

## Immunization for the production of high titre hepatitis-B immunoglobulin: a study of two plasma derived vaccines.

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*The production of hepatitis B immunoglobulin requires a high titre of anti HBs in the starting plasma which could be obtained by booster vaccination to blood donors with known immunity to hepatitis B virus through natural infection. The study was carried out during March 1988-July 1989. Two plasma derived vaccines were used in this study, one of which was heat inactivated (Hepaccine B), the other was chemical inactivated (Hevac B Pasteur). The study consisted of 510 volunteered regular male donors who had not received vaccination before. They were randomly divided into 5 groups to receive Hepaccine-B (3 mcg) and Hevac-B Pasteur (5 mcg) in different schedules of vaccination. Blood samples were drawn from all study population before vaccination and after vaccination at 1, 3, 6, 9, 12, 15 months consecutively to detect antibody titres. The result showed that existing anti-HBs before vaccination determined the antibody response and hence played an important role in the production of high titre HBIG. Only donors who had anti-HBs titres of 0.62 IU/ml and up gave high yield of anti-HBs response after vaccination with either of the two vaccines, the study also showed that Hepaccine B gave slightly higher peak of anti-HBs than Hevac B pasteur in the same schedule (vaccination at 0, 1, 2, 9 months). Therefore hepaccine-B should be the first choice in the production of HBIG since its cost is lower.*

**Key words:** HBIG, Hevac B Pasteur, Hepaccine B

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 การผลิตอิมมูโนโกลบูลินต่อไวรัสตับอักเสบบีให้ได้ไตเตอร์สูงโดยพลาสมาดีโรพวักซันต่อไวรัสตับอักเสบบี  
 2 ชนิด. จุฬาลงกรณ์เวชสาร 2534 กรกฎาคม; 35(7): 423-428

การผลิตอิมมูโนโกลบูลินต่อไวรัสตับอักเสบบี (HBIG) เพื่อให้ได้ไตเตอร์สูงนั้น จำเป็นต้องฉีดวัคซีน  
 ไวรัสตับอักเสบบีในผู้ที่ภูมิคุ้มกันต้านต่อไวรัสตับอักเสบบี (Anti-HBs) สูงอยู่ก่อนแล้วโดยธรรมชาติ วัคซีนที่ใช้ใน  
 การศึกษานี้คือวัคซีนชนิดผลิตจากพลาสมาและทำให้เชื้ออ่อนแรงโดยใช้ความร้อนซึ่งได้แก่วัคซีน Hepaccine-B  
 และอีกชนิดหนึ่งโดยใช้สารเคมี คือ Hevac-B Pasteur กลุ่มศึกษาได้แก่ผู้บริจาคโลหิตประจำ ที่ศูนย์บริการโลหิต  
 สภากาชาดไทย ระหว่าง มีนาคม 2531 - กรกฎาคม 2532 เพศชาย จำนวน 321 คน อายุระหว่าง 20-55 ปี  
 ระดับฮีโมโกลบินไม่ต่ำกว่า 12.5 กรัม % แบ่งกลุ่มศึกษาเป็น 5 กลุ่มโดยวิธีสุ่มเลือกเพื่อรับวัคซีนแต่ละชนิด  
 ตามกำหนดการให้วัคซีนต่าง ๆ กัน (Randomized allocation) วัคซีนที่ใหม่มี 2 ชนิดคือ Hepaccine-B  
 (3 mcg) และ Hevac-B Pasteur (5 mcg) ฉะเก็บโลหิตก่อนฉีดวัคซีน และหลังฉีดวัคซีนในเดือนที่ 1, 3,  
 6, 9, 12 และ 15 เพื่อทำการตรวจระดับ Anti-HBs โดยวิธี Enzyme-linked immunosorbent assay  
 (ELISA) ผลปรากฏว่าการที่จะผลิต HBIG ในไตเตอร์ที่สูงนั้นจะขึ้นอยู่กับระดับ Anti-HBs ของกลุ่มศึกษา  
 ที่มีอยู่เดิมก่อนวัคซีนคือต้องมีระดับสูงตั้งแต่ 0.6 IU/ml ขึ้นไป และพบว่ากลุ่มที่ได้รับ Hepaccine-B (ในกำหนด  
 เดือนที่ 0, 1, 2 และ 9 ที่มีกำหนดการฉีดแบบเดียวกับฉีดในเดือนที่ 0, 1, 2, 9) จะมีระดับ Anti-HBs สูงกว่า  
 กลุ่มที่ได้รับ Hevac-B Pasteur เล็กน้อย เมื่อพิจารณาราคาของวัคซีนทั้งสองชนิดเพื่อการผลิต HBIG ที่ได้  
 ผลดีเท่ากันก็ควรพิจารณาการใช้ Hepaccine-B ซึ่งราคาถูกกว่า

