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Production of shared procedures and choice of the QC material(s) for quality monitoring of analytical phases

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ABSTRACT

One of the major aims of WP4 is to establish the analytical Standard Operating Procedures (SOPs) for a decision criteria suited for an application in clinical practice. Here we report a summary of the evaluated quality assurance programs in order to develop and implement a diagnostic “dyad” including both the assay method and its quality assurance program.

STATEMENT OF ORIGINALITY

This deliverable contains original unpublished work except where clearly indicated otherwise. Acknowledgement of previously published material and of the work of others has been made through appropriate citation, quotation or both.

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1 Introduction

Quality Management (QM) is a set of principles for coordinating management and improvement activities to ensure that an organization continuously meets its customers' requirements [1].

In operational practice, Quality Management System (QMS) are consensus-driven structured frameworks built on the principles of statistical process control (SPC) which is based on Quality Control (QC) and External Quality Assurance (EQA) systems [2, 3].

The International Organization for Standardization (ISO) has developed a QMS, ISO 9001, which is at the core of many standards, including ISO 15189 which mandates that medical laboratories use QC procedures and participate in EQA to ensure that the results of the tests they report are fit for purpose. No laboratory can improve its performance without an independent third party EQA [1, 3, 4].

Quality Management (QM) has to be considered also when laboratory methods are used for research purposes. In the case of research focused at discovery or mechanisms, the adoption of QM procedures should be tailored case by case mainly to ensure that obtained results may be reproducible. On the other hand, laboratory methods used in clinical research must undergo regular QM. In addition to ensuring data reliability for clinical decisions for patients, QC and EQA may be used to harmonize results obtained in different laboratories, or being pooled for research purposes.

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2 Analytical methods

2.1 General principles

The phase of sample analysis, encompassing all laboratory activities connected to the preparation and measurement of a sample, marks an important step in the life cycle of biological samples, especially in the scope of assessing their suitability for use.

Sample analysis can be performed manually by laboratory personnel and/or via automated platforms. Process outputs are defined as 'results'. Results can be either quantitative or qualitative.

Although many analytical methods/procedures exist and can be applied within the scope of the project, a brief description of the general types of measurements is provided below:

- Qualitative measurements or identification tests are employed to identify an analyte in a sample. This is normally achieved by comparison of a property of the sample (e.g., spectrum, chromatographic behaviour, chemical reactivity, etc.) to that of a reference standard. In the context of this document, the term 'test' denotes the qualitative measurement of component(s) in a sample;
- Quantitative measurements or assays are used to determine the amount of an analyte present in a given sample. In the context of this document, the term 'assay' denotes the quantitative measurement of main analyte(s);
- Assessing the amount of impurities in a sample can be either done with a quantitative assay or a limit test. Either measurement is intended to accurately reveal the purity of a sample. Both types of analyses commonly require different validation.

The aim of this document is to define the general principles applied to all analytical methods (tests and assays) with the scope of biological material analysis and Quality Control (QC) production and/or acquisition [5, 6].

Analytical method specifications should contain:

- scope;
- principle of the method;
- safety requirements;
- definition of assayed/tested items;
- parameters or quantities and measured ranges;
- equipment;
- reagents and consumables;
- reference standards / reference material (quality control material);
- environmental conditions;
- process execution;
- data analysis and management;
- acceptance criteria;
- uncertainty of the method;
- recording requirements (so-called 'Forms', 'Sheets' and 'Reports').

In order to optimally fulfil the above requirements, the analytical laboratory should:

- implement and maintain analytical protocols to describe specifications and requirements of the analytical testing methods;
- include conformity/non-conformity assessment criteria, where applicable;
- validate important analytical testing methods;

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- execute analytical testing under controlled environmental conditions, using suitable equipment and software, and adequately trained personnel;
- ensure traceability of the analytical testing steps by relevant records;
- communicate results by issuing official reports (if required).

