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Quality Factors in a Molecular Genetics Laboratory

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Genetic testing has steadily become more popular in diagnostics and research use. Because the results of the genetic tests can have a large impact on the treatment and quality of life of an individual, the validity of the results and the quality of the laboratory work is important.

The purpose of the thesis was to investigate possible factors affecting laboratory quality, and to create a small-scale literary review of suitable industry standards. In addition, a small laboratory experiment related to the development of a new test for Autosomal recessive non-syndromic hearing impairment (ARNSHI) was performed in order to observe possible sources of error during molecular laboratory experiments. The observations were collected into a separate document. The thesis was commissioned by Woble Helsinki Oy.

DNA contamination is a large risk factor in polymerase chain reaction (PCR) analyses due to the sensitivity of the method. The biggest source of contamination is the DNA originating from already amplified samples. This has to be taken into account when planning the laboratory's layout, which has to be constructed in a way that allows the work to flow unidirectionally from pre-PCR sections to post-PCR sections. The work areas should also be adequately decontaminated.

Out of the three phases of a laboratory analysis, errors are most commonly made during the pre-analytic phase, and often include sample-patient mismatches and sampling errors. The second most common errors are the ones made during the post-analytic phase, and include errors associated with reporting. The analytic step is the most error-free, most likely due to the high degree of automatization present in analytic instruments. Errors can be prevented with personnel training and precise protocols.

From the standards related to the field, ISO standards ISO 9001, ISO 17025 and ISO 15189, GLP guidelines, OECD Guidelines for Quality Assurance in Molecular Genetic Testing, CLIA federal laws and CLSI guideline MMA-20 were examined. The ISO 17025 and CLSI guideline were deemed most suitable and chosen as the basis of the new laboratory.

Keywords	quality, laboratories, standards

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Geenitestit kasvattavat tasaisesti suosiotaan sairauksien diagnosoinnissa ja tutkimuskäytössä. Koska geenitestien tuloksilla voi olla suuria vaikutuksia yksilön hoitoon ja elämänlaatuun, on tulosten oikeellisuus ja laboratoriotyön laatu ensiarvoisen tärkeää.

Opinnäytetyön tarkoituksena oli selvittää laboratorion laatuun vaikuttavia tekijöitä, sekä tehdä pienimuotoinen kirjallisuusselvitys olemassa olevista laboratorioon soveltuvista laatustandardeista. Tämän lisäksi tehtiin pieni laboratoriokoe liittyen uuden autosomaalista resessiivistä ei-syndromista kuulovammaisuutta (ARNSHI) detektoivan testin kehitykseen, jonka aikana tarkkailtiin laboratorion virhelähteitä. Huomioista tehtiin erillinen dokumentti. Opinnäytetyön toimeksiantaja oli Woble Helsinki Oy.

DNA-kontaminaatio on suuri riskitekijä polymeraasiketjureaktioon (PCR) perustuvissa analyyseissa menetelmän herkkyyden vuoksi. Suurin kontaminaation lähde on toisista, jo monistetuista näytteistä tuleva DNA. Tämä on otettava laboratorion suunnittelusta asti huomioon siten, että työ etenee tiloissa systemaattisesti ja yksisuuntaisesti pre-PCR-vaiheista post-PCR-vaiheisiin. Työpisteet tulee myös dekontaminoida riittävin tavoin.

Kolmesta eri työn vaiheesta pre-analyysivaiheessa tehdyt virheet ovat kaikkein yleisimpiä ja liittyvät usein potilastietojen sekoittumiseen tai virheelliseen näytteenottoon. Toisiksi yleisimpiä ovat post-analyysivaiheessa tehdyt virheet, jotka liittyvät tietojen riittävään raportointiin. Vähiten virheitä tehdään varsinaisessa analyysivaiheessa, todennäköisesti analyysilaitteiden korkeasta automaatiotasosta johtuen. Virheitä voidaan ehkäistä henkilöstön koulutuksella ja tarkoilla protokollilla.

Alaan liittyvistä laatustandardeista tarkasteltiin ISO-standardeja ISO 9001, ISO 17025 ja ISO 15189, GLP-ohjeistusta, OECD Guidelines for Quality Assurance in Molecular Genetic Testing -ohjeistuksia, USA:n CLIA-lakeja sekä CLSI:n MMA-20-ohjeistusta. Standardeista todettiin sopivimmiksi ISO 17025 ja CLSI-ohjeet, ja ne valittiin uuden laboratorion perustaksi.

Avainsanat	laboratoriot, laatu, standardit

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Appendix 1. Suggestions for laboratory operations

Appendix 2. Primer pairs

Abbreviations and terms

ARNSHI	Autosomal recessive non-syndromic hearing impairment
CLIA	Clinical Laboratory Improvement Amendments
CLSI	Clinical and Laboratory Standards Institute
DNA	deoxyribonucleic acid
EU	European Union
FINAS	Finnish Accreditation Service
GJB2	Gene that encodes gap junction beta 2, also known as Connexin-26
GJB6	Gene that encodes gap junction beta 6, also known as Connexin-30
GLP	Good laboratory practice
ISO	International Organization of Standardization, from Greek ‘isos’.
MIQE	Minimum Information for Publication of Quantitative Real-Time PCR Experiments
OECD	Organization for Economic Cooperation and Development
PCR	Polymerase chain reaction
qPCR	Quantitative real time PCR
RT-PCR	Reverse transcriptase PCR
SFS	Suomen Standardisoimisliitto ry, from **F**inlands **S**tandardiserings**f**örbund.
UV	Ultraviolet

1 Introduction

DNA tests are used to detect specific DNA sequences from the sample. In clinical context they are often referred as molecular diagnostics tests and can be used to detect, for example, genetic disorders [1]. The usage of molecular diagnostics tests has been steadily rising, its global market size valued at over 4,8 billion USD in 2014 [2]. The direct-to-consumer genetic testing market is a relatively small portion of it, its global value at around 70 million USD [3]. Since the results may have large impact on the possible treatment and life quality of the patient, the validity of the results is exceptionally important. To ensure the validity of the results, the quality of the laboratory should be high.

Since the factors affecting quality are so diverse, they have been narrowed to the two most prominent ones – DNA contamination, and errors occurring during, before and after the analysis. The sources of both are explored, as are the potential ways to prevent them.

This thesis was done for Woble Helsinki Oy, a company that develops and performs direct-to-consumer DNA tests for detecting hereditary conditions. Currently the company operates in a very small scale laboratory, but is planning to begin working on a quality manual and expand its laboratory functions. The purpose of this thesis was to provide a literary review of factors affecting laboratory quality and possible suitable laboratory standards and guidelines so that the company could decide what would be best suited as the basis of their new quality manual and laboratory expansion. In addition to the literary review, a small laboratory experiment was performed to observe possible sources of error in a molecular testing laboratory. The knowledge gained from the literary review and the observations was also used to prepare a blueprint for the planned laboratory expansion.

2 DNA Contamination

The strength of PCR techniques is their sensitivity, and the high number of amplicons – one PCR run can produce up to 10^{13} amplicon molecules. However, this makes PCR techniques vulnerable to contamination, as even a small amount of contaminating DNA in a sample or the equipment can affect the results of all subsequent tests. Contamination can cause false positives, which can have far-reaching effects in clinical and diagnostic applications. DNA contamination in this context refers to unwanted, non-sample DNA that has found its way into the sample, or any DNA that can be found on the equipment. [4.]

To ensure good operation, it is advisable for the laboratory to have a documented policy for detecting and controlling contamination, in addition to having policies and procedures for interpreting data that may have been affected by contamination, to ensure that the personnel take proper action when the situation arises. Contamination can be detected, for example, by running appropriate control reactions – a reagent blank can be used to detect reagent contamination, and if the laboratory has DNA-profiles of their personnel, contamination originating from them can be easily seen in negative control samples [5].

2.1 Possible sources

DNA contamination can be caused both by primary transfer, where the DNA comes directly from the contaminant, and secondary transfer where a piece of equipment is contaminated and the DNA is subsequently transferred to another sample [6]. The three most common sources of contaminating DNA are DNA from the personnel, DNA from other samples in the lab and DNA fragments of the standard used to determine the size of amplified alleles. Contamination from bacteria or airborne contaminants such as pollen is possible, but generally causes no problems due to most molecular genetics tests being species-specific [5].

DNA can accumulate on the surfaces, tools and equipment within the laboratory, and pose a great risk of contaminating samples through secondary transfer [6]. Especially gloves have been found to contain significant amount of DNA [7], and should be changed

often. While certain guidelines recommend air-pressurized workrooms to prevent airborne contaminants [8, 9], air has been found to be an unlikely source of DNA contamination [6, 10].

