

SAFETY AND IMMUNOGENICITY OF MEASLES VACCINE, DRY POWDER IN RHESUS MONKEYS

.....

**Godin, C. S.¹; Krause, E.¹; Griffin, D.²; Shermer, C.³; Winston, S.⁴;
Sievers, R.⁴; Lin, W-H² Quinn, B.⁴**

.....

¹AVANZA Laboratories, Gaithersburg, MD, USA

*²Molecular Microbiology and Immunology,
Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA*

³BD Technologies, RTP, NC, USA

⁴Aktiv-Dry LLC, Boulder, CO, USA

ABSTRACT

Needle-free aerosol delivery of a dry-powder measles vaccine may provide an effective and low-cost means of immunization of children in developing countries. The advantages over the freeze-dried marketed product are that the dry-powder vaccine requires no reconstitution or use of needles for clinical use. Aktiv-Dry has developed an alternative to freeze drying that results in preservation of viral potency and particle sizes optimal for alveolar deposition. The purpose of this study was to determine the potential toxicity of Measles Vaccine, Dry Powder (Vaccine) when administered twice three weeks apart to rhesus monkeys by inhalation using either the PuffHaler[®] or Solovent[™] dry powder inhalers. Three groups (6/sex/group) received 50 mg of the control article via Solovent[™] inhaler or 50 mg of the Vaccine via PuffHaler[®] or Solovent[™] inhalers. The first half of animals was euthanized 1 week following the first dose, and the remaining animals were euthanized 14 days following the second dose. Administration of Vaccine via PuffHaler[®] or Solovent[™] inhalers was well tolerated. There were no test article-related effects on mortality, clinical observations, body weights, food consumption, body temperatures, respiratory profile, breathing patterns, post dose evaluation of the nasal cavity, mouth, and pharynx, ocular examination, electrocardiogram evaluation, clinical pathology, gross pathology observations, organ weights, and histopathology. Administration of Vaccine to all animals resulted in detection of measles virus RNA in either bronchoalveolar lavage or lung tissue samples after one dose. Serological analysis also confirmed that all animals in the study were measles seronegative prior to the first dose, and that the animals in the control group were not exposed to measles virus during the in-life phase of the study. Therefore, the no-observed-adverse effect level of the dry powder measles vaccine is 50 mg when administered once or twice via PuffHaler[®] or Solovent[™] inhalers.

INTRODUCTION

Measles is a highly contagious human disease caused by the measles virus (MV), and vaccination programs have dramatically reduced its incidence. However, despite the success of global measles vaccination programs, measles was still responsible for an estimated 345,000 deaths in 2005 (Wolfson et al., 2007), with most of these deaths occurring in developing countries. Measles outbreaks also continue to occur in developed countries that have failed to maintain a high level of population immunity (Moss and Griffin, 2006). Global vaccination coverage is approximately 80% but more than 23 million infants did not receive their first dose of measles-containing vaccine in 2007 (WHO, 2008). Although measles control goals (WHO, 2008) can be achieved with the current vaccination strategies, new measles vaccine formulations that are more easily administered, stable at ambient temperatures, easily transported, and cost effective would be beneficial (Sievers et al, 2008). In addition, safety, disposal, and wastage issues associated with using current lyophilized vaccines that require reconstitution and needles for injection remain a concern (John, 2008).

Aerosol delivery of measles vaccine may provide some or all of the required features mentioned above. A number of clinical studies has been performed using aerosol measles vaccination by nebulizing commercial lyophilized formulations after reconstitution, and this route of administration resulted in equal or better immune responses in children greater than 10 months of age compared with injection (Low et al., 2007). Aerosol delivery of measles vaccine appears to be safe because nearly 4 million children in Mexico were immunized using this route of delivery with no reports of significant adverse events (Valdespino-Gomez et al., 2006). Dilraj et al. (2000) reported that the antibody response in school-age children to Edmonston-Zagreb aerosol vaccine was better after 1 year than the response in children vaccinated subcutaneously. In a follow-up study, the antibody levels and the percentage of children who remained seropositive 6 years later were higher in the group that received vaccine by aerosol than the group that received vaccine by injection (Dilraj et al., 2007).

