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UNITED NATIONS INDUSTRIAL DEVELOPMENT ORGANIZATION
Vienna International Centre, P.O. Box 300, 1400 Vienna, Austria
Tel: (+43-1) 26026-0 · www.unido.org · unido@unido.org

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MONGOLIA

Technical report: "Strengthening of Facilities for Lyophilization and Quality Control of Blood Derivatives at the Institute of Biological Products and Blood Transfusion - Ulan Bator"*

Prepared for the Government of the Mongolian People's Republic
by the United Nations Industrial Development Organization,
acting as executing agency for the United Nations Development Programme

Based on the work of Ilina L. Bineva,
Expert in Quality Control and Lyophilization

Backstopping Officer: Dr. Zoltan Csizer, Chemical Industries Branch

United Nations Industrial Development Organization
Vienna

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Table of Contents

Abstract

Project title: Programme "Biomed": Strengthening of facilities for production and quality control of gammaglobulin and albumin at the Institute of Biological Products and Blood Transfusion-Ulan Bator

DP/MON/82/004

The main duties of a four-month mission were to introduce modern methods in quality control so as to ensure meeting of WHO requirements and standards and to achieve the preparation of stable immunoglobulin by putting into action the lyophilization equipment.

The equipment for quality control and lyophilization was installed and put into action.

A training programme was prepared and strictly followed. Local personnel proved capable to apply the newly introduced methods and work with the new equipment provided by UNIDO.

The analysis of the preparations produced by the new technologies and with the new equipment showed that they meet the requirements of WHO including stability of immunoglobulin (this was achieved after the introduction of the lyophilization method in the production).

A plan for future research in the field of the improvement of the quality control and further characterization of the preparations was prepared.

It is essential that in future the needed reagents (diagnostic preparations) for the determination of antibodies' content are regularly supplied. This is also valid for all reagents needed for the future research.

INTRODUCTION

The Blood Fractionation Unit at the Institute of Biological Products and Blood Transfusion was established in 1971. The equipment before the implementation of the Project "Biomed" was inadequate and old fashioned. The quantities of the products albumin and immunoglobulin were insufficient and the qualities did not meet the WHO requirements with regard to sterility and stability.

The main objective of the Project was to expand, modernize and strengthen the Blood Fractionation Unit so that its production capacity is increased and the qualities of the blood derivatives produced are improved.

The author's mission started on 31 October 1986 and finished on the 22 February 1987 (23-28 February 1987 - travel for debriefing in Vienna).

In the course of the mission, the author worked in close cooperation with an expert in blood protein derivatives and a maintenance engineer (till the 22nd of January 1987) and under the guidance of the Chief Technical Adviser of the Project (till the 3rd of January 1987).

The author's duties were to:

1. Introduce modern methods of analysis at the consequtive stages of the fractionation of retroplacental serum and placenta and up-to-date methods of quality control of immunoglobulin and albumin isolated from placental sources in order to achieve the requirements of WHO.

2. Prepare a training programme for local personnel on modern methods of control applied in blood fractionation.

3. Train local personnel on modern methods of quality control of blood preparations isolated from retroplacental serum and placenta.

4. Standardize the blood preparations isolated from placental materials in comparison with international reference preparations.

5.Introduce research and development work for the control and standardization of the preparations and determination of the antibody content of the immunoglobulin preparations.

6.Install the lyophilization apparatus and start trial runs.

7. Determine the optimal parameters of lyophilization of protein derivatives from retroplacental serum and placenta.

8,Prepare a manual of operation for use and maintenance of the lyophilization apparatus.

9.Train local personnel in the methods of lyophilization, use and maintenance of the apparatus.

ACTIVITIES CARRIED OUT

The mission was started with looking over the methods of quality control (already applied routinely) of final containers of immunoglobulin and albumin,produced at the Blood Fractionation Unit. A training programme was prepared for mongolian specialists on the methods of quality control of immunoglobulin and albumin from placental sources and on the problem of lyophilization.

The following methods were performed correctly at the Unit and insured the characterization of the preparations on some basic points. These were:

- Determination of the protein content - by the biuret method or refractometrically;

- Determination of purity - by electrophoresis in agar gel at 10 v/cm,barbitone buffer pH=8,6 , protein concentration 1,5 %. Electrophoregrams were coloured with amido black 10 B. It was found that normally immunoglobulin purity was above 90 % and albumin purity - above 96%.

- Hydrogen ion concentration - pH measured in a solution of the final product diluted to a concentration of 1% in saline. The pH of the solutions according to local requi-

rements was 7,0 ± 0,4 (requirement of WHO 6,9 ± 0,5).

