-T with a lower confidence bound of 30. The study has 99% power to demonstrate significance with a lower confidence bound of 0

MMR Seroconversion Response

Seroconversion rates to the three components of MMR will be compared between subjects receiving concomitant administration of CAIV-T and MMR (Group 1) and those receiving placebo and MMR (Group 2). The non-inferiority evaluation will be based on two-sided 95% confidence intervals for the three differences in proportions. The null hypothesis of inferior immunogenicity response following MMR co-administered with CAIV-T, compared with MMR co-administered with placebo, will be rejected if the lower bounds of all three 95% two-sided confidence intervals, for each difference (Group 1 – Group 2) in seroconversion rates to the measles, mumps and rubella antigens, is greater than –10%.

MMR Serum IgG Specific Antibody

In a secondary analysis, the immune response will be based on the antibody titer expressed on a logarithmic scale. The antibody response to each MMR component will be evaluated in separate ANCOVA models. The log baseline titer of IgG antibody specific to each MMR antigen will be included as a covariate. The effect of baseline serum HAI (to influenza virus strains A/H1N1, A/H3N2, and B) on the MMR immune response may also be evaluated as covariates in the model for log MMR titers. The estimated group mean difference and the confidence interval will then be exponentiated to obtain the GMT (geometric mean titer) ratio (co-admin./reference) and its 95% confidence interval. If the lower limit of each confidence interval

is ≥ 0.5, then the null hypothesis of inferiority with respect to a two-fold criterion will be rejected at α=0.05.

CAIV-T Vaccine Efficacy

Interval estimates of vaccine efficacy (1 – relative risk) will be presented for each influenza illness and AOM endpoint. Interval estimates for the relative risk of incidence rates will be derived from exact method interval estimates for the proportion of all cases that were in Group 1 (CAIV-T and MMR). Results from Bangladesh and other individual countries may be presented, and country differences will be addressed in descriptive summaries. The primary data summary will be based on the summary that gives equal weighting to each case of influenza illness.

Interval estimates for the relative risk of AOM occurrences will be derived from the generalized Cox regression. This regression model may include covariates or stratification factors for baseline HAI and country.

The efficacy of CAIV-T, co-administered with MMR, will be found statistically significant ($p<0.05$ for the two-sided test) if the lower bound of the two-sided 95% confidence interval for vaccine efficacy is greater than 0.

Analysis Populations

The primary analysis of the immunogenicity objective will be based on the immunogenicity evaluable population. These subjects must meet the following criteria:

- The study inclusion criteria are met and no study exclusion criterion is met
- The first blood draw (Pre Dose 1) must have occurred at the day of the first dose but prior to the first dose
- The study vaccines were administered per protocol

The second blood draw must have occurred 35±7 days following the MMR dose.

A secondary analysis of MMR immunogenicity response may be based on the immunogenicity all-available population. These subjects received a dose of MMR concomitant with either CAIV-T or placebo and had pre-dose and post-dose evaluations of the IgG antibody to the MMR antigens.

Summaries of CAIV-T vaccine efficacy will be based on the efficacy all-available population. These subjects received the first dose of CAIV-T or placebo co-administered with MMR. These subjects also had at least four weeks of follow-up surveillance.

Safety summaries will be based on all subjects who received at least one dose of the study vaccine.

Demographics and Clinical Characteristics

The demographic data, including gender, race and age at dose 1, of the immunogenicity, efficacy and safety analysis populations will be summarized. Baseline serum IgG antibody levels will be reported and the subject baseline HAI seronegative status may be applied as a grouping criterion for some immunogenicity and efficacy outcome summaries.

Efficacy/Immunogenicity/Virological Evaluation

Primary Endpoint

The primary immunogenicity endpoint is the proportion of subjects showing seroconversion for each antigen component of MMR. Seroconversion is defined as a change from seronegative to seropositive, as determined by serum IgG specific antibody as measured by ELISA, or a four-fold increase in specific IgG antibody titer if the subject was seropositive prior to vaccination. Subjects with inconclusive results pre-vaccination are considered as seronegative. The primary analysis interval for MMR is 4-6 weeks following the single dose of MMR.

Secondary Endpoints

The secondary endpoint for immunogenicity is the geometric mean titer (GMT) of serum antibody for each antigen component of MMR. The primary analysis interval for MMR is 4 – 6 weeks following the single dose of MMR.

- *Secondary Influenza Illness Endpoint*

The secondary influenza illness endpoint for efficacy is the first episode in a study child of a culture-confirmed influenza illness, caused by any community-acquired subtype, following receipt of the second dose of study vaccine or placebo.

- *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 in a study child. An episode of acute otitis media in a study participant will be included in the efficacy estimations if it complies with the definition of acute otitis media and occurs at least 15 days after receipt of the first dose of vaccine or placebo.

For the purposes of analysis of efficacy against otitis media, an episode of acute otitis media is defined as occurring at least 30 days since the previous episode of acute otitis media, irrespective of etiology.

- *Secondary AOM Efficacy Endpoints*

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 in a study child.

In addition, the following will be investigated: efficacy against all episodes of acute otitis media 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) in a study child.

Safety and Tolerability Evaluation

The safety objective is to assess the safety and tolerability of concomitant administration of CAIV-T with a combination live, attenuated, mumps, measles, and rubella vaccine in healthy children aged 11 to less than 24 months.

The reactogenicity events are:

Fever (axillary temperature ≥ 37.5°C); cough; sore throat, runny nose or nasal congestion; irritability; vomiting; decreased activity; decreased appetite and abdominal pain, and local reaction at MMR injection site.

The proportion of subjects with any reactogenicity event reported during the 11-day period following each dose of study vaccine will be summarized by treatment group. Adverse events that occurred within 11 days or within 28 days following each study vaccination will be summarized by treatment group. Serious adverse events will be listed and summarized.

- *Statistical Methods for Safety Evaluations*

The overall incidence of reactogenicity events will be compared between the CAIV-T with MMR and placebo with MMR groups using two-sided Fisher's Exact Test. Confidence intervals about reactogenicity adverse event frequency differences will be reported. The frequency of specific adverse events will be compared between treatment groups using two-sided Fisher's Exact Test.

Ethical Assurance for Protection of Human Rights

Describe in the space provided the justifications for conducting this research in human subjects. If the study needs observations on sick individuals, provide sufficient reasons for using them. Indicate how subject's rights are protected and if there is any benefit or risk to each subject of the study.

Declaration of Helsinki

This clinical trial will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, and that are consistent with GCP and the applicable regulatory requirements.

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 Institutional Review Board/Ethics Committee (IRB/EC) 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(s)/legal guardian(s) will receive information on the vaccines to be used in the study. The scope of the trial will be explained. By signing the Informed Consent Form, the parent(s)/legal guardian(s) confirms the child's voluntary participation and his/her intention to follow the study protocol and the instructions of the Investigator.

The Investigator is responsible to obtain written informed consent for each subject enrolled. The Informed Consent Form must be signed and dated by the parent(s)/legal guardian(s) prior to study enrollment. Each parent(s)/legal guardian(s) will receive a copy of the signed Informed Consent Form.The study will not be conducted among sick individuals. The objectives of this study cannot be achieved other than among human subjects (an animal model could not adequately address the study questions).

Use of Animals

Describe in the space provided the type and species of animal that will be used in the study. Justify with reasons the use of particular animal species in the experiment and the compliance of the animal ethical guidelines for conducting the proposed procedures.

Animals will not be used in this study.

Literature Cited

Identify all cited references to published literature in the text by number in parentheses. List all cited references sequentially as they appear in the text. For unpublished references, provide complete information in the text and do not include them in the list of Literature Cited. There is no page limit for this section, however exercise judgment in assessing the "standard" length.

1. Maassab HF, Francis, Jr T, Davenport FJ, Hennessy AV, Minuse E, Andern G. 1969. Laboratory and clinical characteristics of attenuated strains of influenza virus. Bull WHO 41:589-592.

2. Maassab HF, Herlocher ML. 1999. Live influenza virus vaccine. In: Plotkin SA and Orenstein W (eds) Vaccines, 3rd Edition WB Saunders Co, Philadelphia, pp. 909-927.

3. Glezen WP. 1982. Serious morbidity and mortality associated with influenza epidemics. Epidemiol Rev 4:25-44.

4. Couch RB, Kasel JA, Glezen WP, et al. 1986. Influenza: Its control in persons and populations. J Infect Dis 153:431-440.

5. Glezen WP, Taber LH, Frank AL, Gruber WC, Piedra PA. 1997. Influenza virus infections in infants. Ped Infect Dis J 16:1065-1068.

6. Izurieta HS, Thompson WW, Kramarz P, Shay DK, Davis RL, DeStefano F, Black S, Shinefield H, Fukuda K. Influenza and the rates of hospitalization for respiratory disease among infants and young children. NEJM 342, 2000: 232-239.

7. Neuzil KM, Mellen BG, Wright PF, Edward FM, Griffin MR. The effect of influenza on hospitalizations, outpatient visits, and courses of antibiotics in children. NEJM 342, 2000: 225-231.

8. Fleming DM. Cohen JM. Experience of European collaboration in influenza surveillance in the winter of 1993-1994. Journal of Public Health Medicine. 1996; 18:133-142.

9. Lina B. Valette M. Foray S. Luciani J. Stagnara J. See DM. Aymard M. Surveillance of community-acquired viral infections due to respiratory viruses in Rhone-Alpes (France) during winter 1994 to 1995. Journal of Clinical Microbiology. 1996; 34:3007-3011.

10. Carlsen KH, Orstavik I, Halvorsen. 1983. Viral infections of the respiratory tract in hospitalized children. A study from Oslo during a 90 months period. Acta Paediatr Scand 72:53-58.

11. Winter GF, Inglis JM. Respiratory viruses in children admitted to hospital in Edinburgh 1972-1985. 1987. J Infect 15:103-107.

12. Stanek J. Heinz F. Berankova M. Pennigerova S. Tumova B. Kunzova D. Fedova D. Hruskova J. Clinical diagnosis and laboratory study of acute respiratory diseases of viral origin in children. Ceskoslovenska Pediatrie. 1990; 45:715-718.

13. Dong-Lu Z, Zi-Jing Z, Zhi-Liang W, et al. 1988. Surveillance of acute respiratory infections in three kindergartens in Beijing. Chinese Med J 101:787-792.

14. Ruutu P, Halonen P, Meurman O, et al. 1990. Viral lower respiratory tract infections in Filipino children. J Infect Dis 161:175-179.

15. Huq F, Rahman M, Nahar N, et al. 1990. Acute lower respiratory tract infection due to virus among hospitalized children in Dhaka, Bangladesh. Rev Infect Dis 12:S982-S987.

16. Doraisingham S, Goh KT, Ling AE, Yu M. 1988. Influenza surveillance in Singapore: 1972-86. Bull WHO 66:57-63.

17. Chew FT, Doraisingham S, Ling AE, et al. 1998. Seasonal trends of viral respiratory tract infections in the tropics. Epidemiol Infect 121:121-128.

18. Joosting AC, Harwin RM, Orchard M, Martin E, Gear JH. Respiratory viruses in hospital patients on the Witwatersrand. A 7-year study. S Afr Med J. 1979; 55:403-408.

19. Schoub BD, Johnson S, McAnerney JM, Martin E, dos Santos IL. Laboratory studies of the 1984 influenza epidemic on the Witwatersrand. S Afr Med J. 1986; 70:815-818.

20. McAnerney JM, Johnson S, Schoub BD. Surveillance of respiratory viruses. A 10-year laboratory-based study. S Afr Med J. 1994; 84:473-477.

21. Schoub BD, Johnson S, McAnerney J, Blackburn NK. Benefits and limitations of the Witwatersrand influenza and acute respiratory infections surveillance programme. S Afr Med J. 1994; 84:674-678.

22. Avila M, Salomon H, Carballal G, Ebekian B, Woyskovsky N, Cerqueiro MC, Weissenbacher M. Isolation and identification of viral agents in Argentinian children with acute lower respiratory tract infection. Rev Infect Dis. 1990; 12(Suppl 8):S974-S981.

23. Weissenbacher M, Carballal G, Avila M, Salomon H, Harisiadi J. Catalano M, Cerqueiro MC, Murtagh P. Etiologic and clinical evaluation of acute lower respiratory tract infections in young Argentinian children: an overview. Rev Infect Diseases. 1990; 12(Suppl 8):S889-S898.

24. do Nascimento JP, Chaves JR, Ferreira V, Siqueira MM, Krawczuk MM, Deane G de M, Sutmoller F. Influenza surveillance in Rio de Janeiro between 1980-1981: a virological and serological study. Memorias do Instituto Oswaldo Cruz. 1984; 79:169-173.

