

the IONA[®] test - update on dynamic Fetal Fraction

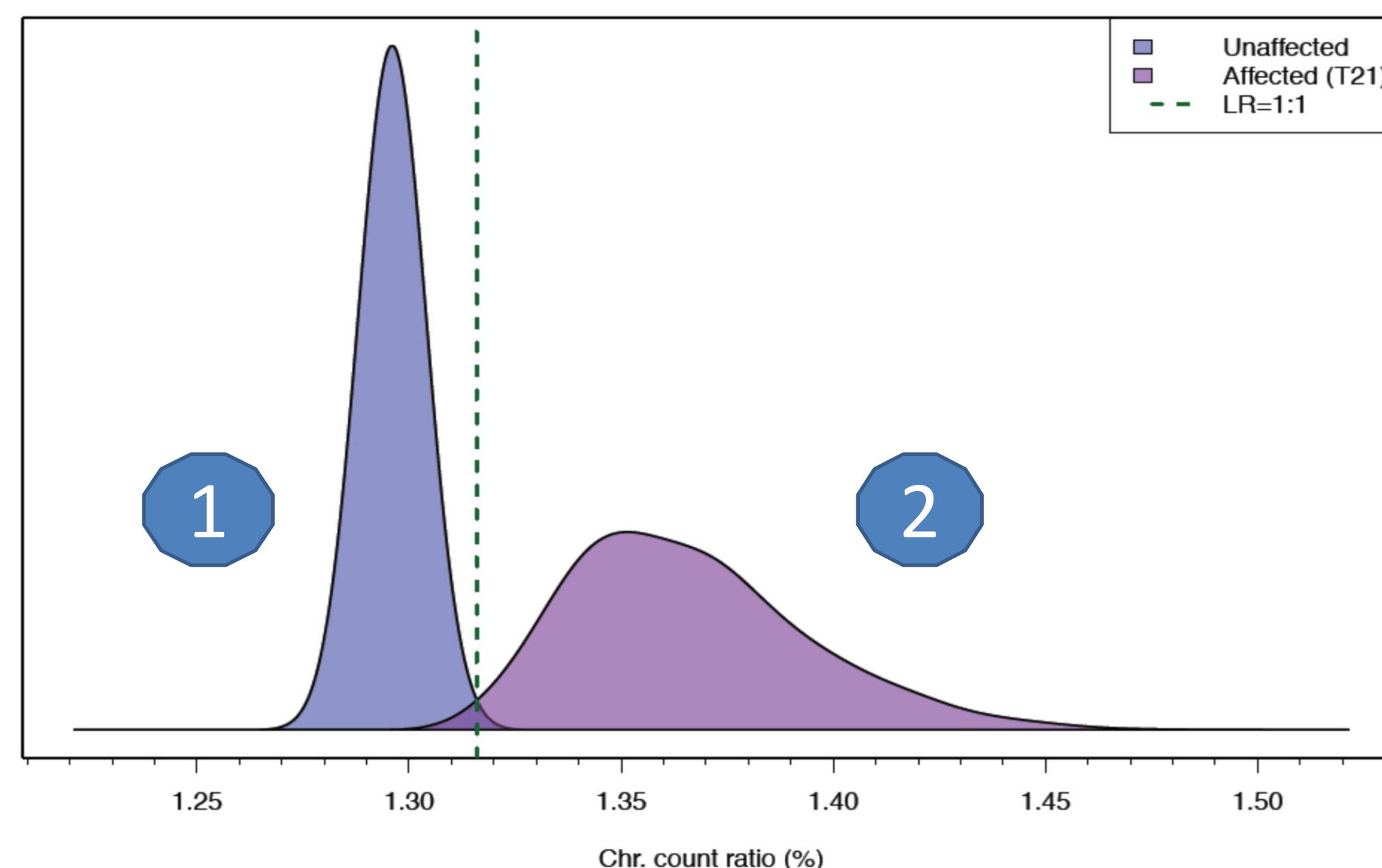
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Objective:

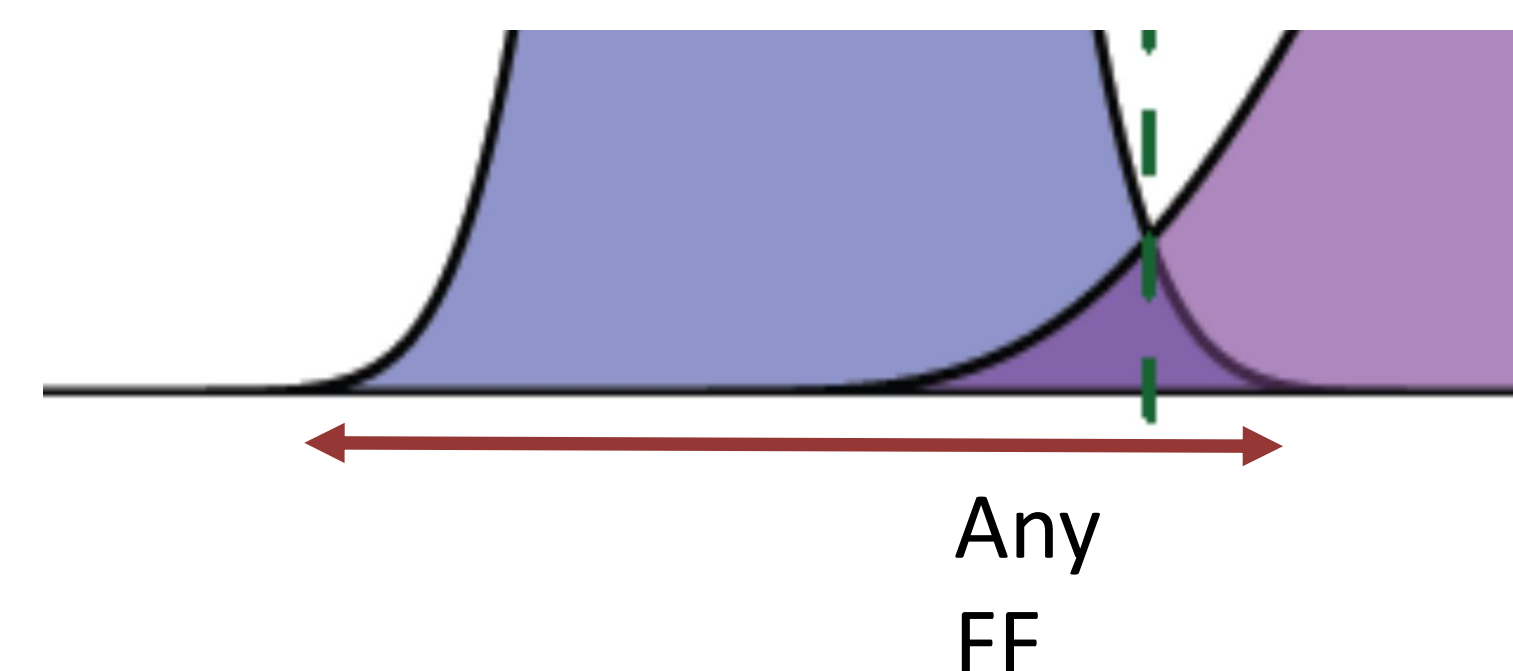
The IONA[®] test is a complete sample to result system for non-invasive prenatal testing. A key component of the IONA test is an automated analysis pipeline that has been developed to maximise the use of the data output from the NGS based workflow. This includes highly optimised methods for sequence alignment, GC correction and content checks, likelihood ratio calculation and fetal fraction estimation and assessment. The purpose of this study was to validate the optimised fetal fraction estimation and QC check.