2.2 General process for the execution of analytical methods

Execution of analytical testing methods is performed under controlled conditions:

- application of relevant laboratory analytical protocol;
- use of suitable equipment and software, meant as:
  - equipment is implemented, qualified, maintained and calibrated (on a day-to-day basis)
  - software is validated
- definition and control of environmental conditions;
- execution by trained personnel;
- traceability of samples to be tested/assayed ;
- recording of the process execution, including documentation about the use of critical consumables and test conditions;
- establishment of a method uncertainty (if not known);
- data analysis and verification of results against pre-defined internal acceptance criteria is performed and recorded, concluding with a pass/fail decision;
- production of a formal report, documenting the outcome of an analysis and including the measurement uncertainty (if known) prior to communication of the results.

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3 Types of Quality Control

Two types of quality control for the analytical phase should be implemented:

- internal quality assessment;
- external quality assessment.

Internal quality assessment is based on the selection/preparation of control materials (as described below, refer to the section 4) and the construction of Levey-Jennings control charts which are updated with results obtained by the analysis of control materials in every routine run [7, 8]. Internal quality assessment is used to validate each assay run AND to perform quality control of the method itself by analysis of the statistics of the cumulative quality control results. Run, or analytical run, is a procedure in which a group of samples are analyzed at the same time under the same conditions of measurement. Internal quality assessment has to be performed by qualified personnel in order to be recorded on the control charts.

QC strategy [3] provides a comprehensive plan for assay control involving all of the following:

- setting of quality standards;
- selection of QC materials;
- selection of concentrations;
- setting (and re-setting) of QC targets;
- setting (and re-setting) of QC limits;
- selection of rules;
- frequency of running QC;
- response to out of range results.

External quality assessment is based on external quality assurance programs and /or proficiency testing (PT) programs (refer to section 5) provided by independent organizations. Whenever available and relevant the participation in internationally recognized external quality assurance programs or PT programs is ensured once a year at least [9, 10].

EQA schemes [3] may present different design features dependent on the type of program. These include:

- the number of samples tested over time (frequency);
- the number of samples tested at the same time (a testing episode);
- the range of concentrations assessed;
- the number of samples across the concentration range;
- the number of samples combined for statistical analysis;
- definition of target values and acceptable performance;
- the number of peer groups for comparison.

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4 Quality control material to monitor analytical methods

4.1 Scope and principles

Biobanks and/or clinical/research laboratories processing biospecimen and producing fluid, cellular, or molecular derivatives, can validate their processing and analytical methods for sample suitability, reproducibility, robustness, homogeneity, and stability, according to *"ISO FDIS 21899:2020 Biotechnology—General Requirements for the validation and verification of processing methods for biological materials in biobanks"* [10]. As we will see in the following sections, these are essentially the same performance characteristics that have to be assessed during Quality Control (QC) implementation.

Many biobanks and/or clinical/research laboratories perform more or less extensive QC checks and characterization assays on the specimens they produce.
QC may include [11] the measurements of:

- molecular concentration, integrity and purity;
- cell count, viability, functionality and purity.

Characterization assays [12] may include some of the following:

- cell composition of whole blood;
- haemolytic, lipemic, and icteric indices of serum or plasma;
- histological composition of tissue samples;
- strip results from urinalysis.

4.2 Sources

Control material is a sample "which is analysed solely for quality control purposes, not for calibration" [13]. Depending on the method, one, two or three control materials are either internally prepared and characterized or purchased as certified and characterized controls from recognized or official suppliers.
QC material may be either purchased from reference material producers (e.g., National Institute of Standards and Technology/NIST) or in-house produced [12]. In the latter case, it may originate from commercial cell lines, or from other biological sources including human specimens. The donor must have therefore consented to the use of his/her own samples aimed at developing QC material, or new QC methods.
Any material labelled as 'QC' must comply with the quality assurance paradigm and display an HIV and HCV negative serological status.

4.3 Identification and record keeping

Certified reference materials (CRM) provided by a competent supplier such as reference material producers (e.g., NIST) should be used as QC material and implemented in routine laboratory processes where applicable. The purchased CRM should be accompanied by a document, issued by the supplier, and should provide values of specific properties with associated uncertainty and metrological traceability, using validated procedures [14, 15].