25. de Arruda E, Hayden FG, McAuliffe JF, *et al.* Acute respiratory viral infections in ambulatory children of urban northeast Brazil. J Infect Dis. 1991; 164:252-258.

26. Nascimento JP, Siqueira MM, Sutmoller F, Krawczuk MM, de Farias V, Ferreira V, Rodrigues MJ. Longitudinal study of acute respiratory diseases in Rio de Janeiro: occurrence of respiratory viruses during four consecutive years. Revista do Instituto de Medicina Tropical de Sao Paulo. 1991; 33:287-296.

27. Sutmoller F, Ferro ZP, Asensi MD, Ferreira V, Mazzei IS, Cunha BL. Etiology of acute respiratory tract infections among children in a combined community and hospital study in Rio de Janeiro. Clin Infect Dis. 1995; 20:854-860.

28. Ceruti E, Diaz A, Vicente M, *et al.* Etiology of lower respiratory infections in hospitalized infants. Revista Chilena de Pediatria. 1991; 62:155-166.

29. Rennels MB, Edwards KM, Harry L, *et al.* Safety and immunogenicity of four doses of *Neisseria meningitidis* group C vaccine conjugated to CRM_{197} in United States infants. Pediatr Infect Dis J, 2001;20:153-9.

30. Belshe RB. Mendelman PM. Treanor J. King J. Gruber WC. Piedra P. Bernstein DI. Hayden FG. Kotloff K. Zangwill K. Iacuzio D. Wolff M. The efficacy of live attenuated, cold-adapted, trivalent, intranasal influenzavirus vaccine in children. N Engl J Med. 1998; 338:1405-1412.

31. Nichol KL, Mendelman PM, Mallon KP, Jackson LA, Gorse GJ, Belshe RB, Glezen WP, Wittes J. Effectiveness of live, attenuated intranasal influenza virus vaccine in healthy, working adults: a randomized controlled trial. JAMA. 1999; 282:137-144.

32. American Academy of Pediatrics. Corticosteroids. In: Pickering LK, ed. 2000 Red Book: Report of the Committee on Infectious Diseases. 25th ed. Elk Grove Village, IL: American Academy of Pediatrics 2000: 61-62.

33. Karma P, Palva T, Kouvalainen K, Karja J, Makela PH, Prinssi VP, Ruuskanen O, Launiala K. 1987. Finnish approach to the treatment of acute otitis media. Report of the Finnish Consensus Conference. Annals of Otology, Rhinology and Laryngology - Supplement 129:1-19.

Dissemination and Use of Findings

Describe explicitly the plans for disseminating the accomplished results. Describe what type of publication is anticipated: working papers, internal (institutional) publication, international publications, international conferences and agencies, workshops etc. Mention if the project is linked to the Government of Bangladesh through a training programme.

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 thirty (30) days prior to presentation or submission for publication for review and approval, to insure that confidential information is not disclosed. Investigators can publish this data as long as the multi-centre nature of the study is mentioned in the publication.

It is anticipated that the findings of the multi-center study will be published with participation of ICDDR,B lead investigators. Leadership for publishing the results of the multi-center study will be the responsibility of WLV. Specific and notable findings from the component of the study involving participation of Bangladeshi subjects will be published in an appropriate journal focused on vaccines and/or influenza. If appropriate the findings may also be reported in a local bulletin, and will likely be presented before an international conference.

Collaborative Arrangements

Describe briefly if this study involves any scientific, administrative, fiscal, or programmatic arrangements with other national or international organizations or individuals. Indicate the nature and extent of collaboration and include a letter of agreement between the applicant or his/her organization and the collaborating organization. **(DO NOT EXCEED ONE PAGE)**

This study will be a multi-center collaborative study with WLV serving as the sponsor and leader of the conduct of the collaboration. Other collaborating institutions will be from countries within Asia, Europe and the Americas. WLV is the sponsor of this trial and will provide necessary fiscal support to conduct the trial.

Biography of the Investigators

Give biographical data in the following table for key personnel including the Principal Investigator. Use a photocopy of this page for each investigator.

1. Name: **Robert F. Breiman, M.D.**

2. Present position: Associate Director, ICDDR,B and Head, Health Systems and Infectious Diseases Division (HSID)

3. Educational background :
(last degree and diploma & training relevant to the present research proposal)

Institution and Location	Degree	Year	Field of Study
University of Arizona	**B.A.**	**1975**	**Political Science**
University of Arizona	**M.D.**	**1979**	**Medicine**
UCLA Affiliated Hospitals	**Residency**	**1979-1982**	**Internal Medicine**
UCLA Affiliated Hospitals	**Chief Resident**	**1982-1983**	**Internal Medicine**
UCLA	**Fellowship**	**1984-1987**	**Infectious Diseases**
CDC	**Fellowship**	**1987-1989**	**EIS Program**

4. List of ongoing research protocols
(start and end dates; and percentage of time)

4.1. As Principal Investigator

Protocol Number	Starting date	End date	Percentage of time
2000-023	10/00	12/02	15%
2000—011	1/02	6/02	20%

4.2. As Co-Principal Investigator

Protocol Number	Starting date	End date	Percentage of time
2001-021	1/02	9/04	5%
2001-029	1/01	6/03	5%

4.3. As Co-Investigator

Protocol Number	Starting date	Ending date	Percentage of time
2001-14	5/02	10/02	10%
2002-10	4/02	7/02	2%
2002-28	10/01	9/03	10%

5. Publications

Types of publications	Numbers
a) Original scientific papers in peer-review journals	90
b) Peer reviewed articles and book chapters	9
c) Papers in conference proceedings	>50
d) Letters, editorials, annotations, and abstracts in peer-reviewed journals	10
e) Working papers	
f) Monographs/books	6

6. Five recent publications including publications relevant to the present research protocol

1) Feikin DR, Elie CM, Goetz MB, Lennox JL, Carlone GM, Romero-Steiner S, Holder PF, O'Brien WA, Whitney CG, Butler JC, **Breiman RF**. Randomized trial of the quantitative and functional antibody responses to a 7-valent pneumococcal conjugate vaccine and/or 23-valent polysaccharide vaccine among HIV-infected adults. *Vaccine*. 2001;20:545-53.

2) **Breiman RF,** Keller DW, Phelan MA, Sniadack DH, Stephens DS, Rimland D, Farley MM, Schuchat A, Reingold AL Evaluation of effectiveness of the 23-valent pneumococcal capsular polysaccharide vaccine for HIV-infected patients. *Arch Intern Med.* 2000;160:2633-8.

3) Lieu TA, Ray GT, Black SB, Butler JC, Klein JO, **Breiman RF**, Miller MA, Shinefield HR. Projected cost-effectiveness of pneumococcal conjugate vaccination of healthy infants and young children. *JAMA*.2000;283(11):1460-8.

4) Metlay JP, Hofmann J, Cetron MS, Fine MJ;,Farley MM, Whitney C, **Breiman RF.** Impact of penicillin susceptibility on medical outcomes for adult patients with bacteremic pneumococcal pneumonia. *Clin Infect Dis* 2000;30:520-528.

5) Nuorti JP, Butler JC, Farley MM, Harrison LH, McGeer A, Kolczak MS, **Breiman RF.** Cigarette smoking and invasive pneumococcal disease. *N Engl J Med* 2000;342:681-689.

1. Name: W. Abdullah Brooks, MD, MPH

2. Present Position

Health & Child Survival Advisor, Health & Child Survival Fellows Program, International Centre for Diarrhoeal Disease Research, Bangladesh

Research Associate , Department of International Health, Johns Hopkins University School of Hygiene and Public Health

3. Educational Background

Institution/Location	Degree	Year Conferred	Field of Study
Stanford University	MD	1991	Medicine
The New York Hospital / Cornell Medical Center	Diploma	1994	Pediatrics
Johns Hopkins University	MPH	1995	International Health
Johns Hopkins University	Diploma	1996	Preventive Medicine

4. Experience and Appointments

Year	Activity
1985-1986	Principal Investigator: Identification of heat-shock proteins in *F.hepatica*, Stanford University
1986	Investigator: Isolation of *L. major* attachment proteins, National Institutes of Health
1990	Epidemiology Intelligence Service Medical Student Clerkship, Enteric Branch, Centers for Disease Control and Prevention
1992	Investigator: Community-based dysentery intervention urban slum children, Salvador, Bahia, Brazil
1995	Multicentre Study on Lower Osmolar ORS, International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B)
1994 – 1997	Paediatric On-Call Physician, Kennedy-Krieger Institute, Johns Hopkins
1995 – 1997	Paediatric consultant: Urban school-based clinics for Baltimore County
1995 – 1997	Paediatric Emergency Room Attending, St. Joseph's Hospital, Baltimore
1996	Epidemiologist: Consultants in Epidemiology and Occupational Health, Washington, DC
1996	EPI Technical Advisor: National measles vaccination campaign children 12 - 59 months, PAHO, EPI/SVI, Georgetown, Guyana
1996 – 1997	Chief Resident Preventive Medicine Program, Johns Hopkins University
1997 – Present	Paediatric Consultant, US Embassy, Dhaka
1997 – Present	Regional Medical Consultant: AEA International, Singapore
1997 – Present	Principal Investigator: Hospital-based study to test efficacy zinc in acute watery diarrhoea in children less than 6 months old
1998 – Present	Principal Investigator: Community-based study to prevent pneumonia/diarrhoea with zinc in children less than 2 years old
1999 – Present	Principal Investigator: Hospital-based study of efficacy zinc as adjuvant therapy in management severe pneumonia, hospitalised children < 2 y/o

5. Protocals under Development

Projected Start Date	Intervention	Stage of Development
2000	Identification of Primary Bacterial and Non-bacterial Pathogens of Pneumonia and Their Patterns of Antimicrobial Resistance Among Urban Slum Children 2 – 59 Months of Age Through Community-based Active Surveillance	Proposal, possible donor
2000	Comparison of antimicrobial outpatient management regimens for pneumonia and their effects on antimicrobial resistance among urban slum children < 5 y/o	Proposal, possible donor

6. Publications Related to Present Work

Brooks WA, Fuchs G. Recent advances in research on zinc and child health in developing countries. J Ind Pediatr 1998;35:1173-76.

Detailed Budget for New Proposal

Project Title: : **A Prospective, Randomized, Double Blind, Placebo-Controlled, Multi-Centre Trial to Assess Safety, Efficacy, Tolerability and Immunogenicity of Influenza Virus Vaccine, Trivalent, Types A & B, Live Cold-Adapted, Liquid Formulation (CAIV-T), Administered Concomitantly with a Combination Live, Attenuated, Mumps, Measles, and Rubella Vaccine in Healthy Children Aged 11 – 24 Months.**

Name of PI: Robert F. Breiman, M.D.

Protocol Number: Name of Division: Health Systems and Infectious Diseases

Funding Source: Wyeth-Lederle Vaccines Amount Funded (direct): Total:
Overhead (%)

Starting Date: September 1, 2002 Closing Date: August 31, 2003

Strategic Plan Priority Code(s):

Sl. No	Account Description	Salary Support			US $ Amount Requested		
	Personnel	**Position**	**Effort%**	**Salary**	**1st Yr**	**2nd Yr**	**3rd Yr**
	Robert F. Breiman, MD	PI	20%	165000	0		
	W. Abdullah Brooks, MD	Co-PI	20%	150345	30,069		
	Research Investigator	NO-B	33%	9552	3,152		
	Medical Officer	NO-A	100%	7872	7,872		
	Nurse	GS-5	100%	4344	4,344		
1	Field Research Officer	GS-5	100%	4344	4,344		
2	Field Research Assistant	GS-3	100%	2796	5,592		
1	Data Manager	GS-6	10%	5640	564		
1	Lab technician	GS-4	33%	3228	1,065		
1	Project/Office Manager	NO-A	33%	7872	2,598		
1	Data Entry Technician	GS-4	50%	3348	1,674		
1	Senior Attendant	GS-2	33%	3300	1,089		
2	Health Workers		100%	936	1,872		
	Sub Total				**64,236**		
	Consultants						
	Local Travel				**2,000**		
	International Travel						
	Sub Total						
	Supplies and Materials (Description of Items)						
	Shipping				700		
	Non-stock drugs				7,500		
	Consumable equipment/supplies				1,560		

	Sub Totals	**9,760**		

	Other Contractual Services			
	Repair and Maintenance			
	Rent (20%)	**4,320**		
	Mobile Phone and utilities	**1,900**		
	Training Workshop, Seminars	**200**		
	Printing and Publication	**600**		
	Staff Development			
	Sub Total			

	Interdepartmental Services	**1st Yr**	**2nd Yr**	**3rd Yr**
	Computer Charges			
	Pathological Tests			
	Microbiological tests			
	Biochemistry Tests			
	X-Rays			
	Patients Study			
	Research Animals			
	Biochemistry and Nutrition			
	Transport			
	Xerox, Mimeographs etc.			
	Miscellaneous	**500**		
	Sub Totals			
	Other Operating Costs			

	Capital Expenditure Office Furniture	600		

TOTAL DIRECT COST	84,116
Total Indirect Cost	21,870
Total	105,989

Budget Justifications

Please provide one page statement justifying the budgeted amount for each major item. Justify use of man power, major equipment, and laboratory services.

