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ORIGINAL ARTICLE

Recommended practice for laboratory reporting of non-invasive prenatal testing of trisomies 13, 18 and 21: a consensus opinion

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ABSTRACT

Objective Non-invasive prenatal testing (NIPT) for trisomies 13, 18 and 21 is used worldwide. Laboratory reports should provide clear, concise results with test limitations indicated, yet no national or local guidelines are currently available. Here, we aim to present minimum best practice guidelines.

Methods All laboratories registered in the three European quality assurance schemes for molecular and cytogenetics were invited to complete an online survey focused on services provided for NIPT and non-invasive prenatal diagnosis. Laboratories delivering NIPT for aneuploidy were asked to submit two example reports; one high and one low risk result. Reports were reviewed for content and discussed at a meeting of laboratory providers and clinicians held at the ISPD 2016 conference in Berlin.

Results Of the 122 laboratories that responded, 50 issued reports for NIPT and 43 of these submitted sample reports. Responses and reports were discussed by 72 attendees at the meeting. Consensus opinion was determined in several areas and used to develop best practice guidelines for reporting of NIPT results.

Conclusions Across Europe, there is considerable variation in reporting NIPT results. Here, we describe minimum best practice guidelines, which will be distributed to European laboratories, and reports audited in subsequent external quality assurance cycles. © 2017 The Authors. *Prenatal Diagnosis* published by John Wiley & Sons, Ltd.

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INTRODUCTION

Non-invasive prenatal testing (NIPT) for aneuploidy is now established as a highly effective prenatal screening test for the major trisomies and is offered in many countries, largely through the commercial sector and private healthcare providers.[1] Some providers also offer sex determination, screening for sex chromosome aneuploidies and screening for other chromosomal rearrangements, for example microdeletion syndromes. Although sensitivity and specificity is high for trisomy 21, the test performance for trisomies 13 and 18 and sex chromosome aneuploidies is more variable.[2]

Non-invasive prenatal testing is based on analysis of circulating cell-free DNA in maternal plasma. After 10 weeks' gestation, 3% to 20% is of fetal origin[3] and is thought to be derived primarily from the placenta.[4] As such, NIPT is a screening test and does not replace the precision obtained with a diagnostic test, for example chorionic villus sampling or amniocentesis. NIPT results that are discordant with the diagnostic test result have a number of reported causes that include statistical/technical false positive/negative, low fetal fraction (FF) and biological factors such as confined placental mosaicism, (vanishing) twin pregnancies, maternal mosaicism[5,6] and maternal malignancy.[7] The FF, the amount of the cell-free DNA in the maternal blood that is of placental/fetal origin, is used by some providers as a quality parameter with some algorithms using a minimum FF below,

which results are not interpreted, whilst others combine FF with other quality metrics to determine if a result is reportable. The FF typically increases with advancing gestational age and is affected by a number of factors that include maternal weight and multiple pregnancies.[8] Furthermore, currently NIPT does not identify the range of chromosome abnormalities detected by karyotyping or microarray analysis of fetal cells obtained following invasive testing.[9] Finally, most performance data reported for NIPT relate to singleton pregnancies, although there is a growing body of data regarding its application to twin pregnancies.[2]

Given these complexities, it can be difficult to provide a comprehensive and clear result that also includes necessary information on test limitations. It is therefore important that reporting guidelines are established to address issues that are specific to NIPT. It is also important that laboratories are able to assess the standard of their service and evidence the standard of testing delivered. This can be achieved by participating in external quality assessment (EQA) schemes and following best practice guidelines, amongst other mechanisms. EQA schemes follow the clinical pathway as closely as possible and assess many of the steps between sample receipt within the laboratory, assay performed, analysis and the final reporting and interpretation of the results.

In Europe, quality standards for laboratories issuing genetic reports, molecular or cytogenetic are provided by three schemes, Cytogenetics External Quality Assessment Service (CEQAS), the European Molecular Quality Network (EMQN) and the UK National External Quality Assessment Service (UK NEQAS) for Molecular Genetics, who work together to set and audit standards in participating laboratories. These providers have delivered EQA schemes for prenatal genetic testing for both monogenic disorders and cytogenetic tests. They have also developed assessments for either rapid aneuploidy testing by quantitative fluorescence PCR (CEQAS and UK NEQAS for Molecular Genetics) or non-invasive prenatal diagnosis (NIPD) for sex determination (EMQN and UK NEQAS for Molecular Genetics). Feedback from participating laboratories, which includes some outside the EU, indicated a demand for EQA for common aneuploidy testing in maternal plasma samples. Any such scheme will require analysis of maternal plasma samples and reporting of results. Sourcing samples will be challenging as the basis of an EQA scheme is to provide the same material to all participants to enable inter-laboratory comparison. However, the availability of patient material does not enable this approach; therefore, the use of alternative EQA material or a different EQA format needs to be explored. Furthermore, the approaches to EQA will be informed by current practice. In order to provide an overview of need and to begin development of an EQA scheme, a survey of current practice and reports issued for NIPT was undertaken. In the absence of reporting recommendations, guidelines need to be established based upon the opinion and experience of those with knowledge of the subject. Consensus opinion is important as it allows different points of views to be expressed and evidence to be heard in a mutually beneficial way to establish best practice. Consensus as a process is central to the validity and applicability of the resulting recommendations.

