# Development of *mRNA* vaccine against *Measles virus* globally

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**Original Article**

## ABSTRACT

**Background:** Nowadays, *Measles* is a dangerous, perhaps fatal viral infection that affects children and newborns in particular. The respiratory tract's mucosal cells are the only area where the infection may first spread before moving on to nearby lymph nodes and eventually, via the blood, to other organs including the skin. Immunity lasts for life.
**Aim of Study:** Widespread utility of the *LNP-mRNA Measles* vaccine to protect against lethal *Measles* viral infection.
**The type of the study:** Screening experimental study.
**Patients and Methods:** In the current work, an envelope spike *Haemagglutinin protein of Measles* vaccine made of lipid nanoparticles was developed. Lipid nanoparticles [*LNPs*] with a particle size of around *100 nanometer* were used as the drug delivery system for the vaccination. By using a hot micro-emulsion process, lipid nanoparticles were created.
**Results:** In *randomized clinical studies* including humans, the vaccine's effectiveness was 76%, while in *preclinical animal research*, it was 90%. Compared to the conventional *live attenuated Measles* vaccination, it demonstrated equivalent biological activity and fewer side effects. The consequence lasted for a long time. In the current investigation, the vaccination proved beneficial as a preventative measure against viral infection. Because fewer amyloid plaques formed in the brain and spinal cord, it did not induce dementia as the earlier immunizations did. This was because it only had trace levels of *mRNA*. The antibody dependent enhancement in this vaccination, which causes the development of non-protective antibodies, was absent. By activating cytokines and the complement cascade through the production of immunological complexes, these non-neutralizing antibodies worsen infection.
The market-purchased killed vaccination demonstrated the antibody-dependent enhancement. Strong antibodies that neutralize *the measles virus's haemagglutinin antigen* were created by the current vaccination. It took roughly 5 mcg/ml of vaccine to immunize 50% of the applicants who got it (*ED 50%*).
**Conclusion:** The capacity to create a preventative lipid nanoparticles mRNA Measles vaccine by bioinformatics, which showed promising immunogenicity reached 76%, enabled the current study to contribute to the worldwide eradication of life-threatening Measles viral infectious illnesses among newborns and young children.

**Key Words:** Infection, Measles virus, mRNA, Prophylaxis, Vaccine.

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## INTRODUCTION

*Measles* is a highly contagious viral infection that can cause catastrophic consequences worldwide (**Parveen, 2020**). Despite the existence of a reliable and effective vaccination, *Measles* continues to be a significant cause of pediatric morbidity and death worldwide (**Caroline and Zeind, 2020**). The first *Measles* vaccine was approved in the United States in 1963 as a reaction to the catastrophic measles outbreak (**Trevor *et al.,* 2021**). The widespread use of *Measles* vaccinations for more than 50 years has decreased *measles* morbidity and mortality globally; yet, the *measles* virus still spreads, infecting people and killing an estimated 400 people daily (**Bardal *et al.,* 2021**). Two doses of the *Measles*, *Mumps*, and *Rubella* [*MMR*] vaccination were 96% effective in preventing *Measles*. In contrast, one dosage was 95% successful in preventing *Measles*, according to a recent Cochrane review of 124 trials evaluating vaccine efficacy (**Olson, 2020**). Measles is one of the infectious illnesses. A very uncommon late consequence is subacute sclerosing panencephalitis (**Levinson, 2021**). *Measles* virus belongs to the viruses composed of an envelope and one single-stranded, negatively polarized fragment of *RNA*. It replicates via a viral *RNA-dependent RNA* polymerase and has 1 *serotype* (**Swanson *et al.,* 2019**). Respiratory droplets are mode of

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the transmission of its infection (**Fisher *et al.*, 2021**). The upper respiratory tract is the initial location of infection. The virus; then infects nearby lymph nodes before moving through the blood to other organs, including the skin (**Dipro *et al.*, 2021**). Encephalitis and giant cell pneumonia can happen (**Golderg, 2020**). Virus-infected vascular endothelial cells in the skin are the target of a cell-mediated immune response by cytotoxic T cells that results in the maculopapular rash (**Wilson, 2019**). It is uncommon to isolate the virus by the conventional laboratory methods. If required, serologic testing is done (**Metting, 2019**). There is no approved antiviral treatment (**Griffin, 2018**). The commercially available vaccine comprises an attenuated, live virus for prevention (**Bailey and Sapra, 2022**). Often administered along with the *Mumps* and *Rubella* vaccinations (**Polloi *et al.*, 2022**). The current study's objective was to develop a lipid nanoparticles vaccination of the *mRNA* that codes for the *Haemagglutinin* protein to combat the lethal infectious illnesses crusaded by the *Measles virus* and avoid their consequences. Figure 1 shows the surface structural proteins of Measles virus including Haemagglutinin (HA).

