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Current practices and harmonization challenges in Alzheimer's disease biomarkers: an EFLM Committee: Harmonization Survey

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Abstract

Objectives: Fluid biomarkers are central to the biological definition and diagnosis of Alzheimer's disease (AD). Despite international recommendations, substantial variability persists in laboratory practices. This survey, promoted by the Committee: Harmonisation (C:H) of the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM), aimed to assess real-world laboratory practices for AD biomarker testing.

Methods: An online survey was distributed to EFLM members. The questionnaire addressed pre-analytical, analytical, and post-analytical practices for cerebrospinal fluid (CSF) and blood-based AD biomarkers. Descriptive statistics were applied.

Results: A total of 316 responses from more than 35 countries were collected. CSF remained the primary matrix for AD diagnostics, although blood-based biomarkers were increasingly implemented. Marked heterogeneity was observed across all phases of testing, including sample handling, assay platforms, biomarker panels, and decisional cut-offs. Cut-off values for core biomarkers varied widely, as well as harmonization of reporting unit and the adoption of SI units preventing a shared approach to interpreting the results.

Conclusions: Despite growing clinical adoption, AD biomarker testing remains highly heterogeneous. Coordinated international harmonization of pre-analytical procedures, analytical methods, and post-analytical interpretation, particularly for blood-based biomarkers, is urgently required to ensure reliable and comparable results.

Keywords: Alzheimer's disease; biomarkers; cerebrospinal fluid; blood biomarkers; harmonization; EFLM

Introduction

Alzheimer's disease (AD) is the leading cause of dementia globally and constitutes a significant public health concern due to its rising prevalence and the substantial socio-economic impact on patients, caregivers, and healthcare systems [1–3]. Advances in the understanding of Alzheimer's disease pathophysiology have facilitated the development of several assays for the measurement of fluid biomarkers that reflect molecular processes allowing for early and accurate detection of AD [4, 5].

In particular, cerebrospinal fluid (CSF) biomarkers reflecting amyloid pathology (Aβ-42 and the Aβ-42/Aβ-40 ratio), tau phosphorylation (phosphorylated tau, p-Tau), and neurodegeneration (total Tau, t-Tau, and Neurofilament Light chain, NfL) have been incorporated into international diagnostic frameworks and are increasingly used in clinical practice [6–8]. To support their reliable clinical implementation, international, evidence-based guidelines for the preanalytical handling of CSF Aβ and Tau biomarkers have been developed, most notably those issued by the Alzheimer's Association, which provide standardized recommendations for lumbar puncture, sample collection, tube material, handling, transport, and storage conditions [9, 10]. These guidelines were derived from multicenter experimental evidence and aim to minimize preanalytical variability, particularly the adsorption-related instability of Aβ peptides, thereby improving inter-laboratory comparability and clinical interpretability. More recently, blood-based biomarkers, including Aβ peptides, p-Tau isoforms, NfL, and glial fibrillary acidic protein (GFAP), have emerged as promising, minimally invasive tools with the potential to broaden access to biomarker testing and enable large-scale

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screening and monitoring [11, 12]. However, in contrast to CSF biomarkers, preanalytical and analytical standardization for blood-based AD biomarkers is still at an early stage, and consensus recommendations remain limited [13].

Despite the availability of international CSF handling guidelines, substantial variability persists across laboratories in pre-analytical workflows, analytical methodologies, instrumentation, and interpretative criteria [14, 15]. Differences in sample collection, handling, storage, assay platforms, and reporting units can profoundly influence biomarker measurements, limiting comparability and challenging clinical decision-making. The rapid expansion of ultrasensitive technologies, such as single molecule array (SIMOA) and fully automated chemiluminescent immunoassays, has further diversified the methodological landscape, underscoring the urgent need for harmonized protocols and consensus-based diagnostic cut-offs [16, 17]. Consequently, the extent to which evidence-based preanalytical recommendations are implemented in routine laboratory practice represents a fundamental issue that remains insufficiently documented. Systematic data on real-world laboratory practices are therefore essential to guide standardization efforts.

