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Research paper

## Evaluation of a novel particle-based assay for detection of autoantibodies in idiopathic inflammatory myopathies

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## ABSTRACT

**Background:** Myositis specific antibodies (MSA) represent not only important diagnostic tools for idiopathic inflammatory myopathies (IIM), but also help to stratify patients into subsets with particular clinical features, treatment responses, and disease outcome. Consequently, standardization of MSA is of high importance. Although many laboratories rely on protein immunoprecipitation (IP) for the detection of MSA, IP standardization is challenging and therefore reliable alternatives are mandatory. Recently, we identified significant variation between IP and line immunoassay (LIA) for the detection of MSA and myositis associated antibodies. In this study we aimed to compare the results from our previous study to the results obtained with a novel fully automated particle-based technology for the detection of MSA and MAA.

**Methods:** A total of 54 sera from patients with idiopathic inflammatory myopathy (IIM) were tested using three methods: IP, LIA (Euroimmun, Germany) and a novel particle-based multi-analyte technology (PMAT, Inova Diagnostics, US, research use only). The analysis focused on antibodies to EJ, SRP, Jo-1, NXP-2, MDA5, TIF1- $\gamma$ , and Mi-2.

**Results:** Significant variations were observed among all methods. Overall, the novel PMAT assays showed slightly better correlation with IP, but the kappa agreement was strongly dependent on the antibody tested. When the results obtained from IP were used as reference for receiver operating characteristic (ROC) curve analysis, good discrimination and a high area under the curve (AUC) value were found for PMAT (AUC = 0.83, 95% confidence interval, CI 0.70-0.95) which was significantly higher ( $p = .0332$ ) than the LIA method (AUC = 0.70, 95% CI 0.56-0.84).

**Conclusion:** The novel PMAT used to detect a spectrum of MSA in IIM represents a potential alternative to IP and other diagnostic assays. Additional studies based on larger cohorts are needed to fully assess the performance of the novel PMAT system for the detection of autoantibodies in myositis.

### 1. Introduction

Myositis specific antibodies (MSA) represent not only important diagnostic tools for idiopathic inflammatory myopathies (IIM), but also help to stratify patients into subsets with particular clinical features, treatment responses and disease outcome (Vulsteke et al., 2019b; Mariampillai et al., 2018). These antibodies even have the potential to be used in classification criteria (Mariampillai et al.,

2018; Bottai et al., 2017; Lundberg et al., 2017b; Lundberg et al., 2018b). Consequently, standardization of detecting MSA is of high importance. Many laboratories rely on protein immunoprecipitation (IP) for the detection of MSA, however, more and more laboratories started using alternative methods, predominantly line immunoassays (LIA) or dot blots (DB). In addition, standardization of IP is challenging. Therefore, reliable alternatives are mandatory (Mahler and Fritzler, 2018). During the past years, a novel particle-based multi-analyte technology (PMAT)

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**Table 1**

Clinical sensitivity for each analyte in different subforms of idiopathic inflammatory myopathies (IIM) and agreement between different methods.

