European Medicines Agency
Pre-authorisation Evaluation of Medicines for Human Use

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OVERVIEW OF COMMENTS RECEIVED ON DRAFT REFLECTION PAPER ON PHARMACOGENOMIC SAMPLES, TESTING AND DATA HANDLING

Table 1: Organisations that commented on the draft Guideline as released for consultation

	Name of Organisation or individual	Country
1	European Federation of Pharmaceutical Industry Associations (EFPIA)	Belgium

7 Westferry Circus, Canary Wharf, London, E14 4HB, UK
Tel. (44-20) 74 18 84 00 Fax (44-20) 74 18 86 13
E-mail: mail@emea.europa.eu http://www.emea.europa.eu

EFPIA

GENERAL COMMENTS - OVERVIEW
Pharmacogenomics and Pharmacogenetics (PG) have the potential of improving development and use of medicinal products. In the coming years, evaluation by regulatory authorities of new medicines may involve increasingly PG components in pre- and post- approval development, and may therefore depend on reliable PG information. It is therefore important that quality criteria of PG samples, assays and generated data are being discussed from a regulatory standpoint. EFPIA welcomes the reflection paper and its emphasis on PG samples, testing and data handling due to our increasing activity in this area. The scope of the document is broad, encompassing genomic DNA, RNA, fixed and lyophilised tissues. At times, the details are too specific because currently, PG is a rapidly and continuously evolving field. The document should reflect on the 'principles' or necessary qualities of developing well controlled, rigorous and reproducible sample handling and testing procedures, without being restrictive as to how this is accomplished. The document also provides a welcome scenario of long-term sample storage and testing. It is welcomed that this reflection paper recognises that PG should be handled in the same manner as other clinical data e.g. elements of informed consent or data security, and directs the reader to already established guidance documents. However, in practice sponsors often experience country-specific difficulties in gaining such informed consent. EFPIA would welcome an EMEA review on this issue. Of note, there are some areas of the paper (sample handling systems, long term storage needs of DNA) where it appears to be setting PG as having exceptional needs so that much of the paper concentrates on the technical aspects of sample (and data) handling which in most areas of drug development would be part of, for example, a company's standard operating procedures. Therefore, it would be useful to understand why the Agency feels this level of detail is needed for PG: have such detailed guidance documents been developed for other technologies such as imaging (or perhaps even more comparable, clinical chemistry) in drug development? With this is mind please note that although the comments below contain some suggested changes regarding such technical matters the overall question is whether such detail is warranted for a future regulatory guideline. **Comment to the comment:** In the revised version of the Reflection Paper it is made clear that the Reflection Paper is addressed also to assessors (for whom PG is new technology), not only to industry (SCOPE, P. 3). For these target readers this level of detail appears appropriate. The paper also touches on most of the steps involved in PG analysis process. Different degrees of details are provided; some are too specific (e.g. storage temperature), while others (most) are too vague. The document needs further clarification noting that PG assays are not unique, DNA sequence variations and RNA levels are measured for other types of research all the time, from discovery to diagnostics, and their validation is (1) technically the same, (2) conceptually the same as other biomarker validation processes and (3) different certification is not needed in many clinical labs. The data analysis part is weak and too short — using proper statistical methodology to analyse data and reach credible conclusions is critical for PG studies and is probably the least well defined step in the process (comparing to sampling and lab analysis). **Comment to the comment:** In the final version of the Reflection Paper the respective parts have been revised, with many of the specific comments (see below) included).

Throughout the document, different PG terms are used: PG testing, PG analysis, PG studies, PG assay, PG data, and PG research. If these terms are used to differentiate specific scenarios or uses of PG, then it is suggested the terms are defined before use. Otherwise it may lead the reader to focus only on studies conducted for the purposes of PG (as in exploratory or hypothesis generating studies with a PG endpoint) in contrast to a PG method conducted as part of a battery of endpoints (composite) to address a specific drug development question in a clinical trial (as in hypothesis confirmation during phase 3 or as a known PG biomarker). For example does 'PG studies' mean those studies with a PG endpoint or simply studies where PG samples are collected? For PG research vs. PG testing it is suggested that the former be used when considering PG research conducted during the exploratory phase and PG testing be confined when results have clinical utility, with potentially the associated use of a validated assay (homebrew or IVD). Thus these points could be re-organized manner to convey these insights clearly and also to be consistent with proposed ICH Topic E15 terminology (draft Note for Guidance on establishing definitions for genomic biomarkers, pharmacogenomics, pharmacogenetics, genomic data and sample coding categories CHMP/ICH/437986/2006).

