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Evaluation of semi-automatic image analysis tools for cerebrospinal fluid electrophoresis of IgG oligoclonal bands

G. Forzy[a,b,*], L. Peyrodie[c], S. Boudet[a], Z. Wang[a], A. Vinclair[b], V. Chieux[a,b]

[a] Faculté de Médecine et de Maieutique, Lille, France
[b] Groupement Hospitalier Institut Catholique de Lille, France
[c] HEI Yncréa Hauts de France, UTSB, Lille, France

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ABSTRACT

Background: IgG concentrations in cerebrospinal fluid generally range from 20 to 45 mg/L. In multiple sclerosis immune reactions lead to intrathecal synthesis of specific IgGs that can be detected in biological fluid samples both quantitatively and qualitatively by isoelectric focusing of supplementary oligoclonal IgG bands.
Method: A simple tool, using the MATLAB application, to facilitate and improve isoelectric focusing profile analysis is presented and evaluated in terms of its sensitivity, repeatability and reproducibility. A comparison between human readers and semi-automatic method has also been performed.
Results: Results from the semi-automatic method were found to be equivalent or superior to generally employed laboratory methods. Repeatability analysis for semi-automatic processing yielded coefficients of variation (CVs) in the 3–7% range, and using a sample with an estimated IgG concentration of 200 mg/L, four bands were still visible after dilution to 5 mg/L, corresponding to band concentrations of 1.1–1.6 mg/L. Discordances between visual inspection and automatic analysis only appear at threshold levels for interpretation (the gray zone).
Conclusion: The semi-automatic method has acceptable performance for routine implementation.

1. Introduction

The incidence of Multiple Sclerosis (MS) in France is one per 1000, making it the leading cause of acquired non-traumatic severe disability in young patients. MS involves inflammatory lesions of white matter in the central nervous system (CNS) that spread over time. Positive evidence of MS can be obtained with Magnetic Resonance Imaging (MRI) demonstrating the occurrence of 3 out of 4 Barkhof brain and spinal cord criteria [1]. The combination of two suggestive MRI lesions associated with a positive cerebrospinal fluid (CSF) analysis also indicates disease [1]. Detection of oligoclonal bands (OCBs) in CSF by isoelectric focusing (IEF) is a common diagnostic tool [2].

Chromogenic staining of immunoblots facilitates detection. The higher the concentration of the IgG bands, the greater the intensity of the colors and the more readable the profile, making interpretation easier. Analyzing a profile involves counting the number of visible IgG bands. A profile with at least two IgG bands in CSF which are not present in a serum sample taken at the same time is said to be oligoclonal.

Chromogen interaction with the immunoblot support determines the baseline or background noise. Other forms of noise (white and dark spots [salt and pepper], blots, stain irregularities) may appear on the profile, increasing the chance of misinterpretation for

* Corresponding author at: Faculté de Médecine et de Maieutique, Lille, France.
E-mail addresses: forzy.gerard@ghicl.net (G. Forzy), laurent.peyrodie@yncrea.fr (L. Peyrodie).

