Clinical Biospecimens: Reference Materials, Certified for Nominal Properties?

Fay Betsou

This report makes the case for clinical biospecimens to be certified for nominal properties, in particular the diagnosis, and to attain the level of Reference Materials. Clinical certified biospecimens that are collected, processed, characterized, stored, and distributed by biobanks are urgently needed to facilitate diagnostic test development, evaluation, and quality assurance. Four examples are provided to illustrate this purpose and the certification approaches that could be applied are proposed.

Introduction

RESEARCH BASED ON CLINICAL BIOSPECIMENS plays a critical role in understanding and improving human health conditions. These specimens are acquired, validated, processed, stored and distributed to academic or private research end-users by professional biobanks, but with no established process to certify their characteristics, in particular the diagnosis. Despite this, biospecimens, such as serum, urine, solid tissue, cell lines, are analyzed to identify and validate clinically relevant biomarkers, including potential prognostic, predictive, and diagnostic markers, and drug targets. The In Vitro Diagnostic (IVD) industry particularly needs accurately characterized biospecimens, and regulations on IVD submissions are increasingly demanding.^{1,2}

Biospecimens must have appropriate annotation to ensure the reliability of research results, especially when it comes to clinical validation. These annotations contain patient-related clinical and biological information and specimen-related processing (also referred to as preanalytical) information. Relevant annotations for one biospecimen often concern information obtained on a paired but different type of specimen. Recently, the essential preanalytical variables, according to the types of biospecimens collected, were defined and their reporting was standardized.^{3–5} Accurate clinical, biological, and anatomic-pathological annotations, as well as precise pre-analytical records are required, especially by diagnostic and pharmaceutical industry end-users, in order to avoid artifactual biomarkers and false discoveries.⁶

Until now, Certified Reference Materials (CRM) have essentially concerned pure chemical substances, certified for their purity and concentration (Table 1). Reference Material (RM) producers must comply with ISO Guide 34 requirements,⁷ which cover production planning, homogeneity, stability testing, and value assignment (Table 1), calculation of the total uncertainty of the assigned value, issue of a

certificate, and post-distribution service. Value assignment typically requires testing by an accredited laboratory. However, certification of a clinical biospecimen corresponds, not to the characteristics of the material, but mainly to its original clinical, biological, and pathological diagnosis. In this context, the focus of certification is not the chemical substance, but the clinical origin of the material. Therefore, application of ISO Guide 34 to biospecimens certified for a clinical nominal property (Table 1) implies a fundamental shift from the substance purity to diagnostic accuracy and related biospecimen characterization. Definition of a biospecimen as Reference Material would require accurate information on the following parameters: processing, purity, characterization, fitness-for-purpose, homogeneity, and stability. Characterization concerns both the specimen originating case (diagnosis based on clinical, biological, or histopathological characteristics) and the biospecimen itself (molecular and cellular characteristics).

The question we are trying to answer in this article is if and how clinical biospecimens can become Reference Materials (RMs), certified for a nominal property, according to ISO Guide 34.^{7,8}

Clinical Biospecimen Examples, as Reference Materials, Potentially Certified for a Nominal Property

Acute Chlamydia trachomatis infection serum (Example A)

The bacterial sexually transmitted infection (STI) pathogen *Chlamydia trachomatis* can cause serious reproductive complications such as pelvic inflammatory disease, ectopic pregnancies, and tubal infertility. Serological diagnostic tests are important in measuring past and current infection with genital chlamydial infection in assessment of women

TABLE 1. DEFINITIONS ACCORDING TO DRAFT ISO GUIDE 30:2013 (REFERENCE MATERIALS—SELECTED TERMS AND DEFINITIONS)

Reference Material	Material, sufficiently homogeneous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process
Certified Reference Material	RM characterized by a metrologically valid procedure for one or more specified properties, accompanied by a certificate that states the value of the specified property, its associated uncertainty, and a statement of metrological traceability
Characterization of a RM	Determination of the property values or attributes of a reference material, as part of the production process
Value assignment	Process by which RM property values or attributes obtained by characterization are combined and expressed in accompanying RM documentation
Property value of a RM	Value corresponding to a quantity representing a physical, chemical or biological property of a [certified] reference material
Nominal property value of a RM	Value corresponding to a qualitative attribute representing a physical, chemical or biological property of a [certified] reference material

presenting to infertility clinics. Assessment of the performance of a serological assay for chlamydial infection is based on analysis of serum samples, collected from infected patients. How can these serum samples be certified?

