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Verification, implementation and harmonization of automated chemiluminescent immunoassays for MPO- and PR3-ANCA detection

Abstract

Objectives: Antineutrophil cytoplasmic antibody (ANCA) testing assists clinicians diagnose ANCA-associated vasculitis (AAV). We aimed to verify and harmonize chemiluminescent immunoassays for the detection of myeloperoxidase (MPO)- and proteinase 3 (PR3)-ANCA.

Methods: An in-house ELISA, a capture ELISA, and a chemiluminescent assay QUANTA Flash on a BIO-FLASH analyzer were used to detect MPO- and PR3-ANCA in sera from 39 patients with AAV, 55 patients with various non-AAV, and 66 patients with connective tissue diseases. The results of the assays were evaluated, and their clinical performance was assessed. The precision and linearity of the QUANTA Flash assays were determined, and likelihood ratios (LRs) for AAV at diagnosis were calculated.

Results: The precision and linearity of the QUANTA Flash assays were confirmed. Overall agreement between 97.5 and 98.8 % and Cohen's kappa coefficients between 0.861 and 0.947 were observed for the results of the QUANTA Flash assays and ELISAs. The diagnostic sensitivity, specificity, and ROC analysis of the assays for AAV were statistically similar (in-house ELISA 89.7 %, 95.0 %, and 0.937; capture ELISA 92.3 %, 98.3 %, and 0.939; and QUANTA Flash 89.7 %, 95.9 %, and 0.972). For the QUANTA Flash assay results, the interval-specific LRs for AAV at diagnosis were: 0–8 CU had LR 0.08, 8–29 CU had LR 1.03, 29–121 CU had LR 7.76, 121–191 CU had LR 12.4, and >191 CU had LR ∞.

Conclusions: The QUANTA Flash MPO and PR3 assays provide precise and consistent results and have comparable clinical utility for AAV. The calculated LRs were consistent with published LRs, confirming the utility of LRs for harmonization of ANCA results.

Keywords: antineutrophil cytoplasmic antibodies (ANCA); proteinase 3 (PR3); myeloperoxidase (MPO); ANCA-associated vasculitis (AAV); chemiluminescent immunoassay; likelihood ratios (LRs)

Introduction

Antineutrophil cytoplasmic antibodies (ANCA) targeting proteins in primary granules of neutrophils are crucial for the identification and classification of ANCA-associated vasculitides (AAV), including granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA), and eosinophilic granulomatosis with polyangiitis (eGPA) [1–4].

According to the international consensus on AAV classification criteria of the Chapel Hill Consensus Conference [5], the presence of ANCA is considered a valuable laboratory marker for AAV diagnosis, but only when there is sufficient clinical suspicion to avoid false positive diagnoses. The 1999 international consensus statement [6] recommended the use of indirect immunofluorescence (IIF) as the initial screening method for the detection of ANCA. Cytoplasmic ANCA (c-ANCA) and perinuclear ANCA (p-ANCA) are the two patterns seen at ANCA IIF. Positive samples should then be analyzed with antigen-specific immunoassays to determine the specificity of the ANCAs. The c- and p-ANCA in patients with AAV are mainly directed against proteinase 3 (PR3) and myeloperoxidase (MPO), respectively [1]. The majority of patients with GPA are PR3-ANCA positive, whereas the majority of patients with MPA are MPO-ANCA positive [1, 4, 7, 8]. The diagnostic performance of this testing algorithm has recently been questioned, although it remains widely used.

According to the multicenter study [9], the diagnostic performance of antigen-specific immunoassays is equal to or

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even better than the diagnostic performance of ANCA IIF in distinguishing AAV from disease controls. Based on these findings, a new international consensus on ANCA testing was reached in 2017 [10], suggesting that high-quality immunoassays can be used as a primary screening method without the categorical need for IIF. Furthermore, in patients with high clinical suspicion of AAV or low antibody levels, performing a second immunoassay has been suggested to increase the sensitivity or specificity of the test. Nevertheless, there is currently no consensus on how ANCA testing should be performed to monitor patients with AAV [11].

