

Diagnostic Strategies for Early Detection of Alzheimer's Disease Through Advanced Bioengineering

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Abstract: *Alzheimer's disease (AD) is a progressive neurodegenerative disorder with a long preclinical phase marked by early molecular alterations. Early detection of these changes is critical for timely intervention, yet current diagnostic methods are limited by invasiveness and cost. This study synthesizes recent advances in molecular diagnostic strategies enabled by bioengineering, including blood and cerebrospinal fluid biomarkers, extracellular vesicles (EV), circulating nucleic acids, and proteomic and glycomic profiling with post-translational modifications (PTMs). A structured literature review was conducted focusing on analytical validity, clinical relevance, and translational potential. The findings indicate that emerging technologies such as ultrasensitive immunoassays, nanotechnology-based biosensors, and artificial intelligence (AI)-driven multi-omics integration enable detection of central nervous system-derived biomarkers in peripheral fluids with high sensitivity and specificity, with reported diagnostic performance approaching $AUC \geq 0.90$. These approaches support the development of scalable and minimally invasive diagnostic systems. Continued standardization and validation are required to facilitate clinical implementation and improve early detection of AD.*

Keywords: Alzheimer's disease, molecular diagnostics, bioengineering, biosensors, blood biomarkers, amyloid-beta, phosphorylated tau, multi-omics, artificial intelligence, nanotechnology, early detection

1. Introduction

Alzheimer's disease (AD) represents the most common cause of dementia worldwide and continues to impose a growing burden on healthcare systems due to increasing life expectancy. The disease is neuropathologically characterized by the accumulation of extracellular amyloid- β plaques, intracellular neurofibrillary tangles composed of hyperphosphorylated tau protein, synaptic dysfunction, and progressive neuronal loss. A defining feature of AD is the existence of a prolonged preclinical phase during which pathological processes evolve silently before the onset of measurable cognitive decline [1]. During this stage, molecular alterations including protein aggregation, synaptic injury, and neuroinflammation accumulate gradually, creating a critical window for early detection and intervention.

Despite advances in neuroimaging and cerebrospinal fluid diagnostics, current clinical approaches remain insufficient for widespread early detection. Positron emission tomography (PET) imaging and CSF biomarker analysis provide high diagnostic accuracy but are limited by cost, invasiveness, and accessibility. Consequently, there is an urgent need for minimally invasive, scalable, and highly sensitive diagnostic tools capable of detecting early molecular signatures of disease [2].

Recent developments in bioengineering have significantly advanced the field of molecular diagnostics. Technologies such as ultrasensitive immunoassays, microfluidic platforms, nanotechnology-based sensors, and AI-driven analytical frameworks have enabled the detection of rare biomarkers in peripheral biofluids [3]. These innovations have shifted the paradigm from symptom-based diagnosis to molecular-level detection, allowing for earlier identification of disease processes. The purpose of this review is to synthesize current

molecular diagnostic strategies for early detection of Alzheimer's disease and evaluate their integration with advanced bioengineering technologies.

2. Methods

The methodology for this study utilizes a rigorous systematic literature synthesis to evaluate the integration of bioengineering and molecular diagnostics in the context of Alzheimer's disease. To ensure methodological transparency and academic reproducibility, the review process was conducted in accordance with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) framework.

The investigative process began with a comprehensive search of leading electronic databases, including PubMed/MEDLINE, ScienceDirect, IEEE Xplore, and Google Scholar. The search strategy employed a combination of MeSH terms and Boolean operators to bridge the disciplines of clinical neurology and engineering, utilizing strings such as ("Alzheimer's disease" OR "AD") AND ("molecular diagnostics" OR "biomarkers") AND ("bioengineering" OR "nanotechnology"). To ensure the findings reflect the current "state-of-the-art" in rapidly evolving fields like digital proteomics and microfluidics, the temporal scope heavily focused on peer-reviewed research published between January 2018 and March 2026.

