ACE BRIEF FOR NEW AND EMERGING HEALTH TECHNOLOGIES

The PrecivityAD Test for the Prognosis of Alzheimer's Disease

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Summary of Key Points

- Alzheimer's disease (AD) is a neurodegenerative disorder whose main pathogenesis is attributed to senile plaques formed by beta-amyloid (Aβ) and neurofibrillary tangles in the hippocampus, which is exacerbated by risk factors including advanced age, family history and apolipoprotein E (ApoE) genotype.
- Currently, the diagnosis of AD involves the assessment of Aβ levels through positron emission tomography (PET) scans or lumbar puncture for cerebrospinal fluid (CSF) analysis, which are either costly, invasive or less practical in resource-limited settings.
- PrecivityAD is a minimally invasive blood test based on the mass spectrometry (MS) platform. It quantifies plasma Aβ42/40 ratio and determine ApoE genotype, which in combination with age provides an Amyloid Probability Score (APS) that predicts the likelihood of brain amyloidosis. It is intended for individuals aged 60 years and older who are experiencing cognitive impairment.
- The PrecivityAD test was shown to be a promising screening test for brain amyloidosis. Although no studies reported on the safety of the PrecivityAD test, no major safety concern was expected related to the procedure. However, there may be potential harms associated with false positive and negative test results, which are difficult to quantify.
- The PrecivityAD test demonstrated good analytical accuracy, as well as good discriminative accuracy using Aβ-PET or CSF Aβ as reference standards.
    - Plasma Aβ42/40 ratio had a good discriminative accuracy of Aβ status (80% to 89%) in individuals of a wide age range and varying cognitive performance, which was further improved up to 94% with the inclusion of age and ApoE genotype.
    - The discriminative accuracy of the test remained consistent in the target population with slightly better performance in individuals with cognitive impairment than those without.
    - Plasma Aβ42/40 ratio may predict brain amyloidosis earlier than Aβ-PET scans.
- Despite good accuracy, its clinical utility in terms of patient's health outcome and cost-effectiveness remains unclear.
- The results were limited by a relatively small sample size of the target population, potential verification bias, time lag bias, non-standardised plasma Aβ42/40 cut-off point across studies, uncertain impact of diurnal variability in Aβ levels and the use of brain amyloidosis as a proxy for AD.
- The PrecivityAD test has a list price of US$1,250 in the United States. It is prohibitively costly as a triage test as it would account for an additional cost of 40% on top of a confirmatory Aβ-PET scan locally.
- Key implementation considerations include the complex workflow in a clinical setting, requirements of skilled operators, high cost, infrastructure requirements and lack of automation of the MS platform. The anticipation of curative treatments for AD may increase the demand for diagnostic testing, which may accelerate the introduction of blood-based biomarkers to clinics.
- Various ongoing developments on plasma biomarker tests for AD were identified.

I. Background

Alzheimer's disease (AD) is a neurodegenerative disorder with insidious onset characterised by progressive cognitive and behavioural impairment that significantly interferes with social and occupational functioning.[1] It is the most common type of dementia, accounting for 60% to 80% of all dementia cases.[2] The main pathogenesis of AD is attributed to senile plaques formed by beta-amyloid (Aβ) and neurofibrillary tangles made of phosphorylated tau protein in the hippocampus.[2] The key risk factors contributing to the pathological development of AD include advanced age, family history, vascular risk factors and apolipoprotein E (ApoE) ε4 isoform (ApoE4) genotype.[3] The clinical manifestations of AD depend on the stage of disease, with hallmark symptoms that include memory loss, impairment in problem solving and executive functioning. In later stages of AD, social withdrawal, psychosis, dyspraxia and extrapyramidal motor signs can occur.[1] Early diagnosis, particularly of patients with mild cognitive impairment (MCI), would be beneficial as patients would be more amendable to treatment advances.[3]

The number of people with AD in Singapore was estimated to be 45,000 in 2015 and is anticipated to rise to 241,000 by 2050 considering the ageing population.[4] A previous estimate found between 75,000 to 100,000 individuals afflicted with MCI locally.[3] The survival period of patients with AD ranges between three to 10 years following diagnosis.[5] During this period, patients with AD live through years of morbidity along with disease progression before their demise.[6] As a result of the detrimental effect of AD on cognition and function, patient's quality of life deteriorate progressively. AD represents a leading cause of disability and morbidity in the United States, with patients experiencing complications such as immobility, swallowing disorders and malnutrition, which increases the risk of serious acute conditions that can lead to death.[6] In addition, AD imposes a huge burden on caregivers and the healthcare system.[7] It is a costly disease, with an annual cost of S$793 million in Singapore for dementia, or S$28,341 per patient, owing to cost of pharmacotherapy, home care and productivity loss.[8]

The pathophysiological hallmarks of AD, such as Aβ, are currently detected by amyloid positron emission tomography (PET) scan which is costly with limited access, as well as lumbar puncture for cerebrospinal fluid (CSF) analysis which is complicated, invasive and time-consuming.[9,10] These methods are also considered to be less practical in primary care settings where resources are limited.[11] Moreover, the diagnosis of AD through cognitive assessment is of low accuracy, resulting in many patients with delayed or no diagnosis for AD.[10] As such, there is a clinical unmet need to identify cost-effective biomarkers that can be obtained through a less invasive manner and can be monitored repeatedly overtime.[12] This is further exemplified by the recent availability and ongoing development of disease-modifying drugs for AD, where early diagnosis of AD is crucial to stop or reverse the disease progression.[10]

II. Technology

The PrecivityAD test (C_2N Diagnostics, LLC) is a minimally invasive blood test that predicts AD brain pathology in individuals aged 60 years and older who are experiencing cognitive impairment such as memory decline or other cognitive issues related to MCI or dementia. It

is an immunoprecipitation liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay based on the company's proprietary Stable Isotope Spike Absolute Quantification (SISAQ) methodology that quantifies plasma Aβ42 and Aβ40 concentrations, as well as determine ApoE genotype based on the presence or absence of plasma ApoE isoform-specific peptides.

