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Quality and uncertainty in screening assays from taking the sample to issuing the result

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PHE Screening

Outline

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- Ensuring that the testing operates smoothly as a programme not just a test
- Sample quality
- Assay quality and population monitoring
- Sample transport
- Reporting results and confirmatory testing

The quality of the spot

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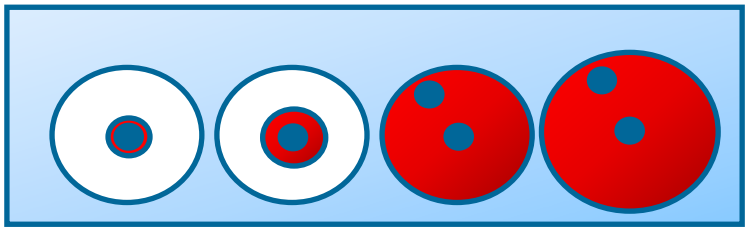
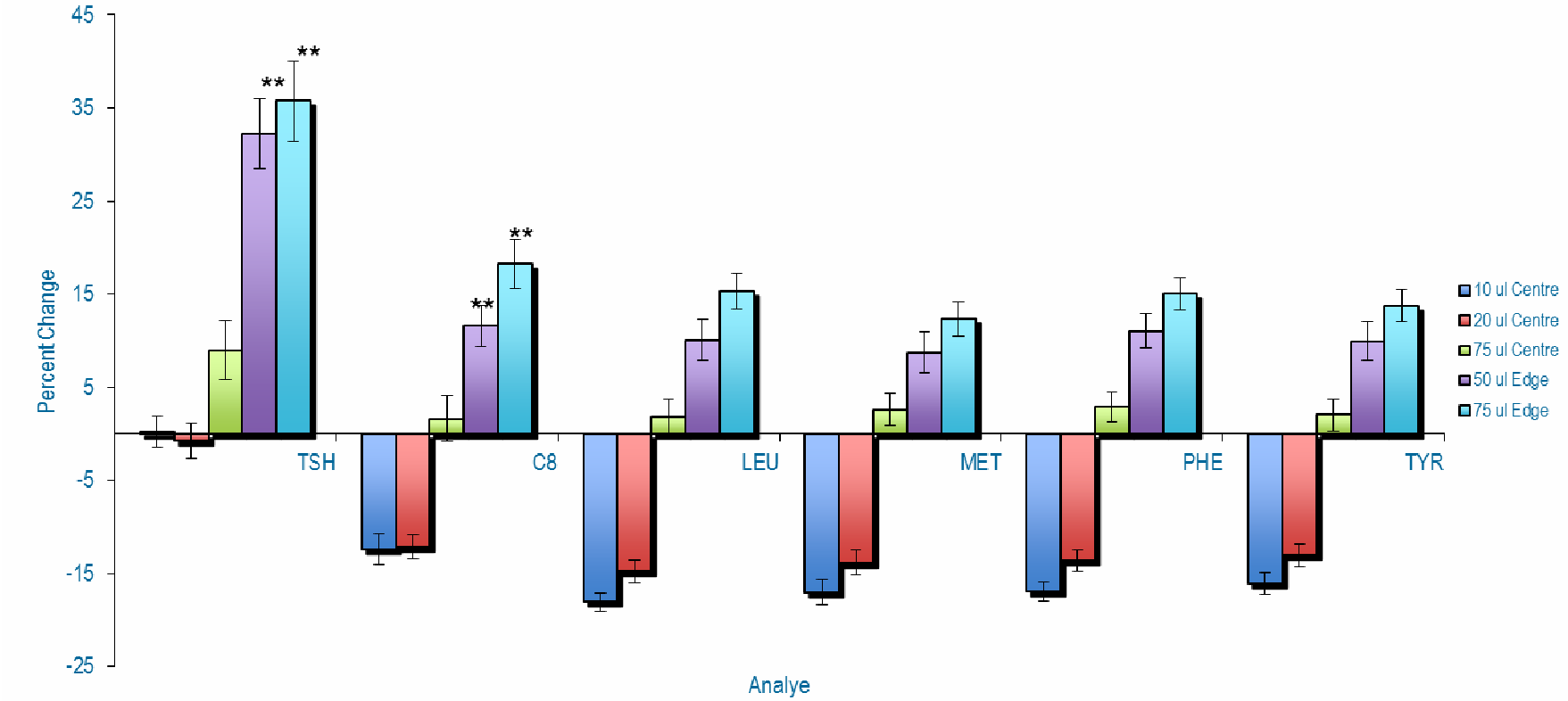


