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A RANDOMISED, OPEN LABEL, SINGLE-PERIOD, SINGLE-TREATMENT, CONTROLLED STUDY OF COMPARING SAFETY AND REACTOGENECITY OF LYOPHILISED BCG VACCINE IP (0.1 mg in 0.1 ml) OF GREEN SIGNAL BIOPHARMA PRIVATE LIMITED INDIA WITH BCG VACCINE (0.1 mg in 0.1 ml) OF SERUM INSTITUTE OF INDIA LIMITED (SIIL),INDIA

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ABSTRACT

The study was conducted with the objective to compare the safety and reactogenecity of lyophilized BCG Vaccine of Green Signal Bioharma Private Limited (GSBPL), India (Test) with lyophilized BCG vaccine which was available in the market (of Serum Institute of India (Reference)) in 120 healthy children.

The above study was conducted in two centers (Chennai & Bangalore). The study was conducted as per the protocol approved by DCGI and Madras ethical Committee.

A single dose was administered to all the subjects and it was inferred that all the 120 subjects vaccinated were safe. Further, the reactogenecity was confirmed after 90th day by PPD (Purified protein derivative) administration to all the subjects. Based on the above observations it was well identified that Test vaccine can be safely administered to children and it is well tolerated and accepted by the subjects. More over statistically it is inferred that there is no significant variation between the test and Reference vaccine.

INTRODUCTION – BCG VACCINE (FREEZE DRIED):

Bacillus Calmette Guérin (BCG) vaccines are live attenuated vaccines originally derived from a strain of Mycobacterium bovis by Calmette and Guerin at the Pasteur Institute, France. Mycobacterium bovis, has lost its virulence in humans by being specially cultured in an artificial medium for years. This is the source of the vaccine. The bacilli have retained enough strong antigenicity to become a somewhat effective vaccine for the prevention of human tuberculosis. At best, the BCG vaccine is 80% effective in preventing tuberculosis for duration of 15 years; however, its protective effect appears to vary according to geography.

Except in neonates, a tuberculin skin test should always be done before administering BCG. A reactive tuberculin skin test is a contraindication to BCG. If someone with a positive tuberculin reaction is given BCG, there is a high risk of severe local inflammation and (S)ring. People found to have reactive tuberculin skin tests should be screened for active tuberculosis.

BCG is given as a single intradermal injection at the insertion of the deltoid. If BCG is accidentally given subcutaneously, then a local abscess may form that may ulcerate and often requires treatment with antibiotics. However, it is important to note that an abscess is not always associated with incorrect administration, and it is one of the more common complications that can occur with the vaccination. Numerous medical studies on treatment of these abscesses with antibiotics have been done with varying results, but the general consensus of opinion is that once pus is aspirated and analysed, providing there are no unusual bacilli present, the abscess will generally heal in a matter of weeks if let as it is.

BCG vaccination leaves a characteristic raised (S) that is often used as proof of prior immunization. The (S) of BCG immunization must be distinguished from that of small pox vaccination which it may resemble. BCG vaccination is widely used through out the world to prevent tuberculosis, but has only been shown consistently to protect against disseminated tuberculous infection (tuberculous meningitis and miliary diseases).

STUDY DESIGN:

This study was an open label, randomized, Single treatment , single period controlled clinical multi center trial comparing the BCG Test Vaccine of Green signal Biopharma Ltd with that of the Reference vaccine of Serum Institute of India Ltd in order to assess the safety and Reactogenecity.

Name of the Sponsor	Green Signal Biopharma Private Limited, Pappankuppam, Gummidipoondi, Chennai- 601201.
Name of the Finished Product	Lyophilized BCG vaccine IP (0.1 mg in 0.1ml)
Name of the Active Ingredient	*Mycobacterium bovis* BCG (Bacillus Calmette Guerin), Danish strain 1331.

Title of the Study	A randomized, open label, single-period, single-treatment, controlled multi center phase III study of comparing Safety and Reactogenecity of lyophilized BCG vaccine IP (0.1 mg in 0.1 ml) of Green Signal Biopharma Private Limited, India with BCG vaccine (0.1 mg in 0.1 ml) of Serum Institute of India Limited (SIIL), India in 120 healthy children.
Study duration	216 days
Objective	To compare Safety and Reactogenecity of BCG vaccine of Green Signal Biopharma Private Limited India with lyophilized BCG vaccine which was available in the market (Serum Institute of India (Reference)) in 120 healthy children.
Methodology	This study was designed as a randomized, open label, single-period, single-treatment, controlled multi center phase III study. The study was conducted in two sites. According to the randomization schedule, sixty subjects were administered with single dose of BCG vaccine (0.1 mg in 0.1 ml) of Serum Institute of India Limited (SIIL), India and other sixty subjects with single dose of lyophilized BCG vaccine IP (0.1 mg in 0.1 ml) of Green Signal Biopharma Private Limited, India The vaccination dose was 0.05 ml for children under one year of age including the new born, and 0.1 ml for children of 1-14 yrs. The vaccine was given intradermally with a tuberculin syringe fitted with 25G/26G sterile needle. Development of Erythema, (P), ulcer and (S) at the vaccination site was observed at 30 minutes, 72 hours, 10th day, 30th day, 60th day and 90th day. Mantoux test was done on 90th day and the results of the test were read after 72 hours (93rd day). During each visit safety of the study vaccines were evaluated. The enrolled subjects were further followed up on 26th week for (S) formation and adverse events.

	The study was carried out as per the Independent Ethics Committee (Madras Ethical Committee, Chennai-600035) approved protocol and informed consent documents (BCGV/034/08, version 00 dt. 29/02/08).
Number of Subjects	One hundred and twenty (120) Healthy children up to 01 month of age were enrolled for the study. A total of 4 subjects did not visit for the follow up on day 90. Post vaccination Mantoux test was performed to 116 subjects on day 90. Among 116 subjects, 2 subjects did not report for assessment of reactogenicity on day 93. And hence the Mantoux test response was assessed for a total of 114 subjects.
Main inclusion criteria	1. Subjects in the age group of 0 – 14 years. 2. Parent(s) who were able to understand and sign the informed consent form after being explained by the investigator. 3. Ability to comply with the schedule of treatment and follow-up 4. Absence of BCG (S) 5. No evidence of any infection 6. No evidence of skin disease

Main Exclusion Criteria	7. History or presence of significant: Cardiovascular, pulmonary, hepatic, renal, hematological, gastrointestinal, endocrinal, immunologic, dermatologic, neurological or psychiatric diseases. 8. More specifically, history or presence of significant: • Low birth weight babies (<2.5 Kg) • Malignancy • Tuberculin positive • Hodgkin's disease • Corticosteroid therapy • Generalised Eczema • Infective dermatosis • Hypogammaglobulinemia • Immunosuppressed • Above 14 years of age • On anti-tubercular drugs • Chest X ray evidence of TB in children.
Investigational Products	
Test (T)	
Product	Lyophilized BCG Vaccine IP
Manufactured by	Green Signal Biopharma Private Limited, Chennai, India
Method of Administration	According to the randomization schedule, a total of sixty subjects (30 subjects in Chennai center and 30 in Bangalore center) were administered with single dose of Test vaccine intradermally. The skin was stretched between thumb and forefinger and sterile needle (25 G or 26 G) inserted bevel upwards for about 2mm into superficial layers of the dermis (almost parallel with the skin) and the injection was made. The site of injection was at the insertion of the deltoid muscle into the humerus.

Reference (R)	
Product	BCG Vaccine IP
Manufactured by	Serum Institute of India Limited, Pune, India
Method of Administration	According to the randomization schedule, the sixty subjects (30 subjects in Chennai center and 30 in Bangalore center) were administered with single dose of Reference Vaccine intradermally. The method of administration was similar to that of the Test vaccine.
Evaluation criteria for Reactogenicity	Development of (P), ulcer and (S) were observed at 30 minutes, 72 hours, 10th day, 30th day, 60th day and 90th day. Mantoux test was done on 90th day and the results of the test were read after 72 hours (93rd day). The subjects were further followed up on 26th week for (S) formation at the site of vaccination.
Safety evaluation	After administration of vaccine, all children were followed up at 30 minutes, 72 hours, 10th day, 30th day, 60th day and 90th day for adverse reactions. The subjects were further followed up on 26th week for adverse events.
Statistical Methods	The statistical analysis was done using SAS, version 9.2. The data of the subjects whoever reported for the follow up visits were taken for analysis. No data was excluded from the analysis. The size of the (P), ulcer and (S) were considered for statistical analysis. Summary statistics of the data was done for test and Reference vaccines and comparison was done using t test. Post vaccination Mantoux test results were analyzed. Summary statistics of the size of transverse indurations induced by Test vaccine and Reference vaccine were done and comparison was done using t test. Demographic details such as age, sex and weight were analysed for summary statistics. Comparison of age was done using Wilcoxon Rank sum test, sex was done using Chi square test and weight was done using One way ANOVA.

RESULTS:	
One hundred and twenty healthy children were screened for the study and the inclusion & exclusion criteria were applied. After thorough scrutinization of the eligibility criteria, they were enrolled and the investigational products were administered as per randomization schedule. The evaluation criteria such as reaction at vaccination site and safety were assessed at 30 minutes, 72 hours, 10^{th} day, 30^{th} day, 60^{th} day, 90^{th} day and on 26^{th} week. At 30 minutes, the data of all the 120 subjects were evaluated.	
Reactogenicity	The response for the post vaccination Mantoux test was assessed on day 93 for 114 subjects. The mean response of Test and Reference vaccines was evaluated statistically for any significant difference. There was no statistically significant difference between the Test & Reference vaccines as the p value is equal to 0.99, indicating the investigational vaccines induce similar immune response.
Safety	All the subjects well tolerated the Investigational Products. No deaths, no serious adverse events and no other adverse event were experienced and subjects were normal till the completion of the study.

DATA ANALYSIS:

The raw data of the subjects including the demographic profile, the reactions at vaccination sites and post vaccination Mantoux test response are as given below.

