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Multi-laboratory analysis of the variability of shipped samples for proteomics following non-cooled international transport

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ABSTRACT

Transporting biological samples such as cells or tissues is complicated by the need to maintain integrity and minimise modification and degradation, but this is economically costly as the samples must be shipped in a frozen state. This multi-laboratory study investigated sample variability introduced by non-cooled transport of dried peptide samples for proteomic analysis using mass spectrometry. Human cancer cell tryptic lysates were proteolysed and dried in Australia and shipped by air to Europe and China. Samples were measured using label free mass spectrometry on similar LC-MS systems at all three sites. Preparation and analysis of the specimens in this manner resulted in only minor differences in protein identification and showed high quantitative reproducibility amongst the participating laboratories. We examined any impact on peptide chemical modification and report no discrepancies compared to the starting, non-shipped sample. We conclude that transport of non-cooled, dried peptides has negligible effect on sample integrity for downstream LC-MS analysis and therefore represents a cost-effective option to facilitate international proteomic collaborations.

Data is available via ProteomeXchange with identifier PXD008160.

Introduction

Proteomic analysis using mass spectrometry is being more frequently used in clinical and biochemical studies, often in tandem with other -omics approaches [1]. Because there is often insufficient expertise in one laboratory to cover all study aspects there is a growing need to establish cooperation between laboratories. The inter-laboratory transport of biological samples, especially tissue samples, which are often declared as biological hazards, is complicated as each country has their own import and quarantine laws, which can represent considerable obstacles for researchers. Another aspect is the integrity of the biological samples such as tissues, which must be protected from degradation or other chemical modifications, and therefore transport of frozen specimens requiring constant cooling with dry ice is necessary. It is expensive and often unpredictable to ensure a stable cold chain for transport from one laboratory to the other because of possible shipping delays or delays due to regulatory inspections [2]. A possible solution in the proteomics field, which commonly applies LC-MS/MS of peptides, would be to proteolytically digest the sample and ship only the dried peptides. In many cases this would circumvent the declaration of biological hazardous material because peptides obtained through proteolytic digestion generally do not retain the biological activity from their protein of origin. This would also simplify the cooling problem as general wisdom suggests that dried peptides are generally more stable than any biological sample or tissue at room temperature. The idea of digesting biological samples and sending peptides is by no means a new one; for example, commercial suppliers offer cell digests as standards for mass spectrometry. These commercially available cell digests are delivered lyophilized and cooled (using cooling-packs). The stability of these lyophilized peptides is specified to be $>$ 2 years when stored at -20 °C or lower [3]. These specifications seem to be purely empirical as to our knowledge there is no recent literature that focuses on the integrity of dried peptides derived from a complex biological sample and analysed by mass spectrometry.

Some studies from the past decade have shown that there are indeed problems in ensuring reproducibility between different laboratories

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conducting proteomics [2,4]. In these studies however, different LC-MS systems were used as well as individual protocols for sample preparation. In a more recent larger-scale cross-laboratory study Tabb et al. reported a median reproducibility for a yeast digest of about 40% on the peptide level and 70% on protein level using Orbitrap mass spectrometers from a distributed yeast digest [5]. In another large-scale multi-laboratory study Percy et al. assessed the quantitative reproducibility across different laboratories and different mass spectrometers [6]. They applied targeted MS/MS (MRM) to quantify different proteins in human plasma. They reported that the difference in instrumentation had a smaller effect on reproducibility than the skill of the researcher preparing the samples. Percy et al. used plasma samples that were digested and desalted in one laboratory and the peptides distributed to the others. They reported the main difficulty was the addition of internal standard peptides, which had to be added separately in each laboratory. Other than these factors, the inherent randomness of Data-Dependant acquisition (DDA) precursor ion selection has to be taken into account which can result into differences even between technical replicates. This is especially problematic when conducting label free quantification as this results in so called “missing values” for a given peptide across runs [7].

In the current study we investigated sample variability following inter-laboratory international transport of dried complex peptide mixtures without cooling. Besides assessing the integrity of the sample, this provided an opportunity to test the reproducibility of the measurements across laboratories in different countries. We centralized sample preparation and data analysis in one laboratory and used identical MS platforms and near-identical LC systems for data acquisition. We investigated reproducibility of peptide and protein identification, quantitation and chemical modifications from samples acquired in three laboratories and compared the results to a non-shipped control sample. Our study provides evidence to support the non-cooled shipping of peptides for proteomic mass spectrometry analysis to support international collaborations.

