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A human IgG anti-Vi reference for *Salmonella typhi* with weight-based antibody units assigned

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Abstract

Recent data showing the high incidence of typhoid fever in young children, the demonstration of safety and efficacy of a Vi conjugate for this age group, the safety and similar immunogenicity in infants when administered concurrently with EPI vaccines, together with the interests of manufacturers and investigators in studying such conjugate vaccines prompted us to prepare a human IgG anti-Vi standard to facilitate this work. Volunteers were injected with an investigational Vi-recombinant *Pseudomonas aeruginosa* exoprotein A (Vi-rEPA) conjugate vaccine. Plasmas with the highest levels of IgG anti-Vi were pooled. The IgG anti-Vi content of this preparation, assayed by precipitin analysis with purified Vi, was 33µg/ml. Accordingly, the estimated IgG anti-Vi protective level of 3.5 ELISA unit/ml, derived from our efficacy trial of Vi-rEPA in 2-to-5 years old children, is equivalent to 4.3µg/ml. This reagent is suitable for comparison of immune response of Vi conjugate vaccines or for other purposes requiring anti-Vi titration.

1. Introduction

Typhoid fever, a serious and common disease in developing countries, has become difficult to treat because of increasing resistance of *Salmonella typhi* to commonly used antibiotics [1-3]. Typhoid causes considerable morbidity and mortality, especially in young children [4,5]. A unique complication of typhoid is prolonged asymptomatic gall bladder infection, which may lead to malignancy and serve as a source of infection to others [6,7].

Vi capsular polysaccharide (Vi) is a licensed vaccine in more than 90 countries; its production includes western and Asian manufacturers [8-11]. However the efficacy of Vi in children < 5 year-olds is still questionable [12, unpublished data]. A Vi protein conjugate, Vi-rEPA, was shown to be safe and confer ~90% efficacy for 4 years in 2-to-5 years old children in a high endemic region of Vietnam [13-16]. A protective level of 3.5 ELISA Units (EU) anti-Vi IgG was estimated from this study based on the geometric mean (GM). Additional studies in this age group showed the dosage of 25 µg of Vi-rEPA to be optimal

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[16]. A recent study of Vi-rEPA, administered to healthy infants in Vietnam concurrently with their routine EPI vaccines (DTP+ OPV+ HepB), elicited protective levels of IgG anti-Vi antibody in approximately 95% of the vaccinees. No interference with responses to the EPI vaccines was found [17]. These findings raised interest in investigation and production of Vi-conjugates [18-21].

To evaluate new Vi-based vaccines, it is necessary to quantitate accurately serum Vi antibodies. ELISA has been the most common method used to determine levels of anti-Vi [22-24]. Evaluating vaccine trials successfully depends on the availability of a standardized human reference. Here we describe the preparation and characterization of a human reference, whose level of IgG anti-Vi was determined by quantitative precipitation with a purified Vi, as was done for *Haemophilus influenzae* type b, *Streptococcus pneumoniae* and *Neisseria meningitidis* [25-28].

2. Materials and Methods

2.1 Vaccination

The study protocol was approved by the Internal Review Board of Lanzhou Institute of Biological Products (LIBP), China. Vi conjugated to recombinant exoprotein A of *Pseudomonas aeruginosa* (Vi-rEPA, lot 060901) was prepared by LIBP. The vaccine contained 24µg Vi, bound to 26µg rEPA, in 0.5 ml dose and passed the required safety tests by the Chinese Sino Food Drug Administration. Eight healthy volunteers, ages 18-40, were recruited, the purpose of the study explained, informed consent signed and blood samples taken for screening. All volunteers were negative for hepatitis B surface and core antigens, had no antibodies to hepatitis C, gonorrhea, syphilis, human T-cell leukemia virus type 1 and human immunodeficiency virus 1 and 2. Before injection, a physician or a nurse gave physical examinations to the volunteers and measured their temperatures. Those, whose temperature was $\leq 37.5^{\circ}\text{C}$ and were not ill, received an IM injection of Vi-rEPA and their temperatures and local reactions recorded at 30 min, 6, and 24 h post immunization.

