Safety & Immunogenicity Results Using Needle Free Injection Systems

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Summary of Safety Study & Immunogenicity Study Results from Clinical Studies and Research

Summary of 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.
   - The immunogenicity levels using the needle free injection was at par or better than the conventional hypodermic needle.
   - 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 and 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 on day 35 are as shown in table below:

Vaccine component		Day 35	
		DSJI (n = 161)	N-S (n = 157)
Measles	Seropositive subjects (%) 2-Sided 95% CI	157 (97.5) (93.8, 99.3)	155 (98.7) (95.5, 99.8)
Mumps	Seropositive subjects (%) 2-Sided 95% CI	159 (98.8) (95.6, 99.8)	155 (98.7) (95.5, 99.8)
Rubella	Seropositive subjects (%) 2-Sided 95% CI	159 (98.8) (95.6, 99.8)	157 (100.0) (97.7, 100.0)

   - Conclusion: MMR vaccination via DSJI is as immunogenic as vaccination by N-S.

4. **Immunogenicity and Tolerance Study, USA (McAllister L, 201)**
   - Influenza vaccine - Afluria
   - Vaccine was delivered by needle-free injection and compared with standard syringe injection to 1247 adults and immunogenicity results are as follows –

Immunogenicity for different type of influenza vaccine and AEs (28 days post vaccination)

Seroconversion rate, %	**NFJI**	**N-S**	**Rate difference (95%CI)**
H1N1	37.5	38.4	0.8 (-4.8, 6.5)
H3N2	43.8	45.1	1.3 (-4.5, 7.1)
B	34.9	35.2	0.3 (-5.5, 5.9)
GMT	**NFJI**	**N-S**	**Rate ratio (95%CI)**
H1N1	282.9	280.6	0·99 (0·8–1·12)
H3N2	247.3	265.9	1·08 (0·96–1·21)
B	42.5	39.7	0·94 (0·83–1·06)
AEs	**NFJI**	**N-S**	**Rate difference (95%CI)**
Local AEs on day 0, %	47.3	17.2	Not reported
Solicited AEs, day 0-6, %	95.1	85.0	Not reported
Systemic AEs	Not reported	Not reported	No significant difference

   - The immune response to influenza vaccine given with the jet injector device was non-inferior to the immune response to influenza vaccine given with needle and syringe.
   - Moreover, jet injection needle free administration addresses needle fear and the safety risks for patients and health-care providers associated with traditional administration of vaccines by needle and syringe.

5. **Immunogenicity and Tolerance Study, USA (Simon JK, 2011)**
   - Sixty healthy adults received one 0.5 mL intramuscular dose of the 2009–2010 seasonal, trivalent, inactivated influenza vaccine (TIV) in randomized, double-masked fashion by either DSJI (n = 30) or N–S (n = 30)
   - DSJI delivery of TIV is well-tolerated and immunogenic and results are as follows –

H1N1	DSJI	N-S	p^a
GMT[b] day 0 (95% CI[c])	17(11–26)	30(18–50)	0.1
GMT day 28 (95% CI)	213(127–357)	199(131–301)	0.8
Seroconversion: Percent ≥4-fold rise from day 0 to day 28 (95% CI)	80(65–95)	63(45–81)	0.3
Seroprotection: Percent HI titer ≥40 on day 28 (95% CI)	83(69–97)	90(79–100)	0.5
H3N2			
GMT[b] day 0 (95% CI[c])	31(18–52)	30(16–54)	0.9
GMT day 28 (95% CI)	426(253–717)	301(177–511)	0.3
Seroconversion: Percent ≥4-fold rise from day 0 to day 28 (95% CI)	80(65–95)	67(49–84)	0.4
Seroprotection: Percent HI titer ≥40 on day 28 (95% CI)	100(NA)	93(84–100)	0.5
B			
GMT[b] day 0 (95% CI[c])	14(9–19)	19(14–28)	0.1
GMT day 28 (95% CI)	111(71–175)	131(83–206)	0.6
Seroconversion: Percent ≥4-fold rise from day 0 to day 28 (95% CI)	73(57–90)	57(38–75)	0.3
Seroprotection: Percent HI titer ≥40 on day 28 (95% CI)	77(61–92)	87(74–99)	0.5

6. **Immunogenicity and Tolerance Study, Australia (Petrovsky N, 2013)**
   - 46 predominantly elderly subjects were randomized 1:1 to receive Fluvax 2012 trivalent inactivated influenza vaccine via prefilled N–S or DSJI
   - A high frequency of subjects in the DSJI group (77.3%) reported no anxiety or fear.
   - The device generated good vaccine immunogenicity, was easy to use with minimal training, and was well accepted by the majority of subjects.
   - Immunogenicity results are as follows:

Table 2
Vaccine efficacy.

	N–S (95% CI)	DSJI (95% CI)
A/California/7/2009 (H1N1)		
GMT (pre/post)	29.1/75.5	34.2/80.0
Seroconversion	33.3% (14.4–52.2)	31.8% (12.3–51.3)
Seroprotection	79.2% (63.0–95.4)	86.4% (72.1–100)
GMT fold increase	2.6 (1.4–3.8)	2.3 (1.3–3.4)
A/Perth/16/2009 (H3N2)		
GMT (pre/post)	23.8/42.4	23.4/49.9
Seroconversion	12.5% (0.7–25.7)	31.8% (12.3–51.3)
Seroprotection	66.7% (47.8–85.6)	72.7% (54.1–91.3)
GMT fold increase	1.8 (1.0–2.6)	2.1 (1.1–3.2)
B/Brisbane/60/2008		
GMT (pre/post)	11.2/16.8	15.5/22.0
Seroconversion	4.1% (3.8–12.0)	4.5% (4.2–13.2)
Seroprotection	16.7% (1.8–31.6)	18.2% (2.1–34.3)
GMT fold increase	1.5 (0.9–2.1)	1.4 (0.7–2.2)

7. 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 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	
	DSJI (n = 61)	**N-S (n = 67)**	**DSJI (n = 61)**	**N-S (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.

Confidentiality Statement

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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.

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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

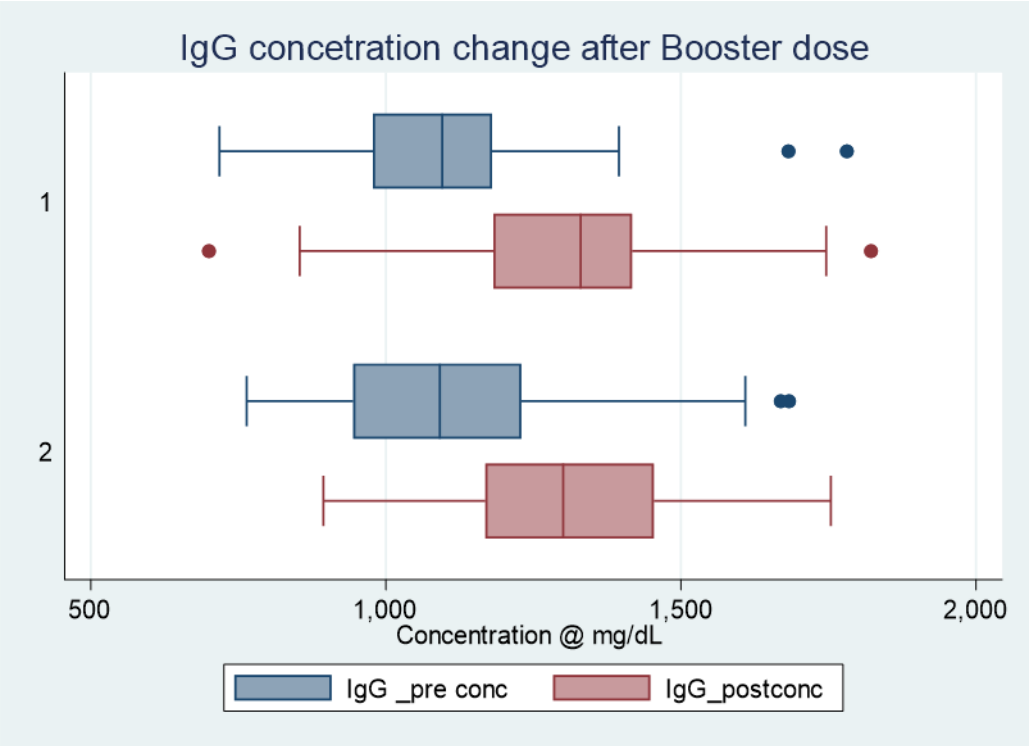
fall concentration around 1100mg/dl and 900 mg/dl for q1(left hand side of the box) and 1250mg/dl for q2(right hand side of the box), however post IgG concentration has been increased from pre concentration as median fall around 1300 mg/dl.

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

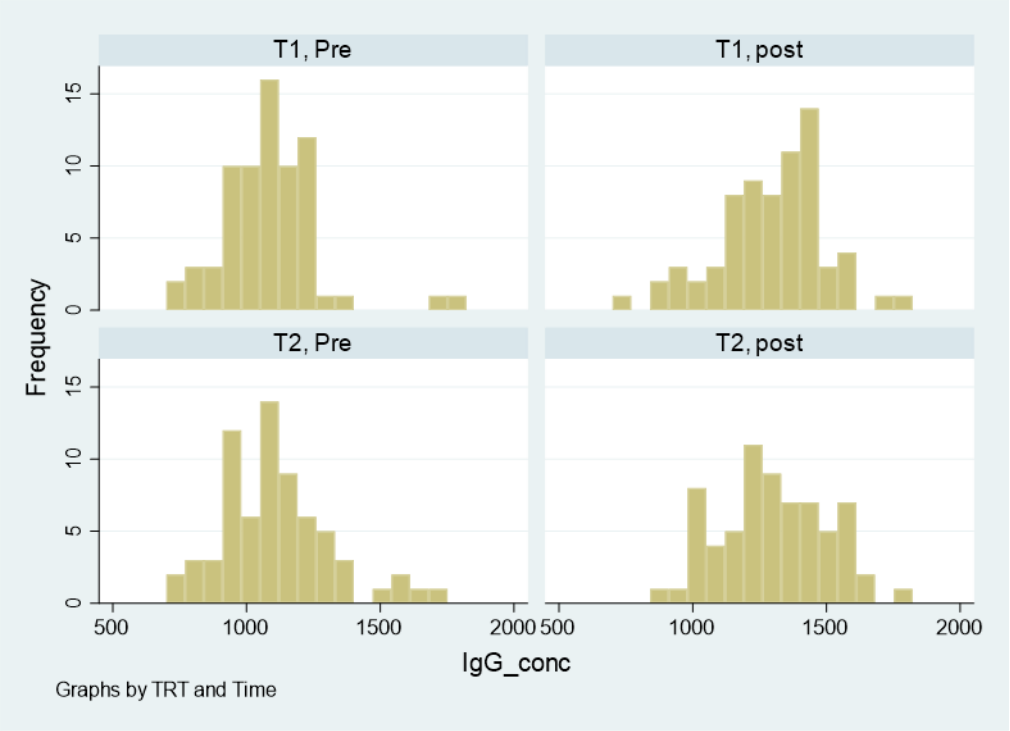
It is also observed that in T2 group (needle less vaccination) some of the cases achieved increased concentration level of IgG, IgA and IgM after vaccination as compared to T1 group. (Shown in Graph 1 A, Graph 2 A, Graph 3 A)

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

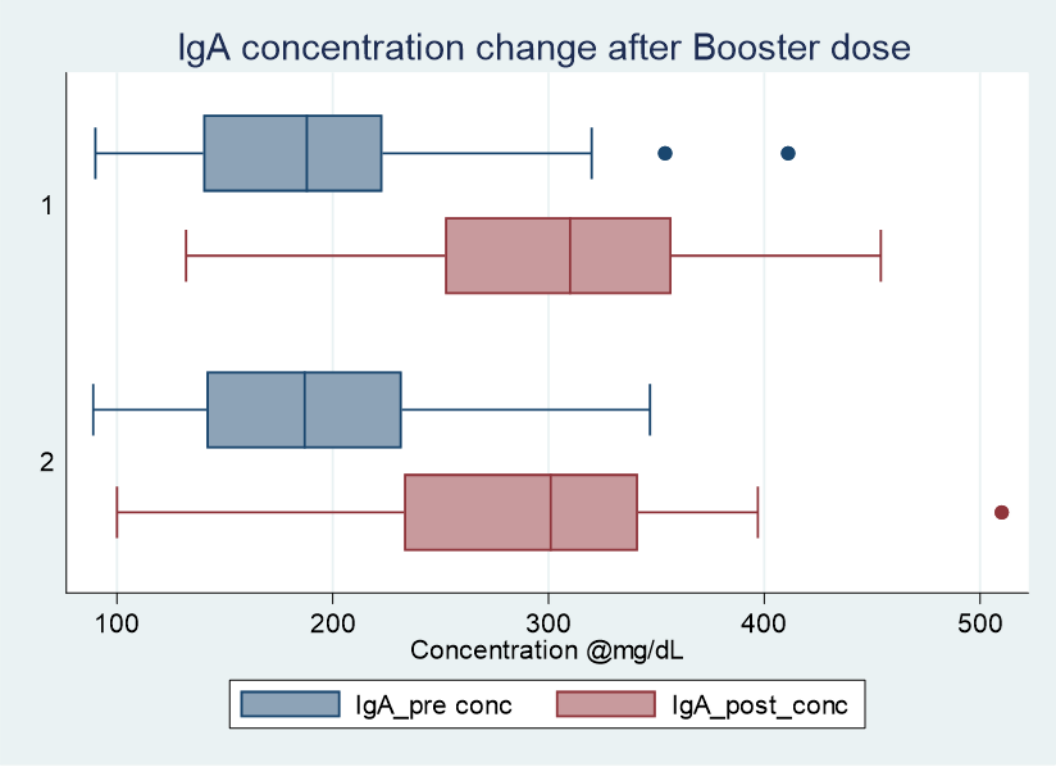
Graph 1



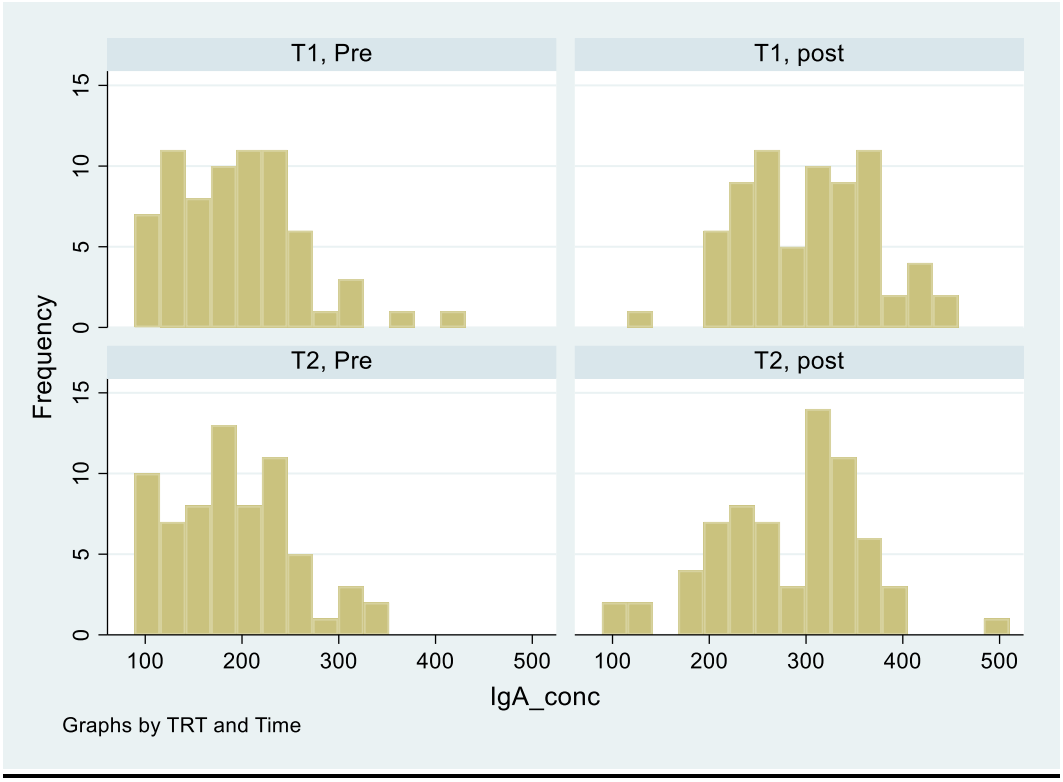
Graph 1 A : <u>Frequency distribution of IgG pre and post concentration of both groups:</u>