Rhesus macaques are considered the best experimental model for studying human measles disease and are often used to test novel measles vaccines prior to clinical studies (Wyde, et al., 2001; Auwaerter et al., 1999). Following either natural exposure or challenge with wild-type MV strains, macaque viremia occurs and the infected animals exhibit all the signs of measles commonly seen in humans (Remfry, 1976). Vaccination of macaques by inhalation of an Edmonston-Zagreb-

derived measles dry powder vaccine produced by milling was attempted by de Swart et al. (2007). Though measles virus was detected in bronchoalveolar lavage (BAL) samples taken 6 days after vaccination, the virus neutralizing antibody response in these animals was much lower than in animals vaccinated by intra-muscular injection. Because vaccination with aerosolized liquid measles vaccine proved effective in a previous study with macaques, it is likely that the limited immune response observed with the dry powder vaccine may have been caused by the aerosol properties of the dry powder formulation or the method of delivery to the lungs (de Swart et al., 2006).

The purpose of this study was to determine the potential toxicity of a Measles Vaccine, Dry Powder manufactured by a CO₂-Assisted Nebulization with a Bubble Dryer® process and delivered by inhalation on Study Day (SD) 1 and 22 to male and female rhesus monkeys using either PuffHaler® or Solovent™ dry powder inhalers. Parameters evaluated included mortality, clinical and cageside observations, body weights and body weight changes, qualitative food consumption, body temperatures, respiratory profile data, evaluation of breathing patterns postdose, evaluation of the nasal cavity, mouth, and pharynx, ophthalmologic examination, electrocardiogram evaluation, clinical pathology (chemistry, hematology, coagulation, and urinalysis), immunogenicity, bronchoalveolar lavage analysis, gross pathology observations, organ weights, and histopathology.

MATERIALS AND METHODS

Test Animals and Husbandry

A total of 18 Rhesus monkeys per sex weighing between 2.9 and 4.3 kg were obtained from Harlan Sprague Dawley (Indianapolis, IN). Animals were screened for measles and herpes B antibodies prior to arrival, and all animals assigned to study had negative antibody titers.

Animals were acclimated to laboratory conditions for 41 days prior to the first dose. During that time, animals had two negative TB tests and were observed for general health and suitability of testing. Certified Global Harlan Teklad Monkey Diet 2055C or Purina Primate Diet 5048C was provided twice daily and water was provided *ad libitum*. Environmental controls for the animal room were set to maintain a temperature of 18 to 29°C, a relative humidity of 30 to 70%, and a 12-hour light/12-hour dark cycle. In addition to standard husbandry procedures, animals were provided with environmental enrichment consisting of visual contact with other non-human primates, listening to music and/or watching television, toys, and fruit and/or other primate treats.

Test and Control Articles

Table 1. Test Materials

Name	Description	Potency
Measles Vaccine, Dry Powder capsules (for use in Solovent™ device)	Capsules filled with dry powder and packaged in a foil overwrap	4.250 log ₁₀ CCID ₅₀ per 10 mg
Measles Dry Powder Placebo (for use in Solovent™ device)	Capsules filled with dry powder and packaged in a foil overwrap	0
Measles Vaccine, Dry Powder blisters (for use in PuffHaler® device)	Aluminum blisters filled with dry powder and packaged in a foil overwrap	4.100 log ₁₀ CCID ₅₀ per 10 mg

The test and control articles were manufactured by Serum Institute of India, Ltd. and provided in a ready-to-use formulation. Duplicate blisters and capsules of the test and control articles were collected on SD 22 and analyzed for stability and potency.

Experimental Design

Animals were acclimated to the chair restraint and the delivery devices for one week prior to initiation of dosing. Animals of each sex were randomized by body weight and assigned to the study as presented in Table 2.

Table 2. Study Design

Group	Treatment	Nominal Dose	Number of Animals/Sex
1	Control Article/Solvent™ pulmonary administration	50 mg dry powder	6
2	Test Article/PuffHaler® pulmonary administration	50 mg dry powder	6
3	Test Article/Solvent™ pulmonary administration	50 mg dry powder	6

All animals were dosed once on Study Day (SD) 1 and half of the animals in each group were dosed again on SD 22. On each day of dosing, animals were exposed to five doses of the test article using either the PuffHaler® (Figure 1A) or the Solvent™ (Figure 1B) inhaler. Each dose consisted of 10 mg of either the test or control article (contents of a single capsule or blister over an interval of approximately 30 seconds).