Analyte Method	DM (n = 20)	PM (n = 18)	OS (n = 11)	ASS (n = 4)	UM (n = 1)	Total (n = 54)	PMAT vs. IP	LIA vs. IP	PMAT vs. LIA
							NPA/PPA/TPA	NPA/PPA/TPA	NPA/PPA/TPA
							Kappa (95% CI)	Kappa (95% CI)	Kappa (95% CI)
<b>Jo-1</b>									
IP	<b>0 (0.0%)</b>	<b>4 (22.2%)</b>	0 (0.0%)	<b>4 (100.0%)</b>	0 (0.0%)	<b>8 (14.8%)</b>	91.3/100.0/92.6	89.1/37.5/81.5	87.0/75.0/85.2
PMAT	<b>1 (5.0%)</b>	<b>5 (27.8%)</b>	<b>2 (18.2%)</b>	<b>4 (100.0%)</b>	0 (0.0%)	<b>12 (22.2%)</b>	0.76 (0.53–0.98)	0.27 (–0.07–0.60)	0.51 (0.23–0.80)
LIA	<b>1 (5.0%)</b>	<b>2 (11.1%)</b>	<b>3 (27.3%)</b>	<b>2 (50.0%)</b>	0 (0.0%)	<b>8 (14.8%)</b>			
<b>MDA5</b>									
IP	<b>4 (20.0%)</b>	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	<b>4 (7.4%)</b>	100.0/75.0/98.1	98.0/75.0/96.3	100.0/75.0/98.1
PMAT	<b>3 (15.0%)</b>	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	<b>3 (5.6%)</b>	0.85 (0.55–1.00)	0.73 (0.37–1.00)	0.85 (0.55–1.00)
LIA	<b>3 (15.0%)</b>	<b>1 (5.6%)</b>	0 (0.0%)	0 (0.0%)	0 (0.0%)	<b>4 (7.4%)</b>			
<b>NXP-2</b>									
IP	<b>4 (20.0%)</b>	<b>2 (11.1%)</b>	<b>1 (9.1%)</b>	0 (0.0%)	0 (0.0%)	<b>7 (13.0%)</b>	100.0/71.4/96.3	97.9/57.1/92.6	95.9/60.0/92.6
PMAT	<b>2 (10.0%)</b>	<b>2 (11.1%)</b>	<b>1 (9.1%)</b>	0 (0.0%)	0 (0.0%)	<b>5 (9.26%)</b>	0.81 (0.56–1.00)	0.63 (0.29–0.96)	0.56 (0.17–0.94)
LIA	<b>4 (20.0%)</b>	<b>1 (5.6%)</b>	0 (0.0%)	0 (0.0%)	0 (0.0%)	<b>5 (9.26%)</b>			
<b>SRP</b>									
IP	0 (0.0%)	<b>1 (5.6%)</b>	<b>2 (18.2%)</b>	0 (0.0%)	0 (0.0%)	<b>3 (5.6%)</b>	100.0/33.3/96.3	88.2/0.0/83.3	97.9/0.0/87.0
PMAT	0 (0.0%)	<b>1 (5.6%)</b>	0 (0.0%)	0 (0.0%)	0 (0.0%)	<b>1 (1.9%)</b>	0.49 (–0.11–1.00)	–0.08 (–0.15–0.01)	–0.03 (–0.09–0.02)
LIA	<b>3 (15.0%)</b>	<b>1 (5.6%)</b>	<b>1 (9.1%)</b>	<b>1 (25.0%)</b>	0 (0.0%)	<b>6 (11.1%)</b>			
<b>Mi-2</b>									
IP	<b>2 (10.0%)</b>	<b>1 (5.6%)</b>	0 (0.0%)	0 (0.0%)	0 (0.0%)	<b>3 (5.6%)</b>	100.0/100.0/100.0	88.2/100.0/88.9	100.0/33.3/88.9
PMAT	<b>2 (10.0%)</b>	<b>1 (5.6%)</b>	0 (0.0%)	0 (0.0%)	0 (0.0%)	<b>3 (5.6%)</b>	1.00 (1.00–1.00)	0.45 (0.11–0.80)	0.45 (0.11–0.80)
LIA	<b>2 (10.0%)</b>	<b>4 (22.2%)</b>	<b>1 (9.1%)</b>	<b>2 (50.0%)</b>	0 (0.0%)	<b>9 (16.7%)</b>			
<b>TIF1-γ</b>									
IP	<b>4 (20.0%)</b>	0 (0.0%)	<b>1 (9.1%)</b>	0 (0.0%)	0 (0.0%)	<b>5 (9.3%)</b>	100.0/100.0/100.0	98.0/80.0/96.3	98.0/80.0/96.3
PMAT	<b>4 (20.0%)</b>	0 (0.0%)	<b>1 (9.1%)</b>	0 (0.0%)	0 (0.0%)	<b>5 (9.3%)</b>	1.00 (1.00–1.00)	0.78 (0.48–1.00)	0.78 (0.48–1.00)
LIA	<b>3 (15.0%)</b>	0 (0.0%)	<b>2 (18.2%)</b>	0 (0.0%)	0 (0.0%)	<b>5 (9.3%)</b>			
<b>EJ</b>									
IP	<b>1 (5.0%)</b>	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	<b>1 (1.9%)</b>	100.0/100.0/100.0	100.0/100.0/100.0	100.0/100.0/100.0
PMAT	<b>1 (5.0%)</b>	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	<b>1 (1.9%)</b>	1.00 (1.00–1.00)	1.00 (100.0–1.00)	1.00 (1.00–1.00)
LIA	<b>1 (5.0%)</b>	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	<b>1 (1.9%)</b>			

