ISBER ENDORSED
IBBL BIOSPECIMEN PROFICIENCY TESTING 2017 PROGRAMME

PARTICIPANT'S MANUAL

Proficiency Testing Provider:

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Abbreviations

CD40L	CD40 Ligand
cfDNA	Cell-Free DNA
CSF	Cerebrospinal Fluid
DI	Dry ice
IBBL	Integrated BioBank of Luxembourg
PIIS	Processing Item Information Sheet
PT	Proficiency testing
QC	Quality control
RT	Room temperature
TIIS	Test Item Information Sheet

1 Introduction

1.1. Who We Are

The provider of this Proficiency Testing (PT) Programme is IBBL, the Integrated BioBank of Luxembourg, based in 6 rue Nicolas-Ernest Barblé, L-1210 Luxembourg, Luxembourg. IBBL is a biorepository, biorefinery, and technology centre that serves Luxembourg and its partners to collect, store and redistribute biospecimens and their related clinical data, and produces analytes suitable for analyses by state-of-the-art genomics and proteomics platforms. The major focus for IBBL's in-house research is Biospecimen Research. IBBL is ISO 9001 and NF S96-900 certified, and ISO 17025 accredited. The scope of ISO 17025 accreditation covers the DNA quantification and purity by spectrophotometry, the DNA quantification by spectrofluorometry, the RNA quantification and purity by spectrophotometry, the RNA integrity assessment, the DNA functionality and amplifiability (cross-linking) assessment by PCR, the 16S rRNA gene sequencing.

The PT Programme is endorsed by ISBER, the International Society for Biological and Environmental Repositories, based at 375 West 5th Avenue, Suite #201, Vancouver, British Columbia, Canada V5Y 1J6. ISBER is the only international forum that addresses the technical, legal, ethical, and managerial issues relevant to repositories of biological and environmental specimens. ISBER was founded in 1999 as an educational forum for discussion of repository management and dissemination of information on operational issues. Careful management ensures that specimen collections are available for study, as new biomarkers emerge and more sensitive measurement technologies become pertinent. ISBER has more than 850 institutional and individual members worldwide.

1.2. Scope

Biospecimen Proficiency Testing (PT), as defined in ISO/IEC 17043:2010 (the International Standard on "Conformity assessment – General requirements for proficiency testing"), is seen as a powerful tool to help laboratories/repositories demonstrate their competence in biospecimen processing and characterisation to researchers, industry, and accreditation bodies. PT enables laboratories/repositories to monitor their Quality Control (QC) tests over time, identify longer term trends, and consider any necessary corrective actions.

The scope of this Programme is to develop, coordinate and implement PT Programmes for biospecimen processing, QC assays and biomolecular characterisation. The PT Programmes include assays performed by repositories and/or end-users for the validation/characterisation of biospecimens and their cellular and molecular derivatives. The PT Programmes also include processing methods for the extraction of such derivatives.

1.3. Vision

This Programme is expected to improve the quality management system of repositories through PT of their processing methods and Quality Control (QC) assays. PT Programmes are designed to promote the quality and the economic health of the particular industry of biorepositories by diminishing the actual "asymmetric information" gap between biospecimen providers and biospecimen end-users. Thus, the PT Programme here proposed represents an essential infrastructural development in the field of biomarker identification and validation.

1.4. Inter-laboratory Proficiency Testing

The PT Programme belongs to the category of inter-laboratory comparison Schemes involving simultaneous participation of Laboratories in different countries in the world. Randomly selected aliquots from a source

material prepared at IBBL (the Processing Items and the Test Items) are distributed simultaneously to Participants for concurrent processing or testing.

In Processing Schemes (e.g. nucleic acid extraction), the Participants' processed "output materials" (e.g. DNA, RNA) are sent back to IBBL for isochronous testing. The results are used to assess Participants' processing efficiency.

After completion of the testing, the Participants' results are returned to the PT provider (IBBL) and compared with the assigned value(s) derived from the reference laboratories to give an indication of the performance of the individual Participants and of the group as a whole.

A "split-level" design is implemented in some quantitative Schemes, which means that similar (but not identical) analyte concentration levels are included in two or three separate aliquots of Test Items. This is used to assess Participant's analytical accuracy. Multiple measurements are used to assess Participant's analytical precision.

1.5. Organisation

ISBER and IBBL collaborate to implement the PT Programme.