S. No.	Subject No.	Age (days)	Sex (Male-M / Female-F)	Weight (Kg)	Site
01	001	1	M	2.75	Chennai
02	002	3	F	3.20	Chennai
03	003	1	F	3.50	Chennai
04	004	2	M	2.60	Chennai
05	005	2	M	3.30	Chennai
06	006	0	M	3.50	Chennai
07	007	3	F	3.80	Chennai
08	008	2	F	2.90	Chennai
09	009	2	F	2.80	Chennai
10	010	3	M	3.00	Chennai
11	011	2	F	3.20	Chennai
12	012	3	M	2.70	Chennai
13	013	3	M	2.80	Chennai
14	014	2	M	2.90	Chennai
15	015	0	M	3.10	Chennai
16	016	1	M	3.50	Chennai
17	017	0	F	3.70	Chennai
18	018	1	M	3.10	Chennai
19	019	1	M	2.90	Chennai
20	020	1	F	3.40	Chennai

S. No.	Subject No.	Age (days)	Sex (Male-M / Female-F)	Weight (Kg)	Site
21	021	0	F	3.60	Chennai
22	022	1	F	3.10	Chennai
23	023	1	M	2.70	Chennai
24	024	0	M	2.80	Chennai
25	025	1	M	2.60	Chennai
26	026	1	F	2.70	Chennai
27	027	1	M	2.60	Chennai
28	028	0	F	2.90	Chennai
29	029	0	M	3.80	Chennai
30	030	0	F	2.60	Chennai
31	031	1	M	2.90	Chennai
32	032	0	M	2.60	Chennai
33	033	1	M	4.00	Chennai
34	034	0	M	3.20	Chennai
35	035	0	F	3.50	Chennai
36	036	1	M	3.70	Chennai
37	037	0	M	3.20	Chennai
38	038	1	F	3.10	Chennai
39	039	2	F	3.50	Chennai
40	040	3	F	2.80	Chennai

S. No.	Subject No.	Age (days)	Sex (Male-M / Female-F)	Weight (Kg)	Site
41	041	2	M	2.60	Chennai
42	042	1	M	3.00	Chennai
43	043	1	M	2.90	Chennai
44	044	0	F	3.30	Chennai
45	045	0	F	3.60	Chennai
46	046	1	M	3.20	Chennai
47	047	0	F	2.60	Chennai
48	048	0	F	2.70	Chennai
49	049	0	F	2.70	Chennai
50	050	0	M	2.90	Chennai
51	051	0	M	3.20	Chennai
52	052	0	F	3.50	Chennai
53	053	0	F	3.60	Chennai
54	054	0	F	3.10	Chennai
55	055	0	F	3.00	Chennai
56	056	0	M	2.60	Chennai
57	057	0	M	2.80	Chennai
58	058	0	F	2.70	Chennai
59	059	0	M	2.70	Chennai
60	060	0	F	2.60	Chennai

S. No.	Subject No.	Age (days)	Sex (Male-M / Female-F)	Weight (Kg)	Site
61	001	0	F	3.50	Bangalore
62	002	1	M	3.80	Bangalore
63	003	1	F	2.90	Bangalore
64	004	0	M	2.80	Bangalore
65	005	1	M	3.00	Bangalore
66	006	0	M	3.10	Bangalore
67	007	0	M	3.50	Bangalore
68	008	1	M	3.70	Bangalore
69	009	1	F	3.10	Bangalore
70	010	1	M	2.90	Bangalore
71	011	0	M	3.40	Bangalore
72	012	0	F	3.60	Bangalore
73	013	0	M	3.10	Bangalore
74	014	0	F	2.70	Bangalore
75	015	0	M	2.80	Bangalore
76	016	1	M	2.60	Bangalore
77	017	2	M	3.00	Bangalore
78	018	0	M	2.90	Bangalore
79	019	0	F	3.30	Bangalore
80	020	1	M	3.60	Bangalore

S. No.	Subject No.	Age (days)	Sex (Male-M / Female-F)	Weight (Kg)	Site
81	021	1	M	3.20	Bangalore
82	022	0	M	2.60	Bangalore
83	023	0	F	2.70	Bangalore
84	024	2	F	2.70	Bangalore
85	025	2	M	2.90	Bangalore
86	026	0	M	3.20	Bangalore
87	027	2	M	2.60	Bangalore
88	028	0	F	2.80	Bangalore
89	029	0	F	2.70	Bangalore
90	030	1	F	2.70	Bangalore
91	031	1	M	2.60	Bangalore
92	032	0	F	3.50	Bangalore
93	033	1	M	2.60	Bangalore
94	034	1	M	3.30	Bangalore
95	035	0	F	3.50	Bangalore
96	036	1	M	2.60	Bangalore
97	037	1	M	3.00	Bangalore
98	038	0	F	2.90	Bangalore
99	039	1	F	3.30	Bangalore
100	040	0	F	3.60	Bangalore

S. No.	Subject No.	Age (days)	Sex (Male-M / Female-F)	Weight (Kg)	Site
101	041	0	M	3.20	Bangalore
102	042	0	M	2.60	Bangalore
103	043	1	M	2.70	Bangalore
104	044	1	F	2.70	Bangalore
105	045	0	F	2.60	Bangalore
106	046	0	F	3.20	Bangalore
107	047	0	F	2.60	Bangalore
108	048	1	M	2.70	Bangalore
109	049	0	M	2.70	Bangalore
110	050	0	F	2.90	Bangalore
111	051	0	M	3.20	Bangalore
112	052	2	F	3.50	Bangalore
113	053	0	M	2.90	Bangalore
114	054	0	F	3.10	Bangalore
115	055	3	F	3.50	Bangalore
116	056	0	M	3.70	Bangalore
117	057	1	F	3.10	Bangalore
118	058	0	F	2.90	Bangalore
119	059	0	F	3.40	Bangalore
120	060	0	M	3.60	Bangalore

Demographic data: (Table-1)

S. No	Subject No.	Treatment	Size of the Papule (P) / Scar (S) / Ulcer (U)					
			30 minutes	72nd hours	Day 10	Day 30	Day 60	Day 90
01	001	T	Normal	2 mm (P)	2 mm (P)	3 mm (P)	3 mm (S)	4 mm (S)
02	002	T	Normal	1 mm (P)	1 mm (P)	2 mm (P)	2 mm (S)	3 mm (S)
03	003	R	Normal	1 mm (P)	2 mm (P)	2 mm (P)	4 mm (P)	4 mm (U)
04	004	R	Normal	NR	NR	3 mm (P)	NR	NR
05	005	T	Normal	1 mm (P)	2 mm (P)	2 mm (P)	3 mm (P)	3 mm (S)
06	006	R	Normal	No lesion	2 mm (P)	2 mm (P)	4 mm (S)	4 mm (S)
07	007	R	Normal	2 mm (P)	2 mm (P)	2 mm (P)	3 mm (P)	3 mm (U)
08	008	T	Normal	1 mm (P)	1 mm (P)	1 mm (P)	2 mm (S)	NR
09	009	R	Normal	1 mm (P)	1 mm (P)	1 mm (P)	3 mm (S)	4 mm (S)
10	010	T	Normal	Erythema	1 mm (P)	1 mm (P)	4 mm (P)	3 mm (S)
11	011	R	Normal	No lesion	1 mm (P)	1 mm (P)	2 mm (P)	4 mm (P)
12	012	T	Normal	1 mm (P)	2 mm (P)	2 mm (P)	3 mm (P)	3 mm (S)
13	013	R	Normal	1 mm (P)	1 mm (P)	1 mm (P)	4 mm (S)	5 mm (S)
14	014	T	Normal	NR	NR	2 mm (P)	4 mm (S)	5 mm (S)
15	015	T	Normal	No lesion	1 mm (P)	1 mm (P)	NR	4 mm (P)
16	016	R	Normal	1 mm (P)	2 mm (P)	2 mm (P)	4 mm (P)	3 mm (U)
17	017	R	Normal	No lesion	1 mm (P)	2 mm (P)	4 mm (S)	5 mm (S)

S. No	Subject No.	Treatment	Size of the Papule (P) / Scar (S) / Ulcer (U)					
			30 minutes	72nd hours	Day 10	Day 30	Day 60	Day 90
18	018	T	Normal	2 mm (P)	2 mm (P)	2 mm (P)	4 mm (S)	4 mm (S)
19	019	R	Normal	No lesion	1 mm (P)	2 mm (P)	3 mm (P)	2 mm (U)
20	020	T	Normal	1 mm (P)	1 mm (P)	2 mm (P)	3 mm (S)	3 mm (S)
21	021	T	Normal	2 mm (P)	2 mm (P)	3 mm (P)	4 mm (P)	3 mm (S)
22	022	T	Normal	1 mm (P)	2 mm (P)	2 mm (P)	3 mm (S)	4 mm (S)
23	023	R	Normal	1 mm (P)	2 mm (P)	2 mm (P)	4 mm (S)	4 mm (S)
24	024	R	Normal	Erythema	1 mm (P)	2 mm (P)	2 mm (P)	4 mm (S)
25	025	T	Normal	1 mm (P)	1 mm (P)	2 mm (P)	5 mm (P)	4 mm (S)
26	026	R	Normal	1 mm (P)	1 mm (P)	2 mm (P)	4 mm (P)	5 mm (S)
27	027	R	Normal	1 mm (P)	2 mm (P)	3 mm (P)	3 mm (S)	4 mm (S)
28	028	T	Normal	1 mm (P)	2 mm (P)	3 mm (P)	4 mm (S)	5 mm (S)
29	029	R	Normal	3 mm (P)	3 mm (P)	3 mm (P)	4 mm (S)	4 mm (S)
30	030	T	Normal	No lesion	1 mm (P)	2 mm (P)	3 mm (S)	3 mm (S)
31	031	R	Normal	1 mm (P)	1 mm (P)	1 mm (P)	3 mm (P)	3 mm (S)
32	032	T	Normal	1 mm (P)	1 mm (P)	3 mm (P)	4 mm (S)	4 mm (S)
33	033	R	Normal	No lesion	1 mm (P)	2 mm (P)	4 mm (P)	2 mm (U)
34	034	T	Normal	2 mm (P)	2 mm (P)	3 mm (P)	5 mm (P)	3 mm (S)
35	035	T	Normal	2 mm (P)	2 mm (P)	4 mm (P)	5 mm (S)	5 mm (S)
36	036	R	Normal	2 mm (P)	2 mm (P)	3 mm (P)	4 mm (S)	4 mm (S)

S. No	Subject No.	Treatment	Size of the Papule (P) / Scar (S) / Ulcer (U)					
			30 minutes	72nd hours	Day 10	Day 30	Day 60	Day 90
37	037	R	Normal	No lesion	1 mm (P)	2 mm (P)	3 mm (S)	4 mm (S)
38	038	T	Normal	1 mm (P)	2 mm (P)	3 mm (P)	3 mm (P)	4 mm (P)
39	039	R	Normal	1 mm (P)	1 mm (P)	2 mm (P)	4 mm (P)	2 mm (S)
40	040	T	Normal	2 mm (P)	2 mm (P)	3 mm (P)	4 mm (S)	4 mm (S)
41	041	T	Normal	2 mm (P)	2 mm (P)	2 mm (P)	3 mm (P)	3 mm (U)
42	042	T	Normal	1 mm (P)	1 mm (P)	2 mm (P)	4 mm (S)	4 mm (S)
43	043	R	Normal	2 mm (P)	2 mm (P)	4 mm (P)	5 mm (P)	3 mm (S)
44	044	R	Normal	1 mm (P)	1 mm (P)	2 mm (P)	3 mm (S)	4 mm (S)
45	045	T	Normal	2 mm (P)	2 mm (P)	3 mm (P)	2 mm (S)	3 mm (S)
46	046	R	Normal	1 mm (P)	2 mm (P)	3 mm (P)	4 mm (S)	4 mm (S)
47	047	R	Normal	2 mm (P)	2 mm (P)	2 mm (P)	4 mm (P)	3 mm (S)
48	048	T	Normal	1 mm (P)	1 mm (P)	1 mm (P)	3 mm (P)	3 mm (U)
49	049	R	Normal	No lesion	1 mm (P)	NR	NR	2 mm (U)
50	050	T	Normal	1 mm (P)	2 mm (P)	3 mm (P)	3 mm (P)	2 mm (U)
51	051	R	Normal	2 mm (P)	1 mm (P)	3 mm (P)	4 mm (P)	3 mm (S)
52	052	T	Normal	No lesion	1 mm (P)	2 mm (P)	4 mm (P)	3 mm (S)
53	053	R	Normal	1 mm (P)	1 mm (P)	3 mm (P)	5 mm (P)	3 mm (U)
54	054	T	Normal	1 mm (P)	1 mm (P)	2 mm (P)	3 mm (S)	4 mm (S)
55	055	T	Normal	2 mm (P)	2 mm (P)	3 mm (P)	4 mm (S)	4 mm (S)

