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RESEARCH ARTICLE

PERFORMANCE EVALUATION OF BIOLOGICAL REFERENCE PREPARATIONS (BRP) HUMAN ALBUMIN AND HUMAN IMMUNOGLOBULIN FOR ELECTROPHORESIS (PROTEIN COMPOSITION) AFTER RECOMMENDED PERIOD OF ITS USE IN TESTING LABORATORIES

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Abstract: A study was conducted in our laboratory along with the routine regulatory testing to evaluate the performance of Biological Reference Preparations (BRP) Human Albumin for Electrophoresis BRP (Code: H0900000, Batch 2, EDQM, France) and Human Immunoglobulin for Electrophoresis BRP (Code: H1000000, Batch 3, EDQM, France) after recommended period i.e fourteen days after reconstitution. These reference preparations are recommended as reference controls for Protein Composition, the test in European Pharmacopoeia (EP)/ Indian Pharmacopoeia (IP) monographs for quality control evaluation of Human Albumin and Human Immunoglobulin preparations intended for therapeutic use. Protein composition by Horizontal Zone Electrophoresis using cellulose acetate membrane strips is a test for determination of purity in these preparations as per EP/IP. The aliquots of these BRP are stored at recommended temperature (20°C – 80°C) after reconstitution and verified for its performance after fourteen days of recommended period. From the results obtained, it was observed that all data points are within the stated limit for both BRP and significant effect of time period was not observed during the study period, i.e. up to 406th day for human albumin BRP up to 350th day for Immunoglobulin BRP. The %RSD calculated for both BRP is less than 1%. From this study, it is found that these BRP can be used even after fourteen days of the recommended period, if reconstituted and stored as per the instructions of EDQM, France, to avoid wastage of precious certified international reference material.

KEYWORDS: Human Albumin, Human Immunoglobulin, BRP, Horizontal Electrophoresis, Protein Composition, Quality Control.

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INTRODUCTION:

Human Albumin and Human Immunoglobulin preparations manufactured from human plasma are very precious Bio-therapeutics used in critical patient care. Quality control of these products is very important in view of patient safety. Purity is one of the test parameters to check quality. Protein Composition by Horizontal Zone Electrophoresis using Cellulose Acetate membrane strips is the recommended method for determination of purity in these preparations as per European Pharmacopoeia (EP)/ Indian Pharmacopoeia (IP), respective monographs [1-7]. A study was conducted along with the routine regulatory testing to evaluate the performance of Biological Reference Preparations (BRP) Human Albumin for Electrophoresis BRP (Code:H0900000, Batch 2, EDQM, France) and Human Immunoglobulin for Electrophoresis BRP (Code:H1000000, Batch 3, EDQM, France) after recommended period i.e fourteen days after reconstitution for its use. Our laboratory is involved in Quality Control testing of various blood Products and on average every year we test, about more than 200 batches of Human Albumin and more than 100 batches of various types of Immunoglobulin. These biological reference preparations are recommended for Protein Composition test in EP/IP as reference controls for quality control evaluation of various plasma derived products like Human Albumin and Human Immunoglobulin. Use of international reference preparations is very essential for any testing laboratory to report quality-assured test results and to meet international working standards like ISO/IEC 17025:2017, to present their laboratory performance on a Global platform for universal acceptance. Biological Reference Preparations (BRP) are very precious materials and their preparation involves a lot of hard work, skill and finances. Their use enables the achievement of consistency in the measurement of key attributes of Biologicals. The timely development of new reference materials is a critically important aspect of harnessing new scientific developments for application in the form of safe and effective biological and securing improved world health [8]. This concept of using well-characterized preparations as references against which batches of biological products are assessed remains fundamental to ensuring the quality of biological products as well as the consistency of production and is essential for the establishment of appropriate clinical dosing. International reference preparations are intended to use for the characterization of the activity of secondary reference preparations (national or working standards). They are made for use in laboratory assays only and should not be administered to humans [22-24]

MATERIAL & METHOD:

Reagents:

Barbital buffer was prepared by dissolving 1.38g of Barbital, 8.76g of barbital sodium and 0.38g of calcium lactate to a final volume of 1000ml in a volumetric flask, pH: 8.6. For staining of the nitrocellulose strips, a 0.5% w/v amido black staining solution was prepared by dissolving 0.5g of amido black 10B in 90ml methanol and 10ml 5M glacial acetic acid. For destaining a mixture of 90ml methanol and 10ml 5M glacial acetic acid is used.