Budget Justification

Personnel

- **Dr Robert Breiman** will lead the investigation as a vaccinologist and the senior epidemiologist.
- **Dr W. Abdullah Brooks** will co-lead the investigation as the field epidemiologist and paediatrician. The time input will average 1.5 hours per day of a five-day workweek or 20% time.
- **Research Investigator** will provide tactical and logistical management and supervision on a daily basis to field operations. The RI will provide 100% time for the first two months (16.7% time), 20% time for 7 months (11.7% time) and 0.25% for the terminal 3 months of the study (6.2%) = 34.6% time for the year.
- **Medical Officers:** There will be 150 patients enrolled in the time period. There are an average of 660 patient visits per week. Children < 5 years old comprise 11.2% of the total population (2,240 children out of 20,000 population), but account for 70.1% of all clinic visits (conservatively). Among these about 50% are in the 9 month – 3 year age group. Thus, 0.35 * 660 = 231; X/150 = 231/2240. Solving for x gives 16 patients/week or 3 – 4 patients per day. Each patient requires about two hours of physician time for history and physical, blood draw, vaccination, observation and completion of the CRF per visit. Thus, each child = 6 – 8 hours per day, or 100% time for one physician.
- **Nurses:** The functional unit of the clinic is a triad of physician/nurse/health worker. Thus, if we require one physician, we require one nurse.
- **Health Workers:** The same applies to the health workers as does the physicians and nurses.
- **Field Research Officer:** This position requires conducting the IVRS randomisation every morning at a site 40 minutes distant from the field site, tracking and monitoring of the randomisation lists, filing the IVRS reports at the end of each day, supervision of the FRAs in the field, checking the patient diary cards, and coordinating activities between the medical team and FRAs, particularly for patient follow-up. The FRO must also compile and submit daily and monthly summary reports on the performance of the field surveillance activities to the senior project staff as a component of monitoring and quality assurance.
- **Field Research Assistants:** The duties of the FRAs will be to consent and enrol the study participants, to obtain background demographic data, arrange for and ensure clinic visits of the patients, and conduct daily home visits to obtain data on adverse events, reactogenicity, obtain axillary temperature, and make referrals to clinic as needed for the duration of the study. Our experience with the previous CAIV-T study indicates that one FRA can follow-up to 15 children per day or 75 per week, thus accounting for two full-time FRAs' for the present study, in which we plan to follow 150 children.
- **Project Manager:** A part-time manager will be required to receive phone calls, process requests, maintain a filing system including photocopies of all documents, co-ordinate shipments, and monitor expenses. This should average 2.5 hours/day or 12.5 hours/week = 12.5hours/40 hours/work week = **33% of workweek**.
- **Senior Attendant:** This individual will assist with procurements, logistical placement of materials in the field including retrieval of vaccines from storage and conveying them to the field in timely manner, bringing lab samples from the field to the lab for processing, bring medicines and other supplies to the field office and bring reports from the field to the Centre. This will average 2.5 hours/day or 12.5 hours/week/40 hours/work-week = **33% workweek**.
- **Lab Technician:** We will require the services of one on-site lab technician to separate, spin, label and transport serum to the main site per day. This will comprise full time activity for approximately 8 weeks at the beginning and another 8 weeks at the end of the study, or 33% time.

Travel

- **Domestic:** This includes transport of materials to and from the field (twice daily 5X/week), the use of Centre transport (driver and petrol) twice daily 5days/week X 2 weeks/month for Quintiles monitoring, and transport of study personnel between field and office for 12 month period. Average Tk500/day X 5 days/week X 4.2 week/month X 12 month = **US $2,500.**

Supplies and Material

- **Shipping:** This is to cover the cost of shipping of CAIV-T study related materials, mainly printed matter, via DHL. The average cost of shipping a package from Dhaka to anywhere in the region is approximately US $50.00. During the past study of 6 months duration, there was about one such shipment per month. Thus for a 12 month study, we anticipate at least 14 such shipments, or US $700.00.
- **Non-stock Drugs:** The monthly cost of providing medicine, including antibiotics, anti-scabies and anti-fungal drugs to the population served by the field clinic averages over US $4.00 per study subject. These costs also include the cost of treating family members for communicable infectious diseases, like scabies, which the clinic must provide on a humanitarian basis. Over the anticipated 12 months of study set-up, data collection, and closure, each child will consume over US $50 worth of treatment, or US $7,500.
- **Consumable Equipment:** During the previous CAIV-T study, each child consumed approximately US $0.87 worth of consumable materials in the form of gloves, syringes, needles, cotton swabs, etc per month. For a target of 150 children we should anticipate US $130 of consumables per month, or US $1,560.

Laboratory Tests and Others

- **Centrifugation:**

Contractual Services

- **Photocopying:** These costs include photocopying and faxing, and cover the cost of copying correspondence between our office and Quintiles, making copies of key documents for the site file and/or key study personnel including the PI's, making copies of reports and patient records. These costs will be higher at the beginning and end of the study. Our projections, based on the previous study, is that our averaged copying costs will be about US $50.00/month.
- **Training and Workshop:** All study personnel will require training. The training focuses on the consent and data collection activities for the field staff, including practice sessions and standardisation exercises to ensure both accuracy (correct method) and precision (reproducibility and close inter-observer agreement). The medical officers and nurses will require training on the history and physical exam for the study, as well as emergency resuscitation methods for anaphylaxis. The cost of training, including the printing of materials, production of overhead transparencies, test materials, pens, paper etc = $20/day X 10 days training (average for training and standardisation exercises) = **US $200**. These costs are normally higher, but since we have just completed another similar study, we can re-use some training materials.
- **Hospitalisation:** During the last study, there were 3 hospitalisations among 349 children over the three study months. We project to enrol 150 children (43% of the previous population). If we had 3 hospitalisations among approximately 350 children during a three-month period, then we should have (x hospitalisations/12 months = 3 hospitalisations/3 months =) approximately 5 hospitalisations during this study. The mean length of hospital stay for pneumonia or sepsis is 5 days, at a base cost of US $25/day or US $625 for the duration of the study. X-rays cost US $2.00/patient, labs average US $10, and are recorded accordingly in the budget.

Other Support

Describe sources, amount, duration, and grant number of all other research funding currently granted to PI or under consideration. (DO NOT EXCEED ONE PAGE FOR EACH INVESTIGATOR)

Dr. Breiman is supported 100% through secondment from CDC-Atlanta.

APPENDIX

International Centre for Diarrhoeal Disease Research, Bangladesh
Voluntary Consent Form

Title of the Research Project: **A Prospective, Randomized, Double Blind, Placebo-Controlled, Trial to Assess Safety, Efficacy, Tolerability and Immunogenicity of Influenza Virus Vaccine, Trivalent, Types A & B, Live Cold-Adapted, Liquid Formulation (CAIV-T), Administered Concomitantly with a Combination Live, Attenuated, Mumps, Measles, and Rubella Vaccine in Healthy Children Aged 11 – 24 Months Protocol No. D153 P522**

Principal Investigator: Robert F. Breiman, M.D.

Before recruiting into the study, the study subject must be informed about the objectives, procedures, and potential benefits and risks involved in the study. Details of all procedures must be provided including their risks, utility, duration, frequencies, and severity. All questions of the subject must be answered to his/ her satisfaction, indicating that the participation is purely voluntary. For children, consents must be obtained from their parents or legal guardians. The subject must indicate his/ her acceptance of participation by signing or thumb printing on this form.

Parent's Information and Informed Consent Form for an Influenza ('Flu) Vaccine Study given with a Measles, Mumps and Rubella (MMR) Vaccine in Children Aged 11 months to 24 Months

We invite you and your child to participate in an international research study involving about 1200 children in several countries. This study looks to see if an investigational influenza ('flu) vaccine given as a nasal spray (CAIV-T) and measles mumps and rubella vaccine (MMR) are safe and effective when given together. Your child has been asked to take part because he or she is between 11 months and 24 months old, and is due to receive their first measles vaccination.

We will now read this form to you. Please ask us if there is anything you do not understand.

What is known about flu vaccines?

Influenza is a virus that causes high fever, sore throat, headache, dry cough, stuffy nose, weakness, muscle pain and loss of appetite in children and adults. It is different from the common cold. Mild cases may last 1 to 2 weeks. However, complications such as ear infections and pneumonia may occur.
In a study performed in young children in the US using a different formulation of this vaccine, the incidence of influenza was reduced in those subjects given the vaccine compared with those given placebo. The efficacy of the liquid formulation being evaluated in this study has not been determined in this age group.

What are alternative vaccines?

Several 'flu vaccines are in general use already and are given once a year in many countries. However, they are normally only used in children or adults at high risk of getting 'flu with complications. These vaccines are given by injection only (through a needle). At present, protection from 'flu by immunisation is not normally available for healthy children. Flu vaccine is not available in Bangladesh.

What is MMR?

MMR is a vaccine, given routinely in many countries, that has been shown to protect against three diseases measles, mumps and rubella. They are caused by three different viruses that are spread from person to person. The typical complications of measles are ear infection, pneumonia and encephalitis. Mumps may cause swelling of the face and neck, fever, headache and tiredness. Rubella causes rash, fever and swelling of the lymph nodes. It may also cause severe birth defects if a woman has not been immunised and is infected for the first time while pregnant. A vaccine directed against all three viruses is available and the first dose is routinely given between 11 and 24 months of age in North America and Europe with a second dose later in childhood. In Bangladesh, MMR is licensed but not routinely given. However, the measles vaccine part is the same as in the standard measles vaccine, which is offered routinely to children in Bangladesh beginning at nine months of age.

What is the purpose of this study?

Both MMR and the nasal spray influenza vaccines are safe and effective given separately. We need to know if they can be given safely and effectively together. In the future, children who are due to get their MMR vaccine may also want to be protected against flu by having the nasal spray flu vaccine.

Please note that the vaccine being used in this study will not provide protection against the common cold.

To answer this question, we will compare a group of children who receive MMR with CAIV-T to a second group who receive MMR with a dummy nasal spray (called a placebo). Children will be placed in one of these groups by lottery. Our staff will not know which group your child is in, but if the doctor needs this information in an emergency he/she can obtain it. Every child will get the MMR vaccine.

Why are we asking you to take part?

Your child is being asked to take part because he/she is 11 to 24 months old, healthy, is due to receive the first dose of measles vaccine and has never received any influenza vaccination.

What happens in the study?

All children will participate in the study for approximately 7 to 9 months from approximately September 2002 to May 2003 and will attend the ICDDR,B Dhalpur Field Clinic about four times. You will not be charged for any study-related procedures or required travel.

Taking part in this research study means that the parent(s)/legal guardian(s) agree to:

- **Visit 1 (Initial visit)**

The study doctor will review the details of the study with you, answer your questions, and if you agree to participate, will ask you to sign a consent form. The doctor will then ask about your child's health, and physically examine your child to make sure your child should receive the study vaccine. Your child will then be randomly assigned to one of the two treatment groups. Before vaccination, we will draw 5ml of blood. Then your child will receive the 'flu vaccine (or the nasal spray placebo), as well as MMR vaccine. After vaccine administration your child will be observed for at least 30 minutes. It is anticipated that the entire visit will take one and a half to two hours.

- **Visit 2**

A second visit to the Clinic will be arranged approximately 4 to 6 weeks later. This visit will be identical to the first (including receiving the nasal spray vaccine or placebo and having the blood sample taken), except that your child will not receive MMR vaccine. This visit will take about one hour.

If the vaccination cannot be performed during a visit (for example, your child has fever) it may be delayed and a new visit will be arranged with you.

- **Visit 3**

Approximately 4 to 6 weeks later, we will arrange a third visit to ask about your child's health since the second vaccination. This visit should take less than one hour. A blood sample will not be needed during this visit for the purposes of the study.

- **Final Contact**

We will arrange a final clinic visit 7-9 months later in approximately May 2003 to ask about your child's health since the previous visit. A blood sample will not be needed during this visit for the purposes of the study. Your child's participation will then be complete and no further procedures will be necessary.

What do you have to do?

We will provide you with a digital thermometer and instructions for its use. On the day of each vaccination, and for the next 10 days, a field research assistant will come to your home and assist you to record your child's temperature, general condition and whether you have had to visit the doctor or take medications.

