Non-invasive fetal RHD genotyping 4268DK 02/2023

The report consists of this letter and additional graphical information showing each laboratory's results compared with all results, as explained below ("Interpretation of reports").

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This report contains:
• This letter
• Individual results

Approved by MP
21.12.2023

Next round
04.03.2024

DANAK
PT Reg.No. 591
Member of EA MLA

Number of participants

49 laboratories from 21 different countries participated. All participants have returned results for one or more investigations.

Sample material

The sample shipment was delayed due to the detection of non virus-free material in the first batch, forcing us to make a new batch. We apologize for any inconvenience caused and appreciate your understanding.
The material is plasma pools from RhD negative, pregnant women. Blood was drawn at 24-26 gestational weeks.
The material has been tested negative for HIV and Hepatitis.
Stability of fetal *RHD* copies/mL has been demonstrated during transport at ambient temperature, with only a slight increase in total DNA copies/mL [1]. The material was processed and tested as described [1].
Notably, the material does not represent clinical samples from earlier gestation than 25 weeks. From earlier experience, the level of total DNA may be higher than some laboratories typically observe from their clinical samples. Notably, some assays may be vulnerable to high background levels of total DNA.

Target values

Fetal *RHD*

'Sample A' was *RHD* negative, and 'Sample B' was *RHD* positive. The samples were made from two different pools. Based on triplicate testing in the Laboratory of Blood Genetics, Department of Clinical Immunology, Copenhagen University Hospital, 'Sample A' was tested *RHD* negative for exons 4, 5, 7, and 10. 'Sample B' was tested *RHD* positive for exons 4, 5, 7, and 10.

Total DNA

'Sample A' and 'Sample B' were both tested positive for *GAPDH*.

Quantification of DNA

'Sample A' was found *RHD* negative, and total DNA was estimated to 7204 geq/mL.
'Sample B' was found *RHD* positive with an estimated level of 74 geq/mL (mean Ct-value of 34.8), with total DNA estimated to 2435 geq/mL. The fetal fraction was approximately 3 %.

Outliers

Outliers are classified based on expert opinion.

Results and comments

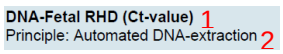
Procedures

OBS: Everyone was instructed to report the Ct-value for a *RHD* negative sample as 50. Most did this, but some laboratories did report 0. A few have reported the number of cycles where they stop the PCR.

Since it is possible to report individual Ct-values for each exon-target, all Ct-values for Fetal *RHD* were merged in the data treatment, regardless of exon target. The mean value of each laboratory's Fetal *RHD* Ct-values are used for drawing the histogram and calculating statistics and are plotted in the graphic as ◆. At the same time, Ct-values from all Fetal *RHD* exon targets are shown ✕.
The summarized distribution for the specific targets for both Fetal *RHD* and Total DNA can be seen in the table.
Please note that precaution should be taken when comparing Ct-values across laboratories, as differences in the methodologies may affect Ct-values.

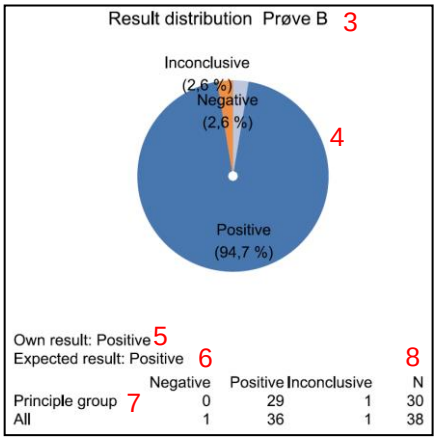
Interpretation of reports

The graphical part of the report is structured with a header above each row.



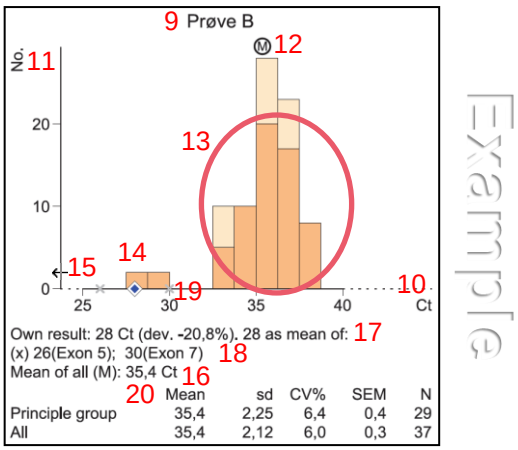
1. The component
2. The principle you have stated also determines the principle group you are in.

For the clinical conclusion, a pie plot shows the distribution of positive, negative and inconclusive results



3. The sample name
4. The distribution of results
5. Your result
6. The expected result
7. Numerical data grouped by your own principle and 'All' participants.
8. N is the total amount of results in each group.