This article scopes the need for a formal EQA scheme in Europe and provides an early consensus opinion of the Berlin workshop participants to set out recommendations for the future reporting of NIPT results.

METHODS

In February 2016, 2726 laboratories registered with CEQAS, EMQN and UK NEQAS for Molecular Genetics were invited to complete an online survey focused on services provided for NIPT and NIPD. Laboratories were asked to submit two example reports; one for a high-risk aneuploidy result and the other reporting a low-risk aneuploidy result as part of a review of current report practice.

The content of the reports was reviewed by an NIPT EQA Specialist Advisory Group and discussion points collated for the workshop. These were presented to the workshop attendees, and a participant discussion was facilitated. Consensus opinion was obtained and minimum guidelines agreed for the reporting of trisomies 13, 18 and 21 testing of cell-free fetal DNA (cffDNA) in maternal plasma.

RESULTS

There were 72 people registered for the workshop at ISPD, which included 28 commercial sector delegates and clinicians, laboratory scientists and healthcare policy makers from 19 countries.

Survey responses

One hundred and twenty-two responses were received (4.8% return rate), with one duplicate response leaving a total of 121 laboratories responding. The majority of laboratories invited to participate did not perform NIPT/NIPD. Three laboratories also reported that they delivered a service for NIPD, one of which is currently validating the service and one just offered NIPD for achondroplasia and two for fetal RHD genotyping. Because of the small numbers, these have not been considered further here. Seventy-five (61.5%) of responding laboratories performed NIPT for aneuploidy at the time of the survey, and 43 submitted sample reports. Two laboratories provided data analysis reports, and these were not included in the survey data. A further 25 laboratories were in the process of validating an NIPT assay, and 17 were offering NIPT on a research basis.

For those laboratories currently delivering an NIPT service, 19 performed between 0 and 50 tests per month, 8 more than 1000 per month and the others between 50 and 100 (Table S1). The methods used for NIPT varied, the majority (73.9%) was based on dosage obtained from next-generation sequencing (NGS), the single nucleotide polymorphism-based sequencing approach was used in 11.4% and other methods, including Sanger sequencing, array-based MLPA and TaqMan, in the remaining 14.8%. Of those using an NGS platform, 70.6% used a whole genome and the others a targeted approach. The platforms used included the Illumina MiSeq (17.9%), HiSeq (26.9%) and NextSeq (34.6%),and the ThermoFisher PGM

(17.9%) and Ion Proton (19.2%). The FF was measured by 60.9%. The turnaround time for reporting results varied from 3 to 21 days with the majority reporting in 4 to 7 (n = 25) or 8 to 14 (n = 40) days (Table S2).

A total of nine laboratories (22%) outsourced the testing, with 75.8% of these stating they would check that the provider participates in an EQA scheme, with two others stating that they would if an EQA scheme existed or the laboratory was accredited anyway, so it did not matter. The majority (71.9%) of those who would enquire about participation in EQA would request sight of the results. Most laboratories (62.5%) outsourcing NIPT would like to receive EQA samples and send them to their provider to participate in EQA.

Fetal sex was always reported by 22.7% of laboratories, only when requested by 48.9% and not reported by 28.4%. Less than half (46.1%) of the laboratories were tested for sex chromosome abnormalities. Some laboratories commented that these were only reported if requested, and two stated that these results would be conveyed to parents by a specialist trained in genetics. Trisomies other than 13, 18 and 21 and sub-chromosomal abnormalities were tested for by 37.8% of laboratories.

Responses to questions regarding a potential EQA scheme showed that 64.4% would like to submit anonymised reports to help develop an EQA scheme and 13.5% would be able to submit maternal plasma samples for analysis in an EQA scheme, with a further 38.9% saying this could be a possibility. Approximately 27.8% stated that their methodology was not amenable to reporting results obtained from pooled plasma samples.

