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TOXICITY, STERILITY AND BIOCHEMICAL TESTING OF NOVEL DTP GROUP OF VACCINES

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ABSTRACT

Conventionally, toxicogenic strains of C. diphtheria, C. tetani and B. pertussis are grown on a media of animal origin for production of DTP group of vaccines. This media poses various risks such as Bovine Spongiform Encephalopathy (BSE), microbial contamination and allergic reactions. To avoid such risks, media containing nutrients of vegetative origin are substituted. The present study involves the toxicity, biochemical and sterility testing of DTP group of vaccines produced on such vegetative media to determine their quality and safety. Toxicity tests are based on the observation that there are body weight changes characteristic to each vaccine and such standardized changes can be used as references for evaluating test vaccines. Sterility test was performed on the final bulk and lot to confirm that the product is free of bacterial and mycotic contamination. Biochemical tests were also carried out to determine the content of aluminium, thiomersal and formalin. The results confirmed that the DTP vaccine samples met the criteria set by I.P., 2007 for toxicity, sterility and safety.

Key words: DTP vaccine, Toxicity, Sterility, Thiomersal, Aluminium, Formalin

INTRODUCTION

Vaccines are biological preparations of antigenic material, which are administered with an objective of inducing in the recipient a specific and active immunity against the infectious agent or toxin produced by it. Vaccination as a deliberate attempt to protect humans against diseases has long been attempted. Of all the branches of modern medicine, vaccinology can claim to be the one that has contributed most to relief of human misery and the spectacular increase in the life expectancy. These vaccines thus have to be tested for toxicity, sterility and safety. [1, 2]

Specific toxicity test is the test for irreversibility which is used to detect the presence of toxin in vaccine sample. [3, 4] In specific toxicity test, each final bulk or final lot should be tested for the presence of toxin. [5, 6, 7] Abnormal toxicity test is used to detect any unknown contamination of vaccine from added chemicals. Toxicity tests are performed on laboratory animals. [7, 8] Although, the immunogenicity of a vaccine in an experimental host may be paralleled exactly by immunogenicity in humans, there is no doubt that preparations that are protective in animals also protect humans. [9, 10]

Sterility test is performed on DTP group of vaccines to ensure that it's free from microbial contamination. [7] The tests must be carried out under conditions designed to avoid accidental contamination of the product during test. The probability of detecting viable microorganisms in the tests for sterility increases with the number present in a given amount of the preparation being examined and varies according to the species of microorganism present. Greater assurance of sterility must come from reliable manufacturing procedures and compliance with Good Manufacturing Practice. The safety of the product is assured by employing appropriate biochemical tests which includes test for estimation of aluminium,

thiomersal and formaldehyde. [11] Aluminium phosphate is used as an immunologic adjuvant in order to accelerate, prolong, or enhance antigen-specific immune responses when used in combination with specific vaccine antigens. As per I.P. 2007, aluminium content in vaccine must not exceed 1.25 mg/ml; If it exceeds this limit then it starts depositing in various tissues, including bone, brain, liver, heart, spleen and muscle and leads to morbidity and mortality. [7, 12, 13, 14]

Thiomersal is an organomercurial salt complex that has been widely used as a preservative in vaccines. [15] As per I.P. 2007 there is a range set for permissible thiomersal content, which is between 0.005- 0.02%. If this limit is exceeded then hypersensitivity reactions to thiomersal may occur. These include redness and swelling at injection site and may cause neurodevelopmental disorders, local necrosis, acute hemolysis, disseminated intravascular coagulation, acute renal tubular necrosis and central nervous system injury including coma and death. [7, 15, 16]

Formalin or formaldehyde is used as a detoxification agent in vaccines and is estimated by qualitative analysis. [17] The formalin content must not exceed 200µg/ml and if it does then it may cause arrhythmia, headache, restlessness, throat infection and hypotension. [7, 18] Generally DTP group of vaccines are prepared by using strains of C. diphtheria, C. tetani and B. pertussis and grown on media of animal origin (eg. casein digest or meat extract). This type of media poses the risk of animal peptide-derived contamination, microbial contamination, allergic reactions and Bovine Spongiform Encephalopathy (BSE), being transmitted into humans in any subsequent therapeutic or prophylactic applications. These risks can be completely removed by substitution of nutrients of vegetative origin, such as proteins from soy beans, cotton seeds, potatoes, etc.
The aim of the present study was to evaluate the safety, toxicity and sterility of new group of DTP vaccines produced using vegetative media. The potency testing of these vaccines has already been carried out. [19]