2.2 Preventive methods

Due to DNA contamination being invisible to naked eyes and sometimes tricky to detect, not to mention hard to get rid of, preventing the contamination from occurring is critical.

To reduce the risk of sample contamination by epithelial cells during DNA extraction and sample processing, personnel should wear protective clothing, including mouth masks. To prevent secondary transfer of contaminants, every section should have its own dedicated equipment and reagents, and sterile and/or disposable consumables should be used. To prevent unnecessary movement between sections, all sections should also have their own storage cabinets, reagents, and fridges. Reagents should be stored in as small aliquots as possible, to minimize damage if a reagent container were to be contaminated. [9.] In the same vein, it is important to document which reagent containers were used for each analysis [5].

To prevent contamination by PCR products, there should be adequate separation between ‘clean’ pre-PCR functions (e.g. sample processing and master mix preparation) and ‘dirty’ post-PCR functions (e.g. PCR itself and any subsequent analysis). Exceptional care should be taken to separate post-PCR functions from the rest, due to the high concentrations of the potentially contaminating DNA. [8.]

Simple way to accomplish this is physical separation, ideally by having a multiple room setup (Figure 1), or by having some other form of physical barrier between functions if a multi-room setup is not possible. Ideally, the laboratory should also be arranged in a way that allows for unidirectional workflow, to lessen the chances of PCR products ending up “upstream”. [8.]

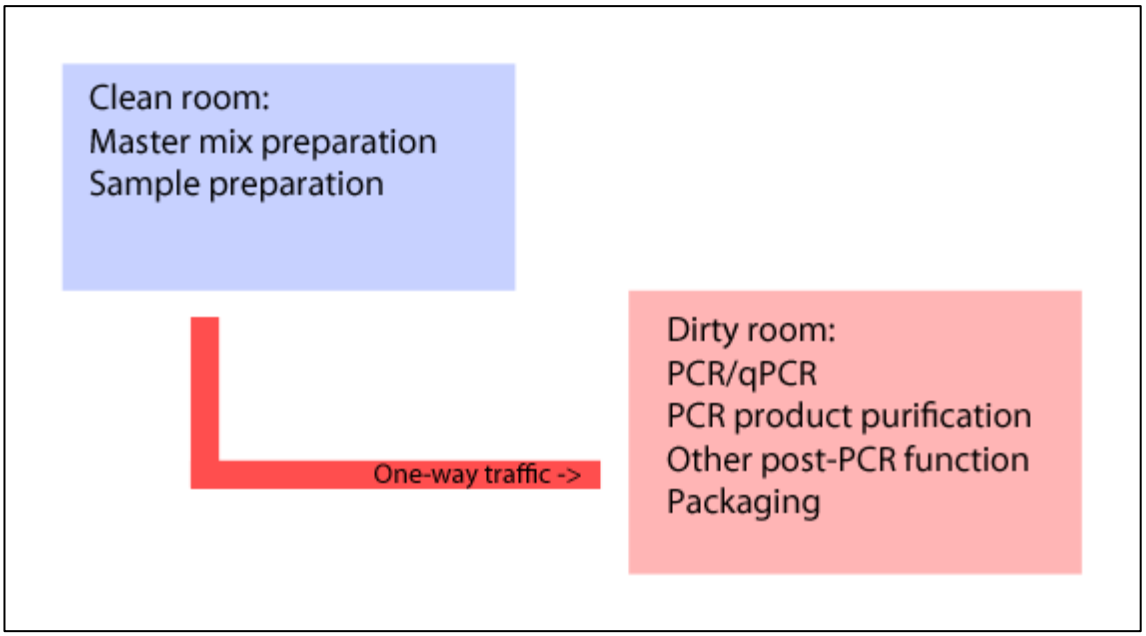

Figure 1. Two-room option for a laboratory layout, modified from one presented by Raquel V. Viana and Carole L. Wallis [8].

In the clean area, the space should be further divided between sample processing and master mix preparation. A boxed PCR workstation or similar solution can be used during master mix preparation to minimize the risk of the currently processed sample contaminating the reagents, and to prevent foreign, non-sample DNA contaminating the master mix. [8.]

The dirty area should ideally be separated into a PCR area and post-PCR area, which includes PCR product purification and any other possible post-amplification functions [8]. If the post-PCR analysis, such as sequencing, is performed elsewhere due to outsourcing or other reasons, the outgoing sample packaging area should also be in the dirty area. Care should be taken to prevent the amplified DNA from contaminating the PCR equipment, for it is in contact with all the samples. In the case that the laboratory only uses qPCR methods, the dirty area can be a lot smaller since certain steps such as PCR product purification can be skipped.

To ensure smooth operation, the laboratory should have documentation on how the laboratory contamination prevention plan is executed in practice and make sure this is understood by the personnel [9].

2.3 Decontamination methods

In addition to separation between functions, cleaning and decontaminating the work area between sample batches is crucial in preventing DNA carry-over contamination from other samples [8]. Therefore, a cleaning protocol and schedule should also be established, documented and enforced [9]. There are two main ways to decontaminate surfaces and equipment: UV-light and chemicals. Heat can also be used to decontaminate equipment, but since the widespread use of disposable consumables renders it often unnecessary, it is not covered here.

2.3.1 UV-light

UV-light degrades DNA-molecules by causing the thymine molecules to react and form a dimer [11]. UV-light can be used efficiently to sterilize a small working space, such as that of a PCR workstation, where master mix preparation or similar sensitive steps are performed to proactively prevent contamination [8]. It can also be used to decontaminate a larger work surface, but doing so requires the entire workstation to be equipped with special illumination. Decontamination also takes a considerable amount of time, since 95 % degradation may require even 1 hour of irradiation, depending on the intensity of the light and the distance of the light source from the surface. UV-light can also be used to decontaminate reagents and consumables; however, this has been found to decrease the performance of Taq–polymerases. Ideally, UV-light should be used together with chemical decontamination methods. [4.]

2.3.2 Chemical

There are several chemical decontamination methods, both generic substances and specific commercial products. Since the active ingredients in commercial solutions are rarely disclosed, only generic substances are discussed here. In general, chemical decontamination methods are slightly more efficient than UV-light for routine decontamination of working surfaces and equipment [4]. They can also be more easily applied to uneven surfaces, such as door handles.

One of the most common chemical decontaminating methods used in laboratories is ethanol, which is most commonly used as a 70% solution to disinfect surfaces by wiping.

Ethanol based commercial disinfectants are myriad, and they are used by even the most high-end laboratories, such as Finland's NBI forensic laboratories [12]. Despite ethanol being very effective at disinfecting surfaces from microbes and other such contaminants, it does not degrade DNA – it merely causes it to precipitate [13]. Hence, while ethanol is an excellent tool to upkeep good general hygiene, it should be used in tandem with other decontamination methods.

Sodium hypochlorite, also known as bleach, is commonly used as 10 % chlorine bleach solution to disinfect surfaces by wiping. It is very effective, achieving up to 95,4 % DNA degradation [4]. On the other hand, the self-life of bleach is only 6 months, and 10 % solutions must be prepared every day for they lose their effectiveness after 24 hours [14]. Bleach solutions can also corrode stainless steel instruments and can produce chlorine gas if in contact with acids.

Hydrogen peroxide is also a very efficient disinfectant, demonstrating 97 % effectiveness when used as a 3 % water solution [15]. Unlike bleach, hydrogen peroxide is non-corrosive and does not release toxic fumes.

Copper complex solutions, such as copper-bis-(phenanthroline)-sulfate/H_20_2 solution, can also be used very effectively to disinfect surfaces by wiping. Copper complex solutions are roughly as effective as bleach, achieving 95,5 % DNA degradation [4]. However, they are not widely commercially available, possibly due to the carcinogenic properties of copper.

3 Sources of errors in a molecular genetics laboratory

In addition to DNA contamination, there are a multitude of other errors that can affect the quality of the laboratory results. Unfortunately, no clear line can be drawn to where laboratory errors begin and pre-laboratory errors end, since the clerical errors made during the sampling or ordering of the test also affect the practical outcome of the analysis. However, distinction can be made between errors made exclusively, physically inside the laboratory, and laboratory errors caused by organizational problems outside the laboratory, for example, sample-patient mismatch during sampling. [16.]

3.1 Different phases of process

The analytical process can be split into three phases: pre-analytic, analytic and post-analytic phase. Most errors occur in the pre-analytic phase and the least in the analytical phase [17]. While the precise numbers vary; in general, the distribution between the phases is very similar in many testing areas, including molecular genetic testing [18].

