

# Characterization of an isoelectric focusing method for detection of IgG oligoclonal banding in paired cerebrospinal fluid and serum specimens

S. Hosseini<sup>1</sup>, P. Beatty<sup>2</sup>, A. Remaley<sup>2</sup>, S. Duh<sup>1</sup>, R.H. Christenson<sup>1</sup>

1. University of Maryland School of Medicine, Baltimore, MD

2. National Institutes of Health, Bethesda, MD

E-mail: saman.hosseini@umm.edu

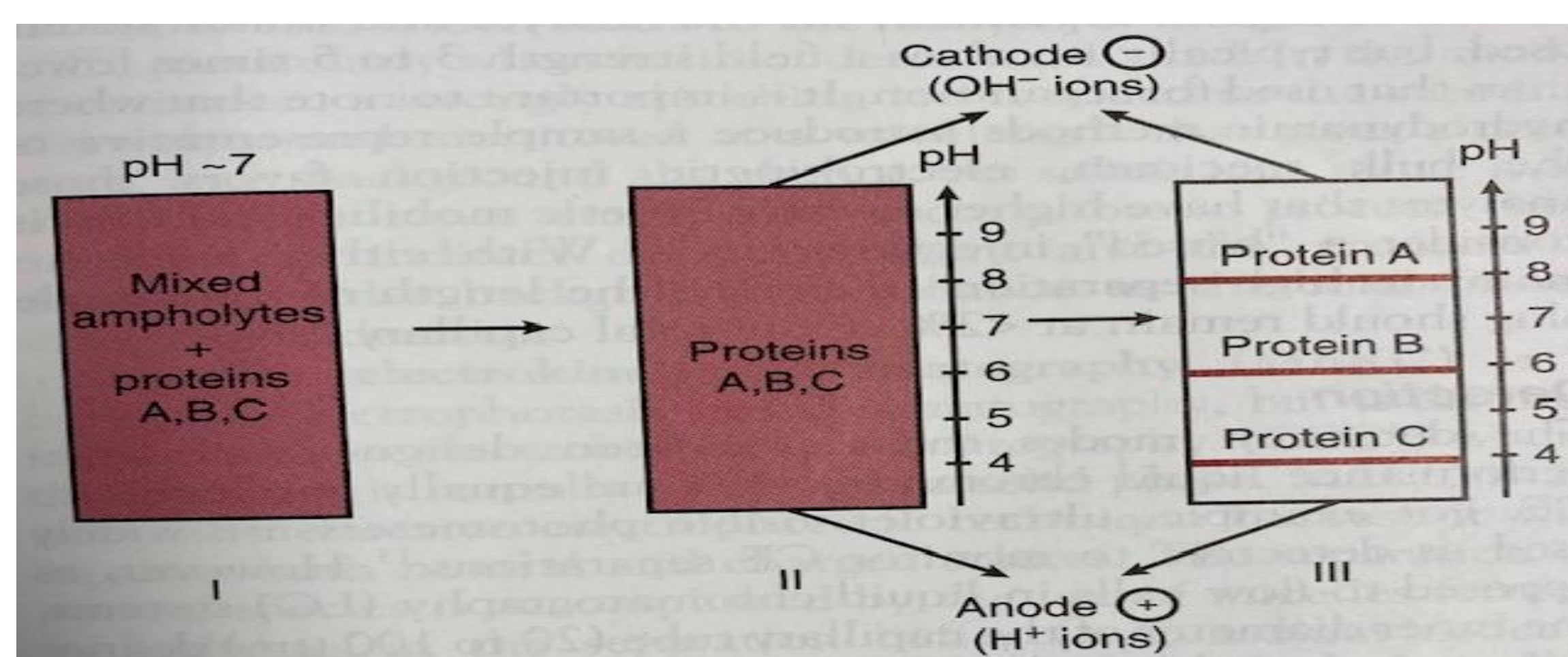


## BACKGROUND

We investigated the qualitative assessment of routinely employed IgG-specific oligoclonal banding (OCB) in paired serum and cerebrospinal fluid (CSF) samples as a tool in the diagnosis of inflammatory central nervous system (CNS) diseases such as multiple sclerosis (MS). OCB is not diagnostic or specific for MS. Our study focused on evaluating the performance of IgG-specific methods utilizing isoelectric focusing on an agarose gel matrix to detect OCB in paired serum and CSF samples. In this study, we compared the diagnostic effectiveness of isoelectric focusing (IEF) combined with IgG immunoblotting (Helena Lab Corp.) against IEF combined with immunofixation using an enzyme (peroxidase)-development technique (Sebia Group) for detecting intrathecal synthesis of IgG.