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Either preparing QCs from a commercial source (if not 'ready to be used') or from in-house material, the laboratory processing steps and QC material production records should be documented. These records include, where relevant:

- reference to the sampling and/or production method used;
- date and time of sampling and/or production;
- data to identify and describe the sample (e.g. number, amount, name);
- identification of the personnel performing sampling and/or production;
- identification of the equipment used, including the software and firmware version;
- environmental or transport conditions;
- storage location;
- deviations, additions to or exclusions from the sampling and/or production method described in the current work instruction.

All QC aliquots must be labelled and stored in monitored storage devices.

4.4 Validity period

QC material, reference standards and/or CRM must have a systematic control of their validity and correspondent certification.
Reference standards and/or CRMs, normally, have a short shelf life. Before expiry date, the laboratory must then acquire new reference standards and/or CRMs or, when possible, get re-certification from the supplier via provision of annotated records to justify extended use.
For some materials, due to their stable properties, the validity of their certification can be indefinite and therefore it is not included in their certificates. In such case, this reference material is valid indefinitely, as long as it is stored and handled according to its certificate.
When applicable, monitoring and validity of QC material is recorded routinely in control charts, which are a valuable tool to examine its overall status and to identify potential trends that may affect test results [16].

4.5 Registry

A registry of all QCs should be kept by the laboratory for a defined period of time period (for example, at least 3 years).

Where appropriate, the general overview of all QC material shall include, but shall not be limited to:

- date of production;
- operator/s involved in the production steps;
- raw material;
- processing method;
- number of aliquots produced;
- location of aliquots;
- assigned value.

4.6 Value assignment

All QC materials need to be assayed under routine operating conditions to characterize the expected measurement variation and distribution of values.

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When no reference measurement procedure exists, the value assignment is performed by appropriately characterized measurement procedures either in the same laboratory where the production took place, or in other laboratories.
The different measurement procedures should be thoroughly described, and known measurement uncertainty should be recorded.
For calculation of the assigned value and its uncertainty, *"ISO 13528:2015 Statistical methods for use in proficiency testing by interlaboratory comparison"* should be applied [9, 17].

Value assignment is done "per method" (e.g. value assignment is done for "DNA quantification BY spectrophotometry").
For quantitative assays, at least eighteen measurements should be performed in routine operating conditions, on different days, using different batches, and by different operators.
For qualitative assays, at least nine assays should be performed in routine operating conditions, on different days, using different batches, and by different operators.

Different parameters (i.e.: temperature, humidity, etc.) can have significant influence on the assigned value or its uncertainty, therefore measurement procedures should be calibrated with standards with metrologically traceable values [15].

A list of reference materials (RM) and reference measurement procedures can be found at: http://www.bipm.org/jctlm/

4.7 Purity assessment

When applicable, purity assessment should be performed. For example, the absence of blood and lipid contamination in plasma is assessed by the Haemolysis Lipaemia Icterus (HLI) indexes.
This characterization is important because haemolytic or lipemic values higher than the minimum index make circulating miRNA measurements uninterpretable.
Lipid contamination can be estimated by triglyceride concentration (< 300mg/dL) and blood contamination can be estimated by haemoglobin concentration (< 0.3 g/L).
Purity of isolated monocytes, which should be higher than 90%, can be determined by flow cytometry with anti-CD14 and anti-CD45 antibodies High purity is important for the accuracy of gene expression measurements [12].

4.8 Homogeneity assessment

Assessment of QC material homogeneity ensures its usability. Homogeneity is important in QC material production because all end users should be using exactly the same material.
In the majority of cases, homogeneity is evaluated through a stratified random sampling plan across the QC material production, consisting of at least eight (ideally ten) different aliquots of QC material (plasma, serum, DNA or RNA).
When QC material is produced in multiple batches, the equivalence of the batches shall be demonstrated or property values shall be assigned to each batch separately.
For example, homogeneity of plasma and serum samples can be assessed by microparticle counting. Total microparticle counting can be performed by an impedance-based method.
Plasma/serum homogeneity testing, relative to at least one of the critical disease-related biomarkers (such as C-reactive protein (CRP) as inflammation marker), measured by a validated assay, must also be performed.
Homogeneity of DNA and RNA samples can be assessed by spectrophotometry [12].