MATERIALS AND METHODS

Vaccine Samples: DTP, DT, TT, Pentavalent (DTP/IPV/Hib) and Quadruple vaccine (DTP/IPV) were obtained from Central Drug Laboratory (CDL) of Central Research Institute (CRI), Kasauli (H.P.). All DTP groups of vaccines were prepared by growing strains of C. diphtheria, C. tetani and B. pertussis on media containing proteinaceous material of vegetative origin such as proteins from soy beans, cotton seeds, potatoes, etc. for 38-48 hours. When optimal level of toxin was reached it was harvested and stored at 37°C for 4 – 6 weeks with formalin (0.6%) for complete detoxification of toxin. Crude toxoid obtained was concentrated and purified and final product was obtained. All vaccines were stored at temperature of 2- 8°C.

Chemicals: Acetate buffer, Ammonium acetate solution, Aqueous solution of thiomersal, Casamino acid solution, Chloroform, Concentrated Hydrochloric acid, Concentrated Nitric Acid, Concentrated Sulphuric Acid, Copper Sulphate, Di-sodium ethylene diamine tetra acetic acid, Dithizone, Formaldehyde (40%), Formalin standard, Methyl orange indicator solution, Phenyl hydrazine hydrochloride, Potassium ferrocyanide, Pyridiazo-naphthol indicator, Sodium hydroxide, Sterile Normal Saline and Thiomersal saline; and Growth Media (Fluid Thioglycollate medium and Soyabean casein digest medium) were obtained from Central Drug Laboratory (CDL) of Central Research Institute (CRI), Kasauli (H.P.).

Animals: Guinea pigs (250-350 g) and Swiss albino mice (13-22 g) were used for the potency testing of vaccines. They were housed under standard conditions (Temperature: 28 ± 2ºC, Relative humidity: 50 ± 2%, 12 hr light / dark cycle) and provided with standard pellet diet and water *ad libitum.* The study was conducted in the Central Drug Laboratory, Central Research Institute, Kasauli, Dist. Solan, Himachal Pradesh. All the procedures were approved and carried out as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi.

METHODS

1. Toxicity Tests:

Specific Toxicity Test for Diphtheria Component: Animals were divided into two groups each containing five guinea pigs. Guinea pigs in group I served as control animals and was inoculated subcutaneously with 2.5 ml of normal saline; whereas animals in group II were inoculated subcutaneously with 2.5 ml of DTP vaccine samples (a quantity equivalent to at least five single human doses). The animals were then observed daily for 42 days (6 weeks) for any abnormality and signs of diphtheria toxemia. At weekly intervals and at the end of the test period the weight of animals was recorded. As per the requirement of I.P., 2007 none of the animals

should die or show signs of diphtheria toxemia within the observation period or lose weight at the end of the observation period. Also, at least 80% of the animals should survive at the end of the test period. [3, 7]

Specific Toxicity Test for Tetanus Component: Animals were divided into two groups each containing five guinea pigs. Guinea pigs in group I served as control animals and was inoculated subcutaneously with 2.5 ml of normal saline; whereas animals in group II were inoculated subcutaneously with 2.5 ml of DTP vaccine samples (a quantity equivalent to at least five single human doses). The animals were observed daily for 21 days (3 weeks) for any abnormality and signs of tetanus toxemia. Also at weekly intervals and at the end of test period animals were weighed. As per the criteria set by I.P., 2007; (i) none of the animals should die or show signs of tetanus toxemia within the observation period or lose weight at the end of the observation period, (ii) no symptoms of paralysis or any other signs of tetanus must be observed, and (iii) at least 80% of animals should survive at the end of the test. [3, 7]