- Sterility was tested on 4 media: thyoglycollate, soya-casein, Sabourau medium and meat-peptone. The media were observed for 14 days as required. After the introduction of the membrane sterile filtration in a laminar flow hood no problems with sterility were observed. During training special attention was paid to correct manipulations ensuring sterility.

- Freedom from undue toxicity was tested on 5 mice each injected with 0,5 ml of the solution and two guinea pigs each injected with 5 ml of the solution. The animals were observed for 5 days; the preparation passed the test if none of the animals showed signs of ill health. If the latter happened the test was repeated. Normally the preparations passed the test.

- Heme-like content was determined in albumin preparations only. The solution was diluted in saline to a concentration of 1%. The extinction at 403 nm in a 1 cm cell was measured. Normally the absorbance was not greater than 0,15.

- Freedom from HBsAg was determined by the immunodiffusion method at the Blood fractionation Unit. This method is of low sensitivity but the control laboratory (central for the Institute) had some ELISA kits (SEVAC - Czechoslovakia) and kits for the detection of HBsAg by inhibition of hemagglutination and the preparations were tested by these methods also. It is recommended that the Blood Fractionation Unit is supplied with enough ELISA kits (SEVAC) for the detection of HBsAg so that this test is also performed there.

The pyrogen test, however, was carried out by a modification of the method which could give falsely low results. The mean value of the temperatures of the rabbits over a period of three days was considered initial and after the tested solution was injected the temperature was measured every 30 min three times. This test had to be revised and now it is performed according to the procedure given in

the International Pharmacopoeia, WHO, Geneva, 1980, v.I, p.155-157. Short description of the method is the following: rabbits (2-3 kg each) in good health are used, the temperature is measured by means of a standard electric tester. One hour before the injection of the tested solution the rabbits are placed in a special stand and the temperatures are measured each 30 min (twice). The initial temperature is the mean value between the two measurements (they should not differ by more than 0,2 ^{o}C). Rabbits with temperatures below 38 ^{o}C and above 39,6 ^{o}C are not used. Three rabbits are used for each tested solution which is injected in the marginal ear vein (1ml/kg for immunoglobulin and 3 ml/kg for concentrated albumin solution). The temperature is registered over a period of 3 hours (every hour). The maximum temperature rise is read, it should not be above 0,6 ^{o}C and the substance passes the test if the sum of the three maximum temperature rises is less than 1,8 (value accepted by the Mongolian control authorities at present). Normally the preparations of albumin and immunoglobulin produced by the Blood Fractionation Unit give a result which meets the requirement of the International Pharmacopoeia (the sum to be less than 1,4).

In the course of the fractionation the quality assurance system included regular control of the parameters temperature, pH, ionic strength, ethanol concentration, protein concentration. The measuring of the pH now (after the implementation of the project) is carried out with a very accurate autocal pH-meter (Radiometer).

Beside the above tests the WHO Expert Committee on Biological Standardization requires that the preparations are stable. Before the introduction of the lyophilization of immunoglobulin and the ultrafiltration of albumin the 10 % solution of immunoglobulin and the 20 % solution of albumin could not withstand the stability test. Now, after the implementation of the project this requirement is also met as it is possible to remove the ethanol completely by the above methods.

The determination of the molecular composition of the preparations became possible after putting into action of the set of tools for analytical chromatography. The determination of the aggregated and fragmented molecules was performed on a Sephadex G-200 column 2,6/100 cm (for immunoglobulin). More than 20 batches of immunoglobulin were analysed this way and it was found that in few batches aggregates were present (a low amount - less than 5%) and in one batch only a more substantial fragmentation had taken place (about 25% of fragments). Practically all batches (except the one with high proportion of fragments) meet the requirement of the European Pharmacopoeia that the quantity of aggregated molecules is less than 10% and the fragments are less than 5%.

The molecular composition of the albumin preparations was also studied. The column 2,6/100 cm was filled with Sephadex G-150. The percentage of aggregated albumin was normally below 5-6%. Despite the fact that there is not a special requirement to this end, it is preferable to have a preparation with low content of aggregated molecules because the latter leave the organism very quickly and thus the efficiency of the preparation could be decreased.

Further on a test for the determination of the anticomplementary activity of immunoglobulin preparations was introduced in the laboratory practice there. The study of the presence of aggregated molecules in immunoglobulin preparations was closely related to the study of the anticomplementary activity. Aggregates are considered the cause for the anticomplementary activity and side reactions to immunoglobulins. That is why special attention was paid to the application of the method of anticomplementary activity in the characterization of immunoglobulin. The principle of the method is to determine the amount of protein which binds $2CH_{50}'$ units of complement. The needed reagents hemolysin, complement and lamb erythrocytes were available and it was possible to study the produced by the Blood Fractionation Unit immunoglobulin preparations. It was found that the

anticomplementary activity was not high (0,5 or 1 mg protein bound 2 CH_{50} units of complement) and this corresponds to the low amount of aggregates in the preparations. The ability to perform this method of analysis is essential in the future research on the technology of production of immunoglobulin for intravenous use at local conditions.