For the next 8 months after the first vaccination, we will visit your home once a week to enquire about your child's health. If your child develops any symptoms of 'flu or ear infection we will refer you to the clinic, where a nurse will take a small mucous sample from your child's nose using a cotton swab and a doctor will examine your child's ears. The sample will be tested for respiratory viruses including the flu virus.

Please tell the study doctor/nurse if your child has experienced any problems with any previous vaccine or if your child is allergic to eggs.

What are the medications that should not be given to your child?

During the study your child may not be given any aspirin or products that contain aspirin (acetylsalicylic acid), as these could be harmful. Paracetamol-containing products, for example, *Calpol*, can be given. Our study team can advise you, if you have any questions or concerns.

Your child should not receive any other vaccines one month before and after he/she has received the study vaccines.

What are the benefits for participation in the trial?

Your child may receive a 'flu vaccine and may be protected against influenza for this season. Your child will also receive the MMR vaccine.

Possible side effects of the vaccinations

The side effects of the nasal spray may include running nose, headache, muscle ache, irritability, fever or mild 'flu like symptoms. There may be other risks, which we don't know about now, but if new information about this vaccine becomes available, we will inform you and discuss whether to continue your child in the study.

The side effects for MMR vaccination include pain, swelling and tenderness at the injection site for a short while; fever; skin rash; sore throat; malaise; nausea, vomiting or diarrhoea; muscle ache and headache. These symptoms are usually mild, if they occur.

Possible side effects of blood sample collection

During the study, two blood samples (total 10 mL or 1 teaspoon each visit) will be taken from each child. This may be uncomfortable, and can occasionally cause minor pain and bruising. These blood samples are necessary to examine your child's response to the vaccines. However, we will not have the results until after the study is complete and the data have been analysed.

Possible side effects of nasal swab collection
The nasal swab may cause mild discomfort when it is being taken.

Is participation in the trial voluntary?

Your child's participation is entirely voluntary. If you decide to allow your child to participate, you may withdraw your child at any time. The study medical officer or the study sponsor may also withdraw your child. Whether or not your child participates, your access to the clinic and care will not be affected in any way.

Insurance

Your child is covered for any injury that arises due to taking part in this study by insurance paid by the manufacturer of the vaccine, Wyeth. Compensation will follow the guidelines of the Association of the British Pharmaceutical Industry (ABPI). Copies of these guidelines are available, or you may talk to the study doctor or nurse.

Illness During the Study

Your child will be treated, free of cost, at our clinic for any and all study-related illnesses throughout the duration of the study. If you withdraw your child from the study before it is completed, your child will still have access to our clinic. If your child requires hospitalisation, all costs pertaining to hospitalisation and transportation to hospital will be paid for.

Confidentiality of data

Your child's identity will remain confidential at all times. No information that identifies your child will leave our facility. The information collected may be used in reports, scientific presentations, or supplied to regulatory authorities. The data could also be used for future medical research to answer questions that may arise after this study.

Although your child's identity will remain confidential, regulatory authorities or representatives of ICDDR,B or Wyeth may examine the records to see that they are documented properly and that there is no problem with the study.

If Wyeth sends study information to its associated companies in other countries, Wyeth will take all reasonable steps to protect your child's privacy.

Additional Information

At the end of the study the nasal spray vaccine (CAIV-T) will not be available straight away. The results of the study will be used to apply for a license to use the vaccine in a variety of countries, possibly including Bangladesh.

If you have any concerns or complaints, you may contact, the Secretary of the Centre's Ethics Committee, Mr. Bejoy Saha, at 8811751 Ext 2115.

The study has been organised and funded by Wyeth who will also fund ICDDR,B for taking part in this study.

The study will be managed and conducted by Quintiles Singapore on behalf of Wyeth.

Contact Details

If you have questions about the rights of your child or need more information about the study please contact any of our study staff at our field office.

Study Doctors

Robert F. Breiman, MD
Health Systems and Infectious Diseases Division
ICDDR,B
988-1761

W. Abdullah Brooks, MD, MPH
Health Systems and Infectious Diseases Division
ICDDR,B
988-1761

Dr. Doli Goswami
Health Systems and Infectious Diseases Division
ICDDR,B
988-1761

Informed Consent

I hereby confirm that I have been informed about the nature, conduct, benefits and risks of this clinical study. I have also received, read, and understood the above written information (Patient Information Leaflet and Informed Consent) regarding this clinical study.

I am aware that the results of the study, including my child's sex, age, date of birth, initials, and diagnosis will be anonymously processed into a study report.

I may, at any stage, without prejudice, withdraw my consent for my child's participation in the study.

I have had sufficient opportunity to ask questions and agree to my child's participation in this study.

_____ _____
Child's Name Subject Number

During Home Visit

Name of Household Head Present: _____

Parent/Legal Guardian Signature: _____ Date:_____
(written by Parent/Legal Guardian)

Name of Parent/Legal Guardian: _____ Relationship_____

If both parents/legal guardians present:

Parent/Legal Guardian Signature: _____ Date:_____
(written by Parent/Legal Guardian)

Name of Parent/Legal Guardian: _____ Relationship_____

Signature of Witness: _____ Date_____
(if Parent/Legal Guardian is unable to read) (written by witness)

Name of Witness: _____

Signature of Study Staff: _____ Date_____
(written by study staff)

Name of Study Staff: _____

During Clinic Visit: The parent/legal guardian has had any questions related to the study answered by the study doctor and they have reconfirmed willingness for their child to participate in the study.

Signature of Witness: _____ Date_____
(written by witness)

Name of Witness: _____

Study Doctor's Signature: _____ Date: _____
(written by Study Doctor)

Study Doctor's name: _____

You will be given a copy of this form and the information sheet to keep.

CENTRE FOR HEALTH AND POPULATION RESEARCH
Mail : ICDDR,B, GPO Box 128, Dhaka-1000, Bangladesh
Phone : 8811751-60, Telex : 642486 ICDD BJ
Fax : 880-2-8823116, 8826050, 8811568, 8811686, Cable : Cholera Dhaka

CENTRE
FOR HEALTH AND
POPULATION RESEARCH

পরিশিষ্ট

আন্তর্জাতিক উদরাময় গবেষণা কেন্দ্র, বাংলাদেশ

সম্মতিপত্র

গবেষণা প্রকল্পের শিরোনামঃ ১১ থেকে ২৪ মাস বয়সী সুস্থ শিশুদের জন্য প্রয়োগতব্য এবং মামপ্স, হাম ও রুবেলার জীবন্ত ও দূর্বলীকৃত মিশ্র টীকার সাথে যৌথভাবে দেয় ত্রিমূখী, এ ও বি ধরনের শৈত্য সহনশীল, তরল প্রকৃতির (CAIV-T) ইনফ্লুয়েঞ্জা টীকার নিরাপত্তা, কার্যকারিতা, সহনশীলতা এবং প্রতিষেধক ক্ষমতা যাচাইয়ের জন্য একটি পরীক্ষামূলক, দৈবচয়নভিত্তিক, ডাবল ব্লাইন্ড এবং প্লাসিবো পদ্ধতির গবেষণা।

প্রধান গবেষকঃ রবার্ট ব্রাইম্যান, এমডি

গবেষণায় অন্তর্ভূক্ত করার আগে অংশগ্রহনকারীকে গবেষণার উদ্দেশ্য, পদ্ধতি এবং এর সাথে সংশ্লিষ্ট সুবিধা ও ঝুঁকিগুলো সম্পর্কে জানাতে হবে। গবেষণা প্রক্রিয়াগলোর বিস্তারিত বিবরন, যেমন, এর সম্ভাব্য ঝুঁকি, প্রয়োজনীয়তা, স্থায়ীত্বকাল, সংখ্যা ও তীব্রতা সম্পর্কে অবশ্যই অবহিত করতে হবে। অংশগ্রহনকারীর সকল প্রশ্নের সন্তোষজনক জবাব দিতে হবে এবং তাকে বোঝাতে হবে যে তার অংশগ্রহন সম্পূর্নভাবে স্বেচ্ছামূলক। শিশুদের ক্ষেত্রে তাদের পিতামাতা বা আইনানুগ অভিভাবকের সম্মতি নিতে হবে। এই সম্মতিপত্রে স্বাক্ষর বা টিপ সইয়ের মাধ্যমে অংশগ্রহনকারী অবশ্যই তার সম্মতি জানাবেন।

---

১১ থেকে ২৪ মাস বয়সী শিশুদের জন্য হাম, মামপ্স ও রুবেলা (এমএমআর) এর সাথে দেয় ইনফ্লুয়েঞ্জা টীকার গবেষণার উদ্দেশ্যে পিতামাতার জন্য তথ্য এবং সম্মতিপত্র

একটি আন্তর্জাতিক গবেষণা কার্যক্রমে অংশগ্রহন করার জন্য আপনাকে এবং আপনার সন্তানকে আমন্ত্রন জানাচ্ছি; পৃথিবীর কয়েকটি দেশে প্রায় ১২০০ শিশু এ কার্যক্রমে অংশ নিচ্ছে। ইনফ্লুয়েঞ্জা (ফ্লু) এর জন্য নাকে স্প্রে-এর মাধ্যমে দেয়া পরীক্ষামূলক এক টীকা (CAIV-T) আর হাম, মামপ্স ও রুবেলা টীকা (MMR) এক সাথে ব্যবহার করা নিরাপদ এবং কার্যকর কিনা সেটা যাচাই করা এ গবেষণার উদ্দেশ্য। আপনার শিশুকে গবেষণায় অংশ নেয়ার জন্য বলা হচ্ছে এ কারনে যে তার বয়স ১১ থেকে ২৪ মাসের মধ্যে এবং এর মধ্যেই তার হামের প্রথম টীকাটি নেবার কথা।

আমরা এখন এ ফরমটি আপনাকে পড়ে শোনাবো, কোন বিষয় বুঝতে না পারলে দয়া করে জানাবেন।

ফ্লুর টীকা সম্পর্কে আমরা কি জানি?

ইনফ্লুয়েঞ্জা ভাইরাসের কারনে শিশু এবং প্রাপ্ত বয়স্কদের তীব্র জ্বর, গলাব্যথা, মাথাব্যথা, শুকনা কাশি, নাক বন্ধ হয়ে যাওয়া, দূর্বলতা, মাংসপেশীতে ব্যথা, ক্ষুধামান্দ্য ইত্যাদি উপসর্গ দেখা দেয়। এটি সাধারন সর্দি থেকে ভিন্ন। সাধারন ফ্লু ১ থেকে ২ সপ্তাহ পর্যন্ত থাকতে পারে। কোন কোন সময় কানের সংক্রমন এবং নিউমোনিয়াও হতে পারে। ভিন্ন পদ্ধতিতে প্রদত্ত একই টীকা দিয়ে মার্কিন যুক্তরাষ্ট্রের কম বয়সী শিশুদের উপর পরিচালিত এক সমীক্ষায় দেখা গেছে যে, টীকা দেয়া শিশুদের মাঝে ইনফ্লুয়েঞ্জার হার প্লাসিবো দেয়া, তথা টীকা না দেয়া শিশুদের চেয়ে তুলনামুলকভাবে কম। উল্লিখিত শিশুদের মাঝে বর্তমান গবেষণার তরল ধরনের টীকার কার্যকারিতা কতটুকু তা মিমাংসিত হয়নি।

বিকল্প টীকাগুলো কি?

ফ্লুর একাধিক ধরনের টীকা বিভিন্ন দেশে ইতোমধ্যেই চালু হয়ে গেছে, এটি বছরে একবার দেয়া হয়। তবে এগুলো সাধারনতঃ পূর্বে উল্লিখিত জটিল ধরনের ফ্লু'র জন্য ঝুঁকিপূর্ন শিশু ও প্রাপ্ত বয়স্কদেরকেই কেবল দেয়া হয়। এই টীকা কেবলমাত্র ইনজেকশনের মাধ্যমেই দেয়া হয়। বর্তমানে সুস্থ শিশুদের ব্যবহারের জন্য ফ্লুর কোনরূপ টীকা সাধারনতঃ পাওয়া যায় না। বাংলাদেশে এই টীকা পাওয়া যায় না।

এমএমআর (MMR) কি?

