

# Assays for Qualification and Quality Stratification of Clinical Biospecimens Used in Research: A Technical Report from the ISBER Biospecimen Science Working Group

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This technical report presents quality control (QC) assays that can be performed in order to qualify clinical biospecimens that have been biobanked for use in research. Some QC assays are specific to a disease area. Some QC assays are specific to a particular downstream analytical platform. When such a qualification is not possible, QC assays are presented that can be performed to stratify clinical biospecimens according to their biomolecular quality.

**Keywords:** quality control, biospecimen, qualification, tissue, cells, biological fluid

## Introduction

CLINICAL BIOSPECIMENS USED in research are subject to two types of laboratory analyses. The first of these is

the analysis of established clinical biology/pathology parameters where reference ranges are usually known and methods are validated (e.g., CLIA or ISO15189 accreditation). Results of these analyses are necessary to support any

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research on novel clinically relevant biomarkers (definition of true positive and negative cases, use as a reference method). The second type is analysis of research parameters where there are usually no established reference ranges, and often methods are not validated by the laboratory as extensively as clinical biology/pathology methods.<sup>1</sup> Results of these analyses are used to discover novel clinical endpoint correlates (biomarkers).

*In vivo* and *in vitro* pre-analytical variations have a more or less significant impact on the output of analyses, depending on the biospecimen type, the pre-analytical variable, and the analyte of interest. According to the type of analysis above, the word “significant” has a different meaning. In the first type—the analysis of clinical biology/pathology parameters—“significant” means clinically consequential at the diagnostic level. In the second type—analysis of research parameters—“significant” means statistically significant. Examples illustrating this concept are shown in Table 1.

In some cases, the impact may be molecule- and even epitope-specific, for example tissue ischemia time may influence specific phospho-epitopes differently. A standard biospecimen research experimental protocol has been proposed for this type of research.<sup>2</sup>

Therefore, in all research comparing different groups of samples for biomarker discovery, it is critical that all samples are of comparable quality to avoid the introduction of uncontrolled variables and increase the power of analysis of biomarkers. There are two approaches to this end: either sample collections with careful pre-analytical annotations (SPREC),<sup>3</sup> or retrospective collections with appropriate quality control (QC) and sample qualification or quality stratification. A combination of the two approaches to control compliance of procedures with specified SPRECs is also possible.

Biobanks underpin all three layers of biomarker discovery, validation, and use in clinical practice. In the biomarker discovery phase, biospecimens collected and processed with one Standard Operating Procedure (SOP), and corresponding to one quality category, should be used in order to avoid pre-analytical bias and increase the power of research. However, in the biomarker validation phase, biospecimens collected and processed with more than one known and documented SOPs and corresponding to more than one quality category should be used in order to validate the robustness of a bio-

marker to relevant pre-analytical variations. Finally, in the biomarker clinical implementation phase, biospecimens collected and processed via validated SOPs should be used in order to ensure successful and accurate clinical diagnostic results. For these reasons, during recent years, biobank managers, auditors, and funding bodies have been asking what assays can be performed in order to assess the quality of biospecimens objectively. This technical review provides answers to this question. Although gaps exist, this review shows that many tools are already available and can be used for specimen qualification.

## Methods

For the purposes of this technical report, the members of the International Society for Biological and Environmental Repositories (ISBER) Biospecimen Science Working Group held face-to-face meetings and teleconferences between 2013 and 2015. The chair of the Working Group performed a thorough literature review and compiled a list of relevant and effective QC attributes for different categories of biospecimens. This list was reviewed and complemented by members of the Working Group. When the information is based on published evidence, the corresponding reference is given. When no reference is given, the information corresponds to current practice or to the corresponding author’s opinion.

The following definitions were used:

- *Biospecimen*: any biological specimen, which may be a:
  - *Primary sample*: specimen directly collected from the donor (e.g., whole blood, urine, solid tissue);
  - *Simple derivative*: sample prepared through a simple laboratory manipulation (e.g., after centrifugation of collection tubes or mechanical disruption of tissues) without the addition of chemical substances, and without cell disruption or cell selection as part of a multi-step process; or
  - *Complex derivative*: derivative whose isolation requires usage of multiple steps and/or addition of chemical substances (e.g., nucleic acids, proteins, lipids, sorted cells, cultured cells, immortalized cells).
- *Qualification*: process of examination of a biospecimen or a collection of biospecimens, and verification, based on

TABLE 1. EXAMPLES ILLUSTRATING THE PROBABLE IMPACT OF PRE-ANALYTICAL CONDITIONS ON THE ANALYSIS OF CLINICAL OR RESEARCH PARAMETERS

