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...
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. 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.

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), 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 SPREC 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 biomarker 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

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**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        | Serum            | Clinical antibodies (e.g., anti-EBV IgG) | Non-significant (clinically) |
| conditions               | Serum            | Research cytokines (e.g., IL-8)            | Significant (statistically) |
| Pre-centrifugation        | Citrate plasma   | Research cytokines (e.g., IL-8)            | Non-significant (statistically) |
| 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 re-
search use, either in a specific disease area or on a specific
downstream analytical platform.

- **Quality stratification**: process of examination of a biospe-
cimen 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** | **Measurement method** |
| Serum | Brain natriuretic peptide (BNP), NT-proBNP<sup>6</sup> | Cardiovascular | EIA |
| | Angiopoietin-like 3 (ANFPTL3) | | ECLIA/EIA |
| | Creatinine kinase MB isoenzyme (CK-MB) | | EIA |
| | Endothelin 1 (ET-1) | | EIA |
| Heparin plasma, serum | Matrix metalloproteinase-3 (MMP-3), matrix metalloproteinase-9 (MMP-9) | | EIA |
| All plasma, serum | Troponin I & T | Lipid metabolism | ECLIA/EIA |
| All plasma | Vasoactive intestinal peptide (VIP) | | EIA |
| All plasma | Cholesterol ester transfer protein activity (CETP) | | Fluoroimmunoassay |
| Serum, all plasma | Alanine aminotransferase (ALT)<sup>7</sup> | Liver | Enzymatic assay |
| Serum, all plasma | Tumor necrosis factor alpha (TNF-α) | Autoimmune, inflammatory | Sensitive EIA |
| Serum | Insulin C peptide<sup>8</sup> | Endocrinology and diabetes | Fluoroimmunoassay, |
| All plasma | Glucagon-like peptide 1 (cleared by DPP4)<sup>9</sup> | | EIA/RIA |
| All plasma, serum | Aldosterone | | ECLIA/RIA |
| Citrate plasma | Anti-factor Xa | Coagulation | Clot detection |
| | Fibrinogen | | EIA |
| | Prothrombin fragments 1&2 | | EIA |
| | 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, immunology | Nephelometry, EIA |
| All plasma, serum | Interstitial adhesion molecule 1 (ICAM-1) | | EIA |
| Citrate/heparin plasma, serum | TNF-α | | EIA |
| Serum | M65 EpiDeath | Oncology | EIA |
| Heparin plasma, serum | Vascular adhesion molecule 1 (VCAM-1) | | EIA |
| Serum | Mid-osteoecalcin, osteocalcin, calctonin | Musculoskeletal | ECLIA, EIA |
| | Parathyroid hormone, intact (PTH) | | ECLIA, EIA |
| All plasma, serum | Telopeptide C terminal, type 1 collagen | | ECLIA, EIA |
| Serum | Vitamin B12 | Nutritional | ECLI |
| CSF, serum, all plasma | Amyloid Ab42 | Neurodegenerative | Kryptor immunoassay, EIA |
| Serum, CSF | Neuron-specific enolase<sup>10</sup> | | |

<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.
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$^{11}$ | Cf DNA genotyping | Microfluidic electrophoresis |
| Cf miRNA         | Extraction efficiency   | Spike in miRNA control (www.qiagen.com/lu/resources/resourcedetail?id=710c0168-e408-408b-95af-91df5b5b1dd6&lang=en) miRNA 16 or other ubiquitous miRNA target | Cf miRNA analysis | qRT PCR |
| Stool DNA        | Inhibitors              | Bacterial DNA content | PCR applications | qPCR |
|                  |                         | Human DNA content | Human DNA analysis | qPCR |
| Whole-blood cell DNA | Inhibitors | SPUD$^{12}$ | PCR applications | qPCR |