In 2020, a new updated document was published concluding that antigen-specific ANCA targeting PR3 and MPO should be tested by solid-phase immunoassays in all patients with clinical features suggestive of AAV and in all patients with anti-GBM disease, idiopathic interstitial pneumonia, and infective endocarditis associated with nephritis. In cases of suspected autoimmune hepatitis type I without conventional autoantibodies or diagnostic uncertainty regarding the distinction between ulcerative colitis and Crohn's disease, ANCA should be tested with IIF because the target antigens are not characterized in these diseases [12].

Three types of enzyme-linked immunosorbent assays (ELISAs) are widely used in laboratories for ANCA testing: first-generation direct ELISA (antigen is directly coated on the plate), second-generation capture ELISA (antigen is indirectly coated through a specific capture monoclonal antibody), and third-generation anchor ELISA (antigen is bound with a peptide linker). In recent years, automated chemiluminescent immunoassays (CLIA) have been developed for ANCA testing and are already used in daily laboratory practice [2, 13–16].

Because of the importance of ANCA testing in AAV and the wide range of assays available today, numerous attempts have been made to standardize ANCA values. A number of manufacturers use reference standards for PR3- and MPO-ANCA to calibrate their ANCA assays. Recently, Institute for Reference Materials and Measurements (IRMM)-certified reference materials for MPO- and PR3-ANCA (IS2720 #15 Anti-MPO-ANCA and IS2721 #16 Anti-PR3-ANCA, from the Centers for Disease Control and Prevention) have become available, and these reference materials were evaluated in the study by Bossuyt et al. [17]. However, despite the availability of reference materials, there is still a need for standardization of ANCA measurements.

The recommendation to harmonize the clinical interpretation of ANCA test results by reporting test result-specific likelihood ratios (LRs) (probability of a specific result in patients divided by the probability of the same result in controls) has been published along with LRs for different assays in use [18, 19]. For all assays, test result-specific LRs increased significantly with increasing antibody levels. Two principals have been proposed: first, reporting LRs for test result intervals; or second, reporting the threshold value in conjunction with LRs of 0.1, 1, 10, and 30. However, the presented principles for reporting results have not yet been routinely adopted.

The new European *In Vitro* Diagnostics Regulation 2017/746 (IVDR), which applies to *in vitro* diagnostic devices, impacts both clinical laboratories and manufacturers, so autoantibody testing practices will need to change in the coming years. These changes will impact many laboratories worldwide, including ours, where current ANCA testing procedures include elements of the 1999 and 2017 international consensus statements. In our practice, this means that all samples currently tested with IIF ANCA and the in-house direct ELISA, and in some cases even with a more sensitive capture ELISA, will then need to be tested with diagnostic medical devices that comply with the new EU Regulation 2017/746.

Therefore, the main objective of this study was to verify, implement, harmonize, and clinically apply CLIA assays (QUANTA Flash MPO and PR3 on BIO-FLASH, Inova Diagnostics, Werfen, San Diego, CA, USA).

Our objective was, first, to evaluate the analytical performance of CLIA assays for the detection of MPO- and PR3-ANCA antibodies, second, to compare the results with those of in-house ELISA (first generation) and capture ELISA (second generation), third, to determine the diagnostic value of MPO- and PR3-ANCA detected by either CLIA assays or in-house or capture ELISAs for the diagnosis of AAV, and fourth, to compare our QUANTA Flash assay interval-specific LRs with previously published LRs [18].

Materials and methods

Patients

Serum samples from patients with AAV (MPA and GPA), from patients with non-AAV vasculitis (IgA vasculitis, cryoglobulinemic vasculitis), and from patients with connective tissue diseases (CTD) (systemic lupus erythematosus (SLE), systemic sclerosis (SSc), Sjögren's disease (SjS), and rheumatoid arthritis (RA)) were collected at the Department of Rheumatology, University Medical Centre Ljubljana, Slovenia, and analyzed in the immunology laboratory. The study was performed according to the guidelines of the Declaration of Helsinki and approved by the National Medical Ethics Committee, Ljubljana, Slovenia (0120-55/2019/5).