Lung adenocarcinoma frozen tissue-extracted DNA (Example B)

Lung cancer is the leading cause of cancer mortality worldwide. Comprehensive genomic analysis of lung adenocarcinoma samples is expected to facilitate identification of new therapeutic targets. Tumor whole genome sequencing, study of mutation patterns, and tumor DNA rearrangements are based on analysis of DNA samples, collected from tumors of patients with adenocarcinoma. How can these DNA samples be certified?

Parkinson's disease cerebrospinal fluid (CSF) (Example C)

Parkinson's disease (PD) is a chronic and progressive neurodegenerative disease of the central nervous system (CNS), and usually of the enteric nervous system (ENS), affecting mainly dopaminergic neurons. The two hallmarks of PD are: the loss of dopaminergic neurons in the substantia nigra with loss of dopamine in the striatum, and the presence of intracellular inclusion bodies in the stroma (Lewy bodies) or in neurites (Lewy neurites). The diagnosis of PD is currently made based on clinical symptoms. This is relatively late in the disease progression as symptoms occur only when about 80% of the dopaminergic neurons are lost. There is evidence that PD is a heterogeneous disorder, as the presentation symptoms and rate of decline are variable. Thus there is a need for efficient tools to diagnose the disease earlier (before appearance of motor symptoms) and to define subtypes. Diagnostic biomarkers would decrease the cost, time, and effort of diagnosis and different types of biospecimens (e.g., cerebrospinal fluid, serum, and urine) are required for identification of such biomarkers. How can PD samples be certified?

Pancreatic cancer cell line (Example D)

Pancreatic adenocarcinoma (PA) is an aggressive disease with very poor prognosis due in part to a lack of molecular information on disease development. Pancreatic cancer cell

lines are useful tools to investigate the underlying molecular events. How can these cell lines be certified?

Certification Approaches Applied

For each of these four materials, the CRM producer should be certified for the core biobanking activities, and accredited for immunological, molecular biological, and histopathological assays.

Processing of the Materials—Required Documentation

Examples A and C

For serum and CSF specimens, the following preanalytical variables should be documented: type of collection tube, pre-centrifugation delay and temperature, centrifugation conditions (speed, time, temperature, brake), post-centrifugation delay and temperature, storage container and temperature. Freeze-thaw cycles should also be documented. For CSF specimens, the site of lumbar puncture, the needle diameter and type, the syringe and collection tube material and reference should also be documented.⁹

Example B

For snap frozen tumor tissue specimens, the following preanalytical variables should be documented: type of collection (e.g., biopsy, surgery, autopsy), warm and cold ischemia times, storage container and temperature.^{3,5}

Example D

The original tissue specimen from which the cell line has been derived should be documented with data on type of collection (e.g., biopsy, surgery, autopsy), warm and cold ischemia times, and transport medium composition. The primary culture record should contain information on the method of tissue dissociation (trypsin, collagenase, primary explants culture), composition of culture medium (animal derived serum, cryoprotectant), batch numbers, passages, seeding details (cell concentration, split ratio, volume per flask), composition of freezing medium, and freezing protocol. The cell culture aliquots that are used for the different characterization assays described below should be traceable to the passage/flask ID.

Purity Assessment

Example A

Serum samples are used for the development and validation of serological diagnostic kits. The interpretation of results from serum antibodies is often difficult because of the need to ensure that each serum sample is well-characterized with regard to wide-spread contact with other chlamydial species, such as *Chlamydophila pneumoniae* and because of potential cross reactions to common chlamydial antigens. Therefore, it is important to assess the purity of a serum sample relative to antibodies specific to other chlamydial species. For this purpose, serological assays based on recombinant antigens from the different species can be used.¹⁰

Example B

The purity of tumor specimens corresponds to the percentage of non-necrotic tumor cells in the specimen. It can be defined by consensus between two certified pathologists and should be at least 80% of the whole area of the tissue section, excluding the stroma and inflammatory cells.

Example C

Absence of blood contamination in CSF is assessed by hemoglobin measurement or red blood cell count (a red blood cell count higher than $5 \times 10^6/L$ renders other quantitative measurements uninterpretable).

