Safety & Immunogenicity/ Seroconversion Results Using Needle Free Injection Systems

Index

Summary of Immunogenicity/ Seroconversion Results from Clinical Trials/Literature Studies

Safety, Tolerability and Immunogenicity / Seroconversion results from clinical trials / literature studies conducted using Needle Free Injection System.

1. **Safety, Tolerability and Acceptability Study of Needle-Free Injection System Vs. Conventional Hypodermic Needle, India**
   - Saline was administered to approximately 60 volunteers.
   - IntegriMedical's Needle Free Injection System (NFIS) is safe, tolerable & acceptable.
   - No significant difference in terms of tenderness, redness, induration, vital & systemic examination parameters.
   - Following is the table comparing Vas Score for Needle-Free injection and Convention Hypodermic Needle Injections.

Pain Score (Vas Score)		**NF Injection (N=30)**	**CHN Injection (N=30)**
		No. (%) of Subjects	
None	(0)	77%	30.0%
Mild	(1,2, or 3)	23%	70.0%
Moderate	(4,5, or 6)	0.0%	0.0%
Severe	(7,8,9, or 10)	0.0%	0.0%

2. **Immunogenicity & Safety Study, India**
   - Covid Vaccine (Covishield)
   - Vaccine was administered in adults and children using the needle-free injection system for approximately 220 volunteers. The immunogenicity levels using the needle free injection was at par or better than the conventional hypodermic needle.
   - The safety, acceptability and tolerability were observed in children and 47% children experienced zero pain using the needle free injections
   - Following is the table comparing the immunogenicity levels –

Table 4: Summary statistics of concentration of IgG, IgA, and IgM

Immunological Parameters		**Group T1 (N=71)**			**Group T2 (NFIS) (N=67)**		
		Pre Dose	**Post Dose**	**P-value (paired t-test)**	**Pre Dose**	**Post Dose**	**P-value (paired t-test)**
IgG concen.	Mean	1083.32	1296.77	0.000	1107.93	1306.75	0.000
	STDEV	174.86	198.32		211.61	197.35	
IgA concen.	Mean	193.24	304.08	0.000	188.88	282.95	0.000
	STDEV	64.32	66.74		63.11	77.02	
IgM concen.	Mean	119.80	197.01	0.000	124.24	189.37	0.000
	STDEV	50.32	55.42		57.10	49.24	

*** Group T1 - Hypodermic Needle,
Group T2 – Needle Free Injection System

3. **Immunogenicity & Safety Study, India (Bavdekar 2018) –**
   - MMR Vaccine
   - Randomized, parallel group, non-inferiority trial
   - Multicentric clinical study was conducted for administration of MMR vaccine in India using needle-free injections and conventional needle syringe (N-S). MMR Vaccine was administered subcutaneously in the anterolateral aspect of the thigh region.
   - On evaluation of the immunogenicity results, it was observed that at baseline, seropositivity rates were similar between both the groups for all three antigens. On day 35, seropositivity rates in the DSJI and N-S groups were 97.5% and 98.7% for measles; 98.8% and 98.7% for mumps; and 98.8% and 100% for rubella.
   - Similar studies were conducted in Brazil on 582 volunteers (de Menezes Martins Reinaldo 2015)

4. **Immunogenicity and Tolerance Study, France & Africa (Isabelle Parent du Chfitelet 1997)**
   - DTP Vaccine
   - Vaccine was delivered by needle-free injection and compared with standard syringe injection to infants and immunogenicity results are as follows –

Type of Antigen	**Diphtheria**		**Tetanus**		**Pertussis**	
	Imule (Needle Free)	Syringe	Imule (Needle Free)	Syringe	Imule (Needle Free)	Syringe
Pre-vaccinal GMT (IU ml-‘)	0.05 (0.04-0.68)	0.07 (0.06-0.08)	0.09 (0.07-0.12)	0.17 (0.12-0.23)	10.6 (7.6-14.5)	9.7 (7.1-13.3)
Post-vaccinal GMT (IU ml-‘)	0.55 (0.44-0.69)	0.34 (0.27-0.41)	2.27 (2.12-2.43)	1.46 (1.29-l .65)	1434 (1188-l 732)	1188 (965-l 465)
Seroconversion %	79.2%	56.7%	88.7%	70.3%	94.4%	94.6%

*GMT = Geometric Mean Titter

   - Similar study was conducted on Pentavalent diphtheria, tetanus, pertussis (whole cell), hepatitis B (rDNA), and Haemophilus influenzae type b conjugate vaccine administered with needle-free injections. Seropositivity rates for the DSJI and N-S groups in the per-protocol population at baseline and at day 84 post vaccination appeared comparable, by descriptive statistics, for all vaccine components.
   - Table below provides Seroprotection/Seropositivity at days 0 and 84 after vaccination
   -

Vaccine component	**Day 0**		**Day 84**	
	Disposable-syringe jet injector (n = 61)	**Needle and syringe (n = 67)**	**Disposable-syringe jet injector (n = 61)**	**Needle and syringe (n = 67)**
Diphtheria	4 (6.6%)	7 (10.4%)	61 (100.0%)	64 (95.5%)
Tetanus	61 (100.0%)	66 (98.5%)	61 (100.0%)	66 (98.5%)
Pertussis	3 (4.9%)	1 (1.5%)	36 (59.0%)	41 (61.2%)
Hepatitis B	9 (14.8%)	9 (13.4%)	60 (98.4%)	66 (98.5%)
Haemophilus influenzae type B (long- term protection)	21 (34.4%)	24 (35.8%)	56 (91.8%)	62 (92.5%)
Haemophilus influenzae type B ≥0.15 µg/mL (short-term protection)	48 (78.7%)	55 (82.1%)	61 (100.0%)	65 (97.0%)

Safety, Tolerability and Acceptability Study of Needle-Free Injection System Vs. Conventional Hypodermic Needle

Clinical Study Report

Title: An open label study to investigate safety, tolerability and acceptability of Needle Free Injection System (NFIS) in healthy volunteers in comparison to conventional needle-based system.

Principal Investigator: Dr. Almas Pathan

Sponsor: IntegriMedical

Sponsor Authorized Signatory: Scott McFarland

Date Study Initiated: 20 Jan 2021

Date Study Completed: 01 Nov 2021

Date of Report: 14th March 2022

Version: 1.0

Prepared By: Jehangir Clinical Development Centre Pvt. Ltd.

This study was performed in compliance with ICH E6R2 "Guidance on Good Clinical Practice", Indian Good Clinical Practices Guideline, National Ethical Guidelines for Biomedical and Health Research involving Human Participants, ICMR 2017, Declaration of Helsinki and relevant SOPs of Jehangir Clinical Development Centre, Pune, Maharashtra, India.

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List of Tables

1. Title

An open label study to investigate safety, tolerability and acceptability of needle free injection system in healthy volunteers in comparison to conventional needle-based system.

Name of investigational medical device: Needle Free Injection System

Indication Studied: The present study is the first in human assessment of IM-NFIS that compares safety, tolerability and acceptability of needle free injection system in healthy volunteers with conventional hypodermic needle-based system at 5 different sites of administration (forearm, abdomen, thigh, buttocks and arm).

Name of the Sponsor: IntegriMedical.

Protocol identification: IM/NFIS/01, Version 3.0

Study Initiation Date: 20 Jan 2021

Date of early study termination, if any: Not Applicable

Study Completion date (last patient completed): 01 Nov 2021

Name and affiliation of Principal Investigator: Dr. Almas Pathan, Jehangir Clinical Development Centre Pvt Ltd, Jehangir Hospital Premises, 32 Sassoon Road, Pune 411001, Maharashtra, India

This study was performed in compliance with ICH E6R2 “Guidance on Good Clinical Practice”, Indian Good Clinical Practices Guideline, National Ethical Guidelines for Biomedical and Health Research involving Human Participants, ICMR 2017, Declaration of Helsinki and relevant SOPs of Jehangir Clinical Development Centre, Pune, Maharashtra, India.

Date of Clinical Study Report: 14th March 2022

2. List of Abbreviations of Terms

Abbreviations	Full Name
AE	Adverse Event
CRF	Case Report Form
CRO	Contract Research Organization
ICF	Informed Consent Form
ICH-GCP	International conference of Harmonization – Good Clinical Practice
ICMR	Indian Council of Medical Research Ethical Guidelines for Biomedical Research on Human Subjects
IEC	Institutional Ethics Committee
IMD	Investigational Medical Device
IRB	Institutional Review Board
MGRS	Multicenter Growth Reference Study
SAE	Serious Adverse Event
SOP	Standard Operating Procedure
WHO	World Health Organization

3. Ethics

3.1 Institutional Ethics Committee (IEC)

The protocol and consent form was reviewed and approved by the Institutional Ethics Committee of JCDC. The EC is registered with the CDSCO (Registration No.-ECR/352/tnst/MW2013/RR-19 and accredited by Association for the Accreditation of Human Research Protection Program(AAHRPP). The Ethics Committee is accredited by National Accreditation Board for Hospitals and Health Care Providers (NABH) (Certificate No. EC-CT-2018-0023).

3.2 Ethical Conduct of the Study

This study was performed in compliance with ICH E6R2 "Guidance on Good Clinical Practice", Indian Good Clinical Practices Guideline, National Ethical Guidelines for Biomedical and Health Research involving Human Participants, ICMR 2017, Declaration of Helsinki and relevant SOPs of Jehangir Clinical Development Centre, Pune, Maharashtra, India.

3.3 Patient Information and Consent

The informed consent was obtained from the subject/LAR of the subject by the Principal Investigator. Subject/LAR of the subject provided written consent to participate in the study after having been informed about the nature and purpose of the study, participation/termination conditions, risks, burdens and benefits of treatment. Personal data from subjects enrolled in this study were limited to those necessary to investigate the safety and tolerability of the investigational study device used in this study.

4. Investigator and Study Administrative Structure

Principal Investigator: Dr. Almas Pathan

Sponsor: IntegriMedical

Clinical Laboratory: Jehangir Hospital

5. Introduction and Background Information

Drug delivery is an important technology in the healthcare sector that uses different systems or approaches to deliver any pharmaceutical to achieve its intended therapeutic effect (1). Drug delivery involves different routes of administration which includes but is not limited to parenteral, inhalation, transdermal, oral etc. Certain pharmaceuticals cannot be delivered orally due to susceptibility to enzymatic degradation and poor absorption due to their molecular size. Such pharmaceuticals are administered through the parenteral route by using hypodermic needle and a syringe. The use of hypodermic needles is very common and the oldest way to overcome the physical barrier. Ideally, a solution of a drug is forced under piston stress straight into the bloodstream or exact tissue. This necessitates skin perforation using a needle, which is associated with trauma and pain. To overcome these drawbacks, other alternative methods have been investigated like jet injections, dermabrasion, thermal ablation, laser, tape stripping, etc. (2) Reduction of the pain and time of injections may lead to improved patient satisfaction and compliance, as well as reduced anxiety in populations of patients who require frequent or ongoing injections to treat their medical conditions. A needle-free delivery system offers the potential to address such issues. They may enhance safety, improve dosing accuracy, and increase patient compliance, particularly in self-administration settings. The needle free injection technology does not involve the use of needles for delivery of pharmaceutical and instead is delivered via a high-pressure stream of liquid which penetrates the site of injection (3). The needle free injection technology has been reported to overcome some of the risks of needles including reduced risk of needle stick injury, eliminated risk of disease transmission from reused needles, reduce scar tissue at the injection site caused by needle damage to the tissue, easier self-administration, etc. The working principle of needle free injection works on different technologies including spring system, gas propelled system, etc. (4) The newly designed needle free injection systems have overcome most of the risks posed by needles by incorporating disposable cartridges to avoid infection, introducing adjustable parameters selected according to skin site properties and thickness as well as the desired depth level intended to deliver the medication. IntegriMedical® Needle Free Injection System (IM-NFIS) is intended to deliver drugs and biologics through intradermal, intramuscular, or subcutaneous sites. Typical doses range from 0.1 ml to 0.5 ml and are delivered to various injection depths. The energy for the device comes from compressed spring which when released propels the plunger forward delivering the medication at high speed thus penetrating the skin.

6. Study objectives

6.1 Primary Objectives

• To investigate safety of needle free injection system

6.2 Secondary Objectives

• To understand the acceptability and tolerability of needle free injection system

6.3 Primary endpoints

• Injection site reactions as assessed according to the toxicity scale provided by US FDA guidance with grading 0-4

6.4 Secondary endpoints

• Pain assessment using 100-mm VAS scores (0 mm = no pain at all; 100 mm = a lot of pain) immediately after each administration (before needle removal)

• Acceptability of needle free injection using a questionnaire

7. Investigational Plan

7.1 Overall Study Design

This was a 5-day open label study to investigate safety, tolerability and acceptability of needle free injection system in 30 healthy volunteers (5 cohorts with 6 subjects in each cohort) in comparison to conventional needle-based system. Prospective healthy volunteers were identified for the study by the study investigator/study team after the screening procedure and qualifying the study in-/exclusion criteria. All study procedures began only after obtaining signed informed consent from the subjects/legally acceptable representatives (LARs). Subjects were randomized for the five sites of injection (forearm, abdomen, thigh, buttocks and arm). Each subject acted as a test (Saline delivery through Needle free injection) and control arm (Saline delivery through Hypodermic needle) for the allocated site of injection. Each site was divided into areas for receiving test and control product as given below:

Cohort 1

Forearm Right: Saline delivery through Needle free injection system

Forearm Left: Saline delivery through Hypodermic needle

Cohort 2

Abdomen area divided into two halves,

Right Half: Saline delivery through Needle free injection system

Left Half: Saline delivery through Hypodermic needle

Cohort 3

Thigh Right: Saline delivery through Needle free injection system

Thigh Left: Saline delivery through Hypodermic needle

Cohort 4

Buttocks side Right: Saline delivery through Needle free injection system

Buttocks side Left: Saline delivery through Hypodermic needle

Cohort 5

Arm Right: Saline delivery through Needle free injection system

Arm Left: Saline delivery through Hypodermic needle

Each participant received two injections (once for the test device and second time for the control device) within an interval of 5-10 minutes in between (5). Participants were evaluated for site reactions, pain level and acceptability separately after each injection for Needle free injection system and conventional hypodermic needle.

The study included a screening period (0 day) and a 4-day study period. The study included 5 time points: Visit 1/ Time point 1 (Baseline/screening visit/Day 0), Visit 2/ Time point 2 (at day 1 from baseline/Enrolment/Administration of product using needle free injection and hypodermic needle), Time point 3 (at day 2 from baseline/Telephonic follow-up), Time point 4 (at day 3 from baseline/Telephonic follow-up) and Time point 5 (at day 4 from baseline/Telephonic follow-up/EOS).

At visit 1 following laboratory investigations were performed for screening of participant:

Complete blood count

Urine pregnancy

Serum creatinine

Chest X ray

Subjects were randomized as per site of injection at Visit 2 during enrolment. Following the randomization each participant in cohort 2 to 5 received known 0.5 ml volume of saline using a needle free injection system at the designated site and areas of abdomen, thigh, buttocks and arm. Participants in cohort 1 received 0.1ml volume of saline using a needle free injection system in the designated forearm. Within a time interval of 5-10 minutes, participants received second injection of known volume

of saline (0.5 ml volume for cohorts 2 to 5 and 0.1 ml volume for cohort 1) using conventional hypodermic needle at the designated site and area.

Participants reported the pain level separately after each injection. Pain assessment was done using a VAS score (6). Investigator also performed an assessment of injection sites at 2 min and between 20 and 30 min following each injection. Injection site reactions were assessed according to the toxicity scale provided by FDA guidance with grading 0-4 (7). Participants were also trained to measure the local site reactions. Photos of injection site were taken by the principal investigator at 2 min and between 20 and 30 min following each injection for record purpose. For visits 3 to 5 participants were requested to report the local site reactions and systemic reactions telephonically and send the photos of injection site to the Principal Investigators. Participants complaining of site reactions were called at the site for further evaluation. Study coordinators masked the identity of the participants. Acceptability questionnaire (8) was completed by the participant before leaving from the study site. A follow phone contact was made with the participant at 24hr, 48hr and 72 h after the injections to assess for injection site reactions and adverse events.

Inclusion/Exclusion Criteria

Inclusion Criteria

- Male or female in the age group 18 to 45 years both inclusive
- Able and willing to sign the informed consent form
- Physical examination without clinically significant findings
- Hemoglobin in the opinion of a PI as clinically not significant
- WBC and differential in the opinion of a PI as clinically not significant
- No history of liver disorders in past 3 months
- No history of kidney disorders in past 3 months
- No history of cardiovascular disorders in past 3 months
- No history of neurological disorders in past 3 months
- Negative human chorionic gonadotropin (beta-HCG) pregnancy test (urine) on day of enrollment
- In good general health without clinically significant medical history and based on clinical judgement of principal investigator

Exclusion criteria

- Breast-feeding women
- More than 10 days of systemic immunosuppressive medications or cytotoxic medications within the 4 weeks prior to enrollment or any within the 14 days prior to enrollment
- Blood products within 16 weeks prior to enrollment
- Bleeding disorder history (e.g. factor deficiency, coagulopathy, or platelet disorder requiring special precautions) or significant bruising or bleeding difficulties with IM, SC injection or blood draws
- Investigational research products within 4 weeks prior to enrollment or planning to receive investigational products while on the study
- Asthma that is not well controlled
- Diabetes mellitus (type I or II)
- Evidence of autoimmune disease or immunodeficiency
- Idiopathic urticaria within the past year
- Hypertension that is not well controlled
- Malignancy that is active or history of malignancy
- Any medical, psychiatric, social condition, occupational reason or other responsibility that, in the judgment of the investigator, is a contraindication to protocol participation or impairs a volunteer s ability to give informed consent

Table 1: Schedule of Assessments

Procedures	**Day 0+3 days/ Screening/ /Visit 1/Time point 1**	**Day 1/ Eligibility/ Enrolment/ /Administration of product using needle free injection and hypodermic needle/ Visit 2/ Time point 2**	**Day 2/ Telephonic follow-up/ Time point 3**	**Day 3/ Telephonic follow-up/ Time point 4**	**Day 4/ Telephonic follow-up/ Time point 5/EOS**
Informed consent	X				
Demographics	X				
Medical history	X				
Prior medication (if any)	X				
Current/concomitant medication	X				
General Physical examination, including height, weight, BMI BP, pulse	X				
Laboratory Investigations					
CBC	X				
Urine pregnancy		X			
Serum creatinine	X				
Chest X ray	X				
Eligibility		X			
Randomization		X			
Product administration		X			
AE/SAE: Local and Systemic		X	X*	X*	X*
Pain assessment using VAS scale		X			
Acceptability using questionnaire		X			

*AE/SAE assessment only for those parameters which are mentioned in Table 1 and 3 will be done telephonically using FDA toxicity scale and photo of the injection site will be send to the Principal Investigator.

7.2 Treatment

7.2.1 Treatments Administered and Identity of Investigational Product(s)

Investigational medical device - IntegriMedical® Needle Free Injection System (IM-NFIS)

Mode of administration – Five sites of injection (forearm, abdomen, thigh, arms and buttocks) for intra dermal, intramuscular and subcutaneous route.

Administration schedule – Subjects were randomized for the five sites of injection (forearm, abdomen, thigh, arms and buttocks). Each subject acted as a test (Saline delivery through Needle free injection) and control arm (Saline delivery through Hypodermic needle) for the allocated site of injection. Each site was divided into areas for receiving test and control product as given below:
Forearm Right: Saline delivery through Needle free injection system

Forearm Left: Saline delivery through Hypodermic needle

Abdomen area divided into two quadrants,

Quadrant Right: Saline delivery through Needle free injection system

Quadrant Left: Saline delivery through Hypodermic needle

Thigh Right: Saline delivery through Needle free injection system

Thigh Left: Saline delivery through Hypodermic needle

Buttocks side Right: Saline delivery through Needle free injection system

Buttocks side Left: Saline delivery through Hypodermic needle

Arm Right: Saline delivery through Needle free injection system

Arm Left: Saline delivery through Hypodermic needle

7.2.2 Method of Assigning Subjects to Treatment Groups

Subjects were randomized for the five sites of injection (forearm, abdomen, thigh, arms and buttocks). Each subject acted as a test (Saline delivery through Needle free injection) and control arm (Saline delivery through Hypodermic needle) for the allocated site of injection. within an interval of 5-10 minutes in between. After each injection participants were evaluated for site

reactions, pain level and acceptability of Needle free injection system in comparison to conventional hypodermic needle.

7.2.3 Selection of volume of saline administered

Following the randomization each participant in cohort 2 to 5 received known 0.5 ml volume of saline using a needle free injection system at the designated site and areas of abdomen, thigh, buttocks and arm. Participants in cohort 1 received 0.1ml volume of saline using a needle free injection system in the designated forearm. Within a time interval of 5-10 minutes, participants received second injection of known volume of saline (0.5 ml volume for cohorts 2 to 5 and 0.1 ml volume for cohort 1) using conventional hypodermic needle at the designated site and area.

7.2.4 Blinding (If Applicable)

Not Applicable

7.3 Analysis of Safety and tolerability Measurements

Safety evaluation includes assessment of Injection site reactions as assessed according to the toxicity scale provided by US FDA guidance with grading 0-4. Acceptability and Tolerability was determined using a questionnaire and a VAS score respectively.

7.4 Data Quality Assurance

A representative of the independent quality assurance team at JCDC monitored the study to assess the compliance with approved protocol and ICH-GCP guidelines and relevant SOPs of Jehangir Clinical Development Centre, Pune, Maharashtra, India.

7.5 Statistical Analysis

Statistical analysis was performed using the SPSS Version 20 software. All available data was used in the analyses.

7.6 Protocol Deviations

There were no protocol deviations noted in the conduct of the study. All 30 volunteers complied to the various trial related procedures and the study was conducted in compliance with the study protocol.

8. Subject Disposition

8.1 Study Subjects

A total of 30 healthy volunteers providing consent and found eligible for participation in the study were enrolled in the five-day study (5 cohorts and 6 subjects in each cohort). Subjects in the first 4 cohorts (forearm, abdomen, buttock, and thigh) were enrolled and completed the study during

the last week of January 2021. Volunteers in the fifth cohort (arm) were studied in the last week of October 2021. All 30 volunteers successfully completed the stipulated five-day study period. Data generated on these 30 healthy volunteers who received both the intervention and control injections form the basis of this report.

8.2 Demographics

The demographic and patient characteristics of the study subjects are summarized in Table 2. The mean age of the 30 male subjects enrolled in the study was 26.2 years (median 22.5 years; range 18 to 43 years). Mean weight was 61.6 Kg (SD 11.6 kg) and mean height was 169.2 cm (SD 7.6 cm). All except one subject were non-smokers and non-alcoholics. Subject-wise listing of demographic characteristics are tabulated in Appendix A.

Table 2: Demographic characteristics of the 30 subjects at baseline

Demographic characteristic		Mean (SD) / No. (%) (N = 30)	
Age (years)	n	30	
	Mean	26.2	
	SD	8.5	
	Median	22.5	
	Min	18	
	Max	43	
Age group	18 - 20 years	13	**43.3%**
	21 - 30 years	9	**30.0%**
	31 years or above	8	**26.7%**
Gender	Female	0	**00.0%**
	Male	30	**100.0%**
Ethnicity	Indian	30	**100.0%**
Race	Asian	30	**100.0%**
Weight (Kg)	n	30	
	Mean	61.6	
	SD	11.6	
	Median	61.5	
	Min	44	
	Max	89	
Height (cm)	n	30	
	Mean	169.2	
	SD	7.6	
	Median	170.0	
	Min	146	
	Max	181	
Smokers	No	29	**96.7%**
	Yes	1	**03.3%**
Alcoholic	No	29	**96.7%**
	Yes	1	**03.3%**

8.3 Past and Current Medical History

None of the study subjects reported any past / current medical history (Appendix B).

8.4 Vital Signs

Vital signs of the study subjects at screening are summarized in Table 3. Subject-wise listings are tabulated in Appendix C. The study subjects had ‘normal’ body temperature, heart rate, respiratory rate, and blood pressure at the time of screening.

Table 3: Subject characteristics at baseline - Vital signs

Vital signs		Mean (SD) / No. (%)	Normal	Abnormal, clinically not significant	Abnormal, clinically significant	Not done
		(N = 30)	Mean SD/No (%)			
Temperature (axillary) (^{0} F)	n	30	30 (**100%**)	0 (**0%**)	0 (**0%**)	0 (**0%**)
	Mean	97.7				
	SD	0.7				
	Median	97.8				
	Min	96.2				
	Max	98.8				
Heart rate (beats / min)	n	30	30 (**100%**)	0 (**0%**)	0 (**0%**)	0 (**0%**)
	Mean	79.6				
	SD	6.5				
	Median	78.0				
	Min	70				
	Max	98				
Respiratory rate (breath / min)	n	30	30 (**100%**)	0 (**0%**)	0 (**0%**)	0 (**0%**)
	Mean	16.6				
	SD	1.8				
	Median	17.5				
	Min	12				
	Max	20				
Systolic BP (mm / Hg)	n	30	30 (**100%**)	0 (**0%**)	0 (**0%**)	0 (**0%**)
	Mean	116.6				
	SD	10.6				
	Median	113.0				
	Min	100				
	Max	140				
Diastolic BP (mm / Hg)	n	30	30 (**100%**)	0 (**0%**)	0 (**0%**)	0 (**0%**)
	Mean	75.0				
	SD	7.3				
	Median	75.0				
	Min	60				
	Max	88				

8.5 General and Systemic Examination

General and systemic examination data of the study subjects at screening are summarized in Table 4. Subject-wise listings are tabulated in Appendix D. None of the subjects had any complications. All subjects in both the groups were 'normal' with respect to general appearance, head, ENT, eyes, skin, neck, abdomen, cardiovascular, respiratory, musculoskeletal, neurological, and lymphatic systems.