Study selection followed a structured protocol based on predefined inclusion and exclusion criteria. Priority was given to primary research and meta-analyses that reported objective, quantitative performance metrics—such as Area Under the Curve (AUC) and Limit of Detection (LoD)—specifically within human peripheral biofluids or advanced in vitro bioengineered models. Conversely, the synthesis excluded

case reports, non-English publications, and studies focusing exclusively on late-stage symptomatic cohorts, thereby maintaining a focus on the engineering challenges of preclinical detection.

Out of an initial pool of relevant identified records, a tiered screening of titles, abstracts, and full texts resulted in the selection of the soundest seminal studies for final synthesis. Each included work was evaluated across three critical dimensions: analytical validity (technical sensitivity and specificity), clinical validity (correlation with established PET or CSF pathology), and clinical utility (the potential to shift diagnostic or therapeutic trajectories). Finally, a comparative analysis was performed to identify synergistic trends between disparate platforms—such as the fusion of CRISPR-based sensors with AI-driven analytics—to establish a comprehensive framework for the clinical translation of next-generation Alzheimer’s diagnostic systems.

3. Results

Molecular Biomarker Modalities

Biomarker science has transitioned from single-analyte testing to multi-dimensional molecular profiling. Current strategies encompass proteins, nucleic acids, extracellular vesicles, and post-translational modifications (PTMs) (figure 1A-C). Blood-based biomarkers offer high scalability for population-level screening. The plasma amyloid-β (Aβ) 42/40 ratio reflects the balance of amyloid production and clearance. Reductions in this ratio correlate directly with cerebral amyloid deposition.

CSF Aβ42, pTau and tTau are core validated biomarkers of AD pathology and associated neurodegeneration

