CPAL
Central **P**ennsylvania **A**lliance **L**aboratory

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SM
- Updated Assay –

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Ordering Information:

Mnemonics:	**Anti -Sm**	**ENA ABS**	**SM/RNP Abs**
Test Name:	Anti SM	ENA ABS	Anti-SM/RNP group
Test Number:	3000810	3000822	3000818
Specimen:	1 ml serum, refrigerated up to 48 hours, frozen up to 30 days Avoid repeat freezing and thawing. Lipemic, hemolyzed, or microbially contaminated samples may give poor results and should not be used.		

Ordering Information (Pinnacle Health):

Mnemonics:	Anti Sm IgG Ab	Not orderable-Component of Anti Sm IgG Ab	Not orderable-Component of Anti Sm IgG Ab	SM/RNP GRP	**ENA GRP**
Test Name:	Anti SM IgG Ab	Sm IgG (numeric)	Anti Sm (alpha)	Anti-SM/RNP group	ENA GRP
Test Number:	1758010	1758014	3000810	1758004	1758000
Specimen:	1 ml serum, refrigerated up to 48 hours, frozen up to 30 days Avoid repeat freezing and thawing. Lipemic, hemolyzed, or microbially contaminated samples may give poor results and should not be used.				

Effective Date: Testing will begin on Friday, May 29, 2015 beginning with samples received Wednesday, May 27, 2015 afternoon.

Performed: Monday, Wednesday, and Friday

Reference Range: Negative

Background:
The determination of antinuclear antibodies (ANA) is of central importance for the clinical diagnosis of systemic lupus erythematosus (SLE). Sm antibodies, and particularly those against the **SmD** component, offer a highly specific, but comparatively insensitive, clinical marker for SLE. Indeed, their presence constitutes one of the revised ACR criteria for diagnosis, even though their overall prevalence ranges from 20% to 30% in SLE. Anti-Sm antibodies react with the proteins BB' and D. However, tests which include the antigens BB' fail to differentiate patients with SLE from those with other autoimmune diseases. Only SmD is considered the most

SLE-specific antigen. The ability of SmD-based antibody tests to differentiate between SLE and other autoimmune diseases can even be improved by using an SmD peptide as antigen.

EliA SmD^P is intended for the in vitro semi-quantitative measurement of IgG antibodies directed to SmD_3 in human serum and plasma (heparin, EDTA) as an aid in the clinical diagnosis of SLE in conjunction with other laboratory and clinical findings. EliA SmD^P uses the EliA IgG method on the instrument Phadia 250.

Principle of Test:
The EliA SmD^P Wells are coated with human recombinant SmD_3 protein. If present in the patient's specimen, antibodies to SmD_3 bind to their specific antigen. After washing away non-bound antibodies, enzyme-labeled antibodies against human IgG antibodies (EliA IgG Conjugate) are added to form an antibody-conjugate complex. After incubation, nonbound conjugate is washed away and the bound complex is incubated with a Development Solution. After stopping the reaction, the fluorescence in the reaction mixture is measured. The assay directly measures the amount of antibody of interest bound to the antigen coating the EliA well, therefore the higher the value of fluorescent signal detected by the instrument, the higher the amount of antibody bound and detected in the sample tested. To evaluate test results, the response for patient samples is compared directly to the response for calibrators.

Results Interpretation:
Phadia 250 measures specific IgG concentrations in µg/L. By using a conversion factor given by the lot-specific code of the EliA SmD^P well, the results are automatically converted to EliA U/ml. There are no international standards for the SmD antibodies. The manufacturer's unit of EliA U/ml is arbitrary and will be reported as U/ml.

Negative: <=6.9 U/ml
Equivocal: 7-10 U/ml
Positive: >=10.1 U/ml

The measuring range (detection limit, upper limit) for EliA EliA SmD^P is from 0.8 to $\geq$ 480 U/ml. Results less than 0.8 will be reported as <0.8 and results greater than 480 will be reported as > 480.

Limitations:
1. A definitive clinical diagnosis should not be based on the results of a single diagnostic method, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
2. In rare cases, interference due to extremely high titers of antibodies to streptavidin can occur.

Validation Data:
Precision:
To assess within-run precision, EliA Negative and EliA ANA Positive controls were run in the same run ten times each. To assess between-run precision, EliA Negative and EliA ANA Positive controls were run ten times each on two different days. Performance was within manufacturer's precision claims of <10% CV. (Table 1)

Table 1: EliA SmD^P Precision

Phadia SN	Within Run Precision				Between Run Precision			
	Mean	CV	Mean	CV	Mean	CV	Mean	CV
N01926	< 0.8 U/mL	0%	103.6 U/mL	4.88%	< 0.8 U/mL	0%	93.3 U/mL	3.50%
N01778	< 0.8 U/mL	0%	98.3 U/mL	2.35%	< 0.8 U/mL	0%	100.5 U/mL	3.81%

Method Comparison
A total of 21 specimens were processed utilizing the EliA Sm and EliA SmD^P assays. The specimens were run on both Phadia 250 instruments for the EliA SmD^P assay.

Figure 1: SmD^P vs Sm Method Comparison

Statistical Summary

	Negative Reference	Positive Reference	Total
Negative Test	16	1	17
Positive Test	2	2	4
Total	18	3	21

Number excluded or missing: 0

There were a total of 3 discordant Sm results. Two of the three discordant specimens were positive using the EliA SmD^P assay, but negative when tested using the EliA Sm assay. The EliA SmD^P shows an improved sensitivity over the current EliA Sm assay, 23% vs 9%. (See Figure 2.) The results are within expected parameters considering the improved sensitivity.

Figure 2: SmD^P vs Sm product claims

	NEW EliA SmD^P	EliA Sm
Sensitivity	23.0%	9.0%
Specificity	98.4%	98.8%
PPV	85.2%	75.0%
NPV	76.2%	73.1%

The third specimen was positive using the EliA Sm assay, but negative using the EliA SmD^P assay. The specimen was repeated and the results verified. The specimen was run by IFA using ImmunoConcepts' HEp-2000 IgG Fluorescent ANA-Ro Test System. The IFA result was negative indicating that this was a false positive Sm result. Common anti-Sm antibody assays (such as the current EliaA Sm) may not only contain SmD, but also SmB,B' antigens. Therefore, it is likely that the current Sm assay detected SmB,B' in this specimen. The SmD^P assay is specific for the SmD^P antigen, which accounts for the negative result.

There is a 100% negative agreement between the two Phadia 250 instruments with the new assay. (Figure 3)

Figure 3: SmD^P Two Instrument Comparison

Statistical Summary

	Negative Reference	Positive Reference	Total
Negative Test	17	--	17
Positive Test	--	4	4
Total	17	4	21

Number excluded or missing: 0

References:

1. EliA SmD^P package insert, August 2014.