Specific Toxicity Test for Pertussis Component (Mouse Weight Gain Test): Animals were divided into two groups each containing ten mice. Mice in group I served as control animals and were inoculated with thio-saline (0.05%) and animals in group II were inoculated with DTP vaccine sample. Each mouse in group II was injected 0.5ml of the test vaccine (intraperitoneally) but not less than single human dose. Control group mice were inoculated with 0.5ml of physiological saline, preferably containing the amount of antimicrobial preservatives equivalent to that present in the vaccine under test. The weight of animals was recorded prior to inoculation, 72 hours post inoculation and at the end of 7 days after injection. As per I.P., 2007 the vaccine passes the test if it meets the following requirements:

i) At the end of 72 hours, the total weight of the mice in the vaccine group is not less than that preceding the injection.

ii) At the end of 7 days, the average increase in the weight/ mouse in the vaccine group is not less than 60% of that of the mice in the control group.

iii) Not more than 5% of the inoculated mice should die during the test. [4, 7]

Abnormal Toxicity Test: Animals were divided into two groups each containing five mice. Mice in group I were inoculated with DTP vaccine and animals in group II were inoculated with TT vaccine. One human dose i.e. 0.5ml was injected intraperitoneally into each of the five adult mice weighing 17- 22 g. Mice were observed and weighed daily for seven days for any signs of ill health after inoculation. The animals were then weighed at the end of 7th day. [7, 8]

2. Sterility Testing: Fluid Thioglycollate Medium (FTM) and Soyabean Casein Digest Medium (SCDM) bottles (which have already been tested for growth promoting quality) each containing 100 ml of the medium were taken[7]. Media bottles were checked for contamination or any leakage from the cork. Each bottle was marked with details like name of product, date, temperature of incubation (30- 35°C or 20-22°C) and bottle no./ sterility no. for identification. All the bottles were kept in plastic crates and these were placed in the sterility chamber. Ultraviolet lights were switched on for one hour before performing the test. Hands were washed thoroughly with soap and water. Sterile clothes (Operation coat, mask, cap and gloves) were worn taking all aseptic precautions. Ultra violet lights were switched off before entering the chamber. Under strict aseptic conditions, sample of product to be tested (1ml) was taken with a sterile syringe fitted with a long needle. Appropriate volume (1ml per bottle) was transferred into each of the eight bottles containing FTM and eight bottles of SCDM medium. All bottles of FTM and SCDM were kept at 35°C and 22°C, respectively. Three bottles of each medium were kept un-inoculated as medium control. After 3 and 14 days of incubation the bottles were observed for microbial growth, if any.

Biochemical Tests:

Estimation of Aluminium content by Complexometric method: Aluminium content in vaccine samples was estimated by complexometric method. In this method, 1.0 ml sample of vaccine was taken in duplicate in Erlenmeyer flask (250 ml capacity). 2.0 ml distilled water was added in each flask followed by addition of 1.0 ml Conc. H_2SO_4 and 0.2 ml of Nitric acid. The flasks were heated until dense white fumes evolved. Flasks were cooled and 10 ml distilled water was added carefully. One drop of methyl orange was added to each flask and titrated with 50% w/v sodium hydroxide (NaOH) solution till yellow coloured end point was obtained. 25 ml of distilled water, 10 ml of M/100 EDTA and 10 ml acetate buffer (containing 68g of sodium acetate, 38.5g of ammonium acetate, 125ml of glacial acetic acid in total volume of 500ml; pH 4.4) was added in each flask. The contents were heated till boiling for three minutes. Five drops of pyridylazonaphthol indicator (0.1% solution in 95% ethanol) was added in each flask. Each hot solution was then titrated with M/100 copper sulphate solution

until a purple-brown end point was obtained. The volume of copper sulphate used up in the titration was noted. Simultaneously, a blank determination (distilled water) was also carried out. [7]

Estimation of Thiomersal Content by Chemical Method: Thiomersal is an organomercurial salt complex of sodium ethyl mercuriothiosalicylate containing ethylmercury that has been widely used as a vaccine preservative. [15, 16] Estimation of thiomersal content of the vaccine was done using UV Spectrophotometer. In this method, separating funnels were washed with concentrated nitric acid and rinsed with distilled water.