S. No	Subject No.	Treatment	Size of the Papule (P) / Scar (S) / Ulcer (U)					
			30 minutes	72nd hours	Day 10	Day 30	Day 60	Day 90
56	056	R	Normal	1 mm (P)	2 mm (P)	2 mm (P)	4 mm (S)	5 mm (S)
57	057	R	Normal	2 mm (P)	2 mm (P)	2 mm (P)	2 mm (S)	2 mm (S)
58	058	T	Normal	1 mm (P)	2 mm (P)	2 mm (P)	4 mm (S)	4 mm (S)
59	059	T	Normal	2 mm (P)	2 mm (P)	3 mm (P)	4 mm (P)	3 mm (U)
60	060	R	Normal	2 mm (P)	2 mm (P)	1 mm (P)	3 mm (P)	3 mm (S)

Vaccination Site Observation data – Chennai site (Table-2)

NR – Not Reported

S. No.	Subject No.	Treatment	30 minutes	72nd hours	Day 10	Day 30	Day 60	Day 90
01	001	T	Normal	No lesion	NR	4 mm (P)	NR	2 mm (S)
02	002	T	Normal	1 mm (P)	2 mm (P)	4 mm (P)	2 mm (S)	3 mm (S)
03	003	R	Normal	1 mm (P)	1 mm (P)	3 mm (P)	2 mm (S)	2 mm (S)
04	004	T	Normal	4 mm (P)	2 mm (P)	No lesion	2 mm (S)	2 mm (S)
05	005	T	Normal	No lesion	3 mm (P)	3 mm (P)	3 mm (S)	3 mm (S)
06	006	R	Normal	No lesion	No lesion	No lesion	2 mm (S)	3 mm (S)
07	007	R	Normal	1 mm (P)	3 mm (P)	1 mm (P)	1 mm (S)	3 mm (S)
08	008	R	Normal	No lesion	2 mm (P)	2 mm (P)	2 mm (S)	3 mm (S)
09	009	R	Normal	2 mm (P)	3 mm (P)	4 mm (P)	4 mm (S)	4 mm (S)
10	010	T	Normal	No lesion	2 mm (P)	No lesion	2 mm (P)	5 mm (S)

S. No.	Subject No.	Treatment	30 minutes	72nd hours	Day 10	Day 30	Day 60	Day 90
11	011	R	Normal	No lesion	1 mm (P)	1 mm (P)	1 mm (P)	3 mm (S)
12	012	T	Normal	1 mm (P)	1 mm (P)	1 mm (P)	1 mm (P)	3 mm (S)
13	013	R	Normal	2 mm (P)	2 mm (P)	3 mm (P)	2 mm (U)	4 mm (S)
14	014	T	Normal	2 mm (P)	4 mm (P)	2 mm (P)	2 mm (U)	3 mm (S)
15	015	T	Normal	No lesion	3 mm (P)	4 mm (P)	4 mm (U)	2 mm (S)
16	016	R	Normal	No lesion	No lesion	No lesion	3 mm (P)	4 mm (S)
17	017	R	Normal	No lesion	No lesion	2 mm (P)	3 mm (U)	3 mm (S)
18	018	T	Normal	No lesion	No lesion	No lesion	No lesion	4 mm (S)
19	019	R	Normal	No lesion	No lesion	3 mm (P)	3 mm (S)	3 mm (S)
20	020	R	Normal	No lesion	No lesion	2 mm (P)	2 mm (P)	3 mm (S)
21	021	T	Normal	No lesion	No lesion	2 mm (P)	2 mm (U)	5 mm (S)
22	022	T	Normal	Erythema	1 mm (P)	3 mm (P)	3 mm (P)	3 mm (S)
23	023	R	Normal	No lesion	No lesion	2 mm (P)	2 mm (S)	3 mm (S)
24	024	T	Normal	1 mm (P)	1 mm (P)	2 mm (P)	1 mm (S)	3 mm (S)
25	025	R	Normal	No lesion	No lesion	1 mm (P)	1 mm (S)	2 mm (S)
26	026	T	Normal	2 mm (P)	2 mm (P)	3 mm (P)	3 mm (S)	5 mm (S)
27	027	R	Normal	1 mm (P)	1 mm (P)	3 mm (P)	2 mm (S)	4 mm (S)
28	028	T	Normal	2 mm (P)	3 mm (P)	3 mm (P)	3 mm (P)	5 mm (S)
29	029	R	Normal	1 mm (P)	1 mm (P)	2 mm (P)	3 mm (P)	4 mm (S)
30	030	T	Normal	2 mm (P)	2 mm (P)	1 mm (P)	2 mm (S)	5 mm (S)

S. No.	Subject No.	Treatment	30 minutes	72nd hours	Day 10	Day 30	Day 60	Day 90
31	031	T	Normal	2 mm (P)	2 mm (P)	2 mm (P)	2 mm (U)	4 mm (S)
32	032	T	Normal	2 mm (P)	2 mm (P)	3 mm (P)	2 mm (S)	4 mm (S)
33	033	R	Normal	1 mm (P)	1 mm (P)	3 mm (P)	3 mm (P)	5 mm (S)
34	034	T	Normal	No lesion	No lesion	No lesion	2 mm (S)	3 mm (S)
35	035	T	Normal	No lesion	No lesion	4 mm (P)	1 mm (S)	2 mm (S)
36	036	R	Normal	3 mm (P)	3 mm (P)	3 mm (P)	3 mm (U)	4 mm (S)
37	037	R	Normal	1 mm (P)	2 mm (P)	2 mm (P)	3 mm (S)	4 mm (S)
38	038	R	Normal	No lesion	No lesion	1 mm (P)	1 mm (S)	4 mm (S)
39	039	R	Normal	3 mm (P)	4 mm (P)	3 mm (P)	3 mm (S)	5 mm (S)
40	040	T	Normal	3 mm (P)	3 mm (P)	3 mm (P)	2 mm (U)	3 mm (S)
41	041	R	Normal	1 mm (P)	1 mm (P)	2 mm (P)	2 mm (U)	2 mm (S)
42	042	T	Normal	1 mm (P)	1 mm (P)	2 mm (P)	2 mm (U)	3 mm (S)
43	043	R	Normal	1 mm (P)	1 mm (P)	3 mm (P)	3 mm (S)	3 mm (S)
44	044	T	Normal	4 mm (P)	4 mm (P)	4 mm (P)	4 mm (P)	4 mm (S)
45	045	T	Normal	No lesion	1 mm (P)	2 mm (P)	2 mm (U)	2 mm (S)
46	046	R	Normal	3 mm (P)	3 mm (P)	2 mm (P)	2 mm (U)	4 mm (S)
47	047	R	Normal	3 mm (P)	3 mm (P)	NR	NR	NR
48	048	T	Normal	4 mm (P)	4 mm (P)	3 mm (P)	4 mm (S)	5 mm (S)
49	049	R	Normal	1 mm (P)	1 mm (P)	3 mm (P)	3 mm (S)	3 mm (S)
50	050	R	Normal	3 mm (P)	3 mm (P)	3 mm (U)	3 mm (S)	5 mm (S)

S. No.	Subject No.	Treatment	30 minutes	72nd hours	Day 10	Day 30	Day 60	Day 90
51	051	T	Normal	3 mm (P)	3 mm (P)	NR	NR	NR
52	052	T	Normal	4 mm (P)	4 mm (P)	4 mm (U)	5 mm (S)	5 mm (S)
53	053	T	Normal	3 mm (P)	3 mm (P)	1 mm (P)	1 mm (S)	2 mm (S)
54	054	T	Normal	No lesion	1 mm (P)	No lesion	3 mm (S)	3 mm (S)
55	055	R	Normal	No lesion	No lesion	No lesion	1 mm (U)	3 mm (S)
56	056	R	Normal	1 mm (P)	1 mm (P)	1 mm (P)	1 mm (S)	3 mm (S)
57	057	R	Normal	1 mm (P)	1 mm (P)	1 mm (P)	1 mm (S)	4 mm (S)
58	058	T	Normal	4 mm (P)	4 mm (U)	3 mm (U)	3 mm (U)	4 mm (S)
59	059	R	Normal	2 mm (P)	2 mm (P)	1 mm (U)	3 mm (S)	4 mm (S)
60	060	T	Normal	2 mm (P)	2 mm (P)	3 mm (P)	3 mm (U)	4 mm (S)

NR – Not Reported

Vaccination Site Observation data – Bangalore site (Table-3)

S. No.	Subject No.	Treatment	Measurement of indurations (mm)	Result (+ve/ -ve)
1.	001	T	--	--
2	002	T	13	+ve
3	003	R	18	+ve
4	004	R	--	--
5	005	T	16	+ve
6	006	R	19	+ve

S. No.	Subject No.	Treatment	Measurement of indurations (mm)	Result (+ve/ -ve)
7	007	R	20	+ve
8	008	T	--	--
9	009	R	15	+ve
10	010	T	17	+ve
11	011	R	8	+ve
12	012	T	22	+ve
13	013	R	19	+ve
14	014	T	14	+ve
15	015	T	19	+ve
16	016	R	20	+ve
17	017	R	18	+ve
18	018	T	18	+ve
19	019	R	18	+ve
20	020	T	18	+ve
21	021	T	17	+ve
22	022	T	19	+ve
23	023	R	17	+ve
24	024	R	16	+ve
25	025	T	16	+ve
26	026	R	19	+ve

S. No.	Subject No.	Treatment	Measurement of indurations (mm)	Result (+ve/ -ve)
27	027	R	18	+ve
28	028	T	15	+ve
29	029	R	20	+ve
30	030	T	19	+ve
31	031	R	19	+ve
32	032	T	18	+ve
33	033	R	7	-ve
34	034	T	18	+ve
35	035	T	17	+ve
36	036	R	20	+ve
37	037	R	21	+ve
38	038	T	17	+ve
39	039	R	19	+ve
40	040	T	22	+ve
41	041	T	20	+ve
42	042	T	18	+ve
43	043	R	17	+ve
44	044	R	15	+ve
45	045	T	16	+ve
46	046	R	16	+ve

