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REFLECTION PAPER ON PHARMACOGENOMIC SAMPLES, TESTING AND DATA HANDLING

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Reflection Paper on PG samples, testing and data handling

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1. INTRODUCTION

Pharmaco-genomics (PG)[1] offer a potential for better understanding the mechanisms of diseases and optimizing the development and use of medicinal products. PG information may allow Regulators to optimize the benefit/risk balance evaluation and provide focussed information as guidance to prescribers and patients. In addition, the PG evaluation may become a valuable tool in risk management and pharmacovigilance strategies.

It is therefore envisaged that in the future PG testing in clinical trials and large-scale epidemiological studies will be increasingly considered in pre- and post- approval development and assessment of medicinal products.

2. SCOPE

The potential of PG analysis is strongly dependant on the reliability of PG information. Reliability of PG information again will strongly depend on the overall quality of the test sample, validation of the assay and methods, the reproducibility of data and their association with the clinical phenotype of interest, e.g. therapeutic response.

This paper addressed reflections on some aspects surrounding pre-analytical, analytical and post-analytical steps surrounding PG samples, testing and data handling, key for the scientific reliability of PG data submitted for regulatory evaluation.

3. PRE-ANALYTICAL ASPECTS

3.1 Samples handling

The availability of nucleic acids (DNA, RNA) of suitable quality is essential for PG studies where the genetic background of individuals or expression profiles are investigated. Nucleic acids from different tissues or other biological origin are used to address different questions. Nucleic acid quality is assured by the combination of appropriate handling when taking the sample for PG studies, sample storage, fixation and nucleic acid extraction procedures.

For expression profiling fast processing of biological materials (e.g. immediate storage, fixation or nucleic acid extraction) is recommended since expression patterns may change significantly shortly after bringing cells or organisms into a new environment.

The potential effects of incubation time and temperature on expression patterns may have to be evaluated and the handling process validated.

3.2 Storage

Biological samples may contain varying amounts of nucleases with different activities under different conditions. Many protocols foresee storage of biological samples at -70°C to -80°C temperature at which no significant effects on stability of nucleic acids are expected over time. Storage at -20°C has been described to be associated with continuous degradation of nucleic acids with low kinetics (e.g. half time of HIV-RNA in human plasma is 40 weeks).

Lyophilisation of tissues has been shown to support long-term storage under moderate temperatures (4°C and -20°C). Biological specimens for PG analysis may be also stored as extracted nucleic acid samples.

[1] RE ICH- E15 EWG definition: The investigation of variations of DNA and RNA characteristics as related to drug response.

It is recommended to keep DNA samples in solution, as dried samples could sometimes be difficult to dissolve. In addition, even after nucleic acid extraction residual amounts of nucleases may still be present sufficient to interfere with subsequent analysis.

Suitable integrity of the nucleic acids under the chosen storage conditions should be checked and verified at least for the primary target regions and for potential control target regions. Furthermore proper controls shall be performed for the sequence identity of the amplified DNA and the identity of the analysed mRNA.

3.3 Fixation

For fixation of tissues and other materials different protocols exist which may be followed after suitable validation of appropriateness for the purpose of the planned studies.

Potential interference of fixation with subsequent nucleic acid detection, identification or quantification procedures should be investigated. Potential interference may occur on different levels: by inhibition of subsequent reactions (e.g. reverse transcription of RNA, amplification of nucleic acid target regions, labelling of nucleic acids, specific hybridization to probes) by fixation reagents or by potential degradation or chemical modification of nucleic acids by the fixation process.

Influence of the fixation process on nucleic acid presence, stability and extractability from fixated tissues is an issue, which should be taken into consideration.

For qualitative analysis of nucleic acids simple fixation protocols, e.g. dried blood on filter paper may be sufficient. Furthermore, reliability of results is strongly increased by their confirmation on a different platform for which equivalent validation of the fixation process should be performed.

3.4 Nucleic acid extraction and quantification

Several different DNA and RNA extraction procedures are available.

Compounds of the biological materials other than nucleic acids may be co-purified with the nucleic acids and residual amounts of extraction reagents may still be present in purified nucleic acids. Potential interference with subsequent steps of PG analysis should be analysed for the combinations sample matrix, chosen extraction protocol and PG analysis method(s).

Validation of consistent extraction efficiency may become an issue when absolute quantitation of nucleic acids is an integral part of the PG analysis.

Integrity and purity of extracted nucleic acids may be analysed by a number of methods, including e.g. gel electrophoresis or determination of ODs at different wavelengths.

The accuracy and precision of estimates of DNA concentration are critical factors for efficient use of DNA samples in high-throughput genotype and sequence analyses. In the application of a specific DNA quantification method to a particular molecular genetic laboratory protocol must take into account the accuracy and precision of the specific method, as well as the requirements of the experimental workflow with respect to sample volumes and throughput.

4. ANALYTICAL ASPECTS

4.1 Test performance

Different platforms for PG analysis are characterized by different techniques. Probes consist of oligonucleotides of different lengths manufactured by organic chemistry or of in vitro synthesized cDNAs stretches.

The validity of the oligonucleotide probes to be used should be checked using appropriate software. It is of critical importance that the identities of the products of the PCR reactions from genomic DNA are ensured by sequencing. Impurities in the DNA preparations may lead to improper annealing or improper hybridization in specific samples.