2.2 Plasma preparation

Six weeks after immunization, a blood sample was taken from each volunteer and anti-Vi IgG measured by ELISA (vide infra). Volunteers with levels higher than 30 ELISA units (EU) were asked for a plasmapheresis within 10 days. Five plasmas with the highest IgG anti-Vi were pooled, de-identified, freeze-dried and stored at -70°C (Lot 091010). The plasma pool was screened again at the NIH and confirmed to be free of blood transmissible viral diseases by molecular testing [29].

2.3 IgG purification

The freeze-dried plasma was reconstituted in pyrogen-free water (Baxter) at 100 mg/mL, centrifuged twice at $15,200 \times g$ (RC6 SS-34, Sorvall,) at 4°C for 2h, the precipitate discarded and the supernatant stored at -70°C . IgG was purified by octanoic acid precipitation (minimum 99%, Sigma Aldrich) followed by DEAE-cellulose chromatography (Sigma Aldrich) [30,31]. The DEAE-cellulose eluent (20 mM Na-phosphate buffer at pH7.5) was assayed for IgG anti-Vi by ELISA and the first peak, containing the antibody was pooled, sterile filtered (0.45µm low protein binding, Millipore), stored at 4°C and designated as Vi-IgG_{R1}.

For final container bottling, 1% sucrose was added to reduce water sorption, stabilize protein structure integrity, prevent physical collapse during freeze drying cycle and for ease of solubility upon reconstitution. Ten percent sucrose was sterile filtered (0.22µm low protein binding, Millipore) and added to Vi-IgG_{R1} to final concentration 1% sucrose, sterile filtered,

aliquot in 1.12 ml and freeze dried. Each vial contains 1 ml of Vi-IgG_{R1} and 0.1 ml sucrose. The extra 0.02 ml was for possible adherence to the vials.

2.4 Vi preparation

To avoid the potential interference of group D lipopolysaccharide from *S. typhi*, Vi was purified from *Citrobacter freundii* strain WR7011, whose capsular structure is identical to that of the Vi from *S. typhi* [13]. Vi purified contained <1% protein and nucleic acids, had no detectable endotoxin (<0.0001 endotoxin unit per mg) and stored as a freeze-dried powder at -40°C [11,18]. Before use, the Vi was freeze-dried again for 3 days to remove residual moisture. A stock solution was prepared by swelling the Vi with a small amount of PBS at 4°C overnight, brought to 2.0 mg/ml in PBS, and stored at 4°C.

2.5 Quantitative Precipitation with Vi polysaccharide

Quantitative precipitin analysis followed published procedures [32]. Briefly 3 ml aliquots of plasma were delivered to conical glass vials and mixed with 100 µl of PBS containing 1 to 200 µg Vi polysaccharide. Controls were 100 µg Vi in 3.1 ml of PBS or plasma alone plus 100 µl of PBS. All samples were in duplicates. The vials were capped and placed at 37°C for 1 h, then 4°C for 5 days during which they were gently mixed twice daily. The vials were centrifuged (2,800 × g, Sorvall Legend RT) at 4°C for 1 h, the supernatants decanted and inverted to drain for 1 h. The precipitates were washed 3 times with 1 ml cold sterile saline, centrifuged at 2,800 × g for 1 h and the supernatants decanted and tested for residual Vi antibody (vide infra). The precipitates were dried at room temperature and 1.0 ml of 1% sodium dodecyl sulfated added to each vial followed by gentle mixing. The precipitates were allowed to dissolve at room temperature until no particles were visible. The protein concentration was determined by UV absorption at 280 nm using the extinction coefficient =13.8 for human immunoglobulin and by colorimetric reaction with bicinchoninic acid (Pierce) using bovine serum albumin (BSA) (Pierce) as a reference [25,26,33].

