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Technical Note

Usage of electrostatic eliminator reduces human keratin contamination significantly in gel-based proteomics analysis

Bo Xu[a,*], *Ying Zhang*[a], *Zongjiang Zhao*[a], *Yutaka Yoshida*[a], *Sameh Magdeldin*[a,b], *Hidehiko Fujinaka*[a,c], *Tamer Ahmed Ismail*[a,d], *Eishin Yaoita*[a], *Tadashi Yamamoto*[a]

[a]*Department of Structural Pathology, Institute of Nephrology, Graduate School of Medical and Dental Sciences, Niigata University, Niigata, Japan*
[b]*Department of Physiology, Faculty of Veterinary Medicine, Suez Canal University, Egypt*
[c]*Institute of Clinical Research, Niigata National Hospital, Kashiwazaki, Japan*
[d]*Department of Physiology, Faculty of Veterinary Medicine, Zagazig University, Egypt*

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ABSTRACT

In the field of bottom-up proteomics, heavy contamination of human keratins could hinder the comprehensive protein identification, especially for the detection of low abundance proteins. In this study, we examined the keratin contamination in the four major experimental procedures in gel-based proteomic analysis including gel preparation, gel electrophoresis, gel staining, and in-gel digestion. We found that in-gel digestion procedure might be of importance corresponding to keratin contaminants compared to the other three ones. The human keratin contamination was reduced significantly by using an electrostatic eliminator during in-gel digestion, suggesting that static electricity built up on insulated experimental materials might be one of the essential causes of keratin contamination. We herein proposed a series of methods for improving experimental conditions and sample treatment in order to minimize the keratin contamination in an economical and practical way.

In the bottom-up proteomics field, a proteolytic digestion of the proteins in a complex sample is performed prior to MS/MS analysis. Information about the proteins, such as the identification, post-translation modification discovery, and relative abundance, is then inferred, based on the MS/MS data [1]. In-gel digestion is one of the popular proteolytic approaches due to its main advantages of allowing the sample pre-fractioning and the removal of harmful compounds to mass spectrometers, such as detergent and salts [2]. However, the commonly used in-gel digestion protocol is time consuming and very susceptible to human keratin contamination, which could affect the detection of other proteins, especially low abundance proteins, as the mass spectrometer usually prefers detecting precursor ions with higher intensity [3,4]. In this study, we put emphasis on this procedure to reduce human keratin contamination in the following three ways: 1) improve the experimental conditions for a clean and static electricity free space; 2) avoid to bring any contaminants directly from the human body (the operator) to test tubes while sample handling and 3) minimize the gel sample processing time by

* *Corresponding author at: Department of Structural Pathology, Institute of Nephrology, Niigata Graduate School of Medical and Dental Sciences, 1-757 Asahimachi-dori, Chuo-Ku, Niigata 951-8510, Japan.* Tel.: +81 25 227 2152; fax: +81 25 227 0768.
E-mail addresses: kyonami@med.niigata-u.ac.jp, xubo19701976@yahoo.com.hk (B. Xu).

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Table 1 – Experiments performed for detection of keratin contamination in the step of in-gel digestion using human glomerular protein samples.

Experimental group	In-gel digestion	
	Electrostatic eliminator	Protocol
1	+	Standard
2	–	Standard
3	+	Standard
4	+	Rapid