For validation of the method used for SNP detections positive and negative controls should be used. This includes tubes with water and tubes containing DNA from subjects' known to have the mutation in question being either homozygously mutated, heterozygously mutated or wild type.

These control samples should be analysed at every event. If the genetic variant is very rare and no genomic standard samples can be obtained, it is of great importance that the identities of the DNA amplified from the samples are confirmed by sequencing.

Lack of reproducibility between different platforms is an issue, which may decrease reliability of PG test results. Non-accurate test results may be generated by different means like cross-hybridisation of targets with some sequence homology or by background signal in the absence of any sequence homology or by secondary structures (folding) of target and/or probe stretches preventing specific binding.

4.2 Quality assurance

Internal quality assurance

Assay standardization. Internationally accepted standard or reference materials for PGs are currently not available. At present, the use of suitable platform- specific reference materials for assay standardization and calibration is recommended. Independent verification of individually determined test results may include highly specific, sensitive and accurate test systems such as validated real time (RT) PCRs.

Assay Controls. Appropriate assay controls are important quality control measures. Controls may include spike-in controls where the hybridization and detection process is controlled by spiking of known amounts of characterized nucleic acids into the extracted nucleic acids to assess the accuracy of the test system. For gene expression studies absolute measurements of gene expression is associated with higher variation compared to relative measurements. Constitutively expressed genes may be chosen for normalisation.

External Quality assurance

External proficiency testing programs using well-characterized test samples have already been introduced for the molecular diagnosis of some genetic markers. These programs reveal the degree of inter- and intra-laboratory variation of respective test results and may illustrate the potential need for corrective measures.

Currently there is no proficiency-testing program for PGs available. Once such systems are introduced for PGs regular participation in suitable testing programs is considered as an important quality control measure.

5. POST-ANALYTICAL ASPECTS

5.1 Sample handling systems

Adequate physical storage and an effective labelling and inventory management system are essential. Labelling of samples so that they are efficiently tracked and retrieved can be done with validated electronic data management programs.

Bar coding of biological specimens allows automation of the banking system and error-proof operation. A unique barcode ID is given to each sample, generating a system of easily tracked

specimens. The characteristics of each sample and related epidemiological and clinical information are linked to the barcode ID in the database system.

Innovative programs for the long-term storage of DNA that combines purification processes proven to produce archival quality DNA, chain of custody documentation through a proprietary Laboratory Information Management System (LIMS), sample security and retrieval efficiency have been developed and are available.

Key features of this process include (non fully exhaustive list):

- GLP-compliant facility
- Interactive, consultative development of customized archiving programs
- LIMS supported, documented chain of custody and sample management processes
- Secure, redundant storage systems
- Efficient and flexible retrieval and shipping
- Documented quality control

5.2 Storage duration and associated data handling

An inherent value of the PG samples is an opportunity for longitudinal research on genomic factors involved in therapeutic drug response and adverse effects. This is only possible via linking the clinical information to the results of the genetic analysis. On a case-by-case basis these longitudinal studies may be appropriate for the regulatory approval of the medicinal products and/or for the post-approval follow-up or monitoring studies.

The full development of medicinal products takes years. Therefore, for the purposes of PG and drug development, long-term storing of the samples and of identification codes may be considered.

A basic component in data collection and handling is informed consent that should cover all relevant issues important to the subject as per Good Clinical Practices Guidelines and relevant European legislation.

The consent has to be sufficient to cover the goals of the trial and/or to give opportunity for re-contacting the subject for new informed consent for additional sample and data collection and research. Although the majority of the trials and sample collections still are using narrow focused informed consent models, current international trends are indicating an increased shift to broader scope of the consent. This is particularly important considering the large scale of trials and the fact that most current studies are of exploratory nature, the result of which might need confirmation in the future.

Trial subject has irrevocable right at any time to withdrawn his/ her consent for participating at the trial and the provision of samples, including for PG testing. However, subject's personal decision autonomy to withdraw the informed consent can have practical value in the existence of the sample/data identification code(s) only.

The data obtained from genetic analysis prior to the withdrawal might continue to be used after the consent withdrawal, depending on the specifics of the informed consent. The general data security measures have to correspond to the Directive 95/46/EC.

6. GLOSSARY

The following definitions refer to features relevant to PG assays and variation of PG data

Accuracy describes the degree of conformity of measured results in one test system with the true (actual) value.

Precision expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogenous material under the prescribed conditions. Precision may be considered at three different levels: repeatability, intermediate precision and reproducibility.

Repeatability expresses the precision under the same operating conditions over a short interval of time (intra-assay precision).

Intermediate precision describes the within-laboratory variation of repeat test results with one test system (different days, different analysts, different equipment).

Reproducibility is the inter-laboratory precision, which may be determined as the variation of repeat test results for one test system in different laboratories (intra platform variation) or for different test systems (inter-platform precision).

Analytical specificity is the ability to unequivocally assess the target nucleic acid in the presence of other nucleic acids or other components, which may be expected to be present.

The **linear range** of a quantitative assay describes the concentration range of targets, which is consistent with accurate results.

The **analytical sensitivity** defines the detection limit, which is the lowest amount of nucleic acid, which can be specifically detected by a PG assay.

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