APPLICATION NOTE

Evaluation of cell free fetal DNA (cffDNA) extraction on the chemagic™ 360 instrument with the FetoGnost® Kit RhD to determine fetal RhD status

Authors

- Irina Korschineck
  Ingenetix GmbH
- Tim Weißbach, Serhan Önder, Catherine Goh
  Revvity

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Introduction

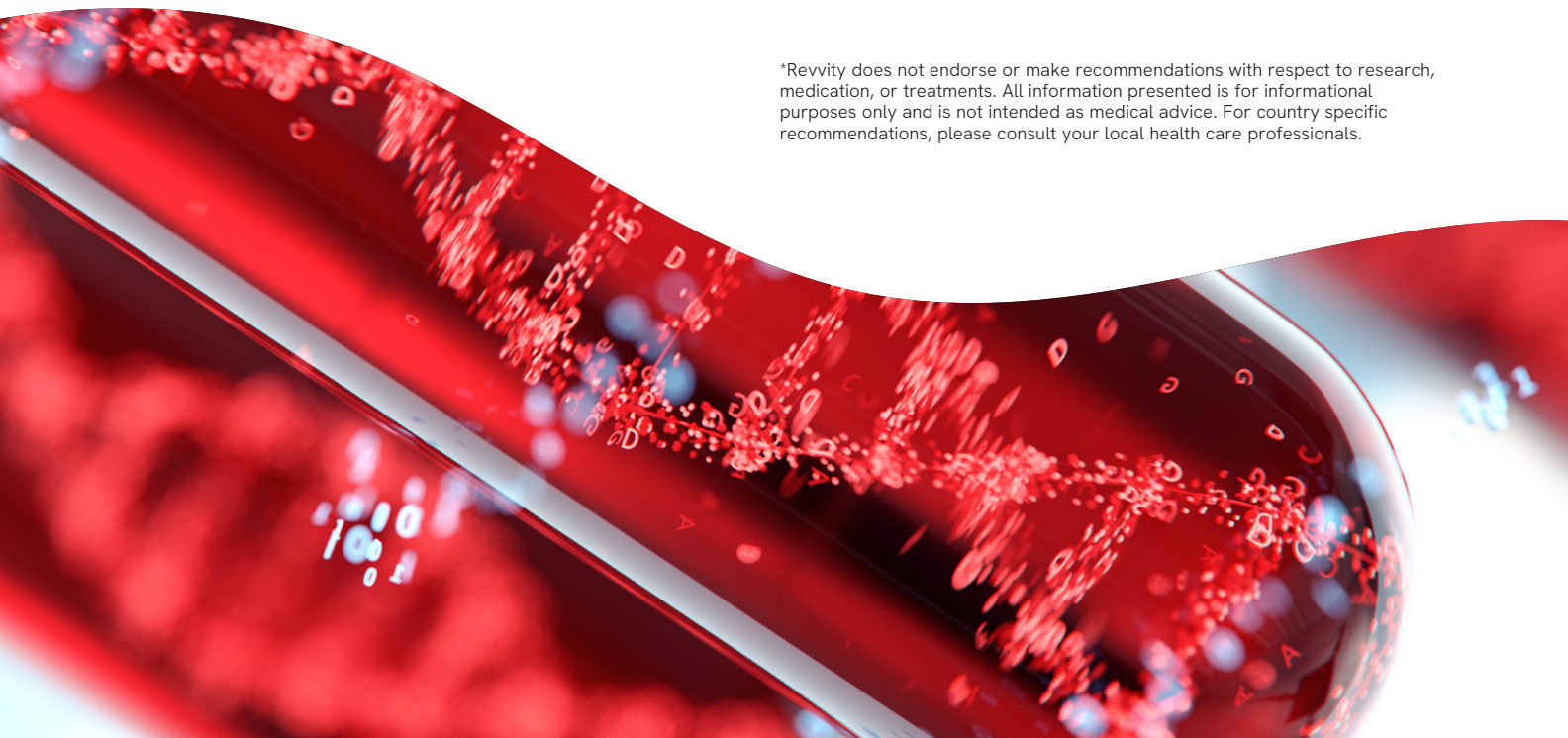
Fetal Rhesus Factor D (RhD) genotyping is typically performed through non-invasive prenatal testing and is important in identifying RhD incompatibility between mother and child. Non-invasive prenatal testing avoids risks associated with invasive procedures such as amniocentesis or chorionic villus sampling and is performed with a simple blood draw to analyze cell free fetal DNA (cffDNA) present in the mother's blood.

The FetoGnost® Kit RHD* from Ingenetix allows rapid, sensitive, and non-invasive fetal RhD genotyping of samples purified from maternal plasma of RhD-negative pregnant women, based on real-time PCR technology. The test detects exons 5, 7 and 10 of the RHD gene from RHD-positive fetuses. An Internal Positive Control (IPC) is included in the kit which checks the integrity of the reagents and serves as a control for cffDNA extraction and possible real-time PCR inhibition.