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In practical terms, all aliquots of plasma/serum or DNA/RNA material should be homogeneous based on the concentration of the measurements discussed above.

4.9 Stability assessment

Assessment and verification of QC material stability should be done before its use, unless there is an evidence of stability (prior experience of stability from similar QC materials, CRM certificate, etc.). Stability assessment should be performed under applied storage conditions (if necessary including pre-treatment, packaging, transport, repeated sampling, etc.) by trialling. Monitoring the QC material stability should include both short and long term plans, which will assess prompt and/or delayed detection of stability changes and, potentially, advise on the possible rate of change.
Where QC material is produced in multiple batches that are not individually tested for stability, the stability of a sufficient number of different batches should be assessed experimentally in order to provide confidence in overall batch stability.
For example, short- and long-term stability studies of plasma, relative to specific miRNA targets, measured by RT-qPCR assays, or better by reference method (droplet digital PCR) can be performed.
Stability studies of plasma, relative to extracellular vesicles, can also be performed following extracellular vesicle isolation by a validated method, enumeration by a method such as qNano, and measurement of specific surface epitopes by ELISA or a flow cytometry-validated method.
Short- and long-term stability studies of DNA and RNA, relative to specific downstream applications (e.g., DNA and RNA sequencing) can be performed [18].
In practical terms, different aliquots of the QC material (plasma, serum or the isolated DNA/RNA aliquots) are put at their long-term storage conditions and are periodically analyzed.

Stability is important because the end user of QC material should know until when the QC material can be used with confidence. Stability data should be therefore included in all QC instruction of use.

4.10 Suitability for use

QC users should be confident about the assigned nominal values, therefore the following items should accompany the material [16]:

- description of the material origin and procedures for minimizing contamination: reference to the procedures of the biobank related to traceability and chain of custody that reduce the risk of specimen mix-up;
- method of choice for value assignment: technical specificities of the method, and of the laboratory methods used to measure the parameters of the complementary characterization of the QC material;
- information on reference data used to support value assignment;
- a qualitative or quantitative indication of confidence in the assigned value: for example, the test results provide very strong evidence for the assigned value of QC material. A quantitative statement of confidence in assigned nominal values is, however, not required. In other words, although each laboratory method, used to measure every particular analyte, has an analytical uncertainty that can be expressed in terms of coefficient of variation (CV %), it is not necessary to provide a statement of confidence of the type.

Different biomarker determination and reporting would add value to the QC material. For example, citrate plasma specimens are suitable sources of circulating miRNAs and for extracellular vesicle analyses when prepared appropriately with:

- gentle inversion of the blood tube;

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- tube kept in an upright position;
- a 30-min precentrifugation time at room temperature;
- a centrifugation without brake;
- storage at -80°C;
- no repeated freeze-thaw cycles.

The suitability of plasma for downstream miRNA analyses can be demonstrated by the absence of haemoglobin contamination (ELISA or spectrophotometry) [12].
The suitability of plasma for downstream extracellular vesicle analyses can be confirmed by the characterization of the isolated extracellular vesicles, based on extracellular vesicle-ubiquitous surface markers, such as CD9 and CD63, and platelet-derived extracellular vesicle markers, such as CD61 and CD42.
The suitability of DNA and/or RNA for downstream sequencing or gene expression microarray studies can be demonstrated by measurement of different quality attributes (www.findmyassay.com) [18].
The QC suitability for use can be extended to the assessment of the clinical validity of kits whose context of use is the diagnosis of clinical conditions.

4.11 Preparation of Control Charts

Control chart is a graphical method for displaying control results and evaluating whether an assay protocol is in-control or out-of-control. Control results are plotted versus time or sequential run numbers. Control charts are plotted either for value assignment and continual method surveillance or for day to day routine run acceptance evaluation.