Table 4: Subject characteristics at baseline – General and systemic examination

	Mean (SD) / No. (%) (N = 30)							
Physical examination	**Normal**		**Abnormal, clinically not significant**		**Abnormal, clinically significant**		**Not done**	
General appearance	30	**100%**	0	**0%**	0	**0%**	0	**0%**
Head	30	**100%**	0	**0%**	0	**0%**	0	**0%**
ENT	30	**100%**	0	**0%**	0	**0%**	0	**0%**
Eyes	30	**100%**	0	**0%**	0	**0%**	0	**0%**
Skin	30	**100%**	0	**0%**	0	**0%**	0	**0%**
Neck	30	**100%**	0	**0%**	0	**0%**	0	**0%**
Abdomen	30	**100%**	0	**0%**	0	**0%**	0	**0%**
Cardiovascular system	30	**100%**	0	**0%**	0	**0%**	0	**0%**
Respiratory system	30	**100%**	0	**0%**	0	**0%**	0	**0%**
Musculoskeletal system	30	**100%**	0	**0%**	0	**0%**	0	**0%**
Neurological system	30	**100%**	0	**0%**	0	**0%**	0	**0%**
Lymphatic system	30	**100%**	0	**0%**	0	**0%**	0	**0%**

8.6 Prior and Current Medications

None of the study subjects reported any past / current medical history (Appendix E).

8.7 Inclusion Criteria and Exclusion Criteria

Subject-wise, details of inclusion criteria and exclusion criteria data are listed in Appendix F and G respectively.

8.8 Study Subjects' Conclusion

All the 30 male adult healthy volunteers recruited for the study had 'normal' findings at screening with respect to anthropometric parameters, vital signs, and physical examination. None of them had any medical history and were not on any concomitant medications in the past and at the time of enrolment into this clinical study.

9. Safety Evaluation (Results and Discussion)

9.1 Administration of Study Products & Time to Assessments

Injection Sites

All 30 subjects received both injections. Subjects were first administered saline with needle free injection (NF Injection) system followed by conventional hypodermic needle injection (CHN Injection). NF injection was given in the right side and the CHN injection on the left side. Five injection sites were used - forearm, abdomen, buttock, thigh, and arm, six subjects in each group.

Time to Pain Assessment Using VAS Score Post Injections

Post NF injection, VAS pain score was recorded within 1 min for all subjects (Table 5). In the case of CHN injection, VAS pain score was recorded within 2 min for 28 subjects; for remaining 2 subjects the measurement was completed in 3 min.

Time to FDA Toxicity Assessments Post Injections (02 min & 20-30 min)

FDA toxicity assessments were done within 02 min for all subjects post NF injection and CHN injection. Toxicity assessments were repeated post 20-30 min of each injection; median time was 28 min post NF injection and 27 min post CHN injection (Table 5). Individual subject-wise details of actual time of each of these assessments showing compliance to protocol are presented in Appendix H and I, for the NF injection and CHN injection, respectively.

Table 5: Time to VAS pain assessment and FDA toxicity assessments

		NF Injection (N = 30)	CHN Injection (N = 30)
Time to VAS pain score assessment (min) post injection	Median	1	2
	Min	1	1
	Max	1	3
Time to FDA toxicity assessment (02 min) post injection	Median	2	2
	Min	2	2
	Max	2	2
Time to FDA toxicity assessment (20-30 min) post injection	Median	28	27
	Min	20	20
	Max	30	30

9.2 FDA Toxicity Scale Assessments

9.2.1 Local Reactions (2 min and 20-30 min post injections)

Data on local reactions at 2 min and at 20-30 min following NF injection and CHN injection are summarized in the following Table 6. Post 2 min, one subject (receiving NF injection in Arm) and three subjects post CHN injection (two in Arm and one in Abdomen) reported Grade 1 pain (does not interfere with activity). Grade 1 (mild discomfort to touch) tenderness was reported by two each, NF injection (both Forearm) and CHN injection (one Forearm and one Abdomen) subjects. None reported erythemia / redness or induration. At 20-30 min post injections no local reaction was reported in for both injection methods. Subject-wise listings are provided in Appendix J-M.

At 2 min post injection, 29 subjects receiving NF injection reported no pain compared with 27 in CHN injection group. However, the higher number in NF injection group is not statistically significant (P = 0.3006). Tenderness, redness, and induration was reported by equal number of subjects in both groups at post 2 min and post 20-30 min.

Table 6: Summary of local reactions post injections (2 min and 20-30 min)

Signs & Symptoms		NF Injection At 2 min (N = 30)	CHN Injection At 2 min (N = 30)	P Value	NF Injection At 20 - 30 min (N = 30)	CHN Injection At 20 - 30 min (N = 30)	P Value
Pain	**No**	29	27	>0.2	30	30	>0.2
	Yes	1	3		0	0	
	Grade 1	1	3		NA	NA	
Tenderness	**No**	28	28	>0.2	30	30	>0.2
	Yes	2	2		0	0	
	Grade 1	2	2		NA	NA	
Erythema / Redness	**No**	30	30	>0.2	30	30	>0.2
	Yes	0	0		0	0	
	Grade	NA	NA		NA	NA	
	Size	NA	NA		NA	NA	
Induration	**No**	30	30	>0.2	30	30	>0.2
	Yes	0	0		0	0	
	Grade	NA	NA		NA	NA	
	Size	NA	NA		NA	NA	

9.2.2 Vital Signs (2 min and 20-30 min post injections)

Vital signs including body temperature, heart rate, blood pressure, and respiratory rate were measured after administration of NF injection and CHN injection post 2 min and post 20-30 min. Table 7 below summarizes the data for both these groups at two time points defined. Appendix N-Q lists the subject specific vital data points.

Mean vital signs parameters at 2 min post NF injection were not statistically (P >0.2; paired t-test) different from similar measurements taken post CHN injection. This conclusion was valid across both groups at 20-30 min post injections also.

Table 7: Summary of vital signs post injections (2 min and 20-30 min)

Vitals		NF Injection At 2 min (N = 30)	CHN Injection At 2 min (N = 30)	P Value	NF Injection At 20 - 30 min (N = 30)	CHN Injection At 20 - 30 min (N = 30)	P Value
Body temperature (in F)	n	30	30		30	30	
	Mean	97.3	97.3	>0.2	97.2	97.2	>0.2
	SD	0.84	0.70		0.72	0.66	
	Median	97.5	97.2		97.2	97.2	
	Min	95.2	96.2		95.4	95.2	
	Max	98.6	98.9		98.4	98.4	
Fever	No	30	30	>0.2	30	30	>0.2
	Yes	0	0		0	0	
	Grade	NA	NA		NA	NA	
Heart rate (bpm)	n	30	30		30	30	
	Mean	80.3	78.4	0.2	77.8	79.6	0.2
	SD	7.30	6.79		7.34	7.07	
	Median	80.5	80.0		77.5	78.0	
	Min	62	62		62	63	
	Max	91	90		95	90	
Tachycardia	No	30	30	>0.2	30	30	>0.2
	Yes	0	0		0	0	
	Grade	NA	NA		NA	NA	
Bradycardia	No	30	30	>0.2	30	30	>0.2
	Yes	0	0		0	0	
	Grade	NA	NA		NA	NA	
Blood pressure – Systolic (mm / Hg)	n	30	30		30	30	
	Mean	115.3	115.3	>0.2	116.9	118.5	>0.2
	SD	9.87	11.70		10.64	10.44	
	Median	114.0	112.5		117.5	118.0	
	Min	100	100		100	96	
	Max	140	144		140	140	
Blood pressure – Diastolic (mm / Hg)	n	30	30		30	30	
	Mean	75.2	74.5	>0.2	73.7	73.3	>0.2
	SD	8.02	7.96		7.65	7.95	
	Median	75.5	75.0		74.5	72.0	
	Min	57	56		60	60	
	Max	86	88		88	88	
Hypertension (Systolic)	No	30	30	>0.2	30	30	>0.2
	Yes	0	0		0	0	
	Grade	NA	NA		NA	NA	
Hypertension (Diastolic)	No	30	30	>0.2	30	30	>0.2
	Yes	0	0		0	0	
	Grade	NA	NA		NA	NA	
Hypotension (Systolic)	No	30	30	>0.2	30	30	>0.2
	Yes	0	0		0	0	
	Grade	NA	NA		NA	NA	
Respiratory rate (per min)	n	30	30		30	30	
	Mean	16.4	16.0	0.2	16.5	15.9	0.1
	SD	1.54	1.71		1.72	1.62	
	Median	16	16		16	16	
	Min	14	12		14	14	
	Max	20	18		20	20	

9.2.3 Systemic Examination (2 min and 20-30 min post injections)

Systemic examination carried out for after NF and CHN injections administration at 2 min and 20-30 min. The data summarized in Table 8 demonstrates that none of the subjects in both injection types reported any difficulties. Subject wise data for all the parameters are listed in Appendix R-U.

Systemic examination parameters (nausea, diarrhea, headache, fatigue, and myalgia) at 2 min post NF injection were not statistically (P >0.2; chi-square test) different from similar measurements taken post CHN injection. This conclusion was valid across both groups at 20-30 min post injections also.

Table 8: Systemic examination post injections (2 min and 20-30 min)

Parameters		NF Injection At 2 min (N = 30)	CHN Injection At 2 min (N = 30)	P Value	NF Injection At 20 - 30 min (N = 30)	CHN Injection At 20 - 30 min (N = 30)	P Value
Nausea / Vomiting	No	30	30	>0.2	30	30	>0.2
	Yes	0	0		0	0	
	Grade	NA	NA		NA	NA	
Diarrhoea	No	30	30	>0.2	30	30	>0.2
	Yes	0	0		0	0	
	Grade	NA	NA		NA	NA	
Headache	No	30	30	>0.2	30	30	>0.2
	Yes	0	0		0	0	
	Grade	NA	NA		NA	NA	
Fatigue	No	30	30	>0.2	30	30	>0.2
	Yes	0	0		0	0	
	Grade	NA	NA		NA	NA	
Myalgia	No	30	30	>0.2	30	30	>0.2
	Yes	0	0		0	0	
	Grade	NA	NA		NA	NA	

9.2.4 Local Reactions (24-, 48- and 72-hours post injections)

All 30 study subjects (who received both NF and CHN injections) were telephonically contacted post 24, 48, and 72 hours of injections. Data on signs and symptoms reported by them are tabulated in Table 9 below. Subject-wise (and day-wise) listings for individual signs and symptoms are

included in Appendix V-X. None of the subjects reported any kind of complaints on all the three instances of telephonic follow up.

Table 9: Summary of local reactions post injections (24, 48 and 72 hours)

Signs & Symptoms		At 24 hours (N = 30)	At 48 hours (N = 30)	At 72 hours (N = 30)
Pain	No	30	30	30
	Yes	0	0	0
Tenderness	No	30	30	30
	Yes	0	0	0
Erythema/Redness	No	30	30	30
	Yes	0	0	0
Induration/Swelling	No	30	30	30
	Yes	0	0	0

9.2.5 Systemic Examination (24-, 48- and 72-hours post injections)

Systemic examination carried out for all subjects telephonically post 24, 48, and 72 hours after injections. The data summarized in Table 10 demonstrates that none of the subjects reported any difficulties. Subject wise data for all the parameters are listed in Appendix Y, Z, and AA.

Table 10: Systemic examination post injections (24, 48 and 72 hours)

Parameters		At 24 hours (N = 30)	At 48 hours (N = 30)	At 72 hours (N = 30)
Nausea / Vomiting	No	30	30	30
	Yes	0	0	0
Diarrhoea	No	30	30	30
	Yes	0	0	0
Headache	No	30	30	30
	Yes	0	0	0
Fatigue	No	30	30	30
	Yes	0	0	0
Myalgia	No	30	30	30
	Yes	0	0	0

9.3 VAS Pain Assessment Score (2 min post injections)

Pain score was assessed within 2 min following the NF injection and CHN injection. 76.7% of the subjects reported no pain post NF injection compared with 30.0% in the CHN injection recipients (Table 11). The percentage of those who reported no pain post NF injection (77%) was significantly higher as compared with CHN injection group (P <0.01; Chi square test). Mean pain score for the NF injection was 0.23 and for CHN injection it was reported as 1.07. The lower pain score post NF injection as compared with CHN injection was statistically significant (P <0.01; paired-t test for comparison of 2 means). Hence, tolerability of NFIS was proven through this study. Individual pain scores are listed in Appendix AB.

Table 11: VAS pain score assessment following NF and CHN injections

Pain score		**NF Injection (N = 30)**		**CHN Injection (N = 30)**		**P Value**
		Number (%) of subjects				
None	(0)	23	**76.7%**	9	**30.0 %**	**P <0.01**
Mild	(1, 2, or 3)	7	**23.3%**	21	**70.0 %**	
Moderate	(4, 5, or 6)	0	**0.0%**	0	**0.0%**	
Severe	(7, 8, 9, or 10)	0	**0.0%**	0	**0.0%**	
	n	30		30		
	Mean	0.23		1.07		P <0.01
	SD	0.43		1.01		
	Median	0.00		1.00		
	Min	0.00		0.00		
	Max	1.00		3.00		

9.4 FDA Toxicity Scale Assessment Conclusion

Toxicity assessments were carried out on all 30 subjects post 2 min and 20-30 min administration of needle free injection system (NF injection) and followed by conventional hypodermic needle injection (CHN injection). These assessments did not highlight any safety concern. Only one subject complained of pain post NF injection after 2 min, three following CHN injection. Tenderness was reported by two subjects for both injection types after 2 min. No other local reactions were noted. Vitals remained stable post NF injection and systemic examination did not highlight any complaints. Toxicity assessments carried out telephonically post 24, 48, and 72 hours did not bring out any complaints. None of the subjects reported any specific adverse events

following the administration of injections during the entire planned follow up period. VAS pain assessment scores demonstrated that the NF injection induced (statistically) significantly lower pain scores as compared with CHN injection. NF injection was well tolerated as that of the convention injection (CHN injection). Hence, tolerability of NFIS was proven through this study.

9.5 Acceptability Assessments

Acceptability Questionnaire Responses Analysis

Acceptability questionnaire was administered to the study subjects post administration of NF injection and CHN injection. Responses given by the subjects separately for the two injections are tabulated in Table 12. Individual subject responses are listed in Appendix AC and AD. The NF injection was generally acceptable with many questions responded as ‘not at all’ by all subjects.

Significantly higher percentage (90%) responded that they did not feel anxious about receiving the NF injection as compared with 43.3% with the CHN injection ($P < 0.01$; 2x2 chi-square test with continuity correction). All the subjects (100%) were not bothered by pain during the NF injection as compared with 43.3% in the CHN injection ($P < 0.01$; 2x2 chi-square test with continuity correction).

Table 12: Acceptability responses given by subjects' post NF and CHN injections

Question		NF Injection (N = 30)		CHN Injection (N = 30)		P Value
		Number (%) of subjects				
1. Just before your injection, did you feel anxious about receiving your injection?	Not at all	27	**90.0%**	13	**43.3%**	<0.01
	A little	3	**10.0%**	14	**46.7%**	
	Moderately	0	**0%**	3	**10%**	
	Very	0	**0%**	0	**0%**	
	Extremely	0	**0%**	0	**0%**	
2. How bothered were you by pain during the injection?	Not at all	30	**100%**	13	**43.3%**	<0.01
	A little	0	**0%**	16	**53.3%**	
	Moderately	0	**0%**	1	**3.3%**	
	Very	0	**0%**	0	**0%**	
	Extremely	0	**0%**	0	**0%**	
3. How bothered were you by redness at the injection site?	Not at all	30	**100%**	29	**96.7%**	>0.2
	A little	0	**0%**	1	**3.3%**	
	Moderately	0	**0%**	0	**0%**	
	Very	0	**0%**	0	**0%**	
	Extremely	0	**0%**	0	**0%**	
4. How bothered were you by swelling at the injection site?	Not at all	30	**100%**	29	**96.7%**	
	A little	0	**0%**	1	**3.3%**	
	Moderately	0	**0%**	0	**0%**	
	Very	0	**0%**	0	**0%**	
	Extremely	0	**0%**	0	**0%**	
5. How bothered were you by itching at the injection site?	Not at all	30	**100%**	29	**96.7%**	>0.2
	A little	0	**0%**	1	**3.3%**	
	Moderately	0	**0%**	0	**0%**	
	Very	0	**0%**	0	**0%**	
	Extremely	0	**0%**	0	**0%**	
6. How bothered were you by hardening (a bump) at the injection site?	Not at all	30	**100%**	28	**93.4%**	>0.2
	A little	0	**0%**	2	**6.6%**	
	Moderately	0	**0%**	0	**0%**	
	Very	0	**0%**	0	**0%**	
	Extremely	0	**0%**	0	**0%**	
7. How bothered were you by bruising at the injection site?	Not at all	30	**100%**	28	**93.4%**	>0.2
	A little	0	**0%**	2	**6.6%**	
	Moderately	0	**0%**	0	**0%**	
	Very	0	**0%**	0	**0%**	
	Extremely	0	**0%**	0	**0%**	
8. How acceptable was/were your local reaction(s)?	Totally acceptable	27	**90.0%**	21	**70.0%**	0.1
	Very acceptable	3	**10.0%**	5	**16.7%**	
	Moderately acceptable	0	**0%**	4	**13.3%**	
	A little acceptable	0	**0%**	0	**0%**	
	Not at all acceptable	0	**0%**	0	**0%**	
9. How acceptable was your pain?	Totally acceptable	28	**93.3%**	19	**63.3%**	0.01
	Very acceptable	2	**06.7%**	10	**33.3%**	
	Moderately acceptable	0	**0%**	1	**3.3%**	
	A little acceptable	0	**0%**	0	**0%**	
	Not at all acceptable	0	**0%**	0	**0%**	
10. How satisfied were you with the injection system that was used to administer the product?	Very satisfied	24	**80%**	17	**56.7%**	0.1
	Satisfied	6	**20%**	13	**43.3%**	
	Neither satisfied nor dissatisfied	0	**0%**	0	**0%**	
	Dissatisfied	0	**0%**	0	**0%**	
	Very dissatisfied	0	**0%**	0	**0%**	

9.6 Acceptability Conclusions

Needle free injection system was well accepted. None of the subjects receiving this injection complained of pain, redness, swelling, itching, hardening, and bruising at the injection site. More than 90% of the respondents indicated that the local reaction and pain was totally acceptable. NF injection had a significantly higher satisfaction percentage compared with the CHN injection administration.

9.7 Overall Conclusion

Findings of the study state that there is no significant difference in terms of tenderness, redness, and induration for 2 groups. Thus, the study concludes that NFIS is well tolerated just like CHN. Also, mean vital signs parameters and systemic examination parameters (nausea, diarrhea, headache, fatigue, and myalgia) at 2 min post NF injection were not statistically different from similar measurements taken post CHN injection. This finding was valid across both groups at 20-30 min post injections also. None of the subjects reported any kind of complaints on all the three instances of telephonic follow up. This indicates NFIS to be similar to CHN with respect to safety of device. The percentage of those who reported no pain post NF injection (77%) was significantly higher as compared with CHN injection group ($P < 0.01$; Chi square test). Further, significantly higher percentage (90%) responded that they did not feel anxious about receiving the NF injection as compared with 43.3% with the CHN injection. All the subjects (100%) were not bothered by pain during the NF injection as compared with 43.3% in the CHN injection ($P < 0.01$; 2x2 chi-square test with continuity correction). Hence, acceptability of NFIS was proven through this study.

10. Reference List

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11. Signature Page

Clinical Study Report Prepared By:	
I have prepared and read this report and confirm that to the best of my knowledge it accurately describes the conduct and results of the study	
Senior Medical Writer	Name: Dr. Arati Ranade Jehangir Clinical Development Center, Pvt. Ltd. Jehangir Hospital Premises, 32, Sasoon Road, Pune 411001 A N Ranade Date and Signature: 14-Mar-2022
Bio- Statistician	Name: Dr. B. Kishore Kumar B. Kishore Kumar Date and Signature: 14-Mar-2022
Clinical Study Report Approved By:	
I have read this report and confirm that to the best of my knowledge it accurately describes the conduct and results of the study.	
Sponsor Representative	Name: Address: Date and Signature:
Investigator	Name: Dr. Almas Pathan Jehangir Clinical Development Center, Pvt. Ltd. Jehangir Hospital Premises, 32, Sasoon Road, Pune 411001 Date and Signature: 14/MAR/2022

Immunogenicity & Safety Study of Covid Vaccine in Adults, India

Title: A pilot open label, randomized study to investigate the performance of the IntegriMedical Needle Free Injection System by assessment of Immunogenicity in subjects receiving booster dose of COVID-19 in comparison to subjects receiving booster dose of COVID-19 using a conventional hypodermic needle.

CONFIDENTIALITY STATEMENT

This confidential document is the property of Sponsor- IntegriMedical. No published and unpublished information contained herein may be disclosed to third parties without prior written approval from IntegriMedical.

TABLE OF CONTENT

Principle investigator:	Dr. Rajnish Nagarkar
Sponsor:	IntegriMedical
Sponsor Authorized Signatory:	Scott McFarland
Study initiated on:	26 Feb 2022
Date of early study termination if any:	Not Applicable
Study completed on:	20 Apr 2022
Version:	1.0
Name of investigational medical device:	IntegriMedical Needle Free Injection System
Name of COVID-19 booster dose used:	COVISHIELD
Protocol identification:	NFIS.2022, Version 1.0
Name of affiliation of principle investigator:	Dr. Rajnish Nagarkar, Manavata Clinical Research Institute, Behind Shivang Auto Mumbai Naka , Nashik -422002,Maharashtra, India
Date of clinical study report:	16th May 2022
Prepared by:	Manavata Clinical Research Centre, Nashik.

1. LIST OF ABBREVIATIONS OF TERMS

Abbreviations	Full Name
AE	Adverse Event
CRF	Case Report Form
CRO	CRO Contract Research Organization
ICF	ICF Informed Consent Form
ICH-GCP	International conference of Harmonization – Good Clinical Practice
ICMR	Indian Council of Medical Research Ethical Guidelines for Biomedical Research on Human Subjects
IEC	Institutional Ethics Committee
IMD	Investigational Medical Device
IRB	Institutional Review Board
MGRS	Multicentre Growth Reference Study
NFIS	IntegriMedical Needle Free Injection System
SAE	Serious Adverse Event
SOP	Standard Operating Procedure
WHO	World Health Organization
LAR	Legally Acceptable Representative

2. INDICATION STUDIED

Patient immunogenic response to COVID-19 booster dose when administered using the IntegriMedical Needle Free Injection System compared with conventional hypodermic needle.

3. INVESTIGATOR AND STUDY ADMINISTRATIVE STRUCTURE

Principal Investigator:	Dr. Rajnish Nagarkar
Sponsor:	IntegriMedical
Clinical Laboratory:	Metropolis Lab
Clinical Study Site:	Manavata Clinical Research Institute, Behind Shivang Auto, Mumbai Naka, Nashik – 422002 Maharashtra, India

4. ETHICS

4.1. INSTITUTIONAL ETHICS COMMITTEE (IEC)

The protocol and consent form were reviewed and approved by the Institutional Ethics Committee of MCRI. The EC is registered with the CDSCO (Registration No.-ECR/500/Inst/MH/2013/RR-17) and accredited by Association for the Accreditation of Human Research Protection Program (AAHRPP). The Ethics Committee is accredited by National Accreditation Board for Hospitals and Health Care Providers (NABH) (Certificate No. EC-CT-2020-0146).

4.2. ETHICAL CONDUCT OF THE STUDY

This study was performed in compliance with ICH E6R2 “Guidance on Good Clinical Practice”, Indian Good Clinical Practices Guideline, National Ethical Guidelines for Biomedical and Health Research involving Human Participants, ICMR 2017, Declaration of Helsinki and relevant SOPs of Manavata Clinical Research Institute, Nashik, Maharashtra, India.

4.3. PATIENT INFORMATION AND CONSENT

The informed consent was obtained from the subject or LAR of the subject by the Principal Investigator. Subject / LAR provided written consent to participate in the study after having been informed about the nature and purpose of the study, participation/termination conditions, risks, burdens, and benefits of treatment. Personal data from subjects enrolled in this study were limited to those necessary to investigate the safety and tolerability of the investigational study device used in this study.

5. INTRODUCTION AND BACKGROUND INFORMATION

Drug delivery refers to the technology utilized to present the drug to the desired body site for drug release and absorption, or the subsequent transport of the active ingredients across the biological membranes to the site of action. A drug delivery system is a formulation or a device that enables the introduction of a therapeutic substance in the body and improves its efficacy and safety by controlling the rate, time, and place of release of drugs in the body.

