Inspection of the Plasma Fractionation Laboratory (Oxford)
(Blood Products Laboratories)
Churchill Hospital
Oxford OX3 7LT

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1. Introduction

The Oxford Plasma Fractionation Laboratory (PFL) is part of the Blood Products Laboratories with Dr R S Lane as the overall Director.

PFL produces all of the factor IX required by the UK and factor VIII is produced from plasma supplied by Oxford RTC and Wessex RTC.

Discussions regarding the long term plans for PFL Oxford have recently been formulated (see letter of Dr Lane of 16th June for details).

This was the first inspection of PFL Oxford and followed an introductory visit to discuss background matters with Dr Bidwell, the Head of PFL.

2. Staff at Oxford

Dr Ethel Bidwell - Head of PFL Oxford (retiring July 1981)
Mr Snape - Scientist I/C Analytical Quality Control
Dr Smith - Scientist I/C Production (Also responsible for part of BPL, Elstree)
Mr Dike - Chief Technical Officer
Mr Evans - Asst Scientist, Production
Mr Stone - Chief Technician, Production
Mr Haddon - Chief Technician (QC)
Mr Sims - Senior Technician (QC)
Total staff comprises of 30 staff, 5 of whom are part time.

Dr Bidwell personally releases all products, with all analytical results and documentation being overseen by Mr Snape. Mr Snape's staff also carry out final labelling of vials.

Dr Smith has production responsibilities at BPL Elstree also and he divides his time between both sites.

Mr Dike is the third person on this second management strata, and he oversees various technical services including engineering, engineering development and also safety, and administration.

Commissioning of autoclaves is carried out by Oxford AHA and Mr Miles (Hospital Engineer) was present on the 24th June.

3. Product Range

Factor VIII ("Intermediate Purity")
Factor IX Complex

Occasional production of Factor VII takes place. Some batches of factor IX contains therapeutic levels of factor XI. Concentrates of factor XIII and III are under development.

4. Production Facilities and Equipment

The main part of the facility, comprising of the production area is joined to but administratively separate from the Haemophilia Centre.

There is a separate Chemical/Physical Testing Laboratory, a Coagulation Testing Laboratory housed in Portakabins, a Research and Development Laboratory and an electrical mechanical workshop. (see sketch plan for details).

The final labelling of the vials is carried out in the Portakabins, and there is also a cold room and issuing store in a nearby building.

4.1 Plasma Reception and Storage

There is a receiving bay at the back of the main building. Fresh frozen plasma is received as single donations separated from whole blood or taken by plasmapheresis. This is stored at - 40°C in a cold room and ACD and CPD bags are kept separate. The cold room has a temperature gauge, a recorder chart, and an alarm system on 24 hour surveillance.

If this cold room is being defrosted then plasma has to be stored in the cold store inside the main room (preparation area)

Upon receipt Plasma is booked onto a goods receiving sheet and a check is carried out that the HB_sAg tests have been performed at the Regional Blood Transfusion Centre.

The batch number of the plasma pool that it is going into is always entered on the receipt sheet at the appropriate time before pooling, and ACD and CPD donations are processed separately.

4.2. Staff Entry

Staff enter the General Processing area from the opposite end of the room to plasma entry and at present there is no proper changing lobby. Plans are being agreed to alter this, but this will still involve staff passing through the wash up area. This has had to be shared with the other staff at the Oxford Haemophilia Centre which brings other people unnecessarily into the locality.

4.3. General Processing Areas

In order to process plasma, the bags are taken from the - 40°C Cold room, though autoclave area and past the engineers room. The engineers room is to be relocated and the present space used as a production control office. The processing areas are not in separate rooms, and thus plasma pooling is carried out in a section of the preparation area. The preparation area also contains the stills, a wash-up section and work such as reclamation is carried out here.

There is no filtered air supply to the general processing areas and the main room was exceedingly warm.

The louvered windows overhead are kept shut but one set had opened and was jammed.

4.4. Plasma Pooling

Fresh frozen plasma (FFP) is kept separate for pooling both regarding source eg Wessex or Oxford and depending on whether ACD or CPD blood bags were used.

FFP is either softened overnight in the 2-5°C cold room for 16 hours maximum or softened on the morning of processing. 75-150 kg of FFP is processed per batch.

