

# Creation of Inhalable Dry Powder Vaccine and Pharmaceutical Aerosols

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## ABSTRACT

**Purpose.** The goal of this work was to learn whether fine dry powder inhalable aerosols can be made by CAN-BD and retain activity through processing and storage. Additional goals were to disperse and deliver the aerosol using a simple dry powder inhaler, and to enhance the emitted dose with the careful selection of device materials.

**Methods.** Emitted dose and cascade impaction with gravimetric and/or total organic carbon (TOC) detection were used to characterize the dry powder and the Aktiv-Dry PuffHaler® DPI performance. CO<sub>2</sub>-Assisted Nebulization with a Bubble Dryer® (CAN-BD) was used to manufacture dry powders from liquid bulk live virus measles vaccine provided by the Serum Institute of India. Viral potency was measured by a 50% cell culture infectious dose assay. Plaque reduction neutralization assays provided measures of immune responses in Rhesus macaques vaccinated with inhaled dry powder. Sprague-Dawley rats were used to test the toxicology of the new myo-inositol excipient for inhalation. Tuberculosis antibiotic dry powders of capreomycin and kanamycin were also prepared by the CAN-BD process.

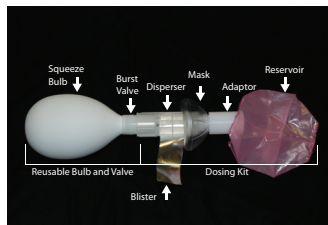
**Results.** A robust immune response was generated in the macaques administered dry powder measles vaccine by inhalation. A challenge study with wild-type measles was conducted 14 months after vaccination. The toxicology of the new myo-inositol stabilizer for inhalable formulations indicated acceptability with no adverse events in a standard battery of observations. Use of an anti-static aerosol reservoir reduced microparticle loss to reservoir surfaces by about half. Coating the surfaces with Tween-80 was also helpful, but somewhat less effective. A solution of capreomycin and leucine (4 to 1 weight ratio) was effectively nebulized and dried by CAN-BD to yield micro-spheres with 59% (by mass) < 5.8 µm in aerodynamic diameter and 33% < 3.3 µm.

**Conclusion.** Live-attenuated measles virus vaccine aerosol was shown effective in test animals in producing a robust immune response. Materials have been chosen for the PuffHaler reservoir to reduce aerosol loss. Myo-inositol appears to be a promising new candidate excipient for stabilization and particle formation with no inhalation toxicity.

## GMP CAN-BD SYSTEM



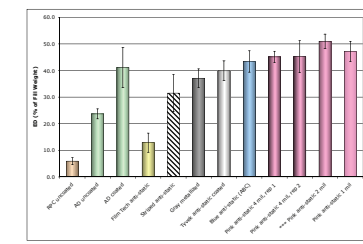
GMP CAN-BD powder manufacturing equipment installed at the Serum Institute (Pune, India) for the production of myo-inositol based inhalable dry powder measles vaccine for the NHP toxicity study and Phase I clinical trial.



The PuffHaler® dry powder inhaler

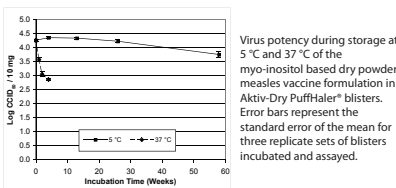


Powder emitting from PuffHaler reservoir  
Emitted dose with PuffHaler is typically 40% - 50%.



Assessment of different materials of construction for the PuffHaler reservoir

## Stability Study of Dry Powder Measles Vaccine



Virus potency during storage at 5°C and 37°C of the myo-inositol based dry powder measles vaccine formulation in Aktiv-Dry PuffHaler® blisters. Error bars represent the standard error of the mean for three replicate sets of blisters incubated and assayed.

## IMMUNOGENICITY STUDY OF DRY POWDER MEASLES VACCINE IN RHESUS MACAQUE

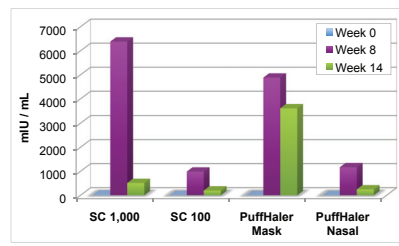
- Demonstrate immunogenicity of measles dry powder vaccine. Measure both cellular and humoral responses.
- Deliver vaccine by inhalation with a mask using the Aktiv-Dry PuffHaler® dry powder inhaler.
- Deliver vaccine by direct nasal delivery with a nasal prong using the PuffHaler®.