Certain pharmaceuticals cannot be delivered orally due to susceptibility to enzymatic degradation and poor absorption due to their molecular size. Such pharmaceuticals are administered through the parenteral route by using hypodermic needle and a syringe. The use of hypodermic needles and syringes is very common and the oldest way to overcome the physical barrier, wherein, the solution of a drug is forced under piston stress straight into the bloodstream or tissue. This necessitates skin perforation using a needle, which is associated with trauma and pain. To overcome these drawbacks, other alternative methods have been investigated like jet injections, dermabrasion, thermal ablation, laser, tape stripping, etc. Reduction of the pain and time of injections may lead to improved patient satisfaction and compliance, as well as reduced anxiety in populations of patients who require frequent or ongoing injections to treat their medical conditions. A needle-free delivery system offers the potential to address such issues. They may enhance safety, improve dosing accuracy, and increase patient compliance, particularly in self administration settings. The needle free injection technology does not involve the use of needles for delivery of pharmaceutical, instead it is delivered via a high-pressure stream of liquid which penetrates the site of injection. The needle free injection technology has been reported to overcome some of the risks of needles including reduced risk of needle stick injury, eliminated risk of disease transmission from reused needles, reduce scar tissue at the injection site caused by needle damage to the tissue, easier self-administration, etc. The needle free injection works on different technologies including spring system, gas propelled system, etc. The newly designed IntegriMedical Needle Free Injection Systems have overcome most of the risks posed by needles by incorporating disposable cartridges to avoid infection, introducing adjustable parameters selected according to skin site properties and thickness as well as the desired depth level intended to deliver the medication. IntegriMedical® Needle Free Injection System (NFIS) is intended to deliver drugs and biologics through intramuscular, or subcutaneous sites. Typical doses range from 0.1 ml to 0.5 ml and are delivered to various injection depths. The energy for the device comes from the compressed spring. When the compressed spring is released, it propels the plunger forward delivering the medication at high speed thus penetrating the skin.

6. STUDY OBJECTIVES AND ENDPOINTS

6.1. STUDY OBJECTIVES

6.1.1. PRIMARY OBJECTIVE

To investigate the performance of the IntegriMedical Needle Free Injection System in subjects receiving COVID-19 booster dose to demonstrate non-inferiority as compared to subjects receiving the same booster dose with a conventional hypodermic needle and syringe.

6.1.2. SECONDARY OBJECTIVES

To understand the tolerability of the IntegriMedical Needle Free Injection System in terms of pain and comfort and to demonstrate non-inferiority of the needle free injection as compared to subjects receiving the same booster dose using a conventional hypodermic needle.

6.2. ENDPOINTS

6.2.1. PRIMARY ENDPOINTS

Change in immunoglobulin levels (IgG, IgA, and IgM) at 2 weeks of receiving booster dose of COVID-19 vaccine in comparison to baseline.

6.2.2. SECONDARY ENDPOINTS

Pain assessment using 100-mm VAS scores (0 mm = no pain at all; 100 mm = a lot of pain) immediately after each administration (before needle removal)**.**

7. INVESTIGATIONAL PLAN

7.1. OVERALL STUDY DESIGN

7.1.1. VISIT 1 – BEGINNING OF STUDY – DAY 0

1. A written informed consent will be given to the subject.
2. Eligibility criteria shall be verified.
3. Pre-work activities shall be conducted within 3 days prior to the commencement of the study. Following pre-work activities shall be performed after obtaining a written informed consent from the subject.
    a. Demographic parameters like age, sex, height and weight will be recorded.
    b. Medical history will be recorded.
    c. The vital signs (including heart rate, respiratory rate, SpO2, blood pressure, and body temperature) and clinical examination of body systems shall be performed and recorded.
    d. Urine pregnancy test will be performed for female participants of childbearing potential.

e. A blood sample will be collected to determine immunoglobulin (IgG, IgA and IgM) concentration before vaccination.

4. The study shall be commenced with the following activities.
   a. Vaccine booster dose will be administered using one of the administering methods. This will be denoted as DAY 0.
   b. VAS Score worksheet shall be given to the patient to indicate the pain assessment.
   c. The subject will be kept under observation for 30 mins after vaccination.
   d. Adverse reactions observed by the subject or the doctor during the post vaccination observation period will be recorded.
   e. A diary card will be issued to record local and systemic adverse reactions observed in the post vaccination observation period.
   f. The subject will be instructed to bring the diary at the next visit.

7.1.2. VISIT 2 – END OF STUDY – DAY 14 (TOLERANCE OF +3 DAYS)

1. Recording the vital signs (including heart rate, respiratory rate, SpO2, blood pressure, and body temperature) and clinical examination of body systems was performed.
2. Adverse reactions observed by the subject or the doctor during the post vaccination observation period were reported.
3. A blood sample was collected to determine immunoglobulin (IgG, IgA and IgM) concentration after vaccination.

7.2. INCLUSION / EXCLUSION CRITERIA

7.2.1. INCLUSION CRITERIA:

1. Healthy subject of either gender ≥ 18 years of age.
2. Subjects who have completed 2 doses of vaccines were eligible for booster dose of vaccination as per CoWIN registration.
3. Subjects who were able to provide consent.
4. Subjects willing to allow storage and use of biological samples for future research.

7.2.2. EXCLUSION CRITERIA:

1. Known SARS-CoV-2 positive (RTPCR).
2. History of contact with a confirmed active SARS-CoV-2 positive patient within 14 days.
3. Febrile illness (temperature ≥ 38°C or 100.4°F) or any acute illness or infection within 4 weeks of enrolment.
4. Subjects with confirmed immunosuppressive or immunodeficiency disorder; or subjects on any immunosuppressive or immunostimulant therapy.

5. Subjects who have administered blood, blood containing products or immunoglobulins within the last 3 months or planned administration during the study.
6. Any other vaccine administration within the last 30 days or planned to be administered during the study period.
7. Pregnant and lactating women.
8. Hypersensitivity reaction or any serious adverse event after any vaccination
9. Uncontrolled Co-morbidities.
10. History of drug / alcohol abuse.
11. Covid-19 sign and symptoms.
12. History of skin diseases or chronic eczema and any coagulation disease.

Table 1: SCHEDULE OF ASSESSMENTS

Sr. No.	Assessment	Visit – 1	Visit – 2
1	Informed consent process	☒	☐
2	Eligibility criteria	☒	☐
3	Demographics (Age, Sex, Height, Weight and BMI)	☒	☐
4	Medical history	☒	☐
5	Clinical examination	☒	☒
6	Vital signs	☒	☒
7	Vaccination (Booster Dose)	☒	☒
8	Immunogenicity (IgG, IgA and IgM)	☒	☒
9	VAS Pain Score Assessment	☒	☐

7.3. TREATMENT PLAN

7.3.1. TREATMENTS ADMINISTERED AND IDENTITY OF INVESTIGATIONAL PRODUCT(S)

7.3.1.1. INVESTIGATIONAL MEDICAL DEVICE:

IntegriMedical® Needle Free Injection System (NFIS).

7.3.1.2. MODE OF ADMINISTRATION:

The COVID-19 booster doses will be administered through the Intramuscular route for both methods of administration.

7.3.1.3. ADMINISTRATION SCHEDULE:

Subjects will be randomly selected to receive the booster dose of COVID-19 vaccine, from which 50% of population got Covid-19 vaccines by hypodermic needle while 50% of population by NFIS.

7.3.2. METHOD OF ASSIGNING SUBJECTS TO TREATMENT GROUPS

METHODOLOGY: In this study, subjects will be randomly assigned to receive a booster dose of COVID-19 vaccine by one of the following methods:

1. Group T1: Hypodermic Needle and Syringe
2. Group T2: IntegriMedical Needle Free Injection System

A target of 80 subjects (with a minimum of 60 subjects) will be assigned to Group T1 and another 80 subjects (with a minimum of 60 subjects) will be assigned to Group T2. The randomization schedule will be generated using SAS® software (Version: 9.4 or higher; SAS Institute Inc., USA).

7.3.3. ANALYSIS OF TOLERABILITY MEASUREMENTS:

Tolerability shall be determined using a VAS score methodology.

7.3.4. STATISTICAL ANALYSIS:

Statistical analysis was performed using the SPSS Version 25 and Stata 15 software. All available data was used in the analysis.

8. PROTOCOL DEVIATIONS:

There were no protocol deviations noted in the conduct of the study. All volunteers complied to the various trial related procedures and the study was conducted in compliance with the study protocol.

9. CLINICAL STUDY RESULTS:

9.1. STUDY SUBJECTS:

160 healthy volunteers provided consent and were found eligible for participation in the study. All 160 participants were enrolled. However, only 138 volunteers were successfully administered with the booster dose due to various reasons unrelated to the study. Data generated on these 138 healthy volunteers who received both the intervention and control injections was analysed and forms the basis of this report.

9.2. DEMOGRAPHICS:

A total of 138 subjects received the booster dose under the study. 71 participants received booster dose by method T1 (Hypodermic Needle and Syringe), and 67 participants received the booster dose by method T2 (NFIS). The demographics of the participants for each group is as shown in

Table 2.

Table 2: Demographic distribution of subjects.

Demographics		Group T1		Group T2 (NFIS)	
		N (71)	%	N* (67)	%
Age	Mean (Years)	56.34		52.66	
	Range (Years)	24 - 87		20 - 85	
Sex	Female	38	53.5	36	53.7
	Male	33	46.5	31	46.3
Weight	Mean (Kg)	66.2		65.0	
	Range (Kg)	36.6 – 89		40.4 – 91	
Height	Mean (cm)	155.8		155.1	
	Min (cm)	143		132	
	Max (cm)	182		184	
BMI	Below 18.5	2	3.0	3	4.5
	18.5-24.9	18	26.9	21	31.3
	25.0-29.9	30	44.8	20	29.9
	Above 30.0	17	25.4	23	34.3

**Height and weight was not captured for 2 subjects. Hence, they were excluded from all analyses.*

9.3. PAST AND CURRENT MEDICAL HISTORY:

None of the study subjects reported any past / current medical history (Appendix B).

9.4. VITAL SIGNS:

Vital signs of the study subjects at screening are summarized in **Table 3**. The study subjects had 'normal' body temperature, heart rate, respiratory rate, and blood pressure at the time of screening.

Table 3: Subject characteristics at baseline - Vital signs.

Vital signs		Group T1	Group T2 (NFIS)
Systolic Blood Pressure (mm Hg)	Mean	125.25	124.61
	SD	3.72	5.32
	Min	118	105
	Max	132	137
	Interpretation Normal	**100%**	**100%**
Diastolic Blood Pressure (mm Hg)	Mean	75.92	76.94
	SD	6.07	6.58
	Min	67	65
	Max	89	96
	Interpretation Normal	**100%**	**100%**
Body Temperature (ºF)	Mean	97.6	97.5
	SD	0.8	0.9
	Min	95	94
	Max	99.1	99.4
	Interpretation Normal	**100%**	**100%**
Pulse Rate (bpm)	Mean	77.7	78
	SD	7.7	8.5
	Min	65	66
	Max	89	103
	Interpretation Normal	**100%**	**100%**
Respiratory Rate (bpm)	Mean	17.85	17.87
	SD	0.87	1.09
	Min	16	16
	Max	20	20
	Interpretation Normal	**100%**	**100%**

9.5. IMMUNOLOGY DATA AND ANALYSIS

For Hypodermic Needle and IntegriMedical Needle Free Injection System, pre and after dose Mean value of concentration of IgG, IgA and IgM is given in ***Table 4***.

Table 4: Summary statistics of concentration of IgG, IgA, and IgM

Immunological Parameters		**Group T1 (N=71)**			**Group T2 (NFIS) (N=67)**		
		Pre Dose	**Post Dose**	**P-value (paired t-test)**	**Pre Dose**	**Post Dose**	**P-value (paired t-test)**
IgG concen.	Mean	1083.32	1296.77	0.000	1107.93	1306.75	0.000
	STDEV	174.86	198.32		211.61	197.35	
IgA concen.	Mean	193.24	304.08	0.000	188.88	282.95	0.000
	STDEV	64.32	66.74		63.11	77.02	
IgM concen.	Mean	119.80	197.01	0.000	124.24	189.37	0.000
	STDEV	50.32	55.42		57.10	49.24	

Change in immunoglobulin levels ($p<0.05$, paired t-test) of IgG, IgA, and IgM concentration, before and after vaccination was found to be increased in both groups.

Distribution of immunoglobulin levels shown in Box plot (Shown in Error! Reference source not found.**, Graph 2, Graph 3**)

Interpretation of Box plot (Error! Reference source not found.**, Graph 2, Graph 3**) Box plots are used to show overall patterns of response for a group. They provide a useful way to visualise the range and other characteristics of responses for a large group. The middle “box” represents the middle 50% of the group. The range of concentration value from lower to upper quartile is referred to as the inter-quartile range. The middle 50% of population fall within the inter-quartile range.

The minimum is the far-left hand side of the graph, at the tip of the left whisker. Q1 is represented by the far-left hand side of the box, The median is represented by the vertical bar. The maximum is the end of the “whiskers”. Small circles or Filled circles are used for known outliers.

In Error! Reference source not found., IgG pre vaccination concentration was found to have a similar distribution for T1 and T2 group as median (middle of “box”) has

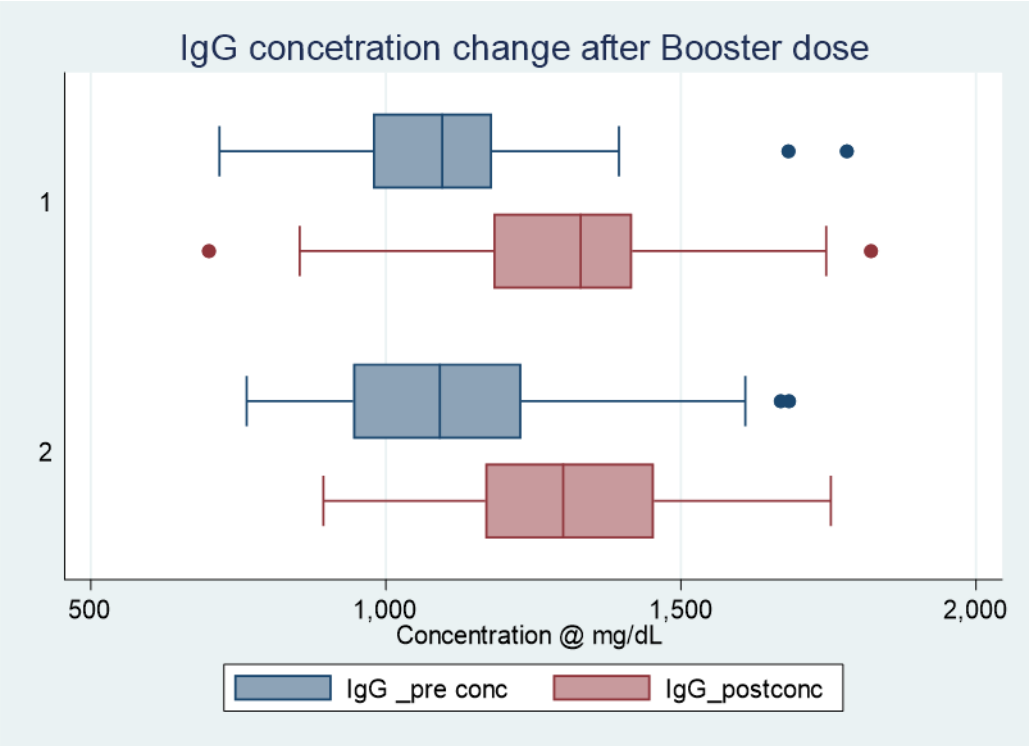
fall concentration around 1100mg/dl and 900 mg/dl for q1(left hand side of the box) and 1250mg/dl for q2(right hand side of the box), however post IgG concentration has been increased from pre concentration as median fall around 1300 mg/dl.

Similarly, IgA and IgM concentration level has been increased from pre to post vaccination for both groups.

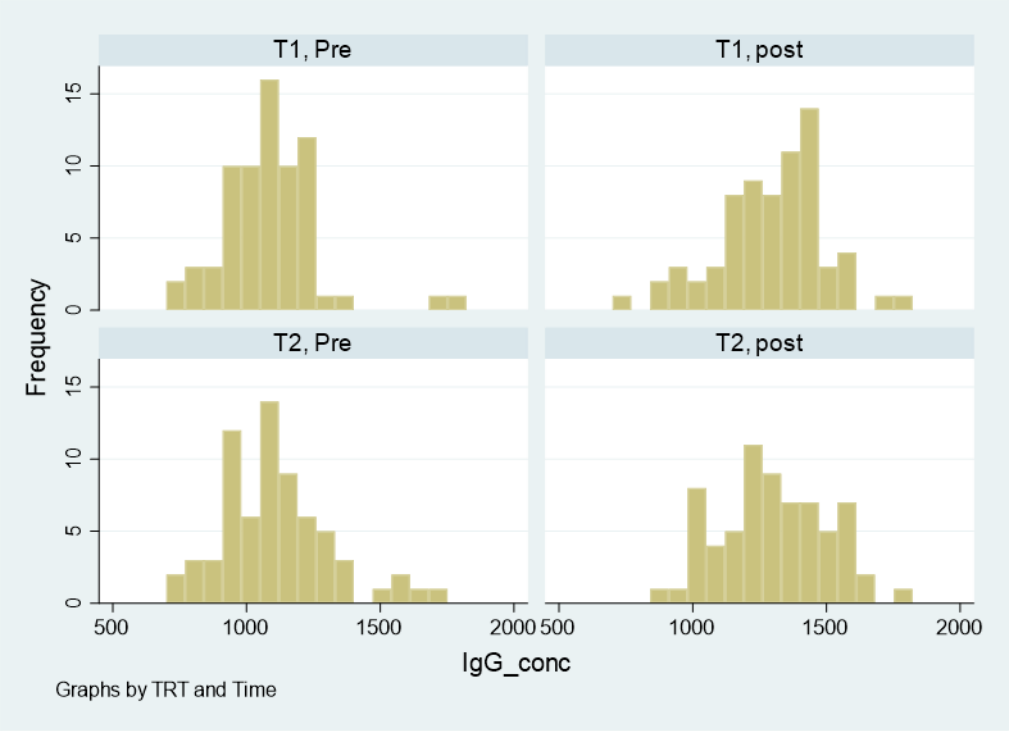
It is also observed that in T2 group (needle less vaccination) some of the cases achieved increased concentration level of IgG, IgA and IgM after vaccination as compared to T1 group. (Shown in Graph 1 A, Graph 2 A, Graph 3 A)

*Overall, the Immunoglobulin levels were at par or better than the hypodermic needle.

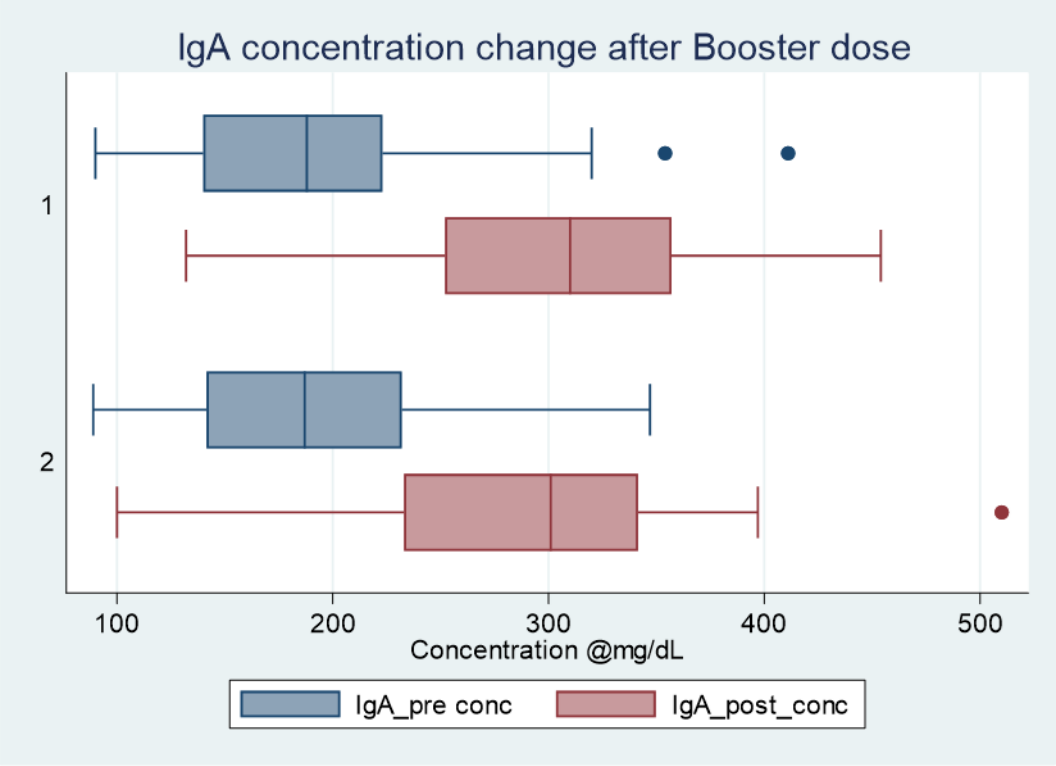
Graph 1



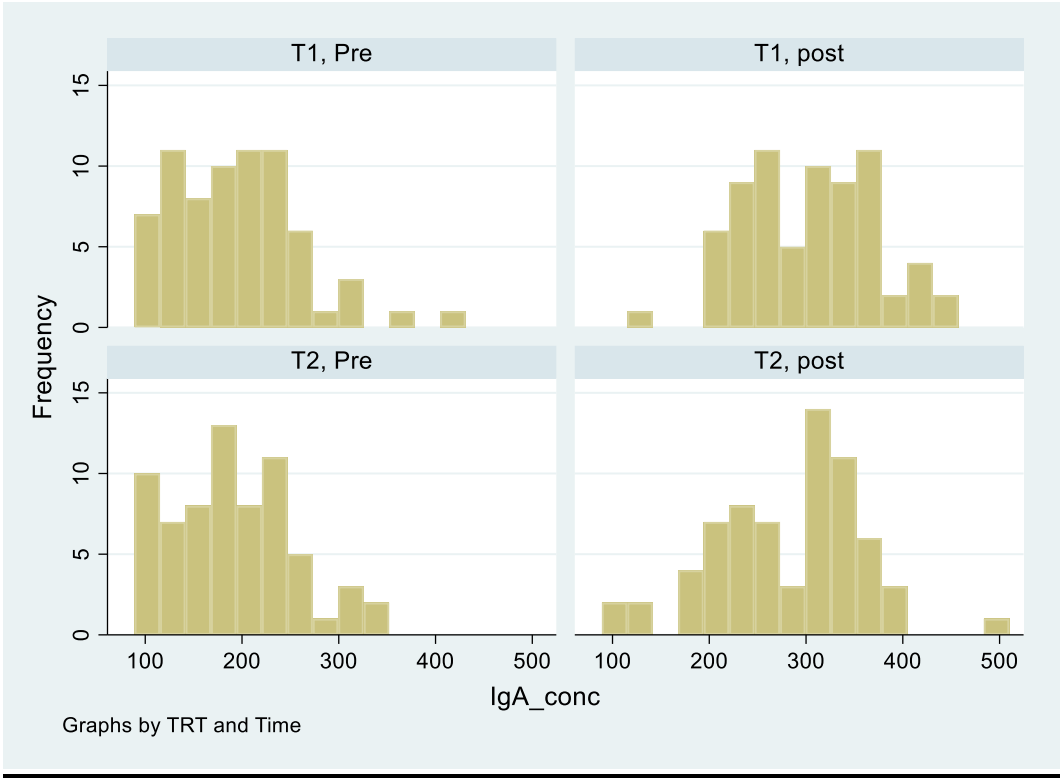
Graph 1 A : <u>Frequency distribution of IgG pre and post concentration of both groups:</u>