Pre-analytical condition	Biospecimen type	Analyzed parameter	Probable impact on the output of analyses
Pre-centrifugation conditions	Serum	Clinical antibodies (e.g., anti-EBV IgG)	Non-significant (clinically)
Pre-centrifugation conditions	Serum	Research cytokines (e.g., IL-8)	Significant (statistically)
Pre-centrifugation conditions	Citrate plasma	Research cytokines (e.g., IL-8)	Non-significant (statistically)
Pre-centrifugation conditions	Citrate plasma	Coagulation parameters (e.g., factor V, factor VIII)	Significant (clinically)
Formalin fixation time	Lung tissue	IHC clinical antibodies (e.g., CK7)	Non-significant (clinically)
Formalin fixation time	Lung tissue	Mutation analysis by next-generation sequencing (e.g., allele frequency <10%)	Significant (not detectable mutation)
Alcohol fixation time	Lung tissue	Mutation analysis by next-generation sequencing (e.g., allele frequency <10%)	Non-significant (detectable mutation)

CK7, cytokeratin 7; EBV, Epstein–Barr virus; IgG, immunoglobulin G; IHC, immunohistochemistry; IL8, interleukin 8.

objective analytical evidence, of their suitability for research use, either in a specific disease area or on a specific downstream analytical platform.

- **Quality stratification:** process of examination of a biospecimen or a collection of biospecimens, and their classification, based on objective analytical evidence, into distinct categories, each category corresponding to a specific *in vivo* biological characteristic (e.g., level of inflammation, % tumor, protein content) or to a specific *ex vivo* pre-analytical condition (e.g., pre-centrifugation conditions).
- **Biomolecular integrity:** quality status of a biospecimen, reflecting whether biomolecules of interest have not un-

dergone either statistically or clinically significant changes relative to their *in vivo* state/levels.

- **Commutability:** equivalence of analytical methods, based on objective evidence.

The term “qualification” is used qualitatively. Therefore, a biospecimen is or is not qualified for use in research in a specific disease area or on a specific analytical platform.

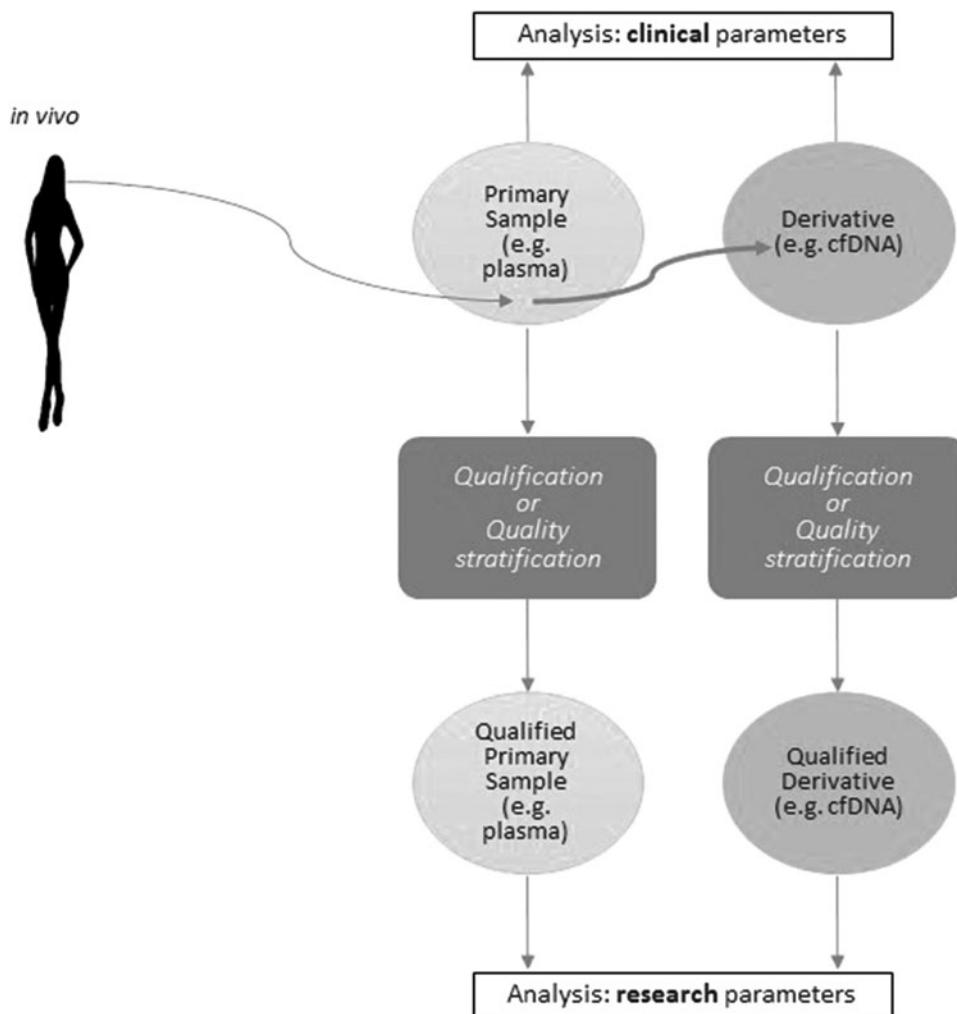
The term “quality stratification” is used quantitatively. Therefore, one or more thresholds apply in order to stratify biospecimens in two or more quality categories. These quality categories correspond to defined *in vivo* or *in vitro* conditions.