3.1.1 Pre-analytic phase

The pre-analytical phase describes all activities from test ordering to the time the sample arrives in the laboratory [19], including test selection done by the customer or physician, patient identification and preparation, various specimen collection procedures and the transport, and possibly including the transferring of patient information to the laboratory's computer systems [17, 19].

The pre-analytical phase is very vulnerable, with up to 61,9 % of all errors being made during this phase [17]. The types of errors made in this phase are diverse, but most of them are related to incorrect sampling. Other error sources outside the laboratory are inappropriate test requests and order entry errors, misidentification of the patient before and/or after sample is taken, and improper labeling of sample containers. Errors made within the laboratory include errors related to sample logistics and labeling. [20.]

3.1.2 Analytic phase

The analytic phase refers to the actual analysis performed by the laboratory personnel, including the selection of test methods, performance of test procedures, and monitoring and verification of the test results [18]. During the last 40 years the rate of errors made during this phase has decreased dramatically, with only 15 % of errors made during the phase. This development is most likely due to the widespread automatization of analyzers that reduce the chance of human error [17, 19]. Integrated computer technology has also greatly aided accurate and consistent specimen identification and handling, and test result reporting [19]. However, equipment malfunction, sample mix-ups, interference and undetected failures in quality control persist as sources of error during the analytical phase [20].

3.1.3 Post-analytic phase

The post-analytic phase consists of reporting the test results to either the clinician or the customer who ordered the test, and archiving records, reports and tested specimens. The major sources of error are patient-result mismatch, and the receiver – either a customer or a physician - misinterpreting the results. Misinterpretation may in some cases be caused by insufficiently informative test reports, as physicians of many medical specialties consider reports that include information beyond that specified by the national requirements to be more helpful, than those that contain the bare minimum. [18.]

3.2 Error prevention

In the general sense, the laboratory quality management system should have policies and procedures for detecting, monitoring and reporting errors. Any errors found should be reviewed and the cause investigated. Any actions taken to solve the problem should be documented, and the effectiveness of the actions should be monitored. [21.]

In order to reduce the prevalence of technical errors during the analytical phase, the training and experience of the personnel is important [16]. Therefore, the laboratory should also have policies and procedures to ensure that the staff has the training and qualifications needed to perform their assigned tasks. Since laboratory methods always evolve, sometimes rapidly, there should also be procedures to ensure the staff receives education and training during their employment. Adequate training is also important in the pre-analytical phase if automatization, such as electronic forms or barcode scanners, are used to reduce human error during the phase.

To reduce the errors caused by instrument malfunction, the staff should be adequately trained to operate them, and the laboratory should have policies and procedures to ensure the equipment is properly maintained and calibrated [21].

4 Standards and guidelines considered

In order to verify that their laboratory meets the basic benchmarks of quality discussed in earlier chapters, molecular genetics laboratories can use and seek accreditation to

several quality standards. Operating in accordance to an internationally accepted standard tells potential customers that the laboratory meets the requirements needed for high quality molecular genetics testing.

4.1 ISO standards

ISO/IEC standards are issued by the International Organization of Standardization (ISO), which is formed by an international network of accreditation bodies [22]. In Finland, ISO standards are issued through Suomen Standardisoimisliitto SFS ry (SFS) and laboratories can seek ISO accreditation through Finnish Accreditation Service (FINAS) [23, 24].

4.1.1 ISO 9001

ISO 9001 is a general quality management standard that can be applied to any organization regardless of their size or scope. The standard focuses on quality management system implementation, management responsibilities, resource management and how the principles of continuous improvement are to be implemented. The standard does not contain any technical requirements. [25.]

ISO 9001 encourages businesses to adopt a “process approach” to how the quality management system is implemented, meaning that the whole system is divided into small sub-processes, “process” here being anything that transforms input into output. The standard also requires businesses to continually improve their management systems through user input and internal audits, or at least construct their management in a way that allows this.

While many of the management principles of ISO 9001 can and should be applied to laboratory work, the standard does not include technical requirements and thus it cannot be used to prove the laboratory’s technical competence. In addition, while the standard mentions that the “product” can also mean a service, it still seems to have been written with the manufacturing industry in mind.

The implementation of ISO 9001 also requires a lot of administrative work that small companies may not have the resources for. The standard’s requirements for every procedure, decision and policy to be extensively documented, while extremely good and

useful, require a very robust document handling system that small companies may lack. The standard may also be challenging to implement without any external guidance, due to the very generic nature of the requirements. In summation, while the standard is not unsuitable for laboratories, the effort may be better spent in implementing a standard that is better tailored for the laboratory industry.

4.1.2 ISO 17025

ISO 17025 is a generic laboratory quality standard that specifies the general requirements for the competence to carry out tests and/or calibrations. The standard is applicable to all laboratories regardless of their size or scope of activities, be it commercial, industrial or federal. ISO 17025 also includes the requirements of ISO 9001 that are relevant to the field. [21.]

The standard is comprised of management requirements and technical requirements. The management requirements are very similar to those found in ISO 9001, encompassing organizational structure, management system and document handling. Most importantly, the standard requires the laboratory to have a quality manual and details what it must contain. The ISO 17025, however, does not mention the “process approach” encouraged in ISO 9001 [25], possibly because the scope of this standard is already so much narrower that dividing the laboratory to sub-processes is unnecessary.

In addition to the management requirements, ISO 17025 also contains technical requirements not found in ISO 9001. Due to the need for the standard to be applicable to any laboratory regardless of field, the technical requirements are very general in nature and consist mainly of requirements for personnel qualifications, data control, and test report contents and procedures.

The standard is internationally accepted, and an accreditation to ISO 17025 is a widely accepted quality indicator for laboratories. However, the standard does not contain any specific requirements for molecular genetics testing laboratories, and designing a laboratory without external guidance may be difficult due to the lack of specialized requirements for the field. Similarly to the case with ISO 9001, a small company may not have the resources for the administrative work and documentation that the successful implementation of ISO 17025 requires, as useful as they are. Nevertheless, if these resource

limitations can be overcome ISO 17025 is a solid, if a slightly barebones option, for molecular genetics testing laboratories.

4.1.3 ISO 15189

ISO 15189 is a standard that contains managerial and technical requirements for medical laboratories. The standard defines medical laboratories as laboratories that run examinations of materials derived from the human body for the purposes of health assessment or diagnosis, treatment or prevention of disease. [26.]

The standard is comprised of management requirements and technical requirements and includes relevant requirements from ISO 17025. The management and technical requirements are very similar to those in ISO 17025, with added emphasis on patient care and well-being. In addition, technical requirements have been categorized into pre-examination, examination and post-examination processes, as is common with clinical standards.

Even though a laboratory developing and performing molecular genetics tests could fall under the definition of a medical laboratory, ISO 15189 is intended to be used in a medical laboratory that operates within a hospital or in close co-operation with the health care industry As a direct result of this, the standard assumes that laboratory service requests are made by a clinician, which can be a cause of confusion if the standard is implemented in a direct-to-consumer genetics laboratory. Like ISO 17025, ISO 15189 lacks any requirements specific to molecular testing industry, and the administrative work and documentation required may be beyond a small company's resources. In summation, while the standard is by no means unsuitable, other standards such as ISO 17025 should be considered instead, unless the company is certain that they are a medical laboratory.

4.2 OECD guidelines

Organization for Economic Cooperation and Development (OECD) is an organization that designs and publishes policies and standards for many fields. The policies are made with the aim of delivering positive effects on the economic and social well-being of people. OECD originated as a European organization, but in the present day has 34 member countries worldwide, including Finland. [27.]

4.2.1 OECD Guidelines for Q. Assurance in Molecular Genetic Testing

In 2002, OECD carried out a survey to measure the availability of molecular genetic testing and to document existing quality assurance practices. The results showed that while all participating OECD countries had mechanisms to assure the quality of molecular genetic testing procedures, these regulatory procedures had not been adopted in laboratories consistently. Since one of the identified causes was that the regulations had not been designed for molecular genetic testing specifically, OECD set out to create and publish a guideline titled *OECD Guidelines for Quality Assurance in Molecular Genetic Testing* in 2007. [28.]

The OECD guidelines are designed to provide principles and best practices for quality assurance in molecular genetic testing for clinical purposes. The principles are directed primarily at government and regulatory bodies, whereas the best practices are aimed for laboratories and other establishments involved in the provision of molecular genetic testing. Testing carried out only for research purposes is not addressed. The guidelines are also partially relevant and applicable to aspects of biochemical genetic testing.

