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PROGRAMME "BIOMED"

DP/MON/82/004/11-02

MONGOLIA

Technical report: "Strengthening of Facilities for Production 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

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United Nations Industrial Development Organization
Vienna

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ABSTRACT

Programme "Biomed": Strengthening of Facilities for Production and Quality Control of Gammaglobulin and Albumin at the Institute of Biological Products and Blood Transfusion

Project Number DP/HON/82/004/II-02

The examination of the supplied equipment for the implementation of the project showed that most of it corresponded to the requirements of the new technologies to be introduced with the exception of the CEPA-Technicum centrifuge TZ-3.

There was some delay in the fulfilment of the fractionation activities because some essential installations (electric, cooling) and production premises needed reconstruction work. This did not influence significantly the achievement of the objectives.

A programme for the theoretical and practical training of local personnel on the methods of fractionation was strictly followed. Local personnel has proved capable to carry out the technological work unsupervised.

New up-to-date technologies for the production of gammaglobulin and albumin from placental sources were introduced. The new technological equipment supplied for the project was used in the fractionation.

By means of the new technologies and equipment the production capacity of the Blood Fractionation Unit was increased. The gamma-globulin and albumin preparations meet the WHO requirements on quality.

A programme for research in the field of the fractionation of human serum proteins from placental materials was prepared. Directions for the fulfilment of the programme were given.

INTRODUCTION

Before the implementation of the Project at the Institute of Bioproducts and Blood Transfusion the Blood Fractionation Unit had production capabilities to process 1500 l retroplacental serum, collected in Ulan Bator and several regional (aimaks) centers and produce gamma-globulin and albumin solutions (5 and 10%). The production was performed by means of old fashioned and worn out equipment. The fractionation technology was a modified Cohn's cold ethanol fractionation method 6+9 adapted according to the Mongolian conditions and capabilities of personnel. The quantities of gamma globulin and albumin which were produced were far less than the real needs of the country to carry out its health programme. Besides, the produced blood derivatives by the old technology and equipment did not meet completely the requirements of the WHO.

In connection with the purpose of the project "Development of technological capabilities and skills for the local production of blood derivatives at the Institute of Biological Products and Blood Transfusion" being an expert in blood derivatives production the author's duties were to:

- prepare a programme for lectures and practical work for training local personnel on fractionation of placental materials;
- carry out experimental work at local conditions with local material to improve the technologies presently used for the fractionation of retroplacental serum at the Institute;
- be in charge and participate in the performance of large scale production of immunoglobulin and albumin from retroplacental serum and placenta;
- special attention to be paid to the specific problem on the removal of hempigments which present the main difficulty in the fractionation of placental materials;
- give continuous attention to theoretical and practical training of personnel - in service in the technological processes in the fractionation of retroplacental serum and placenta;
- start research in the field of fractionation of blood

proteins from placental materials.

These duties were fulfilled as described further on.

ACTIVITIES CARRIED OUT

The author's mission started with examining of the available production premises together with the Chief Technical Adviser of the project and the maintenance engineer. It was found that the premises were not completely reconditioned for the purpose of the enlarged production. That is why measures were undertaken to organize and carry out the needed reconstruction work in the rooms where the new equipment was to be installed. Also it became obvious that some of the essential installations (electric,refrigeration) were inadequate.

The examination of the supplied equipment for the implementation of the project showed that some essential items were not delivered at that time (mohnopump,decanting centrifuge). One of the important apparatuses - the ultrafiltration module "Sartocon" of the firm Sartorius was delivered with a number of missing parts. This was a serious obstacle for putting the apparatus into action. The other items of the supplied equipment for the fractionation purposes were in good state and corresponding completely to the aims of the project - to strengten and modernize the production performance by the introduction of up-to date technologies and considerable enlargement of processing facilities.

At the beginning of the mission the complete number of personnel was not appointed and there were not present all the needed persons who were to be trained in the new technologies.

In spite of the above obstacles,in cooperation with the maintenance engineer the author started working on the installation of the fractionation equipment and translated into Russian (the only language understood by local personnel) the operating instructions of this equipment. After the completion of recruitment of personnel the author began the theoretical training of the workers following a programme which was prepared in advance.