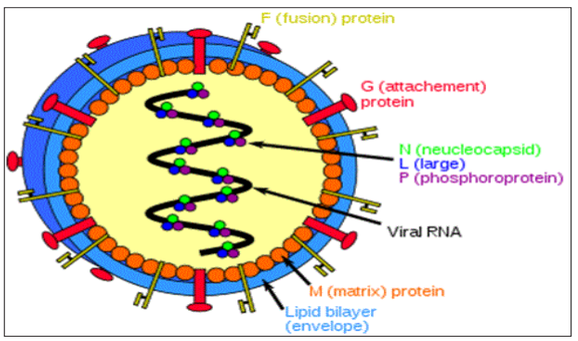


**Figure 1:** It shows the surface structural proteins of *Measles virus* including *HA*.

## PATIENTS AND METHODS:

### *Ethical statement:*

For the current investigation, all applicable national, institutional, and/or international rules for using both people and animals were postdated. By the recommendations of the Weather-all report, the local government, the Ethical Committee for Human and Animal Handling at Cairo University (*ECAHCU*), and the Faculty of Pharmacy at Cairo University, Egypt, all approved all procedures used in the study, including those involving people and animals, with approval number *P-32-11-2020* and randomized human clinical trials phases 1/2 registration number was *NCT00000734*. The human volunteers (infants, young children and adolescents) and the suffering of the animal models used in the study were both minimized. The protocol followed ethical justification for non-human primates in research: the weather all report revisited according to Arnason 2018 study published in journal of medical ethics. *2018 May; 44(5): 328-331*.

### *Date and of place study:*

This screening experimental study between *November 2020* and *September 2023*, this study was conducted at Microbiology and Immunology department, Cairo University's pharmacy faculty in Egypt.

### *Material:*

All chemical and biochemical materials were purchased from Algomhoria pharmaceutical company, Cairo, Egypt and Alnasr pharmaceutical company, Abo zabal Alkhanka, Qalyobia, Egypt. *Riboblock RNase inhibitor* [40 U/µl] with catalog number EO0384 was obtained from ThermoFisher Scientific, USA. *T7 RNA Polymerase* [20 U/µl] with catalog number EP0112 was purchased from ThermoFisher Scientific, USA. Pyrophosphatase, inorganic [0.1 U/µl] with catalog number EF0221 was purchased from ThermoFisher Scientific, USA. *TrancriptAid T7 High Yield Transcription kit* with catalog number K0441 was obtained from ThermoFisher Scientific, USA.

### *Source of animal models:*

They were obtained and legalized from pharmacology and toxicology department of faculty of pharmacy,Cairo university, Egyp.

### *The type of the study:*

Screening experimental study.

### *Inclusion and exclusion criteria for animal models:*

An infection with *the Measles virus* was administered to 100 adult male *transgenic mice* weighing around 50 g. To humanize *transgenic mice*, a lung human cell line was utilized, which increased viral protein expression and strongly induced humoral and cell-mediated immunity. *Human lung epithelial BEAS-2B cells* that exhibited characteristics of mesenchymal stem cells were purchased from AcceGen biotechnology, Fairfield, United States. Transgenic mice that were adult males and weighed around 50g were able to acquire *pneumonia* and *maculopapular rash* after getting an intranasal dosage of infectious *Measles virus* that was at a level of 100-300 viral units. Symptoms began after the incubation phase of two weeks. The lethal dose during this experiment was greater than 500 infectious agent units. Young, pregnant female mice and male mice weighing less than 40gm were excluded from the study.

### *Collection of the samples:*

Three millilitre venous blood sample was taken from each sample of 100 blood samples collected from newborns and young children infected with the Measles virus [MCV], recruited from different regions of Egypt. Sample size was calculated according to Yamane's formula. The blood samples were frozen and stored at -20$^{o}$C.

## METHODS

The potential open reading frames of *mRNA* of envelope spike *haemagglutinin [HA]* protein of *Measles virus* was identified by bioinformatics exploiting *NCBI* website.

The *mRNA* of envelope spike *haemagglutinin protein* was then extracted and purified through an organic extraction method (**Fan and Gulley, 2001**). Afterwards, the purified mRNA of *HA* was enclosed with lipid nanoparticles bubbles. The vaccine drug delivery system was lipid nanoparticles with particle size approximately 100 nanometer synthesized using hot micro-emulsion technique (**Mao *et al.*, 2003**). The immunogenicity was determined in animal models by injecting the purified *mRNA* of *HA* of *Measles* vaccine in 100 transgenic mice by intraperitoneal route of administration. During the immunogenicity evaluation in preclinical trials animal testing; as well as, randomized human clinical trials phases 1/2, the pathogenic *1 valent Measles serotype* consisted of one serotype.

