Quality Assurance in Blood Cell Labelling

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Overview

• What is QA
• QA in Blood Cell Labelling
  – Staff
  – Facilities and Equipment
  – Methods and Protocols
• Specific Methods for labelled cells
  – Erythrocytes
  – Leukocytes
  – Platelets
• Conclusion
Quality Assurance

Definition:
• The sum of all measures taken to obtain the required quality

Quality in medicine:
• Highest possible degree of safety
• Best possible care for each individual patient
QA Aspects in Cell Labelling

- Staff
- Facilities and Equipment
- Products: Radiopharmaceuticals / labelled cells
- Methods and protocols
- Documentation
- ...


Staff

• Defined responsibilities
• Correct qualifications
• Training for specific tasks / aspects of work
• Concept of hygiene: general and radiation
• Continued training
• Evaluate techniques regularly
Facilities and Equipment

- Aspects
  - Correct for purpose
  - Maintenance
  - Quality control
- Facilities and Equipment
  - Clean room (ISO class 7 / grade C)
  - LAF cabinets / Isolators (ISO class 5 / Grade A)
  - Dose calibrators
  - Centrifuges
  - Refrigerators and freezers
Clean rooms:

- Air monitoring for viable and non-viable particles
- Passive air monitoring (settle plates)
- Contact plates
- Air pressure differentials between rooms
LAF cabinets / Isolators

• Regular checks:
  • filter integrity
  • air velocity
  • air flow patterns
  • particle counts
• HEPA filters cannot be cleaned: Replace
• UV lights
LAF cabinets (cont.)

- Filter integrity
  - dioctyl phthalate into intake - measure by smoke photometer
- Air velocity and flow patterns
- Microbial counts: CFU
  - sterile settle plates in laminar flow unit
  - expose 2 h
  - incubate at 37°C
  - observe for growth and count colonies
- Active air sampling
Centrifuge

- Regular service
  - Calibration of speed selector
  - Wear of parts
Methods and Protocols

• Process validation:
  All methods should be validated before first use and
  before introduction of each new variation.
  Extensive range of tests

• Operator validation:
  After training, extensive testing of product, e.g. cell
  viability
  Re-evaluation at regular intervals (6 months)
  Include settle plates to monitor air quality during
  labelling process
Labelling technique

• Practice runs
• Microscopy of labelled cells (Haematology)
• Mock labelling techniques to test aseptic technique
  – Substitute sterile soy cassein broth for blood and reagents (ACD-A, Hespan)
  – Follow labelling protocol, including centrifuging
  – Incubate broth
  – Test for microbiological growth
• Maintenance of clean environment in cabinet
  – Sterile settleplates in LAF cabinet during labelling
  – Incubate and observe for growth
Radiopharmaceuticals

Quality control of radiolabelled cells includes:
1. Sterility – testing in radiolabelled blood products?
   Use media fills
2. Labelling efficiency
3. Cell viability, clumping etc
4. Biodistribution

New techniques require more testing,
   e.g. elution of activity from cells

Reagents and solutions
Radiolabelled Erythrocytes: QC

- Labelling efficiency
  - Centrifuge sample
  - Determine activity / counts in cells and in supernatant
- Imaging of Tc-99m RBC
  - No free pertechnetate
Labelled Leukocytes: QC

Aspects:

– HMPAO
– Visual inspection: routine
  no clumps, clots, aggregates
– Labelling efficiency: routine
– Cell viability: periodically
– Cell subset recovery tests: initial validation
– Efflux of Tc-99m from cells: initial validation
– In vivo appearance: routinely
HMPAO Chromatography

- 3-strip miniature method
  - attention to detail!
  - small droplets
  - do not let droplet dry
  - strips may not adhere to sides of vials
Cell Labelling efficiency

• Labelling efficiency:
  – measure radioactivity of cells and supernatant

\[
\text{\% LE} = \frac{\text{Activity cells}}{\text{Activity supernatant} + \text{cells}} \times 100
\]

40 – 80 %
Cell viability

Cell viability – in vitro: Trypan blue exclusion test

- Mix trypan blue solution with labelled WBC suspension
- Haemocytometer
- Phase contrast microscope, 100 x magnification
  - Clumps / aggregates?
  - Count cells, determine % of blue cells

< 4 %
Cell subset recovery test

• Count number of different cell subsets
• During separation and labelling:
  after each crucial step: 1 drop of cell suspension in 1 ml PBS
  haemocytometer
  optical microscope
• Final cell suspension:
  • RBC/WBC < 3
  • Platelet/WBC < 1
Efflux of radiolabel from cells

- Damaged leukocytes may release more radioactivity
- Aliquots of cells at 37 °C
- After 1 h, 4h and 24 h:
  - Centrifuge
  - Count radioactivity in pellet and in supernatant
- < 10% at 1 h is acceptable
In vivo lung uptake

1. Rapid transit, disappearance of radioactivity from lungs within 5 min
2. Delayed transit but complete clearance within 30 min
3. Prolonged focal or diffuse retention of lung activity, disappears within 3 h
4. Delayed transit with increased liver activity (> spleen)

1 and 2 are normal

Some disease processes also show late lung activity
Labelled Platelets: QC

- Platelet viability
- Platelet recovery
- Imaging
Platelets: Viability

- Platelet viability
  Mix 50 µl platelet susp + 50 µl 5 µM ADP on microscope slide.
  Visually observe aggregation under microscope

- Comment:
  - Waiting until viability testing has been finished may reveal normal viability, but the stored labelled cell population may have dramatically lost functional capacity.
  - Therefore, well trained staff needs to establish the performance of labelling on a routine base.
Platelets: Recovery

• Recovery:
  1. Collect 5 ml of venous blood at 60 min post inj.
  2. Calculate the recovery by the formula:

\[
\text{Recovery (\%)} = \frac{\text{blood act/ml} \times \text{blood volume} \times 100}{\text{injected dose}}
\]

% of injected dose (radiolabelled platelets) remaining cell bound in the circulation for 60 min.

Normal values: 55% - 72%
(pooling of about one-third of the platelets in the spleen and, especially, the liver.)
Platelet QC: Heart and liver imaging

- Perform a dynamic acquisition during the injection, with liver and heart in the field of view.
- The time activity curves, obtained by drawing a ROI over both organs, must show an almost parallel exponential decrease.
QA: Record Keeping

• Record of products and tests required
• **COMPLETION** of necessary forms
• Compare results with standards / criteria and act on deficiencies
• Examples
  – Radiopharmacy:
    • Receipt of materials / products
    • Elution of generators
    • Preparation of radiopharmaceuticals
    • Supply of radiopharmaceuticals (doses dispensed)
    • Quality control (equipment and products)
Conclusion

- Quality Assurance more than a few tests on finished product
- Cell labelling more than only labelling efficiency
- Initial validation of procedures and operators important
- Not all tests done routinely
- Our responsibility to protect our patients and ourselves
References

• A consensus for the minimum requirements of a blood cell radiolabeling facility. Eur J Nucl Med Mol Imaging 2009
Thank you