The PVC bags are cracked open by plunging into liquid nitrogen and the FFP "slugs" transferred to closed softening trays. This "stripping" is carried out in an area that is curtained off whilst processing takes place. The trays containing plasma may be stored further at - 40°C or transferred to the 2-5°C room for softening overnight.

There is a wash-up and preparation area across the room, and DEAE cellulose reclamation also takes place nearby.

The outline flow chart for Factor VIII is as follows:

1. FFP in Single Donations (ex - 40°C Cold room)
2. Thaw, and strip (preparation area)
3. Pool (preparation area)
4. Centrifuge Cryoprecipitate (cold room 1)
5. Extract using Tris Buffer (fractionation lab)
6. Alhydrogel Absorption (fractionation lab)
7. Cold Step Purification (cold room 1)
8. Centrifugation (cold room 1)
9. Salt and pH Adjustment (frac lab)
10. Freeze in Bulk (if required)
11. Thaw Bulk (if applicable)
12. Pool Batches (if applicable)
13. Filter Sterilise (sterile room)
14. Fill into vials (sterile filling room)
15. Freeze Dry - (sterile area)
16. Label (portakabin)

4.5 Cold Room (+4°C)

A separate operator takes the bulk product into this area.

The product is weighed on a large scale (the tare of the vessel is known), and processed via the Sharples centrifuges.

The air is not filtered, but low bacterial counts are normally found.

The lagging to various pipes is mediocre and not easily washed down, but the room is otherwise sound.

The cryoprecipitate is scraped out of the centrifuge bowls. After extraction of factor IX complex and possibly factor VII, supernatant from the centrifuges is sent to BPL Elstree for recovery of Albumin etc

All contact parts of the centrifuges are autoclaved before use.

4.6. Tris Extraction onwards

This is carried out in the area adjacent to the sterile area and the air is cleaned up to some extent by filtered air leaving the sterile area.

4.7. Autoclaves etc

Mr Dike and Mr Stone have a monitoring function regarding technical aspects but routine maintenance is by the Hospital Engineer. Mr Miles is the Hospital Engineer and attended for part of 24th June.

The current HTM 10 has yet to be implemented by Oxford AHA and present maintenance is not yet to this level.

A Sterilising Engineer has now been appointed by Oxford AHA to implement the standards required by HTM 10.

Six point recordings are taken every three months to check the autoclave which is a 21 cubic foot Drayton Castle. This is a 'one off' special adaption of the MK 5.

Empty Vials, and wads are sterilised in the autoclave.

No special monitoring or cleaning up of the steam takes place.

There is no extraction over the autoclave.

A log book of maintenance carried out is kept by PFL, but general maintenance and detailed schedules are the responsibility of the Hospital Engineer.

4.8. Aseptic (Sterile) Area

Vials are washed at BPL Elstree and supplied clean. They are double wrapped the day before use on a table alongside the plasma pooling area, but this is not done during pooling.

A large 'pass-thru' hatch is to be completed to upgrade existing arrangements for entry of the vials into the aseptic area.

Filters to the area have not been DOP tested. The terminal HEPA filters are changed routinely every three months, which is probably not necessary, and could be reduced by detailed filter monitoring.

Coats hung up in the changing rooms were over sterile packs.

Although cramped, the changing area is adequate and leads into a reception corridor.

The small filling room has LAF, and due to space requirements, a sliding door is present. Recesses etc were however clean. A 'house' label and not the final label is applied at this stage.

There is a freeze drying room past the filling room, and the condition of this is poor eg

1. crudely lagged flexible pipe from freezer
2. no coving
3. some wall finishes requiring repair
4. ceiling lights not flush fitting
5. Freezer not fitted either flush with wall or far enough away to facilitate cleaning.

The new Virtis freezer dryer is still not functioning properly despite a great deal of effort from PFL staff.

The Virtis engineer was present on 24 June and it appears progress has now been made to assure satisfactory conditions and monitoring inside the unit.

In the past, some vials have been brought out of the aseptic area and stored in a cold room before final drying and sealing of the freeze dried product. This was due to the problems with the Virtis equipment.

4.9. Distilled Water

This is kept in a heated tank at 80-90°C, and take off points are routinely sampled and tested. The Limulus pyrogen test is carried out as an in process test.

Any tests for bacterial levels are carried out at BPL, Elstree

4.10. Labelling

This is carried out in one of the Portakabins by QC staff after the final assay has been completed and the product released for packaging.

