SYNTap® Biomarker Test – CSF
Performance Characteristics

Accurate Detection of Pathogenic α-Synuclein

The SYNTap Biomarker Test-CSF is a first-in-class Laboratory Developed Test (LDT) for accurate and reliable detection of misfolded aggregates of α-synuclein in cerebrospinal fluid (CSF). The SYNTap Test was developed and validated by Amprion, and is performed in Amprion's CLIA-certified, CAP-accredited Clinical Laboratory in San Diego, CA (CLIA ID 05D2209417; CAP # 8168002).

Intended Use

The SYNTap Test is intended to aid the diagnosis of synucleinopathies such as Parkinson's Disease (PD) and Lewy Body Dementia (LBD/DLB). Test results may also be used at the discretion of the clinician to aid the diagnosis of Multiple System Atrophy (MSA) and the Lewy body variant of Alzheimer's Disease. SYNTap Test results are used alongside other clinical and diagnostic findings for patient case management. A "Detected" result indicates the presence of misfolded α Synuclein protein aggregates in the patient sample and is consistent with diagnosis of a synucleinopathy. A "Not Detected" result is inconsistent with a neuropathological diagnosis of a synucleinopathy at the time of the test. MSA follows a distinct profile that is recognized and reported by the director as a comment supported by limited publication data.

Clinical Performance

Accuracy: Clinical Diagnosis Confirmed by Dopamine Transporter (DAT) SPECT as Comparator

An analytical/clinical accuracy study was performed using biobank samples provided by the Michael J. Fox Foundation (MJFF) Parkinson's Progression Markers Initiative (PPMI) repository. A total of 164 samples were included in the analysis: 55 PD, and 109 with no diagnosed neurological disease. All samples were analyzed blinded, and accuracy assessed by comparing test results to sample PPMI clinical cohort assignments. Overall accuracy of SYNTap Test in this study was 93.9% (87.3% sensitivity, 97.2% specificity).

Clinical/Analytical Accuracy		**Expected Result (Clinical Diagnosis Confirmed by DAT SPECT)**		
		Detected	**Not Detected**	**Total**
SYNTap® Biomarker Test- CSF	**Detected**	48	3	51
	Not Detected	7	106	113
	Total	55	109	164
Sensitivity:		87.3% (95% CI: 0.755 – 0.947)		
Specificity:		97.2% (95% CI: 0.922 – 0.994)		
Overall Accuracy:		93.9% (95% CI: 0.891 – 0.970)		
Positive Predictive Value:		94.1% (95% CI: 0.838 – 0.988)		
Negative Predictive Value:		93.8% (95% CI: 0.877 – 0.975)		

Abbreviations: CI = confidence interval; CSF = cerebrospinal fluid

SYNTap® Biomarker Test – CSF
Performance Characteristics

Accuracy: Clinical Diagnosis Alone as Comparator

A separate analytical/clinical accuracy study was performed using biobank samples provided by the National Institutes of Health (NIH), National Institute of Neurological Diseases and Stroke (NINDS), Parkinson's Disease Biomarker Program (PDBP) repository. A total of 118 samples were included in the analysis: 41 PD, 20 LBD, and 57 with no diagnosed neurological disease. All samples were analyzed blinded, and clinical diagnosis was used as the comparator. Overall accuracy of SYNTap Test in this study was 83.9% (78.7% sensitivity, 89.5% specificity). The accuracy of clinical diagnosis of PD and LBD are estimated at approximately 80% (Rizzo, Copetti et al. 2016, Rizzo, Arcuti et al. 2018). Therefore, the lower accuracy of the SYNTap Test in this evaluation compared with the accuracy evaluation described above likely reflects the reduced accuracy of the study comparator (i.e., clinical diagnosis alone vs. clinical diagnosis confirmed by DAT SPECT).

Clinical/Analytical Accuracy		**Expected Result (Clinical Diagnosis)**		
		Detected	**Not Detected**	**Total**
SYNTap® Biomarker Test- CSF	**Detected**	48	6	54
	Not Detected	13	51	64
	Total	61	57	118
Sensitivity:		78.7% (95% CI: 0.66 – 0.88)		
Specificity:		89.5% (95% CI: 0.78 – 0.96)		
Overall Accuracy:		83.9% (95% CI: 0.76 – 0.90)		
Positive Predictive Value:		88.9% (95% CI: 0.77 – 0.96)		
Negative Predictive Value:		79.7% (95% CI: 0.68 – 0.89)		

Abbreviations: CI = confidence interval; CSF = cerebrospinal fluid

Analytical Performance

Precision (Repeatability and Reproducibility)

A study was performed to evaluate repeatability (within run) and reproducibility (variability across operators, reagents, and instrument sets) of the SYNTap Biomarker Test. Five samples (two negative, one high positive, one medium positive, and one low positive) were tested 24 times each over five days. The high positive sample was an undiluted sample from a patient diagnosed with PD. The medium and low positive samples were created by diluting the high positive sample with control CSF. The two negative samples were pools from subjects with no known diagnosed neurological disease.

REPEATABILITY: Each of the five samples was tested eight times by one operator on one day. Each replicate for each sample gave the expected result.

Repeatability: SYNTap® Biomarker Test - CSF

Sample	Neg 1	Neg 2	Low Pos	Med Pos	High Pos
Correct Call	100%	100%	100%	100%	100%

REPRODUCIBILITY: Each of the five samples was tested in duplicate over five days by two operators using two reagent sets and two analytical instrument sets. Reproducibility was 87.5% for the low positive sample, and 95.8% or higher for the other four samples.

