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Comparative Study of the VI Polysaccharide Typhoid Vaccines Effectiveness from Local Strain with Commercial Vaccine

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Abstract
Typhoid fever, which is caused by *Salmonella enterica* subspecies *enterica* serovar Typhi (*S.* Typhi) is an exclusively human enterically transmitted, range from mild gastroenteritis to severe systemic disease and spread by the fecal-oral route via contaminated food and water.In current study done production of polysaccharide typhoid vaccine against typhoid fever from local strain (*S.* Typhi). Identification of *S.* Typhi (local strain) by traditional methods of diagnosis as microscopic examination and biochemical tests as Api20E system diagnosis, Vitek-2 and serological test .All confirmed the identity of the bacteria belonging to the *Salmonella* Typhi. In current study, polysaccharide was used as immunogen to production typhoid vaccine from local strain and evaluation these vaccine biological, chemical, physical and Immunological with Commercial vaccine and comparative the results, the results of biological test as sterility, safety and pyrogenicity test were conforms. A result of chemical test (phenol content and residual formalin) by using HPLC and GC, PH value and protein content were safety side comparative WHO protocol. On the other hand, the results of physical test present particles size of local typhoid vaccine was less than Com. V. the particle, the smaller the particle size of vaccine supports the immune response increased immunization. The result Immunological test by ELISA was showed that the titration of PS V. local strain group was high immune response and significantly differences ($P<0.5$) in the sera of treated guinea pigs between first and booster dose, control group was Non-significant. The result of cellular immune response that present in total white blood corpuscles (WBCs) count showed no significant different of Com. Vaccine and control group between first and booster dose but, there significant different ($P<0.05$) of PS vaccine between first and booster dose. In challenge test the result was showed 80% and 70% ratio protected in PS and Com groups vaccines respectively and resisted with the virulent enhance challenge exposure against *S.* Typhi organism after give 100LD50 dose (10^8) to those animal.In conclusion, can reproduction of Iraqi typhoid vaccine (Killed vaccine) that was produced in VIS and stopped since 2003 by using new vaccine that recommended by WHO for humane immunization against typhoid fever, also possibility use of method evaluation for evaluates commercial typhoid vaccine.
Keywords: (HPLC) High-performance liquid chromatography, (GC)gas chromatography.

1. Introduction

Typhoid fever, which is caused by *Salmonella enterica* subspecies *enterica* serovar Typhi (*S.* Typhi) is an exclusively human enterically transmitted ,range from mild gastroenteritis to severe systemic disease (Bnesaru,2015). The extra-intestinal infections caused by *Salmonella* are very fatal (Marathe,2012). *S.* Typhi has a combination of characteristics that make it an effective pathogen. This species contains an endotoxin typical of gram negative organisms, as well as the Vi antigen which is thought to increase virulence (Lillehoj and Okamura, 2003). Available vaccine for typhoid fever has less-than-desired efficacy and certain unacceptable side effects, making it pertinent to search for new immunogen suitable for vaccine formulation (Sagi, *et al*., 2006; Hamid and Jain, 2008). Al-Shammari *et al*., (2010) recorded that Vi vaccine in boosting dose after 8 days from vaccination is the best efficient and gave highest mean in phagocytosis, Ab titration assay and significant increase in delayed hypersensitivity. World Health Organization in 2008 was recommended that typhoid vaccination be considered for the control of endemic disease and outbreaks, but programmatic use remains limited. (Date *et al*., 2014). However, the efficacy of both the vaccines (Ty21a and ViCPS) was approximately (65-70)% (WHO, 2014). In Iraq, typhoid fever was recorded (21,005-22,280) infect among 2013-2014 (Iraq CDC ,2015) , and typhoid vaccine (killed Ty21a) was producing till 2003 by Vaccines and Serums Institute / Iraqi Health Ministry as 1.500.000 dose, divided 500.000 dose for Ministry of Defense and 1.000.000 to Public Health Offices (VSI, 2015). Now Iraq will depend on importing vaccine to meet the needs, and the development of improved typhoid vaccines is a high global public health priority.

2.Methods

- Bacterial strain

Local strain of S. Typhi supplied by the National Center of Drugs Control and Research (NCDCR) /Iraqi

Ministry of Health was used in the current study.

- Identification and Confirmation of local S. Typhi:

Morphological colonies characteristics were recorded on the media that used for primary identification as blood agar, XLD agar and SS agar of S. typhi (Jawetz et al. , 2010). Gram's stain was used to examine the isolated bacteria for studying the microscopic properties, depending on the color and shape and the presence of a single or series (Isenberg and Garcia, 2004). Biochemical Tests according to MacFaddin method (MacFaddin, 2004).

