Evaluating the icIEF 400 Cartridge: Enhanced Throughput for Maurice Systems

The Maurice™ platform enables imaged capillary isoelectric focusing (icIEF) for rapid and precise charge heterogeneity analysis of proteins, vaccines, and viruses. This study evaluates the two icIEF cartridges available for Maurice systems: the industry-validated Maurice cIEF cartridge, capable of up to 200 injections, and the newly launched icIEF 400 cartridge, designed for extended performance running up to 400 injections. To compare analytical capabilities between the two cartridges, a diverse set of molecules was analyzed on the Maurice system, including USP mAb 001 and 002, Mosunetuzumab, Belimumab, Abatacept, and AAV8.

Materials

TABLE // 01

Materials and reagents used in this study.

Material	Vendor	Catalog #
USP mAb 001	USP	1445539-MAB1
USP mAb 002		1445547-MAB2
Lunsumio® (Mosunetuzumab)	Genentech	N/A
Benlysta® (Belimumab)	GlaxoSmithKline	N/A
Orencia® (Abatacept)	Bristol Myers Squibb	N/A
AAV8	Virovek	N/A
Maurice System	Bio-Techne	**090-000**
Maurice cIEF Cartridge		**PS-MC02-C**
Maurice icIEF 400 Cartridge		**PS-MC02-400C**
Maurice cIEF Method Development Kit*		**PS-MDK01-C**
Biolyte 3-10	Bio-Rad	1631112
Biolyte 4-6		1631143
Phosphate Buffer Saline (PBS)	Sigma-Aldrich	P5493
Dithiothreitol (DTT)	Bio-Techne	N/A
DNase I Reaction Buffer (10X)	New England Biolabs	B0303S
Acetone	Millipore Sigma	100014
Iminodiacetic acid (IDA)		220000
Benzonase® Nuclease		E8263-5KU

*The Maurice cIEF Method Development Kit contains all the necessary reagents for icIEF runs on Maurice/MauriceFlex systems.

Methods

The results section lists details for each sample and their corresponding datasets. Data were generated using both absorbance and native fluorescence (NF) modes of detection (except Abatacept and AAV8, which were analyzed only with NF), and all data were analyzed with Waters™ Empower Chromatography Data System (CDS).

Results

USP mAbs 001 and 002

Each of the USP mAb samples was prepared at a final concentration of 0.15 mg/mL in an ampholyte solution containing 0.35% MC, 2 M urea, 4% Pharmalyte (3:1 8-10.5:5-8), 10 mM arginine, and pI Markers 6.14 and 10.17 before analysis with both cartridges. Samples were focused for 1 min 1500 V, and 10 min 3000 V.

Figure 1 compares the charge profiles of both USP mAb1 and mAb2, generated with the Maurice icIEF 400 and Maurice cIEF cartridges. Both cartridges demonstrate comparable performance, showcasing similar peaks for each mAb analyzed. Further evidence of comparability is highlighted in **Table 2**, with data on the percent peak area (%PA).

FIGURE // 01

Analysis of USP mAb 1 and USP mAb 2 with the Maurice icIEF 400 and Maurice cIEF cartridges. The profiles show similar resolution for charge variants and near-identical peak distribution, confirming comparable performance.

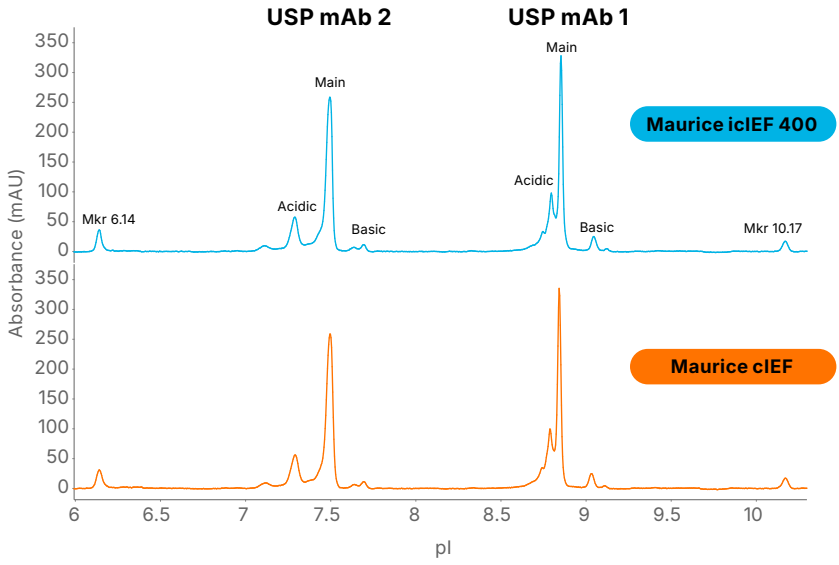

TABLE // 02

Tabulated %PA values for USP mAbs 001 and 002, showing comparable results across the Maurice cIEF and icIEF 400 cartridges for all major peaks.