Material and methods

Study material and design

In this study SW480 colon cancer cells were proteolytically digested in the laboratory at Macquarie University, as described previously [8]. Briefly, cells were lysed in 1% sodium deoxycholate (SDC) buffer (1% w/v sodium deoxycholate in 0.1 M triethylammonium bicarbonate). After lysis proteins were reduced using 20 mM dithiothreitol (DTT) in SDC buffer at 56 °C for 60 min. After reduction the samples were cooled to room temperature and cysteines were blocked using 60 mM 2-iodoacetamide (IAA) in SDC buffer in the dark at 36 °C for 60 min. Digestion was carried out overnight using trypsin (Promega, WI) in an enzyme to protein ratio of 1:100. Peptides were desalted using a reversed phase column (SepPak C18 cartridge, Waters) as per the manufacturers guidelines and aliquoted to 100 µg/tube. Peptides were lyophilized using a vacuum centrifuge (SpeedVac, Labconco).

Three aliquots of the dried peptides were retained at Macquarie University (control). Three aliquots of dried peptides were transported by air without cooling to the University Medical Center Hamburg-Eppendorf (UKE), Germany, further referred to as HH-Shipped and Fudan University, Shanghai, China (FU-Shipped) respectively. Further three aliquots were transported to the UKE site, then returned to Macquarie University (MQ-Shipped).

Dried peptides were kept at −20 °C in each laboratory for over 12 months before analysis. The analysis of the samples in Hamburg and Shanghai took place only a few days apart from each other, whereas the analysis in Sydney took place 3 months later.

Mass spectrometry

Measurement of the samples was carried out in all three institutes on a tandem mass spectrometer (QExactive, Thermo Fisher Scientific) using the same analytical column (from the same production batch) and the same LC configuration with minor changes in Macquarie, here peptides were directly injected onto the analytical column without prior trapping. The same LC and MS method was used in each laboratory. The setup used in Hamburg is described.

Samples were analysed on a nano-ultra-pressure-liquid chromatography system (Ultimate 3000 RSLCnano, Thermo Fisher Scientific) coupled to a tandem mass spectrometer (QExactive, Thermo Fisher Scientific) with a nano-spray source. Peptides were trapped on a reversed phase trap column (2 cm × 75 µm ID; Acclaim PepMap trap column packed with 3 µm beads, Thermo Fisher Scientific) and separated on a reversed phase column (25 cm × 75 µm ID, Acclaim PepMap, 3 µm beads, Thermo Fisher Scientific). Column temperature was kept at 40 °C. Peptides were separated using a 120 min stepped gradient starting at 5% Buffer B (100% acetonitrile (ACN) and 0.1% formic acid (FA)) to 22% in 100 min, increasing to 32% in 10 min and ramping to 90% in 10 min at a flow rate of 300 nL/min. Data were acquired in data dependent mode. Spray voltage was set to 2600 V and the transfer capillary temperature set to 275 °C. All data were acquired in positive mode using a dynamic exclusion for precursor ions of 30 s. Fullscan spectra were acquired using a resolution of 70000 with a scan range of 400–1220 m/z. Automatic gain control (AGC) target was set to 1×10^6 with a maximum injection time of 120 ms. All Fullscan spectra were acquired in profile mode. The top 12 precursor ions were selected for fragmentation with a minimum intensity of 1×10^5. Signals with unassigned, singly charged or with 8 or higher charges were excluded from fragmentation. Peptide match option was turned off. Ions were isolated using a 2.0 m/z window and fragmented using higher energy collisional dissociation (HCD) with stepped normalized collision energy (22.5, 25 and 27.5). Fragment spectra were acquired using a resolution of 17500 with a scan range from 200 to 2000 m/z. AGC target was set to 5×10^5 with a maximum injection time of 60 ms. All fragment spectra were acquired in profile mode.

Data analysis

The resulting 12 data files were processed together using the MaxQuant software [9,10] version 1.5.8.3. Spectra were searched against a human database, obtained in October 2014 from UniProt containing 20193 entries, and a contamination database provided by MaxQuant. Cysteine carbamidomethylation was set as fixed modification. Oxidation on methionine, acetylation on protein N-terminus and cyclization of N-terminal glutamine to pyroglutamic acid (pyro-glu), which may be favoured during elevated temperatures [11], were set as variable modification. The maximum amount of modifications per peptide was set to 5 and a minimum peptide length of 6 amino acids was chosen. Trypsin was selected as specific enzyme and two missed cleavages were allowed. Peptide and protein identification were filtered to a false-discovery rate (FDR) of 1%. Orbitrap was set as instrument type, the first search was conducted with 20 ppm tolerance and the main with 4.5 ppm, MS/MS tolerance was set to 20 ppm and 10 ppm for *de novo* matching. Label-free quantification (LFQ) option was activated using the Fast LFQ algorithm and an LFQ minimum ratio count of 2 was selected.

Post-processing of the MaxQuant output files was done using the Perseus [12] software version 1.5.8.5 and Microsoft Excel 2016. The analysis was done on protein- and peptide-level respectively. After filtering out the proteins only identified by one unique peptide, contaminants, reverse hits and, at protein-level, the proteins, which were only identified by site, a comparison of all 4 sample groups (Control, Macquarie, UKE and Fudan) was conducted. Reproducibility on protein-level was investigated using the coefficient of variation as well as