### Rhesus Immunogenicity Study Design

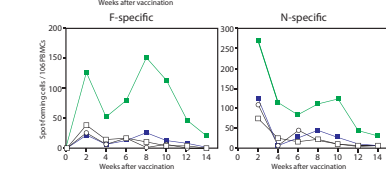
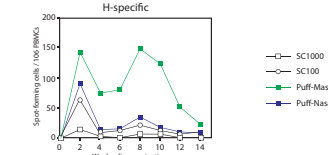
- Rhesus macaques were administered measles vaccine dry powders with the PuffHaler® dry powder inhaler. Control animals were administered commercial SI measles vaccine by subcutaneous injection.
- Inhalation was by free-breathing from a mask. Vaccine dry powders were also delivered by direct nasal application.

Group	Treatment	Nominal Loaded Dose	# of Animals
1	SI measles vaccine Subcutaneous	1000 PFU	2
2	SI measles vaccine Subcutaneous	100 PFU	2
3	PuffHaler-inhalation	50 mg powder 3086 PFU / 10 mg 4.72 log CCID50 / 10 mg	3
4	PuffHaler-nasal	50 mg powder 3086 PFU / 10 mg 4.72 log CCID50 / 10 mg	3

### PRNT Following Vaccination



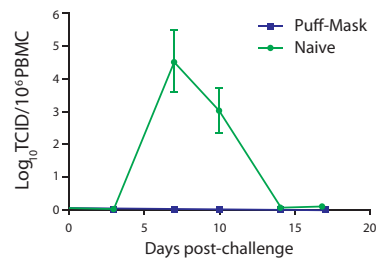
Virus neutralizing antibody levels in plasma as determined by plaque reduction neutralization endpoint titration (mIU/mL)



Measurement of Hemagglutinin (H), Fusion (F), and Nucleoprotein (N) protein-specific interferon-gamma producing T cells was done with ELISPOT assay

### Challenge Study of Rhesus Macaque Immunized with Dry Powder Measles Vaccine

14-16 months following immunization the animals were challenged with wild-type measles virus (Bilthoven).



Determination of viremia after challenge with wild-type measles virus. Measles-naive or previously immunized (by inhalation with a PuffHaler and mask) macaques were challenged. Peripheral blood mononuclear cells (PBMCs) were co-cultured with Vero-Slam cells

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## SAFETY OF INHALED MYO-INOSITOL



- Aktiv-Dry conducted a GLP study in rats of myo-inositol powder delivered by inhalation.

- Rats were exposed to a maximum of 30 mg/day for 3 consecutive days.

- Endpoints include clinical pathology, histopathology, body and organ weights, clinical and cageside observations, and ophthalmology.

- No myo-inositol-related effects or delayed onset of toxicity were observed.

### BACKGROUND RELEVANT TO SAFETY OF MYO-INOSITOL

- Myo-inositol (*cis*-1,2,3,5-*trans*-4,6-cyclohexanehexol) is a six-carbon cyclic polyol ubiquitous in nature.

- Commercially derived from rice, and also commonly found in nuts, grains, fruits, and melons.

- Normal concentration of myo-inositol in human plasma is about 4-5 mg/liter.

- Intracellular concentration is 5 to 500x higher than in plasma.

- Average dietary intake is 1 gram in the form of inositol hexaphosphate or myo-inositol in phospholipids.

- Human milk contains about 450 mg/liter of myo-inositol.

- Myo-inositol is on the FDA's Generally Regarded as Safe (GRAS) list of food substances as a nutrient and/or dietary supplement.

- Lam et al. have completed a Phase I clinical trial of orally ingested myo-inositol powder with no SAEs reported following intake of up to 18 g per day for one month; Phase II clinical trials are beginning in Canada.

- Inhalation of dry powder measles vaccine by Rhesus macaques in a pilot immunogenicity study was well tolerated with no deaths or adverse events.

- An inhalation toxicology study in Rhesus macaques is planned in 2009.

- A Phase I clinical trial in India is planned in 2010.