In this context, C:H of the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) promoted a survey to assess the current state of practice in laboratories measuring AD biomarkers. The primary objective was to document real-world procedures and strategies employed across different countries, with particular attention to the biological matrices used, biomarker panels selected, assay technologies implemented, pre-analytical and analytical protocols, and decisional cut-offs adopted for clinical interpretation. By mapping existing heterogeneity and identifying areas of convergence or divergence, this survey aims to support ongoing harmonization initiatives and contribute to the development of standardized, evidence-based recommendations for fluid biomarker testing in the field of Alzheimer's disease.

Materials and methods

Survey design

The survey was designed to collect comprehensive information on current laboratory practices for the measurement of AD biomarkers in CSF and blood. The SurveyMonkey platform (SurveyMonkey Inc.), used to administer the questionnaire, was accessible from July to November 2025. The questionnaire was developed by members of C:H and structured into 5 sections addressing: (i) general information; (ii) introduction; (iii) pre-analytical; (iv) analytical; (v) post-analytical (Figure 1).

The survey consisted of both multiple-choice and open-ended questions, allowing respondents to provide quantitative data as well as additional comments or specifications regarding laboratory protocols. The survey results were anonymized, and participation was voluntary.

Data handling and quality control

Data were anonymized and exported from the online platform into a structured database for further processing. Responses were screened for internal coherence and completeness. No personal identifiers were collected, ensuring compliance with privacy and ethical requirements for survey-based research.

Open-ended responses were examined individually and grouped into thematic categories when appropriate. For questions involving multiple biomarkers or methodological options, respondents were allowed to select more than one answer, and results are reported as frequencies of selections.

Statistical analysis

The study employed descriptive statistics to summarize the information provided by respondents. Frequencies and counts were calculated for categorical variables, including biomarker types, analytical platforms, sample matrices, pre-analytical procedures, and decisional cut-offs. Due to the exploratory and descriptive nature of the survey, no inferential statistical analyses were performed.

Results

A total of 316 responses were collected. Of these, 15 % were fully completed, while 85.4 % were partially completed. All responses, including incomplete ones, were included in the descriptive analysis to maximize the representativeness of current laboratory practices. For each survey item, analyses were conducted using all available responses for that specific variable, without imputation of missing data; consequently, the number of respondents varies across analyses, including for key variables such as biomarker cut-offs and sample storage conditions.

General information

Responses were obtained from laboratories across more than 35 countries. The highest number of respondents came

