Comparison of Three IgG Purification Methods for M-protein Detection Using MALDI-TOF Mass Spectrometry

Proteomics (9a)

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Introduction

Detection of M-protein from serum using MALDI-TOF MS

- Recently, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has emerged as a sensitive method to detect small amounts of M-protein.
- Dominantly, IgG is the most common type of monoclonal protein (M-protein) observed in patients with plasma cell disorders (PCDs).
- There are various techniques for IgG purification, such as Melon™ gel IgG spin purification kit, magnetic beads consist of protein G, and IgG affinity beads.(Figure 1)
- We compared those three methods to find the most effective IgG purification approach for detecting M protein.
- We sought to find out the most effective method to purify IgG for detecting M-protein by MALDI-TOF MS.

Methods

Different methods of sample preparation for IgG purification

- A normal and an abnormal patient's serum samples were selected from residual samples from immunofixation electrophoresis (IFE) tests on HYDRASIS 2 (Sebia, Fulda, German).
- In the IFE result, a patient serum showed IgG and kappa type of monoclonal antibody.
- To observe analytical sensitivity, daratumuamb was serially diluted into a normal serum at concentration of 0, 0.05, 0.1, 0.2, 0.5, 1.0 g/dL.
- C4 ZIPTIP was used for desalting after Melon™ Gel IgG Spin Purification Kit (Thermo Fisher Scientific Inc., MA, USA). For beads based methods, Dynabeads™ magnetic beads (Thermo Fisher Scientific Inc., MA, USA) and CaptureSelect™ affinity beads (Thermo Fisher Scientific Inc., MA, USA) were used.
- For each purification method, samples were spotted five times onto a 96-well plate. Each set of replicates was measured by MALDI-TOF MS (Bruker Daltonics, Bremen, Germany) for five days.
- We used α-Cyano-4-hydroxycinnamic acid (CHCA) matrix, and 500 shots were summed up

Results

IgG affinity beads showed a superior outcome in this study

- Peaks indicating reduced light chains (LC) with single and double charge of purified IgG were observed in mass spectrum within the 22000-25000 and 11000- 12500 range of mass-to-charge ratio (m/z), respectively.
- Polyclonal peak consisting of kappa light chain and lambda light chain was observed in normal patient, while monoclonal peak was observed with higher intensity in abnormal patient. Coefficients of variation of m/z values for all the methods were in less than 20%.
- Limit of detection of IgG affinity beads was 0.1 g/dL, which was the lowest among three methods. Melon kit with C4 ZIPTIP was 0.2 g/dL, and magnetic beads showed 0.5 g/dL. IgG purification using CaptureSelect™ affinity beads showed a superior outcome compared to Melon kit or magnetic beads for detection of monoclonal protein.
- Melon kit might be considered as an alternative method for IgG Purification.

Conclusions

CaptureSelect™ IgG affinity beads was the best method for IgG purification to detect M-protein using MALDI-TOF MS

- Among three methods, IgG affinity beads showed the best outcome to purify IgG for identifying M-protein using MALDI-TOF MS.

Serum sample preparation

Residual samples
- from immunofixation electrophoresis (IFE)
- normal and abnormal serum (IgG/kappa type)

Three different IgG purification methods

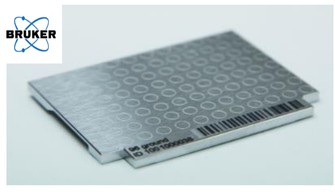
Melon™ Gel IgG Purification Kit

ZipTip with 0.6 µL C_4 resin

Dynabeads™ Protein G for Immunoprecipitation

CaptureSelect™ FcXL Affinity Matrix

Reduction and elution by 20mM TCEP



MALDI target plate (96 positions)

Sample loading
- CHCA sandwich method
- CHCA, sample, CHCA
- 1µL for each

Bruker Daltonics - microflex LT MALDI-TOF MS

MALDI-TOF MS performance
- 500 shots summation
- Mass spectrum
- Peak range of 1+ LC: 22000-25000 m/z
- Peak range of 2+ LC: 11000- 12500 m/z
- Data interpretation

Normal
Polyclonal peaks of normal serum
- A: 2^+ LC
- B: 1^+ LC

Abnormal
Monoclonal peaks of abnormal serum with higher intensity
- C: 2^+ LC
- D: 1^+ LC

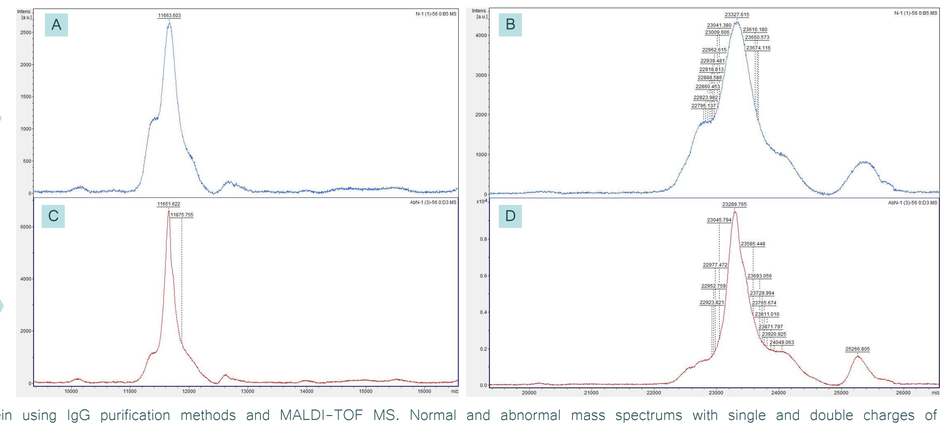


Figure 1. Procedure of detecting serum M-protein using IgG purification methods and MALDI-TOF MS. Normal and abnormal mass spectrums with single and double charges of light chains are shown in A~D. (A) Polyclonal peak of normal serum in 10000~16000 range of m/z, (B) Polyclonal peak of normal serum in 20000~26000 range of m/z, (C)Monoclonal peak of abnormal serum in 10000~16000 range of m/z, (D) Monoclonal peak of abnormal serum in 20000~26000 range of m/z. **Abbreviation:** MALDI-TOF MS, Matrix-assisted laser desorption/ionization-time of flight; CHCA, α-Cyano-4-hydroxycinnamic acid matrix; LC, light chains; m/z, mass-to-charge ratio