### STUDY DESIGN

- Three groups of Sprague-Dawley rats (10/sex/group): 20 total rats per group

- Daily exposure to the following for three consecutive days:

- > Group 1: Low dose test article (10 mg of myo-inositol powder)

- > Group 2: High dose test article (30 mg of myo-inositol powder)

- > Group 3: Control (30 mg of lactose powder)

- Powder delivered using the Becton Dickinson Solovent® DPI:

- > About 10 mg of powder dispersed from a capsule into a cardboard spacer

- > Nose inserted into spacer and rat allowed to breath at liberty ≥ 1 minute

- > For rats receiving 30 mg of powder, this process repeated twice more

- On study day four, 5 rats/sex/group sacrificed; remaining animals sacrificed on day 15.

- Animals monitored for mortality, clinical and cage-side observations, body weights, food consumption, functional observation battery, ophthalmology, clinical pathology, gross pathology, organ weights and histopathology.

## STUDY RESULTS

- **NO** test article-related effects
- **NO** delayed onset of toxicity observed
- **NO** observed effect level for inhaled myo-inositol of 30 mg/day for 3 consecutive days

## REPRESENTATIVE VACCINES, BIOLOGICS, AND PHARMACEUTICALS PROCESSED BY CAN-BD

**Vaccines:** live attenuated measles virus vaccine, influenza live virus vaccine, hepatitis B surface antigen (HBsAg) vaccine, human papilloma virus (HPV) vaccine

**Oligonucleotides:** single- and double-stranded siRNA and DNA, aptamers, amides

**Antibodies:** PRIMATIZED® anti-CD4, human IgG, anti-human lambda light chain

**Enzymes:** α<sub>1</sub>-antitrypsin, trypsinogen, lactate dehydrogenase, lysozyme, insulin, alkaline phosphatase

**Antibiotics:** capreomycin, kanamycin, moxifloxacin hydrochloride, tobramycin sulfate, amoxicillin, doxycycline, cefazolin, ciprofloxacin hydrochloride, amikacin, rifampin

**Other:** phytosterols, PEG, PVP, hydrolyzed gelatin, sodium chloride, DPPC, salbutamol

**Sugar excipient stabilizers:** myo-inositol, trehalose, mannitol, sorbitol, lactose, sucrose

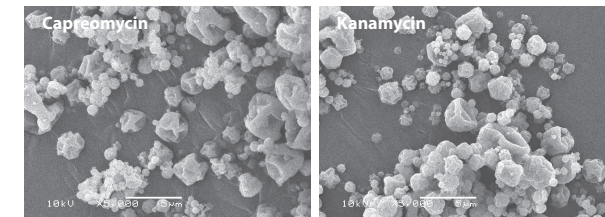
**Components in formulations:** buffers (tricine, sodium or potassium phosphate, sodium acetate, sodium citrate), surfactants (palmitic acid, stearic acid, Tween 20, Tween 80, Pluronic F68), amino acids (arginine, alanine, histidine, leucine, methionine), and metal chelating agents (EDTA, DTPA)

## POSSIBLE BENEFITS OF AEROSOL DELIVERY OF ANTIBIOTICS TO THE LUNGS

- Higher doses delivered locally to treat lung infections
- Reduced whole body burden of antibiotics
- Longer persistence at higher concentrations in lung air space and tissue

### TB Antibiotic Microparticles for Inhalation

- Formulation Details
  - 80% capreomycin sulfate, 20% L-leucine (CL)
  - 80% kanamycin sulfate, 20% L-leucine (KL)
  - Solutions of 5% (CL) and 5.625% (KL) total dissolved solids processed by CAN-BD



TB Antibiotic	Fine Particle Fractions (Average Mass % ± S.D.)		Emitted Dose (mass % ± S.D.)	Moisture Content (mass % ± S.D.)		
	Dispersion from Aerolizer® FPF < 3.3 µm	Dispersion from PuffHaler® FPF < 5.8 µm				
Capreomycin	33 ± 5	59 ± 9	23 ± 2	39 ± 4	40 ± 3	1.5 ± 0.1
Kanamycin	32 ± 5	67 ± 12	18 ± 1	41 ± 7	55 ± 7	2.7 ± 0.4

For references see [www.AKTIV-DRY.COM](http://www.AKTIV-DRY.COM)

## ACKNOWLEDGEMENTS



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