এটি একটি টীকা যা হাম, মামপ্স এবং রুবেলা (rubella) এই তিনটি রোগের প্রতিষেধক হিসেবে বহু দেশে নিয়মিতভাবে ব্যবহার করা হয়। তিনটি ভিন্ন ধরনের ভাইরাসের মাধ্যমে এই রোগগুলো এক ব্যক্তি থেকে আরেক ব্যক্তির মধ্যে ছড়ায়। হামের কারনে সাধারনতঃ কানের সংক্রমন, নিউমোনিয়া এবং মস্তিস্কের প্রদাহ রোগ দেখা দেয়। মামপ্সের কারনে রোগীর মুখমন্ডল ও গলা ফুলে যেতে পারে এবং রোগীর জ্বর, মাথা ব্যথা ও অবসাদবোধ হতে পারে। আর রুবেলা বা জার্মান মিজ্‌ল্‌স্ হলে রোগীর ছোট লাল লাল ফুসকুড়ি দেখা দেয়, তার জ্বর হয় এবং লসিকা গ্রন্থি ফুলে যায়। গর্ভাবস্থায় প্রথমবারের মত রুবেলা হলে এবং টীকা দেয়া না থাকলে সন্তান বিকলাঙ্গ হয়ে জন্মাতে পারে। উল্লিখিত তিনটি ভাইরাসের বিরুদ্ধে সমানভাবে কার্যকর একটি টীকা বর্তমানে উত্তর আমেরিকা এবং ইয়োরোপে নিয়মিতভাবে ব্যবহৃত হচ্ছে। এর প্রথম মাত্রা (dose) দেয়া হয় শিশুর ১১ থেকে ২৪ মাস বয়সে, ২য় মাত্রা আরো কিছু কাল পর। এমএমআর বাংলাদেশে লাইসেন্সকৃত কিন্তু নিয়মিতভাবে দেয়া হয় না। তবে এ টীকার হাম অংশের মান স্বীকৃত হামের টীকার অনুরূপ এবং এটি বাংলাদেশে শিশুর ৯ মাস বয়স থেকে নিয়মিতভাবে দেয়া হয়।

এই গবেষণার উদ্দেশ্য কি?

আলাদাভাবে দিলে এমএমআর এবং ফ্লু'র জন্য নাকের স্প্রে উভয়ই নিরাপদ। আমরা জানতে চাই এই দুটো টীকা নিরাপদ এবং কার্যকরভাবে একসাথে দেয়া যায় কিনা। ভবিষ্যতে যে সকল শিশুর এমএমআর টীকা নেয়ার সময় হবে তারা ফ্লু থেকে নিরাপদ থাকার জন্য নাকের স্প্রের মাধ্যমে নেয়া ফ্লু'র টীকাও চাইতে পারে।

এখানে দয়া করে লক্ষ্য করুন যে এই গবেষণায় ব্যবহৃত টীকা সাধারন সর্দির জন্য কোন উপকারে আসবেনা।

এই গবেষণায় আমরা শিশুদের দুটো গ্রুপকে তুলনা করে দেখতে চাই। প্রথম গ্রুপ হল যারা এমএমআর এর সাথে সিএআইভি-টি (CAIV-T) (নাকের স্প্রে) নিয়েছে আর দ্বিতীয় গ্রুপ হল যারা এমএমআর-এর সাথে একটা নকল স্প্রে (এটাকে প্লাসিবো বলা হয়) নিয়েছে। শিশুদের এই গ্রুপগুলো করা হবে লটারীর মাধ্যমে। আপনার শিশু কোন গ্রুপে আছে তা আমাদের গবেষকরা জানবেন না তবে জরুরী কোন প্রয়োজনে ডাক্তার যদি তা জানতে চান তিনি জানতে পারবেন। প্রতিটি শিশু অবশ্য এমএমআর টীকা পাবে।

আপনাকে আমরা কেন অনুরোধ করছি?

এ গবেষণায় আপনার শিশুর অংশগ্রহনের বিষয়ে আমরা অনুরোধ করছি কারন তার বয়স ১১ থেকে ২৪ মাসের মধ্যে এবং তার স্বাস্থ্য স্বাভাবিক। আরো কারন এই যে, তার হামের টীকার ১ম মাত্রা নেয়ার সময় হয়ে গেছে এবং সে কখনও ইনফ্লুয়েঞ্জার টীকা নেয়নি।

গবেষণায় কি কি কার্যক্রম আছে?

সকল শিশু আনুমানিক ৭ থেকে ৯ মাস এ গবেষণার সাথে যুক্ত থাকবে যা সেপ্টেম্বর ২০০২ নাগাদ শুরু হয়ে মে ২০০৩ পর্যন্ত চলবে। এই সময় সীমায় উক্ত শিশুরা আইসিডিডিআরবি'র ধলপুরের মাঠ পর্যায়ের ক্লিনিকে প্রায় চার বার যাবে। গবেষণা সংক্রান্ত বিষয়ে বা সংশ্লিষ্ট যাতায়াত বাবদ আপনার কাছ থেকে কোনরূপ খরচ নেয়া হবে না।

গবেষণা কার্যক্রমে অংশগ্রহনের মাধ্যমে পিতা/মাতা বা আইনানুগ অভিভাবক নিম্নবর্নিত বিষয়গুলোর সাথে সম্মত বলে ধরে নেয়া হবেঃ

❋ **প্রথম সাক্ষাৎকার**

আমাদের ডাক্তার গবেষণার বিষয়ে আপনার সাথে বিস্তারিত আলোচনা করবেন, আপনার প্রশ্নের উত্তর দেবেন এবং আপনি অংশগ্রহনে সম্মত হলে আপনাকে একটি সম্মতিপত্রে স্বাক্ষর করতে অনুরোধ করবেন। ডাক্তার এরপর আপনার শিশুর স্বাস্থ্য সম্পর্কে জিজ্ঞাসাবাদ করবেন এবং তাকে ব্যক্তিগতভাবে পরীক্ষা করে নিশ্চিত হবেন যে সে গবেষণার টীকা গ্রহন করার উপযুক্ত কিনা। আপনার শিশুকে এরপর দৈবচয়নের ভিত্তিতে উল্লিখিত দুটি গ্রুপের একটিতে অন্তর্ভূক্ত করা হবে। টীকা নেয়ার আগে আমরা ৫ মিলি রক্ত নেব, এরপর দেয়া হবে এমএরআর এবং সত্যিকার ফ্লু'র টীকা বা প্লাসিবো স্প্রে। টীকা দেয়ার পর আপনার শিশুকে কমপক্ষে আধা ঘন্টা ধরে পর্যবেক্ষন করা হবে। ক্লিনিকের এই প্রথম সাক্ষাৎকারে দেড় ঘন্টা থেকে ২ ঘন্টার মত সময় লাগতে পারে।

❋ **দ্বিতীয় সাক্ষাৎকার**

২য় বার আপনি ক্লিনিকে যাবেন ৪ থেকে ৬ সপ্তাহ পর। এটি হবে প্রথম সাক্ষাতের মতই, পার্থক্য এই যে এবারে স্প্রে টীকা বা প্লাসিবো দেয়া এবং রক্তের নমুনা নেয়া হলেও আপনার শিশুকে কোনরূপ এমএমআর টীকা দেয়া হবে না। এ সাক্ষাতে সময় লাগবে প্রায় ১ ঘন্টা।

সাক্ষাৎকালে কোন টীকা না দেয়া গেলে (যেমন - আপনার শিশুর জ্বর থাকলে) এটি দেরীতেও নেয়া যাবে এবং আপনার জন্য ক্লিনিকে আলাদা সাক্ষাতের ব্যবস্থা করা হবে।

❋ **তৃতীয় সাক্ষাৎকার**

দ্বিতীয়বার টীকা দেয়ার প্রায় ৪ থেকে ৬ সপ্তাহ পর আপনার শিশুর স্বাস্থ্যের খবর নেয়ার জন্য আমরা তৃতীয় সাক্ষাতের আয়োজন করব। এক ঘন্টারও কম সময় লাগবে এতে এবং এ সাক্ষাতে কোনরূপ রক্ত সংগ্রহ করার প্রয়োজন হবে না।

❋ **শেষ যোগাযোগ**

টীকা দেবার পর আপনার শিশুর স্বাস্থ্যের সর্বশেষ অবস্থা জানার জন্য ৭ থেকে ৯ মাস পর, ২০০৩ সালের মে-এর দিকে আমরা আয়োজন করব চূড়ান্ত সাক্ষাৎকারের। গবেষণার জন্য এ সাক্ষাতে কোনরূপ রক্তের নমুনার প্রয়োজন হবে না। গবেষণায় আপনার শিশুর অংশগ্রহন শেষ হয়ে যাবে, আর কোন কার্যক্রমের আবশ্যক হবে না।

আপনাকে কি কি করতে হবে?

আমরা আপনার জন্য ব্যবহার পদ্ধতিসহ একটি ডিজিটাল থার্মোমিটারের ব্যবস্থা করব। প্রতিবার টীকা দেয়ার দিন থেকে শুরু করে পরবর্তী ১০ দিন পর্যন্ত একজন মাঠ গবেষণা সহকারী আপনার বাড়ীতে গিয়ে আপনার শিশুর শরীরের তাপ এবং অন্যান্য শারিরীক অবস্থা লিপিবদ্ধ করার বিষয়ে এবং ডাক্তারের কাছে যেতে হবে কিনা বা কোনরূপ ঔষধপত্র লাগবে কিনা সে বিষয়ে সহায়তা করবেন।

প্রথম টীকা দেবার পরবর্তী ৮ মাস পর্যন্ত আপনার শিশুর স্বাস্থ্যের খবর নেয়ার জন্য আমরা সপ্তাহে ১ বার আপনার বাড়ীতে যাব। আপনার শিশুর ফ্লু বা কানের সংক্রমনের লক্ষণ দেখা গেলে আমরা তাকে ক্লিনিকে নিয়ে যাবার পরমর্শ দেব। সেখানে একজন নার্স তুলা দিয়ে আপনার শিশুর নাক থেকে শ্লেষ্মার সামান্য একটু নমুনা নেবেন এবং ডাক্তার তার কান পরীক্ষা করবেন। উল্লিখিত শ্লেষ্মা ব্যবহার করা হবে ফ্লু ভাইরাসসহ শ্বাস সংক্রান্ত অন্যান্য ভাইরাস পরীক্ষার জন্য।

পূর্ববর্তী টীকা দেয়ার সময় আপনার শিশুর কোনরূপ সমস্যা হয়েছিল কিনা বা ডিম খেলে আপনার শিশুর এলার্জি হয় কিনা দয়া করে তা গবেষণার সাথে যুক্ত ডাক্তার বা নার্সকে জানান।

কোন কোন ঔষধ আপনার শিশুকে দেয়া যাবে না?

গবেষণা চলাকালে আপনার শিশুকে এ্যাসপিরিন (acetylsalicylic acid) বা এ্যাসপিরিন আছে এমন কোন ওষুধ দেয়া যাবে না, এগুলো ক্ষতিকারক হতে পারে। তবে প্যারাসিটামলজাতীয় ওষুধ, যেমন ক্যালপল (calpol), দেয়া যেতে পারে। এ ব্যাপারে আপনার কোন প্রশ্ন বা জানার থাকলে আমাদের গবেষণা কর্মীরা তা জানাতে পারবে।

আমাদের টীকা গ্রহন করার ১ মাস আগে পরে আপনার শিশু অন্য কোন টীকা নিতে পারবেনা।

এই গবেষণায় অংশগ্রহন করার সুফল কি?

আপনার শিশু ফ্লুর টীকা পেতে পারে এবং এই মওসুমে ইনফ্লুয়েঞ্জার সংক্রমন থেকে রক্ষা পেতে পারে। এ ছাড়া আপনার শিশু এমএমআর টীকাও পাবে।

টিকা নেয়ার সম্ভাব্য পার্শ্ব প্রতিক্রিয়া

নাকের মাধ্যমে স্প্রে গ্রহন করার ফলে সর্দি, মাথাব্যথা, মাংসপেশীর ব্যথা, খিটখিটেভাব, জ্বর বা কিছুটা ফ্লু'র মত হতে পারে। এই মুহুর্তে আমাদের জানা নেই এমন কিছু অসুবিধাও হতে পারে, তবে, এই টীকা সম্পর্কে নতুন কোন তথ্য পাওয়া গেলে আমরা তা জানাব এবং সেই প্রেক্ষিতে আপনার শিশুকে গবেষণা কার্যক্রমে রাখা না রাখার ব্যাপারে আপনার সাথে আলোচনা করব।

এমএমআর টীকার কারনে ইনজেকশনের জায়গাটি অল্প কিছুক্ষনের জন্য ফুলে যেতে পারে এবং এখানে ব্যথা হতে পারে। এছাড়া জ্বর, ত্বকে ছোট লাল লাল ফুসকুড়ি, গলাব্যথা, অস্থির ও বমিবমিভাব, বমি বা ডায়রিয়া, মাংসপেশীর ব্যথা এবং মাথাব্যথা হতে পারে। এসব লক্ষণগুলো হলেও সাধারনতঃ মৃদু ধরনের হয়।

রক্তের নমুনা সংগ্রহের সম্ভাব্য পার্শ্বপ্রতিক্রিয়া

গবেষণা চলাকালে প্রতিটি শিশুর কাছ থেকে দুবারে মোট ১০ মিলি (প্রতিবারে ১ চা চামচের সম পরিমান) রক্ত সংগ্রহ করা হবে। এটি অস্বস্থিকর হতে পারে এবং সামান্য ব্যথা ও একটু কালচে দাগও পড়তে পারে। টীকাগুলোর প্রতি আপনার শিশু সাড়া দিচ্ছে

কিনা সেটি পরীক্ষা করার জন্য এই রক্তটুকু প্রয়োজন। যদিও গবেষণা শেষ হবার পর সমস্ত তথ্য বিশ্লেষন করার আগে পর্যন্ত আমরা উক্ত পরীক্ষার ফলাফল জানতে পারব না।

শ্লেষ্মা সংগ্রহের সম্ভাব্য পার্শ্বপ্রতিক্রিয়া

নাকের শ্লেষ্মা নেয়ার সময় সামান্য অস্বস্থিবোধ হতে পারে।

গবেষণায় অংশগ্রহন কি স্বেচ্ছামূলক?