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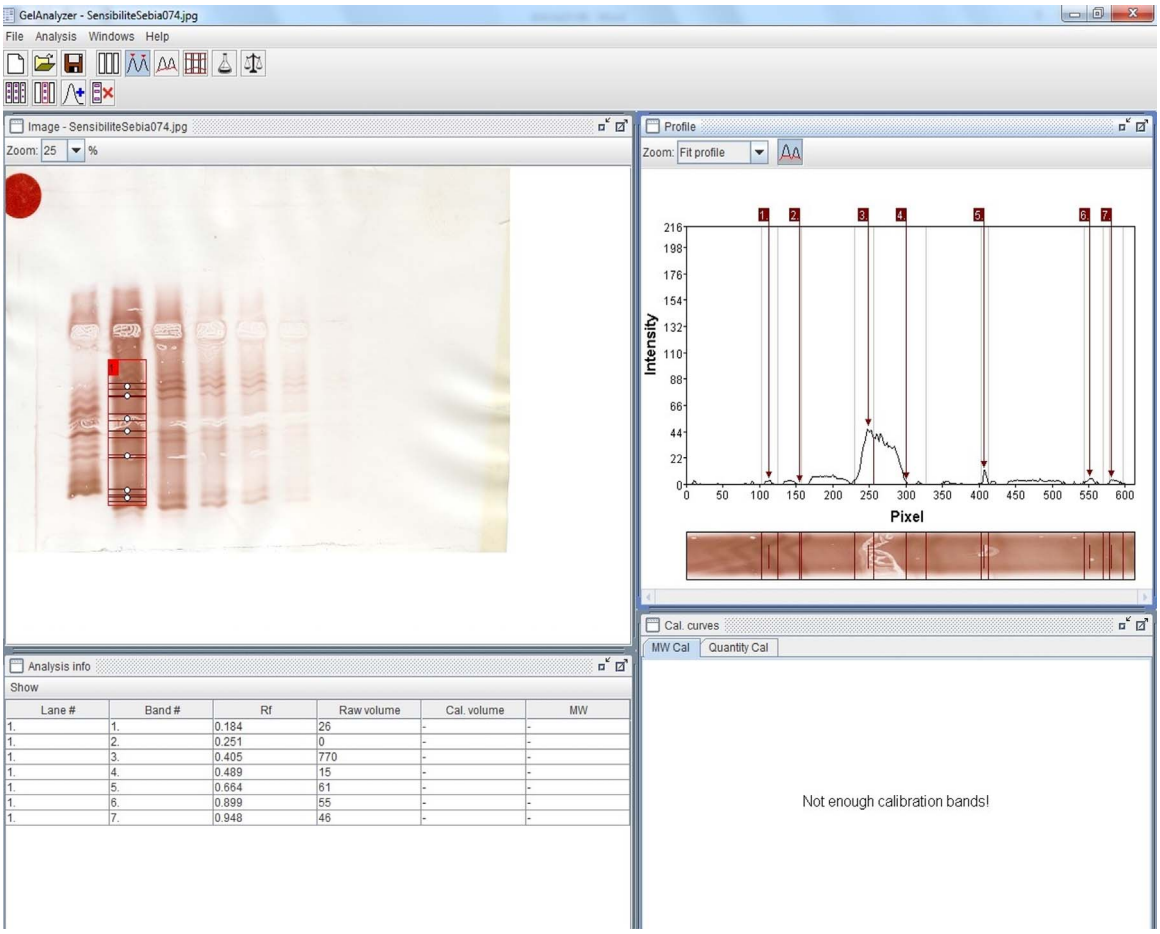

Lane #	Band #	Rf	Raw volume	Cal. volume	MW
1.	1.	0.184	26	-	-
1.	2.	0.251	0	-	-
1.	3.	0.405	770	-	-
1.	4.	0.489	15	-	-
1.	5.	0.664	61	-	-
1.	6.	0.899	55	-	-
1.	7.	0.948	46	-	-

Fig. 1. Analysis of a manual ROI CSF profile by GelAnalyser software.

the operator who must distinguish peaks as band intensity versus background and noise.

Patients with MS exhibit a wide range of IgG electrophoretic profiles in CSF samples, and the skill in interpretation consists of distinguishing false-negatives and positives in samples to correctly classify the profile as oligoclonal or not in the presence of background noise. Counting bands is not an easy task and the operator frequently needs help. New technology based on computerized analysis can provide an important tool to supplement human reading. Automated analysis can also determine other properties such as surface area, band location and the intensity of the band. This information may be useful for further studies and patient follow-up.

In the past decade, methods for filtering, segmenting, and detecting gel bands, as well as rectifying geometries, have been described [3,4]. Major applications and interfaces to fine tune DNA band analysis have been developed [5,6] based on Gaussian mixture models [7] and wavelet transformation [8]

However, semi-automatic techniques require manual setting of sensitivity thresholds. Problems with grayscale intensity also occur and end-users must often adjust parameters for individual bands.

Other methods automatically filter and smooth grayscale intensities, which can cause true bands to disappear while false bands remain. Blob areas may compute as rectangular markers, decreasing reproducibility. Performance assessment of these kinds of tools and associated interfaces is, to our knowledge, absent from the literature. Use of a DNA Gel Analyzer interface for CSF profiles does not give satisfactory results (Fig. 1). Methods designed to process DNA electrophoresis need to be adapted to be relevant to IgG isoelectric focusing. OCB analysis does not require fine band tuning, but contrast enhancement and elimination of false readings and artefacts.

Existing software can, however be adapted to IgG isoelectric focusing. This article briefly describes a tool based on Gaussian adaptation (GA) that we have developed [9] to enhance peak detection. These tools were then assessed (accreditation process) according to good laboratory practice (GLP), in terms of reproducibility [10].