

Inadvertent use of a D variant cord donation in a Feto-Maternal Haemorrhage EQA sample: Investigation and Lessons Learned

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BACKGROUND AND AIMS

- In July 2018, UK NEQAS BTLP created an EQA sample designed to mimic an FMH bleed of approximately 3mL.
- All material used passed pre-acceptance serological testing; samples were dispatched to 298 participants in 17 countries.
- Post-dispatch testing by flow cytometry (FC) using an anti-D marker showed a bleed volume of 1.1mL so an investigation was initiated to determine the cause of the unexpectedly low bleed volume and what lessons could be learnt.

METHODS

- Production methodology and results of pre-acceptance testing were reviewed.
- FC testing was repeated, plots examined, and the FMH Scientific Advisory Group consulted for advice.
- Further FC testing was performed at WBS using alternative markers, and the material used was investigated at IBGRL.
- Participant results were examined to determine if the sample should be withdrawn from scoring.
- A questionnaire on how results were managed was sent to the 36 participants using FC with an anti-D marker; responses were received from 16.

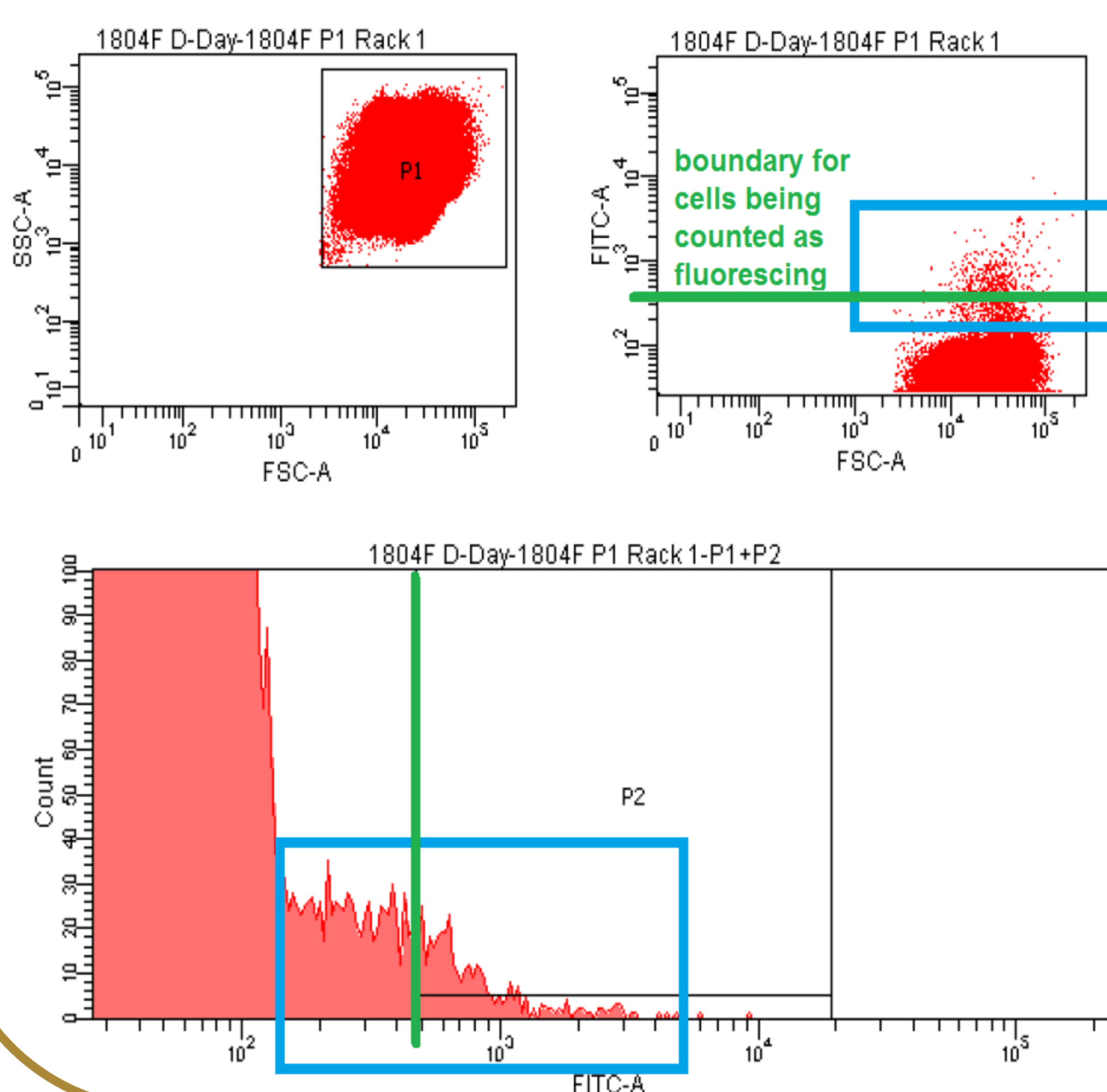
Production methodology

- No issues seen with production of material
- Cord D types retested using three technologies