### *Construction of mRNA transcripts of haemagglutinin spike protein:*

[Dynabeads™ *mRNA* purification kits with Catalog number: 61006 were obtained from Invitrogen Thermo Fisher Scientific, USA].

### *Principle of mRNA in-vitro transcription:*

In the present study linearized *DNA* template of the gene of interest coding *spike haemagglutinin protein* that present on the envelope of *Measles virus* was obtained using restriction endonuclease type II enzymes such as *EcoR I* and *Hind* I. This was followed by cloning using *polymerase chain reaction [PCR]* technique. *PCR cycle*: The required reagents and template were added to *PCR* tubes. Afterwards blended and spined for 3 minutes at 300 rpm. The mineral oil was added to prevent evaporation in a thermal cycler without a heated lid. Then, amplification per thermocycler and primer parameters was achieved. Using agarose gel electrophoresis and ethidium bromide staining, the amplified *DNA* was examined. *PCR cycles* included: Initial denaturation at 94°C for 2 minutes: The double-stranded *DNA* template strand was heated during this initiation stage to the point where the strands began to denaturize and the hydrogen bonds between the nucleotide base pairs ruptured. At 94 to 98°C, the first denaturation stage was carried out. The primers were then annealed for 30 seconds at 55°C: Within this temperature range, the forward and reverse primers were stable enough to anneal to each of the single-stranded *DNA template* strands. Additionally stable enough to attach to the primer *DNA sequence* was the *DNA polymerase*. Then, extension of *DNA* for 1 minute at 72°C was done: The *Taq polymerase* possess an optimal temperature around 70-75°C so this step enabled the *DNA* polymerase to synthesize and elongate the new target *DNA* strand accurately and rapidly. Repeating previous steps was achieved 25-30 times. On the other hand, final Extension for 5 minutes was performed at 72°C to fill-in any protruding ends of the newly synthesized strands. *PCR* kits which were utilized during the present study included *PureLinkR™ PCR* Purification kit with Catalog number K310001 which was purchased from ThermoFisher scientific company, USA. *RNA* polymerase was then, mixed with *DNA* template and ribonucleotides for in vitro *mRNA* transcription. The invitro *mRNA* transcription lasted about 2 hours; then an *RNAase inhibitor* was added to the mixture to aid in the purification of *mRNA* extract. The post in vitro transcription comprised the addition of *DNAase* for the digestion of *DNA* template. As well as, *proteinase K* was added to aid in the cleaning up of any protein contamination. Table 1 shows the components of in vitro mRNA transcription reaction mixture.

**Table 1:** It shows the components of in vitro mRNA transcription reaction mixture:

| Ingredient | Volume |
|---|---|
| 5X Transcription buffer | 10 µl |
| ATP/GTP/CTP/UTP Mix, 10 mM each | 10 µl [2mM final concentration] |
| Linearized DNA template | 1 µg |
| RiboLock™ RNAase inhibitor | 1.25 µl [50 U] |
| T7/T3/SP6 RNA polymerase | 1.5 µl [30 U] |
| DEPC-treated water | Up to 50 µl |
| **Total volume** | 50 µl |

### *Procedure for in vitro transcription:*

Using the following process, more than 10µg of *mRNA transcript* could be produced from a 1µg *DNA* template. The TranscriptAid™ *T7* High Yield Transcription kit was utilized for high-yield transcription, producing up to 200µg of *mRNA*. Thawed and combined frozen reagents under 300rpm centrifuge for three minutes. Nucleotides and enzymes were kept cold. At room temperature, the reaction buffer was retained. At room temperature, the following combination for the reaction was created:

The *mRNA* transcription reaction mixture was then incubated at 37°C for 2 hours. 2µl [2 U] of *DNAase* I, *RNAase- free* were added, mixed and incubated at 37°C for 15 minutes in order to remove *DNA* template. The reaction was obstructed aside the addition of 2µl 0.5M *EDTA* at PH 8.0 and incubation at 65°C for 10 minutes. *mRNA* was subjected to hydrolysis in the absence of a chelating agent such as *EDTA* (**Van *et al.*, 2020**).

### *Purification of mRNA transcripts:*

This was done using the *liquid-liquid extraction* method known as *AGPC*, which stands for *Acid Guanidinium Thiocyanate-Phenol-Chloroform*. Chloroform reagent solutions were manufactured and consisted of 96% *chloroform* and 4% *isoamyl alcohol*. They could be combined with an equivalent volume of *phenol* to create a *25:24:1 solution. RNases* were made inactive by *isoamyl*

*alcohol*, which also helped to minimize foaming. The *phenol* utilized in this intervention was delivered as a water-saturated solution with a *Tris buffer* made up of 50% *phenol*, 48% *chloroform*, and 2% *isoamyl alcohol solution*.