Two aliquots of a standard 0.01% aqueous solution of thiomersal (0.5 and 1.0 ml) and two samples of the test solution (1ml) were added to individual separating funnels. The volume was adjusted to 10 ml with 1% solution of ammonium acetate (pH 6.0). 10 ml of a 1 in 10 dilution of a fresh solution of 0.01% dithizone in chloroform was added to each funnel. The contents were shaken vigorously for 45 seconds. The chloroform layer was then separated carefully. [7] The spectrophotometer was set at 490nm using the diluted dithizone solution and a scan was taken of the test solution from 470-520nm. The transmission at 520nm was plotted against the thiomersal concentration of the standards on semi-logarithmic paper and the concentration of thiomersal in the sample was determined.

Estimation of Formalin Content: Formalin or formaldehyde is used as a detoxification agent that converts the toxin of the antigen into toxoid. [18] The left free formalin content of the test vaccine was performed as per I.P., 2007. Briefly, test tubes were labeled as Blank, Standard and Sample.

To the test tube marked as 'Blank' 3 ml of distilled water was added. Formalin standards of various concentrations ranging from 0.5 to 40 µg/ml were prepared and 1 ml of each was added to different test tubes marked as 'Standard'. 2 ml of distilled water was then added to each of these tubes.

To the test-tubes marked as 'Sample' 1 ml of vaccine sample and 2 ml of distilled water was mixed. 1 ml of phenyl hydrazine hydrochloride (1%) and 0.5 ml of potassium ferrocyanide (5%) was added to the Blank, Standard and Sample test tubes. 1 ml of conc. HCl was added to all the above test- tubes and the contents were mixed well. Color of the samples was qualitatively matched with the color of the formalin standards. [7]

RESULTS

1. Toxicity Tests:

Specific Toxicity Test for Diphtheria: Animals in group I and II, which were inoculated with normal saline (control) and DTP vaccine, respectively showed an initial average body weight of 281.6 gms and 280.8 gms, respectively which increased to 291.8 and 296.6 gms on the 6th week, resulting in an average weight gain of 10.2 and 15.8 gms (Table 1). Also the guinea pigs did not show any symptoms of specific intoxication within 6 weeks of injection.

Specific Toxicity Test for Tetanus: The animals in saline-treated group I (Control) and DTP immunized group II, showed an initial average body weight of 288.8 and 286.6 gms which increased to 296.4 and 293.0 gms on 3rd week, resulting in an average weight gain of 7.6 and 6.4 gms at the end of 3rd week, respectively (Table 2). The guinea pigs also did not show any symptoms of paralysis or any other signs of tetanus within 3 weeks of injection.

Specific Toxicity Test for Pertussis: Animals in group I and II were inoculated with thio-saline (control) and DTP vaccine, respectively. The initial average weight of animals of group I and II was recorded as 15.0 and 15.3 gms which increased to 16.0 and 16.1 gms at the end of 72 hours and 19.3 and 18.1 gms at the end of the 7th day, resulting in an average weight gain of 4.3 and 2.9 gms, respectively.

Thus at the end of 72 hours, the total weight of the group was not less than that preceding the injection. Also at the end of 7th day, the weight gain per animal of Group II was found to be not less than 60% (2.5 gms) and not more than 150% (6.4 gms) of average weight gain of control group animals (Table 3).

Test For Inocuity (Abnormal Toxicity): Animals in group I and II, which were immunized with DTP and TT vaccine, respectively recorded an initial average body weight of 21.4 and 22.0 gms which was found to increase to 22.6 and 23.2 gms on 7th day, resulting in an average weight gain of 1.2 gms, in both the groups (Table 4). Also none of the animals showed signs of ill health during the observation period of 7 days.

Sterility Test: Sterility of DPT group of vaccines was determined by Direct Inoculation Method. No growth was found in any bottle after inoculation.