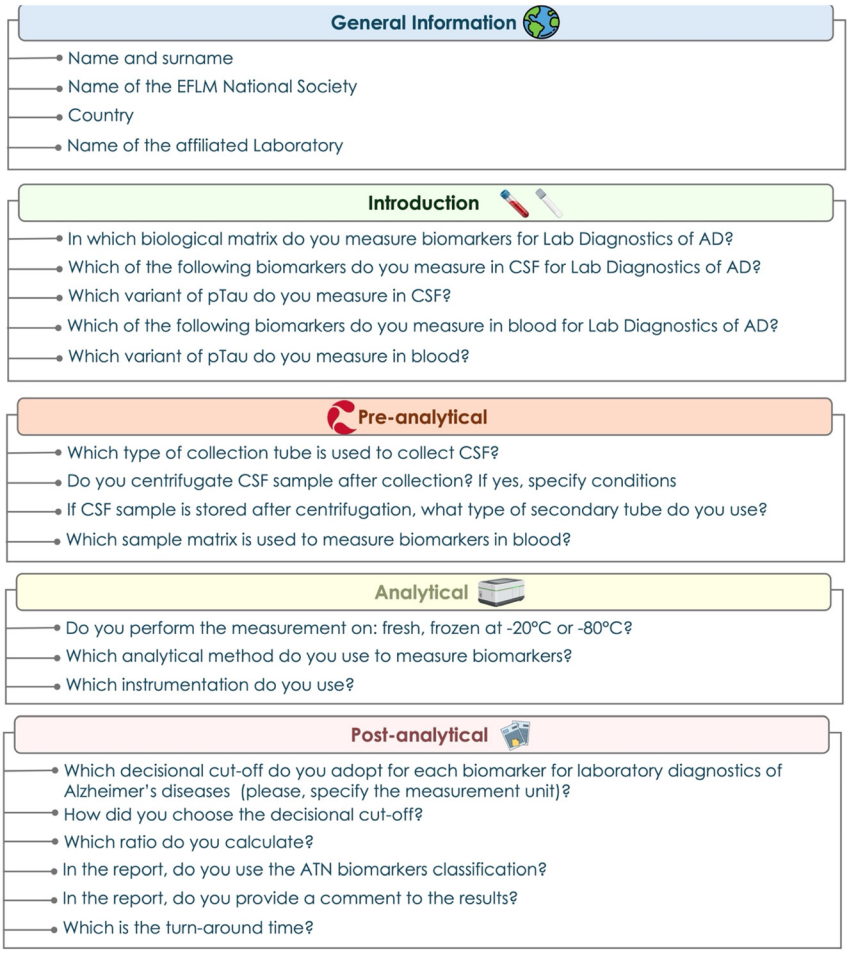

Figure 1: Survey questionnaires.

from Spain (n=22), followed by the United Kingdom (n=9), Italy (n=8), Romania (n=7), Belgium (n=7), France (n=6), and the Netherlands (n=6). We did not receive any response from Armenia, Cyprus, Iceland, Latvia, Luxembourg, Moldova, Montenegro, Norway, Poland, and Slovak Republic. Interestingly, responses were obtained from three independent laboratories in Georgia, all of which answered "no" to all survey questions. These concordant responses suggest, and we had the confirmation, that, at present, none of the laboratories measure AD biomarker in Georgia.

Introduction

CSF emerged as the principal biological matrix used for AD laboratory diagnostics. Most laboratories reported measuring biomarkers exclusively in CSF (50 %) or in both CSF and blood (32 %), whereas a smaller proportion (16 %) relied solely on blood-based testing. A limited number of respondents (2 %) indicated that biomarker measurements were outsourced to referral laboratories or that plasma biomarkers were still under validation and not yet implemented in routine diagnostics.

Overall, these data indicate that, despite growing interest in blood biomarkers, CSF continues to represent the reference biological matrix for AD biomarker assessment in routine laboratory practice.

Sixty-four percent of laboratories reported measuring a comprehensive CSF biomarker panel including Aβ-42, Aβ-40, phosphorylated tau (p-Tau), and total tau (t-Tau). This combination was by far the most frequently adopted approach, consistent with current diagnostic frameworks [6]. Nevertheless, a small proportion (7 %) of laboratories reported not measuring Aβ-40, potentially limiting the use of Aβ-42/Aβ-40 ratios.

Beyond the core AD biomarkers, 19 % of laboratories measures Neurofilament Light chain (NfL), indicating its growing role as a biomarker of neurodegeneration. In contrast, glial fibrillary acid protein (GFAP) and 14-3-3 protein were rarely included in routine CSF testing (<1 % of the responders).

Regarding tau phosphorylation sites, p-Tau 181 was the dominant isoform measured in CSF (73 %). P-Tau 217 and

p-Tau 231 were used by <1 % of laboratories, while a small proportion (9 %) reported not measuring p-Tau at all.

Blood-based biomarker testing showed substantially greater heterogeneity than CSF testing. NfL was the most frequently reported blood biomarker, followed by p-Tau 217. Only 22 % of laboratories reported measuring full Amyloid and Tau panels in blood, reflecting the early stage of the clinical implementation of these assays. Notably, 24 % of respondents reported that no AD biomarkers were measured in blood. Among laboratories assessing p-Tau in blood, p-Tau 217 was widely preferred (40 %), whereas p-Tau 181 and p-Tau 231 were measured by a small number of laboratories (5 and 4 %, respectively). A large proportion of laboratories (51 %) did not measure blood p-Tau at all.