S. No.	Subject No.	Treatment	Measurement of indurations (mm)	Result (+ve/ -ve)
47	047	R	19	+ve
48	048	T	7	-ve
49	049	R	13	+ve
50	050	T	--	--
51	051	R	20	+ve
52	052	T	15	+ve
53	053	R	19	+ve
54	054	T	17	+ve
55	055	T	22	+ve
56	056	R	17	+ve
57	057	R	18	+ve
58	058	T	20	+ve
59	059	T	15	+ve
60	060	R	16	+ve

Post vaccination Mantoux Test Observation data – Chennai site (Table-4)

S. No.	Subject No.	Treatment	Measurement of indurations (mm)	Result (+ve/ -ve)
1.	001	T	9	-ve
2	002	T	10	+ve
3	003	R	10	+ve

S. No.	Subject No.	Treatment	Measurement of indurations (mm)	Result (+ve/ -ve)
4	004	T	12	+ve
5	005	T	12	+ve
6	006	R	10	+ve
7	007	R	11	+ve
8	008	R	10	+ve
9	009	R	14	+ve
10	010	T	10	+ve
11	011	R	8	-ve
12	012	T	9	-ve
13	013	R	11	+ve
14	014	T	14	+ve
15	015	T	10	+ve
16	016	R	10	+ve
17	017	R	13	+ve
18	018	T	12	+ve
19	019	R	12	+ve
20	020	R	15	+ve
21	021	T	12	+ve
22	022	T	9	-ve
23	023	R	8	-ve

S. No.	Subject No.	Treatment	Measurement of indurations (mm)	Result (+ve/ -ve)
24	024	T	10	+ve
25	025	R	13	+ve
26	026	T	10	+ve
27	027	R	9	-ve
28	028	T	11	+ve
29	029	R	12	+ve
30	030	T	13	+ve
31	031	T	9	-ve
32	032	T	8	-ve
33	033	R	11	+ve
34	034	T	12	+ve
35	035	T	13	+ve
36	036	R	9	-ve
37	037	R	13	+ve
38	038	R	10	+ve
39	039	R	13	+ve
40	040	T	15	+ve
41	041	R	12	+ve
42	042	T	10	+ve
43	043	R	13	+ve

S. No.	Subject No.	Treatment	Measurement of indurations (mm)	Result (+ve/ -ve)
44	044	T	13	+ve
45	045	T	14	+ve
46	046	R	10	+ve
47	047	R	--	--
48	048	T	13	+ve
49	049	R	9	-ve
50	050	R	10	+ve
51	051	T	--	--
52	052	T	13	+ve
53	053	T	10	+ve
54	054	T	14	+ve
55	055	R	11	+ve
56	056	R	12	+ve
57	057	R	12	+ve
58	058	T	11	+ve
59	059	R	9	-ve
60	060	T	10	+ve

+ve – Positive -ve – Negative

Post vaccination Mantoux Test Observation data – Bangalore site (Table-5)

SUBJECT DISPOSITION
Disposition of the entire study population:

72 hrs data was available for 118 subjects, as 2 subjects, one subject each in Test group (Subject No. 014) and Reference group (Subject No. 004) did not turn up for the visit. Both the defaulters were from the Chennai site.

10th day data was available for 117 subjects, as 3 subjects, two subjects in Test (subject no. 014 in Chennai & subject no. 001 in Bangalore) and one in Reference (Subject no. 004 in Chennai) did not turn up for the visit.

30th day data was available for 117 subjects, as 3 subjects, one subject in Test (subject no. 051 in Bangalore) and two in Reference (Subject no. 049 in Chennai and Subject No. 047 in Bangalore) did not turn up for the visit.

60th day data was available for 114 subjects, as 6 subjects, 3 subjects in Test (subject no. 015 in Chennai and Subject no. 001 & 051 in Bangalore) and 3 in Reference (Subject no. 004 & 049 in Chennai and Subject No. 047 in Bangalore) did not turn up for the visit.

90th day data was available for 116 subjects, as 4 subjects, 2 subjects in Test (subject no. 008 in Chennai and Subject no. 051 in Bangalore) and 2 in Reference (Subject no. 004 in Chennai and Subject No. 047 in Bangalore) did not turn up for the visit.

The 116 subjects reported on day 90 were given Mantoux injection (In Chennai site – Subject Nos. 004, 008 and in Bangalore site – Subject Nos. 047, 051 did not report on day 90 and were not given Mantoux injection). 2 subjects did not visit for the assessment of Mantoux response on day 93. Hence the data was collected from 114 subjects (In Chennai site – Subject Nos. 001 & 050 did not report on day 93 for Mantoux assessment).

26th week data was available for 110 subjects. Subject Nos 001, 004, 008, 019, 033 & 050 in Chennai site and 012, 034, 047 & 051 in Bangalore site did not turn up for the visit.

Disposition of the study population in Chennai site
At 30 minutes, the data was available for all 60 subjects vaccinated.

72 hrs data was available for 58 subjects, as 2 subjects, one subject each in Test group (Subject No. 014) and Reference group (Subject No. 004) did not turn up for the visit.

10th day data was available for 58 subjects, as 2 subjects, one subject each in Test group (subject no. 014) and Reference group (Subject no. 004) did not turn up for the visit.

30th day data was available for 59 subjects, as Subject no. 049 did not turn up for the visit.

60th day data was available for 57 subjects, as 3 subjects, 1 subject in Test (subject no. 015) and 2 in Reference (Subject no. 004 & 049) did not turn up for the visit.

90th day data was available for 58 subjects, as 2 subjects, one subject each in Test (subject no. 008) and Reference (Subject no. 004) did not turn up for the visit. These 58 subjects were given Mantoux injection. In this 2 subjects (subject Nos. 001 & 050) did not visit for the assessment of Mantoux response on day 93. Hence the data was collected from 56 subjects.

26th week data was available for 54 subjects. Subject Nos 001, 004, 008, 019, 033 & 050 did not turn up for the visit.

Disposition of the study population in Bangalore site
At 30 minutes, the data was available for all 60 subjects vaccinated.

72 hrs data was available for all 60 subjects.

10th day data was available for 59 subjects, as subject no. 001 in Test group did not turn up for the visit.

30th day data was available for 58 subjects, as 2 subjects, one subject each in Test group (subject no. 051) and Reference group (Subject No. 047) did not turn up for the visit.

60th day data was available for 57 subjects, as 3 subjects, 2 subjects in Test group (Subject nos. 001 & 051) and 1 in Reference (Subject No. 047) did not turn up for the visit.

90th day data was available for 58 subjects, as 2 subjects, one subject each in Test group (Subject no. 051) and Reference (Subject No. 047) did not turn up for the visit. These 58 subjects were given Mantoux injection. All the 58 subjects visited for the assessment of Mantoux response on day 93. Hence the data of all 58 subjects were collected.

26th week data was available for 56 subjects. Subject Nos 012, 034, 047 & 051 did not turn up for the visit.

Demographic and other Baseline Characteristics:
Demographic profile such as Date of birth, Age, Sex, Weight, Race were obtained from the study subjects and were documented.

The data of all one hundred and twenty subjects were included for statistical analysis of the demographic profile. The total number of subjects was divided into Test and Reference groups that contained 60 subjects each.

Among the 60 subjects in the Test group, 30 were females and the remaining 30 were males. In the Reference group, 25 subjects (41.7%) were females and 35 (58.3%) were males.

The mean age in the Test group was 0.67 days ± 0.91 SD and in Reference group it was 0.75 days ± 0.86 SD. This indicates that most of the subjects were vaccinated on the day of birth (0 day).

Regarding the weight of the subjects, the mean weight was 3.09 kg ± 0.40 SD and 3.03 kg ± 0.34 SD in Reference and Test groups respectively.

All the subjects recruited were Indians by Racial origin.

SAFETY, EFFICACY/ REACTOGENECITY:

Events that happened during the study - Table 6.

Procedure	Screening	Vaccination	Observation after vaccination							
			30 Minutes	72 hrs	10th day	30th day	60th day	90th day	93rd day	26th week
Informed consent for screening and study	X									
Demography, Personal history, family history	X									
Physical examination	X		X	X	X	X	X	X		X
Serology	X									
Vaccine administration		X								
Mantoux test								X		
Adverse events			X	X	X	X	X	X		X
Vaccination site observation			X	X	X	X	X	X		X

1) Analysis of efficacy/reactogenicity:

The response for the Mantoux test performed on day 90 was assessed after 72 hrs on day 93. The horizontal measurement of the induration in millimetres was taken into consideration and if the measurement was more than/equal to 10 mm, it was regarded as positive reaction indicating good immune response or reactogenicity. Among 120 children vaccinated, 116 were given Mantoux injection (In Chennai site – Subject Nos. 004, 008 and in Bangalore site – Subject Nos. 047, 051 did not report on day 90 and were not given Mantoux injection). 2 subjects did not visit for the assessment of Mantoux response on day 93. Hence the data was collected from 114 subjects (In Chennai site – Subject Nos. 001 & 050 did not report on day 93 for Mantoux assessment).

Among the 114 children who were assessed for the Mantoux response on day 93, 100 children had the induration that was more than / equal to 10 mm. 6 subjects in the Test vaccine group and 8 subjects in the Reference group had the induration that was less than 10 mm.

The following subjects who were vaccinated with Test vaccine had less than 10 mm induration. In Chennai site – subject Nos 048 (7 mm) and in Bangalore site – Subject Nos. 001 (9 mm), 012 (9 mm), 022 (9 mm), 031 (9 mm) and 032 (8 mm).

The following subjects who were vaccinated with Reference vaccine had less than 10 mm induration. In Chennai site – subject Nos 011 (8 mm) and 033 (7 mm) and in Bangalore site – Subject Nos. 011 (8 mm), 023 (8 mm), 027 (9 mm), 036 (9 mm), 049 (9 mm) and 059 (9 mm).

The reaction at vaccination site was assessed at 30 minutes, 72 hours, 10th day, 30th day, 60th day, 90th day and on 26th week.

At 30 minutes, the vaccination site was normal for all the subjects.

72 hrs data was available for 118 subjects, as 2 subjects, one subject each in Test group (Subject No. 014) and Reference group (Subject No. 004) did not turn up for the visit. Both the defaulters were from the Chennai site. In the 72 hrs assessment, 3 subjects had erythema, 31 subjects had no lesions and 84 subjects had (P)s.

10th day data was available for 117 subjects, as 3 subjects, two subjects in Test (subject no. 014 in Chennai & subject no. 001 in Bangalore) and one in Reference (Subject no. 004 in Chennai) did not turn up for the visit. In this assessment, 13 subjects had no lesions, one subject had ulcer and the remaining 103 subjects had (P)s.