2. Biochemical Tests

Test for Aluminium Estimation: For three lots of DTP and TT vaccines, mean aluminium content was calculated to be 0.8000, 0.854 & 0.791 mg/ml and 0.881, 0.692 & 0.854 mg/ml, respectively (Table 5). All the vaccines were found to have aluminium content less than 1.25 mg/ SHD.

Test for Thiomersal Estimation: The mean thiomersal contents of samples of DPT, TT and DT vaccine were found to be 0.0107, 0.0100 and 0.0096%, respectively (Table 6).

Estimation of Free Formalin Content: Three samples each of two different lots of DTP, DT and TT test vaccines showed mean free formalin content of 2.0 and 4.0, µg/ml, 1.0 and 2.0µg/ml and 2.0 and 1.0 µg/ml, respectively (Table 7).

DISCUSSION

Toxicity, sterility and biochemical testing of DTP group of vaccines produced on vegetative media showed the best result in terms of linearity, accuracy, precision and quality rather than the previous methods of production. [3, 8, 11, 15, 18] Specific toxicity test is performed to check whether vaccine sample contains any toxin which may cause toxic effects.

The specific toxicity of diphtheria and tetanus component was tested for diphtheria and tetanus toxin. In the specific toxicity test for diphtheria component the guinea pigs did not show any symptoms of specific intoxication within 6 weeks of injection and none of the animals lost weight at the end of test (rather recorded a gain in weight of 15.8 gms). So, the DTP vaccine sample passed the test as per I.P., 2007 requirements, according to which the animals must not show any symptoms of specific intoxication within 6 weeks of injection and none of the animals should lose weight at the end of the test. In the specific toxicity test for tetanus component, the guinea pigs did not show any symptoms of paralysis or any other signs of tetanus within 3 weeks of injection; and none of the animals lost weight at the end of test period (rather gained a weight of 6.4 gms by the end of 3rd week).

Thus the vaccine sample passed the test as per the requirements set by I.P., 2007, according to which the animals must not show any symptoms of paralysis or any other signs of tetanus within 3 weeks of injection and none of the animals should lose weight at the end of the test. In the specific toxicity test for pertussis component, which was performed by the Mouse Weight Gain test, the test vaccine passes the test only if it meets the three criteria's set by I.P., 2007, namely, a) at the end of 72 hours, the total weight of the inoculated group is not less than that preceding the injection; b) at the end of 7 days, the weight gain per mouse in the inoculated group is not less than 60% and not more than 150% of that of control group of mice, and c) none of the animals died during the test. [7] The vaccines under test showed an increase in weight at the end of 72 hours. Also at the end of 7th day, the weight gain per animal of the inoculated group was found to be not less than 60% (2.5 gms) and not more than 150% (6.4 gms) of average weight gain of control group animals and reported no mortality.

Thus the samples passed the test as per I.P., 2007 requirements. Abnormal toxicity test is performed to check whether the vaccine contains any abnormal constituent or not. As per I.P., 2007 the vaccine sample passes the test if none of the animals show any signs of ill health in the period of 7 days of observation and none of the animals lose weight following the inoculation. These criteria were met by the vaccine samples under consideration and thus confirm the absence of any abnormal constituents. Sterility test is performed on a product to check it for any microbial contamination. Direct inoculation method was used for determining the sterility of final product.

The inoculated media bottles were observed after 14 days of incubation and there was no evidence of bacterial and fungal growth in any of the media bottles inoculated by the test vaccine under test. The negative controls were found to be negative. The samples thus showed compliance with the requirements of I.P., 2007 which requires no growth of microorganisms upon incubation; and thus passed the test for sterility. Vaccines contain additives like aluminium, thiomersal and formalin, which if in excess may pose health hazards. Thus these need to be estimated and for which the I.P. has set permissible limits.