Serological conformation of local S. Typhi isolate was done according to Koneman et al., (1992) ,the isolate is doing with polyvalent O and H antisera by using slide agglutination test. The tested local strain S. Typhi with the 20 biochemical tests and confirmed by API 20E (BioMerieux, France) and confirmed by Vitek-2(BioMerieux, France)**.**

- Preparation of Seed Lot, Vi purified lot and vaccine:

Production of Vi polysaccharide typhoid vaccine (Vi PSTV) shall be based on a seed lot system (WHO, 1994). Preparation cells by growing bacteria in group of vials containing 25 ml of brain heart infusion broth at 37 ºC for (18-24) hr. The fresh cultures were used to inoculate nutrient agar distribute in culture bottle, 200 ml each this bottle. The inoculated bottle was incubated at 37ºC for (18-24) hrs. Then been prepared slides of gram stain of each culture bottle to avoid any contamination (Silipo et al., 2002). Phenol 0.05% and heat was used to kill the organism modified method WHO, (1994). After then the washing process and sedimentation for the harvest using the cooling ultracentrifuge at 13,000 rpm/min for 10 min. using phosphate buffer solution with every time, until get clear solution by use Nalgene bottle, then the extraction and purification of S. Typhi Vi PS (local strain) was done according to WHO, (1994) and Cui et al., (2010).

- Biological, Chemical, physical and Immunological tests of Vi PS Vaccines:

A. Biological Tests of vaccines:

- Sterility test: Each final bulk shall be test, Sample of prepared PS , LPS vaccines by cultured on nutrient and MacConkey agar for bacterial sterility then incubated at 37ºC for (24-72) hrs., and cultured on Sabouraud Dextrose Agar (SDA) then incubated at 25°C for 72 hrs. for mycotic sterility (WHO, 1973 ;Robbins and Robbins,1984; Guzman et al., 2006).

- Safety test or abnormal toxicity: Each final lot were tested by injection of one human dose into each of two guinea pig, weight between (250-350) g and five mice weight between (17-22) g into i.p. (Wang et al., 2005 ; Guzman et al., 2006).

- Pyrogenicity Test: Modified method of WHO, (1979) was done for a pyrogenicity test using two groups of healthy New Zealand white rabbits, each group consist of three rabbits, weighing (2–2.5) kg of each one, by ear vein intravenous injection of 0.025µg of Vi PS typhoid and per ml/kg body weight, a temperature was measured each 2hr. post 3hrs. from vaccination by medical thermometer through enter it into the rectal to more than half of the thermometer painted after his introduction with paraffin for easy entry and keep the thermometer for (3-5) min. , then withdraw it to recorded the temperature (WHO, 1979 ;WHO, 2015)

B. Chemical test:

- Molisch's test: A sensitive chemical test for the presence of carbohydrates was done according Admin , (2014); Robyt and White, (1987)

-Protein content: Estimate of total protein in Vi P.S. vaccines with com. vaccines by kjeldahl method to certificate purification of vaccines (Pearson, 1970).

- Phenol content and Residual Formalin: Estimation of the phenol content in the local and LPS vaccines by High-performance liquid chromatography (HPLC) and gas chromatography (GC).

- PH Measure: The PH of each final lot measured by PH meter at (7± 0.5) (WHO, 1994).

C. Physical test:

- Inspection of final containers: Inspection of the vial in each final lot and to showing if there are any abnormalities as particle.

- Shaped molecule vaccine: Using Olympus Microscope to determinate particle size in prepared of Vi P.S. vaccine.

D. Immunological test and evaluation Immune Response:

- Determination dose and Immunogenicity studies:

Purified Vi PS V. 25 µg in 0.5 ml was injected as first dose one group of two G. pigs each intramuscular (i.m.) into. Group 2 was injected 0.5 ml i.m of PBS as control. Each groups were received a booster dose post 15 days from first dose. The challenge test after 3 weeks from boosting dose.

- Blood samples collection: At (0, 15 and 30) day from vaccination, 3ml of blood samples were collected from G. pigs via intracardiac puncture , each sample divided into two portions. First was treated with anticoagulant was used for estimated the total WBC count. The second portion left until clotting then centrifuged of supernatant to separate the serum and then transferred to suitable plane tube for serological tests (Lewis, 2001).

- Detection of antibody titration in the animal's serum:

A. Evaluation of Humeral Immune Response Titration:
Estimated titration of humeral immunity in the sera of immunized and control group to G. pigs by enzyme-linked immunosorbent assay (ELISA), using the manufacture kit that preparation into university of London / King of London College and done those test in Thi-Qar Poison Consultation Center.

B. Evaluation of cellular Immune Response:
Total leukocytes count: Blood samples were collected by heart puncture using a disposable syringe 2ml pre-coated with heparin. The method was done according to Haen ,(1995) .

2.5. Lethal dose of half animal (LD50) and challenge test

A. Lethal dose of half animal (LD50): The lethal dose (LD50) of S. Typhi was calculated for challenge, according to Reed and Muench, (1938). S. Typhi grown in nutrient agar and incubated at 37 ºC for (18-24) hrs, then preparation the harvest. The culture suspension was serially diluted, each group (10) mice were divided into five groups and inject infection dose according McFarland tube (10^3,10^4, 10^5, 10^6 and 10^7) cfu/ml, mice were injected 0.25 ml/mice of S. Typhi harvest using i.p. route and sterile normal saline NS was injected to control group (Venkatesan et al., 2011). All the mices were monitored to check recorded the number survived or dead during 15 day period (Robbins and Robbins 1984).