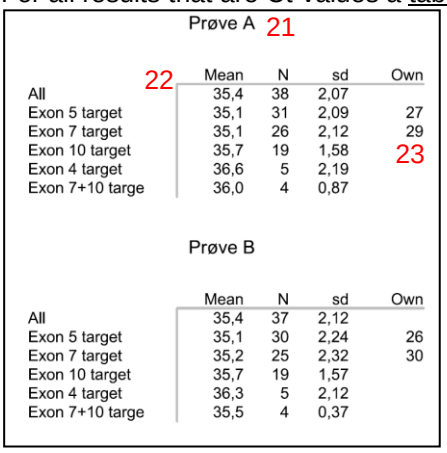
For all results that are Ct-values a histogram is shown:



9. The sample name
10. The x-axis shows the Ct-value
11. The y-axis shows the number of laboratories in each column in the histogram
12. (M) show the location of the mean value of all results
13. Rosa color indicates results with the same principle-group as you
14. ◆ Blue dot show the location of your result.
If more than one target was used: The mean value of results from different exon targets are shown
15. An arrow indicates an outlier
16. The mean value of all results
17. Your result and the deviation from the mean value of all results (16). If more than one target was used, your result is the mean value of your results from different targets.

18. If more than one target was used: your results for the individual targets
19. If more than one target was used: ✕ is the individual results of your targets
20. Numerical data grouped by your own principle and 'All' participants. sd is the standard deviation, CV% is the relative standard deviation, SEM is the standard error on the mean and N is the total amount of results in each group.

For all results that are Ct-values a table is shown



21. The sample Name
22. Numerical data grouped by target. Mean is the average Ct-value. N is the total amount of results And sd is the standard deviation.
23. 'Own' is your individual results for a given target

Components

Clinical Conclusion

Almost all clinical conclusions from all participants were correct, except for a false positive result and an inconclusive result for Sample A. For Sample B a single false negative result was reported.
The reported *RHD* Ct-value for the inconclusive result in Sample A was high (average of 46.8 [range = 43.1–50]), markedly lower than the Ct-value reported for Sample B (35.9).
The reported false-positive and false-negative results came from the same laboratory. The *RHD* positive result was clear (Ct = 34.6); and the negative result was clear. The lab found Sample A to contain less total DNA than Sample B (opposite of expected). The relatively high level of fetal DNA in Sample B in general may suggest that failure to detect fetal DNA should not be considered the likeliest reason for a false-negative result.

Fetal *RHD*

In general, as judged from the Ct-values only, the detection of fetal DNA was satisfactory.

Total DNA

In general, as judged from the Ct-values only, participants reported expected levels of total DNA in 'Sample A' and 'Sample B'.

Method differences

All participants used real-time PCR for amplifying fetal *RHD* targets and a target for total DNA (with one exception using no target for total DNA). Five different targets were used for total DNA.
A couple of participants also used ddPCR.
39 laboratories used automated DNA extraction, 11 laboratories used manual DNA extraction, and 1 lab used direct amplification without DNA extraction.

Next round

The first round of 2024 will be shipped the 4th of March 2024.

Feedback

We welcome any kind of feedback you think might improve the scheme in the future. Please contact frederik.banch.clausen@regionh.dk and morten.pedersen@deks.dk.

Yours sincerely
Frederik Banch Clausen (Copenhagen University Hospital),
Lisbeth Nielsen (DEKS) and
Morten Pedersen (DEKS)

References

1. Clausen FB, Hellberg Å. External quality assessment of noninvasive fetal *RHD* genotyping. Vox Sang 2020; 115: 466-471.

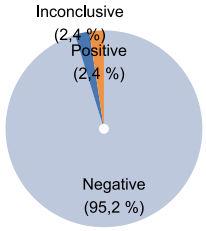
4268 DK - Non-invasive fetal RHD genotyping - EKSEMPEL RAPPORT

Udsendelse 2 - 2023. Laboratorie nr. 500, resultat id. 500

DNA- Clinical conclusion

Principle: Automated DNA-extraction

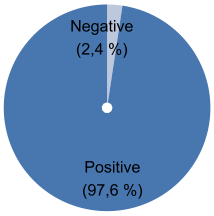
Result distribution Sample A



Own result: Negative
Expected result: Negative

	Negative	Positive	Inconclusive	N
Principle group	34	1	0	35
All	40	1	1	42

Result distribution Sample B



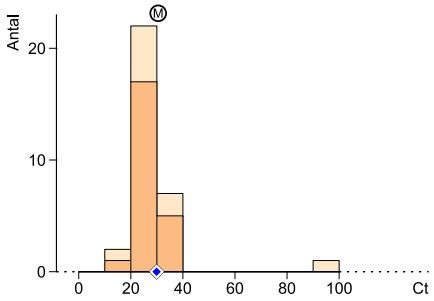
Own result: Positive
Expected result: Positive