30th day data was available for 117 subjects, as 3 subjects, one subject in Test (subject no. 051 in Bangalore) and two in Reference (Subject no. 049 in Chennai and Subject No. 047 in Bangalore) did not turn up for the visit. In this assessment, 8 subjects had no lesions, 4 subjects had ulcer and the remaining 105 subjects had (P)s.

60th day data was available for 114 subjects, as 6 subjects, 3 subjects in Test (subject no. 015 in Chennai and Subject no. 001 & 051 in Bangalore) and 3 in Reference (Subject no. 004 & 049 in Chennai and Subject No. 047 in Bangalore) did not turn up for the visit. In this assessment, 1 subject had no lesion, 61 subjects had (S), 15 subjects had ulcer and the remaining 37 subjects had (P)s.

90th day data was available for 116 subjects, as 4 subjects, 2 subjects in Test (subject no. 008 in Chennai and Subject no. 051 in Bangalore) and 2 in Reference (Subject no. 004 in Chennai and Subject No. 047 in Bangalore) did not turn up for the visit. In this assessment, 11 subjects had ulcer, 102 subjects had (S), and the remaining 3 subjects had (P)s.

26th week data was available for 110 subjects. Subject Nos 001, 004, 008, 019, 033 & 050 in Chennai site and 012, 034, 047 & 051 in Bangalore site did not turn up for the visit. All the subjects who visited for this follow up were having (S) at the site of vaccination.

Multicenter Studies

In this study of 120 children, sixty were enrolled from Bangalore site and other sixty from Chennai site. The data and analysis discussed are for the entire 120 subjects for the two sites are discussed here.

Chennai site

Among 60 children vaccinated, 58 were given Mantoux injection, as subject Nos. 004 and 008 did not report on day 90 and were not given Mantoux injection. 2 subjects (subject Nos. 001 & 050) did not visit for the assessment of Mantoux response on day 93. Hence the data was collected from 56 subjects.

Among the 56 children who were assessed for the Mantoux response on day 93, 53 children had the induration that was more than / equal to 10 mm. One subject in the vaccinated group using Test vaccine and 2 subjects in the Reference group had the induration less than 10 mm. Subject No. 048 vaccinated with Test vaccine had the induration of 7 mm and subjects nos. 011 and 033 had 8 mm & 7 mm respectively.

The Mantoux test reaction of 56 subjects was included for the statistical analysis. The mean induration induced by the Reference product was 17.28 mm ± 3.27 SD and for Test product 17.22 mm ± 3.09 S.D. The difference in the response of Test & Reference products was analyzed for statistical significance using t test. The analysis yielded the p value of 0.95, indicating there was no significant difference between the Test and Reference products.

The reaction at vaccination site was assessed at 30 minutes, 72 hours, 10th day, 30th day, 60th day, 90th day and on 26th week.

At 30 minutes, the vaccination site was normal for all the subjects.

72 hrs data was available for 58 subjects, as 2 subjects, one subject each in Test group (Subject No. 014) and Reference group (Subject No. 004) did not turn up for the visit. In the 72 hrs assessment, 2 subjects had erythema, 10 subjects had no lesions and 46 subjects had (P)s.

10th day data was available for 58 subjects, as 2 subjects, one subject each in Test group (subject no. 014) and Reference group (Subject no. 004) did not turn up for the visit. In this assessment, all the 58 subjects had (P)s.

30th day data was available for 59 subjects, as Subject no. 049 (Reference vaccine) did not turn up for the visit. In this assessment, all the 59 subjects had (P)s.

60th day data was available for 57 subjects, as 3 subjects, 1 subject in Test (subject no. 015) and 2 in Reference (Subject no. 004 & 049) did not turn up for the visit. In this assessment, 30 subjects had (S) and the remaining 27 subjects had (P)s.

90th day data was available for 58 subjects, as 2 subjects, one subject each in Test (subject no. 008) and Reference (Subject no. 004) did not turn up for the visit. In this assessment, 11 subjects had ulcer, 44 subjects had (S), and the remaining 3 subjects had (P)s.

26th week data was available for 54 subjects. Subject Nos 001, 004, 008, 019, 033 & 050 did not turn up for the visit. All the subjects who visited for this follow up were having (S) at the site of vaccination.

The transverse measurements of (P), ulcer and (S) in millimetres were analysed for Test and Reference products.

The mean size of (P) observed in the subjects given Test vaccine was 1.40 mm on 72 hour, 1.59mm on day 10, 2.30mm on day 30 & 3.67mm on day 60 and in the subjects given Reference vaccine was 1.43mm on 72 hours, 1.52mm on day 10, 2.14mm on day 30 & 3.60mm on day 60. Analysis was done using t test for assessing the significance of the difference between Test and Reference products that indicated the p value of 0.86 (for 72 hours), 0.63 (for day 10), 0.41 (for day 30) and 0.84 (for day 60), which implies that there was no significant variation between the Test and Reference vaccines.

The mean size of (S) observed in the subjects given Test vaccine was 3.41 mm on day 60 and 3.70 mm on day 90 and in the subjects given Reference vaccine it was 3.54 mm on day 60 and 3.76mm on day 90. Analysis was done using t test for assessing the significance of the difference between Test and Reference products that indicated the p value of 0.65 (for 60 day) and 0.79 (for day 90), which implies that there was no significant variation between the Test and Reference vaccines.

The mean size of ulcer observed in the subjects given Test vaccine was 2.75 mm on day 90 and in the subjects given Reference vaccine it was 2.71 mm on day 90. Analysis was done using t test for assessing the significance of the difference between Test and Reference products that indicated the p value of 0.93 (for day 90), which implies that there was no significant variation between the Test and Reference vaccines.

Bangalore Site

Among 60 children vaccinated, 58 were given Mantoux injection, as Subject Nos. 047 and 051 did not report on day 90 and were not given Mantoux injection. All the 58 subjects visited for the assessment of Mantoux response on day 93. Hence the data of all 58 subjects were collected.

Among the 58 children who were assessed for the Mantoux response on day 93, 47 children had the induration that was more than / equal to 10 mm. 5 subjects in the Test vaccine group and 6 subjects in the Reference group had the induration of less than 10 mm.

Subject nos 001 (9 mm), 012 (9 mm), 022 (9 mm), 031 (9 mm) and 032 (8 mm) who were vaccinated with Test vaccine had the induration less than 10 mm. Similarly, Subject Nos. 011 (8 mm), 023 (8 mm), 027 (9 mm), 036 (9 mm), 049 (9 mm) and 059 (9 mm) who were vaccinated with Reference vaccine also had the induration less than 10 mm.

The Mantoux test reaction of 58 subjects was included for the statistical analysis. The mean induration induced by the Reference product was 11.03 mm ± 1.80 SD and for Test product 11.31 mm ± 1.87 S.D. The difference in the response of Test & Reference products was analyzed for statistical significance using t test. The analysis yielded the p value of 0.57, indicating there was no significant difference between the Test and Reference products in the Mantoux test reaction. The reaction at vaccination site was assessed at 30 minutes, 72 hours, 10th day, 30th day, 60th day, 90th day and on 26th week.

At 30 minutes, the vaccination site was normal for all the subjects. 72 hrs data was available for all 60 subjects.

In the 72 hrs assessment, 1 subject had erythema, 21 subjects had no lesions and the remaining 38 subjects had (P)s.

10th day data was available for 59 subjects, as subject no. 001 in Test group did not turn up for the visit. In this assessment, 13 subjects had no lesions, one subject had ulcer and the remaining 45 subjects had (P)s.

30th day data was available for 58 subjects, as 2 subjects, one subject each in Test group (subject no. 051) and Reference group (Subject No. 047) did not turn up for the visit. In this assessment, 8 subjects had no lesions, 4 subjects had ulcer and the remaining 46 subjects had (P)s.

60th day data was available for 57 subjects, as 3 subjects, 2 subjects in Test group (Subject nos. 001 & 051) and 1 in Reference (Subject No. 047) did not turn up for the visit. In this assessment, 1 subject had no lesion, 31 subjects had (S), 15 subjects had ulcer and the remaining 10 subjects had (P)s.

90th day data was available for 58 subjects, as 2 subjects, one subject each in Test group (Subject no. 051) and Reference (Subject No. 047) did not turn up for the visit. In this assessment, all the 58 subjects had (S).

26th week data was available for 56 subjects. Subject Nos 012, 034, 047 & 051 did not turn up for the visit. All the subjects who visited for this follow up were having (S) at the site of vaccination.

The transverse measurements of (P), ulcer and (S) in millimetres were analysed for Test and Reference products.

The mean size of (P) observed in the subjects given Test vaccine was 2.47 mm at 72 hours, 2.33mm on day 10, 2.68mm on day 30 & 2.60mm on day 60 and in the subjects given Reference vaccine it was 1.68mm on 72 hours, 1.90mm on day 10, 2.21mm on day 30 and 2.40mm on day 60. Analysis was done using t test for assessing the significance of the difference between Test and Reference products that indicated the p value of 0.02 (for 72 hours), 0.17 (for day 10), 0.10 (for day 30) and 0.77 (for day 60). Except for the 72 hours there was no statistically significant difference between the Test and Reference product.

The mean size of (S) observed in the subjects given Test vaccine was 2.38 mm on day 60 and 3.48 mm on day 90 and in the subjects given Reference vaccine was 2.22mm on day 60 and 3.48mm on day 90. Analysis was done using t test for assessing the significance of the difference between Test and Reference products that indicated the p value of 0.69 (for 60 day) and 1.0 (for day 90), which implies that there was no significant variation between the Test and Reference vaccines.

The mean size of ulcer observed in the subjects given Test vaccine was 4.00 mm on day 10, 3.50 mm on day 30 and 2.44 mm on day 60 and in the subjects given Reference vaccine was 2.00 mm on day 30 and 2.17 mm on day 60. Analysis was done using t test for assessing the significance of the difference between Test and Reference products that indicated the p value of 0.35 (for day 30) and 0.49 (for day 60), which implies that there was no significant variation between the Test and Reference vaccines.

STATISTICAL ANALYSIS:

Statistical plan

The statistical analysis was done using SAS, version 9.2.

The data of the subjects whoever reported for the follow up visits were taken for analysis. No data was excluded.

Post vaccination Mantoux test results were analyzed. Summary statistics of the size of transverse indurations induced by Test vaccine and Reference vaccine were done and comparison was done using t test.

The size of the (P), ulcer and (S) were considered for statistical analysis. Summary statistics of the data was done for Test and Reference vaccines and comparison was done using t test.

Demographic details such as age, sex and weight were analysed for summary statistics. Comparison of age was done using Wilcoxon Rank sum test, sex was done using Chi square test and weight was done using one way ANOVA.