TABLE 2. QC MEASURANDS FOR QUALIFICATION FOR USE IN SPECIFIC DISEASE AREAS

Biospecimen type	Measurand	Scope of qualification (disease area)	Measurement method
Serum	Brain natriuretic peptide (BNP), NT-proBNP <sup>6</sup> Angiopoietin-like 3 (ANFPTL3) Creatinine kinase MB isoenzyme (CK-MB) Endothelin 1 (ET-1)	Cardiovascular	EIA ECLIA/EIA EIA
Heparin plasma, serum	Matrix metalloproteinase-3 (MMP-3), matrix metalloproteinase-9 (MMP-9)		EIA
All plasma, <sup>a</sup> serum	Troponin I & T		ECLIA/EIA
All plasma	Vasoactive intestinal peptide (VIP)		EIA
All plasma	Cholesterol ester transfer protein activity (CETP)	Lipid metabolism	Fluoroimmunoassay
Serum	Alanine aminotransferase (ALT) <sup>7</sup>	Liver	Enzymatic assay
Serum, all plasma	Tumor necrosis factor alpha (TNF- $\alpha$ )	Autoimmune, inflammatory	Sensitive EIA
Serum	Insulin C peptide <sup>8</sup>	Endocrinology and diabetes	Fluoroimmunoassay, EIA/RIA
All plasma	Insulin-like growth factor II precursor Glucagon-like peptide 1 (cleared by DPP4) <sup>9</sup>		EIA/RIA
All plasma, serum	Adenocorticotrophic hormone (ACTH)		ECLIA/RIA
Citrate plasma	Aldosterone Somatomedin C		EIA
Citrate plasma	Anti-factor Xa Fibrinogen Prothrombin fragments 1&2	Coagulation	Clot detection
	Plasminogen activator inhibitor type 1 activity or antigen		EIA
	Thrombin generation assay		Fluoroimmunoassay
	Tissue-type plasminogen activator antigen (TPA antigen)		EIA
Urine	Beta 2 microglobulin	Nephrology	Nephelometry, EIA/RIA
All plasma, serum	Complement C3	Inflammation,	Nephelometry, EIA
All plasma, serum	Intercellular adhesion molecule 1 (ICAM-1)	immunology	EIA
Citrate/heparin plasma, serum	TNF- $\alpha$		EIA
Serum	M65 EpiDeath	Oncology	EIA
Heparin plasma, serum	Vascular adhesion molecule I (VCAM-1)		EIA
Serum	Mid-osteocalcin, osteocalcin, calcitonin	Musculoskeletal	ECLIA, EIA
	Parathyroid hormone, intact (PTH)		ECLIA, EIA
All plasma, serum	Telopeptide C terminal, type 1 collagen		ECLIA, EIA
Serum	Vitamin B12	Nutritional	ECLIA
CSF, serum, all plasma	Amyloid Ab42	Neurodegenerative	EIA
Serum, CSF	Neuron-specific enolase <sup>10</sup>		Kryptor immunoassay, EIA

<sup>a</sup>All plasma refers to all EDTA, citrate, and heparinized plasma.

CSF, cerebrospinal fluid; DPP4, dipeptidylpeptidase 4; ECLIA, electrochemiluminescent immunoassay; EIA, enzyme immunoassay; QC, quality control; RIA, radioimmunoassay.