Review of reports

Individual information

All reports included the patient/mother's name, and the majority (35/43, 81.4%) also provided the mother's date of birth. Some reports (8/43, 18.6%) only provided the mother's age and a small number (2/43, 4.7%) stated the weight and height of the individual (Figure 1). ISO 15189 standards[10] recommend that at least two unique patient identifiers are used on a report, and this should include the patient's full name and date of birth. The consensus was that these standards should be adhered to.

Sample information

ISO 15189 Standard 5.8.3[10] requires the type of primary sample and the date of primary sample collection. However, sample information on forms was variable. Some reports (21/43, 48.8%) provided the sample type being tested, which is essential information for the interpretation of the results and may be helpful for traceability. Slightly more (27/43, 62.7%) included the date of sample collection, and 33/43 (76.7%) provided the date of sample receipt in their laboratory (Figure 1). Following discussion and in view of the fact that maternal cell lysis over time reduces the proportion of cffDNA (the fetal fraction),[11] the consensus view was that the sample type, date of sample collection and date the sample is received within the laboratory should be stated on the report.

Clinical information

There was considerable variation in the clinical information provided in reports (Figure 2), with most laboratories (33/43, 76.7%) reporting gestational age. Consensus opinion was that it is recommended that the gestational age as defined by fetal ultrasound scan at the time when the sample is taken must be stated on the report. There was no majority view on the other pieces of clinical information stated, for example screening results, indication of the number of fetus present, any abnormal scan findings or whether or not *in vitro* fertilization had been performed.

Reporting information

Several laboratories failed to provide the contact details of the referrer or the destination of the report, or the report date. (Figure 1). As ISO 15189, Standard 5.8.3d[10] requires these details, the group recommended that the report must state the date of issue and the details of the individual requesting the test should also be included on the report.

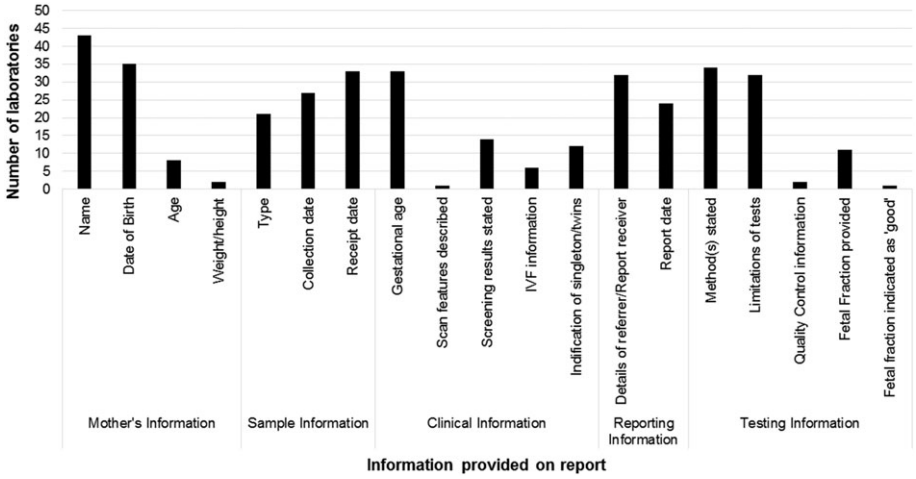

Figure 1 Summary of information stated in the example reports (the horizontal bar shows the total number of laboratories that provided reports for evaluation)