ANALYSIS OF DEMOGRAPHIC DATA

Analysis of the entire study population (120 subjects)

The data of all one hundred and twenty subjects were included for statistical analysis of the demographic profile. The summary statistics of the demographic profile is given below.

Age (days)			
	Reference	**Test**	**Both Test and Reference**
N	60	60	120
Mean	0.75	0.67	0.71
Standard Deviation	0.86	0.91	0.88
Minimum	0.0	0.0	0.0
Maximum	3.0	3.0	3.0

Age (days)			
	Reference	**Test**	**Both Test and Reference**
Sex			
F	25 (41.7%)	30 (50.0%)	55 (45.83%)
M	35 (58.3%)	30 (50.0%)	65 (54.17%)
Weight (Kgs)			
N	60	60	120
Mean	3.09	3.03	3.06
Standard Deviation	0.40	0.34	0.37
Minimum	2.6	2.6	2.6
Maximum	4.0	3.8	4.0
Race			
All the subjects enrolled were Indians by race			

Table 7:

The difference between the mean data of Test and Reference groups was compared for statistical significance. Comparison of age was done using Wilcoxon Rank sum test, of sex was done using Chi square test, weight was done using one way ANOVA. The p value for age is 0.40, for sex 0.35 and for weight it is 0.37.

Analysis of the demographic data of Chennai site

The data of all 60 subjects were included for statistical analysis of the demographic profile. The summary statistics of the demographic profile is given below.

Age (days)			
	Reference	**Test**	**Both Test and Reference**
N	30	30	60
Mean	0.83	0.90	0.86
Standard Deviation	0.91	1.09	0.99
Minimum	0.0	0.0	0.0

Age (days)			
	Reference	**Test**	**Both Test and Reference**
Maximum	3.0	3.0	3.0
Sex			
F	12 (40.0%)	16 (53.3%)	28 (46.67%)
M	18 (60.0%)	14 (46.7%)	32 (53.33%)
Weight (Kgs)			
N	30	30	60
Mean	3.12	3.01	3.06
Standard Deviation	0.44	0.31	0.38
Minimum	2.6	2.6	2.6
Maximum	4.0	3.6	4.0
Race			
All the subjects enrolled were Indians by race			

Table 8: Summary statistics of demographic data - Chennai site

The difference between the mean data of Test and Reference groups was compared for statistical significance. Comparison of age was done using Wilcoxon Rank sum test, sex was done using Chi square test and weight was done using one way ANOVA. The p value for age is 0.97, for sex 0.30 and for weight it is 0.23.

Analysis of the demographic data of Bangalore site

The data of all 60 subjects were included for statistical analysis of the demographic profile. The summary statistics of the demographic profile is given below.

Age (days)			
	Reference	**Test**	**Both Test and Reference**
N	30	30	60
Mean	0.67	0.43	0.55
Standard Deviation	0.80	0.63	0.72
Minimum	0.0	0.0	0.0
Maximum	3.0	2.0	3.0
Sex			
F	13 (43.3%)	14 (46.7%)	27 (45%)
M	17 (56.7%)	16 (53.3%)	33 (55%)
Weight (Kgs)			
N	30	30	60
Mean	3.05	3.05	3.05
Standard Deviation	0.35	0.38	0.36
Minimum	2.6	2.6	2.6
Maximum	3.7	3.8	3.7
Race			
All the subjects enrolled were Indians by race			

Table 9: Summary statistics of demographic data – Bangalore site

The difference between the mean data of Test and Reference groups was compared for statistical significance. Comparison of age was done using Wilcoxon Rank sum test, sex was done using Chi square test and weight was done using one way ANOVA. The p value for age is 0.26, for sex 0.80 and for weight it is 0.97.

Analysis of Post vaccination Mantoux test results

Analysis of the study population in both the sites:

Among the 114 children who were assessed for the Mantoux response on day 93, 100 children had the induration that was more than / equal to 10 mm. 6 subjects in the Test vaccine group and 8 subjects in the Reference group had the induration less than 10 mm.

The following subjects who were vaccinated with Test vaccine had less than 10 mm induration. In Chennai site – subject Nos 048 (7 mm) and in Bangalore site – Subject Nos. 001 (9 mm), 012 (9 mm), 022 (9 mm), 031 (9 mm) and 032 (8 mm).

The following subjects who were vaccinated with Reference vaccine had less than 10 mm induration. In Chennai site – subject Nos 011 (8 mm) and 033 (7 mm) and in Bangalore site – Subject Nos. 011 (8 mm), 023 (8 mm), 027 (9 mm), 036 (9 mm), 049 (9 mm) and 059 (9 mm).

The summary statistics of the induration caused by the Test and Reference vaccines is given below.

S. No.	Vaccine	No. of subjects	Mean induration (mm)	SD (mm)	Minimum (mm)	Maximum (mm)
1	R	58	14.16	4.09	7	21
2	T	56	14.16	3.90	7	22

Table 10: Summary statistics of post vaccination Mantoux results

The difference in the response of Test & Reference products was analyzed for statistical significance using t test. The analysis yielded the p value of 0.99.

Analysis of the Mantoux response in Chennai site

Among the 56 children who were assessed for the Mantoux response on day 93, 53 children had the induration that was more than / equal to 10 mm. One subject in the Test vaccine group and 2 subjects in the Reference group had the induration less than 10 mm. Subject No. 048 vaccinated with Test vaccine had the induration of 8 mm and subjects nos. 011 and 033 had 8 mm & 7 mm respectively.

The summary statistics of the induration caused by the Test and Reference vaccines is given below.

S. No.	Vaccine	No. of subjects	Mean induration (mm)	SD (mm)	Minimum (mm)	Maximum (mm)
1	R	29	17.28	3.27	7	21
2	T	27	17.22	3.09	7	22

Table 11: Summary statistics of post vaccination Mantoux results – Chennai site

The difference in the response of Test & Reference products was analyzed for statistical significance using t test. The analysis yielded the p value of 0.95.

Analysis of the Mantoux response in Bangalore site

Among the 58 children who were assessed for the Mantoux response on day 93, 47 children had the induration that was more than / equal to 10 mm. 5 subjects in the Test vaccine group and 6 subjects in the Reference group had the induration less than 10 mm.

Subject nos 001 (9 mm), 012 (9 mm), 022 (9 mm), 031 (9 mm) and 032 (8 mm) who were vaccinated with Test vaccine had the induration less than 10 mm. Similarly, subject Nos. Subject Nos. 011 (8 mm), 023 (8 mm), 027 (9 mm), 036 (9 mm), 049 (9 mm) and 059 (9 mm) who were vaccinated with Reference vaccine also had the induration less than 10 mm.

The summary statistics of the induration caused by the Test and Reference vaccines is given below.

S. No.	Vaccine	No. of subjects	Mean induration (mm)	SD (mm)	Minimum (mm)	Maximum (mm)
1	R	29	11.03	1.80	8	15
2	T	29	11.31	1.87	8	15

Table 12: Summary statistics of post vaccination Mantoux results – Bangalore site

The difference in the response of Test & Reference products was analyzed for statistical significance using t test. The analysis yielded the p value of 0.57.

The analysis of vaccination sites for the type of reaction at intervals studied in both the sites (figure 1 to 5):

At 30 minutes, the vaccination site was normal for all 120 subjects. 72 hrs data was available for 118 subjects. In this, 3 subjects had erythema, 31 subjects had no lesions and 84 subjects had (P)s.

10th day data was available for 117 subjects. In this assessment, 13 subjects had no lesions, one subject had ulcer and the remaining 103 subjects had (P)s.

30th day data was available for 117 subjects, and among them, 8 subjects had no lesions, 4 subjects had ulcer and the remaining 105 subjects had (P)s.

60th day data was available for 114 subjects and in this, 1 subject had no lesion, 61 subjects had (S), 15 subjects had ulcer and the remaining 37 subjects had (P)s.

90th day data was available for 116 subjects and here 11 subjects had ulcer, 102 subjects had (S), and the remaining 3 subjects had (P)s.

26th week data was available for 110 subjects and all of them had (S) at the site of vaccination.

The transverse measurements of (P), ulcer and (S) in millimetres were analysed for Test and Reference products.

The mean size of (P) observed in the subjects given Test vaccine was 1.86 mm on 72nd hour, 1.92mm on day 10, 2.46mm on day 30, 3.35mm on day 60 & 4.00 mm day 90 and in the subjects given Reference vaccine it was 1.55mm on 72nd hours, 1.68mm on day 10, 2.17mm on day 30, 3.30mm on day 60 & 4.00mm on day 90. Analysis was done using t test for assessing the significance of the difference between Test and Reference products that indicated the p value of 0.10 (for 72nd hours), 0.14 (for day 10), 0.08 (for day 30) and 0.88 (for day 60).

The mean size of (S) observed in the subjects given Test vaccine was 2.97 mm on day 60 and 3.58 mm on day 90 and in the subjects given Reference vaccine it was 2.77mm on day 60 and 3.60mm on day 90. Analysis was done using t test for assessing the significance of the difference between Test and Reference products that indicated the p value of 0.49 (for 60 day) and 0.90 (for day 90).

The mean size of ulcer observed in the subjects given Test vaccine was 4.00 mm on day 10, 3.50 mm on day 30, 2.44 mm on day 60 & 2.75 mm on day 90 and in the subjects given Reference vaccine it was 2.00 mm on day 30, 2.17 mm on day 60 & 2.71 mm on day 90. Analysis was done using t test for assessing the significance of the difference between Test and Reference products that indicated the p value of 0.35 (for day 30), 0.49 (for day 60) and 0.93 (for day 90).

Analysis of reaction at vaccination site in Chennai site (Figure 6 to 10):
At 30 minutes, the vaccination site was normal for 60 subjects.

72 hrs data was available for 58 subjects. In this, 2 subjects had erythema, 10 subjects had no lesions and 46 subjects had (P)s.

10^{th} day data was available for 58 subjects. In this assessment, all the 58 subjects had (P)s.

30^{th} day data was available for 59 subjects and in this assessment, all the 59 subjects had (P)s.

60^{th} day data was available for 57 subjects and in this, 30 subjects had (S) and the remaining 27 subjects had (P)s.

90^{th} day data was available for 58 subjects and here 11 subjects had ulcer, 44 subjects had (S), and the remaining 3 subjects had (P)s.

26^{th} week data was available for 54 subjects and all of them had (S) at the site of vaccination.

The mean size of (P) observed in the subjects given Test vaccine was 1.40 mm on 72^{nd} hour, 1.59mm on day 10, 2.30mm on day 30 & 3.67mm on day 60 and in the subjects given Reference vaccine was 1.43mm on 72^{nd} hours, 1.52mm on day 10, 2.14mm on day 30 & 3.60mm on day 60. Analysis was done using t test for assessing the significance of the difference between Test and Reference products that indicated the p value of 0.86 (for 72^{nd} hours), 0.63 (for day 10), 0.41 (for day 30) and 0.84 (for day 60).