10 µL 20 µL 50 µL 75 µL

xLeu: The edge of a large spot vs the centre of a small spot, approx 35% difference at 400 µmol/L ie +/- 70 µmol/L



K Hall : 2014, personnel Communication

Blood Spot Quality

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Effect of Dried Bloodspot Quality on Newborn Screening Analyte Concentrations and Recommendations for Minimum Acceptance Criteria for Sample Analysis

Roanna S. George[1]* and Stuart J. Moat[1,2]

CONCLUSIONS: All bloodspots containing ≤20 μL (bloodspot diameter <8 mm), those in which blood has not fully penetrated the filter paper, and all samples with evidence of compression should be rejected, since there is a risk of producing false-negative results.

Table 1. Effect of punch location on measured analyte concentration for different sample volumes.*

	20 μL			50 μL			75 μL			100 μL		
Analyte	Central	Peripheral	P	Central	Peripheral	P	Central	Peripheral	P	Central	Peripheral	P
Phenylalanine, μmol/L	206 (5.9)	219 (9.2)	<0.001	239 (8.5)	247 (8.2)	<0.05	256 (10.6)	258 (11.3)	NS	265 (8.2)	268 (10.2)	NS
Tyrosine, μmol/L	165 (5.1)	169 (5.9)	NS	183 (6.5)	187 (6.6)	NS	194 (7.9)	193 (7.4)	NS	196 (5.5)	199 (7.3)	NS
Leucine, μmol/L	505 (15.0)	525 (24.7)	<0.05	594 (22.5)	597 (23.3)	NS	635 (26.4)	624 (29.8)	NS	660 (21.7)	648 (25.0)	NS
Methionine, μmol/L	38 (1.1)	39 (1.6)	NS	43 (1.6)	44 (2.3)	NS	46 (2.5)	45 (1.9)	NS	47 (1.4)	47 (1.8)	NS
C8, μmol/L	0.39 (0.01)	0.42 (0.02)	<0.001	0.46 (0.02)	0.47 (0.02)	<0.05	0.48 (0.02)	0.50 (0.03)	NS	0.50 (0.02)	0.52 (0.03)	NS
C10, μmol/L	0.51 (0.02)	0.54 (0.03)	<0.05	0.57 (0.03)	0.60 (0.03)	<0.05	0.61 (0.03)	0.63 (0.04)	<0.05	0.63 (0.02)	0.64 (0.05)	NS
C5DC, μmol/L	0.52 (0.02)	0.53 (0.02)	NS	0.59 (0.02)	0.58 (0.03)	NS	0.62 (0.03)	0.62 (0.03)	NS	0.63 (0.02)	0.64 (0.03)	NS
C5, μmol/L	1.42 (0.04)	1.48 (0.06)	<0.05	1.70 (0.06)	1.70 (0.07)	NS	1.81 (0.07)	1.81 (0.09)	NS	1.89 (0.06)	1.89 (0.08)	NS
TSH, mU/L	NA	NA	NA	11.8 (0.57)	12.6 (0.69)	<0.001	11.9 (0.60)	12.6 (0.87)	<0.001	12.5 (0.60)	12.8 (0.66)	NS
IRT, ng/mL	NA	NA	NA	61 (3.1)	65 (3.1)	<0.05	65 (3.2)	66.2 (3.9)	NS	71 (2.9)	72.1 (4.3)	NS

* Data are mean (SD). NA, not analyzed; NS, not significant.