Labelling is to be moved shortly to a converted store room.

Joined sheets of labels from Label Research Ltd are used, and an absolute reconciliation can be done.

Mr Snape wishes to continue to carry out this work since his staff are also checking each vial, and in practice this seems reasonable.

4.11. Finished Goods Stores

This is in an adjacent building. Some vials were very near the cold room evaporator and in danger of becoming wet. (These were subsequently moved)

Factor VIII usually goes directly to the Haemophilia Centres once released and Factor IX is issued from here.

4.12. Proposals for Present Facility

These are proposals to upgrade and alter the present facilities. These include the following:-

1. Step-over acess/dressing areas at front and rear of clean processing arear to be provided.
2. Workshop to be moved,
   Conversion of present workshop to office area
3. Recycling of DEAE cellulose to be transferred to BPL Elstree
4. Two LAF units for the fractionation laboratory, plus one overhead unit to protect component assembly, and one overhead unit to protect freeze-dry loading.
5. Frozen plasma opening/crushing/thawing machine (bags to be opened without touching). This is at an advanced state of development.
6. Forced ventilation to some areas
7. TFS room to be moved
8. Cold room (1) - lagging to be upgraded
9. 'Pass-thru' - large hatch to clean room to be double doored.

5. Documentation and Procedures

Comprehensive process documents are now used and a great effort has been made to document all procedures. Examples of standard process documentation and standard procedures are filed.

A master check list is used to ensure that all documents are present and have been checked before product release occurs.

Quality Assurance

6.1. General Background

Mr Snape is in charge of analytical quality control and reports to Dr Bidwell, Head of the Laboratory.

Facilities are available on site for analytical control and assay of all coagulation factors. Some testing and/or reading of results is done by BPL at Elstree (eg hepatitis testing and the reading of sterility test results).

Factor VIII and IX produced here is of "intermediate purity". Higher purity material can be made but yields would be affected adversely. The National Reporting System for adverse reactions with haemophiliacs is based at the Churchill Hospital. The clinician responsible for the Haemophilia Centre (Dr Rizza) advised the following:

1. There was little evidence of auto-immune induced reactions in haemophiliacs (and those that were unlikely to be caused by the treatment).

2. Additional testing for tumour inducing viruses was thought unnecessary.

3. Potential screening for "Non A - Non B" hepatitis and hepatitis A by excluding plasma drawn from patients with raised liver enzyme activity was thought unnecessary and impractical.

4. Close control of dosage seems unnecessary - patients receiving bottle(s) rather than exact units. Patients undergoing surgery at this hospital are checked for clotting ability. The haemophilia centre also checks the potency of batches of Factor VIII and IX to be used (including those originating at the PFL Oxford).

5. The "intermediate purity" product is clinically effective.

6.2. The role of the PFL, Oxford

The laboratory processes pooled plasma obtained from two centres - Wessex and Oxford. The main products are Factor VIII and IX with occasional batches of Factor II, VII, XI, and XIII.

PFL Oxford itself exercises no direct control over the plasma supplied, which is its main raw material. Reliance is placed on the dialogue which occurs between BPL, Elstree and the Transfusion Directors.

6.3. The Role of the Quality Control Department

1. This is essentially an analytical service as opposed to a true quality assurance role. The unsatisfactory production facilities have not been upgraded to modern standards by the active involvement of a QC department.

2. Unusually the QC department is directly responsible for labelling and inspection of finished vials. Providing sufficient assurance can be given that adequate independent checks are incorporated, so that labelling errors will be identified and corrected, this situation may be allowed to continue.

3. The limulus test for pyrogens is not used for bulk or finished product. The test does appear to be used elsewhere in conjunction with the rabbit test and does result in informative data.

4. A manual of Standard Operating Procedure is available and this appeared comprehensive.

6.4. Laboratory Facilities

Analytical Control Laboratory

Testing is carried out for electrolytes, moisture, pH, conductivity and protein estimation (by the Biuret method).

A UV Spectrophotometer is available.

All raw materials are tested at BPL, Elstree.

Hydrochloric acid is identified at Oxford but tested at Elstree.

6.5 Documentation used

These included the instruction method, a laboratory work book and batch sheets for products.

6.6 Sterility testing

Whilst the Oxford laboratory "sets-up" the test, it is the Microbiology Laboratory at BPL, Elstree who adjudicate and "read" the media at Elstree.