Background

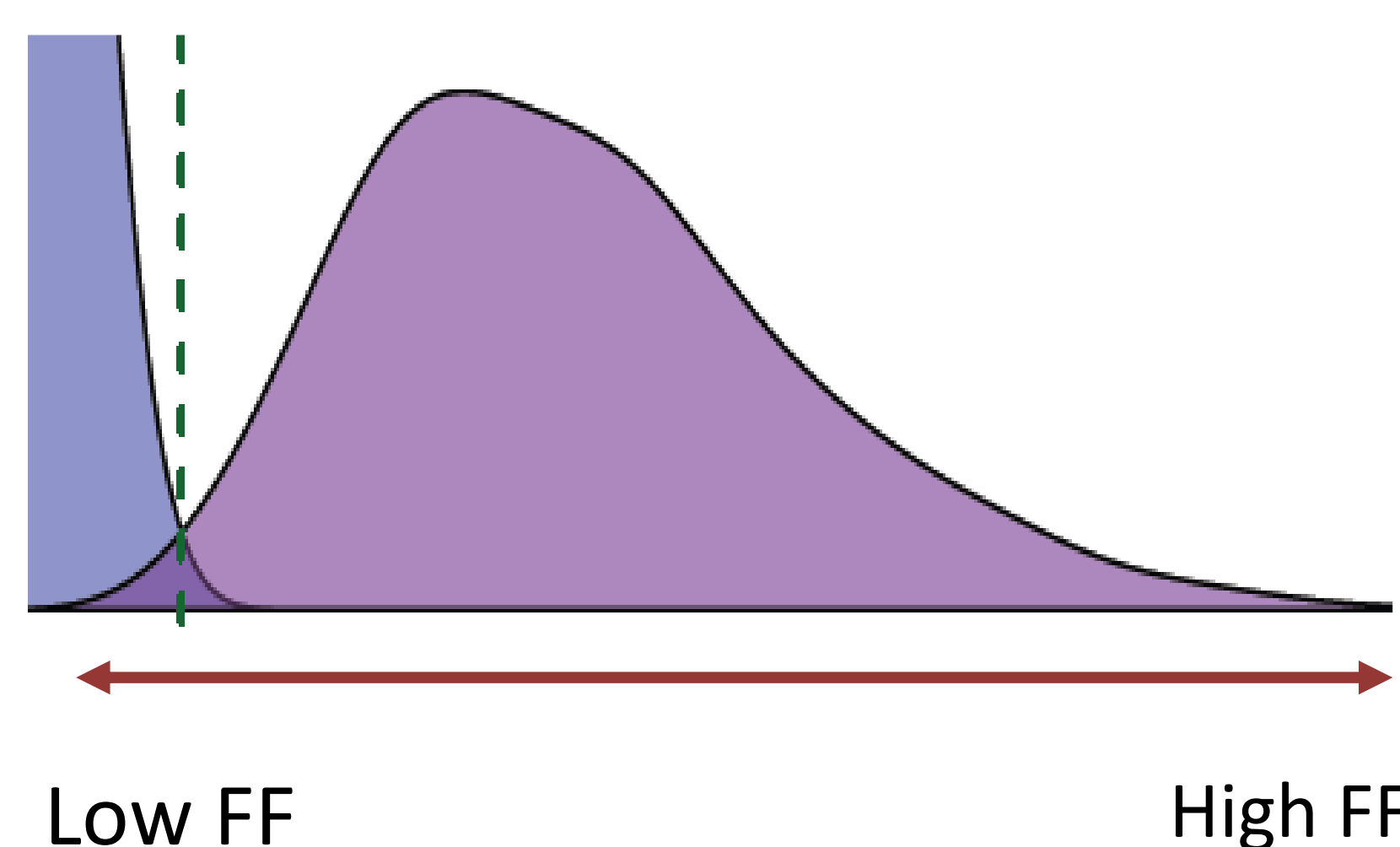
- The importance of measuring fetal fraction is well understood: **to reduce the risk of false negative results** in samples with low amounts of cfDNA originating from the placenta
- No matter how deep the sequencing there will always be some overlap between the affected and unaffected sample distributions



- With unaffected samples, the data output is dependent on the number of counts



- Trisomy sample data output is dependent on the number of counts AND the fetal fraction



Methods:

Data from clinical samples were processed to demonstrate the accuracy of the results and the test failure rate. All of the available data from the sequencing run was utilized and a novel approach employed to how the data was interpreted. First, a hard cut-off of 2% was used to invalidate samples with the greatest risk of presenting a false negative result. Next, a 'dynamic' cut-off was used as a second check for samples with low fetal fraction ($\geq 2\%$ fetal fraction) that appear where the unaffected and trisomy distributions overlap. This dynamic fetal fraction estimate is based on all of the available sequencing information available and determines the minimum amount of fetal fraction required for each sample in question.