**FIG. 1.** Flow diagram illustrating sample preparation and qualification for use in research.

When qualification is not possible because of lack of relevant assays, then quality stratification can be made. In some cases, qualification can be achieved for biomarker research in a specific disease area (Table 2) or on a specific downstream analytical platform. For primary

samples, qualification depends on their biomolecular integrity. For simple or complex derivatives, qualification depends both on the biomolecular integrity of the primary sample from which the derivative has been extracted and on the efficiency/performance of the extraction, culture,

TABLE 3. QC MEASURANDS FOR QUALIFICATION OF FLUID BIOSPECIMENS AND THEIR DERIVATIVES

Biospecimen type	Qualification parameter	Measurand	Scope of qualification	Measurement method
Cf DNA	Contamination by blood cell DNA	DNA fragment size 100–300 bp <sup>11</sup>	Cf DNA genotyping	Microfluidic electrophoresis
Cf miRNA	Extraction efficiency	Spike in miRNA control ( <a href="http://www.qiagen.com/lum/resources/resourceDetail?id=710c0168-e408-408b-95af-91df5b5b1dd6&amp;lang=en">www.qiagen.com/lum/resources/resourceDetail?id=710c0168-e408-408b-95af-91df5b5b1dd6&amp;lang=en</a> )	Cf miRNA analysis	qRT PCR
		miRNA 16 or other ubiquitous miRNA target	Cf miRNA analysis	qRT PCR
Stool DNA	Inhibitors	SPUD <sup>12</sup>	PCR applications	qPCR
	Extraction efficiency	Bacterial DNA content	Bacterial DNA analysis	qPCR
		Human DNA content	Human DNA analysis	qPCR
Whole-blood	Inhibitors	SPUD <sup>12</sup>	PCR applications	qPCR
	cell DNA			

Cf, cell free; qRT PCR, quantitative reverse transcription polymerase chain reaction.

TABLE 4. QC MEASURANDS FOR QUALITY STRATIFICATION OF FLUID BIOSPECIMENS AND THEIR DERIVATIVES

Biospecimen type	Quality stratification parameter	Quality stratification parameter category	Measurand	Quality stratification threshold	Measurement method and reference
Serum	Pre-centrifugation conditions	>8 h 4°C	Transferrin receptor	>300 IU/mL	ELISA <sup>13</sup>
	Post-centrifugation conditions	>24 h RT	sCD40L	<4 ng/mL	ELISA <sup>14</sup>
	Coagulation conditions	Not effectively coagulated	Fibrinogen	>100 mg/mL	ELISA
	Hemolysis	Hb contaminated	Hb	>50 mg/L	ELISA, spectrophotometry (www.ifcc.org/ifccfiles/docs/130401002end.pdf) Nephelometry, ELISA <sup>15</sup> Architect instrument
Rapid serum (RST) EDTA plasma	Inflammation Pre-centrifugation conditions	Inflamed >48 h 4°C	C-reactive protein (CRP) Progastrin-releasing peptide (proGRP)	>10 mg/L <30 pg/mL	Enzymatic assays <sup>16</sup> GC MS <sup>17</sup>
	Pre-centrifugation conditions	<3 h RT	Lacrascore	<5	MxP score ≥90, 89–70, <70
	Post-centrifugation conditions	<2 h, 2–6 h, >6 h RT	Metanomics	<0.3 ng/mL	ELISA (Betsou, unpublished)
	>24 h RT	sCD40L	Complement component 3 peptide (C3f), complement component 4 (C4) Platelets	C4, 1896.1 m/z C3f, 2021.1 m/z <10 <sup>4</sup> /mL	MALDI-TOF-MS LC-ESI-MS <sup>18,19</sup>
<sup>402</sup> All plasma <sup>a</sup>	Post-centrifugation conditions	>4 h RT	β-thromboglobulin (βTG) Hb	>200 ng/mL >20 mg/L	Cell count ( <a href="https://en.wikipedia.org/wiki/Platelet-poor_plasma">https://en.wikipedia.org/wiki/Platelet-poor_plasma</a> ) ELISA, spectrophotometry <sup>21</sup> (www.ifcc.org/ifcc/vol13no4/13041002.htm)
	Platelet contamination	Platelet poor			Nephelometry, ELISA Coagulation activity assay <sup>22</sup>
	Platelet activation	Activated platelets			
	Hemolysis	Hb contaminated			
Citrate plasma	Inflammation Pre-centrifugation conditions	Inflamed >26 h 4°C	CRP F VIII:C activity	>10 mg/L <50 IU/dL	Coagulation activity assay <sup>23</sup>
	Post-centrifugation conditions	>9 years –80°C	Protein S activity	<50%	Enzymatic assay <sup>24</sup>
	Freezing	>6 months –20°C	Alkaline phosphatase activity	<0.1 IU/mmol creatinine	ELISA <sup>25</sup>
Urine	Protein content	Low, intermediate, high, very high protein content	Creatinine Cystatin C	10, 50, 100 mg/dL 10, 50, 100 ng/mL	pH
	acidity	Alkaline		>8	pH paper

(continued)

TABLE 4. (CONTINUED)