Example D

Purity of a cell line is established if it is not contaminated by a different cell line or by microbial contaminants. Absence of contamination by a different cell line can be assessed by STR profiling on eight different STR loci and the amelogenin locus; this profiling can reveal differences of only one or two alleles.¹¹ The species of origin can be identified, if necessary, by isoenzyme analysis, or species-specific antibodies.¹² Testing for absence of fungi, yeast, and bacterial contamination is done by visual inspection, by culturing a sample of the cell medium in nutrient broth or agar, or by PCR methods (16S, 18SrRNA genes). Mycoplasma contamination is assessed by Hoechst 33258 staining and by polymerase chain reaction (PCR), using validated commercial kits.¹³ Viruses are detected by PCR.

Nominal Property Value Assignment and Characterization (or, How to Certify that a Clinical Sample Is Really What It Is Diagnosed As)

While nominal property value assignment and characterization are required to define the diagnosis of the specimen's originating case, information such as gender, age, ethnicity, medication taken at the time of specimen collection, and informed consent should also be reported.

Serum from *C. trachomatis* acute infection (A)

Value assignment. To certify that a serum sample corresponds to acute infection, direct diagnosis of a paired urogenital specimen is performed by an accredited laboratory. *Chlamydia trachomatis* can be directly diagnosed by cul-

ture, direct antigen detection tests, or nucleic-acid based tests with or without amplification. Nucleic Acid Amplifications Tests (NAATs) have replaced culture as the reference method for the laboratory diagnosis of *C. trachomatis*. Amplification and detection of nucleic acids from *C. trachomatis* in urine specimens or in urethral/cervical swabs, based on technologies such as PCR, ligase chain reaction, strand displacement amplification, or transcription-mediated amplification can be used to confirm "acute infection." This property value is assigned to the urogenital, and by extension, to the paired serum specimen. Stringent negative controls should be included in the NAATs to avoid false positive results. Positive results by one NAAT should be confirmed using another NAAT. Alternatively, the same NAAT targeting another gene can be used.¹⁴ If only one NAAT is available, and has x% specificity, then the uncertainty of the material characterization is $(100 - x)\%$.

For past genital chlamydial infection, it is important that the serum sample is used in a serology assay that contains antigens or whole elementary bodies of different species of *Chlamydia*. Cross reactivity with different *Chlamydia* species may increase the uncertainty of its property value.

Complementary characterization. The patient origin of the serum can be verified by human DNA fingerprinting between DNA extracted from the serum and from the paired urine sample.^{11,15}

Screening of the serum for HIV, HBV, and HCV must be performed for biosafety reasons. Hemolytic and lipemic indexes are recorded.

DNA from lung adenocarcinoma tissue (B)

Value assignment. Characterization/classification has historically been based on histological and immunohistochemical analysis of tumor tissues. Molecular characterization of tumor samples is now also required. Lung adenocarcinoma, for example, can be histologically subdivided into several subtypes. These subtypes can be further subdivided based on the presence of *EGFR*, *KRAS*, *BRAF*, and *HER2* mutations and *ALK* translocations. In order to certify a tissue sample as an adenocarcinoma, clinical and anatomical indications (peripheral localization of the tumor) are documented, but the diagnostic gold standard remains histopathology. The anatomic location of the tumor (right or left lung; superior, middle or inferior lobe; segment) is documented. The histological definition of an adenocarcinoma is "a malignant epithelial tumour with glandular differentiation or mucin production, showing acinar, papillary, bronchioloalveolar (or lepidic growth pattern), or solid with mucin growth patterns, or a mixture of these patterns."¹⁶ Two certified and experienced pathologists must classify the tumor as an adenocarcinoma (WHO 8140/3) and agree on an exact histological type of adenocarcinoma, by microscopic examination of a mirror FFPE (formalin fixed paraffin embedded) sample, according to WHO¹⁶ and ICD-O codes (Table 2).

The adenocarcinoma must also be classified according to the international TNM system for pathological (pTNM) and clinical (cTNM) staging.^{17,18} Histological grading of pulmonary adenocarcinomas is based on conventional histological criteria; three grades are used: well (grade 1), moderate (grade 2), and poorly differentiated (grade 3) tumors. cTNM staging is mainly performed with imaging technologies (CT scan and MRI).