Serum collection

Samples were collected in routine clinical settings. Blood samples were centrifuged at 1,800×*g* for 10 min within 2 h of collection, and serum was separated directly from the cell pellet. Samples were stored at 4 °C and analyzed the next day, or aliquoted into tubes and stored at –80 °C for later analysis.

Assays for the measurement of MPO- and PR3-ANCA

MPO- and PR3-ANCA antibodies were tested with three immunoassays: an in-house direct ELISA, a capture ELISA, and a CLIA QUANTA Flash on the BIO-FLASH analyzer according to the manufacturer's guidelines. The characteristics of the different assays are shown in Table 1 and Figure 1.

Verification of CLIA assays

As part of the verification process, we evaluated the precision and linearity of the CLIA QUANTA Flash MPO and PR3 assays. Assays were performed in accordance with the relevant Clinical and Laboratory Standards Institute (CLSI) guidelines, EP5-A3. Two samples were tested

Table 1: Characteristics of the MPO- and PR3-ANCA assays.

MPO-ANCA			
Assay principle	**Direct ELISA**	**Capture ELISA**	**CLIA (QUANTA Flash)**
Manufacturer	In-house	Svar Life Science (formerly Euro Diagnostica AB), Malmö, Sweden	Inova Diagnostics, San Diego, CA, USA
Antigen	MPO on the plate	MPO on the MoAb carrier on the plate	MPO on the beads
Units	U/mL	IU/mL	IU/mL or CU
Cut-off	10	5	6 or 20
Range	10–320	5–200	6–221.94 or 20–739.8
Traceability	/	IS2720 #15 anti-MPO-ANCA	IS2720 #15 anti-MPO-ANCA
PR3-ANCA			
Manufacturer	In-house	Svar Life Science (formerly Euro Diagnostica AB), Malmö, Sweden	Inova diagnostics, San Diego, CA, USA
Antigen	PR3 on the plate	PR3 on the MoAb carrier on the plate	PR3 on the beads
Units	U/mL	IU/mL	IU/mL or CU
Cut-off	10	5	5 or 20
Range	10–320	5–200	5.0–821.3 or 20–3,285.5
Traceability	/	IS2721 #16 anti-PR3-ANCA	IS2721 #16 anti-PR3-ANCA

five times daily for 5 days. To assess linearity, positive samples and their dilutions (1:2, 1:4, 1:8, 1:16) were tested in duplicate, and linear regression was calculated.

Comparison of assays

The overall agreement, negative agreement, and positive agreement between the results of the different assays were calculated. The spearman's rank correlation coefficient was used to analyze the quantitative correlations, and the Cohen's kappa test was performed to determine the qualitative agreement between the results of the different assays using the cut-off values shown in Table 1. p-Values of less than 0.05 were considered significant.

Measurement of the diagnostic value

The diagnostic sensitivity, specificity, and receiver operating characteristic (ROC) analysis of MPO/PR3-ANCA (the highest reactivity level from MPO- or PR3-ANCA determination was selected) were determined to evaluate the diagnostic performance of QUANTA Flash, in-house ELISA, and capture ELISA for AAV.

Likelihood ratios (LRs)

We calculated LRs (probability of a specific result in patients divided by the probability of the same result in controls) as suggested in publication on harmonization of ANCA testing [18]. Interval-specific LRs associated with AAV at diagnosis were calculated for QUANTA Flash MPO and PR3 results. The highest reactivity level from MPO- or PR3-ANCA determination was selected for analysis of LRs for AAV.

Statistical programs

Statistical analyses were performed using Analyse-IT for Microsoft Excel (Analyse-IT Software Ltd., Leeds, UK).