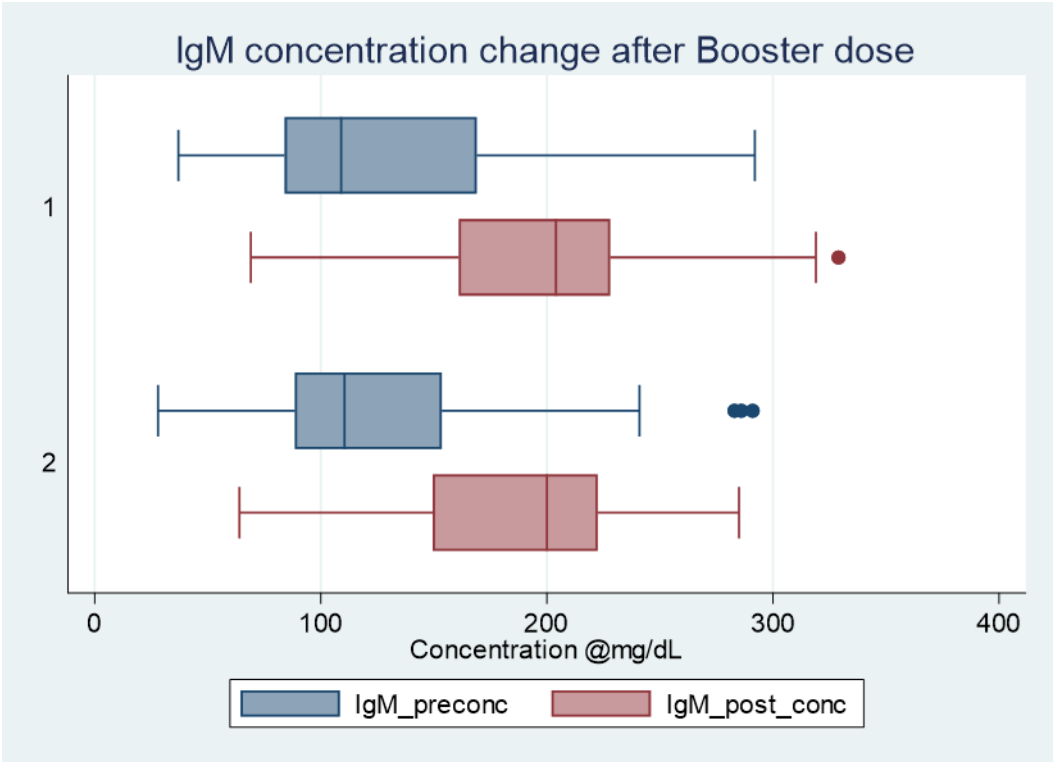
Graph 2



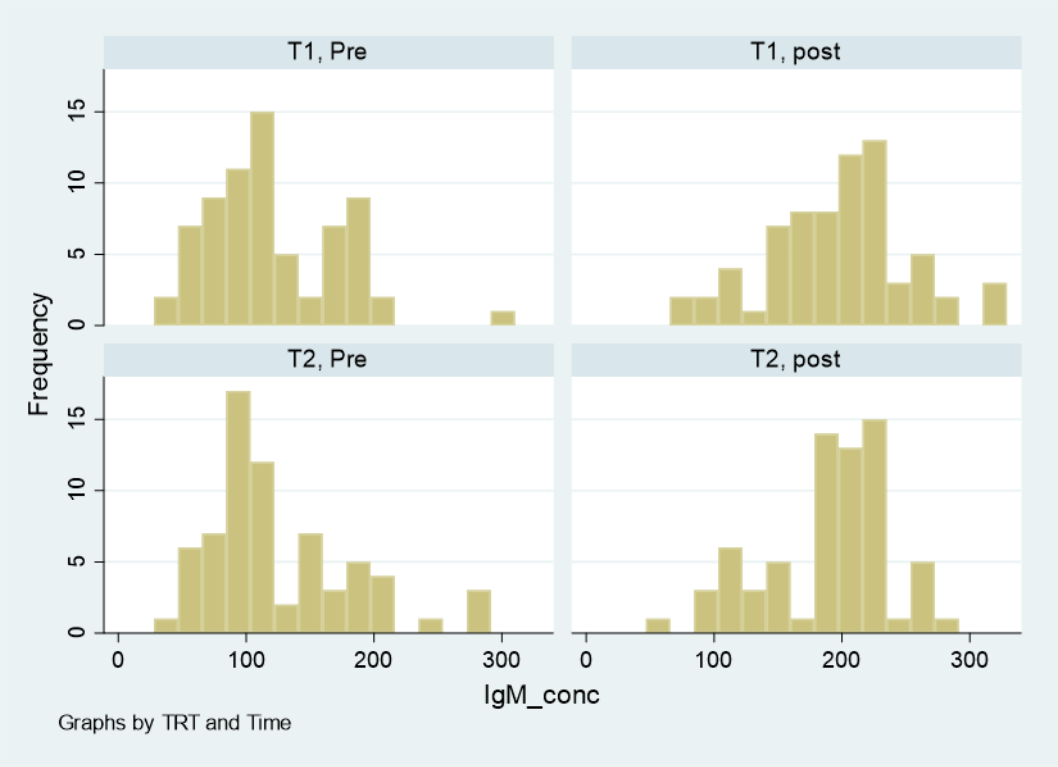
***Graph 2 A*: Frequency distribution of IgA pre and post concentration of both groups:**



Graph 3



***Graph 3 A*: Frequency distribution of IgM pre and post concentration of both groups:**



9.6. STATISTICAL INFERENCE FOR SIGNIFICANCE OF CHANGE:

To evaluate statistical significance in change found between Immunoglobin level after 2 weeks of booster dose. Mixed model has been fitted for immunoglobin level (concentration of IgG, IgA, and IgM) with respect to time (pre dose and post dose) and study population (T1 Vs T2). It was found that there was significant difference($P<0.05$) between pre dose and post dose concentration level of Immunoglobins IgG, IgA and IgM. IgG mean concentration of post dose was greater than 0.1754 units to pre dose concertation. IgA mean post dose concentration was found to be greater than 0.4561 units to pre dose concentration and IgM post dose concentration was greater than 0.5099 unit to mean pre dose concentration. The results show that for IgG concentration mean of T2 is 0.0124 unit greater than T1, and for IgM concentration mean of T2 is 0.0018 unit greater than to T1. Although Concentration of IgG, IgA and IgM are not significantly different for T1 and T2 group ($p>0.05$) (given in Table 5). Hence, it could be concluded that Increased change in immunoglobin level after 2 weeks of booster dose has been found similar for subjects dosed with Hypodermic Needle and IntegriMedical Needle Free Injection System.

Table 5: Model Estimation to show comparison of Change in Concentration of IgG, IgA, and IgM with respect to Dosing Method and baseline (Pre) concentration value.

Parameters compared	IgG concentration		IgA concentration		IgM concentration	
	Estimate	p-value	Estimate	p-value	Estimate	p-value
Dosing method T1 vs Dosing method T2	0.01248	0.528	-0.0525	0.159	0.0018	0.968
Time pre vs post	0.1754	0.000	0.4561	0.000	0.5099	0.000

10. SAFETY EVALUATION (RESULTS AND DISCUSSION):

Vital signs including body temperature, heart rate, blood pressure, and respiratory rate were measured after administration of needle free injection and needle injection post 30 min.

Table 6 below summarizes the data for both these groups at two time points defined.

Mean vital sign parameters after 30 mins of injecting with IntegriMedical Needle Free Injection System were not statistically (P >0.05) different from similar measurements taken after injecting with needle injection, except for systolic blood pressure that is because of pain due to injection with needle while no pain in needle less injection.

10.1. VITAL SIGNS (POST VACCINATION -After 30 min of Vaccination)

Table 6:

Demographics		Group T1	Group T2 (NFIS)
Systolic Blood Pressure (mm Hg)	Mean	129.83	128.18
	SD	2.76	3.72
	Min	122	118
	Max	138	136
Diastolic Blood Pressure (mm Hg)	Mean	81.07	80.07
	SD	4.5	4.88
	Min	72	72
	Max	90	88
Body Temperature (oF)	Mean	97.58	97.86
	SD	0.67	0.58
	Min	95	96.8
	Max	98.7	99
Pulse Rate (bpm)	Mean	80.81	81.19
	SD	6.10	5.92

	Min	68	70
	Max	90	98
Respiratory Rate (bpm)	Mean	17.77	17.71
	SD	0.68	0.92
	Min	17	16
	Max	20	20

10.2. VAS Pain Assessment Score (2 min post injections):

Pain score was assessed within 2 min following the Needle free injection and needle injection (**Table 7**). The percentage of those who reported no pain post needle free injection (86%) was significantly higher as compared with needle injection group (P <0.01). Mean pain score for the Needle free injection was 0.16 and for needle injection was found to be 2.6. The lower pain score measured post Needle free injection as compared with needle injection was statistically significant (P <0.01; t test for comparison of 2 means). Hence, tolerability of needle free injection was determined through the VAS Pain Assessment score.

Table 7: VAS pain score assessment following Needle Injection and Needle free injections.

	Group T1 (N=71)	**Group T2 (NFIS) (N=67)**	**P-value**
0.00 (No pain)	0(0%)	59(86.76%)	0.000
1.00	5(7.14%)	8(11.76%)	
2.00	23(32.85%)	0(0%)	
3.00	37(52.85%)	1(1.4%)	
4.00	5(7.14%)	0(0%)	
Mean	2.6	0.16	
SD	0.73	0,47	
Min	1	0	
Max	4	3	

11. References

Barolet D, B. A. (2018). Current trends in needle-free jet injection: an update. *Clin CosmetInvestig Dermatol*.

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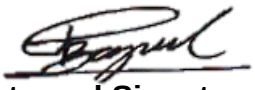
Dias C, A. B. (2015). Tolerability of High-Volume Subcutaneous Injections of a Viscous Placebo Buffer: A Randomized, Crossover Study in Healthy Subjects. *AAPS PharmSciTech*.

Dinesh K. Mishra, V. P. (2019). Fundamentals of Drug Delivery Advances in Pharmaceutical Product Development and Research. *Chapter 15 - Cutaneous and Transdermal Drug Delivery: Techniques and Delivery System*, Pages 595-650.

Kojic N, G. P. (2017). An Innovative Needle-free Injection System: Comparison to 1 ml Standard Subcutaneous Injection. *AAPS PharmSciTech*.

Ravi AD, S. D. (2015). Needle free injection technology: A complete insight. Int J Pharm Investig. *Int J Pharm Investig*.

12. Signature Page:

Clinical Study Report Prepared By:	
I have prepared and read this report and confirm that to the best of my knowledge it accurately describes the conduct and result of the study.	
Medical Writer	**1) Name:** Dr. Shrikant Suryavanshi Manavata Clinical Research Institute, Behind Shivang Auto, Mumbai Naka, Nashik, 422002  **Date and Signature: 18-May-2022** **2) Name:** Mr. Roshan Patil Manavata Clinical Research Institute, Behind Shivang Auto, Mumbai Naka, Nashik, 422002.  **Date and Signature**: **18-May-2022**
Biostatistician	**Name:** Mr. Dilesh Bagul  **Date and Signature**: **18-May-2022**
Clinical Study Report Approved By:	
I have read this report and confirm that to the best of my knowledge it accurately describes the conduct and result of the study.	
Sponsor Representative	**Name:** Scott McFarland **Address:** INTEGRIMEDICAL LLC 805 N,4th AVENUE, UNIT 903, PHOENIX. ARIZONA 85003 **Date and Signature:**

Immunogenicity & Safety Study of Covid Vaccine in Children, India

Title: A pilot open label, randomized study to investigate the tolerability, acceptability and safety of IntegriMedical Needle Free Injection System in subjects receiving dose of COVID-19 Corbevax Vaccine.

CONFIDENTIALITY STATEMENT

This confidential document is the property of Sponsor- IntegriMedical No published and unpublished information contained herein may be disclosed to third parties without prior written approval from IntegriMedical.

TABLE OF CONTENTS

Principle Investigator:	Dr. Rajnish Nagarkar
Sponsor:	IntegriMedical
Sponsor Authorized signatory:	Scott McFarland
Date of study initiated:	12 Apr 2022
Date if study completed:	25 Apr 2022
Date of early study termination if any:	Not applicable
Date of Clinical study report:	05 June 2022
Name of investigational medical device:	IntegriMedical Needle Free Injection System
Name of COVID-19 vaccine:	Corbevax
Version:	1.0
Protocol identification:	NFIS.2022,02 Version 1.0
Name and affiliation of principle investigator:	Dr. Rajnish Nagarkar, Manavata Clinical Research Institute, Behind Shivang Auto Mumbai Naka, Nashik -422002, Maharashtra, India
Prepared by:	Manavata Clinical Research Centre, Nashik

1. LIST OF ABBREVIATIONS OF TERMS

Abbreviations	Full Name
AE	Adverse Event
CRF	Case Report Form
CRO	CRO Contract Research Organization
ICF	ICF Informed Consent Form
ICH-GCP	International conference of Harmonization – Good Clinical Practice
ICMR	Indian Council of Medical Research Ethical Guidelines for Biomedical Research on Human Subjects
IEC	Institutional Ethics Committee
IMD	Investigational Medical Device
IRB	Institutional Review Board
SAE	Serious Adverse Event
SOP	Standard Operating Procedure
WHO	World Health Organization
LAR	Legally Acceptable Representative
NFIS	Needle Free Injection system

2. INDICATION STUDIED

Patient's tolerance to COVID-19 Corbevax vaccine when administered using the IntegriMedical Needle Free Injection System.

3. INVESTIGATOR AND STUDY ADMINISTRATIVE STRUCTURE

Principal Investigator:	Dr. Rajnish Nagarkar
Sponsor:	IntegriMedical
Clinical Laboratory:	Metropolis Lab
Clinical Study Site:	Manavata Clinical Research Institute, Behind Shivang Auto, Mumbai Naka, Nashik – 422002 Maharashtra, India

4. ETHICS

4.1. INSTITUTIONAL ETHICS COMMITTEE (IEC)

The protocol and consent form were reviewed and approved by the Institutional Ethics Committee of MCRI. The EC is registered with the CDSCO (Registration No.-ECR/500/Inst/MH/2013/RR-17 and accredited by Association for the Accreditation of Human Research Protection Program (AAHRPP). The Ethics Committee is accredited by National Accreditation Board for Hospitals and Health Care Providers (NABH) (Certificate No. EC-CT-2020-0146).

4.2. ETHICAL CONDUCT OF THE STUDY

This study was performed in compliance with ICH E6R2 "Guidance on Good Clinical Practice", Indian Good Clinical Practices Guideline, National Ethical Guidelines for Biomedical and Health Research involving Human Participants, ICMR 2017, Declaration of Helsinki and relevant SOPs of Manavata Clinical Research Institute, Nashik, Maharashtra, India.

4.3. PATIENT INFORMATION AND CONSENT

The informed consent was obtained from the subject/LAR of the subject by the Principal Investigator. Subject/LAR provided written consent to participate in the study after having been informed about the nature and purpose of the study, participation/termination conditions, risks, burdens, and benefits of treatment. Personal data from subjects enrolled in this study were limited to those necessary to investigate the safety and tolerability of the investigational study device used in this study.

5. INTRODUCTION AND BACKGROUND INFORMATION

Drug delivery refers to the technology utilized to present the drug to the desired body site for drug release and absorption, or the subsequent transport of the active ingredients across the biological membranes to the site of action. A drug delivery system is a formulation or a device that enables the introduction of a therapeutic substance in the body and improves its efficacy and safety by controlling the rate, time, and place of release of drugs in the body.

Certain pharmaceuticals cannot be delivered orally due to susceptibility to enzymatic degradation and poor absorption due to their molecular size. Such pharmaceuticals are administered through the parenteral route by using hypodermic needle and a syringe. The use of hypodermic needles is very common and the oldest way to overcome the physical barrier. A solution of a drug is forced under piston stress straight into the bloodstream or tissue. This necessitates skin perforation using a needle, which is associated with trauma and pain. To overcome these drawbacks, other alternative methods have been investigated like jet injections, dermabrasion, thermal ablation, laser, tape stripping, etc. Reduction of the pain and time of injections may lead to improved patient satisfaction and compliance, as well as reduced anxiety in populations of patients who require frequent or ongoing injections to treat their medical conditions. A needle-free delivery system offers the potential to address such issues. They may enhance safety, improve dosing accuracy, and increase patient compliance, particularly in self administration settings. The needle free injection technology does not involve the use of needles for delivery of pharmaceutical and instead is delivered via a high-pressure stream of liquid which penetrates the site of injection. The needle free injection technology has been reported to overcome some of the risks of needles including reduced risk of needle stick injury, eliminated risk of disease transmission from reused needles, reduce scar tissue at the injection site caused by needle damage to the tissue, easier self-administration, etc. The needle free injection works on different technologies including spring system, gas propelled system, etc. The newly designed needle free injection systems have overcome most of the risks posed by needles by incorporating disposable cartridges to avoid infection, introducing adjustable parameters selected according to skin site properties and thickness as well as the desired depth level intended to deliver the medication. IntegriMedical® Needle Free Injection System (NFIS) is intended to deliver drugs and biologics through intramuscular, or subcutaneous sites. Typical doses range from 0.1 ml to 0.5 ml and are delivered to various injection depths.

6. STUDY OBJECTIVE AND ENDPOINTS

6.1. STUDY OBJECTIVE

To investigate the tolerability, acceptability, and safety of IntegriMedical Needle Free Injection System to demonstrate its performance.

6.2. STUDY ENDPOINTS

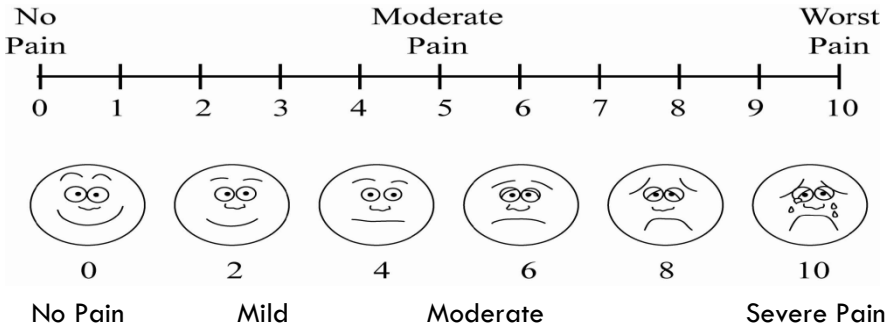
6.2.1. PRIMARY ENDPOINTS

Injection site monitoring including Redness, Swelling, Itching.

6.2.2. SECONDARY ENDPOINTS

Pain assessment using 10-mm VAS scores (0 mm = no pain at all; 10 mm = a lot of pain) immediately after each administration (before needle removal)

Visual Analogue Scale (VAS)



Correlation between Visual and verbal scale:

1-3 = mild pain; minimal impact on ADL's

4-6 = moderate pain; moderate impact on ADL's

7-10 = severe pain; major impact on ADL's

7. INVESTIGATIONAL PLAN

7.1. OVERALL STUDY DESIGN

7.1.1. VISIT 1 – BEGINNING OF STUDY (DAY 0):

1. A written informed consent will be given to the subject.
2. Eligibility criteria will be verified.
3. Pre-work activities shall be conducted prior to the commencement of the study. Following pre-work activities shall be performed after obtaining a written informed consent from the subject.
   a. Demographic parameters like age, sex, height and weight will be recorded.
   b. Medical history will be recorded.
   c. Vital signs (including heart rate, respiratory rate, SpO2, blood pressure, and body temperature) recording and clinical examination of body systems will be performed.
4. The study shall be commenced with the following activities.
   a. Covid 19 Vaccine dose will be administered using IntegriMedical NFIS device.
   b. VAS Score worksheet shall be given to the patient to indicate the pain assessment.
   c. Subject will be observed for 30 minutes after vaccination.
   d. Adverse reaction either volunteered by the subject or noticed by the doctor during the post vaccination observation period will be reported.
   e. A diary card will be issued to record local and systemic adverse reactions that are observed during the post vaccination observation period.
   f. The subject will be instructed to bring the diary card during subsequent visit.

7.1.2. VISIT 2 (DAY 3):

1. Vital signs (including heart rate, respiratory rate, SpO2, blood pressure, and body temperature) recording and clinical examination of body systems will be performed.
2. Adverse reaction either volunteered by the subject or noticed by the doctor during the post vaccination observation period will be reported.
3. Vaccination site monitoring will be conducted.

7.1.3. VISIT 3 (DAY 7):

1. Vital signs (including heart rate, respiratory rate, SpO2, blood pressure, and body temperature) recording and clinical examination of body systems will be performed.
2. Adverse reaction either volunteered by the subject or noticed by the doctor during the post vaccination observation period will be reported.
3. Vaccination site monitoring will be conducted.

7.1.4. VISIT 4 (DAY 11):

1. Telephonic follow up
2. Overall health status reported
3. Adverse Events (Local injection site)
    a. Pain
    b. Redness
    c. Swelling
    d. Itching
4. Adverse Events (Systemic)
    a. Fever
    b. Headache
    c. Tiredness
    d. Nausea
    e. Vomiting

7.2. INCLUSION/EXCLUSION CRITERIA

7.2.1. INCLUSION CRITERIA

1. Healthy subject of either gender 12 to 14 years of age group.
2. Must be eligible for 1st and 2nd of Corbevax vaccination as per Cowin registration.
3. Ability to provide consent.

7.2.2. EXCLUSION CRITERIA

1. Known SARS-CoV-2 positive (RTPCR).
2. History of contact with a confirmed active SARS-CoV-2 positive patient within 14 days.
3. Febrile illness (temperature ≥ 38°C or 100.4°F) or any acute illness or infection within 4 weeks of enrolment.
4. Subjects with confirmed immunosuppressive or immunodeficiency disorder; or subjects on any immunosuppressive or immunostimulant therapy
5. Subjects administered blood, blood containing products or immunoglobulins within the last 3 months or planned administration during the study.
6. Any other vaccine administration within the last 30 days or planned to be administered during the study period.
7. Hypersensitivity reaction or any serious adverse event after any vaccination
8. Uncontrolled Co-morbidities.
9. History of drug / alcohol abuse.
10. Covid-19 sign and symptoms.
11. History of skin diseases or chronic eczema and any coagulation disease.

Table 1: SCHEDULE OF ASSESSMENTS

Sr. No	Assessment	Visit-1	Visit-2	Visit-3	Visit-4
1	Informed consent process	☒	☐	☐	☐
2	Eligibility criteria	☒	☐	☐	☐
3	Demographics (Age, Sex, Height, Weight and BMI)	☒	☐	☐	☐
4	Medical history	☒	☐	☐	☐
5	Clinical examination	☒	☒	☒	☐
6	Vital signs including SpO2 a	☒	☒	☒	☐
8	Vaccination (Corbevax vaccine)	☒	☐	☐	☐
9	Vaccination site Monitoring	☒	☒	☒	☐
10	Overall, Health status	☐	☐	☐	☒

7.3. TREATMENT

7.3.1. TREATMENTS ADMINISTERED AND IDENTITY OF INVESTIGATIONAL PRODUCT(S)

7.3.1.1. INVESTIGATIONAL MEDICAL DEVICE

IntegriMedical® Needle Free Injection System (NFIS).

7.3.1.2. MODE OF ADMINISTRATION

Intramuscular route.

7.3.1.3. ADMINISTRATION SCHEDULE

Subjects were randomized for receiving dose of COVID-19 vaccine, considering all Subjects will get Covid_19 vaccines by NFIS.

7.3.2. METHODOLOGY

This protocol describes tolerability, acceptability and safety of IntegriMedical Needle Free Injection System in subjects receiving dose of COVID-19 Corbevax vaccine.

7.3.3. ANALYSIS OF TOLERABILITY MEASUREMENTS-

Tolerability was determined using a VAS score respectively.

7.3.4. STATISTICAL ANALYSIS

Statistical analysis was performed using the SPSS Version 25 and Stata 15 software. All available data was used in the analysis.

7.3.5. PROTOCOL DEVIATIONS

There were no protocol deviations noted in the conduct of the study. All volunteers complied to the various trial related procedures and the study was conducted in compliance with the study protocol.

8. SUBJECT DISPOSITION

8.1. STUDY SUBJECTS

Study subjects a total of 60 healthy volunteers providing consent and found eligible for participation in the study were enrolled.

All 60 volunteers successfully completed the study with Vaccination. Data generated on these 60 healthy volunteers who received both the intervention and control injections form the basis of this report.

8.2. DEMOGRAPHICS

Total 60 Subjects has been received Corbevax vaccine under the study. 48% of subjects are 13 years old while 21.7% are 12 years old and 30.0% are from 14 years old age group. 60% female and 40% male were administered with the vaccine through IntegriMedical Needle Free Injection System. (Presented in **Table *2***)

Table 2 . Demographic distribution of subjects.

Demographics		N (60)	%
Age	Mean	13.08	
	SD	0.72	
Age group	12 Years	13	21.7
	13 years	29	48.3
	14 years	18	30.0
Sex	Female	36	60
	Male	24	40
Weight(kg)	Mean	42.20	
	SD	6.64	
	Min	26.6	
	Max	59.9	
Height(cm)	Mean	147.44	
	SD	7.29	
	Min	130	
	Max	162	

8.3. PAST AND CURRENT MEDICAL HISTORY

None of the study subjects reported any past / current medical history

8.4. VITAL SIGNS

Vital signs of the study subjects at screening are summarized in **Table 3**. The study subjects had 'normal' body temperature, heart rate, respiratory rate, and blood pressure at the time of screening.

Table 3: Subject characteristics at baseline - Vital signs (Pre vaccination).

Vital signs		Summary Statistics
Systolic Blood Pressure (mm Hg)	Mean	123.82
	SD	5.21
	Min	105
	Max	137
	Interpretation normal	**100%**
Diastolic Blood Pressure (mm Hg)	Mean	76.62
	SD	6.42
	Min	65
	Max	96
	Interpretation normal	**100%**
Body Temperature (ºF)	Mean	97.69
	SD	0.76
	Min	95
	Max	99.4
	Interpretation normal	**100%**
Pulse Rate (bpm)	Mean	77.86
	SD	9.60
	Min	65
	Max	103
	Interpretation normal	**100%**
Respiratory Rate (bpm)	Mean	17.85
	SD	0.82
	Min	16
	Max	20
	Interpretation normal	**100%**

9. SAFETY EVALUATION (RESULTS AND DISCUSSION)

Vital signs including body temperature, heart rate, blood pressure, and respiratory rate were measured after administration of vaccine using IntegriMedical Needle Free Injection System post 30 min, at visit 2 and visit 3 to know the overall clinical status of subjects.

9.1. VITAL SIGNS (POST VACCINATION)

Table 4 below summarizes the data for vital sign at different time point after vaccination.

Table 4: Post Vaccination Vital Signs

Vital signs		After 30 min of vaccination	Follow up Visit2	Follow up Visit 3
Systolic Blood Pressure (mm Hg)	Mean	128.07	121.47	121.85
	SD	5.21	2.77	2.72
	Min	118	117	117
	Max	144	126	128
	Interpretation Normal	**100%**	**100%**	**100%**
Diastolic Blood Pressure (mm Hg)	Mean	80.95	80.35	77.6
	SD	4.95	4.07	3.82
	Min	72	72	72
	Max	88	89	85
	Interpretation Normal	**100%**	**100%**	**100%**
Body Temperature (ºF)	Mean	97.8	97.29	96.94
	SD	0.55	1.11	1.36
	Min	96.8	95	91
	Max	98.8	99.6	98.9
	Interpretation Normal	**100%**	**100%**	**100%**
Pulse Rate (bpm)	Mean	82.53	81.28	80.58
	SD	6.55	4.31	4.42
	Min	68	73	74
	Max	98	89	89
	Interpretation Normal	**100%**	**100%**	**100%**
Respiratory Rate (bpm)	Mean	17.7	18.0	18.6
	SD	0.76	1.15	1.03
	Min	17	16	17
	Max	20	20	20
	Interpretation Normal	**100%**	**100%**	**100%**

9.2. VAS PAIN ASSESSMENT SCORE (2 MIN POST INJECTION)

Pain score was assessed within 2 min following the IntegriMedical Needle Free Injection System.