## METHODS

We analyzed 20 paired, concurrently collected CSF and serum patient samples using the Helena Lab IgG IFE-10 kit (SPIFE<sup>®</sup> Touch) at our institution and compared the results to the Sebia (HYDRASYS 2 System) assay utilized by the National Institutes of Health (NIH) Diagnostic Laboratories (Figure 1). Two independent individuals, blinded to each other's evaluations, interpreted the results. A positive result was defined as two or more bands in the CSF but absent in the serum pattern. Analytical sensitivity of the Helena Lab IgG IFE-10 kit (SPIFE<sup>®</sup> Touch) was 0.45 mg/dL, as estimated by serial dilution of a CSF sample positive for OCB. These outcomes were compared with NIH results to assess agreement, calculating Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA). Concordance was also calculated for total number of samples.



**Figure 1.** Schematic of IFE procedure that was utilized for Helena Lab SPIFE IgG IEF-10 and Sebia (HYDRASYS 2 System) assay. [1]

### Helena Lab SPIFE IgG IEF-10

- Applied 5  $\mu$ L neat CSF and 5  $\mu$ L pre-diluted serum (1:300) to agarose IEF gel. Electrophoresed for 45 min at 700 V and 15°C.
- Transferred proteins to nitrocellulose membrane
- Detected IgG using goat anti-human IgG peroxidase-labeled antibody.