Results

Patients

Samples from 160 patients were included in our study. The AAV group comprised 39 patients with newly diagnosed AAV (17 patients with MPA and 22 patients with GPA, median age [IQR] 66 years [54–77], 53.8 % females), the non-AAV vasculitis control group comprised 55 patients with other vasculitides (IgA vasculitis, cryglobulemic vasculitis, skin organ vasculitis, median age [IQR] 58 years [50–80], 43.6 % females), and the CTD control group comprised 66 patients with SLE (n=19), SjS (n=17), SSc (=12), or RA (n=18) (median age [IQR] 60 years [48–72], 80.3 % females).
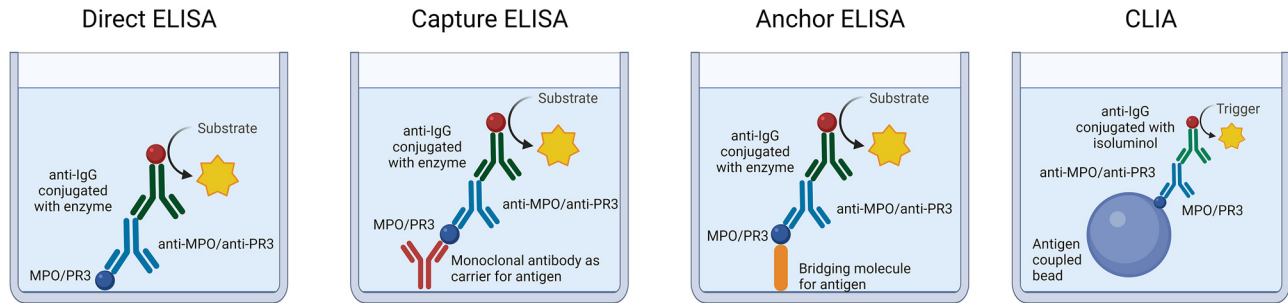
Figure 1: The principle graphical representation of direct in-house ELISA, capture ELISA, anchor ELISA and CLIA.

Analytical performance – precision and linearity of QUANTA Flash MPO and PR3

Within-run coefficient of variation (CV) ranged from 4.7 % (low positive sample, overall mean 48.6 CU) to 5.1 % (high positive sample, overall mean: 281 CU) for MPO-ANCA and from 3.9 % (low positive sample, overall mean: 46.6 CU) to 5.3 % (high positive sample, overall mean: 321.3 CU) for PR3-ANCA. Between-run CV ranged from 5.7 % (high positive sample) to 5.8 % (low positive sample) for MPO-ANCA and from 5.2 % (low positive sample) to 8.9 % (high positive sample) for PR3-ANCA.

QUANTA Flash MPO had a regression slope of 1.003 (95 % CI 0.986–1.049) and QUANTA Flash PR3 had a regression slope of 1.061 (95 % 0.910–1.243).

Qualitative agreement and quantitative correlation between QUANTA Flash and ELISAs

The number of positive samples analyzed with three different assays is shown in Table 2 for each patient group.

Table 2: The number of positive samples in each patient group analyzed with three different assays.

Groups		Assay (number of positive samples)		
		In-house ELISA	Capture ELISA	QUANTA Flash
MPA (n=17)	MPO	17	16	16
	PR3	0	0	0
GPA (n=22)	MPO	3	3	3
	PR3	15	17	16
Non-AAV (n=55)	MPO	3	2	3
	PR3	0	0	1
CTD (n=66)	MPO	3	0	1
	PR3	0	0	0

Agreement for MPO-ANCA

The agreement between in-house ELISA and QUANTA Flash results was 98.1 %, with a Cohen's kappa coefficient of 0.928 (95 % CI 0.847–1.000) (Table 3). Three mismatches (3/160 samples) were detected that were classified as negative by QUANTA Flash and positive by in-house ELISA. All three were negative by capture ELISA. Two of them were from the CTD group and one from a patient with MPA. The Spearman's rank correlation coefficient of the positive MPO-ANCA results between the in-house ELISA and QUANTA Flash was 0.809, p<0.001, indicating that there was a significant and strong positive relationship between the results of the two tests.

There was excellent agreement between the results of the capture ELISA and QUANTA Flash (Cohen's κ=0.947, 95 % CI 0.875–1.000) with an overall agreement of 98.8 %. Two mismatched samples (2/160 samples) were positive by QUANTA Flash and negative by capture ELISA (samples were positive by in-house ELISA). These two samples were from patients with CTD. The Spearman's rank correlation coefficient between the capture ELISA and QUANTA Flash results was 0.914, p<0.001, indicating that there was a significant and strong positive relationship between the results of both tests.