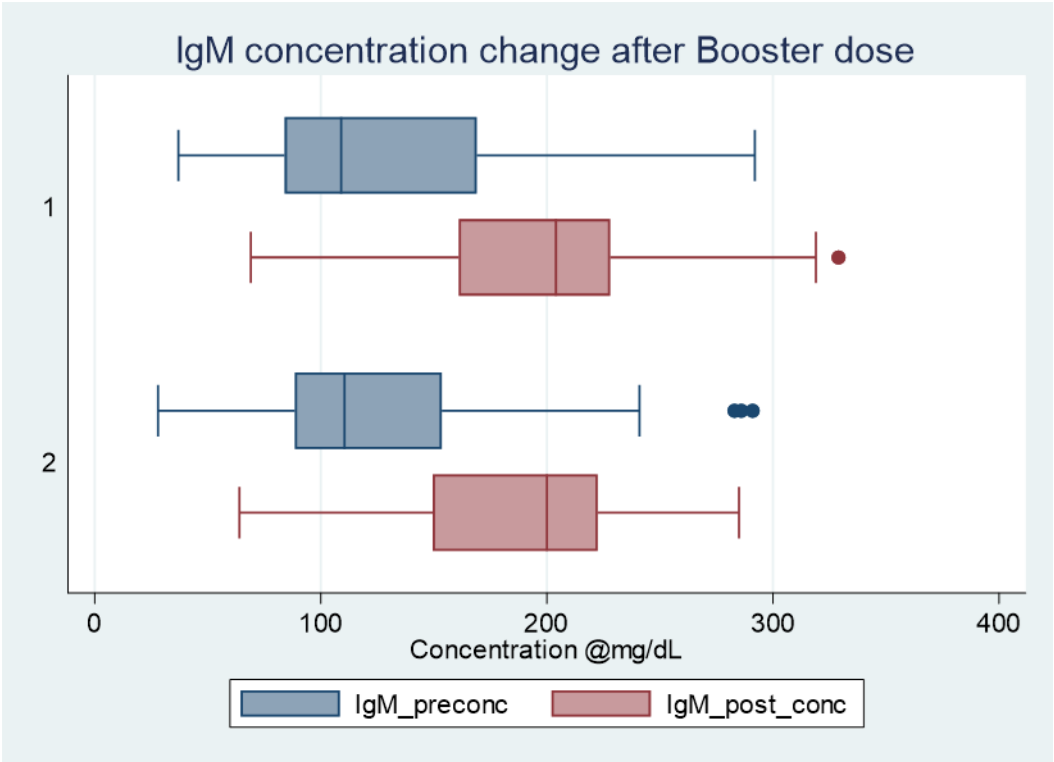
Graph 2



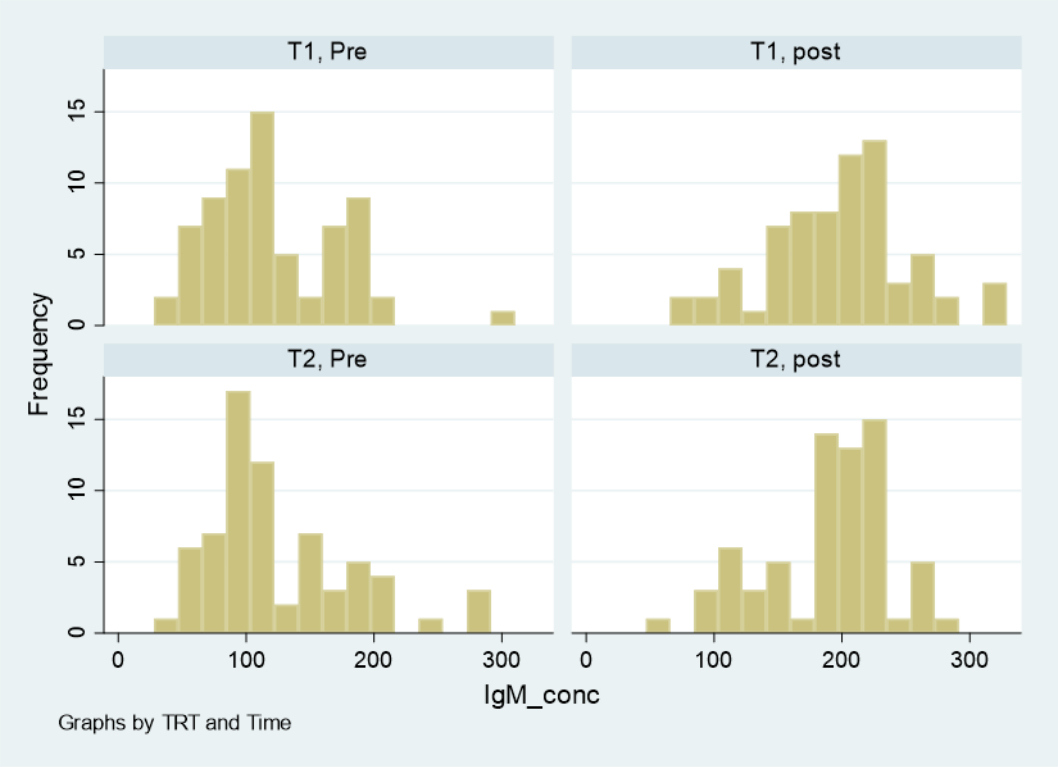
***Graph 2 A*: Frequency distribution of IgA pre and post concentration of both groups:**



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*.

CBER. (2007). FDA Guidance for Industry. Toxicity grading scale for healthy adult and adolescent volunteers enrolled in preventive vaccine clinical trials.

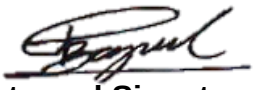
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12. Signature Page:

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.

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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

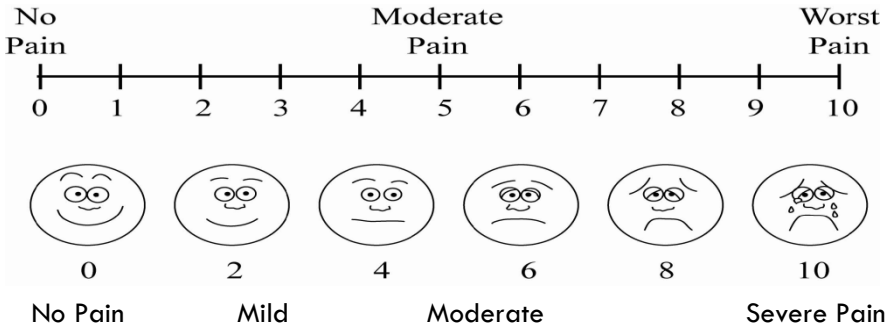
6.2.1. PRIMARY ENDPOINTS

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

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

CBER. (2007). FDA Guidance for Industry. Toxicity grading scale for healthy adult and adolescent volunteers enrolled in preventive vaccine clinical trials.

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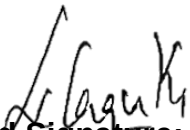
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Ravi AD, S. D. (2015). Needle free injection technology: A complete insight. Int J Pharm Investig. *Int J Pharm Investig*.

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:**

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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

Ashish Bavdekar [a], Jitendra Oswal [b], Padmasani Venkat Ramanan [c], Chandrashekhar Aundhkar [d], P. Venugopal [e], Dhananjay Kapse [g], Tara Miller [h], Sarah McGray [i], Darin Zehrung [i], Prasad S. Kulkarni [g,*], SII MMR author group [1]

[a] KEM Hospital Research Centre, Pune, India
[b] Bharti Vidyapeeth Deemed University Medical College, Pune, India
[c] Sri Ramachandra Medical Centre, Chennai, India
[d] Krishna Institute of Medical Sciences, Karad, India
[e] Andhra Medical College and King George Hospital, Visakhapatnam, India
[g] Serum Institute of India Pvt. Ltd., Pune, India
[h] PharmaJet, Golden, USA
[i] PATH, Seattle, USA

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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.
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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

* Corresponding author at: Serum Institute of India Pvt. Ltd., 212/2, Hadapsar, Pune 411028, India.
E-mail address: drpsk@seruminstitute.com (P.S. Kulkarni).
[1] See complete list of the SII MMR author group co-authors in the online version.

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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.

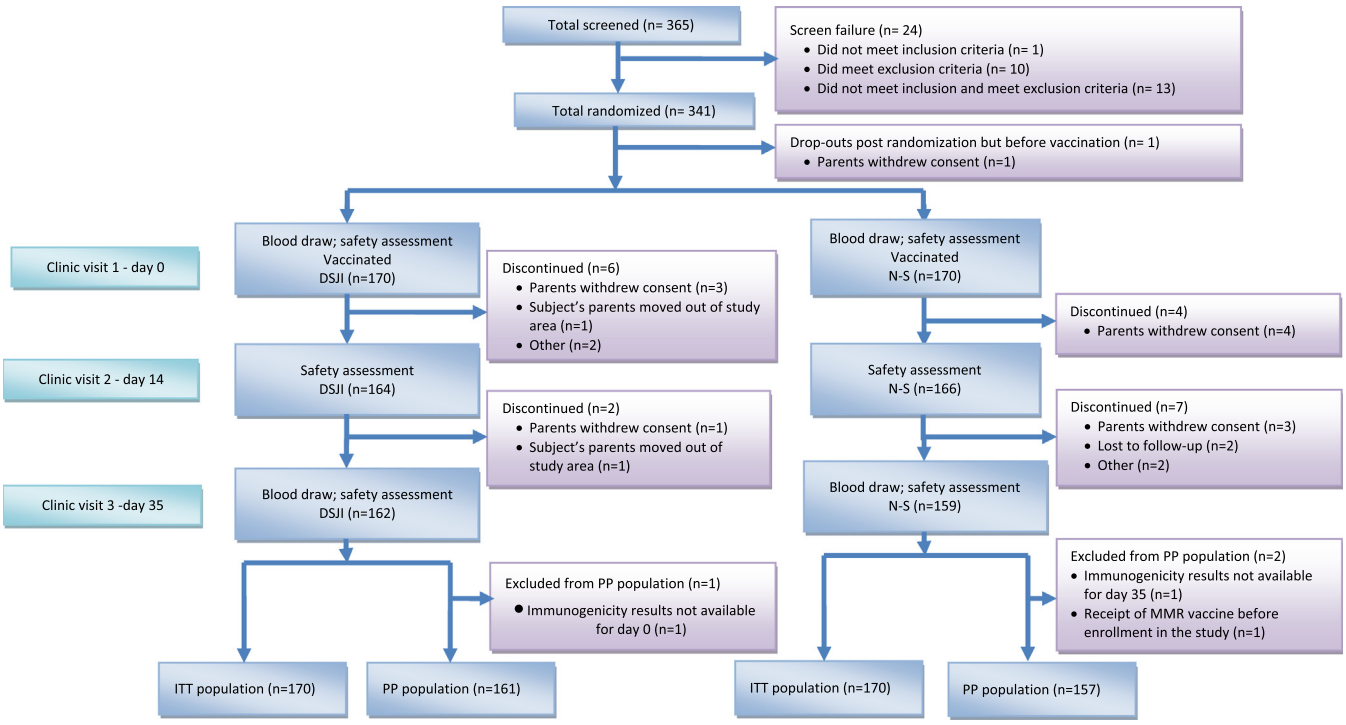
3. Results

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 & Tolerance Study of Influenza Vaccine (Afluria), USA

Needle-free jet injection for administration of influenza vaccine: a randomised non-inferiority trial

Linda McAllister, Jonathan Anderson, Kristen Werth, Iksung Cho, Karen Copeland, Nancy Le Cam Bouveret, David Plant, Paul M Mendelman, David K Cobb

Summary

Background Administration of vaccines by needle-free technology such as jet injection might offer an alternative to needles and syringes that avoids the issue of needle phobia and the risk of needle-stick injury. We aimed to assess the immunogenicity and safety of trivalent influenza vaccine given by needle-free jet injector compared with needle and syringe.

Methods For this randomised, comparator-controlled trial, we randomly assigned (1:1) healthy adults (aged 18–64 years) who attended one of four employee health clinics in the University of Colorado health system, with stratification by site, to receive one dose of the trivalent inactivated influenza vaccine Afluria given either intramuscularly with a needle-free jet injector (Stratis; PharmaJet, Golden, CO, USA) or with needle and syringe. Randomisation was done with a computer-generated randomisation schedule with a block size of 100. Because of the nature of the study, masking of participants was not possible. Immunogenicity was assessed by measurement of the hemagglutination inhibition antibody titres in serum for the three viral strains included in the vaccine. We included six coprimary endpoints: three strain-specific geometric mean titre ratios and the absolute differences in three strain-specific seroconversion rates. The immune response of the jet injector group was regarded as non-inferior to that of the needle and syringe group if both the upper bound of each of the three 95% CIs for the strain-specific geometric mean titre ratios was 1·5 or less, and the upper bound of the three 95% CIs for the strain-specific seroconversion rate differences was less than 10 percentage points. We used *t* test for group comparison. This study is registered with ClinicalTrials.gov, number NCT01688921.

Findings During the 2012–13 influenza season of the northern hemisphere, we allocated 1250 participants to receive vaccination by needle-free jet injector (n=627) or needle and syringe (n=623). In the intention-to-treat immunogenicity population, all participants with two serum samples were included (575 in the jet injector group and 574 in the needle and syringe group). The immune response to Afluria when given by needle-free jet injector met the criteria for non-inferiority for all six coprimary endpoints. The jet injector group met the geometric mean titre criterion for non-inferiority for the A/H1N1, A/H3N2, and B strains (upper bound of the 95% CI for the geometric mean titre ratios were 1·10 for A/H1N1, 1·17 for A/H3N2, and 1·04 for B strains). The jet injector group met the seroconversion rate criterion for non-inferiority for the A/H1N1, A/H3N2, and B strains (upper bound of the 95% CI of the seroconversion rate differences were 6·0% for A/H1N1, 7·0% for A/H3N2, and 5·7% for B strains). We recorded serious adverse events in three participants, none of which were study related.

Interpretation The immune response to influenza vaccine given with the jet injector device was non-inferior to the immune response to influenza vaccine given with needle and syringe. The device had a clinically acceptable safety profile, but was associated with a higher frequency of local injection site reactions than was the use of needle and syringe. The Stratis needle-free jet injector device could be used as an alternative method of administration of Afluria trivalent influenza vaccine.

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PharmaJet, Golden, CO, USA (L McAllister MD, K Werth MS, I Cho MS); **bioCSL, Parkville, VIC, Australia** (J Anderson MBChB, N Le Cam Bouveret MD, D Plant PhD); **Boulder Statistics, Boulder, CO, USA** (K Copeland PhD); **838 Esplanada Way, Stanford, CA, USA** (P M Mendelman MD); **and Medical Center of the Rockies, Loveland, CO, USA** (D K Cobb MD)

Correspondence to: Dr Linda McAllister, PharmaJet, Golden, CO 80401, USA **linda.mcallister@pharmajet.com**

Introduction

Since 2010, the US Centers for Disease Control and Prevention (CDC) Advisory Committee on Immunization Practices (ACIP) has recommended annual influenza vaccination for all people in the USA aged 6 months or older who do not have contraindications.[1] Present coverage falls short of this goal, especially in young adults for whom immunisation rates were less than 25% for the 2012–13 flu season.[2] Needle fear can be a big barrier[3–5] and standard administration carries the risk of needle-stick injuries for both patients and health-care workers.[6]

Needle-free vaccine delivery has the potential to improve compliance with annual influenza immunisation by providing an alternative to people with needle fear and by improving safety.[7] Needle-free vaccine delivery has been recommended as an alternative by WHO,[8] the Global Alliance for Vaccines and Immunization,[9] the CDC,[10] the Occupational Safety and Health Administration,[11] and the American Nurses Association.[12]

Jet injectors are needle-free devices that use a high-pressure narrow jet of liquid drug to penetrate tissue and deliver the drug to the desired depth. First developed in

the 1860s, the technology came into use in the 1940s, was introduced by the military and used widely in mass immunisation campaigns.[13,14] An outbreak of hepatitis B infection was linked to the use of the multi-use nozzle jet injectors[14–16] leading to the present jet injectors, which use one-use, disposable cartridges.[17,18]

An extensive body of clinical literature (including findings of several studies on trivalent influenza vaccine) has shown that vaccines given by jet injection generate immune responses that are often similar to those induced by conventional needle and syringe administration.[17–21] Presently, no influenza vaccines in the USA are labelled to be given with a jet injector device.[22]

The present study was undertaken in response to a US Food and Drug Administration (FDA) practice directive of October, 2011,[22] and at the request of the Center for Biologics Evaluation and Research (CBER) of the FDA to compare the safety and show the non-inferior immunogenicity of a trivalent inactivated influenza vaccine given by jet injector versus needle and syringe.