<i>Biospecimen type</i>	<i>Quality stratification parameter</i>	<i>Quality stratification parameter category</i>	<i>Measurand</i>	<i>Quality stratification threshold</i>	<i>Measurement method and reference</i>
CSF	Post-centrifugation conditions	>32 h 4°C >3 months –20°C	Transthyretin (TTR) isoforms Cystatin C (CycC) truncation	Unmodified TTR-Cys10 peak <60% Intact CycC>truncated CysC peak	ESI-MS <sup>26</sup>
Stool	Hemolysis	Hb contaminated	Hb	>15 ng/mL	MALDI-TOF-MS, SELDI MS <sup>27,28</sup>
	Inflammation	Inflamed	Calprotectin	>50 mg/kg	ELISA <sup>29</sup>
	Whole blood cell DNA	Double-strandedness	Spectrofluorimetry	>70%	Spectrophotometry, spectrofluorimetry
	Integrity	No degraded	MW	≥30 kb	Gel electrophoresis
		With no strand breaks	Long-range amplifiability	15 kb	PCR
	Purity	Not protein contaminated	A260/A280 ratio	≥21.5	Spectrophotometry
403	Damage (oxidation, deamination, alkylation)	TBD	Apurinic/apyrimidinc sites	TBD	Colorimetric detection (aldehyde reactive probe-based)
	Post-bisulfitation quality	Of high DNA integrity	PCR amplicon size	≥600 bp	Multiplex PCR <sup>30</sup>
	rRNA integrity	Of high integrity	RIN	>7	Microfluidic electrophoresis
	mRNA integrity	Not 5' degraded	mRNA index	ΔCt <1	qRT PCR <sup>31</sup>
	purity	Not protein contaminated	A260/A280 ratio	>1.6	Spectrophotometry
	Pre-centrifugation conditions	>24 h RT	Gene targets <sup>b</sup>	TBD	qRT PCR <sup>32,33</sup>
	WBC subpopulation composition	Normal composition	Lymphocytes, granulocyte, monocyte numbers	Neutrophils: 2.5–7.5 × 10 <sup>9</sup> /L Lymphocytes: 1.5–3.5 × 10 <sup>9</sup> /L Monocytes: 0.2–0.8 × 10 <sup>9</sup> /L	Blood count <sup>34</sup> ( <a href="http://emedicine.medscape.com/article/2085133-overview">http://emedicine.medscape.com/article/2085133-overview</a> )

<sup>a</sup>All plasma refers to all EDTA, citrate, and heparinized plasma.<sup>b</sup>Under investigation by the International Society for Biological and Environmental Repositories (ISBER) Biospecimen Science Working Group.  
ELISA, enzyme-linked immunosorbent assay; Hb, hemoglobin; LC-ESI-MS, liquid chromatography electrospray ionization mass spectrometry; MALDI-TOF-MS, matrix-assisted laser desorption/ionization time of flight mass spectrometry; RT, room temperature; SELDI MS, surface-enhanced laser desorption/ionization mass spectrometry; TBD, to be defined; WBC, white blood cell.

TABLE 5. QC MEASURANDS FOR QUALIFICATION OF TISSUE BIOSPECIMENS AND THEIR DERIVATIVES

Biospecimen type	Qualification parameter	Measurand	Scope of qualification	Measurement method
Frozen tissue	Freeze–thaw	Cell lysis	IHC, RNA-based analyses	H&E staining
Viable frozen tissue	Sterility	Absence of contaminants	Tissue culture	Growth on agar; mycoplasma testing
	Cryopreservation conditions	Post-thaw viability		Growth in flasks

H&E, hematoxylin and eosin.

cryopreservation, or other laboratory manipulation (e.g., cfDNA from plasma; Fig. 1).

## Results

The results are presented in the form of Tables for fluid (Tables 3 and 4), tissue (Tables 5 and 6), and cytological biospecimens and their derivatives.

Table 2 includes information on QC measurands for qualification for use of samples in specific disease areas.<sup>4,5</sup> The measurands in this table are molecules that are recognized biomarkers in the respective disease areas and are also known to be labile. Detection of the measurand above the method's level of detection is necessary (though not always sufficient) for qualification of a sample. As an example for reading Table 2, if Aβ42 is undetectable in CSF samples, then these samples cannot be qualified for research in the area of neurodegenerative diseases.

Tables 3, 5, and 7 include information that can be used for the qualification of fluid, tissue, or cytological specimens, respectively, in the scope of different types of downstream analyses. In these tables, “qualification parameter” is the quality aspect of the biospecimen that is being evaluated; “measurand” is the molecule, or the morphological or functional characteristic that is being measured and whose positive or negative result is necessary for the qualification; “scope of qualification” is the type of downstream analysis for which the biospecimen is being qualified as fit-for-purpose; and “measurement method” is the type of method that is used to measure the measurand.