IBBL acts as the PT Provider and has the responsibility for coordinating all of the activities involved in the operation of the PT Programmes and Schemes, in compliance with standard ISO/IEC 17043:2010.
IBBL has the responsibility of the production and shipment of Processing Items and Test Items.

ISBER members with detailed technical knowledge and experience are part of the **ISBER PT Advisory Group**, which supports the operations of the PT Programmes and Schemes.

2 Design of the PT Schemes

The PT Programme for 2017 includes the following Testing Schemes:

- DNA Quantification and Purity
- RNA Integrity
- RNA Quantification and Purity
- Cell Viability
- Tissue Histology
- CD154 (sCD40L) Quantification in Serum
- Haemoglobin Quantification in Plasma
- Haemoglobin Quantification in CSF

The PT Program for 2017 includes the following Processing Schemes:

- DNA Extraction from Whole Blood
- RNA Extraction from Whole Blood
- DNA Extraction from FFPE cells
- RNA Extraction from FFPE cells
- Viable PBMC Isolation
- Microbial DNA Extraction from Stool
- Microbial DNA Extraction from Saliva
- DNA Extraction from Frozen Tissue
- Total RNA Extraction from Frozen Tissue
- Cell-Free DNA (cfDNA) Extraction from Whole Blood
- Cerebrospinal fluid (CSF) Aliquoting

2.1. The "DNA Quantification and Purity" Scheme Design

The DNA used for this Scheme is extracted from whole blood obtained from a healthy donor. Three different Test Items containing DNA at a different concentration and 260/280 ratio (i.e. Tube A, Tube B and Tube C) are provided to each Participant. The Test Items are shipped at room temperature and should be stored at -80°C or lower until analysis. No processing is required at reception.

For each Test Item (Tube A, Tube B and Tube C), the Participant will need to measure the DNA concentration (ng/µl) and 260/280 ratio following his/her usual routine method. The results are collected under the following methods: Spectrophotometry, Spectrofluorometry, Microfluidic LabOnchip, Trinean Spectrophotometry (with cDROP Software) or Other.

2.2. The "RNA Integrity" Scheme Design

The RNA used for this Scheme is extracted from a Jurkat cell line by a Qiagen RNeasy mini kit method. Three different Test Items containing RNA at a different level of integrity (i.e. Tube A, Tube B and Tube C) are provided to each Participant. The Test Items are shipped on dry ice and should be stored at -80°C or lower until analysis. No processing is required at reception.

For each Test Item (Tube A, Tube B and Tube C) the RNA Integrity is measured following the Participant's usual routine testing method(s). The results are collected under the following methods: Agilent Bioanalyzer (RIN), Biorad Experion (RQI), ScreenTape R6K (SDV), QIAxcel System (RIS), Fragment Analyzer (RQN), Caliper LabChip (RQS), or Other (RIN, RQI or SDV).

2.3. The "RNA Quantification and Purity" Scheme Design

The RNA used for this Scheme is extracted from a Jurkat cell line by a Qiagen RNeasy mini kit method. Three different Test Items containing RNA at a different concentration and 260/280 ratio (i.e. Tube A, Tube B and Tube C) are provided to each Participant. The Test Items are shipped on dry ice and should be stored at -80°C or lower until analysis. No processing is required at reception.

For each Test Item (Tube A, Tube B and Tube C), the Participant will need to measure the RNA concentration (ng/µl) and 260/280 ratio following his/her usual routine method. The results are collected under the following methods: Spectrophotometry, Spectrofluorometry, Microfluidic LabOnchip, Trinean Spectrophotometry (with cDROP Software) or Other.

2.4. The "Cell Viability" Scheme Design

The cells used for this Scheme are a Jurkat cell line that is grown in culture in RPMI1640 10% FBS at a concentration of 0.1 to 1.5 x 10^6cells/mL in T175 cm^2 in a humidified incubator at 37°C, 5% CO_2. Cells are then frozen in an animal protein-free, serum-free and defined cryopreservation medium containing 10% dimethyl sulfoxide (DMSO).

Three different Test Items containing cells at a different level of viability (i.e. Tube A, Tube B and Tube C) are provided to each Participant. The Test Items are shipped on dry ice and should be stored in LN_2 until analysis. No processing is required at reception.

