

IMMUNOPATH UPDATE™

PM-Scl Antibody

For better diagnosis of Polymyositis-Scleroderma and its overlap

INTRODUCTION

Anti-nuclear antibodies (ANA) are a characteristic feature in the serum of patients suffering from connective tissue diseases (CTD) such as polymyositis (PM), dermatomyositis (DM), systemic sclerosis (SSc) and systemic lupus erythematosus (SLE). A subset of ANAs, namely anti-nucleolar antibodies, are directed against autoantigens located in the nucleolar compartment of the cell. This includes antibodies to the PM-Scl complex, also known as the human exosome complex, which are found in patients with polymyositis-scleroderma (PM-SSc) overlap syndrome and related diseases. Anti-PM-Scl antibodies represent a specific serological marker for a subset of patients with scleroderma (Scl) and polymyositis (PM), and especially with the PM-Scl overlap syndrome. Anti-PM-Scl reactivity is found in 24% of PM-Scl overlap patients and is found in 3–10% of Scl and PM patients.

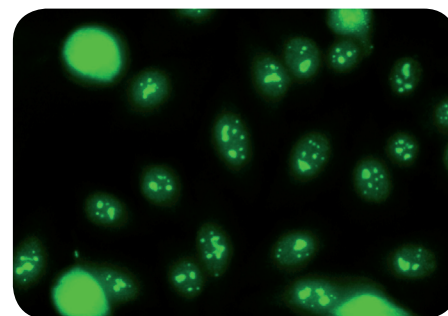
DISEASE

Scleromyositis or PM-Scl overlap syndrome is a complex autoimmune disease. Patients with scleromyositis have symptoms of both polymyositis and systemic scleroderma, and therefore this is considered an overlap syndrome. The incidence of the PM-Scl is estimated in the range of 0.5-8.4 cases per million in the US.

Polymyositis is an idiopathic autoimmune inflammatory joint and muscle disorder. In polymyositis, the inflammatory cells of the immune system directly attack muscles and related tissue, causing weakness in the hips, thighs, upper arms, shoulders, back and neck. Scleroderma affects the connective tissue of the body, skin, and internal organs, and also attacks muscles, joints and blood vessels. Sclerosis or “hardening” of the organs may occur, causing dysfunction.

PM-SCL ANTIBODY DIAGNOSIS

Several studies have used a variety of techniques to detect anti-PM-Scl antibodies in CTD. Most studies have used nucleolar staining in indirect immunofluorescence (IFA) on HEp-2 cells as a screening test and then follow this with an assay such as double immunodiffusion (ID), immunoprecipitation (IP) or enzyme linked immunosorbent assay (ELISA) to confirm anti-PM-Scl reactivity. Historically, the presence of PM-Scl antibodies was monitored by IFA on HEp-2 cells, ID with calf thymus extract, immunoblotting (IB) using extractable nuclear antigens and/or by IP from radioactively labeled cell extracts. The detection of PM-Scl antibodies by IB and IFA, however, is difficult, due to subjective and sometimes weak reactivity on IB and interference of other ANA reactions in IFA. The limitation of this method is that it is subjective and requires significant skills for the identification of a specific reaction pattern.



**IMMCO HEp-2:
Nucleolar Pattern 60x Magnification**

The antigens to which PM-Scl antibodies react to are PM-Scl-75, one of the nine core complex proteins of the human exosome, as well as to an associated protein, PM-Scl-100. Recently, a specific epitope to which the PM-Scl antibodies react has been identified. The identification of specific antigens has resulted in the availability of immunoassays by immunodiffusion and ELISA that are easy to perform. However, there is significant variability in the specificity and sensitivity of these immunoassays. Using a proprietary method, IMMCO's scientific team has developed an ELISA for detecting PM-Scl antibodies that is specific as well as sensitive.

INCIDENCE OF PM-SCL ANTIBODIES (Mahler et al., 2005)

Disease Group	n	n Positive	% Positive
PM-Scl Group			
Polymyositis/Scleroderma	40	22	55
Polymyositis	40	3	7.5
Scleroderma	205	27	13
Disease Controls			
Rheumatoid Arthritis	69	0	0
Systemic lupus erythematosus	114	3	2.5
Rheumatic disease controls	452	33	7

ImmuliTM PM-SCL ASSAY

Test data was collected from subjects suspected of PM-Scl associated connective tissue disorders. Subjects were identified as positive by IFA screening using HEp-2. The positive specimens were further tested using three PM-Scl antibody ELISA assays. The correlation between the results obtained from IFA on HEp-2 and the three PM-Scl antibody ELISAs were then evaluated.