USP mAbs Percent Peak Area						
	Maurice icIEF 400			Maurice cIEF		
	ACIDIC	MAIN	BASIC	ACIDIC	MAIN	BASIC
USP mAb 001 (n=96)						
Average	37.40	55.18	7.42	37.11	55.50	7.38
%RSD	1.22	0.91	2.47	1.78	1.32	2.52
USP mAb 002 (n=96)						
Average	23.28	72.39	4.33	23.27	72.53	4.21
%RSD	1.11	0.37	2.56	1.17	0.38	3.33

Mosunetuzumab

Mosunetuzumab (brand name Lunsumio®) was prepared at a final concentration of 0.15 mg/mL in an ampholyte solution containing 0.35% MC, 2 M urea, 4% Pharmalyte (3:1 8-10.5:5-8), 10 mM arginine, and pI Markers 6.14 and 10.17 before the samples were analyzed with the Maurice icIEF 400 and Maurice cIEF cartridges, respectively. Samples were focused for 1 min 1500 V, and 10 min 3000 V.

Figure 2 shows an overlay of representative electropherograms of Mosunetuzumab analyzed with both cartridges. The charge profiles are consistent with each other, demonstrating only negligible variations. The consistent performance between cartridges is further highlighted by the percent peak area values in **Table 3**.

FIGURE // 02

Charge heterogeneity analysis of Mosunetuzumab with both cartridges. The overlaid profiles demonstrate comparable charge variant resolution and peak distribution, highlighting the consistent performance between the two cartridge types.

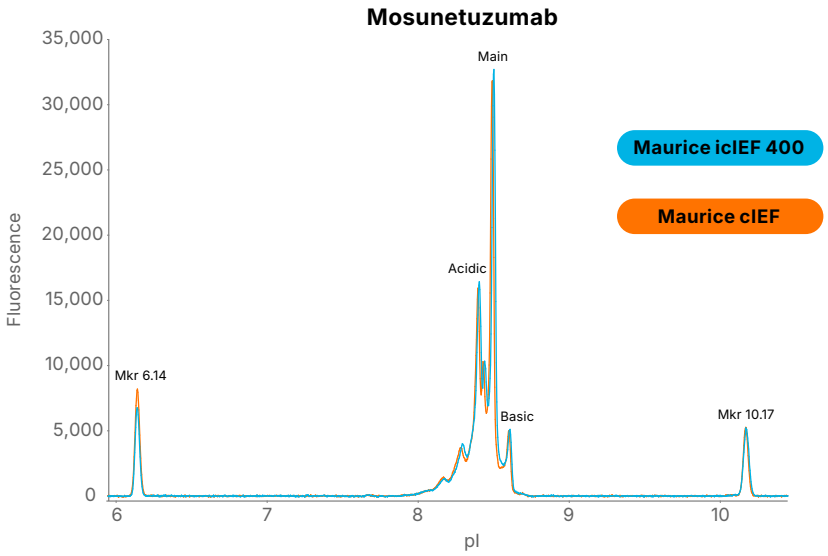

TABLE // 03

Summary and comparison of %PA for Mosunetuzumab charge variants. Consistent results are observed between the icIEF 400 and Maurice cIEF cartridges for each peak. Furthermore, comparable performance of both cartridges was observed under both native fluorescence and absorbance detection modes.

Mosunetuzumab Percent Peak Area (n=96)						
	Maurice icIEF 400			Maurice cIEF		
	ACIDIC	MAIN	BASIC	ACIDIC	MAIN	BASIC
Absorbance						
Average	54.10	37.61	8.10	51.72	39.61	7.82
%RSD	1.27	1.56	4.25	0.91	1.18	4.47
Native Fluorescence						
Average	54.05	37.89	8.05	54.00	37.25	8.19
%RSD	0.89	1.23	3.03	0.84	1.51	6.64

Belimumab

Belimumab (brand name Benlysta®) was prepared at a final concentration of 0.15 mg/mL in an ampholyte solution containing 0.35% MC, 2 M urea, 4% Pharmalyte (3:1 8-10.5:5-8), 10 mM arginine, and pI Markers 6.14 and 10.17 before the samples were analyzed with both cartridges. Samples were focused for 1 min 1500 V, and 10 min 3000 V.