Control charts are necessary and serve for Value Assignment and/or Continual Method Surveillance. Control charts are used for Run Acceptance Evaluation either.

Day to day use and interpretation of control charts can help to:

- understand the variations that are always present in processes. Variations within the established control limits which indicate that the process is working. Variations that spike outside of the control limits indicate problems that need to be corrected;
- see when something is going wrong (or may go wrong). These problem indicators let you know that corrective action needs to be taken;
- notice patterns within plotted points. The patterns indicate possible causes, which can help to find possible solutions;
- predict future performance;
- generate new ideas for improving quality based on the selected analysis.

Specific rules of QC are applied for analytical methods, based on the Quality Control charts and the first of these rules determines rejection of an analytical run.

The laboratory should select a QC algorithm to ensure good error detection of critical shifts in performance using well-defined QC rules, which will have at least a probability of error detection (P_{ed} of 0.9). Westgard and Westgard [19] have constructed sigma tools giving QC rules and QC sample frequencies for assays with σ of 4, 5, and 6 with a P_{ed} of 0.9 [3].

The following rules can be implemented [13]:

- 1_{3s} corresponds to a Levey-Jennings chart having control limits set as the mean plus or minus 3 standard deviations. An analytical run is rejected when a single control measurement exceeds a 3s control limit.
- 2_{2s} refers to the control rule that is used with a Levey-Jennings chart when the control limits are set as the mean plus or minus 2 standard deviations. This rule can be used as a warning. When two

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consecutive control measurements exceed the same 2s control limit, this indicates the beginning of a potential systematic bias.

Control limits (are “mean ± 3s”) and warning limits (are “mean ± 2s”) are lines drawn on a control chart to provide graphical criteria for assessing whether a method is in-control or out-of-control. These control limits are either provided by the supplier in the form of a certificate of analysis or calculated from the mean and SD, determined for a given control material during internal value assignment (see section 4.6). The operator plots the control results in the relevant Control Chart with every run.

If the control material measurements fall within the expected distribution, the internal QC is successful and the run acceptance criterion linked to the QC passes. If the control value falls outside the control limits, the related run is rejected. Review of control charts is performed regularly and can be “for cause” or periodic. Laboratories need to have a documented procedure for dealing with QC failure caused by recalibration, reagent or instrument error, start-up or maintenance issues [3]. Different examples and flowcharts for dealing with a QC failure have been published [3, 8, 16, 20].

4.12 Overview of QCs implemented.

Overview of QCs implemented at each site listed in Annex 1.

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5 Proficiency Testing as external quality assessment

Proficiency Testing (PT) is a requirement for accreditation to ISO/IEC 17025 and ISO 15189 and there are different PT schemes across the food, beverage, environmental, clinical, pharmaceutical, consumer safety, forensic and petroleum sectors.

A list of PT programs and providers can be found at: https://www.eptis.bam.de/eptis/WebSearch/main

Clinical PT schemes support laboratories in their crucial role helping healthcare providers to diagnose and treat patients. More detailed information can be found at: https://www.lgcstandards.com/LU/en/Proficiency-Testing/Clinical-Schemes/cat/280743

Laboratories working with biospecimens, including biorepositories, clinical laboratories, research organisations and bioservice providers can rely on a unique PT programme to verify the precision and accuracy of testing analytical methods, and the efficiency of processing methods. It can be found following the link: https://www.ibbl.lu/ibbl-bioservices/biospecimen-proficiency-testing/

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6 Monitoring the validity of sample analytical method

Monitoring the validity of sample characterization methods is performed to ensure the validity of the methods applied and guarantee the cogency of the results obtained with those methods [5, 8]. This monitoring is achieved by:

- use of internal quality materials or purchased external reference materials (refer to section 4);
- use and periodic evaluation and update of relevant quality control charts (refer to the Sections 4.11 of this document) ;
- participation in intra-laboratory comparisons (EQA) such as annual PT (refer to Section 5 of this document) ;
- regular review (QC check) of reported method results according to the schedule implemented by each laboratory;
- use of calibrated instrumentation under regular maintenance to provide traceable results;
- correlation of results for different characteristics (for example, during annual QC).