B. Challenge test:
The challenge test was conducted after 15 days from the booster dose. The 100 LD50 of S. Typhi were injected 0.25 ml/G.pig of harvest using i.p. route each vaccinated groups (Vi PS, Com.) and control group (that was injected PBS). All the mice were monitored to recorded the clinical signs and the number survived or dead during 3 days period (Maslog ,1997).

2.6. Statistical Analysis:
The Statistical Analysis System- SAS (2012) was used to effect of different factors in study parameters. Least significant difference-LSD test was used to significant compare between means in the study.

3. Results and Discussion

3.1. Identification and Confirmation of local strain
Identification of S. Typhi (local strain) showed that strain was positive result based on the morphological and cultural characteristics of the colonies on SS agar, XLD agar and blood agar, as mentioned by Jawetz et al., (2010).

Microscopic examination of cultures showed the bacteria as mentioned by Muthiadin et al., (2015)

The results of biochemical tests referred the isolate was S. Typhi as mentioned by MacFaddin, (2004) and Jawetz et al., (2010).

The local isolate that showed conformity with the S. Typhi in slide agglutination test and API 20E test showed excellent identification confidence with the S. Typhi organism in Vitek-2 with the identification values or probability 99%.

3.2. Result of Biological, Chemical, physical and Immunological tests of local Vaccines

A. Biological test:
- Sterility test: The result were confirm the purity of the harvest, free from bacterial and mycotic organisms, and there haven't any contamination, these will as mentioned by (Robbins and Robbins, 1984; WHO, 1994; Guzman et al., 2006).
-Safety test or Abnormal toxicity: Pathological signs doesn't appear on the animals after 72 hrs. of injection by the prepared vaccines that indicate the safety of these vaccines and the animals survived for at least 7 days without weight loss, those agree with (Wang et al., 2005 ; Guzman et al., 2006).
- Pyrogenicity test: The results of pyrogenicity test were as the following table:

Table (3-1): The results of pyrogenicity of prepared and commercial vaccines.

Type of Vaccine	Mean ± SE			
	Initial temp. (A)	Maximum temp. (B)	(B-A)	Summation of (B)-(A)
PS	38.77 ± 0.62	39.17 ± 0.81	0.40 ± 0.06	0.90 ± 0.07
Com	38.79 ± 0.80	39.17 ± 0.57	0.38 ± 0.05	0.85 ± 0.087
LSD value	1.392 NS	1.168 NS	0.120 NS	0.209 NS
NS: Non-significant.				

There were significant (P<0.05) differences in summation of (B)-(A) between of the purified local Vi polysaccharide and Com vaccine. Vaccines safety because its ≤ 1.4 º C (WHO, 2015) and whose safety, immunogenicity and efficacy most that the agreement with Fanning, (1997).

B. Chemical test:

- Protein content: According to requirement for Vi PS typhoid vaccine of WHO, 1994 the protein content of local and com. Com. vaccine was less than 10 mg per gram of PS, the current result of Vi PS vaccine was 0.50 mg per gram of PS. These differences in protein content were due to used of different method in extraction and purification of PS. The WHO has identified the prescribed percentage of protein and whether the protein was less than 10mg per gram increased proportion of them will be considered as contamination in vaccine.

- Phenol content: The Phenol content in preservative determination by two technique (HPLC and GC) according to WHO, 1994 protocol that should be each single dose content about (0.92-1.25) mg/dose. The result of phenol content by GC for Com. vaccine 1.20 mg/dose and local typhoid PS vaccine 1.15mg/dose while result in HPLC were Com. vaccine 1.17 mg/dose and local typhoid PS vaccine 1.14 mg/dose respectively**. Figure (3- 1 and 2)**. These results were slightly similar as mentioned in WHO protocol in typhoid vaccine (as preservative).