Comment to the comment: In the final version of the Reflection Paper the term PGx is used with reference to the ICH-E15 EWG definition included. Different parts (e.g. SCOPE, P.3) have been have been revised, with many specific comments (see below) included).

In addition, it could be generally useful to distinguish recommendations for DNA from recommendations for RNA as these may differ substantially in some cases. It would also be very useful to extend the recommendations regarding DNA handling and testing of samples which require specific care, such as tumour samples, as an independent section. Finally, considerations related to quality control of samples (pre-analytical) could be summarized in a separate section.

Comment to the comment: In the final version of the Reflection Paper some respective parts have been revised, with many of the more specific comments (see below) included). RNA and DNA are now covered by separate subsections in the different chapters.

In conclusion, PG approaches can be used for a number of purposes, from basic research to clinical practice. Samples handling, assay characteristics and data handling can be quite different depending on which setting PG is being applied. EFPIA welcomes that PG biomarkers may increasingly be viewed with other biomarkers as part of a battery of variables used to gain insight into drug response The document also provides a welcome scenario of long-term sample storage and testing. However, there are many available methods for PG sample preparation and testing. Others appear every year, making it very difficult to list them all, and impossible to propose practical recommendations that could apply to any possible situation. This seems to be reflected in the current version of the document; in many cases it is too specific, in other cases too unspecific for research and clinical application in the continuously evolving PG field. Therefore, this paper should only discuss general principles of PG in drug development and the document should reflect on the necessary qualities of developing well controlled, rigorous and reproducible sample handling and testing procedures, without being to be restrictive as to how this is accomplished.

GUIDELINE SECTION TITLE		
Line no. + paragraph no.	**Comment and Rationale**	**Outcome**

Section 1 Line 1	Unclear why a Pharmaco-genomics are split by hyphen?	Pharmacogenomics Proposed change included
Section 1 Line 6	Since this reflection paper is focused in the research setting it is suggested that term 'PG testing' is not appropriate as this infers some level of clinical utility: As outlined in the general section above it is suggested that terms are defined and then used as appropriate throughout the document.	Suggest replacing with PG studies or PG research as required. Introduction and Scope has been revised. It should be clear that the main focus is not the basic research setting but the pre- and post approval development and the assessment of medicinal products. (see INTRODUCTION and SCOPE p. 3).
GUIDELINE SECTION TITLE: 2. SCOPE		
Line no. + paragraph no.	**Comment and Rationale**	**Outcome**
Section 2	Although it is stated that 'This paper addressed reflections on some aspects surrounding pre-analytical, analytical and post analytical steps…' more clarity around the objective and the desired impact would be useful.	Wording of SCOPE has been changed to make it clearer
Section 2 Paragraph 2	It is stated "…key for scientific reliability of PG data submitted for regulatory evaluation." Does the reflection paper relate only to those PGx samples and data which might be submitted for regulatory evaluation? Are there different reflections applying to samples and data which are obtained in a research setting, and which will not be used for regulatory purposes?	It should be clear that the main focus is not the basic research setting but the pre- and post approval development and the assessment of medicinal products. (see INTRODUCTION and SCOPE p. 3).
GUIDELINE SECTION TITLE: 3. PRE-ANALYTICAL ASPECTS		
Line no. + paragraph no.	**Comment and Rationale**	**Outcome**
Section 3 in general	It is unclear why there is felt to be a need to define in such a detail sample handling, storage, fixation and extraction requirements? Examples include stating storage temperatures (-70C to –80C), and providing a list of how to store samples (which appears to be incomplete). If these specifications are to be included in the final version, they should be extensively referenced, but better to not be so specific due to the difficulty in being inclusive of all	References have been extended, level of detail appears appropriate for target readers

