ANALYTICAL PERFORMANCE CHARACTERISTICS OF A PROTOTYPE IMMUNOASSAY FOR QUANTIFICATION OF α-SYNUCLEIN IN HUMAN CEREBROSPINAL FLUID

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INTRODUCTION

α-Synuclein is a pre-synaptic protein whose aggregation is the main characteristic of a sub-group of neurodegenerative disorders called α-synucleinopathies, including but not limited to Parkinson's disease (PD) and dementia with Lewy bodies (DLB). CFS α-Synuclein levels reflect the presence of Lewy bodies in the brain and have been shown to be decreased in PD and DLB patients as compared to healthy aging controls. Robust assays with good lot-to-lot consistency are required for integration into clinical trials. The data presented in this poster documents the analytical performance characteristics of a novel colorimetric ELISA for α-Synuclein measurement in biological fluids (focus: CSF).

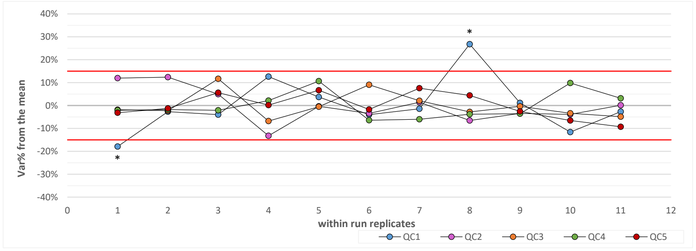
PRECISION AND ACCURACY

MAXIMUM CV% THRESHOLD < 15%

Five Quality Control samples (QC; neat CSF) covering the dynamic range of the assay (90 – 6142 pg/mL) were used. For the determination of Intra-Assay variation, QCs were repeated at least 11 times. For Inter-Assay variation, QCs were measured in 4 to 6 different assays on 4 to 5 different days.

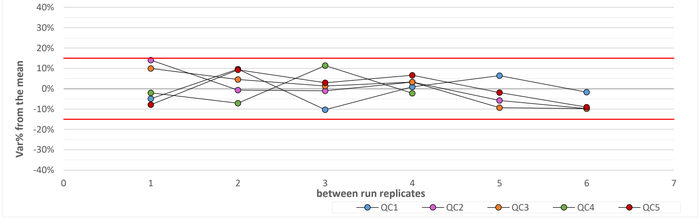
	QC1	QC2	QC3	QC4	QC5
Target (pg/mL)	200	650	1500	2000	3800
Intra CV%	11.9	7.6	5.7	5.8	5.9
Inter CV%	7.3	8.2	8.0	**7.9**	7.6

INTRA-ASSAY VARIATIONS



*QC1 is close to the first standard and the Lower Limit of Quantitation, which explains its higher variation from the mean on two replicates.

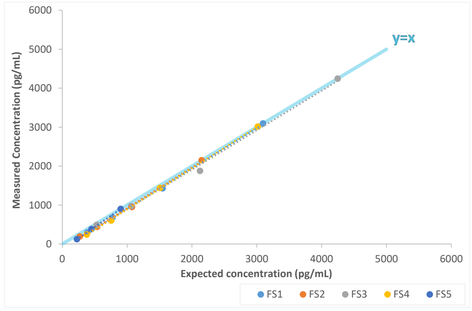
INTER-ASSAY VARIATIONS



PARALLELISM AND SPIKE-RECOVERY

ACCEPTABLE RECOVERY RANGE: 80 - 120%

PARALLELISM



Five different samples with mid-high values were diluted subsequently until 1:16 in sample buffer. The recovery percentages are then calculated as followed:

$$Recovery\% = \frac{(measured\ value\ X\ dilution\ factor)}{Expected\ value} \times 100$$

Recommended maximum dilution: 1:4

SPIKE - RECOVERY

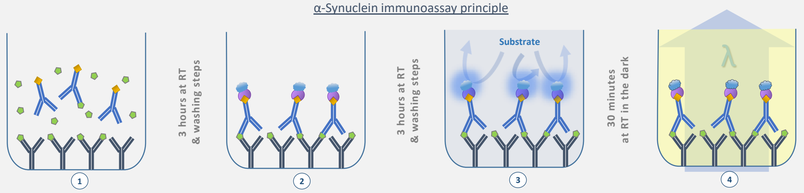
Two CSF samples with different concentrations were spiked with 5 different concentrations of calibrators in a 75:25 ratio (sample:calibrator). The calculated value is then compared to the expected value.