2.6 ELISA for anti-Vi

Vi (1 mg/ml in saline) was stored at -40°C. Plates (Nunc Maxisorp, ThermoFisher) were coated with Vi (100µl of 2µg/ml in PBS pH7.4), sealed, incubated at RT overnight, and washed (0.85% NaCl, 0.1% Brij 35, 0.02% NaN₃). The coated plates were blocked at 25°C for 2 h with 1% BSA in PBS, filtered through 0.45 µm SFCA membrane (Millipore). Serum samples were diluted in dilution buffer (1% BSA, 0.1% Brij35, PBS). An in-house Vi reference serum (Vi-IgG_{NIH}) from a volunteer injected with Vi was assayed in parallel on each plate [14-17]. Ten serial 2-fold dilutions of each serum were carried out on the plate. The plates were covered and left at 25°C overnight. Murine MAb specific for human IgG (HP6043, CDC), IgM (HP6083, CDC) and IgA (HP6104, CDC) were used as the second antibodies; diluted 1:4000 for IgG, 1:2000 for IgM and IgA. The plates were incubated at 25°C for 5 h, 100 µl/well of alkaline phosphatase labeled rat anti-mouse IgG (Jackson Immuno Research) added, and incubated at 25°C overnight. Lastly the substrate, 4-nitrophenyl phosphate disodium salt hexahydrate (Fluka) in 1 M Tris, 3 mM MgCl₂, pH 9.8 was added.

Plates were read at 405 nm after 20 to 40 minutes or when the OD reading of the highest concentration reached approximately 2.0 to 3.0. IgG anti-Vi levels were computed with an ELISA data processing program provided by the Biostatistics and Information Management Branch of the Centers for Disease Control and Prevention [34]. The titration curves, used for computation by linear regression fit, were compared with the in-house Vi reference Vi-IgG_{NIH} assigned a value of 75 EU [14-17]. Antibody concentrations with a mean standard deviation < 15% were accepted.

2.7 Characterization of Vi-IgG_{R1}

Immunodiffusion between Vi (10 μ l at 200 μ g/ml) and reconstituted plasma (20 μ l) was conducted in 1% agarose (Invitrogen) in PBS (pH 7.2) and stained with Coomassie blue.

The molecular weight of the Vi-IgG_{R1} (0.07 μ g to 0.7 μ g/loading) was analyzed by 10% SDS-PAGE Bis-Tris gels, stained with Coomassie Blue and compared to protein molecular standards (Invitrogen). Western blots with goat anti-human IgG (Sigma Aldrich) was performed for identification of IgG.

2.8 Verification of antibody concentration of Vi-IgG_{R1} in the final container

Five vials of bottled Vi-IgG_{R1} were reconstituted in pyrogen free water (1 ml/vial). Samples were removed from each of the 5 vials and analyzed for IgG anti-Vi concentration by ELISA, repeated 12 times per vial on 6 separate plates on different dates. The levels of IgG anti-Vi in the vials were evaluated, mean standard deviation calculated, and the GM compared with those obtained before and after addition of 1% sucrose, before and after bottling. The excessive amount of reconstituted Vi-IgG_{R1} was aliquot into micro tubes with rubber-seal caps to prevent lost of moisture and stored at -35°C .

Samples of Vi-IgG_{R1} from the final container stored at 4°C for 9 months and from aliquots stored at -35°C for 6 months were removed for preliminary stability study by ELISA.

3. Results

3.1 Quantitative precipitation and ELISA of Vi antibodies

Vi-IgG_{R1} showed a characteristic precipitation curve (Fig.1). Maximal precipitation occurred at 1.6 μ g Vi/ml measured by both protein assays. The concentration of IgG anti-Vi at the equivalence zone was 33 μ g/ml.

Assayed by ELISA along side with our in-house standard Vi-IgG_{NIH}, Vi-IgG_{R1} contained 26.57 ± 1.2 EU of IgG anti-Vi. Thus 1 EU is equal to 1.24 μ g/ml. Vi-IgG_{R1} had non-detectable IgM and IgA at the dilution used for the IgG ELISA measurements.