TABLE 2. WHO HISTOLOGICAL CLASSIFICATION OF ADENOCARCINOMAS

Adenocarcinoma	/8140/3
Adenocarcinoma, mixed subtype	/8255/3
Acinar adenocarcinoma	/8550/3
Papillary adenocarcinoma	/8260/3
Bronchioalveolar carcinoma	/8250/3
Nonmucinous	/8252/3
Mucinous	/8253/3
Mixed nonmucinous and mucinous or indeterminate	/8254/3
Solid adenocarcinoma with mucin production	/8230/3
Fetal adenocarcinoma	/8333/3
Mucinous (colloid) carcinoma	/8480/3
Mucinous cystadenocarcinoma	/8470/3
Signet ring adenocarcinoma	/8490/3
Clear cell adenocarcinoma	/8310/3

Complementary characterization. Complementary immunohistochemical analysis, done on the same FFPE block as the one used for histology, can be performed for the following antigens (standard profile is indicated in parentheses, although the profile depends on the subtype of adenocarcinoma and can vary from one case to another): CK7 (+), TTF1 (+), **napsin A**(+), CK5/6 (-), **p40** (-), p63 (-), chromograninA (-), CD56 (-), synaptophysin (-). The antigens in **bold** correspond to a minimum panel, which can be tested to discriminate poorly differentiated adenocarcinoma from poorly differentiated epidermoid carcinoma.

Analysis and documentation of *EGFR* mutation and *ALK* translocation are now mandatory, and are based on analysis of FFPE tumor-extracted DNA. *K-ras* oncogene, *p53* gene, and *c-erbB2* oncogene mutations can complement the molecular characterization of a pulmonary adenocarcinoma.

Cytogenetic analysis (the mean chromosome number is near the triploid range¹⁹) and CGH analysis can also complement the characterization of the adenocarcinoma sample (frequently observed imbalances are deletions on chromosomes 3p, 4q, 5q, 6q, 8p, 9, and 13q, and gains on 5p, 8q, and 20q).

The DNA sample, once extracted from the adenocarcinoma tissue, is characterized by quantification by either spectrophotometry or spectrofluorimetry. Its purity (absence of protein contamination) is assessed by the spectrophotometric ratio A260/A280 (expected to be >1.6). Its integrity is assessed by agarose gel mentioned and the composition of elution buffer is specified. The correspondence of the DNA to the patient of origin can be confirmed by DNA fingerprinting comparison between the adenocarcinoma tissue-extracted and paired whole blood-extracted DNA.^{11,20}

CSF from Parkinson's Disease (C)

Value assignment. Diagnosis of PD is based on clinical symptoms only (UK Brain Bank criteria)²¹ and these are documented in a clinical report form (CRF). The three main symptoms are bradykinesia (slow and difficult movements), muscular hypertonia (rigidity, known as “extrapyramidal”), and tremors (typically at 4 to 7 cycles per second when still). Up to 80% of PD patients develop dementia. In addition, other symptoms such as olfactory dysfunction, pain, cramps, constipation, pins and needles, orthostatic hypotension,

frequent urination, visual dysfunction, sweating, excessive production of saliva, anxiety, REM sleep behavior disorder (RBD), depression, irritability, and manias are characteristic.²² PD clinical diagnosis must be established by two independent neurologists.

To determine the stage of the disease and follow its course, neurologists use a rating scale. The most commonly used scale is the Unified Parkinson's Disease Rating Scale (UPDRS). The first four sections of UPDRS must be documented:

Part I: evaluation of mentation, behavior, and mood;

Part II: self-evaluation of the activities of daily life including speech, swallowing, handwriting, dressing, hygiene, falling, salivating, turning in bed, walking, cutting food;

Part III: clinician-scored motor evaluation;

Part IV: Hoehn and Yahr staging of severity of Parkinson's disease. (H&Y is a scale of 0 to 5; 0 being a stage without signs of disease and 5, a stage with total loss of autonomy).

A new improved version has been developed by the Movement Disorder Society (MDS) and published in 2008. The MDS-UPDRS has four parts: I. Non-motor Experiences of Daily Living; II. Motor Experiences of Daily Living; III. Motor Examination; IV. Motor Complications. Twenty questions are also completed by the patient/caregiver.²³

All the above data items are part of the value assignment and must be reported. The Parkinson subtype must also be reported: postural instability gait difficulty (PIGD), tremor dominant (TD), or indeterminate.

Complementary characterization. Tomodensitometry (TDM) and magnetic resonance imaging (MRI) are not always used to diagnose idiopathic PD when the clinical picture is clear. Likewise, olfactory tests and transcranial sonography (TCS) are not used routinely in diagnosis. However, when the clinical picture is unclear, it is necessary to eliminate other potential causes of parkinsonian syndrome (iatrogenic, vascular, toxic). For this purpose, complementary examinations can be applied, for example, DaTSCAN to differentiate essential tremor from PD, TDM, or MRI when vascular parkinsonian syndromes are suspected.