- Tube anti-D showed weaker reactions than the +ve control

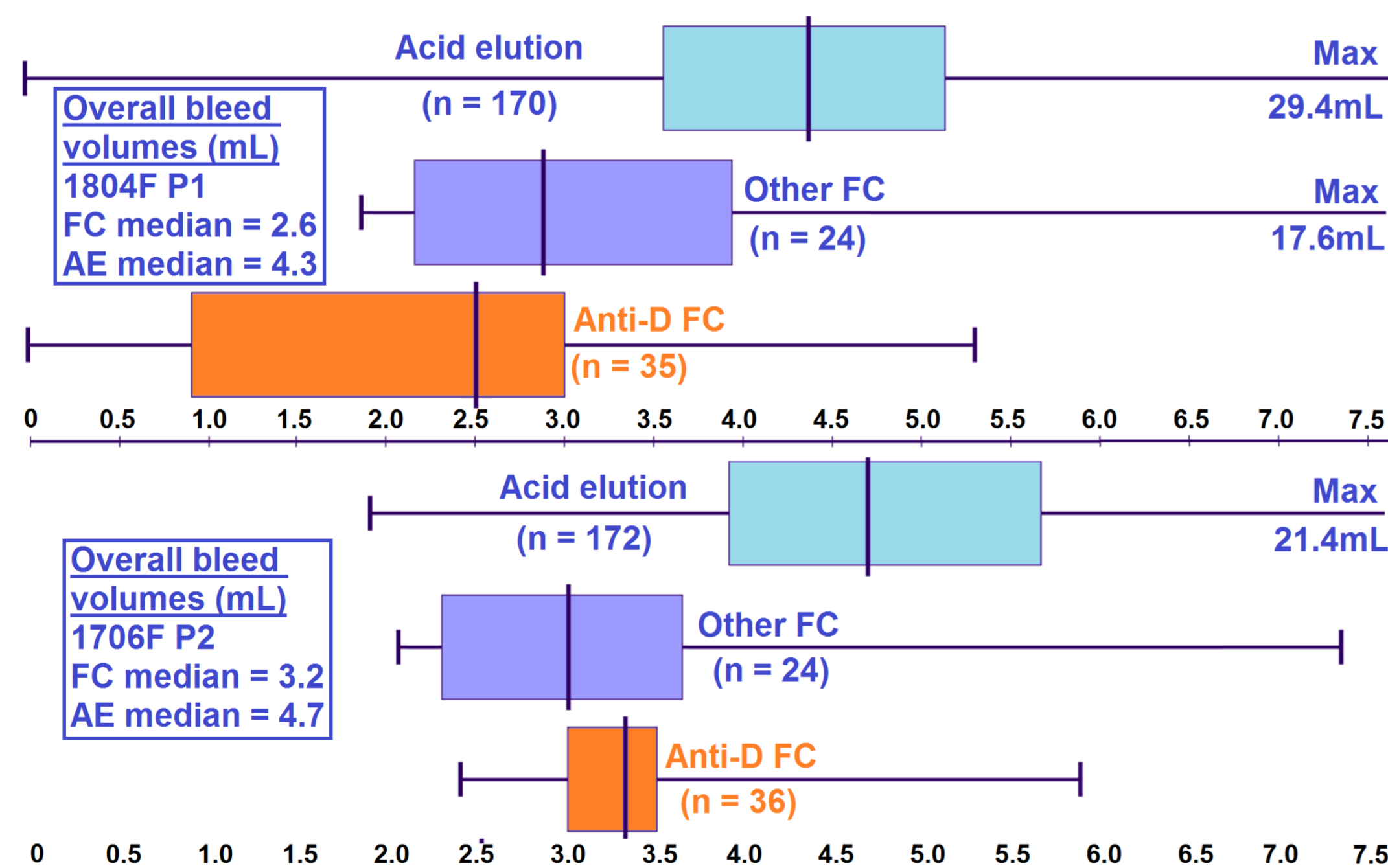
FC examination



- Repeat testing gave similar results (1.2mL)
- Examination of plots showed a “shoulder” of cells after the negative peak
- Less fluorescence on cord cells than normal indicates less binding of the anti-D reagent
- Less binding could be due to by fewer D antigen sites
- Results indicate the cord may be a D variant

Participant's results

- The IQR of results was compared to the last similar sample
- FC-anti-D spread was more broad for 1804FP1 than 1706FP2
- Spread for other methods was similar for 1804FP1 and 1706FP2



- 1804FP1 was not scored as the “correct” result is based on the FC median which had been skewed by the D variant cord

Reference testing

- FC testing using an anti-HbF marker indicated that the correct amount of cord material had been added
- FC testing using anti-D showed reduced binding of the anti-D
- The cord material was tested against 17 different anti-D reagents, 4/17 showed reactions weaker than the control
- Genotyping of the cord material showed that the cord material was *RHD*DVII*

Questionnaire

- 16/16 respondents examine FC plots routinely
- 8/16 noticed unusual plots
 - 3/8 did not adjust the gates
- 12/16 have back up plan for when results cannot be interpreted
 - 9 test using a different method or refer the sample
 - 2 repeat the testing
 - 1 matches the gating to the positive control

CONCLUSION

The use of a D variant cord in an EQA sample was not planned, but allowed UK NEQAS to highlight some important learning points:

- Thorough examination of FC plots is essential to avoid underestimation of FMH
- A controlled procedure should be in place if modification of gates is required
- Access to the cord/neonatal blood to allow serological investigation may be useful in similar clinical situations
- It is important to have a back-up plan for issuing anti-D Ig in the event of an uninterpretable FMH result