Biological Reference Preparation: Human Albumin for Electrophoresis BRP (Code: H0900000, Batch 2, EDQM, France) and Human Immunoglobulin for Electrophoresis BRP (Code: H1000000, Batch 3, EDQM, France) are used as per instructions of EDQM, France.

Equipment:

Horizontal electrophoresis Unit suitable for cellulose acetate electrophoresis (Cat No. CSLCELLAS) consists of a tank, a applicator, a bridge & power pack, a Flatbed scanner and a PC with Turboscan software (Cat No. CSLSCAN) for analysing the bands was used in the study.

Method:

Protein Composition in Human Albumin and Immunoglobulin samples is done by zone electrophoresis as described in Pharmacopeia using

Cellulose Acetate Membrane strips as supporting media and Barbital buffer as electrolyte solution [9, 10]. Horizontal electrophoresis systems are designed to run cellulose acetate strips immersed in running buffer maintained at a definite pH and electrophoretic separation conducted for a definite time, under the influence of an electrical field. Samples introduced to an electric field disperse in an electrolyte solution towards anode or cathode depending on their inherent charge. The sample is separated into distinct fractions as per their differential migration rates according to size, shape and charge resulting from the opposing interactions of the electrical force and molecular sieving [11-13]. The components of the sample are isolated into distinct bands after the separation is completed and each band represents constituents in the samples that possess similar or identical characteristics. Cellulose acetate strips possess larger pores than agarose and polyacrylamide gels. The mobility of the components in the sample during cellulose acetate electrophoresis is largely based on the overall charge rather than the size of the component [14-16].

Cellulose acetate membrane strips are submerged in the running buffer and each end of the strip overlaps with a filter paper wick that reaches into the buffer in an electrophoresis tank. Samples are applied on the surface of the cellulose acetate membrane using an applicator at half or one third of the length of the strip. The separation begins when an electric current is applied to each end of the strip, which acts as an anode and cathode. After completion of an electrophoresis run, separated components separate as different bands are stained, followed by de-staining for visualization purposes and for further quantitative analysis.

In this study, 2% protein concentration of Human Albumin & 3% protein concentration of Human Immunoglobulin (IV/IM) samples and their respective BRP (BRP H0900000, Batch 2 & BRP H1000000, Batch 3) were prepared. Approx. 500ml of Barbital buffer was poured into the electrophoresis chamber and the cellulose acetate strips were wetted in the buffer before loading the sample. 5 µl of the sample was applied on the strips with the help of an applicator. The sample was gently loaded at the cut side of the strips left 2-3 cm from the edge of the bridge, making sure that the sample was evenly loaded. On another strip, the reference solution was loaded in the same manner. Now the bridge was placed in the horizontal electrophoresis chamber filled with a barbital buffer, ensuring that both ends of the strips were properly dipped from both sides of the bridge in the buffer. The electrophoresis was carried out at 175V for 45 minutes so that the sample applied on the strip migrates at least 30 mm from the spot of application. After completion of electrophoresis, the strips were stained with 0.5% w/v amido black stain solution for 2-3 minutes. The strips were then destined to clear the background. Using Turboscan software, the strips were analysed to find the percentage of the principal band and bands other than the main band present in samples and BRP.

RESULTS:

In our laboratory, after use for a recommended time period, the left over material of both BRP are aliquoted, stored at recommended temperature (2^0-8^0C) as per the instructions of EDQM, France [17, 18] and were used along with routine testing to verify their performance.

I. Performance of Human Albumin for Electrophoresis BRP H0900000, Batch 2:

The study was done on random basis ranging from 32nd day to 406th day after reconstitution. The reconstituted material was distributed as three sets (reconstituted at different days) and twenty tests (n=20) performed with each sample set at different time intervals randomly. In all tests the results obtained are within the stated value i.e., 96.7% to 99.2% of Principal Band. The results are statistically analysed by calculating mean, SD and % RSD. The % RSD for all three sets was less than 1%. Results are given in **Table.1** and sample performance trend are graphically represented in **Figure.1 (a-d)**.

