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# STUDIES ON HEPATITIS B VACCINES

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## ABSTRACT

HBs vaccines were prepared by three different methods: 1) Heat inactivation (Krugman, et.al; 1971);2) Ammonium sulphate precipitation followed by column chromatography (Sephadex G-200); 3) PEG-6000 (polyethylene glycol) precipitation followed by column chromatography using Sephadex G-200.

Their efficacy was studied in guinea pigs, and the results compared with commercial Japanese vaccine (Green Cross Corporation, Osaka, Japan). We conclude that vaccine prepared by PEG-6000 precipitation gives better results.

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## INTRODUCTION

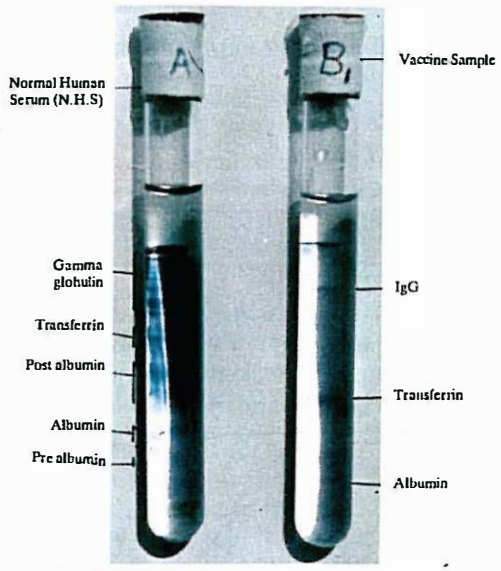
About 200 million people of the world's population are estimated to be infected with hepatitis B virus (HBV).[1] Prevention of hepatitis B is therefore of great international importance. In the absence of successful replication of HBV in cell culture, one of the present sources of antigenic material to prepare the vaccine is plasma of human carriers of hepatitis B surface antigen (HBsAg).[2-5] We have used the following methods for the preparation of vaccine:

1) Heat inactivation vaccine by Krugman's method;
2) Ammonium sulphate precipitation vaccine;
3) Polyethylene glycol (PEG) 6000 precipitation vaccine.

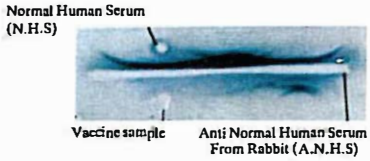
## MATERIAL AND METHODS

High titre HBsAg-positive plasma was used for all methods of preparation of HBs vaccine. Detection of HBsAg was carried out by counterimmunoelectrophoresis (CIEP)[6] and reserve passive hemagglutination (RPHA) commerical test kit by Green Cross of Japan. We have used the following methods for preparation of vaccine.

Photographs Show Purity of HBs Vaccine Prepared By Heat Inactivation Method.



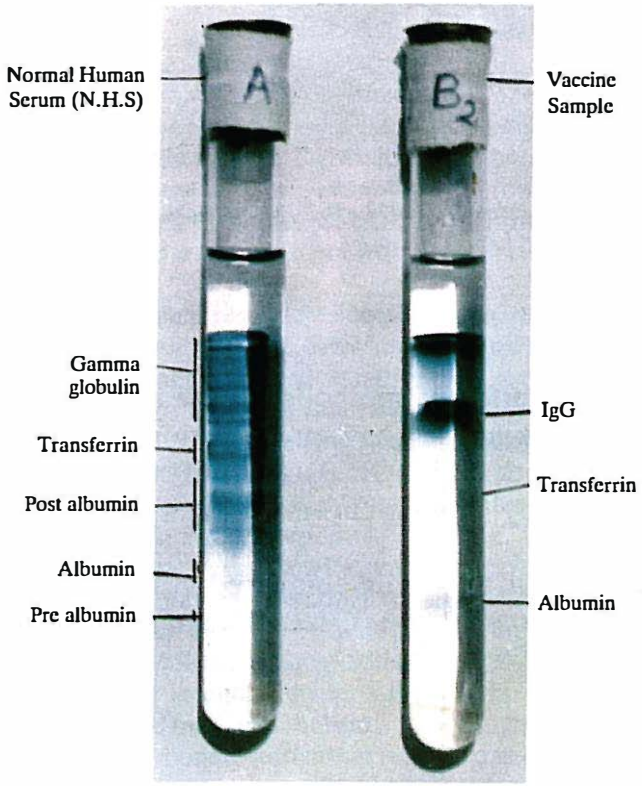

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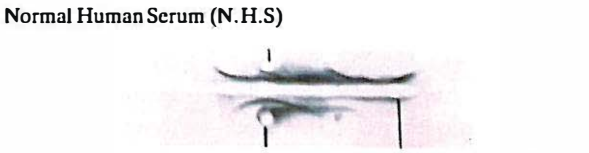



