INTRODUCTION

Despite the fact that the core cerebrospinal fluid (CSF) biomarkers (Tau, Amyloid) reflect on-going pathology in the brains of subjects with Alzheimer’s Disease (AD), new biomarkers are needed to monitor other hallmarks, such as synaptic degeneration. This study documents the analytical performance characteristics of a novel ELISA, targeting C-terminal P75 truncated Neurogranin, which has been shown to be the most abundant Neurogranin isoform in CSF.

METHOD

Neurogranin is quantified in human CSF with a manual sandwich ELISA developed by ADx NeuroSciences (Gent, Belgium), and manufactured by EUROMIMMUN (Lübeck, Germany).

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 samples used for the validation are CSF provided by 4 different centers. All CSF samples were stored at -70°C. The sample concentration is based upon duplicate analysis. When additional tubes are required, pre-rinsed LoBind tubes from Eppendorf are used.

ANALYTICAL PERFORMANCE IN HUMAN CSF

PARALLELISM AND SPIKING RECOVERY

ACCEPTABLE RECOVERY RANGE: 80 - 120%

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

Recovery % = \frac{\text{measured value} \times \text{dilution factor}}{\text{expected value}} \times 100

Expected concentration (pg/mL)

CSF-Neurogranin - Parallelism

CSF Sample (pg/mL) Calibrator (pg/mL) Spiked samples (pg/mL) Recovery %
R1 115.9 50.0 278 96
R2 353.7 50.0 89 97
Mean 95

Two CSF samples with different concentrations have been spiked with 3 different concentrations of calibrators in a 1:0:10 ratio (sample:calibrator). The assay value is then compared to the expected value.

PARALLELISM AND SPIKING RECOVERY

ACCEPTABLE RECOVERY RANGE: 80 - 120%

Three CSF samples with different concentrations have been 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 timepoint, the levels of Neurogranin have been determined the same day on the same run, and the results have been compared to the reference value (TO).

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Freeze/Thaw cycles effects

Five CSF samples have been consecutively thawed and frozen 4 times. No effect on 4 Freeze/Thawing has been detected on Neurogranin concentrations. Percentages of variation when compared to TO were all below 10%.

ANALYTICAL PERFORMANCE IN HUMAN EDTA PLASMA

PARALLELISM AND SPIKING RECOVERY

ACCEPTABLE RECOVERY RANGE: 80 - 120%

Six plasma samples have been diluted until 1:60. Percentages of recovery range from 84 to 104% (mean 93% ± 6%).

Six plasma samples have been spiked with a low concentration of Neurogranin. Percentages of recovery range from 84 to 96% (mean 94% ± 4%).

TAKE HOME MESSAGE

The analytical performances of the Neurogranin Trunc P75 colorimetric ELISA meets all internal acceptance criteria for CSF samples testing and represents an additional tool for synaptic damage assessment in clinical trial.

The assays shows also satisfying preliminary validation results on EDTA plasma.