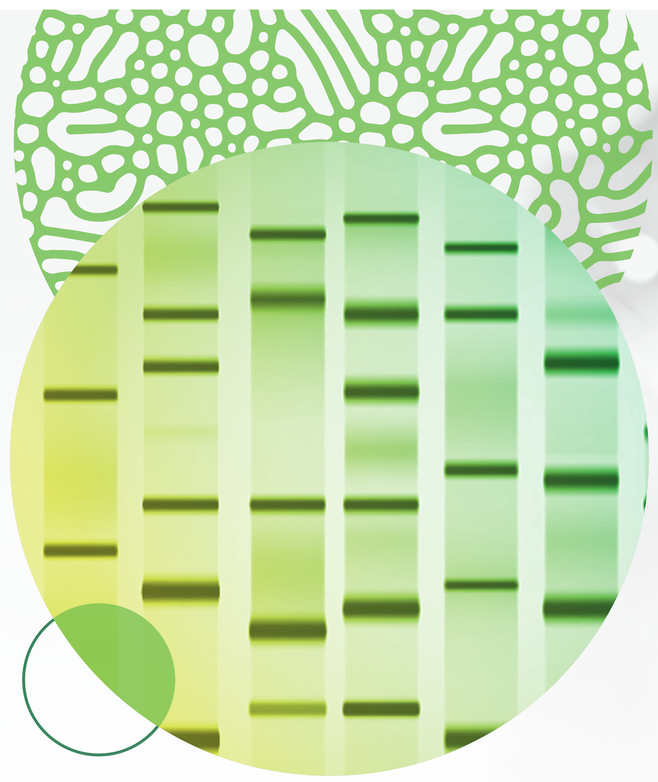
A Review of Oligoclonal Band Counts in an External Quality Assurance Program

D Tran, P Graham

The Royal College of Pathologists of Australasia Quality Assurance Programs (RCPAQAP), St Leonards, NSW, 2065, Australia



Introduction

Oligoclonal bands (OCBs) in Cerebrospinal fluid (CSF) are a crucial diagnostic tool in neurology, especially for inflammatory and autoimmune diseases of the central nervous system. OCB counting can also help with diagnosis, prognosis, and assessment of disease severity or progression in Multiple sclerosis patients.

In 2022, the RCPAQAP introduced a band counting option to the CSF Oligoclonal Bands program as a potential guide to method differences and sensitivities, based on a suggestion from the CSF Advisory Committee. We sought to assess whether the option to record band counts had influenced any practice and method performance changes between 2022 and 2025.

Method

A set of 84 CSF and 84 serum samples, spiked with varying levels of monoclonal paraproteins or from patients with known OCBs, were distributed to participating laboratories between January 2022 and June 2025. In addition, 'Challenge' samples, spiked only with haemoglobin (2022, 2023 and 2025) and with both haemoglobin and oligoclonals (2024) were also included each year to check for potential haemolysis interference. Each sample was analysed on four occasions over 12 months. The returned band counts from a total of 84 CSF and 84 serum samples were reviewed.

Participants were asked to count all visible bands, including faint ones, in each isoelectric focusing (IEF) track. No target range was provided; counts were for informational purposes only.

Results and Discussion

Between January 2022 and June 2025, 84 CSF and 84 serum samples were reviewed, with 65 and 16 expected positives, respectively. Of the 28 participating laboratories, 10 consistently reported their oligoclonal band counts when present in CSF (Figure 1) and serum (not shown).

Three main measurement systems were used: Manual Method, Helena SPIFE Electrophoresis, and Sebia Hydrasys. Six variations in analytical and staining techniques were noted. Band counts varied widely, reflecting differences in method sensitivity (Figure 1).

The Manual Method consistently produced the highest band counts, followed by the Sebia Hydrasys. Helena SPIFE Electrophoresis showed greater variability and generally lower counts (Figure 1). We noted, two participants transitioned from older systems (Bio-Rad Model 111 and Interlab Easyfix G26) to Sebia Hydrasys and Helena SPIFE Electrophoresis respectively. Aside from sample A22, majority of the participants reported absent when no oligoclonal bands were present in samples A23, A24 and A25 (Figure 1).