Figures 3A and **3B** present the charge profiles of Belimumab analyzed with the Maurice icIEF 400 and Maurice cIEF cartridges, respectively. Both profiles align closely, with only minor variations observed. The comparability in performance between the cartridges is further supported by the percent peak area data summarized in **Table 4**.

FIGURE // 03

Charge profiles of Belimumab analyzed using the Maurice icIEF 400 cartridge (A) and the Maurice cIEF cartridge (B). Both profiles exhibit comparable charge variant resolution and peak distribution, demonstrating consistent performance across the two cartridge types.

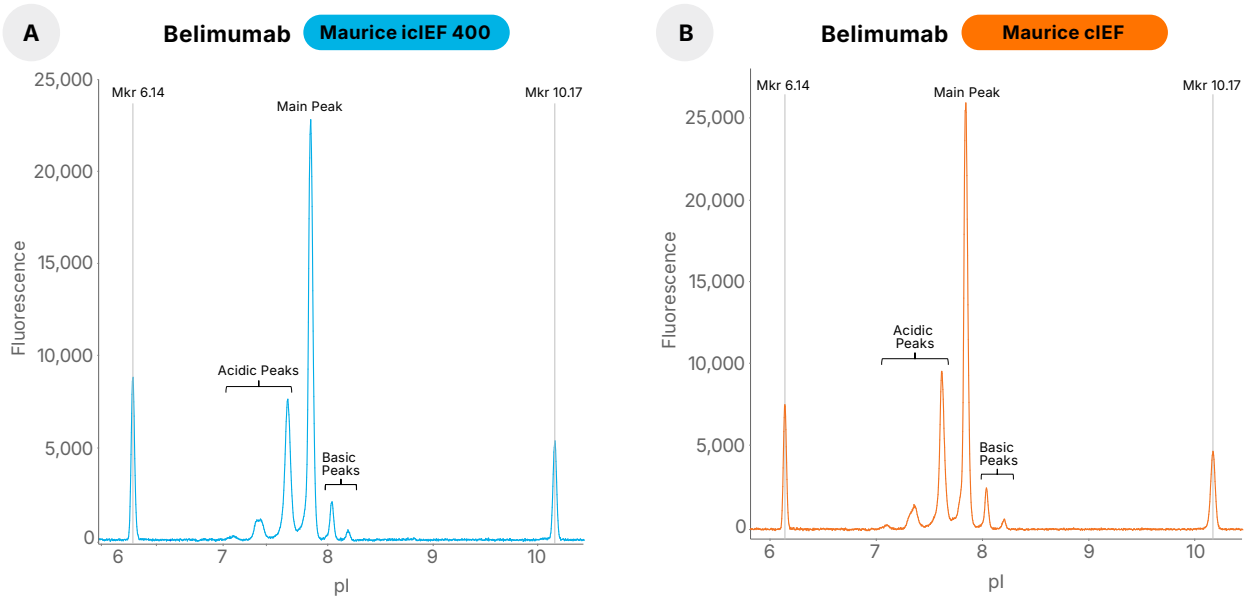

TABLE // 04

Comparison of %PA values for Belimumab charge profiles. The results demonstrate similar performance between the Maurice icIEF 400 and Maurice cIEF cartridges for each peak. Additionally, similar performance was noted for both cartridges under absorbance and native fluorescence detection modes.

Belimumab Percent Peak Area (n=96)						
	Maurice icIEF 400			Maurice cIEF		
	ACIDIC	MAIN	BASIC	ACIDIC	MAIN	BASIC
Absorbance						
Average	35.07	58.36	6.57	33.33	60.70	5.96
%RSD	1.15	0.77	3.65	1.15	0.73	5.05
Native Fluorescence						
Average	34.87	58.59	5.27	34.95	58.83	5.05
%RSD	1.26	0.70	2.81	0.98	0.62	4.76

Abatacept

250 mg of Abatacept (brand name Orencia®) was dissolved in 2 mL dH_2O and stored at -80°C in aliquots of 125 mg/mL. Abatacept was prepared at a final concentration of 0.2 mg/mL in an ampholyte mixture containing 0.35% MC, 10% SimpleSol, 4% Biolyte (3:1 4-6:3-10) 10 mM arginine, pI Markers 4.05 and 6.14. The samples were analyzed on the Maurice system with the Maurice icIEF 400 and Maurice cIEF cartridges respectively, and separated for 1 min at 1500 V, then 12 min at 3000 V.