The National Blood Centre, Thai Red Cross Society has been successfully producing good quality albumin and globulin for clinical use since 1985, but on a small scale (60 litres of plasma per batch). The production of specific immunoglobulins, i.e. human rabies immunoglobulin (HRIG) and hepatitis immunoglobulin (HBIG) was initiated a year afterward.<sup>(1)</sup> The production of HBIG requires a high titre of anti-HBs of more than 5 IU/ml which was generally obtained by anti-HBs titre screening of donated blood using counter immunoelectrophoresis-this approach is however a tedious one. To facilitate increased volume of high titre plasma collection (HBIG of 200 IU/ml), the study on anti-HBs response after hepatitis-B vaccination in donors who had different anti-HBs level (from natural infection) was conducted by using 2 types of plasma derived vaccines (H.B Pasteur and Hepaccine B). This is to define an immune criteria before vaccination, so that proper donor selection for the production of high titre HBIG could be made.

### Material and Method

The study population consisted of 510 male volunteered blood donors who had pre-existing anti-HBs as detected by ELISA. The criteria for selection were as follows : healthy regular male donors of the National Blood Centre (at least 7 donations), age between 20-55 years old, Hb of 12.5 gm %, no history of overt hepatitis B disease nor H.B vaccination. They were randomised to receive one of two kinds of hepatitis-B vaccine : 3 mcg of

Hepaccine-B in the schedules of 0,1,2,9 month; 0,1,3,9 month; 0,2,6,12 month; 0,1,6,12 month and 5 mcg of Hevac-B Pasteur in the schedule of 0,1,2,9 month. The type of vaccine and the schedule were randomised by the personnel of the blood collection section during March 1988 to July 1989. After the first vaccination 15 ml of blood was drawn from each subject at 1,3,6,9,12 and 15 months.

### Testing of anti-HBs antigen

Blood collected were tested for anti-HBs titre by radioimmunoassay using the reagent of the Dupont Co. Ltd. The result was read in the quantity of anti-HBs that has the standard value from 0-150 mIU/ml. If the anti-HBs value is more than 150 mIU/ml, the serum will be diluted, retested and the result calculated result calculated to give an average by using the geometric mean titre (GMT) of the anti-HBs responses.

### Results

Among 510 volunteered regular donors who participated in the study program, 34.9 % had the anti-HBs level of 0.01-0.59 IU/ml before vaccination. Those who had anti-HBs of less than 0.01 and of 0.6 IU/ml and up were 43.7 % and 21.4 % respectively (Table 1). There was somewhat similar in the distribution of donors with various levels of pre-existing anti-HBs (< 0.01, .01-0.59, .60-1.0 and > 1.0 IU/ml) in each group of the vaccine types and schedules. (Table 2).

Table 1. Pre-existing Anti-HBs level in volunteered regular donors before vaccination.

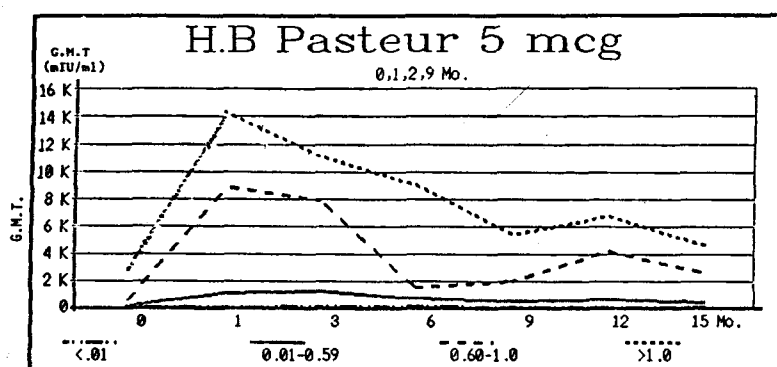
Pre-existing Anti-HBs level (IU/ml)	No.	%
< .01	223	43.7
.01 - .59	178	34.9
.60 - 1.0	28	5.5
> 1.0	81	15.9
<b>Total</b>	<b>510</b>	<b>100.0</b>

**Table 2.** Percentage of donors with various pre-existing anti-HBs level (IU/ml) before vaccination by type of vaccine and schedule.