Methods

Study design and participants

We did this randomised, comparator-controlled trial during the 2012–13 northern hemisphere influenza season (the first participant was recruited on Oct 15, 2012, and the last on Dec 20, 2012) in individuals who presented to the employee health influenza immunisation clinics of the Medical Center of the Rockies (Loveland, CO, USA; University of Colorado health system). All health-centre employees were required to receive an influenza vaccination. Additionally, we recruited friends and family of employees. Participants were adult volunteers (aged 18–64 years) who had stable health status with no exclusionary medical or neuropsychiatric disorders and were mainly health-care workers. The appendix lists exclusion criteria.

See Online for appendix

The study was approved by the institutional review board 1–biomedical of Poudre Valley Hospital (CO, USA) health system and was done in accordance with the principles established by the 18th World Medical Association General Assembly (Helsinki 1964) and subsequent amendments and clarifications used by the General Assemblies; present FDA regulations; Good Clinical Practice; and local ethical and regulatory requirements. All participants provided written informed consent before enrolment in the trial.

Randomisation and masking

Participants were randomly assigned (1:1) to the jet injector group or the needle and syringe group with a computer-generated randomisation schedule created by a statistician who had no involvement in the rest of the trial. The randomisation was stratified by investigational site with a block size of 100. Because of the nature of the study, patients were not masked to the type of injection device.[19] Different study staff gave the vaccine and assessed safety after the injection. The safety assessor was masked to the device used for injection. Staff who did the haemagglutination inhibition antibody titre assays were masked to the device, participant identity, and day of sampling.

Procedures

The jet injector (Stratis; PharmaJet, Golden, CO, USA) was cleared for sale and use by the Center for Devices and Radiologic Health of the FDA in 2011 (FDA 510(k) number K111517), for 0·5 mL intramuscular and subcutaneous injections of liquid vaccines and drugs. The influenza vaccine (Afluria, bioCSL, Parkville, VIC, Australia; lot 08249221A) was formulated to meet the recommendations for the 2012–13 northern hemisphere influenza season and contained 45 µg of haemagglutinin: 15 µg each of A/California/7/2009 (H1N1), A/Victoria/361/2011 (H3N2), and B/Hubei-Wujiagang/158/2009. The vaccine was supplied without adjuvant in 5 mL multidose vials.[23]

Participants received one intramuscular injection of the trivalent influenza vaccine (0·5 mL) in the lateral deltoid of the non-dominant arm or the arm preferred by the participant. Participants in the jet injector group who had a wet shot (in which liquid remains on the skin after injection, suggesting incomplete delivery of vaccine) were revaccinated with needle and syringe and continued in the study only for safety endpoints.

We collected blood samples before vaccination (day 0) and 28 days after vaccination. Safety assessments comprised immediate complaints (noted within 30 min after vaccination), solicited adverse events (local and systemic) from the evening of day 0 through the evening of day 6 after vaccination as recorded on a 7-day diary card, assessment on day 28, and spontaneously reported adverse events during the study.

Immediate complaints solicited from participants after vaccination included pain, tenderness, itching, redness, swelling, and bruising at the vaccination site. Participants recorded solicited local reactions (pain, tenderness, itching, redness, swelling, and bruising) and solicited systemic adverse events (fever, headache, malaise, myalgia, chills, nausea, and vomiting) on the 7-day diary card. Spontaneously reported adverse events were recorded separately on a 28-day diary card.

We assessed immunogenicity by measurement of serum concentrations of haemagglutination antibodies specific for the three virus strains included in the vaccine. We did haemagglutination inhibition assays with Focus Diagnostics (Cypress, CA, USA) in triplicate with egg-derived antigen. Titres that were undetectable at the starting dilution were reported as less than 10 and were analysed as 5. We derived geometric mean titre ratios for each virus strain. We established three strain-specific seroconversion rates. Seroconversion was defined as achieving a four times increase in titre after immunisation when the baseline titre was 10 or higher or a titre after immunisation of 40 or higher when the baseline titre was

less than 10. We calculated geometric mean fold rise in haemagglutination inhibition titre by taking the anti-logs of the mean of the log-transformed fold increase in titre after vaccination over titre before vaccination for each of the three virus strains. Seroprotection was defined as a haemagglutination inhibition titre of 40 or higher.

With the exception of fever, all solicited local reactions (immediate complaints and local reactions on days 0–6) and systemic adverse events on days 0–6 were judged as grade 1 if they did not interfere with activity, grade 2 if they interfered with activity, and grade 3 if they prevented activity (severity scale for fever: ≥100·4°F to <101·1°F [≥38·0°C to <38·4°C] for grade 1; ≥101·2°F to <102·0°F [≥38·4°C to <38·9°C] for grade 2; ≥102·1°F to <104·0 °F [≥38·9°C to <40·0°C] for grade 3).

Outcomes

The primary objective was to show that six coprimary immunogenicity endpoints, the three strain-specific geometric mean titre ratios and the absolute differences in three strain-specific seroconversion rates, met the criteria for non-inferiority of jet injector compared with needle and syringe. The non-inferiority analysis was based on the criteria outlined by the FDA for accelerated approval for licensure of seasonal influenza vaccines.[24] The secondary outcome was to compare the safety profiles of the vaccine given by needle-free jet injector or needle and syringe based on specifically solicited local and systemic reactions through 7 days after vaccination and adverse events spontaneously reported through day 28 after vaccination.

Statistical analysis

We did statistical calculations with SAS (version 9.2.2). To confirm power calculations, we used PASS (version 8.0.15). We analysed safety in all participants who received the vaccination and for whom any follow-up safety data were available for specific safety analyses. We used Fisher's exact test to compare the proportions of participants with adverse events between the two treatment groups. The analysis of severity grade was done for all grades combined and for severe (grade 3) complaints. We did all tests with a two-sided significance level of 0·05, without adjustment for multiple comparisons.

We analysed immunogenicity in two populations: the immunogenicity population, which included all participants who completed the study with no major protocol deviations judged likely to interfere with immune response to the study vaccine, and the intent-to-treat

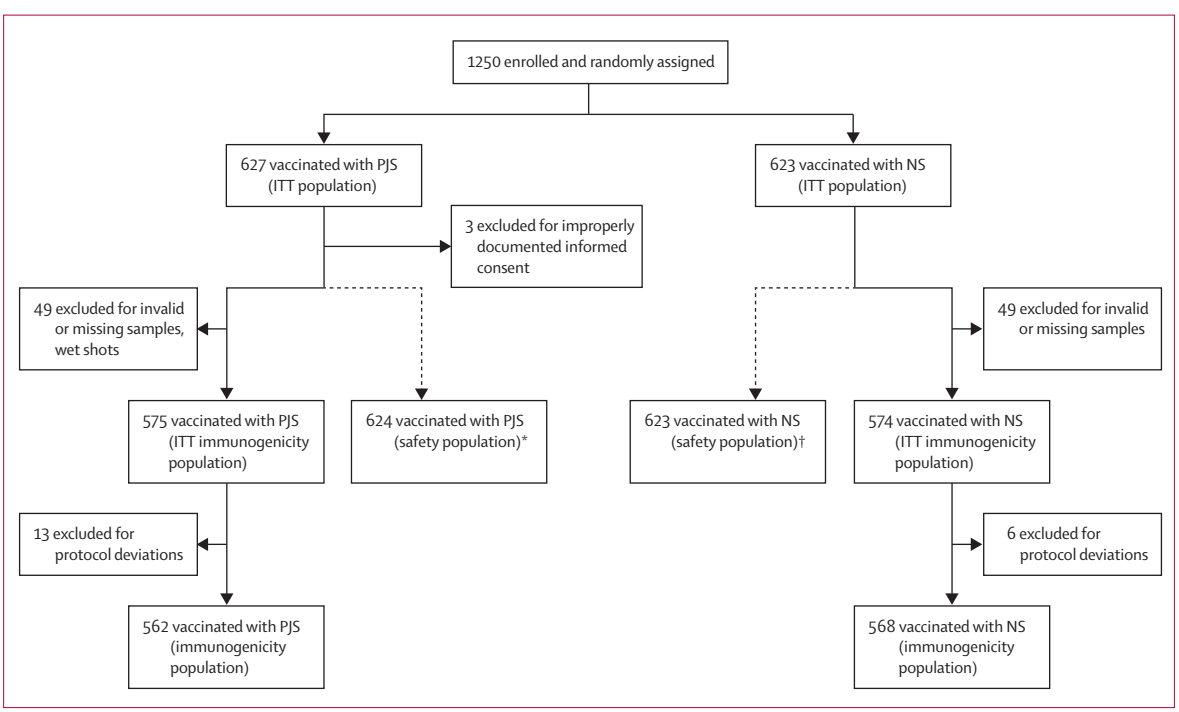


Figure 1: **Trial profile**

The safety population included individuals who received the vaccination and for whom any follow-up safety data were available for a specific safety analysis. The ITT population included all study participants; however, only those with two serum samples could be analysed for immunogenicity (ITT immunogenicity population). The immunogenicity population included all participants who completed the study with no major protocol deviations judged likely to interfere with immune responses in the study vaccine. Reasons for exclusion: 49 participants in the NS group and 43 in the PJS groups were excluded from the ITT population for invalid or missing samples; an additional six who received wet shots in the PJS group were excluded from immunogenicity analysis but were included in the safety analysis. 13 participants in the PJS group and six in the NS group were excluded from the immunogenicity population for the following protocol deviations: second sample collected outside of 28±3 days (PJS n=9 and NS n=4); two sample failures (PJS n=1 and NS n=1); receipt of an additional vaccine (PJS n=2 and NS n=1); failure to meet an inclusion criterion (PJS n=1 and NS n=0). PJS=Pharmajet Stratis needle-free jet injector. NS=needle and syringe. ITT=intention to treat. *n=616 for solicited adverse events days 0–6 because of missing 7-day diary card. †n=607 for solicited adverse events days 0–6 because of missing 7-day diary card.

	ITT and safety population			Immunogenicity population		
	PJS group (n=624)	NS group (n=623)	Total (n=1247)	PJS group (n=562)	NS group (n=568)	Total (n=1130)
Age (years)						
Mean (SD)	41·3 (12·92)	41·5 (12·49)	41·4 (12·70)	41·6 (12·77)	41·8 (12·53)	41·7 (12·64)
Median	41·0	42·0	41·0	42·0	42·0	42·0
Range	18–64	18–64	18–64	18–64	18–64	18–64
Sex						
Men	175 (28·0%)	191 (30·7%)	366 (29·4%)	152 (27·0%)	173 (30·5%)	325 (28·8%)
Women	449 (72·0%)	432 (69·3%)	881 (70·6%)	410 (73·0%)	395 (69·5%)	805 (71·2%)
Race or ethnic origin						
White	595 (95·4%)	600 (96·3%)	1195 (95·8%)	537 (95·6%)	546 (96·1%)	1083 (95·8%)
Not Hispanic or Latino	547 (87·7%)	529 (84·9%)	1076 (86·3%)	495 (88·1%)	480 (84·5%)	975 (86·3%)
Hispanic or Latino	40 (6·4%)	60 (9·6%)	100 (8·0%)	34 (6·0%)	55 (9·7%)	89 (7·9%)
Asian	11 (1·8%)	9 (1·4%)	20 (1·6%)	8 (1·4%)	9 (1·6%)	17 (1·5%)
Black or African American	4 (0·6%)	4 (0·6%)	8 (0·6%)	4 (0·7%)	3 (0·5%)	7 (0·6%)
American Indian or Alaskan Native	3 (0·5%)	1 (0·2%)	4 (0·3%)	2 (0·4%)	1 (0·2%)	3 (0·3%)
Native Hawaiian or other Pacific Islander	2 (0·3%)	0 (0·0%)	2 (0·2%)	2 (0·4%)	0 (0·0%)	2 (0·2%)
Other	9 (1·4%)	9 (1·4%)	18 (1·4%)	9 (1·6%)	9 (1·6%)	18 (1·6%)
Body-mass index						
<25 kg/m² (underweight and normal)	278 (44·6%)	278 (44·6%)	556 (44·6%)	244 (43·4%)	256 (45·1%)	500 (44·2%)
≥25 kg/m² (overweight and obese)	344 (55·1%)	345 (55·4%)	689 (55·3%)	316 (56·2%)	312 (54·9%)	628 (55·6%)
Missing	2 (0·3%)	0 (0·0%)	2 (0·2%)	2 (0·4%)	0 (0·0%)	2 (0·2%)

Data are mean (%) unless otherwise stated. ITT=intention to treat. PJS=PharmaJet Stratis needle-free jet injector. NS=needle and syringe.

Table 1: **Baseline characteristics**

immunogenicity population, which included all participants for whom two serum samples were available, irrespective of protocol deviations. The second blood draw to assess immune response was taken day 28 plus or minus 3 days after vaccination.

The study success was based on achievement of six co-primary endpoints without multiplicity adjustment.[25] Geometric mean titres were summarised by study population, by treatment group, and by visit. Two-sided 95% CIs for the ratios of strain-specific geometric mean titres after vaccination were based on the log normal distribution. We used the natural $\log_e$ values to construct a CI using *t*-distribution for the mean difference between the two groups. We exponentiated the mean difference and the corresponding CI limits to obtain the geometric mean titre ratio and the corresponding CI. We used the same statistical method to construct CIs for the geometric mean fold rise ratios (needle and syringe/needle-free jet injector).

We summarised seroconversion rates for treatment groups. To show non-inferiority, we established the upper bound of the 95% CI for the proportion seroconversion rate differences with the Newcombe–Wilson score method.[26] We used the same statistical method to construct the CIs for the seroprotection rates.

Immune response of the jet injector group was regarded as non-inferior to that of the needle and syringe group if both the upper bound of each of the three 95% CIs for strain-specific geometric mean titre ratios after vaccination was 1·5 or less, and the upper bound of the three 95% CIs for strain-specific seroconversion rate differences after vaccination was less than 10 percentage points.

This study is registered with ClinicalTrials.gov, number NCT01688921.

Roles of the funding sources

BARDA and PATH are supportive of publication as a means to further their objectives to serve public health but otherwise had no direct involvement in the study or publication. PharmaJet served as the sponsor and took main responsibility for design and execution of the study; bioCSL collaborated closely on the protocol development, study implementation, results analysis, and development of the manuscript. LM, JA, IC, and DKC had full access to all the data in the study and final responsibility for the decision to submit for publication.

Results

Between Oct 15, 2012, and Dec 20, 2012, 1250 participants were enrolled and were randomised to receive vaccination by jet injector (n=627) or needle and syringe (n=623; figure 1). Three participants were excluded from the jet injector group because of improperly documented informed consent. In the intention-to-treat immunogenicity population, we included all participants with

two serum samples (575 in the jet injector group and 574 in the needle and syringe group). Six participants in the jet injector group received wet shots and were excluded from analysis of immune response but were included in the jet injector safety analysis. The immunogenicity population comprised 562 participants in the jet injector group and 568 participants in the needle and syringe group.

Table 1 shows the baseline characteristics of the two groups. In the intention-to-treat population, median age was 41 years for the jet injector group and 42 years for the needle and syringe group (range 18–64 years); participants were mainly women (72% in the jet injector group and 69% in the needle and syringe group); and were mainly white, non-Hispanic (88% in the jet injector group and 85% in the needle and syringe group).

After one vaccination, both types of administration elicited a similar immune response to the three influenza virus strains contained in the study vaccine (immunogenicity population; figure 2). The geometric mean titre ratio for each influenza strain was about 1. The jet injector group met the geometric mean titre criterion for non-inferiority because the upper bound of the 95% CI of each ratio for the A/H1N1, A/H3N2, and B strains was less than 1·5 (1·12 for A/H1N1, 1·21 for A/H3N2, and 1·06 for B strains). The geometric mean titre ratios in the intention-to-treat immunogenicity population also met the criterion for non-inferiority (upper bound of the 95% CI 1·10 for A/H1N1, 1·17 for A/H3N2, and 1·04 for B strains). The baseline geometric mean titre for both A strains was high in both study groups (figure 2).