Chloroform was stabilized with small quantities of amylene or ethanol, because exposure of pure chloroform to oxygen and ultraviolet light produces *phosgene gas*. This method relied on phase separation by centrifuge of a mixture of the aqueous sample and a solution containing water-saturated phenol and *chloroform*, resulting in an upper aqueous phase and a lower organic phase (mainly *phenol*). *Guanidinium thiocyanate, a chaotropic agent*, was added to the organic phase to aid in the denaturation of proteins (such as those that strongly bind nucleic acids or those that degrade *RNA*). The nucleic acids (*RNA* and/or *DNA*) partitioned into the aqueous phase, while protein partitioned into the organic phase. The pH of the mixture determined which nucleic acids got purified. While *RNA* stayed in the aqueous phase under acidic conditions (pH 4-6), *DNA* partitioned into the organic phase. Both *DNA* and *RNA* partitioned into the aqueous phase at neutral pH levels (7-8). The nucleic acids were eventually extracted from the aqueous phase using *2-propanol* precipitation. After washing the 2-propanol with 70% *ethanol*, the pellet was quickly air-dried before being dissolved in *TE buffer*. While *phenol*, *isopropanol*, and *water* had low solubility, 1ml of 4M (50%) *guanidinium thiocyanate* denatured proteins, including *RNases*, and separated *rRNA* from ribosomal *proteins*. A clear, upper aqueous phase, which contains the nucleic acids, and a lower phase, which contains the proteins dissolved in *phenol* and the lipids dissolved in chloroform, completely separated in the presence of *chloroform* or *BCP* (*bromochloropropane*). PH was maintained at about 4, which preferentially retained *mRNA* in the aqueous phase for *mRNA* transcript purification (**Nic, 2019**).

### *Northern blot technique for the confirmation of the purification of mRNA transcripts coding for the haemagglutinin spike protein:*

The sizes and amounts of the various mRNA transcripts for the 1 valent serotype of *Measles virus* that code for the haemagglutinin spike protein formation were measured using *the Northern blot method*. Denaturing gel was originally employed in Northern blot to separate mRNA transcripts based on size. Then, with the same distribution as in the gel, mRNA was transferred into a nylon membrane. In order to hybridize the immobilized mRNA, a la-belled probe complementary to the target gene was added after the mRNA transcripts were fixed to the membrane. After that, the loosely bound probes were rinsed away. The solid membrane was then dried, made visible, and was subjected to examination with the probe precisely bound to the target *mRNA* transcripts. *The Northern blot* measured the quantities and sizes of the target *mRNA* transcripts (**Cho *et al.,* 2009**).

### *Inclusion of Measles virus mRNA vaccine with lipid nanoparticles:*

Bubbles made of lipid nanoparticles were added. 50 mcg of *dimethyl dioctadecyl ammonium bromide lipid* (*DDAB*) were used to create these lipid nanoparticles. A Quaternary ammonium lipid called *DDAB* formed vesicles that contained *mRNA* transcripts when it complexed with *mRNA* to trigger innate immunity. The delivery system for the vaccine made of lipid nanoparticles included particles that were around 90 nm in size. It was accomplished throughout this investigation to create lipid nanoparticles with various cationic and solid lipid types and to assess their suitability as *LNP-mRNA* delivery systems. This was accomplished by using a hot micro-emulsion process to combine cationic lipids such *stearylamine, DOMTA*, or *DDAB* with solid lipids like *Compritol 888 ATO [C] or cetyl palmitate 15[CP 15]* to create a sequence of lipid nanoparticles. The created *cationic solid nanoparticles systems (CSLNS)* were examined using a *Malvern NanoZS analyzer* [obtained from Biotechne, USA] to determine their particle size, size distribution, and zeta potential. Comparing and assessing the cytotoxicity of *CSLNS* systems was done using the Resazurin test. For the examination of the *CSLNS systems*, which effectively bound *mRNA* transcripts and shown minimal cytotoxicity, in-vitro cellular uptake [fluorescence microscopy] and gene silencing [*Northern blot*] experiments were used. On the other hand, high Performance size exclusion chromatography (*HPSEC*) was exploited to assess the particle configuration of *mRNA* transcripts. Later on loading on a *CSLNS* formulation, the transition temperature [*Tm*] of the *mRNA* transcripts was deliberated exploiting *differential scanning fluorimetry (DSF)* (**Riguero *et al.,* 2023**).

### *In vivo evaluation of vaccine on transgenic animals (mice):*

Transgenic mice are those whose genes are altered by recombinant *DNA* technology using tissue culturing technique (**Mina *et al.,* 2017**). A transgenic animal is one that has integrated a gene of *DNA* sequence (a trans-gene) which has been transferred by human intervention into the genome of a cell.100 transgenic mice were injected with the vaccine. They received two doses, 28 days were apart from each other. The first dose was half the second booster dose to enhance immunity (**Chen and Tang, 2020**).