6.7 Potency testing

Biological potency testing is done by the laboratory at Oxford. For Factors VII, VIII and IX two operators assay two bottles against two ampoules of a standard. Low potencies are not routinely re-assayed because of confidence in the system. Some low potency batches were noted (205 units; 210 units). An explanation for the low potencies was normally available (eg plasma collected into ACD rather than CPD).

For factors II and X a single stage assay is used and for factors VIII and VII a two stage assay is employed.

6.8 Hepatitis testing HB_sAg

The BPL RIA test is used to test the final product.

In addition sub pools are also tested from the supernatant of two different columns used to absorb the Factor VIII.

6.9 Defects/Adverse reactions

A file of adverse reactions is maintained. These appear to be followed up as far as possible.

6.10 Summary of tests carried out on products

Physico-chemical

Appearance
Solubility
Total Protein
Fibrinogen
Electrolytes
Moisture
Protease Activities (Prekallikrein activator)

Biological Safety Tests

Sterility
Pyrogen
Abnormal Toxicity

Specific Tests of Product Safety

Potential thrombogenicity
HB_sAg
Allo Haemaglutinens (Anti-A). Each batch of factor VIII, 1 in 10 of factors VII, IX).

Other tests

Factor VIII related antigen (electrophoresis method)
Factor VIII clotting antigen (RIA - as a measure of the destructiveness of the manufacturing process).

6.11 Microbial monitoring

Viable counts have been carried out on process fluids. Some (eg the back wash from the Sharples centrifuge(have been high - though later results have been substantially improved.

It should be noted that the sample size is small (1 ml out of a total volume of 100 litres) and anaerobes may not be shown up.

Settle plates are used in the sterile area whenever filling takes place. Results are given as "per foot" of agar rather than per plate because different sized plates are used.

A slit sampler is also used on occasions.

No broth filling trials have been carried out.

7. Summary and Conclusions

This small fractionation facility in general is staffed by people of a high academic standard.

The Head of the Laboratory, Dr Bidwell, is retiring shortly and Mr Snape will be the Scientist in Charge Additional QC staff will therefore be necessary.

The facility suffers from poor basic design and lay-out and has not been brought up to modern standards, and at this stage upgrading plans do not include installation of filtered air to the general processing areas. Other plans to alter processes eg stripping and pooling are nearing implementation.

It is essential that the Management and nominated Quality Controller review the total Quality Assurance system including the requirements for better flow of work, separation of areas, microbiological monitoring of plasma from source, etc.

The use of a non BP procedure for the test for sterility is an example of where the new Quality Controller must co-ordinate matters. Tests and methodology controlled by Oxford are basically of a high standard.

This unit could be brought up to acceptable standards of GMP, probably at a reasonable cost but it is emphasised that cost effectiveness is not gone into for the purpose of this report.

The present facility does not meet the necessary standards of GMP that would be required of a commercial operation, and if licensable products are to be produced, a detailed schedule of upgrading must be agreed in the immediate future.

8. Recommendations to Licensing Authority

Refer report to the Management of the Laboratory for comments and for proposals.

GRO-C

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(P.F.L. Oxford)

List of Deficiencies

1. The general production areas are not provided with filtered air.

If localised LAF protection is supplied to critical areas, as scheduled, then the background filtered air could be at 5 micron level.

2. Greater separation/protection to critical areas should be provided.

Wherepossible, washing, recycling etc should be relocated.

It is noted that some relocation of work to BPL Elstree is scheduled.

3. PFL staff should use separate wash-up facilities from other non PFL staff eg the Wash-Up area.

4. Autoclaves should be commissioned and maintained as per HTM10

5. The sterile area where freeze drying takes place requires general upgrading to surface finishes.

6. Validation that the present system of washing vials at BPL Elstree is adequate is necessary. A better procedure is to wash and sterilise at Oxford or failing this vials should be resterilised at Oxford after transferring in closed containers from Elstree.

7. The nominated Quality Controller should ensure that all tests conform to compendial requirements eg the Ph Eur Test for Sterility

8. The present and proposed systems for the stripping of plastic blood bags, the pooling of plasma, and fractionation processes should be routinely monitored to ensure the plasma is not unnecessarily contaminated by these processes.

9. Plasma supplied to PFL Oxford should be routinely assessed for microbial contamination levels.

P.F.L. (OXFORD)
(Sketch Plan)