আপনার শিশুর অংশগ্রহনের বিষয়টি সম্পূর্ন আপনার ইচ্ছার উপর নির্ভর করে। আপনার শিশু গবেষণায় অংশ নেয়ার পরও আপনি যখন ইচ্ছে তাকে প্রত্যাহার করতে পারেন। গবেষণায় নিয়োজিত ডাক্তার বা গবেষণার উদ্যোক্তারাও আপনার শিশুকে প্রত্যাহার করতে পারেন। গবেষণায় আপনার বাচ্চা থাকুক আর নাই থাকুক, আমাদের ক্লিনিকের সেবা থেকে সে বাদ যাবে না।

বীমা

গবেষণায় অংশগ্রহন করার করনে আপনার শিশুর কোনরূপ ক্ষতি হলে সে এই টীকা প্রস্তুতকারী প্রতিষ্ঠান ওয়ায়েথ এর কাছ থেকে বীমা সুবিধা পাবে। ক্ষতিপূরনের ক্ষেত্রে দি এসোসিয়েশন অব দি ব্রিটিশ ফার্মাসিউটিক্যাল ইন্ডাস্ট্রি (ABPI) এর নীতিমালা অনুসরন করা হবে। এই নীতিমালার অনুলিপি আমাদের কাছে আছে, আপনি আমাদের ডাক্তার বা নার্সের কাছে এটি চাইতে পারেন।

গবেষণাকালীন অসুস্থতা

গবেষণার পুরো সময়টিতে গবেষণা সংক্রান্ত যেকোন এবং সকল অসুস্থতার জন্য আপনার শিশুকে বিনামূল্যে আমাদের ক্লিনিকে চিকিৎসা করা হবে। গবেষণা শেষ হবার আগেও যদি আপনি আপনার শিশুকে প্রত্যাহার করে নেন তাহলেও আপনার শিশু আমাদের ক্লিনিকে যেতে পারবে। আপনার শিশুকে হাসপাতালে রাখার প্রয়োজন হলে হাসপাতালে নেয়ার এবং হাসপাতালে রাখার যাবতীয় খরচ শোধ করা হবে।

তথ্যের গোপনীয়তা

আপনার বাচ্চার পরিচয় সব সময়ের জন্য গোপন থাকবে। আপনার শিশুর পরিচিতি সম্বলিত কোন তথ্য আমাদের প্রতিষ্ঠান থেকে বাইরে যাবে না। গবেষণায় সংগৃহিত তথ্য বিভিন্ন প্রতিবেদনে বা বৈজ্ঞানিক উপস্থাপনায় ব্যবহৃত হতে পারে অথবা নিয়ন্ত্রনকারী কর্তৃপক্ষের বরাবরে পাঠানো হতে পারে। এই সমীক্ষার কারনে উদ্ভূত বিভিন্ন প্রশ্নের সমাধানের জন্য ভবিষ্যৎ কোন গবেষণায় এই তথ্য ব্যবহার হতে পারে।

আপনার শিশুর পরিচিতি গোপন রাখা হলেও বিভিন্ন নিয়ন্ত্রনকারী কর্তৃপক্ষ বা আইসিডিডিআরবি অথবা ওয়ায়েথ এর প্রতিনিধিরা এই গবেষণার যথার্থতা যাচাই করার জন্য সংশ্লিষ্ট নথিপত্র পরীক্ষা করে দেখতে পারেন।

ওয়ায়েথ গবেষণা সংক্রান্ত কোন তথ্য অন্যান্য দেশের সহযোগী প্রতিষ্ঠানের কাছে পাঠানোর সময়ও আপনার শিশুর পরিচিতি গোপন রাখার সম্ভাব্য সব ব্যবস্থা গ্রহন করবে।

অন্যান্য তথ্য

গবেষণা শেষ হবার পর নাকের স্প্রের মাধ্যমে দেয়া টীকা তাৎক্ষনিকভাবে পাওয়া যাবেনা। গবেষণার ফলাফল বিভিন্ন দেশে টীকা ব্যবহারের লাইসেন্সের আবেদন করার কাজে ব্যবহার করা হবে, এর মধ্যে সম্ভবতঃ বাংলাদেশও অন্তর্ভূক্ত থাকবে।

আপনার কোন প্রশ্ন বা অভিযোগ থাকলে আপনি আইসিডিডিআরবি'র এথিক্স কমিটির সচিব জনাব বিজয় সাহার সাথে যোগাযোগ করতে পারন, তার ফোন নম্বর ৮৮১১৭৫০ এক্সটেনশন নং ২১১৫। এই গবেষণার উদ্যোক্তা এবং অর্থায়নকারী ওয়ায়েথ, গবেষণায় অংশ নেয়ার জন্য তারা আইসিডিডিআরবিকেও অর্থায়ন করবে। ওয়ায়েথ এর পক্ষে গবেষণার ব্যবস্থাপনা এবং পরিচালনা করবে কুইনটাইল্স সিংগাপুর।

যোগাযোগের বিবরণঃ

আপনার শিশুর অধিকার সম্পর্কে আপনার কোন প্রশ্ন থাকলে বা এই গবেষণা সম্পর্কে আরো জানতে চাইলে দয়া করে আমাদের ফিল্ড অফিসের নিম্নবর্ণিত গবেষণাকর্মীদের যে কারো সাথে যোগাযোগ করুনঃ

গবেষণাকারী ডাক্তার

রবার্ট এফ ব্রাইম্যান, এমডি
হেল্‌থ সিস্টেম্‌স এন্ড ইন্‌ফেকশাস ডিজিজেস ডিভিশন
আইসিডিডিআরবি
ফোনঃ ৯৮৮১৭৬১

ডব্লিউ আব্দুল্লাহ ব্রুক্‌স, এমডি, এমপিএইচ
হেল্‌থ সিস্টেম্‌স এন্ড ইন্‌ফেকশাস ডিজিজেস ডিভিশন
আইসিডিডিআরবি
ফোনঃ ৯৮৮১৭৬১

ডাঃ ডলি গোস্বামী
হেল্‌থ সিস্টেম্‌স এন্ড ইন্‌ফেকশাস ডিজিজেস ডিভিশন
আইসিডিডিআরবি
ফোনঃ ৯৮৮১৭৬১

১১ থেকে ২৪ মাস বয়সী শিশুদের জন্য হাম, মাম্‌প্‌স ও রুবেলা (এমএমআর) এর সাথে দেয় ইনফ্লুয়েঞ্জা টীকার গবেষণার উদ্দেশ্যে পিতামাতার জন্য তথ্য এবং সম্মতিপত্র

অবগত সম্মতিপত্র

আমি এই মর্মে প্রত্যয়ন করছি যে, আমাকে এই চিকিৎসা সংক্রান্ত গবেষণা কার্যক্রমের প্রকৃতি, পদ্ধতি, সুযোগ/সুবিধা এবং ঝুঁকি সম্পর্কে অবহিত করা হয়েছে। এ ছাড়াও আমি এ গবেষণা সম্পর্কিত লিখিত তথ্য (রোগীদের তথ্য পুস্তিকা এবং অবগত সম্মতিপত্র) পেয়ে তা পড়েছি এবং বুঝেছি।

আমি অবগত আছি যে, আমার সন্তানের লিংগ পরিচয়, বয়স, জন্ম তারিখ, নাম এবং রোগের বর্ননাসহ এই গবেষণার ফলাফল কোনরূপ নাম/পরিচয় উল্লেখ ছাড়াই গবেষণা প্রতিবেদন আকারে প্রস্তুত করা হবে।

গবেষণার যে কোন পর্যায়ে, কোনরূপ বাধা বা আপত্তি ছাড়াই আমি এ গবেষণায় আমার সন্তানের অংশগ্রহনের সম্মতি প্রত্যাহার করতে পারব।

এ গবেষণায় আমার সন্তানের অংশগ্রহনের বিষয়ে রাজী হবার আগে প্রশ্ন করার জন্য আমার যথেষ্ট সুযোগ ছিল।

_____ _____

শিশুর নাম বিষয় নম্বর

<u>বাড়ীতে স্বাক্ষাৎকারের সময়ঃ</u>

উপস্থিত পরিবার প্রধানের নামঃ .....

পিতা/মাতা বা আইনানুগ অভিভাবকের স্বাক্ষরঃ..... তারিখঃ .....

[পিতা/মাতা বা আইনানুগ অভিভাবকের হস্তাক্ষরে]

পিতা/মাতা বা আইনানুগ অভিভাবকের নামঃ ..... সম্পর্কঃ .....

<u>পিতা/মাতা বা আইনানুগ অভিভাবকের উভয়ে উপস্থিত থাকলেঃ</u>

পিতা/মাতা বা আইনানুগ অভিভাবকের স্বাক্ষরঃ..... তারিখঃ .....

[পিতা/মাতা বা আইনানুগ অভিভাবকের হস্তাক্ষরে]

পিতা/মাতা বা আইনানুগ অভিভাবকের নামঃ ..... সম্পর্কঃ .....

স্বাক্ষীর স্বাক্ষরঃ ..... তারিখঃ .....

[পিতা/মাতা বা আইনানুগ অভিভাবক পড়তে অক্ষম হলে] [স্বাক্ষীর হস্তাক্ষরে]

স্বাক্ষীর নামঃ .....

গবেষণা কর্মীর স্বাক্ষরঃ ..... তারিখঃ .....

[গবেষণা কর্মীর হস্তাক্ষরে]

গবেষণা কর্মীর নামঃ .....

<u>ক্লিনিকে সাক্ষাৎকারের সময়ঃ</u> গবেষণার বিষয়ে পিতা/মাতা বা আইনানুগ অভিভাবকের কোনরূপ প্রশ্ন থাকলে গবেষণায় নিয়োজিত চিকিৎসক তার উত্তর দেবেন এবং পিতা/মাতা বা আইনানুগ অভিভাবক গবেষণায় শিশুর অংশগ্রহনের বিষয়ে পুনরায় সম্মতি জ্ঞাপন করবেন।

স্বাক্ষীর স্বাক্ষরঃ ..... তারিখঃ .....

[স্বাক্ষীর হস্তাক্ষরে]

স্বাক্ষীর নামঃ .....

গবেষণা চিকিৎসকের স্বাক্ষরঃ ..... তারিখঃ .....

[গবেষণা চিকিৎসকের হস্তাক্ষরে]

গবেষণা চিকিৎসকের নামঃ .....

সংরক্ষনের জন্য এই ফরম এবং তথ্যপত্রের একটি কপি আপনাকে দেয়া হবে।

ম ফজলে রাব্বী
কো-অর্ডিনেশন ম্যানেজার
এইচএসআইডি
Date: 5 August 2002

Check List

After completing the protocol, please check that the following selected items have been included.

1. Face Sheet Included ☐
2. Approval of the Division Director on Face Sheet ☐
3. Certification and Signature of PI on Face Sheet, #9 and #10 ☐
4. Table on Contents ☐
5. Project Summary ☐
6. Literature Cited ☐
7. Biography of Investigators ☐
8. Ethical Assurance ☐
9. Consent Forms ☐
10. Detailed Budget ☐

MEASLES MUMPS & RUBELLA VACCINES

WHAT YOU NEED TO KNOW

1 Why get vaccinated?

Measles, mumps, and rubella are serious diseases.

Measles

- Measles virus causes rash, cough, runny nose, eye irritation, and fever.
- It can lead to ear infection, pneumonia, seizures (jerking and staring), brain damage, and death.

Mumps

- Mumps virus causes fever, headache, and swollen glands.
- It can lead to deafness, meningitis (infection of the brain and spinal cord covering), painful swelling of the testicles or ovaries, and, rarely, death.

Rubella (German Measles)

- Rubella virus causes rash, mild fever, and arthritis (mostly in women).
- If a woman gets rubella while she is pregnant, she could have a miscarriage or her baby could be born with serious birth defects.

You or your child could catch these diseases by being around someone who has them. They spread from person to person through the air.

