Development of an Improved Artificial Matrix

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Introduction:

- Who are UK NEQAS
- Background to FIT EQA
- Challenges of developing EQA for FIT testing
- Validation of the buffer
- Conclusion- findings from study using the new EQA material
- I will be available at the end of the meeting, if you have any questions

Who are UK NEQAS

- UK NEQAS- United Kingdom National External Quality Assessment Service
- Since 1969, UK NEQAS has provided a comprehensive world-wide service that enables laboratories to fulfil quality goals
- UK NEQAS now comprises a network of 390 EQA schemes operating from 26 centres- one being Birmingham Quality

Background to FIT EQA

- Birmingham Quality has run the Colorectal Cancer Screening EQA program for over three years
- Initially the scheme was mostly gFOBt cards, however, during this time FIT testing has dramatically increased
- But, the transition from gFOBt to FIT wasn't easy from EQA perspective...

Background to FIT EQA

- EQA material for gFOBt based on bran.
- Weighed amount of bran and binding agent are mixed with set volume of water (spiked with blood of known haemoglobin concentration).
- The amount of Hb spiked is then divided by the total mass of EQA material produced, to give the concentration of ug Hb/g faeces

Challenges of developing EQA for FIT testing

- Validation of gFOBt cards proved the stability of that product for at least two months.
- The same EQA material used for gFOBt was trialled in a pilot for the FIT EQA Scheme, the results were not promising:
- Up to half the Hb concentration was lost within 24 hours.

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Challenges of developing EQA for FIT testing

- I was given the task of developing and validating a buffer to keep the Hb stable in the FIT EQA material for at least two to three weeks.
- I used both current techniques from our other EQA schemes and extensive research into Hb/protein stability to develop the new buffer.

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Challenges of developing EQA for FIT testing

- Current stabilisation/preservation techniques used by Birmingham Quality include:
- Antibacterial agents
- Antifungal agents
- Protease inhibitors
- UK NEQAS always aims to keep EQA specimens as close to a patient's sample as possible- these agents are not added to all schemes, only if required.

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Challenges of developing EQA for FIT testing

- Initially, these antimicrobials/protease inhibitors were added to the original gFOBt material. This slightly improved the stability, but, it still didn't give the two-three weeks required.
- These poor results lead us to turn our attention to the bran component..

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Challenges of developing EQA for FIT testing

- The bran used also contained some sugar (and other ingredients) that may either aid microbial growth or affect the Hb.
- Other agents, such as rice flour, were trialled, however, feedback wasn't positive and results didn't improve.

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Challenges of developing EQA for FIT testing

- As these techniques didn't give the required stability, additional controls were clearly needed.
- Research showed "fixation" could greatly improve Hb stability.
- "Fixed" whole blood plus antimicrobials/ protease inhibitors were trialled and showed excellent results. This formulation was then moved into formal validation..

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Validation of the buffer

- The basic aim was to prove the EQA specimens gave the same result following despatch through the post and potentially after being stored at 2-8c for up to three weeks before being analysed.
- To validate this, a HM-JACKarc analyser was loaned from Alpha Laboratories and EQA samples were produced as normal then sent through the post back to Birmingham Quality.

Validation of the buffer

- One of the EQA specimens was tested upon receipt, then the remaining ones were stored at 2-8c and re-tested at regular intervals over the three weeks. The results were very pleasing and the new buffer showed excellent stability.

Validation of the buffer

	Results at Time of Prep.	Results Upon Receipt*	Results After 1 Week	Results After 2 Weeks	Results After 3 Weeks
Hb Conc.	145ug Hb/g	130ug Hb/g	153ug Hb/g	154ug Hb/g	135ug Hb/g
Percent. Recovery	N/A	90%	106%	107%	93%

Validation of the buffer

- After reviewing the postal validation data, Birmingham Quality has decided to dispatch the EQA material to participants 3 days after production, once the matrix has stabilised.
- Participants will then be given two weeks to report the results but advised to store the material in the refrigerator if it cannot be analysed immediately upon receipt.

Validation of the buffer

- The final part of the validation involved testing the homogeneity of the material. Each one of the remaining twenty tubes was sampled using the collection pickers supplied with the analyser and tested on the HM-JACKarc.
- The standard deviation and average haemoglobin were then calculated across the twenty samples. The between sample agreement expressed as percentage CV, was found to be 5.1%. Taking into account the potential sampling error, the material appears to be suitably homogeneous.

Findings from study using the new EQA material

- In September, a selection of laboratories were sent ten sets of the new material for analysis.
- The main finding is that sample preparation is key to getting the right results.

Findings from study using the new EQA material

- The first finding is the importance of ensuring that the tip of the collection device is clean.
- Rubber collar trims excess faecal material from edges of collection picker, however, material can still remain on the tip.
- Depending on the consistency of the faeces, the additional amount transferred can be quite sizable.

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Tip with excess material

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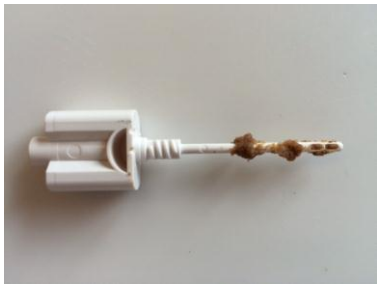
Clean tip



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Findings from study using the new EQA material

- To highlight the affect of excess material on tip, the following are actual participant results from:

Same person, same material, same machine

Vial	Unclean Tip (ug Hb/g)	Clean Tip (ug Hb/g)
1	63.8	119.1
2	67.8	116
3	45	107.2
4	70.3	108.5
5	58.3	110.7
6	35.6	109.9
7	44.4	99.7
8	46.7	103.2
9	39.4	103.6
10	41.9	112.8
Mean	51.3	109.1
SD	12.6	5.98
% CV	24.5	5.5

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Findings from study using the new EQA material

- As can be seen from this data, the % CV score goes from 24.5 with unclean tip Vs 5.5 for clean tip!

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Findings from study using the new EQA material

- The second finding relates to the time taken for the material to leave the "dimples" in the collection picker and fully disperse into the solution.
- We recommend leaving an hour from sampling the EQA material to analysing it. In the next set of results, the hour was timed from taking first sample (vial 1 specimen A):

Vial 1 Specimen A sampled first, then Vial 1 Specimen B, and so on until Vial 10 Specimen C

Vial	Specimen A (ug Hb/g)	Specimen B (ug Hb/g)	Specimen C (ug Hb/g)
1	20.4	102.8	170.4
2	20.2	118.8	177.4
3	24.6	102.1	169.8
4	19.6	103.5	215.8
5	16.8	102	203.5
6	18.1	97.9	202.3
7	16.9	86	164.3
8	14.7	79.9	138.3
9	15.7	75.5	212.8
10	14.2	61.5	134.3

Looking closer at Specimen A

Vial	Specimen A (ug Hb/g)	Timings
1	20.4	Start of the timed hour
2	20.2	
3	24.6	
4	19.6	
5	16.8	
6	18.1	
7	16.9	
8	14.7	
9	15.7	
10	14.2	Sampled last

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Findings from study using the new EQA material

- As could be seen from first table, there were thirty samples to be tested, even allowing a minute for taking each one, this would significantly eat into the hour.
- It can be seen the results drop from 20.4ug Hb /g stool in the first sample to 14.2ug Hb/g in the last.

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Findings from study using the new EQA material

- Both sets of data highlight that sample preparation is key to getting the right results.
- Thank you for listening!