Automated cfDNA Isolation for the Clarigo Workflow

Abstract

Agilent’s Clarigo non-invasive prenatal test for aneuploidy screening of chromosomes 21, 18 and 13 was evaluated with the QIAsymphony SP instrument for cell free DNA (cfDNA) isolation.

The data using the QIAsymphony instrument and corresponding cfDNA extraction kit are equivalent to the current validated manual cfDNA extraction method for the tested sample set.

Introduction

Clarigo is a CE marked, non-invasive prenatal test (NIPT) based on Agilent’s proprietary Multiplex PCR technology, followed by NGS on Illumina MiSeq, HiSeq or NextSeq systems. The test is intended to screen for fetal aneuploidy status of chromosomes 21, 18 and 13 using cell-free DNA (cfDNA) prepared from a blood sample of a pregnant woman\(^1\).

The current Clarigo Instructions for Use (IFU) provide validated instructions to isolate cfDNA from blood using the manual QIAamp Circulating Nucleic Acid Kit (Qiagen; Cat No./ID: 55114), processing up to 24 QIAamp Mini columns on the QIAvac 24 Plus.

To minimize sample batch variation and human error, an automated cfDNA isolation method from blood can be performed by using the QIAsymphony DSP Circulating DNA Kit (Qiagen; Cat No./ID: 937556) on the QIAsymphony SP instrument. This magnetic-particle based technology can process up to 96 samples in a single run in 4 batches of each 24 samples.

To compare the performance of the manual with the automated cfDNA extraction method, an equivalence study was performed in two independent labs.
Materials & methods

Samples
A total of 192 fresh blood samples from 96 pregnant women with a normal pregnancy were collected. Per patient, 20 ml blood was drawn in two Streck Cell-Free DNA BCT blood collection tubes. Plasma isolation was performed within 48 hours of blood draw as per Clarigo IFU. Next, plasma was pooled per patient and then equally split before performing the cfDNA extraction with both the manual and automated method (Figure 1).

Since the automated QIAsymphony protocol requires a volume of 4.5 ml plasma, PBS was added to the split plasma samples in case total plasma volume was less than 9 ml.

cfDNA extraction
Manual cfDNA extractions from plasma samples were processed according to the Clarigo IFU. The only deviation from the IFU when using the QiaAmp Circulating Nucleic acid kit occurred during the elution step where 60 µl of buffer AVE was used instead of 50 µl to elute the cfDNA.

Automated cfDNA extractions were processed with the QIAsymphony DSP Circulating DNA Kit according to the manufacturer’s instructions (HB-2309-002 1103177 157018501 03/2017) and eluted in 60 µl of elution buffer.

Clarigo workflow
To minimize intra-run variation in the final results, the Clarigo* protocol was performed on 12 manually extracted cfDNA samples and the corresponding 12 automatically extracted cfDNA samples. The srMID primers were selected in such a way that the amplicon libraries contained unique and compatible combinations.

Two batches of 96 amplicon libraries processed simultaneously were sequenced in two separate NextSeq runs as per Clarigo IFU and analyzed with Clarigo Reporter v2.0.0. Gender calling was not evaluated in this study since this is optional.

Results
Analysis of the 192 FastQ files with Clarigo Reporter v2.0.0 showed that a total of 6 samples could not be processed due to Quality Control (QC) issues, resulting in 186 FastQ files for further analysis. Two samples are rejected because they did not meet the QC parameters using both the manual and QIAsymphony cfDNA extraction method. Additionally, 1 QC rejected sample was observed for each individual extraction method.

Fetal fraction
Comparison between the calculated fetal fractions of all QC passed samples between the manual and the QIAsymphony protocol is shown in Figure 2. Regression R² values are above 95 % and the regression coefficient does not differ statistically from 1 (95 % confidence interval).

Trisomy calls
All samples which have sufficient fetal fraction (> 3 %) in both protocols under investigation were compared. Final trisomy calls using the QIAsymphony instrument are identical to final trisomy calls using manual protocol. A total of 6 not automatically called samples (NACs) were observed. With the manual method 4 NACs were identified whereas only 2 NACs with the QIAsymphony method.

Performance summary
Combining all parameters, Clarigo results for the studied samples are equivalent when the cfDNA extraction was performed using the QIAsymphony (Table 1). The overall number of automatically called samples after QC is 3.2 % higher when samples were prepared with the QIAsymphony method.

* Clarigo data is analyzed with Clarigo v2 algorithm.
Conclusion
Based on the data presented in this application note, QIAsymphony instrument and manual method show equivalent results for the studied sample set regarding QC parameters, fetal fraction and trisomy calling.

Disclaimer
The use of QIAsymphony DSP Circulating DNA Kit and the QIAsymphony SP instrument shown here demonstrates the feasibility of automating the cfDNA extraction for running the Clarigo kit. However, considering the limited number of samples and study parameters, data presented are not sufficient to include this solution as an alternative step in the validated Clarigo workflow, laid out in the IFU.

References
1. Additional Clarigo information: www.clarigo.com

Table 1. Performance summary showing the number of failed samples after QC.

<table>
<thead>
<tr>
<th></th>
<th>Acceptance criteria*</th>
<th>Manual</th>
<th>QIAsymphony</th>
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<tbody>
<tr>
<td>LFF</td>
<td>≤ 1.41 %</td>
<td>1/186 (0.54 %)</td>
<td>0/186 (0.00 %)</td>
</tr>
<tr>
<td>NAC 21</td>
<td>≤ 2.13 %</td>
<td>3/186 (1.61 %)</td>
<td>1/186 (0.54 %)</td>
</tr>
<tr>
<td>NAC 18</td>
<td>≤ 1.37 %</td>
<td>0/186 (0.00 %)</td>
<td>1/186 (0.54 %)</td>
</tr>
<tr>
<td>NAC 13</td>
<td>≤ 2.88 %</td>
<td>1/186 (0.54 %)</td>
<td>0/186 (0.00 %)</td>
</tr>
<tr>
<td>FP call</td>
<td>≤ 0.26 %</td>
<td>0/186 (0.00 %)</td>
<td>0/186 (0.00 %)</td>
</tr>
<tr>
<td>FN call</td>
<td>≥ 0.00 %</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>% Automatically called samples after QC **</td>
<td>≥ 92.66 %</td>
<td>94.6 %</td>
<td>97.8 %</td>
</tr>
</tbody>
</table>

Legend: LFF: Low Fetal Fraction; NAC: Not Automatically Called; FP: False Positive; FN: False negative.

*Acceptance criteria are based on the results of the Clarigo v2 performance evaluation study.
**Automatic call for every chromosome.