The plasma Aβ42/40 ratio, together with ApoE genotype and age, are incorporated into a logistic regression model and is presented as the Amyloid Probability Score (APS). The APS predicts the likelihood of brain amyloidosis and provides an estimated probability that the patient will be amyloid positive on an Aβ-PET scan. It ranges from zero to 100, with a higher score indicating a higher likelihood of brain amyloidosis. The APS is stratified into three classifications, which depicts a low, intermediate or high likelihood of the presence of amyloid plaques. Details of the APS classification were summarised in Table 1. The PrecivityAD test is also expected to be part of a future Brain Health Panel offered by C_2N Diagnostics that measure multiple pathological markers of AD and related disorders.

Table 1: Classification and interpretation of the APS

APS classification	Interpretation
Low APS (0 to 35)	A low score is consistent with a negative amyloid PET scan result and, thus, a low likelihood of amyloid plaques. Absence of amyloid plaques is inconsistent with an Alzheimer's disease diagnosis and indicates other causes of cognitive symptoms should be investigated.
Intermediate APS (36 to 57)	An intermediate score does not distinguish between the presence or absence of amyloid plaques and indicates further diagnostic evaluation may be needed to assess the underlying cause(s) for the patient's cognitive symptoms.
High APS (58 to 100)	A high score is consistent with a positive amyloid PET scan result and, thus, a high likelihood of amyloid plaques. Presence of amyloid plaques is consistent with an Alzheimer's disease diagnosis in someone who has cognitive decline, but alone is insufficient for a final diagnosis; clinical presentation and other factors should be considered along with the APS.
Abbreviations: APS, amyloid probability score; PET, positron emission tomography.	

A blood-based test for Aβ to measure brain amyloidosis offer advantages over CSF or PET imaging, owing to its less invasive nature, reduced cost, and lower burden to patients and the healthcare system (with reference to tracer costs and scanning time).[12] Furthermore, the availability of a blood-based test for patients with MCI is opportune given the ongoing development of new and emerging disease-modifying therapies that promise to slow down the progression of AD. In particular, the US Food and Drug Administration (FDA) approved aducanumab as a disease-modifying therapy for AD and granted Breakthrough Therapy Designation for three other similar drugs in 2021, although market authorisation of aducanumab was rejected by the European Medicines Agency due to safety and efficacy concerns. Nevertheless, such blood-based biomarker tests are limited by the lack of anatomical information and the extent of pathologies, which can be gained from confirmatory neuroimaging tests such as Aβ-PET scans.[11]

III. Regulatory and Subsidy Status

The PrecivityAD test received a CE mark in December 2020. In the United States, it is commercially available as a Laboratory Developed Test (LDT) under the Centers for Medicare

and Medicaid Services (CMS) Clinical Laboratory Improvement Amendments (CLIA) program. In addition, it was granted the Breakthrough Device Designation by the FDA in 2019 and is currently seeking FDA approval.

IV. Stage of Development in Singapore

☒	Yet to emerge	☐	Established
☐	Investigational / Experimental (subject of clinical trials or deviate from standard practice and not routinely used)	☐	Established *but* modification in indication or technique
☐	Nearly established	☐	Established *but* should consider for reassessment (due to perceived no/low value)

V. Treatment Pathway

Based on local practice, individuals suspected of cognitive impairment are subjected to dementia assessment through a multifaceted approach for the diagnosis of AD.[3] First, acute causes of cognitive impairment, such as delirium, has to be excluded. For those with chronic cognitive impairment, other causes such as depression or late-onset psychiatric disorders have to be ruled out, followed by the diagnosis of dementia through subjective means such as the DSM-IV criteria or objective means such as mental status or neuropsychological test.[3] Individuals with chronic cognitive impairment who fulfil the clinical criteria for dementia would undergo further evaluation to determine the aetiology of dementia. This involves an assessment of the patient's history, physical examination, blood and urine tests to rule out reversible causes of dementia such as metabolic abnormalities or neoplastic causes.[3] For patients with such causes ruled out, further confirmatory test for AD includes biomarker analysis by CSF or PET scans.[3]

While the PrecivityAD test is not considered as a confirmatory test for cerebral Aβ, its introduction may serve as a triage test for individuals presenting with MCI in the initial diagnostic workup. It may be used in a specialist memory clinic in addition to neurological examination, neuropsychological investigation and imaging, or as a screening tool together with cognitive tests in a primary care setting for referral to a specialist clinic for further confirmation of AD pathology by Aβ-PET scan or CSF analysis.[12] This may streamline the diagnostic process, avoiding unnecessary costly or invasive testing for patients with a low likelihood of amyloid plaques.

VI. Summary of Evidence

This assessment was conducted based on the Population, Intervention, Comparison and Outcome (PICO) criteria presented in Table 2. The evidence base, the inclusion and exclusion criteria were listed in Table A1 (Appendix A). Six studies[13-18] were included in this brief, of which one is an analytical validation study[14] while five are predictive accuracy studies[13,15-18].

Among the predictive accuracy studies, one small study[15] (n=41) had a prospective design while the other four[13,16-18] had a retrospective design. Of note, the included studies comprised of individuals with a wide age range (45 to 93.1 years old) and included both cognitively impaired and cognitively normal individuals. Details of the included studies were summarised in Table A2 (Appendix A).