Tables 4, 6, and 8 include information that can be used for the quality stratification of a fluid, tissue, or cytological biospecimen, respectively. In these tables, “qualification parameter” is the quality aspect of the biospecimen for which the biospecimen is being stratified; “measurand” is the molecule, or the morphological or functional characteristic that is being measured and whose level is used to stratify the biospecimens in categories; “quality stratification thresholds” are the levels of the measurand, which are critical for the quality stratification; and “measurement method” is the type of method that is used to measure the measurand. The quality stratification thresholds listed in Tables 4, 6, and 8 classify the biospecimens into the categories of the qualification parameter given. The “time xxx/temperature yyy” categories correspond to available experimental data, but they should be understood as “time xxx/temperature yyy or equivalent conditions.” The quality stratification thresholds listed in Tables 4, 6, and 8 are those corresponding to the measurement methods described in the

references. Application of a threshold with a measurement method that is different from the method that has been used for the establishment of the threshold requires previous demonstration of the commutability of the methods.

### Tissue type specificities

Assays for tissue qualification or quality stratification may be tissue type-specific. Some examples are given below. Fixation conditions have a significant impact on P-Akt and P-Erk1/2 in breast cancer tissue.<sup>35</sup> Ischemia has a significant impact on estrogen and progesterone receptors in breast tissue.<sup>36,37</sup> A Tissue Quality Index has been proposed for formalin-fixed, paraffin-embedded breast tissue in order to assess its cold ischemia time by immunohistochemistry.<sup>38</sup> Stathmin<sup>2–20</sup> has been proposed as indicator of degradation in brain tissue by matrix-assisted laser desorption/ionization time of flight mass spectrometry.<sup>39</sup> AKT-P has been proposed as indicator of postmortem conditions in brain tissue by western blot.<sup>40</sup> Superoxide dismutase in the liver and peptidyl-prolyl-cis-trans isomerase and insulin C-peptides in the pancreas have been associated with postmortem delay and assessed by two-dimensional difference in gel electrophoresis.<sup>41</sup>

## Discussion

This article proposes a biospecimen QC strategy, based on current state of knowledge, in the form of summary tables (Fig. 2).

The qualification and quality stratification assays presented in this technical report do not aim for an absolute assessment of the quality of samples, since a sample can be of high enough quality (fit-for-purpose) for one type of analysis (e.g., antibody analysis), but not for other types of analyses (e.g., metabolite analysis). Therefore, scientists should devote time and effort to understand and define what sample quality is needed to obtain consistent results with a given downstream analytical platform. As can be seen from Tables 3, 5, and 7, there are several gaps in the area of biospecimen qualification for use on specific analytical platforms. These include, for example, urine, saliva, or frozen tissue qualification for use in proteomic analyses, serum, plasma, or other body fluid qualification for use in miRNome analyses, or DNA qualification for use in methylation analyses. In the absence of such knowledge, this technical report offers a strategy for sample quality stratification so that bias due to samples of inconsistent quality levels can be minimized.

TABLE 6. QC MEASURANDS FOR QUALITY STRATIFICATION OF TISSUE BIOSPECIMENS AND THEIR DERIVATIVES

Biospecimen type	Quality stratification parameter	Quality stratification parameter category	Measurand	Quality stratification threshold	Measurement method and reference
Tumor	% tumor	Tumor-rich	Tumor	>70%	H&E staining, digital pathology
FFPE	Fixation time NBF Fixation conditions Cold ischemia Cold ischemia Fixation conditions (cross-linking); extraction efficiency DNA integrity	>72h NBF (no acidic formalin) >12h >12h Highly deaminated CGH compatible WGA compatible Of good integrity	None to date <sup>a</sup> Size range RT PCR None to date <sup>a</sup> None to date <sup>a</sup> qPCR ΔCt PCR amplicon size WGA score	TBD ~250 bp TBD TBD ΔCt ≥1.5 ≥200bp, ≥300 bp ≥3 µg yield	qRT PCR RT PCR qRT PCR qRT PCR Illumina FFPE QC kit Agilent NGS FFPE QC kit or equivalent <sup>42</sup> Multiplex PCR <sup>43,44</sup> WGA ( <a href="http://www.enzolifesciences.com/ENZ-42440/bioscore-screening-and-amplification-kit-20-reactions">www.enzolifesciences.com/ENZ-42440/bioscore-screening-and-amplification-kit-20-reactions</a> ) Microfluidic electrophoresis qRT PCR <sup>31</sup>
Frozen tissue FFPE DNA			Of good integrity Extremely 5' degraded Of good mRNA integrity >72h >12h TBD With no strand breaks	DIN mRNA index Size range RT PCR Gene targets <sup>a</sup> Gene targets <sup>a</sup> Phospho-Tyrosine (P Tyr 100) Long range PCR	>7  ΔCt >8 ~250 bp TBD TBD TBD 15kb
FFPE RNA	mRNA integrity Fixation time Ischemia time Ischemia time Processing/storage conditions; extraction efficiency	Of high integrity Of high integrity conditions; extraction efficiency rRNA integrity	RIN RIS DV200 or equivalent	>6	Microfluidic electrophoresis ( <a href="http://www.agilent.com/cs/library/applications/5989-1165EN.pdf">www.agilent.com/cs/library/applications/5989-1165EN.pdf</a> ), ( <a href="http://www.qiagen.com/gb/shop/automated-solutions/dna-analysis/qiaxcel-advanced-system/">www.qiagen.com/gb/shop/automated-solutions/dna-analysis/qiaxcel-advanced-system/</a> ), ( <a href="http://www.aati-us.com/product/fragment-analyzer/download_dv200_metric">www.aati-us.com/product/fragment-analyzer/download_dv200_metric</a> ) qRT PCR <sup>31</sup> Spectrophotometry Western blot <sup>46</sup>
Frozen tissue RNA	Processing/storage conditions; extraction efficiency				ΔCt <1 >1.6 285 kDa >150 kDa
Frozen tissue proteins	mRNA integrity Purity Postmortem interval/ ischemia	Not 5' degraded Not protein contaminated >48 h cold ischemia	mRNA index A260/A280 ratio αII spectrin cleavage (no 285 kDa, only 150 kDa)		