The reconstruction work in the premises, the equipment installation and the repair of essential installations to make them suitable for the work on the enlarged scale and with new technologies continued till the 10-th of December 1986 when the regular production was started.

Despite the delay, the needed conditions were created to process under my direct supervision 400 l retroplacental serum (more than 1/4 of the collected and processed serum per year before the implementation of the project) in the course of two weeks.

Thus all available at the Blood Fractionation Unit retroplacental serum (collected in months) was processed.

A number of changes in the technologiy for the production of gamma-globulin (Annex 1) were introduced. These changes brought on the one end to a double increase of the processing capability of the production equipment (for example concentrated ethanol was applied in the fractionation stages) and on the other end an improvement in the standartization of the fractionation (control of the salt concentration in the different stages and strict following of the temperature requirements).

In the production of human serum albumin a new (Annex 2) unknown in Mongolia technology ensuring a better control of the parameters of the production stages was introduced. Special attention was paid to the problem of the decrease of the hempigments' content. The technology proved very suitable for the fractionation of the retroplacental serum obtained locally.

Besides, all procedures for the preparation of sterile solutions and their filling into final containers were carried out in an up-to-date manner (unknown in Mongolia till present) by means of the new equipment ensuring sterility, safety and facilitating the work of the personnel (membrane filtratration devices, laminar flow hood, ampoule filling and sealing machine etc.).

The yields of gamma-globulin (30 ampoules, 1,5ml each 10% solution fron one litre of serum) and albumin (50ml 5% solution from one litre of serum) correspond to the yields obtained

in other countries processing similar raw materials by means of up-to-date technologies.

In the course of regular production of gamma-globulin and albumin from retroplacental serum the new fractionation equipment supplied for the implementation of the project was utilized and on-the-job training for the local specialists and workers was carried out both theoretically and practically on the application of the new technologies and on the correct work with the supplied up-to-date technological equipment (Annex 5). The results of the training were positive.

Detailed descriptions of the technologies were prepared. The needed amounts of reagents and matherials were calculated and the technological instruction were handed over to the Mongolian specialists.

The Mongolian side proposed that the fractionation of placentae (placental extract) envisaged by the project be carried out on a semi-production scale because of local difficulties with the supply of the necessary quantities of ethanol and the relatively high tax on this product in the country. Indeed placental extract (this additional raw material for the production of gamma-globulin and albumin) contains relatively small amounts of the important serum proteins but large volumes are to be processed and these large volumes need the addition of great quantities of ethanol to precipitate the different fractions and isolate gamma-globulin and albumin. 1900 l 96% ethanol are needed for the processing of 1000 kg placentae.

300 Kg placentae were processed and gamma-globulin and albumin were obtained. There were serious difficulties in the preparation of the placental extract (the extraction of blood out of the placental tissue) because of the inadequacy for the purpose of the supplied by Renka-Export centrifuge CEPA-Technicum TZ-3 (this point would be discussed further in the report). The extract had to be prepared by means of heavy, manual labour of low productivity which did not correspond to the purposes and the requirements of the project. In spite of this the needed extract was obtained and it was possible to

carry out the work planned.

New, unknown in Mongolia, technologies were introduced in the production of gamma-globulin and albumin from placental extracts (Annexes 3 and 4). These technologies ensure the removal of the hempigments and the preparation of products meeting the WHO requirements of the present day.

The equipment supplied for the project was used in the course of the fractionation procedures with placental materials and on-the-job training of local specialists and workers on the new technologies and the correct uses of the newly delivered equipment was carried out.

It could be concluded that local personnel engaged in the fractionation is capable of unsupervised work. In order to achieve this, beside the theoretical and practical training, detailed technological instructions with all calculated amounts of needed reagents were written down.

The technologies introduced in the fractionation of placental extract give the possibility to produce out of one kilogramme of placenta 2,5 ampoules 1,5 ml each 10% solution of gamma-globulin and 12,5 ml 20% solution of albumin. These yields correspond to the possibilities of the technologies of fractionation and to the yields obtained in other countries from the same raw material.