CI = confidence interval; PPA = positive percent agreement; NPA = negative percent agreement; TPA = total positive agreement. AAS = anti-synthetase syndrome; DM = dermatomyositis; PM = polymyositis; OS = Overlap syndrome; UM = Undifferentiated myositis. Bold indicates numbers and percentages that were greater than 0 (0.0%).

system has been developed and evaluated in several studies (Richards et al., 2019; Mahler et al., 2019). Recently, we identified significant variation between IP and LIA for the detection of MSA and myositis associated antibodies (MAA) (Cavazzana et al., 2016). In this study we aimed to compare the results from our previous study to the results obtained with the novel fully automated PMAT assays for the detection of MSA and MAA (Cavazzana et al., 2016).

## 2. Methods

A total of 54 sera from patients with IIM most of whom had dermatomyositis (DM,  $n = 20$ ), followed by polymyositis (PM,  $n = 18$ ), overlap syndromes ( $n = 11$ ), anti-synthetase syndrome (ASS,  $n = 4$ ), and undifferentiated myositis (UM,  $n = 1$ ) were tested using three methods: IP, LIA (not FDA approved; OJ, EJ, PL-12, PL-7, SRP, Jo-1, Ro52, PM/Scl75, PM/Scl100, Ku, SAE1, NXP-2, MDA5, TIF1-γ, Mi-2β, Mi-2α; Euroimmun, Germany) and a novel particle-based multi-analyte technology (PMAT, research use only; OJ, EJ, PL-12, PL-7, SRP, Jo-1, SAE, NXP-2, MDA5, TIF1-γ, Mi-2β, HMGCR; Inova Diagnostics, USA). The 54 sera were from adult European Caucasian patients with myositis followed up in the Rheumatology Unit in Brescia (Spedali Civili, Brescia, Italy) between 2010 and 2012 for this retrospective study. Polymyositis (PM) and dermatomyositis (DM) were defined according to Bohan and Peter's criteria (Bohan and Peter, 1975b; Bohan and Peter, 1975a); the anti-synthetase syndrome was defined as the triad of arthritis, myositis, and interstitial lung disease associated with ARS (Imbert-Masseau et al., 2003). Patients' clinical data were independently re-viewed by two authors (M.F. and F.F.), in order to

confirm or change the diagnoses previously made. The study was approved by the Institutional Review Board of the Hospital. This study meets, and is in compliance with, all ethical standards of medicine, and informed consent was obtained from all patients in accordance with the Helsinki Declaration of 1975/83/2013.