<sup>a</sup>Under investigation by the ISBER Biospecimen Science Working Group.  
FFPE, formalin-fixed, paraffin-embedded; NBF, normal buffered formalin.

TABLE 7. QC MEASURANDS FOR QUALIFICATION OF CYTOLOGICAL BIOSPECIMENS

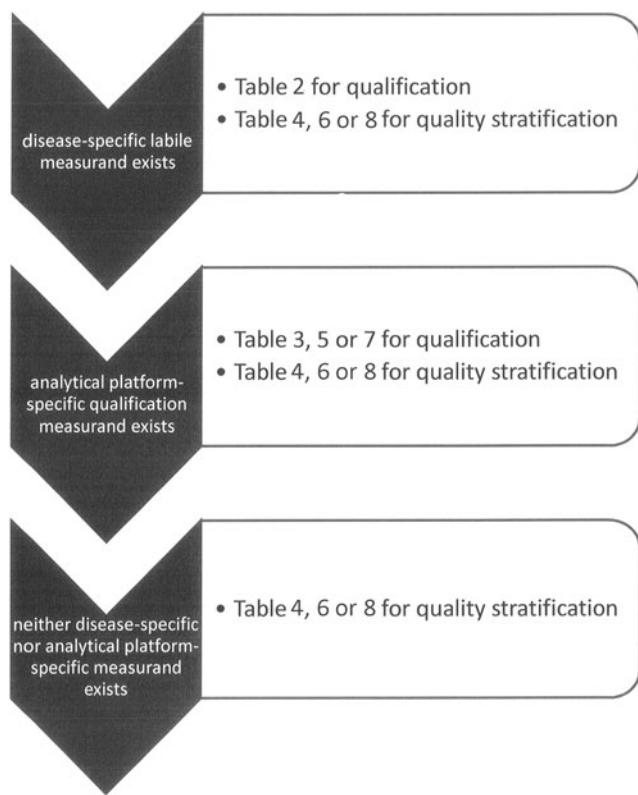
<i>Biospecimen type</i>	<i>Qualification parameter</i>	<i>Measurand</i>	<i>Scope of qualification</i>	<i>Measurement method</i>
All cell suspensions	Sterility	Absence of contaminants	Culture	Growth on agar: mycoplasma testing ICC, ELISA, FC
	Identity	Protein markers	Any type of downstream analysis	PCR, STR genotyping, FISH, karyology
	Genetic identity	Genetic identity	Any type of downstream analysis	ICC, ELISA, FC
	Purity	Absence of protein markers	Any type of downstream analysis	ICC, ELISA, FC
Genomic stability	Genomic stability	Absence of cellular impurities	Any type of downstream analysis	FC
		Chromosomal stability	Any type of downstream analysis	FC-banding, ICC, FC, microscopy
		Phenotypic stability	Any type of downstream analysis	PCR, karyology/FISH, sequencing/arrays
Cell line 406 Stem cells	STR, karyotype, SNP fingerprint <sup>47</sup>	Absence of contaminants	Culture, functional assays	Growth on agar: mycoplasma testing, HIV, HBV, HCV, EBV, CMV, syphilis, fungus, bacteria, endotoxin
	Identity	Karyotype	Any type of downstream analysis	G-banding
	Sterility	Match parent cells	Any type of downstream analysis	STR
		C-Myc, P53, p21, p16 absence of expression	Any type of downstream analysis	Immunostaining, gene expression
Lymphoblastoid cell lines (LCL)	Normal karyotype	Karyotype	Any type of downstream analysis	G-banding
	EBV transformation	EBV gene expression	Any type of downstream analysis	RT PCR <sup>48</sup>
	Cancer phenotype	EpCam+, CK8+, 18+, 19+, CD45-	Any type of downstream analysis	Immunostaining <sup>49</sup>
Cytomegalovirus; FC, flow cytometry; FISH, fluorescent <i>in situ</i> hybridization; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; ICC, immunocytochemistry; SNP, single nucleotide polymorphism; STR, short tandem repeats.				