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

**FIG. 1.** Flow diagram illustrating sample preparation and qualification for use in research.
| 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[^13]                      |
|                  | Post-centrifugation conditions   | >24 h RT                                  | sCD40L     | <4 ng/mL                       | ELISA[^14]                      |
|                  | 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) |
|                  | Inflammation                     | Inflamed                                  | C-reactive protein (CRP) | >10 mg/mL                      | Nephelometry, ELISA[^14]         |
|                  | Rapid serum (RST)                | Pre-centrifugation conditions             | Progastrin-releasing peptide (proGRP) | <30 pg/mL                      | Architect instrument[^15]        |
|                  | EDTA plasma                       | Pre-centrifugation conditions             | Lacascore | <5                             | Enzymatic assays[^16] GC MS[^17] |
|                  | All plasma[^a]                   | Pre-centrifugation conditions             | Complement component 3 peptide (C3f), complement component 4 (C4) | C4, 1896.1 m/z, C3f, 2021.1 m/z | MALDI-TOF-MS, LC-ESI-MS[^18,19]  |
|                  | Platelet contamination            | Platelet poor                             | Platelets  | <10¹⁰/mL                       | Cell count (https://en.wikipedia.org/wiki/Platelet-poor_plasma) ELISA[^20] |
|                  | Platelet activation              | Activated platelets                       | β-thromboglobulin (βTG) | >200 ng/mL                     | ELISA, spectrophotometry[^21] (www.ifcc.org/ejifcc/vol13no4/13041002.htm) |
|                  | Hemolysis                        | Hb contaminated                           | Hb         | >20 mg/L                       | Coagulation activity assay[^22] |
|                  | Inflammation                     | Inflamed                                  | CRP        | >10 mg/L                       | Coagulation activity assay[^23]  |
| Citrate plasma   | Pre-centrifugation conditions    | >26 h 4°C                                  | F VIII:C activity | <500 IU/dL                     | Nephelometry, ELISA             |
|                  | Post-centrifugation conditions   | >9 years −80°C                             | Protein S activity | <50%                          | Coagulation activity assay[^23]  |
| Urine            | Freezing                         | >6 months −20°C                            | Alkaline phosphatase activity | <0.1 IU/mmol creatinine        | Enzymatic assay[^24] ELISA[^25]  |
|                  | Protein content                  | Low, intermediate, high, very high protein content | Creatinine | 10, 50, 100 mg/dL creatinine  | pH paper                         |
|                  | acidity                           | Alkaline                                  | pH         | >8                             | (continued)                     |
| Biospecimen type | Quality stratification parameter | Quality stratification parameter category | Measurand | Quality stratification threshold | Measurement method and reference |
|------------------|----------------------------------|------------------------------------------|-----------|---------------------------------|----------------------------------|
| CSF              | Post-centrifugation conditions   | >32 h 4°C                                | Transthyretin (TTR) isoforms | Unmodified TTR-Cys10 peak <60% | MALDI-TOF-MS, SELDI MS^27,28       |
|                  |                                  | >3 months –20°C                           | Cystatin C (CycC) truncation | Intact CycC >truncated CysC peak |                                   |
| Stool            | Hemolysis                        | Hb contaminated                           | Hb        | >15 ng/mL                        | ELISA^28                          |
| Whole blood      | Inflammation                     | Inflamed                                 | Calprotectin | >50 mg/kg                       | ELISA^29                          |
| cell DNA         | Double-strandedness              | Highly double stranded                    | Spectrophotometry | >70%                            | Spectrophotometry, spectrofluorimetry |
| Whole blood      | Integrity                        | No degraded                              | MW        | ≥30 kb                           | PCR                              |
| cell RNA         | Purity                           | With no strand breaks                    | Long-range amplifiability | 15 kb                           | Spectrophotometry                  |
| Whole blood      | Damage (oxidation, deamination, alklylation) | TBD                                       | Apurinic/apyrimidinic sites | TBD                             | Colorimetric detection (aldehyde reactive probe-based) |
| cell RNA         | Post-bisulfitation quality       | Of high DNA integrity                    | PCR amplicon size | ≥600 bp                          | Multiplex PCR^30                   |
|                  | rRNA integrity                   | Of high integrity                        | RIN       | >7                               | Microfluidic electrophoresis      |
|                  | mRNA integrity                   | Not 5’ degraded                          | mRNA index | | qRT PCR^31                        |
|                  | purity                           | Not protein contaminated                  | A260/A280 ratio | ≥1.5                            | Spectrophotometry                  |
| Whole blood      | Pre-centrifugation conditions    | >24 h RT                                 | Gene targets^b | TBD                             | qRT PCR^32,33                      |
| cell RNA         | WBC subpopulation composition   | Normal composition                       | Lymphocytes, granulocyte, monocyte numbers | Neutrophils: 2.5–7.5×10⁹/L | Blood count^34 (http://emedicine.medscape.com/article/2085133-overview) |
|                  |                                  |                                          |           | Lymphocytes: 1.5–3.5×10⁹/L      |                                   |
|                  |                                  |                                          |           | Monocytes: 0.2–0.8×10⁹/L        |                                   |

