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Quality assurance and standardization in view of non-invasive prenatal testing (NIPT)

Qualitätssicherung und Standardisierung in Bezug auf nicht-invasive Pränataltestung (NIPT)

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Abstract: The development and utilization of non-invasive prenatal tests have provided new and exciting challenges for quality assurance. Quality managers, scientists and technicians have been faced with the question of appropriate validation and quality controls for these innovative tests. Guidelines on quality assurance and quality control are still lacking and the need is growing inexorably. To integrate non-invasive prenatal tests into existing guidelines, attention must be paid to ISO standard 15189 which describes the requirements for medical laboratories and therefore diagnostic molecular genetics laboratories. Performing the test in an accredited molecular genetics laboratory according to ISO 15189 ensures the adherence to, and compliance with, all important principles. In this article, an overview of quality requirements applied to non-invasive prenatal testing is given from a quality manager's point of view.

Keywords: cell-free fetal DNA; guidelines; ISO 15189; non-invasive prenatal test (NIPT); quality assurance; quality control; validation.

Zusammenfassung: Die Entwicklung und Durchführung von nicht-invasiven pränatalen Tests stellte die Qualitätssicherung vor neue, spannende Herausforderungen. Qualitätsmanagementbeauftragte, Wissenschaftler und technisches Personal wurden mit der Frage nach geeigneten Validierungen und Qualitätskontrollen für diese innovativen Tests konfrontiert. Qualitätssicherungs- und Qualitätskontroll-Richtlinien sind noch immer nicht verfügbar und das Bedürfnis danach wächst unaufhaltsam. Um nicht-invasive Pränatal-Tests in bestehende Richtlinien einzuordnen, muss die ISO 15189-Richtlinie beachtet werden, die die Anforderungen an medizinische und somit auch an diagnostische, molekulargenetische Labore beschreibt. Die Durchführung des Tests in einem akkreditierten molekulargenetischen Laboratorium nach ISO 15189 gewährleistet die Beachtung und Einhaltung aller wichtigen Voraussetzungen. In diesem Artikel wird ein Überblick der Qualitätsanforderungen an nicht-invasive Pränatal-Tests aus der Sicht eines Qualitätsmanagementbeauftragten gegeben.

Schlüsselwörter: ISO 15189; Nicht-invasiver Pränatal-Test (NIPT); Qualitätssicherung; Qualitätskontrolle; Richtlinien; Validierung; zellfreie fetale DNA.

Introduction

The discovery and utilization of new analytes and new analytical methods often gives rise to new challenges for quality assurance in a medical laboratory. With the appearance of cell-free fetal DNA analysis, the interpretation and implementation of quality assurance parameters such as quality controls and validation has to be made by a molecular genetics laboratory. To harmonize and improve the quality of new methods, best practice guidelines have to be developed over time [1–4].

In this manuscript the general principles of quality assurance in a molecular genetics laboratory shall be reviewed and applied to non-invasive prenatal testing.

Cell-free DNA

The existence of cell-free DNA was first described by Mandel and Metals in 1948 [5]. Currently, different kinds

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of cell-free DNA are known, cell-free fetal DNA (cffDNA) originating from the placenta [6], from solid tumors [7, 8] or solid organ transplants [9]. The presence of cffDNA in maternal blood was first shown in 1997 [10]. This led to new possibilities in prenatal diagnosis, and the use of cell-free fetal DNA for non-invasive prenatal testing/screening (NIPT/NIPS – hereafter referred to as NIPT) has evolved quickly [11]. This has given rise to new challenges for quality assurance.

Quality assurance and standardization in a diagnostic molecular genetics laboratory

Put generally, standardization is essential for health care providers. The goal of standards in diagnostic environments is to ensure the highest quality and safety for patients. It must be guaranteed, that the correct test result is given to the correct patient and that everything which constitutes part of the health care is performed accurately [12, 13].