For each Test Item (Tube A, Tube B and Tube C) the percentage of viable cells and/or apoptotic cells is measured following the Participant's usual routine testing method(s). The results are collected under the following methods: Trypan Blue Staining or Flow Cytometry.

2.5. The "Tissue Histology" Scheme Design

The tissues used for this Scheme are taken from tumoural and non-tumoural specific tissue types (as defined by ISBER Advisory Group). Web access to five different photos (Test Item A, Test Item B, Test Item C, Test Item D, Test Item E) is granted to each Participant.

The tissue characterization/mapping is done through the assessment of the percentage of Viable Tumor, Normal Tissue, and Other (necrotic tissue, tumor stroma).

2.6. The "CD154 (CD40L) Quantification in Serum" Scheme Design

The serum used for this Scheme is aliquoted from CAT blood obtained from healthy donors. Two Test Items containing CD40L (i.e. Tube A, Tube B) are provided to each Participant. The Test Items are shipped on dry ice and should be stored in -80°C or lower until analysis. No processing is required at reception.

The CD40L concentration is measured following the Participant's usual routine testing method. The results are collected under the following method: ELISA.

2.7. Haemoglobin Quantification in Plasma

The plasma used for this Scheme is aliquoted from EDTA blood obtained from healthy donors. Two Test Items containing haemoglobin (i.e. Tube A, Tube B) are provided to each Participant. The Test Items are shipped on dry ice and should be stored at -80°C or lower until analysis. No processing is required at reception.

For each Test Item (Tube A and Tube B), the haemoglobin concentration is measured following the Participant's usual routine testing method. The results are collected under the following methods: ELISA, Spectrophotometry, Luminescent terbium complexes, Hemoglobind, Other.

2.8. Haemoglobin Quantification in CSF

Artificial CSF (diluted plasma from EDTA blood obtained from healthy donors) is used for this Scheme. Two Test Items containing haemoglobin (i.e. Tube A, Tube B) are provided to each Participant. The Test Items are shipped on dry ice and should be stored at -80°C or lower until analysis. No processing is required at reception.

For each Test Item (Tube A and Tube B), the haemoglobin concentration is measured following the Participant's usual routine testing method. The results are collected under the following methods: ELISA, Other.

2.9. The "DNA Extraction from Whole Blood" Scheme Design

One PAXgene tube of stabilized whole blood is shipped to the Participant. The Processing Item is shipped at room temperature and should be stored at room temperature or lower until analysis. No processing is required at reception.

DNA is extracted following the Participant's routine procedures (Magnetic Bead-Based, Silica Membrane-Based, Salting Out or Other) and is sent back to IBBL at room temperature for isochronous testing.

Each Participant will receive a performance report with summary statistics highlighting his individual results, in terms of total and double-stranded DNA yield per mL of whole blood, DNA purity, DNA integrity (DIN) and DNA amplifiability.

2.10. The "RNA Extraction from Whole Blood" Scheme Design

One PAXgene tube of stabilized whole blood is shipped to the Participant. The Processing Item is shipped on dry ice and should be stored at -80°C or lower until analysis. No processing is required at reception.

RNA is extracted following the Participant's routine procedures (Magnetic Bead-Based, Silica Membrane-Based, Guanidium Thiocyanate-Based or Other) and is sent back to IBBL at room temperature immediately after extraction for isochronous testing.

Each Participant will receive a performance report with summary statistics highlighting his individual results, in terms of RNA yield per mL of whole blood, RNA purity and RNA integrity.

2.11. The "DNA Extraction from FFPE Cells" Scheme Design

Two sections of FFPE Jurkat cell pellet of 20 µm thickness are shipped to the Participant. The Processing Item is shipped at room temperature and should be stored at room temperature or lower until analysis. No processing is required at reception.

DNA is extracted following the Participant's routine procedures (Magnetic Bead-Based, Silica Membrane-Based, Salting Out or Other) and is sent back to IBBL at room temperature for isochronous testing.

Each Participant will receive a performance report with summary statistics highlighting his individual results, in terms of total and double-stranded DNA yield per 20 µm section, DNA purity, DNA integrity (DIN), cross-linking and amplifiability (multiplex PCR, whole genome amplification quality assessment).

2.12. The "RNA Extraction from FFPE Cells" Scheme Design

Two sections of FFPE Jurkat cell pellet of 20 µm thickness are shipped to the Participant. The Processing Item is shipped at room temperature and should be stored at room temperature or lower until analysis. No processing is required at reception.