Determination of immunogenicity of *LNP-mRNA Measles vaccine* involved Protection tests which were used to determine the potency of vaccine. For boosting viral protein expression and inducing potent humoral and cell-mediated immunity, transgenic mice were made human by lung human cell line [*Human lung epithelial BEAS-2B cells*, which showed features of mesenchymal stem cells, bought from Accegen, USA]. After being inoculated with *Measles virus* at an infectious dosage of 100-300 viral units via an intranasal method of administration, transgenic mice, which were adult male mice weighing 40–50g,

were infected with lower respiratory tract infections such pneumonia (**Wilder-Smith and Qureshi, 2020**).

***Active:***

Following immunization of 100 transgenic mice with the mRNA vaccine that was being tested via intraperitoneal route of vaccination, groups of transgenic mice were challenged with increasing numbers of infectious microorganisms. The lowest number of microorganisms lethal for 50% of animals (i.e., LD 50%) was determined and compared to LD 50% in non-vaccinated transgenic in order to measure the protective power of the vaccine (**Moss, 2017**).

***Passive:***

Graded amounts of serum from immunized individuals were transferred to 100 normal transgenic mice via intraperitoneal route of immunization, which were ;then challenged with the increasing graded doses of infectious agent. The highest dilution of serum which was effective at protecting 50% of animals (i.e.ED 50%) was determined as a measure of the efficacy [immunogenicity] of the *mRNA* vaccine (**Conis, 2019**).

***Formulation of LNP-mRNA Measles vaccine:***

The dose form of lipid nanoparticles vaccination with mRNA was administered as intramuscular injection. It consisted of a sterile solution of the *mRNA* transcripts of the genes encoding the proteins promoting the synthesis of *Measles haemagglutinin spike protein*. Each 1ml dose contained 10 mcg of the *mRNA* transcript for each cluster gene of *Measles virus* that codes for *spike haemagglutinin protein* of 1 valent Measles pathogenic serotype, 50 mcg of the lipid *dimethyl dioctadecyl ammonium bromide (DDAB*), and 0.9 mg of aluminium hydroxide. Additionally, 0.873 mg of sodium dihydrogen phosphate dihydrate and 9 mg of sodium chloride were included in each dose.

***Randomized human clinical trials phases 1/2:***

*Vaccine immunogenicity evaluation was carried out through human randomized clinical trials phases 1/2*. Spread of infection among volunteers (infants, young children and adolescents) was achieved through contact with infected nasal or throat secretions (coughing or sneezing) or breathing the air that was breathed by someone with *Measles*. Recruitment of volunteers was finished through online discussion boards. Three groups of human participants were employed in the current experiment. 100 people were split among each group. Group 1 (the negative control group) received an intramuscular injection of the placebo. Injections of the standard *1-valent Measles live attenuated* vaccine were given intramuscularly to Group 2 (the positive control group). Group 3 (the test group) received an intramuscular injection of the *Measles LNP-mRNA test* vaccine. The three groups were exposed to graded amounts of the infectious 1 serotype of *Measles virus* and after two weeks to enhance the production of protective neutralizing antibodies (this was ethical and approved to determine the effectiveness of the test vaccination). Following a 21-day period, intramuscular injection booster doses were administered to the three groups. The degree of protection provided by the test vaccination was evaluated over a three-year period. In contrast to the protective *cell-mediated immunity*, which was assessed using *a flow cytometry method*, the *protective antibodies* were identified using an *enzyme-linked immunosorbent assay (ELISA)*.

***ELISA***

***ELISA for detection of neutralizing antibodies to the test vaccine:***

[*Invitrogen coated and instant ELISA kits with product number 37581 obtained from Thermo Fisher scientific company, USA*].

Each well received 10µl of the antigen suspension by passive adsorption, which was then given an hour to incubate. Bovine serum albumin served to inhibit the extra binding sites. Three *PBS-T* washes were applied to the plates to remove unattached molecules. Biotinylated IgG was incubated for 15 minutes after being mixed with 50µl of *horseradish peroxidase (HRP)* in each well. The wells were once again washed with *PBS-T* to remove any unattached molecules. The enzyme and then the antigen could be identified after each well received 50µl of *chromophore substrate (TMB)* for 15 minutes (**Hughes *et al.*, 2020**).