Figure 1A. Assembled PuffHaler® Device

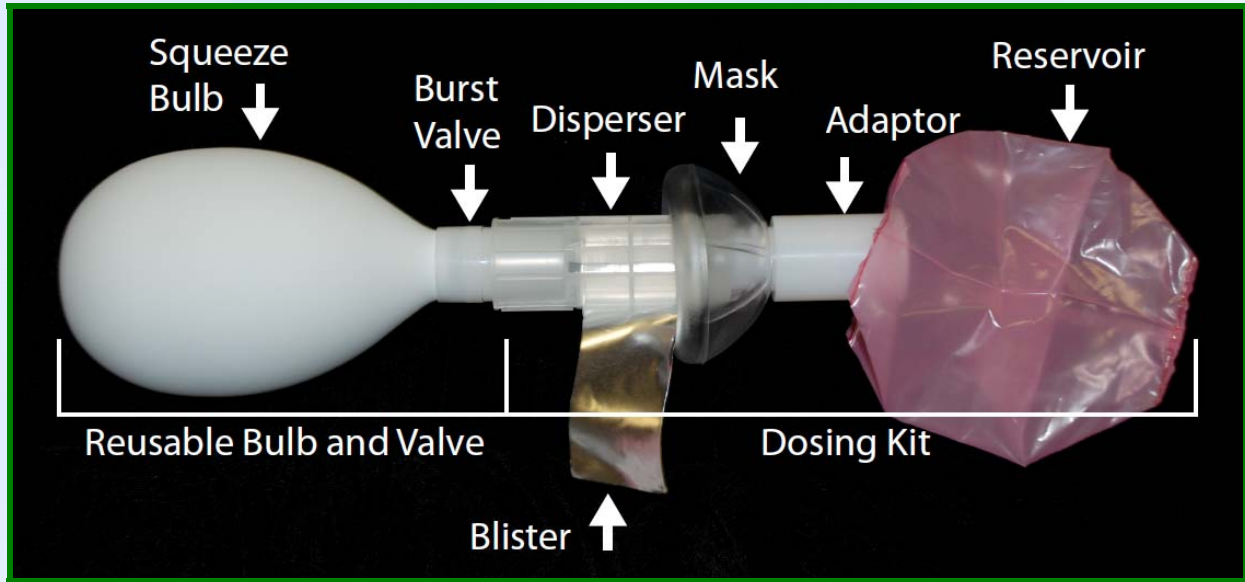


Figure 1B. Assembled Solovent™ Device



Animals were observed as shown in Table 3.

Table 3. Animal Observations/Measurements

Procedure	Frequency of Testing
Cageside Observations	≥ 2 Daily
Physical Examinations	Twice daily: Unscheduled observations were recorded
Body Weight	Animals necropsied on SD 8: Prior to dosing on SD 1, on SD 7 (unfasted) and 8 (fasted) Animals necropsied on SD 36: Prior to dosing on SD 1 and 22, on SD 8, 15, 29, 35 (unfasted), and SD 36 (fasted)
Food Consumption	Daily qualitative (adjusted as necessary for study-related events)
Body Temperatures	Five intervals, one immediately prior to each dose and at approximately 24, 48, 72, and 168 hours after each dose
Evaluation of Respiration	The respiratory rate was recorded at the end of each full dose and breathing patterns were evaluated
Evaluation of Nasal Cavity, Mouth, and Pharynx	Prior to dosing and 1 day after each dose
Ophthalmologic Examination	Prior to dosing on SD 1 and on SD 8 and 36 (all surviving animals at each interval)
Electrocardiogram (ECG) and Respiratory Assessment	SD -1 and within 30 ± 10 minutes following completion of dosing on SD 1 and 22

Cageside observations included observation for mortality, moribundity, general health and signs of toxicity. Physical examinations included evaluation of skin and fur characteristics, eye and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, and somatomotor and behavior patterns. Respiratory assessment included respiration rates, end tidal carbon dioxide, saturated blood oxygen. Additionally, breathing patterns were evaluated for signs of coughing, wheezing and/or rales and respiration rates measured following dosing. Evaluation of the nasal cavity, mouth and pharynx included redness, discoloration, and general signs of irritation. Daily qualitative food consumption was evaluated using the following scale: poor = 1–4 biscuits eaten, fair = 5–8 biscuits eaten, good = 9–12 biscuits eaten. Body temperatures were collected using a rectal probe.