The principles and best practices defined in the guideline cover general information, quality assurance systems, proficiency testing, quality of test reporting, and educational standards for laboratory personnel. Since the principles are aimed at government bodies, only best practices are covered here.

The best practices related to quality assurance systems include requirements for the terminology used, accreditation, documentation and periodical review of analytical validity. For proficiency testing, there are requirements for how it should be arranged and structured. The best practices related to quality of test reporting include requirements for the minimum information that should be in a report, and that the reports should take in account that the recipient may not be a specialist health care professional. The educational requirements include that the laboratory personnel should have relevant qualifications and receive continuing education.

The guideline is narrower than the ISO standards are, but they are similar in content. The statements in the guideline are very brief and general in nature, making it very hard

to utilize as the main base for building a laboratory quality system. It is, however, distributed free of charge and thus can be easily used to supplement other standards, such as the field-nonspecific ISO 17025.

4.2.2 GLP guidelines

Good laboratory practice (GLP) is a standard by which laboratory studies are designed, implemented and reported and has been widely adopted due to the GLP principles published by OECD in 1998. OECD principles on GLP are meant to be applied to any non-clinical safety testing of synthetic or organic chemicals in pharmaceutical, pesticide or cosmetic products, food additives and industrial chemicals [29]. GLP is also integrated into the EU-directives. Within EU, unless specifically exempted by national legislation, GLP applies to all health and safety studies required by regulations that are performed for the purpose of registering or licensing products mentioned above. [30.] In Finland, GLP monitoring is conducted by Fimea [31].

The requirements defined in GLP principles include, but are not limited to, requirements for the test facility organization and personnel, the laboratory's quality assurance programme, facilities, materials and equipment, test systems and reference items, standard operating procedures, and how the test data is reported to authorities [29].

While accordance to GLP is required only of GLP-certified research laboratories, most GLP principles can be applied to any industry in which laboratory work is performed. That said, GLP does not contain any specific requirements for molecular laboratories. It is available free of charge, however, and thus can be used as a complimentary piece while building a quality management system.

4.3 MIQE guidelines

Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) were published in 2009 and authored by Stephen A. Bustin *et al*. The guidelines are the third most cited paper published in Clinical Chemistry [32], with over 4000 citations according to Google Scholar [33].

The guideline aims to unify qPCR data reporting to the point that all papers adhering to the guidelines provide the reader enough information to judge whether the data is sound, or to even repeat the test. The guidelines are presented as a checklist for authors to use when submitting the initial manuscript to the publisher. The guideline also defines certain vocabulary, such as whether RT-PCR refers to real-time or reverse-transcript PCR, to make sure that scientific publications can be as unambiguous as possible. [34.]

The checklist items listed in the guide and their contents are given below:

- Experimental design, namely the sizes of experimental and control groups
- Sample description, processing and storage conditions
- Nucleic acid extraction information, such as the method and kit used
- Reverse transcription information, such as complete reaction conditions and volume
- qPCR target information, such as gene accession number and amplicon length
- Information on qPCR oligonucleotides, such as primer sequences
- qPCR protocol information, such as complete reaction conditions and volume
- qPCR validation information, such as specificity, calibration curves and PCR efficiency
- Data analysis information, such as the analysis program used and justification for number and reference genes.

The checklist items are divided to either *essential information* that is to be submitted with the manuscript, or *desirable information* that should be submitted if available.

MIQE Guidelines are very useful, for they contain very specific guidelines for writing scientific papers. Due to its format it does not, however, go into great detail about the specifics of each checklist item, which can make certain items hard to understand. For this reason, it is advisable to couple the guidelines with a guide of some sort. The guideline is free of charge and widely accepted; thus, there is not much reason to not use it.

4.4 CLIA federal regulations

Clinical Laboratory Improvement Amendments (CLIA) are federal standards applicable to all facilities in U.S that test human specimens for health assessment or to diagnose, prevent or treat disease [35].

CLIA regulations include requirements for laboratory's management system and technical requirements for its operations. The requirements vary significantly according to the category the laboratory belongs to; the category, in turn, is assigned according to the category of the tests performed. [36.]

CLIA categorizes tests by simplicity. A test can be considered a waived test if it fulfills the following requirements:

- It employs simple laboratory examinations and procedures that have been cleared by FDA for home use.
- It uses methodologies that are accurate and simple enough to make the likelihood of error minimal, OR…
- It employs methods that pose no reasonable risk of harm to the patient if the test is performed incorrectly.

The categories for tests of moderate complexity and high complexity are determined by a scoring system that measures the following:

- knowledge required to perform tests
- training and experience required to perform tests
- complexity of reagents and materials (i.e. do they need to be prepared)
- characteristics of operational steps
- complexity and importance of correct calibration to the results
- quality control and proficiency testing materials
- complexity of equipment troubleshooting and maintenance
- amount of interpretation and judgement the tests need

Each attribute is given a score from 1 to 3. If the test system's score is above 12, it will be categorized as high complexity - otherwise, the test is categorized as moderate complexity [36]. At a glance, most molecular genetic tests tend to fall under high complexity.

CLIA regulations are extensive and include useful and specific instructions for the facilities of a molecular diagnostics laboratory. However, successful implementation of CLIA regulations requires the laboratory to asses all its test systems, which may be overly arduous for a small-scale laboratory. In addition, CLIA compliance is recognized only in the U.S. Therefore, for a non-U.S based laboratory, other standards and guidelines should be considered.

4.5 CLSI guideline MM20-A

Clinical and Laboratory Standards Institute (CLSI) is a non-profit organization that publishes voluntary consensus standards and guidelines, reports and companion products such as Quick Guides. The laboratory testing standards are developed on the basis of input from and consensus among industry, government, and health care professionals [37]. CLSI participates in the development of international ISO standards as the ISO TC 212, which is responsible for clinical laboratory testing and *in vitro* diagnostics test systems [38].

For establishing a quality management system for a molecular genetics testing laboratory, CLSI offers guideline MM20-A. MM20-A is intended for use by medical laboratories that perform molecular genetic testing for inherited or acquired conditions. Even though many quality system principles described in MM20-A are applicable to most medical laboratories, it is not intended to address non-clinical molecular testing or direct-to-consumer laboratory services. [39.]

MM20-A contains instructions for organizational structure, personnel qualifications and document control. For technical requirements, MM20-A separates functions to pre-analytical, analytical and post-analytical activities similar to ISO 15189. MM20-A also offers a list of related CLSI reference materials and examples of quality manuals and several forms.

MM20-A is an in-depth guideline for molecular laboratories. It is, however, a voluntary guideline and laboratories cannot seek accreditation to it. Due to the close ties CLSI has to governments around the world and the ISO, the guideline is most likely in line with ISO 17025 and ISO 15189 standards, making them viable accreditation goals for a laboratory that follows MM20-A.

5 Additional documents

In addition to the literary review, two documents were prepared as a part of this thesis. The first one is a list detailing possible sources of error in a molecular genetics laboratory and ways to avoid them, based on observations made during a laboratory experiment. The list is to be used as the starting point of the company's quality manual. The second document is a blueprint for the planned laboratory expansion that utilizes knowledge gained from the literary review.

5.1 Laboratory experiment

A small laboratory experiment was conducted as a part of the thesis. The main focus of the experiment from this thesis's perspective was to observe possible sources of error and how the laboratory should be arranged to ensure a smooth workflow. These observations were used together with the standards and other sources to create a document called *Suggestions for laboratory operations* (Appendix 1), which will be used as the starting point for the company's quality manual. The document groups the suggestions by analytical phases, as pictured with example observations in Table 1.

Table 1. Examples of observations recorded in *Suggestions for laboratory operations*.

Phase	**Notes**
General	There should be an adequate supply of laboratory consumables. Wearable protection should be adequately sized.
Pre-PCR phase	All sample tubes should be clearly marked. To prevent contamination from PCR products, there should be no in-flow from post-PCR area.
PCR and post-PCR	The post-PCR area should have its own consumables The post-PCR area should have its own protective equipment The protective equipment should be marked to prevent accidental usage in pre-PCR areas

The experiment itself was part of the development process of a new molecular diagnostics test for autosomal recessive non-syndromic hearing impairment (ARNSHI), and it was performed in the company's pre-expansion laboratory, which is described in chapter 5.2.