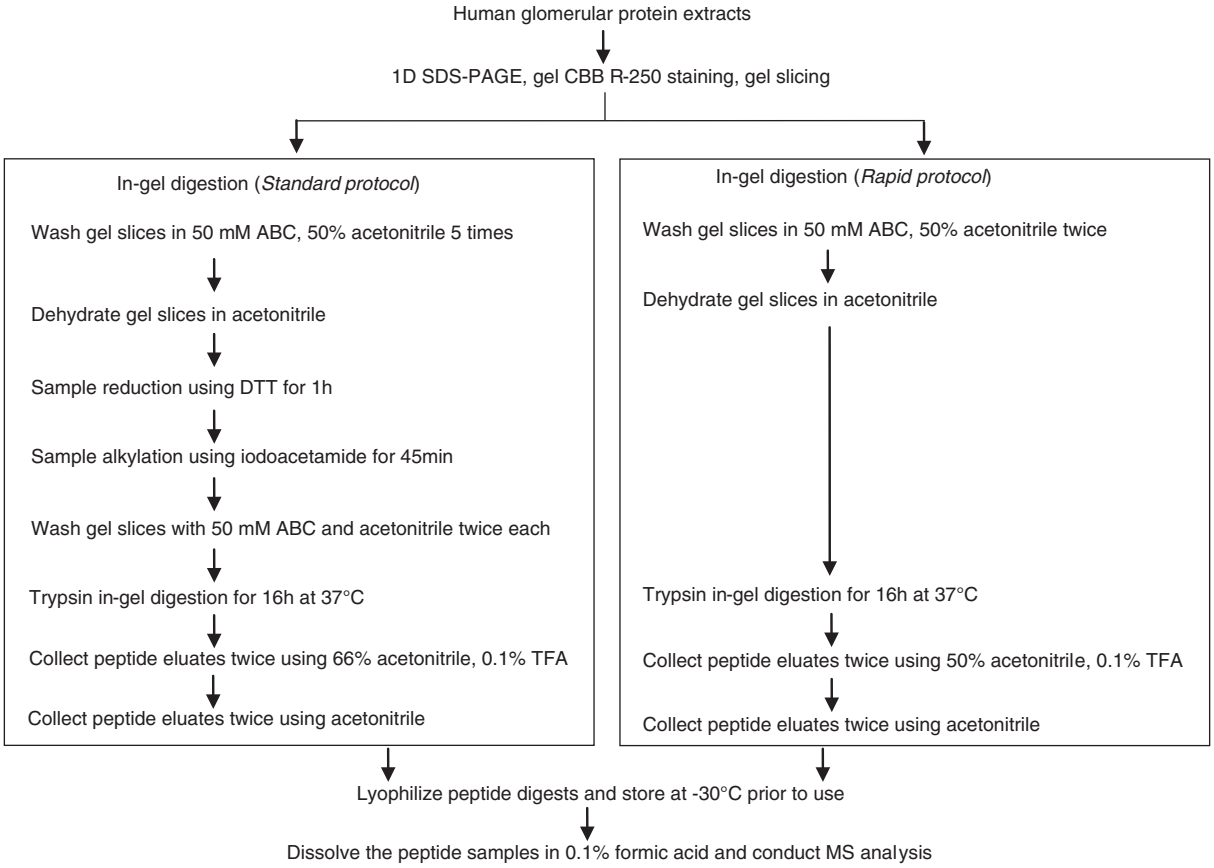

Fig. 1 – Experiment workflow for in-gel digestion using either the standard protocol or the rapid one. CBB R-250, Coomassie Brilliant Blue R-250; ABC, ammonium bicarbonate; DTT, dithiothreitol and TFA, trifluoroacetic acid.

the usage of dispenser pipettes and vacuum suction for liquid treatment. We also tried a rapid protocol of in-gel digestion as an alternative to the standard method to compare the keratin contamination. In addition to in-gel digestion, we investigated the keratin contamination during the other three experimental procedures including gel preparation, gel electrophoresis and gel staining.

In this study, we used normal human and rat glomerular protein extracts, in which there is little cytokeratin expression [5,6], and also blank gel slices as samples for in-gel trypsin digestion and mass spectrometry analysis to detect keratin contamination. This work was approved by the Ethics Committees and the Animal Committee of Niigata University Faculty of Medicine. The normal human tissue was obtained from a patient with his consent who underwent nephrectomy due to renal cell carcinoma. Male Wistar rats (Charles River Japan) were used at the age of 8 weeks. Both human and rat glomeruli were isolated using the standard sieving method. The isolated glomeruli were dissolved in the lysis buffer containing 9.8 M urea and 2% NP-40 as described in the previous report [7]. Mass spectrometry analysis was performed by using Agilent 6300 Series Ion Trap LC/MS systems combined with Agilent 1100Series nanoHPLC systems, and MS/MS raw data were searched against IPI human database ver.3.51 by using Agilent SpectrumMill workbench algorithm (Rev A.03.03.081 SR1a). The similar amino acid sequence coverage (AA %) of standard bovine serum albumin (BSA) digest was confirmed before and after sample analysis to ensure the stable working state of LC–MS/MS instrument. For protein identification, carbamidomethylation on cysteine was set as fixed modification, with oxidation on methionine and deamidation on asparagine as variable modifications.