The mean size of (S) observed in the subjects given Test vaccine was 3.41 mm on day 60 and 3.70 mm on day 90 and in the subjects given Reference vaccine it was 3.54 mm on day 60 and 3.76mm on day 90. Analysis was done using t test for assessing the significance of the difference between Test and Reference products that indicated the p value of 0.65 (for 60 day) and 0.79 (for day 90).

The mean size of ulcer observed in the subjects given Test vaccine was 2.75 mm on day 90 and in the subjects given Reference vaccine it was 2.71 mm on day 90. Analysis was done using t test for assessing the significance of the difference between Test and Reference products that indicated the p value of 0.93 (for day 90).

Analysis of reaction at vaccination site in Bangalore site (Figure 11 to 15):
At 30 minutes, the vaccination site was normal for all 60 subjects.

72 hrs data was available for all 60 subjects. In this, 1 subject had erythema, 21 subjects had no lesions and the remaining 38 subjects had (P)s.

10th day data was available for 59 subjects. In this assessment, 13 subjects had no lesions, one subject had ulcer and the remaining 45 subjects had (P)s.

30th day data was available for 58 subjects and in this assessment, 8 subjects had no lesions, 4 subjects had ulcer and the remaining 46 subjects had (P)s.

60th day data was available for 57 subjects and in this 1 subject had no lesion, 31 subjects had (S), 15 subjects had ulcer and the remaining 10 subjects had (P)s.

90th day data was available for 58 subjects and here all the 58 subjects had (S).

26th week data was available for 56 subjects and all of them had (S) at the site of vaccination

The mean size of (P) observed in the subjects given Test vaccine was 2.47 mm on 72nd hour, 2.33mm on day 10, 2.68mm on day 30 & 2.60mm on day 60 and in the subjects given Reference vaccine it was 1.68mm on 72nd hours, 1.90mm on day 10, 2.21mm on day 30 and 2.40mm on day 60. Analysis was done using t test for assessing the significance of the difference between Test and Reference products that indicated the p value of 0.02 (for 72nd hours), 0.17 (for day 10), 0.10 (for day 30) and 0.77 (for day 60).

The mean size of (S) observed in the subjects given Test vaccine was 2.38 mm on day 60 and 3.48 mm on day 90 and in the subjects given Reference vaccine was 2.22mm on day 60 and 3.48mm on day 90. Analysis was done using t test for assessing the significance of the difference between Test and Reference products that indicated the p value of 0.69 (for 60 day) and 0.10 (for day 90). The mean size of ulcer observed in the subjects given Test vaccine was 4.00 mm on day 10, 3.50 mm on day 30 and 2.44 mm on day 60 and in the subjects given Reference vaccine was 2.00 mm on day 30 and 2.17 mm on day 60. Analysis was done using t test for assessing the significance of the difference between Test and Reference products that indicated the p value of 0.35 (for day 30) and 0.49 (for day 60).

Charts for the Reactions at vaccination site of the entire study population in both the sites

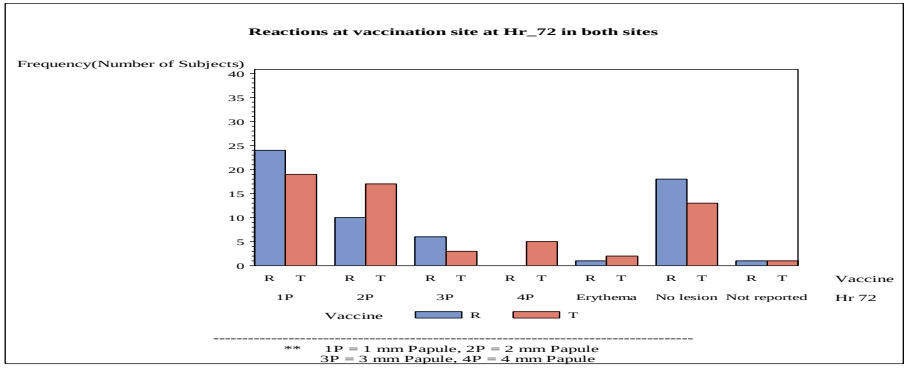


FIGURE 1

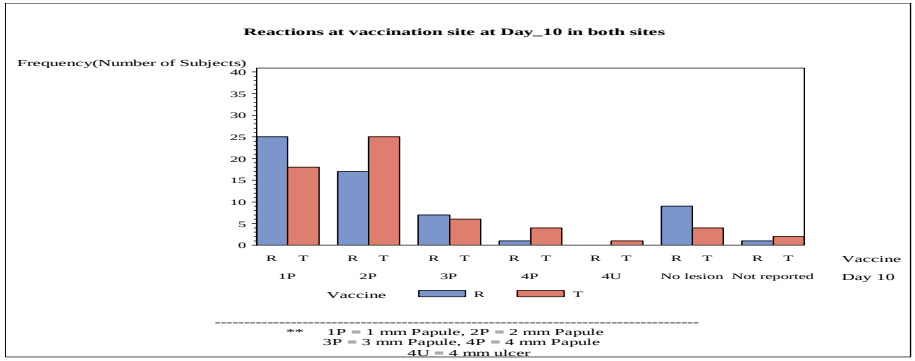
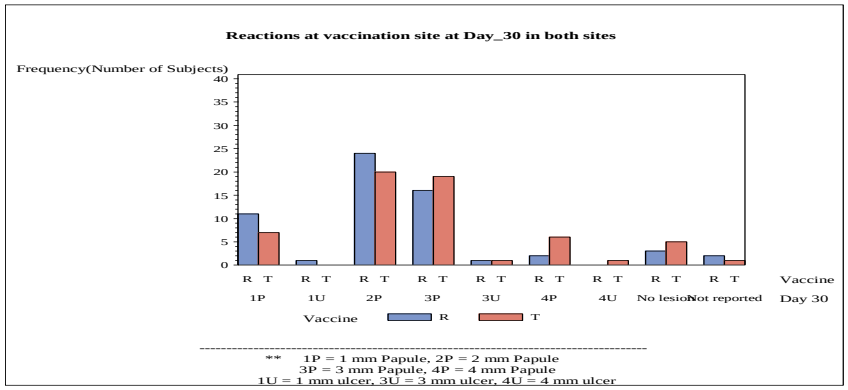


FIGURE 2



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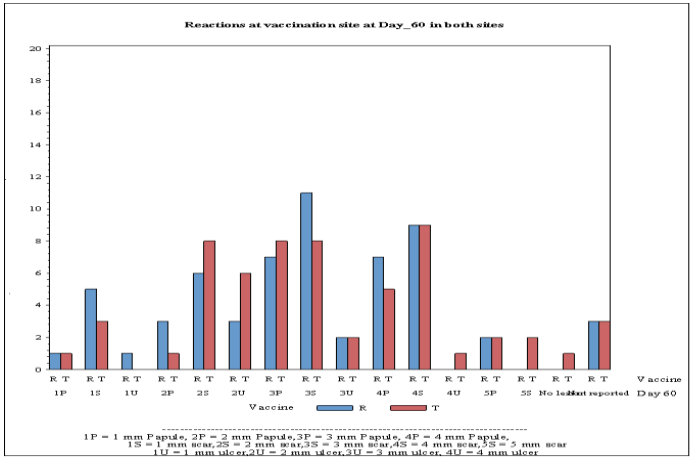