The Parkinson cognitive status can be reported: normal, MCI (mild cognitive impairment), or dementia. Cognitive markers recorded closest to biospecimen collection can be documented: MMSE (Mini Mental State Examination) total score; CDR (Clinical Dementia Rating) overall; CDR sum of boxes.

Neuropsychological tests can be performed and documented as part of the characterization: 1. a word list test (e.g., CERAD 10-word learning test) with documentation of the sum learning trials raw score, the sum learning trials z-score, the delayed recall raw score, and the delayed recall z-score; 2. A type story recall test with documentation of the sum immediate recall raw score, the sum immediate recall z-score, the delayed recall raw score, and the delayed recall z-score; 3. A type visuoconstruction test, with documentation of the copy figures raw score, the copy figures z-score; 4. A type verbal fluency test; 5. An attention/executive function test with documentation of the Trail Making Tests TMTA and TMTB raw and z-scores. If any of the following imaging has been performed within 12 months of biospecimen collection, the results must be documented: MRI, single photon emission computed tomography (SPECT), positron emission tomography (PET), fluorodeoxyglucose (FDG)

PET. If any genotyping data among the following are available, these can be reported: *SNCA*, *LRRK2*, *VPS35*, *EIF4G1*, *PARK2*, *PINK1*, *DJ-1*.

The correspondence of the CSF to the patient of origin can be confirmed by DNA fingerprinting comparison between the CSF-extracted and paired whole blood-extracted DNA.^{11,20} CSF samples are characterized by protein concentration, glucose concentration, white cell count, CSF index (CSF IgG to CSF albumin ratio compared to the serum IgG to serum albumin ratio), and oligoclonal banding.^{24,25} The most important protein biomarkers for CSF characterization, in the scope of neurodegenerative diseases, are α -synuclein, T-tau, P-tau, NF-L, A β ₁₋₄₂, and DJ-1.²⁶

Cell line from pancreatic cancer (D)

Value assignment. Characterization of pancreatic cell lines includes information on clinical history, *in vitro* and *in vivo* growth characteristics, phenotypic characteristics (adhesion, invasion, migration, tumorigenesis, all of which can be compared to a well established and commercially available cell line, such as Capan-1), and genotypic characteristics.

Specific factors include:

1. Site of derivation of the initial tumor specimen, exact histological characterization, pTMN, cTNM classification, ICD-O classification, is documented by two independent certified pathologists.
2. Adhesive ability of the cell line to different extracellular matrix proteins (fibronectin, collagen I, collagen IV, laminin) must be tested and documented. Documentation includes the percent adherence and the length of time allowed for adhesion, but also technical details on the cell quantification method used, the cell culture conditions and extracellular matrix handling.
3. Invasive ability of the cell line, monitoring cell movement through Matrigel (a mixture of laminin, type IV collagen, entactin, and heparin sulfate) are tested and documented. Invasive ability is expressed as mean invaded cells per high-powered field, cells/chamber, cells/cm², or percent of added cells having completely migrated into the bottom chamber. The length of time allowed for invasion, the cell culture conditions, the cell quantification method, and the invasiveness assay or commercially available kit should be documented.

Levels of pro-angiogenic factors (COX-2, PGE2, VEGF, IL-1 α , IL-8) secreted by the cell line are used as a surrogate measure of the angiogenic potential. Pro-angiogenic factors are preferably expressed in absolute units (pg/mL, pg/mg protein, pg/10⁶ cells) and again, the exact experimental conditions should be documented. Tumorigenicity, which describes a cancer cell line's ability to produce tumors *in vivo*, is measured after injecting a suspension of pancreas cancer cells into the subcutaneous tissue of immunocompromized mice and allowing a tumor to grow. Tumorigenicity is expressed as tumor volume and/or mass (mm³) formed from a specified injected number of cells (e.g., 10⁶ cells) after a specified time period (20–50 days).

Complementary characterization. Genotypic analysis should include mutation analysis of the *KRAS*, *TP53*, *CDKN2A*, and *SMAD4* genes, which are frequently mutated in pancreatic cancers (<http://pathology2.jhu.edu/pancreas/geneticsweb/profiles.htm>).