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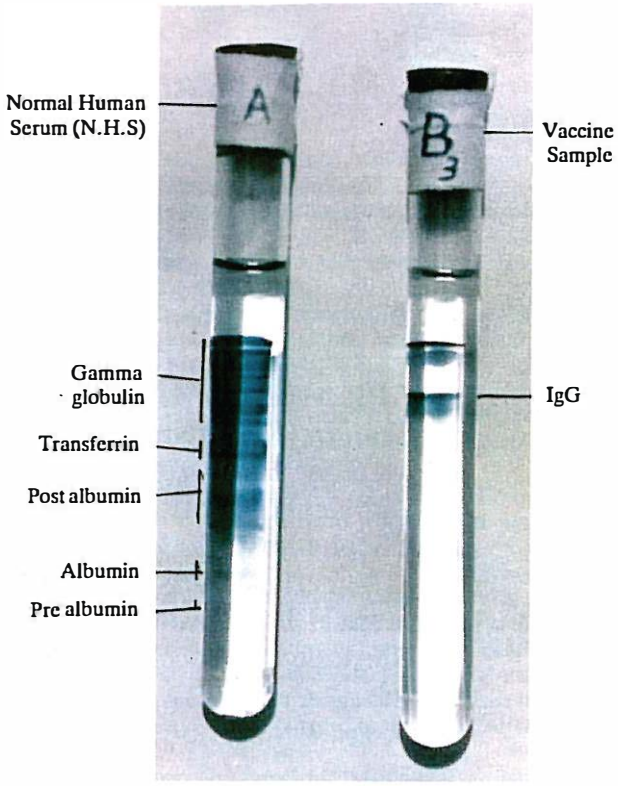
Photographs Show Purity of HBs Vaccine Prepared By polyelene glycol (PEG) 6000 precipitation.



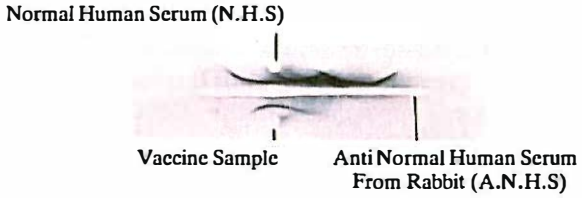

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Photographs Show Purity of HBs Vaccine Prepared By Ammonium Sulphate Precipitation Method.




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**Heat-inactivation vaccine**

High-titre, HBsAg was heated at 98°C for one minute according to Krugman's method.[7]

Purity of vaccine was checked by polyacrylamide gel electrophoresis (PAGE)[8] and immunoelectrophoresis (IE).[9]

**Dose of vaccine**

We have adjusted 0.5 ml of vaccine to 1:32 titre by CIEP and 1:32768 by RPHA. Thus, each of the five guinea pigs were immunized intramuscularly with 0.5 ml of vaccine without $AlPO_4$ as adjuvant.

**Preparation of HBs vaccine by ammonium sulphate precipitation**

HBsAg-positive plasma was treated with low concentration (13.4%) ammonium sulphate followed by high concentration ammonium sulphate (16.6%).[4] Inactivation was carried out by heat and formaline treatment.[4] The final purification was carried out by column of Sephadex G-200.

**Schedule of immunization**

In our schedule one dose of vaccine without $AlPO_4$, and one dose vaccine with $AlPO_4$ were administered intramuscularly in guinea pigs.

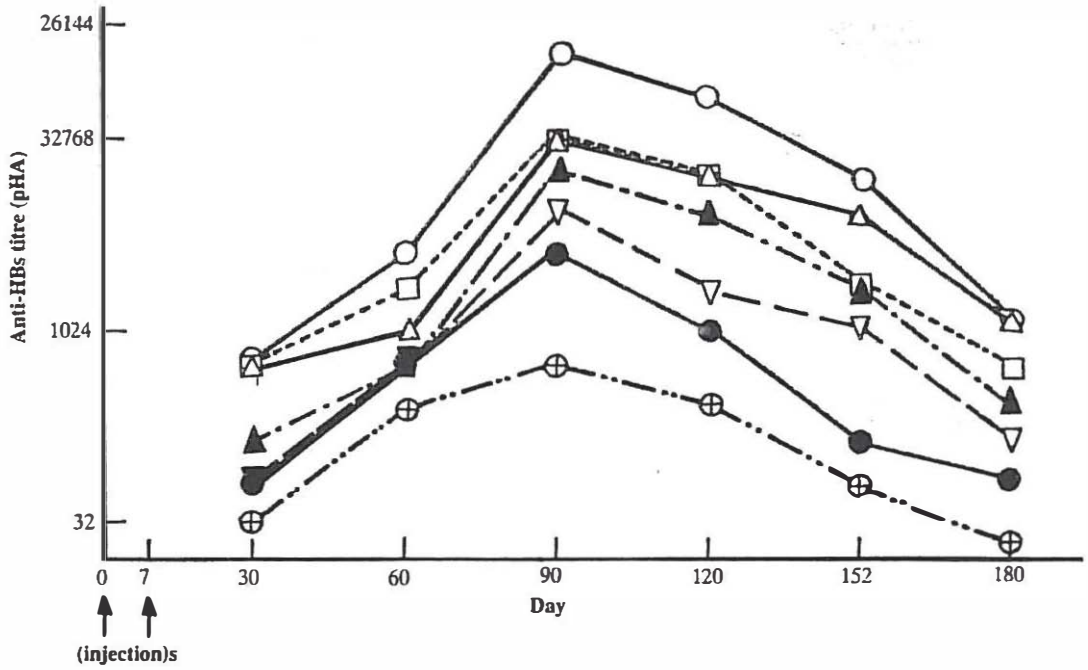
**Preparation of HBs vaccine by PEG-6000 precipitation**