Pre-analytical

Considerable variability was observed in pre-analytical handling of both CSF and blood samples. CSF was most collected in polypropylene tubes, with Sarstedt tubes being the most frequently reported (80 %). Low-binding tubes, including protein low-binding and false-bottom tubes, were used by a minority (20 %) of laboratories.

Post-collection centrifugation of CSF was performed by 58 % laboratories, typically at room temperature, while the remaining laboratories (42 %) did not centrifuge CSF samples.

For blood-based biomarkers, plasma was the preferred matrix, predominantly collected in EDTA tubes. Both K_2-EDTA and K_3-EDTA were widely used, while serum was less frequently (10 %) employed.

Sample storage conditions varied markedly. Biomarker measurements were performed on both fresh and frozen samples, with frozen samples stored at temperatures ranging from −20 °C to −80 °C. CSF samples were more frequently stored frozen than blood samples, although no uniform storage strategy emerged from the survey data.

Analytical

Chemiluminescent enzyme immunoassays represented the most used analytical approach, particularly on fully automated platforms: Fujirebio platforms dominated both CSF and blood testing (58 and 70 %, respectively). ELISA methods were still widely used, indicating ongoing reliance on semi-automated or manual techniques in a significant proportion of laboratories. Ultra-sensitive methods, such as SIMOA and mass spectrometry, were reported by only a small number of respondents (9 %).

Post-analytical

Marked heterogeneity was observed in decisional cut-offs for all biomarkers. For CSF Aβ-42, reported cut-off values varied widely, ranging from 500 to over 1,000 ng/L. Cut-offs for Aβ-40 were often not defined independently, as 54 % laboratories reported using this biomarker exclusively for ratio-based interpretation.

Similarly, CSF p-Tau cut-offs showed wide dispersion, with reported values spanning more than a two-fold range (from 27 to 70 ng/L) In addition, measurement units were not consistently harmonized, with some laboratories reporting values in pg/mL and others in ng/L. Comparable variability was observed for CSF t-Tau, with laboratories adopting fixed cut-offs, age-adjusted thresholds, or multi-category interpretative schemes.

For NfL, both in CSF and blood, age-dependent cut-offs were frequently applied; however, the numerical values differed substantially between laboratories according to the analytical method adopted. In contrast, decisional cut-offs for blood-based amyloid and tau biomarkers were rarely reported (<1 %), and when provided, they showed no apparent consistency. No laboratory reported decisional cut-offs for GFAP in either CSF or blood.

Most laboratories (61 %) provide the Aβ-42/Aβ-40 ratio applying a decisional cut-off of 0.07 and 29 % calculates also p-Tau/Aβ-42 ratio. Finally, only 26 % includes ATN classification and 47 % provides interpretative comments in the report. Finally, the turn-around time (TAT) varied from one week (16 %), two weeks (34 %), three weeks (18 %), one month (24 %), and up to six months (8 %).

The relevant critical issues in the total testing process of measuring Alzheimer's biomarkers based on the survey results are summarized in Figure 2.

Discussion

This survey provides a comprehensive snapshot of current laboratory practices for AD biomarker testing and reveals a pervasive lack of harmonization across all phases of the analytical process. Although biochemical biomarkers are increasingly central to the biological definition of AD, their implementation in routine laboratory practice remains highly fragmented.

First, the survey yielded 316 responses, 46 of which (15 %) were fully completed. The high proportion of incomplete questionnaires itself suggests variability in local experience and implementation of AD biomarker testing.