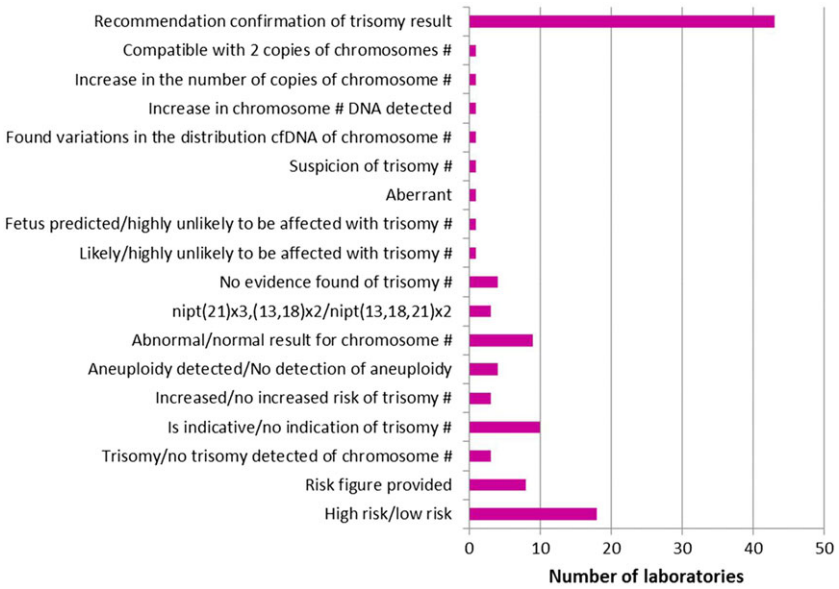

Figure 2 Summary of the terminology used to report the result. [Colour figure can be viewed at wileyonlinelibrary.com]

Laboratory testing information

In order to interpret the results reported, it is important not just to be aware of the test performed but also to know the basis of the test performed. Nine laboratory reports did not include any information on the test(s) being reported (Figure 1). It is recommended that the report must state the methodology used, for example NGS.

Eleven laboratories did not include any information with regard to the limitations of the test (Figure 1). This is particularly important when no abnormality has been detected, so the recipient is able to interpret and act upon the result appropriately. It is recommended that the report must state the limitations of the test.

Other information such as internal laboratory quality control information was included in a small number of reports (Figure 1). After discussion, it was agreed that this information should be available from the laboratory if requested but is not required on the issued report.

A long discussion was held on the merits of (1) measuring FF and (2) reporting the fetal fraction. Eleven laboratories out of the 43 submitting example reports stated the percentage of cffDNA present in the sample, with a further laboratory reporting FF was 'good'. Workshop delegates could not agree on whether or not FF should be measured or reported. As no consensus was reached, no clear guidance has been agreed.

Reporting the result

The terminology used to report the result is very important. It must be easy to find within the report, clear and not open to any misinterpretation. The review of the submitted reports is summarised in Figure 1 and demonstrates significant variation in reporting the same result. The delegates deemed the use of the Internal System for Cytogenomic Nomenclature to be inadvisable.[11] The group considered that as the use of the universal chromosomal abnormality nomenclature implies that a diagnostic test has been performed, this should not be used. This is consistent with the fact that the latest Internal System for Cytogenomic Nomenclature[11] does not reference NIPT. Similarly, as the use of the term 'abnormal' implies a diagnostic result is being reported, this approach was not recommended.

The most commonly used phrasing was 'high-risk/low-risk' result (Figure 2). Following discussion, the group recommended that to minimise the chance of the result being misreported, the results are reported as 'high risk/low risk' of aneuploidy.

All laboratories recommended that a high-risk trisomy result must be confirmed. The group unanimously agreed that reports clearly state that it is recommended that a high-risk result is confirmed by invasive testing.

Other issues discussed included the merits of providing a risk figure on the report and whether negative and positive predictive values should be included, but no consensus was reached.

DISCUSSION

The survey carried out as part of this evaluation demonstrates that the NIPT testing strategy varies widely, particularly with respect to fetal gender analysis, sex chromosome aneuploidy and sub-chromosomal abnormality such as copy number variation. Because this is to some extent governed by national policy or local legislation, we do not attempt to provide guidance on the extent of testing but rather on how this should be reported to provide a clear and unambiguous result. Similarly, a variety of platforms and protocols is used for testing, and this diversity provides resilience, particularly when objectively assessed though an EQA scheme. It should be noted however that any test must be thoroughly validated and limitations made clear in accordance with accrediting body standards.

The work presented here demonstrates the diversity in reporting NIPT for aneuploidy, with many reports lacking basic

information such as details of referrers, date of testing, gestational age and so on, thus clearly indicating the need for standardisation and audit. These are clear standards already mandated for laboratories seeking or holding ISO15189 accreditation, the current standard in Europe. As such, consensus was readily achieved on many issues (Table 1). There was considerable debate over the inclusion of FF on the report, possibly because this is not readily measured by some platforms, whilst others use it as a quality marker and do not report results if the FF falls below a certain cut-off.[12] Measuring and reporting FF may minimise false-negative results secondary to very low levels of cffDNA in a sample but may also result in an increase in test failure. As some trisomies are associated with lower levels of cffDNA[13] including the measurement in the laboratory methodology and report may have value. The cut-off used by some providers is 4%,[12] but this may vary with platform used. Also, the minimal FF needed for providing an adequate NIPT result (i.e. 'limit of detection') is not only related to the specific approach but also to the sequencing depth.[14–16] However, whilst the group could not reach consensus on reporting fetal fraction, it was decided that if reported, the significance in relation to the test result should be made clear.