The overall rate of seroconversion with jet injector was similar to that with needle and syringe for all three vaccine strains (immunogenicity population; figure 3). In both groups, seroconversion was highest for the A/H3N2 strain and lowest for the B strain (figure 3). The non-inferiority criterion for the difference in seroconversion rate between jet injector and needle and syringe was met for all three strains. The upper bound of the 95% CI on the difference in seroconversion rates for A/H1N1, A/H3N2, and B strains did not exceed 10 percentage points (6·5% for A/H1N1, 7·1% for A/H3N2, and 5·9% for B strains). The seroconversion rate differences were very similar in the intention-to-treat immunogenicity population and met the criterion for non-inferiority (upper bound of the 95% CI 6·0% for A/H1N1, 7·0% for A/H3N2, and 5·7% for B strains)

The geometric mean fold rise in haemagglutination inhibition titres (before and after vaccination) was similar between the two groups. Additionally, the proportion of participants with seroprotection was similar between the two groups. Nearly 100% of the participants in the immunogenicity population achieved a haemagglutination inhibition titre of ≥40 after vaccination for the two A strains in both treatment groups (data not shown for intention-to-treat population). A haemagglutination inhibition titre of ≥40 after vaccination for the B strain was achieved by 63·7% in the jet injector group and 60·2% in the needle and syringe group (appendix). Before vaccination, participants had high seroprotection rates against both A strains (about 80% had titres ≥40). However, only 20% had titres ≥40 for the B strain before vaccination (appendix).

Participants in the jet injector group reported more immediate complaints (table 2) and more solicited adverse events (days 0–6, p<0·001) than did those in the needle and syringe group. These adverse events were mostly grade 1 or 2 and generally resolved within 3 days.

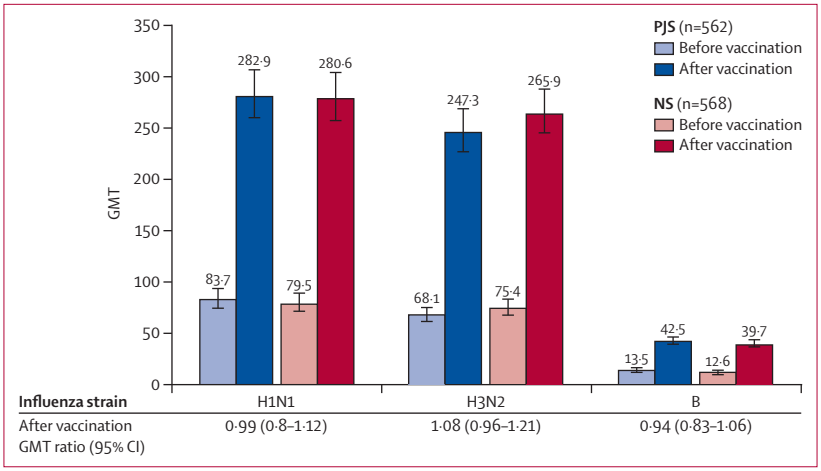


Figure 2: **Geometric mean titres of participants in the immunogenicity population before and after vaccination by study group**
GMT before vaccination equals day 0 and after vaccination equals day 28. GMT ratio is the strain-specific GMT NS group/GMT PJS group. Error bars show 95% CIs. GMT=geometric mean titre. PJS=PharmaJet Stratis needle-free jet injector. NS=needle and syringe.

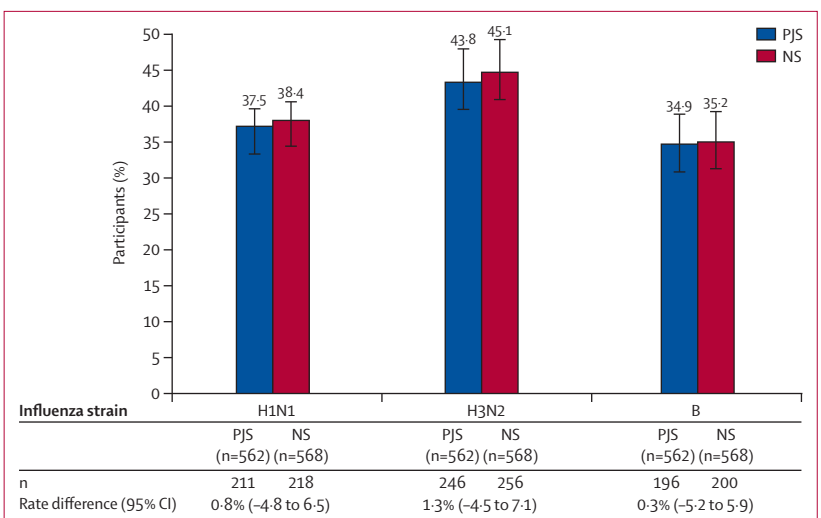


Figure 3: **Seroconversion rates of participants in the immunogenicity population by study group**
The percentage of participants who seroconverted for each virus strain is shown. Seroconversion rate was defined as the proportion of participants with either a haemagglutination inhibition (HI) titre <10 before vaccination and achieving an HI titre ≥40 after vaccination or with an HI titre ≥10 before vaccination and achieving a four-fold or greater increase in HI titre after vaccination. Error bars show 95% CIs. GMT=geometric mean titre. PJS=PharmaJet Stratis needle-free jet injector. NS=needle and syringe.

	PJS group		NS group	
	n/N	%	n/N	%
At least one immediate local reaction (day 0)	295/624	47·3%	107/622*	17·2%
At least one solicited adverse event (days 0–6)	586/616†	95·1%	516/607†	85·0%
At least one spontaneous adverse event (days 0–28)	90/624	14·4%	67/623	10·8%
At least one vaccine-related spontaneous adverse event (days 0–28)	43/624	6·9%	31/623	5·0%
At least one spontaneous severe adverse event (days 0–28)	9/624	1·4%	14/623	2·2%
At least one vaccine-related severe spontaneous adverse event (days 0–28)	3/624	0·5%	4/623	0·6%
At least one serious adverse event	1/624	0·2%	2/623	0·3%

All solicited local reactions were regarded as related to the study procedures. For all solicited systemic adverse events and all spontaneous adverse events, the investigator made a clinical judgment about whether the adverse event was related to the study. The degree of certainty with which an adverse event was attributable to the study or an alternative cause was assessed by the investigator on the basis of three factors: temporal relation to the vaccination or cessation of treatment, reactions of a similar nature previously recorded in the individual or others after needle-free jet injector administration of the vaccine or the vaccine given without the injection device, and safety profiles in the published scientific literature. PJS=PharmaJet Stratis needle-free jet injector. NS=needle and syringe. *Day 0 data were not provided for one participant. †Eight participants in the jet injector group and 16 participants in the needle and syringe group did not return the 7-day diary card.

Table 2: **Safety summary**

	Immediate complaints (≤30 min after vaccination)						Days 0–3 after vaccination						Days 4–6 after vaccination					
	PJS grade ≥1	NS grade ≥1	p value	PJS grade 3	NS grade 3	p value	PJS grade ≥1	NS grade ≥1	p value	PJS grade 3	NS grade 3	p value	PJS grade ≥1	NS grade ≥1	p value	PJS grade 3	NS grade 3	p value
Pain	163/622 (26·2%, 22·8–29·8)	69/622 (11·1%, 8·7–13·8)	<0·001	0/622 (0·0%, <0·1–0·6)	0/622 (0·0%, <0·1–0·6)	NA	397/616 (64·4%, 60·5–68·2)	299/606 (49·3%, 45·3–53·4)	<0·001	5/616 (0·8%, 0·3–1·9)	4/606 (0·7%, 0·2–1·7)	1·00	30/616 (4·9%, 3·3–6·9)	14/606 (2·3%, 1·3–3·8)	0·02	0/616 (0·0%, <0·1–0·6)	0/606 (0·0%, <0·1–0·6)	NA
Tenderness	104/622 (16·7, 13·9–19·9)	36/622 (5·8%, 4·1–7·9)	<0·001	0/622 (0·0%, <0·1–0·6)	0/622 (0·0%, <0·1–0·6)	NA	551/616 (89·4%, 86·7–91·8)	471/606 (77·7%, 74·2–81·0)	<0·001	13/616 (2·1%, 1·1–3·6)	6/606 (1·0%, 0·4–2·1)	0·16	79/616 (12·8%, 10·3–15·7)	25/606 (4·1%, 2·7–6·0)	<0·001	1/616 (0·2%, <0·1–0·9)	0/606 (0·0%, <0·1–0·6)	1·00
Itching	63/622 (10·1%, 7·9–12·8)	17/622 (2·7%, 1·6–4·3)	<0·001	0/622 (0·0%, <0·1–0·6)	0/622 (0·0%, <0·1–0·6)	NA	146/540 (27·0%, 23·3–31·0)	49/527 (9·3%, 7·0–12·1)	<0·001	0/540 (0·0%, <0·1–0·7)	1/527 (0·2%, <0·1–1·1)	0·49	31/540 (5·7%, 3·9–8·0)	8/527 (1·5%, 0·7–3·0)	<0·001	0/540 (0·0%, <0·1–0·7)	0/527 (0·0)	NA
Redness	113/624 (18·1%, 15·2–21·4)	11/621 (1·8%, 0·9–3·1)	<0·001	0/624 (0·0%, <0·1–0·6)	1/621 (0·2%, <0·1–0·9)	0·50	366/609 (60·1%, 56·1–64·0)	115/599 (19·2%, 16·1–22·6)	<0·001	8/609 (1·3%, 0·6–2·6)	2/599 (0·3%, <0·1–1·2)	0·11	65/609 (10·7%, 8·3–13·4)	11/599 (1·8%, 0·9–3·3)	<0·001	1/609 (0·2%, <0·1–0·9)	0/599 (0·0%, <0·1–0·7)	1·00
Swelling	5/624 (0·8%, 0·3–1·9)	0/622 (0·0%, <0·1–0·6)	0·06	0/624 (0·0%, <0·1–0·6)	0/622 (0·0%, 0·1–0·6)	NA	392/605 (64·8%, 60·8–68·6)	118/599 (19·7%, 16·6–23·1)	<0·001	10/605 (1·7%, 0·8–3·0)	1/599 (0·2%, <0·1–0·9)	0·011	54/605 (8·9%, 6·8–11·5)	12/599 (2·0%, 1·0–3·5)	<0·001	2/605 (0·3%, <0·1–1·2)	0/599 (0·0%, <0·1–0·6)	0·50
Bruising	0/623 (0·0%, <0·1–0·6)	0/622 (0·0%, <0·1–0·6)	NA	0/623 (0·0%, <0·1–0·6)	0/622 (0·0%, <0·1–0·6)	NA	104/607 (17·1%, 14·2–20·4)	30/599 (5·0%, 3·4–7·1)	<0·001	1/607 (0·2%, <0·1–0·9)	0/599 (0·0%, <0·1–0·6)	1·00	50/607 (8·2%, 6·2–10·7)	11/599 (1·8%, 0·9–3·3)	<0·001	0/607 (0·0%, <0·1–0·6)	0/599 (0·0%, <0·1–0·6)	NA

Data are n/N (%, 95% CI). The frequency and severity of solicited local reactions after vaccination within 30 min, days 0–3, and days 4–6 are shown. Solicited local reactions were regarded as grade 1 if they did not interfere with activity, grade 2 if they interfered with activity, and grade 3 if they prevented daily activity. Severity grade between treatment groups was compared with Fisher's exact test. 95% CIs were calculated with the Clopper–Pearson (exact) method. NA=not applicable. PJS=PharmaJet Stratis needle-free jet injector. NS=needle and syringe.

Table 3: **Solicited injection site adverse events**

The percentage of participants who reported at least one spontaneous adverse event through day 28 was 14·4% (90/624) for the jet injector group and 10·8% (67/623) for the needle and syringe group (p=0·06). The most frequently reported (≥1% of participants) spontaneous adverse events were injection site erythema (1·4% [9/624] in the jet injector group and 0/623 in the needle and syringe group), injection site haematoma (1·8% [11/624] and 0·2% [1/623], respectively), headache (3·7% [23/624] and 2·2% [14/623] respectively), and oropharyngeal pain (0·5% [3/624] and 1·0% [6/623], respectively). No participants withdrew from the study because of adverse events. Serious adverse events were reported by three participants, none of which were deemed to be related to the study (appendix).

More participants in the needle-free jet injector group reported immediate complaints of pain, tenderness, itching, and redness reported within 30 min after

vaccination than did those in the needle and syringe group; all were grade 1 or 2 except for one report of grade 3 redness in the needle and syringe group (table 3). The differences between treatment groups in immediate reports of pain, tenderness, itching, and redness were significant (p<0·001), but reports of swelling and bruising were not significantly different. On days 0–3 and days 4–6, solicited local reactions remained more common in the jet injection group than in the needle and syringe group. The overall severity tended to be greater in the needle-free jet injector group (6·0% [37/616] grade 3) than in the needle and syringe group (3·5% [21/607] grade 3). Fewer local reactions were reported on days 4–6, most resolved within 3 days.

Solicited systemic adverse events were headache, malaise, myalgia, chills, nausea, and vomiting. The occurrence of these adverse events on days 0–3 and days 4–6 was similar in the two groups, with a higher occurrence on days 0–3 that then decreased over time (appendix). Most participants had no fever (ie, temperatures <100·4°F [<38·0°C]) after vaccination (99·7% in both groups; 610/612 needle-free jet injector, 602/604 needle and syringe). Two participants in each group (0·3%) had mild fever; no participants had moderate or severe fever.

Discussion

This is the first definitive non-inferiority study of jet injection versus needle and syringe for influenza vaccine delivery (panel). Findings of previous clinical studies comparing influenza vaccination by jet injection with vaccination by needle and syringe have shown similar immunogenicity with the two methods.[17–19] Additional reports describe the favourable performance of jet injection for administration of various vaccines.[14]

This study used robust endpoints that are recommended by the FDA for accelerated approval for licensure of seasonal influenza vaccines.[23] The jet injection device met the criteria for non-inferiority for both haemagglutination inhibition geometric mean titres and rates of seroconversion for each of the three virus strains contained in the vaccine.

The study population was mainly health-care workers who had previously received annual influenza vaccinations. The prevaccination titres, especially for the A strains, were high, leading to a low rate of seroconversion. Although the seroconversion rates for the A strains were low, the seroprotection rates after vaccination were very high (>98%). An inverse correlation between titre before vaccination and seroconversion rate has been described previously.[27–29]

Both the jet injection and the needle and syringe methods of administration induced modest responses to the B strain (seroprotection rate 63·7% in jet injection group and 60·2% in needle and syringe group) compared with the A strains (for H1N1, 98·8% in the jet injection group *vs* 98·6% in the needle and syringe group and for H3N2, 98·8% in jet injection group and 98·8% in the needle and syringe group). These results are consistent with bioCSL's 2012–13 yearly re-registration study, which met the European Medicines Agency's Committee for Medicinal Products for Human Use criteria for licensure of annual influenza vaccine formulations.[30] Lower seroconversion to B strains has also been recently reported with other influenza vaccines.[31]

The jet injection device had an acceptable safety and tolerability profile in this study, although we recorded increased rates of local reactions in participants vaccinated with the jet injection device. Jet injection probably leaves trace amounts of vaccine in the layers of the skin as it penetrates to the intramuscular target leading to inflammation and irritation along the injection path.[14] Additionally, jet injection might cause more tissue damage than does needle and syringe. Other clinical studies have also reported that jet injection is associated with a higher frequency of local reactions than is needle and syringe.[14,17,19] We noted no significant difference in systemic adverse events between the two groups. All reported systemic adverse events were consistent with typical influenza vaccination adverse events.

Limitations of this study include the absence of a data and safety monitoring board to oversee the analyses of adverse event causality and the lack of masking of the participants to the method of vaccination. In the setting of a high-volume employee influenza vaccination clinic, masking of participants was impractical. Additionally, participants were health-care workers who, because of their compliance with annual influenza immunization leading to pre-existing immunity, might not provide the same measure of immune responses as a more general population with lower levels of pre-existing immunity. Despite this limitation, the vaccine was immunogenic and the study endpoints were met.

In conclusion, the results from this study support the use of the jet injection device as an acceptable method for administration of Afluria. Moreover, jet injection needle-free administration addresses needle fear and the safety

***Panel:* Research in context**

Systematic review

On Dec 26, 2013, we searched PubMed for reports published with the terms "influenza vaccine jet injection clinical trial". Between Jan 1, 1979, and Dec 26, 2013, only three clinical trials of liquid influenza vaccine had been published.[17–19] Although findings of these studies show that immunogenicity was similar with the two methods of administration, they were not designed or adequately powered to establish whether administration of influenza vaccine by jet injection was non-inferior to administration by needle and syringe. Additional reports[14] describe the favourable performance of jet injection for administration of various vaccines.

Interpretation

This is the first definitive non-inferiority clinical study of jet injection versus needle and syringe for influenza vaccine delivery. These data support the use of the jet injection device as an acceptable method for administration of Afluria.

risks for patients and health-care providers associated with traditional administration of vaccines by needle and syringe. These qualities might contribute to the reduction of barriers to immunisation in the US adult population to help reach CDC goals for annual influenza vaccine coverage.