(**Table *5***). The percentage of those who reported no pain post Needle Free Injection System was 46.7% while 26% felt mild pain with VAS score 1.00 and remaining 26.7% felt mild pain with VAS score 2.00 and 3.00.

Table 5: VAS pain score assessment following Needle free injections

	N	Percentage
00(No Pain)	28	46.7
1.00	16	26.7
2.00	12	20.0
3.00	4	6.7
Mean	0.87	
SD	0.96	
Min	0	
Max	3	

9.3. ACCEPTABILITY ASSESSMENTS

As per VAS Pain Assessment of 2 min post injections. The percentage of those who reported no pain post needle free injection was 46.7% while 53.3% felt mild pain (**Table *5***). Also, vaccination site Monitoring was done on visit-2(day-3) and visit-3(Day-7). For 93.3% subjects, injection site monitoring was observed normal. On Visit-4 / day-11 Telephonic visit for overall health status recorded that all the subjects were found normal. There was not any AE noted, and all subjects were stable.

9.4. ACCEPTABILITY CONCLUSIONS

IntegriMedical Needle free injection system was well accepted. None of the subjects receiving this injection complained of any moderate and severe pain, redness, swelling, itching at the injection site. More than 90% of the respondents indicated that the local reaction and pain was totally acceptable. As per verbally communicated by subjects during this subsequent visits NFIS injection had a significantly higher satisfaction percentage. A higher percentage (94%) responded that they did not feel anxious about receiving the IntegriMedical Needle Free Injection System.

10. References

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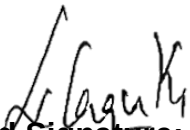
11. SIGNATURE PAGE

Clinical Study Report Prepared By:	
I have prepared and read this report and confirm that to the best of my knowledge it accurately describes the conduct and result of the study.	
Medical Writer	**1) Name: Dr.** Shrikant Suryavanshi Manavata Clinical Research Institute, Behind Shivang Auto, Mumbai Naka, Nashik, 422002. **Date and Signature:** 07-Jun-2022 **2) Name:** Mr. Roshan Patil Manavata Clinical Research Institute, Behind Shivang Auto, Mumbai Naka, Nashik, 422002. **Date and Signature:** 07-Jun-2022
Biostatistician	**Name:** Mr. Dilesh Bagul **Date and Signature:** 07-Jun-2022
Clinical Study Report Approved By:	
I have read this report and confirm that to the best of my knowledge it accurately describes the conduct and result of the study.	
Sponsor Representative	**Name:** Scott McFarland **Address**: Integri Medical LLC,805 N,4th, Avenue,Unit 903,Phoenix. Arizona 85003**.** **Date and Signature:**

Investigator	**Name:** Dr. Rajnish Nagarkar Manavata Clinical Research Institute, Behind Shivang Auto, Mumbai Naka, Nashik, 422002.  **Date and Signature:**

Immunogenicity & Safety Study of MMR Vaccine, India

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Immunogenicity and safety of measles-mumps-rubella vaccine delivered by disposable-syringe jet injector in India: A randomized, parallel group, non-inferiority trial

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ABSTRACT

Background: We conducted a randomized, non-inferiority, clinical study of MMR vaccine by a disposable-syringe jet injector (DSJI) in toddlers in India in comparison with the conventional administration.
Methods: MMR vaccine was administered subcutaneously by DSJI or needle-syringe (N-S) to toddlers (15–18 months) who had received a measles vaccine at 9 months. Seropositivity to measles, mumps, and rubella serum IgG antibodies was assessed 35 days after vaccination. Non-inferiority was concluded if the upper limit of the 95% CI for the difference in the percent of seropositive between groups was less than 10%. Solicited reactions were collected for 14 days after vaccination by using structured diaries.
Results: In each study group, 170 subjects received MMR vaccine. On day 35, seropositivity for measles was 97.5% [95% CI (93.8%, 99.3%)] in the DSJI group and 98.7% [95% CI (95.5%, 99.8%)] in the N-S group; for mumps, 98.8% [95% CI (95.6%, 99.8%)] and 98.7% [95% CI (95.5%, 99.8%)]; and for rubella, 98.8% [95% CI (95.6%, 99.8%)] and 100% [95% CI (97.7%, 100.0%)]; none of the differences were significant. The day 35 post-vaccination GMTs in DSJI and N-S groups were measles: 5.48 IU/ml [95% CI (3.71, 8.11)] and 5.94 IU/ml [95% CI (3.92, 9.01)], mumps: 3.83 ISR [95% CI (3.53, 4.14)] and 3.66 ISR [95% CI (3.39, 3.95)] and rubella: 95.27 IU/ml [95% CI (70.39, 128.95)] and 107.06 IU/ml [95% CI (79.02, 145.06)]; none of the differences were significant.
The DSJI group reported 173 solicited local reactions and the N-S group reported 112; most were mild grade. Of the total of 156 solicited systemic adverse events, most were mild, and incidence between the two groups was similar.
Conclusions: MMR vaccination via DSJI is as immunogenic as vaccination by N-S. Safety profile of DSJI method is similar to N-S except for injection site reactions which are more with DSJI and are well-tolerated.
Registration
US National Institutes of Health clinical trials identifier – NCT02253407.
Clinical trial registry of India identifier – CTRI/2013/05/003702

1. Introduction

Worldwide, measles caused 89,780 deaths in 2016, mostly among children under age five [1]. Rubella is generally a mild viral infection in children, but in pregnant women it may cause fetal

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death or severe congenital defects [2]. As a result, the World Health Organization (WHO) recommends measles and rubella vaccines for all the children in the world [3]. In 2012, several global agencies led by the WHO set the goal of eliminating measles and rubella in at least five WHO regions by 2020 [4]. Currently, global measles immunization coverage is at 85%, but, to achieve elimination, at least 95% coverage with two doses of vaccine is required [5]. Though measles vaccine is used universally, in many developing countries, vaccines against mumps and rubella are not used in immunization programs.

Measles-mumps-rubella (MMR) vaccine generally is administered as a subcutaneous injection with needle and syringe (N-S). However, vaccine delivery with needles can cause needle-stick injuries and cross-infection, and it also creates dangerous sharps waste in communities. N-S delivery also complicates the logistics of immunization campaigns, since the requirement for proper sharps disposal can limit their reach and coverage. An alternative to N-S for vaccine delivery is the jet injector, a device that creates a fine stream of pressurized liquid that penetrates the skin to deposit vaccine without using a needle [6].

Disposable-syringe jet injectors (DSJIs) that use a sterile, single-dose, disposable syringe for each patient were introduced in the 1990s, and a number of models have been approved in the United States and Europe for different uses, including vaccinations [6].

The risks associated with DSJI use include failure to deliver the correct dose; pain, bleeding, or swelling at the injection site; and user error in positioning the injector to deliver the dose to the correct layer of tissue—however, most of these risks also apply to vaccination by N-S [7].

Vaccination by jet injection has been shown to induce immunity similar to that provided by N-S injection and to have a similar safety profile for a number of vaccines, including typhoid, cholera, smallpox, hepatitis A and B, influenza, whole cell pertussis-diphtheria-tetanus, polio, yellow fever, and tetanus. [6] A previous study comparing a DSJI with N-S for administering MMR vaccine several years ago met the requirement for rubella but failed to demonstrate non-inferiority of the DSJI to N-S for the measles and mumps vaccines [8]. However, that study used a different jet injector than the one used in the present study as well as a vaccine from a different manufacturer.

We conducted a phase IV, randomized, observer-blind, non-inferiority, parallel-group, multicentric clinical study of MMR vaccination in infants in India to compare immunogenicity and safety of the vaccine when administered by a DSJI to administration by conventional N-S method. A result of non-inferiority for the DSJI would support use of vaccination with a jet injector, offering a needle-free alternative for country immunization programs.

2. Methods and materials

The study sponsor was the Serum Institute of India Pvt. Ltd. (SIIPL, Pune, India). DiagnoSearch Life Sciences (Mumbai, India) was delegated by the sponsor for site monitoring, project management, clinical data management, and statistical analysis of the data. Approvals were obtained from the Drug Controller General of India, the institutional ethics committees of all study centers, and the Western Institutional Review Board in the United States. The study was carried out in accordance with the Declaration of Helsinki and the ICH Harmonized Tripartite Guideline for GCP (E6) 1996; the GCP Guidelines in India; and the Ethical Guidelines for Biomedical Research on Human Subjects, issued by Indian Council of Medical Research in 2006.

2.1. Vaccine

MMR vaccine (SIIPL, India) was used in the study. It is presented as a single-dose of lyophilized vaccine and is provided with a sterile diluent (0.5 ml of water for injection) in a separate container. The vaccine is reconstituted by adding the diluent to the vial containing the lyophilized pellet. A single dose of 0.5 ml contains live attenuated strains of Edmonston-Zagreb measles virus (not less than 1000 CCID50), Leningrad-Zagreb mumps virus (not less than 5000 CCID50), and Wistar RA 27/3 rubella virus (not less than 1000 CCID50). The same batch of the vaccine (MMR batch 013N4017A expiry May2016 and diluent batch 064Q40330Z expiry April 2016) was used throughout the study. It was stored at 2–8 °C. The dose was 0.5 ml by both delivery methods.

2.2. Injection devices

The investigational product for this study was the MMR vaccine administered subcutaneously by the Stratis DSJI (PharmaJet, Golden, Colorado, USA) (Fig. 1). This device is licensed for use in the United States and in the European Economic Area; it is also prequalified by WHO [9,10.11]. The Stratis needle-free injection system delivers 0.5 ml fluid volumes either intramuscularly or subcutaneously by means of a precise narrow fluid stream, which penetrates the skin in about a 1/10th of a second and delivers the medicine or vaccine into the body. Energy to propel the fluid is supplied by a hand-held, spring-powered injector, designed to be reused a minimum of 20,000 times. A disposable syringe containing medicine or vaccine is attached to the injector and placed in contact with the patient's skin. The fluid is then expelled through a very small orifice in the face of the syringe.[10] The batch numbers for the Stratis devices used in the study were 25854275 and 23436455. The reference product was the same MMR vaccine administered subcutaneously via N-S.

2.3. Study populations and settings

The study was conducted at six sites across India from September 2014 through December 2015. Eligible participants were healthy children aged 15–18 months who had received a measles vaccine at 9 months of age. Children with a past history of measles, mumps, or rubella infection; significant abnormality; any neoplasm or blood disorder; or a history of allergy to any of the vaccine components and those who had previously received the MMR vaccine were not eligible. After written informed consent from their parents, 5 ml blood was drawn for immunogenicity testing from eligible subjects, and the MMR vaccine was administered

Fig. 1. Stratis SC/IM (0.5 ml fixed dose).

subcutaneously in the anterolateral aspect of the thigh on day 0 by either of the two techniques.

Parents were issued subject diaries and educated to fill in the solicited adverse reactions as well as other reactions for 14 days and asked to return to the study site for follow up visits on day 14 (2nd visit) and day 35 (3rd visit).

2.4. Randomization and blinding

A block randomization scheme was used to allocate eligible subjects in a 1:1 ratio to receive MMR vaccine either by DSJI or N-S. Each block consisted of six subjects. The randomization list was generated using SAS® statistical software version 9.2 in SAS Enterprise Guide 4.2 (SAS Institute, Cary, North Carolina, USA). The list of randomization numbers and the group allocations covered with scratch labels were provided to all sites. Subjects were allocated to groups by scratching the label corresponding to the randomization number in the list by the vaccinator, just before vaccine administration. Investigator site personnel—except for staff administering the vaccine—and laboratory staff were not aware of the allocation.

2.5. Immunogenicity evaluations

A blood sample was collected from each subject at baseline and on day 35 after vaccination. Paired serum samples were tested using ELISA IgG kits (Trinity Biotech, Bray, Ireland) at Quest Diagnostics (Gurgaon, India). Seropositivity for each vaccine component was defined as IgG antibody titers $\geq$1.10 immune status ratio (ISR). For measles and rubella, antibody titers were converted from ISR to IU/ml per instructions in the ELISA kits. For mumps, the ISR values were used. Geometric mean titers (GMTs) were calculated for the secondary endpoint.

2.6. Safety evaluations

At each visit, subjects were examined by the study physician and a history was taken for adverse events (AEs) and concomitant medications. AEs were coded using the Medical Dictionary for Regulatory Activities (MedDRA), Version 18.0 [12]. Parent diaries for solicited local and systemic AEs occurring over 14 days post-vaccination were transcribed into the case report form. All solicited events were recorded for maximum severity and relatedness to treatment. The solicited local reactions were pain, redness, swelling, or bruising. The solicited systemic AEs were fever, rash, parotitis, lymphadenopathy, and loss of appetite. Unsolicited events and serious events were also collected from subjects throughout their entire participation in the study. All adverse events were categorized into mild, moderate or severe based on pre-defined severity criteria. As the investigational product was the combination of delivery device with vaccine, it was not possible to categorize AEs by component (vaccine or device related).

2.7. Delivery evaluations

Data on injection quality were recorded immediately post injection. The absence or presence and severity of injection site trauma was recorded and residual wetness remaining on the skin was measured using blotting paper. The absence or presence of crying and duration of cry was also noted.

2.8. Statistical analyses

All statistical analyses were performed using SAS statistical software, version 9.2. Sample size was determined under the assumption that 90%of individuals in the control group would become seropositive, and that 10% would withdraw from the study. A total of 340 subjects were enrolled to provide at least 80% power to rule out a difference in percentage seropositivity of greater than 10% between groups, using a one-sided significance level of 0.025 for each vaccine component.

The intention-to-treat (ITT) population was used for baseline and safety analysis. The per-protocol (PP) population (those with no major protocol deviations impacting immunogenicity analysis and who completed all three visits with evaluable blood samples on day 0 and day 35) was used for immunogenicity analysis.

Percent seropositivity was calculated as the percentage of subjects for whom the day 35 post-vaccination titer was $\geq$1.10 ISR. The percentage and 95% confidence interval (CI) of seropositivity for measles, mumps, and rubella between the two groups was compared using the Farrington and Manning method [13]. Non-inferiority was concluded if the upper limit of the 95% CI for the difference in the percent seropositive between groups was less than 10%.

The GMTs of antibodies between DSJI and N-S groups were compared between groups using a two sample *t*-test. Pre- and post-dose seropositivity and GMTs within each group were compared using McNemar's chi-square test and a paired *t*-test, respectively. Safety endpoints were the proportion of solicited local and systemic AEs, unsolicited AEs, and serious AEs (SAEs) throughout the study. The intention-to-treat population was used for safety analyses. Further details are provided in the supplementary material.

A post hoc immunogenicity analysis was performed for measles seropositivity at day 35 in subjects who were measles seronegative at baseline. The percentage and 95% CI were compared between groups using the Farrington and Manning method.

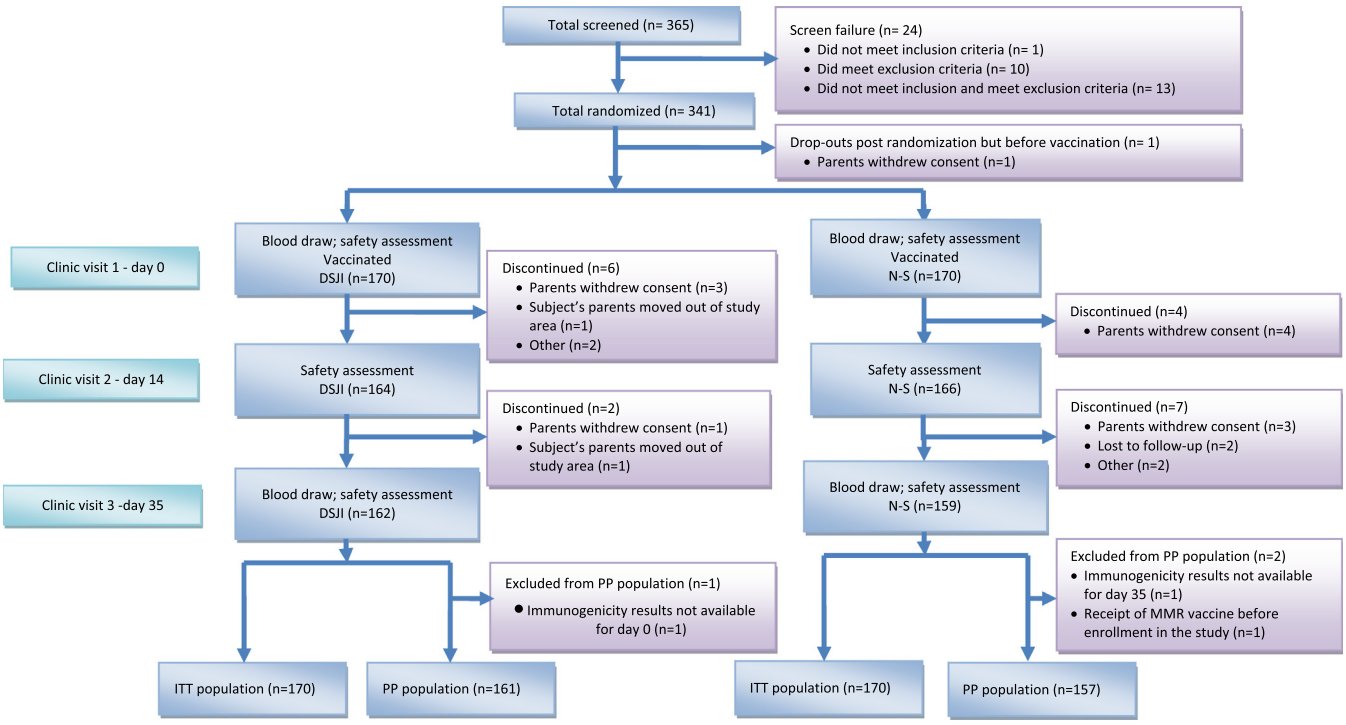
3. Results

A total of 365 subjects were screened and 341 eligible subjects were randomized. The parents of one subject withdrew consent after randomization but before vaccination; thus, a total of 340 subjects received study vaccine, 170 in each group (Fig. 2). At baseline, the DSJI and N-S groups were similar in age, weight, and height; however, there were more males in the DSJI group (Table 1, p = .039).

3.1. Immunogenicity results

At baseline, seropositivity rates were similar between both the groups for all three antigens (Table 2). On day 35, seropositivity rates in the DSJI and N-S groups were 97.5% [95% CI (93.8%, 99.3%)] and 98.7% [95% CI (95.5%, 99.8%)] for measles; 98.8% [95% CI (95.6%, 99.8%)] and 98.7% [95% CI (95.5%, 99.8%)] for mumps; and 98.8% [95% CI (95.6%, 99.8%)] and 100% [95% CI (97.7%, 100.0%)] for rubella. All seropositivity rates were comparable between the two groups. In addition, there was a significant rise in the proportions of seropositive subjects from baseline to day 35 within each group for all three components (p = .0001, chi-square test, data not shown). In subjects who were seronegative for measles at baseline, more than 95% were seropositive at day 35 in both groups, and the difference between groups was not significant (data not shown).

GMTs were not significantly different between the two groups at baseline (Table 3). At day 35 after vaccination, in the DSJI group, GMTs were 5.48 IU/ml, 3.83 ISR, and 95.27 IU/ml for measles, mumps, and rubella, respectively. As for the comparable values in the N-S group at day 35, GMTs were 5.94 IU/ml, 3.66 ISR, and 107.06 IU/ml (p > .05). There was a significant rise in GMTs for all three antigens from baseline to day 35 in both groups (Table 3).



Abbreviations: ITT, intention-to-treat; PP, per-protocol; N-S, needle and syringe; DSJI, disposable-syringe jet injector; MMR, measles-mumps-rubella.

Fig. 2. Study flowchart.

Table 1
Baseline characteristics: Intention-to-treat population.[#]

Characteristic[*]	DSJI (n = 170)	N-S (n = 170)
Age (months)		
Mean (SD)	16.4 (1.1)	16.3 (1.1)
Height (cm)		
Mean (SD)	77.5 (3.3)	77.9 (2.7)
Weight (kg)		
Mean (SD)	9.5 (1.2)	9.4 (1.1)
Gender[^]		
Male, n (%)	97 (57.1%)	77 (45.3%)
Female, n (%)	73 (42.9%)	93 (54.7%)

[#] The intention-to-treat population is all participants who received the study vaccine.
[*] All subjects were of Indian ethnicity.
[^] Numbers of males and females in the study groups were significantly different (p = .039, Fisher's exact test).

For subjects who were **seronegative** for measles at baseline, GMTs for measles were 0.04 IU/ml [95% CI (0.04, 0.05)] at Day 0 and 2.92 IU/ml [95% CI (1.45, 5.89)] at Day 35 in DSJI group; while in N-S group it was 0.05 IU/ml [95% CI (0.04, 0.05)] at Day 0 and 3.59 IU/ml [95% CI (1.78, 7.23)] at Day 35. For subjects who were **seropositive** for measles at baseline, GMTs for measles were 0.49 IU/ml [95% CI (0.35, 0.69)] at Day 0 and 8.04 IU/ml [95% CI (5.10, 12.69)] at Day 35 in DSJI group; while in N-S group it was 0.31 IU/ml [95% CI (0.24, 0.40)] at Day 0 and 7.51 IU/ml [95% CI (4.48, 12.61)] at Day 35.

3.2. Safety results

A total of 285 solicited local reactions were reported, with173 in the DSJI group and 112 in the N-S group, a statistically significant difference (Table 4). Pain was the most frequently reported reaction in both groups (44.7% in DSJI group and 35.3% in N-S group). In the DSJI group, 54.7% of subjects had mild intensity local reactions and 8.8% of subjects had moderate intensity local reactions; in the N-S group, the proportion was 40.6% and 5.9% respectively. Only one subject (0.59%) had severe intensity local reaction i.e. pain in the DSJI group. No severe local reaction was reported in N-S group. All reactions resolved without sequelae.

Out of 156 solicited systemic AEs, 86 were reported in the DSJI group and 70 in the N-S group. The most commonly reported were loss of appetite, fever, and rash (Table 4). In the DSJI group, loss of appetite was reported in 20% of subjects, fever in 11.2%, and rash in 7.6%, compared with 17.1%, 11.8%, and 7.1%, respectively, in the N-S group. The incidence of solicited systemic AEs between the two groups was similar.

A total of 371 unsolicited AEs (including SAEs) were reported in 185 subjects across both the groups (178 in DSJI group and 193 in N-S group—data not shown). Nine events (four injection site haemorrhage; one lymphadenopathy; one parotitis; and three upper respiratory tract infections) in DSJI group and seven events (one injection site haemorrhage; one injection site induration; four upper respiratory tract infections; and one vomiting) in N-S group were related to investigational or reference product (investigational product is the combination of delivery method and vaccine; causality is not attributable to the separate components). Incidence of unsolicited AEs was comparable between the two groups, and most of these AEs were of mild intensity. Four SAEs were reported during the study, two in each of the study groups, with the seriousness criteria of hospitalization. All were unrelated to the study vaccination, and none of the subjects was discontinued from the study. Further details are in the supplementary material. All local and systemic AEs reported during the study period resolved without sequelae.

Most injections for both groups resulted in a single drop of residual fluid at the site after injection: for the DSJI group, this

Table 2
Seropositivity* at day 0 and at day 35 after vaccination in the per-protocol population.β

Vaccine component		Statistic			Day 35		
		DSJI (n = 161)	N-S (n = 157)	Two-sided p-value by Fisher's Exact Test	DSJI (n = 161)	N-S (n = 157)	Difference in percentage^
Measles	Seropositive subjects (%)	100 (62.1)	107 (68.2)	–	157 (97.5)	155 (98.7)	1.2
	2-Sided 95% CI	(54.1, 69.6)	(60.3, 75.4)	0.2902	(93.8, 99.3)	(95.5, 99.8)	(−4.0, 6.4)
Mumps	Seropositive subjects (%)	13 (8.1)	10 (6.4)	–	159 (98.8)	155 (98.7)	−0.1
	2-Sided 95% CI	(4.4, 13.4)	(3.1, 11.4)	0.6665	(95.6, 99.8)	(95.5, 99.8)	(−5.0, 4.9)
Rubella	Seropositive subjects (%)	7 (4.3)	6 (3.8)	–	159 (98.8)	157 (100.0)	1.2
	2-Sided 95% CI	(1.8, 8.8)	(1.4, 8.1)	1.0000	(95.6, 99.8)	(97.7, 100.0)	(−3.7, 6.2)

* IgG antibody titers were determined by ELISA (Trinity Biotech) for each vaccine component. Seropositivity was defined as IgG antibody titers ≥1.10 immune status ratio (ISR), according to the levels given in the Trinity Biotech kit. For measles and rubella, antibody titers were converted from ISR to IU/ml per instructions in the Trinity Biotech kits. For mumps, the ISR values were used. All samples were tested in duplicate and the mean of the two values was used. Repeat testing was performed on samples with equivocal results.

β The per-protocol population consisted of all subjects who had no major protocol violations and who completed all three clinic visits, with evaluable blood samples at day 0 and day 35.

^ Two-sided 95% CI is estimated for the difference between proportions using the Farrington and Manning method. The upper limit of the two-sided 95% CI for the percentage of seropositivity for all vaccine components was less than 10%; thus, the seropositivity of the MMR DSJI group was non-inferior to that of the MMR N-S group.