Table 2: Summary of PICO criteria

Population	Patients aged 60 years and older who are experiencing cognitive impairment, such as memory decline or other cognitive issues related to mild cognitive impairment or dementia
Intervention	PrecivityAD
Comparison	Quantification of beta-amyloid (Aβ) by positron emission tomography (PET) scan or cerebrospinal fluid (CSF) analysis
Outcome	Safety, effectiveness (test accuracy, clinical utility), cost effectiveness

Safety

There were no studies identified that reported on the safety of the PrecivityAD test. As phlebotomy is a common procedure that is routinely performed, no major safety concern is expected. However, potential harm arising from false test result may occur with the use of the PrecivityAD test, although it may not be easy to assess such effects. Briefly, a false positive result may lead to unnecessary psychological stress and anxiety for the patient, while a false negative result may lead to delayed intervention.

Effectiveness

Accuracy

The technical performance of the PrevicityAD test was mainly reported in one study by Kirmess et al. (2021)[14]. The results indicated good accuracy of the test in quantifying the true level of plasma Aβ40 and Aβ42, with a recovery rate of the spike-and-recovery assay falling within the acceptable range (90% to 110%).[14] The test was also precise in quantifying the Aβ isotypes, where it demonstrated good repeatability (within-day variability) and reproducibility (within-lab variability) with a coefficient of variation (CV) below 10%.[14] This was supported by other studies, where low CV values were similarly reported.[13,16] Notably, the test was able to detect small differences (11% to 14.3%) in plasma Aβ42/40 ratio between individuals with and without brain amyloidosis with a high degree of significance ($p<0.0001$), further corroborating the high precision and accuracy of the assay.[15,16,18] These findings should be considered in view of the 10 to 100-fold lower analyte concentrations of beta-amyloid in blood compared to CSF as a consequence of the blood brain barrier.[11] Moreover, the PrecivityAD test was also highly accurate in determining ApoE genotype with a positive percent agreement (PPA) of 100% with its proteotype.[14]

Along with a good analytical accuracy, the PrecivityAD test also demonstrated good discriminative accuracy quantified as area under receiving operator curve (AUC) in classifying individuals with or without brain amyloidosis across five studies[13,15-18]. In individuals of a wide age range and varying cognitive performance, the plasma Aβ42/40 ratio classified Aβ status as determined by the Aβ-PET or CSF Aβ reference standards with a discriminative accuracy of 80% to 88% and 85% to 86% respectively (Table 3).[13,15-18] Regardless of the reference used,

the overall discriminative accuracy ranged from 80% to 89%.[13,15-18] The discriminative power of plasma Aβ42/40 ratio was also reflected by the PPA and negative percent agreement (NPA) of 88% and 76% respectively in one study[16] and an overall percent accuracy of 81% in another study.[18] The inclusion of age and ApoE genotype augmented the classification performance of the plasma Aβ42/40 ratio, further improving the discriminative accuracy to 90% to 94%.[16,18] Markedly, the test accuracy remained consistent with heterogenous participant demographics, plasma collection and Aβ determination protocols similar to real world conditions, indicating robustness of the classifier performance (AUC, 0.86 for plasma Aβ42/40 ratio; 0.90 for APS).[18] Furthermore, the plasma Aβ42/40 ratio was moderately to strongly correlated with Aβ-PET Centiloid and CSF Aβ42/40 ratio values (Figures B1 and B2 in Appendix B).[15,16]

In addition, the classifier performance remained consistent in the target population. In cognitively impaired individuals aged above 60 years (n=86), the plasma Aβ42/40 ratio predicted Aβ-PET status with a discriminative accuracy of 87%.[17] Notably, the test performed better in predicting Aβ-PET status in individuals with cognitive impairment than those without in both the plasma Aβ42/40 ratio (AUC, 0.87 *vs.* 0.80; PPA, 79% *vs.* 64%; positive predictive value, 79% *vs.* 69%) and APS (Figure B3 in Appendix B) classifiers.[17] Similarly, the discriminative accuracy was consistent in those aged above 60 years with varying cognitive performance (AUC range, 0.85 to 0.89).[13,15,16] These findings were also supported by early data from some abstracts that reported the ability of plasma Aβ42/40 ratio to predict Aβ status in higher risk cohorts.[19,20]

Taken together, the PrecivityAD test demonstrated good analytical accuracy in determining the levels of Aβ40/Aβ42 and ApoE genotype. The clinical validity of the test was ascertained by the robust discriminative accuracy of plasma Aβ42/40 ratio and APS classifiers in predicting brain amyloidosis as determined by either Aβ-PET or CSF Aβ in a population of a wide age range and varying cognitive performance, both in the target population and under heterogenous study conditions. However, these results should be considered in view of non-standardised cut-off points used to determine plasma Aβ42/40 ratio positivity across studies (Table B1 in Appendix B).[15,16,18] It should also be noted that Aβ-PET scans and CSF Aβ analysis do not provide a definitive diagnosis of AD, which requires a post-mortem neuropathological examination of the brain.[21]

Table 3: Predictive accuracy of the PrecivityAD test

Study	N	Reference test(s)	Cognitive status	Age (years)	Outcome	Plasma Aβ42/40	Plasma Aβ42/40, age and ApoE genotype (APS)
Schindler et al. (2019)[16]	158	Aβ-PET	Varied	46.1 to 86.9	AUC PPA NPA	0.88 (0.82 to 0.93) 0.88 (0.75 to 0.96) 0.76 (0.67 to 0.83)	0.94 (0.90 to 0.97) – –
	101*			>60	AUC	0.87 (0.80 to 0.94)	–
	152	CSF p-tau181/ Aβ42	Varied	46.1 to 86.9	AUC^	0.85 (0.79 to 0.92)	–
West et al. (2021)[18]	414	Aβ-PET or CSF Aβ42/40	Varied	45.0 to 93.1	AUC† Accuracy†	0.86 (0.82 to 0.90) 0.81	0.90 (0.87 to 0.93) 0.86