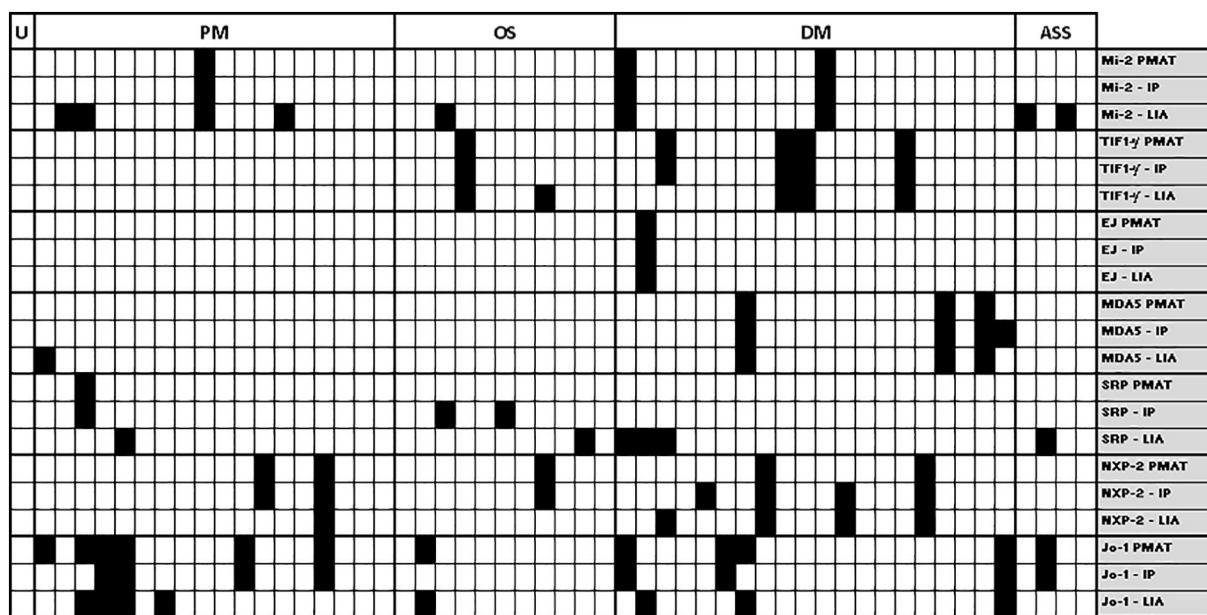
The analysis focused on MSA with a higher number of positives in the cohort and ability of measuring those antibodies by the three methods, namely antibodies to EJ, SRP, Jo-1, NXP-2, MDA5, TIF1-γ, and Mi-2. The principle of the PMAT system has been previously described (Richards et al., 2019). In brief, antigens are coupled to paramagnetic particles that carry unique signatures and incubated with diluted patients' sera. After 9.5 min incubation at 37 °C, particles are washed and incubated (9.5 min incubation at 37 °C) with anti-human IgG conjugated to phycoerythrin (PE). Finally, after another washing cycle, particles are analyzed through digital imaging technology.

Receiver operating characteristic (ROC) curve analysis of LIA and PMAT against IP as binary classifier was used to assess agreement of methods intended from cut-off. IP result as positive or negative was generated based on analytes included in the analysis (EJ, SRP, Jo-1, NXP-2, MDA5, TIF1-γ, and Mi-2). Combined scores for PMAT and LIA were generated based on cumulative quantitative values for analytes included in the analysis. For LIA, semi-quantitative results (1–3) and for PMAT (MFI) were summed up and used for further analyses of the combined scores in comparison to IP. Area under the curve (AUC) values were calculated.

The data was statistically evaluated using the Analyze-it software (Leeds, UK). For method comparison, data were normalized by dividing each patient's results by cut-off value. Chi-square, Spearman's