Type of vaccine and schedule	% of donors with various pre-existing antiHBs level (IU/ml)			
	<.01	.01 – .59	.60 – 1.0	>1.0
Hevac B Pasteur 5 mcg 0, 1, 2, 9 mo. (n = 104)	41.3	32.7	9.6	16.4
Hepaccine 3 mcg 0, 1, 2, 9 mo. (n = 98)	42.9	35.7	6.1	15.3
Hepaccine 3 mcg 0, 1, 3, 9 mo. (n = 98)	42.9	33.7	4.1	19.3
Hepaccine 3 mcg 0, 2, 6, 12 mo. (n = 105)	47.6	37.2	3.8	11.4
Hepaccine 3 mcg 0, 1, 6, 12 mo. (n = 105)	43.8	35.2	3.8	17.2

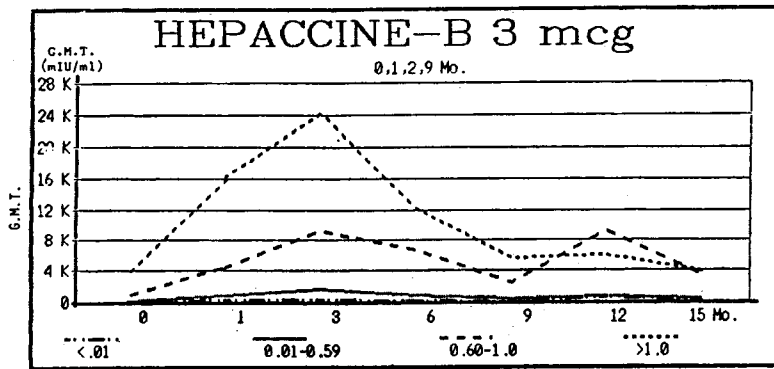
Each figure represents the full picture of the pre-antibody level and post-antibody response after vaccination of four pre-existing antibody subgroups in each vaccine type & schedule. In the group of Hevac B. Pasteur 5 mcg, in the schedule of 0,1,2,9 mo (fig.1), the response of the subgroups with pre-existing antibody level of >1.0 and 0.6-1.0 were highest in the first month (14.3 and 8.9 IU/ml respectively), whereas the group that received Hepaccine B 3 mcg, in the same schedule (fig.2), the highest response of both subgroups were noted in the

third month (24.3 and 9.2 IU/ml respectively). In fact the subgroup with pre-existing antibody of >1.0 in figure 2 also showed high antibody response in the first month (16.3 IU/ml) and even higher than the H.B Pasteur group. Post vaccination antibody level in these two subgroups gradually declined after its peak, but however the level was still higher than 5 IU/ml, particularly in the >1.0 subgroup. They also showed that after the booster dose in the ninth month, the antibody response rose again in the twelfth month and declined afterwards.



(K = 10<sup>3</sup>)

**Figure 1**

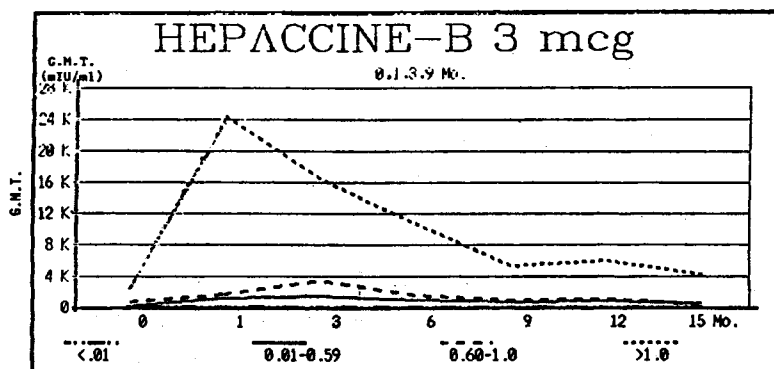


(K = 10<sup>3</sup>)

Figure 2

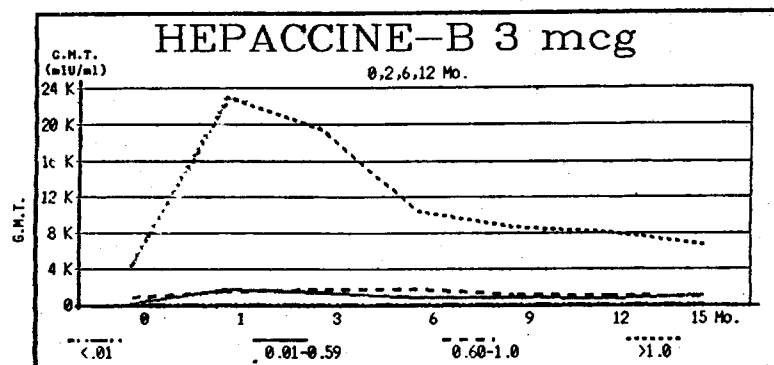
Among those who received Hepaccine B 3 mcg, in the schedule of 0,1,2,9 month (fig.3) and in the schedule of 0,2,6,12 mo (fig.4), the antibody response higher than 5 IU/ml was shown only in the subgroup with pre-existing antibody level of >1.0. The highest antibody responses were found in the first month as that of H.B Pasteur group and declined in later months. In the group of Hepaccine B 3 mcg, in the schedule of 0,1,6,12 mo (fig.5), the peak antibody responses in the >1.0 and 0.6-1.0 subgroups were similar to the group of H.B Pasteur. Exceptional