Any cell line can be characterized by short tandem repeat (STR) profiling with previously published STR primers.¹⁰ It is advisable to submit any cell line to a recognized central cell bank (ATCC, DSMZ, ECACC, JCRB, RIKEN) and to submit the STR profiling data to a central database (Cell Line Data Base, <http://bioinformatics.istge.it/hypercldb/>, Cell Line Integrated Molecular Authentication database, <http://bioinformatics.istge.it/clima/>).²⁷ The same STR analysis can be used to connect the cell line to its original patient, by DNA fingerprinting on the cell line and the paired whole blood-extracted DNA.^{11,15,28}

Confirmation of the tissue of origin can be made by immunocytochemistry with tissue specific markers on fixed cells, for example, carboxypeptidase B for pancreas specificity.

Finally, cell concentration and post-thaw cell viability must be reported.

Suitability Study (or How to Certify That a Clinical Sample Is Fit-For-Purpose)

Example A

If the downstream application is related to serum immunoglobulins, any serum sample, with documented standard pre-analytical code (SPREC) and stored at –80°C is fit-for-purpose. Presence of anti-*C. trachomatis* IgG, IgA, and/or IgM antibodies in the serum sample can be confirmed by a CE-marked or FDA-approved immunosorbent assay (ELISA).

The preanalytical quality of the serum can be assessed by measuring soluble CD40L.²⁹ Concentration of sCD40L should be higher than 6 ng/mL. Using this cut-off provides a sensitivity of 97.5% in eliminating serum samples that have been exposed to room temperature for over 48 h.

Example B

The suitability of the DNA sample for downstream whole genome amplification (WGA) or comparative genome hybridization (CGH) studies can be assessed by multiplex PCR³⁰ and by assessment of inhibition of amplification by the SPUD assay.³¹

Example C

The suitability of CSF for downstream proteomic applications can be assessed by the absence of hemoglobin (ELISA).

Example D

The suitability of a cancer cell line, for any kind of downstream application, can be assessed by testing the phenotypic characteristics described above.

Homogeneity Study

Samples A and C

Homogeneity of serum or CSF samples can be assessed by microparticle counting. Total microparticle counting can be performed by an impedance-based method. Serum homogeneity testing, relative to anti-*C. trachomatis* antibodies, measured by immunosorbent assay, must also be performed.

TABLE 3. ASSAYS USED FOR NOMINAL (CLINICAL) PROPERTY VALUE CERTIFICATION AND COMPLETE CHARACTERIZATION OF FOUR EXAMPLES OF BIOSPECIMEN TYPES

Immunological	Cell biology/Functional	Molecular biology	Histopathology	Imaging	Other
ELISA IgG, IgA, IgM HIV, HBV, HCV ELISA ELISA pro-angiogenic factors ELISA sCD40L ELISA hemoglobin ELISA A β ₁₋₄₂ , T-tau, P-tau, α -synuclein, NF-L, DJ-1 Isoelectric focusing and immunoblotting	Cell counting Cell viability Adhesive ability Invasive ability Tumorigenicity Flow cytometry	STR profiling NAAT microbial DNA PCR 16S, 18SrRNA PCR Mycoplasma Genotyping Karyotyping SPUD assay DNA methylation	Histology Immunohistochemistry Immunocytochemistry	MRI TDM SPECT PET	Isoenzyme analysis Spectrophotometry Spectrofluorimetry Proteomics Metabolomics

Sample B

Homogeneity of DNA samples can be assessed by spectrophotometry.

Sample D

Homogeneity of pancreatic cancer cell lines cannot be assessed. Pancreatic cancer cell lines should be considered as heterogeneous populations of cells because of genetic drift and the presence of different tumor cell subtypes. Although it sounds inconsistent to certify a reference material without homogeneity assessment, this reflects the actual clinical situation.³² Homogeneity can still be assessed relative to attributes such as viable/apoptotic cell subpopulation percentages, or different immunophenotypic cell subpopulation percentages, by flow cytometry.

Stability Study

Sample A

Short- and long-term stability studies of serum, relative to anti-*C. trachomatis* IgG, IgA, and IgM antibodies, measured by immunofluorescence and/or immunoenzymatic assays, should be performed.

Sample B

Short- and long-term stability studies of DNA, relative to specific downstream applications (e.g., whole genome sequencing, methylation analysis) should be performed.

Sample C

Short- and long-term stability studies of CSF, relative to specific downstream applications (e.g., proteomics, metabolomics) should be performed.