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ANNEX I

A survey on the Quality Controls (QC) and External Quality Assurance (EQA) programs implemented by the WP4 partners (for other details please refer to the above text).

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			Quality Controls (QC)				External Quality Assurance (EQA) programs				
Analytes	**Method/platform**	**Are test results used regularly for clinical-decision making? (yes/not)**	**Do you perform internal QC in every assay? (Yes/no)**	**Do you use a QC strategy ? (Yes/no)**	**Which control material do you use? (Commercial - please specify/In house)**	**Are you available to share QC results in the framework of Revert project to harmonize results from different labs? (Yes/no)**	**Do you take part in an EQA program(s)? (Yes/no)**	**EQA scheme name(s)**	**Number of EQA runs per year**	**Are you available to share EQA results in the framework of Revert project to harmonize results from different labs? (Yes/no)**	**Notes**
CEA	Non-competive immunoassay, electrochemiluminescence / Elecsys, Roche	Yes	Yes	Yes	Included in commercial kit. Liquichek Immunoassay Plus Control, Biorad	Yes	Yes	Spanish Society of Clinical Chemistry (SEQC)	NR	Yes	NR
	Abbott				Commercial			Riqas Randox	4		NR
	COBAS 600 */ 800 Roche *				Biorad / Roche			CRR Regione Toscana	12		* Before 2019 / ** after 2019
CA19.9	Non-competive immunoassay, electrochemiluminescence / Cobas 8000, Roche	Yes	Yes	Yes	Included in commercial kit. Liquichek Tumor Marker Control, Biorad	Yes	Yes	Spanish Society of Clinical Chemistry (SEQC)	NR	Yes	NR
	Abbott				Commercial			Riqas Randox	4		NR
	COBAS 600*/ 800 Roche **				Biorad/ Roche			CRR Regione Toscana	12		NR
VIT D	chemiluminescence immunoassay (CLIA) / Liason XL	No	Yes	Yes	Included in commercial kit. LIAISON ® 25 OH Vitamin D total	Yes	No	NR	NR	Yes	NR
	Abbott	yes			Commercial		yes	Riqas Randox	4		NR
	VISTA* / COBAS 800 Roche **	yes			Biorad/ Roche		yes	CRR Regione Toscana	12		NR

To be continued

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Analytes	Method/platform	Are test results used regularly for clinical-decision making? (yes/not)	Do you perform internal QC in every assay? (Yes/no)	Do you use a QC strategy ? (Yes/no)	Which control material do you use? (Commercial - please specify/In house)	Are you available to share QC results in the framework of Revert project to harmonize results from different labs? (Yes/no)	Do you take part in an EQA program(s)? (Yes/no)	EQA scheme name(s)	Number of EQA runs per year	Are you available to share EQA results in the framework of Revert project to harmonize results from different labs? (Yes/no)	Notes
Hematology parameters (*)	Cytometry / XN-9100™, Sysmex	Yes	Yes	Yes	Included in commercial kit.	Yes	Yes	Spanish Society of Hematology and Hemotherapy (SEHH)	NR	Yes	NR
	Dasit				Commercial			Centro Ricerca Biomedica	4		NR
	Sysmex XN 9100				Dasit			CRR Regione Toscana	12		NR
Clinical chemistry parameters (**)	Different methods / Cobas, Roche	Yes	Yes	Yes	Included in commercial kit. Liquid Assayed Multiqual Bio-Rad	Yes	Yes	Spanish Society of Clinical Chemistry (SEQC)	NR	Yes	NR
	Abbott				Commercial			Riqas Randox	4		NR
	VISTA * / COBAS800 **				Biorad / roche			CRR Regione Toscana	12		* Before 2019 / ** after 2019
Coagulation parameters (***)	Clotting, chromogenic and immunological / ACL-TOP 550 CTS Instrumentation Laboratory	Yes	Yes	Yes	Included in commercial kit.	Yes	Yes	Spanish Society of Hematology and Hemotherapy (SEHH)	NR	Yes	NR
	Werfen				Commercial			UK Neqas	4		NR
	Werfen ACL TOP				Werfen			CRR Regione Toscana	12		NR
HbA1c	Capillary electrophoresis / Capillarys 3Tera	Yes	Yes	Yes	Included in commercial kit. HbA1c Capillaire Multisystemes levels 1 y 2 (SEBIA)	Yes	Yes	Spanish Society of Clinical Chemistry (SEQC)	NR	Yes	NR
	Tosoh				Commercial			Centro Ricerca Biomedica	4		NR
	ARKRAY Menarini				Menarini			CRR Regione Toscana	12		NR