FIGURE 4

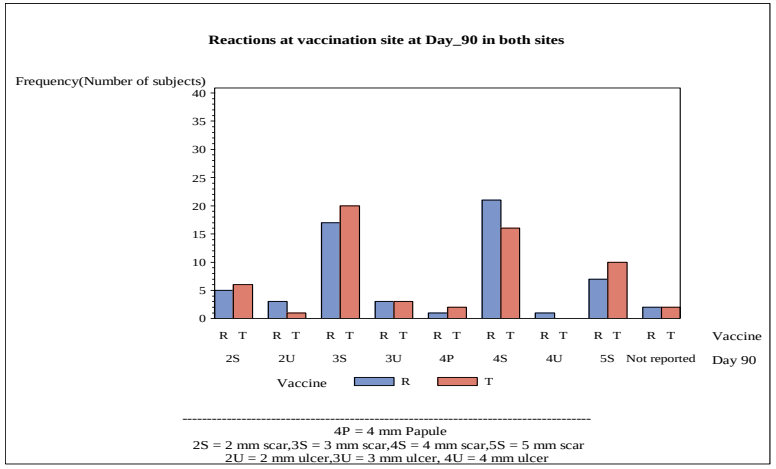

FIGURE 5

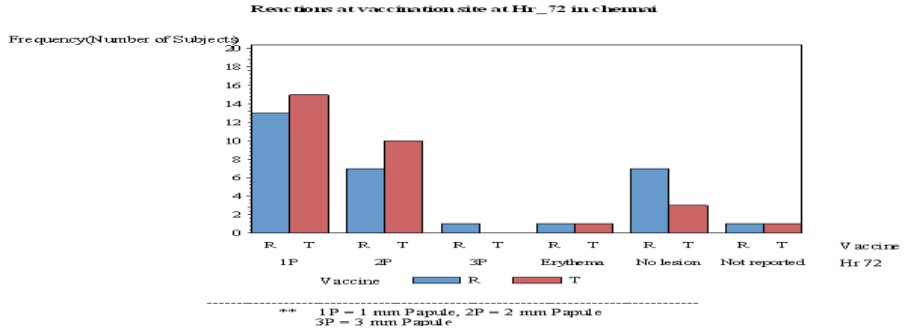

FIGURE 6

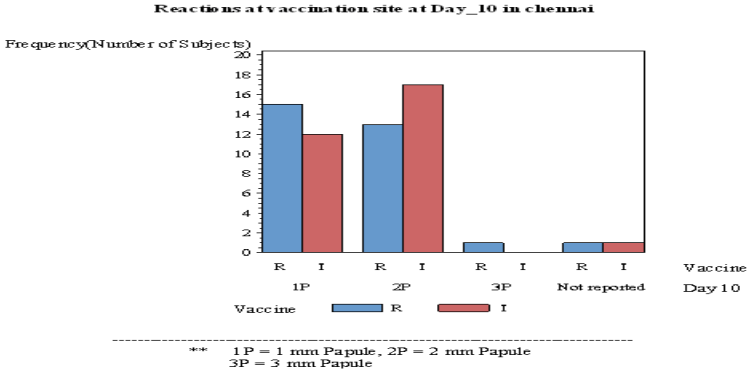

FIGURE 7

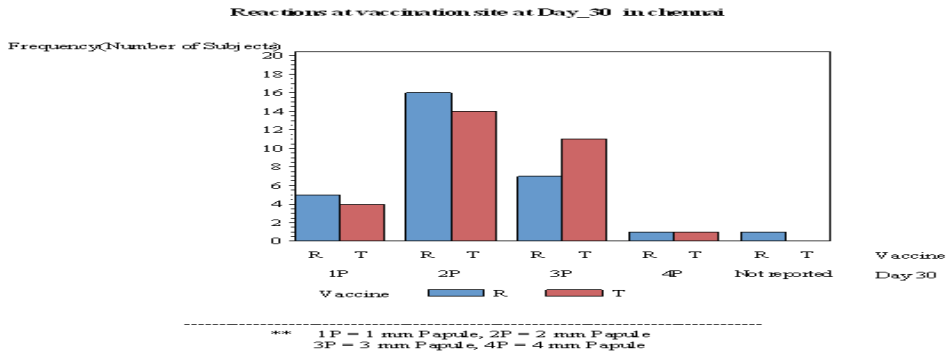

FIGURE 8

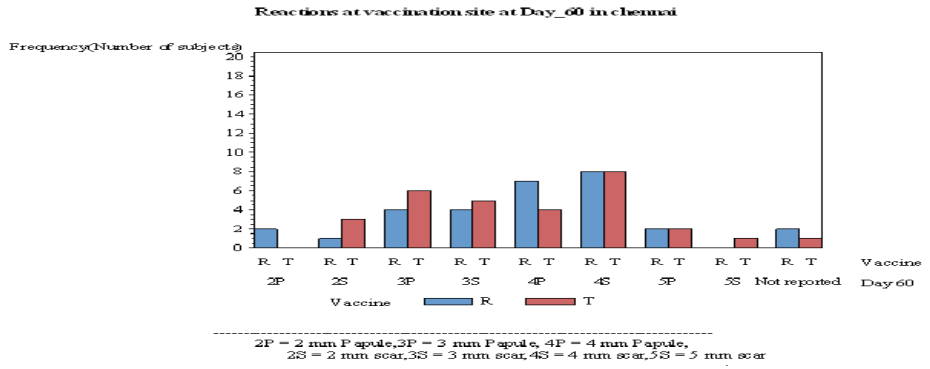

FIGURE 9

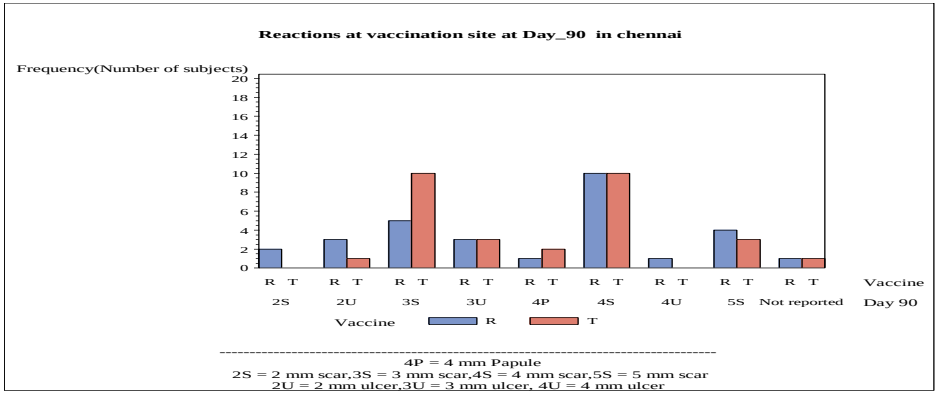

FIGURE 10

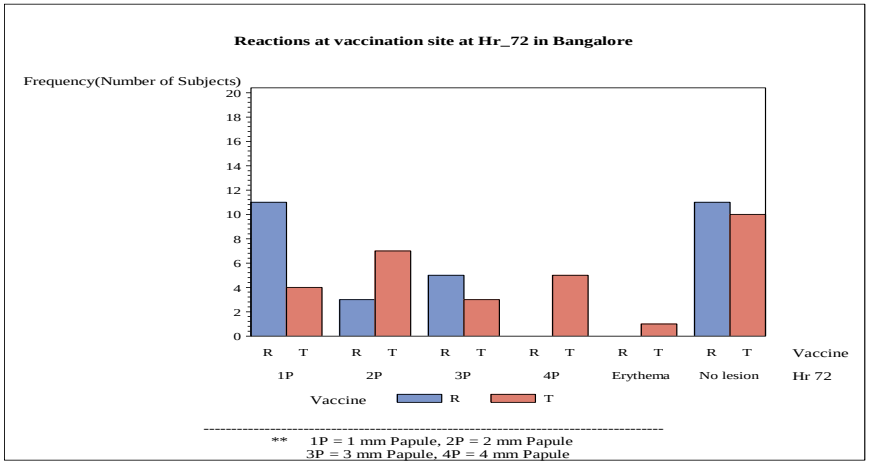

FIGURE 11

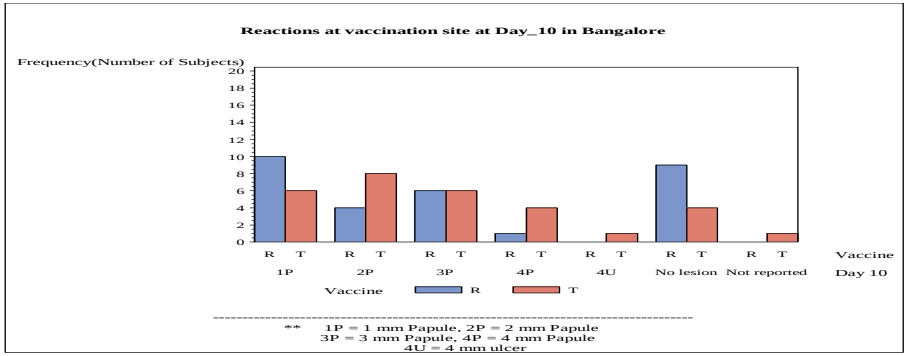

FIGURE 12

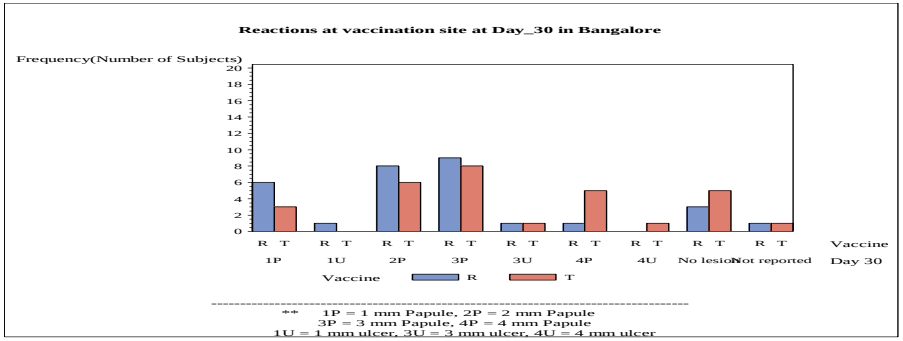

FIGURE 13

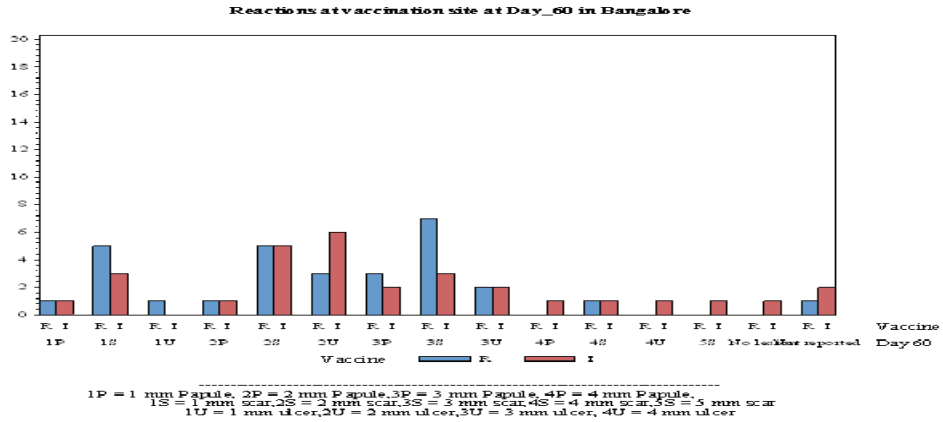

FIGURE 14

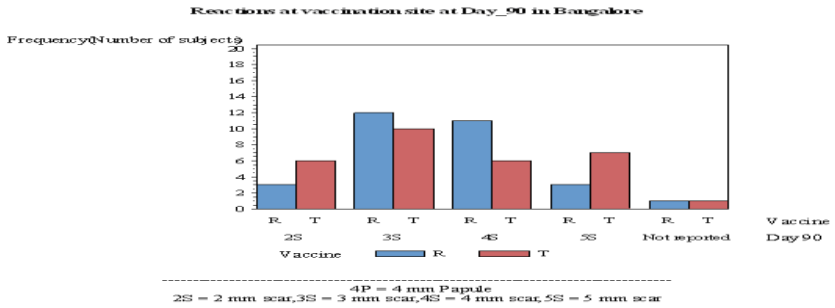

FIGURE 15

DISCUSSION

One hundred and twenty (120) children in the age of less than 04 days were enrolled in the study. This study was conducted in two centers namely KC General Hospital, 3rd cross, Malleswaram, Bangalore and Arya Vysya Maternity Home and Child Welfare Center, 178/158 Govindappa Naicken street, Parrys, Chennai. Sixty children were vaccinated in each center.

Out of one hundred and twenty children (120), sixty (60) children received BCG vaccine of Green Signal Biopharma Private Limited, Chennai (Test product) and sixty (60) children received BCG vaccine of Serum Institute of India Limited, Pune (Reference product).

After administration of vaccine, all children were followed up at 30 minutes, 72 hours, 10th day, 30th day, 60th day, 90th day and 26th week for any serious adverse event or adverse events and for excessive reaction at the site of vaccination. At 90th day, post vaccination Mantoux test was done and its response was assessed on day 93.

A total of 4 subjects did not visit for the follow up on day 90 and Post vaccination Mantoux test was performed to 116 subjects on day 90. Among 116 subjects, 2 subjects did not report for assessment of reactogenicity on day 93. And hence the result of Mantoux test was assessed for a total of 114 subjects. The mean response of Test and Reference vaccines was evaluated statistically for any significant difference. There was no statistically significant difference between the Test & Reference vaccines as the p value is equal to 0.99, indicating the investigational vaccines induce similar immune response.

All the subjects well tolerated the Investigational Products. No deaths, no serious adverse events and no other adverse event were experienced and subjects were normal till the completion of the study.

CONCLUSION

Based on the results, it is concluded that the Test vaccine of Green Signal Biopharma Private Limited, Chennai is as well tolerated as the Reference vaccine of Serum Institute of India Limited. The immunogenicity induced by the Test vaccine is similar to that of the Reference vaccine. There were no death/ serious adverse event/ adverse event was observed, no safety issues emerged during observation period. The Lyophilized BCG vaccine IP of Green Signal Biopharma Private Limited, Chennai is a safe and effective vaccine.

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