Contributors

All authors contributed to the study concept and design, and analysed and interpreted data. LM did the literature search, collected clinical data, and approved the report. DKC supervised the study and acquired data. IC and KC provided statistical analyses. LM, JA, DP, and NLCB contributed to the drafting and revision of the report. All authors approved the final version.

Declaration of interests

LM, KC, and PMM are compensated consultants of PharmaJet; JA and DP are employees of bioCSL; KW is an employee of PharmaJet; NLCB was a compensated consultant of bioCSL; IC was a compensated consultant of PharmaJet, a consultant to PATH at the time of study unblinding and analysis, and is currently employed by PATH; and DKC received support from PharmaJet for this study. We declare no other competing interests.

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Safety, tolerability, and immunogenicity of inactivated trivalent seasonal influenza vaccine administered with a needle-free disposable-syringe jet injector

Jakub K. Simon[a,b,*], Mihaela Carter[a], Marcela F. Pasetti[b], Marcelo B. Sztein[a,b], Karen L. Kotloff[a,b], Bruce G. Weniger[c], James D. Campbell[b], Myron M. Levine[a,b]

[a] Division of Geomedicine, Department of Medicine, Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD, United States
[b] Division of Infectious Disease and Tropical Pediatrics, Department of Pediatrics, Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD, United States
[c] National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention, Atlanta, GA, United States

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ABSTRACT

Background: Jet injectors (JIs) avoid safety drawbacks of needle–syringe (N–S) while generating similar immune responses. A new generation of disposable-syringe jet injectors (DSJIs) overcomes the cross-contamination risk of multi-use-nozzle devices used in 20th-century campaigns. In the first study in humans, the newly-US-licensed LectraJet® model M3 RA DSJI was compared to N–S.
Methods: Sixty healthy adults received one 0.5 mL intramuscular dose of the 2009–2010 seasonal, trivalent, inactivated influenza vaccine (TIV) in randomized, double-masked fashion by either DSJI ($n = 30$) or N–S ($n = 30$). Adverse reactions were monitored for 90 days after injection, and serologic responses assayed by hemagglutination inhibition (HI) at days 28 and 90.
Results: There were no *related* serious adverse events (SAEs), nor differing rates of unsolicited AEs between DSJI and N–S. Solicited erythema and induration occurred more often after DSJI, but were transient and well-tolerated; a trend was noted for fewer systemic reactions by DSJI. Pre-vaccination HI geometric mean titers (GMT) increased by 28 days for H1N1, H3N2, and B antigens by 13-, 14-, and 8-fold via DSJI, and by 7-, 10-, and 7-fold for N–S, respectively. No trending differences in GMT, seroconversion, or seroprotection were noted; sample sizes precluded non-inferiority assessment.
Conclusions: DSJI delivery of TIV is well-tolerated and immunogenic.
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1. Background

Needle-free vaccine delivery has the potential to lead to significant advances in immunization, including improved safety for the vaccinator and vaccinee, better compliance with immunization schedules, decreased fear of injection and needles, easier and speedier vaccine delivery, and reduced cost [1]. For these reasons, needle-free vaccine delivery has been supported by the World Health Organization [2], the Global Alliance for Vaccines and Immunization [3], and the Centers for Disease Control and Prevention [4].

Needle-free jet injectors (JI) are devices that use pressure to deliver the drug or vaccine into different parts of the human body, such as skin, muscle, or fat. They have been used since the late 1940s to deliver millions of vaccine doses [5]; the first devices were multiple-use nozzle jet injectors "MUNJIs" whereby the vaccine was delivered through the same fluid stream and nozzle for multiple patients. After an outbreak of hepatitis B virus infection in a weight reduction clinic in 1985 [6], and upon a growing body of evidence for possible cross-contamination, such devices are no longer used in public health [7]. More recent development efforts have resulted in disposable syringe jet injectors, "DSJI", which provide a completely non-reusable fluid pathway so that splash back of blood cannot occur [7].

We report a first human safety and immunogenicity study of trivalent inactivated influenza vaccine (TIV) administered by the LectraJet® M3 RA DSJI, cleared for sale and use by the U.S. Food and Drug Administration in 2009 (FDA 510(k) #K090959) based on substantial equivalence to legally marketed predicate devices [8].

Abbreviations: AE, adverse event; CBC, complete blood count; CDC, centers for Disease Control and Prevention; CHMP, Committee for Human Medicinal Products; CI, confidence interval; DSJI, disposable-syringe jet injection/injector; EMEA, European Medicines Agency; FDA, U.S. Food and Drug Administration; FE, Fisher's exact; GAVI, Global Alliance for Vaccines and Immunization; GMT, geometric mean titers; HBV, hepatitis B virus; HCV, hepatitis C virus; IM, intramuscular; HIV, human immunodeficiency virus; HI, hemagglutination inhibition; JI, jet injector/injection; mm, millimeter; MUNJI, multi-use-nozzle jet injector; NA, not applicable; N–S, needle–syringe; PATH, Program for Appropriate Technology in Health; SAE, serious adverse event; TIV, trivalent (inactivated) influenza vaccine; URI, upper respiratory infection; USAID, United States Agency for International Development; WHO, World Health Organization.

* Corresponding author. Current address: NanoBio Corporation, MS 2311 Green Road Suite A, Ann Arbor, MI 48105, United States. Tel.: +1 734 302 4000.
E-mail address: jakub.simon@nanobio.com (J.K. Simon).

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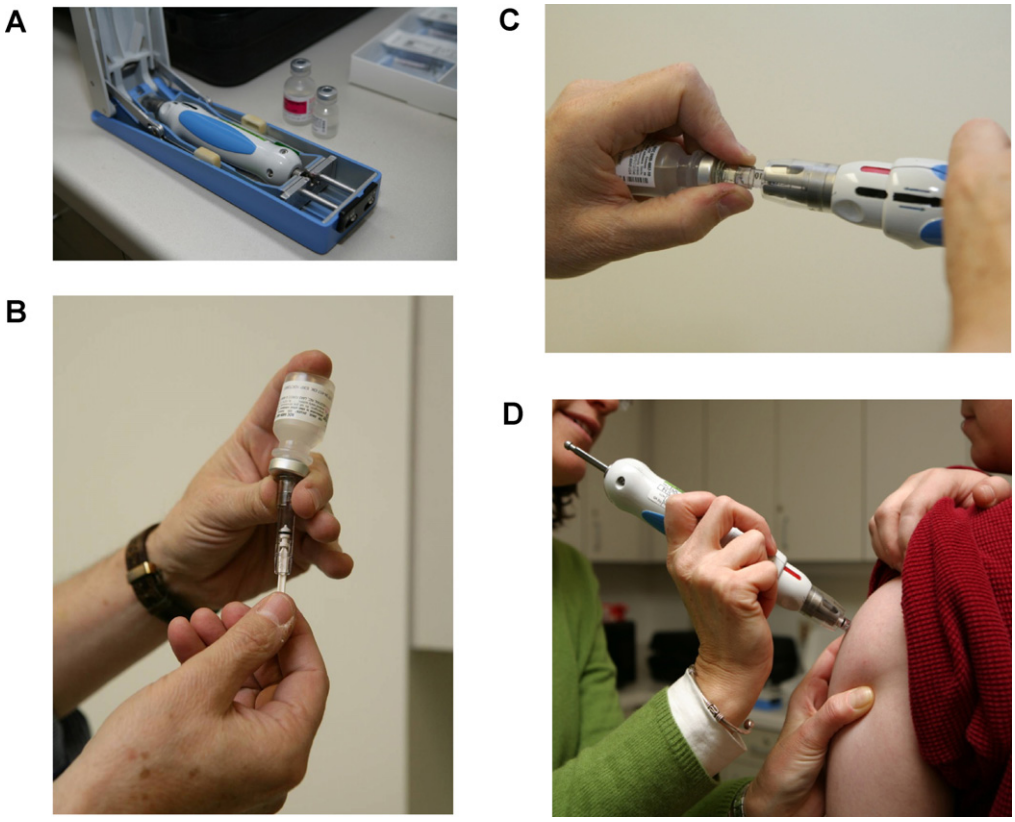


Fig. 1. (A) The LectraJet® M3 RA in its combination storage case and reset station. Upon closing in reset mode, the metal spring of the device is compressed to power the next injection. (B) Manual filling of the LectraJet syringe using a sterile vial adaptor. (C) Engagement of the filled syringe to the injector, before removal of the vial and vial adaptor, which can also be separated beforehand. (D) The injection is activated when the device reaches a pre-set pressure upon application against the limb.

2. Methods

2.1. Subjects

Healthy male and female volunteers aged 18–49 years were recruited from the Baltimore/Washington, DC area for this randomized, controlled, double-masked clinical trial. Volunteers were required to be in good health as evidenced by medical history and physical examination, if indicated. Ineligible were those who received an influenza vaccine in the 2008–2009 or 2009–2010 influenza seasons, received a live-attenuated vaccine within 30 days prior to enrollment or a killed vaccine within 14 days prior to enrollment, were dependent on alcohol or illicit drugs, or were females of childbearing potential with a positive pregnancy test. There were no restrictions on race, ethnic origin, religion, or any social or economic qualifications. Volunteers who satisfied eligibility criteria were randomly allocated into two groups of 30 each to receive licensed, seasonal TIV, either by conventional N–S or DSJI. Investigational Review Board approval, ClinicalTrials.gov registration (identifier NCT00987350), and informed consent of subjects were obtained prior to initiation of enrollment. Good Clinical Practice was utilized throughout the trial.

2.2. Vaccine

All subjects received one 0.5 mL dose from a single lot (U 239 AA) of U.S.-licensed TIV (Fluzone®, Sanofi Pasteur Inc., Swiftwater, PA, USA). The vaccine was non-adjuvanted, formulated as a liquid, and packaged in latex-free 5 mL multi-dose vials containing 10 doses each. It was labeled for the 2009–2010 (Northern Hemisphere) season, and contained 15 μg each of an A/Brisbane/59/2007 (H1N1)-like virus, an A/Brisbane/10/2007 (H3N2)-like virus, and a B/Brisbane/60/2008-like virus. The vaccine was stored refrigerated as per manufacturer's instructions with cold-chain maintained and documented throughout the protocol.

2.3. Double masking

Vaccine was injected into the deltoid muscle either by DSJI or N–S. To prevent volunteers from using sound, sight, or feel to identify their study group, during vaccination, the vaccinees: (1) wore ear earphones playing music loud enough to mask the sound of a jet injector, (2) inserted their non-dominant arm through a medical screen that blocked their vision of the injection event and (3) had the needle inserted through the center of a plastic hollow ring held against the skin for those receiving vaccine by N–S. The ring's diameter was equal to the nozzle of the jet injector syringe, in order to mimic contact by the DSJI syringe. Randomization was performed by an unmasked statistician who provided the allocation code to an unmasked vaccinator not involved in subsequent clinical or immunologic assessments.

2.4. Delivery

Vaccinations by the LectraJet® DSJI (Fig. 1) were performed in accordance with its *Instructions for Use* provided by its manufacturer (D'Antonio Consultants International, Inc., East Syracuse, NY, USA). To use the LectraJet®, the vaccinator manually operates a separate "reset" station which provides mechanical advantage to compress the injector's metal spring (Fig. 1A). Vaccine is loaded into the syringe by attaching it via an adaptor to the vaccine vial, pulling back on its plunger to fill its chamber, then breaking off the distal end of the plunger (Fig. 1B). The filled syringe is then inserted into the injector until its grasping jaws lock onto the flanges of the

Table 1
Demographic characteristics of volunteers assigned to TIV injection by needle and syringe (N–S) and disposable-syringe jet injector (DSJI) administration.

	DSJI (n^a = 30)	NS (n = 30)	Total (n = 60)
Age, mean in years (95% CI[b])	20.6 (19.7–21.4)	20.4 (19.3–21.6)	20.5 (19.8–21.2)
Female, % (95% CI)	43 (25–62)	50 (31–69)	47 (34–60)
Black, % (95% CI)	10 (0–21)	20 (5–35)	15 (6–24)
Asian, % (95% CI)	30 (13–47)	27 (10–23)	28 (17–40)
White, % (95% CI)	60 (42–78)	53 (35–72)	57 (44–70)

[a] Number of volunteers.
[b] Confidence interval.

syringe (Fig. 1C). The injector is then held against the deltoid muscle (Fig. 1D). Upon actuation to release the spring, a rod rapidly pushes the syringe piston/plunger forward, pressurizing and ejecting vaccine through the syringe orifice (diameter: 0.01 in. [0.254 mm]), effecting the injection. The vaccine is delivered to intramuscular tissue as based on performance criteria including injection force and duration as well as depth and dispersion in homogenous substrate (data not shown).

Those in the N–S group were injected intramuscularly using a 25 gauge, 1 in.-long [25.4 mm] needle on a conventional disposable syringe.

2.5. Clinical assessment

Volunteers were observed in the clinic for at least 15 min after inoculation, and their injection sites were photographed. Vital signs including oral temperature (in degrees Fahrenheit) were recorded. The largest diameter of any erythema, induration, and bruising were measured in millimeters (mm) and graded (mild = 1–29 mm, moderate = 30–119 mm, severe ≥ 120 mm). Local symptoms including pain and tenderness were recorded, as were systemic symptoms such as headache, myalgia, and fatigue. Symptoms were graded as mild (does not interfere with activities), moderate (somewhat interferes with activities), and severe (markedly interferes with or prevents activities for ≥24 h). Volunteers were trained to measure and report signs and symptoms. Fifteen minutes after vaccination, volunteers were asked whether they would rate the experience of getting this vaccine as “poor”, “acceptable”, or “excellent”.

Instructions were provided to volunteers to maintain a memory aid to record both prompted and unsolicited local and systemic adverse events (AEs) for 3 days after immunization and to return to the clinic on days 1 (window 1–2 days), 3 (window 3–6), and 7 (window 7–10) after vaccination. Loss of appetite (anorexia) was graded separately as mild (eating typical amount), moderate (eating less than typical amount), and severe (unable to eat for ≥24 h).

Upon return on days 1–2, 3–6, and 7–10 after vaccination, the injection site was again photographed, the memory aid was reviewed by investigators, and a targeted physical examination performed, if indicated. Volunteers returned on days 28 (window 26–36) and 90 (window 80–100) to obtain serum and record intervening unsolicited AEs.

2.6. Measurement of antibody responses

Seed viruses matching the vaccine strains [A/Brisbane/59/2007 (H1N1), A/Brisbane/10/2007 (H3N2) and B/Brisbane/60/2008] and reference antisera were kindly provided by Dr. Alexander Klimov, CDC. Virus stocks were produced in embryonated chicken eggs, as previously described [9]. Hemagglutination inhibition (HI) antibody levels to the three vaccine components were measured by incubating serially diluted serum samples (starting at 1:8) with 4 hemagglutination units of each antigen and turkey erythrocytes, following standard techniques [10]. Positive reference antisera [Influenza A(H1N1) FR-42; Influenza A(H3N2) FR-44; Influenza B FR-48] and negative controls were included in the assays. Sera were pre-treated with receptor-destroying enzyme (Denka Seiken Co., Tokyo, Japan) to inactivate nonspecific inhibitors of viral hemagglutination. HI titers were calculated as the inverse of the highest dilution that inhibited hemagglutination. Seroconversion was defined as achieving a fourfold or greater rise in HI titer with a post-vaccination titer ≥40 if a pre-vaccination titer was ≤10. Seroprotection was defined as achieving an HI titer ≥40 (dilution ≥1:40)

2.7. Statistical analysis

Clinical data were collected on coded, scannable clinical reporting forms that are subsequently scanned using Teleform™ software (Autonomy, Inc. [Cardiff Software], Vista, CA, USA). Data management and statistical analyses were performed with Office Excel 2007 (Microsoft, Seattle, WA, USA), GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, CA, USA), and STATA 9.0 (StataCorp LP, College Station, TX, USA). Point estimates and 95% confidence limits were calculated for the proportion of subjects in the DSJI and N–S groups experiencing any grade of local and systemic AEs within three days after vaccination. Point estimates and 95% confidence limits were calculated for the HI geometric mean titer (GMT) for each influenza antigen. A Fisher’s exact (FE) test was used to compare the proportion of subjects in the two study groups with any AE, as well as the proportion achieving seroconversion and seroprotection at each time point. A Student’s t test was used to compare the measured continuous variables, as well as natural-log-transformed titers on days 0, 28, and 90 between DSJI and N–S variables that were normally distributed; the Mann–Whitney test was used for variables that were not normally distributed. All hypotheses were evaluated using two-sided tests. Demographic information and adverse event reporting were descriptive. A sample size of 30 volunteers in each group provides 80 power at alpha 0.05 (two-sided) to detect a 48% lower HI seroconversion against H1N1 and H3N2 as well as a 53% lower HI seroconversion against B on day 28 after vaccination.