RNA is extracted following the Participant's routine procedures (magnetic bead-based, silica membrane-based, salting out or Other) and is sent back to IBBL at room temperature for isochronous testing.

Each Participant will receive a performance report with summary statistics highlighting his individual results, in terms of RNA yield per 20 µm section, RNA purity, RNA integrity, and mRNA quality.

2.13. The "Viable PBMC Extraction" Scheme Design

The Participant will proceed to the collection of one blood tube (any anti-coagulant permitted) from a healthy donor. The Participant will then extract the PBMC following its routine procedure. The Participant will measure the number of PBMC (cells/mL) and prepare PBMC aliquots. Expected viability and cell concentration are the following:

- Viability: minimum 80%
- Cell concentration: between $5*10^6$ and 10^7 cells/mL of medium.

The Participant will proceed to the freezing of the PBMC aliquots following its routine procedure. As soon as possible, the Participant will send all the aliquots generated to IBBL on dry ice for isochronous testing.

Each Participant will receive a performance report with summary statistics highlighting his individual results, in terms of number of viable PBMC extracted per mL of blood, PBMC viability (%) and early apoptosis/necrosis (%), PBMC function (ELISPOT).

2.14. The "Microbial DNA Extraction from Saliva" Scheme Design

One Omnigene.Oral OM-501 tube of stabilized saliva will be provided to each Participant. The Processing Item is shipped at room temperature and should be stored at room temperature or lower until analysis. No processing is required at reception.

DNA is extracted following the Participant's routine procedures (Magnetic Bead-Based, Silica Membrane-Based, Salting Out or Other) and is sent back to IBBL at room temperature for isochronous testing.

Each Participant will receive a performance report with summary statistics highlighting his individual results, in terms of total DNA yield per mL saliva, and DNA purity. IBBL will also conduct a qPCR for bacterial DNA and for human DNA, will assess the presence of PCR inhibitors and perform 16S rRNA gene sequencing.

2.15. The "Microbial DNA Extraction from Stool" Scheme Design

One Omnigene.Gut OMR-200 tube of stabilized stool will be provided to each Participant. The Processing Item is shipped on dry ice and should be stored at -80°C or lower until analysis. No processing is required at reception.

DNA is extracted following the Participant's routine procedures (Magnetic Bead-Based, Silica Membrane-Based, Salting Out or Other) and is sent back to IBBL at room temperature for isochronous testing.

Each Participant will receive a performance report with summary statistics highlighting his individual results, in terms of total DNA yield per mg stool, and DNA purity. IBBL will also conduct a qPCR for bacterial DNA and for human DNA, will assess the presence of PCR inhibitors and perform 16S rRNA gene sequencing.

2.16. The "DNA Extraction from Frozen Tissue" Scheme Design

One cryoExtract core of frozen pig liver tissue (10 – 20 mg) will be provided to each Participant. The Processing Item is shipped on dry ice and should be stored at -80°C or lower until analysis. No processing is required at reception.

DNA is extracted following the Participant's routine procedures (Magnetic Bead-Based, Silica Membrane-Based, Salting Out, Trizol or Other) and is sent back to IBBL at room temperature for isochronous testing.

Each Participant will receive a performance report with summary statistics highlighting his individual results, in terms of total and double-stranded DNA yield per mg tissue, and DNA purity. IBBL will also assess the DNA integrity (DIN) and the presence of PCR inhibitors.

2.17. The "Total RNA Extraction from Frozen Tissue" Scheme Design

One cryoExtract core of frozen pig liver tissue (10 – 20 mg) will be provided to each Participant. The Processing Item is shipped on dry ice and should be stored at -80°C or lower until analysis. No processing is required at reception.

RNA is extracted following the Participant's routine procedures (Magnetic Bead-Based, Silica Membrane-Based, Salting Out, Trizol or Other) and is sent back to IBBL at room temperature for isochronous testing.

Each Participant will receive a performance report with summary statistics highlighting his individual results, in terms of RNA yield per mg tissue, RNA purity, and RNA integrity.

2.18. CSF Aliquoting

The material used for this scheme is human cerebrospinal fluid (CSF). In this scheme two "Processing Items" (frozen and freeze-dried CSF aliquots) are shipped to each Participant on dry ice and should be stored at -80°C or lower until processing. Each Participant reconstitutes the freeze-dried aliqout with deionized water, and aliquote both Processing Items following his/her usual routine operating procedures. The CSF aliquots are shipped back on dry ice in labelled tubes.