	possibilities. If the aim is to ensure a 'quality' product it may be more appropriate for the reflection paper to look at how quality may be monitored and assessed and allow researchers to define their own protocols on how to achieve this.	
Section 3.1, **title**	**Samples**	Sample Changed to "Sampling"
Section 3.1, **title**	The title of this section is confusing: Is the aim to look at how samples are processed? Sampling handling could in itself mean the categories for sample and data coding. But sample handling is a much broader issue to address than sample processing. The content of this section in the current form focus mostly on sample processing of biological material for expression profiling. What about DNA? What about principles of robust sample tracking?	Changed to "Sampling" RNA and DNA are covered
Section 3.1 Paragraph 2	Additional processing methods should be mentioned.	For expression profiling fast processing of biological materials (e.g. immediate storage, fixation, nucleic acid extraction, or adequate pre-analytical procedure such as preservation of blood in …….blood RNA tubes, or preservation of skin samples in …….) is recommended since expression patterns may change significantly shortly after bringing cells or organisms into a new environment. Proposed change included (without reagent or product names).
Section 3.1 Paragraph 2	Fast processing of samples for expression profiling is indeed critical for successful studies. The first example given in brackets simply states "immediate storage". This is technically vague and would be better stated as "immediate flash frozen storage".	Propose sentence should read…"For expression profiling fast processing of biological materials (e.g. immediate flash-frozen storage, fixation or nucleic acid extraction) is recommended since expression patterns may change significantly shortly after bringing cells or organisms into a new environment." Proposed change included
Section 3.2 in general	Within this section on storage it would be useful to discuss the need/duration for short and long-term storage and/or to cross reference to the sampling section below. The desire is not to develop additional guidance in this area since procedures for the conduct of clinical studies	Suggest adding the additional sentence to paragraph 4 With the appropriate consent, PG samples may be stored beyond the duration of the clinical trial, potentially for the duration of a clinical

	already exist. However a reference to the fact that samples may be stored for longer then the trial duration, and that samples may have utility beyond a single trial and perhaps even for the duration of a development program would be extremely useful.	development program and beyond. Therefore suitable integrity of the nucleic acids…… Proposed change included
Section 3.2 Paragraph 1	It would be helpful to differentiate between storage of starting material (blood/tissue) and storage of purified material (nucleic acids) -80^{o}C storage is optimum for biological samples collected for nucleic acid extraction. However this is not always possible at some clinical sites. Recommendations concerning storage temperatures should therefore not restrict the possibility of performing multi-center studies. Rather, the best storage alternatives should be suggested. In addition, the usual temperature storage range is rather: -65°C to -80°C. Also, it might be useful to differentiate temperature requirements according to collection matrix (e.g.: EDTA tubes, …… tubes, fixed tissues or …… cards may have different requirement in term of short and long term storage). A mention on the effect of the number of freeze/thaw cycles should be added, as these can have a major impact on the quality of the samples. Finally, a precise reference for the low kinetics degradation of HIV-RNA should be indicated.	Many protocols foresee storage of biological samples at -65°C to -80°C temperature at which no significant effects on stability of nucleic acids are expected over time. Proposed change included Proposed statement included **New** reference for RNA stability included
Section 3.2 Paragraph 3, line 1	The level of scientific direction may be inappropriately too detailed; rather it is the responsibility of the sponsor to demonstrate that storage conditions do not affect assay results.	