Results over the 3.5-year period (Figure 1) and for the same sample in 2024 (Figure 2) further highlight method variability. The Manual Method with ALP Labelled Antisera showed higher sensitivity compared to the Manual Method with only Labelled Antisera (Figure 2). Sebia Hydrasys results varied due to two analytical principles and three stains in use (Figure 2). Despite the variation due to different stains being used, the bands counted for the Helena SPIFE Electrophoresis did not vary greatly (Figure 2).

There was some evidence of haemoglobin band interference observed in each of the samples spiked with haemoglobin, with four participants reporting bands in samples F22, F23 and F25, which were absent for oligoclonal bands (Figure 1). There was no conclusive evidence of haemoglobin bands being reported in sample F24, which was spiked with both oligoclonals and haemoglobin.

Conclusion

The data from 2022 to 2025 show ongoing variability in band counts between and within methods. This variability should be considered for inclusion in a commentary in the report to assist clinicians who may use them as indicators for diagnosis and/or prognosis for conditions such as multiple sclerosis.

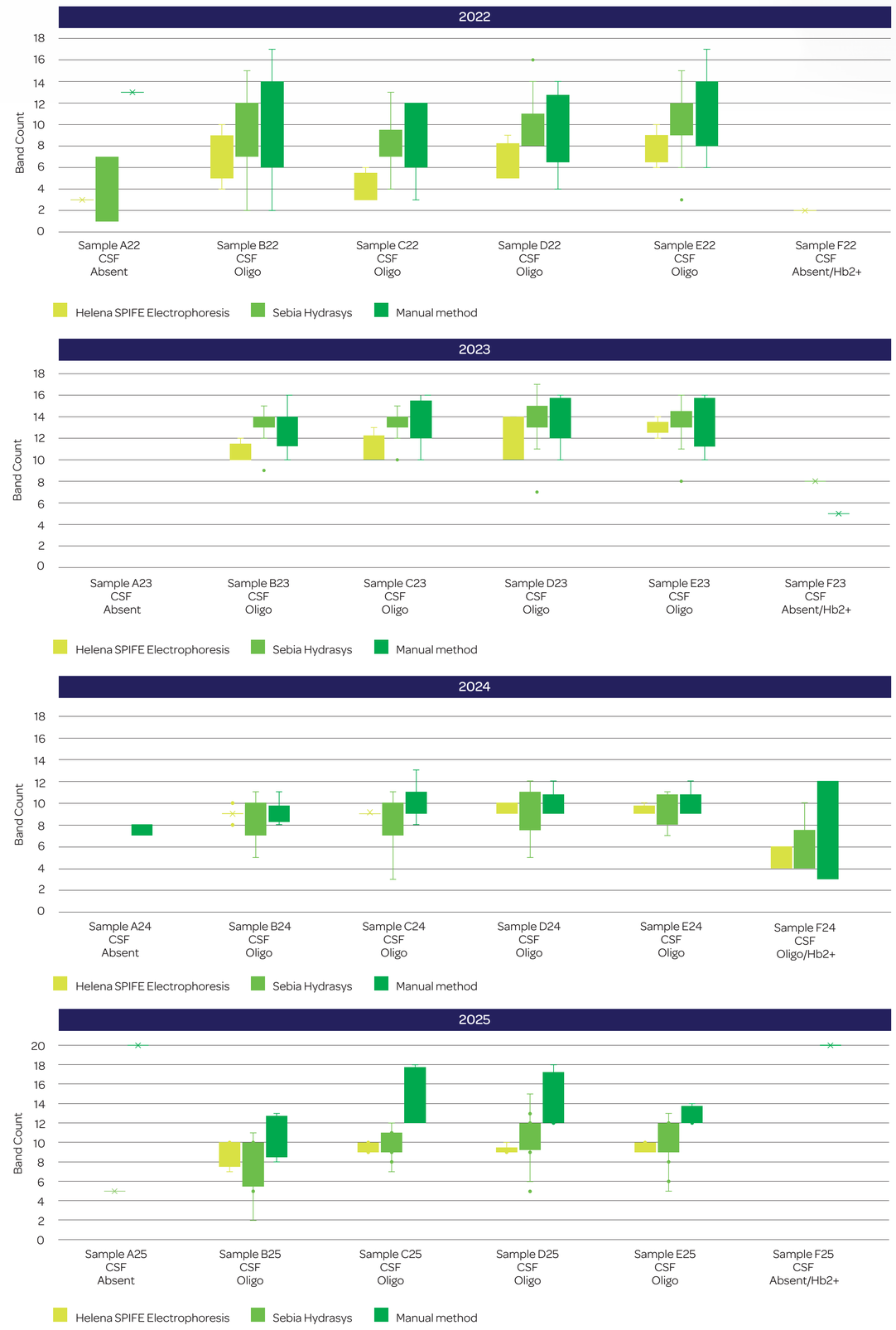
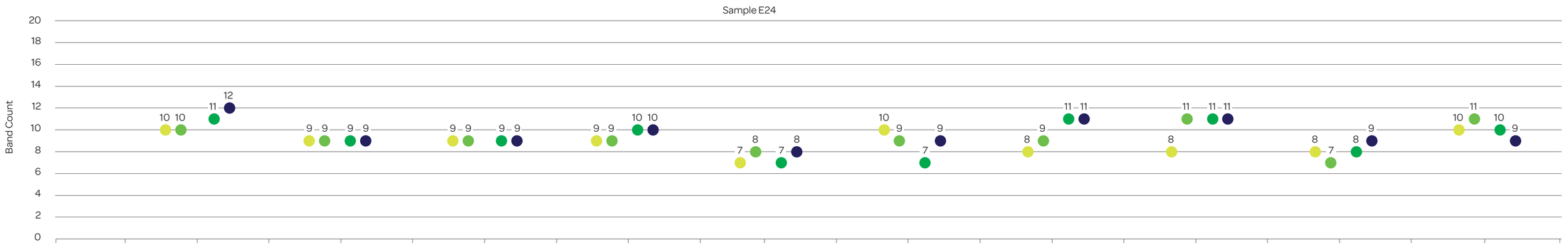