Hearing impairment is a highly heterogenous sensory disorder, caused by genetical factors in 60 % of the cases [40]. In many populations, up to 50 % of all cases of ARNSHI are caused by mutations in the GJB2 gene, which encodes connexin-26. Connexin-26, among other connexins, has been shown to participate in the complex potassium ion cycle in the mammalian cochlea [40]. Molecular testing for GJB2 mutations is the standard of care for diagnosis of non-syndromic hearing impairment of unknown cause. However, a deletion in the neighboring connexin-30 gene, GJB6, was found to be present in up to 50 % of patients affected by ARNSHI [40]. Total of 26 mutations have been found to be associated with connexin-26 [41].

The goal of the experiment was to test several primer pairs for use in the development of a new diagnostics test for ARNSHI. The primer pairs were selected based on the findings of Ram Shankar Mani *et al*. [41] and a study by F J del Castillo *et al*. [40]

In the experiment, a total of 5 primer pairs were tested (appendix 2) using a known sequence, out of which Cx26-2F was additionally used to test 6 samples from patients with no ARNSHI to check against the primers giving false positives. Each was made with a duplicate. A standard 32-cycle PCR procedure was performed with the annealing temperature of 56 °C for 15 seconds and the extension temperature of 72 °C for 40 seconds.

The PCR products were purified using an Illustra Exoprostar kit, which utilizes alkaline phosphatase and exonuclease 1. After purification, the duplicates were used to verify the success of the amplification via gel electrophoresis, which was performed using Invitrogen E-gel precast agarose gels and E-gel loading buffer. The remaining samples were sent to a sequencing laboratory for further analysis and prepared according to the other laboratory's instructions.

5.2 Laboratory Blueprint

Prior to the completion of this thesis, the company operated in a very small scale laboratory. Both the pre-PCR and post-PCR reactions were performed in a single room, on the same workbench, which also housed office supplies. However, the planned expansion was intended to remedy this and improve the quality of the company's laboratory work.

As established in chapter 2.2, the positioning and separation of work areas is crucial in preventing cross-contamination between pre- and post PCR areas in a molecular genetics laboratory. Therefore, to establish good laboratory quality, the laboratory layout has to be carefully considered during construction. For this reason, a blueprint for the laboratory expansion was prepared in addition to the document mentioned in chapter 5.1.

Ideally, a molecular genetics laboratory would be split into two or three separate rooms – a pre-PCR area, a separate PCR area, and a post-PCR area. However, since the blueprint is intended to be used as the basis for the company's new laboratory, the real life limitations of the space have been taken into account. Due to size and budget constraints, the laboratory is planned to be in one room, with different functions being separated by wooden panels (Figure 2). As discussed earlier in chapter 2.1, air is an unlikely source of contamination; therefore, air pressure and air flow have not been taken into account.

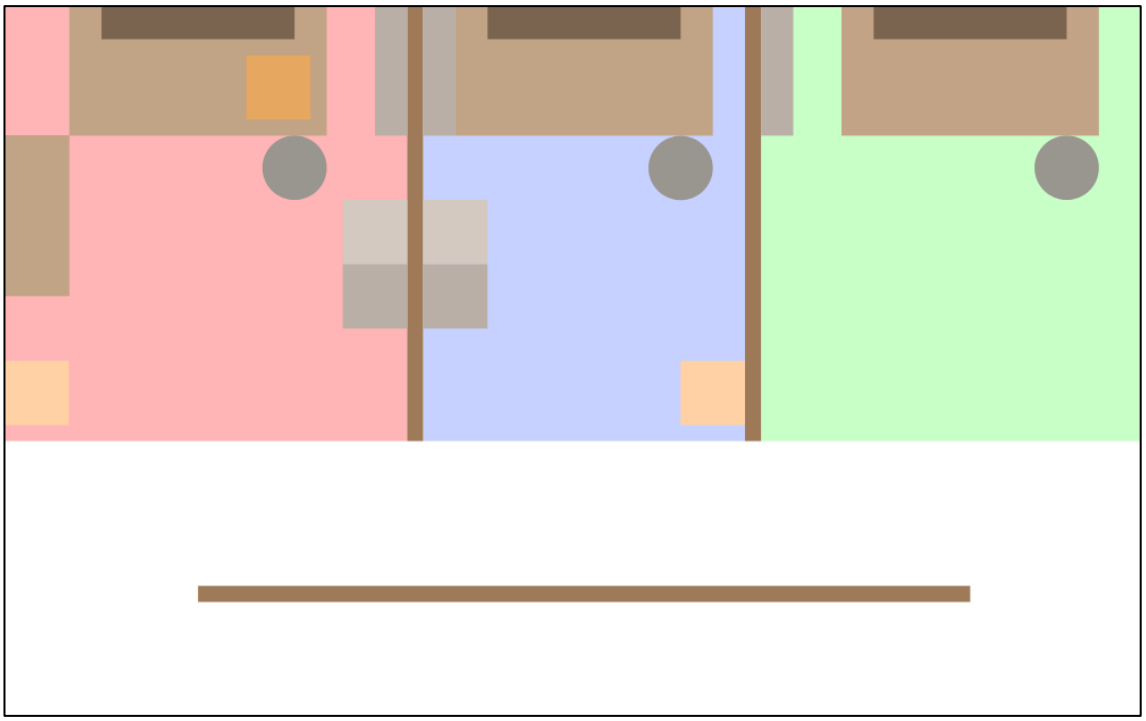

Figure 2. The hypothetical laboratory blueprint, with the different areas color-coded as follows: mailroom = green, pre-PCR = blue, post-PCR = red. The horizontal panel separates the laboratory area from the office space.

The company receives their samples through mail and thus needs a dedicated mail processing area. To avoid patient-sample mismatch, patient information is entered into the electronic system immediately and barcode stickers are used to identify sample tubes throughout the process. Since the mail processing area has the most potential to become cluttered due to envelopes and other packaging materials, and the incoming mail potentially bringing in foreign contaminants, the area is separated from the more sensitive pre-PCR functions.

The mail processing area (Figure 3) has a workbench, surrounded by a cleaning supplies cabinet and waste bin. Laboratory consumables, such as gloves, are stored on an overhead shelf for easy access. The sample envelopes are opened, the patient information is inputted into the electronic system, samples stickered with bar-code stickers and moved forward. The work area is thoroughly cleaned after a sample batch. Since no DNA extraction is performed during this step, protective clothing other than gloves is optional.

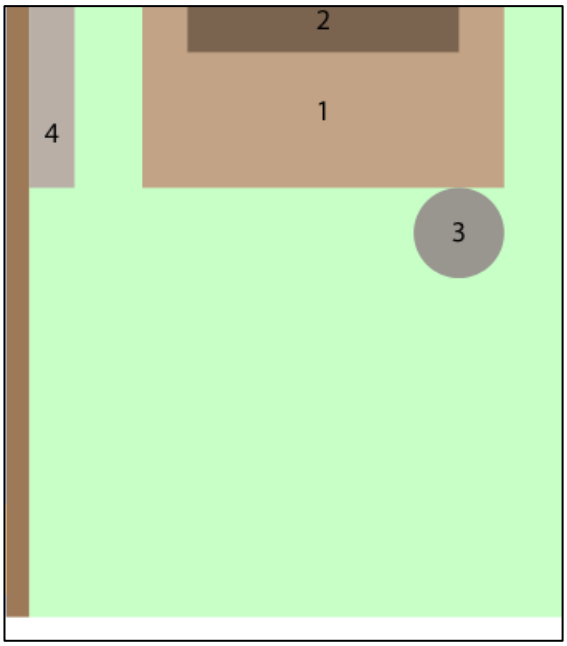

Figure 3. The mail processing area: (1) workbench, (2) overhead shelf for consumables, (3) waste bin, (4) cleaning supplies cabinet.

The sample preparation, DNA extraction and master mix preparation are all combined into one pre-PCR area. To prevent contamination from post-PCR areas, the area has its own dedicated reagents, storage, cold storage, tube racks, gloves and other laboratory consumables. Protective clothing such as coats and face masks are also readily available and should be used to prevent contamination.

Like the other areas, the pre-PCR area (figure 4) has a workbench, surrounded by a cleaning supplies cabinet, an overhead shelf for consumables, and a waste bin. In addition to the items found in the previous area, the pre-PCR area also has a reagent storage cabinet and a fridge/freezer for sample storage and temperature-sensitive reagent storage. A coat rack is located near the entrance, as far away from the workbench as possible, so that protective equipment can be taken off and put on without the danger of kicking up contaminating dust and/or knocking anything down. The work area is thoroughly cleaned between sample batches.