In cooperation with the Chief Technical Adviser of the project, the quality control expert and the National Project Coordinator, the author participated in the preparation of a plan for future research in the field of fractionation of serum proteins in Mongolia. The research programme (Annex 6) included experimental work on the problem of the preparation of some specific immunoglobulins and on immunoglobulin for intravenous application, on the development of a new technology for the production of albumin from placental serum by means of thermodenaturation of balast proteins (thus aiming at lowering of the need for ethanol for the fractionation), experiments for the removal of ethanol and concentration of immunoglobulin solutions by means of ultrafiltration. The first steps in the experimental

work to be carried out according to the research programme were discussed in detail with the Mongolian specialists. It is foreseen that in the future collaboration between Mongolian and Bulgarian specialists would be realized according to an agreement between the Ministries of Health of Mongolia and Bulgaria.

CONCLUSIONS

The duties of an expert in blood derivative production specified in the author's job description were fulfilled:

1. A programme for theoretical and practical training of local personnel on the methods of fractionation of placental materials was prepared by me and strictly followed.

2. Experimental work with local raw materials was carried out. The results of the experiments were applied in the improvement of the technological capabilities in the production of gamma-globulin and albumin.

3. Local personnel has learned well to apply the new equipment in the technological processes.

4. Technological prerequisites were created for the future considerable increase in the production capacity of the Blood Fractionation Unit.

5. The adopted technology in the production of gamma-globulin from retroplacental serum was improved.

6. New, up-to-date technologies were introduced in the production of albumin from retroplacental serum and of gamma-globulin and albumin from placentae. Specific stages for the removal of hempigments were characteristic of these technologies. Local personnel was trained to carry out the technological processes without supervision.

7. A Programme for research in the field of the fractionation of human serum protein from placental materials was prepared. Directions for the fulfilment of the programme were given.

RECOMMENDATIONS

In order fully to develop,consolidate and fully utilize the achieved technological results,it is recommended to:

1. Supply a really decanting centrifuge suitable for the removal of the placental tissue from placental extract. The CEPA-Technicum centrifuge TZ-3 (Purchase order № 15-6-DO497) delivered for the implementation of the project was characterized by the firm as "decanting centrifuge". This term was used in the commercial invoice and the packing list also.The operating instructions of the centrifuge showed that it was not the decanting but the filtering type. This was also proved by the documents which accompanied the centrifuge upon arrival. It was named there: "centrifuga dojarka do mleka" (milk centrifuge). The CEPA TZ-3 centrifuge proved suitable for the clarification of the placental extract and would be applied for this purpose at the Blood Fractionation Unit.

2. It is recommended that the Government authority ensure regularly each year the necessary funds for import of reagents and materials needed for the production.

3. Special attention to be paid to the future training of local personnel and it is recommended periodically to send specialists abroad in countries having well developed production of blood derivatives.

Fractionation scheme in the production of immunoglobulin from blood/plasma

retroplacental serum
or donor plasma
protein 5-6%
ethanol 8%
μ = 0,14
pH = 6,8
T^o = -3 °C

---

supernatant
protein 4%
ethanol 25%
pH = 7,0
μ = 0,09
T^o = -8 °C

precipitate
(discard)

---

supernatant
(for albumin)

precipitate
protein 2%
ethanol 25%
pH = 7,0
μ = 0,09
T^o = -8 °C

---

supernatant
(discard)

precipitate
protein 1,5%
ethanol 13%
pH = 4,8-5,0
μ = 0,024
T^o = -4 °C

---

supernatant
protein 0,5%
ethanol 25%
pH = 7,0
μ = 0,05
T^o = -8 °C

precipitate
(discard)

---

supernatant
(discard)

precipitate

IMMUNOGLOBULIN

Fractionation scheme in the production of albumin from blood serum/plasma

retroplacental serum
or donor plasma

protein 5-6%
ethanol 8%
μ = 0,14
pH = 6,8
T^o = -3°C

---

supernatant
protein 4%
ethanol 25%
pH = 6,9
μ = 0,09
T^o = -8°C

precipitate
(discard)

---

supernatant
protein 2%
ethanol 40%
pH = 4,8
μ = 0,09
T^o = -8°C

precipitate
(for immunoglobulin)

---

supernatant
(discard)

precipitate
protein 3%
ethanol 10%
pH = 4,7
μ = 0,01
T^o = -3°C

---

supernatant
protein 2%
ethanol 35%
pH = 6,0
μ = 0,05
T^o = -8°C

precipitate
(discard)

