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Syn-One Test®

Case #: XXXXXXXX
 Patient: Test, Patient
 Lab Receipt Date: 11/1/2022
 Lab Report Date: 11/20/2022

Syn-One Test® Pathology Report

Patient Information	
Patient:	Test, Patient
Case #:	XXXXXXX
DOB:	5/5/1946
Gender:	Male
Indications:	G20 Parkinsonism

Provider Information	
Clinician:	John Doe, MD
Biopsy Date:	10/31/2022
Account:	CND Life Sciences
Address:	9165 E Del Camino Dr. Ste 101 Scottsdale, AZ 85258
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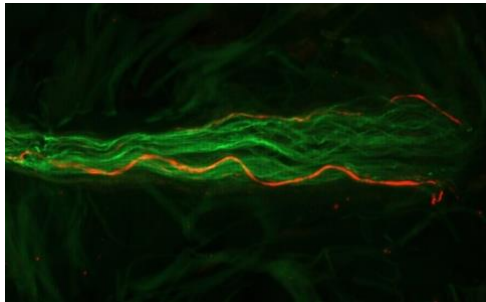
Conclusions	
Synucleinopathy:	There is pathologic evidence of phosphorylated alpha-synuclein deposition within cutaneous nerves. This finding is consistent with a diagnosis of an alpha-synucleinopathy. Clinical correlation is required to distinguish the type of synucleinopathy.
Small Fiber Neuropathy:	There was normal intraepidermal nerve fiber density. A normal intraepidermal nerve fiber density does not exclude a small fiber neuropathy or neurodegenerative process.
Amyloidosis:	There is no pathologic evidence of amyloid deposition in cutaneous nerves. A normal Congo red stain does not exclude a diagnosis of amyloidosis.

Macroscopic Description and Processing
Three (3) skin biopsies in Zamboni fixative were received. The vials were labeled as left posterior cervical, left distal thigh and left distal leg and biopsies were measured to be 3 mm. The biopsies were in adequate condition. They were washed and cryoprotected. The biopsies were sectioned into 50 µm samples. The following stains were performed:
<ol style="list-style-type: none"> Standard dual immunostaining with protein gene product 9.5 and phosphorylated alpha-synuclein Standard Congo Red staining Standard hematoxylin and eosin staining

Microscopic Description	
<p style="text-align: center;">Phosphorylated Alpha-Synuclein</p> <p style="text-align: center;">Abnormal</p> <p>Phosphorylated alpha-synuclein deposition was observed in all biopsies.</p>	<p style="text-align: center;">Intraepidermal Nerve Fibers</p> <p style="text-align: center;">Normal</p> <p>Intraepidermal nerve fiber density was normal in all biopsies.</p>
<p style="text-align: center;">Amyloid Deposition (Congo Red)</p> <p style="text-align: center;">Normal</p> <p>Modified Congo red staining shows no evidence of apple-green birefringence under polarized light in any of the biopsies.</p>	<p style="text-align: center;">Skin Histology (Hematoxylin and Eosin)</p> <p style="text-align: center;">Benign Findings</p> <p>Routine hematoxylin and eosin staining shows benign histopathologic abnormalities in the posterior cervical biopsy (see page 2).</p>

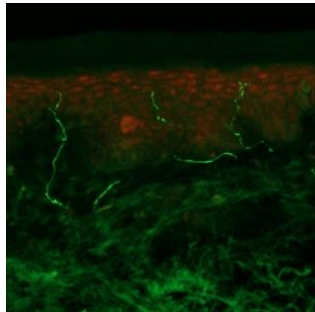
Pathology Results

Phosphorylated Alpha-Synuclein (P-Syn)



Bx Site	P-Syn Deposition	Description
Posterior Cervical (Left)	Abnormal	Two or more colocalized fibers seen across all stained sections.
Distal Thigh (Left)	Abnormal	Two or more colocalized fibers seen across all stained sections.
Distal Leg (Left)	Abnormal	One colocalized fiber seen across all stained sections.

The green regions indicate PGP9.5 immunostained nerve fibers. The regions in red/orange are immunostained regions of phosphorylated alpha-synuclein within nerve fibers. This region displays evidence of phosphorylated alpha-synuclein deposition.