TABLE 8. QC MEASURANDS FOR QUALITY STRATIFICATION OF CYTOLOGICAL BIOSPECIMENS

<i>Biospecimen type</i>	<i>Quality stratification parameter</i>	<i>Quality stratification parameter category</i>	<i>Measurand</i>	<i>Quality stratification threshold</i>	<i>Measurement method and reference</i>
Peripheral blood mononuclear cells (PBMCs)	Cryopreservation Specificity (granulocyte contamination)	Of high viability <12–14 h RT post venipuncture;	Post thaw viability CD15+ granulocytes	>80% <20%	FC <sub>C<sup>50</sup></sub> trypan blue
All cell suspensions	Biological activity	With no T-cell function inhibition Cell type specific	Receptors Secreted proteins mRNA expression Migration	Cell type-specific	ICC, FC, microscopy, FRET microscopy, ELISA, qRT PCR, microarray Dunn, Boyden or Impedance Chamber, Scratch assay, Matrigel invasion assay
Sperm	Concentration, viability	Of high viability	Cell number Viability	>80%	FC, impedance, microscopy
Viable RBC	DNA integrity	Of compromised DNA integrity >4 days 4°C	Acridine Orange staining and acid-induced denaturation 2,3-diphosphoglycerate (2,3-DPG)	COMP <sub>At</sub> <sup>a</sup> >30%	Viability assays
Viable platelets	Storage lesion	With highly activated platelets	Surface P selectin (CD62)	<2 mmol/L	Sperm chromatin <sub>51</sub> structure assay
Activation	Cryopreservation conditions	Efficiently cryopreserved	Colony formation and diameter doubling expression SSEA-4, expression SSEA-1	>70%	Spectrophotometry <sup>52</sup> (340 nm) <sup>52</sup>
Stem cells	Surface antigen expression of stem cell markers	Stem cell positive	Pluripotent	<5 days >80%, <20%	Flow cytometry <sup>53</sup> Colony doubling
Liquid biopsy-based cytology specimens Sorted cells	Pluripotency	Upregulation of genes associated with each of the three germ layers	Number of cells	2-fold compared to control (at least one gene per germ layer) <i>Downstream application-specific</i> Pure	qRT PCR
	Cell concentration	Purity	% of cells with expected immunophenotype, e.g.: T cells (CD3), NK cells (CD16/56), B cells (CD19/20), monocytes (CD14), functional memory B cells (CD19, CD27, CD45, CD38, CD138)	>90%	Cell count <i>application-specific</i> Flow cytometry

<sup>a</sup>COMP, cells outside the main population.

FRET, fluorescence resonance energy transfer; RBC, red blood cell; SSEA, stage-specific embryonic antigen.



**FIG. 2.** Decision tree for any given specimen type.

The information provided in this report is important because its application will enable and support bioprocessing method validation by providing relevant readouts (measurands); assessment of the quality of biospecimens of unknown history; biomarker discovery by ensuring use of qualified biospecimens or biospecimens belonging to a specific quality category; validation of biomarker robustness by using quality-stratified biospecimens belonging to different, defined quality categories; implementation of novel biomarkers in clinical practice; and characterization and production of clinical reference materials.

For the above purposes, QC measurands of clinical biospecimens can be assessed either by the biobanks themselves, or by subcontractors/collaborators who are accredited or successfully participate in relevant Proficiency Testing schemes. The results of the QC can be used by biobanks for qualification of legacy collections (the definition of cutoff values for acceptance of legacy collections or specific samples can be made and disclosed by the biobank), by end users for stratification of samples of different origins, or by funding agencies for assessment of the fitness for purpose of collections to be used in the context of grant allocation.

#### Author Disclosure Statement

F.B. is listed as co-inventor in patent no. 0704237 and in the filed patent 15195301.5-1408 (on sCD40L and LacaScore, respectively).

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