Measles, mumps, and rubella (MMR) vaccine can prevent these diseases.

Most children who get their MMR shots will not get these diseases. Many more children would get them if we stopped vaccinating.

2 Who should get MMR vaccine and when?

Children should get 2 doses of MMR vaccine:

✓ The first at **12-15 months of age**
✓ and the second at **4-6 years of age.**

These are the recommended ages. But children can get the second dose at any age, as long as it is at least 28 days after the first dose.

Some **adults** should also get MMR vaccine: Generally, anyone 18 years of age or older, who was born after 1956, should get at least one dose of MMR vaccine, unless they can show that they have had either the vaccines or the diseases.

Ask your doctor or nurse for more information.

MMR vaccine may be given at the same time as other vaccines.

3 Some people should not get MMR vaccine or should wait

- People should not get MMR vaccine who have ever had a life-threatening allergic reaction to **gelatin**, the antibiotic **neomycin**, or **a previous dose of MMR vaccine**.

- People who are moderately or severely ill at the time the shot is scheduled should usually wait until they recover before getting MMR vaccine.

- Pregnant women should wait to get MMR vaccine until after they have given birth. Women should not get pregnant for 3 months after getting MMR vaccine.

- Some people should check with their doctor about whether they should get MMR vaccine, including anyone who:
  - Has HIV/AIDS, or another disease that affects the immune system
  - Is being treated with drugs that affect the immune system, such as steroids, for 2 weeks or longer.
  - Has any kind of cancer
  - Is taking cancer treatment with x-rays or drugs
  - Has ever had a low platelet count (a blood disorder)

Over . . .

- People who recently had a transfusion or were given other blood products should ask their doctor when they may get MMR vaccine

Ask your doctor or nurse for more information.

4 What are the risks from MMR vaccine?

A vaccine, like any medicine, is capable of causing serious problems, such as severe allergic reactions. The risk of MMR vaccine causing serious harm, or death, is extremely small.

Getting MMR vaccine is much safer than getting any of these three diseases.

Most people who get MMR vaccine do not have any problems with it.

Mild Problems

- Fever (up to 1 person out of 6)
- Mild rash (about 1 person out of 20)
- Swelling of glands in the cheeks or neck (rare)

If these problems occur, it is usually within 7-12 days after the shot. They occur less often after the second dose.

Moderate Problems

- Seizure (jerking or staring) caused by fever (about 1 out of 3,000 doses)
- Temporary pain and stiffness in the joints, mostly in teenage or adult women (up to 1 out of 4)
- Temporary low platelet count, which can cause a bleeding disorder (about 1 out of 30,000 doses)

Severe Problems (Very Rare)

- Serious allergic reaction (less than 1 out of a million doses)
- Several other severe problems have been known to occur after a child gets MMR vaccine. But this happens so rarely, experts cannot be sure whether they are caused by the vaccine or not. These include:
  - Deafness
  - Long-term seizures, coma, or lowered consciousness
  - Permanent brain damage

5 What if there is a moderate or severe reaction?

What should I look for?

Any unusual conditions, such as a serious allergic reaction, high fever or behavior changes. Signs of a serious allergic reaction include difficulty breathing, hoarseness or wheezing, hives, paleness, weakness, a fast heart beat or dizziness within a few minutes to a few hours after the shot. A high fever or seizure, if it occurs, would happen 1 or 2 weeks after the shot.

What should I do?

- Call a doctor, or get the person to a doctor right away.
- Tell your doctor what happened, the date and time it happened, and when the vaccination was given.
- Ask your doctor, nurse, or health department to file a Vaccine Adverse Event Reporting System (VAERS) form, or call VAERS yourself at **1-800-822-7967.**

6 The National Vaccine Injury Compensation Program

In the rare event that you or your child has a serious reaction to a vaccine, a federal program has been created to help you pay for the care of those who have been harmed.

For details about the National Vaccine Injury Compensation Program, call **1-800-338-2382** or visit the program's website at **http://www.hrsa.gov/bhpr/vicp**

7 How can I learn more?

- Ask your doctor or nurse. They can give you the vaccine package insert or suggest other sources of information.
- Call your local or state health department's immunization program.
- Contact the Centers for Disease Control and Prevention (CDC):
  - Call **1-800-232-2522** (English)
  - Call **1-800-232-0233** (Español)
  - Visit the National Immunization Program's website at **http://www.cdc.gov/nip**

U.S. DEPARTMENT OF HEALTH & HUMAN SERVICES
Centers for Disease Control and Prevention
National Immunization Program

VIRAL VACCINE DEVELOPMENT TECHNICAL REPORT

Technical Report: TR#10 (AD)

Title: Immunogenicity and Efficacy Testing of Frozen, and Liquid Monovalent FluMist Formulations

Author: Edward A. Chroscinski, Jr V.M.D.
Supervisor, In Vivo Testing

SUMMARY

This study was conducted to determine the immunogenicity and efficacy of frozen and liquid cold adapted (ca) monovalent FluMist formulations. Three different monovalent strains of Cold Adapted Influenza Vaccine (CAIV) were used in the study: ca A/Beijing, ca A/Sydney, and ca B/Yamanashi. Each strain was grouped separately and had both positive and negative controls. Results from this study showed that frozen and liquid CAIV formulations are equally immunogenic and efficacious.

Approvals

	Signature	Date
Author	Ed. Chroscinski Jr MS	3/8/00
Assoc. Director VVD	[signature]	3-9-00
Director VVD	[signature]	3-9-00
VVD Compliance Specialist	[signature]	3-9-00

1.0 STUDY

1.1 Test Articles:

Monovalent Frozen FluMist:
ca A/Beijing CAE088-33
ca A/Sydney CAE090-42
ca B/Yamanashi CAE121-1

Monovalent Liquid FluMist:
ca A/Beijing CB003H
ca A/Sydney CAZ034 TK991104
ca B/Yamanashi CB004H

Monovalent Wild Type (wt):
CDC wt A/Beijing TK990212
CDC wt A/Sydney TK990212
wt B/Yamanashi 11-22-99 B/Y

1.2 Study Initiation/Completion:

Study initiated on 12-13-99/Study completed on 01-17-00

1.3 Protocol:

BDV-00005: Immunogenicity and Efficacy Testing of Frozen and Liquid Monovalent FluMist Formulations

1.4 Deviation(s)

According to BDV Protocol #00005 – Immunogenicity and Efficacy Testing of Frozen and Liquid Monovalent FluMist Formulations, challenge with the corresponding wt influenza virus should be performed on study Day 28. Two-thirds of the test ferrets, those that were tested with A/Beijing and B/Yamanashi and their associated eight negative control ferrets, were challenged with the corresponding wt influenza virus as per protocol on Day 28. The remaining one-third ferrets, those tested with A/Sydney and remaining four negative control ferrets, were challenged on Day 32 instead of Day 28 because the sample was not received in time for testing. This deviation had no impact on the study. Collection of lung lobes and nasal turbinates was still performed 3 days post-challenge as per protocol. EID_{50} testing showed negative results in the test ferrets after challenge with the wt A/Sydney influenza virus, demonstrating efficacy of both the frozen and liquid CAIV FluMist formulations.

2.0 PURPOSE

The purpose of this study is to compare the immunogenicity and efficacy of frozen and liquid cold adapted monovalent FluMist formulations. Three different monovalent strains of Cold Adapted Influenza Vaccine (CAIV) are used in the study, and the test is conducted using ferrets as the animal model.

3.0 RATIONALE

This study was conducted according to BDV Protocol #00005: Immunogenicity and Efficacy Testing of Frozen and Liquid Monovalent FluMist Formulations.

Cold Adapted Influenza Vaccine (CAIV) is a live attenuated vaccine. It is cold adapted (ca) so that the virus will replicate in the relatively cool temperatures of the nose and nasopharynx, and it is temperature sensitive (ts) so that it is unable to replicate efficiently in the warm temperature of the lungs.

Most of the data generated for CAIV has used a frozen vaccine formulation – a stabilized liquid vaccine filled in nasal delivery devices and stored frozen at −15°C. The monovalent bulk for the frozen formulation undergoes no purification and consists primarily of virus-infected egg allantoic fluid. The final product for which registration will be sought is a stabilized liquid formulation stored at 2-8 °C, not frozen. The monovalent bulk for the liquid formulation is purified by high-speed continuous flow centrifugation to concentrate the virus on a sucrose gradient and separate it from the allantoic fluid.

The first part of this study determined the immunogenicity of the frozen and liquid formulations, and the second part characterized and compared the formulations for efficacy against the wt influenza virus challenge. The ferret was selected as the animal model because ferrets are highly susceptible to the influenza virus and have been used extensively in influenza research (1-6).

Three monovalent strains of CAIV were chosen for the study: ca A/Beijing, ca A/Sydney, and ca B/Yamanashi. Frozen, liquid, and wt formulations of the strains were inoculated intranasally into each of four ferrets per formulation per strain. On study Days 2 and 3, nasal washes were collected from each animal and tested by Fluorescent Focus Assay (FFA) potency testing (7) to determine virus replication. On Day 28, blood was drawn from each animal for testing by Hemagglutination Inhibition (HAI) assay (8) to measure immune response, then all animals were challenged with the corresponding wt of influenza virus. Three days post-challenge, lung lobes and nasal turbinates were collected from each animal and were tested by Egg Infectious Dose (EID_{50}) assay (9) to determine the efficacy of the initial inoculations against the wt challenge. Titers were compared from the frozen and liquid formulations, and results showed that both formulations demonstrated immunogenicity and were equally efficacious when challenged with wt influenza virus.

4.0 METHOD

4.1 Test Article Preparation

The FluMist samples and two of the wt samples, A/Sydney and A/Beijing, were prepared by and sent from Avtron in Mountain View, CA, to Wyeth-Ayerst Research (WAR) in Marietta, PA. The third wt sample, B/Yamanashi, was prepared on site by WAR personnel. All wt samples were originally obtained from CDC in Atlanta, GA. WAR personnel diluted all samples to ensure an inoculated dose of 2.0E7 $TCID_{50}$ per animal.

4.2 Test Procedure

The animal procedure was performed according to Protocol BDC99009 – Ferret Reactogenicity/Attenuation Test, approved by WLVP Animal Care and Use Committee (10).

Prior to inoculation, ferrets were pre-bled, and the sera were tested by HAI assay for the presence of anti-influenza virus antibodies. Only ferrets having influenza

virus titers of less than 1:40 were selected for use in the study. The ferrets were housed in micro-isolator or ventilated rack caging systems to prevent any cross contamination of samples.

Four 8-10 week old male castrated ferrets were used for each test group. The test groups were divided as follows:

1) three groups (one per strain) of monovalent frozen FluMist formulations
2) three groups (one per strain) of monovalent liquid FluMist formulations
3) three groups (one per strain) of wt influenza virus as positive controls
4) three groups (one per strain) of uninoculated animals as negative controls

The study protocol consisted of two stages. The first stage was designed to determine the immunogenicity of the frozen and liquid formulations, and the second stage was designed to characterize and compare the frozen and liquid formulations for efficacy against wt influenza virus challenge.

4.2.1 Procedures to Measure Immunogenicity and Efficacy

All ferrets except the negative control animals were anesthetized using isoflurane and inoculated intranasally on Day 0. Frozen FluMist and wt samples were inoculated into four ferrets per strain by giving each ferret a dose of 0.5mL/nostril (2.0E7 $TCID_{50}$/mL). Liquid FluMist samples were inoculated into four ferrets per strain by giving each ferret a dose of 0.2mL/nostril (5.0E7 $TCID_{50}$/mL).

After inoculation, ferrets were observed for any signs of influenza, including sneezing, ocular and/or nasal discharge, and fever. Rectal temperatures were taken from each animal prior to inoculation, daily on Days 1-3, and on final three days of study. On Days 2 and 3, all test ferrets and four randomly selected negative control animals were anesthetized with isoflurane, and nasal washes were collected and tested by FFA assay to determine virus replication.

On Day 28, two-thirds of the test ferrets, those that were tested with with A/Beijing and B/Yamanashi and their associated eight negative control ferrets, were challenged with the corresponding wt influenza virus as per protocol. The remaining one-third ferrets, those tested with A/Sydney and remaining four negative control ferrets, were challenged on Day 32 (See Deviation 1.4). The challenge was performed by anesthetizing the ferrets with isoflurane, collecting a small sample of blood, and then inoculating each animal with 0.5mL/nostril of the wt influenza virus of same CAIV strain that was initially inoculated. The blood sample was tested via HAI to measure immune response. Three days post-challenge, all animals were anesthetized with isoflurane and exsanguinated using a vacutainer collection system into serum separator blood collection tubes. While still under anesthesia, animals were given 1.0mL Pentobarbital intraperitoneally to ensure euthanasia.