^a All plasma refers to all EDTA, citrate, and heparin plasma.
^b 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.
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. 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. Ischemia has a significant impact on estrogen and progesterone receptors in breast tissue. A Tissue Quality Index has been proposed for formalin-fixed, paraffin-embedded breast tissue in order to assess its cold ischemia time by immunohistochemistry. Stathmin has been proposed as indicator of degradation in brain tissue by matrix-assisted laser desorption/ionization time of flight mass spectrometry. AKT-P has been proposed as indicator of postmortem conditions in brain tissue by western blot. Peroxide 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.

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 miRNAome 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.
| 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 |
| Fixation time NBF| NBF (no acidic formalin)        | None to date                             | TBD      | qRT PCR                       |
| Cold ischemia    | >12 h                            | None to date                             | TBD      | qRT PCR                       |
| Frozen tissue FFPE DNA | Fixation conditions (cross-linking); extraction efficiency; DNA integrity | Highly deaminated                         | qPCR ΔCt | ΔCt ≥1.55                    |
| Fixation conditions | NBF (no acidic formalin) | Size range RT PCR                       | ~ 250 bp | Multiplex PCR^43,44           |
| Cold ischemia    | >12 h                            | None to date                             | TBD      | Illumina FFPE QC kit          |
| Frozen tissue DNA | Processing/storage conditions; extraction efficiency | With no strand breaks                  | PCR amplicon size ≥200bp, ≥300 bp | Microfluidic electrophoresis |
| mRNA integrity  | Extremely 5' degraded            | DIN                                      | >7       | qRT PCR                       |
| Fixation time    | Of good mRNA integrity           | mRNA index                               | | RT PCR                        |
| Ischemia time    | >72 h                            | Gene targets                             | TBD      | qRT PCR                       |
| FFPE proteins FFPE RNA | mRNA integrity                         | Of high integrity                       | DIN      | Microfluidic electrophoresis |
| Ischemia time    | >12 h                            | Gene targets                             | TBD      | (www.agilent.com/es/library/applications/5989-1165EN.pdf), (www.qiagen.com/gb/shop/automated-solutions/dna-analysis/qiaxcel-advanced-system/), (www.aati-us.com/product/fragment-analyzer/download_dv200_metric) |
| Frozen tissue DNA | Processing/storage conditions; extraction efficiency; rRNA integrity | Of high integrity                       | RIN      | qRT PCR^41                     |
| mRNA integrity  | Not 5' degraded                  | mRNA index                               | | RT PCR                        |
| Purity           | Not protein contaminated          | A260/A280 ratio                          | | Agilent NGS FFPE QC kit or equivalent^43 |
| Frozen tissue proteins | Postmortem interval/ischemia     | zII spectrin cleavage                    | >1.6     | PCR 46                        |
| mRNA integrity  | Not 5' degraded                  | mRNA index                               | | Spectrophotometry             |
| Purity           | Not protein contaminated          | A260/A280 ratio                          | | Western blot^46                |