Agreement for PR3-ANCA

The agreement between the results of in-house ELISA and QUANTA Flash was 97.5 %, with a Cohen's kappa coefficient of 0.861 (95 % CI 0.728–0.995) (Table 3). Four (4/160 samples) discrepancies were found, two of which were found to be positive by QUANTA Flash and negative by in-house ELISA (but positive by capture ELISA). These two patients were diagnosed with GPA. One patient with IgA vasculitis had only a positive QUANTA Flash, and one patient with GPA had only a positive in-house ELISA. The Spearman's rank

Table 3: Qualitative agreement between assays.

		In-house ELISA vs. QUANTA Flash	Capture ELISA vs. QUANTA Flash
MPO-ANCA	Total % agreement	98.1	98.8
	Negative agreement (95 % CI)	100 (97.2–100)	98.6 (94.9–99.6)
	Positive agreement (95 % CI)	88.5 (71.0–96.0)	100 (84.5–100)
	Cohen's kappa coefficient (95 % CI)	0.928 (0.847–1.000)	0.947 (0.875–1.000)
	Spearman's rho (ρ)	0.809 (<0.001)	0.914 (<0.001)
PR3-ANCA	Total % agreement	97.5	98.8
	Negative agreement (95 % CI)	97.9 (94.1–99.3)	99.3 (96.1–99.9)
	Positive agreement (95 % CI)	93.3 (70.2–98.8)	94.1 (73.0–99.0)
	Cohen's kappa coefficient (95 % CI)	0.861 (0.728–0.995)	0.934 (0.844–1.000)
	Spearman's rho (ρ)	0.451 (0.1)	0.674 (0.005)

correlation coefficient of positive PR3-ANCA results between in-house ELISA and QUANTA Flash was 0.451, p=0.100, indicating that there was no correlation between the results of the two tests.

The agreement between the results of capture ELISA and QUANTA Flash was 98.8 % with a Cohen's kappa of 0.934 (95 % CI 0.844–1.000). Here we found two samples whose results differed (2/160 samples). One, from a patient with IgA vasculitis, was positive with QUANTA Flash and negative with the capture ELISA, and the other from a GPA patient had an opposite result. Both samples were negative with the in-house ELISA. The Spearman's rank correlation coefficient between the capture ELISA and QUANTA Flash results was 0.674, p=0.005, indicating that there was a significant positive relationship, between the results of both tests.

Diagnostic performance of MPO/PR3-ANCA for AAV measured with the QUANTA Flash assays compared with in-house and capture ELISAs

MPO- and PR3-ANCA results and predefined cut-offs (in-house ELISA 10 U/mL, capture ELISA 5 U/mL, and QUANTA Flash 20 CU) were used to determine diagnostic sensitivity, specificity, and ROC curves for AAV. The diagnostic sensitivity and specificity of all three assays were similar for AAV diagnosis (Table 4). The diagnostic performance of MPO/PR3-ANCA for the diagnosis of AAV was evaluated using ROC curves. The areas under the curve (AUC) of each assay were comparable and not statistically significantly different. ANCA correlated significantly with AAV, regardless of which assay was used for its detection. The diagnostic accuracy for AAV of the in-house ELISAs, capture ELISAs, and QUANTA Flash assays ranged from 0.937 to 0.972 (Table 4).

Table 4: Clinical significance of MPO/PR3-ANCA for AAV detected with different assays.

