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Quality Control in the Production of an Immunoglobulin for Intravenous Use

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Summary. This paper briefly surveys the control measures and tests which are carried out before, during and after manufacture of an i.v. immunoglobulin in order to assure the quality of the preparation. The quality requirements are determined by both the standards expected by physicians and patients and by the specifications of the registration authorities in the various countries.

A great deal of know-how and considerable technical investment is required to match these requirements. Furthermore a comprehensive quality assurance programme is compulsory: It begins with the careful collection and analysis of each single blood donation, continues through all the manufacturing stages and ends with the rigorous testing of every batch of the end product – an examination comprising 23 different tests.

Key words: Immunoglobulin preparation – i.v. application of immunoglobulin – Quality control standardization of IgG preparation

Introduction

In manufacturing an immunoglobulin preparation for intravenous use great care must be devoted to the planning and performance of quality control and quality assurance, mainly for the following three reasons:

1. Blood as starting material is heterogeneous because it is obtained from many individual blood donors. It has to be carefully handled since blood and the plasma obtained from it are excellent substrates for bacterial growth. In addition it may be contaminated by pathogenic organisms, notably hepatitis B virus.
2. The manufacture of parenteral products in general requires a high level of pharmaceutical production expertise. Special problems arise in the manufacture of large volume parenterals, which must be distributed into containers and freeze-dried under aseptic conditions.
3. The IgG molecule is a complex and labile structure having many biochemical functions and reactivities. In order to prevent denaturation of the protein and damage

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to these special functions very mild processing conditions are required, together with comprehensive in-process controls and quality assurance measures.

This paper surveys the quality requirements which must be observed in order to ensure that the product will meet the high standards set.

Quality Requirements

The *quality* of a batch is defined as the degree to which it matches the prototype batches – those batches which were exhaustively tested, which were used in the clinical trials and which formed the basis for drafting the specifications and compiling the registration file.

Quality assurance comprises all the measures taken during the collection of the raw material and the production process in order to *assure* the quality of each individual batch. These assurance systems are summarized [1, 2] under "Good Manufacturing Practice" (GMP Guidelines).

Quality control is one arm of quality assurance, and comprises all the tests carried out on raw materials, intermediates and the end product which are intended to *confirm* that the product quality meets the standards described by specifications.

These specifications must be outlined in such a way that they provide a definite verdict on the quality of the batch. Thus we do not have to perform every conceivable test on every batch. This quality standard is defined taking into account the expectations and demands of physicians, patients and the health authorities.

The physician and the patient expect the preparation to be well tolerated, safe and efficacious, as described in the package leaflet and in the manufacturer's literature.

The authorities who grant permission to market pharmaceutical products require the safety and efficacy claimed for it to be demonstrated and documented by means of a number of in vitro studies and controlled trials. They also require detailed information on the production facilities, the manufacturing process and the test methods. The manufacturer has to show that he is in a position to produce batches of consistent quality ("lot to lot" consistency), and must submit complete data on the stability of the products from the date of manufacture to the expire date.

An immunoglobulin preparation for intravenous administration which meets every theoretically possible requirement is so far unavailable; however, we may draw up an "ideal" specification against which an immunoglobulin product can be compared (Table 1). There is no easy way to attain this ideal; various routes have been explored, and accordingly the preparations now available have different characteristics.

Quality Assurance During Production

The starting material for most preparations is still the standard immunoglobulin which is isolated from plasma by Cohn's alcohol fractionation method. This process guarantees that the preparation will not transmit hepatitis; any departure from it would involve extremely costly testing programmes to demonstrate that the immunoglobulin produced was free from contamination with hepatitis virus.

Table 1. Requirements in an ideal preparation vs properties of our immunoglobulin preparation