HBsAg-positive plasma was treated with low concentration of PEG-6000 (3.5%) followed by high concentration of PEG-6000 to 10%.[3] Inactivation was carried out by heat and formaline treatment.[4]

The same schedules of immunization were followed as given above; only two doses of this vaccine were



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One injection without $AIPO_4$ prepared by heat inactivated vaccine
One injection with $AIPO_4$ prepared by ammonium sulphate precipitation vaccine
One injection without $AIPO_4$ prepared by ammonium sulphate precipitation vaccine
One injection with $AIPO_4$ prepared by PEG-6000 precipitation vaccine
Two injections without $AIPO_4$ prepared by PEG-6000 precipitation vaccine
One injection without $AIPO_4$ prepared by PEG-6000 (without mannitol) vaccine
One injection with $Al(OH)_3$ by Green Cross Corporation (Japan) vaccine

**Figure 1.** Anti-HBs titre in guinea pigs immunized with different HBs vaccines

administered with $AIPO_4$.

**Standard vaccine**

One dose containing 20 $\mu$g of Green Cross commerical vaccine were administered to each of five guinea pigs.

**Antibody detection**

CIEP and PHA (Passive Hemagglutination) tests used for detection of anti-HBsAg were prepared and standardized according to PHA test kit marketed by Green Cross Corporation, Japan.

## RESULTS

**Table I.**
**Recovery of HBS vaccine by different methods.**

| Type of vaccine | Heat inactivated | Ammonium Sulphate ppt. | PEG6000 ppt |
|---|---|---|---|
| % of Recovery | 15.82 | 37.5 | 80.55 |

It was observed from Table I that the recovery of HB vaccine is better by PEG-600 method.

Table II reveals that treatment with PEG-6000 gives a pure vaccine.

**Table II.**
**Purity of vaccines by polyacrylamide gel electrophoresis and immunoelectrophoresis.**

| Type of vaccines | Protein contaminations | | Protein contaminations |
|---|---|---|---|
| Heat Inactivated | IgG | Transferrin | Albumin |
| Ammonium Sulphate ppt. | IgG | Transferrin | Albumin |
| PEG 6000 Prectipitation | IgG | | |

Single dose of vaccine (prepared by PEG-6000) with $AIPO_4$ showed antibody titre 1:512 on 30th day. Its peak antibody reached 1:13107 (1:16 by CIEP) on 90th day. It gradually dropped to 1:1024 on 180 th day.

## ACKNOWLEDGEMENTS

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**Table III. Anti-HBs titre by CIEP and PHA by different immunized HBs vaccines in guinea pigs.**

| DAY | | 0 | 30 | 60 | 90 | 120 | 150 | 180 |
|---|---|---|---|---|---|---|---|---|
| **Titre** | | | | | | | | |
| Heat-inactivated | CIEP | - | - | - | - | - | - | - |
| vaccine | PHA | - | 1:32 | 1:256 | 1.512 | 1.256 | 1.64 | 1.16 |
| Ammonium sulphate | CIEP | - | - | - | 1:1 | - | - | - |
| ppt. vaccine with $AIPO_4$ | PHA | - | 1:128 | 1:1024 | 1:16384 | 1:8192 | 1:2048 | 1:256 |
| Ammonium sulphate | CIEP | - | - | - | - | - | - | - |
| ppt. vaccine without $AIPO_4$ | PHA | - | 1:64 | 1:512 | 1:8192 | 1:2048 | 1:1024 | 1:128 |
| PEG 6000 ppt. | CIEP | - | - | - | 1:16 | 1:8 | 1:1 | - |
| vaccine with $AIPO_4$ | PHA | - | 1:512 | 1:4096 | 1:131072 | 1:65536 | 1:16384 | 1:1024 |
| PEG 6000 ppt vaccine | CIEP | - | - | - | - | - | - | - |
| without $AIPO_4$ | PHA | - | 1:64 | 1:512 | 1:4096 | 1:1024 | 1:128 | 1:64 |
| Two dose PEG 6000 | CIEP | - | - | - | 1:4 | 1:1 | - | - |
| ppt without $AIPO_4$ | PHA | - | 1:512 | 1:2048 | 1:32768 | 1:16384 | 1:4096 | 1:512 |
| GCC vaccine | CIEP | - | - | - | 1:4 | 1:1 | - | - |
| | PHA | - | 1:512 | 1:1024 | 1:32768 | 1:16384 | 1:8192 | 1:1024 |

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*Comment:*

*This article is an academic research conducted on production of hepatitis*

*of a vaccine must be proven with standardized procedures which conform to the guidelines of the World Health Organization.*


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