3.2 Characterization of Vi-IgG_{R1}

There was a faint line of precipitation between the plasma and Vi in immunodiffusion (not shown). The molecular weight of Vi-IgG_{R1} was ~ 150 KD in SDS-PAGE (Fig. 2a). At 10 times the loading concentration, another faint band (at > 188 KD) was visible. Both bands reacted with goat anti-human IgG by western blot.

3.3 Validation of the antibody concentration in the Vi-IgG_{R1} final container

After reconstitution with pyrogen free water, the Vi-IgG_{R1} in the final container dissolved instantly and the solution appeared clear. The GM IgG anti-Vi of the 5 vials tested was 32.79 ± 1.21 μ g/mL, similar to that before bottling (32.95 ± 1.49 μ g/mL). The variation among vials were $< 9\%$ which is within the experimental error (Table 1). The GM IgG anti-Vi level before and after addition of 1% sucrose were 32.95 μ g/mL and 31.91 μ g/mL respectively, also within the experimental error. Thus the addition of 1% sucrose or freeze-drying did not change the level of Vi antibody in Vi-IgG_{R1}.

The preliminary stability study of Vi-IgG_{R1} in the final container stored at 4°C for 9 months or in aliquots stored at -35°C for 6 months showed no obvious degradation in the level of IgG anti-Vi (Table 1)

4. DISCUSSION

In line with human anti-capsular reference sera for Hib, pneumococcal or meningococcal vaccines, we prepared for *S. typhi* anti-capsular human reference. This new global reference should facilitate immunogenicity comparison and evaluation for typhoid Vi conjugate vaccine. We prepared Vi human reference anti-IgG (Vi-IgG_{R1}) from pooled plasma of volunteers immunized with Vi-rEPA. Quantitative precipitin analysis showed that Vi-IgG_{R1} contained 33 µg/ml of anti-Vi. Calibrating with the NIH in-house reference serum used in our Vi conjugate clinical trials, whose IgG anti-Vi was measured by ELISA, the converting factor is 1 EU=1.24 µg. Accordingly, the estimated protective level of 3.5 EU/ml IgG anti-Vi, derived from the geometric mean of the 2-3 years old group in our efficacy trial of Vi-rEPA in Vietnam, is 4.34 µg/ml [15,16]. This value is likely over estimated since there were no cases of typhoid fever in the 2-3 year-olds (the younger age group) during the last 2 years of the trial and nearly half of the children had less than this geometric mean level, yet with the efficacy was >84%. This level is higher than the proposed protective level of 0.6 to 2.0 µg/ml based on the Vi polysaccharide trial in South Africa measured by radioimmunoassay [8]. The discrepancy is probably due to different serologic assays, antibody standards, and over estimation from the efficacy trial in Vietnam [16].

Asymptomatic *S. typhi* carriers remain to be a source of transmission and their detection is important for typhoid control [35]. Carriers harbor *S. typhi* in the biliary tract for prolonged periods and have high levels of anti-Vi. Measuring serum anti-Vi level is a reliable way to screen for carriers. Most investigations used passive hemagglutination or ELISA to estimate the level of Vi antibodies [35-37]. Without referring to a common reference, analyses done in different laboratories may not be comparable and there is no consensus on the range of Vi antibodies in carriers. Quantitative analyses of serum IgG anti-Vi levels from individuals with acute and chronic *S. typhi* infections should provide more reliable information.

A standard serum with an assigned weight-based antibody concentration would allow inter-laboratory comparisons of the levels and duration of “natural”, disease- and vaccine-induced serum IgG anti-Vi or to correlate with other assays such as functional assay [38].