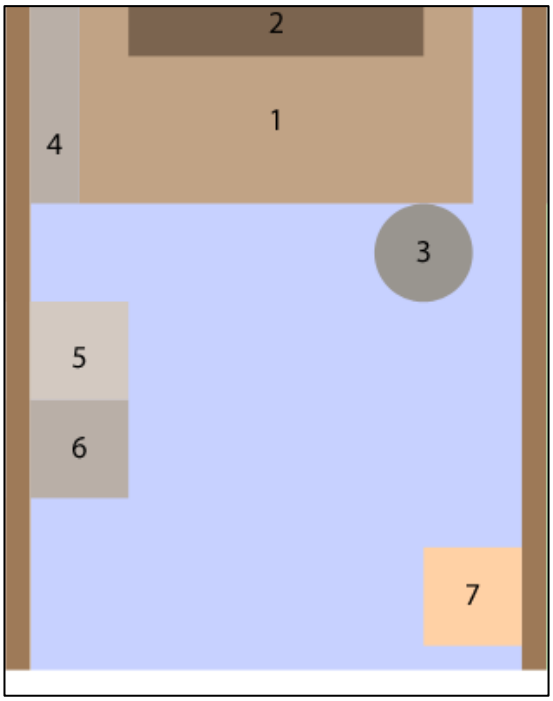

Figure 4. The pre-PCR area: (1) workbench, (2) overhead shelf for consumables, (3) waste bin, (4) cleaning supplies cabinet, (5) storage, (6) cold storage, (7) coat rack.

Ideally, the PCR functions should have their own room, but due to limited space, both the PCR and post-PCR functions are placed in the third area. To prevent the PCR equipment from becoming contaminated, the machine has a dedicated, marked space where post-PCR functions are forbidden. Since the company sends certain samples by mail to be further analyzed, the outgoing mail packaging area is also located in the third area. To prevent potentially contaminated equipment or supplies from moving to more sensitive areas, the area has its own reagents, storage and laboratory consumables. The area also has its own protective equipment, clearly marked to prevent mix-up or other accidental usage in other areas.

The post-PCR area (Figure 5) has a workbench surrounded by an overhead shelf for consumables, a cleaning supply cabinet and a waste bin. The post-PCR area also has a reagent storage and a fridge/freezer. A coat rack is located near the entrance, but on the opposite side of the pre-PCR area, to minimize the chances of contamination through air or any mix-up between pre- and post-PCR coats. The work area is thoroughly cleaned between sample batches, and the PCR products are either analyzed and disposed, or packaged and sent forward.

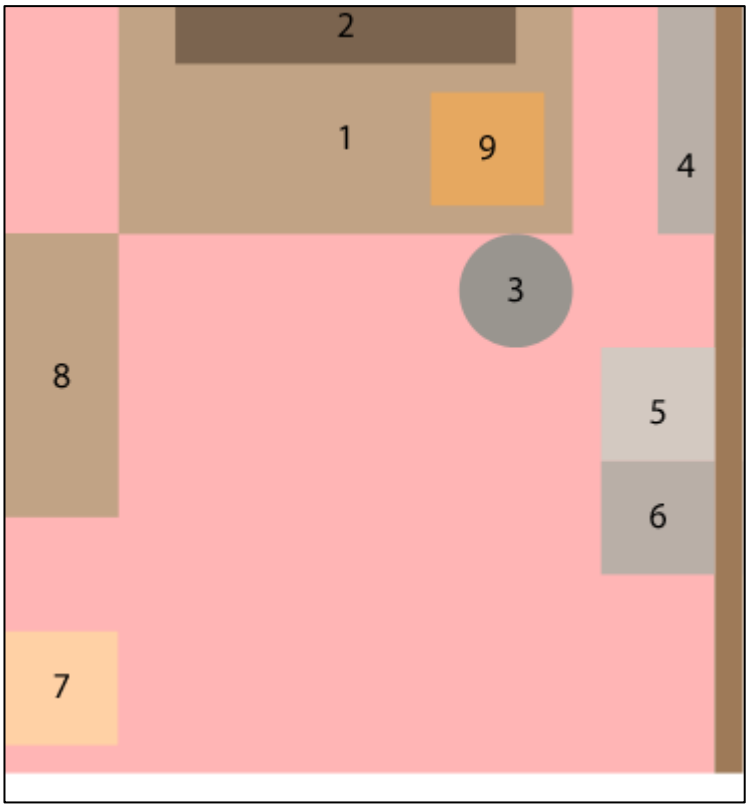

Figure 5. The PCR and post-PCR area: (1) workbench, (2) overhead shelf for consumables, (3) waste bin, (4) cleaning supplies cabinet, (5) storage, (6) fridge, (7) coat rack, (8) outgoing mail packaging area, (9) marked PCR area.

The workflow and general layout are designed to be unidirectional, to reduce the risk of contamination from post-PCR products (Figure 6). In addition to PCR cleanliness, care was taken to reduce human error by making the laboratory layout as ergonomic and easy to use as possible by making sure waste bins, reagent storage and consumable storage are all close to the working areas.

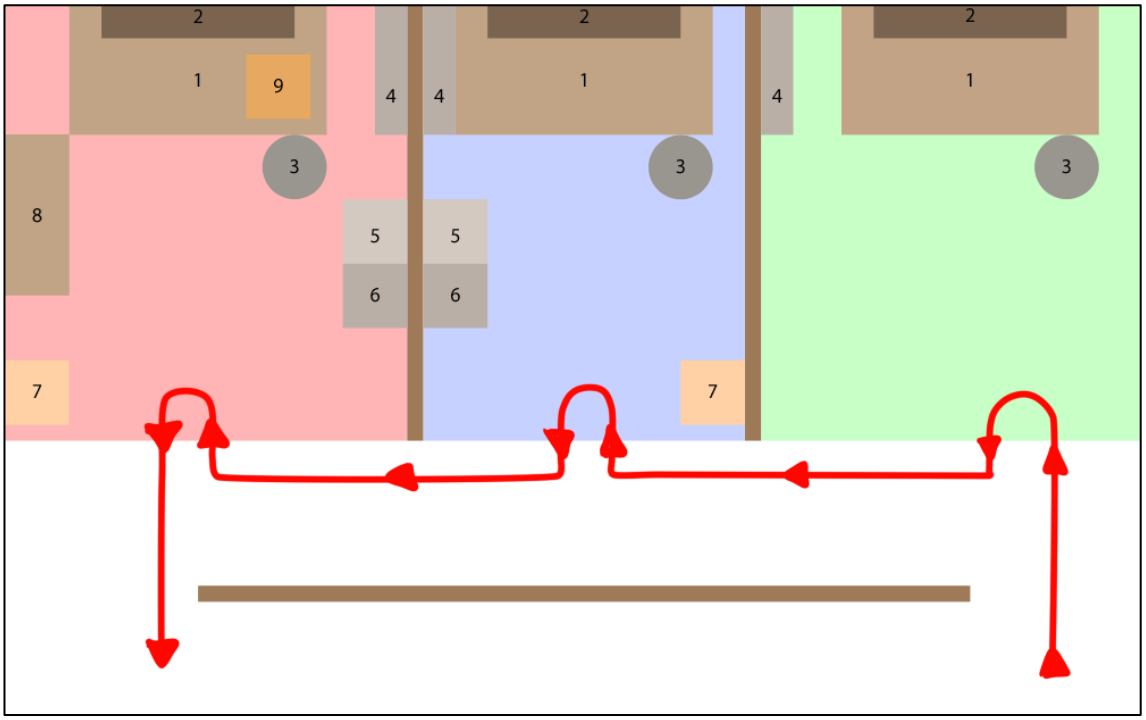

Figure 6. The laboratory layout, with the workflow illustrated. The sample enters from the right, travels through the areas, and exits from the left, usually either in a waste bin or in a mail package. Personnel are only allowed to walk from right to left.

6 Conclusions

The purpose of the thesis was to investigate the factors affecting the quality of molecular laboratories. The factors affecting laboratory quality are diverse, but the most important factor in molecular testing is its vulnerability to contamination. Therefore, contamination prevention should be taken into account during every step of setting up the laboratory, starting from the facility design and continuing to the design of analysis protocols and day-to-day cleaning protocols.

The second factor affecting quality is the possible clerical and analytical errors performed before, during, and after the analysis. Since many molecular tests are used to detect mutations for research or diagnostics purposes, errors can have surprisingly far-fetching consequences. Most of the errors are performed in the pre- and post-analytical steps, and for this reason these steps should be the main focus when designing error-preventing protocols, as opposed to the analytical step.

