to consider the thromboplastin source when making dosage adjustments for patients with high INRs.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

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References

Jasper A. Remijn*
Bertil Wildeboer
Jeroen D.E. van Suijlen
Henk J. Adriaansens

Department of Clinical Chemistry and Laboratory Hematology
Gelre ziekenhuizen
Apeldoorn/Zutphen, the Netherlands

* Address correspondence to this author at:
Department of Clinical Chemistry and Laboratory Hematology
Gelre ziekenhuizen
P.O. Box 9014
7300 DS Apeldoorn, the Netherlands
Fax +31-55-581-1235
E-mail j.remiijn@gelre.nl

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Noninvasive Prenatal Diagnosis of a Case of Down Syndrome due to Robertsonian Translocation by Massively Parallel Sequencing of Maternal Plasma DNA

To the Editor:

There has been much recent interest in the use of massively parallel sequencing of maternal plasma DNA for the detection of fetal Down syndrome, or trisomy 21 (1–4). DNA fragments in maternal plasma were sequenced at random to determine if an additional dose of chromosome 21 (chr21)1 sequences was contributed by the fetus. This approach has been shown to be highly robust in distinguishing trisomic and euploid cases. In these studies, however, all retracted trisomy 21 cases possess supernumerary whole chr21, as confirmed by karyotyping. This approach has not been formally shown to be applicable to other forms of the condition. For example, although the ratio of fetal to maternal DNA in maternal plasma has been shown to remain relatively constant across the entire genome for normal chromosomes (5), it is unknown whether an aberrant chromosome (e.g., one containing a chromosomal translocation) would exhibit an atypical genomic representation in the plasma. As a first step in addressing this issue, we applied the sequencing approach to a case of familial robertsonian translocation.

This study was approved by the local research ethics committee. Maternal peripheral blood samples from pregnant study participants were collected into EDTA-containing blood tubes before invasive procedures were conducted. A woman pregnant with a male fetus affected by an unbalanced translocation (46,XY,der(14;21)(q10;q10),+21) was recruited with informed consent. The mother carried a balanced translocation between chr14 and chr21 [45,XX,der(14;21)(q10q10)], and a blood sample was obtained before chorionic villus sampling. For comparison, we recruited 7 pregnant women with singleton euploid fetuses (4 males and 3 females). The chromosomal status of the 8 fetuses was confirmed by full karyotyping. The median gestational age was 13.3 weeks (Table 1).

Library construction from maternal plasma DNA was performed as previously described (4). Massively parallel sequencing (36 cycles) was performed on an Illumina Genome Analyzer IIx according to the manufacturer’s protocol. Real-time image processing and base calling were performed with the Genome Analyzer Sequencing Control Software (ver-
pregnancy with a female fetus
The chr21 and chrX rober
tsonian case (case 5) were

Clinical Chemistry

Clinical details and z scores for chr21 and chrX for 8 maternal plasma samples.

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Fetal karyotype</th>
<th>Fetal sex</th>
<th>Gestation, weeks</th>
<th>chr21 z score</th>
<th>chrX z score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>46,XY</td>
<td>Male</td>
<td>12.6</td>
<td>0.06</td>
<td>1.31</td>
</tr>
<tr>
<td>2</td>
<td>46,XY</td>
<td>Male</td>
<td>13.3</td>
<td>1.14</td>
<td>−1.12</td>
</tr>
<tr>
<td>3</td>
<td>46,XY</td>
<td>Male</td>
<td>13.5</td>
<td>−1.30</td>
<td>−0.16</td>
</tr>
<tr>
<td>4</td>
<td>46,XY</td>
<td>Male</td>
<td>13.6</td>
<td>0.10</td>
<td>−0.03</td>
</tr>
<tr>
<td>5</td>
<td>46,XY,der(14;21) (q10;q10), +21</td>
<td>Male</td>
<td>13.3</td>
<td>17.76</td>
<td>1.32</td>
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<tr>
<td>6</td>
<td>46,XX</td>
<td>Female</td>
<td>13.2</td>
<td>−3.39</td>
<td>2.91</td>
</tr>
<tr>
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<td>Female</td>
<td>13.3</td>
<td>−4.07</td>
<td>2.41</td>
</tr>
<tr>
<td>8</td>
<td>46,XX</td>
<td>Female</td>
<td>13.4</td>
<td>−1.48</td>
<td>3.46</td>
</tr>
</tbody>
</table>

We obtained a median of 2.33 × 10^6 unique mappable single-ended reads from each of the 8 samples. The percentage of genomic representation of each human chromosome (%GR) was calculated by dividing the number of sequenced reads from a specific chromosome by the total number of sequenced reads, excluding reads from chrY. The 4 euploid pregnancies involving male fetuses (cases 1–4) were chosen as the control group. The mean and SD values for the chr21 %GR for these samples were calculated and used to determine the chr21 z scores of the remaining samples. The chr21 z score for a test case is the difference between the chr21 %GR for the test case and the mean chr21 %GR of the control group, divided by the SD. A z score >3 indicates a trisomy 21 pregnancy, whereas a z score >1.67 for chrX indicates a pregnancy with a female fetus (1). The chr21 and chrX z scores for the robertsonian case (case 5) were 17.76 and 1.32, respectively; the chr21 z scores for the 3 euploid female cases (cases 6–8) were −3.39, −4.07, and −1.48, respectively, and they all had z scores >1.67 for chrX (Table 1). The highly negative chr21 z scores for cases 6 and 7 could be attributed to the small number of control cases that had been used to provide the reference values for calculating the z scores.