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References

1. Crump JA, Luby SP, Mintz ED. The global burden of typhoid fever. Bull World Health Organ. 2004;346–53. [PubMed: 15298225]
2. Ochai RL, Acosia CJ, Danovaro-Holiday MC, Baiqing D, Bhattacharyya SK, Agini MD, et al. the DOMI Typhoid Study Group. A study of typhoid fever in five Asian countries: disease burden and implications for control. Bull WHO. 2008; 86:260–8. [PubMed: 18438514]
3. Parry CM, Ho VA, Phuong le T, Bay PV, Lanh MN, Tung le T, et al. Randomized controlled comparison of ofloxacin, azithromycin, and an ofloxacin-azithromycin combination for treatment of multidrug-resistant and nalidixic acid-resistant typhoid fever. Antimicrob Agents Chemother. 2007; 51:819–25. [PubMed: 17145784]
4. Sinha A, Sazawal S, Kumar R, Sood S, Reddaiah VP, Singh B, et al. Typhoid fever in children ages less than 5 years. Lancet. 1999; 354:734–7. [PubMed: 10475185]
5. Brooks WA, Hossain A, Goswami D, Nahar K, Alam K, Ahmed N, et al. Bacteremic typhoid fever in children in an urban slum, Bangladesh. Emerg Infect Dis. 2005; 11:326–9. [PubMed: 15752457]

6. Kelly-Hope LA, Alonso WJ, Thiem VD, Anh DD, Canh do G, Lee H, et al. Geographical distribution and risk factors associated with enteric diseases in Vietnam. *Am J Trop Med Hyg.* 2007; 76:706–12. [PubMed: 17426175]
7. Dutta U, Garg PK, Kumar R, Tandon RK. Typhoid carriers among patients with gallstones are at increased risk for carcinoma of the gallbladder. *Amer J Gastroenterol.* 2000; 97:784–7. [PubMed: 10710075]
8. Klugman KP, Koornhof HJ, Robbins JB, Le Cam NN. Immunogenicity, efficacy and serological correlate of protection of *Salmonella typhi* Vi capsular polysaccharide vaccine three years after immunization. *Vaccine.* 1996; 14:435–43. [PubMed: 8735556]
9. Acharya IL, Lowe CU, Thapa R, Gurubacharya VL, Shrestha MB, Bryla DA, et al. Prevention of typhoid fever in Nepal with the Vi capsular polysaccharide of *Salmonella typhi*: A preliminary report one year after immunization. *N Engl J Med.* 1987; 317:1101–4. [PubMed: 3657877]
10. Yang HH, Wu CG, Xie GZ. Efficacy trial of Vi polysaccharide vaccine against typhoid fever in south-western China. *Bull World Health Organ.* 2001; 79:625–31. [PubMed: 11477965]
11. Requirements for Vi polysaccharide typhoid vaccine. World Health Organization Technical Report Series. 1994; (840)
12. Khan MI, Soofi SB, Ochiai RL, Habib MA, Sahito SM, Nizami SQ, et al. DOMI typhoid Karachi Vi effectiveness study group. Effectiveness of Vi capsular polysaccharide typhoid vaccine among children: A cluster randomized trial in Karachi, Pakistan. *Vaccine.* Jun 15.2012 in print.
13. Szu SC, Taylor DN, Taylor AC, Trofa JD, Clements JD, Shiloach JC, et al. Laboratory and preliminary clinical characterization of Vi capsular polysaccharide-protein conjugate vaccines. *Infect. Immun.* 1994; 62:4440–4. [PubMed: 7927707]
14. Kossaczka Z, Lin F-Y, Ho VA, Thuy NTT, Bay PV, Thanh TC, et al. Safety and immunogenicity of Vi conjugate vaccines for typhoid fever in adults, teenagers, and 2- to 4-year-old children in Vietnam. *Infect Immun.* 1999; 67:5806–10. [PubMed: 10531232]
15. Lin F-YC, Ho VA, Khiem HB, Trach DD, Bay PV, Thanh TC, et al. The efficacy of a *Salmonella typhi* Vi conjugate vaccine in two-to-five-year-old children. *N Engl J Med.* 2001; 344:1263–9. [PubMed: 11320385]
16. Lanh MN, Bay PV, Ho VA, Thanh TC, Lin F-YC, Bryla DA, et al. Persistent efficacy of Vi conjugates against typhoid fever in young children. *N Eng J Med.* 2003; 349:1390–1.
17. Thiem VD, Lin F-YC, Canh DG, Son NH, Anh DD, Mao D, et al. The Vi conjugate typhoid vaccine is safe, elicits protective levels of IgG anti-Vi, and is compatible with routine infant vaccines. *Clin Vaccine Immunol.* 2011; 18:730–5. [PubMed: 21411598]
18. van Damme P, Kafaja F, Anemona A, Basile V, Hilbert AK, De Coster I, et al. Safety, immunogenicity and dose ranging of a new Vi-CRM conjugate vaccine against typhoid fever: randomized clinical testing in healthy adults. *PLoS One.* 2011; 6:e25398. [PubMed: 21980445]
19. Micoli F, Rondini S, Pisoni I, Proietti D, Berti F, Costantino P, et al. Vi-CRM 197 as a new conjugate vaccine against *Salmonella Typhi*. *Vaccine.* 2011; 29:712–20. [PubMed: 21115057]
20. Cui CF, Carbis R, An SJ, Jang H, Czerkinsky C, Szu SC, et al. The physical chemical characterization and immunologic properties of *Salmonella* ser. *Typhi* capsular polysaccharide-diphtheria toxoid conjugates. *Clin Vaccine Immunol.* 2010; 17:73–9. [PubMed: 19889941]
21. Guide book on immunization. Individual Vaccines. Indian Academy of Pediatrics Vaccine. 2009:56–8. Chapter 11.
22. Ferry BL, Misbah SA, Stephens P, Sherrell Z, lythgoe H, Bateman E, et al. Development of an anti-*Salmonella typhi* Vi ELISA: assessment of immunocompetence in healthy donors. *Clin Exp Immunol.* 2004; 136:297–303. [PubMed: 15086394]
23. Pulickal AS, Gautam S, Clutterbuck EA, Thorson S, Basynat B, Adhikari N, et al. Kinetics of the natural, humoral immune response to *Salmonella enterica* serovar *typhi* in Kathmandu, Nepal. *Clin Vaccine Immunol.* 2009; 16:1413–9. [PubMed: 19710294]
24. Staats HF, Kirwan SM, Whisnant CC, Stephenson JL, Wagener DK, Majumder PP. Development of a bead immunoassay to measure Vi polysaccharide-specific serum IgG after vaccination with the *Salmonella enterica* serovar Typhi Vi polysaccharide. *Clin Vaccine Immunol.* 2010; 17:412–9. [PubMed: 20107010]