	Negative	Positive	Inconclusive	N
Principle group	1	34	0	35
All	1	41	0	42

DNA- Total DNA (Ct-value)

Principle: Automated DNA-extraction

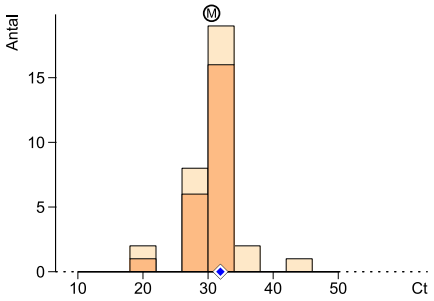
Sample A



Eget resultat: 29,9 Ct (afv. -2,0%)
Middelværdi af alle (M): 30,5 Ct

	Middel	sd	CV%	SEM	N
Principgruppe	28,7	2,53	8,8	0,5	23
Alle	30,5	11,59	38,0	2,0	32

Sample B



Eget resultat: 31,9 Ct (afv. 4,4%)
Middelværdi af alle (M): 30,5 Ct

	Middel	sd	CV%	SEM	N
Principgruppe	30,2	2,75	9,1	0,6	23
Alle	30,5	4,18	13,7	0,7	32

Sample A

	Middel	N	sd	Eget
Alle	30,5	32	11,59	
CCR5	35,19	11	18,79	29,9
GAPDH	29,86	10	1,45	
Albumin	29,26	3	1,15	
HGH	18,30	2	0,20	
beta-Globin	26,57	2	2,50	
Beta-actin	28,82	1		
EIF2C1	27,20	1		
No target	29,20	1		
SOD	27,80	1		

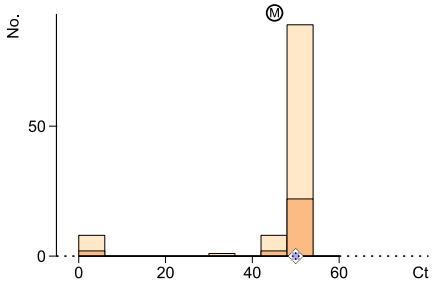
Sample B

	Middel	N	sd	Eget
Alle	30,5	32	4,18	
CCR5	32,7	11	4,50	31,9
GAPDH	31,0	10	2,04	
Albumin	30,9	3	1,08	
HGH	20,0	2	0,33	
beta-Globin	28,3	2	2,52	
Beta-actin	29,2	1		
EIF2C1	28,8	1		
No target	30,8	1		
SOD	29,5	1		

DNA-Fetal RHD (Ct-value)

Principle: Exon 10 target

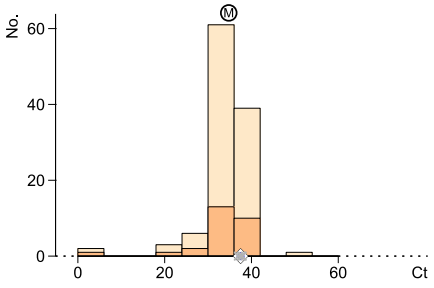
Sample A



Own result: 50 Ct (dev. 10,8%). 50 as mean of:
(x) 50(Exon 10); 50(Exon 5); 50(Exon 7)
Mean of all (M): 45,1 Ct

	Mean	sd	CV%	SEM	N
Principle group	45,8	13,54	29,6	2,7	26
All	45,1	14,13	31,3	2,1	47

Sample B



Own result: 37,5 Ct (dev. 7,9%). 37,5 as mean of:
(x) 36,98(Exon 5); 37,63(Exon 10); 37,88(Exon 7)
Mean of all (M): 34,8 Ct

	Mean	sd	CV%	SEM	N
Principle group	33,6	7,78	23,2	1,5	27
All	34,8	6,19	17,8	0,9	50

Sample A

	Mean	N	sd	Own
All	45,1	47	14,13	
Exon 5 target	45,6	37	13,78	50
Exon 7 target	46,5	31	12,46	50
Exon 10 target	45,8	26	13,54	50
Exon 7+10 target	48,1	8	5,46	
Exon 4 target	36,3	4	24,30	

Sample B

	Mean	N	sd	Own
All	34,8	50	6,19	
Exon 5 target	34,0	39	6,48	36,98
Exon 7 target	34,6	33	3,58	37,88
Exon 10 target	33,6	27	7,78	37,63
Exon 7+10 target	37,6	8	5,04	
Exon 4 target	36,7	5	0,56	