



# THE PM/DM 4 ELISA, A TEST THAT IS TWICE AS SENSITIVE AS Jo-1 FOR AUTOANTIBODIES IN PATIENTS WITH POLYMYOSITIS AND DERMATOMYOSITIS

Tyler T. Webb<sup>1</sup>, Noreen Fertig<sup>2</sup>, Chester V. Oddis<sup>2</sup>, Peter Charles<sup>3</sup> and Rufus W. Burlingame<sup>1</sup>

<sup>1</sup>INOVA Diagnostics, Inc., San Diego, CA; <sup>2</sup>University of Pittsburgh School of Medicine, Pittsburgh, PA;

<sup>3</sup>Hammersmith Hospitals NHS Trust, London, UK

## ABSTRACT

**Objective:** The objective was to develop an assay with the highest sensitivity to help diagnose patients with polymyositis (PM) and dermatomyositis (DM), including those at risk for the anti-synthetase syndrome and interstitial lung disease. Therefore, we used a mixture of four antigens, Jo-1, PL-7, PL-12 and SS-A/Ro 52 (SS-A 52), in a high performance ELISA.

**Methods:** Purified native bovine Jo-1, and recombinant human SS-A 52, PL-7 and PL-12 antigens were mixed together in the same well of a polystyrene microwell plate under conditions that preserve them in their antigenic state. In two studies, a total of 131 patients with PM or DM were tested on the ELISA. Additionally, over 250 blood donors and control samples with other diseases were tested, as well as 33 patients known to be positive for anti-PL-7 or PL-12 by immunoprecipitation.

**Results:** Forty one (31%) of the patients with PM/DM were positive on the PM/DM 4 ELISA. In one of the groups where individual antibody specificities were measured, 15 samples from PM/DM patients were positive on the screen. Just 6 of 15 (40%) were positive for Jo-1, with or without other reactivities. Five samples (33%) were monospecific for anti-SS-A 52, two were monospecific for anti-PL-7, one for PL-12 and the remaining sample was positive for both anti-SS-A 52 and PL-12. The PM/DM 4 ELISA was greater than 99% specific in normal blood donors and patients with rheumatoid arthritis. However, patients with autoimmune diseases such as lupus, systemic sclerosis and Sjögren's Syndrome were positive in the PM/DM 4 at a rate of 15% to 60% because they are positive for anti-SS-A 52. Thirty-two of the thirty-three samples known to be positive for anti-PL-7 or PL-12 were positive on the respective ELISAs.

**Conclusion:** The new PM/DM 4 ELISA is more than twice as sensitive for detecting patients with PM/DM than a test for anti-Jo-1 alone. Some of the added sensitivity comes in the important group of patients with the anti-synthetase syndrome. However, the test is not as specific as Jo-1 because patients with other autoimmune diseases such as lupus and Sjögren's syndrome produce autoantibodies to the SS-A 52 portion of the PM/DM 4 ELISA. A positive result caused by the non-specificity of anti-SS-A 52 should not by itself lead a physician into a diagnosis of polymyositis. Clinical features such as rash (in the case of DM), muscle weakness, elevated muscle enzymes and an abnormal muscle biopsy are necessary to make a diagnosis of myositis.

## INTRODUCTION

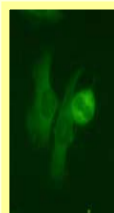
It is important for disease treatment to diagnose patients who have PM/DM<sup>1</sup>. Timely diagnosis of the subset of PM/DM patients with anti-synthetase syndrome is particularly important because they often need to be treated aggressively with immunosuppressive drugs<sup>2,3</sup>. Patients with anti-synthetase syndrome have autoantibodies that react with certain aminoacyl transfer RNA synthetases such as histidyl (Jo-1)<sup>4</sup>, threonyl (PL-7)<sup>5</sup> and alanyl (PL-12)<sup>6</sup> tRNA synthetases, and occasionally other tRNA synthetases<sup>7</sup>. Most patients with these antibodies have similar symptoms such as interstitial lung disease, Raynaud's phenomenon, and other clinical features<sup>8,9</sup>. These autoantibodies are called myositis specific antibodies because they are rarely present except in myositis patients. Anti-SS-A/Ro 52 are myositis related autoantibodies because they are found both in patients with PM/DM<sup>10</sup> as well as other autoimmune diseases such as lupus, systemic sclerosis and Sjögren's syndrome<sup>9,10</sup>. The group of PM/DM Patients that exhibit synthetase syndrome have a large increase in anti-SS-A 52 autoantibody reactivity compared to those without synthetase syndrome, and usually are negative for anti-SS-A-60 autoantibodies<sup>11</sup>.