	Ideal preparation	Our immunoglobulin preparation
Safety and Tolerance	Free from impurities (including bacteria, pyrogens, PKA, proteases, suspended matter)	Assured for each batch
	No spontaneous anticomplementary activity	Assured for each batch
	Free from aggregates and fragments	Aggregates less than 3%, fragments less than 10%; assured for each batch
	Free from pathogenic organisms (especially hepatitis B virus)	HBs antigen negative, assured on each batch HBs antibody detectable, assured on each batch
	Tolerated intravenously at any desired dose	Tolerance demonstrated [9, 10]
	No neoantigenicity	No neoantigenicity detectable
	Stable	Stability demonstrated
Efficacy	Monomeric intact IgG (7S)	Assured for each batch
	Broad spectrum of activity resulting from production from a large plasma pool	Over 8000 blood donors per batch
	Normal sub-class distribution	Demonstrated [11]
	Normal kappa/lambda ratio	Demonstrated [12]
	Normal IgG antibody structure and functions, in particular	
	– antigen binding	Demonstrated [13]
	– passage through biological membranes	Demonstrated [14]
	– normal biological half life	Demonstrated [14]
	– activation of the complement system in the classical way following antigen-antibody reaction	Demonstrated [13]
	– enhancement of phagocytosis	Demonstrated [13]
	– enhacement of antibody-dependent cytotoxicity (ADCC)	Demonstrated [13]

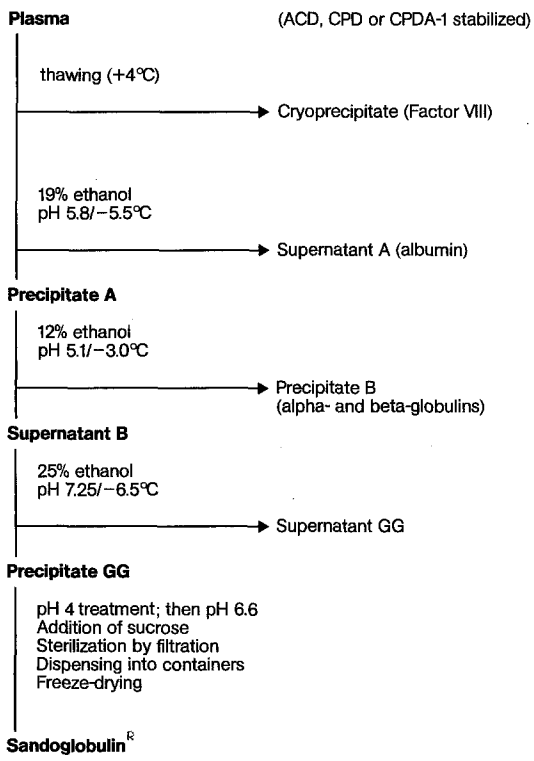

Fig. 1. The manufacture of immunoglobulin for intravenous use

The disadvantage is that the immunoglobulin isolated by the Cohn process, unlike that which circulates in the bloodstream, has spontaneous anticomplementary activity. The object of all efforts to produce the ideal preparation is to obtain a preparation free from spontaneous anticomplementary activity without changing the structure of the IgG molecule. This is no easy task, since we do not know precisely how the anticomplementary activity arises. It is assumed that the cause lies in the formation of aggregates; it is known, however, that not all aggregates are anticomplementary.

An *intact* antibody concentrate suitable for intravenous administration was first described by Barandun et al. [3] in 1962, by treatment of a standard immunoglobulin preparation at pH 4. The pH 4 process, particularly if minute amounts of pepsin were added for stabilisation, was a great step forward in the quest for of the ideal preparation. After a further time-consuming period of research we finally succeeded in the development of the preparation as characterized in Table 3[1].

Figure 1 is a summary of the manufacturing process, and the associated quality controls are shown in Table 2. The production of the i.v. preparation did not necessitate any fundamental change in the fractionation process. However, the larger volumes processed made it necessary to adopt more rigorous GMP regulations and also to modify the plant and equipment. Still more stringent quality control was also insti-

[1] Immunoglobulin SRK i.v., identical with Sandoglobulin®

Table 2. Manufacture and quality control of our immunoglobulin preparation

Material	Manufacturing step	Quality controls
Whole blood	Separation of the cellular components	HBs antigen in the blood unit
–	Pooling of the plasma	Protein content, bacterial count, limulus test
Ethanol Distilled water Chemicals Filter aids	Fractionation pH4 treatment Bulk manufacture (solution for filling containers)	Monitoring of the environment (germ count in air and on surfaces) Raw material testing (identity, purity, limulus test) Water testing (conductivity, germ count, limulus test) Physicochemical measurements, (pH, protein concentrations, temperature, density, weights)
Sterile filters, vials, stoppers	Sterilization of the materials	Testing the materials
	Aseptic filling and insertion of stoppers	Monitoring the sterilization conditions Monitoring the environmental conditions (germ counts in air and on surfaces) Checking the filling weight (spot checks) Checking the integrity of the sterile filter before and after filling
–	Freeze-drying	Control of freeze-drying conditions
–	–	Tests on final product (see table 3)