Blood spot quality

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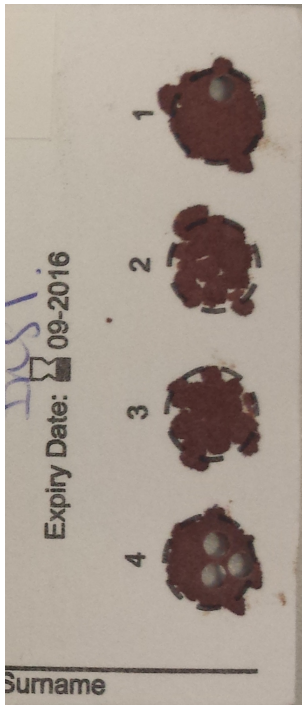
Baby X Card 1

- Date of Birth 17/10/15
- Date of Specimen 22/10/15 (Day 5)
- Received in laboratory 23/10/2015 (Friday) and processed.
- Results reviewed on 26/10/2015 (Monday).
- Poor quality spot (probably spot 1).
- Result: C8= 0.39

Front



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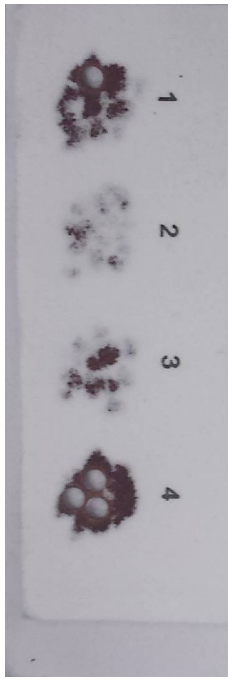

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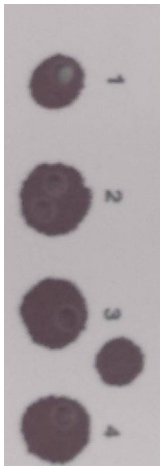
Baby X Card 2

- An urgent repeat sample was requested by phone.
- Received and analysed 26/10/15.
- Results:
  C8= 0.66
  C10= 0.3
  C8:C10 = 2.2
- Referral was made 18:49 26/10/15.
- Baby was seen on the 27/10/2015.

Front

Back



Method performance

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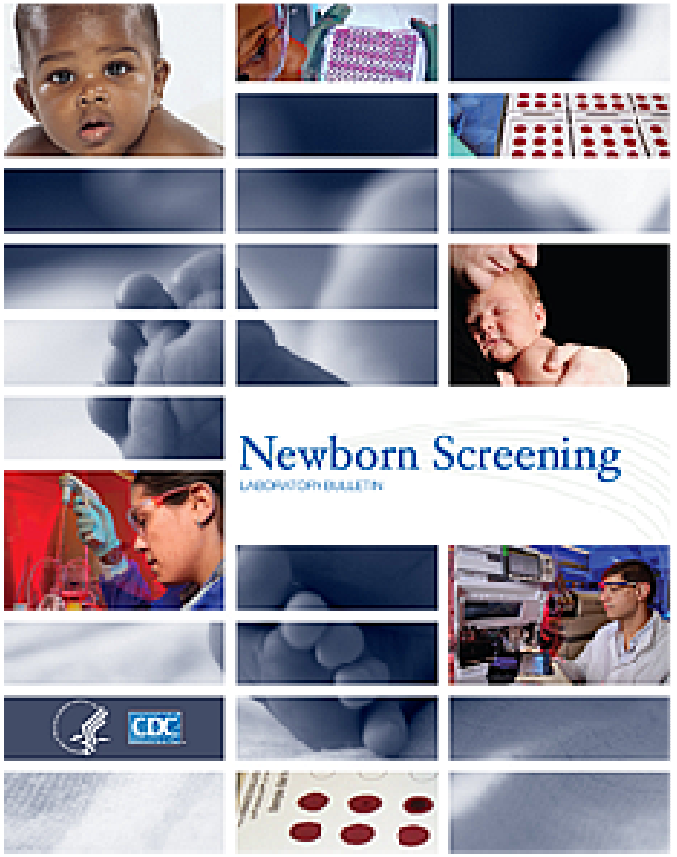
EQA experience