Each Participant will receive a performance report with summary statistics highlighting his individual results, in terms of concentrations of albumin, amyloid β peptide 1-42 (Aβ1-42) and phosphorylated Tau 181 (pTau181).

2.19. The "Circulating Cell-Free DNA (cfDNA) Extraction from Whole Blood" Scheme Design

One Cell Free DNA BCT tube (Streck) of stabilized blood spiked with ultramers will be provided to each Participant. The Processing Item is shipped at room temperature and should be stored at room temperature or lower until extraction. No processing is required at reception.

DNA is extracted following the Participant's routine procedures (Magnetic Bead-Based, Silica Membrane-Based, Salting Out, or Other) and is sent back to IBBL at room temperature for isochronous testing.

Each Participant will receive a performance report with summary statistics highlighting his individual results, in terms of cfDNA yield per mL plasma. IBBL will also assess the extracted cfDNA contamination by gDNA and size distribution by microfluidic electrophoresis.

3 Criteria for Participation

3.1. General Requirements

Participation is entirely voluntary, provided the following requirements are met:

- The required facilities to perform the test are in place at the Participant site. You can check what facility/equipment you require by reviewing the relevant PIIS (Processing Item Information Sheet) and TIIS (Test Item Information Sheet), which contain basic information on the sample preparation, safety and handling instructions. PIIS and TIIS for each scheme are downloadable on IBBL website (http://www.ibbl.lu/ibbl-bioservices/biospecimen-proficiency-testing/).
- The laboratory operator at the site has the technical competence to perform the test, or has been appropriately delegated to conduct the test. Your Institution will ensure the technical competence is covered.
- The test shall be conducted under the Participant routine conditions. Directions will not be provided on the method to use for processing or analysis to avoid any deviation from your normal processing or testing conditions. Recommendations on conditions to perform the test may be available on certain Schemes.

3.2. Registration Instructions

Register directly online on the website http://biospecimenpt.ibbl.lu/. Registration for the programme 2017 will be open between May 2nd and August 11th. No registration will be accepted outside these dates.

3.2.1. Participant Coding System

Participants are being assigned a specific code to ensure confidentiality of the data they are providing. The coding is structured as follows:

- The Laboratory Number is the letter L followed by a three-digit number (L015...), unique to each Participant. Only the IBBL Project Manager knows the link between the Laboratory Number and the Participant identity.
- The Password is assigned to every individual from an Organisation. You will use the Laboratory Number and the password to log in to the website (see Section 4) to submit your results and to access your report and the global statistical analysis of all results for the specific Round/Scheme.

Once the registration is complete, you will receive an email with your Laboratory Number and password to be able to access the website to report your results.

NOTE: In case of recurrent participation, Participant will use the login information received during his/her first registration to register for next programs. Using the same account every year will allow a history of z-scores to be established, enabling the Participant to monitor consistently his/her performance over time.

3.2.2. Instructions for Participants

For all Schemes, a detailed "*Test Item Information Sheet*" (TIIS) or "*Processing Item Information Sheet*" (PIIS) is provided to all Participants with the PT Items and is also available on IBBL website (http://www.ibbl.lu/ibbl-bioservices/biospecimen-proficiency-testing/). TIIS and PIIS describe all relevant information related to the preparation of the samples, testing conditions, biohazard information and all administrative aspects to consider.

Participants have to read these instructions carefully before undertaking any operation on the Items.

Please note that concentration of the Test Items and/or nature of the samples are not provided to the Participants to avoid any bias in the performance of the test. Those details may be disclosed along with the Participant Report.

3.2.3. Timelines

Each Scheme will follow specific timelines for the shipment of PT Items, the testing phase of the Items, the return of the results by each Participant and the availability of the reports. Those timelines are indicated in the relevant PIIS and TIIS that is provided along with the Processing Items and Test Items, respectively.

Failure to comply with those timelines will result in inability to perform statistical analysis of Participant results. Results cannot be submitted after the deadline set-up for each Scheme.