Tosun et al. (2021)[17]	87	Aβ-PET	Cognitively normal	66.6 to 79.9	AUC PPA NPA Accuracy PPV NPV	0.80 (0.65 to 0.94) 0.64 ± 0.18 0.77 ± 0.13 0.72 ± 0.07 0.69 ± 0.12 0.76 ± 0.08	Refer to Figure B3 in Appendix B#
	86		Cognitively impaired	62.4 to 80.1	AUC PPA NPA Accuracy PPV NPV	0.87 (0.75 to 0.99) 0.79 ± 0.08 0.75 ± 0.10 0.77 ± 0.06 0.79 ± 0.07 0.76 ± 0.08	Refer to Figure B3 in Appendix B#
Ovod et al. (2017)[15]	41	Aβ-PET or CSF Aβ42	Varied	>60	AUC	0.89	–
Janelidze et al. (2021)[13]	286‡	Aβ-PET	Varied	67.0 to 77.0	AUC	0.83 (0.79 to 0.88)	–
		CSF Aβ42/40			AUC	0.86 (0.81 to 0.90)	–
	122§	Aβ-PET	Varied	65.7 to 77.5	AUC	0.85 (0.77 to 0.92)	–

Note:
1. Data in parentheses are presented as 95% confidence interval.
2. Reference test indicates the diagnostic test used to determine brain amyloidosis.
3. Varied cognitive status refers to a study cohort that includes both cognitively normal and cognitively impaired individuals.

* A subcohort of individuals aged 60 years and above.

^ Based on the CSF Elecsys p-tau181/Aβ42 cut-off of 0.0198.

† Overall percent accuracy and AUC values after adjusting for inter-cohort differences with a logistic regression model.

Numerical outcome values not reported but were presented in a graphical format.

‡ Based on the BioFINDER1 cohort.

§ Based on the Alzheimer Disease Neuroimaging Initiative (ADNI) cohort.

Abbreviations: Aβ; beta-amyloid; ApoE, apolipoprotein E; APS, amyloid probability score; AUC, area under receiving operator curve; CSF, cerebrospinal fluid; NPA, negative predictive agreement; NPV, negative predictive value; PET, positron emission tomography; PPA, positive predictive agreement; PPV, positive predictive value.

Besides its use to predict prevailing Aβ status, baseline plasma Aβ42/40 ratio was also found to predict future conversion of Aβ-PET status. In a subcohort of individuals (n=100) with longitudinal Aβ-PET data, Schindler et al. (2019)[16] reported that Aβ-PET negative individuals with a positive baseline plasma Aβ42/40 ratio were 15 times more likely to convert to Aβ-PET positive compared to individuals with a negative baseline plasma Aβ42/40 ratio (p=0.01; Figure B4 in Appendix B).[16] This indicates that abnormal changes in plasma Aβ42/40 ratio may precede positive Aβ-PET results, which has been previously demonstrated.[11,22] Besides Aβ-PET, early results from an abstract showed that individuals with a positive baseline plasma Aβ42/40 ratio but a negative baseline CSF Aβ42/40 had a five times likelihood of converting to CSF positive within an average of 6.9 years.[19]

Clinical utility

Despite good analytical and discriminative accuracy of the PrecivityAD test, its translation into clinically useful improvement of patient's health outcome remains to be seen. However, in the context of screening for potential Aβ-PET positive participants for a preventive drug trial

for AD in a cohort of cognitively normal individuals, it was estimated that screening with the PrecivityAD test reduced the number of Aβ-PET scans by 62% compared to no screening, thus saving time and cost for trial recruitment (Table 4).[16] Nevertheless, its clinical utility remains unclear in a symptomatic cohort presenting with cognitive impairment.

Table 4: Predicted clinical utility of PrecivityAD in an asymptomatic cohort

ApoE status	Age (years)	Aβ-PET positive rate (%)	Probability of Aβ-PET positive if plasma Aβ42/40 positive (%)	Aβ-PET scans required to find 100 Aβ-PET positive individuals		Percentage of Aβ-PET scans saved by using blood test screening (%)
				Without plasma Aβ42/40	With plasma Aβ42/40	
ε4+	65-75	44	84	227	119	48
	75-85	68	98	147	102	31
ε4-	65-75	17	69	588	145	75
	75-85	27	95	370	105	71
Overall	65-85	30	80	333	125	62

Note:
1. The frequency of Aβ-PET positivity as a function of age and ApoE status was estimated based on data from the Anti-Amyloid Treatment in Asymptomatic Alzheimer's Disease (A4) Prevention Study.
2. The probability of a positive amyloid-PET scan for individuals with a positive blood test was based on a logistic regression model generated with data from the study by Schindler et al. (2019)[16].

Table and data adapted from Schindler et al. (2019)[16].

Early evidence from two abstracts also reported on the clinical utility of the PrecivityAD test. Briefly, the APS classification was found to impact physician-rated likelihood of AD and the prescription of anti-AD drugs, with a higher APS leading to greater AD diagnosis and therapeutic management post-test as compared to pre-test.[23] It was also reported as a potential tool to track treatment effects of disease-modifying therapy for AD.[22]

Cost effectiveness

No studies on cost effectiveness were identified. Although the PrecivityAD test may potentially be cost-effective as a triage test to rule in or rule out individuals for confirmatory Aβ-PET scan or CSF analysis which are costly and time-consuming, this is yet to be determined.