To be continued

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Analytes	Method/platform	Are test results used regularly for clinical-decision making? (yes/not)	Do you perform internal QC in every assay? (Yes/no)	Do you use a QC strategy ? (Yes/no)	Which control material do you use? (Commercial - please specify/In house)	Are you available to share QC results in the framework of Revert project to harmonize results from different labs? (Yes/no)	Do you take part in an EQA program(s)? (Yes/no)	EQA scheme name(s)	Number of EQA runs per year	Are you available to share EQA results in the framework of Revert project to harmonize results from different labs? (Yes/no)	Notes
KRAS mutations	qPCR / Idylla KRAS mutation Test	Yes	Yes	Yes	Included in commercial kit (KRAS control sequence amplification)	Yes	Yes	Spanish Society of Pathology (SEAP)	NR	Yes	NR
	Real-Time PCR/Pyrosequencer / Agilent				Commercial			EMQN	4		NR
	Myriapod Colon Status SQ010 (Diatech) on MassArray System Agena Bio (Sequenom)				Commercial			EMQN	NR		NR
NRAS mutations	qPCR / Idylla NRAS-BRAF mutation Test	Yes	Yes	Yes	Included in commercial kit (NRAS/BRAF control sequence amplification)	Yes	Yes	Spanish Society of Pathology (SEAP)	NR	Yes	NR
	Real-Time PCR/Pyrosequencer / Agilent				Commercial			EMQN	4		NR
	Myriapod Colon Status SQ010 (Diatech) on MassArray System Agena Bio (Sequenom)				Commercial			EMQN	NR		NR
BRAF mutations	qPCR / Idylla NRAS-BRAF mutation Test	Yes	Yes	Yes	Included in commercial kit (NRAS/BRAF control sequence amplification)	Yes	Yes	Spanish Society of Pathology (SEAP)	NR	Yes	NR
	Real-Time PCR/Pyrosequencer / Agilent				Commercial			EMQN	4		NR
	Myriapod Colon Status SQ010 (Diatech) on MassArray System Agena Bio (Sequenom)				Commercial			EMQN	NR		NR
EGFR mutations	qPCR / Idylla EGFR mutation Test	No	Yes	Yes	Included in commercial kit (EGFR control sequence amplification)	Yes	Yes	Spanish Society of Pathology (SEAP)	NR	NR	NR
	Real-Time PCR/Pyrosequencer / Agilent	yes			Commercial	yes	yes	EMQN	4	NR	NR