3. Results

Sixty-five volunteers were recruited and sixty enrolled and vaccinated in February and March of 2010. Four recruits were removed from the study prior to vaccination because of the exclusion criteria of current use of antimicrobials (two recruits), a positive pregnancy test (one), and a history of a bleeding disorder (one). Another eligible volunteer changed her mind prior to being vaccinated. All thirty volunteers randomly assigned to the DSJI study arm received the vaccine by that route; all thirty in the N–S group also received their vaccine by the designated route (Table 1). Written memory aids and serology for all planned time points were obtained for all sixty enrollees.

3.1. Clinical assessment

There were no related serious adverse events (SAEs) throughout the clinical trial. One volunteer had an unrelated SAE, a 2-day hospitalization for acute appendicitis with onset 1-day after vaccination.

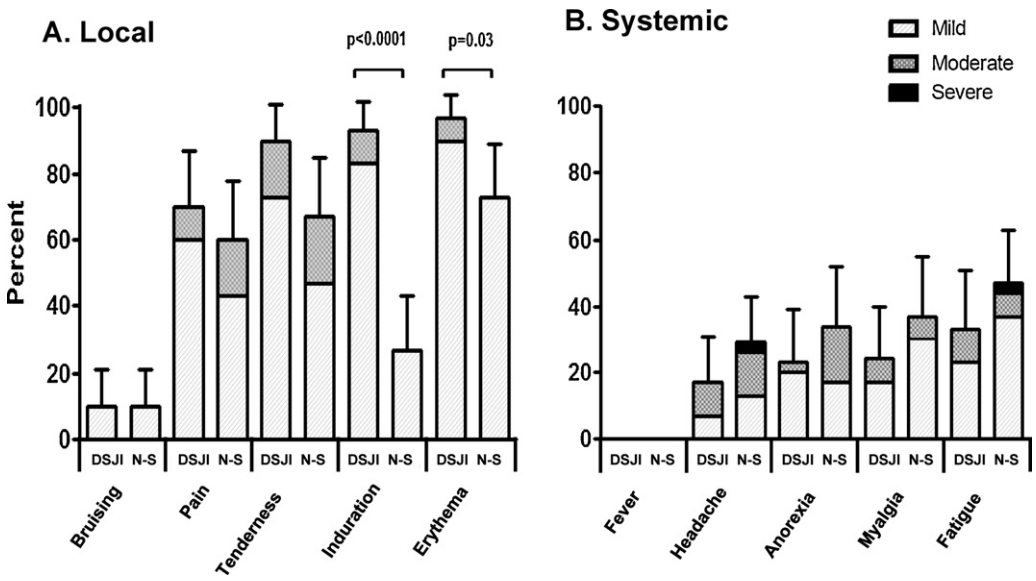


Fig. 2. Comparison of maximal solicited local (A) and systemic (B) reactions during the first three days after vaccination between volunteers receiving licensed 2009–2010 seasonal trivalent inactivated influenza vaccine (TIV) by disposable-syringe jet injection (DSJI) and by needle–syringe (N–S). Error bars indicate the upper 95% confidence interval (CI). The statistically significant *p* values shown derive from two-sided Fisher's exact tests.

A total of 39 unsolicited AEs were reported by 34 volunteers: 25 reported upper respiratory infection (URI), 7 reported unsolicited headache, 3 gastrointestinal upset, 3 musculoskeletal injury, and one urinary tract infection.

Twenty unsolicited AEs were reported by 17 volunteers in the N–S group and 19 unsolicited AEs were reported by 17 volunteers in the DSJI group, without any significant difference between the groups for any unsolicited AE category.

All solicited AEs were mild or moderate, with the exception of severe headache and fatigue on day 2 post-vaccination in a recipient vaccinated by N–S, who had been exposed to contacts with illness, and who developed a URI on day 1 after vaccination.

The most common local AE during the 3 days post-vaccination was erythema, which was seen or reported in 97% of DSJI vaccinees, but in only 73% of the N–S group ($p = 0.03$ by FE). Induration was next in reported frequency, found in 93% of the DSJI group, but in only 27% of the N–S group ($p < 0.0001$ by FE) (Fig. 2A). The mean diameter of the maximum erythema measured in subjects, or among those with any erythema, was 10.4 mm in the DSJI group and 3.5 mm in the N–S group ($p = 0.001$ by Mann–Whitney).

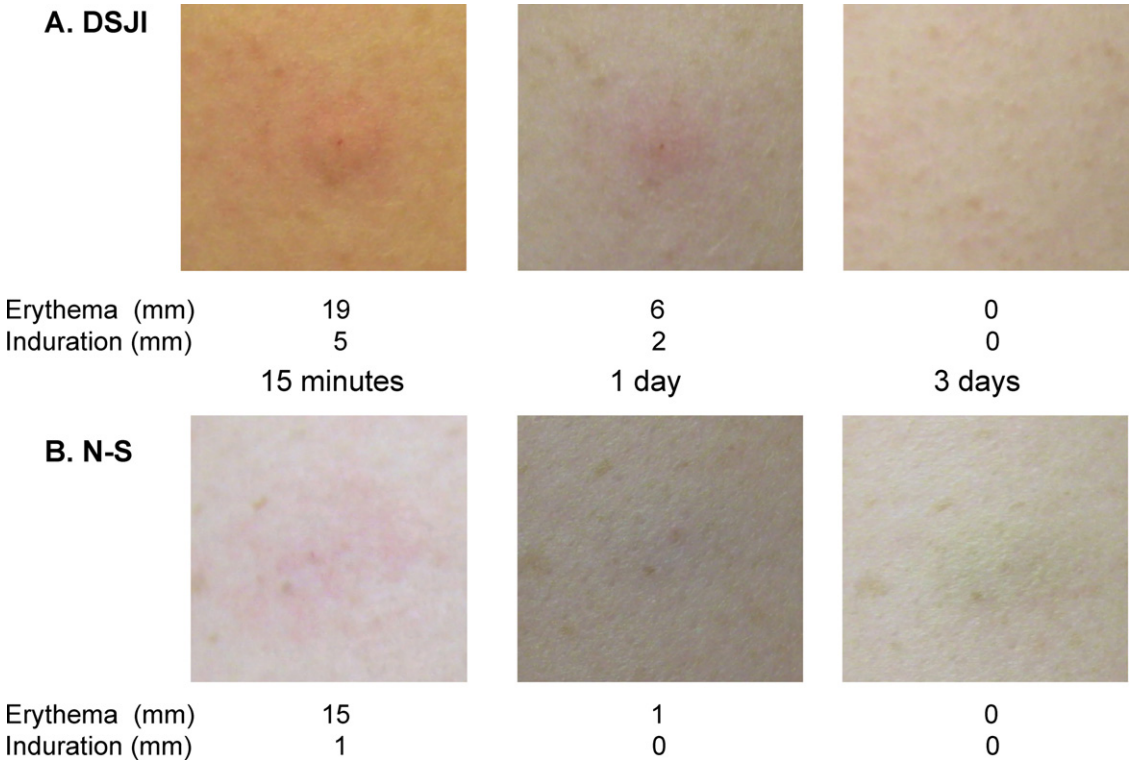


Fig. 3. Serial photographs representing approximately 20 mm × 20 mm sections of skin around injection sites taken of representative volunteers 15 min, one day and three days after receiving vaccine by DSJI (A) and N–S (B). Reported diameters of erythema and induration are reported for the longest axis of measurement, in millimeters (mm).

Table 2
Immunologic responses by hemagglutination–inhibition (HI) assay to antigens of licensed 2010 seasonal trivalent influenza vaccine (TIV), by disposable-syringe jet injector (DSJI) or needle–syringe (N–S) delivery route.

	H1N1			H3N2			B		
	DSJI	N–S	p^a	DSJI	N–S	p^a	DSJI	N–S	p^a
GMT[b] day 0 (95% CI[c])	17 (11–26)	30 (18–50)	0.1	31 (18–52)	30 (16–54)	0.9	14 (9–19)	19 (14–28)	0.1
GMT day 28 (95% CI)	213 (127–357)	199 (131–301)	0.8	426 (253–717)	301 (177–511)	0.3	111 (71–175)	131 (83–206)	0.6
Seroconversion: Percent ≥4-fold rise from day 0 to day 28 (95% CI)	80 (65–95)	63 (45–81)	0.3	80 (65–95)	67 (49–84)	0.4	73 (57–90)	57 (38–75)	0.3
Seroprotection: Percent HI titer ≥40 on day 28 (95% CI)	83 (69–97)	90 (79–100)	0.5	100 (NA)	93 (84–100)	0.5	77 (61–92)	87 (74–99)	0.5

[a] Two-sided independent Student's *t* test on natural log-transformed HI titers for GMT. Two-sided Fisher's exact test for percentages of NS vs. DSJI.
[b] Geometric mean titer.
[c] Confidence interval.

Similarly, the mean diameter of maximum induration was 11.6 mm among DSJI vaccines and 0.8 mm among N–S vaccinees ($p < 0.0001$ by Mann–Whitney). Frequencies of reported local pain, tenderness, and bruising were not significantly different between DSJI and N–S groups, although there was a trend toward slightly higher local pain and tenderness in the DSJI group.

The most common systemic AE was fatigue, reported by 33% of the DSJI group and 47% in the N–S ($p = 0.4$ by FE) (Fig. 2B). Second-most common was myalgia: 23% in DSJI, 37% in N–S ($p = 0.4$ by FE). In contrast to local reaction rates, statistically non-significant trends toward more frequent systemic reactions were reported in the N–S group (Fig. 2B). No volunteers in either group had a temperature greater than 99.6 °F (37.6° C).

All AEs were transient, well-tolerated, and resolved completely. The size of measurable reactions and the severity of graded ones – both local and systemic – peaked on the day of vaccination (day 0) and began improving by day 1 (Fig. 3). No volunteers rated their vaccination experience as "poor". Among the DSJI group, 40% rated it as "acceptable", as did 27% of the N–S group (p = 0.4 FE). An "excellent" experience was described by 60% and 73% of DSJI and N–S groups, respectively ($p = 0.4$ FE).

3.2. Measurement of antibody responses

HI GMT for H1N1, H3N2, and B antigens increased by 13-, 14-, and 8-fold at day 28 post-vaccination for DSJI vaccinees, and by 7-, 10-, and 7-fold for N–S vaccinees, respectively (Table 2). Titers for all antigens decreased by day 90 in both study arms (Fig. 4). The percent of vaccinees that seroconverted by exhibiting a fourfold or greater rise in HI titer from day 0 to 28 among the DSJI group was 80% for H1N1, 80% for H3N2, and 73% for B, and for the N–S group 63% for H1N1, 67% for H3N2, and 57% for B by N–S. The percent of DSJI vaccinees that were seroprotected by achieving an HI titer ≥40 on day 28 were 83% for H1N1, 100% for H3N2, and 77% for B, and for N–S 90%, 93%, and 87%, respectively. There were no statistically significant differences between DSJI and N–S for any of the measured immunogenicity endpoints.

4. Discussion

In this first human clinical study using LectraJet® M3 RA to administer TIV vaccination, injection using the DSJI was found to be well-tolerated and immunogenic. Higher rates of transient local reactogenicity were seen in volunteers vaccinated with the LectraJet device compared to N–S, although a trend toward fewer systemic reactions was also noted.

The traditional administration of vaccines via needles poses safety risks for patients, healthcare providers, and the community [11–13]. An estimated 3 million healthcare workers worldwide are injured annually by N–S and other medical sharps contaminated with hepatitis C virus (HCV), hepatitis B virus (HBV), or human

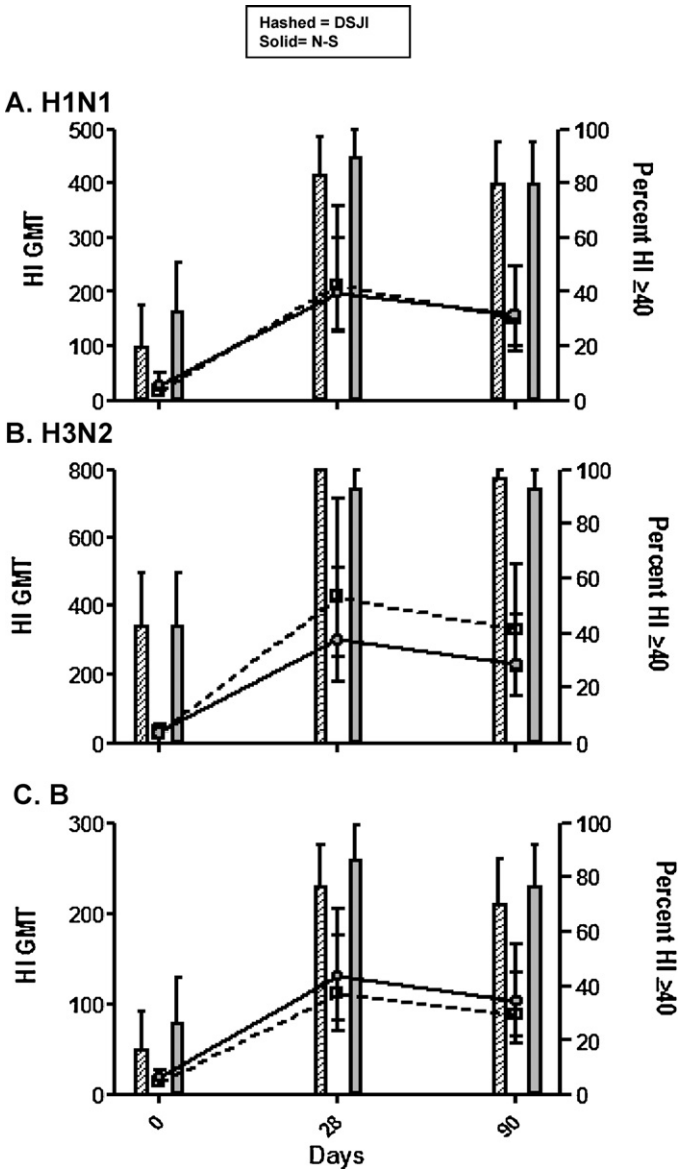


Fig. 4. Geometric mean titers (GMT) (left *y*-axis) of hemagluttination inhibition (HI) assays against H1N1 (A), H3N2 (B), and B (C) vaccine antigens for volunteers vaccinated by DSJI (dashed lines) and by N–S (solid lines). Percentages (right *y*-axis) of DSJI-group (dashed bars) and N–S-group (solid bars) volunteers with HI titers ≥40. The *x*-axis represents the number of days after vaccination. Error bars indicate 95% confidence intervals (CI).

immunodeficiency virus (HIV) resulting in an estimated 16,000 HCV, 66,000 HBV, and 1000 HIV infections [14]. The risk for needlestick injury may substantially increase during the haste of mass vaccination campaigns in response to a bioterrorism emergency or a natural pandemic [15]. Use of DSJIs to administer vaccine obviates the need for needles and thus mitigates this risk and the associated complications. Future high-speed models would allow for delivery of up to 600 vaccinations per hour [8,16], perhaps at reduced overall cost [1,16,17]. The manually powered LectraJet® M3 RA DSJI model used in this study is unique in that it shares its disposable syringes with the still-investigational motorized high speed model.

The medical literature reports that immune responses such as antibody titers and seroconversion rates after vaccination by JI (either MUNJI or DSJI) are sometimes greater than by N–S [7], but the underlying reasons are not known. Theories include wider dispersal of vaccine into immunologically relevant tissues than that achieved by N–S, as well as residual vaccine in the skin and along the track of the jet injector route to its site of primary deposition in fat or muscle [7,16–18]. Such dispersion may enhance contact with antigen presenting cells, lymphocytes, and other effector cells of the immune system. On the other hand, a comparative study of DSJIs vs. N–S for influenza vaccination with sample size groups similar to ours also found no significant differences in immune responses by method of vaccination [19].

Another general finding reported in the JI literature is that this method of vaccination often produces higher rates of local reactions than N–S [7], as borne out in this study, as well. Jackson, et al. found erythema, swelling, and pain more frequent among their two DSJI study arms when compared to the control N–S arm [19]. Another study of JI immunization using the measles, mumps, and rubella vaccine found no difference in pain [20], but its sample size was small and trended toward more pain. These and other studies, including ours, found the increased reactogenicity to be tolerable and not of a severity that would be clinically significant or contraindicate use. Indeed, similar numbers of volunteers in both groups of this study found the vaccination experience “acceptable” or “excellent”.

On the other hand, however, a DSJI study of aluminum-adjuvanted hepatitis B vaccine produced local pain reactions sufficiently obnoxious to cause some volunteers to refuse subsequent doses [21], and another study of adjuvanted hepatitis A virus vaccine found ten times more frequent local reactions among the DSJI group than N–S [18], suggesting that chemical irritability of the product injected may be a major factor in local reactions.