Ongoing clinical trial

There were no ongoing clinical trials investigating the accuracy of the PrecivityAD test identified from the ScanMedicine database (NIHR Innovation Observatory). However, a manuscript evaluating the discriminative accuracy of the PrecivityAD test on Aβ-PET status in the target population is currently in preparation (Table 5).[24] There are also some ongoing trials on AD management involving PrecivityAD as a screening test for trial inclusion identified from ClinicalTrials.gov (Table C1 in Appendix C).

Table 5: Ongoing clinical trials of the PrecivityAD test

Study (Trial ID)	Estimated enrolment	Brief description	Current status
PARIS study as an add-on study to the IDEAS study (NCT02420756)	18,488 participants for the IDEAS study; unclear enrolment for	The IDEAS study recruited a cohort of individuals aged above 65 years with progressive unexplained MCI, or dementia of uncertain etiology. The PARIS add-on to the IDEAS study is a blinded cross-sectional two-phase study that collects blood samples from eligible IDEAS participants. The plasma Aβ42/40 ratio will be	Manuscript in preparation for the first phase of the PARIS study that

	the PARIS add-on study	quantified by C_2N Diagnostics for evaluation of its concordance with Aβ-PET status after unblinding.	established the cut-off values.
Abbreviations: Aβ, beta-amyloid; IDEAS, Imaging Dementia-Evidence for Amyloid Scanning; MCI, mild cognitive impairment; PARIS, Plasma test for Amyloidosis RIsk Screening; PET, positron emission tomography.			

Summary

Overall, the PrecivityAD test was shown to be a promising screening test for brain amyloidosis. It was anticipated to be safe with no major issues expected from phlebotomy procedures, although potential harm may arise from false test results. It had a good accuracy in quantifying Aβ42/Aβ40 levels and in determining ApoE genotype. The test also demonstrated a discriminative accuracy of 80% to 89% on plasma Aβ42/40 ratio to predict Aβ-PET status regardless of the reference standards in individuals of a wide age range and varying cognitive performance, however it performed slightly better in individuals aged above 60 years with cognitively impairment than those without (AUC, 0.87 *vs.* 0.80 for plasma Aβ42/40). Both age and ApoE genotype had an additive effect on plasma Aβ42/40 ratio, further improving the discriminative accuracy up to 94% in the prediction of Aβ-PET status. Furthermore, there were some indications that the plasma Aβ42/40 ratio may predict brain amyloidosis earlier than Aβ-PET scans. Despite good accuracy, the clinical utility of the PrecivityAD test in terms of patient's health outcome as well as its cost-effectiveness remains unclear, although early findings showed potential in reducing Aβ-PET scans required and guiding clinician's decision.

These results should be interpreted with caution, with small sample size for the target population (n=86). Further studies are warranted in patients with MCI or mild dementia, where the use of a plasma Aβ test would bring value. The evidence was also limited by potential verification bias where non-standardised tracers and assay platforms were used for Aβ-PET and CSF analysis respectively. Similarly, the cut-off points for plasma Aβ42/40 positivity were not standardised across studies. Determination of Aβ status may also be influenced by time lag bias, where there was a delay of up to 18 months between the administration of the plasma analysis of Aβ42/40 and the reference tests, which may consequentially lead to changes in brain amyloidosis status. Besides, the impact of diurnal variation in Aβ levels on the test is not known and may affect its discriminatory power. In addition, given that Aβ pathology is also present in other neurodegenerative conditions such as Lewy body disease, the clinical utility of the test may be better informed by studies that evaluate the concordance between the PrecivityAD test with symptomatic AD instead of brain amyloidosis.[25]

VII. Estimated Costs

The PrecivityAD test has a list price of US$1,250 in the United States and is not currently covered by Medicare or Medicaid. It is prohibitively costly as a triage test as it would account for an additional cost of 40% on top of a confirmatory Aβ-PET scan locally (Personal communication: Neurologist from National Neuroscience Institute, 15 January 2022).

In the United States, patients who are eligible for the financial assistance programme offered by the company (C_2N Diagnostics, LLC) would have an out-of-pocket cost between US$25 to US$400.

VIII. Implementation Considerations

The PrecivityAD test is performed on the LC-MS/MS platform, which is a highly complex equipment that involves routine maintenance.[26] The inherent technical, process, cost and infrastructure challenges associated with the use of LC-MS/MS platform in the hospital setting need to be considered.

Compared to other plug-and-play tests that can be readily deployed in a hospital setting, the LC-MS/MS platform involves complex workflows that does not readily fit into the typical clinical laboratory. The complexity of the technology requires highly skilled operators to run and interpret the test. This is coupled with limited options for front end automation of the LC-MS/MS system where manual sample preparation is required, which can be both time consuming and labour intensive.[27] Also, the LC-MS/MS system involves a high capital cost which may be prohibitive in some healthcare institutions. In the United States, the test is performed at a central laboratory. While this ensures consistency in test performance and economies of scale, infrastructure considerations may arise with the setup of a central laboratory. Additionally, there may be a longer turnaround time and increased cost associated with shipping of plasma samples.

Furthermore, the availability of the PrecivityAD test may potentially lead to earlier diagnosis of AD. Consequentially, this may increase the demand for medical therapies. While existing drug therapies for AD provide symptomatic relief, the FDA recently approved aducanumab (Aduhelm, Biologic Inc.) which is a disease-modifying therapy aimed at reducing Aβ plaques. Several other disease-modifying therapies for AD are currently in late-stage development. Of note, the current list price of aducanumab is an average of US$28,200 a year per patient.[28] Although the cost of other emerging disease-modifying therapies for AD remains unclear, similar high costs could impose significant financial burden on the healthcare system given the anticipated rising prevalence of AD in Singapore.