	1 in-house ELISA	2 capture ELISA	3 QUANTA Flash	p-Value
Sensitivity	89.7	92.3	89.7	1 vs. 2 ns
(95 % CI)	(76.4–95.9)	(79.7–97.3)	(76.4–95.9)	1 vs. 3 ns
				2 vs. 3 ns
Specificity	95.0	98.3	95.9	1 vs. 2
(95 % CI)	(89.6–97.7)	(94.2–99.5)	(90.7–98.2)	0.05
				1 vs. 3 ns
				2 vs. 3 ns
AUC	0.937	0.939	0.972	1 vs. 2 ns
(95 % CI)	(0.886–0.988)	(0.871–1.006)	(0.937–1.008)	1 vs. 3 ns
				2 vs. 3 ns

Thresholds for defined specificity

Following the recommendations for harmonization of clinical interpretation of ANCA test results [18], thresholds for the QUANTA Flash immunoassay were calculated in our group of AAV patients. Thresholds corresponding to specificities of 95.0, 97.5, 99.2 and 100 % were determined. The LRs for a positive test result increased with increasing threshold (Table 5). LRs were 18.62, 36.20, and 93.08 for a threshold corresponding to 95.0, 97.5, and 99.2 % specificity, respectively. Sensitivity (proportion of patients tested positive) ranged from 92.3 % corresponding to a 95.0 % specificity to 76.9 %, corresponding to 99.2 % specificity.

Interval-specific LRs

Next, we defined test result intervals using the above thresholds. For each test result interval, we calculated the fraction of patients and controls who had a result within that

Table 5: Thresholds corresponding to predetermined specificities.

Threshold, CU	Sensitivity, % (95 % CI)	Specificity, % (95 % CI)	LR (+) (95 % CI)	LR (−) (95 % CI)
8	92.3 (79.7–97.3)	95.0 (89.6–97.7)	18.62 (8.819–40.449)	0.08 (0.028–0.214)
29	89.7 (76.4–95.9)	97.5 (93.0–99.2)	36.20 (12.68–106.43)	0.11 (0-04-0.24)
121	76.9 (61.7–87.4)	99.2 (95.5–99.9)	93.08 (16.85–529.52)	0.23 (0.13–0.39)
191	66.7 (61.0–79.4)	100 (96.9–100.0)	∞ (21.63–∞)	0.33 (0.21–0.49)

Table 6: LRs corresponding to a specific interval.

Specificity, % intervals	Interval, CU	Fraction of patients	Fraction of controls	LR (95 % CI)
<95.0	0–8	0.077	0.950	0.08 (0.03–0.21)
95.0–97.5	8–29	0.026	0.025	1.03 (0.42–2.25)
97.5–99.2	29–121	0.128	0.017	7.76 (3.43–21.06)
99.2–100	121–191	0.103	0.008	12.41 (5.49–73.18)
>100	191–1,577	0.667	0.000	∞

interval and the interval-specific LR for AAV. The interval-specific LRs for AAV are shown in Table 6.

Applying the 97.5–99.0 % specificity interval, we found that the same number of AAV patients (35/39) were selected, regardless of the way of interval determination (calculated from the current study [29–121 CU] or published interval [23.8–78.2 CU]).

Discussion

ANCA testing is nowadays almost indispensable in the diagnosis and monitoring of patients with AAV. These assays play an enormous role in medical decisions, treatment plans, and patient care. As recommended by many AAV experts, solid-phase immunoassays to detect MPO/PR3-ANCA could be used adjunctively in all patients with clinical features suggestive of AAV without the use of IIF [9]. To ensure that assays are accurate, reliable, and provide consistent results, they need to be verified, implemented, and harmonized among laboratories. Importantly, the new European *In Vitro* Diagnostic (IVD) Regulation 2017/746 imposes stricter requirements on the use of in-house-developed tests than the previous directive.

In this study, we verified, implemented, harmonized and clinically applied the ANCA CLIA assays (QUANTA Flash MPO and PR3 assays on the BIO-FLASH analyzer). In the first step of the verification process, we confirmed that the precision and linearity claimed by the manufacturer were also achieved in our laboratory environment. Coefficients of variation (CV) were below 10 % for within-run repeatability and below 15 % for between-run repeatability, which is considered broadly acceptable CV. The reported regression slopes of 1.003 (95 % CI 0.986–1.049) for MPO-ANCA and 1.061 (95 % 0.910–1.243) for PR3-ANCA were fully comparable to those reported by the manufacturer, confirming the linearity of the QUANTA Flash MPO and PR3.