3.3. Subscription to Multiple Schemes

The current fees for each Scheme are indicated below in EUR. You can participate in one or more PT Schemes, at the following prices**:

Schemes	Code	2016	2017	2018
DNA Quantification and Purity*	DNAQ17R1			
RNA Integrity*	RNAI17R1			
RNA Quantification and Purity*	RNAQ17R1			
Cell Viability*	CELL17R1	€ 399	€ 369	€ 339
CD40L Quantification in Serum	CD4017R1			
Haemoglobin Quantification in Plasma*	PLHB17R1			
Haemoglobin Quantification in CSF*	CSHB17R1			
Tissue Histology	THIS17R1	€ 299	€ 277	€ 254
CSF Aliquoting	CSAL17			
Viable PBMC Isolation***	PBMC17R1			
DNA Extraction from Whole Blood	DNABLD17			
DNA Extraction from FFPE Cells	DNAFFC17			
RNA Extraction from Whole Blood	RNABLD17			
RNA Extraction from FFPE Cells	RNAFFC17			
Microbial DNA Extraction from Saliva	DNASAL17	€ 199	€ 184	€ 169
Microbial DNA Extraction from Stool	DNASTL17			
DNA Extraction from Frozen Tissue	DNAFRT17			
Total RNA Extraction from Frozen Tissue	RNAFRT17			
Cell-Free (cfDNA) Extraction from Whole Blood	cfDNA17			

ISBER Members will benefit from a 20% discount (prices indicated above are prices for non-members). In order to receive the member discount, Organizational members will need to have their assigned delegate or alternate register. The number of Organizational member discounts will be limited to the total number of delegates and alternates held by the Organization.

Additionally, Participants who register for 2 or 3 consecutive years to the same Scheme will benefit from a discount of 7.5% or 15%, respectively.

* For these schemes, you may test multiple methods in the same run according to the lists of accepted methods. You will however only be able to submit one result per method. For DNAQ16R1, RNAQ16R1, RNAI16R1, CELL16R1, THIS16R1, PLHB16R1, CSHB16R1 and CD4016R1, you may enter replicate measurements.

**Prices are subject to change. Terms and conditions apply.

***Shipping costs are excluded for new participants.

4 Shipment of PT Items

4.1. Shipment Organization

IBBL is responsible for the preparation of the Processing Items and Test Items that will be shipped to each Participant.

Once the registration is complete and Subscription fees are received at IBBL, the PT Items required for the Scheme participation that have been requested will be prepared for shipment. Once materials are ready, Participants will be informed about the date of shipment and the estimated delivery date.

Processing Items and Test Items are prepared and packed according to the ICAO/IATA regulations and any additional local regulation applicable in the country where the Participant is located. Transporter airway-bill numbers for tracking purposes may be requested if needed.

Test Items are shipped along with the relevant TIIS. Processing Items are shipped along with the relevant PIIS.

Participants will be notified when shipping will commence so they know approximately when the PT Items will arrive. It is essential that the Participant communicate immediately to IBBL if any delay occurs in the delivery of the PT Items.

4.2. Shipment Temperatures

Depending on the conditioning of the samples to be shipped, the Schemes are categorized as either RT (Room Temperature) or DI (Dry Ice):

- DNA Quantification and Purity: RT
- RNA Integrity: DI
- RNA Quantification and Purity: DI
- Cell Viability: DI
- CD154 (CD40L) Quantification in Serum: DI
- Haemoglobin Quantification in Plasma: DI
- Haemoglobin Quantification in CSF: DI
- Tissue Histology: Shipment Temperature Not Applicable
- CSF Aliquoting: DI
- Viable PBMC isolation: Shipment Temperature Not Applicable
- DNA Extraction from Whole Blood: RT
- RNA Extraction from Whole Blood: DI
- DNA Extraction from FFPE Cells: RT
- RNA Extraction from FFPE Cells: RT
- Microbial DNA Extraction from Saliva: RT
- Microbial DNA Extraction from Stool: DI
- DNA Extraction from Frozen Tissue: DI
- Total RNA Extraction from Frozen Tissue: DI
- Circulating Cell-Free DNA (cfDNA) Extraction from Whole Blood: RT

4.3. Import/Export License

For some countries (e.g. Australia, China, South Africa), an import/export licence is required to receive/send biological samples. When registering to the PT programme, the Participant has to verify with their competent national authority whether such import/export license is required.

In such cases, the Participant has to apply him/herself to obtain this import/export permit. Once the application is complete, the Participant provides a copy or the references of his/her import/export permit to IBBL.

NOTE: The application process for an import/export license can take several months.