Ophthalmologic observations were conducted while the animal was sedated with Telazol or Ketamine (predose eye exam only) using indirect ophthalmoscopy and slit-lamp biomicroscopy (as needed) following administration of 1% Tropicamide[®] mydriatic solution.

Nine-lead electrocardiograms, including leads I, II, III, aVR, aVL, aVF, V₁, V₂, and V₃, were recorded while the animal was sedated with Telazol and evaluated by a board-certified veterinary cardiologist. Heart rate, QT, and RR intervals were measured from the representative ECG waveform at each time point during ECG evaluation. The RR interval was calculated and reported from the measured heart rate. QTc was calculated based on the QT interval and heart rate measured from representative ECG waveforms by using the Fridericia correction.

On SD 6 animals were anesthetized and placed in dorsal recumbancy. Using a swab, the oropharyngeal tonsils of each animal were swabbed, and the swabs were sent for PCR analysis at Johns Hopkins University. Following swabbing, the mouth was opened using a laryngoscope to expose the glottis and tracheal pathway. To minimize coughing, 0.1 mL of 2% lidocaine was administered topically to the region. A size 3-10 French catheter was advanced until lodged into a subsegmental bronchus. Approximately 3 to 5 mL of saline was infused, the chest area was massaged, and, while massaging, the saline was gently aspirated into a syringe. The procedure was repeated three times and the recovered lavage fluid was placed into appropriately sized tubes and centrifuged at 300g for 15 minutes at 2-8°C. The supernatant was aliquoted into 1-mL or 5-mL portions, and the supernatant and cell pellets were then frozen at $-75 \pm 15^{\circ}\text{C}$ and then sent for PCR analysis at Johns Hopkins University.

Blood and urine samples for clinical pathology evaluation (clinical chemistry, hematology, coagulation, and urinalysis) were collected prior to the first dose and on SD 8, 15, 22, and 36. Additional blood samples for plaque-reduction assays and measles-specific enzyme immunoassays were collected prior to the first dose and on SD 8, 22, 29, and 36 and analyzed by Johns Hopkins University.

Termination and Postmortem Procedures

On SD 8, the first 3 animals/sex/group and on SD 36 the remaining 3 animals/sex/group were euthanized by injection of sodium pentobarbital followed by exsanguination. Following exsanguination of each animal, the lung was removed and weighed. A small incision was made in a main bronchus leading to the left diaphragmatic lobe and the bronchi to other lobes were ligated. The diaphragmatic lobe was lavaged by administration of five consecutive 10-mL aliquots of Sterile Saline for Injection, USP. The infused saline was gently aspirated into a syringe and carefully ejected into a labeled conical 50-mL polypropylene tube. Samples were maintained on wet ice until delivered to Johns Hopkins University by courier on the day of collection.

Following the bronchoalveolar procedure, a gross necropsy, which included examination of the external surface of the body, all orifices, the cranial, thoracic, and abdominal cavities, and their contents was performed. Fresh samples of mediastinal lymph nodes, lung (from the lobes not used for histopathology or lavage), trachea, spleen (medial), and oropharynx were collected and placed in sterile vials containing sterile PBS and stored on wet ice. Additional samples of the mediastinal lymph nodes, lung, trachea, spleen (medial), and oropharynx were collected into sterile vials and snap frozen in liquid nitrogen. Frozen samples were maintained on dry ice. Both sets of samples were sent on the day of collection on wet or dry ice as appropriate to Johns Hopkins University for detection of measles virus by PCR analysis.

A standard panel of organ weights were collected, and tissues were preserved in 10% neutral buffered formalin (NBF) with the exception of eyes (with optic nerve and lacrimal glands) and testes (with epididymides) which were preserved in modified Davidson's fixative and subsequently transferred to 10% NBF. Two bone marrow smears were prepared from the sternum of all animals. The following tissues were embedded in paraffin, sectioned, stained with hematoxylin and eosin and examined by a board-certified veterinary pathologist: brain, bronchi (primary and secondary), heart, kidney, liver, lung, mediastinal lymph node, nasoro-pharynx, nasal turbinates, ovary, testis, and all gross lesions.

