A novel gel electrophoresis technique for rapid biomarker diagnosis via mass spectrometry

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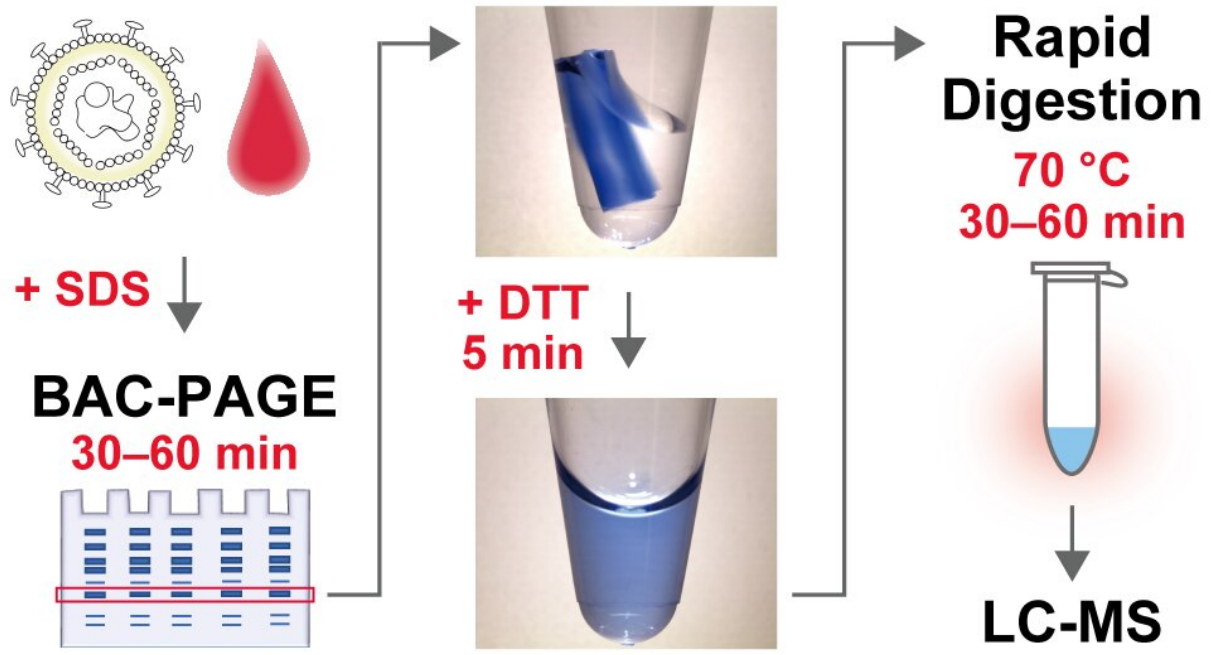

Dissolvable BAC cross-linked gels allow rapid and lossless recovery of protein biomarkers separated by SDS-polyacrylamide gel electrophoresis and facilitate analysis by mass spectrometry. Credit: *Journal of Proteome Research*, American Chemical Society (ACS)

Polyacrylamide gel electrophoresis enables high-resolution separation of proteins extracted from biological samples, but it requires more than one

day of pretreatment to recover the separated proteins trapped inside the gel for detection by mass spectrometry. BAC-DROP, our novel electrophoresis technology, uses a dissolvable form of polyacrylamide gel, which allows sample pretreatment to be completed in about five hours. The developed technology will enable the rapid diagnosis of viruses and disease protein markers.

Mass spectrometry (MS) is a powerful method for biomarker analysis because it enables highly sensitive and accurate measurement of target molecules in clinical samples. The application of MS to clinical diagnosis, such as neonatal metabolic screening, has been progressing with a focus on metabolite markers. MS measurement of proteins is currently mainly used for novel marker discovery studies, but there is a growing interest in its application in clinical marker diagnosis as an alternative to immunoassays.

MS-based quantification of protein biomarkers is mainly performed by a bottom-up approach using peptide fragments obtained by enzymatic protein digestion with trypsin. Standard digestion protocols require a reaction time of more than 20 hours, which is a rate-limiting factor in sample preparation workflows.

Although protein quantification by MS is highly sensitive, plasma and serum proteome are highly complex, and interference by other components poses a significant challenge. For high-precision detection of target markers, approaches for pre-removal of major serum protein components such as albumin or selective enrichment of target markers using antibody columns have been reported, but the off-target effect on quantitative results and the difficulty of processing multiple samples remain obstacles.

In this study, we focused on dissolvable polyacrylamide gels using N,N'-Bis(acryloyl)cystamine (BAC) as a cross-linker to solve these

problems. BAC cross-linked polyacrylamide gels readily dissolve by reduction treatment, allowing the recovery of proteins that have escaped into the solution. We found that the recovered proteins were suitable for rapid trypsin digestion under high temperature conditions, and we succeeded in establishing a high-throughput sample preparation method for MS-based biomarker quantification, which we named BAC-DROP (BAC-Gel Dissolution to Digest PAGE-Resolved Objective Proteins).

High-resolution proteome fractionation with BAC-DROP is particularly effective for MS quantification of targeted trace marker proteins derived from clinical samples. By introducing BAC-DROP into the MS-based quantification workflow of the inflammatory biomarker C-reactive protein (CRP), we were able to complete the sample pretreatment in only 5 hours and successfully quantified CRP from a 0.5 μL human serum sample. We also succeeded in a serological diagnosis of hepatitis B virus (HBV) infection by HBsAg quantification combined with BAC-DROP and MS. Recently, interest in MS diagnosis of viral infections has been rapidly increasing, as exemplified by the diagnosis of COVID-19. The high-throughput sample preparation approach by BAC-DROP shown in this study will be applicable not only to HBV but also to other infectious viral disease samples.

More information: Ayako Takemori et al. BAC-DROP: Rapid Digestion of Proteome Fractionated via Dissolvable Polyacrylamide Gel Electrophoresis and Its Application to Bottom-Up Proteomics Workflow, *Journal of Proteome Research* (2020). DOI: 10.1021/acs.jproteome.0c00749

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