Table 3
Geometric mean titers of anti-measles, anti-mumps, and anti-rubella antibody concentrations* on day 0 and day 35 after vaccination in the per-protocol population.

Vaccine component	Statistic	Day 0		Day 35	
		DSJI (n = 161)	N-S (n = 157)	DSJI (n = 161)	N-S (n = 157)
Measles (IU/ml)	Geometric mean titer (GMT)	0.19	0.17	5.48	5.94
	Two-sided 95% CI	(0.15, 0.26)	(0.13, 0.21)	(3.71, 8.11)	(3.92, 9.01)
	p-Value (between groups$^{\alpha}$)	.4571		.7813	
	p-Value (within group$^{\beta}$)			<.0001	<.0001
Mumps (ISR)	GMT	0.29	0.29	3.83	3.66
	Two-sided 95% CI	(0.25, 0.32)	(0.25, 0.33)	(3.53, 4.14)	(3.39, 3.95)
	p-Value (between groups$^{\alpha}$)	.9821		.4293	
	p-Value (within group$^{\beta}$)			<.0001	<.0001
Rubella (IU/ml)	GMT	3.25	3.04	95.27	107.06
	Two-sided 95% CI	(2.73, 3.86)	(2.77, 3.34)	(70.39, 128.95)	(79.02, 145.06)
	p-Value (between groups$^{\alpha}$)	.5150		.5914	
	p-Value (within group$^{\beta}$)			<.0001	<.0001

* IgG antibody titers were determined by ELISA (Trinity Biotech) for each vaccine component.

$^{\alpha}$ For comparing GMTs between the two study groups, the two-sample *t*-test was used.

$^{\beta}$ For comparing GMTs within each group between day 0 and day 35, the paired *t*-test was used. The p-value is shown in the day 35 columns.

proportion was 78%, and for the N-S group, it was 64%. In the DSJI group, 15% had a completely dry site, and in the N-S group, the proportion was 35% (see supplementary material).

4. Discussion and conclusions

The seropositivity following MMR vaccine administered using the DSJI was non-inferior to that for vaccine administered via N-S for all three components of the vaccine with a non-inferiority margin of 10%; thus, the primary efficacy endpoint of the study was met. Also, differences in Day 35 post-vaccination GMTs between two groups for each component of vaccine were not statistically significant. A booster effect was seen for measles with MMR vaccination in both the groups in subjects who were seropositive at baseline. There was more increase in seropositivity from baseline to Day 35 post-vaccination for mumps and rubella in N-S group as compared to DSJI group, and the opposite was seen for measles. Similarly, there was more rise in GMTs from baseline to Day 35 post-vaccination for measles and rubella in N-S group as compared to DSJI group, and the opposite was seen for mumps. However, these apparent small differences were not statistically compared as they were not part of statistical analysis plan. Injection site reactions were more in DSJI group as compared to N-S group and this difference between two groups was statistically significant. However all injection site reactions resolved without any sequelae. Similarly, in a previous study of influenza vaccination, higher frequency of local injection site reactions were reported with DSJI than with the use of needle and syringe [14]. Systemic adverse reactions were comparable between the two study groups. Nine unsolicited adverse events (four injection site haemorrhage; one lymphadenopathy; one parotitis; and three upper respiratory tract infections) in DSJI group and seven unsolicited adverse events (one injection site haemorrhage; one injection site induration; four upper respiratory tract infections; and one vomiting) in N-S group were related to investigational or reference product (investigational product is the combination of delivery method and vaccine; causality is not attributable to the separate components). All reported systemic adverse events were consistent with typical MMR vaccination adverse events.

Since all subjects had received a measles vaccination at 9 months of age, around 65% were measles seropositive at the beginning of the study. However, for mumps and rubella, the baseline seropositivity was less than 10%. After vaccination, the proportions of seropositives in both the groups increased significantly, to a level of 98–100% for all three antigens, indicating that administration with the DSJI results in immunogenicity similar to that after injection with N-S.

Table 4
Solicited local reactions and systemic adverse events by study groups, intention-to-treat population.

	DSJI (n = 170)			N-S (n = 170)			p-Value[α]
	No. subjects	No. events	% subjects (95% CI)	No. subjects	No. events	% subjects (95% CI)	
Local adverse events							
Pain	76	81	44.7 (37.1, 52.5)	60	61	35.3 (28.1, 43.0)	.096
Redness	40	42	23.5 (17.4, 30.6)	22	22	12.9 (8.3, 18.9)	.016
Swelling	47	48	27.6 (21.1, 35.0)	27	27	15.9 (10.7, 22.3)	.012
Bruising	2	2	1.2 (0.1, 4.2)	2	2	1.2 (0.1, 4.2)	1.00
At least one local reaction	97[*]	173 (Mild: 155; Moderate: 16; Severe: 1) Severity for one local reaction i.e. redness is missing	57.1 (49.3, 64.6)	75[*]	112 (Mild: 99; Moderate: 13; Severe: Nil)	44.1 (36.5, 51.9)	.02
Systemic adverse events							
Loss of appetite	34	44	20.0 (14.3, 26.8)	29	35	17.1 (11.7, 23.6)	.58
Fever	19	19	11.2 (6.9, 16.9)	20	20	11.8 (7.3, 17.6)	1.00
Rash	13	15	7.6 (4.1, 12.7)	12	13	7.1 (3.7, 12.0)	1.00
Lymphaden-opathy	4	5	2.4 (0.6, 56.0)	2	2	1.2 (0.1, 4.2)	0.25
Parotitis	3	3	1.8 (0.4, 5.1)	0	0	0 (0.0, 2.2)	0.68
At least one event	51[*]	86 (Mild: 68; Moderate: 14; Sever: 4)	30.0 (23.2, 37.5)	46[*]	70 (Mild: 56; Moderate: 11; Severe: 3)	(20.5, 34.4)	0.63

[α] p-Value for number of subjects calculated using Fisher's exact test.
[*] Total number of subjects with at least one local reaction or systemic adverse event is less than the sum of the numbers for that column because some subjects experienced more than one event.

As noted earlier, jet injectors have worked well with many licensed vaccines, with the exception of a study of MMR vaccine conducted in Brazil that failed to demonstrate non-inferiority of the DSJI to N-S for measles and mumps vaccines [6,8]. The device used in that study was discontinued by the manufacturer and replaced with the Stratis device used in the current study. One hypothesis for the outcome of the Brazil study was that the pressures and shear forces generated during jet injection might have affected the viability of the live viruses in MMR vaccine; however, subsequent laboratory studies found that this was not the case [15]. Another possibility was that vaccine left on the surface of the skin might have contributed to the reduced immunogenicity after DSJI delivery. The different manufacturers' MMR vaccines also could have contributed to the difference in results. Thus, ours is the first study that demonstrates that MMR vaccine can be given by a jet injector with equivalent immunogenicity as that with conventional N-S. Also, MMR vaccination by jet injector is as safe as vaccination by N-S except for injection site reactions, in particular redness and swelling, which are more with DSJI. The cause of the increased injection site reactions with DSJI is not proven, but may be due to the mechanism of action of the DSJI, which deposits residual amounts of vaccine at each layer of the skin as it penetrates to the correct delivery depth.

Limitations of this study include the lack of masking of the study participants and their parents to the method of vaccination and unequal gender distribution in the two study groups. An unequal gender distribution was purely a random occurrence. The use of block randomization can introduce bias, particularly with smaller block sizes.

To conclude, subcutaneous MMR vaccination via DSJI is as immunogenic as vaccination by N-S. MMR vaccination by DSJI demonstrates a clinically acceptable safety profile and is similar to vaccination by N-S except for injection site reactions which are more with DSJI and are well-tolerated. Results of this study support use of the DSJI for MMR vaccination and provide information for regulatory authorities, immunization program managers, and clinicians who make decisions about safe clinical practice standards. Using the DSJI can reduce the risks of needle-stick injuries and the burden of sharps waste disposal, which can streamline logistics and contribute to improved coverage in low-resource settings, helping to reach the goal of preventing these diseases and their serious sequelae.

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Conflict of interest

DK, BG, AC and PSK are employed by Serum Institute of India Pvt. Ltd., which manufactures the MMR vaccine that was used in the study.

TM is employed by PharmaJet, which manufactures the devices used in the study.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.vaccine.2018.01.006.

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Immunogenicity & Safety Study of MMR Vaccine, Brazil

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Immunogenicity and safety of measles–mumps–rubella vaccine delivered by disposable-syringe jet injector in healthy Brazilian infants: A randomized non-inferiority study

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ABSTRACT

This study aimed to determine if immunogenicity to measles–mumps–rubella vaccine delivered to infants via a disposable-syringe jet injector (DSJI) was non-inferior to that administered by needle and syringe (NS). Vaccination safety was evaluated, as were the use, performance, and acceptability of each delivery method. The DSJI was the PharmaJet® 2009 generation-1 device (G1) and the vaccine was measles–mumps–rubella vaccine from Bio-Manguinhos. Five hundred eighty-two healthy Brazilian infants were randomized to receive vaccine via G1 or NS. Seroconversion rates against measles and mumps viruses in the G1 treatment group did not meet non-inferiority criteria when compared with the NS group; however, responses in the G1 group to rubella virus were non-inferior to those of NS vaccinees. Most adverse events were mild or moderate. Crying after injection was more frequent in the NS group, and local skin reactions were more common in the G1 group. Five serious adverse events were judged causally unrelated to treatment and all resolved. Parents/guardians expressed a strong preference for G1 over NS for their children. Vaccinators found the G1 easy to use but noted incomplete vaccine delivery in some cases. Although the G1 has been superseded by an updated device, our results are important for the continued improvement and evaluation of DSJIs, which have the potential to overcome many of the challenges and risks associated with needle-based injections

Abbreviations: DSJI, disposable-syringe jet injector; G1, PharmaJet® 2009 generation-1 device; NS, needle and syringe; MMR, measles–mumps–rubella vaccine; AE, adverse event; SC, subcutaneous; FDA, (United States) Food and Drug Administration; ANVISA (from Portuguese abbreviation), Brazilian Health Surveillance Agency; CONEP (from Portuguese abbreviation), Brazilian National Research Ethics Commission; ITT, intention-to-treat; PP, per-protocol; ELISA, enzyme-linked immunosorbent assay; NT, neutralizing titer; PRNT, plaque reduction neutralization test; IgG, immunoglobulin G; GMC, geometric mean concentration; SeroC, seroconversion; CI, confidence interval; CRF, case report form; OR, odds ratio.

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worldwide. Recommendations for future DSJI clinical studies include rigorous training of vaccinators, quantitative measurement of wetness on the skin following injection, and regular monitoring of device and vaccinator performance.

1. Introduction

Disposable-syringe jet injectors (DSJIs) are needle-free devices that employ a sterile, single-use syringe to administer vaccines with a fine stream of pressurized, high-velocity liquid that penetrates the skin [1,2]. The liquid typically is propelled by release of a piston powered by a compressed spring or gas. DSJIs were developed to address risks associated with a type of device used earlier, the multiple-use nozzle jet injector (MUNJI), after evidence of cross-contamination between patients [1,3–5]. Prior to this, MUNJIs had been widely used in mass immunization campaigns [6,7] and in the military.

Several DSJIs have been approved and marketed in the United States and Europe [1]. They are capable of delivering all injectable vaccines used in immunization programs, whether into intradermal, subcutaneous, or intramuscular tissues, and have the potential to overcome many of the challenges and risks associated with needle-based injections and sharps waste. DSJIs that are particularly attractive for use in developing-country programs are low cost and use manually compressed springs rather than compressed gas.

Antibody responses to vaccines administered by DSJIs generally have been reported as comparable or superior to those induced by needle and syringe (NS). Vaccines shown to induce immunity when given by DSJI include typhoid, diphtheria, pertussis, hepatitis A [8,9], influenza [10–13], and measles–mumps–rubella (MMR) [14]. To date, no immunogenicity data have been published for MMR vaccine administered with the DSJI tested in this study.

The reported rates of local adverse events (AEs) (e.g., edema, erythema, tenderness) have been higher for DSJI delivery than for NS [15], but events are generally mild [8,16,17]. Some studies have found the pain during jet injection to be equivalent to or less than that associated with injection using a conventional NS [15], although other studies reported higher levels of pain with DSJIs [10,12].

For DSJI technology to be adopted globally, data demonstrating immunogenicity and safety of vaccination are important. Another consideration includes acceptability of DSJIs by patients or their parents/guardians and by vaccinators. To provide an initial clinical evidence base for the potential use of DSJIs in immunization programs, we conducted a randomized, controlled trial in which healthy Brazilian infants aged 12 to 18 months received MMR vaccine via either DSJI or NS. The primary aim of the study was to determine if the immunogenicity of the vaccine delivered via DSJI was non-inferior to that administered by NS. The comparison was made for each vaccine antigen separately. Secondary aims were to collect safety data and to survey parents and vaccinators for their perceptions of the specific DSJI evaluated. We also recorded insights on operational aspects of this study that will be useful in future clinical trials of DSJIs.

2. Methods

2.1. Vaccine

MMR vaccine from Bio-Manguinhos used in this study was formulated according to procedures transferred by GlaxoSmithKline to Bio-Manguinhos.[2] Each reconstituted 0.5-mL dose contained the following:

- ≥1000 $CCID_{50}$ of measles live attenuated virus (Schwarz strain),
- ≥5000 $CCID_{50}$ of mumps live attenuated virus (RIT 4385 strain, derived from the Jeryl Lynn strain),
- ≥1000 $CCID_{50}$ of rubella live attenuated virus (Wistar RA 27/3 strain).

2.2. Injection devices

The DSJI used for subcutaneous (SC) vaccination in this study was the first-generation PharmaJet system (PharmaJet; Golden, CO, USA) shown in Fig. 1 and referred to hereafter as the G1. The system consisted of two injectors: a blue device described by the manufacturer's instructions as suitable for adults and children aged two years and older, and a purple injector suitable for infants and for children up to two years old. The G1 had the United States Food and Drug Administration (FDA) (510(k) number K081532, 26 February 2009) and Brazilian Health Surveillance Agency (Agência Nacional de Vigilância Sanitária [ANVISA]) regulatory clearance at the time of the study. The blue injector was used for two pilot studies and the purple injector was used for the infant study reported here. These injectors are spring-powered. Both G1 injectors (blue and purple) were superseded by the PharmaJet Stratis device in late 2011 and are no longer available.

The vaccine in the NS treatment group was administered with sterile, single-use, disposable needles (13 × 4.5, Brazilian scale; 26 gauge, 5/8 in., US scale) and 3-mL-capacity, sterile, single-use, disposable syringes (BD).

2.3. Study populations and setting

The study was conducted at three public health immunization clinics operated by the Health Secretary of Rio de Janeiro: Guadalupe, Irajá, and Rocinha. Adult and pediatric pilot studies

[2] This vaccine is currently used exclusively in the Brazil National Immunization Program.

Fig. 1. The first-generation PharmaJet system (G1) for vaccine delivery with charging station, vial adapter, and syringe.

were conducted to train vaccinators on the use of the G1 and to assess immediate injection-site results. These studies included ten healthy adult males aged 18 to 50 years and 15 healthy children aged four to six years (data not shown). The study reported here was conducted on healthy infants aged 12 to 18 months from August 2010 through March 2011.

Eligibility criteria required that participants be in good health and not enrolled in another research study. They were not to have received their first dose of MMR vaccine and were required to be up to date on all other routine vaccines included in Brazil's Basic Child Vaccination Schedule. They were not to have received any other injectable vaccines within 28 days prior to the study. Written informed consent was obtained from all participants or parents/guardians. The study was approved by the research ethics committee of the Municipal Health Department of Rio de Janeiro, the Brazilian National Research Ethics Commission (Comitê de Ética em Pesquisa [CONEP]), and the PATH Research Ethics Committee, and was registered as International Standard Randomized Controlled Trial 4280032 [18].

The intention-to-treat (ITT) population included all enrolled infants who received an SC injection of MMR vaccine and had safety data recorded immediately and 60 min following injection. All members of the ITT population were included in safety and tolerability analyses.

The per-protocol (PP) population comprised those infants in the ITT population (thus meeting all eligibility criteria listed above) who received an SC injection of MMR vaccine and had a post-vaccination blood sample taken within 35 to 56 days after vaccination. A subject was included in the PP population for analysis of any antibody for which he or she was negative at baseline. For example, a subject who had not previously received MMR and was baseline-positive for antibody against the measles antigen (due to prior exposure) but negative for the other two antibodies was excluded from the PP population for the analysis of measles antibody. However, that individual was still a member of the PP population for the analysis of mumps and rubella antibody responses.

2.4. Vaccinations and study visits

The treatment consisted of a 0.5-mL dose of MMR vaccine administered SC in the left deltoid area, using either the G1 or NS. Infants were randomized 2:1 to receive vaccine via G1 or NS, respectively. This allocation ratio emphasizes the experimental group, allowing better use of resources to generate more data for the G1 [19]. There were three clinic visits. The first visit included a baseline blood draw, vaccination, and monitoring of AEs immediately and at 60 min following vaccination. The second visit occurred within days 8 to 28 after the day of vaccination and included the collection of AEs recorded in diaries, plus a review of delayed local and systemic AEs. The third visit occurred within days 35 to 56 after the day of vaccination and included the post-vaccination blood draw and evaluation of delayed local or systemic AEs.

2.5. Immunogenicity assessment

Enzyme-linked immunosorbent assays (ELISAs) were performed at the Respiratory Virus Laboratory of Instituto Oswaldo Cruz (Fiocruz, Rio de Janeiro). The plaque reduction neutralization test (PRNT) was performed at the Virologic Technology Laboratory of Bio-Manguinhos (LATEV, Fiocruz, Rio de Janeiro). The geometric mean concentration (GMC) for antibodies to each antigen was also calculated for the two treatment groups. Parents/guardians of infants in either study arm who failed to seroconvert to any of the three vaccine antigens were contacted after the study and offered an additional MMR dose delivered by NS.

The immunogenicity of the MMR vaccine was assessed primarily as a percentage of baseline-negative infants who seroconverted for antibodies against each of the three vaccine components—measles, mumps, and rubella viruses. Seroconversion (SeroC) was calculated separately for each vaccine antigen as the percentage of baseline-negative vaccinees having a post-vaccination antibody level greater than or equal to the following cutoff levels:

- Anti-measles neutralizing titer (NT): ≥200 milli-international units per mL (mIU/mL) by PRNT (methods described in reference [26]).
- Anti-mumps Immunoglobulin G (IgG): ≥231 units/mL by ELISA, or if <231 units/mL by ELISA (Enzygnost® anti-parotitis-virus/IgG, Siemens-Behring) and retested by PRNT, then a positive test at a dilution ≥1:10.
- Anti-rubella IgG: ≥4 IU/mL by ELISA (Enzygnost® anti-rubella-virus/IgG, Siemens-Behring).

Non-inferiority was defined a priori as a difference of less than 10% on the upper limit of the 95% confidence interval (CI) for the difference in SeroC rates between the two treatment groups ($SeroC_{NS}$–$SeroC_{G1}$). The sample size was calculated with Power Analysis & Sample Size Software (PASS) 2008 (Number Cruncher Statistical Systems, Kaysville, Utah). Data were analyzed using SPSS predictive analytics software, version 16.0 (SPSS, Inc., Chicago, Illinois). With an allocation ratio G1: NS of 2:1, it was calculated that the sample should be 348 G1

recipients plus 174 NS recipients to equal 522 total subjects. With these group sizes, if both true proportions were 85%, then the power to find the G1 statistically non-inferior to NS would be 88%. To allow for 10% loss of data due to causes such as subject withdrawal and some subjects having detectable pre-vaccination titers, the targeted sample size was increased to a total of 388 plus 194 to equal 582. We also calculated a 95% CI for the ratio of the GMCs (separately for antibody to each of the vaccine antigens) among subjects in the NS and G1 treatment groups (GMC_{NS}/GMC_{G1}). If the upper limit of the 95% CI for the ratio was <1.5, then the null hypothesis of inferiority of the G1 treatment group would be rejected.

For post hoc analyses on immunogenicity, a series of univariate and multivariate regression analyses were done to assess the importance of several independent variables (e.g., age and gender of vaccinees, duration of injector use, or loss of vaccine at the injection site) regarding SeroC rates or $\log_{10}$ of the titer of antibodies against measles, mumps, and rubella viruses as dependent variables. For analyses of incomplete delivery or loss of vaccine, data were gathered from vaccinators' qualitative observations of the injection as prompted by specific fields and open-ended comment sections in the case report form (CRF).

2.6. Safety assessment

The safety and tolerability of vaccination was assessed in terms of the following AEs: 1) local injection-site reactions and systemic AEs observed immediately upon vaccination as well as 60 min later by a clinic physician blinded to the method of injection, 2) local injection-site reactions and systemic AEs recorded by parents on a diary card for days 1 through 10 or otherwise ascertained by study staff during the second clinic visit, and 3) delayed local injection-site reactions and systemic AEs ascertained by study staff during the third clinic visit. The possible, probable, or definite relationship of AEs to treatment (vaccine, injection device, or other aspect of treatment) was determined by the principal investigator. Parents/guardians and study staff were aware of the injection method. Pearson's chi-square test or Fisher's exact test was used, as appropriate for the comparison (Fisher's exact test was used when an observed cell was <5), to evaluate the statistical significance of the differences in AEs between the treatment groups, with $p \leq 0.05$ defined as significant. p-Values were not adjusted for multiple comparisons and were calculated for reference purposes only.

Pre-specified local injection site signs and symptoms included pain, laceration, bruising, induration, swelling, erythema, warmth, and pus or drainage. Pre-specified systemic AEs included anaphylaxis, swelling under the jaw line, rash, irritability/crying, loss of appetite, sleepiness, and fever (axillary temperature ≥37.5 °C).

2.7. Performance, acceptability, and usability of injection devices

Qualitative information regarding the use, performance, and acceptability of the two methods of injection—by G1 or conventional NS—was collected, with emphasis on the following assessments: 1) the incomplete delivery of vaccine, which could have implications for immune response; 2) the perceptions of the provider and parent/guardian of the subject regarding use and acceptability; and 3) ease of use and human factors. Injection performance and human factor data were recorded by vaccinators in the CRF after each vaccination to document delivery of the vaccine. Following each injection, parents/guardians of infants were asked by the vaccinator to rate qualitatively the injection experience (poor, acceptable, or excellent) and indicate whether they would like to have their child receive a future vaccination using the same mode of injection. Monitoring of vaccinators during the study showed that they were employing the recommended techniques for G1 and NS injections.

3. Results

3.1. Study populations

The ITT population consisted of 582 healthy infants. There were no significant differences between subjects in the two treatment groups with respect to the ratio of males to females, age, weight, height, or skin color. Median age was 13 months (range 12.0–18.8; four subjects from 18.1 to 18.8 months of age, all in the G1 group, were considered to meet eligibility requirements); median weight was approximately 10 kg (range 6.6–17.0). Of the 582 subjects, 573 had a blood sample of sufficient volume for determination of antibodies against the three viral antigens. However, 21 of these had the sample taken outside the pre-specified window of days 35 to 56 and so were not eligible for inclusion in the PP population for the analysis of antibody responses by treatment group, leaving a PP population of 552. In addition, five pre-vaccination blood samples contained antibodies at a level above the designated cutoff for antibodies against measles virus (≥200 mIU/mL); antibody levels in samples from four individuals were above the designated cutoff for antibodies against mumps virus (≥231 units/mL); and one sample was too small to permit necessary retesting for antibodies against mumps virus. Thus, 547 infants met the criteria for inclusion in the PP population for analysis of antibodies against measles and mumps viruses; for rubella the PP population was 552.

3.2. Immunogenicity assessment

Table 1 shows by treatment group the SeroC rates for antibodies to measles, mumps, and rubella viruses among baseline-negative infants in the PP population 35 to 56 days after receiving an injection of MMR vaccine. For antibodies to rubella virus, the upper limit of the 95% CI for the difference $SeroC_{NS}$–$SeroC_{G1}$ was <10%, meeting the criterion for non-inferiority. For antibodies against measles and mumps viruses, the upper limits of the 95% CI were >10%; thus, the responses in the G1 treatment group did not meet non-inferiority criteria.

GMCs of serum antibodies against the three vaccine components also were estimated. For antibodies to both measles and mumps viruses, the upper limit of the 95% CIs for the ratio GMC_{NS}/GMC_{G1} for subjects in the PP population exceeded the protocol-defined limit of 1.5, while for antibodies against rubella virus it was less than 1.5 (Table 1). Thus, the GMCs for measles and mumps vaccine components delivered by the G1 did not meet the non-inferiority definition for comparison with NS, but those for rubella virus were non-inferior, mirroring the results of the SeroC rates. Families of all infants who did not mount an adequate response were notified, and all of these infants subsequently were re-vaccinated.

Table 1
Seroconversion (SeroC) rates and geometric mean concentrations for antibodies against measles, mumps, and rubella viruses by treatment group among baseline-negative subjects following an injection of measles–mumps–rubella vaccine (per-protocol [PP] population).