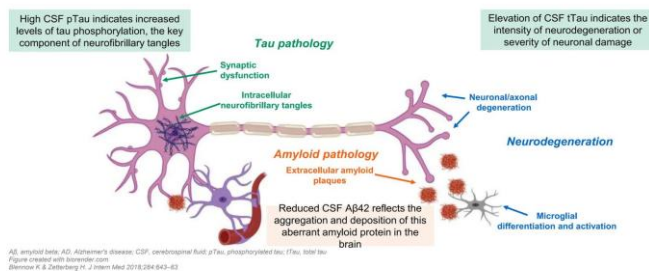


Figure 1A

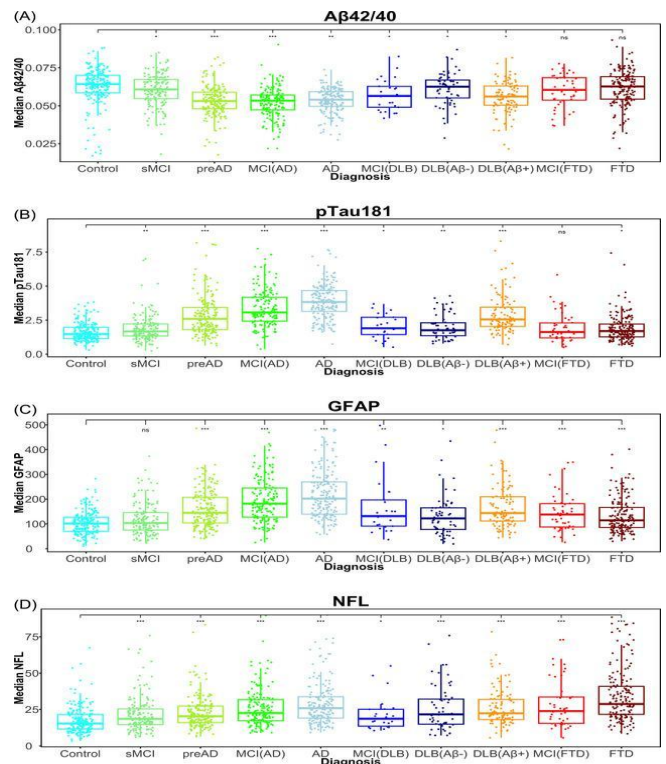


Figure 1B

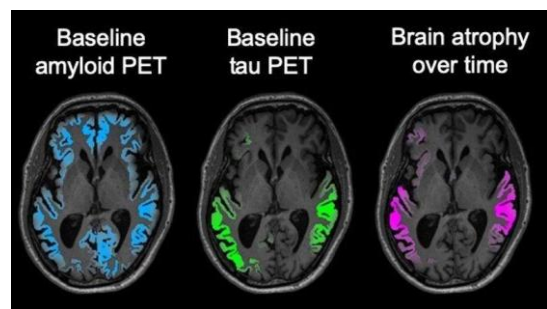


Figure 1C

Figure 1A-C Schematic representation of major molecular biomarker classes in Alzheimer’s disease. Blood-based biomarkers include amyloid-β (Aβ42/40), phosphorylated tau (p-tau181, p-tau217), and neurofilament light chain (NfL). Cerebrospinal fluid (CSF) biomarkers reflect central nervous system pathology more directly. Extracellular vesicles (EVs) transport neuron-derived proteins and nucleic acids across the blood–brain barrier. Molecular imaging modalities, including amyloid and tau positron emission tomography (PET), provide spatially resolved pathological confirmation. Together, these modalities enable early detection across preclinical and symptomatic stages.

The precision of Aβ quantification has improved through immunoprecipitation coupled with mass spectrometry (IP–LC–MS/MS). These methods detect subtle physiological deviations from healthy baselines [5, 6]. Phosphorylated tau isoforms, specifically p-tau217 and p-tau181, show strong concordance with CSF markers and PET imaging. Plasma p-tau217 distinguishes amyloid-positive individuals with AUC values near 0.90 [7].

Neurofilament light chain (NfL) serves as a marker for neuroaxonal injury. While not specific to AD, it provides prognostic data regarding neuronal damage. Elevated plasma

NfL levels correlate with the conversion from mild cognitive impairment (MCI) to AD dementia [4]. NfL remains a primary dynamic indicator of neurodegeneration.

Cerebrospinal fluid (CSF) remains the reference standard for CNS biochemistry. The core panel includes A β 42, total tau, and phosphorylated tau. Decreased CSF A β 42 indicates plaque formation, while increased tau reflects neuronal injury. Expanded panels now incorporate synaptic markers like neurogranin and SNAP-25. Inflammatory markers, such as YKL-40 and soluble TREM2, further characterize microglial activation.

Neuron-derived extracellular vesicles (NDEVs) capture CNS signals through minimally invasive sampling [8]. NDEVs are isolated from plasma using immunoaffinity techniques targeting neuronal surface markers. These vesicles transport microRNAs and messenger RNAs related to amyloid processing and synaptic function. Dysregulated miRNAs in APP processing pathways act as early indicators of pathology.

Cell-free DNA (cfDNA) offers an additional layer of information through epigenetic modifications. DNA methylation patterns help infer the tissue-of-origin for circulating DNA. Digital PCR and next-generation sequencing detect these low-abundance signals with high sensitivity [9]. Distinguishing CNS-derived cfDNA from peripheral sources remains a technical challenge.

Proteomic and glycomic profiling capture the complexity of protein networks. Tau PTMs, including truncation and nitration, drive protein aggregation and toxicity. Mass spectrometry allows for site-specific characterization of these modifications [10]. Altered glycan structures on plasma proteins serve as complementary diagnostic indicators.

Molecular imaging provides spatial context for biochemical changes. PET imaging uses specific radioligands to visualize

amyloid and tau deposits in vivo. Recent tracer developments have improved binding kinetics and specificity. Emerging affinity reagents, such as aptamers and nanobodies, offer engineered alternatives for targeted imaging and therapy.

Molecular Diagnostic Technologies

Advanced technologies now detect trace-abundance biomarkers in peripheral biofluids (figure 2A-C). These innovations address the extreme dilution of CNS-derived molecules in systemic circulation.