Figure 4 displays stacked charge profiles of Abatacept obtained using both cartridges. Several peaks were detected by each cartridge, revealing charge profiles like those published by Wu *et al.* in their work on developing a platform icIEF method for analyzing fusion proteins[1]. The profiles demonstrate strong alignment with minimal variations across the two cartridges, as further shown in **Table 5**.

FIGURE // 04

A comparison of charge profiles of Abatacept, analyzed with the Maurice icIEF 400 and Maurice cIEF cartridges. The peaks are divided into groups, all of which are detected by both cartridges, demonstrating comparability between them.

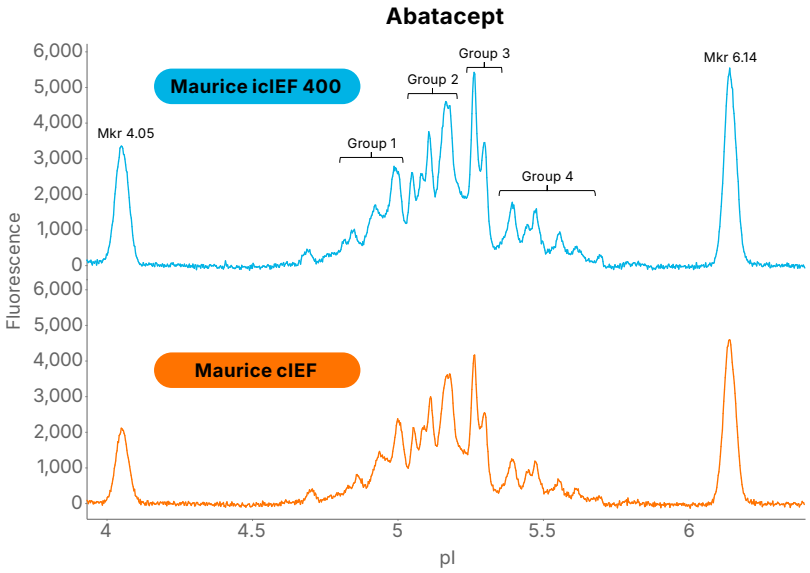

TABLE // 05

A comparison of the percent peak area for Abatacept. Results between both cartridges are consistent for each peak group detected.

Abatacept Percent Peak Area (n=96)								
	Maurice icIEF 400				Maurice cIEF			
	GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 1	GROUP 2	GROUP 3	GROUP 4
Average	24.04	39.58	18.84	17.55	24.58	39.77	18.60	17.04
%RSD	0.91	1.15	2.41	1.31	1.00	1.37	2.31	1.62

AAV8

Denatured AAVs Analysis

10 µL of AAV8 was incubated for 30 min at 37°C. After incubation, cold acetone (5X the sample volume) was added, followed by incubation for 1 hour at -80°C. The sample was centrifuged at 13,200 rpm for 10 min, and the resulting pellet was air-dried. The pellet was then resuspended in a 10 µL solution containing DMSO, 20 mM histidine/ 30 mM acetate buffer, and DTT in a 7:2:1 ratio. The mixture was incubated at 70°C for 5 min. Finally, 10 µL of the prepared sample mixture was added to an ampholyte mixture (50 µL) containing 0.35% MC, 3% Pharmalyte: 3-10, 5 mM DTT, 5 mM IDA, 4.5 mM arginine, pI Markers 5.85 and 8.4, 4M urea, and 17% formamide. The samples were analyzed with both cartridges, and separated for 1 min at 1000 V, 1 min at 2000 V, and finally 12 min at 3000 V. Data were generated through NF detection.

Figures 5A and **5B** show the representative charge profile of the denatured AAV8, detected with the Maurice icIEF 400 and Maurice cIEF cartridges, respectively. These profiles are consistent with previously reported AAV8 charge heterogeneity[2,3]. Both profiles consist of similar peaks, indicating similar performance of both cartridges. The quantitative results of AAV8 analysis are shown in **Table 6**.