These were estimated by different methods i.e. simple spectrophotometry and derivative UV-spectrophotometry. UV-spectrophotometric method of analysis is more economic and simpler. Under computer controlled instrumentation it plays a very important role in the analysis of vaccine samples. Aluminium is added as an adjuvant to vaccines in order to enhance the immune response. Aluminium content was thus determined by complexometric method. [11, 12] As per I.P. 2007, aluminium content in vaccine must not exceed 1.25 mg/ml. The aluminium

content of the vaccine samples were found to be 0.800, 0.854, 0.791, 0.881, 0.692 and 0.854 mg/ml, which were all within permissible limits, that is less than 1.25 mg/ SHD. Thus, the vaccine passed the test, as per the requirements set by I.P., 2007.

Thiomersal is added as a preservative in vaccine to prevent microbial growth. Thiomersal content was determined by chemical method [15, 16] which is more rapid, economic and simpler than previous microbiological method. In chemical method, UV-spectrophotometer is used and gives more accurate results than instruments like colorimetric titration and simpler spectrophotometer described in Indian Pharmacopoeia. As per I.P. 2007 there is a range set for permissible thiomersal content, which is between 0.005- 0.02%[7]. The thiomersal content of samples was found to be 0.0107%, 0.0100% and 0.0096%, which is within the range specified. Thus, the vaccine passed the test, as per the requirement of I.P., 2007.

In vaccines, the formalin content must not exceed 200µg/ml as it may cause side effects. Thus, the residual free formalin content needs to be biochemically tested. In the method used, phenyl hydrazine hydrochloride forms a complex with the free formalin and the potassium ferrocyanide acts as an indicator which gives color of different shades on the addition of conc. hydrochloride. This method of formalin estimation was rapid and showed accurate results. The formalin content was found to be within the acceptable limits i.e. less than 200µg/ml. Thus, the vaccine samples passed the test as per the requirements of I.P., 2007.

CONCLUSION

In the present study the toxicity, sterility and biochemical tests used to control the quality of vaccines are more economical, simpler and show the best result in terms of linearity, accuracy, precision and quality for the vaccines. After analyzing the tests employed in the present project, the results were found to be well within the acceptable limits mentioned in I.P., 2007. The results obtained thus fulfilled the requirements for safe and efficacious vaccines, which can be used in vaccination programs or in therapeutic treatments.

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Table 1: Effect of test vaccine on weight of animals following vaccine inoculation for determination of specific toxicity for diphtheria component

Product Details	Group No.	Initial Weight (gms)	Weight (gms)						Weight gain at the end of 6th week (gms)
			1st week	2nd week	3rd week	4th week	5th week	6th week	
Normal Saline (Control)	I	278	280	284	289	290	299	296	18
		276	278	279	280	282	283	284	08
		280	281	282	284	285	286	287	07
		285	287	289	290	291	293	295	10
		289	290	291	292	294	295	297	08
	Mean	**281.6**	**283.2**	**285**	**287**	**288.4**	**291.2**	**291.8**	**10.2**
DTP	II	275	280	282	290	292	295	294	19
		280	274	276	276	284	286	289	09
		271	278	280	282	288	286	283	12
		290	290	288	294	299	304	308	18
		288	290	295	294	298	300	309	21
	Mean	**280.8**	**282.4**	**284**	**287**	**292**	**294**	**296.6**	**15.8**

n= 5 per group; DTP- Diptheria, Tetanus and Pertussis

Table 2: Effect of test vaccine on weight gain of animals following inoculation of vaccine for determination of specific toxicity of tetanus component

Product Details	Group No.	Initial Weight (gms)	Weight (gms)			Weight gain at the end of 3 weeks (gms)
			1st week	2nd week	3rd week	
Normal Saline (Control)	I	280	286	288	294	**14**
		292	294	295	297	**05**
		287	289	290	292	**05**
		295	297	299	301	**06**
		290	293	295	298	**08**
	Mean	**288.8**	**291.8**	**293.4**	**296.4**	**7.6**
DTP	II	250	255	260	260	**10**
		264	261	268	270	**06**
		250	248	253	257	**07**
		333	338	342	338	**05**
		336	339	345	340	**04**
	Mean	**286.6**	**288**	**294**	**293.0**	**6.4**

n= 5 per group; DTP- Diptheria, Tetanus and Pertussis

Table 3: Effect of test vaccine on weight gain of animals following vaccine inoculation for determination of specific toxicity for pertussis component