Antibody	Treatment group (Total N subjects[a])	Subjects sero-converting N (%)	$SeroC_{NS}$–$SeroC_{G1}$ (95% CI)	Geometric mean concentration (GMC)	GMC_{NS}/GMC_{G1} (95% CI)
Anti-measles NT	NS (182)	182 (100.0)	9.3 (5.9, 12.7)	4996.75 mIU/mL	1.40 (1.19, 1.64)
	G1 (365)	331 (90.7)		3563.20 mIU/mL	
Anti-mumps IgG[b]	NS (183)	140 (76.5)	14.4 (6.1, 22.7)	661.20 U/mL	1.57 (1.27, 1.92)
	G1 (364)	226 (62.1)		422.27 U/mL	
Anti-rubella IgG	NS (184)	183 (99.5)	0.3 (−1.5, 2.1)	43.05 IU/mL	1.01 (0.87, 1.18)
	G1 (368)	365 (99.2)		42.47 IU/mL	

[a] The number of subjects for the anti-rubella PP population was 552. Because four subjects had high pre-vaccination antibody blood levels and one had an inadequate blood sample, the number for anti-measles and anti-mumps PP populations was 547.
[b] Includes two subjects in the G1 group negative by ELISA but positive by PRNT retest.

Because the post-vaccination SeroC rates for antibodies against measles and mumps viruses among subjects in the G1 treatment group did not meet non-inferiority criteria compared with those of infants in the NS treatment group, we conducted a number of post hoc analyses to identify factors that may have contributed to the diminished antibody responses in the G1 treatment group. Several variables were found to have a significant effect on SeroC rates among subjects in the G1 treatment group for antibodies against measles and/or mumps virus but not for antibodies against rubella virus.

In the G1 group, female gender ($p = 0.032$, Pearson's chi-square) and children 12 to <13 months ($p = 0.016$, Pearson's chi-square), were associated with a lower SeroC rate for measles antibody, and in univariate regression analysis female gender was marginally associated with a lower SeroC rate for mumps antibody (odds ratio [OR] 0.656, $p = 0.052$). Lower body weight also exhibited a lower, but not statistically significant, SeroC rate for measles antibody (OR 0.498, $p = 0.063$).

In a further multivariable regression analysis, we looked for evidence of possible incomplete delivery of vaccine, noted by vaccinators on the CRF as "failure to inject 0.5 mL,""liquid or vaccine at the injection site," or "spray at injection." These observations were reported for 13% of vaccinations in the NS treatment group and 58% in the G1 treatment group. Incomplete delivery was significantly associated with a reduced SeroC rate for mumps antibody ($p = 0.044$) and lower measles GMCs in the G1 treatment group ($p = 0.047$).

3.3. Safety assessment

Vaccination was generally well tolerated by infants in both treatment groups, but there were statistically significant

Table 2
Number of subjects with local and systemic adverse events (AEs) observed in at least 4% of vaccinees in either treatment group immediately, 60 min, and 1 to 10 days following an injection of measles–mumps–rubella vaccine (intention-to-treat [ITT] population).

Time of observation	Type of AE	NS	G1	p-Value[c]
Immediately	Injection-site AE			
	• Blood at injection site	9/194 (4.6%)	8/388 (2.1%)	0.082
	• Papules	1/194 (0.5%)	36/388 (9.3%)	<0.001
	Systemic AE			
	• Short cry	153/194 (78.9%)	152/388 (39.2%)	<0.001
	• Inconsolable cry	21/194 (10.8%)	6/388 (1.5%)	<0.001
Total with immediate AEs[a]		174 (89.7%)	183 (47.2%)	<0.001
60 min	Injection-site AE			
	• Erythema	10/194 (5.2%)	92/388 (23.7%)	<0.001
	Systemic AE			
	• Sleepiness	9/194 (4.6%)	17/388 (4.4%)	0.877
Total with AEs at 60 min[a]		24 (12.4%)	112 (28.9%)	<0.001
Days 1 10	Injection-site AE			
	• Pain	25/193 (13.0%)	33/384 (8.6%)	0.100
	• Erythema	16/192 (8.3%)	80/384 (20.6%)	<0.001
	• Swelling	17/192 (8.9%)	80/383 (20.9%)	<0.001
	Systemic AE			
	• Fever			
	Any (≥37.5 °C)	85/153[b] (55.6%)	109/277 (39.4%)	0.001
	High fever (≥39 °C)	22/153[b] (14.4%)	23/277 (8.3%)	0.049
	• Loss of appetite	81/193 (42.0%)	153/384 (39.8%)	0.624
	• Sleepiness	37/193 (19.2%)	75/384 (19.5%)	0.918
	• Irritability	41/193 (21.2%)	72/384 (18.8%)	0.476
	• Rash	12/193 (6.2%)	24/384 (6.3%)	0.988
Total with AEs in days 1 10[a]		137 (80.6%)	264 (78.8%)	0.640

[a] Numbers in "Total" rows refer to the total number of subjects with adverse events, and some subjects experienced more than one AE; in this table we included only those AEs reported in ≥4% of subjects.
[b] Note that denominators are lower in some cases. This is because a smaller number of parents/guardians recorded this information.
[c] Pearson's chi-square test or Fisher's exact test was used, as appropriate for the comparison.

differences in the frequencies of certain AEs. Table 2 shows the frequencies of pre-specified and other local and systemic AEs noted immediately, at 60 min, and 1 to 10 days after injection. Overall, the percentage of subjects with any AE immediately following vaccination was significantly greater among subjects in the NS treatment group compared with the G1 treatment group (89.7% versus 47.2%, $p < 0.001$). Local AEs (mainly papules) were more frequent among G1 vaccinees, while crying following injection was more frequent in the NS treatment group.

By 60 min, the proportion of subjects in the NS and G1 treatment groups with an AE declined to 12.4% and 28.9%, respectively (Table 2). The difference between groups after 1 h was largely due to the higher rate of local AEs (mostly erythema at the injection site) in the G1 treatment group.

During days 1 through 10 (Table 2), the proportions of subjects with one or more pre-specified AEs were similar in both treatment groups (78.8% in the G1 group; 80.6% in the NS group). G1 vaccinees continued to experience more local AEs (mostly erythema and swelling at the injection site), while NS vaccinees had slightly more systemic AEs, such as fever. No AE reported during this time was rated as serious. One NS vaccinee had a local AE rated as severe (Grade 3 pain), while a small proportion of subjects in both treatment groups (9.0% G1 to 13.5% NS) experienced at least one severe systemic AE. All other local and systemic AEs were graded as mild or moderate (Grade 1 or 2).

By the second clinic visit, the overall frequency of any delayed AEs was 13.5% for the G1 group, compared with 15.0% for the NS group, and by the third clinic visit, the frequency declined to 6.5% compared with 4.2%, respectively. In addition to these AEs, persistent injection site stigmata (e.g., scars, hypochromia, macula, and papules) were noted as minor events more prevalent among G1 compared with NS treatment group subjects at both the second clinic visit (9.9% G1 versus 0.5% NS, $p < 0.001$, Fisher's exact test) and third clinic visit (3.5% G1 versus 0.0% NS, $p = 0.006$, Fisher's exact test).

Five subjects in the study experienced a serious AE but all events resolved and none were judged to have been related to treatment. In the G1 treatment group, two were cases of pneumonia and one was thought to be dengue. In the NS treatment group, there was one case of a subgaleal hematoma and one of meningitis. Among nine other significant AEs (including five cases of pneumonia), two cases of pneumonia with onset ten days after G1 vaccination were described as possibly related and probably related to treatment; the other events were judged not related.

3.4. Acceptability assessment

In the G1 treatment group 90.2%, 9.5%, and 0.3% of parents/guardians rated the injection experience as excellent, acceptable, and poor, respectively. The ratings for NS were 7.2%, 62.9%, and 29.9% for these descriptors. When asked about future injections, 96.1% of families of infants vaccinated with the G1 indicated they would prefer it when their child needed an injection, and 92.3% of families of infants injected with NS indicated they would prefer an alternate mode of injection.

At each of the three study sites, one primary vaccinator administered most of the injections. Filling the needle-free syringe from the vaccine vial using the vial adapter was rated by the vaccinators as easy for 99% of the injections. In approximately 4% of cases, the vaccinator had to obtain another syringe package or reattach/realign the syringe to the device. The G1 was rated as easy to use for 99% of injections, and less than 5% of the injections were noted as causing slight hand/arm strain for the vaccinator. There were no reports of any vaccinator injury related to the G1.

4. Discussion

Our study compared the immunogenicity of a MMR vaccine administered via the G1 with that of the vaccine administered via NS. While the results showed non-inferiority of SeroC rates for G1 delivery of the rubella component of the vaccine, SeroC and GMCs for the measles and mumps components did not meet non-inferiority criteria. Our post hoc analyses showed that some characteristics such as female gender and younger age (12 to <13 months) were associated with a lower SeroC rate for measles antibody, although the mechanisms by which female gender or infant age might lead to lower SeroC are not known. Incomplete delivery of vaccine was associated with lower SeroC rates for mumps and lower measles GMCs. A small amount of liquid on the surface of the skin is common following DSJI injections; however, the high rate of incomplete injections observed in our study and the relationship with lower immune responses suggests that the G1 was not optimized for this age group. Incomplete delivery was observed visually as wetness on the skin, a spray in the air at the time of injection, or reflux of vaccine from the puncture site. Visual observation is a subjective method; use of a quantitative method for measuring liquid not injected might have strengthened the correlation between volume of vaccine delivered and immune responses.

In the NS group, the performance of the measles and rubella vaccine components was excellent, and the SeroC and GMCs were comparable to previous reports [20–24]. In contrast, mumps immunogenicity by NS was poor, although the results of this study are consistent with the immunogenicity variation observed in several studies using the same MMR vaccines and laboratory methods [20–26]. The reasons for these variations in immunogenicity have not yet been explained. It should be noted that in one of these studies [27], vaccines from two different MMR producers were used, and both had similar mumps SeroC rates of around 70%.

The PharmaJet G1 used in this study has been superseded by the PharmaJet Stratis and is no longer available. The Stratis was designed to improve injection quality by reducing incomplete injections and simplifying operation, helping to reduce training requirements. The Stratis and another DSJI, the Lectrajet® from D'Antonio Consultants International, Inc., have recently been evaluated for delivery of trivalent inactivated influenza vaccine, and results showed that vaccination with DSJIs in these studies produced immune responses non-inferior to those from vaccination with NS [11,13]. Bench testing of both the G1 and Stratis to assess whether jet injection affects the viability of the live measles, mumps, and rubella viruses in the vaccine found minimal loss of vaccine potency (written communication, April 25, 2014: Melissa Coughlin, Marcus Collins, and Paul Rota, all of United States Centers for Disease Control and Prevention). Studies of MMR vaccine delivery to infants with the Stratis device are needed to assess non-inferiority to NS for this vaccine.

Vaccination was generally safe and well tolerated by infants who were administered MMR vaccine using either the G1 or NS; G1 vaccinees had more minor local injection-site AEs while NS vaccinees had more crying, irritability, and fever. Vaccinators found the G1 very easy to load and use but noted a problem with incomplete delivery of vaccine in a significant proportion of vaccinees. Parents/guardians expressed a strong preference for the G1 over NS as a mode of injection for their children, which may be related to the significantly lower degree of crying observed during and after injection with the G1.

Although the G1 system has been discontinued, our study demonstrates the importance of evaluating new DSJIs and provides insights for future studies. We suggest including the following activities for any trial evaluating immunizations via DSJIs:

- Include an NS control group receiving the same vaccine at the same dose and depth of delivery as the DSJI group.
- Work closely with the DSJI manufacturer to train vaccinators on the use of the device and to monitor the performance of the devices used.
- Include quantitative measurement of loss of vaccine during immunization. Methods for quantifying the volume of liquid on the exterior of the skin and the device have been developed and used in other clinical studies, including weight-based and absorption-based procedures [27,28], but qualitative observation is the only known method for reporting vaccine sprayed in the air.
- Conduct interim analyses of injection performance to identify and correct any device malfunctions or additional training needs as they occur.
- Create a plan for re-immunization of subjects in any study arm who do not exhibit an adequate immune response.

5. Conclusions

The DSJI is a promising technology with potential for use in mass immunization campaigns and for routine immunization programs in low- and middle-income countries. The use of a sterile, single-dose, disposable, non-reusable syringe in these devices eliminates the risk of blood-borne infections that can be associated with the use of a needle and syringe, and the use of a spring to power the injection makes the DSJI attractive for settings that lack access to other power sources. Parents found the G1 highly acceptable and vaccinators considered it easy to use. While the specific DSJI used in this study cannot be endorsed for use in immunization programs, and has been discontinued, our experiences and recommendations may inform future evaluations of newer DSJIs for routine infant immunizations.

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Immunogenicity & Tolerance Study of DTP and Other Vaccines, France & Africa

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Clinical immunogenicity and tolerance studies of liquid vaccines delivered by jet-injector and a new single-use cartridge (Imule®): comparison with standard syringe injection

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A new needleless jet-injector, Mini-Imojet®, was developed that administers liquid vaccines from a single-use, pre-filled cartridge named Imule® , which avoids the risk of cross-contamination. We conducted clinical trials in several settings in France and West Africa to compare the immunogenicity and tolerance of five vaccines (influenza vaccine, Vi capsular polysaccharide typhoid vaccine, tetanus toxoid vaccine, diphtheria–tetanus–whole cell pertussis vaccine, and inactivated hepatitis A vaccine) administered with the Imule® system vs standard syringe technique. In each vaccine study, all subjects of either group were tested for serum antibody titres to calculate the geometrical mean titres and seroconversion rates after complete vaccination. Immediate local reactions were noted after each injection, and local and general reactions were evaluated during a predetermined period of follow-up. When delivered by the Imule® technique, all the administered vaccines were of equivalent or superior immunogenicity, compared to the syringe technique. The tolerance to vaccines injected by the Imule® system was acceptable in all studies. The most frequently observed reactions were mild (e.g. minor bleeding, superficial papules, erythema and induration) and could be considered to be inherent to the injection technique. The technical and safety advantages of the Mini-Imojet®/Imule® system, compared to sterilizable, standard disposable or autodestruct syringes and to classical multi-dose vial jet-injectors, reinforces the interest of this new injection technique for collective immunizations. © 1997 Elsevier Science Ltd.

Keywords: jet-injector; clinical trials; review; vaccines; immunogenicity; tolerance.

The first jet injectors (Ped-O-Jet®), initially developed by the US Army in the context of bacterial warfare, were used in 1954 for mass administration of diphtheria and tetanus toxoids[1]. The principle consists of injecting vaccines subcutaneously (s.c.) by a thin, high-pressure jet of fluid. The jet is created and directed on the skin surface by a nozzle, which is resterilized at the end of each session. Previous studies showed equal or better vaccine serological responses from jet-injection compared to the standard syringe technique[2,3]. Owing to the mechanics of the self-contained system, jet injectors have several advantages: less manipulation is required; there are no needles or syringes to sterilize; there is no risk of accidental puncture; and it is possible to immunize large groups of people rapidly because of the jet injector's high speed of operation.

Despite these important advantages, many concerns remain about routine use of jet-injectors. Transfer of virus from a chronic carrier to a healthy vaccinee through reflux of blood from the nozzle has been demonstrated, when vaccines are delivered in multi-dose vials[4–6]. Since the onset of the human

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immune-deficiency virus (HIV) pandemic, the risk of transmitting it, and other agents such as the hepatitis B virus, is a major concern. Coupled with the availability of single-use and resterilizable syringes and needles through the United Nations Children's Fund (UNICEF) and the World Health Organization (WHO), jet injectors have come into disfavour[7]. However, even in experienced immunization programs, the security of syringe injections remains a problem[8].

Pasteur-Mérieux sérums et vaccins (P.M. sv.) has developed a jet injector (Mini-Imojet®) that administers liquid vaccine from a single-use, pre-filled cartridge of vaccine (Imule®), with a single-use nozzle to prevent cross-contamination. As the Imule® system uses the same sterile, pre-filled cartridge for transport and administration of the vaccine, the vaccine is guaranteed to be contamination free and chemically stable.

This report describes the results of clinical tolerance and immunogenicity trials conducted in France and West Africa to compare the Imule® system with standard syringe administration techniques for five different liquid vaccines manufactured by P.M. sv.: influenza vaccine (Vaxigrip®), Vi capsular polysaccharide typhoid vaccine (TyphimVi®), tetanus toxoid vaccine (Tetavax®), diphtheria–tetanus–whole cell pertussis (DTP) vaccine (DTCoq®) and inactivated hepatitis A vaccine (Avaxim®).

MATERIAL AND METHODS

The Mini-Imojet® jet-injector and the Imule® system

The single-use Imule® cartridge is a 0.5 ml polypropylene cylinder containing one dose of vaccine. The pressure required for injection (300 bars) is produced by a spring powered piston which is released by a trigger. The vaccine is ejected through the delivery canal at a speed of 120 m. s^{-1} through the different layers of the skin, but mainly into the deep s.c. tissue layer. Only the cartridge nozzle comes into contact with the skin surface, and this cartridge is changed after each patient[9] (*Figure 1*).

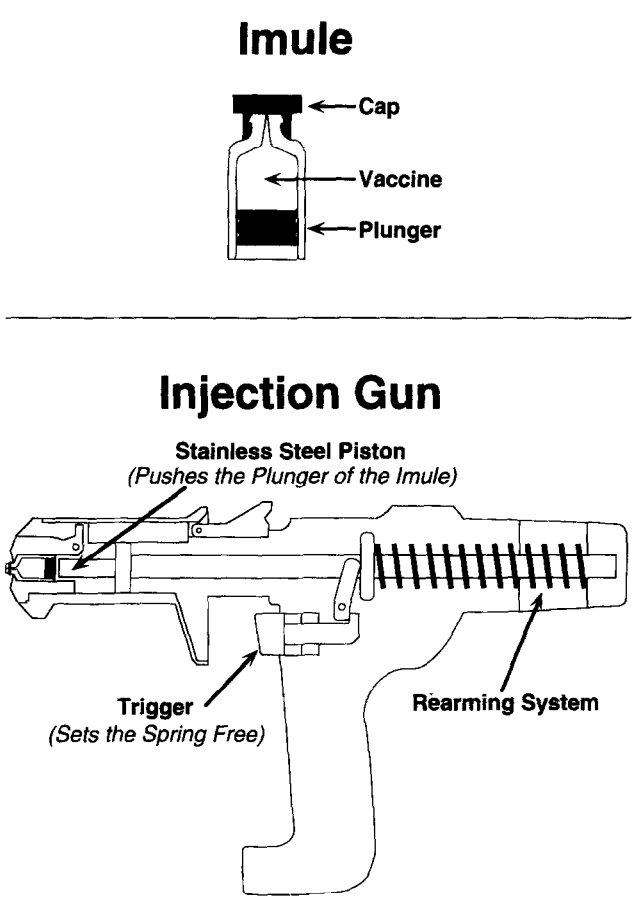

Figure 1 Mini-Imojet® and the Imule® system

Clinical studies

These studies were conducted between June 1990 and July 1994 in institutional settings in France (influenza, typhoid, and hepatitis A vaccines), or in West Africa (tetanus and DTP vaccines) as part of mass immunization campaigns or Expanded Programme on Immunizations (EPI) activities, and were run and monitored under European or WHO standards of good clinical practice. All immunogenicity trials were controlled studies (i.e. jet-injector vs syringe). The hepatitis A vaccine study was conducted on three groups: jet-injector; intramuscular (i.m.) injection; or s.c. injection. The different vaccines, populations and methods are summarized in *Table 1*.

Inclusion criteria included: unvaccinated subjects (in particular, subjects who had not been vaccinated for 5 years and had no history of typhoid fever) for the Vi typhoid vaccine study; male subjects without history of previous tetanus immunization for the tetanus toxoid vaccine immunogenicity study; adults, excluding women of childbearing age who are immunized according to the tetanus EPI schedule as documented by an immunization card, for the tetanus toxoid vaccine tolerance study; unimmunized infants with no history of diphtheria and pertussis for the DTP study; and subjects seronegative for hepatitis A virus (HAV) for the hepatitis A vaccine study. [HAV serologies were performed with the ELISA Wellcozyme anti-HAV assay (Murex Biotech ltd, Dartford, UK).]

Vaccines

All vaccines were provided by P.M. sv. The composition and volume for one dose (0.5 ml) of each vaccine was similar, whether injected by the Imule® system or by syringe. The route of administration with syringe technique was s.c. for Vaxigrip®, i.m. for Typhim Vi® and DTCoq®, deep-s.c. for Tetavax®, and i.m. or s.c. for Avaxim®.

Evaluation criteria

Immunogenicity

All serum titrations were performed blindly according to lists pre-coded by the control laboratory of P.M. sv. Evaluation criteria for each antigen were the percent of seroconversion and the geometric mean titre (GMT) after immunization.

Influenza. Anti-haemagglutinin antibodies directed against the three strains contained in the vaccine were measured using the micromethod of haemagglutination-inhibition recommended by WHO, and results are expressed as inverse titres. Seroconversion is defined by at least a fourfold rise in antibody titre at 21 days after vaccination.

Table 1 Populations and methods for immunogenicity and tolerance studies

	Influenza immunogenicity	Influenza tolerance	Typhoid	Tetanus immunogenicity	Tetanus tolerance	Diphtheria–Tetanus–Pertussis	Hepatitis A
Country and trial period	France—1990	France—1990	France—1990	Burkina Faso—1991	Senegal—1993	Burkina Faso—1992	France—1994
Design of the study	Randomized, controlled, monocentric	Non-randomized, controlled, multicentric	Non-randomized, controlled, monocentric	Randomized, controlled, monocentric	Non-comparative, monocentric	Randomized, controlled, multicentric	Randomized, controlled, multicentric
Population and context	Adults males (≥18 years), firemen	Adults (≥18 years), military	Adults (≥18 years), students	Adults males (≥18 years), health center/mass immunization	Adults (≥15 years), medical center/mass immunization	2–3-months-old infants, rural health centers/EPI	Adults (18–60 years), hospital
Sample size							
Imule® group	120	151	65	122	218	74	48
Syringe group	119	211	62	111	—	71	50 (i.m.) and 49 (s.c.)
Immunization schedule	One injection	One injection	One injection	Two injections given at a 3 month interval	One injection	Three injections given at a 1 month interval	Two injections given at a 6 month interval
Sera	Week 0, week 3	—	Week 0, week 4	Week 0, week 30	—	Week 0, week 21	Week 0, 4, 24 and 28
Immunogenicity criteria	% of seroconversion GMTs		% of seroconversion GMTs	% of seroconversion GMTs	—	% of seroconversion GMTs	% of seroconversion GMTs
Positivity threshold for antibody titre	Neutralizing antibodies ≥1/40	—	≥1.5 μg ml^{-1}	≥0.01 IU ml^{-1}	—	≥0.01 IU ml^{-1} for tetanus and diphtheria[a]	≥20 mUI ml^{-1}
Follow-up duration for tolerance	—	4 days (self-monitoring form)	5 days (self-monitoring form)	—	Medical examination at day 3	Medical examination at days 2 and 4 after the first injection	5 days (self-monitoring form)

[a]No validated data for pertussis positivity threshold

Typhoid. Serum antibodies were measured by radioimmunoassay (RIA), and the GMTs are expressed in μg ml^{-1}. Seroconversion is defined by at least a fourfold rise of the initial antibody titre 28 days after vaccination.

Tetanus. Serum antibodies were determined using a RIA method, and the GMTs are expressed in IU ml^{-1}. Seroconversion is defined to be at least a fourfold rise in antibody titre or as a rise beyond the previously defined minimal positive threshold 120 days after the second dose in the tetanus toxoid study, and 90 days after the third dose in the DTP study.

Diphtheria. Serum antibodies were measured by RIA, and the GMTs are expressed in IU ml^{-1}. Seroconversion is defined as at least a fourfold rise in antibody titre, or is taken to be a rise beyond the previously defined minimum positive threshold 90 days after the third dose of DTP.

Pertussis. Serum antibody concentrations were measured by an agglutination assay, and the GMTs expressed as the inverse of dilution. Seroconversion is at least a fourfold rise in antibody titre 90 days after the third dose of DTP.

Hepatitis A. Serum antibodies were measured by RIA[10], modified to increase the sensitivity[11], using a commercial kit (HAVAB®, Abbott Laboratories, North Chicago, IL, USA), and results were converted into International Units by comparison with a reference curve generated from WHO Reference HAV Globulin. The detection cut-off was 10 mIU ml^{-1}. Seroconversion is defined as an antibody titre that rises from below 20 mIU ml ^{1}, initially, to $\geqslant$20 mIU ml^{-1} after vaccination, and GMTs are expressed in mIU ml^{-1}.

Tolerance

Immediate local reactions were evaluated by the investigators 3–15 min after the injection. A follow-up period was defined for each study; delayed local and general reactions were evaluated through a self-monitoring form filled out by the vaccinees or through an active surveillance medical team (*Table 1*).

Statistical analysis

The biometry department of P.M. sv. performed all statistical analysis using SAS software (SAS Institute Inc., Cary, NC, USA).

For the influenza immunogenicity study, parametric tests were done using Student's *t*-test on the inverse titres, after logarithmic transformation, to compare pre- and post-vaccination GMTs for all three influenza strains. A χ^2 test or Fischer's exact test allowed seroconversion rate comparisons.

The test of significance for immunogenicity studies of influenza, typhoid and hepatitis A vaccines, generally administrated on an individual basis, was bilateral and based on demonstration of a difference between the routes of administration (conventional significance testing).

For tetanus toxoid or DPT vaccine, generally administrated on a collective basis like EPI setting where operational aspects are important, the objective was to demonstrate that Imule® technique did not significantly decrease immunogenicity compared to syringe. The test of significance for immunogenicity studies of these vaccines was unilateral and based on demonstration of an equivalence between the two administration routes (Imule® and syringe) rather than a difference[12]. The null hypothesis was expressed as:

(1) [(seroconversion rate in syringe group) $\geqslant$ (seroconversion rate in Imule® group)+15%] for seroconversion criteria; and
(2) [(GMT in syringe group) $\geqslant$ 1.5 (GMT in Imule® group)] for GMT.

