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SAAmplify™–αSYN (CSF)

Intended Use: SAAmplify™–αSYN (CSF) is an in vitro diagnostic test for qualitative detection of aggregates of misfolded α-synuclein in cerebrospinal fluid (CSF) intended for use in adult patients with clinically uncertain cognitive decline or clinically uncertain Parkinsonian syndromes. Results are used to aid diagnosis of synucleinopathies such as Parkinson's disease (PD), dementia with Lewy bodies (DLB), Alzheimer's disease with Lewy body pathology (AD-LB), and multiple system atrophy (MSA). The results must be interpreted in conjunction with other patient clinical information. The test is for professional use only.

An Important Note on Comparators Used for Accuracy Studies: There are no approved diagnostic tests available for use as a comparator for determination of SAAmplify™–αSYN accuracy. Therefore, accuracy is determined by performing blind testing of patient specimens and comparing results to clinical diagnosis, or to pathology. Comparison to pathology is considered the gold standard as it is a direct comparison of test result to autopsy-confirmed disease status. Clinical diagnosis represents a less ideal comparator since it is known to be inaccurate as often 30% to 50% of the time for PD/DLB and MSA, respectively (see references section). However, accuracy of clinical diagnosis is improved when confirmed by brain imaging. The comparator used for each accuracy study used to assess performance is identified below.

Tabulated Summary of Validation Results[1]

Validation Element		Results		
%Accuracy: Autopsy as Comparator (95% CI)		**LB Pathology (n=69)**	**Sensitivity**	**Specificity**
		None (n=33)	NA	97.0 (84.2-99.9)
		Neocortical – diffuse and brainstem predominant (n=19)	100 (82.4 – 100.0)	NA
		Limbic – transitional, amygdala predominant, and olfactory bulb (n=17)	64.7 (38.3-85.8)	NA
%Accuracy: Clinical Diagnosis as Comparator (95% CI)	**Cohort[2]**	**Accuracy**	**Sensitivity**	**Specificity**
	PPMI	94.1 (90.1-96.8)	96.4 (91.1-99.0)	91.6 (84.6-96.1)
	MSA	86.0 (73.3-94.2)[3]	80.6 (62.5-92.6)[3]	92.9 (66.1-99.8)[3]
	PDBP	84.6 (79.9-88.6)	PD: 88.0 (80.3-93.4) DLB: 68.7 (56.2-79.4)	92.3 (85.4-96.6)
Overall Precision		**Reproducibility**	**Repeatability**	
		92.2%	87.0%	
Analytical Specificity (Interfering Substance)		**Interferent**	**Results**	
		Conjugated Bilirubin	Interference observed with visible discoloration and spiked bilirubin ≥6.7mg/dL	
		Hemoglobin	Interference observed with visible discoloration and hemoglobin ≥67.14mg/dL	
		Albumin	No interference	
		Red Blood Cells	Interference observed with visible discoloration and ≥2060 RBC/µL	
CSF Specimen Stability		**Storage Condition**	**Stability**	
		≤ -65°C	Long term	
		2–8°C	Up to 14 days	
		18–25°C	Up to 7 days	
		Heat (Approx. 42°C)	Up to 72 hours	
		Freeze/Thaw	Up to 3 cycles	
In-Use Reagent Stability		**Reagent**	**Storage Condition**	**Stability**
		Reaction Mixture	18–25°C 2–8°C	Up to 32 hrs Up to 56 hrs
		PIPES Buffer	2–8°C	Up to 12 months
		Assay Controls	18–25°C 2–8°C	Up to 32 hrs Up to 56 hrs
Limit of Detection (Analytical Sensitivity)		~110 fg/mL (determined using synthetic aggregated α-synuclein protein)		

Tabulated Summary of Glass Bead Verification Results[4]

Verification Element		Results		
Accuracy[5] (% Agreement)	**Cohort**	**% Agreement**	**95% CI**	
	PD/DLB[6]	100.0	69.2–100.0	
	Control[7]	100.0	76.8–100.0	
Precision (%) (95% CI)	**Cohort**	**Reproducibility**	**Repeatability**	
	PD/DLB[6]	100.0 (86.3-100.0)	100.0 (79.7-99.9)	
Accuracy[8] (%) (95%CI)	**Cohort**	**Accuracy**	**Sensitivity**	**Specificity**
	MSA[9]	58.1% (42.1% - 73.0%)	PD: 83.3 (35.9-99.6) MSA: 32.0[10] (15.0-53.5)	100.0 (73.5-100.0)

Table Abbreviations: CI, Confidence Interval; DLB, dementia with Lewy bodies; MSA, multiple system atrophy; PD, Parkinson's disease; PDBP, Parkinson's Disease Biomarker Program; PPMI, Parkinson's Progression Markers Initiative; RBC, red blood cell.