25. Gotschlich EC, Rey M, Triaux R, Sparks KJ. Quantitative determination of the human immune response to immunization with meningococcal vaccines. *J Clin Invest.* 1972; 51:89–96. [PubMed: 4621363]
26. Robbins JB, Whisnant JK, Schneerson R, Parke JC Jr. Quantitative measurement of the “natural” and immunization-induced *Haemophilus influenzae* type b capsular polysaccharide antibodies. *Pediatr Res.* 1973; 7:103–10. [PubMed: 4120458]
27. Quataert SA, Rittenhouse-Olson K, Kirch CS, Hu B, Secor S, Stong N, et al. Assignment of weight-based antibody units for 13 serotypes to a human antipneumococcal standard reference serum, Lot 89-S(F). *Clin Diag Lab Immunol.* 2004; 11:1064–9.
28. Holder PK, Maslanka SE, Pais LB, Dykes J, Plikaytis BD, Carlone GM. Assignment of *Neisseria meningitidis* serogroup A and C class-specific anticapsular antibody concentrations to the new standard reference serum CDC1992. *Clin Diag Lab Immun.* 1995; 2:132–7.
29. Epstein JS. Alternative strategies in assuring blood safety: an overview. *Biologicals.* 2010; 38:31–5. [PubMed: 20110174]
30. Harkins, KR. Antibody purification methods. In: Howard, GC.; Bethell, DR., editors. *Basic Methods in Antibody Production and Characterization.* CRC Press; 2000. p. 141-166.pp.141-66
31. Steinbuch M, Audran R. The isolation of IgG from mammalian sera with the aid of caprylic acid. *Arch Biochem Biophys.* 1969; 134:279–84. [PubMed: 4982185]
32. Kabat; Mayer. *Experimental Immunochemistry.* 2nd ed.. Charles C. Thomas; Springfield, IL: 1971. p. 72-5.
33. Brown R, Jarvis KL, Hyland KJ. Protein measurement using bicinchoninic acid: elimination of interfering substances. *Anal Biochem.* 1989; 180:136–9. [PubMed: 2817336]
34. Plikaytis BD, Goldblatt D, Frasch CE, Blondeau C, Bybel MJ, Giebink GS, et al. An analytical model applied to a multicenter pneumococcal enzyme-linked immunosorbent assay study. *J Clin Microbiol.* 2000; 38:2043–50. [PubMed: 10834951]
35. Gupta A, My Thanh NT, Olsen SJ, Sivapalasingam S, My Trinh TT, Phuong Lan NT, et al. Evaluation of community-based serologic screening for identification of chronic *Salmonella typhi* carriers in Vietnam. *Inter J Infect Dis.* 2006; 10:309–14.
36. Lanata CF, Ristola C, Jimenez L, Garcia G, Levine MW, Black RE, et al. Vi serology in detection of chronic *Salmonella typhi* carriers in an endemic area. *Lancet.* 1983; 1:441–2. [PubMed: 6131167]
37. Engleberg NC, Barrett TJ, Fisher H, Porter B, Hurtado E, Hughes JW. Identification of a carrier by using Vi enzyme-linked immunosorbent assay serology in an outbreak of typhoid fever on an Indian reservation. *J Clin Microbiol.* 1983; 18:1320–2. [PubMed: 6655039]
38. Saha SK, Baqui AH, Hanif M, Darmstadt GL, Ruhulamin M, Nagatake T, et al. Typhoid fever in Bangladesh: implications for vaccination policy. *Pediatr Infect Dis J.* 2001; 20:521–524. [PubMed: 11368111]