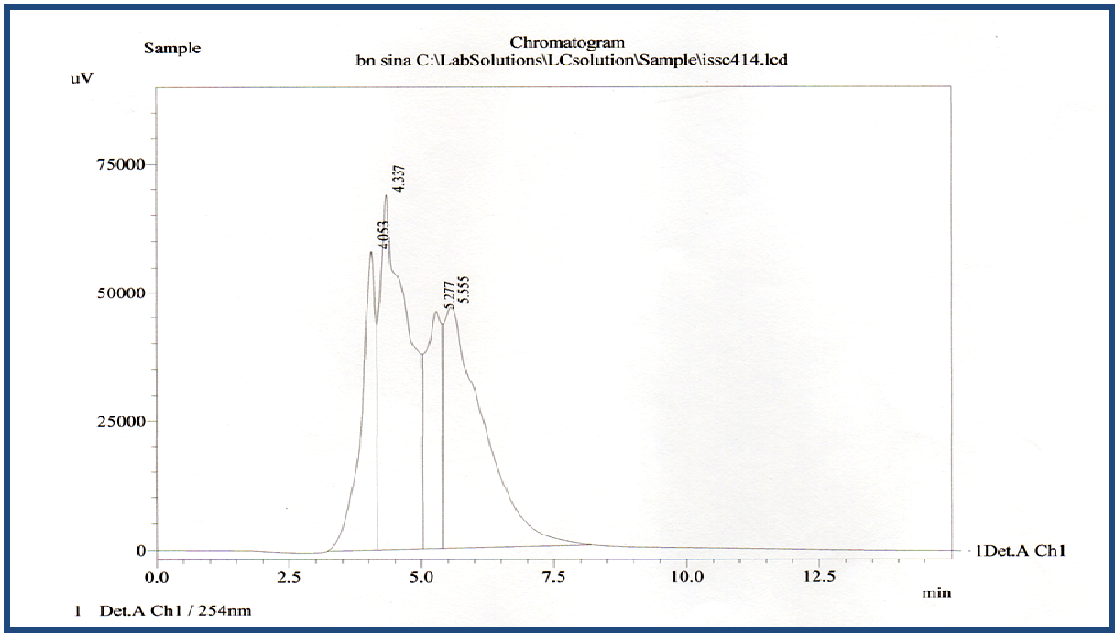

Figure (3-1): The result of phenol content in local typhoid vaccine using HPLC technique.

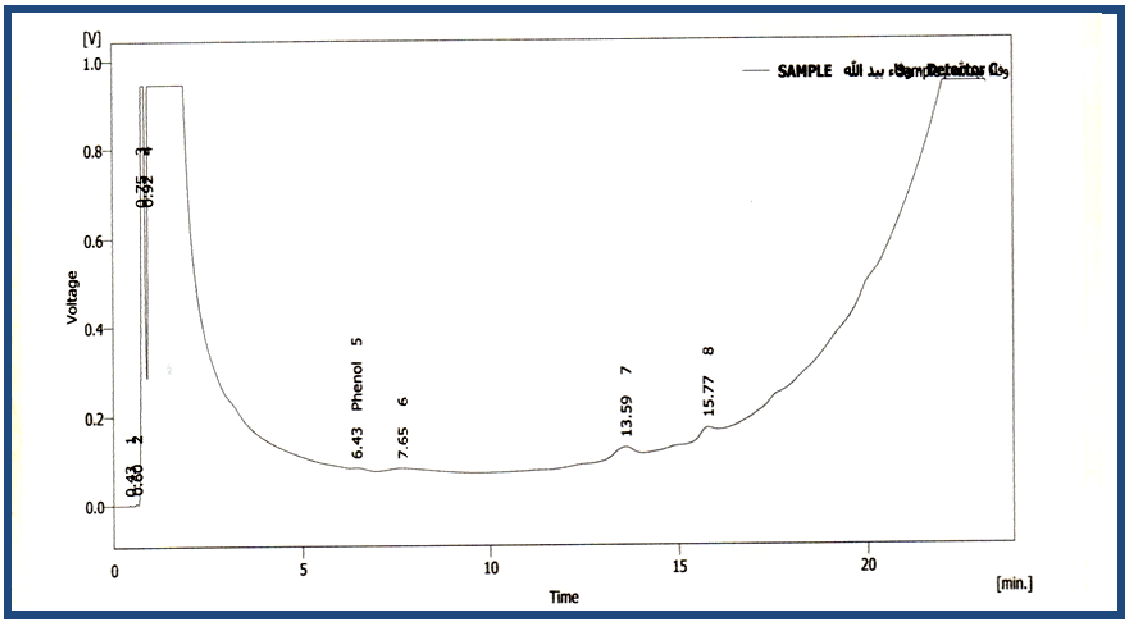

Figure (3-3): The phenol content in Com. vaccine by GC.