A key limitation to this and similar studies is the small number of participating subjects, which makes difficult any statistically based conclusions on similarities, differences, and non-inferiority between study groups. Instead, our study was intended as a first analysis of the safety and tolerability of a new needle-free system for vaccine delivery, and its ability to satisfy accepted standards for immune response to influenza vaccine.

In 2007, the FDA issued a Guidance for Industry for the clinical data needed to support licensure of seasonal inactivated influenza vaccines [22]. The recommendation is that HI GMT and rates of seroconversion (defined as percentage of subjects with either a pre-vaccination HI titer <10 and post-vaccination titer $\geq$40 or a pre-vaccination titer $\geq$10 and a fourfold or greater rise in post-vaccination titer) be used as immunogenicity endpoints [22]. For adults <65 years of age, the lower bound of the two-sided 95% CI for the percent of subjects achieving seroconversion should meet or exceed 40%, and the lower bound of the two-sided 95% CI for the percentage of subjects achieving a titer $\geq$40 should meet or exceed 70%. This study was not powered for such criteria and thus has wide confidence intervals. However, seroconversion and seroprotection were high enough to satisfy the FDA criteria for four of six (4/6) assessments in the DSJI group and five of six (5/6) assessments for the N–S group; the lower bounds of the 95% CI of the FDA cutoff is missed by 1% for H1N1 and 9% for B in the DSJI group and by 2% for B in the N–S group. It is to be noted that satisfying FDA guidance for TIV licensure is not required for licensed medical devices such as the LectraJet® M3 RA, nor the licensure of N–S devices. It is reassuring, however, that the DSJI immunogenicity reported here is similar to that for N–S delivery, and close to satisfying FDA guidelines despite the small size of this study.

In conclusion, needle-free jet injection of TIV by the newly-licensed LectraJet® M3 RA device appears to have an acceptable safety profile and induce satisfactory immune responses. Future studies should be powered to confirm the observed trends for potential differences and non-inferiority with respect to the N–S route.

Acknowledgments

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Immunogenicity & Tolerance Study of Influenza Vaccine (Fluvax), Australia

Trials in Vaccinology 2 (2013) 39–44

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Original Article

A randomized controlled study to assess the immunogenicity and tolerability of a 2012 trivalent seasonal inactivated influenza vaccine administered via a disposable syringe jet injector device versus a traditional pre-filled syringe and needle ☆

Nikolai Petrovsky [a,b,d,*], Yoshikazu Honda-Okubo [a], Michael Royals [c], Kylie Bragg [b], Dimitar Sajkov [d]

[a] Vaxine Pty Ltd., Flinders Medical Centre, Bedford Park, Adelaide, SA 5042, Australia
[b] Department of Endocrinology, Flinders Medical Centre/Flinders University, Adelaide, SA 5042, Australia
[c] PharmaJet Inc., 400 Corporate Circle, Golden, CO 80401, USA
[d] Australian Respiratory and Sleep Medicine Institute, Flinders Medical Centre, Bedford Park, Adelaide, SA 5042, Australia

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ABSTRACT

The Stratis® disposable syringe jet injection (DSJI) system (PharmaJet Inc., Denver, USA) delivers vaccine utilizing a spring powered energy source to create a fine high-velocity jet of liquid that directly penetrates the skin without using a needle. We performed a study to collect data on the effect of the Stratis DSJI device on influenza immunization in 46 predominantly elderly subjects (28M, 18F; mean age 61 years) who were randomized 1:1 to receive Fluvax 2012 trivalent inactivated influenza vaccine via prefilled N–S or Stratis DSJI. H1N1 seroprotection was not significantly different for vaccine delivered by DSJI (86.4%, 95% CI 72.1–100) or N–S (79.2%, 95% CI 63.0–95.4), and likewise for H3N2 and B strains. The DSJI had a ~2-fold higher mean injection pain score (DSJI: 3.0 versus N–S 1.58, $p = 0.045$) plus increased rates of swelling and tenderness but this was offset by a lower rate of elicited systemic reactions, particularly the frequency of post-immunization headaches (DSJI: 9% vs N–S: 33.3%). This study suggests that subject to confirmation of non-inferiority in an appropriately powered study, the Stratis DSJI is a viable alternative strategy for the administration of seasonal influenza vaccines with particular appeal for individuals with needle phobia. Australia New Zealand Clinical Trials Register: ACTRN12612000709842.

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1. Introduction

Influenza is a highly contagious and potentially deadly viral disease, particularly in the elderly, those with chronic disease and young children. Seasonal trivalent inactivated influenza virus (TIV) vaccines are used to boost neutralizing antibody titers and thereby help prevent severe disease. TIV vaccines are most commonly administered either by intramuscular or deep subcutaneous injection using a needle and syringe (N–S) method. Although this is a reliable and well-tested method, downsides include the risk of needle stick injuries to the operator [1] and avoidance of immunization by subjects suffering from needle phobia [2]. Alternative approaches for TIV vaccine administration include aerosol delivery or mucosal application [3,4], intradermal injection [5], and transdermal patch [6]. In particular, recent advances in intradermal delivery devices, e.g. the Soluvia™ id-needle system (Becton-Dickenson, USA) has enabled intradermal influenza vaccines to be developed for the first time as commercial products, e.g. Intanza® (Sanofi Pasteur, Lyon, France). Another major advance in vaccine delivery has been in the area of jet injector devices [7] including the recently-developed Stratis disposable syringe jet injection (DSJI) device. The Stratis DSJI delivers vaccine using a hand-held, spring-powered, energy source to create an extremely fine high-velocity jet of liquid that directly penetrates through the skin without the need for a needle. The Stratis DSJI is approved as a medical device for human use by the US Food and Drug Administration (FDA) and employs a single-use, sterile, auto-disable needle-free syringe for vaccine delivery. The delivery of TIV vaccine through this needle-free device could offer a superior experience compared to N–S injection, particularly for needle

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* Corresponding author. Address: Department of Diabetes and Endocrinology, Flinders Medical Centre, Adelaide, SA 5042, Australia. Tel.: +61 8 82044572; fax: +61 8 82045987.
E-mail address: nikolai.petrovsky@flinders.edu.au (N. Petrovsky).

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phobic subjects, with an added advantage being removal of needle stick injury risk.

Vaccine labeling is clear on the route of parenteral administration (IM, SC, ID), but is most often silent on the method of administration. While N–S is most commonly used, DSJI devices have been used for over 50 years across a wide range of vaccines [7–10]. However, on October 26th, 2011 the US FDA issued a communication to "inform the public that inactivated influenza vaccines labeled for IM injection are intended for administration using a sterile needle and syringe." This recommendation was based, in part, on the fact that safety and effectiveness information submitted to the FDA by vaccine manufacturers in support of the influenza vaccine approvals were acquired with N–S, and not DSJI. This delivery method-specific approach to vaccine approval signals a significant departure on the part of the FDA from previous practice.

The primary aim of this pilot clinical study was to test the utility of the Stratis DSJI for delivery of TIV vaccine as compared to a preloaded N–S with the aim of collecting data to inform the design of a larger, appropriately powered, immunogenicity non-inferiority study. A secondary aim was to collect preliminary data on the frequency and severity of local and systemic side effects between the two administration approaches and to assess subject satisfaction with the Stratis DSJI device.

2. Methods

2.1. Study site

The study was conducted as a single site study sponsored by Vaxine Pty Ltd. and performed within the Australian Respiratory and Sleep Medicine Institute at Flinders Medical Centre (FMC), Adelaide, Australia with recruitment undertaken between March 30, and August 24, 2012. The study was approved by the Flinders Clinical Research Ethics Committee. The study was registered on the publicly accessible Australia New Zealand Clinical Trials Register (http://www.anzctr.org.au) under the trial record number ACTRN12612000709842.

2.2. Study subjects

After obtaining informed consent adult volunteers were randomized to receive their TIV vaccine through either the Stratis DSJI or a preloaded N–S on a 1 to 1 basis. Randomization was performed by use of blinded envelopes containing randomization codes prepared independently using the randomization program available at http://www.radomization.com. The study population included ambulatory subjects and did not exclude those of older age or with chronic disease. Inclusion criteria included males or females 18 years or older, able to provide written informed consent and willing to comply with the protocol for the study duration. Exclusion criteria included history of vaccination with 2012 seasonal influenza vaccine or of serious vaccine or egg allergy, women of childbearing potential unless using a reliable and appropriate contraceptive method, pregnant or lactating women, receipt of another investigational agent within 14 days preceding initiation of treatment or any other serious medical, social or mental condition which, in the opinion of the investigator, would be detrimental to the subjects or the study.

2.3. Study vaccine and devices

Fluvax 2012 (CSL Ltd., Parkville, Australia) was purchased as a single dose preloaded plastic syringe with staked needle containing 15 ug hemagglutinin (HA) dose of each of A/California/7/2009 (H1N1pdm09), A/Perth/16/2009 (H3N2) and B/Brisbane/60/2008 in a volume of 0.5 ml. In the control needle and syringe (N–S) group the vaccine was administered as supplied by the manufacturer in a prefilled syringe with 1/2′ needle for deep subcutaneous/intramuscular injection into the deltoid muscle. For the Stratis DSJI group, immediately prior to use 2 vials of Fluvax 2012 vaccine were dispensed into a sterile 2 ml vial and then using the filling adaptor supplied by PharmaJet, 0.5 ml of the vial contents were aspirated into the DSJI syringe which was then loaded onto the Stratis DSJI device prior to injection into the deltoid muscle. Due to the very different nature of the delivery devices, neither subjects nor clinical staff were blinded to the study arm to which subjects were randomized.

2.4. Study visits

After assessment for eligibility criteria and providing informed consent, study subjects had a baseline clinical assessment, including medical history. Blood samples were drawn for measurement of baseline titers of anti-influenza antibodies. Each eligible subject was then randomized to receive vaccination in one of the study groups. Immediately following immunization subjects recorded their pain score using a visual analogue chart. Subjects were observed for 30 min following immunization and then rescored their pain with the same visual analogue chart. Those subjects who received their vaccine via the Stratis DSJI were asked to complete a copy of a questionnaire to assess their perceptions of the injection. Subjects were then provided with a diary to take home with them to record any symptoms or adverse events. Day 1 post-immunization subjects were telephoned to check their condition and to enquire about any adverse reactions. They then returned on day 28 post-immunization for collection of the subject diary, recording of any additional adverse events, and to provide a final blood sample for immunogenicity studies. Adverse events were graded using the "FDA/CBER toxicity grading scale for healthy adult and adolescent volunteers enrolled in preventative vaccine clinical trials" (www.fda.gov/cber/gdlns/toxvac.htm). Those subjects who received their vaccine via the Stratis DSJI were also asked at this final visit to complete a second copy of the questionnaire they had completed previously. The CONSORT Flow Diagram for the study is shown in Fig. 1.

2.5. Influenza assays

Red blood cell hemagglutination inhibition assays were performed in the Dept. of Endocrinology, Flinders Medical Centre using previously described techniques [11]. They were performed against each of the vaccine strains, namely A/California/7/2009 (H1N1), A/Perth/16/2009 (H3N2), and B/Brisbane/60/2008 on serum samples of all subjects taken prior to immunization and at 28 days post-immunization.

2.6. Statistical evaluation

The analysis included listings of objective response according to currently pertaining WHO influenza vaccine criteria using the hemagglutination inhibition (HI) assay, including seroconversion = 4-fold increase in titer over baseline, seroprotection = post-immunization titer of 1:40 or greater and potency = Geometric Mean of Titer (GMT) Ratio. Exact binomial confidence intervals were reported for all proportional end points. Reported p-values are two-sided, with no adjustment for multiple testing; $p \leqslant 0.05$ was considered significant. GMT and 95% confidence intervals were computed by taking the exponent of the mean and of the lower and upper limits of the 95% confidence intervals of the $\log_e$-transformed titers. Differences in mean values were compared

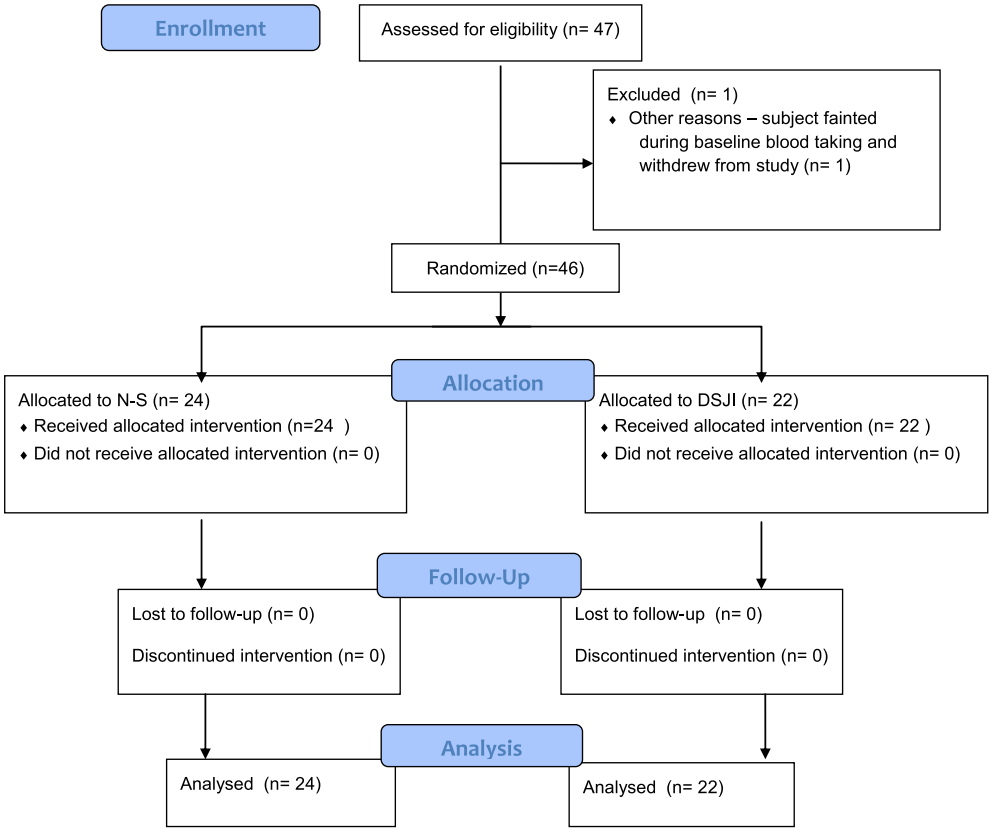


Fig. 1. CONSORT 2010 Flow Diagram –2012 Influenza Vaccine DSJI Study.

with Student *t*-test and for proportions using the Fisher's exact test using the GraphPad online calculator. As this was a pilot study, no formal sample size estimation was performed. A minimum sample size of 20 subjects per group was chosen in order to provide adequate evaluation of the study endpoints and allow estimates of treatment effect to be generated to guide power calculations for design of a future larger non-inferiority study.

3. Results

3.1. Demographics

Twenty-four subjects received Fluvax by staked needle and syringe (N–S group) and 22 subjects received the same Fluvax vaccine via the Stratis DSJI (DSJI group). Overall, the two groups were well matched, although there was a slightly higher mean age and ratio of male to female subjects in the DSJI group (Table 1). This was an older population with mean age over 60 years, and the majority of subjects (>90%) were taking one or more medications for chronic medical conditions. All subjects completed the study protocol with no withdrawals due to post-immunization adverse events and no subjects being lost to follow up.

Table 1
Subject demographics.

	N–S	DSJI
Number of subjects	24	22
Male: *n* (%)	13/24 (54%)	15/22 (68%)
Female: *n* (%)	11/24 (46%)	7/22 (32%)
Age: mean (SD)	59.5 (16.1)	62.5 (9.8)
Age: 18–59 years: *n* (%)	6 (25%)	7 (31.8%)
Age: 60–78 years: *n* (%)	18 (75%)	15 (68.2%)
Caucasian (%)	24/24 (100%)	22/22 (100%)
Previous flu vaccine *n* (%)	23/24 (96%)	22/22 (100%)
On chronic medication *n* (%)	22/24 (92%)	20/22 (91%)

3.2. Vaccine immunogenicity

Vaccine efficacy as measured by hemagglutination inhibition (HI) responses to immunization was modest (Table 2), which likely reflected the elderly demographics of the study population, with such subjects known to have reduced TIV vaccine responses [12]. For the H1N1 vaccine strain, the proportion of subjects achieving a seroprotective titer was not significantly different between the DSJI group (86.4%, 95% CI 72.1–100%) and the N–S group (79.2%,

Table 2
Vaccine efficacy.

