Quality Assurance in Blood Cell Labelling

Sietske Rubow
Radiopharmacist
Nuclear Medicine
Tygerberg Hospital and Stellenbosch University
smr@sun.ac.za

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
  - Refrigerator s and freezers

Clean rooms:

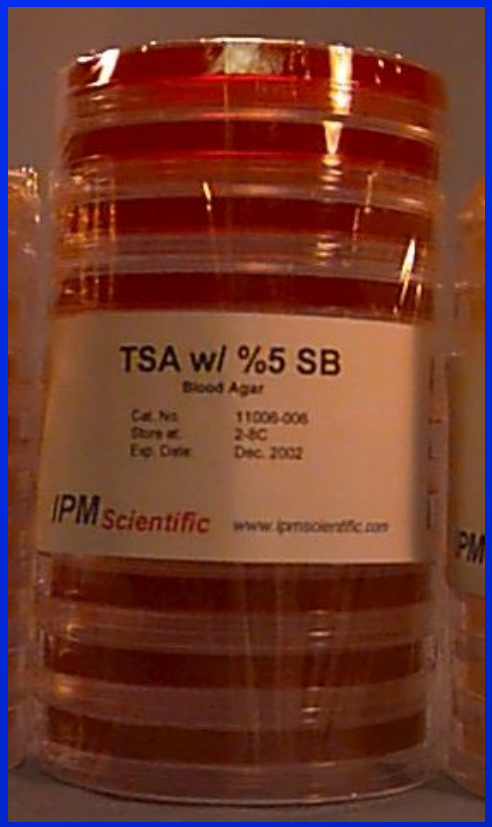
- Air monitoring for viable and non-viable particles
- Passive air monitoring (settle plates)
- Contact plates
- Air pressure differentials between rooms

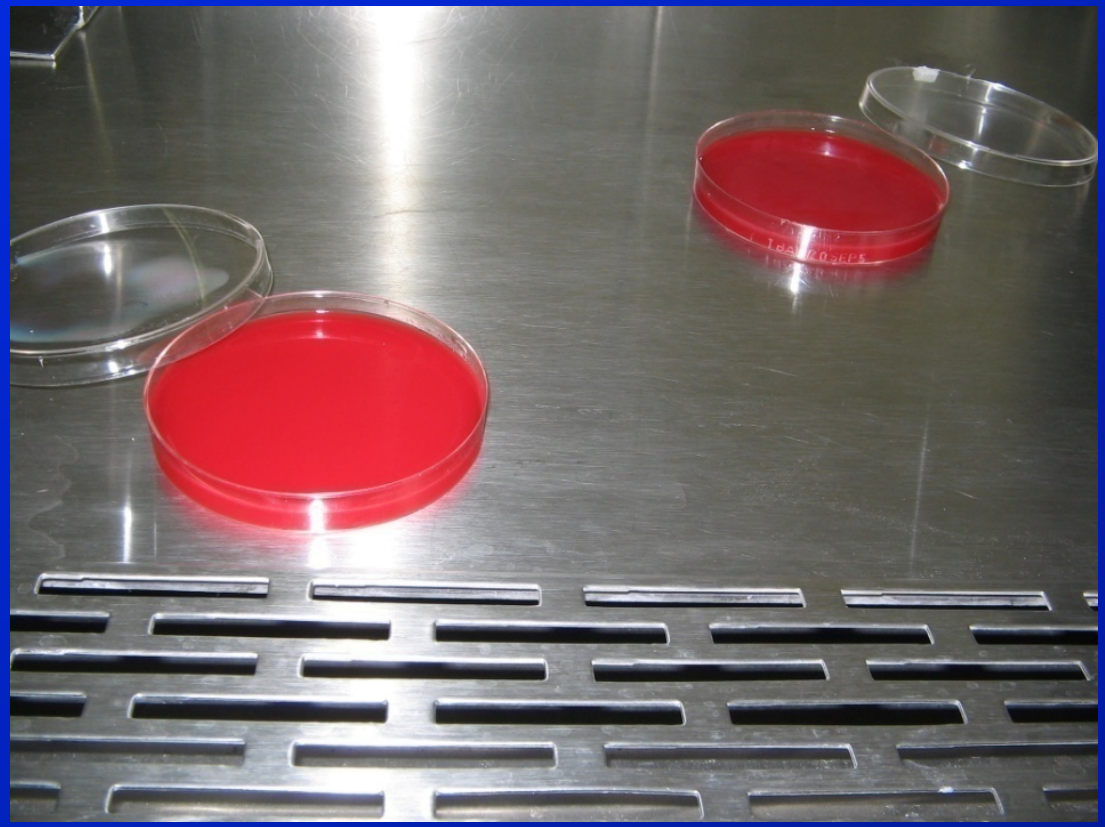
LAF cabinets / Isolators

- 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 phtalate 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