High lights

- A *S. Typhi* anti-capsular human reference serum (IgG anti-Vi) established
- An estimated protective level in $\mu\text{g/mL}$ against typhoid fever proposed
- The reference serum is available to regulatory agencies and global manufactures
- The human reference serum can facilitate Vi conjugate vaccine development.

Fig. 1.

Quantitative precipitin curve of Vi polysaccharide and IgG purified from plasma of volunteers immunized with Vi-*r*EPA. Anti-Vi concentration was determined by absorption at 280 nm. The peak value, using percent extinction coefficient =13.8 for immunoglobulin, is equivalent to 33µg/ml.

Fig. 2. Molecular weight and subclass antibody analysis of Vi-IgGR1 in precipitation with Vi*
* 1% SDS solution of Vi-IgGR1 and Vi precipitation at the peak of the quantitative precipitin analysis. SDS-Page of 10%Bis-Tris gel was loaded with 0.07 μ g (lane 2) or 0.7 μ g (lane 3) of the anti-Vi and molecular weight reference (lane 1); (2a) Coomassie blue stain, (2b) western blot with goat anti-human IgG, (2c) western blot with goat anti-human IgM.

Table I

Concentration of IgG anti-Vi of the Vi human reference serum Vi-IgG_{Ri} in the final container compared with those in the bulk measured before, during and after the bottling process

Final container (Vial #)	IgG Anti-Vi (µg/mL)
1	31.36 [*]
2	31.91 [*]
3	33.01 [*]
4	33.31 [*]
5	34.36 [*]
Average±SD	32.79±1.21
Bulk (no sucrose)	32.95 ⁺ ±1.49
Bulk (with 1% sucrose)	31.91 ⁺
Bulk (with 1% sucrose, centrifuged)	31.40 ⁺
Vial stored for 9 months, 4°C	32.61 ⁺
Aliquot store for 6 months, -35°C	31.22 [^]

^{*} Each represents GM of 12 ELISA repeat runs of the same sample

⁺ Each represents GM of 4 ELISA repeat runs of the same sample

[^] GM of 4 ELISA repeat runs of 3 aliquot samples