Sample D

Stability of a cancer cell line should be assessed relative to potential genetic drift over sequential passages. STR profile, karyotype (development of aneuploidy and heteroploidy), and gene mutations should be assessed regularly over passages.

Conclusion on Clinical Biospecimens as RMs Certified for Nominal Properties

The diagnostic and pharmaceutical industries need CRMs to raise the standards of regulatory submissions based on clinical biospecimen analysis. A few Reference Materials exist that are certified for nominal properties, essentially in microbiology.³³ Now, the certification process needs to be extended to many more clinical biospecimen types, provided by professional biobanks. Different examples of simple (serum, CSF) and complex (DNA extracted from tissue, cell line derived from tissue) derivatives of clinical specimens were used to illustrate a potential RM certification process. The properties for which these materials can be certified are nominal: *C. trachomatis* acute urogenital infection, lung adenocarcinoma, Parkinson's disease, or pancreas cancer. The collection of the data necessary for this value assignment is part of the biobank's activities. Compliance to biobank Best Practices³⁴ is necessary to ensure consistency,

robustness, and traceability in the chain of biobanking processes, including data collection. Moreover, we show that for these nominal properties to be accurate, various clinical, biological, histopathological, immunological, cell biology, and molecular biology data have to be collected and reported. Although the certified value is nominal, some of the certification attributes (e.g., homogeneity and stability) may need to be assessed by quantitative assays. The need for accurate performance of many assays (Table 3) is the reason that not only compliance to Best Practices, but also accreditation is required for biobanks to operate as CRM producers.³⁵ Certainly not all biobanked samples should be characterized to this level of detail. However, we believe that biobanks, accredited in the scope of the relevant characterization assays, can become producers of reference materials, certified for nominal clinical properties.

Acknowledgments

I am grateful to Laurent Antunes, Pieter Visser, Barbara Parodi, and Rosanna Peeling for review of the manuscript in their respective areas of expertise.

Author Disclosure Statement

No competing financial interests exist.