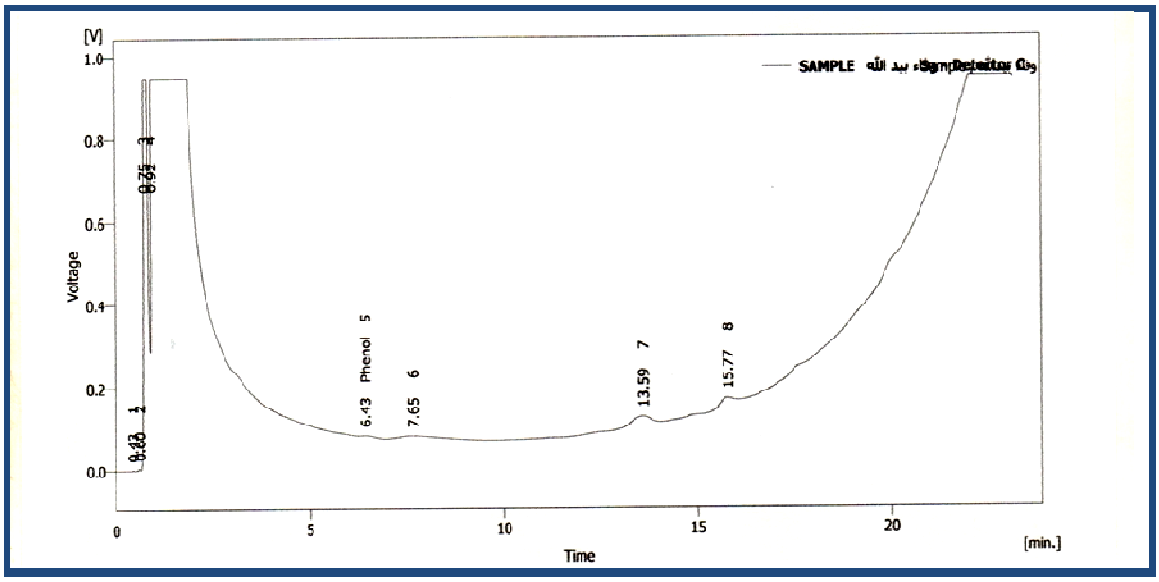

Figure (3-4): Result of phenol standard by GC.

- Residual formaldehyde: The **Figures (3-5, 6)** were showed results of residual formaldehyde content in Com. and local typhoid PS vaccine was < 0.17µg/dose into com. vaccine while free residual from formaldehyde on local typhoid PS vaccine, because of company of Com. vaccine using formaldehyde while the current study using phenol for killing bacteria to obtained Vi antigen for production local typhoid vaccine. The result agree with WHO protocol that safety rate of residual formaldehyde in the vaccine should be ≤100 µg/dose (WHO1994).

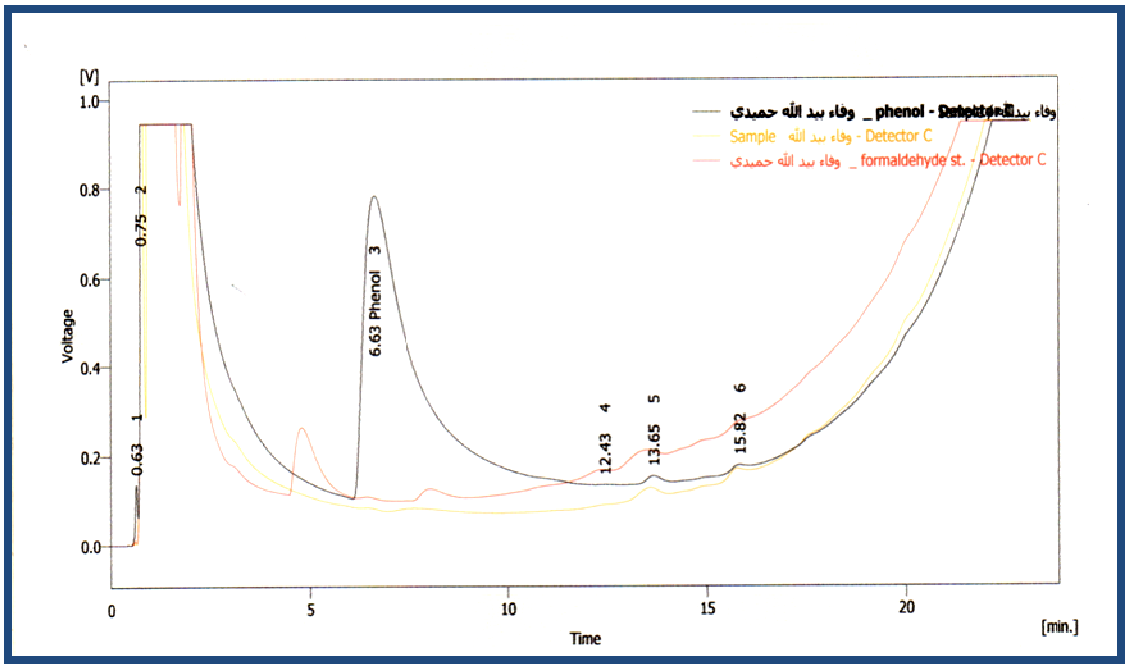

Figure (3-5): The result of standard Formalin and Phenol comparative with local typhoid PS vaccine by GC.

Gudlavalleti *et al*.,(2015) mentioned that the ability to accurately measure and report trace amounts of residual formaldehyde impurity in a vaccine product is not only critical in the product release but also a regulatory requirement, in many bacterial or viral vaccine manufacturing procedures , formaldehyde is used either at a live culture inactivation step or at a protein de-toxification step or at both.

Although there is no clear guidance limit on HCHO in human vaccines, USFDA has recently published a risk assessment on residual formaldehyde in infant vaccines by a remarkable pharmacokinetic modeling approach, Mitkus *et al*., (2013) indicating exogenously applied formaldehyde content (anywhere

between (0.4 and 100) µg per 0.5 ml dose) of current infant vaccines is safe.

Moreover since formaldehyde is not easily ionizable , sensitive techniques such as gas chromatography or mass spectrometry could not be applied to micro-analyze this molecule (Gudlavalleti *et al*., 2015).

A reverse phase HPLC method for the determination of low level formaldehyde in a solid drug substance was reported by (Soman *et al*., 2008).