Figure 1 Box and Whisker plots comparing CSF band count across three different measurement systems over four years (2022-2025). Each graph includes six samples per year (A-F), the year (2022-2025), with sample type (CSF) and whether CSF bands were Absent, Oligo (Oligoclonal), Absent/Hb2+ or Oligoclonal/Hb2+.



Participant:	1	2	3	4	5	6	7	8	9	10
Measurement System:	Manual Method	Manual Method	Helena SPIFE Electrophoresis	Helena SPIFE Electrophoresis	Sebia Hydrasys	Sebia Hydrasys	Sebia Hydrasys	Sebia Hydrasys	Sebia Hydrasys	Sebia Hydrasys
Analytical Principle:	IEF with Immunoblotting	IEF with Immunoblotting	IEF with Immunoblotting	IEF with Immunoblotting	IEF with Immunoblotting	IEF with Immunofixation	IEF with Immunofixation	IEF with Immunofixation	IEF with Immunofixation	IEF with Immunofixation
Stain:	ALP Labelled Antisera	Labelled Antisera	Labelled Antisera	Protein Dye and Labelled Antisera	Protein Dye and Labelled Antisera	TTF1	TTF1	TTF1	Labelled Antisera	Labelled Antisera

Figure 2 Illustrates the variation in band counts for Sample E24 as reported by 10 different participants. The x-axis represents individual participants (1–10), each using a combination of measurement systems, analytical principles, and staining techniques. The y-axis shows the band count (0–20). The four different coloured dots correspond to each replicate of Sample E24 that was run in 2024.

Acknowledgement

We acknowledge the AACB/RCPAQAP CSF Advisory Committee for their guidance and support in the development of this program.