RESULTS

Formulation Analysis

Analysis of the test and control articles showed the materials to be stable during the conduct of the study. The analysis also showed that the vaccine potency was unchanged during the conduct of the study.

Animal Disposition and Physical Examinations

Treatment with Measles Vaccine, Dry Powder had no effect on mortality, physical examinations or cageside observations.

Body Weight and Body Weight Changes

Treatment with Measles Vaccine, Dry Powder had no effect on body weights or body weight changes. Mean body weights are presented graphically in Figure 2A (males) and Figure 2B (females).

Figure 2A: Mean Body Weights – Male

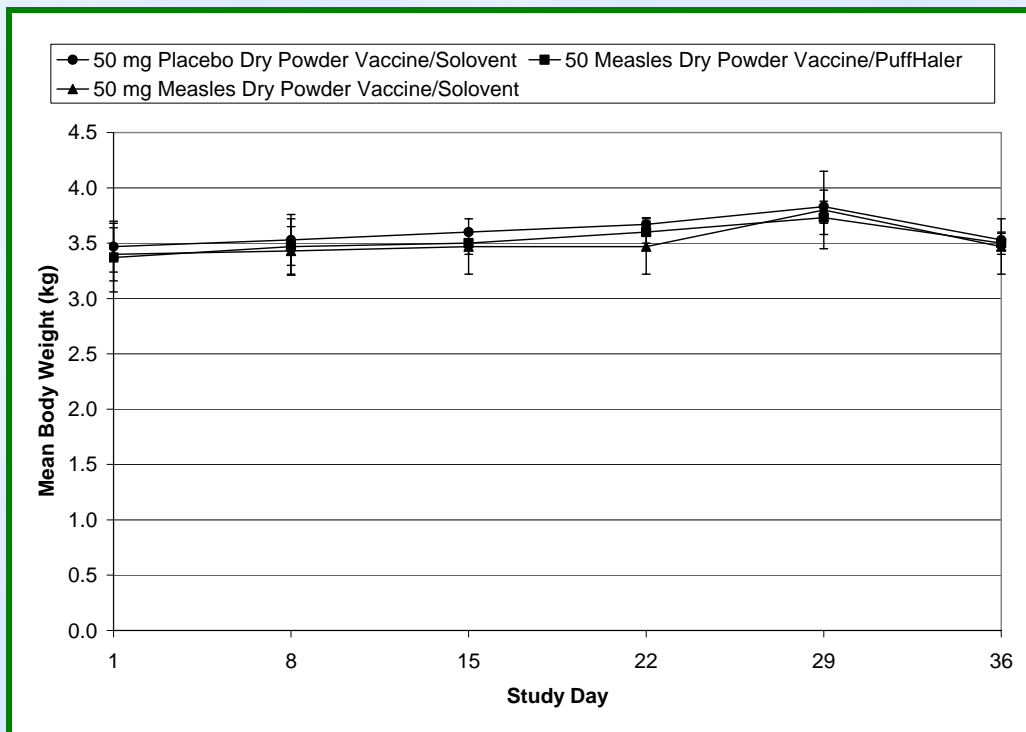
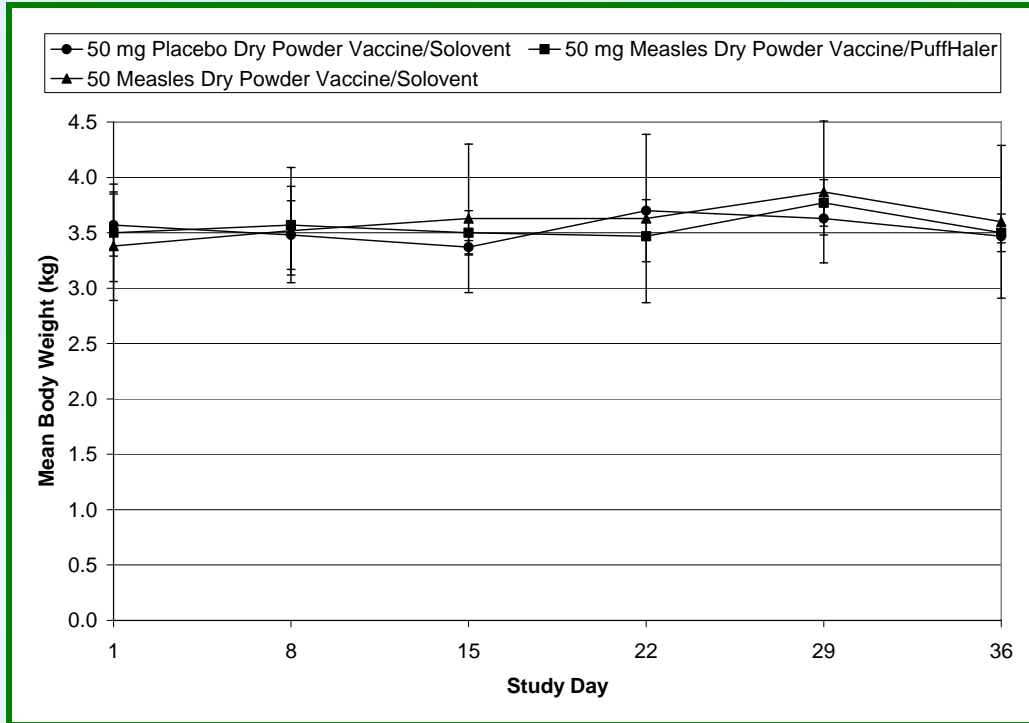