Another method which was introduced in the practice of the Unit was the determination of the quantities of the immunoglobulin classes by the radial immunodiffusion method. Antisera and standards (Behringwerke) were available. It was found that the immunoglobulin preparations contain mainly IgG (as expected), the amounts of IgA and IgM were below 1% of the immunoglobulin content.

Due to the fact that the raw materials for the production of immunoglobulin and albumin are of placental origin it was expected that blood group substances could be present. The preparations were studied by means of the method of inhibition of hemagglutination (anti-A and anti-B sera were used) of human erythrocytes with the respective antisera by the blood group substances. There were no standard substances A and B and so only the titres of the inhibition of agglutination were recorded. This method is also to be applied in future research.

Also with regard to future research attention was paid to the method of ion exchange chromatography of immunoglobulin from placental sources. 500 ml of immunoglobulin from placental extract was purified with DEAE-Sephadex A-50 and was analyses by the above methods. The immunoglobulin proved to be of high purity and so if in future normal immunoglobulin preparations would be produced by this method from placental extract they would meet the quality standards of WHO.

It was decided to use the greater part of the immunoglobulin isolated from placental extract for experimental work aiming at the preparation of immunoglobulin for intravenous application. Residual proteolytic activity is present in the immunoglobulin preparation from placental extract and this would be used to reduce the anticomplementa-

ry activity of the preparation (because of the partial splitting).

The methods of the determination of antibody content were discussed mainly theoretically because most of the needed diagnostic preparations were not available. Only the determination of the titre of antistaphylococcus antibodies (by the neutralization of the hemolytic properties of staphylococcus toxin alpha-hemolysine) could be performed. It is recommended that the needed diagnostic preparations are supplied in order to characterize fully and routinely the antibody content of the immunoglobulin preparations produced in Mongolia.

Local personnel was trained in modern methods of quality control of blood preparations and the training programme was followed (Annex 1). In a course of lectures (each Tuesday from 16 to 17 hours) the different topics of the programme were discussed. Also practical training was carried out on-the-spot with professional and assisting personnel. The author also lectured at the course of the leaders of blood transfusion centres on the qualities of immunoglobulin and albumin.

Research programme was prepared. This programme includes experimental work in the field of the characterization and quality control of blood derivatives (Annex 2).

The author was also responsible for putting into action of the lyophilization equipment. The freeze dryer was unpacked on 19 November 1986 and it was found that it had suffered substantial damage: the door of the vacuum chamber had fallen off and the plastic window was torn out of its holding bolts. The front cover of the machine behind the door was bent inwards. It was found that the bolt which holds the vacuum chamber to the upper lid was bent and practically out of place. The tube of the reservoir of the thermal medium was torn out of its place and that is why the thermal medium was spilled away. All this could be due to a great shock because of negligent transportation. The needed repair work was done. Fortunately the apparatus was delivered with

essential spares (for example the temperature controller had to be changed) and it was repaired successfully, installed and put into action. In trial runs the optimal parameters of lyophilization of immunoglobulin were found. A manual of operation was prepared and further on freeze-drying was introduced as routine process in the production of immunoglobulin. The lyophilized preparation was analysed in respect to molecular composition and it was found that at the mild conditions selected aggregates were not formed. Local personnel was trained in the method of lyophilization both theoretically and practically. A manual of operation and maintenance of the appartus was prepared and handed over to the specialists who would work with it.

The method of ultrafiltration was applied in the production of 20% albumin solutions and thus the native form of the molecules was preserved and the high quality standards of WHO were met.

Beside the above activities, after the 3rd of January 1987 (the date Dr.V.Velev - Chief Technical Adviser of the project left Ulan Bator) the author took over the responsibilities of CTA till the end of my mission.

CONCLUSIONS

As a result of the implementation of the project improved quality control facilities were created. New up-to-date equipment for the control of the production stages and control of the final preparations (autocal pH meters,set of tools for automatic gel chromatography,laminar flow hoods, electric pyrogen tester,etc.) were installed and put into action.

New quality control procedures and methods so as to study all parameters according to the requirements of WHO, were introduced. It was found that the preparations produced by the new technologies with the modern equipment from retroplacental serum and placentae meet the WHO quality requirements.

The qualities of the produced blood derivatives in re-

lation to stability, sterility and pyrogenicity were improved by means of ensuring sterile conditions of work under the protection of laminar flow hoods, membrane filtration, etc.