The other purpose of this thesis was to collect and review industry standards and guidelines that could be used for a molecular genetics laboratory. The standards chosen were three ISO standards, ISO 9001, ISO 17025 and ISO 15189, GLP and Quality Assurance Guidelines for Molecular Genetic Testing by OECD, MIQE guidelines, CLIA federal regulations and the CLSI guideline MMA-20.

Out of the three ISO standards, the general laboratory quality standard ISO 17025 was found to be the most applicable. ISO 9001 is a general quality standard and, while not exactly unsuitable, does not provide tools to improve a laboratory's technical competence. ISO 15189 on the other hand is designed for medical laboratories and thus includes many clauses that are not applicable for this particular business setup.

GLP guidelines by OECD were also considered, but were dismissed since they are intended to be used in research laboratories. *Quality Assurance Guidelines for Molecular Genetic Testing* is also published by OECD, and while intended for the exact purpose the company seeks a standard for, is too brief to be used as the basis of a quality management system on its own.

MIQE guidelines are different from the other standards, since they are intended solely for improving the quality of published qPCR data. They do succeed in this very well however, and are widely used.

CLIA federal regulations differ from international standards due to their complexity based ranking system and ranking-based requirements. Unlike the very general ISO standards, the regulations offer more specific and in-depth requirements for laboratories. There are no glaring faults in the regulations; however, the greatest issue in designing a quality management system based on them is the fact that they are only recognized in the U.S. The CLSI guideline MMA-20, on the other hand, also contains specific instructions for molecular laboratories, and unlike the OECD guidelines on the same subject, is very extensive.

On the basis of the findings during this thesis, the company concluded that it would begin by implementing OECD and CLSI standards, with accreditation to ISO 17025 planned at a later date. Due to the CLSI accounting for other industry standards and the co-operation with ISO standards, there should not be much modifying needed to accommodate ISO 17025 later. At the time of the writing, the new laboratory expansion has also been built roughly in accordance with the blueprint prepared during this thesis project.

References

1 Klieche, F. 2016. What is Molecular Diagnostics? GoMolecular. Web Document. <https://www.gomolecular.com/discover/what_is_molecular_diagnostics.html> Read 25.5.2016.

2 Molecular Diagnostics Market Analysis By Product (Instruments, Reagents), By Test Location (Point of Care, Self Test, Central Laboratories), By Application (Oncology, Breast Cancer, Prostate Cancer , Colorectal Cancer Pharmacogenomics, Infectious Disease, MRSA, Clostridium Difficile, Vancomycin-resistant Enterococci, Carbapenem-resistant Bacteria Testing, Flu, Respiratory Syncytial Virus (RSV), Candida, Tuberculosis and drug-resistant TB), By Technology And Segment Forecasts To 2022. 2015. Grand View Research. Web document. <http://www.grandviewresearch.com/industry-analysis/molecular-diagnostics-market> Read 25.5.2016.

3 Direct-to-Consumer Genetic Testing Market By Channel (Direct sales, Retail sales, Doctors office), Business Model (Genome data bank material model, Individual health planning model, Comprehensive genome tests model, Medical precision tests model, Restricted trait tests model) - Growth, Share, Opportunities & Competitive Analysis, 2016 – 2022. 2016. Credence Research. Web document. <http://www.credenceresearch.com/report/direct-to-consumer-genetic-testing-market> Read 4.7.2016.

4 Champlot, S. et al. 2010. An Efficient Multistrategy DNA Decontamination Procedure of PCR Reagents for Hypersensitive PCR Applications. PLoS ONE 5(9): e13042.

5 Sundquist T, Bessetti J. 2005. Identifying and Preventing DNA Contamination in a DNA-Typing Laboratory. PDF. < https://www.promega.com/~/media/Files/Resources/Profiles %20In %20DNA/802/Identifying %20and %20Preventing %20DNA %20Contamination %20in %20a %20DNA %20Typing %20Laboratory.ashx > Read 31.5.2016

6 Vandewoestyne M, et al. 2011. Sources of DNA Contamination and Decontamination Procedures in the Forensic Laboratory. Journal of Forensic Research S2:001.

7 Poy, A. 2006.Trace DNA Presence, Origin, and Transfer within a Forensic Biology Laboratory and Its Potential Effect on Casework. Journal of Forensic Identification 4/2006 558-576.

8 Viana R, Wallis C. 2011. Good Clinical Laboratory Practice (GCLP) for Molecular Based Tests Used in Diagnostic Laboratories. PDF. <http://www.intechopen.com/books/wide-spectra-of-quality-control/good-clinical-laboratory-practice-gclp-for-molecular-based-tests-used-in-diagnostic-laboratories> Read 31.5.2016

9 Contamination prevention guidelines. 2010. European Network of Forensic Science Institutes. PDF. <http://www.enfsi.eu/sites/default/files/documents/dna_contamination_prevention_guidelines_for_the_file_contamantion_prevention_final_-_v2010_0.pdf> Read 31.5.2016

10 Witt, N et al. 2009. An assessment of air as a source of DNA contamination encountered when performing PCR. Journal of Biomolecular Techniques 20/2009 236-240

11 How does ultraviolet light kill cells? 1998. Scientific American. Web document.<http://www.scientificamerican.com/article/how-does-ultraviolet-ligh> Read 13.5.2016.

12 Pulkkinen, E. 2016. Technician, DNA investigations, NBI Forensic laboratory, Vantaa. Email conversation. 18.8.2016.

13 Zumbo, P. Ethanol Precipitation. Weil Cornell Medical College. PDF. <http://physiology.med.cornell.edu/faculty/mason/lab/zumbo/files/ETHANOL_PRECIPITATION.pdf> Read 18.8.2016

14 Comparison Study of Disinfectants for Decontamination. 2011. National Forensic Science Technology Center. PDF. <https://www.nfstc.org/wp-content/files//Decontamination_Study_-_Revised_247.pdf> Read 31.5.2016

15 Sallom A, Collins M. 2007. Use of dilute hydrogen peroxide to remove DNA contamination. Patent US20070289605 A1.

16 Bonini P. et al. 2002. Errors in Laboratory Medicine. Clinical Chemistry 48/2002 691-698

17 Carraro P, Plebani M. 2007. Errors in a Stat laboratory: Types and Frequencies 10 Years Later. Clinical Chemistry 53/2007 1338-1342

18 Chen B et al. 2009. Good Laboratory Practices for Molecular Genetic Testing for Heritable Diseases and Conditions. MMWR Recommendations and Reports 58/2009 1-29.

19 How reliable is laboratory testing? 2015. Lab Tests Online. Web document <https://labtestsonline.org/understanding/features/reliability/start/2> Read on 15.4.2016.

20 Abdollahi A, Saffar H, Saffar H. 2014. Types and Frequency of Errors during Different phases of Testing at a Clinical Medical Laboratory of a Teaching Hospital in Tehran, Iran. North American Journal of Medical Sciences 6/2014 224-228.

21 ISO 17025:2005 - General requirements for the competence of testing and calibration laboratories. 2005. 2nd edition. International Organization for Standardization.

22 Structure and Governance. International Organization for Standardization. <http://www.iso.org/iso/home/about/about_governance.htm> Read 14.4.2016.

23 Standardien laadinta. Suomen Standardoimisliitto SFS Oy. <http://www.sfs.fi/standardien_laadinta> Read 10.4.2016.

24 Akkreditointialueet. 2016. Finnish Accreditation Service. Web Document. <https://www.finas.fi/akkreditointi/Akkreditointialueet/Sivut/default.aspx> Read 28.4.2016.

25 ISO 9001:2008 – Quality Management Systems – Requirements. 2008. 4th edition. International Organization for Standardization.

26 ISO 15189:2012: - Medical laboratories — Requirements for quality and competence. 2012. 3rd edition. International Organization for Standardization.

27 About the OECD. OECD. Web document. <http://www.oecd.org/about/> Read 13.5.2016.

28 OECD guidelines for quality assurance in molecular genetic testing. 2007. OECD. PDF. < http://www.oecd.org/science/biotech/38839788.pdf> Read 31.5.2016

29 OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring. 1998. OECD.

30 Directive 2004/9/EC of the European Parliament and of the Council of 11 February 2004 on the inspection and verification of good laboratory practice (GLP). 2004. EUR-Lex. < http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32004L0009> Read 31.5.2016

31 GLP-valvonta. Fimea. Web Document. <http://www.fimea.fi/valvonta/glp-valvonta> Read 14.5.2016

32 Most cited articles. 2016. Clinical Chemistry. Web document. <http://www.clinchem.org/reports/most-cited> Read 14.5.2016.