**Table 2**  
List of discrepant samples between methods.

Sample ID	Discrepant Analyte(s)	PMAT	IP	LIA	Other Ab(s)	Diagnosis
		(Analyte, MFI, neg/pos)	Analyte	(Analyte, Grading)		
Sample 1	MDA5	MDA5, 49, neg	MDA5 pos	MDA5, 0	None	DM
Sample 2	Jo-1	Jo-1, 10,445, pos	Jo-1 pos	Jo-1, 0	None	PM
Sample 3	SRP	SRP, 33, neg	negative	SRP, 1	PM/Scl, IP	OS
					PM/Scl, LIA	
Sample 4	Mi-2	Mi-2, 53, neg	negative	Mi-2, 1	U1RNP, IP	PM
Sample 5	NXP-2	NXP-2, 567, pos	NXP-2 pos	NXP-2, 0	PL-12, LIA	PM
Sample 6	Jo-1	Jo-1, 11,460, pos	negative	Jo-1, 3	None	OS
Sample 7	Mi-2	Mi-2, 39, neg	negative	Mi-2, 1	Jo-1, IP	ASS
					Ku, LIA	
Sample 8	NXP-2, TIF1- $\gamma$	NXP-2, 2377, pos	NXP-2 pos	NXP-2, 0	None	OS
		TIF1- $\gamma$ , 44, neg	TIF1- $\gamma$ neg	TIF1- $\gamma$ , 1		
Sample 9	Jo-1	Jo-1, 69, neg	negative	Jo-1, 3	None	OS
Sample 10	Jo-1	Jo-1, 12,389, pos	Jo-1 pos	Jo-1, 0	None	PM
Sample 11	SRP	SRP, 32, neg	SRP pos	SRP, 0	None	OS
Sample 12	SRP, Mi-2	SRP, 32, neg	SRP pos	SRP, 0	None	OS
				Mi-2, 1		
Sample 13	NXP-2	NXP-2, 239, neg	NXP-2 pos	NXP-2, 1	None	DM
Sample 14	Jo-1	Jo-1, 8861, pos	negative	Jo-1, 3	None	OS
Sample 15	Jo-1	Jo-1, 9505, pos	Jo-1 pos	Jo-1, 0	None	PM
Sample 16	SRP	SRP, 59, neg	negative	SRP, 3	PM/Scl, IP	PM
Sample 17	SRP	SRP, 31, neg	negative	SRP, 1	HMGCR, PMAT	ASS
					Jo-1, IP	
					U1RNP, IP	
Sample 18	NXP-2	NXP-2, 196, neg	NXP-2 pos	NXP-2, 0	None	DM
Sample 19	Jo-1	Jo-1, 66, neg	negative	Jo-1, 1	PL-7, LIA	DM
Sample 20	SRP, Mi-2	SRP, 3135, pos	SRP pos	SRP, 0	PL-7, LIA	PM
		Mi-2, 23, neg	Mi-2 neg	Mi-2, 1		
Sample 21	Mi-2	Mi-2, 22, neg	Mi-2 neg	Mi-2, 2	Jo-1, IP	ASS
					Jo-1, LIA	
Sample 22	Mi-2	Mi-2, 26, neg	Mi-2 neg	Mi-2, 2	Jo-1, IP	PM
					Jo-1, LIA	
Sample 23	MDA5	MDA5, 35, neg	negative	MDA5, 1	Jo-1, LIA	PM
Sample 24	NXP-2, SRP, EJ, TIF1- $\gamma$	NXP, 108, neg	NXP-2 neg	NXP-2, 3	None	DM
		SRP, 34, neg	SRP neg	SRP, 1		
		EJ, 24, neg	EJ pos	EJ, 0		
		TIF1- $\gamma$ , 276, pos	TIF1- $\gamma$ neg	TIF1- $\gamma$ , 0		
Sample 25	SRP	SRP, 60, neg	negative	SRP, 1	EJ, all	DM
Sample 26	SRP	SRP, 36, neg	SRP neg	SRP, 1	Mi-2, all	DM



**Fig. 1.** Reactivity profile of 54 idiopathic inflammatory myopathy (IIM) patients tested by three methods. Qualitative results derived from immunoprecipitation (IP), particle-based multi-analyte technology (PMAT) system and line immunoassay (LIA) are displayed stratified by IIM subform. AAS = anti-synthetase syndrome; DM = dermatomyositis; PM = polymyositis; OS=Overlap syndrome; U = Undifferentiated myositis.