National skills in the field of quality control and lyophilization were developed through theoretical lectures and practical in-service training. The fact that capabilities and skills were developed was confirmed by the demonstrated ability of local personnel to perform the quality control and lyophilization procedures without the need to be supervised.

RECOMMENDATIONS

1. In order to characterize fully the immunoglobulin preparations produced by the Blood Fractionation Unit it is recommended that a regular supply with diagnostic reagents for antibodies' determinations is arranged. The antibody content of each lot must be thoroughly studied and thus the immunoglobulin preparations would be directed for the propnylaxis or treatment of definite diseases and be used more efficiently.

2. The started research in the field of quality control must be supported by periodically (e.g. once in two years) sending members of professional staff abroad to get acquaintance with new developments of these methods.

3. Spare parts for the lyophilization equipment to be timely ordered and supplied.

Annex 1

PROGRAMME FOR TRAINING MONGOLIAN SPECIALISTS ON QUALITY CONTROL METHODS FOR THE CHARACTERIZATION OF IMMUNOGLOBULIN AND ALBUMIN FROM PLACENTAL MATERIALS AND ON THE PROBLEM OF LYOPHILIZATION OF IMMUNOGLOBULIN

1. Looking over the adopted system of control of immunoglobulin and albumin preparations at the Institute of Biological Products and Blood Transfusion and comparison with the WHO requirements	3-6.11.1986 and during fractionation and production of final preparations
2. Preparation of a list of needed reagents for the introduction of the methods given below	3-6.11.1986
3. Introduction of up-to-date methods of analysis which would be the basis for the development of experimental research work aiming at the improvement of the quality control of the preparations	November-1986 January 1987
3.1. Determination of molecular composition by gel filtration on Sephadex G-150 and Sephadex G-200	10.11 - 30.11.1986
3.1.1. Theoretical training on gel filtration	
3.1.2. Preparation of analytical columns	
3.1.3. Installation and putting into action of the set of tools for chromatography	
3.1.4. Analysis of immunoglobulin and albumin preparations produced in Mongolia	
3,2. Determination of immunoglobulin classes in the preparations	XI - XII.1986
3.2.1. Theoretical training on the method of immunoprecipitation and its different modifications	
3.2.2. Practical application of the method of radial immunodiffusion	
3.3. Determination of anticomplementary activity	1.12 - 30.12.1986
3.3.1. Theoretical and practical training	
3.3.2. Study of the immunoglobulin preparations produced in Mongolia	
3.4. Determination of blood group substances in the preparations of placental origin by the method of inhibition of hemagglutination	XII.1986 - I.1987

4. Performance of analytical (experimental, research) work on the comparison of the qualities of immunoglobulin and albumin prepared from retroplacental serum and placental extract. Studies on stability, sterility, pyrogenicity, heme-like content, blood group substances, anticomplementary activity, molecular composition.	XII.1986 - II.1987
5. Introduction of the method of ion-exchange chromatography in the practice of the unit for the purification of immunoglobulin from placental extract	I-II.1987
6. Theoretical training of local personnel on methods for antibodies' determination	II.1987
7. Reconstruction works (putting of a new door and bringing three-phase electricity in the room for lyophilization and installation of the USIFROID Apparatus.	17.XI - 30.XI.1986
8. Repair works on the lyophilization equipment	till 15.XII.'86
9. Trial runs and determination of the optimal parameters of lyophilization of gamma-globulin	till end of December 1986
10. Practical training of local personnel on the method of lyophilization	I-II.1987
11. Preparation of a manual of operation and maintenance of the lyophilization equipment	II.1987

The following members of local personnel took part in this training programme:

Control methods:	Lyophilization:
Dar	Ganbat
Erdenetuia	Jamsranjav
Narantuia	Ganbold
Sainchimeg	Soldbaatar
Ojunchimeg	

Annex 2

PROGRAMME FOR RESEARCH ON THE IMPROVEMENT OF THE QUALITY CONTROL OF THE PREPARATIONS FROM HUMAN BLOOD PROTEINS

1987 - 1990

I. Improvement of laboratory quality control and further characterization of immunoglobulin and albumin preparations

a) study of the molecular composition of immunoglobulin and albumin preparations by means of gel chromatography

b) determination of residual proteolytic activity in immunoglobulins isolated from different raw materials

c) methods for the stabilization of the physical properties, fragmentation processes and the specific activity of the immunoglobulin preparations

d) study of the immunoglobulin classes in the preparations

e) study on the presence of blood group substances in immunoglobulin and albumin preparations from placental sources

f) determination of antibodies in immunoglobulin preparations

g) determination of the anticomplementary activity of the immunoglobulin preparations