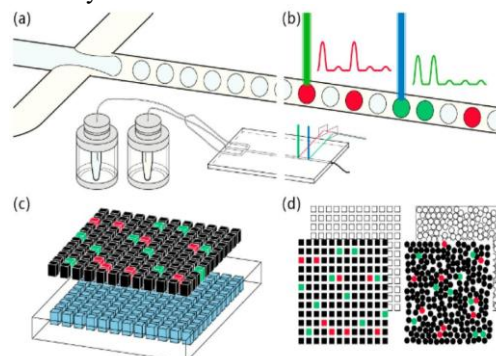


Figure 2A

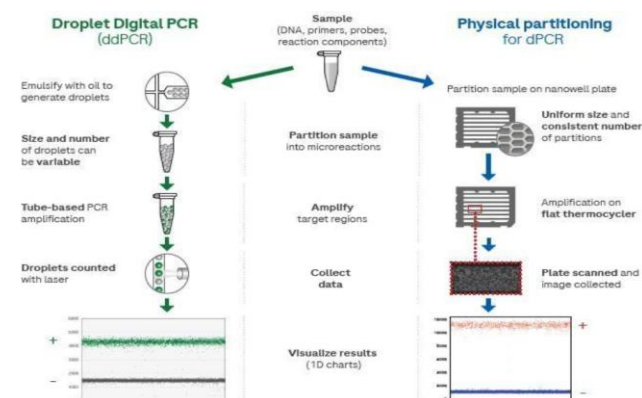


Figure 2B

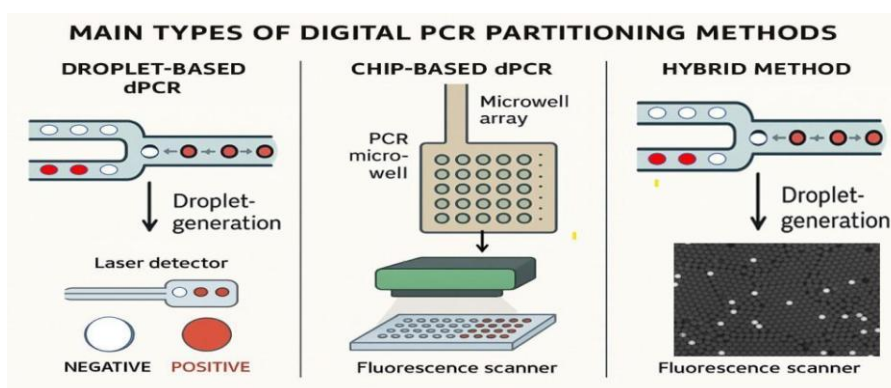


Figure 2C

Figure 2A-C: Overview of advanced molecular diagnostic technologies enabling detection of low-abundance biomarkers. Digital ELISA (Simoa) isolates single molecules in femtoliter wells. Mass spectrometry provides high-specificity proteomic profiling. CRISPR-based diagnostics enable rapid nucleic acid detection via collateral cleavage. Digital PCR partitions samples into thousands of reactions for absolute quantification.

Immunoassays remain a cornerstone of protein biomarker detection, with recent innovations significantly enhancing sensitivity and specificity. Digital ELISA, specifically the Simoa platform, achieves femtomolar detection limits. It isolates individual immunocomplexes within microreactors to convert analog signals into digital readouts [11]. This

precision allows for the quantification of trace proteins like p-tau and NfL. Improved antibody design has further reduced cross-reactivity in these assays.

Mass spectrometry provides high specificity for structural characterization. Multiple reaction monitoring (MRM)

enables targeted quantification of peptides with high precision. Data-independent acquisition (DIA) allows for broader proteomic profiling across samples. Integrating immunoprecipitation (IP-MS) enhances sensitivity by enriching target proteins [12].

Nucleic acid diagnostics utilize digital PCR (dPCR) for absolute quantification. Samples are partitioned into thousands of microreactions to reduce amplification bias [13]. CRISPR-based platforms, including SHERLOCK and DETECTR, leverage Cas enzymes for rapid detection [14]. These systems achieve attomolar sensitivity and support point-of-care applications.

Microfluidic platforms integrate sample preparation and analyte detection into a single device. This reduces volume requirements and total assay time. Multiplex detection allows for the simultaneous measurement of various biomarkers. SERS-based microfluidics have demonstrated ultra-sensitive detection of protein aggregates [15].

