

Meeting report series

Report of the 3rd DSC Working Group on Sequencing teleconference

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Organization

Organized by: IRDiRC Scientific Secretariat
Teleconference

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REPORT

Two issues in sequencing needed to be discussed in two separate teleconferences. In this teleconference, the EuroGentest guidelines will be discussed. In the next teleconference that will take place on October 13, 2014, other topics will be discussed such as certifications, the issue in using sequencing in diagnosing rare disorders or in diagnostics in general.

Review of the EuroGentest guidelines for diagnostic next-generation sequencing

The members of the WG reviewed a PowerPoint document summarizing the main points of the guidelines.

Rationale for the guidelines

- ▶ Novel diagnostic tests should not be introduced into diagnostics without a proper validation. The ISO 15189 norm which specifies the quality management system requirements particular to medical laboratories states that tests should be validated, but does not precise how validation should be realized.
- ▶ A EuroGentest guideline was issued in 2010 outlining the principles of validation and verification in the context of clinical human molecular genetic testing¹. Some national accreditation bodies have officially adopted this document which gives legal validity to it. However, it does not answer to the question “How many times is it necessary to repeat a test to validate it?”
- ▶ Three workshops were organized in order to elaborate the EuroGentest guidelines. The first one set the scene, the second one allowed to draft the guidelines and the third one allowed to comment the guidelines.
- ▶ EuroGentest is building its guidelines on existing guidelines. Any disagreement or point missing in the existing guidelines is highlighted.
- ▶ The paper layout is divided in 6 chapters. Feature of the guidelines is the 3-5 statements composing each chapter.

Chapter 1- Introduction

- ▶ The introduction, which explains the rationale for the guidelines, has 2 statements. The Statement 1.2 highlights the importance for the laboratory to clearly states whether the test offered may be used to exclude a diagnosis or to confirm a diagnosis.
- ▶ The guidelines are limited to genes panel and does not involve whole genome. This document is also not adapted to somatic testing and quantitative testing is missing. The guidelines could be expanded later. The ambition is for the guidelines to be adopted by the national accreditation bodies.

¹ A standardized framework for the validation and verification of clinical molecular genetic tests, Mattocks CJ et al, European Journal of Human Genetics, 2010 ; 18(12):1276-88

Chapter 2 Diagnostic/Clinical utility

- ▶ The first 2 statements of this chapter focus on the diagnostic routine and the diagnostic utility, based on the definition of the Dutch guidelines.
- ▶ Statement 2.3 defines what core disease gene lists are, i.e. genes that should be tested minimally, optional genes and genes that should be left out as controversial genes. An US WG is working on core disease gene lists for each group of diseases. There is an economical aspect in panel genes as some countries may decide to only reimburse minimal core gene panel.
- ▶ EuroGentest tried to define a scoring system for gene panels and exomes on the basis of coverage and diagnostic yield, which would allow comparison of the diagnostic testing offer between laboratories. An economical aspect is involved. Three levels were defined:
 - Type A test: 99% reliable reference or variant calls of the coding region and flanking intronic sequences; fills all the gaps with Sanger sequencing. This represents perfectly covered test.
 - Type B test: the laboratory describes exactly which regions are sequenced at > 99 % and some of the gaps with Sanger sequencing.
 - Type C test: the test solely relies on the quality of NGS sequencing, while no additional Sanger sequencing is offered.

Chapter 3- Informed consent

- ▶ This section is written from the standpoint of the laboratories. It is important for the customers to be informed of the laboratory policy on informed consent, the risk of secondary findings, and opt-in, opt-out procedures. The scope is not the ethic of consents.

Chapter 4- Validation

- ▶ This chapter is more technical with explanation on how to validate the bioinformatics pipeline, the platforms, the different methods and the procedure. *“The quality of a sample can/should be evaluated at 3 levels: technical target, clinical target, list of transcripts”*. Laboratories should communicate to doctors or in reports what are their clinical targets and what percentage of the target is covered. This can be translated to exomes with lists of transcripts as the definition of clinical targets and the sequencing technologies may differ from one center to another.
- ▶ The laboratory has to ensure that all versions are saved in case of pipeline version changes.
- ▶ How much coverage is needed for NGS? Examples of coverage are presented for whole exome sequencing for recessive and dominant disorders.
- ▶ To avoid people rushing to NGS, it is necessary to validate accuracy and precision of the platform, as well as the bioinformatic pipeline.
- ▶ Statement 4.7 states that the diagnostic laboratory should keep track of all the variants it has encountered to build a local database and then eventually an international database where disease variants frequencies as well as unclassified, polymorphic and silent variants would be available. Statement 4.8: *“The diagnostic laboratory has to take steps for long-term storage of all relevant datasets”*. There is no international consensus on long-term storage.
- ▶ Statements 4.9 and 4.10 focus on the definition and requirement for the ‘reportable range’, i.e. the portion of the ‘region of interest’.