	Sample value (pg/mL)	Added values (pg/mL)	Expected values (pg/mL)	Assay values (pg/mL)	Recovery %
R1	2394.5	6142	3331	2 979	89%
		4095	2820	2 617	93%
		2047	2308	2 059	89%
		896	2020	1 797	89%
		448	1908	1 703	89%
R2	2906.0	6142	3715	3 188	86%
		4095	3203	2 716	85%
		2047	2691	2 298	85%
		896	2404	2 044	85%
		448	2292	1 900	83%
				Mean :	**88%**

METHOD

α-Synuclein is quantified in human CSF with a (manual) sandwich ELISA developed by ADx NeuroSciences (Gent, Belgium).

α-Synuclein immunoassay principle



1. Simultaneous incubation of sample (25 µL undiluted) with biotinylated detector antibody (100 µL) on antibody-coated plates.
2. Addition of streptavidin peroxidase conjugate.
3. Addition of the peroxidase substrate
4. Stop of the reaction by the addition of sulfuric acid, followed by reading the absorbance at the appropriate wavelengths.

Bioclinica LAB analytical validation method is designed to ensure the accuracy and reliability of the results. The validation process, the criteria applied, and the parameters chosen are optimized based on current guidelines, field experience and method requirements. The Laboratory is CAP accredited (College of American Pathologists).

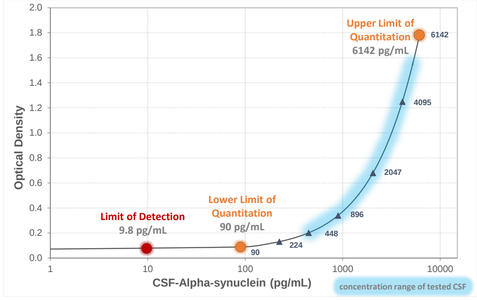
CSF samples were used for the validation, provided by 2 different centers (Cerba Specimen & AMS bio). All CSF samples were stored at -70°C. The sample concentration is calculated upon duplicate analysis. When additional tubes were required, LoBind tubes from Eppendorf were used (ref. 0030108094).

WORKING RANGE & LIMITS

LLOQ ≥ FIRST CALIBRATOR | CV% from 15 to 20%

α-Synuclein concentrations were back-calculated following a seven points calibration curve (4-PL curve fitting).

CALIBRATION CURVE



LOWER LIMIT OF QUANTITATION (LLOQ)

90.0 pg/mL (first standard)

LLOQ was determined with three CSF samples with low values and was measured at least 11 times within the same run. The lowest mean concentration with a CV% between 15 and 20% should be considered as the lower limit of quantitation.

LIMIT OF DETECTION (LOD)

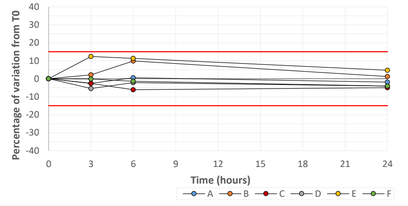
9.8 pg/mL

LOD was determined by adding three SD (standard deviation) to the mean optical density (OD) of 22 replicates of the zero calibrator. The concentration is then back-calculated with the equation of the curve.

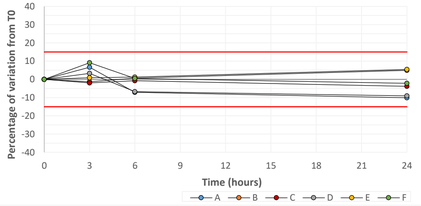
SAMPLE STABILITY

MAXIMUM TOLERATED FROM REFERENCE VALUE: ±15%

STABILITY AT ROOM TEMPERATURE



STABILITY AT +4°C



Six CSF samples with different concentrations were thawed and placed 3, 6 and 24 hours at ambient temperature (+22°C ± 2°C) or in a fridge (+4°C ± 2°C). For each time point, the levels of α-Synuclein were determined the same day on the same run. The results were compared to the reference value (T0).

Freeze / Thaw cycles effects

Six CSF samples were consecutively thawed and frozen 4 times. No effect on 4 freeze/thaw cycles was detected on α-Synuclein concentrations. Percentages of variation, when compared to T0, were all below 10%.

TAKE HOME MESSAGE

The analytical performance of the α-Synuclein colorimetric ELISA shows good repeatability and reproducibility; samples can be diluted until 1:4 without showing any matrix interference. The samples tested fall within the dynamic range of the assay. The α-synuclein protein remains stable after 4 freeze/thaw cycles or after storage for 24 hours at room temperature or +4°C.

This assay meets all internal criteria for CSF sample testing and represents an additional tool for patient management in research clinical trials.