- PH of vaccine: The PH result to Com. and local Typhoid PS vaccine ranges between 7± 0.5 according as mentioned in WHO protocol typhoid vaccine, (1994). pH (acidity and alkalinity) is from the factors affecting stability of the vaccines, and used to help the vaccine maintain its effectiveness during storage, vaccine stability is essential, particularly where the cold chain is unreliable, therefore instability can cause loss of antigenicity and bacterial vaccines can become unstable due to hydrolysis and aggregation of protein and carbohydrate molecules, agree with Sun and Köditz, (2012); WHO, (2014).

C. Physical test:

Inspection of final vial**:** the local typhoid PS vial vaccine in each final lot after closing was a clear, colorless liquid and free from visible particles, these results agreed with requirement WHO of typhoid vaccine. (WHO,1994**).**

- Shaped molecule vaccine: The determination of shaped molecule vaccine of particle size by Olympus under 100Xto compare between local typhoid PS vaccine and com. The result showed diffirences in the particale sizes between them ,that all particles were regular shaped in local typhoid PS vaccine, while irregular shaped in com. vaccine .**Figure (3-8) and Figure (3-9)** respectively.

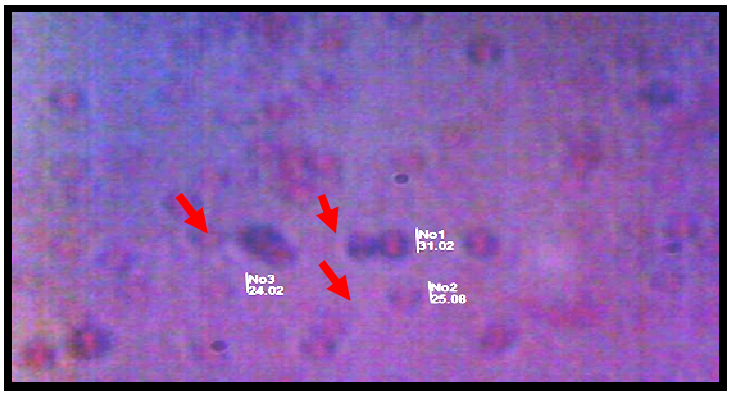

Figure (3-8): Partical size Vi PS. typhoid vaccine under 100X.

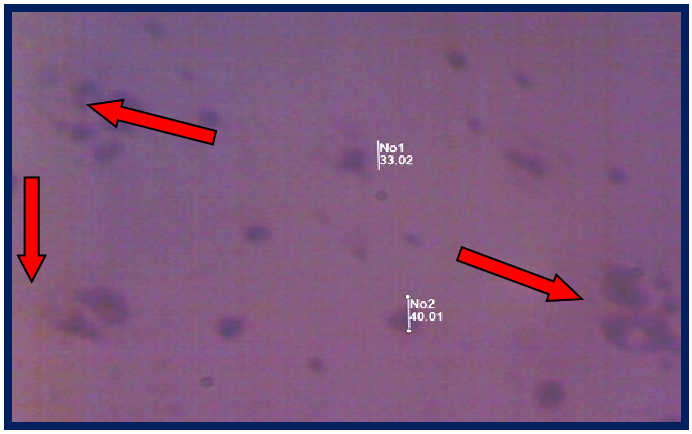

Figure (3-9): Partical size of Com. vaccine under 100X.

These results were due to using high speed centrifugation during extraction of Vi PS that causes adherence in particle vaccine with themselves, that's notice when preparation local vaccine then doing treatment by using sonicator. The smaller PS particles used to deliver antigen and the stronger antigen-specific against typhoid fever and effective to the same degree as immune adjuvant, Therefore the particles of local vaccine were uniform shaped, these mentioned by (Oyewumi *et al.* 2010; Joshi *et al.*, 2013).

D. Result of Immunological test

1. Slid agglutination test: Slid agglutination test was used by polyvalent sera against antigens of Salmonella, as the results showed that the bacteria belonging to the genus *S.* Typhi gave a positive result for the agglutination test react through one min. indicate that positive reaction, as it is characterized by serological diagnosis performed easily and speed of the results obtained this agreed with (Collins *et al*., 1987; Wattiau *et al*., 2008).

2. Evaluation of Immune Response

A. Humeral immune response: All immunized groups (5 guinea pigs from each group) were revealed humeral response in varied degrees as shown in **Table (3-2).** Antibody titers between group PS vaccine against *S.* Typhi showed significantly differences (P<0.05), that means of antibodies titers were increased immunized between groups and significantly (P<0.05) differences between doses respectively**.**

Then good significant differences (P<0.05) was shown between first and booster dose in the local Typhoid PS V. titration as (5520.20 ± 342.13) and (9513.40 ± 881.85) respectively. **Figure (3-10)**

Com. vaccine was shown less titration than the local vaccine (Typhoid PS) as (3920.80 ± 3889.78) and (5578.40 ± 568.68) in first and booster dose respectively **Figure (3-11)**. Control group that injected PBS haven't immune response (223.20 ± 27.91) and (242.80 ± 48.31) at first and booster dose respectively. **Figure (3-12)**

Table (3-2): The result of humeral immune response using ELISA

Groups	Mean ± SE		LSD value
	After 15 days from 1st Dose	After 15 days from Booster Dose	
local Typhoid Vi PS V.	5520.20 ± 342.13	9513.40 ± 881.85	1092.48 *
Com. Typhoid	3920.80 ± 389.78	5578.40 ± 568.68	972.41 *
Control (PBS)	223.20 ± 27.91	242.80 ± 48.31	32.95 NS
LSD value	921.84 *	1392.69 *	----
* (P<0.05), NS: Non-significant.			