Estimating the Fetal Fraction

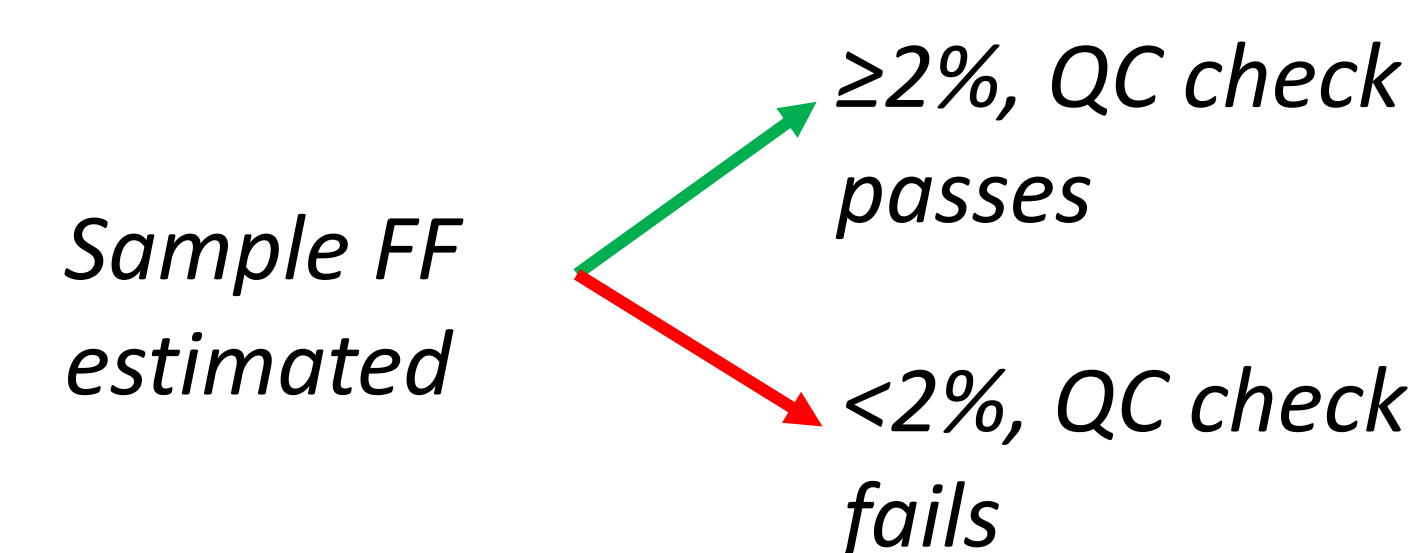
The IONA[®] test measures fetal fraction using two complementary methods:

- One of the methods is an X-chromosome assessment
- The second method utilises fragment size information from the sample data

How does the IONA[®] test use FF?

The FF estimate needs to be applied carefully and with consideration of (i) other available data and (ii) the technology used

- The IONA[®] test firstly, uses a hard cut-off of 2%



- Samples are evaluated as to whether a dynamic fetal fraction cut-off is required and, if needed, this is applied

- A valid IONA[®] test result is a balance of fetal fraction, count density and chromosome ratio
- If the dynamic check is required, the number of aligned sequencing reads is assessed (count density) which allows for the required level of fetal fraction to be determined.
- A higher count density means the FF% required can be lower. Conversely, a lower count density will require a higher fetal fraction.