References

- European Medicine Agency. Reflection paper on co-development of pharmacogenomic biomarkers and assays in the context of drug development. EMA/CHMP/641298/2008.
- European Parliament. Proposal for a Regulation of the European Parliament and of the Council on *in vitro* diagnostic medical devices. COM 2012/0267.
- Betsou F, Lehmann S, Ashton G, et al. Standard pre-analytical coding for biospecimens: Defining the sample PREanalytical Code (SPREC). *Cancer Epidemiol Biomarkers Prev* 2010;19:1004–1011.
- Moore HM, Kelly A, Jewell SD, et al. Biospecimen reporting for improved study quality. *Biopreserv Biobank* 2011;9:57–70.
- Lehmann S, Guadagni F, Moore H, et al. Standard preanalytical coding for biospecimens: Review and implementation of the Sample PREanalytical Code (SPREC). *Biopreserv Biobank* 2012;10:366–374.
- Diamandis EP. Cancer biomarkers: Can we turn recent failures into successes? *J Natl Cancer Inst* 2010;102:1462–1467.
- ISO General requirements for the competence of reference materials producers, International Organization for Standardization, Geneva, Switzerland, ISO Guide 34:2009.
- Nordin G, Dybkaer R, Forsum U, Fuentes-Arderiu X, Schadow G, Pontet F. An outline for a vocabulary of nominal properties and examinations—Basic and general concepts and associated terms. *Clin Chem Lab Med* 2010;48:1553–1566.
- Teunissen CE, Petzold A, Bennett JL, et al. A consensus protocol for the standardization of cerebrospinal fluid collection and biobanking. *Neurology* 2009;73:1914–1922.
- Radouani F, Maille J, Betsou F. Serological profiling with Chlamycheck, a commercial multiplex recombinant antigen Western blot assay of chlamydial infections. *Can J Microbiol* 2007;53:1360–1368.
- Dirks WG, Frexler HG. Authentication of scientific human cell lines: Easy-to-use DNA fingerprinting. *Methods Mol Biol* 2005;290:35–50.
- Hay RJ. Cell Quantitation and characterization. In: *Methods of Tissue Engineering*, Atala A, Lanza RP (eds), Academic Press, San Diego: pp. 55–84, 2002.
- European Pharmacopoeia 2007; 5.8–2.6.7.
- Chernesky MA. The laboratory diagnosis of Chlamydia trachomatis infections. *Can J Infect Dis Med Microbiol* 2005;16:15–25.
- Reid Y, Mintzer J. The current state of cell contamination and authentication—and what it means to biobanks. *Biopreserv Biobank*. 2012;10:236–238.
- WHO Classification of Tumours. Tumours of the lung, pleura, thymus and heart. IARC Press 2004.
- UICC, International Union Against Cancer (UICC): *TNM Classification of Malignant Tumours*. 6th ed. Wiley and Sons: New York, 2002.
- Rami-Porta R, Crowley JJ, Goldstraw P. The revised classification TNM staging system for lung cancer. *Ann Thorac Cardiovasc Surg* 2009;15:4–9.
- Mitelman F, Johansson B, Mertens F. Mitelman database of chromosome aberrations in cancer, 2003 <http://cgap.nci.nih.gov/chromosomes/mitelman>. Accessed 26 March 2014.
- Kofanova O, Betsou F. DNA fingerprinting: A quality control case study for human biospecimen authentication. *Biopreserv Biobank*. 2014;17:151–153.
- Hughes AJ, Daniel SE, Kilford L, Lees AJ. Accuracy of clinical diagnosis of idiopathic Parkinson's disease. A clinico-pathological study of 100 cases. *JNNP* 1992;55:181–184.
- Berg D, Lang AE, Postuma RB, Maetzler W, Deuschl G, Gasser T, Siderowf A. Changing the research criteria for the diagnosis of Parkinson's disease: obstacles and opportunities. *Lancet Neurol* 2013;12:514–524.
- Goetz GC, Tilley BC, Shaftman SR, et al. Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS): Scale presentation and clinimetric testing results. *Movement Dis* 2008;23:2129–2170.
- Reiber H, Peter JB. Cerebrospinal fluid analysis—Disease related data patterns and evaluation programs. *J Neurol Sci* 2001;184:101–122.
- Reiber H, Otto M, Trendelenburg C, Wormek A. Reporting cerebrospinal fluid data—Kknowledge base and interpretation software. *Clin Chem Lab Med* 2001;39:324–332.
- Parnetti L, Castrioto A, Chiasserini D, Persichetti E, Tambasco N, El-Agnaf O, Calabresi P. Cerebrospinal fluid biomarkers in Parkinson disease. *Nature Rev Neurol* 2013; 9:131–140.
- Romano P, Manniello A, Aresu O, Armento M, Cesaro M, Parodi B. Cell line Data Base: Structure and recent improvements towards molecular authentication of human cell lines. *Nucleic Acids Res* 2009;37:D925–D932.
- Freshney RI. Cell line provenance. *Cytotechnology* 2002; 39:55–67.
- Lengellé J, Panopoulos E, Betsou F. Soluble CD40-Ligand as a biomarker for storage-related preanalytic variations of human serum. *Cytokine* 2008;44:275–282.
- van Beers EH, Joosse SA, Ligtenberg MJ, Fles R, Hogervorst FBL, Verhoef S, Nederlof PM. A multiplex PCR predictor for aCGH success of FFPE samples. *Br J Cancer* 2006;94:333–337.
- Nolan T, Hands RE, Ogunkolade W, Bustin SA. SPUD: A quantitative PCR assay for the detection of inhibitors in nucleic acid preparations. *Anal Biochem* 2006;351: 308–310.

32. Deer EL, Gonzalez-Hernandez J, Coursen JD, et al. Phenotype and genotype of pancreatic cancer cell lines. *Pancreas* 2010;39:425–435.
33. Van Iwaarden P, Meeus N, Corbisier P. Certification of a Reference Material of purified genomic DNA from *Campylobacter jejuni* (NCTC 113351)–IRMM448, 2007 Publications Office of the European Union, LU, EUR 22504 EN, ISBN 92-79-03469-3.
34. ISBER Best Practices for Repositories: Collection, storage, retrieval and distribution of biological materials for research. Third Edition. *Biopreserv Biobank* 2012;10: 79–161.
35. Betsou F, Luzergues A, Carter A, et al. Towards norms for accreditation of biobanks for human health and medical

research: Compilation of existing guidelines into ISO certification/accreditation norm-compatible format. *Qual Assur J* 2007;11:219–292.

Address correspondence to:

Dr. Fay Betsou
IBBL (Integrated Biobank of Luxembourg)
6 rue Ernest Barblé
1210 Luxembourg
Luxembourg

E-mail: fay.betsou@ibbl.lu