The animals were then placed one at a time on sterile disposable surgical drapes inside a Biosafety cabinet. Using sterile instruments and techniques, the lower left lung lobe was removed from each ferret. The animal was then placed sternally to collect nasal turbinates. Each lung lobe and turbinate sample was placed into an individually numbered and labeled sterile container. Lung lobes were homogenized using a sterile, stainless steel chamber assembly and homogenizer using Modified 2X

Eagles Media with concentrated antibiotics. The chamber assembly was placed in ice while homogenizing. Turbinates were placed in separate vessels containing the same media and shaken vigorously. All samples were centrifuged in refrigerated centrifuges. The supernatant was decanted and collected using pipettes and sterile technique under a biosafety cabinet. The supernatant was tested by EID_{50} to determine the efficacy of the initial inoculations against the wt challenge.

4.3 Controls

Positive control ferrets were dosed with wt influenza virus on Day 0 of the study. Negative control ferrets remained uninoculated on Day 0 of the study. Both positive and negative control ferrets were challenged with wt influenza virus on Day 28.

4.4 Interpretation of Data

FFA is a potency test used to measure virus replication. Any number indicates a positive result. No number indicates no virus detected.

HAI is a measure of immune response. Any number higher than 1:40 shows response, with a greater number indicating a greater response. A titer of less than 1:40 is considered acceptable for lack of response.

EID_{50} is a hemagglutination test using serial dilutions to reach an end point. It is used to determine the efficacy of the initial inoculations against the wt challenge. Any number indicates a positive result. No response indicates no virus detected.

5.0 RESULTS

5.1 Observation for Signs of Influenza

After inoculation, the ferrets were observed for any signs of influenza, including sneezing, ocular and/or nasal discharge, and fever. Ferrets inoculated with wt viruses did spike fevers of >/=104 °C at the time of dosing, and are noted in Tables 1, 2, and 3. No animals showed any signs of nasal or ocular discharge, excessive sneezing, or any difficulty breathing. At the end of the study, the temperatures of the control animals after inoculation with wt viruses were taken. Fevers of >/=104 °C are noted in Tables 1, 2, and 3.

5.2 FFA Testing of Nasal Wash From Days 2 and 3

All inoculated animals tested positive for influenza virus in both washes tested from study Days 2 and 3. The four randomly selected negative control ferrets all tested negative. FFA results can be seen in Tables 1, 2, and 3.

5.3 HAI Testing of Day 28 Sera

All inoculated ferrets had an HAI titer of 1:320 or higher, indicating an immune response to the vaccine or virus inoculated. All negative control animals had a titer of 1:40 or lower, indicating no new exposure. HAI results can be seen in Tables 1, 2, and 3.

5.4 EID_{50} Test of Lung and Turbinate Tissue 3 Days Post Challenge

All ferrets initially inoculated with either CAIV or wt virus showed no positive results in either lung or turbinate tissue. All negative control animals tested positive in both lung and turbinate tissue, with the exception of one ferret challenged with wt A/Sydney. This animal had a positive response in the turbinates, but a negative response in the lung. This one result appears to be an aberration that may have been caused by a number of factors, including but not limited to improper inoculation, small lung lobe size, and operator error in either homogenization or EID_{50} testing. EID_{50} results can be seen in Tables 1, 2, and 3.

6.0 VALIDITY

The nasal washes conducted on Days 2 and 3 showed virus replication. The challenged test ferrets all tested negative in both lung and turbinate tissue. The challenged negative control animals all tested positive in both lung and turbinate tissue, with the exception of one animal as noted above. Therefore, the test is considered valid.

7.0 CONCLUSIONS

Both the frozen and liquid CAIV FluMist formulations showed immunogenicity and demonstrated efficacy when challenged with wt influenza virus. There does not appear to be a demonstrable difference between the frozen and liquid CAIV FluMist formulations for induction of immunogenicity and protection efficacy.

8.0 REFERENCES

1. Buchman, CA. Swarts, JD. Seroky, JT. Panagiotou, N. Hayden, F. Doyle, WJ. Otologic and systemic manifestations of experimental influenza A virus infection in the ferret. Otolaryngology - Head & Neck Surgery. 112(4):572-8, 1995.

2. Chen, KS. Bharaj, SS. King, EC. Induction and relief of nasal congestion in ferrets infected with influenza virus. International Journal of Experimental Pathology. 76(1):55-64, 1995.

3. Donnelly, JJ. Friedman, A. Ulmer, JB. Liu, MA. Further protection against antigenic drift of influenza virus in a ferret model by DNA vaccination. Vaccine. 15(8):865-8, 1997.

4. Leigh, MW. Connor, RJ. Kelm, S. Baum, LG. Paulson, JC. Receptor specificity of influenza virus influences severity of illness in ferrets. Vaccine. 13(15):1468-73, 1995.

5. Renegar, KB. Influenza virus infections and immunity: a review of human and animal models. Laboratory Animal Science. 42(3):222-32, 1992.

6. Whitaker-Dowling, P. Maassab, HF. Youngner, JS. Dominant-negative mutants as antiviral agents: simultaneous infection with the cold-adapted live-virus vaccine for influenza A protects ferrets from disease produced by wild-type influenza A. Journal of Infectious Diseases. 164(6):1200-2, 1991.

7. SOP 3220.119: Performing the Fluorescent Focus for Monovalent and Trivalent Cold Adapted Influenza Virus Serotype-Specific Potency Assay

8. Standard Operating Procedures for the performance of the Hemagglutination Inhibition Test:

   SOP 527.27-001: Preparing Ferret Antisera For Influenza Virus Strains
   SOP 527.28: Preparing Antisera for Hemagglutination Inhibition Tests
   SOP 527.41: Performing the Hemagglutination Inhibition Test

9. SOP 3220.136: Performing the Egg Infectious Dose (EID50) Assay

10. Protocol BDC99009 – Ferret Reactogenicity/Attenuation Test. Investigator: Dr. Ed Chroscinski. Approved by WLVP Animal Care and Use Committee, expiration 08/31/2000

Table 1: Immunogenicity and Efficacy Study - A/Beijing

Ferret Number	Immunization Virus Strain/Formulation[a]	Fever[b]	Nasal Wash log10 FFU/mL		HAI titer Day 28	Challenge with wt A/Beijing		
			Day 2	Day 3		Fever[b]	NT log10 EID50/mL	Lung log10 EID50/mL
6740	A/B Frozen	-	4.7	<1.4	320	ND[c]	<0.5	<0.5
6755	A/B Frozen	-	5.1	3.7	640	ND	<0.5	<0.5
6742	A/B Frozen	-	4.8	2.6	320	ND	<0.5	<0.5
6758	A/B Frozen	-	4.5	3.9	640	ND	<0.5	<0.5
	Ave		4.7	2.8	480		<0.5	<0.5
6764	A/B Liquid	-	3.7	<1.4	640	ND	<0.5	<0.5
6703	A/B Liquid	-	5.1	3.9	640	ND	<0.5	<0.5
6709	A/B Liquid	-	5.4	4.6	640	ND	<0.5	<0.5
6715	A/B Liquid	-	3.8	4.5	640	ND	<0.5	<0.5
	Ave		4.6	3.6	640		<0.5	<0.5
6773	A/B wildtype	-	5.1	5.2	1280	ND	<0.5	<0.5
6775	A/B wildtype	-	5.6	5.0	1280	ND	<0.5	<0.5
6725	A/B wildtype	+	6.0	5.8	1280	ND	<0.5	<0.5
6772	A/B wildtype	+	5.4	5.9	1280	ND	<0.5	<0.5
	Ave		5.5	5.5	1280		<0.5	<0.5
6710	A/B control	-	-	-	20	+	>/+4.5	3.0
6705	A/B control	-	-	-	20	-	>/+4.5	3.0
6702	A/B control	-	-	-	40	+	>/+4.5	2.7
6730	A/B control	-	-	-	40	+	>/+4.5	1.7
	Ave				30		>/+4.5	2.8

[a] A/B Frozen: ca A/Beijing CAE088-33
A/B Liquid: ca A/Beijing CB003H
A/B wildtype: CDC wt A/Beijing TK990212

[b] >/+ 104 °C on Day 1 and/or Day 2. No fever on Day 3 was detected in any of the ferrets.

[c] Not done

Table 2: Immunogenicity and Efficacy Study - A/Sydney

Ferret Number	Immunization Virus Strain/Formulation[a]	Fever[b]	Nasal Wash log10 FFU/mL		HAI titer Day 28	Challenge with wt A/Sydney		
			Day 2	Day 3		Fever[b]	NT log10 EID50/mL	Lung log10 EID50/mL
6739	A/S Frozen	-	5.7	4.7	1280	ND[c]	<0.5	<0.5
6748	A/S Frozen	-	5.0	3.8	1280	ND	<0.5	<0.5
6757	A/S Frozen	-	5.2	4.7	1280	ND	<0.5	<0.5
6752	A/S Frozen	-	4.6	4.8	1280	ND	<0.5	<0.5
	Ave		5.1	4.5	1280		<0.5	<0.5
6765	A/S Liquid	-	4.1	5.0	1280	ND	<0.5	<0.5
6769	A/S Liquid	-	4.0	5.0	640	ND	<0.5	<0.5
6762	A/S Liquid	-	4.6	4.8	640	ND	<0.5	<0.5
6760	A/S Liquid	-	4.1	4.8	1280	ND	<0.5	<0.5
	Ave		4.2	4.9	960		<0.5	<0.5
6741	A/S wildtype	-	5.0	4.9	2560	ND	<0.5	<0.5
6728	A/S wildtype	+	4.0	5.6	1280	ND	<0.5	<0.5
6743	A/S wildtype	+	4.8	5.8	2560	ND	<0.5	<0.5
6771	A/S wildtype	+	4.5	5.7	640	ND	<0.5	<0.5
	Ave		4.6	5.5	1760		<0.5	<0.5
6704	A/S control	-	-	-	40	+	4.3	<0.5
6706	A/S control	-	-	-	40	+	4.5	2.7
6708	A/S control	-	-	-	40	+	4.0	2.5
6712	A/S control	-	-	-	40	+	5.5	2.5
	Ave				40		4.6	2.6

[a] A/S Frozen: ca A/Sydney CAE090-42
A/S Liquid: ca A/Sydney CAZ034 TK991104
A/S wildtype: CDC wt A/Sydney TK990212

[b] >/+ 104 °C on Day 1 and/or Day 2. No fever on Day 3 was detected in any of the ferrets.

[c] Not done

Table 3: Immunogenicity and Efficacy Study - B/Yamanashi

Ferret Number	Immunization Virus Strain/Formulation[a]	Fever[b]	Nasal Wash log10 FFU/mL		HAI titer Day 28	Challenge with wt B/Yamanashi		
			Day 2	Day 3		Fever[b]	NT log10 EID50/mL	Lung log10 EID50/mL
6701	B/Y Frozen	-	3.7	3.9	320	ND[c]	<0.5	<0.5
6761	B/Y Frozen	-	4.1	4.2	640	ND	<0.5	<0.5
6759	B/Y Frozen	-	4.9	4.0	640	ND	<0.5	<0.5
6768	B/Y Frozen	-	5.1	4.1	640	ND	<0.5	<0.5
	Ave		4.5	4.1	560		<0.5	<0.5
6763	B/Y Liquid	-	5.1	4.6	320	ND	<0.5	<0.5
6717	B/Y Liquid	-	5.6	4.6	640	ND	<0.5	<0.5
6738	B/Y Liquid	-	3.8	3.5	1280	ND	<0.5	<0.5
6747	B/Y Liquid	-	4.1	3.7	1280	ND	<0.5	<0.5
	Ave		4.6	4.1	560		<0.5	<0.5
6776	B/Y wildtype	-	3.8	<1.4	640	ND	<0.5	<0.5
6719	B/Y wildtype	+	5.1	4.6	1280	ND	<0.5	<0.5
6714	B/Y wildtype	+	4.5	4.6	640	ND	<0.5	<0.5
6733	B/Y wildtype	+	5.6	3.9	640	ND	<0.5	<0.5
	Ave		4.8	3.8	800		<0.5	<0.5
3146	B/Y control	-	-	-	10	+	5.0	2.3
86	B/Y control	-	-	-	10	+	4.5	2.3
6711	B/Y control	-	-	-	10	-	5.3	2.0
3198	B/Y control	-	-	-	10	+	5.0	>/=2.5
	Ave				10		5.0	2.2

[a] B/Y Frozen: ca B/Yamanashi CAE121-1
B/Y Liquid: ca B/Yamanashi CB004H
B/Y wildtype: wt B/Yamanashi 11-22-99 B/Y

[b] >/+ 104 °C on Day 1 and/or Day 2. No fever on Day 3 was detected in any of the ferrets.

[c] Not done