Product Details	Group No.	Initial Weight (gms)	Weight (in gms)		Weight Gain at the end of 7th day (gms)
			At 72 hrs	On 7th day	
Thio-Saline (0.05%)	I	14.5	15.8	19.3	**4.8**
		15.0	15.8	17.9	**2.9**
		14.8	15.7	18.5	**3.7**
		14.2	15.8	18.8	**4.6**
		15.5	16.7	19.5	**4.0**
		15.9	16.4	21.9	**6.0**
		14.3	15.5	18.5	**4.2**
		15.1	15.9	20.8	**5.7**
		16.0	16.5	19.0	**3.0**
		14.7	15.9	18.8	**4.1**
	Mean	**15.0**	**16.0**	**19.3**	**4.3** **60% of 4.3 = 2.5** **150% of 4.3= 6.4**
DTP	II	15.0	15.9	17.8	**2.8**
		16.5	18.0	19.1	**2.6**
		15.5	16.4	18.9	**3.4**
		14.5	15.2	17.6	**3.1**
		15.9	16.5	18.8	**2.9**
		14.9	15.6	17.7	**2.8**
		15.5	16.9	18.2	**2.7**
		15.2	16.4	18.5	**3.3**
		15.0	16.0	17.9	**2.9**
		15.0	15.9	17.9	**2.9**
	Mean	**15.3**	**16.2**	**18.2**	**2.9**

n= 10 per group; DTP- Diptheria, Tetanus and Pertussis

Table 4: Effect of test vaccines (DTP and TT) on the weight gain of animals following vaccine inoculation (for determination of abnormal toxicity)

Product Details	Group No.	Initial Weight of animals (gms)	Days of Observation							Weight gain at the end of 7th day (gms)
			1	2	3	4	5	6	7	
DTP	I	21.0	21.0	21.6	21.7	21.5	22.0	22.3	22.6	**1.6**
		21.0	21.0	21.4	21.6	21.6	21.5	21.2	21.6	**0.6**
		21.4	21.4	22.0	22.5	22.6	23.0	22.7	23.4	**2.0**
		22.0	22.0	22.0	22.2	22.2	22.5	22.2	22.5	**0.5**
		21.6	21.6	22.0	22.0	22.1	23.0	22.6	22.9	**1.3**
	Mean	**21.4**	**21.4**	**21.8**	**22.0**	**22.0**	**22.4**	**22.2**	**22.6**	**1.2**
TT	II	22.0	22.3	22.2	22.2	22.2	22.5	23.0	22.9	**0.9**
		22.4	22.2	22.5	23.0	23.0	23.1	23.6	23.6	**1.2**
		22.3	22.4	22.8	22.8	22.8	23.0	23.3	23.4	**1.1**
		21.5	22.0	22.3	22.3	22.3	22.5	22.5	22.5	**1.0**
		21.8	22.1	22.2	22.2	22.2	22.3	22.5	22.5	**1.7**
	Mean	**22.0**	**22.2**	**22.4**	**22.6**	**22.6**	**22.8**	**23.2**	**23.2**	**1.2**

n=5 per group; DTP-Diphtheria, Tetanus and Pertussis; TT- Tetanus Toxoid.

Table 5: Estimation of aluminium content for different lots of test vaccines by complexometric method

Sr. No.	Product	Absorbance of Test	Difference of absorbance between Blank and Test	Conc. of Aluminium (mg/ml)	Mean Conc. of Aluminium (mg/ml)
1.	**DTP**	7.0	3.0	0.8094	**0.800**
		7.1	2.9	0.78242	
		7.0	3.0	0.8094	
2.	**DTP**	6.9	3.1	0.83638	**0.854**
		6.7	3.3	0.89034	
		6.9	3.1	0.83638	
3.	**DTP**	7.2	2.8	0.75544	**0.791**
		7.0	3.0	0.8094	
		7.0	3.0	0.8094	
4.	**TT**	6.7	3.3	0.89034	**0.881**
		6.8	3.2	0.86336	
		6.7	3.3	0.89034	
5.	**TT**	7.6	2.4	0.64752	**0.692**
		7.6	2.4	0.64752	
		7.1	2.9	0.78242	
6.	**TT**	6.9	3.1	0.83638	**0.854**
		6.8	3.2	0.86336	
		6.8	3.2	0.86336	