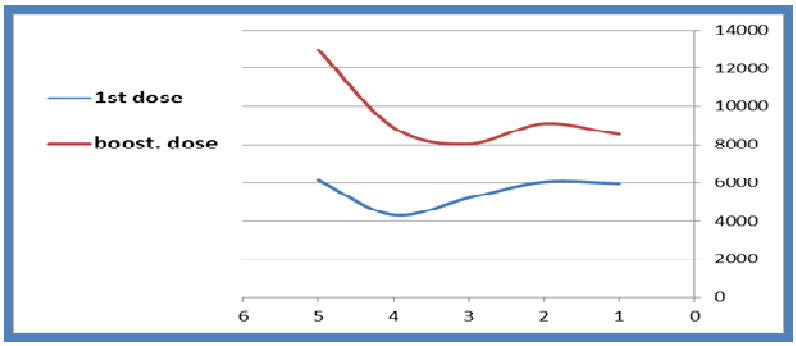

Figure (3- 10): Antibodies' titration after first and booster dose of Typhoid PS local vaccine.

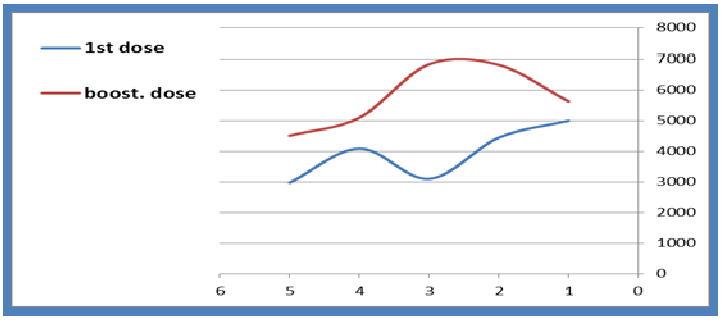

Figure (3- 11): Antibodies' titration after first and booster dose of Com. Vaccine.

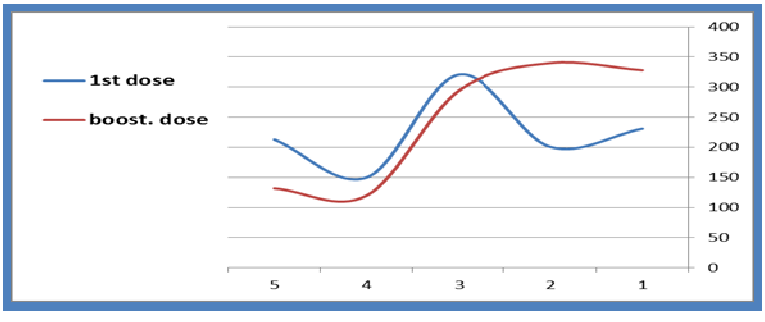

Figure (3- 12): Antibodies' titration after first and booster dose of Control (PBS).

When need explanation the current results, it should be know protective antibodies develop within 15 days after the booster dose PS is a component of the gram-negative eubacterial cell wall which act as a toxin, because it's part of the bacterial cell structure it's called endotoxin, toxicity is associated with the lipid portion of the LPS molecule, although all gram-negative eubacteria have LPS in their cell walls, LPS is not toxic unless its released from the outer layer of the cell. When gram-negative bacteria die, their cell walls disintegrate, releasing the LPS toxin. Some growing gram-negative bacteria also release LPS toxin due to sloughing or blabbing of outer membrane; in these cases, the LPS can have a toxic effects on a host organism (Bates, 2007).

Result of local typhoid PS and com. vaccine, local typhoid PS was few above immune response titration from com. vaccine. This can be because local typhoid PS production from local strain that be stronger than from standard strain that used to production com. vaccine, in addition occurred in Iraq seasonal change (temperature height in summertime), Al-Karawiy, (2008) host resistance, drug resistance strain and particles size of local Typhoid PS vaccine less than the particles size of commercial PS vaccine, the smaller the particle size of vaccine may supports the immune response increased immunization. (Joshi *et al.,*2013).

3. Cellular immune response:

Total white blood cells (WBCs) count:

Table (3-3) was showed results of G. pigs groups post vaccination after 15 days from first dose were (9.260 ± 0.82 and 9.050±0.69) cells /mm^3. Blood in the Typhoid PS local V., Com. V. respectively. The result showed no significant increase in total WBCs count as compared with the control group (6.255± 0.42) cells/ mm^3. Blood, but the results of guinea pigs groups post vaccination after 15 days from booster dose were (10.200 ± 0.749 and 9.800±0.79) cells /mm^3. Blood in the Typhoid PS local V. and Com. V. respectively, while the control group (6.350± 0.51) cells/ mm^3. Blood.