***Flow cytometry***

***Flow cytometry for detection of CD+4 and CD+8 T lymphocytes:***

The *CD+4* and *CD+8* T cells that were specific to the *mRNA* gene cluster vaccination were seen and examined using an *Invitrogen Attune Cytpix flow cytometer*, Thermo Fisher Scientific, Waltham, Massachusetts, USA. The patient's cells were marked in this experiment using a monoclonal antibody. The *CD4* protein, which is used to count T helper cells, is one of the proteins that these antibodies were created against. Fluorescent dyes like *rhodamine* and *fluorescein* were used to label monoclonal antibodies. Individual cells came into contact with the laser beam and lit up. A fluorescence-activated cell Sorter (*FACS*) was used to measure the fluorescence (**Piccrilli *et al.*, 2015**).

***Statistical analysis:***

All experimental samples were conducted in triplets. Their presentation was by means and standard deviation. One way analysis of variance (p value $\leq 0.05$) was used as means for performing statistical analysis and also, statistical analysis based on excel-spreadsheet-software. The F statistical analysis test was utilized during the present study.

## RESULTS

The vaccine resulted in 90% efficacy during preclinical trials (animal testing); while it reached 76% in clinical trials phases 1/2. Nearly 500 viral units were found to be the *RSV* LD50%. The effective dose which killed 50% of animal models [ED 50%] reached 5mcg HA/ml of vaccine suspension. The *mRNA* vaccine formulation contained 5 mcg of *mRNA* transcript of cluster gene of the 1 valent pathogenic *Measles virus* per millilitre. The degree of purity of recombinant *mRNA* transcripts that were purified using *the liquid-liquid extraction technique Acid guanidinium thiocyanate-phenol-chloroform (AGPC)* was demonstrated by the ratio of *mRNA* transcript absorbance at 260 and 280 nm, which was nearly 2 when measured with a *UV spectrophotometer*.

The present *LNP-mRNA Measles* vaccine formulations that fall under the category of *CSLNS* systems displayed small particle sizes of approximately 100nm, a narrow size distribution as measured by *the polydispersity index (PDI)*, which ranged from 0.29 to 0.35, and high zeta potentials of between +21 and +23 mV. Because of its significant cytotoxicity on typical *Vero cell* lines, using *CSLNS* in combination with stearyl amine was deemed unsuitable. Because it displayed the best complexion capacity with mRNA transcripts and the least amount of cytotoxicity on typical *Vero cell* lines during the Resazurin assay, *CSLNS* combined cationic lipid *DDAB* with solid *lipid CP 15*, was the ideal formulation. Only the *DDAB* and *CP 15 combo* was absorbed by *PC-3* cells and showed silencing effectiveness. Following loading on the *CSLNS* formulation, *differential scanning fluorimetry (DSF)* revealed that *the transition temperature [Tm]* of the *mRNA* transcripts augmented for a while between 0.5 and 0.7°C. No deterioration of the *mRNA* transcripts' particle structure was discovered using *High Performance size exclusion chromatography [HPSEC]* studies after electrostatic-ally loading them onto *CSLN*. Bioinformatics analysis: Type II transmembrane proteins with external and intracellular domains were found in the Haemagglutinin spike protein of 1valent pathogenic *Measles virus* serotype.

*The messenger RNA* of the gene cluster that contributes to the production of *the Measles Haemagglutinin spike protein* is shown in Figure 2 using *the Northern blot method*. The purity of the sample was about 86%. The T. lymphocyte count [KU/L] following vaccination with the *LNP-mRNA Measles* vaccine is shown in Table 3. Table 2 demonstrates that the *mRNA Measles* vaccine had a 90% protection power during preclinical animal testing. Figure 3 depicts *the Measles virus's Haemagglutinin (HA) protein in three dimensions*. The absorbance of various serum concentrations of neutralizing antibodies to *the mRNA Measles* vaccination using *ELISA* is shown in Figure 5. Figure 4 shows that the *mRNA Measles* vaccine had a 76% protection power during stages 1 and 2 of clinical testing.

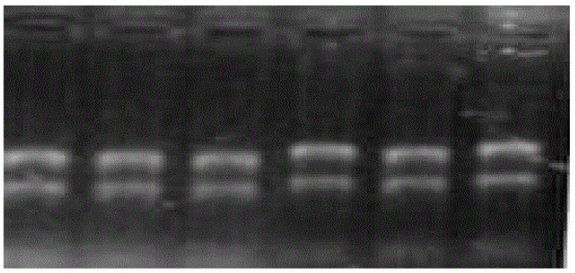

**Figure 2:** It shows the *Northern blot* technique which depicts the messenger *RNA* of gene cluster that helps synthesizes *Measles Haemagglutinin spike protein*. Approximately 86% of the sample was pure.