Figure 2B: Mean Body Weights – Female



Food Consumption

Treatment with Measles Vaccine, Dry Powder had no effect on food consumption.

Body Temperatures

Treatment with Measles Vaccine, Dry Powder had no adverse effect on body temperatures. Generally, animals treated with Measles Vaccine, Dry Powder had lower body temperatures when compared to the control animals. This effect is not toxicologically or biologically significant because the temperatures remained within the range considered normal for rhesus monkeys and did not exceed 40°C.

Ocular Exams

There were no ocular observations that were related to treatment with Measles Vaccine, Dry Powder.

Respiratory Profile Data

Treatment with Measles Vaccine, Dry Powder had no effect on respiratory profile data. Respiration rates after dosing were not affected by treatment with Measles Vaccine, Dry Powder and breathing patterns were all normal except for that of a single Group 1 female on SD 22 that was observed with wheezing and labored respiration after dosing. Additionally, the nasal cavity, mouth, and pharynx were evaluated prior to dosing and one day after each dose. All animals showed no test article-related signs of discoloration, redness, or irritation.

Electrocardiography

There were no effects on electrocardiograms that were related to treatment with Measles Vaccine, Dry Powder.

Clinical Pathology

There were no test article or dosing method effects on clinical chemistry, hematology, coagulation, or urinalysis variables.

Organ Weights and Macroscopic Findings

There were no test article or dosing method effects on organ weight changes or macroscopic findings.

Microscopic Findings

There were no test article- or dosing method-related microscopic abnormalities. Testes were immature in all males; therefore, potential test article effects could not be adequately assessed in this organ. All other microscopic findings were consistent with spontaneous background changes observed in this age and species of monkey.