CSF continues to represent the reference biological matrix; however, the survey demonstrates that laboratories

Biomarkers panel, CSF/blood	• Inconsistent measurement of Aβ-40 • Heterogeneous selection of pTAU isoforms
Preanalytical phase, CSF/blood	• CSF, type of tubes • CSF, centrifugation after collection • Storage temperature
Analytical phase	• Lack of analytical traceability to reference systems
Postanalytical phase, CSF/blood	• Lack of harmonized/clinical validated/age adjusted cut-offs • Blood, lack of defined decisional threshold • Widely use of traditional units (not SI unit)

Figure 2: Critical issues in the process of measuring Alzheimer's biomarkers based on the survey results.

differ substantially in how CSF testing is performed and interpreted. Variability was observed not only in the selection of biomarker panels, but also in the choice of p-Tau isoforms, with p-Tau 181 predominating despite growing evidence supporting alternative phosphorylation sites. Indeed, multiple head-to-head studies and meta-analyses consistently demonstrate that plasma p-Tau 217 outperforms p-Tau 181 in discriminative accuracy for Alzheimer's disease across all major biological reference standards, including amyloid-PET, tau-PET, CSF biomarkers, and neuropathology. Pooled data from over 100 studies show that p-Tau 217 achieves higher sensitivity (88.1 vs. 80.5 %), specificity (88.7 vs. 76.4 %), and area under the receiver operating characteristic curve (AUROC 91.1 vs. 81.5 %) compared to p-Tau 181, with moderate certainty of evidence for p-Tau 217 and low certainty for p-Tau 181 [18, 19]. These findings are robust across different assay platforms and clinical populations, including both cognitively impaired and unimpaired individuals.

A subset of laboratories does not measure Aβ-40, thereby precluding the use of Aβ-42/Aβ-40 ratios, which are increasingly recommended to improve diagnostic accuracy: in fact the ratio corrects for individual and pre-analytical variability in absolute Aβ42 concentrations and more closely reflects brain amyloid pathology than Aβ42 alone [20]. Studies consistently show that the Aβ42/Aβ40 ratio achieves higher sensitivity, specificity, and AUC for detecting amyloid PET or CSF amyloid positivity compared to Aβ42 alone [21–24]. Without Aβ40 measurement, laboratories are limited to reporting Aβ42 alone, which is more susceptible to confounding by peripheral production, plasma protein binding, and pre-analytical factors, resulting in lower diagnostic accuracy and reduced clinical utility [25, 26].

Additional new biomarkers, such as NfL, are implemented inconsistently, while GFAP and 14-3-3 protein were rarely measured. These findings highlight the absence of consensus regarding extended biomarker panels beyond the core AD markers.

Harmonization challenges are even more pronounced for blood-based biomarkers. While scientific evidence supporting plasma biomarkers is rapidly expanding, their translation into routine diagnostics remains uneven. Laboratories differ widely in whether blood biomarkers are implemented at all, which biomarkers are selected, and which analytical platforms are used. The predominance of p-Tau 217 among laboratories measuring blood p-Tau contrasts with the absence of any consensus regarding cut-offs or clinical decision limits, underscoring the premature nature of clinical implementation in the absence of standardized guidance.

Pre-analytical variability represents another critical source of non-comparability. Differences in tube type, centrifugation practices, sample matrix, and storage conditions were common and often contradictory. Although polypropylene tubes were most used for CSF collection (80 %), a variety of tube types were reported, including different low-binding options. Post-collection centrifugation of CSF was performed by several laboratories (58 %) but omitted by others, indicating a lack of standardized pre-analytical workflows. Furthermore, blood biomarker measurements were performed predominantly in K_2-EDTA plasma, K_3-EDTA plasma. Storage conditions further differed, with laboratories analysing fresh samples or frozen samples stored at −20 °C, −60 °C, or −80 °C. Such variability is known to significantly influence biomarker concentrations, particularly for amyloid and Tau species, and may partly explain the wide dispersion of the reported decisional cut-offs [27–31]. Overall, these discrepancies introduce additional sources of analytical variability and severely restricts results comparability. Importantly, the lack of harmonization observed in the pre-analytical phase does not reflect an absence of guidance. Detailed international recommendations for CSF and blood handling for Alzheimer's disease biomarkers have been published by multiple expert groups [9–13], including consensus protocols addressing sample collection, tube material, centrifugation, storage temperature, freeze–thaw cycles, and transport conditions. These guidelines were specifically developed to minimize pre-analytical variability and to improve inter-laboratory comparability of amyloid, Tau, and neurodegeneration markers. However, the present survey clearly demonstrates that adherence to these recommendations remains highly variable in routine laboratory practice. This gap between available guidance and real-world implementation suggests that the primary challenge is no longer guideline

