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

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

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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

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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

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*).

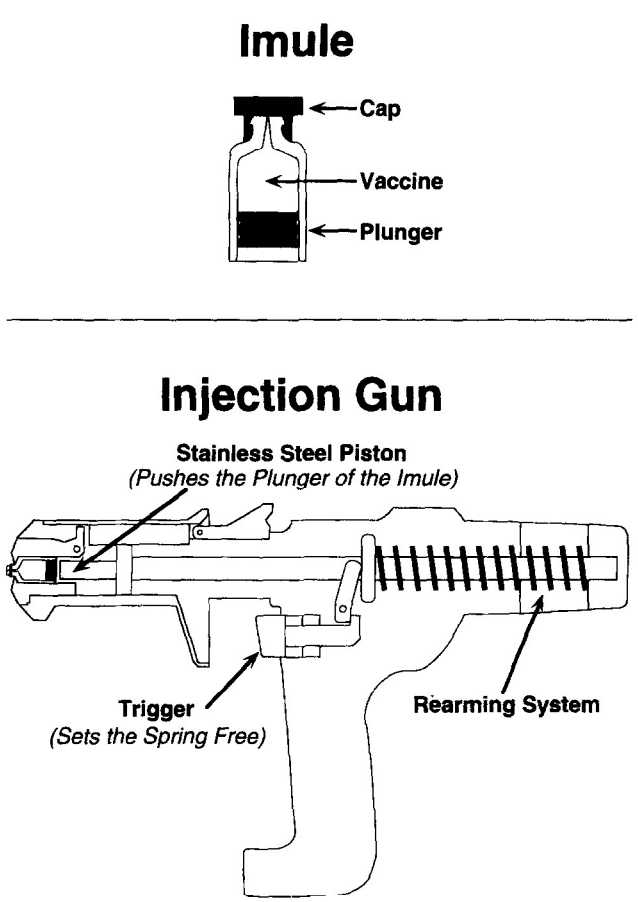

Figure 1 Mini-Imojet® and the Imule® system

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[a]
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 pre-vaccination 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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REFERENCES