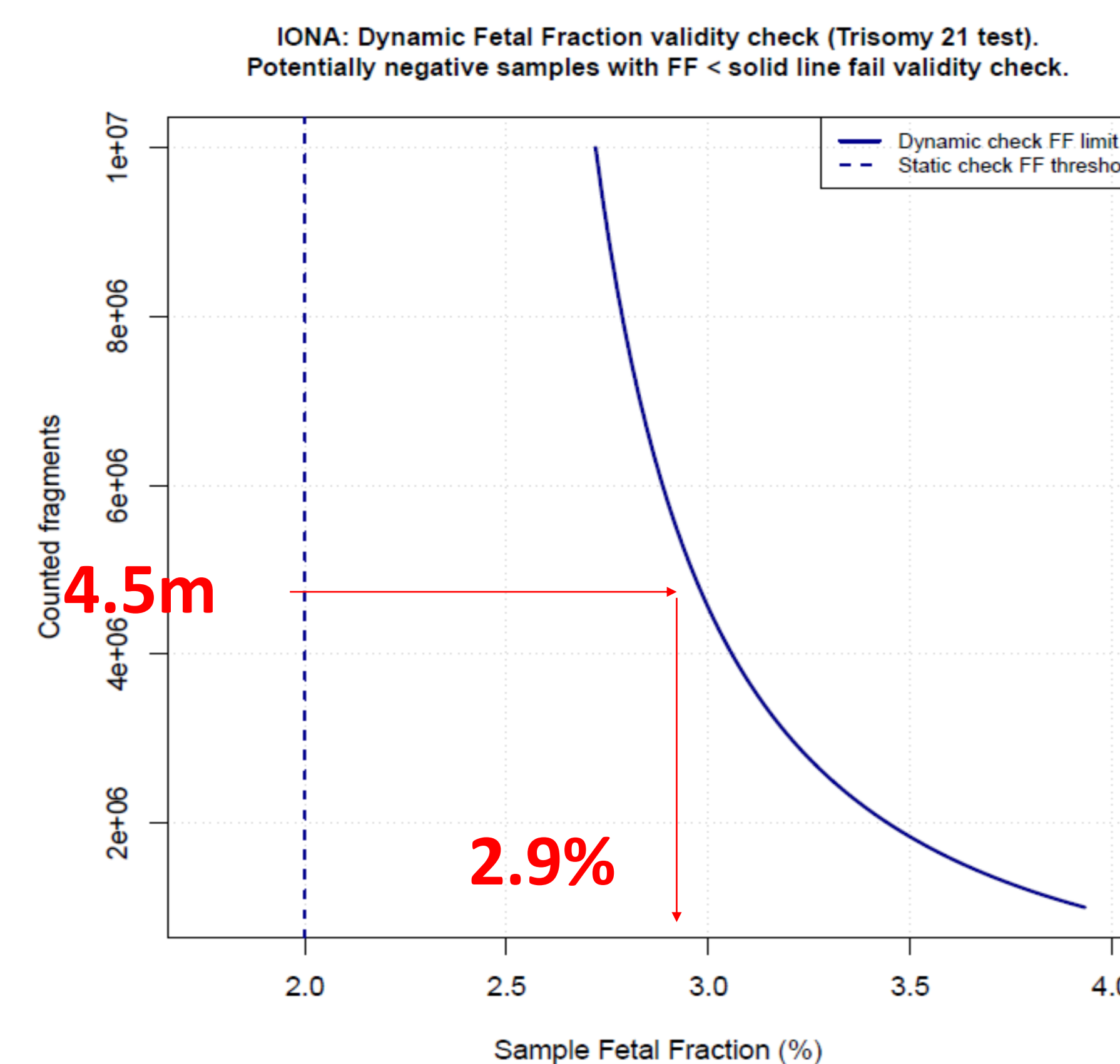
Limitations of a single hard cut-off

- At a 2% FF cut-off, there is still a small overlap between the unaffected and trisomy sample distributions
- A single hard cut-off does not fully address the potential for false negative results due to low FF
- There will be some results where a sample could either be an unaffected sample of any fetal fraction or a trisomy sample with a low fetal fraction (i.e., just above 2%)
- Accounts for <0.5% of the sample population

Benefits of the dynamic method

Example:

- A sample with a fetal fraction of 3% has a chromosome ratio that could be either unaffected or a low fetal fraction trisomy 21 sample
- A test with a 4% cut-off would reject the sample outright.
- After evaluating all other sequencing parameters, the IONA[®] test sets a dynamic cut-off of between approximately 2% and 3.5%, based on count numbers
- The sample FF estimate is assessed against this dynamic cut-off



- The sample is found to require 2.9% fetal fraction to generate a valid result
- The sample is found to have sufficient fetal fraction to generate a screening result and is valid

Results:

All sample results were correct, when assessed against the reference method. Less than 1% of samples presented an invalid result, with no re-tests performed.

Summary

- A total of 409 sample results with known clinical outcomes were evaluated
- Of these, 405 produced a valid result (99.0%)
- The study showed 100% concordance of all results
- There were 4 invalid results (0.98%):
 - 1 x fetal fraction <2% (Unaffected)
 - 2 x fetal fraction < dynamic fetal fraction cut-off (%) (Unaffected)
 - 1 x low counts (Trisomy 21)

	Correct Result	False Positive	False Negative
Trisomy 21	40	0	0
Trisomy 18	9	0	0
Trisomy 13	5	0	0
Unaffected	351	0	0
Total:	405	0	0

- No retests were performed due to limited availability of sample material. The pass rate for retested samples is typically >50%

Conclusion:

- Combining lower percentage hard cut-off and a dynamic fetal fraction assessment keeps the test success rate high
- The IONA[®] test has a re-draw rate of <0.5%
- The samples most at risk of a false negative result benefit from having a second level of check performed; i.e., trisomy samples with a low FF, just above 2%
- This approach maintains excellent sensitivity and specificity
- In combination with the use of a likelihood ratio output, a grey-zone, ambiguous result (or equivalent) is not required

Acknowledgements

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