	N–S (95% CI)	DSJI (95% CI)
A/California/7/2009 (H1N1)		
GMT (pre/post)	29.1/75.5	34.2/80.0
Seroconversion	33.3% (14.4–52.2)	31.8% (12.3–51.3)
Seroprotection	79.2% (63.0–95.4)	86.4% (72.1–100)
GMT fold increase	2.6 (1.4–3.8)	2.3 (1.3–3.4)
A/Perth/16/2009 (H3N2)		
GMT (pre/post)	23.8/42.4	23.4/49.9
Seroconversion	12.5% (0.7–25.7)	31.8% (12.3–51.3)
Seroprotection	66.7% (47.8–85.6)	72.7% (54.1–91.3)
GMT fold increase	1.8 (1.0–2.6)	2.1 (1.1–3.2)
B/Brisbane/60/2008		
GMT (pre/post)	11.2/16.8	15.5/22.0
Seroconversion	4.1% (3.8–12.0)	4.5% (4.2–13.2)
Seroprotection	16.7% (1.8–31.6)	18.2% (2.1–34.3)
GMT fold increase	1.5 (0.9–2.1)	1.4 (0.7–2.2)

95% CI 63.0–95.4%, n.s.). A similar result was obtained for the H3N2 and B vaccine strains (Table 2).

3.3. Vaccine tolerability

Post-immunization pain scores were assessed by visual analogue pain charts completed by study subjects immediately following and then 30 min post-immunization. Overall, both injection methods were well tolerated (Fig. 2). The DSJI was associated with a significantly higher mean pain score at the time of injection (mean pain score – DSJI 3.0 versus N–S 1.58, $p = 0.045$ by Student t-test) with several subjects in the DSJI group reporting pain scores of 8 or more out of 10. Overall, the proportion of subjects recording pain scores of 3 or higher at the time of immunization was higher for the DSJI group (10/22, 45.4%; 95% CI 24.6–66.2%) than the N–S group (4/24, 16.6%; 95% CI 1.7–31.5%, $p = 0.05$ by Fisher's exact test). However, in all cases injection site pain abated rapidly and there was no significant difference between groups in injection site pain scores 30 min post-immunization, with no pain score in the DSJI group greater than 3 out of 10 by this time.

3.4. Local adverse reactions

Local injection site reactions were ascertained by a telephone call to all subjects the day following their immunization, and by records from subject diaries returned on post-immunization day 28. Subjects were asked to record in their diary the occurrence of any local reactions (pain, tenderness, redness, swelling and bruising) and also asked to record its duration and severity (Fig. 3). There were a higher total number of local adverse reactions in the DSJI group (total 43 AE amongst 22 subjects) than in the N–S group (total 19 AE amongst 24 subjects). In particular, there were significant differences in rates of tenderness (DSJI: 17/22, 77%; 95% CI 59.41–94.59% versus N–S: 10/24, 41.7%; 95% CI 21.97–61.43%; $p = 0.02$ Fisher's exact test) and swelling (DSJI: 10/22, 45.5%; 95% CI 24.7–66.3% versus N–S: 1/24, 4.2%; 95% CI −3.83–12.23%, $p < 0.001$) with a non-significant trend towards higher rates of bruising and redness in the DSJI group. However, all of the local reactions in the DSJI group were graded as mild only (Grade 1) with just one case of Grade 2 tenderness occurring in the N–S group. Most local reactions abated within 4 days with the majority lasting less than 2 days.

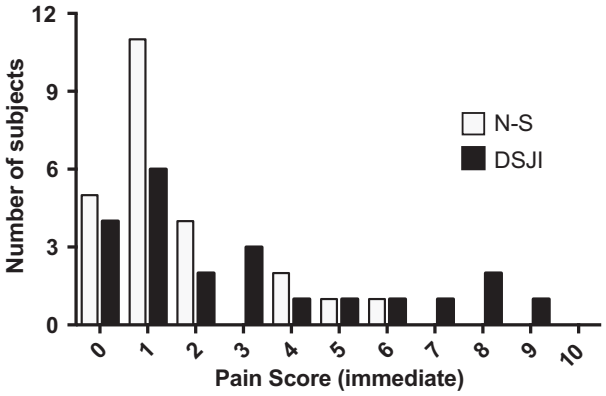


Fig. 2. Post-immunization pain scores of influenza vaccine administered by Stratis DSJI or needle and syringe (N–S). Post-immunization pain scores assessed by visual analogue pain charts recording pain on a scale of 0 (no pain) to 10 (worst imaginable pain) completed by study subjects immediately following influenza immunization.

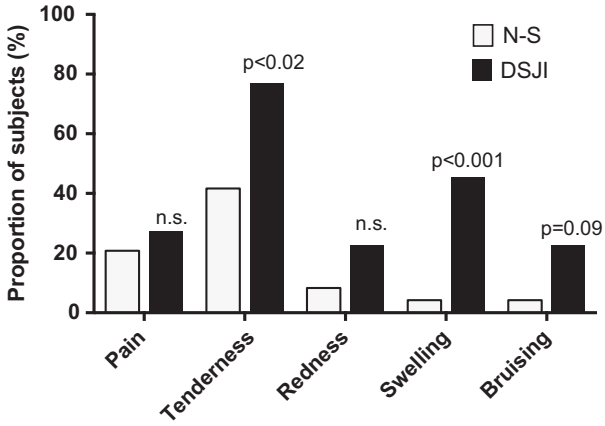


Fig. 3. Local adverse events for influenza vaccine administered by Stratis DSJI or needle and syringe (N–S). Subjects were asked to record any symptoms or adverse events in a diary they took home with them and then returned on day 28 post-immunization.

3.5. Systemic adverse reactions

Systemic adverse reactions were ascertained by a telephone call to all subjects the day following their immunization, and by records from subject diaries returned on day 28. Subjects were asked to record in their diary the occurrence of any systemic reactions (fever/chills, headache, muscle ache, fatigue, nausea, diarrhea) and to record its duration and severity. By comparison to local reactions, which were higher in the DSJI group, there was a significantly lower rate of elicited systemic reactions (fever, headache, muscle ache, fatigue, nausea, diarrhea) in the DSJI compared to the N–S group. Significantly fewer subjects ($p = 0.02$) in the DSJI group (2/22, 9.1%; 95% CI −2.92–21.12%) experienced one or more systemic reactions as compared to the N–S group (10/24, 41.7%; 95% CI 21.97–61.43%). Interestingly, there was a strong trend that did not quite reach statistical significance ($p = 0.07$) to a reduced rate of headaches (Grade 1 or 2) post-immunization in the DSJI group (2/22, 9.1%, 95% CI −2.92–21.12%) compared to the N–S group (8/24, 33.3%, 95% CI 14.44–52.16%).

3.6. Subject perceptions of the stratis DSJI device

A simple questionnaire was administered to all subjects who received immunization via the DSJI, both immediately after immunization and again on the Final Study Visit on day 28 (Table 3). A high frequency of subjects in the DSJI group (77.3%) reported no anxiety or fear about needles. Immediately after immunization 86.4% of subjects reported being moderately to extremely satisfied with having the influenza vaccine through the DSJI and 81.8% still felt this way 4 weeks later. Subject acceptability of the DSJI was high, with over 81.9% expressing a moderate to extreme interest in having their next vaccine using the DSJI. Two subjects (9.1%) reported immediately post-immunization being not at all satisfied with the DSJI which correlated with very high injection pain scores reported by the same subjects (data not shown).

4. Discussion

The study within the limitations of its size and power demonstrated that administration of TIV vaccine via the Stratis DSJI had no adverse effect on TIV immunogenicity when compared to administration via the pre-filled N–S. This is consistent with the findings of a recent comprehensive review by Weniger and Papania of alternative vaccine delivery methods that concluded the

Table 3
Stratis DSJI consumer questionnaire.

Questions	Answers	Day 0 (n = 22)	Day 28 (n = 22)
Q1. Do you have anxiety or a fear of needles?	Not at all	17 (77.3%)	21 (95%)
	Mildly	3 (13.6%)	1 (4.5%)
	Somewhat	1 (4.5%)	0
	Moderately	1 (4.5%)	0
	Extremely	0	0
Q2. Overall, how painful or physically uncomfortable was your needleless injection?	Not at all	8 (36.4%)	10 (45.5%)
	Mildly	7 (31.8%)	7 (31.8%)
	Somewhat	3 (13.6%)	1 (4.5%)
	Moderately	3 (13.6%)	2 (9.1%)
	Extremely	1 (4.5%)	2 (9.1%)
Q3. How satisfied were you with having your flu vaccine via a needleless injection?	Not at all	2 (9.10%)	0
	Mildly	0	1 (4.5%)
	Somewhat	1 (4.5%)	3 (13.6%)
	Moderately	6 (27.3%)	5 (22.7%)
	Extremely	13 (59.1%)	13 (59.1%)
Q4. If it were available, would you want to have your next vaccine via a needleless injection?	Not at all	2 (9.1%)	2 (9.1%)
	Mildly	0	4 (18.2%)
	Somewhat	2 (9.1%)	0
	Moderately	8 (36.4%)	2 (9.1%)
	Extremely	10 (45.5%)	14 (63.6%)
Q5. How likely are you to recommend needleless injections to a friend or family member?	Not at all	3 (13.6%)	1 (4.5%)
	Mildly	0	4 (18.2%)
	Somewhat	2 (9.1%)	2 (9.1%)
	Moderately	9 (40.9%)	4 (18.2%)
	Extremely	8 (36.4%)	11 (50%)

immunogenicity of jet injectors to be usually equal to, if not better than, vaccine administered by N–S [13]. Notably, no studies of TIV vaccine administered via DSJI devices have found a reduction in vaccine immunogenicity [14,15]. The TIV data obtained with at least four different DSJI devices should help reassure regulatory bodies including the FDA that administration of TIV by DSJI should be regarded as equivalent to N–S approaches, with definitive proof on this point soon to be available from a large randomized non-inferiority study by Pharmajet currently underway.

From a tolerability standpoint, there was a significant increase in mild local injection site reactions when TIV was administered using the Stratis DSJI, in accord with findings of other DSJI studies [13–16]. However, local reactions abated within 4 days of immunization, with the majority lasting less than 2 days. Subjects also reported a higher overall pain score immediately post-immunization with the Stratis DSJI, in several cases reaching levels of more than 8 out of 10 on a visual analogue scale but reassuringly this pain was transient and in almost all cases had returned to baseline by 30 min post injection.

Systemic adverse events are more serious than local reactogenicity events from a regulatory standpoint. Interestingly, there was a significant reduction in systemic adverse events, particularly headaches, reported by subjects who were immunized with the Stratis DSJI. This reduction in systemic adverse events with the Stratis DSJI is currently unexplained but could possibly reflect differences in the rate of systemic release of antigen with the different injection techniques. Interestingly, a similar but non-significant trend towards reduced systemic reactions was also reported in the study of TIV administered by the LectraJet DSJI device [15]. We speculate that more superficial deposition of the influenza vaccine when administering vaccine using DSJI devices may delay the systemic release of the TIV antigen, and thereby reduce the systemic side effects consequent upon a bolus of antigen entering the systemic circulation.

Subject acceptability of the Stratis DSJI was high with over 50% expressing a moderate to high interest in having their next influenza vaccine using the DSJI. This was a highly subjective questionnaire administered to gain subjects' perceptions regarding the use of the Stratis DSJI. The subject population is drawn predominantly from elderly subjects, most of whom reported having had regular seasonal N–S influenza immunizations in the past. Only 25% of subjects reported anxiety or fear about needles. A different questionnaire outcome may have been found if the questionnaire was administered to a group with a higher rate of needle phobia. However, such subjects were unlikely to volunteer for this study, as there was a 50% chance they would be randomized to receive the vaccine by standard injection. In fact, many individuals enquiring about participation in the study because of publicity of the Stratis DSJI withdrew their interest when they found that there was no certainty that they would receive the vaccine through the needle free device (data not shown). Despite the study population not being a needle-phobic population, there was nevertheless good acceptance of the device by the majority of subjects. Immediately after immunization 90% reported being moderately to extremely happy with having the influenza vaccine through the Stratis DSJI and 80% still felt this way when asked the same question 4 weeks later. Just two subjects reported not being at all satisfied with the use of the device immediately after they had received the vaccine, which correlated with their high reported pain scores. However, one of these subjects relaxed this assessment when asked the same question 4 weeks later.

Overall, this study highlighted the utility and advantages of the Stratis DSJI when compared to traditional N–S vaccine delivery. The device performed well, generated good vaccine immunogenicity, was easy to use with minimal training, and was well accepted by the majority of subjects. While being associated with an increased rate of mild injection site reactions this was offset by reduced systemic reactions including headaches. In markets where prefilled syringes are not used or not available, the current field-filled DSJI format where the healthcare worker transfers the vaccine from the primary glass container into the needle-free syringe just prior to delivery, works well. The technology could however provide further benefit from bringing a pre-filled syringe option to the market. Whilst this pilot study was not powered to assess non-inferiority of influenza vaccine immunogenicity when administered by the Stratis DSJI device, an appropriately powered study

to address this question is currently underway and when completed should provide reassurance of the suitability of the Stratis DSJI device for routine influenza vaccine delivery.

Disclosures

M.R. is an employee of PharmaJet, the company commercializing the Stratis device.

Acknowledgments

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Immunogenicity & Tolerance Study of Influenza, Typhoid, Tetanus, DTP, & Hepatitis-A 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

Isabelle Parent du Châtelet*, Jean Lang†¶, Martin Schlumberger*, Emmanuel Vidor†, Georges Soula‡, Alain Genet†, Steven M. Standaert*, Pierre Saliou† and Imule® Investigators Group§

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

*Association pour l'Aide à la Médecine Préventive, 3 avenue Pasteur, BP10, 92430, Marnes-la-Coquette, France. †Pasteur-Mérieux Sérums et Vaccins, 58 avenue Leclerc, 69007, Lyon, France. ‡Centre Muraz (OCCGE), B.P. 153, Bobo Dioulasso, Burkina Faso. §Dr A. Gueye (Head of medical District of Velingara, Senegal), Dr H. Julien (Chief Medical Officer, Firemen Brigade of Paris, France), Professor Ch. Lafaix (Villeneuve Saint-Georges Hospital, France), Dr P. Lemardeley (Legouest Army Teaching Hospital, Metz, France), Dr A. Monnereau (AMP, Kolda, Senegal), Dr A. Spiegel (Begin Army Teaching Hospital, Saint-Mandé, France), Dr M. Soke (Head of Medical District of Zoudweogo, Burkina Faso) and Dr J.P. Varichon (Tonkin Clinic, Villeurbanne, France). ¶To whom correspondence should be addressed. (Received 11 March 1996; revised 11 July 1996; accepted 15 July 1996)

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*).

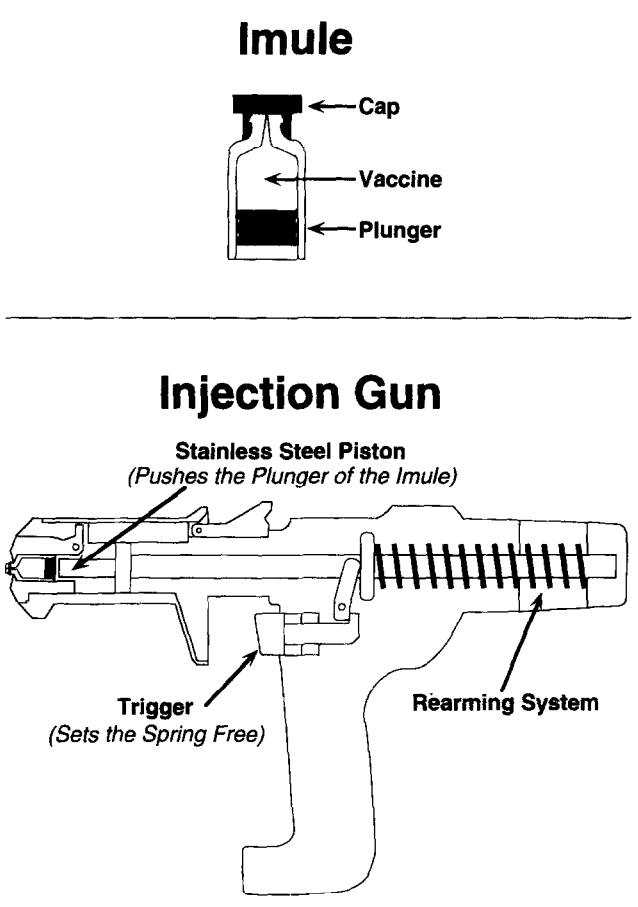


Figure 1 Mini-Imojet® and the Imule® system

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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