Immunological Analyses

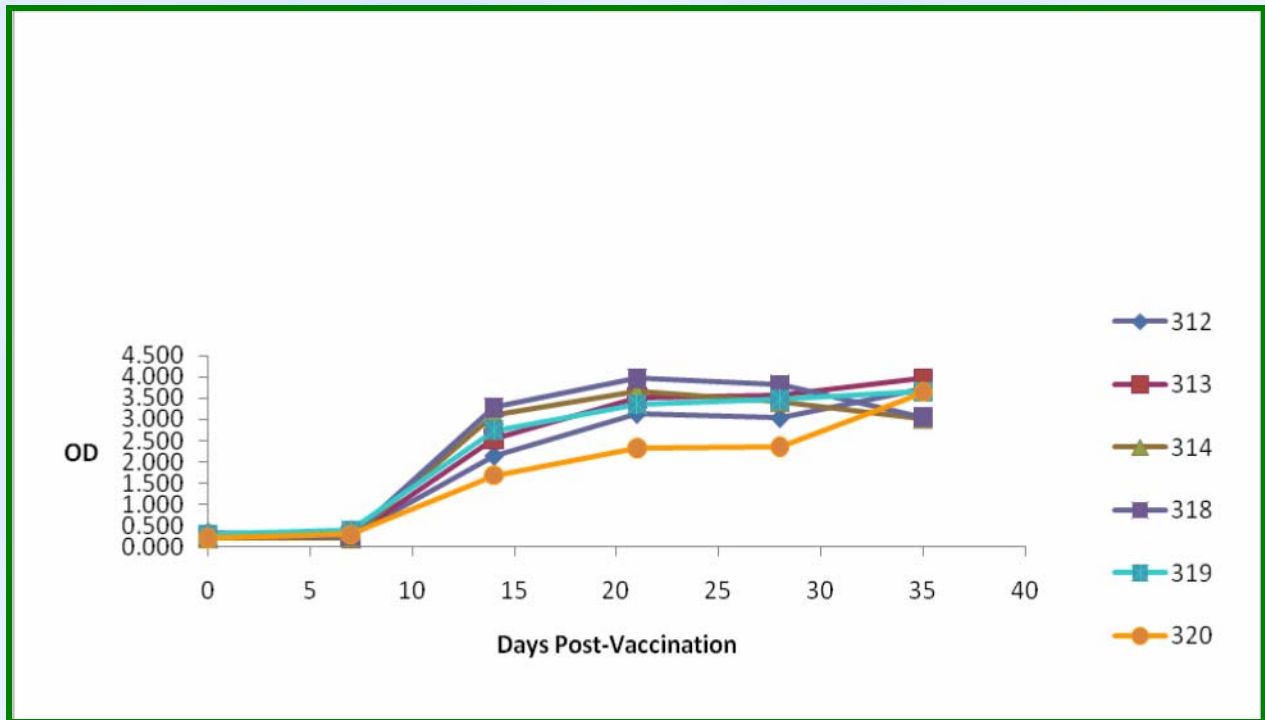
Administration of the Measles Vaccine, Dry Powder to all animals receiving a single dose of the vaccine on SD 1 was confirmed by the detection of measles virus RNA by PCR in bronchoalveolar lavage or lung tissue samples taken at the interim necropsy (data not shown). Administration of Measles Vaccine, Dry Powder to all animals receiving two doses of the vaccine was confirmed by the detection of

measles-specific antibodies in blood samples obtained from the animals sacrificed at the recovery necropsy (SD 36). All animals receiving the vaccine developed titres that were greater than 120 mIU/mL which is considered the minimum protective titre but none of the animals receiving the control article had titres greater than 85 mIU/mL demonstrating the lack of exposure (Table 4). Analysis of serum for measles-specific IgG also confirmed that animals receiving the vaccine by either device were seropositive by SD 15 whereas all animals receiving the control article were seronegative (Figures 3 A-C).

Table 4. Plaque Reduction Neutralization Titers in Plasma Following Administration of Two Doses of Control Article or Vaccine to Rhesus Monkeys

Animal Number	Group	mIU/mL
18288	Control Article/Solvent™	44
18289		67
18290		77
18294		84
18295		85
18296		80
18300	Test Article/PuffHaler®	2125
18301		2818
18302		2611
18306		1949
18307		1914
18308		1624
18312	Test Article/Solvent™	2999
18313		2375
18314		2462
18318		2196
18319		2414
18320		2436

Figure 3C. Enzyme Immunoassay of Measles-Specific IgG Response Following Administration of Two Doses of Vaccine to Rhesus Monkeys with the Solovent™ Device



CONCLUSIONS

Administration of Measles Vaccine, Dry Powder via PuffHaler[®] or Solovent[™] dry powder inhalers was well tolerated by male and female rhesus monkeys. There were no test article-related effects on mortality, clinical and cageside observations, body weights and body weight changes, qualitative food consumption, body temperatures, respiratory profile data, evaluation of post dose breathing patterns, post dose evaluation of the nasal cavity, mouth, and pharynx, ocular examination, electrocardiogram evaluation, clinical pathology (chemistry, hematology, coagulation, and urinalysis), gross pathology observations, organ weights, and histopathology. Therefore, the no-observed-adverse effect level of the dry powder measles vaccine is 50 mg when administered once or twice via PuffHaler[®] or Solovent[™] devices.