In diagnostic laboratories the greatest attention is paid to International Standardization Organization (ISO) standards. General requirements for laboratories are described in ISO 15189 and ISO 17025 [12, 14]. Since ISO 17025 describes general principles for all kind of laboratories [14], this article will focus on ISO 15189, which describes the specific requirements for a medical laboratory [12]. The implementation of ISO 15189 in a medical laboratory ends successfully with an accreditation, a "formal recognition by an authoritative body, that a laboratory has the competence to carry out specific tasks" [12, 13, 15]. Furthermore, the accreditation brings the benefits of international comparability and recognition, and prevents costs resulting from multiple assessments [16].

ISO 15189 gives a framework for all parts that are involved in the diagnostic procedural process. It contains requirements for pre-, intra- and post-analytical steps during the processing of samples. Besides the principles for all technical procedures, ISO 15189 also states laboratory management requirements.

Some important parts of ISO 15189 are stated below and will be looked at in more detail.

Standard operating procedures (SOP): The use of SOP is an important prerequisite for reproducibility of NIPT results, as describing a procedure in a specific SOP ensures it is always performed in the same way. Therefore, a written SOP must be present for all procedures which are performed in the laboratory. This includes technical procedures as well as management operations.

Validation of processes: The validation must comprise all critical steps and ensure that the validated process is suitable for the intended purpose. This could be achieved, e.g. by result confirmation with another validated method or by successful participation in an external quality assessment. Moreover, the validation should also contain procedures for ongoing validation, comprised risks and set the frame in which the test can be provided. A useful guideline for validation of molecular genetic tests is given by Mattocks et al. [17].

Training of personnel: All procedures must be performed exclusively by trained and skilled employees. Personnel training must be described and documented, and should be offered frequently, as well as an accurate on-the-job-training. Using the example of evaluation of genetic data, it is easy to imagine the importance of knowledge and experience.

Internal quality controls (IQC): IQC should be implemented to ensure that a method is working consistently. Furthermore, they are necessary to decide whether a result can be trusted or not [18]. There are different possibilities to introduce IQC. For example, double-checks could be performed or reference materials used, such as a positive control for a PCR. Internally determined quality control (QC) value cut-offs could also be documented as IQC.

External quality assessments (EQA): EQA represent a comparison of test results between laboratories and are essential to ensure results concordance. There are several EQA providers, for example EMQN (The European Molecular Genetics Quality Network), UK NEQAS (United Kingdom National External Quality Assessment Service) or RfB of the DGKL (Reference Institute for Bioanalytics of the Deutsche Vereinte Gesellschaft für Klinische Chemie und Laboratoriumsmedizin e.V.), who provide method- and analyte-specific schemes in which a laboratory can participate. In addition, EuroGentest and the IFCC (International Federation of Clinical Chemistry and Laboratory Medicine) homepage provide databases in which EQA worldwide are listed. The external quality assessments should contain all sections and parts of a specific test, and be performed regularly. In addition, they represent a method to train and educate staff [18]. If no external quality assessments schemes are available, the laboratory

has to consider alternative strategies, such as interlaboratory exchanges [12].

Quality indicators (QI): A laboratory should implement QI, which enables the measurement of quality and detection of potential error sources. QI could be the duration of analysis, error rates or internal audits, with the latter considered a process review [18]. The QI should be evaluated regularly, to improve the quality of all measured units. This could be done in the context of a management review.

As ISO 15189 represents the "gold-standard" of principles for any diagnostic laboratory [13, 18], it also applies to molecular genetics laboratories which perform human DNA-based tests for the determination of genetic properties. It is strongly recommended that a diagnostic molecular genetics laboratory should be accredited according to ISO 15189 standards [18, 19], alternative approaches would prefer a "requirement for accreditation of genetic laboratories, instead of a recommendation", as molecular genetics tests are extremely specific and the quality of these tests could be improved by accreditation [13, 15, 18, 20].