^aUnder investigation by the ISBER Biospecimen Science Working Group. FFPE, formalin-fixed, paraffin-embedded; NBF, normal buffered formalin.
| Biospecimen type     | Qualification parameter | Measurand                             | Scope of qualification          | Measurement method                                                                 |
|---------------------|-------------------------|---------------------------------------|---------------------------------|-------------------------------------------------------------------------------------|
| All cell suspensions| Sterility               | Absence of contaminants               | Culture                         | Growth on agar; mycoplasma testing                                                  |
|                     | Identity                | Protein markers                       | Any type of downstream analysis  | ICC, ELISA, FC                                                                       |
|                     |                         | Genetic identity                      |                                | PCR, STR genotyping, FISH, karyology                                               |
|                     | Purity                  | Absence of protein markers            | Any type of downstream analysis  | ICC, ELISA, FC                                                                       |
|                     | Genomic stability       | Chromosomal stability                 | Any type of downstream analysis  | FC                                                                                   |
| Cell line           | Identity                | STR, karyotype, SNP fingerprint        | Any type of downstream analysis  | PCR, karyology/FISH, sequencing/arrays                                              |
| Stem cells          | Sterility               | Absence of contaminants               | Culture, functional assays       | Growth on agar; mycoplasma testing                                                  |
|                     | Normal karyotype        | Karyotype                             | Any type of downstream analysis  | G-banding                                                                           |
|                     | Identity matching       | Match parent cells                    | Any type of downstream analysis  | STR                                                                                   |
|                     | Non oncogenicity        | C-Myc, P53, p21, p16 absence of       | Any type of downstream analysis  | Immunostaining, gene expression                                                        |
|                     |                         | expression                            |                                |                                                                                      |
| Lymphoblastoid cell| Normal karyotype        | Karyotype                             | Any type of downstream analysis  | G-banding                                                                           |
| lines (LCL)         | EBV transformation      | EBV gene expression                   | Any type of downstream analysis  | RT PCR                                                                               |
| Circulating tumor   | Cancer phenotype        | EpCam+, CK8+, 18+, 19+, CD45−          | Any type of downstream analysis  | Immunostaining                                                                        |
| cells (CTC)         |                         |                                       |                                |                                                                                      |

CMV, cytomegalovirus; FC, flow cytometry; FISH, fluorescent in situ 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

| Biospecimen type | Quality stratification parameter | Quality stratification parameter category | Measurand | Quality stratification threshold | Measurement method and reference |
|------------------|----------------------------------|------------------------------------------|-----------|-------------------------------|---------------------------------|
| Peripheral blood mononuclear cells (PBMCs) | Cryopreservation | Of high viability | Post thaw viability | >80% | FC, trypan blue |
|                  | Specificity (granulocyte contamination) | <12–14 h RT post venipuncture; With no T-cell function inhibition | CD15+ granulocytes | <20% | |
| All cell suspensions | Biological activity | Cell type specific | Receptors | Cell type-specific | ICC, FC, microscopy, FRET microscopy, ELISA, qRT PCR, microarray |
|                  |                   |                                      | Secreted proteins | | |
|                  |                   |                                      | mRNA expression | | |
|                  |                   |                                      | Migration | | |
|                  |                   |                                      | Concentration, viability | >80% | FC, impedance, microscopy |
|                  |                   |                                      | Viability | | |
| Sperm | DNA integrity | Of compromised DNA integrity | Acridine Orange staining and acid-induced denaturation | COMPₐ >30% | Viability assays |
| Viable RBC | Storage lesion | >4 days 4°C | 2,3-diphosphoglycerate (2,3-DPG) | <2mmol/L | Spectrophotometry (340 nm)52 |
| Viable platelets | Activation | With highly activated platelets | Surface P selectin (CD62) | >70% | Flow cytometry53 |
| Stem cells | Cryopreservation conditions | Efficiently cryopreserved | Colony formation and diameter doubling | <5 days | Colony doubling |
|                  | Surface antigen expression of stem cell markers | Stem cell positive | expression SSEA-4, expression SSEA-1 | >80%, <20% | Immunostaining |
|                  | Pluripotency | Pluripotent | Upregulation of genes associated with each of the three germ layers | 2-fold compared to control (at least one gene per germ layer) | qRT PCR |
| Liquid biopsy-based cytology specimens | Cell concentration | Downstream application-specific | Number of cells | Downstream application-specific | Cell count |
| Sorted cells | Purity | Pure | % 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% | Flow cytometry |

COMP, cells outside the main population. FRET, fluorescence resonance energy transfer; RBC, red blood cell; SSEA, stage-specific embryonic antigen.
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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