Reproducibility: SYNTap® Biomarker Test - CSF

Sample	Neg 1	Neg 2	Low Pos	Med Pos	High Pos
Correct Call	95.8%	95.8%	87.5%	100%	95.8%

SYNTap® Biomarker Test – CSF
Performance Characteristics

Analytical Sensitivity (Limit of Detection)

A study was performed to determine the analytical limit of detection (LOD) of the SYNTap Biomarker Test. Known quantities of recombinant human α-synuclein protein aggregate (Abcam; Cambridge, UK) were spiked into control CSF to create the samples used for the determination. Two synthetic positive aggregates were evaluated at five different concentrations. Each sample was tested in triplicate on three days by two operators using two reagent sets and two analytical instrument sets. The positive hit rate vs. concentration was plotted and curve fitted to estimate the cutoff between positive and negative assay classifications for each sample, and the average of the two samples calculated as the LOD.

LOD for detection of recombinant α-synuclein protein aggregates in CSF: **~44 fg/mL**

Analytical Specificity (Interfering Substances)

Positive and negative pooled CSF samples were spiked with a high and a low level of interferent (high level listed in table below). Results indicate the listed substances are not expected to interfere with reported results for the SYNTap Test.

Analytical Specificity: SYNTap® Biomarker Test - CSF

Interferent	Results
Conjugated Bilirubin	No Interference at up to 6.7 mg/dL
Hemoglobin	No Interference at up to 100 mg/dL
Albumin	No Interference at up to 767 mg/dL
Whole Blood	No Interference at levels producing a red sample appearance

Acknowledgments

Research reported in this document was supported by the National Institute of Neurological Disorders and Stroke of the National Instituted of Health under Award Number U44NS111672 and the Michael J. Fox Foundation for Parkinson's Research grant MJFF-020475. The content is solely the responsibility of Amprion and does not necessarily represent the official views of the National Institutes of Health or the Michael J. Fox Foundation.

SYNTap® Biomarker Test – CSF
Performance Characteristics

References

Rizzo, G., et al. (2018). "Accuracy of clinical diagnosis of dementia with Lewy bodies: a systematic review and meta-analysis." J Neurol Neurosurg Psychiatry **89**(4): 358-366.

BACKGROUND: The diagnosis of Dementia with Lewy Bodies (DLB) is based on diagnostic clinical criteria, which were updated over the years. OBJECTIVE: To evaluate, through a systematic review, accuracy of the diagnostic criteria, testing a possible improvement over time. METHODS: We searched on MEDLINE and SCOPUS databases for studies reporting diagnostic parameters regarding the clinical diagnosis of DLB until October 2016. We performed meta-analysis, using a Bayesian approach, on those using pathological examination as gold standard, subclassified based on the different diagnostic criteria used. RESULTS: We selected 22 studies on 1585 patients. Pooled sensitivity, specificity and accuracy were 60.2%, 93.8%, 79.7%, respectively, for criteria antecedents to McKeith 1996. For McKeith 1996-possible, pooled sensitivity, specificity and accuracy were 65.6%, 80.6%, 77.9% in early stages and 72.3%, 64.3%, 66% in late stages, respectively. For McKeith 1996-probable, pooled sensitivity, specificity and accuracy were 19.4%, 95.1%, 77.7% in early stages and 48.6%, 88%, 79.2% in late stages, respectively. McKeith criteria 2005 were evaluated only in late stages: pooled sensitivity, specificity and accuracy were 91.3%, 66.7% and 81.6%, respectively, for possible diagnosis (only one study) and 88.3%, 80.8%, 90.7% for probable diagnosis, decreasing to 85.6%, 77.1% and 81.7% if only considering clinical settings focused on dementia diagnosis and care. CONCLUSIONS AND RELEVANCE: Diagnostic criteria have become more sensitive and less specific over time, without substantial change in the accuracy. Based on current data, about 20% of DLB diagnosis are incorrect. Future studies are needed to evaluate if the recently released revised consensus criteria will improve the diagnostic accuracy of DLB.

Rizzo, G., et al. (2016). "Accuracy of clinical diagnosis of Parkinson disease: A systematic review and meta-analysis." Neurology **86**(6): 566-576.

OBJECTIVE: To evaluate the diagnostic accuracy of clinical diagnosis of Parkinson disease (PD) reported in the last 25 years by a systematic review and meta-analysis. METHODS: We searched for articles published between 1988 and August 2014. Studies were included if reporting diagnostic parameters regarding clinical diagnosis of PD or crude data. The selected studies were subclassified based on different study setting, type of test diagnosis, and gold standard. Bayesian meta-analyses of available data were performed. RESULTS: We selected 20 studies, including 11 using pathologic examination as gold standard. Considering only these 11 studies, the pooled diagnostic accuracy was 80.6% (95% credible interval [CrI] 75.2%-85.3%). Accuracy was 73.8% (95% CrI 67.8%-79.6%) for clinical diagnosis performed mainly by nonexperts. Accuracy of clinical diagnosis performed by movement disorders experts rose from 79.6% (95% CrI 46%-95.1%) of initial assessment to 83.9% (95% CrI 69.7%-92.6%) of refined diagnosis after follow-up. Using UK Parkinson's Disease Society Brain Bank Research Center criteria, the pooled diagnostic accuracy was 82.7% (95% CrI 62.6%-93%). CONCLUSION: The overall validity of clinical diagnosis of PD is not satisfying. The accuracy did not significantly improve in the last 25 years, particularly in the early stages of disease, where response to dopaminergic treatment is less defined and hallmarks of alternative diagnoses such as atypical parkinsonism may not have emerged. Misclassification rate should be considered to calculate the sample size both in observational studies and randomized controlled trials. Imaging and biomarkers are urgently needed to improve the accuracy of clinical diagnosis in vivo.