5 Online Submission of Results

Each Participant will need to login into the PT website http://biospecimenpt.ibbl.lu/ with the Laboratory Number and password received during registration.

Each Participant will complete the form on the website as accurately as possible, within indicated timelines.

The number of significant figures required by Participants is:

- Zero for the Tissue Histology (i.e. "0.", no figure after the delineator);
- One for the DNA concentration (i.e. "0.1", 1 figure after the delineator);
- One for the Cell Viability (i.e. "0.1", 1 figure after the delineator);
- Two for the RNA Integrity (i.e. "0.01", 2 figures after the delineator);
- Three for the DNA ratio (i.e. "0.001", 3 figures after the delineator).

Result Submission Guidelines for each Scheme are available on IBBL website (http://www.ibbl.lu/ibbl-bioservices/biospecimen-proficiency-testing/).

Failure to comply with the timelines will result in inability to perform statistical analysis of Participant results. Results cannot be submitted after the deadline set-up for each Scheme.

6 Availability of Performance Reports

6.1. Statistical Analysis Approach

Statistical procedures used are those proposed by the International Harmonised Protocol for the Proficiency Testing of Analytical Chemistry Laboratories (IUPAC technical Report 2006).

All results provided by Participants will be analysed with the same statistical method and individual results are assessed against an assigned value.

For quantitative Schemes (i.e. DNAQ16R1, RNAI16R1, RNAQ16R1, CELL16R1, CD4016R1, THIS16R1, PBMC16R1, DNABLD16, RNABLD16, DNAFFC16, RNAFFC16, DNASAL16, DNASTL16, DNAFRT16, RNAFRT16, and cfDNA16), the mean, median, standard deviation and range are provided.

For qualitative Schemes, the modes (most common responses) and range (lowest and highest response) are provided. In both cases, graphs are produced with the results from all Participants.

6.2. Evaluation of Performance

A Participant's result x is converted into a z-score according to the equation $z=(x-x_a)/\sigma_p$, where x_a is the Assigned Value and σ_p is the fitness-for-purpose based "standard deviation for inter-laboratory assessment".

The standard deviation for PT is determined by the ISBER PT Advisory Group based on approval of the following coefficients of variation:

- DNAQ16R1:
  - DNA Concentration: 25% for spectrophotometry and other methods (Trinean with c-Drop software)
  - DNA Ratio: 16%
- RNAQ16R1:
  - RNA Concentration: 25% for spectrophotometry and other methods (Trinean with c-Drop software)
  - RNA Ratio: 16%
- THIS16R1: 30% for normal tissue and 15% for viable neoplasm
- CD4016R1: 30%

For the "**DNA Quantification and Purity**", the "**RNA Integrity**", the "**RNA Quantification and Purity**", the "**Cell Viability**", the "**Haemoglobin Quantification in Plasma**", the "**Haemoglobin Quantification in CSF**" and the "**CD40L Quantification in Serum**" Schemes, the Assigned Values will be the robust mean of the Participants and the PT standard deviation will be calculated based on Participants' standard deviation.

For the "**Tissue Histology**", the Assigned Values will be defined by the reference laboratories and the standard deviations will be calculated based on the above CV values.

For **Processing Schemes** (PBMC, DNABLD, RNABLD, DNAFFC, RNAFFC, DNASAL, DNASTL, cfDNA, DNAFRT, RNAFRT, CSAL), the Assigned Values are the average obtained by all the Participants. The standard deviation is the standard deviation of all the Participants. Results are analysed globally (all extraction methods) and also by individual method. The scoring system is based on distance from the Assigned Value.

For most of the parameters measured, the following scoring system is used:

Deviation from assigned value	Consensus Score	Interpretation
< 1 standard deviation	0	"Accurate" or "Very Satisfactory"
≤ 2 standard deviation	1	"Acceptable" or "Satisfactory"
> 2 standard deviation	2	"Questionable"
> 3 standard deviation	3	"Requiring Action"

When the Assigned Value corresponds to the consensus mean of Participants, it can be required to highlight the proficiency of a participant to obtain better results than the Participants mean.
For certain parameters (e.g. nucleic acid extraction yield, RNA integrity…), better results correspond to results higher than the mean. In such cases, the scoring system applied is:

Deviation from assigned value	Consensus Score	Interpretation
< –3 standard deviation	3	"Requiring Action"
> -3 and < –2 standard deviation	2	"Questionable"
> -2 and < –1 standard deviation	1	"Acceptable" or "Satisfactory"
> -1 and < +1 standard deviation	0	"Accurate" or "Very Satisfactory"
> +1 standard deviation	0	"Accurate" or "Very Satisfactory"

For other parameters (e.g. cell apoptosis/necrosis…), better results correspond to results lower than the mean. In such cases, the scoring system applied is:

Deviation from assigned value	Consensus Score	Interpretation
< –1 standard deviation	0	"Accurate" or "Very Satisfactory"
> -1 and < +1 standard deviation	0	"Accurate" or "Very Satisfactory"
> +1 and < +2 standard deviation	1	"Acceptable" or "Satisfactory"
> +2 and < +3 standard deviation	2	"Questionable"
> +3 standard deviation	3	"Requiring Action"

The Advisory Group will review in details all the results falling into the "Questionable" and "Requiring Action" performance outcome, to provide additional advice to Participants.

6.3. Performance Reports

Reports provided to all Participants include the following: summary on the design of the Scheme, recommendations based on the outcome of the Scheme, statistical procedure used, Participant results, statistical data and summaries, including assigned values and ranges of acceptable results, procedures used to establish the standard deviation and comments on Participant performance. History of z-scores is also provided.

Once the statistical analysis and the evaluation of performance are completed, Participants will receive their reports by email.

Classification or ranking of Participants on the basis of their z-scores will not be done.

6.4. How Results Can Be Used

Upon reception of the report, the Participant can decide to use his/her PT results for publication and/or for method validation. IBBL and ISBER can also use the global results for a publication in specialized international journals. Participant identity will never be disclosed to third parties, and performance data will be kept strictly confidential unless a specific request is made by the Participant (to be released, for example, to an accrediting agency).

7 Certificate of Participation

All Participants that submit results will receive a Certificate of Participation to the Scheme(s), which they have participated in. In case of participation in a Scheme with multiple methods, one Certificate of Participation will be issued per method.

When **all** the results are "Very Satisfactory", the Certificate of Participation includes a comment specifying "Results Were Very Satisfactory".

8 Label of Participation

All Participants that submit results to any of the Scheme(s) will receive a Label of Participation. All participants may use the provided label on printed or digital media to showcase their participation in IBBL's Biospecimen Proficiency Testing (PT) programme.

9 Confidentiality

9.1. General Policy

All data related to Participant's subscription, results and performance assessment will be kept confidential and will not be disclosed to individual Participants and/or anyone else outside IBBL office members involved

in the PT Program. Performance data may be shared with regulatory and/or accreditation bodies where appropriate and necessary, but only with explicit written permission from the Participant.

As mentioned in Section 3.2.1, Participant identity and performance results are protected through a specific coding system, which will also avoid any unintentional disclosure of information.

9.2. Data Protection

IBBL will prevent the misuse of personal data held on computers and will conform to all applicable standards, e.g. Data Protection laws in force.

10 Participation Feedback and Complaints

10.1. Use of Comment Section Online

While submitting the results of Participant's tests on the samples directly on the website (http://biospecimenpt.ibbl.lu/), comments on the performance of the test may be added in the comment section. Any comment on specific process and/or material used, as well as any issue, technical or logistic that a Participant may have encountered in this phase, will be reviewed carefully.

10.2. Advisory Service on Demand

For any technical issue that a Participant may encounter and/or for any clarification needed during the testing phase, IBBL support can be contacted at ISBERPT@ibbl.lu.

10.3. Customer Satisfaction Survey

IBBL seeks Participant's feedback, both positive and negative, to be used and analysed to improve its management system, future Schemes, and customer service. At the end of the PT Scheme, Participants are encouraged to complete the Customer Satisfaction Survey, available online. The IBBL Project Manager will send to link to the survey to each Participant once Reports have been issued and received by Participants.

10.4. Complaints

IBBL welcomes the opportunity to discuss informally any problem or query that a Participant may have. A formal complaint regarding the service provided within the PT Programme should be sent by email, fax or letter and will be acknowledged in writing by the same means of communication as used to contact us.

All complaints are reviewed formally and corrective actions will be taken to resolve the complaints. A specific procedure is in place to ensure all complaints are brought to resolution.

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V01	22MAY2017	Olga KOFANOVA	New document for 2017 program

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