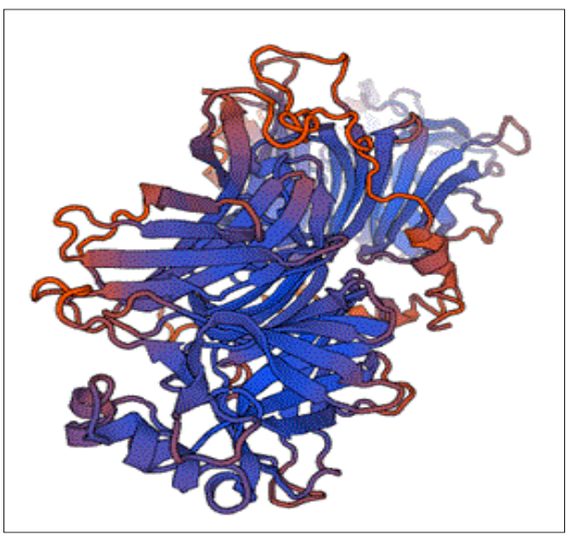

**Figure 3:** It shows 3D structure of [*Haemagglutinin*] *HA* protein of *Measles virus*. It is composed of 617 amino-acids. It is a surface glycoprotein, a receptor-binding attachment protein. It resembles *type II transmembrane proteins*.

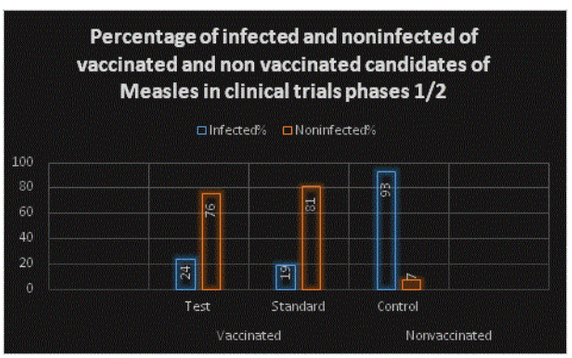


**Figure 4:** It represents that protection power of *mRNA Measles* vaccine was 76% during clinical trials stages 1/2.

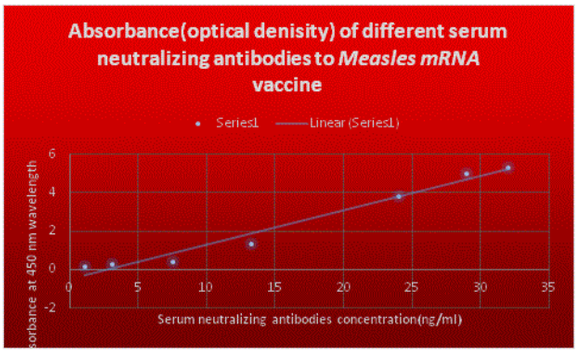


**Figure 5:** It represents the absorbance of different serum concentrations of neutralizing antibodies to *mRNA Measles* vaccine via *ELISA*.

**Table 2:** It shows that the protection power of *mRNA Measles* vaccine during preclinical animal testing was 90%:

| Description | Vaccinated | Non-vaccinated |
|---|---|---|
| Alive | 90 | 12 |
| Dead | 10 | 88 |
| **Total** | 100 | 100 |

**Table 3:** It shows T.lymphocyte count [KU/L] after immunization with *LNP-mRNA Measles* vaccine:

| Description | Vaccinated | Pre-vaccinated |
|---|---|---|
| CD+4 COUNT | 1082 | 1000 |
| CD+8 COUNT | 763 | 600 |
| **Total** | 1845 | 1600 |

## DISCUSSION

The present study comprised the design and the manufacture of *LNP-mRNA Measles vaccine* due to the overwhelming lethal *Measles infectious diseases* and the consequent complications such as *pneumonia* (**Janine *et al.*, 2021**). The novel vaccine in the present study showed comparable biological activity [76% efficacy] to *live attenuated Measles vaccine*. The *mRNA* vaccine showed fewer side effects such as mild pain, headache, redness and swelling at the site of intramuscular injection than other standard vaccines such as live attenuated vaccine. These adverse effects did not exceed 3 days after receiving the vaccine dosage regimen. Its efficacy was long lasting because it persisted to more than 2 years. It did not cause dementia as do the previous vaccines due to less amyloid plaques formation in the brain and the spinal cord (**Griffin *et al.*, 2009**). This was because it contained small amount of RNA. This vaccine was devoid of antibody dependent enhancement which leads to the formation non protective antibodies. These non neutralizing antibodies exacerbate infection by the activation of cytokines and complement cascade through the formation of immune complexes (**Laudati *et al.*, 2008**). The antibody dependent enhancement was shown by the killed Measles vaccine which was drawn from the market (**Winkler *et al.*, 2022**).