Conversely, the prospect of new disease-modifying therapies offered to individuals with MCI suggests that healthcare systems should prepare for the substantial increase in demand for diagnostic testing of AD pathology.[29] It was previously estimated that an additional increase of 100,000 Aβ-PET scans and 100,000 CSF tests would cost £113 million and £48 million respectively in the United Kingdom, including equipment, staff and training costs.[29] The increase in diagnostic testing may also place pressure on radiology staff, skilled nurses, equipment and laboratory facilities.[29] As a result, the incoming burden on the healthcare system in anticipation of curative treatment for patients with AD may accelerate the implementation of blood-based biomarkers in clinics.[12] It was previously estimated that blood-based biomarkers for AD may enter the specialist memory clinics in the next three to five years, and the primary care setting in the next five to 10 years.[12]

Also, as the PrecivityAD test is indicated for use in individuals aged above 60 years, its accessibility to younger patients is limited. This is of particular importance given the rising trend of patients with young onset dementia in Singapore.[30]

IX. Concurrent Developments

There has been an ongoing preponderance of blood-based *in-vitro* diagnostic (IVD) assays to identify individuals at risk of AD. The advent of more sensitive assays, such as immunoprecipitation-mass spectrometry or the single molecule array (Simoa) platform, has resulted in rapid progress in the development of plasma biomarkers for AD which includes plasma Aβ42/40, neurofilament light chain (NfL) and p-tau.[31] Table 6 summarised some of the ongoing development of blood-based IVD assays to prognose individuals at risk of developing AD.

Amidst the rapid development of blood-based IVD assays for AD, a recent head-to-head comparison of eight Aβ assays found that plasma Aβ42/40 determined using mass-spectrometry assays (including PrecivityAD) was more accurate in distinguishing abnormal brain Aβ status than immunoassays.[13] The plasma Aβ42/40 ratio determined by the PrecivityAD test was also found to outperform other plasma biomarkers including p-tau181 and NfL in distinguishing Aβ status.[17]

Table 6: Ongoing development of blood-based *in-vitro* diagnostic assays for AD

Test (Manufacturer)	Brief description	Current developmental status
PreADx (Pre Diagnostics AS)	PreADx measures the clearance of Aβ peptides by analysing patient's monocytes with the Simoa platform.	Completed clinical evaluation in Q1 2020; planned for CE marking in Q2 2021.
OAβ test (PeopleBio, Inc.)	The OAβ ELISA test can measure the oligomerization levels of beta-amyloid, the biomarker of AD, prior to the onset of symptoms.	CE marked and approved by Korean FDA.
AMYBLOOD (ADx Neurosciences)	Simoa plasma assay that measure the full length of Aβ40 and Aβ42 isoforms.	In development.
Simoa phospho-Tau 181 blood test (Quanterix Corporation)	A semiquantitative immunoassay intended for the measurement of pTau-181 concentration in human serum and plasma using the Quanterix HD-X immunoassay system.	Granted FDA Breakthrough Device Designation in October 2021
Blood test for AD (Hong Kong University of Science and Technology)	An ultrasensitive and high-throughput protein measurement technology (proximity extension assay) that distinguish AD patients with a panel of 19 plasma proteins.	Completed clinical evaluation in 2021.
APEX system (NUS Institute for Health Innovation & Technology)	The APEX system detects and analyses aggregated forms of Aβ proteins in blood samples, to enable detection of AD even before clinical symptoms appear and to accurately classify the disease stages.	Completed a feasibility clinical study in 2019; planning for technology commercialisation.
Amyloid MS (Shimadzu Scientific Instruments)	The blood analysis works using a combination of immunoprecipitation and MALDI-TOF mass spectrometry (IP-MS) for early screening of amyloid-positive subjects.	Launched in the United States for research use only.
Alzosure Predict (Diadem srl)	A non-invasive mass spectrometry-based blood test that measures the level of U-p53^{AZ} to predict the early onset of AD with the ability to identify MCI patients before the clinical symptoms are identifiable 6 years in advance of clinical diagnosis.	Expected global launch in 2022.
LUMIPULSE G (Fujirebio Diagnostics, Inc.)	A plasma p-Tau181 detection assay on the fully automated LUMIPULSE G immunoassay platform.	In development with Alzheimer's Drug Discovery Foundation Diagnostics Accelerator.

Abbreviations: Aβ; beta-amyloid; AD, Alzheimer's disease; APEX, Amplified Plasmonic Exosome; CE, Conformité Européenne; ELISA, enzyme-linked immunosorbent assay; FDA, U.S. Food and Drug Administration; IP-MS, immunoprecipitation-mass spectrometry; MALDI-TOF, Matrix-assisted laser desorption/ionization-time of flight; MCI, mild cognitive impairment; NUS, National University of Singapore.

X. Additional Information

Conflict of interest was reported in all the included studies[13-18], where some or most of the authors were either affiliated with C_2N Diagnostics or Washington University where the technology was initially developed.