Administration of Measles Vaccine, Dry Powder to all animals receiving a single dose of vaccine was confirmed by the presence of measles virus RNA by PCR. Vaccine administration was confirmed in animals that received two doses of the vaccine by the detection of measles-specific antibodies in blood samples obtained at the recovery necropsy. Analysis of serum for measles-specific IgG also confirmed that animals receiving the vaccine by either device were seropositive whereas all animals receiving the control article were seronegative.

ACKNOWLEDGMENT

This work was funded by Aktiv-Dry LLC in support of its Grand Challenges in Global Health Initiative grant administered by the Foundation for the National Institutes of Health.

REFERENCES

- Auwaerter P, Rota P, Elkins W, Adams R, Delozier T, Shi Y, Bellini W, Murphy B, Griffin D. Measles Virus Infection in Rhesus Macaques: Altered immune responses and comparison of the virulence of six different virus strains. *J. Infect. Dis.* 1999. 180:950-958.
- de Swart R, LiCalsi C, Quirk A, van Amerongen G, Nodelman V, Alcock R, Yuksel S, Ward G, Hard J, Vos H, Witham C, Grainger C, Kuiken T, Greenspan B, Gard T, Osterhaus A. Measles vaccination of macaques by dry powder inhalation. *Vaccine* 2007. 25: 1183-1190.
- de Swart R, Kuiken T, Fernandez-de Casro J, Papania M, Bennett J, Valdespino J, Minor P, Witham C, Yuksel S, Vos H, Amerongen G, Osterhaus A. Aerosol measles vaccination in macaques: Preclinical studies of immune responses and safety. *Vaccine* 2006. 24:6424-6436.
- Dilraj A, Cutts F, Fernandez de Castro J, Wheeler J, Brown D, Roth C, Coovadia H, Bennett J. Response to different measles vaccine strains given by aerosol and subcutaneous routes to schoolchildren: a randomized trial. *Lancet* 2000. 355:798-803.
- Dilraj A, Sukhoo R, Cutts F, Bennett J. Aerosol and subcutaneous measles vaccine: Measles antibody responses 6 years after re-vaccination. *Vaccine* 2007. 25:4170-4174.
- John, TJ. Death of children after measles vaccination. *Indian Pediatrics* 2008. 45:447-448.
- Low N, Kraemer S, Schneider M, Henao-Restrepo, AM. Immunogenicity and safety of aerosolized measles vaccine: Systematic review and meta-analysis. *Vaccine*. 2007.
- Moss, W.J. and Griffin, D.E. Global measles elimination. *Nat. Rev. Microbiol.* 2006. 4:900-908.
- Remfry J. A measles epizootic with 5 deaths in newly-imported rhesus monkeys (*Macaca mulatta*). *Lab Anim.* 1976. 10:49-57.

Sievers R, Cape S, Kisich K, Bennet D, Braun C, Burger J, Best J., McAdams D, Wolters N, Quinn B, Searles J, Krank D, Pathak P, Bhagwat P, Rebits L. Challenges of developing a stable dry powder live viral vaccine. *Respiratory Drug Delivery* 2008.

Valdespino-Gomez J, de Lourdes Garcia-Garcia M, Fernandez-de-Castro J, Henao-Restrepo AM, Bennet J, Sepulveda-Amor J. Measles Aerosol Vaccination. In: *Mass Vaccination: Global Aspects-Progress and Obstacles, Current Topics In Microbiology and Immunology*, Plotkin, SA (ed) Springer-Verlag, Berlin, Germany 2006. 304:165-193.

Wolfson L.J., Strebel, P.M., Gacic-Dabo M., Hoekstra E.J., McFarland J.W., Hersh B.S., Measles Initiative. Has the 2005 measles mortality reduction goal been achieved? A natural history modeling study. *Lancet* 2007. 369:191-200.

World Health Organization. Progress in global measles control and mortality reduction, 2000-2007. *Weekly epidemiological record*. 2008. No 49. 83:441-448.

Wyde P, Sittlaar K, Osterhaus A, Guzman E, Gilbert B. Use of cotton rat for preclinical evaluation of measles vaccine. *Vaccine* 2001. 9: 42-53.