We have thus demonstrated for the first time that Down syndrome due to unbalanced robertsonian translocation can be detected by sequencing maternal plasma DNA. From this single case, we found no evidence that the chromosome containing the translocation behaves differently with regard to the GR of its fragments in the plasma. If this observation can be confirmed in general, then Down syndrome caused by other cytogenetic abnormalities, such as segmental duplication, could theoretically also be detected by the same approach. We envision that sequencing maternal plasma DNA will play an increasingly important role in the future developments of noninvasive prenatal diagnosis.

**Author Contributions:** All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

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**Employment or Leadership:** None declared.

**Consultant or Advisory Role:** Y.M.D. Lo, Sequenom and Clinical Chemistry, AACC.

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**Honoraria:** None declared.

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**Expert Testimony:** None declared.

**Other Remuneration:** F.M.F. Lun filed patent applications on the detection of fetal nucleic acids in maternal plasma for noninvasive prenatal diagnosis. R. Chiu and Y.M.D. Lo, patents and filed patent applications on aspects of fetal nucleic acid analysis (part of the patent portfolio has been licensed to Sequenom), Illumina (travel support), and Life Technologies (travel support).

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**References**


Primer Sequence Disclosure: A Clarification of the MIQE Guidelines

To the Editor:

The publication of the minimum information for the publication of real-time quantitative PCR experiments (MIQE) guidelines (1) has turned out to be a defining event in the maturing of quantitative real-time PCR (qPCR) technology. The response from instrument and reagent manufacturers has been universally positive. There has been extensive publicity in print, online, and at scientific meetings, and scientific journals are beginning to take note (2). Citations of the MIQE paper are accelerating, with 63 of the 169 citations (as of the end of January 2011) having appeared since September 2010. There is an enormous amount of good will toward this initiative, with many researchers keen to implement the different parameters within their own experimental protocols.

MIQE was never conceived with the intent of imposing an immutable edict, as in the spirit of a regulatory agency. The aim was to provide commonsense guidelines for enhancing the reproducibility and transparency of qPCR assays. MIQE, however, has become a marketing and selling argument (“MIQE compliance”), and this practice places a responsibility on the authors of the guidelines to assess whether the rapidly evolving technology demands refinement of the guidelines to acknowledge researchers’ uncertainty.

Most discussion has concerned the stipulation of primer sequence disclosure. Many commercial qPCR assays are not supplied with the primer/probe sequences because most vendors consider such information commercially sensitive. In addition, there usually are no details provided regarding empirical validation of each individual assay. The increasing use of commercial qPCR assays is creating problems, because it leads to publications that cannot satisfy current MIQE requirements and limits the universal acceptance of MIQE. Consequently, we propose a pragmatic amendment of the original guidelines to require “EITHER primer sequences OR amplicon context sequence.” This proposal is based on our assessment that in the absence of full disclosure of primer sequence, it is possible to achieve an adequate level of transparency, but only if there is an appropriate level of background information and disclosure of validation results regarding the qPCR assay:

- Our key concern is that today’s reports must remain technically accessible in the medium to long term. For that reason, publications must not report assays without reference to sequence data, with invalid Web site references, or with resources obtained from vendors that no longer exist.
- We continue to affirm that full disclosure of the reagents used and validation of their performance are principal requirements for MIQE “compliance.”
- Full primer (and probe) sequence disclosure remains our ideal; however, it may be possible to obtain equivalent results from slightly different assays as long as they target the same region and splicing variants and they take single-nucleotide polymorphisms and secondary structures into account.

Consequently, if primer sequences are not disclosed, a MIQE-compliant publication should provide all of the following:

- The assay identification provided by the commercial vendor.
- The specific amplicon context sequence for the qPCR assay. Preferably, this information is obtained by sequencing the target PCR amplicon; alternatively, it could be supplied by the vendor or approximated by the authors (Fig. 1).
- The same validation criteria used for assays reporting primer/probe sequences. Specifically, when a precise fold change for a transcript is reported, an essential require-

Nonstandard abbreviations: MIQE, minimum information for publication of quantitative real-time PCR experiments; qPCR, quantitative real-time PCR.