TSA w/ %5 SB
Blood Agar
Cat. No. 11006-006
Store at: 2-8C
Exp. Date: Dec. 2002
IPM Scientific
www.ipmscientific.com



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

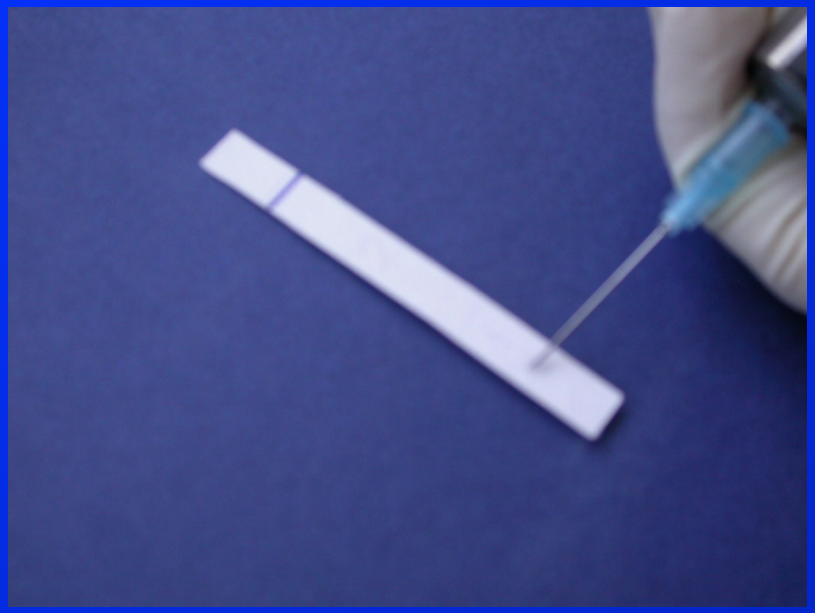
Labelled Leukocytes: QC

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




POWER
SCANNING SPEED (cm/min)
12
+
–
START/STOP

Cell Labelling efficiency

- Labelling efficiency:
  - measure radioactivity of cells and supernatant

40 – 80 %

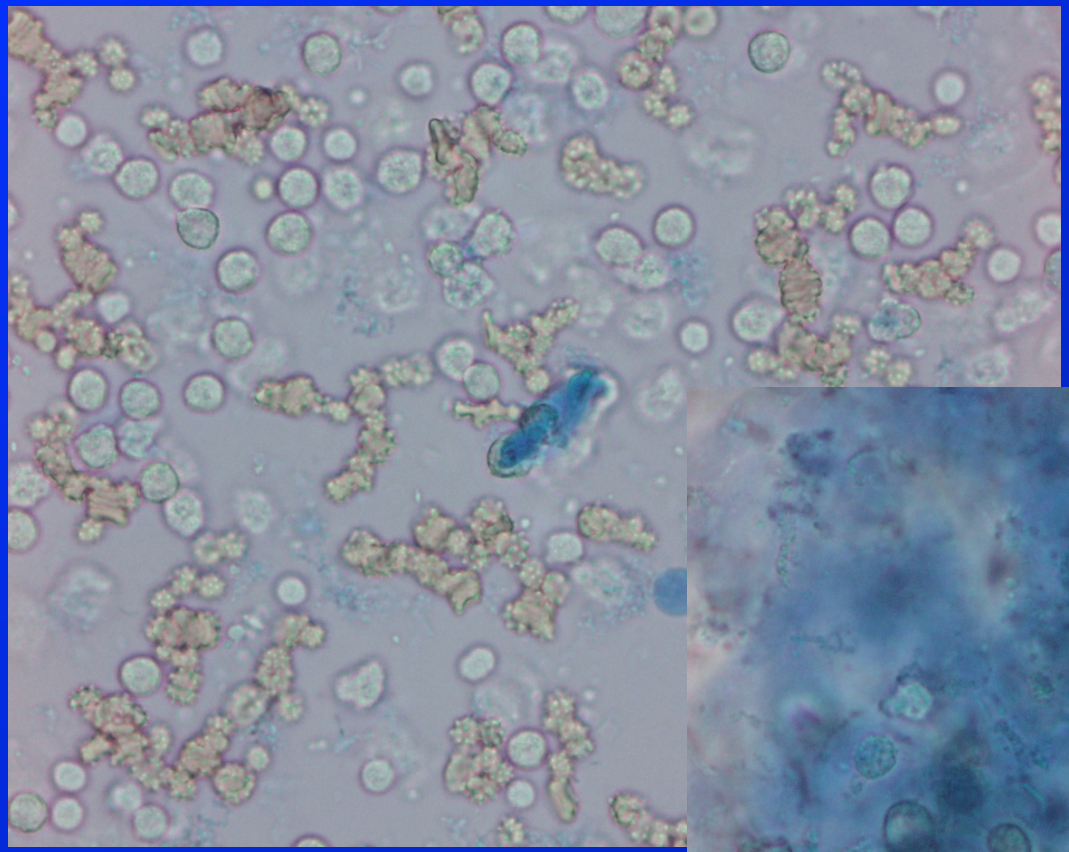
$$\% \text{ LE} = \frac{\text{Activity cells} \times 100}{\text{Activity supernatant + cells}}$$