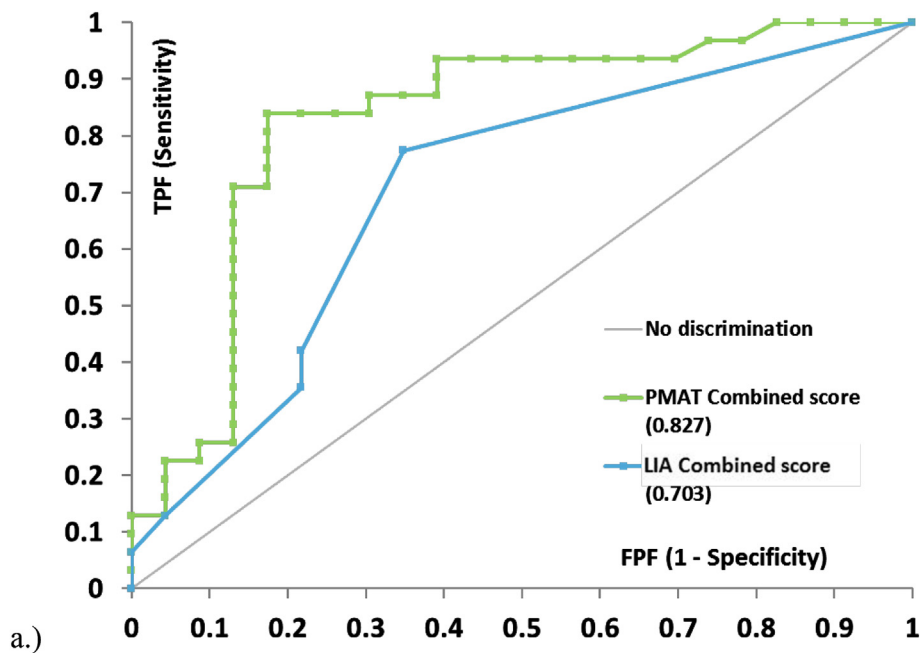
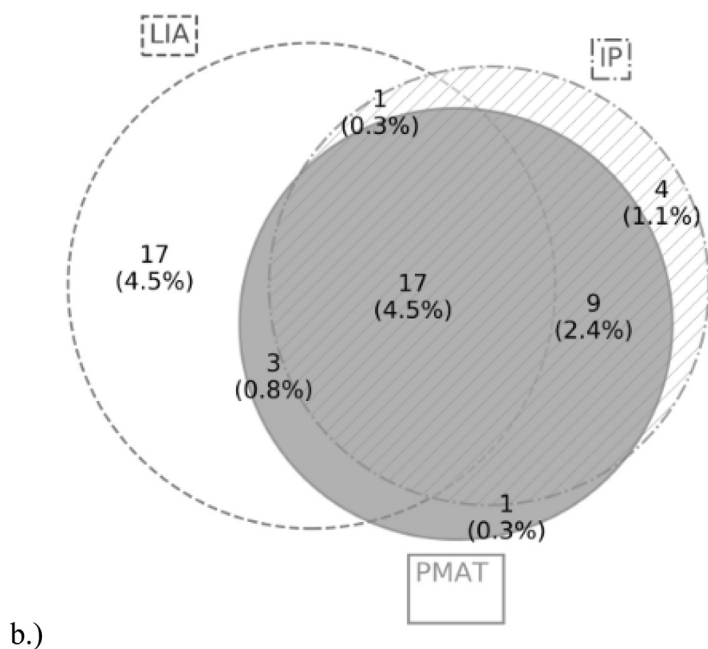