Section 3.2 Paragraph 3	It should be distinguished between DNA and RNA sample storage conditions. The following conditions should be given: The freezing of DNA samples at -20°C is recommended and no significant increase in stability has been observed at temperatures below -20°C. Furthermore, for long-term storage DNA samples are best stored in TE buffer to minimize degradation. **4.2** Repeated cycles of freezing and thawing may also contribute to degradation. Therefore, splitting of samples into multiple aliquots and thawing one at a time should be mentioned.	DNA samples should be stored at -20°C to prevent bacterial contamination and to minimize evaporation. Since pure water lacks buffering capacity, DNA is preferentially stored in 10mM TrisHCl/0.5mM EDTA Buffer to prevent acidic hydrolysis of the DNA. RNA samples are stored in RNAse-free water at -80°C to minimize degradation by nucleases. It is recommended to minimize the freeze-thaw cycles by splitting the DNA samples into multiple aliquots. Proposed sentence included
Section 3.2 Paragraph 4	Depending on the methodology used, different levels of integrity of nucleic acids may be acceptable. This should be mentioned in the text.	Please add: "Depending on the methodology used, different levels of integrity of nucleic acids may be acceptable". Proposed sentence included
Section 3.2 Paragraph 4	'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'. What is the proposed recommendation to check the integrity of the nucleic acids? Is the paper recommending to check ALL the testing samples for integrity or to check some samples to validate the storage conditions in general?	Suggest changing the wording into: 'The integrity of the nucleic acids under the chosen conditions should be checked. Storage condition should be validated using control samples to ensure sample stability and integrity' Validation of storage conditions is essential
Section 3.2 Paragraph 4	'Furthermore proper controls shall be performed for the sequence identify of the amplified DNA and the identity of the analysed mRNA'. It is unclear what recommendation this sentence proposes. Most, if not all, assays will work on amplified material. It would not only be impractical but also questionable scientific value to sequence amplified product for all the samples.	**Suggest deleting:** 'Furthermore proper controls shall be performed for the sequence identify of the amplified DNA and the identity of the analysed mRNA'. Proposed sentence not deleted. It is to be interpreted in the way that identity of amplification products should be validated, not that all amplification products would have to be sequenced.
Section 3.3 Paragraph 2	The paragraph should mention potential structural changes of nucleic acid due to fixation procedures, as this could lead to artefacts in sequencing reaction. It is our understanding that the considerations relating to fixation apply mostly to oncology samples (tumour biopsies). We suggest grouping all these comments in a specific section dealing with such type of samples.	Suggest specific and separate section referring to handling tissue samples, referencing for example such special considerations as for oncology. The considerations are not restricted to oncology samples
Section 3.3 Paragraph 4	Is the need to test reliability of a result on a second platform considered a necessity or a suggestion?	

Section 3.3 Paragraph 4	**Critical comment** The example given for a simple fixation protocol is suitable only for DNA studies but not for RNA. The first sentence of this paragraph implies that it is suitable for nucleic acids in general.	Propose sentence should read…"For qualitative analysis of nucleic acids simple fixation protocols, e.g. for DNA dried blood on filter paper or for RNA the addition of …..may be sufficient." Proposed sentence included without product name
Section 3.4 Paragraph 2	Nucleic acid extraction methods are robust and well established methods, which usually make use of commercially available purifications kits. In most cases, non-nucleic acids contaminations in the purified probes will not have any impact on the PG analysis. Reliability of the PG results should essentially be based on the use of appropriate positive and negative controls. In the rare cases where these contaminations could impact the PG experiment, the interference analysis mentioned in this paragraph should be performed. However, it should be clearly mentioned in the text that this type of analysis will only concern a small number of samples, and should not be systematic, as this will be part of the method validation.	It is now included that this belongs to validation
Section 3.4 Last paragraph	Can more insight be provided on how to determine the accuracy of DNA concentration after extraction from tissue? Is this in reference to absolute quantification? It is not clear how this would be accomplished. Is this considered to be important in qualitative studies (e.g., SNP analysis) where quantitation is not an integral part of the PG analysis?	It is dependant on the type of study, therefore it has been left more general
Section 3.4 Last paragraph	**Important** '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." It is also critical that the DNA is fully in solution, if not, this will lead to inaccuracies in DNA concentration determination and possible genotype/sequencing failures.	'It is essential that the extracted DNA is fully in solution since 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 Proposed sentence included
GUIDELINE SECTION TITLE: 4. ANALYTICAL ASPECTS		
Line no. + paragraph no.	**Comment and Rationale**	**Outcome**
Section 4 in general	In general, many items are too specific. Examples include designating negative control samples as "tubes of water".	See SCOPE

