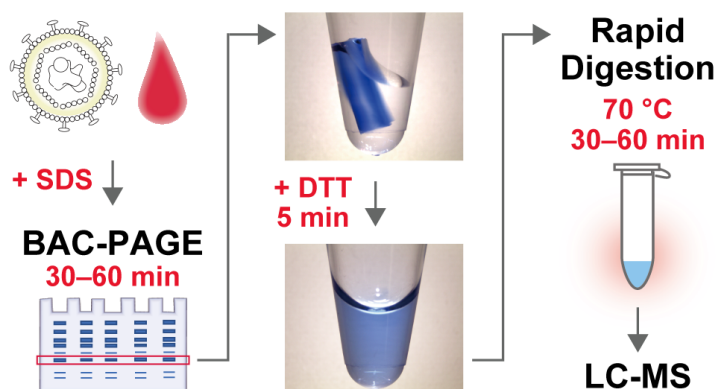


## Japan develops methods for viruses and disease protein markers diagnosis

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### Novel Gel Electrophoresis Technique performs rapid biomarker diagnosis via Mass Spectrometry



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.

A collaborative research group at Japan's Ehime University, Hamamatsu University School of Medicine, and Kitasato University have developed "BAC-DROP", a novel electrophoresis technology, which uses a dissolvable form of polyacrylamide gel, which allows sample pretreatment to be completed in about 5 hours. The developed technology will enable the rapid diagnosis of viruses and disease protein markers.

Mass spectrometry (MS) measurement of proteins is currently mainly used for novel marker discovery studies. Currently, clinical marker diagnosis is 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, and not compatible with time-sensitive complex plasma and serum proteome.

Thus, researchers 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. Recovered proteins were suitable for rapid trypsin digestion under high temperature conditions, and succeeded in establishing a high-throughput sample preparation method for MS-based biomarker quantification, which is 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  $\mu$ L 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.

**Image Caption:** *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*