Clinical Biochemistry 47 (2014) 280–287

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

Tools to assess tissue quality

Veronique M. Neumeister

Department of Pathology, Yale University School of Medicine, 310 Cedar Street, LMP101, New Haven, CT 06520, USA

ARTICLE INFO

Article history:
Received 22 November 2013
Received in revised form 12 February 2014
Accepted 13 February 2014
Available online 22 February 2014

Keywords:
Biospecimens
Tissue quality
Pre-analytical variables
Degradation of proteins and nucleic acids
Tools to assess tissue quality

ABSTRACT

Biospecimen science has recognized the importance of tissue quality for accurate molecular and biomarker analysis and efforts are made to standardize tissue procurement, processing and storage conditions of tissue samples. At the same time the field has emphasized the lack of standardization of processes between different laboratories, the variability inherent in the analytical phase and the lack of control over the pre-analytical phase of tissue processing. The problem extends back into tissue samples in biorepositories, which are often decades old and where documentation about tissue processing might not be available. This review highlights pre-analytical variations in tissue handling, processing, fixation and storage and emphasizes the effects of these variables on nucleic acids and proteins in harvested tissue. Finally current tools for quality control regarding molecular or biomarker analysis are summarized and discussed.

Introduction

Increasing efforts towards personalized and targeted cancer therapy are based on molecular characterization of patients' blood and tissues. Research to identify useful biomarkers for development of new drugs and companion diagnostic tests relies heavily on the availability of human tissue samples and connected databases. Biobanks are systematic collections of samples of human body substances for storage and management of the specimens and of data containing information of the donor [1]. While nearly two thirds of the biobanks in the United States have been established within the past decade, almost 20% of the existing biobanks have been in use since 20 years or longer, with a few of them being even 50 years old [2]. Serum and plasma are stored in 77% of biobanks; solid specimens are the second most commonly stored form of human sample archiving and are reported to be stored in 69% of the biobanks in the United States [2]. The biobanking repositories are poised to expand as human tissue is used for research and development of pharmaceutical and diagnostic tools, and while the number of their tissue specimens was estimated around 300 million samples at the turn of the century, this number increases by 20 million each year [3]. However, concerns about access and availability of high quality human biospecimens for research and discovery purposes are growing and demands for high quality tissue are increasing despite the seemingly abundance of samples stored in biorepositories [3,4].

The variables in tissue handling and processing

Within the past years advances have been made in quality control of the analytical part of human tissue and of the interpretation of specific tests leading to publication of guidelines for biomarker evaluation in breast cancer by the College of American Pathologists (CAP) [5,6]. The critical role of sample preparation and storage has been found to be an important factor for the validity of all human samples, especially if standardization and quality control of specimens are not established [7]. Variability in sample handling and storage has been shown to affect the integrity of stored specimens [8], emphasizing the need for standardization and quality control tools of existing repositories. Specimen collection and storage might ultimately impact the utility of clinical specimens not only for clinical testing but also for research and drug development. The samples stored in biobanks range from frozen tissue to formalin fixed, paraffin embedded (FFPE) tissue, to plasma, blood and cell cultures. Formalin fixation with paraffin embedding is probably the most common and widely used method for tissue preservation and stabilization prior to histological evaluation [9]. Reactivity of formaldehyde with lysyl, argininyl, tyrosyl, aspartyl, histidyl and seryl residues results in protein–nucleic acid and protein–protein cross-linking and therefore biomarker preservation of the specimens [10].

Increasing awareness of the effects of pre-analytical variables during tissue processing on proteins and nucleic acids [11,12] has prompted the need for standardization in biobanking and standard operating procedures (SOPs).

There is a long list of variables that occur during and after surgical procedures, including, but not limited to, removal of the tissue, tissue handling, the time before fixation, time in fixative, time and methods of processing and staining (Table 1). The most attention has been paid

E-mail address: Veronique.neumeister@yale.edu.

http://dx.doi.org/10.1016/j.clinbiochem.2014.02.016
0009-9120/

Table 1
A list of variables, which occur during the pre-analytical and analytical phases of tissue procurement and processing.

Pre-analytical variables; incomplete list	Variables during analysis; incomplete list
Effects of intraoperative conditions and anaesthesia	Antigen retrieval method
Warm ischemic time	Antibody used, antibody titer, incubation time
Specimen size and manipulation	Manual versus automated staining, method of automation
Cold ischemic time	Assay reproducibility and antibody validation methods
Fixative — formula, age, concentration	Method of detection — fluorescence or chromogenic stain
Fixation — volume, methods, light exposure, time of fixation	Method of slide reading — objective or subjective, quantitative or semi-quantitative
Postfixation conditions, storage, processing	
Paraffin impregnation	
Storage of the blocks	
Sectioning and storage of the slides	

to cold ischemic time [13–18], defined as the time of removal of the tissue from the patient until further processing such as snap freezing or formalin fixation. A number of pre-analytical variables such as drugs administered to the patient during surgery, duration of the surgery, warm ischemic time, defined as the time between clamping of the arteries to removal of the tissue from the patient, and the size of the specimen might have an impact on the quality of the harvested tissue. A study by Guendisch et al. has reported the dramatic impact of warm ischemic time on expression levels of several proteins comparing liver biopsies to resection specimens which were processed right after surgical resection therefore limiting pre-analytical variables to warm ischemic time [19]. Warm ischemic time is a function of the surgical procedure. Researchers should be aware of the fact that this and other factors might alter biomarker expression levels of the removed specimen as compared to the in vivo status of the tissue.