Glycomic profiling utilizes HILIC and MALDI-TOF mass spectrometry to analyze glycan structures. These complex analyses require standardized reference libraries to ensure reproducibility. Collectively, these tools enable the detection of disease-associated markers with unprecedented specificity.

Integration with Bioengineering Platforms

Bioengineering platforms provide mechanistic insights beyond simple analytical measurement. Organ-on-chip systems simulate the complex microenvironment of the central nervous system. Brain-on-chip models replicate the neurovascular unit using endothelial cells, neurons, and astrocytes. These platforms demonstrate that p-tau is actively transported across the blood-brain barrier via LRP1 receptors [16].

Nanotechnology has played a critical role in enhancing molecular detection through signal amplification and improved sensitivity. Nanoparticles can be functionalized with antibodies or nucleic acid probes to selectively bind target molecules, while their unique optical and electronic properties enable sensitive signal transduction. For example, gold nanoparticles used in SERS-based sensors amplify Raman signals, allowing for detection of biomarkers at extremely low concentrations. These technologies also enable multiplex detection, facilitating the simultaneous analysis of multiple biomarkers within a single assay [17].

Artificial intelligence and machine learning (ML) have emerged as essential tools for integrating complex, high-dimensional datasets generated by multi-omics analyses. By combining proteomic, glycomic, transcriptomic, and imaging data, AI-driven models can identify patterns and interactions that are not apparent through traditional statistical methods. These models enable the development of predictive molecular signatures that estimate the probability of disease progression, rather than relying on binary classification. Such approaches improve diagnostic specificity and support personalized medicine by identifying distinct disease subtypes and trajectories [18].

Microfluidic and lab-on-chip systems further enhance integration by enabling automated, high-throughput analysis of biological samples. These platforms reduce variability and improve reproducibility by standardizing sample handling and processing. When combined with AI-driven analytics, they provide a powerful framework for real-time diagnostics and monitoring.

The convergence of these bioengineering platforms represents a paradigm shift in molecular diagnostics, moving from static measurements to dynamic, systems-level understanding of disease. This integrated approach not only improves diagnostic accuracy but also provides insights into the underlying mechanisms of Alzheimer's disease, facilitating the development of targeted therapeutic strategies [19].

4. Discussion

The evolution of molecular diagnostic technologies has significantly improved analytical validity, enabling detection of CNS-derived biomarkers in peripheral blood at extremely low concentrations. Advances in assay design have reduced detection limits from picomolar to attomolar ranges, addressing the challenge of detecting brain-derived molecules diluted in systemic circulation.

Clinical validity has also improved substantially through the use of multi-biomarker panels. Combinations of plasma p-tau217 and A β 42/40 ratios have demonstrated high diagnostic accuracy in preclinical populations, with area-under-the-curve values exceeding 0.90. These findings support the feasibility of blood-based screening for early disease detection. Furthermore, integration with neurofilament light chain enhances the ability to predict disease progression and conversion from mild cognitive impairment to Alzheimer's disease [6].

Organ-on-chip studies have reshaped an understanding of biomarker dynamics, indicating that biomarker levels in blood may reflect regulated biological processes rather than passive leakage. This insight has important implications for interpreting diagnostic results and developing targeted therapies. Additionally, AI-driven data fusion generates probabilistic risk scores. This enables the stratification of patients into distinct progression categories, representing a paradigm shift in clinical diagnostics [18].

5. Conclusion

This study demonstrates that molecular diagnostic strategies supported by advanced bioengineering technologies enable highly sensitive detection of early Alzheimer's disease pathology. Integration of blood-based biomarkers, multi-omics profiling, and artificial intelligence-driven analysis provides a scalable and minimally invasive framework for early diagnosis. These approaches show strong diagnostic performance, with several biomarkers achieving $AUC \geq 0.90$ in distinguishing preclinical disease states. Despite these advances, challenges related to standardization, population diversity, and regulatory validation remain. Future research should focus on large-scale validation studies, harmonization of diagnostic protocols, and integration into clinical

workflows to enable reliable early detection and improved patient outcomes.

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