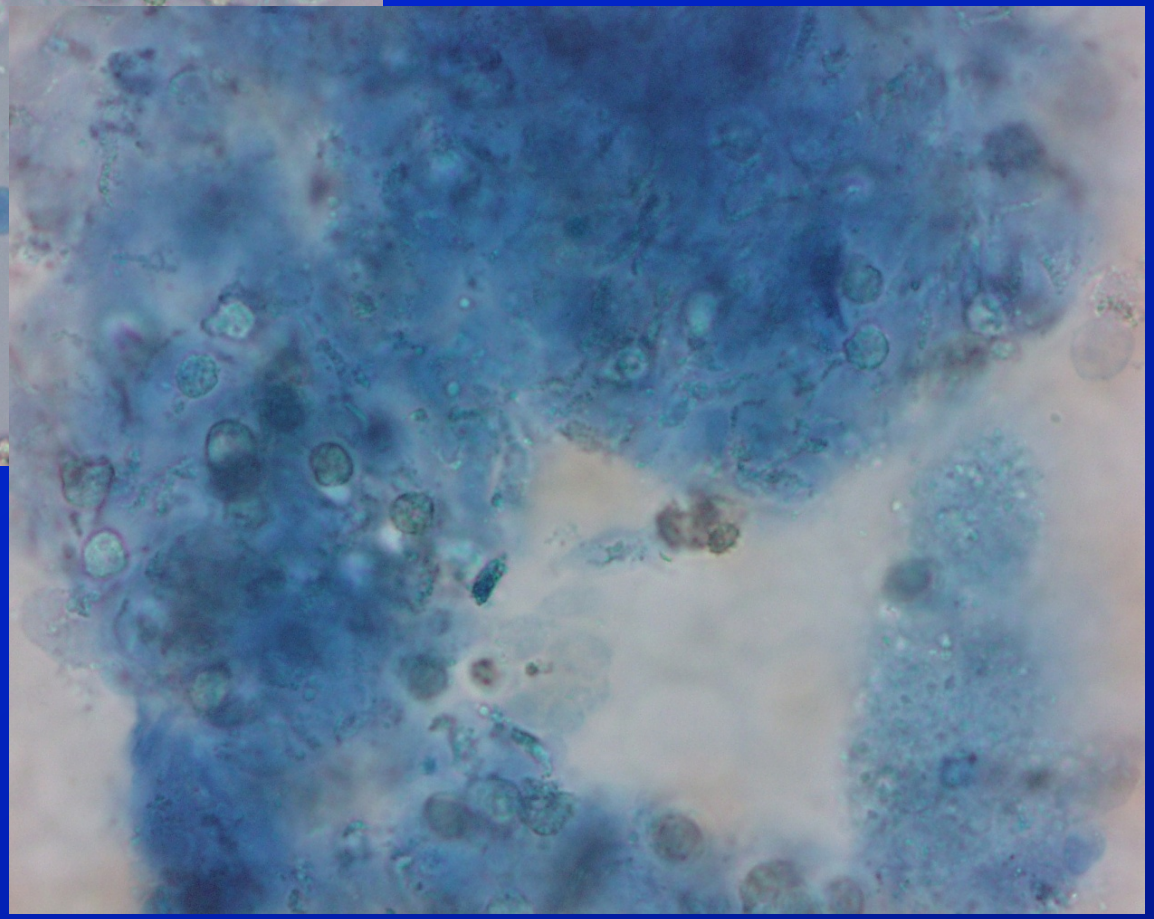
Cell viability

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:

$$\text{Recovery (\%)} = \frac{\text{blood act/ml x blood volume x 100}}{\text{injected dose}}$$

% 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

- Recommended Method for Indium- 111 Platelet Survival Studies ICSH, J Nucl Med (1988) 29:564-566
- Labelling of platelets with indium-111 oxine and technetium-99m hexamethylpropylene amine oxime: suggested methods Rodrigues et al, Eur J Nucl Med (1999) 26:1614–1616
- Guidelines for the labelling of leucocytes with 99mTc-HMPAO, Eur J Nucl Med (2010) 37:842-848
- A consensus protocol for white blood cell labelling with technetium-99m HMPAO, Eur J Nucl Med Mol Imaging (1998) 25:797-799
- A consensus for the minimum requirements of a blood cell radiolabeling facility. Eur J Nucl Med Mol Imaging 2009

Thank you