However, tissue processing after removal of the specimen and storage conditions can be standardized in order to minimize variability and changes, which might occur in the still viable tissue. Efforts have been made to establish standard operating procedures to minimize inconsistencies during the workflow of tissue processing, including use of different fixatives, time of fixation, temperature during fixation, storage condition of the tissue, freeze–thaw cycles, and an additional number of variables during tissue preparation for further analysis.

While this is only an incomplete list of the lack of standardization and therefore variability in tissue processing within and across institutions, there are many more variables in this group that represent opportunities for errors affecting research and patient care. This problem of pre-clinical variation can significantly influence and bias the interpretation of genomic and proteomic testing [20,21], both in the research and the clinical setting.

The effects of variable tissue handling and processing

Prompted through a clinical mishap in Canada with 40% of misclassifications in estrogen receptor (ER) testing of breast cancer patients [22], the American Society of Clinical Oncologists (ASCO) and the College of American Pathologists (CAP) published guidelines for ER testing in breast cancer in the United States emphasizing the importance of limiting cold ischemic time to 60 min or less and also including recommendations for fixation, tissue processing, use of internal positive controls to optimize compliance and accuracy of this test [6]. Similarly guidelines for the evaluation of human epidermal growth factor receptor 2 (Her2) in breast cancer were published and recently revised [5] with the attempt to standardize and optimize the evaluation of Her2 expression levels in breast cancer.

Although both guidelines emphasize the limiting of cold ischemic time to 60 min and efforts are made in a majority of clinical institutions to meet these recommendations, these standards cannot always be met. Not all biopsies or surgeries are performed in centers where onsite processing and pathological evaluation of the tissue is available. Anecdotal reports of tissue being stored either at room temperature or at 4 °C between 4 and 48 h are not rare, considering the fact that biopsies and small surgeries can easily be performed in outpatient units and transport of the tissue for further processing might be delayed over weekends. Several reports have recently been published describing protein degradation of tissue samples as a direct result of increasing cold ischemic time (Fig. 1) [15–17,23]. While phosphorylated epitopes seem to be more labile and epitope degradation can happen within 30 min of cold ischemic time [17,18,23,24], the time course of degradation is protein specific and loss of epitopes of some biomarkers might not happen before 3 or 4 h of cold ischemic time or even beyond this time frame. The situation is even more complex in a way that epitope degradation of the same proteins seems to occur at different rates when tissue samples from different patients are compared to each other. Also, different tissue types reveal variable degradation rates of the same epitopes with increasing cold ischemic time [19]. It has been shown that proteins, involved in hypoxic pathways and posttranslational modification, are upregulated as a response to hypoxia induced stress conditions [17,24]. The expression levels of proteins and their changes/fluctuations have to be understood as a balance of modifications of the translational level and degradation through phosphatases and proteinases as a response to hypoxic conditions.

Modification on a translational level implies upregulation of genes involved in hypoxic pathways, and degradation processes have also been reported to affect gene expression levels after tissue removal. Similar to the changes on the protein level, up- or downregulation of genes does not affect all nucleic acids uniformly. Increased cold ischemic time results in increased RNA fragmentation within the tissue samples leading to statistically significant reduction of RNA integrity (RIN) numbers [25]. Even though it has been shown that expression levels of single genes and multigene signatures currently used in diagnostic settings for breast cancer are not affected by this RNA fragmentation, loss of RNA integrity due to pre-analytical variables has been described. After only 3 to 30 min of cold ischemic time, significant changes of the expression levels of several genes have been reported in a study of healthy and malignant colon tissues by Spruessel et al. [12]. These results were confirmed by a recent study of renal carcinoma by Liu et al., describing that expression of more than 4000 genes was significantly altered by ischemia times or storage conditions [26].

The ASCO and CAP guidelines for processing of breast tissue samples also recommend that time of fixation in 10% neutral buffered formalin (NBF) ranges from 6 to 72 h for ER and PgR and 6 to 48 h for Her2. Insufficient immersion of tissue samples in formalin results in underfixation, leading to detrimental effects on immuno-histochemical and immunofluorescent evaluation of proteins such as ER and Her2 [27–29] and to unreliable results. A common misconception is that the limiting step for fixation is the rate of tissue penetration of formaldehyde, which is relatively fast, approximately 1 mm/h [30]. The chemical reactions responsible for cross-linking and completion of the fixation process take several hours regardless of the specimen size, tissue section thickness or formaldehyde volume [31–33]. Sufficient time of fixation is therefore equally important for larger tissue specimens and for core