Table 3. Final product test

Test (6% solution)	Requirements [a]
1. Safety tests	
Sterility test	Sterile
Pyrogen test	Pyrogen-free
Safety test	Safe
HBsAg	Not detectable
HBs antibody	At least 0.1 IU/ml
Anticomplementary activity	No spontaneous anticomplementary activity
Prekallikrein activator (PKA)	3% (FDA ref. [2])
Isoagglutinins anti-A and anti-B	1:2
Dissolution time	8 min
Particulate matter (spot check)	no visible particles
2. Composition	
Protein content	100–120% of declared value
Sucrose content	100–120% of declared value
pH	6.6
Protein composition	98% IgG
Molecular size distribution of the IgG	Monomers + dimers: 95% Aggregates : 1% Fragments : 4%
Residual moisture content	0.8%
3. Efficacy (antibody content)	
Anti-HBs	0.4 IU/ml
Anti-HAV	30 IU/ml
Anti-polio type 1	55 IU/ml
Anti-measles	100% of FDA reference standard
Diphtheria antitoxin	2.5 IU/ml
Antistreptolysin 0	500 IU/ml
4. Stability	
After storage for one month at 37°C	No spontaneous anticomplementary activity Fragments: 4%

[a] The data shown are representative mean values

tuted, and today the environmental conditions are carefully monitored, bacterial counts of the air, water supply and working surfaces are performed, the staff is continuously supervised and all technical data are automatically monitored.

End-product Controls

Before release the final product is subjected to a comprehensive set of tests (Table 3). Some of the tests are those generally stipulated by the authorities for all biological parenteral products; examples of these are the sterility, pyrogen and toxicity tests.

However, most of the tests and requirements are specifically designed for intravenous immunoglobulins. Since so far the pharmacopoeias do not include a monograph on intravenous immunoglobulins, we have had to develop our own set of tests; the registration authorities imposed further requirements, but also contributed many valuable suggestions. The tests used have been selected in such a way that the results *together* with the manufacturing records containing all the production steps in considerable detail, guarantee the conformity of the quality of each batch to that of the prototype batches; in other words it may be assumed that those properties which are not always tested also conform to the specifications. The validity of this assumption has been proved for the prototype batches during the development phase and documented in the registration file.

It was somewhat difficult to decide which antibodies should be assayed in each batch. The antibodies tested for at present, as listed in Table 3, represent a compromise dictated by the force of circumstances.

The monograph on normal immunoglobulin in the European Pharmacopoeia [4] stipulates that at least one viral antibody and one bacterial antibody shall be determined for which an international reference preparation or standard exists. This requirement alone considerably restricts the choice. The American FDA requires that the antibodies against poliomyelitis, measles and diphtheria be assayed and specifies the minimum content. Although this US requirement is applicable strictly speaking only to the standard preparation [5], it has been adopted for our i.v. preparation, applying a correction factor for the protein concentration; in addition we assay the antibody against hepatitis Bs antigen, the reason being that addition (and therefore the presence) of anti-HBs antibody to plasma products contaminated with hepatitis B virus can prevent an infection [6]. This is important because it has been reported that hepatitis was transmitted by an immunoglobulin lacking anti-HBs [7, 8].

Regular monitoring of the content of the antibodies listed above provides valuable evidence of uniform quality of each batch; moreover, the antibodies against polio and diphtheria are assayed by the neutralization test, and this test provides for direct evidence of clinical efficacy.

Much importance attaches to the size of the plasma pool – i.e. the number of blood units processed in a single batch. A minimum of eight thousand donors contribute to the manufacture of our immunoglobulin preparation by their blood donations. Therefore each batch contains all the humoral IgG antibodies consistently present in the donor population.

If the future should prove that some specific antibody deficiency states – e.g. anti-varicella zoster – or anti-CMV deficiencies – are as successfully treated with a pooled immunoglobulin preparation as with a specific hyperimmune preparation, the contents of these antibodies will also be determined in each batch and its concentration declared.

After release by the Quality Assurance Department the preparation is still not cleared for sale. In several countries a sample of the batch must be submitted to the health authority along with all the analytical results. Only after receipt of the release document from the health authority the product can go out to physicians and patients.

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