---

supernatant
protein 1,5%
ethanol 35%
pH = 4,8
μ = 0,09
T^o = -8°C

precipitate
(discard)

---

supernatant
(discard)

precipitate

ALBUMIN

Fractionation scheme in the production of albumin from placental extract

placental extract
protein 3%
ethanol 20%
μ = 0,12
pH = 6,9
T^o = -8°C

---

supernatant
protein 1%
ethanol 40%
μ = 0,12
pH = 4,8
T^o = -8°C

precipitate
(for immunoglobulin)

---

supernatant
(discard)

precipitate

1. protein 3%
   pH = 2,1
   T^o = 5-10°C

2. pH = 6,5
   ethanol 9%
   T^o = 68°C (30 min)

3. T^o = 35°C
   pH = 4,4
   T^o = 10°C

---

supernatant
protein 0,5%
pH = 4,85
ethanol 40%
T^o = -8°C

precipitate
(discard)

---

supernatant
(discard)

precipitate

ALBUMIN

in the presence of
sodium octanoate

Fractionation scheme in the production of immunoglobulin from placental extract

placental extract
protein 3%
ethanol 20%
μ = 0,12
pH = 6,9
T^o = -8°C

---

supernatant
(for albumin)

precipitate
protein 1%
ethanol 6%
pH = 4,9
μ = 0,8
T^o = -2°C

---

supernatant
protein 0,5%
ethanol 25%
pH = 6,85
μ = 0,08
T^o = -8°C

precipitate
(discard)

---

supernatant
(discard)

precipitate
protein 1%
ethanol 17%
pH = 4,6-5,1
μ = 0,01
T^o = -6°C

---

supernatant
protein 0,5%
ethanol 25%
pH = 7,0
μ = 0,013
T^o = -8°C

precipitate
(discard)

---

supernatant
(discard)

precipitate
protein 8-9%
pH = 7,0
μ = 0,06
DEAE-Sephadex

I.M. IMMUNOGLOBULIN

PROGRAMME FOR THEORETICAL AND PRACTICAL TRAINING OF LOCAL PERSONNEL IN METHODS OF BLOOD FRACTIONATION

Course of lectures (November-December 1986)

1. Retroplacental serum and placental extract - raw materials for the isolation and purification of biopreparations.
2. Methods for the fractionation of blood proteins. Fractionation with cold ethanol.
3. Specific problems of the fractionation of placental materials.
4. The methods of membrane filtration and ultrafiltration.
5. Sterility and non-pyrogenicity. The laminar flow hood. Methods for the removal of pyrogens.
6. Qualities of albumin and immunoglobulin from placental sources.

Practical training (November 1986-January 1987)

1. Mounting of the equipment for the fractionation in the cold room and trial runs.
2. Practical training on the application of the fractionation equipment in the cold room (reactors, pumps, filters, colloid mill etc.).
3. Practical course on the technologies for the production of albumin and gamma-globulin from retroplacental serum.
4. Mounting of the equpment for the preparation of placental extract.
5. Practical training on the application of the equipment for the preparation of placental extract (extractors, decanting centrifuge etc.).
6. Practical training in ultrafiltration. Dialysis and concentration of albumin solutions.
7. Practical training on the technologies for the production of albumin and gamma-globulin from placental extract.
8. Practical training in sterile membrane filtration. Work in a laminar flow hood.

Lectures and discussion were held once a week (every Monday) for one hour.

PLAN FOR RESEARCH IN THE FIELD OF BLOOD DERIVATIVES FROM PLACENTAL SOURCES

1. Study on the methods for the preparation of specific immunoglobulins (anti-staphylococcal, antitetanus).
2. Reserch and work on a technology for the preparation of an immunoglobulin for intravenous application. Introduction of the method of ion-exchange chromatography for the purification of immunoglobulin from non-specific contamination.
3. Research and work on a technology for the production of albumin from placental sources by means of thermal denaturation of ballast proteins. Comparison of the qualities of albumin obtained by the cold ethanol technology and of albumin obtained by thermal denaturation of contaminations.
4. Experiments on the removal of ethanol and concentration of immunoglobulin solutions.