33 Google Scholar search: Papers referencing MIQE Guidelines: minimum information for publication of quantitative real-time PCR experiments. <https://scholar.google.fi/scholar?cites=6338124712618390161&as_sdt=2005&sciodt=0,5&hl=fi> Read 31.5.2016

34 Bustin, S et al. 2009. The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments. Clinical Chemistry 55/2009 611-622

35 Clinical Laboratory Improvement Amendments. 2015. Centers for Disease Control and Prevention. Web document. <http://wwwn.cdc.gov/clia/Default.aspx> Read 30.3.2016

36 Electronic Code of Federal Regulations: Public Health - Chapter IV – Subchapter G – Part 493. 2016. U.S. Government Publishing Office. < http://www.ecfr.gov/cgi-bin/text-idx?SID=1248e3189da5e5f936e55315402bc38b&node=pt42.5.493> Read 30.3.2016

37 Committed to Continually Advancing Laboratory Practices. Clinical and Laboratory Standards Institute. Web document. <http://clsi.org/about-clsi/> Read 14.5.2016

38 ISO/Technical Committee 212. Clinical and Laboratory Standards Institute. <http://clsi.org/standards/iso-standards/technical-committee-212/> Read 14.5.2016

39 Quality Management for Molecular Genetic Testing; Approved guideline. 2012. Clinical and Laboratory Standards Institute.

40 del Castillo FJ et al. 2005. A novel deletion involving the connexin-30 gene, del(GJB6-d13s1854), found in trans with mutations in the GJB2 gene (connexin-26) in subjects with DFNB1 non-syndromic hearing impairment. J Med Genet 42/2005 588-594.

41 Mani RS et al. 2009. Functional Consequences of novel connexin 26 mutations associated with hereditary hearing loss. European Journal of Human Genetics 17/2009 502-509.

Appendix 1: Suggestions for laboratory operation

A list of things to consider in a molecular diagnostics laboratory

General instructions:

- There should be an adequate supply of laboratory consumables
    - E.g. disposable gloves, pipette tips and plastic tubes
- Wearable protection should be adequately sized
- All relevant data is to be written into the laboratory notebook immediately
    - Notes should include: date, author, sample/patient id, mention of procedure used, possible calculations, the kit and/or own reagents used
- The laboratory notebook should always be available at the workbench
    - Possible electronic system should be such that it allows making notes easily while working
- If laboratory-made reagents are used, their recipe should be documented and easily available at the workbench
- Reagent solutions should be well-labeled
    - Labels should include: date, author, dilution ratio, reagent stock lot number, kit lot number if the reagent is from a kit, and buffer used if relevant
- External disturbance should be minimized
    - The workroom should not be in a particularly noisy location
    - The workroom should be free of unnecessary traffic
- Storage solutions should be arranged in an easily accessible way
- Waste disposal bins should be arranged in an easily accessible way
    - Every function should have its own waste bin
    - If the waste bin cannot be reasonably placed near the workbench, there should be a small bin on the workbench for small consumables
- Protocols, procedures, and other such information should be documented and available at the laboratory

- Laboratory housekeeping responsibilities and duties should be documented and available at the laboratory

Sample reception and preparation

- Sample tubes should be clearly marked
    - The markings should include at least the sample name or patient id
- The workbench should have enough space for both laboratory work and note making
    - The workbench should be clear of unnecessary items
    - The laboratory notebook/laptop should have its own reserved space
- The workbench should have a workflow, e.g. from least processed to most processed
- To prevent contamination from PCR products, there should be no in-flow from post-PCR area
    - Sample preparation area has its own consumables
    - Sample preparation area should have its own reagent storage
- Adequate measures should be taken to prevent contamination from the employee(s), e.g. coats and gloves should be worn
    - Since the company processes samples taken from mouth epithelial cells, face masks should be considered

Pre-PCR

- To make sure the correct analysis is done, samples should have relevant information added to them upon registration to the system.
- To make sure the correct reagents needed are chosen, test protocols should be documented and available at the workbench
- The test tubes should be clearly marked, either with a sticker or otherwise
    - If the tube is too small for a full description (e.g. PCR-tubes) and a code is used (e.g. 1,2,3…) the code is documented in the laboratory notebook.

- The workbench should have enough space for both laboratory work and note making
    - The workbench should be clear of unnecessary items
    - The laboratory notebook/laptop should have its own reserved space
- The workbench should have a workflow, e.g. from least processed to most processed
- To prevent contamination from PCR products, there shall be no in-flow from post-PCR area
    - The pre-PCR area has its own consumables, and in the event of them running out, they should not be restocked from the post-PCR area
    - The pre-PCR area has its own lab coats
    - The border between pre- and post-PCR areas should be clearly marked, so the personnel are in the clear where they should change their lab coats etc.
- Adequate measures should be taken to prevent contaminating the reagents with unprocessed DNA-products
    - Reagent supplies should be verified before the analysis begins, to prevent reagents from running out in the middle of the analysis
    - Reagents should be stored as small aliquots
    - Interrupting the current analysis to prepare more reagents increases the risk of human error considerably, and should be avoided
- Pre-PCR area has its own reagent storage and fridge
    - The fridge is to be kept free of any unnecessary items (e.g. food)
- The workbench is cleaned with an adequate cleaning solution (e.g. H_2O_2) between sample batches
- The workbench and surrounding area should be scheduled to be thoroughly decontaminated periodically

PCR and Post-PCR

- PCR procedures should be documented and available at the workbench
- The test tubes should be clearly marked, either with a sticker or otherwise
    - If the tube is too small for a full description (e.g. PCR-tubes) and a code is used (e.g. 1,2,3…) the code is documented in the laboratory notebook.
- The workbench should have enough space for both laboratory work and note making
    - The workbench should be clear of unnecessary items
    - The laboratory notebook/laptop should have its own reserved space
- The workbench should have a workflow, e.g. from least processed to most processed
- The post-PCR area should have its own consumables
- The post-PCR area should have its own protective equipment
    - The protective equipment should be marked to prevent accidental usage in pre-PCR areas
- To prevent the contamination of PCR equipment, it should be clearly separated from the post-PCR work area
- If gel electrophoresis is performed, the well-sample pairs should be written down into the laboratory notebook
    - if traditional (i.e. non-encased) gels are used, the wells should be filled asymmetrically to prevent confusion if the gel is flipped
- If any procedure that requires temperature sensitive reagents is performed, a cold block or similar solution should be prepared before the work begins
- There should be no flow of samples from the post-PCR area
    - Samples are disposed, if they’re not sent out for further analysis
    - There should be a packaging area for outgoing samples
- Outgoing samples are carefully documented to the laboratory notebook
    - The notes should include at least: Packaging date, destination/procedure, sample-order id pair

- If the samples need to be prepared and/or coded in a specific way before being sent out, the procedures/coding should be documented

- The workbench is thoroughly cleaned after each sample batch

- The workbench and surrounding area should be scheduled to be thoroughly decontaminated periodically

Post-analytic phase and test results

- The test results are documented to the laboratory notebook

    - The notes should include: numerical values, procedure, date, author

- Test reports and notes should include patient/sample id

    - Samples that have been sent out for analysis should be marked in a way that prevents mix-up when the results come back

- In case the method chosen produces results that need interpretation, the laboratory should have documented procedures and policies how the results are interpreted

- The results should be reported in a way that accounts for the customer's education, e.g. a layman versus a physician

- If the results are reported electronically, the transmission methods should be secure

- If the results are retained, the retention time should be documented and pre-established

    - Back-up procedures and schedules should be established and documented

Appendix 2: Primer pairs

The primer pairs tested during the laboratory experiment were:

Primer pairs	Sequences
GJB6-1R/BKR-1	(5'-TTT AGG GCA TGA TTG GGG TGA TTT-3')/(5'-CAC CAT GCG TAG CCT TAA CCA TTT T-3')
DelBK1/DelBK2	(5'-TCA TAG TGA AGA ACT CGA TGC TFT TT-3')/(5'-CAG CGG CTA CCC TAG TTG TGG T-3')
Cx30Ex1A/Cx30Ex1B	(5'-CGT CTT TGG GGG TGT TGC TT-3')/(5'-CAT GAA GAG GGC GTA CAA GTT AGA A-3')
Cx26-1F/Cx26-1R	(5'-TCT TTT CCA GAG CAA ACC GC-3')/(5'-GAC ACG AAG ATC AGC TGC AG-3')
Cx26-2F/Cx26-2R	(5'-CCA GGC TGC AAG AAC GTC TG-3')/(5'-GGG CAA TFC GTT AAA CTG GC-3'