A review article on blood-based biomarkers for AD recently published in The Lancet Neurology in November 2021 indicated that more evidence from prospective studies and real world performance as well as investigation on the impact on clinical outcomes will guide the implementation of plasma Aβ biomarkers in clinical practice.[12] This resonated with a Personal View article published in The Lancet Neurology in April 2021, where the International Working Group on AD did not recommend plasma Aβ biomarkers for the clinical diagnosis of AD as it requires further standardisation and validation to establish it as a reliable indicator of AD pathology.[32]

Further, while the prospect of early testing for AD offered by the PrecivityAD test may be clinically useful, there may be ethical implications arising from the testing of an individual with MCI and the subsequent disclosure of his or her risk status. Such information on the risk of developing AD may impose psychological burden on the individual, as they would in turn become a "patient-in-waiting".[33] The uncertainty of AD progression, stigmatisation and anxiety may decrease the benefit accrued from the predictive information of the PrecivityAD test.[33] Given these ethical implications, the availability of novel curative treatments for AD such as disease-modifying therapies may be considered in implementing the PrecivityAD test. In addition, genotyping for ApoE status may also present implications on a person's family members. As a result, the harms and benefits of the test are not confined to the person tested. It has been suggested that the results of predictive test should not be routinely disclosed or be permissible in a direct-to-consumer manner, similar to the "right not to know" framework in genetic testing.[33]

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Appendix

Appendix A: Studies included and study design

Table A1: List of included studies

Type of study	Number of studies included
Predictive accuracy study	5
Analytical validation study	1

Note:
1. Inclusion criteria
   a. Studies that fulfil the PICO criteria listed in Table 1. Of note, studies were also included if a subset of the cohort includes the intended population as listed in Table 1.
2. Exclusion criteria
   a. Studies only available in the abstract form.

Table A2: Characteristics of the included studies

Study	N; Study Design	Study cohort	Reference standard(s)	Cognitive status	Time between plasma collection and reference test
Schindler et al. (2019)[16]	N=158; Retrospective	The study cohort represents a convenience sample of participants of all age and diagnoses who underwent plasma collection within 18 months of Aβ-PET scan and have sufficient plasma sample for analysis.	• Aβ-PET (^{11}C-PiB or florbetapir) • CSF Aβ42/40 (determined by Elecsys)	Mostly cognitively normal (94% CDR=0)	Mean ± S.D.: 0.26 ± 0.35 years Range: 0 to 1.5 years
West et al. (2021)[18]	N=414; Retrospective	Random banked plasma samples of individuals of all age and diagnoses from six independent cohorts, where brain amyloid status was available.	• Aβ-PET (^{11}C-PiB, Amyvid, NeuraCeq) • CSF Aβ42/40 (determined by ELISA or MS)	50% to 75% cognitively normal (CDR=0) amongst the six cohorts	NR
Tosun et al. (2021)[17]	N=173; Retrospective	Participant selection was made *a priori* from all participants in the ADNI study based on the availability of complete cross-sectional data.	• Aβ-PET (florbetapir)	Separate cohorts of cognitively normal and cognitively impaired individuals	Within 6 months
Kirmess et al. (2021)[14]	NA	NA	NA	NA	NA
Ovod et al. (2017)[15]	N=41; Prospective	Carefully selected participants aged above 60 years.	• Aβ-PET (^{11}C-PiB) • CSF Aβ4 (determined by IPMS)	27 cognitively normal (CDR=0) and 14 cognitively impaired (CDR>0)	NR
Janelidze et al. (2021)[13]	N=286; Retrospective	Participants selected from the Swedish BioFINDER-1 cohort.	• Aβ-PET (flutemetamol)	182 cognitively normal and	NR

			• CSF Aβ42/40 (determined by Elecsys)	104 with MCI	
	N=122; Retrospective	Participants selected from the ADNI cohort.	• Aβ-PET (florbetapir)	51 cognitively normal, 51 with MCI and 20 with AD dementia	NR
Abbreviations: Aβ; beta amyloid; AD, Alzheimer's disease; ADNI; Alzheimer Disease Neuroimaging Initiative; CDR, clinical dementia rating; CSF; cerebrospinal fluid; ELISA, enzyme linked immunosorbent assay; IPMS, immunoprecipitation mass spectrometry; MCI; mild cognitive impairment; MS, mass spectrometry; NA, not applicable; NR, not reported; PET, positron emission tomography; PiB, Pittsburgh Compound B.					

Appendix B: Supplementary tables and figures of included studies

Table B1: Plasma Aβ42/40 cut-off values reported in each study

Study	Plasma Aβ42/40 cut-off
Schindler et al. (2019)[16]	0.1218
West et al. (2021)[18]	0.0975
Tosun et al. (2021)[17]	NR
Kirmess et al. (2021)[14]	NA
Ovod et al. (2017)[15]	0.1243
Janelidze et al. (2021)[13]	NR
Abbreviation: NA, not applicable; NR, not reported.	

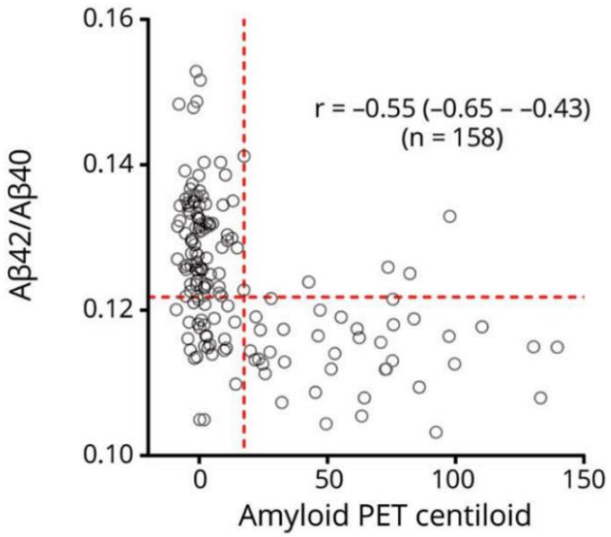

Figure B1: Moderate correlation between plasma Aβ42/40 ratio with Aβ-PET centiloid (Spearman correlation coefficient: -0.55). Adapted from Schindler et al. (2019)[16].

