
To the Editor:

The acceptance of the clinical utility of protein biomarkers for Alzheimer disease diagnosis and their integration into routine clinical testing require extensive standardization at different levels (preanalytical, analytical, postanalytical) (1). The approval of such biomarkers by regulatory authorities is hampered in part by (a) uncertainty regarding the accuracy of values produced with different measurement technologies, (b) the absence of international reference standards, (c) the absence of validated reference methods for measuring absolute analyte concentrations, and (d) a lack of well-defined recommendations for immunoassay validation (2). The present letter provides a solution for harmonization of cutoff concentration values for different technology platforms by performing immunoassays that use calibrators consisting of undiluted cerebrospinal fluid (CSF).1

For this exploratory study, 197 CSF samples had been analyzed previously at the same time (on 5 different immunoassay) by ELISA [INNOTEST® β-Amyloid(1–42); Innogenetics, single analyte, CE version] and LumineX’s xMAP assay system with the INNO-BIA AlzBio3 assay (Innogenetics; multiplexed, research use only assay) (3). Differences in the design of the assays have previously been described (4). The plate layouts for CSF testing for the 2 assay formats were identical. No new test runs were performed for the current study. Kit calibrators consisted of synthetic β-amyloid(1–42) dissolved in a phosphate-buffered solution. After run validation (criteria: out of range, precision, head count), 31 CSF samples (layout of 6 or 7 per plate) were selected for each ELISA plate to cover the β-amyloid(1–42) concentration interval of the assay and to generate CSF-based calibrator series. The assays were compared with the remaining 141 samples. The same CSF calibrator samples were used in the ELISA and xMAP assays. Reactivity values obtained for selected CSF samples in xMAP test runs were used to generate calibration curves for the β-amyloid(1–42) concentrations as measured by ELISA. β-Amyloid(1–42) concentrations in the unknown CSF samples were obtained by using the kit calibrators and the CSF calibration curves. Results were compared according to a modified procedure, as described previously (5).

β-Amyloid(1–42) concentrations in CSF as measured with the kit calibrators were about 3-fold higher with the ELISA assay than with the xMAP assay [ratio of β-amyloid(1–42) concentration obtained with the ELISA to that obtained with the xMAP assay (ELISA/xMAP ratio): median, 3.12; interquartile range, 2.75–3.56; n = 141]. The ELISA/xMAP ratio increased significantly as a function of the β-amyloid(1–42) concentration. When the data were subsequently analyzed with a calibrator series that was prepared with CSF from individual patients and covered a range of β-amyloid(1–42) concentrations as calculated on the basis of the ELISA results, the difference in absolute concentrations was minimized (median ELISA/xMAP ratio, 1.137; interquartile range, 0.90–1.44; n = 141). Furthermore, the increase in the ELISA/xMAP ratio as a function of the β-amyloid(1–42) concentration was eliminated (Fig. 1). This important finding was noted both with the pooled data set and when results from individual immunoassays were compared, pointing to the reproducibility of our approach.

The generation of different output values (concentrations) when different measurement technologies of comparable clinical accuracy are applied represents a major obstacle to full market acceptance. The use of calibrator curves constructed with CSF samples containing different β-amyloid(1–42) concentrations, however, minimizes differences in output values and the dependence on the β-amyloid(1–42) concentration. The solution we have proposed takes into account binding characteristics (affinity) of the monoclonal antibodies with respect to the analyte when different matrices are used for measurements. No artificially produced CSF is currently capable of substituting for actual CSF to harmonize results obtained with different assay formats.

In the future, our approach will facilitate the measurement of β-amyloid(1–42) concentrations around defined cutoff values for a specific context of use. A standardized procedure for preparing large volumes of such CSF samples can be generated by preparing pools of CSF samples with different β-amyloid(1–42) concentrations. Such samples can then be provided to the community as part of a biorepository, so that every vendor will be able to bridge to this cutoff value. The same samples could be helpful for monitoring lot-to-lot consistency of assays, evaluating the effect of changes in critical raw materials, and documenting commutability limitations when preparing buffer-based quality control samples. Of course, long-term stability data for these samples will

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1 Nonstandard abbreviations: CSF, cerebrospinal fluid; ELISA/xMAP ratio, ratio of β-amyloid(1–42) concentration obtained with the ELISA to that obtained with the xMAP assay.
have to be documented. The samples will have to be provided as ready-to-use solutions, so that artifacts related to freeze–thaw cycles or storage conditions can be minimized.

In conclusion, the approach we have described may benefit the interlaboratory comparisons of tests, because it will help determine a laboratory’s capability to conduct specific diagnostic tests, check the performance of operators, and harmonize different test methods. It could ultimately lead to a proficiency-testing program that forms part of the accreditation of the laboratory, as has been done for other biomarkers. Finally, the positive results obtained in the present study support the concept of assay harmonization through the use of certified reference materials with a performance compatible with CSF.

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**References**