1 Fournier, J. Les injecteurs sans aiguille et leur usage en médecine préventive. *Bull. Soc. Path. Ex.* 1973, **66,** 332–339
2 Vibes, J. Efficacité comparée de 2 techniques de vaccination contre la grippe. Taux sérologiques obtenus après administration du vaccin par le Porton Jet et la seringue. *Méd. Mal. Infect.* 1971, **1,** 343–348
3 Edwards, E.A., Johnson, D.P., Pierce, W.E. and Peckinpaugh, R.O. Reactions and serological responses to monovalent aceton-inactivated typhoid vaccine and heat-killed TAB when given by jet injection. *Bull. WHO* 1974, **51**; 501–505
4 Brink, P.R.G., Van Loon, A.M., Trommelen, J.C.N., Grisnau, S.W.J. and Smalenovakova, I.R.O. Virus transmission by subcutaneous jet injection. *J. Med. Microbiol.* 1985, **20,** 393–397
5 Centers for Disease Control and Prevention. Hepatitis B associated with jet gun injection—California. *Morbid. Mortal. Wkly Rep.* 1986, **35**(23), 373–376
6 Zachoval, R., Deinhardt, F., Gütler, L., Eisenberg, J. and Korger, G. Risk for virus transmission by jet injection (letter). *Lancet* 1988, 189
7 World Health Organization. WHO/UNICEF criteria for collaborating in field studies on low workload jet injectors. *WHO/UNICEF/EPI TS/1986.1.* WHO/EPI, Geneva, 1993
8 Aylward, B., Lloyd, J., Zaffran, M., Mc Nair-Scott, R. and Evans, P. Reducing the risk of unsafe injections in immunization programmes: financial and operational implications of various injections technologies. *Bull. WHO* 1995, **73,** 531–540
9 Galy, M., Genet, A. and Saliou, P. Un progrès dans le domaine de l'injection sans aiguille: le système Imule®. *STS Pharma Pratiques* 1992, **2,** 261–266
10 Safford, S. *et al.* Radio immuno assay for detection of antibody to hepatitis A virus. Results of clinical evaluation. *Am. J. Clin. Pathol.* 1980, **74,** 25–31
11 Miller, W.J., Clark, W., Hurni, W. *et al.* Sensitive assays for hepatitis A antibodies. *J. Med. Virol.* 1993, **41,** 201–204
12 Dunnet, C.W. and Gent, M. Significance testing to establish equivalence between treatments, with special reference to data in the form of 2×2 tables. *Biometrics* 1977, **33,** 593–602
13 Ajjan, N., Souvras, M., Duhamel, J. and Rey, M. A study of tetanus immunization among older subjects. *Proc. VI Int. Conf. Tetanus,* Lyon, France, 3–5 December 1981. Fondation Mérieux Collection, Lyon, 1981, pp. 185–198
14 Anonymous. Report of the 1994 Technet meeting. *WHO/EPI/LHIS/94.3.* WHO, Geneva, 1994
15 Evans, P. and Llyod, J. Injection safety in Immunization Programmes. *Proc. IV Int. Sem. Imm. Africa,* Yamoussoukro, Ivory-Coast, 13–16 March 1994. Fondation Mérieux Collection, Lyon, 1994, pp. 14–16
16 World Health Organization. Reducing the risk of unsafe injections in immunization programmes. *WHO/EPI/LHIS/94.2.* WHO, Geneva, 1994
17 Dagnan, S., Kouakou, A., Ekra, K.D., Ouattara, S. and Tagliante-Saracino, J. Sécurité de l'injection vaccinale à Abidjan. *Proc. IV Int. Sem. Imm. Africa,* Yamoussoukro, Ivory-Coast, 13–16 March 1994. Fondation Mérieux Collection, Lyon, 1994, p. 340
18 Spiegel, A., Lemardeley, P., Germanetto, P. *et al.* Mini-Imojet® et vaccination anti-grippale dans les armées françaises. Tolérance immédiate et faisabilité. *Proc. IV Int. Sem. Imm. Africa,* Yamoussoukro, Ivory-Coast, 13–16 March 1994. Fondation Mérieux Collection, Lyon, 1994, p. 344
19 Prost, A., Schlumberger, M. and Fayet, M.T. Response to tetanus immunization in onchocerciasis patients. *Ann. Trop. Med. Parasit.* 1983, **77,** 83–85
20 Dastur, F., Awatramani, V. and Dixit, J.A. Response to a single dose of tetanus vaccine in subjects with naturally acquired tetanus antitoxin. *Lancet* 1981, **2,** 219–221
21 Veronisi, R. *et al.* Naturally acquired tetanus immunity in human and animals from Galapagos Islands. *J. Infect. Dis.* 1983, **147,** 308–311
22 Clemens, J.D., Ferrecio, C., Levine, M.M. *et al.* Impact of *Haemophilus influenza* b polysaccharide–tetanus protein conjugate vaccine on response to currently administered Diphtheria–Tetanus–Pertussis vaccine. *J. Am. Med. Assoc.* 1992, **265,** 673–678
23 Watemberg, N., Dagan, R., Arbelli, Y. *et al.* Safety and immunogenicity of *Haemophilus influenza* type b tetanus conjugate vaccine, mixed in the same syringe with Diphtheria–Tetanus–Pertussis vaccine in young infants. *Pediatr. Infect. Dis. J.* 1991, **10,** 758–761
24 Thellmann, L., Kallinowski, B., Gmelin, K. *et al.* Reactogenicity and immunogenicity of 3 different lots of a hepatitis A vaccine. *Vaccine* 1992, **10,** S132–134
25 Tacket, C.O., Ferrecio, C., Robbins, J.B. *et al.* Safety and immunogenicity of two *Salmonella typhi* Vi capsular polysaccharide vaccines. *J. Infect. Dis.* 1986, **154,** 42–45
26 Tacket, C.O., Levine, M.M. and Robbins, J.B. Persistence of antibody titres three years after vaccination with Vi capsular polysaccharide vaccine against typhoid fever. *Vaccine* 1988, **6,** 307–308
27 Drucker, J., Soula, G., Diallo, O. *et al.* Evaluation of a new combined inactivated DTP-polio vaccine. *Dev. Biol. Standard.* 1986, **65,** 145–151
28 Wiedermann, G., Ambrosch, F., Kollaritsch, H. *et al.* Safety and immunogenicity of an inactivated hepatitis A candidate vaccine in healthy adults volunteers. *Vaccine* 1990, **8,** 581–584