development, but rather effective dissemination, education, and enforcement. Greater promotion of existing pre-analytical standards, their integration into laboratory accreditation processes, and their systematic adoption by diagnostic laboratories are urgently needed to achieve true harmonization of the pre-analytical phase and to ensure the reliability and clinical transferability of AD biomarker measurements.

Analytical heterogeneity further compounds these issues. Although automated chemiluminescent immunoassays are increasingly adopted, a substantial proportion of laboratories still rely on ELISA-based methods. The coexistence of multiple platforms, assay designs, and calibration strategies limits traceability and prevents direct comparison of results across laboratories and countries.

The most striking evidence of insufficient harmonization is the extreme variability in decisional cut-offs. For CSF Aβ-42, reported cut-off values spanned more than a two-fold range. Aβ-40 was frequently used only for the ratio calculations, with no independent cut-offs provided. Similar dispersion was observed for p-Tau and t-Tau, with laboratories adopting markedly different thresholds. In addition to numerical variability, inconsistencies were observed in measurement units adopted. In fact, the manufacturers themselves report in IFU traditional units (pg/mL) instead of SI units (ng/L). It should be underline that pg/mL and ng/L are numerically equivalent units (conversion factor=1), thus differences in reported units reflect variations in reporting conventions rather than true analytical differences. In addition, "Liter" is the recommended SI unit for volume. Therefore, in order to harmonize the measurement units in reporting the results, the adoption of SI units is strongly recommended [32].

Unlike other biomarkers, age-dependent thresholds are reported for NfL although very different values are adopted by various laboratories.

For blood-based biomarkers, decisional cut-offs were rarely reported, and when available, they showed no comparability. No laboratory provided decisional cut-offs for GFAP in either CSF or blood. This heterogeneity severely limits the clinical transferability of laboratory results and represents a major obstacle to the use of AD biomarkers in multicenter studies, clinical trials, and routine patient care.

Overall, the survey reveals a pervasive lack of harmonization across all phases of AD biomarker testing, encompassing choice of biological matrix, biomarker panels, pre-analytical handling, analytical platforms, and decisional cut-offs (Figure 2).

Taken together, these findings indicate that despite widespread clinical interest, AD biomarker testing is currently characterized by fragmented practices rather than standardized diagnostic pathways, without harmonized protocols in all phase of the testing process, limiting their full diagnostic potential and application in clinical practice. From a clinical perspective, increased variability or prolonged turnaround times (as highlighted by the survey results) may delay completion of the diagnostic workup and biological characterization of patients, with potential downstream effects on referral to specialist centers, scheduling of additional investigations (e.g., neuroimaging or neuropsychological assessments), diagnostic disclosure and counselling, and timely access to and initiation of disease-modifying therapies, particularly in care pathways where clinical decision-making is contingent upon laboratory biomarker results.