To be continued

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Analytes	Method/platform	Are test results used regularly for clinical-decision making? (yes/not)	Do you perform internal QC in every assay? (Yes/no)	Do you use a QC strategy ? (Yes/no)	Which control material do you use? (Commercial - please specify/In house)	Are you available to share QC results in the framework of Revert project to harmonize results from different labs? (Yes/no)	Do you take part in an EQA program(s)? (Yes/no)	EQA scheme name(s)	Number of EQA runs per year	Are you available to share EQA results in the framework of Revert project to harmonize results from different labs? (Yes/no)	Notes
HER2	Fluorescence and Silver in situ Hybridization (FISH-SISH) / Leica and Roche	No	No	Yes	Included in commercial kit	Yes	Yes	Spanish Society of Pathology (SEAP)	NR	NR	NR
	FISH Roche	yes	yes	yes	Commercial	yes	yes	NR	NR	NR	NR
CIMP	Methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) / MRC-Holland	No	Yes	Yes	Included in commercial kit (internal and external control)	Yes	No	NR	NR	NR	NR
	Pyrosequencer	yes			Commercial	yes	yes	NR	NR	NR	NR
MSI	Immunohistochemistry MLH1,MSH2, MSH6,PMS2	Yes	Yes	Yes	Normal cells as positive internal control	Yes	No	NR	NR	NR	NR
	Real-Time PCR (Agilent Aria)/Sanger sequencer				Commercial	yes	yes	NR	NR	NR	NR
	Immunohistochemistry of MLH1, MSH2, MSH6 and PMS2 proteins OncoMate™ MSI DNA Test - CE-Marked IVD Medical				Commercial	yes	yes	EMQN	NR	yes	NR

To be continued

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Analytes	Method/platform	Are test results used regularly for clinical-decision making? (yes/not)	Do you perform internal QC in every assay? (Yes/no)	Do you use a QC strategy ? (Yes/no)	Which control material do you use? (Commercial - please specify/In house)	Are you available to share QC results in the framework of Revert project to harmonize results from different labs? (Yes/no)	Do you take part in an EQA program(s)? (Yes/no)	EQA scheme name(s)	Number of EQA runs per year	Are you available to share EQA results in the framework of Revert project to harmonize results from different labs? (Yes/no)	Notes
transcriptomic	Microarray / Whole Human Genome Oligo Microarray Kit Agillent	No	Yes	Yes	Included in commercial kit (internal and external control)	Yes	No	NR	NR	NR	NR
	MACE-Seq/Illumina	NR			in house	yes	no	NR	0	no	GenXPro has in-house quality control
methylome	Microarray / Infinium Illumina HumanMethylation450K BeadChip	No	Yes	Yes	Included in commercial kit (internal and external control)	Yes	No	NR	NR	NR	NR
	Methyl-Seq/Illumina	NR	yes	yes	in house	yes	no	NR	0	no	GenXPro has in-house quality control
Microtranscriptomic	Microarrays / Affymetrix GeneChip miRNA 3.0	No	Yes	Yes	Included in commercial kit (internal and external control)	Yes	No	NR	NR	NR	NR
microbiome	Next Generation Sequencing (NGS) / Illumina 16S Metagenomic Sequencing	No	Yes	Yes	Included in commercial kit (internal and external control)	Yes	No	NR	NR	NR	NR
Immunoscore (CD8, CD3)	Immunohistochemistry / Ventana, roche	No	Yes	Yes	Multi positive control (different tissues)	Yes	No	NR	NR	NR	NR
	Immunohistochemistry/FISH Ventana	NR	NR	NR	NR	NR	yes	NR	NR	NR	NR
PIK3CA mutations	droplet digital PCR / BioRad	No	Yes	Yes	Mutation-positive control and non-template control	Yes	No	NR	NR	NR	NR
	Illumina/ Ventana	Yes	Yes	Yes	Commercial	yes	yes	NR	NR	NR	NR
	Myriapod Colon Status SQ010 (Diatech) on MassArray System Agena Bio (Sequenom)				Commercial	yes	yes	EMQN	NR	yes	NR
PDL1	Immunohistochemistry / Ventana, Roche	Yes	Yes	Yes	ositive (tonsil) and negative (no primary antibody) control	Yes	No	NR	NR	NR	NR
	Immunohistochemistry/Ventana				Commercial	yes	yes	NR	NR	NR	NR

(*) Hb, WBC, Neu, Lymp, Mono, PLT, Neu/PLT, Neu/Lymp

(**) Glyc, BUN, Albumin, ALT, AST, gGT, Bil tot, ALP, LDH, TRIGL, CHOL, HDL, LDL, CRP

(***) PT, aPTT, Fibrinogen, D-dimer

NR - not reported

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End of D4.2

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