Section 4.1 in general	A paragraph should be added to address heterogeneity of oncology samples and sensitivity of sequencing/genotyping platforms.	This paper covers tissues in general, therefore a more general approach has been chosen
Section 4.1 Paragraph 1	Replace "stretches" with "probes" or some more scientific term	Suggest replacing "stretches" with "probes" or some more scientific term. Proposed change included
Section 4.1, Paragraph 1	Probes may consist of oligonucleotides (or not), depending on the methodology used	Probes may consist of oligonucleotides of different lengths manufactured by organic chemistry or of in vitro synthesized cDNA. Proposed sentence included
Section 4.1, Paragraph 2 line 1	'It is of critical importance that the identity of the products of the PCR reactions from genomic DNA are ensured by sequencing'. Is the paper recommending sequencing for each of the PG samples used in the study or only control samples to ensure the accuracy of the assay? It would be impractical to perform sequence reactions of amplified PCR products on all samples. In addition depending on the methodology used, other techniques may be used to verify sequences of PCR products.	It is of critical importance that the sequence identities of the products of the PCR reactions from genomic DNA are verified. Proposed change included
Section 4.1 Paragraph 3	Inappropriate plural (SNP detections)	SNP detection Proposed change included
Section 4.1 Paragraph 3	'….known to have the mutation in question being either homozygously mutated, heterozygously mutated or wild type'. Most of the markers utilised in PG analysis are polymorphisms, not mutations. Recommend to change the term	'...known to have different genotypes of the polymorphism being analysed: homozygous for each of the allele and heterozygous for both alleles' Proposed change included
Section 4.1 Paragraph 3	Can plasmids containing the alleles of interest, or other manufactured constructs, be used as a control, in place of DNA from subjects?	Plasmids may be appropriate
Section 4.1 Paragraph 4	Is it considered necessary that sequencing of DNA to confirm identity be conducted only during validation or for each experimental test?	It is dependant on the type of study, therefore it has been left more general
Section 4.1 Paragraph 4	As paragraph 2 line 1	It is of great importance that the sequence identities of the DNAsamplified from the samples are verified. Proposed change included
Section 4.1 Paragraph 4	**Editorial** '…at every event'	'…at every experiment' Proposed change included
Section 4.1 Paragraph 5	The objective of this paragraph is unclear. Is the focus to highlight the issue of reproducibility between different platforms or to make recommendation to address the issue? If the latter it is suggested that these are broad and flexible.	It is to highlight the issue of reproducibility between platforms

Section 4.1 Paragraph 5 line 2	'like'	means such as cross-hybridisation Proposed change included
Section 4.1 Paragraph 5 line 4	probe stretches	Probe sequence Proposed change included
Section 4.2 in general	The discussion of reference materials overlaps with the FDA concept paper on pharmacogenomic standards and work of the MAQC	Reference the FDA concept paper 'Recommendations for the Generation and Submission of Genomic Data**'** and MAQC FDA concept paper is still in draft and not referenced**;** MAQC included (references)
Section 4.2 Paragraph 1	***1.1*** *RT-PCR is a sensitive and accurate system. However, validation processes for this methodology have not been described to our knowledge. As a consequence, the meaning of "validation" should be clarified with a working definition for this reflection paper*	There is already validation processes defined for some RT-PCR applications, e.g. HCV-RNA detection (Europ. Pharmakopoe)
Section 4.2 Paragraph 2	***1.2*** *Normalization is essential; use of constitutively expressed genes is one of the options, but there may be others. In addition, it should be mentioned that normalization does not apply to experiments involving DNA as starting material.*	Point has been made clearer
Section 4.2	*Fourth paragraph:* Are the envisioned proficiency testing programs considered to be internal or external to a particular organization (e.g., pharmaceutical company)? There should be a distinction made between exploratory research, drug development research, and clinical testing levels of quality assurance and proficiency testing.	See SCOPE
GUIDELINE SECTION TITLE: 5. POST-ANALYTICAL ASPECTS		
Line no. + paragraph no.	**Comment and Rationale**	**Outcome**