TABLE 1: Performance of Human Albumin for Electrophoresis BRP H0900000, Batch 2, EDQM, France

Performance No.	SET – I (%) (Vial-I studied from 0 to 32 days)	SET – II (%) (Vial-II studied from 0 to 300 days)	SET – III (%) (Vial-III studied from 0 to 406 days)
1	98.9	98	98.2
2	99.1	98.5	98.4
3	98.9	99.2	97.2
4	98.4	99.1	97.7
5	99	99	97.3
6	98.6	97.8	97.4
7	98.8	99.1	98.6
8	99	98.3	97.7
9	99.2	97.9	97.1
10	98.7	98.5	97.7
11	98.6	97.7	98
12	99	99.2	98.3
13	99	99.2	97.5
14	99.1	99.2	97.7
15	99	96.9	97.3
16	99.1	97.6	98.9
17	99	97.3	99.2
18	98.5	98.3	97.7
19	97.8	98.2	97.8
20	98.5	98	98
MEAN	**98.81**	**98.35**	**97.885**
SD	**0.33**	**0.69**	**0.56**
%RSD	**0.33**	**0.70**	**0.57**

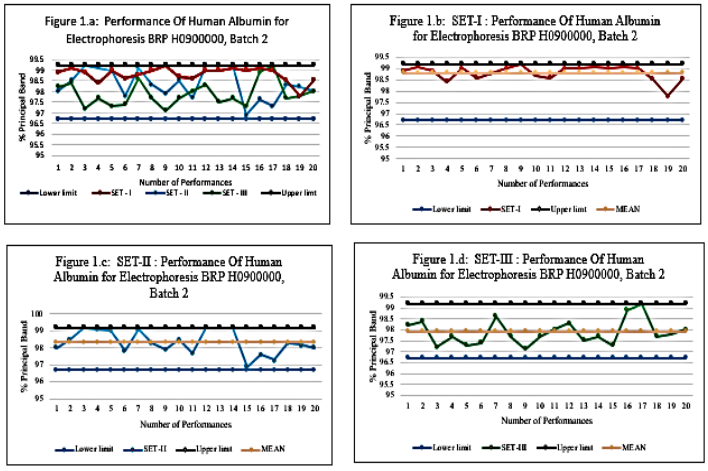


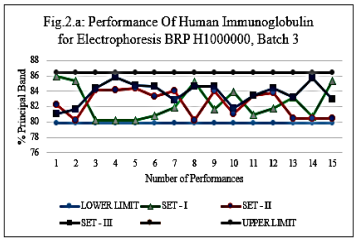
Figure. 1(a - d): Performance of Human Albumin for Electrophoresis. BRP H0900000, Batch 2, EDQM, France

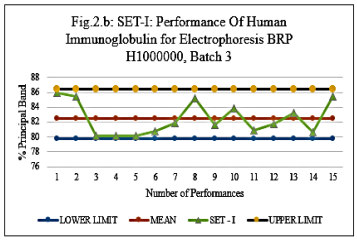
II. Performance Of Human Immunoglobulin for Electrophoresis BRP H1000000, Batch 3:

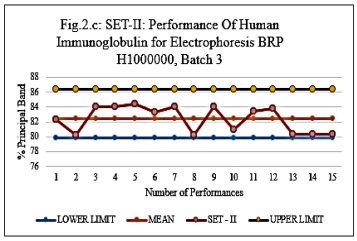
The study was done on random basis ranging from 20th day to 350th day after reconstitution. The reconstituted material was distributed as three sets (reconstituted on different days) and fifteen (n=15) tests performed with each at different time intervals randomly. In all tests the results obtained are within the stated value i.e. 79.8% to 86.4% of Principal Band. The results are statistically analysed by calculating mean, SD and % RSD. The % RSD for all three sets was less than 3%. Results are given in **Table. 2** and sample performance trend is graphically represented in **Figure.2 (a-d).**

TABLE 2: Performance of Human Immunoglobulin for Electrophoresis BRP H1000000, Batch 3

Performance No.	SET – I (%) (Vial-I studied from 0 to 20 days)	SET – II (%) (Vial-II studied from 0 to 200 days)	SET – III (%) (Vial-III studied from 0 to 350 days)
1	86	82.3	81
2	85.4	80.2	81.6
3	80.2	84.1	84.4
4	80.2	84.1	85.8
5	80.2	84.4	84.7
6	80.8	83.3	84.6
7	81.9	84	82.8
8	85.2	80.2	84.6
9	81.6	84	84.6
10	83.9	81	81.8
11	80.9	83.4	83.4
12	81.8	83.8	84.4
13	83.2	80.4	83.2
14	80.7	80.4	85.7
15	85.4	80.4	83
MEAN	**82.49**	**82.4**	**83.71**
SD	**2.08**	**1.68**	**1.41**
%RSD	**2.52**	**2.04**	**1.68**