This survey has several limitations that should be considered when interpreting the findings. First, the questionnaire was distributed exclusively to members of EFLM. Although EFLM represents a broad and highly relevant professional network, this distribution strategy may have introduced a selection bias by excluding laboratories not affiliated with EFLM, particularly those operating in regions with limited representation or outside Europe. Consequently, the results may not fully reflect global laboratory practices for Alzheimer's disease biomarker testing. Second, although 316 responses were collected, the overall response rate was very poor considering the number of laboratories potentially eligible to participate. Moreover, a low number of questionnaires (approximately 15 %) were fully completed, while the majority were partially completed. This criticism entails the lack of information for certain survey sections not allowing the depth of analysis for specific pre-analytical, analytical, and post-analytical aspects. The high proportion of incomplete responses may reflect heterogeneous levels of experience with AD biomarkers, partial implementation of testing, or limited familiarity with some methodological details. Third, the voluntary participation may have encouraged laboratories operating in a clinical setting with a specific interest in neurodegenerative diseases or those already engaged in biomarker testing. Therefore, laboratories with limited or no activity in this field may be underrepresented, potentially leading to an overestimation of the overall uptake of biomarker testing. In addition, all data were self-reported and not independently verified, which introduces the possibility of reporting bias or inaccuracies in the description of local practices. Finally, due to the descriptive nature of the survey and the heterogeneity of responses, no inferential statistical analyses were performed.

Despite these limitations, the survey also has several important strengths. To our knowledge, this is one of the largest and most comprehensive international surveys

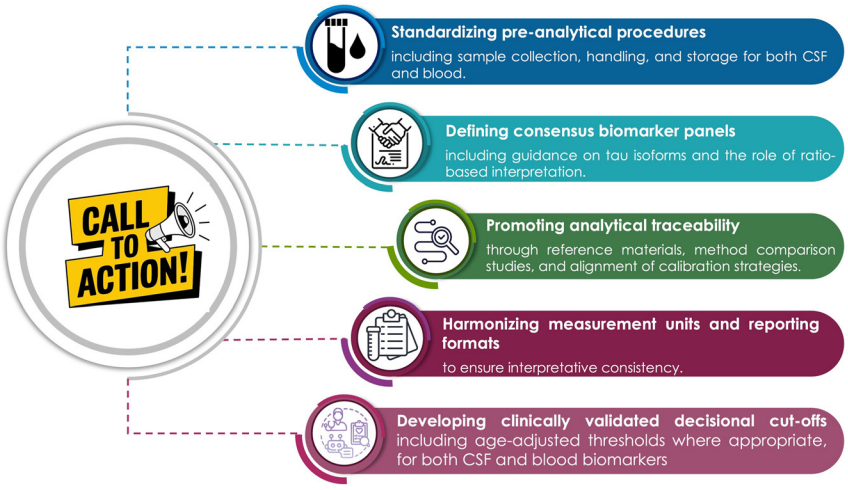

Figure 3: A call to action to promote harmonization in Alzheimer's disease biomarkers.

specifically addressing real-world laboratory practices for both CSF and blood-based Alzheimer's disease biomarkers. The inclusion of responses from laboratories across more than 35 countries provides a broad overview of current practice and highlights substantial inter-laboratory and inter-country variability. Importantly, the decision to include partially completed questionnaires allowed the capture of valuable information from laboratories that may be at different stages of implementation, thereby reflecting real-world conditions more accurately. Rather than representing a highly selected subset of expert centers, the survey encompasses a wide spectrum of laboratory settings, ranging from fully established AD biomarker services to laboratories with limited or emerging activity. Furthermore, the survey comprehensively addressed all phases of the total testing process, including pre-analytical handling, analytical platforms, and post-analytical interpretation. This holistic approach enables the identification of critical sources of variability and provides a solid empirical basis for prioritizing harmonization efforts.

Overall, while the findings should be interpreted with appropriate caution, the survey offers a valuable snapshot of current laboratory practices and clearly demonstrates the urgent need for coordinated, international harmonization initiatives in Alzheimer's disease biomarker testing.

Conclusions

Despite the growing clinical relevance of biomarker-based diagnostics for AD, laboratory practices remain highly heterogeneous. Harmonization of pre-analytical protocols, analytical methodologies, and decisional cut-offs, particularly for blood-based biomarkers, is essential to ensure reliable and comparable results across laboratories.

On the basis of the obtained results, C:H emphasize the urgent need for coordinated international actions essential to support the safe, effective and comparable use of AD biomarkers in clinical practice and to facilitate their integration into international diagnostics frameworks (Figure 3).

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