NHS
Blood and Transplant

Measurement uncertainty for FMH, antibody titration and antibody quantification

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Caring Expert Quality

MU and confidence in results

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- Confidence
  - will the correct result be reported?
  - identify risks potentially affecting
    - accuracy, reproducibility
  - mitigate / control risks
- Uncertainty and ISO15189 (5.5.1.4)
  - what is the MU (dispersion) of quantitative assays?
  - what is the uncertainty of the measurement steps in qualitative tests that can influence the result?

Confidence – some sources

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- Sample quality - CE IVDD tubes, User Guide
- Staff - documented training, PPDR, competency assessment
- Measuring equipment
  - maintained, calibrated to ISO17025 / (UKAS TPS41)
  - metrological traceability of cal. standard / ref method
  - specific standards - pipettes, ISO 8655-2 / 6

Confidence

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- Reagents and analytical equipment
  - CE IVDDs / accessories
  - purchasing specifications
  - traceability – NIBSC stds, Eu CTS, UKBTS G.lines
  - assessed suppliers / service agents
  - IFU / SOP
  - CC, verification / validation
  - reagent BPAT, DAT
  - internal Q/C (sensitivity and specificity)
  - EQA, NEQAS
  - appropriate storage / inventory control
  - post mkt. surveillance / incident reporting to MHRA

Confidence – control over critical test parameters, e.g. Ab titration

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	Validated assay tolerance nominal / range	Controllable limits	Measurement error
Pipetted volumes (25 to 200uL)	Acc. <+/- 0.8uL Prc. < +/- 0.3uL	Acc. <+/- 0.8uL Prc. < +/- 0.3uL (cal. failure)	< +/- 0.1uL
Incubation temp.	37°C +/- 2°C	37°C +/- 1.5°C	+/- 0.1°C
Incubation time	15 mins +/- 10 s	15 mins +/- 5 s	+/- 0.025 s
Red cell % conc.	0.8% +/- 0.2%	0.8% +/- 0.08%	Pipetting + Hct.
Centrifuge speed Dia-Med 24 card head	910 +/- 45 rpm	910 +/- 5	< 1 rpm

MU definition

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- "A parameter, associated with the result of a measurement, that characterises the **dispersion** of the values that could reasonably be attributed to the measurand"
  - *e.g. for anti-D (**analyte**) concentration in plasma (**measurand**)*

    *reported value = **10 IU/mL** **+/- 2 IU/mL***

    Ref: ISO IEC Guide 98-3:2008. GUM.

MU principles

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- Measurement result – only complete when accompanied by a quantitative statement of its uncertainty
- Helps decide:
  - is result fit for its intended purpose?
  - is it consistent with other similar results?
- MU - a core element of the QMS for calibration and diagnostic laboratories

MU methods

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- Type A – assess the dispersion of long term Q/C data (imprecision - SD or CV)
  - *Ref White GH, Francis I. (2004).*
- Type B – sum all individually identified uncertainty components:
  - *UKAS M003*

$$u_c^2(y) = \sum_{i=1}^{N} \left(\frac{\partial f}{\partial x_i} \right)^2 u^2(x_i)$$

Method A - imprecision

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- Assess all operating conditions:-
  - multiple calibrator and reagent batches
  - multiple operators, all equipment
  - seasonal variation.
- Ensure Q/C materials reflect patient specimens
  - beware – diluted, pooled, recombinant materials
- EQAS data not recommended
  - scarcity of data points
- Use Q/C materials close to clinical decision values

Method A - imprecision

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- New methods
  - ideally 30 data points
  - two different batches of reagents and calibrator
- Well established methods
  - six months Q/C data (same batch of control)
  - update calculation annually
- Calculation of MU values

  ± 1.96 x SD; or **± 1.96 x CV**

  E.g. Anti-D = 10 IU/mL **+/- 2 iu / mL** or +/- **20 %**

MU database (e- or paper)

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- Advantages
  - most info. already available in lab
  - easy updating MU values
  - ready access – audits / customers
- Content
  - analytical principle, diagnostic limitations of method
  - sources of error and their control
  - measurand / units
  - traceability to relevant standards (e.g. NIBSC)
  - clinical decision limits
  - imprecision and performance targets