FIGURE // 05

Analysis of denatured AAV8 with Maurice icIEF 400 (A) and Maurice cIEF (B) cartridges. The same peaks are detected by both cartridges.

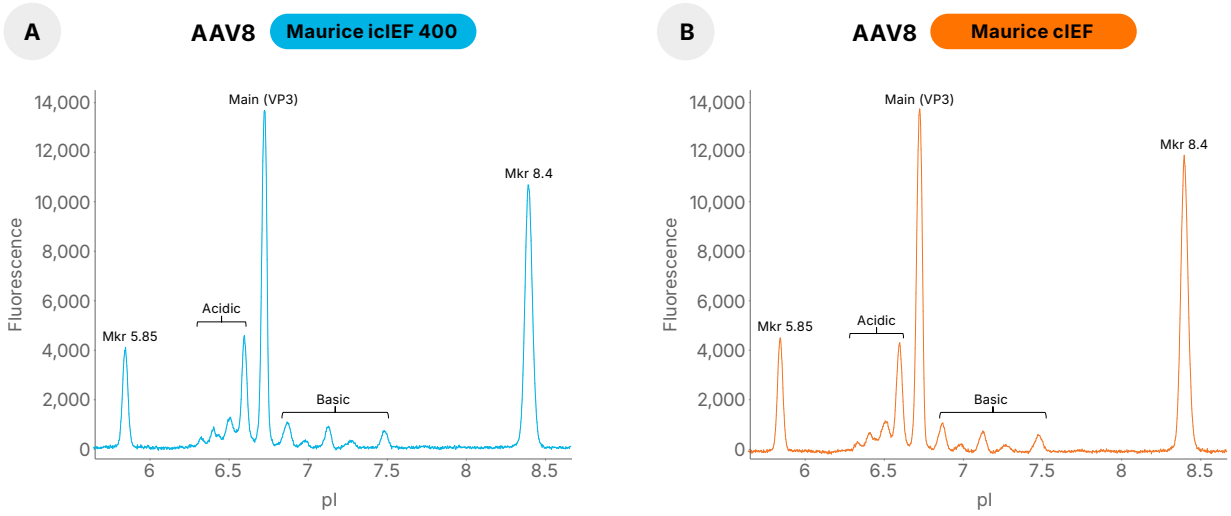

TABLE // 06

Summary of %PA for AAV8, showing overall comparability between both Maurice icIEF 400 and Maurice cIEF cartridges

AAV8 Percent Peak Area (n=96)						
	Maurice icIEF 400			Maurice cIEF		
	ACIDIC	MAIN (VP3)	BASIC	ACIDIC	MAIN (VP3)	BASIC
Average	32.12	50.32	17.58	32.23	49.92	17.83
%RSD	0.46	0.63	1.72	0.73	0.51	0.95

Conclusion

Significant updates to analytical instruments or their consumables for biotherapeutics must demonstrate—in addition to robust performance—comparability to their legacy counterparts. The Maurice icIEF 400 cartridge was designed to improve throughput and efficiency by enabling the analysis of 400 samples. However, as a new cartridge, it is important to measure its performance against the Maurice cIEF cartridge. In this application note, five different molecules were analyzed with both cartridges and their average percent peak area values were compared. Across all molecules, the results were remarkably similar and highlighted that both cartridges are indeed comparable, and either one can be used depending on the requirements of different drug development phases.

Get the Maurice icIEF 400 Cartridge

Scan the QR Code or Visit:
bio-techne.com/icIEF-400

References

1. Wu, G., Yu, C., Wang, W., Zhang, R., Li, M., & Wang, L. (2022). A platform method for charge heterogeneity characterization of fusion proteins by icIEF. Analytical biochemistry, 638, 114505. **https://doi.org/10.1016/j.ab.2021.114505**

2. He, X. Z., Powers, T. W., Huang, S., Liu, Z., Shi, H., Orlet, J. D., Mo, J. J., Srinivasan, S., Jacobs, S., Zhang, K., Runnels, H. A., Anderson, M. M., & Lerch, T. F. (2023). Development of an icIEF assay for monitoring AAV capsid proteins and application to gene therapy products. Molecular therapy. Methods & clinical development, 29, 133–144. **https://doi.org/10.1016/j.omtm.2023.03.002**

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