- **Leu (n=273), mean 168 µmol/L**
  UL(95%) 231, LL (95%) 104
- **C5 (n=268), mean 2.0 µmol/L**
  UL(95%) 2.7, LL (95%) 1.4
- **Met (n=261), mean 21 µmol/L**
  UL(95%) 28, LL (95%) 13
- **C5DC (n=275), mean 2.1 µmol/L**
  UL(95%) 3.7, LL (95%) 0.40



CDC QAP Q3 2014

What have we done – population monitoring?

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- Each lab submits data and receives monthly and quarterly report
- Reports are summarised by analyte
- Snapshot of how one lab compares to another
- Box whisker plots - scaled to analytical cut-off value
- Gives 10th, 50th, 90th and 99th centiles for each lab relative to "all labs" data
- Results split by instrument for each lab
- Useful for identifying any significant bias
- Regular meetings to discuss performance

What do we find - assay quality

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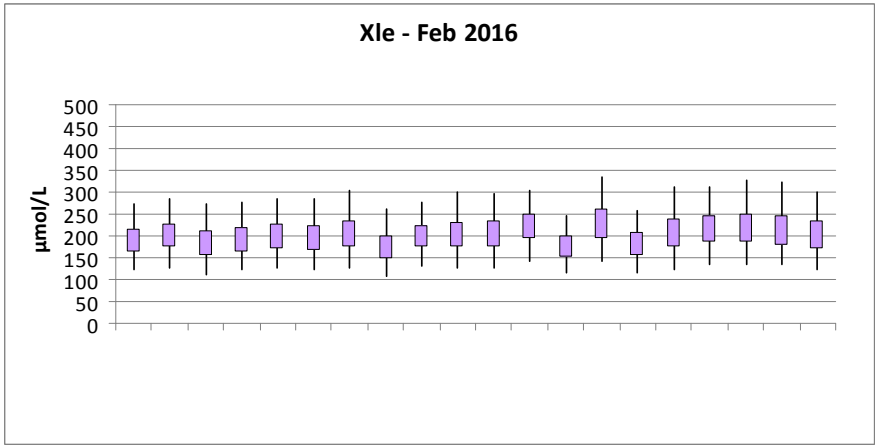