This vaccine strongly stimulated the humoral immunity and weakly stimulated the cell mediated immunity. The humoral immunity was the main body defense mechanism that prevented the infection. The main neutralizing antibodies in blood were IgM2, IgG1 against envelope spike haemagglutinin protein of the virus. Few IgA antibodies against this antigen of the virus were produced because it was not taken by the natural route of infection. This vaccine weakly activated cell mediated immunity against the infection of virus. Advantages include no reversion to virulence is possible. Disadvantages comprises excretion of vaccine virus and transmission to nonimmune contacts is not possible; thus it does not contribute to the development of the herd immunity against this viral infection. Shorter duration of action than live attenuated vaccine. It needs to be stored at -70$^{o}$C in a refrigerator to avoid spoilage and contamination. Phases 1 and 2 of randomized human clinical trials involved 93 infected participants in the negative control group, 19 infected participants in the positive control (standard) group, and 26 infected participants in the test group indicating that the test vaccine showed comparable preventive consequences to the standard commercially available vaccines currently. It demonstrated moderate immunogenicity and fewer adverse reactions than other common vaccinations. The vaccine needs to be updated on a regular basis every 5-10 years despite its long-lasting efficacy to avoid the high pathogenicity liability of overwhelming Measles infectious diseases and their complications especially among infants and young children globally. The high purity of recombinant mRNA transcripts that were purified using the liquid-liquid extraction technique Acid guanidinium thiocyanate-phenol-chloroform (AGPC) was demonstrated by the ratio of mRNA transcript absorbance at 260 and 280 nm, which was almost 2 when measured with a UV spectrophotometer. 90% of animals were protected by the mRNA Measles vaccine during preclinical animal testing. At phases 1/2 of clinical testing, the mRNA Measles vaccine's level of protection was 76%. The percentage of purity of The messenger RNA of the gene cluster that aids in the synthesis of the Measles Haemagglutinin spike protein using the Northern blot technique stood at about 86%. Measles incidence was reduced by 66% (from 145 to 49 cases per million people) and mortality decreased by 73% (from 535 600 to 142 300) globally between 2000 and 2018, significantly lowering the global disease burden (**Hviid *et al.*, 2019**). Routine MCV immunization has been the cornerstone of measles control and prevention. MCV was 97% effective in preventing measles after two doses, making it one of the most effective vaccinations ever created (**Goodson and Seward, 2015**). Immunization with the live, attenuated vaccine is the cornerstone of prevention. The vaccination is safe and has few negative effects. It is commonly administered subcutaneously to children at the age of 15 months, together with the rubella and mumps vaccinations. The vaccination should not be administered to children under the age of 15 months because maternal antibodies in the kid can neutralize the

virus and impair the immune response (**Eygeris *et al.*, 2021**). A booster dosage is recommended since immunity might diminish. Because the vaccine includes live virus, it should not be administered to Immunocompromized people or pregnant women. The vaccination has significantly reduced the number of measles infections in the United States; there were just 138 documented cases of measles in 1997 (**Srugo and Brunell, 2022**). However, outbreaks continue to affect those who have not received vaccinations, such as youngsters in disadvantaged areas and underdeveloped nations. Use of the dead vaccination is not advised. If administered to unimmunized people early in the incubation phase, immune globulin may be utilized to change the illness. This is especially important if the unimmunized people have impaired immune systems.

**Janine *et al.*, 2021** study stated that Age did not appear to alter long-term Measles seroconversion or seropositivity at MCV1, although vaccination efficacy did decline with younger age. The impact of age at MCV1 on immunological blunting could not be examined due to a lack of sufficient data. In the present study the LNP-mRNA Measles vaccination was efficacious for infants aged 9-15 months (**Bailey and Sapra, 2022**).

According to DE *Griffin*'s 2009 study, the first Measles vaccinations were created as a result of Enders and colleagues' 1960s tissue culture measles virus isolation. A vaccination that was inactivated only offered transient immunity and failed to elicit enough T cell responses or affinity-maturing antibodies. This vaccination was removed because it predisposed the body for atypical measles, a more serious form of the disease. With the use of two doses, a live attenuated virus vaccine has been extremely effective in preventing measles and stopping endemic measles virus transmission. Injections of this vaccination are given between the ages of 9 and 15 months. If newborns could be immunized at a younger age, if the vaccine were thermostable, and if the administration didn't call for a needle and syringe, measles control would be made easier (**Bester, 2016**). The current mRNA Measles vaccination in the present study offered long-lasting protection, elicited moderate T-cell responses, and produced antibodies that completed affinity maturation.

## CONCLUSION

mRNA vaccine of Measles envelope spike haemagglutinin protein was efficacious as a prophylaxis agent against the consequences of viral infection with Measles virus. It is recommended in the future to explore its possibility to be combined with with other vaccines such as Mumps and Rubella formulated in lipid nanoparticles mRNA vaccination forms.

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## CONFLICT OF INTEREST

There are no conflicts of interest.

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