Section 5.1 in general	**Important** The objectives of this section seem unclear since the sample handling requirements for PG samples are no different from other clinical sample requirements. It would be useful to understand why it is felt that current regulations do not already cover this area and/or why PG samples are thought to necessitate this level of detail. Overall, it is recommended that the paper is not prescriptive as to exactly how these actions are undertaken. Samples can be handled in a number of diverse laboratory ways and still achieve the same endpoint.	See SCOPE
Section 5.1 in general	The aim of this section seems to be appropriately focused on ensuring that a sample is correctly labelled, tracked and associated with the correct clinical data. However it is recommended that the paper is not prescriptive as to exactly how this is undertaken. Bar codes may be one of many ways this can be achieved. It may also be appropriate here (or even at the beginning of this whole section) to reference ICH E15 and sample coding categories since these will have some impact on traceability of data, clinical monitoring etc. The reference to 'GLP compliant facilities' appears confusing. Since GLP provides a framework for pre/non clinical research clarity around its applicability is requested.	Suggesting adding some wording Adequate physical storage and effective labelling and inventory management systems are essential. Labelling of samples so that they are effectively tracked, retrieved and linked to the appropriate clinical data can be done with validated electronic data management programs. The manner in which the samples (and data) are collected will impact how samples (and data) can be traced back to the subject, the ability to perform clinical monitoring, subject follow up and/or addition of new data. As outlined in ICH E15[1] four general categories of coding can be used and the impact on sample (and data) handling systems should be considered. Whole section has been revised
Section 5.1 Paragraph 1	Looking at the sentence "Adequate physical storage and an effective …." An adequate tracking system is essential for sample collection, PG methods and for the results from the analysis: it would be useful to understand why it is only emphasized in the post-analytical section.	An adequate tracking system is extended to all phases of the process
Section 5.1 Paragraph 1	Looking at "….can be done with validated electronic data management programs" if this level of detail is felt necessary then some definition as to what is meant by validated in this context would be extremely helpful. On the other hand, this section is very specific and focused on only one approach (electronic data management programs and bar coding of samples); other possible systems include paper-based system (especially for a smaller PG effort), sample labels not including barcodes, etc.	Please add a working definition of 'validated' for this specific application regarding electronic data. No standard validation approach is defined at present and shall be defined according to in-house criteria and evaluated on a case by case basis.

Section 5.1 Paragraph 2	It might be useful to add a paragraph on the tracking of Informed Consent attached to each sample.	We will refer to the ESHG statement
Section 5.1 Paragraph 3	Agreement with the outlined chain of custody, but not the designation that an innovative program(s) must be used to accomplish this.	Comment on comment: the point is noted. However the use of innovative programs is supported.
Section 5.1 Paragraph 4	It is stated "Key features of this process include….". Does the term 'this process' refer to long-term storage of DNA or to the 'purification process' as mentioned in the paragraph above? Suggestion to clarify the precise meaning.	Facilities which meet appropriate quality standards Proposed change included
Section 5.1 Paragraph 4	Meaning of "redundant storage systems" should be clarified.	"redundant" deleted
Section 5.1 Paragraph 4	Key features: First bullet indicates a 'GLP-compliant facility'. This phrase could be interpreted as suggesting that sample handling should be performed according to GLP. However, the reflection paper relates only to clinical- and epidemiological studies. Since PGx studies do not necessarily concern safety, one could question a need to perform (part of) such studies under GLP. As it concerns clinical studies, we consider that GCP are applicable.	Replace GLP with GCP Proposed change included
Section 5.2 in general	It is good to see the paper recognizes that samples need to be collected and stored for a period of time before they may be analysed. One of the challenges faced when collecting PG samples during global clinical research/across development programs is the fact that different regions/IRB/EC apply their own rules and regulations. However it would be useful if the paper were clearer in that long-term storage does not necessarily constitute longitudinal research. Longitudinal research implies following subjects for a period of time with the addition of new clinical data. For PG research it is more likely that the samples and data will be collected at some time point during a clinical trial but that a PG experiment may occur some time later, when a PG hypothesis has been identified. It would be extremely useful if the paper could promote a more harmonized approach to collection, research and long term storage of clinical samples: this approach should be developed within the current clinical trials framework, not just be for PG, to provide guidance on the potential value	The full development of medicinal products takes years. Therefore, for the purposes of PG and drug development, long term storing of the samples, and the use of appropriate identification codes, will need to be considered (ref ICH E15) An inherent value of the PG samples is the opportunity to conduct PG research (investigating therapeutic drug response and/or adverse events) at any time across the development and life cycle management of a medicine. This is only possible if samples have been collected and stored long term with the appropriate consent. Proposed change included