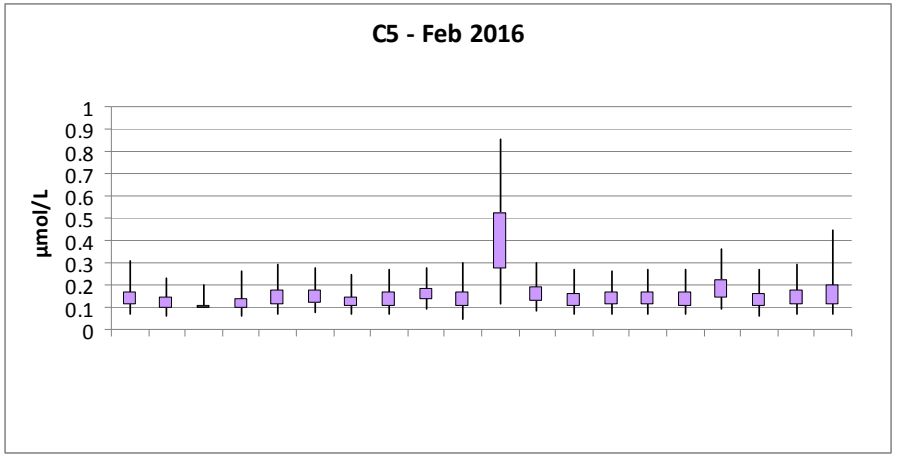

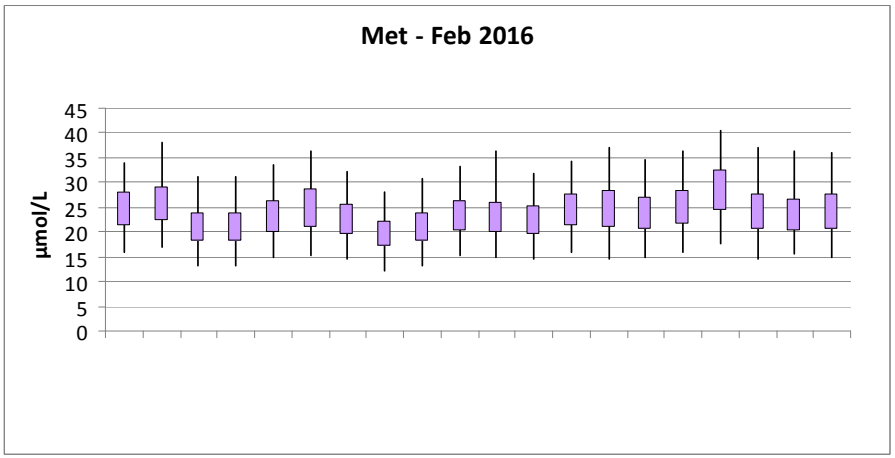

What can we do?

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Conclusions

- The ENBS programme is not unsafe
- Analytical cut-offs generally well removed from 90th centiles
  Methionine is the exception but 2nd tier testing is part of screening protocol
- There is potential for false positives and unnecessary referral of babies and possibly false negatives
- Harmonisation of ENBS should improve to maintain common cut-off values – common internal standard study

- Co-ordination of approach at kit lot change - IRTs

Transport

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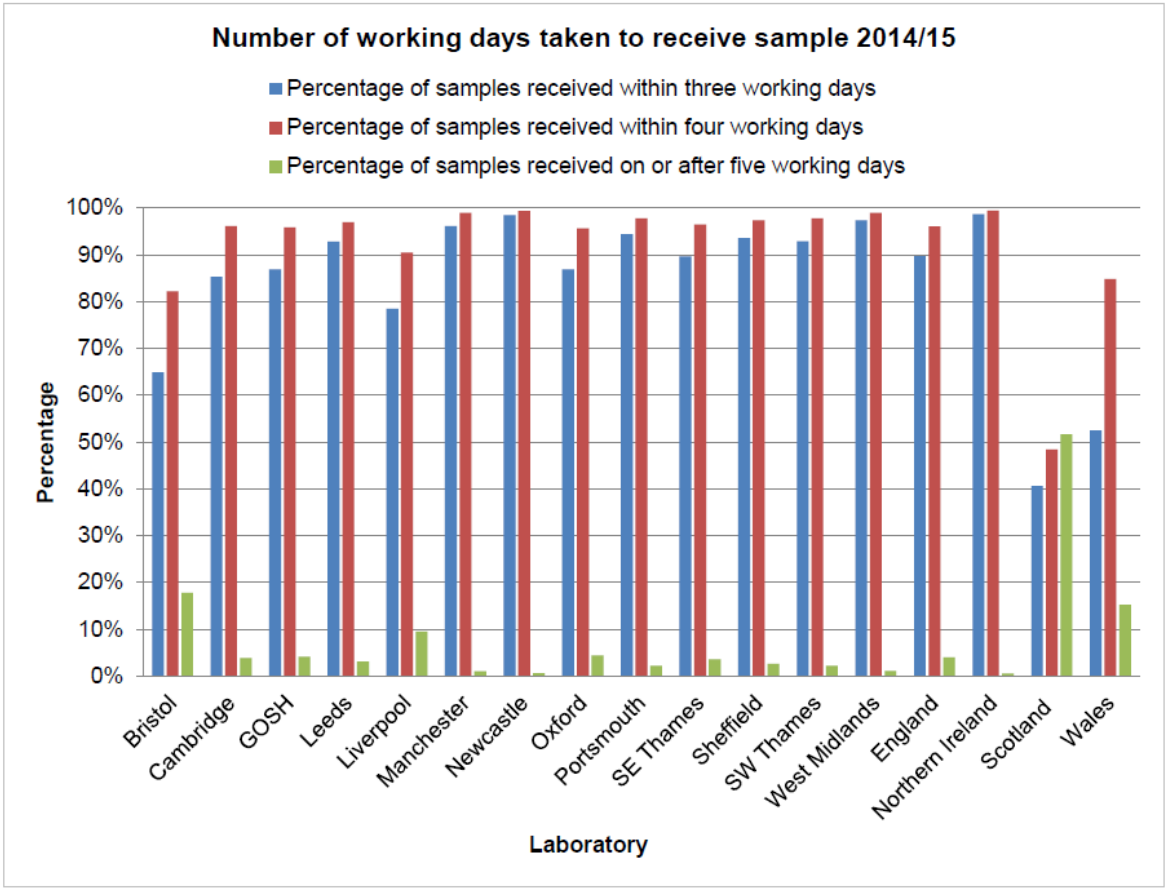