Fig. 2. Agreement between proterin immunoprecipitation (IP), particle-based multi-analyte technology (PMAT) and line immunoassay (LIA). In A.) Receiver operating characteristic (ROC) curve analysis of LIA and PMAT against IP as binary classifier including all samples in the cohort ( $n = 54$ ). IP result as positive or negative was generated based on analytes included in the analysis (EJ, SRP, Jo-1, NXP-2, MDA5, TIF1- $\gamma$ , and Mi-2). Combined scores for PMAT and LIA were generated based on cumulative quantitative values for analytes included in the analysis. Area under the curve values are provided in the figures. The difference was statistically significant ( $p = .0332$ ). Note: LIA results are expressed as grading values (0 = negative, 1–3 = positive according to instructions for use). In B.) Venn diagram is displaying the overall agreement between the methods.



correlation and Cohen's *kappa* agreement test were carried out to analyze the agreement between portions and  $p$  values  $< .05$  were considered significant. Receiver-operating characteristics (ROC) analysis was used to analyze the discriminatory ability of different immunoassays. A Venn diagram was generated using a software library for plotting area-proportional two- and three-way Venn diagrams in Python obtained from the library definition (<https://pypi.org/project/matplotlib-venn/>). Pooled qualitative results from all results were submitted to the code to generate the area-weighted Venn diagrams.

### 3. Results

The prevalences of the individual markers were in line with the expected values based on current knowledge about their occurrence (details see Table 1). When comparing the PMAT assays to LIA and IP, the comparison showed varying qualitative agreement between the

methods (Cohen's *kappa* = 0.08–1.00, Table 1). In general TIF1- $\gamma$ , NXP-2, and Mi-2 showed highest agreements between LIA or PMAT with IP. For, Jo-1, a very established marker for PM, varying agreement was found. The kappa agreements were 0.76 (PMAT vs. IP), 0.27 (LIA vs. IP) and 0.51 (PMAT vs. IP). Very low level of agreement was found for SRP among all methods. Overall, the novel PMAT assays showed slightly better correlation with IP, but the *kappa* agreement was strongly dependent on the antibody tested. Discrepancies between methods for all analytes are summarized in Table 2.

To further analyze the reactivity profiles in light of the clinical phenotype, the results were displayed in a heat-map (see Fig. 1). When the results obtained from IP were used as reference for ROC curve analysis (Fig. 2), good discrimination and a high area under the curve (AUC) value were found for PMAT (AUC = 0.83, 95% confidence interval, CI 0.70–0.95) which was significantly higher ( $p = .0332$ ) than the LIA method (AUC = 0.70, 95% CI 0.56–0.84).

Ab(s) = antibodies; Neg = negative; Pos = positive. MFI = median fluorescent intensity; IP = immunoprecipitation; PMAT = particle-based multi-analyte technology (PMAT); LIA = line immunoassay; AAS = anti-synthetase syndrome; DM = dermatomyositis; PM = polymyositis; OS = Overlap syndrome;

#### 4. Discussion

Careful evaluation of autoantibody assays for the detection of MSA and MAA is of utmost importance since some of these antibodies are included or being considered for IIM classification criteria (Lundberg et al., 2018a; Mariampillai et al., 2018; Bottai et al., 2017; Lundberg et al., 2017a; Malaviya, 2017). The markers are not only relevant for establishing the diagnosis, but also in the stratification into specific disease subsets (Mariampillai et al., 2018; Vulsteke et al., 2019b; Lundberg et al., 2018b; McHugh and Tansley, 2018). However, due to the rarity of IIM and the relatively low prevalence of each MSA within IIM, most studies are rather small and do not allow for a clear conclusion (Mahler and Fritzler, 2018; Espinosa-Ortega et al., 2019).

Historically, most of the clinical associations of MSA and MAA have been established using IP. Recently, LIA became a popular alternative to IP for the detection of MSA. To address the significant subjectivity of interpreting LIA and DB assays, automated scanning systems have been developed and introduced for LIA and DB (Ghirardello et al., 2010; Mahler et al., 2014). A 'Semi-quantitative' approach using scanning systems allows for the analysis of discrepant results considering the antibody levels (titers). Other potential limitations of LIA include the lack of analyte specific controls and proper calibration as well as the temperature sensitivity of the test (Ronnelid et al., 2009).