The other significant area of debate with lack of consensus was around the reporting of positive and negative predictive values with revised individualised risks. Whilst this provides a more specific risk, the group acknowledged that accurate prior risk and data from very large series were required to deliver this and so may not be feasible.

The terminology used in reporting results may warrant further consideration as, although not discussed in detail at the workshop, there has been concern voiced recently by the lay public and Down Syndrome Support groups over the use of the term 'risk' when reporting prenatal screening results, as they feel this has negative connotations.[17] With that in mind, the term 'chance' rather than 'risk' could be considered; however, further discussions will be facilitated by the EQA providers before any decision is made.

Table 1 **Points to be considered for inclusion when reporting NIPT for aneuploidy**

A minimum of two unique patient identifiers should be included, to include the patient's full name and date of birth.	Full consensus
Sample type, date of collection and date of sample receipt in the laboratory	Full consensus
Gestational age, confirmed by fetal ultrasound scan at the time of blood sampling	Full consensus
Date of issue of the report	Full consensus
Details of the individual requesting the test	Full consensus
Test methodology	Full consensus
Test limitations	Full consensus
Fetal fraction	Discussed but no consensus reached
Results are reported as 'high risk/low risk' of aneuploidy[a]	Full consensus
Recommend that 'high-risk' results are confirmed by invasive testing	Full consensus
Provision of revised individualised risks, positive and negative predictive values	Discussed but no consensus reached

NIPT, non-invasive prenatal testing.
[a]Consider changing to 'chance' in view of lay support group opinions following further discussion.

The results of this workshop will inform the standards required for reporting NIPT for aneuploidy in Europe and will be used in the EQA assessments scheduled for 2017. Ultimately, how laboratories deliver NIPT for aneuploidy and report results will need to align with national legislation and guidelines. As such, there is likely to be significant variation in reporting some aspects of this testing, for example reporting fetal gender, screening for sex chromosome aneuploidies and other chromosome rearrangements. However, the basic principles discussed here should apply when reporting results of any NIPT testing. Whilst many national and international bodies have published guidelines on the use of NIPT, most are concerned with the testing strategy rather than reporting guidelines.[18,19] Guidelines published by American College of Medical Genetics and Genomics do include very specific recommendations including reporting high-risk results by a genetic health professionals, the use of personalised positive predictive values and reporting of FF.[20] As discussed here, some laboratories may find it challenging to comply with all of these guidelines at the present time, although these may be optimal standards to work towards. Furthermore, the health professionals delivering Down Syndrome Screening programmes vary across the world highlighting the fact that guidelines need to vary with local practice. However, it is to be hoped that the standard principles of offering and reporting prenatal tests to parents would be adhered to regardless of who delivers results.[21]

The data reported here have also informed development of standards required to perform quality assurance of the laboratory techniques themselves. An initial pilot has been completed by a small number of laboratories using maternal plasma samples collected from pregnancies at risk of aneuploidy with known outcomes, and a second pilot assessment is underway using artificial plasma material for laboratories testing using NGS technologies. However, the EQA approach to sample preparation will need careful consideration as more than a quarter of laboratories stated that their methodology was not amenable to using pooled plasma samples.

In conclusion, we have developed minimum guidelines for reporting laboratory results for NIPT for aneuploidy (Table 1) and have generated useful data to inform development of an EQA scheme to assess laboratory performance; this is ongoing and will be reported elsewhere. As with other EQA schemes, these guidelines will be reviewed annually so that reporting can accommodate changes in service delivery and evolve as those areas where consensus was not reached, use of FF and revised risk scores, for example, become clearer. Most published data relate to singleton pregnancies and the autosomal trisomies, but as use in multiple pregnancies and for other chromosome abnormalities becomes more widespread, EQA schemes will need to be revised to apply to all pregnancies.

WHAT'S ALREADY KNOWN ABOUT THIS TOPIC?

- In Europe, quality standards for laboratories issuing genetic reports are provided by three schemes who deliver external quality assessment schemes for prenatal genetic testing for both monogenic disorders and cytogenetic tests, including non-invasive prenatal diagnosis for sex determination.
- Feedback from participating laboratories indicates a demand for external quality assessment for non-invasive prenatal testing for the major trisomies.

WHAT DOES THIS STUDY ADD?

- There is considerable variation in laboratory reporting of non-invasive prenatal testing results.
- Here, we describe minimum best practice guidelines, which will be distributed to European laboratories, and reports audited in subsequent external quality assessment cycles.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.