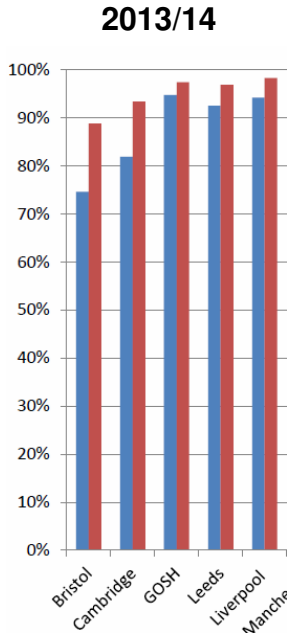

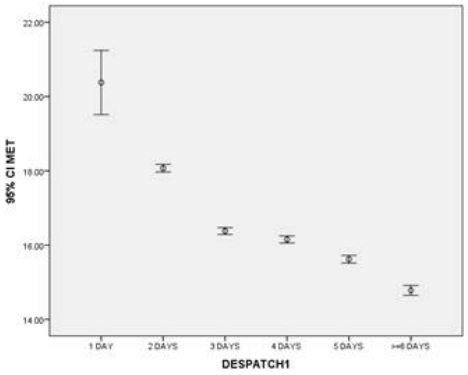

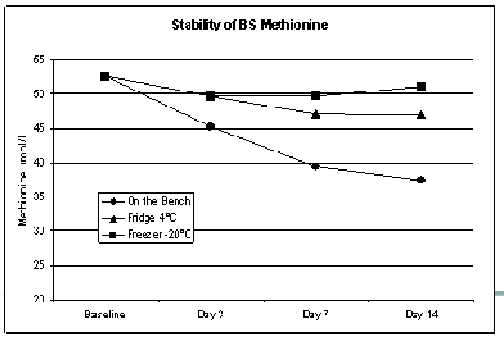

Results reporting

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- Are the diagnostic (confirmatory) tests agreed?
  - Diagnostic protocol
- Are the results timely with clear metrics?
  - Quality dashboard
- Are the qualitative reports eg organic acids clear and unambiguous when they arrive?
  - No agreed standards in terms of layout or content
  - No training
  - No EQA or IQC
  - No user surveys
  - A variety of practice
- Can we support patients more effectively during this time of uncertainty?
  - Provision of information – an App with high quality information that is readily accessed
  - Thought about the processes of information receipt