Chosen Q/C materials

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- Anti - D/c quantification
  - NHSBT Reagents Anti-D - high control ~ 10 IU / mL
  - NIBSC Reagents Anti-c - control ~ 10 IU / mL
- Antibody titration
  - Initial exercise NHSBT Reagents Anti-K - titre 1/32
  - Future exercises – NIBSC Anti-D 07/304
- FMH
  - 'In house' simulated 4 mL FMH (0.2% D+ in D-)
  - CE mark in future

Antibody titration

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- Doubling dilution IAT titration Dia-Med gel cards
- Initial exercise
  - 100 BMS participants across 8 RCI labs
  - Expected titre 1/32
  - KPI 95% within +/- 1 doubling dilutions
    100% within +/- 2 DD
- Results
  - Mode = 1 / 32,
  - range 1/16 to 1/64
  - **Uncertainty = +/- 1 doubling dilution**

Anti- D / c quantification

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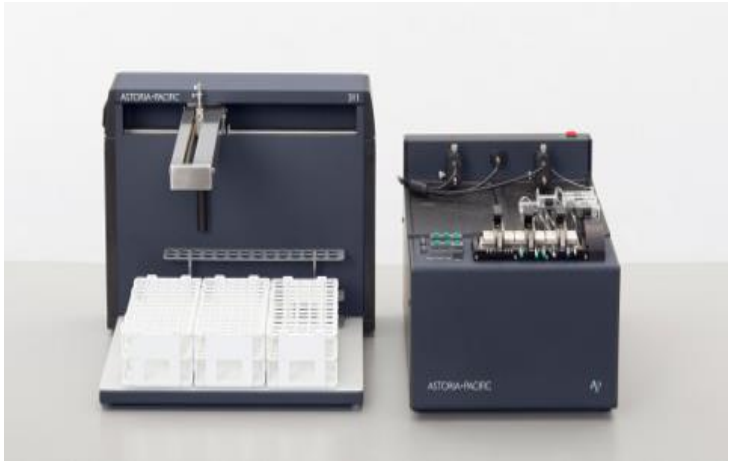
- Astoria Pacific 2 Analyzer BMC continuous flow analysis
- Diluted samples and ref. prep run Vs.
  - Group O bromelin treated red cells
    - Anti-D Vs. R1R1,
    - Anti-c Vs. rr
- Agglutinated cells decanted
  - Non agg. lysed / read 550nm
- FASPacII analytical software
  - sample peak height interpreted Vs. std ref curve.



Anti- D / c quantification

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High anti-D Quant Q/C sample						KPI
Lab	**A**	**B**	**C**	**D**	**E**	
mean	10.06	10.06	9.88	9.79	14.69	
SD	0.95	0.76	1.12	0.71	1.25	% MU < 20%
CV	9.49	7.60	11.32	7.29	8.53	20 - 25%
1.96 x CV	**18.60**	**14.90**	**22.19**	**14.29**	**16.73**	> 25%

Anti-c Quant Q/C sample						KPI
Lab	**A**	**B**	**C**	**D**	**E**	
mean	11.52	10.34	10.20	9.86	9.45	
SD	0.75	1.04	0.66	0.65	0.85	% MU < 20%
CV	6.47	10.01	6.51	6.57	9.00	20 - 25%
1.96 x CV	**12.68**	**19.62**	**12.76**	**12.88**	**17.64**	> 25%

FMH

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- Flow cytometric estimation % D+ fetal cells and FMH (mL)
- IBGRL FMH kit
- FITC- BRAD 3 Anti-D direct staining D+ cells
- FITC AVEZ 5.3 negative control

4 mL FMH clinical decision point sample				KPI
Lab	A	B	C	
mean	5.38	5.37	5.42	
SD	0.16	0.23	0.18	% MU < 15%
CV	2.96	4.25	3.32	15 - 20%
1.96 x CV	**5.80**	**8.32**	**6.51**	> 20%

References

- Medical laboratories - Requirements for quality and competence (ISO 15189:2012)
- *ISO IEC Guide 98-3:2008. MU – Part 3; Guide to the expression of uncertainty in measurement (GUM)*
- *White GH, Francis I. (2004). Uncertainty of Measurement in quantitative medical testing* http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1934961/
- *UKAS M003 The expression of uncertainty and confidence in measurement, UKAS Edition 3, November 2012* http://ndcsb217:8088/upload/controlled_documents/ESD93.pdf

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Thank you –
any questions?