In Table 1, the results of the three PM-Scl antibody ELISA assays show similar correlation with positive nucleolar staining pattern on HEp-2 (i.e., true positives). In Table 2, however, IMMCO's ImmuliTM PM-Scl antibody ELISA assay shows significantly superior ability to detect false positives with negative nucleolar staining pattern on HEp-2. IMMCO's ImmuliTM PM-Scl antibody ELISA assay demonstrates specificity of 92% versus Competitor A (62%) and Competitor B (81%).

The IMMCO PM-Scl antibody ELISA assay offers optimal presentation of the antigen, reducing background without sacrificing sensitivity. The IMMCO PM-Scl antibody ELISA incorporates a peptide rather than the whole molecule antigen used in competitor assays.

Features of the IMMCO detection system including the optimal antigen, HRP conjugate, and five point calibrators combine to deliver the most accurate and reliable PM-Scl antibody ELISA available. In conjunction with IFA using HEp-2 substrates, IMMCO's ImmuliTM PM-Scl assay offers the complete solution for detection and confirmation of PM-Scl antibodies.

SPECIMEN REQUIREMENTS

IMMCO Test Name: PM-Scl

IMMCO Test Code: #052

Methodology: ELISA

Reference Range: Qualitative

CPT Code: 86331

Schedule/Turnaround Time: Assay performed once weekly. Report availability is within one week from the time of specimen receipt.

Specimen Requirements: Specimen need not be refrigerated or frozen. Collect 5-10 ml of blood in a red

HEP-2 POSITIVE FOR NUCLEOLAR ANTIBODIES

	IMMCO	Competitor A	Competitor B
ELISA Positive	12	17	14
Positive Predictive Agreement	71%	100%	82%
Nucleolar Positive = 17			

Incidence of PM-Scl ELISA positive samples with a positive nucleolar staining pattern on HEp-2 cells.

HEP-2 NEGATIVE FOR NUCLEOLAR ANTIBODIES

	IMMCO	Competitor A	Competitor B
ELISA Positive	4	20	10
Negative Predictive Agreement	92%	62%	81%
Overall Agreement	93%	71%	81%
Nucleolar Negative = 53			

Incidence of PM-Scl ELISA positive samples with a negative nucleolar staining pattern on HEp-2 cells.

top or serum separator tube. If possible, separate serum from clot and place into orange tube provided with IMMCO collection kits. Do not puncture top of orange tube. If separation facilities are not available, the blood can be sent in the tube used for collection.

Sample Stability: Sample is stable at ambient temperature during shipment. If sample is stored prior to shipment, it is stable refrigerated (2-8°C) up to five days and frozen (-20°C or lower) up to one year.

SAMPLE SUBMISSION

Specimen collection kits are available free of charge by calling **1.800.537.8378** or e-mail request to **service@immco.com**.

Specimen can be shipped by courier services, U.S. Postal service and overnight carriers free of charge. Results are reported within two business days of the receipt of the specimen via mail, fax and at **immco.com**, a HIPAA compliant patient tracking system.

SELECTED REFERENCES

Buhan A, Peter JB. Polymyositis and dermatomyositis 1975 292:344-347

Limaye VS, Blumbergs P, Roberts-Thomson PJ. Idiopathic inflammatory myopathies. Intern Med J 39:179-190; 2009.

Mahler M, Fritzler MJ. PM1-Alpha ELISA: The assay of choice for the detection of anti-PM/Scl autoantibodies? Autoimmunity Rev 8:373-378; 2009.

Mahler M, Raijmakers R, Novel aspects of autoantibodies to the PM/Scl complex: Clinical, genetic and diagnostic insights. Autoimmunity Rev. 6:432-437; 2007.

Mahler M, Raijmakers R, Dahnrich C, et al. Clinical evaluation of autoantibodies to a novel P/Scl peptide antigen. Arthritis Res Ther 7:R704-R713, 2005.

von Muhlen CA, Tan EM. Autoantibodies in the diagnosis of systemic rheumatic diseases. Semin Arthritis Rheum 24:323-358, 1995.

Ramsperger V, et al. Do we need Immunofluorescence for PM-Scl antibody detection? 7th International Congress on Autoimmunity 2010.

Reichlin M, Maddison PJ, Targoff I, et al. Antibodies to a nuclear/nucleolar antigen in patients with polymyositis overlap syndromes J Clin Immunol 4: 40-44, 1984.

IMMCO Diagnostics, Inc. 800.537.8378
60 Pineview Drive 716.691.0466 Fax
Buffalo, NY 14228 info@immco.com
716.691.0091 www.immco.com

©2010 IMMCO Diagnostics, Inc.
ML 017 REV 5/10