Table Footnotes:

1. Validation data were obtained by performing the assay utilizing 3.2 mm silicon nitride beads.
2. Accuracy is established by comparing assay results to cohort assignment. Cohort assignments are based on clinical diagnosis alone (PDBP, MSA) or clinical diagnosis confirmed by dopamine transporter single photon emission computed tomography (PPMI).
3. Detected-1 and Detected-2 results considered true positives for accuracy calculations; 60% of specimens from clinically diagnosed MSA patients with α-synuclein seeding aggregates detected displayed the amplification profile predominantly found in patients with MSA (Detected-2).
4. Verification data were obtained by performing the validated assay utilizing 2.45 mm borosilicate glass beads in place of silicon nitride beads.
5. Accuracy is determined by calculating percent agreement between results obtained for the same specimen when tested using 2.45 borosilicate glass beads and 3.2 mm silicon nitride beads.
6. Specimens producing a "Detected-1" result (α-synuclein aggregates detected; amplification profile found predominantly in patients with PD or DLB) when tested under validated test conditions (3.2 mm silicon nitride beads).
7. Specimens producing a "Not Detected" result (α-synuclein aggregates not detected) when tested under validated test conditions (3.2 mm silicon nitride beads).
8. Accuracy is established by comparing assay results to expected results based on clinical diagnosis for previously untested specimens, or to known results from previous testing using silicon nitride beads.
9. Cohort primarily comprised of previously untested specimens with majority of clinical diagnosis of MSA (n=27) combined with specimens with known results based on previous testing with silicon nitride beads (n=5 Detected-1, n=11 Not Detected).
10. Due to the low sensitivity for detection of α-synuclein protein aggregates with a Detected-2 profile using glass beads under validated test conditions, a result of Not Detected should be interpreted with caution when a clinical diagnosis of MSA is being considered.

Regulatory Status: SAAmplify™–αSYN (CSF) is offered as a high-complexity Laboratory Developed Test performed in Amprion's CLIA-certified, CAP-accredited Clinical Laboratory in San Diego, CA (CLIA ID 05D2209417; CAP # 8168002). It was developed and its performance characteristics determined by Amprion. It has not been cleared or approved by the US FDA and is not CE marked.

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The ADNI study actively supports the investigation and development of treatments that slow or stop the progression of Alzheimer's disease (AD). Researchers at over 60 clinical sites in the USA and Canada collect data to study the progression of AD in the human brain across normal aging, mild cognitive impairment (MCI), and Alzheimer's disease and dementia. For more information, see ADNI | About ADNI (usc.edu).

PPMI is a public-private partnership funded by the Michael J. Fox Foundation for Parkinson's Research and funding partners. A full list of funding partners can be found at https://www.ppmi-info.org/about-ppmi/who-we-are/study-sponsors.

PDBP is a network of researchers, patients, family members, and healthcare professionals who are dedicated to accelerating the pace of biomarkers research – is funded by the National Institute of Neurological Disorders and Stroke at the National Institutes of Health. See https://pdbp.ninds.nih.gov/about.

Previously untested specimens from a cohort comprised primarily of patients with a clinical diagnosis of MSA for glass bead accuracy verification study provided by University College of London, London WC1E 6BT.

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Summary of Validation Study Designs

Accuracy: Lewy Body Pathology at Autopsy as Comparator: This study was performed to compare assay results to Lewy body pathology status for participants in the Alzheimer's Disease Neuroimaging Initiative (ADNI) study who provided CSF during life, went on to autopsy, and had available pathology results at the time of data analysis. The ADNI study includes patients with Alzheimer's disease (AD), mild cognitive impairment (MCI), and elderly controls (subjects not meeting the clinical criteria for MCI or AD). A total of 69 specimens had pathology results and a definitive assay result; the average time between CSF collection and autopsy was 4 years (range 0-14 years). Accuracy is determined by comparing test result to Lewy body pathology status determined at autopsy, with further division by anatomical location of Lewy body pathology. Due to insufficient volume for repeat testing, specimens producing an Indeterminate result upon initial test were considered "no call" and were not included in accuracy calculations.

Accuracy: Clinical Diagnosis as Comparator: Three accuracy studies using silicon nitride beads were performed using blinded cerebrospinal fluid (CSF) specimens from different well-characterized repositories/sources: MJFF-sponsored Parkinson's Progression Markers Initiative (PPMI) biorepository (n=236; 116 PD, 120 control); NIH, NINDS-sponsored Parkinson's Disease Biomarker Program (PDBP) biorepository (n=300; 112 PD, 71 DLB, 7 MSA, 110 control); and clinician supplied Multiple System Atrophy (MSA) specimens (n=53; 33 MSA, 5 PD, 15 control). Accuracy is determined by comparing assay results to clinical diagnosis. Due to insufficient volume for repeat testing, specimens producing an Indeterminate result upon initial test were considered "no call" and were not included in accuracy calculations. In the MSA accuracy study, Detected-1 and Detected-2 results were considered true positives for MSA specimens; as noted in the table footer, 60% of MSA specimens with detected α-synuclein aggregates displayed the amplification profile predominantly found in MSA (Detected-2). Accuracy of clinical diagnosis is estimated to be approximately 80% for PD and DLB, and 62-79% for MSA (Wenning et al., 2022; Rizzo et al., 2016; Rizzo et al. 2018). Discrepancy between test result and clinical diagnosis is likely due, at least in part, to the high level of uncertainty inherent in clinical diagnosis. Clinical diagnosis for subjects participating in the PPMI study is confirmed by dopamine transporter single-photon emission computed tomography and is considered closer to clinical truth than clinical diagnosis alone.