Abs. of Blank – 10.0

Table 6: Estimation of thiomersal content in different test vaccines by chemical method

Sr. No.	Product	O. D. of Test	Thiomersal Content (%)	Mean Thiomersal Content (%)
1.	**DTP**	0.409	0.0108	
		0.409	0.0108	0.0107
		0.398	0.0105	
2.	**TT**	0.377	0.01	
		0.398	0.0105	0.0100
		0.367	0.0097	
3.	**DT**	0.377	0.01	
		0.367	0.0087	0.0096
		0.387	0.0102	

O.D. *- Optical Density;* ***DTP****- Diphtheria Tetanus and Pertussis;* ***TT****- Tetanus Toxoid;* ***DT****- Diphtheria and Tetanus;* ***O.D. of Standard****= 0.377*

Table 7: Estimation of Mean Formalin content in different lots of test vaccines

S. No.	Product Details	Free Formalin Content (µg/ml)	Mean Formalin content (µg/ml)
1.	**DTP**	2.0	
		2.0	2.0
		2.0	
2.	**DTP**	4.0	
		4.0	4.0
		4.0	
3.	**DT**	1.0	
		1.0	1.0
		1.0	
4.	**DT**	2.0	
		2.0	2.0
		2.0	
5.	**TT**	2.0	
		2.0	2.0
		2.0	
6.	**TT**	1.0	
		1.0	1.0
		1.0	

DTP*-Diphtheria, Tetanus and Pertussis****; DT****- Diphtheria and Tetanus****; TT****- Tetanus Toxoid*

REFERENCES

1. Susan LP. Diphtheria Toxoid. In: Stanley A and Shef M (eds.). Vaccines, 1st ed., New York; McGraw-Hill: 2004, pp. 211-24.
2. Bytchenko B. Excerpta Medica, 1981; 16(2): 28-39.
3. Naito S, Furuhata K. J Biol Stand, 1968; 23(2): 97-123.
4. Kendrick PI, Minser MK. J Public Health, 1947; 37(3): 803-10.
5. Fine PEM, Clarkson JA. Lancet, 1982; 254 (1): 666-9.
6. Jebb WH, Tomilinson AH. J Gen Microbiol, 1959; 17(4): 59-63.
7. Singh GN. Diphtheria and Tetanus and Whole cell Pertussis Vaccine (Adsorbed). I.P., 2007; 3(3): 744-57.
8. Mizukami T, Masumi A. J Biol Stand, 1999; 43(3): 124-60.

9. Bleck TP. *Corynebacterium diphtheria*. In: Mendell GL, Bennett JE and Dolin R (eds.). Principles and Practices of Infectious Diseases, 4th ed., Washington DC; 1995, pp. 2173-8.
10. Taylor EM, Moloney PJ. J Biol Stand, 1980; 12(1): 167-73.
11. Grabenstein JD. Hosp Pharm, 1996; 31(6): 1387-401.
12. Aggerbeck H. Adjuvants and their applications. In: Zanardi L and Shef M (eds.). Vaccination against Diphtheria and Tetanus, 3rd ed., New York; McGraw-Hill: 1998, pp. 81-106.
13. Collier LH, Polakoffs MJ. Lancet, 1979; 254(1): 1364-8.
14. Zackrisson G, Taranger J, Troufors B. J Pediatr, 1990; 116(22): 190-4.
15. Harada M. Crit Rev Toxicol, 1995; 25(3): 1-24.
16. Cox NH, Forsyth A. Contact Dermatitis, 1998; 18(1): 229-33.
17. LaForce FM, Young FM, Bennet JV. J Med, 1969; 280(5): 566-74.
18. Chandler MD, Frerichs GN. J Biol Stand, 1980; 8(1): 145-9.
19. Sharma M, Brahmne HG, Bafna PA. Int J Compr Pharm, 2011; 2(11): 1-9.