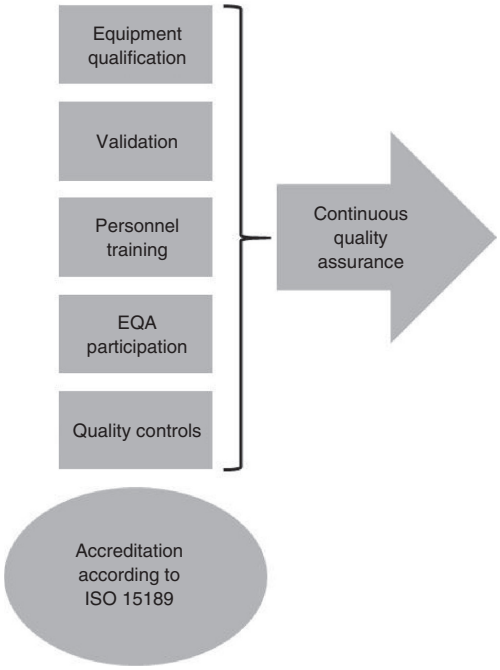

Figure 1: Basic principles to ensure quality of NIPT.
A molecular genetics laboratory performing NIPT has to fulfill at least the stated requirements to receive an accreditation according to ISO 15189 standards for this test. In addition, the laboratory must implement a continuous quality assurance, which comprises the above-mentioned principles to ensure the consistent quality of NIPT.

Non-invasive prenatal test (NIPT)

NIPT represents a method to estimate the risk of fetal aneuploidies by genetically analysing cffDNA [11]. Although NIPT is not regarded as a genetic "test", but as "screening" [2, 11], the whole process of isolating and analysing the cffDNA should be carried out in a human molecular genetics laboratory. Therefore, the requirements outlined above apply to NIPT [1, 2] (Figure 1).

To describe the requirements of ISO 15189 with regards to NIPT, it is necessary to have a more detailed look at the single steps of NIPT. In this context, the required use of written SOP and the performance by trained employees will not be mentioned again, as this is a prerequisite.

Blood draw and transportation

First of all, adequate information about genetic tests should be provided to patients and documented as an informed consent.

The actual NIPT process begins with a blood draw. Consequentially, the quality assurance begins with drawing the blood, too. It is necessary to use a suitable tube containing the essential ingredients for stabilizing the blood sample. For NIPT the Cell-Free DNA Streck™ BCT® tubes were validated as an appropriate choice [21]. Additionally, the correct labeling of the tube with patient's name or another obvious identification characteristic is mandatory. According to ISO 15189, the laboratory is obligated to reject samples which cannot be identified clearly, as well as tubes which do not contain enough blood as considered to be necessary for the analysis [12]. It should be recommended that the laboratory receives enough blood to perform two independent tests, to ensure the possibility of result confirmation.

In summary, it is paramount to give clear and understandable instructions to the patients, the physicians and all other involved persons.

Once the blood is drawn, it should be stored and shipped appropriately. There are studies available which describe the best transportation conditions, with regard to the duration and temperature of transport [21], however the laboratory itself has to investigate and validate the most suitable conditions by performing validation studies of the process. These validation studies could be comparative studies, being performed with non-critical samples. The validation experiments should comprise an evaluation of the effects from different conditions on previously determined quality parameters, such as the amount of cell-free DNA after extraction of an identical sample. The conditions

covered by these studies must be at least temperature and transport duration, as mentioned before. Additionally, validation experiments should cover all pre-analytical variables, considered as critical, especially steps, where operator dependent handling is required. To validate the appropriate transport temperature and duration, temperatures must be monitored, for example, by using a temperature logger, and the duration measured exactly.

Extraction of cell-free DNA

There are different methods available for the extraction of cell-free DNA, and differing results in quantifying the extracted DNA with these methods have been reported [22, 23]. Therefore, it is essential to validate and ensure the suitability of the method chosen. Additionally, standard quality controls should be implemented to check the efficiency of the extraction method. One approach as to how standardized quality controls could look has been published [22].

Quantification of cell-free fetal DNA

After a study revealed that three out of five laboratories reported a NIPT result ("no aneuploidy detected") for women who had not been pregnant, it is necessary to prove the suitability of the sample for NIPT pre-analytically. This is done by a straight forward determination of fetal DNA in the sample [4]. During analysis, the maternal DNA is still present in the same tube as the cffDNA [2]. Thus, it is essential for further analysis to implement strategies to measure the quantity of the fetal DNA [4, 22]. For instance, the fetal fraction could be determined based on differential methylation [24].