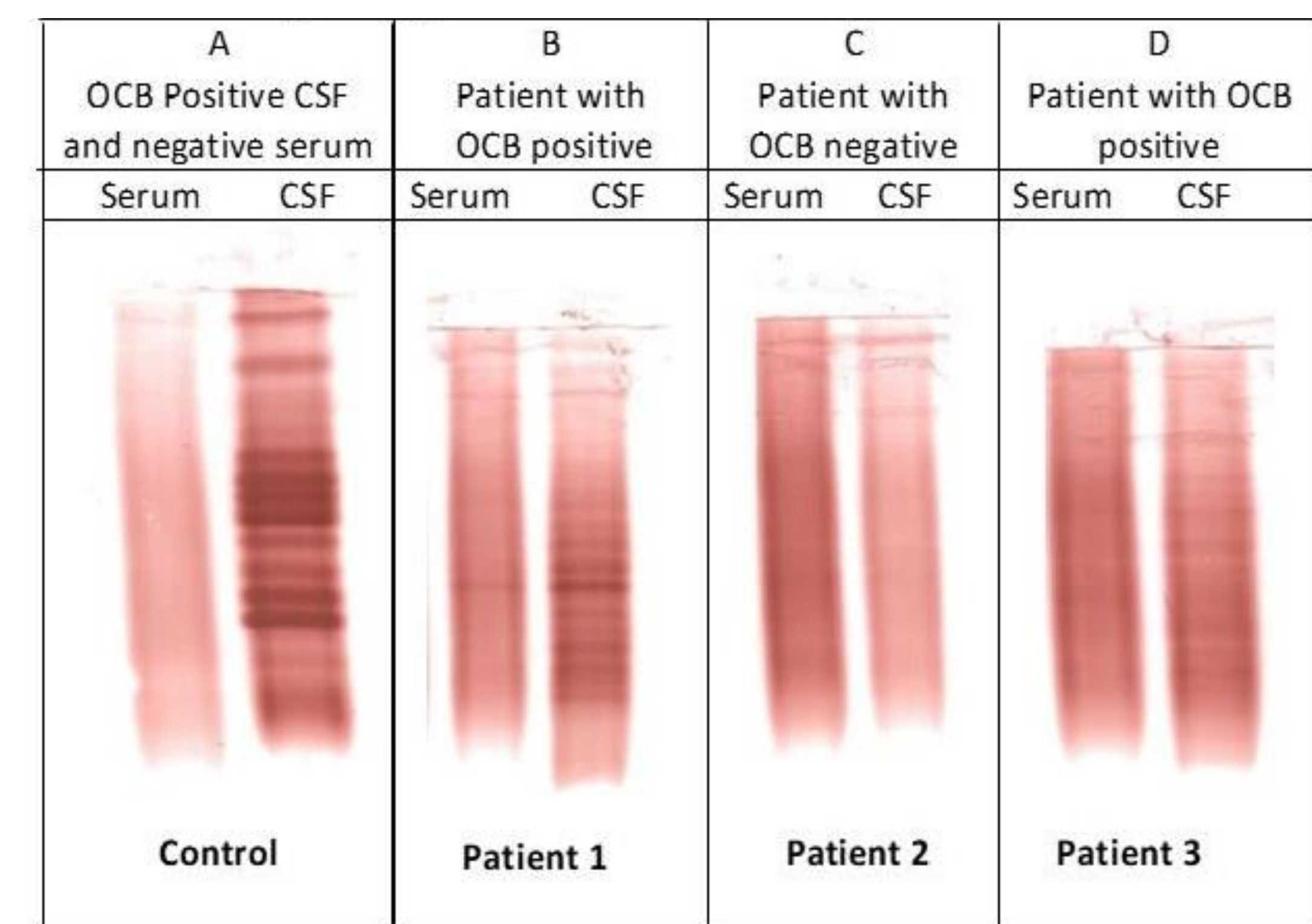
### Sebia (HYDRASYS 2 System) assay:

- Designed to identify and compare IgG oligoclonal bands in CSF and serum
- Performed isoelectric focusing on agarose gel to fractionate proteins in CSF and serum samples.
- Followed by immunofixation using peroxidase-labeled anti-IgG antiserum to detect IgG oligoclonal bands.



## RESULTS

Figure 2 depicts paired serum and CSF samples: control (A), examples of positive OCB patients (B, D), and a negative OCB patient (C). The Helena assay showed agreement with Sebia assay in 11 out of 12 positive OCB patients (PPA 91.6%) and all 8 negative subjects (NPA 100%). The highest dilution that remained positive was 20-fold. Representative gel photos are also shown in Figure 2, highlighting sharp and easily detectable OCBs distributed across the pH gradient on the IEF gel.



**Figure 2.** OCB testing by isoelectric focusing with IgG-specific antisera on agarose gel with immunoblotting (Helena Lab. Corp.)

The Helena and Sebia assays showed 95% concordance across 20 samples. Sebia identified 12 positive and 8 negative OCB samples. Helena agreed with 11 positive and all 8 negative results, with one discordant sample. Clinical diagnoses were unavailable due to lack of patient history.

## CONCLUSIONS

**Detection of oligoclonal bands using paired serum and CSF samples by isoelectric focusing electrophoresis is the "gold standard" method.**

**The Helena IgG IFE-10 assay exemplifies this approach and has demonstrated strong agreement (high PPA and NPA) with the Sebia System. Therefore, both the Helena IFE-10 kit (SPIFE<sup>®</sup> Touch) and the Sebia (HYDRASYS 2 system) assay are dependable for detecting OCBs in serum and cerebrospinal fluid samples. The choice between these methods hinges on specific considerations such as the availability of laboratory equipment, cost considerations, and local laboratory preferences.**