Table (3-3): Total WBCs Cells /mm^3. Blood in vaccinated G. pigs post first and booster dose of vaccines.

uType of Vaccine• Mean Type of Vaccine	Mean of Total WBCs Cells /mm^3. Blood		
	After 15 days from 1st Dose	After 15 days from Booster Dose	LSD value
PS	9.260 ± 0.82	10.200 ± 0.74	0.866 *
Com	9.050 ± 0.69	9.800 ± 0.79	0.891 NS
Control	6.255 ± 0.42	6.350 ± 0.51	0.503 NS
LSD value	1.6628 *	2.1755 *	----
* (P<0.05), NS: Non-significant.			

The result showed no significant different in total W**BC** count of Com. Vaccine and control group between first and booster dose. Each groups of vaccines in first dose or booster dose have significant different (P<0.05). LPS can trigger the complement cascade by the alternative pathway, endotoxin interacts with the blood clotting system and gave physiological effectiveness may result in the inappropriate clotting of blood in the peripheral vasculature, Rietschel and Brade, (1992). In addition WBC are considered as the active cells in starting out the functions of the immune system, both non-specifically and specifically, and their count may give a general picture about the function of the immune system (Lydyard and Grossi, 1998). Due to these diverse immunological functions, the normal counts of WBC total can be deviated by infections (Ad'hiah *et al.,* 2002). In agreement with such them, both counts total of leukocytes were deviated in the present study. In a more recent estimation, the range was (5000-12000) cells/ mm^3.blood (McGarry *et al.,* 2010).

The **Table (3-3)** was showed of counts of WBC total at first dose were less than from booster dose of vaccines after collection blood from vaccines group primed with PS the total WBCs counts was significantly (P<0.05) increased as compared with control and Com. group, Com. V. group (9.800± 0.79 cells/ mm^3. blood) and control group (6.350± 0.51 cells/ mm^3. blood); an observation that may suggest that Typhoid local Vaccine may able to enhance the adaptive immunity. Such suggestion has some support from a study carried out by (Mahieu *et al.,* 2006; Dejager *et al.,* 2010 ; AL- Shibbani and Alkhozai , 2015).

3.3. Result of lethal dose of half animal (LD50) and challenge test:

The result of LD50 of *S.* Typhi was 10^6 CFU/ml as shown in **Table (3-4)**.

Table (3-4): The results of lethal dose (LD_{50}) for *S.* Typhi

Dilutions of *S.* Typhi culture (10^3-10^7) cfu/ml	No. of mice per group	Injected Volume (i.p.) per ml	No. of live and death during 15 days period of observation		Cumulative numbers		mortality %
			Live	Dead	live	dead	
10^3	10	0. 25	10	-	2	8	80
10^4	10	0. 25	8	2	6	14	70
10^5	10	0. 25	6	4	12	18	60
10^6	10	0. 25	4	6	20	20	50
10^7	10	0. 25	2	8	30	20	40
Control*	10	0. 25	10	-	-	-	-
LSD value	---	---	12.794 **	12.794 **	---	---	9.316 **

Control*: Normal Saline. ** (P<0.01)

Post 15 days from booster dose of vaccines, the challenge dose was $100LD_{50}$ (10^8 CFU/ml) that was injected intra-peritoneally into G. pigs groups (vaccination and control groups) as the following table:

Table (3-5): The results of challenge test for local prepared and com. vaccines in G. pigs.

Groups	No. of animal challenged (i.p) per 0.25 ml	No. of survival to challenged and infected /death during 3 days period of observation		survival %
		survival	Death	
Vi PS	10	8	2	80
COM	10	7	3	70
Control	10	0	10	0
Chi-square	---	--	--	14.072 **
** (P<0.01)				

The result were showed 80% and 70% ratio protected in PS and Com groups respectively and resisted with the virulent challenge exposure against *S.* Typhi organism but G. pigs of unvaccinated control group were recorded as dead which was similar to Mastroeni *et al., (*1993); Venkatesan *et al.,* (2011).

Control group showed depression, loss of appetite, severe diarrhea, and positive feacal culture. Before death they showed signs of dehydration, recumbence with shallow respiration and weak pulse. Death occurs within (3) days post challenge as the **Table (3-5)**. Animals of the two groups survived the challenge exposure, and only during the first week post challenge the feacal cultures were positive to *S.* Typhi.

Animals of the four groups that survived the challenge exposure were slightly depressed with normal appetite and normal feaces, and only during the first week post challenge the feacal cultures were positive to *S.* Typhi.

- Conclusions: The possibility of using PS Typhoid Vaccine from local strain as vaccine against typhoid fever increasing the immunization ability more than commercial vaccine. We can reproduction of Iraqi typhoid vaccine (Killed vaccine) that was produced in VSI and stopped since 2003 by using new vaccine that recommended by WHO for humane immunization of typhoid fever.

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