Until recently, the main ways to detect anti-tRNA synthetase autoantibodies other than Jo-1 were by Ouchterlony immunodiffusion, inhibition of a specific tRNA synthetase activity, or immunoprecipitation of radioactively labeled tissue culture cells. None of these techniques are suitable for screening large groups of patients. The availability of recombinant PL-7, PL-12 and SS-A/Ro 52 proteins allowed the development of an ELISA that is sensitive for PM/DM patients, including a high percentage with anti-synthetase syndrome. The ELISA will enable more widespread testing for these autoantibodies.

## METHODOLOGY

### QUANTA Lite™ PM/DM 4 ELISA

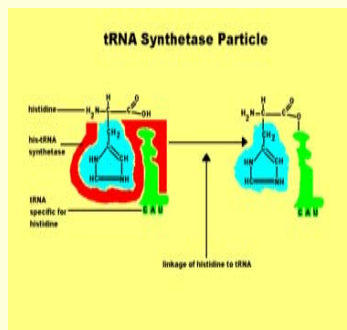
Recombinant SS-A-52, PL-7 and PL-12, and native Jo-1 antigens are bound to the wells of a polystyrene microwell plate under conditions that preserve the proteins in their antigenic states. Jo-1, PL-7 and PL-12 are myositis specific autoantibodies, while SS-A 52 is a myositis related autoantibody. All are diagnostically important.<sup>7,9</sup> Pre-diluted controls and diluted patient sera are added to separate wells, allowing any SS-A-52, Jo-1, PL-7 and PL-12 reactive autoantibodies present to bind to the immobilized antigen. Unbound sample is washed away and an enzyme labeled anti-human IgG conjugate is added to each well. A second incubation allows the enzyme labeled anti-human IgG to bind to any patient antibodies, which have become attached to the microwells. After washing away any unbound enzyme labeled anti-human IgG, the remaining enzyme activity is measured by adding a chromogenic substrate and measuring the intensity of the color that develops. The assay can be evaluated spectrophotometrically by measuring and comparing the color intensity that develops in the patient wells with the color in the control wells.



## Immunofluorescence

Anti-synthetase antibodies yield a characteristic weak fine speckled cytoplasmic pattern on HEp-2 cells, as demonstrated by the anti-PL-7 (threonyl tRNA synthetase) pattern shown here.

## Cartoon of Histidyl tRNA synthetase



## Sensitivity for Defined Autoantibodies

Antibody	Number	PM/DM 4 +	Sensitivity
Jo-1 +	14	14	100%
PL-7 +	18	18	100%
PL-12 +	15	14	93%
SS-A 52 +	14	14	100%

## Clinical Sensitivity and Specificity

Diagnosis	# Samples	PM/DM 4 Positive	Sensitivity
Polymyositis or Dermatomyositis	131	41	31%
Control Samples	# Samples	PM/DM 4 Positive	Specificity
Normal B.D.*	145	1	99%
RA* Patients	20	0	100%
Infectious Disease Samples	114	13	89%
Systemic Sclerosis	61	15	75%
SLE*	50	22	56%
SS*	10	8	20%

\*Abbreviations: B.D. = Blood Donors; RA = Rheumatoid Arthritis; SLE = Systemic Lupus Erythematosus; SS = Sjögren's Syndrome

## Clinical Sensitivity of Individual Tests

Total PM/DM Patients*	PM/DM 4 Positive	Jo-1 Positive	SS-A 52 Positive	PL-7 Positive	PL-12 Positive
41	15	6	5	2	2
% positive	37%**	15%	12%	5%	5%

\*These samples were all from Hammersmith Hospitals, London.

\*\*Note that more than twice as many PM/DM patients are positive for the PM/DM 4 as for Jo-1 alone.

## CONCLUSIONS

- The PM/DM 4 ELISA detects virtually all sera that are positive for either anti-Jo-1, PL-7, PL-12 or SS-A 52 autoantibodies.
- Because it detects all of these autoantibodies, The PM/DM 4 ELISA is more than twice as sensitive at detecting patients with PM/DM as Jo-1 by itself, the most common test.
- For Normal blood donors, people with rheumatoid arthritis and some other controls, the PM/DM 4 is a specific test.
- However, from 25% to 80% of patients with certain rheumatic diseases such as SLE, Sjögren's syndrome and systemic sclerosis will be positive on the PM/DM 4, mostly because they have antibodies reactive with SS-A 52.

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