- ▶ Statement 4.11 *“Whenever major changes are made to the test, quality parameters have to be checked, and samples will have to be re-run. The laboratory should define beforehand what kind of samples and what number of cases will be assayed whenever the method is updated or upgraded”*.

Chapter 5- Reporting

- ▶ In this chapter, minimal content report for laboratories is defined, as well as what laboratories should do with variants classification, incidental findings, and if they should re-contact patients or not.
- ▶ Five classes of variants are defined for laboratories (benign, likely benign, VOUS, likely pathogenic, pathogenic) in order to fill the international databases with the most useful information.
- ▶ Statement 5.1 states that the essential results should be summarized in one page. Classification of genomic variants should be explained to doctors.
- ▶ Statement 5.3 *“For diagnostic purpose, only variants in genes with a known (i.e. published and confirmed) relationship between the aberrant genotype and the pathology, should be reported”*.
- ▶ Statement 5.5 *“The laboratory is not responsible to go back systematically, if the core disease genes panel changes and novel information about the disease may be hidden in the (raw) dataset”*. On the other hand, the laboratory is responsible to systematically go back if a variant changes of class (i.e. pathogenic to neutral).

Chapter 6- Distinction between research and diagnostics

- ▶ Statement 6.5 *“Research results have to be confirmed in an accredited laboratory before being transferred to the referring clinician and patient”*. Nothing should go straight from a research laboratory to medical files without a confirmation.
- ▶ The frequency of all variants detected in healthy individuals should be shared and all reported variants should be submitted to national and/or international databases.

After revision, the EuroGentest guidelines for diagnostic NGS will be posted on the EuroGentest website as a draft for people to comment. Then, it will eventually be published as a formal recommendation by EuroGentest/European Society for Human Genetics in the *European Journal of Human Genetics*.

‘IRDiRC Recommended’

The ‘IRDiRC Recommended’ label aims to highlight tools and standards generated through IRDiRC activities but also tools and standards not generated by but identified by IRDiRC as key resources. Any recommended tools/standards/guidelines will be highlighted on the IRDiRC website. A logo has been designed and a regulatory disclaimer will be posted on the website.

The EuroGentest guidelines on diagnostic NGS should be recommended by IRDiRC. The guidelines should first be circulated to the members of the WG that could not attend the call to ask them if they have any concern of this being recommended by IRDiRC.

Then, the process for adoption would be the following:

- ▶ Diagnostics Scientific Committee to produce 1-2 pages of rationale, including list of other most important tools or standards, and contentious issues.
- ▶ Consultation of the two other Scientific Committees.
- ▶ Submission to the Executive Committee for approval.

If this process could be achieved before the publication of the article, it could certainly be mentioned in the paper that EuroGentest guidelines are recommended by IRDiRC (if the Executive Committee approves it).

Pipeline comparison of various informatics platforms for identification of rare variants

RD-Connect has used a reference material defined by a well-known cell line fully annotated (genome) to compare three different pipelines (France, Spain and Netherlands). Their findings were reported in terms of efficiency (how much variants found compared to the reference variants) and speed. The three pipelines were accurate: 99% of the variants that were filtered were accurately found in the gold standards files. Difference appears in terms of speed between the three pipelines. Netherlands was faster than Spain and France which were very similar. It is still too early to conclude. Other tests still need to be realized. Canada could join this pipeline comparison.

In the next teleconference, the EuroGentest guidelines should be commented by the WG members that could not attend this teleconference. Standards for clinics should be discussed, and then more complicated topics could be reviewed i.e. How do you well defined the research analytics in exome sequencing?

Deliverables

- ▶ Circulate the minutes to the other WG members and highlight the 'IRDiRC Recommended' process for the EuroGentest guidelines
- ▶ Circulate the EuroGentest guidelines draft prior to the next teleconference for all members to read and comment