The quantification of the fetal fraction represents a critical step of the NIPT workflow and must be validated accurately, for example as explained before by using an independent, validated method or by successful participation in an EQA. The implementation of quality controls to ensure the correct assessment of cffDNA amount has to be considered as well.

Analysis of cffDNA with next-generation sequencing (NGS)

The most common method to analyze cffDNA is next-generation sequencing (NGS) [2]. For the implementation of NGS in diagnostics, the need for quality standards was noted years ago and guidelines have already been published [25, 26]. For a better explanation of the quality needs, the analysis with NGS in the given context will be summarized and split into a technical phase and a statistical/bioinformatics phase. Detailed descriptions of NGS workflows are available [25, 27].

The technical phase represents the preparation of a DNA-library and the enrichment of target sequences; this is performed in the laboratory by technical assistants and demands accurate handling. Regarding validation, it is important to detect and describe critical steps which could occur during handling. For example, it could be necessary to perform a quality control step by implementing a four-eye principle to check the correctness of crucial functions such as adapter ligation for which errors cannot be detected subsequently, or establishing appropriate quality controls which illustrate deviations occurring during the processing of samples.

The second part of NGS consists of the actual sequencing run on a suitable sequencer and the evaluation of the produced data. To ensure the correct operation of the sequencer, internal quality controls should be implemented in the sequencing run. The data generated during one single sequencing run are large, since many Gigabytes are accumulated [27]. Computational aid in this case is inevitable, in particular for the data analysis and the backup of raw data. Nevertheless, the validation of this system is essential for producing reliable test results. Software validation in general should be highlighted here. Even the use of CE-marked software could require validation, in particular if the user creates their own analyses workflows in the software.

In the case of NIPT, sequences of cffDNA do not need to be analysed qualitatively in detail, but the amount of determined sequence reads must be compared to the quantity of sequence reads of a euploid reference sample set. Subsequently, an estimation of the fetal set of chromosomes with regards to the current tested trisomies (13, 18, 21) [1, 2] could be given. It is superfluous to emphasize the importance of validation for this purpose.

If whole genome amplification and analysis is being performed, other chromosomes should be blinded, to avoid inadvertent results.

Report

The required contents of a NIPT report that medical laboratories should follow, are described in detail in ISO 15189

[12]. Additionally, a NGS report should be clear, consistent and understandable. The description of the test represents a crucial topic [26].

As the result of NIPT is a risk estimation, additional genetic counseling after the test should be recommended to clarify and discuss further options [28].

Conclusion and outlook

Many articles on the subject of NIPT recommend and claim for the standardization of this method. Moreover, as NIPT comprises many critical steps, specific quality assurance guidelines should be developed [1, 3, 4, 22]. As long as these are lacking, an accreditation according to ISO 15189 is the most appropriate conclusion [1, 3] and should remain, even after publication of required guidelines. A successful accreditation as a medical laboratory covers all critical parts which should be considered by a laboratory performing NIPT. To receive an accreditation for NIPT, the herein described requirements, as well as all others stated in ISO 15189, must be investigated in more detail, as this manuscript introduces only the main principles which should be considered.

Furthermore, it is recommended that the results of NIPT presented in a report are reviewed and validated by a physician who ensures that the results are given in a medically acceptable manner. Nevertheless, genetic counseling, before and after NIPT, should also explain limitations and risks based of this test and whether the test provides a diagnostic or a screening result [28, personal communication]

Additionally, the development of (new) quality assessment schemes for NIPT which represent all phases of the test (optimal plasma separation, DNA extraction, quantification of the fetal DNA and the analysis) should be developed urgently, although EMQN, UK NEQAS and the Cytogenetic External Quality Assessment Service (CEQAS) have already made efforts to provide a suitable EQA. One issue faced in the provision of an EQA for NIPT is the preparation of artificial material, which mimics cffDNA.

As the evolution of non-invasive prenatal tests continues to develop, the implementation of appropriate and feasible standards and guidelines will remain a task for the future.

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