	of many types of biological samples collected in a clinical trial that extend beyond the conduct of that individual trial. Suggest moving the 2nd paragraph up and amending as indicated For terminology on coding suggest referencing the ICH E15 paper	
Section 5.2 Paragraph 1	The general ideas outlined in this paragraph are highly welcomed! However, they do not seem to be aligned with current clinical or regulatory practices throughout the EU region, with strong concerns about subject privacy. Will further guidance be provided on storage duration in this Reflection Paper or other Guidance?	There is no further guidance on storage duration in this document
Section 5.2 Paragraph 1	It is stated, "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." It is not completely clear what is meant here. Could the authorities please clarify when they consider such studies appropriate, or whether they consider it necessary to always discuss this with authorities when submitting a file?	"case-by case" means that it is dependent on which information is already existing and which would have to be generated
Section 5.2 Paragraph 2	The emphasis on long-term sample storage and broad consent is welcomed.	
Section 5.2 Paragraph 3	The requirements for informed consent are clearly articulated in GCP and relevant European legislation: Since the consent requirements for PG are no different from other clinical research it may be more appropriate to simply direct the reader to the appropriate guidance then list only a small proportion of the requirements in the paper. In addition, since PG does not always necessitate a separate consent it would be extremely helpful if the paper reflected these different situations.	Suggest deleting paragraph 3 and replacing with: "If PG research is to be conducted, subjects should be informed via the informed consent process. PG may be included in the main trial consent or it may be handled with a separate consent form, depending on trial design/objectives. Elements of informed consent for PG are the same as the elements for all clinical research, and are clearly articulated as per GCP and relevant European legislation."
Section 5.2 Paragraph 4	Even though the title of the section does not reflect the information discussed, for example the informed consent may better be addressed at the pre-analytical stage or sampling stage, rather than the post-analytical stage.	It is agreed that the undertaking of the informed consent is relevant at the pre-analytical and sampling stage. However the main consequences of the consent for data and sample handling are more evident at a post-analytical stage.

Section 5.2 Paragraph 4	By articulating the fact that consent may need to be broad to allow PG research to be conducted is extremely helpful. This paragraph will have even more impact if the reader understood the hurdles imposed when consents are unnecessarily restricted, which can lead to the utility of the samples being extremely hampered.	Suggest amending paragraph 4 to "The consent obtained has to be sufficient to cover the goals of the trial. The consent process must strike a reasonable balance between sufficiently describing research purposes and not being overly restrictive so that data and samples become limited in use in light of new scientific knowledge and technology. In special circumstances, if the scope of the proposed research is beyond the original consent obtained, subject re-consent may be considered. However, if this is not practicable, alterative routes to ensure appropriate human subject protection, such as later anonymization of the sample(s) will be considered." Section has been revised
Section 5.2 Paragraph 5	Clarification of the following sentence: "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?" If this is meant to say that the ability of a subject to withdraw from a trial and have a sample destroyed depends upon the coding of these samples then see amendment proposed. It is recognized that subjects have the right to withdraw his/her consent from participating in a trial at any time however such subjects may not have the right to request sample destruction and/or to stop analysis providing this situation is outlined in the informed consent. For example, for studies where PG results are integral to the interpretation of a clinical trial (e.g., well defined PG hypothesis are included in the trial objectives and endpoints) then consent withdrawal and destruction of PG samples could compromise the trial objectives. It may be useful to note that for other clinical trial parameters/samples collected during the course of a trial (e.g., blood samples for viral load, development of resistance to medicines etc.) are not subject to such	Suggest delete sentence and use the following: 'Trial participants have the right at any time to withdrawn his/her consent for participating in the trial. Dependent upon the consent obtained and the sample coding system used, withdrawal from trial participation may or may not allow for PG sample destruction. Where PG sample analysis post-withdrawal is required this should be outlined in the consent form. For information on coding refer readers to ICH E15.

	destruction guidelines as these samples are integral to interpretation of trial results.	
Section 5.2 Paragraph 6	**Critical** The sentence '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' seem to run counter to GCP, and appears to be setting PG analysis as different to other clinical analysis. Once data is generated it cannot, and should not, be destroyed as outlined in: GCP section 5.5.3 (c) of 5.5: Trial Management, data handling and record keeping section which states 'Ensure that systems are designed to permit data changes in such a way that the data changes are documented and that there is **no deletion** of entered data (i.e. maintain an audit trial, data trial, edit trial)'	Please amend to 'Data obtained/generated prior to the consent withdrawal may continue to be used. The use, and generation, of data subsequent to consent withdrawal will be guided upon the informed consent obtained.' Proposed change included
GUIDELINE SECTION TITLE: 6. GLOSSARY		
Line no. + paragraph no.	**Comment and Rationale**	**Outcome**
Section 6	Analytical sensitivity & specificity.	Definitions for clinical sensitivity & specificity could be added (in the context of PG analysis). Definitions added
Section 6	Repeatability: we suggest removing the reference to "short intervals".	