Second, we compared the results of QUANTA Flash MPO and PR3 with those of in-house (first generation) and capture (second generation) ELISAs. In this way, we wanted to verify to what extent the new assay would provide similar results to the diagnostic procedures we have been performing in our laboratory for more than a decade. In this way, we would be able to communicate to physicians the comparability of the new and old results. Although QUANTA Flash and ELISA differed in their analytical and technological characteristics, the agreement between the different assays for the detection of MPO- and PR3-ANCA results was excellent (overall agreement between 97.5 and 98.8), considering the manufacturers' thresholds. In particular, QUANTA Flash MPO and PR3 provided more comparable results to capture ELISA than to in-house ELISA, as evidenced by a higher percentage of positive agreement (100 vs. 88.5 % for MPO-ANCA, 94.1 vs. 93.3 % for PR3-ANCA), a higher Cohen's kappa coefficient (0.934 vs. 0.861 for PR3-ANCA), and higher correlation coefficients (0.914 vs. 0.809 for MPO-ANCA, 0.674 vs. 0.451 (ns) for PR3-ANCA). Our results mirror those of previous studies showing high agreement and correlation between QUANTA Flash and commercial ELISAs [15, 16]. Importantly, we also confirmed that novel assays can indeed replace in-house ELISA and be used in daily practice, as the results obtained with QUANTA Flash are very similar to in-house ELISA results.

The next section of this study addressed the evaluation of the clinical utility of QUANTA Flash MPO and PR3. In addition to the analytical comparability of the two QUANTA Flash assays with currently used assays, the comparable clinical utility of CLIA for AAV (MPA/GPA) was also demonstrated. The diagnostic sensitivity and specificity of QUANTA Flash MPO and PR3 for AAV (89.7 and 95.9 %, respectively) are fully comparable to the sensitivity and specificity of in-house (89.7 and 95.0 %, respectively) and capture ELISA (92.3 and 98.3 %, respectively). This is consistent with the manufacturer's reported diagnostic sensitivity and specificity for MPA/GPA (MPO: 79.8 and 96.2 % for MPA; PR3: 68.3 and 97.4 % for GPA, respectively).

Harmonization of diagnostic tests between laboratories is critical to ensure consistent and comparable results. Standardization of methods, reagents, and equipment helps reduce variability and discrepancies in test results. It allows comparison of results between different laboratories. Bossuyt et al. [18, 19] proposed harmonization of ANCA testing by reporting interval and test result-specific LRs. LRs are alternative statistics that indicate the probability of a given result in patients divided by the probability of the same result in controls to summarize diagnostic accuracy. LR values above 10 are considered strong evidence of diagnostic accuracy in most cases. Each test result has its own LR, which summarizes how much more likely or less likely it is that patients with the disease will have that particular result compared with patients without disease.

Therefore, in the final part of our study, we evaluated the previously published LRs in our clinically defined patient population and determined our own LRs corresponding to diagnostic specificities of 95.0, 97.5, 99.2 %, and 100 %. However, because of a different population and a smaller number of patients with AAV, we obtained slightly different intervals and LRs. However, considering either the published intervals or the intervals calculated in our study, the same fraction of patients with AAV (n=35) fell within the interval of 97.5–99 % diagnostic specificity or higher.

This study was an important step in the verification, implementation, and harmonization of QUANTA Flash MPO and PR3 assays in our laboratory to ensure the precision, reliability, and consistency of test results. Nevertheless, the study has its limitations. We included only a relatively small group of patients. And because the study was limited to a single center, the inter-laboratory variation could not be verified.

In this study, we critically examined and evaluated a QUANTA Flash chemiluminescent immunoassays performed on a fully automated analyzer. We confirmed that the analytical precision and linearity claimed by the manufacturer could be achieved in our laboratory. The QUANTA Flash assays provided results that were highly comparable to ELISA results, not only from an analytical perspective but also in terms of clinical utility. Crucially, our work on a different population showed similarity to previously published LRs for ANCA testing for AAV, suggesting that the LRs could be adopted in other laboratories and would be useful for harmonizing ANCA test results.

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