Appendix 1 In-house CBA

Our laboratory constructed an HEK293T cell line transfected with lentivirus containing M1 and M23-AQP4 sequence. M1-M23-AQP4-IgG identified dominant genetic segments of lentivirus pLV-CMV-AQP4-M1-M23-EGFP-Puro. HEK293 cells transfected with the negative control lentivirus pLV-CMV-NC-EGFP-Puro were used as a control. The lentivirus transfection rate of HEK293 cells was detected and identified by flow cytometry. Cells with a transfection rate of more than 95% were collected, and cell smear slides were made, dried, and frozen at –80 ℃. Before detection, the slides were removed and fixed. To determine the appropriate dilution, AQP4-Ab positive serum and Cy3-labelled donkey anti-human IgG (Jackson immune, USA) were diluted at different concentrations and then immunofluorescence staining was used. Finally, we established a monoclonal HEK293 cell line that stably expressed M1-M23-AQP4 and made long-term, preservable smeared cell slides using the Jilt piece technique. Using this kind of frozen slide, the test results were stable even after being frozen for 16 weeks (data not shown), which suggested that this technique with high stability might satisfy the clinical requirement at any time. To verify the reliability of the system, from April 2015 to June 2016, our laboratory tested 110 samples from different patients, and the results are shown in *Table S1*. In this study, only 1 out of 24 patients with LETM was positive for serum AQP4-Ab (titre 1:8); 3 of 18 patients with optic neuritis were seropositive, with titres ranging from 1:4 to 1:8. All 22 patients with other neuroimmune diseases and 16 healthy controls were tested negative. The positive rate of CBA was 73.33% (22/30), which was higher than that of ELISA 63.33% (19/30) (*Table S1*). This result is consistent with previous reports regarding M23-based CBA (4,10). In this group of NMOSD patients, the samples were also sent to Euroimmun, and they used IIF to test the expression of NMO-IgG in the samples. The NMO-IgG positive rate was 60% (18/30), which seemed to be lower than that of AQP4-Ab with CBA of 73.33% (22/30).

Table S1 The essential information of 110 enrolled patients and the results of AQP4-Ab detection.

	HC, n=16	NMOSD, n=30	LETM, n=24	ON, n=18	ONID, n=22
Age (years)	7.95±2.15	38.73±2.42	43.88±2.85	32.11±2.75	42.32±3.65
Gender (Female/male)	8/8	24/6	12/12	10/8	12/10
ELISA-AQP4-Ab (U/L)/ Positive AQP4-Ab (%)	0.68±0.12/0	25.89±4.60/33.33 (19/30)	1.75±0.15/4.17 (1/24)	1.26±0.16/16.67 (3/18)	1.47±0.18/0
CBA-AQP4-Ab/Positive AQP4-Ab (%)	–	1:32–1:1024/73.33 (22/30)	1:2–1:8	1:4–1:8	0–1:2

NMOSD, neuromyelitis optic spectrum disease; LETM, longitudinally extensive transverse myelitis; ON, optic neuritis; ONID, other neural system autoimmune diseases; HC, healthy control.

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