Equivalence (i.e. the alternative hypothesis) was defined in two ways as equivalence tests:

(1) a difference of no more than 15% for seroconversion rates [(seroconversion rate in syringe group) − (seroconversion rate in Imule® group)< 15%]; and
(2) a ratio of no more than 1.5 for GMT levels [(GMT in syringe group)/(GMT in Imule® group)<1.5].

Although all age groups were enrolled, the tetanus toxoid vaccine study had been originally designed to include only persons <40 years of age because there are age dependent differences in the immune response to tetanus toxoid[13]. For this reason, the two age groups were analysed separately. For hepatitis A, a multivariate regression analysis model was used that included all time intervals (weeks 4, 24 and 28) and routes of injection (Imule®, i.m. and s.c.).

All tests accept an alpha error of 5%.

RESULTS

Characteristics of the populations

There were few differences between subjects in each of the study groups (*Table 2*).

Immunogenicity results

The results are summarized in *Table 3* for each antigen.

Influenza. Data on 104 subjects in the Imule® group and 109 subjects in the syringe group were available for final analysis. When a significant difference could be demonstrated, the seroconversion rates and post-vaccination GMTs were higher for each strain in the Imule® group.

Typhoid. Data on 60 subjects in the Imule® group and 61 subjects in the syringe group were available for final analysis. The seroconversion rates and post-vaccination GMT were higher in the Imule® group ($P<0.05$).

Tetanus. In the tetanus vaccine study, data on 108 subjects (49 subjects younger than and 49 subjects older than 40 years of age) in the Imule® group and 99 subjects (51 subjects younger than and 48 subjects older than 40 years of age) in the syringe group were available for final analysis. Subjects younger than 40-years-old had higher seroconversion rates and post-vaccination

Table 2 Summarized population characteristics

Studies		Subjects characteristics	Imule®	Syringe
Influenza immunogenicity		Mean age ±σ (years)	20.5±1.8	20.5±1.8
Influenza tolerance		Mean age ±σ (years)	41.9±10.6	38.6±11.3[a]
		Sex ratio (M/F)	2.0	2.1
		Previous flu immunization	74.1%	78.0%
Typhoid		Mean age ±σ (years)	26.0±9.6	25.8±7.8
		Sex ratio (M/F)	0.7	0.8
Tetanus immunogenicity	≤40 years	Mean age ±σ (years)	32.0±6.1	31.6±6.6
		Tetanus antibody before vaccination ≥0.01 IU ml^{-1}	100%	100%
	≥40 years	Mean age ±σ (years)	55.6±10.0	54.2±9.8
		Tetanus antibody before vaccination ≥0.01 IU ml^{-1}	100%	100%
Tetanus tolerance		Mean age ±σ (years)	35±14	—
		Sex ratio (M/F)	0.8	—
DTP		Mean age ±σ (days)	85.9±21.9	88.6±20.4
		Sex ratio (M/F)	1.5	1.4
		Diphtheria antibody before vaccination <0.01 IU ml^{-1}	5.0%	1.2%
		Tetanus antibody before vaccination <0.01 IU ml^{-1}	2.5%	0.0%
		Pertussis antibody before vaccination ≤1/5	65.8%	69.6%
Hepatitis A		Mean age ±σ (years)	32.8±8.3	i.m.=31.8±8.4 s.c.=29.8±8.2
		Sex ratio (M/F)	0.8	i.m.=0.9 s.c.=0.6

[a]$P<0.01$ (Student's *t*-test)

GMTs with the syringe technique compared to the Imule® system, and, therefore, equivalence between the two methods was not demonstrated ($P>0.05$). In contrast, in the group older than 40 years of age, post-vaccination GMT and seroconversion rates were not significantly inferior for Imule® compared to syringe and, therefore, they were demonstrated to be equivalent ($P<0.01$ for seroconversion rates and $P<0.05$ for GMT).

In the DPT study, data on 71 subjects in the Imule® group and 74 subjects in the syringe group were available for final analysis of tetanus antigen. The pre-vaccination GMTs of tetanus antibody were two times greater in the syringe group ($P<0.01$). The post-vaccination GMT and seroconversion rates were not significantly inferior for the Imule® group compared to syringe and, therefore, they were demonstrated to be equivalent ($P<0.01$ for both GMT and seroconversion rates).

Diphtheria. Data on 72 subjects in the Imule® group and 75 subjects in the syringe group were available for final analysis of diphtheria antigen. The post-vaccination GMT and seroconversion rates were not significantly inferior for the Imule® group compared to syringe and, therefore, they were demonstrated to be equivalent ($P<0.01$ for both GMT and seroconversion rates).

Pertussis. Data on 71 subjects in the Imule® group and 74 subjects in the syringe group were available for final analysis of pertussis antigen. The post-vaccination GMT and seroconversion rates were not significantly inferior for the Imule® group compared to syringe and, therefore they were demonstrated to be equivalent ($P<0.01$ for both GMT and seroconversion rates).

Hepatitis A. For the final analysis, data on 119 subjects (40 subjects in the Imule® group, 39 in the syringe i.m. group, and 40 in the syringe s.c. group) were available for descriptive serological results at week 4; data on 134 subjects (45, 45 and 44 subjects in the Imule®, syringe i.m. and syringe s.c. groups, respectively) were available at week 24 (booster); and data on 128 subjects (43, 43 and 42 subjects in the Imule®, syringe i.m. and syringe s.c. groups, respectively) were available at week 28. Logistic regression analysis on seroconversion rates was not possible because most subjects seroconverted after week 4.

A multivariate regression analysis model of GMT response for all time points (week 4, 24 and 28), established an overall significant effect for route ($P<0.05$) and time ($P<0.01$) that favoured Imule® . Direct comparison between each route indicated that only the difference between Imule® and the s.c. route was significant ($P<0.05$).

Data were not stratified regarding gender except for typhoid and hepatitis A immunogenicity studies and no effect of sex was shown for post-vaccination GMT.

Tolerance results

Immediate reactions and, local and general reactions observed during the follow-up, are summarized in *Table 4*.

Influenza vaccine.

Immediate reactions. There were few differences in the frequency of immediate reactions noted for Imule® and syringe. Drops of blood ($P<0.05$), drops of serous fluid ($P<0.01$), papules ($P<0.01$) (the majority of which were superficial) and erythema ($P<0.01$) were seen more often in the Imule® group. The mean size of erythema was 15 mm in the Imule® group and 9 mm in the syringe group.

Delayed local and general reactions. Persons in the Imule® group more frequently experienced spontaneous

Table 3 Summary of immunogenicity results

	Imule®	Syringe		*P*
Influenza				
A/Guizhou/54/89 (H3N2)				
Pre-vaccinal GMT	1.41 (1.34–1.48)	1.46 (1.40–1.52)		(1)
Post-vaccinal GMT	2.34 (2.27–2.41)	2.22 (2.15–2.29)		<0.05[b]
% of seroconversion	79.8%	78.0%		N.S.[a]
A/Singapore/6/86 (H1N1)				
Pre-vacinal GMT	0.93 (0.86–0.99)	0.99 (0.92–1.07)		(1)
Post-vaccinal GMT	2.26 (2.17–2.34)	2.09 (1.99–2.18)		<0.05[b]
% of seroconversion	94.2%	84.2%		<0.05[a]
B/Yamagata				
Pre-vaccinal GMT	1.04 (0.99–1.08)	1.01 (0.96–1.06)		(1)
Post-vaccinal GMT	2.12 (2.05–2.18)	2.02 (1.94–2.09)		N.S.[b]
% of seroconversion	98.8%	88.9%		<0.01[a]
Typhoid				
Pre-vaccinal GMT (g ml^{-1})	0.18 (0.15–0.21)	0.19 (0.17–0.22)		
Post-vaccinal GMT (g ml^{-1})	2.10 (1.63–2.72)	1.45 (1.15–1.82)		<0.05[b]
% of seroconversion	86.7%	68.9%		<0.05[a]
Tetanus				
≤40 years				
Pre-vaccinal GMT (IU ml^{-1})	0.06 (0.04–0.08)	0.05 (0.04–0.06)		(2)
Post-vaccinal GMT (IU ml^{-1})	0.22 (0.16–0.29)	0.26 (0.18–0.37)		0.2[c]
% of seroconversion	42.9%	58.8%		0.5[c]
≥40 years				
Pre-vaccinal GMT (IU ml^{-1})	0.04 (0.03–0.04)	0.04 (0.03–0.05)		(2)
Post-vaccinal GMT (IU ml^{-1})	0.13 (0.09–0.18)	0.12 (0.09–0.17)		<0.05[c]
% of seroconversion	50.9%	39.6%		<0.01[c]
DTP				
Diphtheria				
Pre-vaccinal GMT (IU ml^{-1})	0.05 (0.04–0.68)	0.07 (0.06–0.08)		(2)
Post-vaccinal GMT (IU ml^{-1})	0.55 (0.44–0.69)	0.34 (0.27–0.41)		<0.01[c]
% of seroconversion	79.2%	58.7%		<0.01[c]
Tetanus				
Pre-vaccinal GMT (IU ml^{-1})	0.09 (0.07–0.12)	0.17 (0.12–0.23)		(2)
Post-vaccinal GMT (IU ml^{-1})	2.27 (2.12–2.43)	1.46 (1.29–1.65)		<0.01[c]
% of seroconversion	88.7%	70.3%		<0.01[c]
Pertussis				
Pre-vaccinal GMT (inverse of dilution)	10.6 (7.8–14.5)	9.7 (7.1–13.3)		(2)
Post-vaccinal GMT (inverse of dilution)	1434 (1188–1732)	1188 (965–1465)		<0.01[c]
% of seroconversion	94.4%	94.6%		<0.01[c]
Hepatitis A				
		i.m. route	s.c route	(1)
Pre-vaccinal GMT (mIU ml^{-1})	5.2 (4.4–6.2)	5.1 (4.3–5.9)	4.5 (3.9–5.2)	
Post-vaccinal GMT (mIU ml^{-1}) at week 4	305 (212–439)	211 (145–306)	166 (118–232)	
Post-vaccinal GMT (mIU ml^{-1}) at week 24	251.3 (199.9–315.8)	157.6 (119.8–207.3)	152.3 (119.4–194.3)	<0.05[d]
Post-vaccinal GMT (mIU ml^{-1}) at week 28	3727.5 (3006.1–4621.9)	3152.6 (2323.2–4278.1)	2082.9 (1572.2–2759.5)	
% of seroconversion at week 4	100%	100%	97.5%	N.S.[a]
% of seroconversion at week 28	100%	100%	100%	N.S.[a]

In parentheses 95% confidence interval; N.S., not significant. [a]χ^2 test, [b]Student's t-test, [c]Student's *t*-test with normal approximation, [d]effect of route [(multivariate regression analysis for all time points (Week 4, 24, and 28)]. (1) Nul hypothesis, H0=not different; (2) nul hypothesis, H0=not equivalent

pain ($P<0.01$), prolonged erythema ($P<0.01$), induration ($P<0.01$) and hematoma ($P<0.01$) on the day of vaccination (day 0). There was no difference in erythema if only lesions $\geqslant 3$ cm were considered. Among these reactions, the only significant difference persisting beyond day 0 was hematoma [7.9% in the Imule® group vs 2.4% on day 1 ($P<0.05$); 6% in the Imule® group vs 0.9% on day 2 ($P<0.05$)].

It was observed that the proportion of vaccinees having white skin was greater in the Imule® group (90.7% vs 82.4%, $P<0.05$), and they also were older ($P<0.01$). Therefore, the analysis was stratified to see if the differences had been influenced by these two variables (i.e. mean age and proportion of white-skinned persons). All the previously noted differences in the reactions persisted other than prolonged erythema, which was noted more frequently by white-skinned persons, and prolonged hematoma, which was noted more frequently by older subjects.

VI typhoid vaccine.

Immediate reactions. Reported pain during Imule® injection consisted of transient tingling, being difficult to distinguish from the perception of the injector on the skin surface. No pain was perceived 3 min later. A superficial skin wound occurred in one subject because of slippage of the jet injector caused by premature triggering.

Delayed local and general reactions. Pain was noted on the day of vaccination by most subjects and lasted <48 h; there was no difference between the two groups. Compared to the syringe group, erythema at the

Table 4 Proportions of local and general reactions after vaccination

Number of subjects:	Influenza at day 0 Imule® 151	Influenza at day 0 Syringe 211	P	Typhoid Imule® 65	Typhoid Syringe 62	P	Tetanus Imule® 213	DTP Imule® 83	DTP Syringe 84	P	Hepatitis A (after first dose) Imule® 46	Hepatitis A (after first dose) Syringe i.m. 46	Hepatitis A (after first dose) Syringe s.c. 46	Hepatitis A (after first dose) Syringe total 92	P (Imule® vs total syringe)
Immediate reactions															
Drop of vaccine (%)	9.9	8.5	N.S.[c]	4.6	—	—	13.1	1.2	0	N.S.[b]	8.7	—	—	—	—
Drop of serous fluid (%)	40.4	7.6	<0.01[c]	6.2	—	—	17.8	N.R.	N.R.	—	13.0	—	—	—	—
Bleeding, drop of blood (%)	19.2	10.9	<0.05[c]	3.1	—	—	1.0	9.6	13.1	N.S.[a]	36.9	—	—	—	—
Papule (%)	98.0	13.3	<0.01[c]	N.R.	—	—	30.0	N.R.	N.R.	—	8.7	—	—	—	—
Superficial (%)	87.2	4.7	<0.01[c]				27.7								
Deep (%)	10.6	8.5	N.S.[c]				2.3								
Pain (%)	7.2	9.4	N.S.[c]	7.7	—	—	63.4	N.R.	N.R.	—	N.R.	—	—	—	—
Superficial (%)	6.0	8.5	N.S.[c]	—	—		16.4								
Deep (%)	1.3	0.9	N.S.[c]	—	—		46.9								
Erythema (%)	46.4	10.4	<0.01[c]	N.R.	—	—	3.8	N.R.	N.R.	—	4.4	—	—	—	—
Hematoma (%)	1.9	0	N.S.[b]	N.R.	—	—	0	N.R.	N.R.	—	0				
Delayed adverse reactions							(*n*=184)								
Pain (%)	43.7	30.3	<0.01[c]	78.1	64.1	N.S.[a]	68.5	34.2	27.4	N.S.[a]	34.7	13.0	26.1	19.6	0.05[e]
Erythema (%)	35.8	14.2	<0.01[c]	62.5	19.3	<0.01[a]	7.7	N.R.	N.R.	—	8.7	0.0	13.0	6.5	N.S.[b]
Induration (%)	25.8	10.5	<0.01[c]	34.4	14.5	<0.01[a]	26.0	68.3	51.2	<0.05[a]	2.2	0.0	0.0	0.0	N.S.[b]
Hematoma (%)	9.3	0.9	<0.01[c]	N.R.	N.R.	—	0.0	N.R.	N.R.	—	6.5	0.0	6.5	3.3	N.S.[b]
Adenopathy (%)	N.R.	N.R.	—	N.R.	N.R.	—	N.R.	1.2	2.4	N.S.[a]	0.0	2.2	2.2	2.2	N.S.[b]
Fever (%)	2.6	4.3	N.S.[c]	4.7	3.2	N.S.[a]	35.9	4.9	2.4	N.S.[a]	10.9	8.7	8.7	8.7	N.S.[b]

N.R., not recorded; N.S., not significant. [a]χ^2 test, [b]Fischer exact test, [c]χ^2 of Mantel–Haenzel

injection site ($P<0.01$) [particularly erythema 2 cm (18.8% vs 6.4%, $P<0.05$)] and induration ($P<0.01$) were noted more frequently in the Imule® group, but all signs disappeared within 72 h.

Tetanus toxoid vaccine.

Immediate reactions to Imule® injections. The most frequent reaction was minor pain at the injection site. The mean size of erythema was 7 mm (ranging from 4 to 10 mm).

Delayed local and general reaction observed after Imule® vaccination. Data on only 184 subjects from the 213 monitored for immediate reactions were available for analysis. Pain at the injection site persisted <48 h, and <24 h in half (54.8%) of the subjects. Mean size of induration was 6 mm (ranging from 3 to 25 mm).

DTP vaccine.

Immediate reactions. The only reactions checked for were bleeding or vaccine leak, and they were unrelated to the method of vaccination.

Delayed local and general. Induration 1 cm was more frequently experienced following the Imule® vaccination ($P<0.05$).

Hepatitis A vaccine.

Immediate reactions. These were monitored in the Imule® group. After the first dose, bleeding was always mild and easily controlled with a small swab, as is commonly applied after injection by needle. Results observed after the booster dose were similar.

Delayed local and general reactions during follow-up. These reactions were checked after each dose of vaccine, but were more frequent after the first dose. For local reaction following the first dose, no significant difference was noted between the Imule® group and the overall syringe group (i.m. and s.c. routes combined), except for pain, which was more frequently reported from the Imule® group ($P=0.05$). Two subjects, both in the s.c. syringe group, developed a local-regional adenopathy.

Systemic reactions. Systemic reactions (e.g., fever, asthenia, headache, myalgia/arthralgia or gastro-intestinal tract signs) also were noted with the same frequency in the three groups.

DISCUSSION

Since the initiation of the EPI, maintaining sterile injection practices has been a high priority for the WHO and UNICEF on a worldwide level. Over half a million portable steam sterilization sets have been provided to developing countries. In addition, auto-destruct syringes have been supplied to those areas where the destruction of disposable single-use syringes cannot be guaranteed. Despite these efforts, recent surveys on injection practices reveal that 30% of EPI injections are not performed satisfactorily in an aseptic manner (i.e. 150 million injections per year are unsafe)[14,15]. Thus, patients, health care workers and entire communities may risk contracting an infectious disease through unsafe syringe injection practices[8]. Because of these concerns, the WHO issued in 1994 a world declaration, called the "Yamoussoukro declaration", aimed at improving the safety of injections[15].

The first available alternative to syringe injections was the classic multidose vial jet-injectors. Although this technique is safest if the head is cleaned with acetone or alcohol[16] after each injection, the risk of patient-to-patient cross infection exists if routine sterilization is not performed between each patient. Nonetheless, if properly used, multidose vial jet-injectors are very convenient for mass immunization campaigns in areas where the sero-prevalence of HIV and hepatitis B virus are low. Unfortunately, even the theoretical risk of cross-contamination may lead to rejection of all immunizations in populations that prefer single-use injection equipment[17].

The technology of single-dose, jet-injector Mini-Imojet® was greatly improved during mass immunization campaigns against tetanus in West Africa and immunization against influenza among Army personnel in France[18]. In Burkina Faso, Mini-Imojets®, when compared to other traditional jet-guns that use multi-dose vials (e.g. Ped-O-Jets®, Imojets®), were found to be more reliable because they required no sterilization, no routine mechanical maintenance and could be operated without repeated unplugging of nozzles and tubes. In addition, nearly 200 persons per hour could be immunized with a Mini-Imojet®. Compared to standard syringe technique, vaccinators in Velingara (Senegal) preferred the Imule® system; it was found to be more rapid and more easily adapted to collective immunization, cold chain and storage of vaccines. There is a clear-cut benefit to this device when 50 subjects a day must be immunized.

In the studies reported here, the five vaccines were demonstrated to be of equivalent or superior immunogenicity when delivered by the Imule® system, compared to standard syringe technique, with few exceptions. The tetanus toxoid vaccine study in Burkina Faso was the only occasion where syringe delivered vaccination was more immunogenic, and this was only seen for persons younger than 40-years-old. The reason for this disparity is unclear, but, in contrast to other vaccine studies, the observed seroconversion rates were low for both the Imule® and syringe groups, irrespective of age. Advanced age[13], and a high prevalence of onchocerciasis parasitosis[19], both of which can decrease immunogenicity to tetanus vaccine, may have contributed to this finding. In addition, our arbitrarily defined seroconversion criteria of a fourfold rise of the initial antibody titre could have underestimated the true conversion rate in this population; all subjects had pre-vaccination antibody titres 0.01 IU ml^{-1} (minimal protective level), which was a somewhat unexpected finding, most likely related to natural immunity in rural populations[20,21].

In contrast, the infants in the DTP study enrolled in either the Imule® or syringe groups had equivalent immune responses to the tetanus toxoid component (as was true for the diphtheria and pertussis components, as well), even though tetanus pre-vaccination antibodies of

maternal origin were higher among the syringe group. Although the absolute value of the anti-diphtheria and anti-tetanus seroconversion rates noted in this study were low, when a common definition of seroconversion is used (titre >20 mIU ml^{-1}), our results matched the 97–100% rates found in previous studies[22–24].

All other vaccines tested with the Imule® system demonstrated excellent results. Vi typhoid vaccine seroconversion rates in subjects vaccinated with jet-injector were similar to the results obtained with syringe vaccinations carried-out during previous studies[25,26], even though the rates were significantly larger than those obtained via syringe in this study. Hepatitis A and influenza vaccines both demonstrated similar, if not superior, immunogenicity profiles compared to syringe. The hepatitis A GMTs were also comparable after the booster dose, indicating that the duration of protection was equivalent.

The tolerance of injection by the Imule® system was quite good in all studies, and all of the local reactions were transient. The most frequent benign reactions, such as superficial papules, minor bleeding, erythema or induration, may even be considered inherent to the Imule® injection technique, rather than as adverse reactions, *per se*. More significant reactions, such as prolonged hematoma following influenza vaccination, were related to the age of the subject rather than the injection technique. In this same study, prolonged erythema was noted more frequently in light-skinned subjects. In the DTP and hepatitis A vaccine studies, the local and general reactions that were observed were those classically reported. Indeed, these reactions were more likely related to the site of vaccine deposition (mainly s.c. for jet-injector vs i.m. for syringe) and to the aluminium adjuventation rather than to the jet-injector itself[22,23,27,28]. For the hepatitis A vaccine, a decrease of systemic reactions with successive doses suggested that the vaccine did not induce hypersensitization.

The improved immunogenicity can be explained in part by the characteristics of jet-injection. The vaccine is delivered to the deep s.c. layer, which provides greater contact of the antigen with immune cells, such as the antigen presenting macrophages and lymphocytes distributed in s.c. dermal and i.m. tissues. Moreover, it is logical to speculate that the penetration of liquid through different layers of skin could cause an inflammatory-like process, which would also recruit immune-competent inflammatory cells.

Although the injection of vaccines by jet-injector is more superficial than i.m. syringe injection, the puncture site that results is larger, and therefore, more frequently leads to the appearance of drops of blood and serous fluid, or erythema at the site of injection. Moreover, the definition of observed bleeding at the injection site was not the same for all the studies. For influenza, DTP and hepatitis A studies, it was defined simply as a drop of blood, whereas, it was defined for typhoid and tetanus studies as running bleeding, appearing within 30 s following the injection, that required the application of a compressive dressing. This latter definition seems to be of more clinical relevance than the previous one. Although the appearance of drops of blood and serous fluid are of great concern, due to the risk of reflux to the nozzle and cross-contamination of vaccinees when multidose jet-injectors are used, this risk does not exist with the Imule® single-dose injection system. Thus minor bleeding can be considered as a benign consequence of vaccination.

Although all the vaccines are authorized for marketing when administered by syringe, clinical trials using standardized methods were required under French law for authorization of the new Imule® container. Nonetheless, certain field conditions led to some limitations. To demonstrate immunogenicity, absence of prevaccination specific antibodies ideally should have been required for inclusion. This condition was only met in the hepatitis A study. However, no subjects would have been enrolled in the tetanus vaccine immunogenicity study, if these conditions had been respected. Because of the existing field conditions, a comparison group was not used in tetanus vaccine tolerance study and only immediate reactions were checked for Imule® injection technique in the hepatitis A and typhoid studies. Although the analysis of the immunogenicity studies was adequately blinded, this was more difficult for the tolerance studies, due to the obvious difference in appearance of the injection site between the two techniques.

The main advantage of the Imule® system is to avoid any risk of cross-contamination, but the use of sterile and pre-filled cartridge does not represent a complete alternative immunization system because it does not permit the injection of lyophilized vaccines (e.g. measles, yellow fever and meningitis vaccines), which are frequently used in mass immunizations and outbreak control. An other approach could be the use of an empty, sterile cartridge as a simple transfer system, which could be filled just before use with any vaccine (liquid or reconstituted). This device would allow use of the "empty Imule® system" in all cases of collective immunization, including lyophilized vaccines. The cost of an empty cartridge would be about the same as for an empty plastic syringe.

A limiting factor could be the manufacturer's cost for either Mini-Imojet® (higher than the price published by WHO for low workload jet-injector[8]) or Imule®, which, because of the manufacturing process, eventually would increase the cost per injection. An economic evaluation of its use as an alternative injection system is needed, particularly in the context of large scale immunization programs. A cost–benefit study comparing syringe and Imule® techniques should take into account not only the manufacturer's cost but also the costs related to safety (sterilization and destruction of syringes), storage, labour and training, and wastage (for syringe technique), as well as the impact in terms of immunization coverage and time invested by the patient. The use of the Imule® system for day-to-day vaccination of children may also prove to be cost effective, given the decreased need for manipulation and sterilization, and because of less waste of vaccines compared to standard syringe injections.

In conclusion, these studies have confirmed that influenza, typhoid, DTP, tetanus toxoid and hepatitis A vaccines delivered by the Mini-Imojet®/Imule® system in collective immunization settings provide equal or superior immunogenicity, and a well acceptable tolerance profile, compared to standard syringe technique. This system is useful and effective for mass immunizations, particularly in developing countries where sterile injection procedures are difficult to maintain; and this device also could be of interest in military settings and in travellers clinics.

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