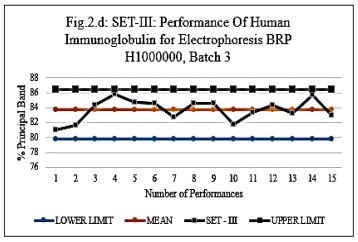


Figure. 2(a - d): Performance of Human Immunoglobulin for Electrophoresis BRP H1000000, Batch 3

From the data given in **Table. 3**, it can be seen that performance of both BRP is satisfactory and within prescribed limit even after fourteen days of recommended period. The overall %RSD for Albumin BRP 0.38% and for Immunoglobulin BRP is 0.72%, which is statistically insignificant.

TABLE 3: Data showing overall performance of both BRP

	BRP H0900000, BATCH 2 (HUMAN ALBUMIN) [96.7% to 99.2%]	**BRP H1000000, BATCH 3 (HUMAN IMMUNOGLOBULIN) [79.8% to 86.4%]**
MEAN	98.35	82.86
SD	0.38	0.59
%RSD	**0.38**	**0.72**

DISCUSSION:

WHO biological reference standards are widely used in the development, evaluation, standardization and control of products in industry, by regulatory authorities and also in biological research in academic and scientific organizations. They play a vital role in facilitating the transfer of laboratory science into clinical practice worldwide and the development of safe and effective Biologicals [22]. The provision of international biological reference standards makes a critically important contribution to the high standards of efficacy, quality, purity and safety of many biological medicines used worldwide in the prevention, treatment or diagnosis of diseases or conditions. Their use supports the application of the numerous biological and immunological assays used in the standardization and control of a wide range of biological, including therapeutics, blood-derived products, vaccines and immunological products of traditional types as well as those derived from modern biotechnological approaches [22-24]. They also have important applications in the standardization of materials and approaches used in medical diagnostics such as diagnosis of disease, monitoring therapy, blood safety and public health applications (e.g., monitoring immune status, screening for disease or susceptibility) or otherwise characterizing biological material from individuals [22-24].

Shelf life of reference material is usually determined by the stability studies conducted by the reference standard producer or material donor [26-29]. It is understood that the shelf-life of fourteen days for this BRP is mentioned by EDQM may be on the safer side to avoid any unsatisfactory performance of the BRP due to handling and storage by user/ testing laboratories, as the conditions of reconstitution and storage cannot be studied during collaborative studies. Generally, it is mentioned by the EDQM that the user can further use the reference material based on their experience in their laboratories. Assigning shelf-life to an international reference preparation is a laborious job, where several factors have to be considered during the study [25].

The results are analysed using Trend analysis statistical method. It is, fundamentally, a method for understanding how and why things have changed – or will change – over time [21]. Here it is used as an approach to analysing data and then attempts to discover patterns/ trends within that data for the purposes of understanding or predicting behaviour. In the figures, the results obtained in the time span mentioned are plotted against the range of values given by the EDQM, France for the respective BRP and it shows that all results are found within the

certified range in the time span mentioned above. RSD or CV is calculated based on the data obtained to verify the statistical significance of results and performance of BRP [30].

From the results obtained, it was observed that all values are within the stated limit for both BRP (Human Albumin BRP - SD=0.38; %RSD=0.38; Immunoglobulin BRP – SD=0.59; %RSD=0.72) when compared with freshly reconstituted sample and no drift of content could be observed during the study period i.e. from 32nd day to up to 406th day for Human Albumin BRP and from 20th day to up to 350th day for Immunoglobulin BRP. From this study, it is found that these BRP can be used even after fourteen days of recommended period, if reconstituted and stored as per instructions of EDQM, France.

Presently, these batches of BRP i.e. Human Albumin batch 2 and Human Immunoglobulin batch 3 have been replaced by EDQM, France due to stock depletion and in new batches i.e. Batch 3 & Batch 4, the shelf-life assigned is 14 days and 4weeks respectively, after reconstitution, following instructions as per leaflet for the same [19,20]. The international reference preparations should be used judiciously. This study will help in guiding testing laboratories to verify the performance of reference preparations in their laboratories, time to time, and if found satisfactory, it will be better to use the material till its satisfactory performance to save and minimize the use of precious reference material.

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