antibody level of <.01 which gave surprisingly highest antibody response (5.3 IU/ml) in the third month, higher and lasted in higher level longer than 0.6-1.0 subgroup. After booster in the sixth month, the antibody response slightly increased in the ninth month and then quickly declined to the lowest level in the fifteenth month. The other subgroups with the pre-existing antibody level of <.01 (fig.1-4) showed slightly increased antibody level after vaccination, though the level did not reach 5 IU/ml, but they went up above the protective level (10 mIU/ml).



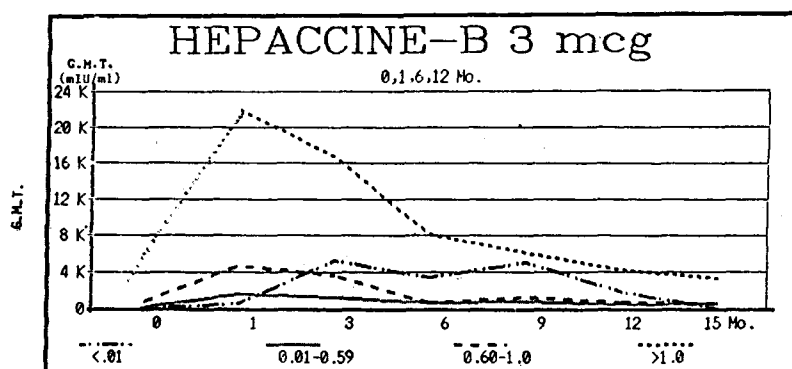
(K = 10<sup>3</sup>)

Figure 3



(K = 10<sup>3</sup>)

Figure 4



(K =  $10^3$ )

Figure 5

## Discussion

Hepaccine B is a heat inactivated plasma derived vaccine which is produced at lower cost. Hevac B Pasteur is also a plasma derived vaccine but is chemically inactivated and cost higher to produce. Production of both vaccines are similar in terms of purification of 22 nanometer hepatitis surface antigen particles from human carriers' plasma, using gradient ultracentrifugation.<sup>(2)</sup> Post vaccination immune response was generally studied in non-immune individuals. A protective anti-HBs level of 10 mIU/ml could be obtained in 95 % of vaccinated individuals by any vaccine.<sup>(3)</sup> This low level is, however, considered not suitable for HBIG production. Higher anti-HBs response is expected to be obtained by vaccination to immune individuals.<sup>(4)</sup> This study did confirm our expectation. The study revealed that people with high pre-existing antibody levels yielded higher antibody response than those who had low pre-existing antibody levels. Good anamnestic response was seen when prevaccination anti-HBs level was at 0.6 IU/ml or higher in any vaccination schedule. The greatest response was obtained when prevaccination anti-HBs was 1.0 IU/ml or above. Peak GMT titre of the Hevac B Pasteur group was lower than that of Hepaccine B group. There was poor anamnestic response when prevaccinated anti-HBs was below 0.01 IU/ml except in the group Hepaccine 3 mcg, in the schedule of 0,1,6,12 month.

It can be concluded from this study that the criteria for donor selection should be those who have anti-HBs before vaccination of 0.6 IU/ml and up, and it will be even better in those who have prevaccination antibody level of  $>1.0$  IU/ml. It was found that there were about 21.4 % of the former and 15.9 % of the latter. This study will result in the reduction of the quantity of vaccine used in HBIG production since only one dose of any types of vaccine given to these recommended donors will give, in one month,

the antibody level many times higher than the expected ( $>5$  IU/ml). Moreover there seems to be no need for a vaccination series.

## Conclusion

The higher prevaccination anti-HBs level, the greater postvaccination anti-HBs response is achieved. Therefore the prevaccination anti-HBs titre of 0.6 IU/ml and up should be considered for donor selection, since they are the ideal patients for the production of high titre HBIG (200 IU/ml). About 44 % of regular donors have anti-HBs below the level of protection before vaccination. Most of them gained the antibody level of more than 10 mIU/ml (the protective level) by a series of vaccination in any schedule. Hepaccine B should be used for the production of HBIG, since it costs less but renders equally or even higher antibody response.

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