In efforts to improve standardization, the fundamental first step is to compare newer technologies such as LIA or PMAT to IP and to understand differences (Lundberg et al., 2018a). Of relevance, in a recent study comparing LIA and IP, we observed poor agreement for several MSA (Cavazzana et al., 2016). This observation does not imply that IP is correct in all instances or that IP should be regarded as the 'gold standard', but comparative data is invaluable. Along these lines, IP has significant limitations including the use of radiolabeled cell lysate, labor-intensiveness and subjectivity in interpretation of results. In addition, no standardized protocol is available which influences the technical quality of IP and the interpretation of results between different laboratories.

The current study expands on the findings from our previous comparison (Cavazzana et al., 2016) and provides promising insights that PMAT might exhibit good agreement with IP. The low agreement for anti-SRP antibodies observed in our study is somewhat inconsistent with previous reports (Picard et al., 2016; Aggarwal et al., 2015; Suzuki et al., 2015). Two recent studies that analyzed the agreement between ELISA using SRP54 as the antigen showed fairly high agreement to IP (Suzuki et al., 2015; Aggarwal et al., 2015). On the other hand, a large international multi-center study showed differences between LIA and IIF (Picard et al., 2016). In addition, due to the challenges with IP, labs have different criteria for classifying anti-SRP positive by IP based on the presence of only the 54kD band. It has been observed that some sera immunoprecipitate the SRP54 but no other components, are positive by anti-SRP54 ELISA, and show cytoplasmic staining by HEp-2 cells in indirect immunofluorescence assay (IFA). The two samples that had disagreement between IP and PMAT only showed the 54kD protein band and may in fact not be conclusively positive by IP method. For anti-Mi-2 antibodies, a moderate agreement was found. The LIA detected more anti-Mi-2 antibody positive patients, but all that were negative for IP and PMAT had a clinical phenotype that was inconsistent with the known clinical association of anti-Mi-2 antibodies (McHugh and Tansley, 2018). This inconsistency is in line with several previous studies indicating the strong clinical association between anti-Mi-2 antibodies is method dependent and might get lost with some assays (Vulsteke et al., 2019a; Ronnelid et al., 2009; Espinosa-Ortega et al.,

2019; Richards et al., 2019). High level of agreement was found for MDA5 and TIF1- $\gamma$  which are both important markers as they characterize severe IIM phenotypes. While MDA5 is associated with severe lung disease, TIF1- $\gamma$  has been shown to increase risk of cancer in IIM patients. Of interest, we found variability between methods for anti-Jo-1 antibody detection which is consistent with recent literature (Espinosa-Ortega et al., 2019; Vulsteke et al., 2019a; Cavazzana et al., 2016). This is potentially related to the known limitation of IP for the detection of anti-Jo-1 antibodies, due to the relatively thin uncharacteristic band in IP (in contrast to other aminoacyl-tRNA synthetases) and to the co-migration with the IgG heavy chain (Satoh et al., 2017). IP analysis of RNA components to confirm the presence of tRNA is helpful; however, it does not tell the tRNA specificity; it only tells IP of "some" tRNA and cannot distinguish between histidyl-tRNA and other tRNAs. Practically, if a band corresponding to a ~50 kD protein is observed on IP, and tRNA by RNA analysis is present, it is reasonable to report anti-Jo-1 antibody positivity. In conclusion, additional studies based on larger cohorts are needed to fully assess the performance of the novel PMAT system for the detection of autoantibodies in myositis.

The novel PMAT used to detect a spectrum of MSA in IIM represents a potential alternative to IP and other diagnostic assays.

#### Declaration of Competing Interest

Mahler M, Seaman A, Richards M, and Bentow C are employees of Inova Diagnostics. The other authors have no competing interests.

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