The chemagic™ automated nucleic acid isolation technology has been previously demonstrated to provide high and consistent yields of cell free DNA (cfDNA) from plasma when compared to manual column procedures. Known for its reliable performance and uniquely wide sample volume range, the chemagic 360 instrument is an ideal solution for labs looking to automate their cfDNA extractions for diverse applications.

Here, compatibility of the FetoGnost® Kit RHD with extracted cffDNA obtained with the chemagic™ 360 instrument was tested using RhD-positive or -negative plasma from 36 blood donors.

*Revvity does not endorse or make recommendations with respect to research, medication, or treatments. All information presented is for informational purposes only and is not intended as medical advice. For country specific recommendations, please consult your local health care professionals.



Methods

Sample Handling

Plasma samples were obtained from 36 blood donors and stored at -20 °C until extraction. Prior to extraction, samples were thawed at room temperature.

Automated Extraction Procedure

Two extraction experiments were performed with 1 ml plasma volume per sample using the chemagic cfDNA 2K Kit H24 (CMG-1302) on the chemagic 360 instrument. A pre-mix of Proteinase K, poly(A) RNA, Lysis Buffer 1 and Internal Positive Control (IPC) target from the FetoGnost(R) Kit RHD was prepared and added to the samples prior to loading on the chemagic 360 instrument for automated cffDNA extraction. Elution was carried out in 80 or 90 µl volumes.

Analysis of RhD Status

The FetoGnost® Kit RHD mastermix was prepared and real-time PCR performed according to the manufacturer's instructions with a QuantStudio™ 5 Real-Time PCR System (Thermo Fisher).

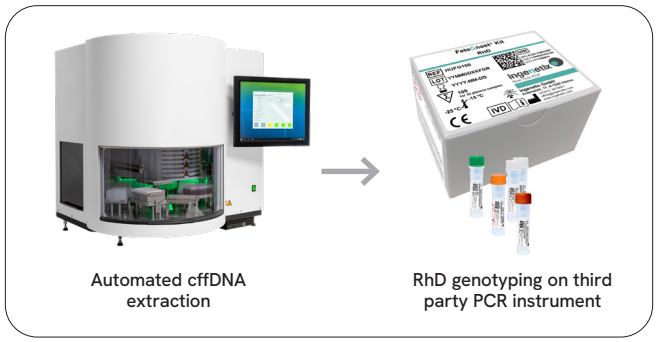


Figure 1: Workflow for fetal RhD genotyping with automated cffDNA extraction using chemagic 360 instrument and downstream PCR using FetoGnost® Kit RHD

Results

Successful identification of RhD positive and negative samples using chemagic automated cffDNA extraction applied with FetoGnost® Kit RHD

Results from positive and negative controls were as expected and validated the test results obtained. Table 1 shows the number of RhD positive and negative reactions obtained for exons 5, 7 and 10 from all donors. Based on the number of positive replicates, a positive RhD genotype was assessable for the 14 RhD-positive samples which is defined as having at least 4 out of 9 replicates positive for all exons analyzed. For RhD-negative donors, a negative RhD genotype was also assessable, defined by having at least 7 out of 9 replicates negative for all exons analyzed. The IPC test showed presence of IPC target in all samples aside from the negative control, indicating kit integrity and extraction performance was sound (Table 2). Based on these results, sensitivity and specificity of the tests were 100% (Table 3).

Table 1: Number of RHD positive and RHD negative reactions of exons 5, 7 and 10 from donors and samples obtained

Total samples tested (n = 36)	Exon 7 determined in triplicate				Exon 5 determined in triplicate				Exon 10 determined in triplicate			
	3/3	2/3	1/3	0/3	3/3	2/3	1/3	0/3	3/3	2/3	1/3	0/3
RhD-postive samples (n=14)	14 (100%)	0	0	0	14 (100%)	0	0	0	11 (79%)	3 (21%)	0	0
	0/3	1/3	2/3	3/3	0/3	1/3	2/3	3/3	0/3	1/3	2/3	3/3
RhD-negative samples (n=22)	19 (86%)	3 21%)	0	0	16 (73%)	6 (16%)	0	0	16 (73%)	6 (16%)	0	0

Table 2: IPC test to evaluate extraction performance and kit integrity

	IPC determined in triplicate			
	3/3	2/3	1/3	0/3
RhD-positive or negative samples (n=36)	108 (100%)	0	0	0

Table 3: Sensitivity and specificity

	IPC determined in triplicate				
	True positive	True negative	False positive	False negative	Cannot be assessed
RhD-positive or negative samples (n=36)	14	22	0	0	0
Sensitivity			100%		
Specificity			100%		

Conclusion

An analytical method has been developed on the chemagic 360 instrument with the extraction kit (chemagic cfDNA 2k Kit H24) for fetal RhD genotyping.

Learn More about the FetoGnost® Kit RHD from Ingenetix

Learn More about cfDNA extraction with chemagic™ technology from Revvity

Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA

(800) 762-4000
www.revvity.com