Intraepidermal Nerve Fiber Density

Bx Site	Observed Measurement (fibers/mm)	Normal Range (fibers/mm)
Posterior Cervical (Left)	28	>20
Distal Thigh (Left)	18.1	>11.5
Distal Leg (Left)	16.4	>7.9

The above photomicrograph represents an intraepidermal nerve fiber density representative of this patient's results. The linear green structures indicate PGP9.5 immunostained nerve fibers. This region displays normal intraepidermal nerve fiber density. Patient-specific images are available upon request.

Additional Comments (if applicable)

Posterior cervical: benign histologic abnormalities: Solar elastosis.
Distal thigh: no significant histologic abnormalities
Distal leg: no significant histologic abnormalities

Signed: Todd Levine, MD
Medical Director, CMO

Guidance for Use

The Syn-One Test® is intended to provide objective pathological evidence to aid in the diagnostic evaluation of patients with clinical features suggestive of a synucleinopathy. The synucleinopathies encompass a group of neurodegenerative diseases that include Parkinson's disease, dementia with Lewy bodies, multiple system atrophy, and pure autonomic failure. An abnormal test that identifies phosphorylated synuclein within cutaneous nerves is highly specific for a diagnosis of a synucleinopathy but cannot distinguish between the synucleinopathies. Physicians should use the results of the Syn-One Test along with other clinical features to help make a more specific diagnosis. For more information, please visit www.cndlifesciences.com.

Stability data is not available for tissue specimens kept in fixative for greater than 120 hours. Prolonged fixative time could result in an artificial decrease in the intra-epidermal nerve fiber density. Stability of phosphorylated synuclein deposition has not been established for prolonged fixation times.

References

For additional information on the skin biopsy technique and detection of phosphorylated alpha-synuclein see:

1. Wang N, Gibbons CH, Lafo J, Freeman R. α-Synuclein in cutaneous autonomic nerves. *Neurology*. 2013 Oct 29;81(18):1604-10.
2. Gibbons, C., Wang, N., Rajan, S., Kern, D., Palma, J. A., Kaufmann, H., & Freeman, R. (2023). Cutaneous α-Synuclein Signatures in Patients With Multiple System Atrophy and Parkinson Disease. *Neurology*, 100(15), e1529–e1539.
3. Gibbons CH, Garcia J, Wang N, Shih LC, Freeman R. The diagnostic discrimination of cutaneous α-synuclein deposition in Parkinson disease. *Neurology*. 2016 Aug 2;87(5):505-12.
4. Kim JY, Illigens BM, McCormick MP, Wang N, Gibbons CH. Alpha-Synuclein in Skin Nerve Fibers as a Biomarker for Alpha-Synucleinopathies. *J Clin Neurol*. 2019 Apr;15(2):135-142.
5. Provitera V, Gibbons CH, Wendelschafer-Crabb G, Donadio V, Vitale DF, Stancanelli A, Caporaso G, Liguori R, Wang N, Santoro L, Kennedy WR, Nolano M. A multi-center, multinational age- and gender-adjusted normative dataset for immunofluorescent intraepidermal nerve fiber density at the distal leg. *Eur J Neurol*. 2016 Feb;23(2):333-8.
6. Freeman, R., Gonzalez-Duarte, A., Barroso, F., Campagnolo, M., Rajan, S., Garcia, J., Kim, J. Y., Wang, N., Orellana, L., & Gibbons, C. (2022). Cutaneous amyloid is a biomarker in early ATTRv neuropathy and progresses across disease stages. *Annals of clinical and translational neurology*, 9(9), 1370–1383.

Immunohistochemistry tests were developed, and their performance characteristics were determined by CND Life Sciences, Phoenix, AZ. These tests have not been cleared or approved by the U.S. Food and Drug Administration. The FDA has determined that such clearance or approval is not necessary. These tests are used for clinical purposes. These tests should not be regarded as investigational or for research. CND is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA-88) as qualified to perform high complexity clinical laboratory testing. All histochemical and immunohistochemical controls are in accordance with quality assurance standards.