Precision: A total of 11 CSF specimens were tested multiple times in two studies to assess assay reproducibility and repeatability. In one study, three unique PD and three unique control specimens were tested 10 times over 10 days using different operators, instruments, and components to evaluate reproducibility, and five times on one day by one operator to evaluate repeatability. In a second study, five specimens (one unique PD, one formulated low positive PD, and three control specimens) were tested similarly, with reproducibility with the same type of plate evaluated over five days. For the two studies combined, overall reproducibility and repeatability were 92.2% and 87.0%, respectively.

Analytical Specificity: Specificity was evaluated by testing CSF specimens (PD, formulated low positive PD, and control) spiked with four potential interferents that may be found in CSF. The tested materials included blood (from blood-contaminated CSF with high red blood cell count), hemoglobin, conjugated bilirubin, and human serum albumin. Human serum albumin did not appear to interfere with result classification. However, results indicate the potential for interference by blood, hemoglobin, and conjugated bilirubin when present at levels causing visible discoloration of specimens. Therefore, tested specimens must be clear and colorless for valid result reporting.

Specimen Stability: Evaluations were performed to assess specimen stability under conditions that may be found during specimen collection and testing. Results support stability of CSF specimens for up to 14 days at 2–8°C, up to seven days at room temperature (18–25°C), up to 72 hours at approx. 42°C, and up to three cycles of freeze/thaw. Accuracy validation studies support long term storage (i.e., several years) in various types of tubes at –65°C or below.

In-Use Reagent Stability: Evaluations were performed to assess in-use stability of reagents prepared for use in the assay. Results support use of prepared reaction mixture stored up to 32 hours at room temperature (18–25°C), and up to 56 hours at 2–8°C; prepared PIPES buffer stored up to 12 months at 2–8°C; and thawed assay controls stored up to 32 hours at room temperature, and up to 56 hours at 2–8°C.

Analytical Sensitivity (Limit of Detection; LOD): A LOD for detection of endogenous α-synuclein aggregates in CSF cannot be directly established because there is no independent method by which the concentration of endogenous aggregates in specimens can be measured. A study was performed to estimate the LOD using control CSF spiked with known concentrations of synthetic α-synuclein aggregates. Using synthetic aggregates as a surrogate, the LOD of the rapid assay for detection of α-synuclein aggregates in CSF is estimated to be approximately 110 fg/mL. While LOD does not apply to qualitative tests in the same manner as quantitative tests, this information can be useful for understanding test characteristics.

Summary of Glass Bead Verification Study Designs

Accuracy: Two accuracy studies were performed using blinded CSF specimens to verify acceptability of using 2.45 mm borosilicate glass beads in place of 3.2 mm silicon nitride beads to perform the assay. The first study determined percent agreement between results when the specimens were tested using both bead types. A total of 22 specimens were tested, including eight specimens with known Detected-1 results (i.e., α-synuclein aggregates detected; amplification profile found predominantly in patients with PD or DLB), 12 specimens with known Not Detected results (i.e., α-synuclein aggregates not detected) and two independent lots of control diluent (expected to produce a Not Detected result). Accuracy is calculated by determining percent agreement between the test result obtained using glass beads and the test result obtained using silicon nitride beads for each specimen.

The second accuracy study was performed blinded using previously untested CSF specimens from a cohort comprised primarily of patients clinically diagnosed with MSA (n=25 MSA, n=1 PD, n=1 PSP) and specimens with known results from previous testing using silicon nitride beads (n=5 Detected-1; n= 11 Not Detected). Accuracy is calculated by comparing glass bead assay results to clinical diagnosis (previously untested specimens) or known test results (specimens previously tested using silicon nitride beads). To perform MSA sensitivity calculations, only Detected-2 is considered true positive for specimens with a clinical diagnosis of MSA, and Indeterminate results are counted as a false result.

Precision: A total of five CSF specimens with known results from testing using silicon nitride beads (two Detected-1, one contrived low-positive Detected-1, and two Not Detected) and two independent lots of control diluent (expected result = Not Detected) were tested five times on one day to evaluate repeatability, and once per day over five days to evaluate reproducibility.

References:

- Rizzo, G., et al. (2016). "Accuracy of clinical diagnosis of Parkinson disease: A systematic review and meta-analysis." Neurology 86(6): 566-576.
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- Wenning G.K., et al. (2022). “The Movement Disorder Society Criteria for the Diagnosis of Multiple System Atrophy.” Movement Disorders 37(6): 1131-1148.