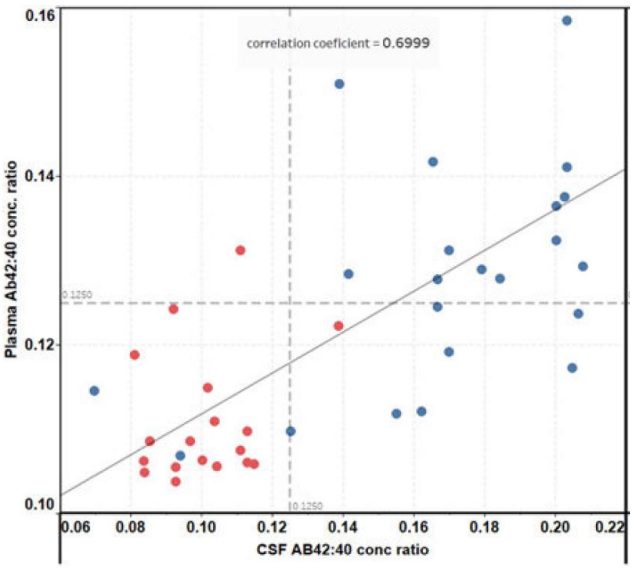

Figure B2: Strong correlation between plasma Aβ42/40 ratio and CSF Aβ42/40 (Spearman correlation coefficient: 0.6999). Adapted from Ovod et al. (2017)[15]

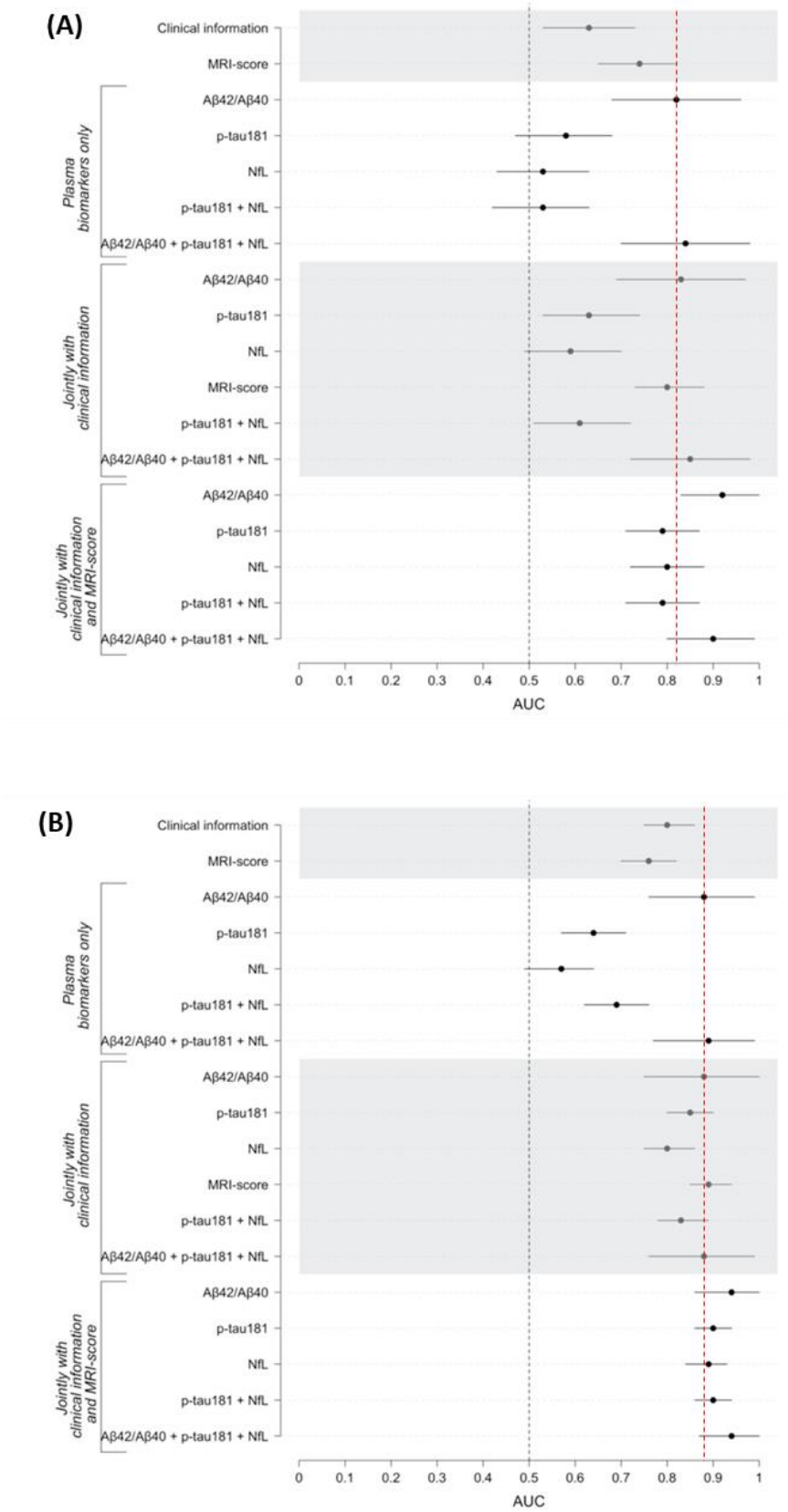

Figure B3: Receiver operating characteristic (ROC) analysis of plasma Aβ42/40 ratio on Aβ-PET status was repeated in the ADNI cohort with clinical information restricted to age and ApoE genotype in (A) cognitively normal individuals and (B) individuals with mild cognitive impairment. Error bars indicate union of 95% CIs from cross-validation iterations. The red dotted line serves as a reference to compare the area under the curve (AUC) value of plasma Aβ42/40 ratio alone and plasma Aβ42/40 ratio with clinical information. Adapted from Tosun et al. (2021)[17].

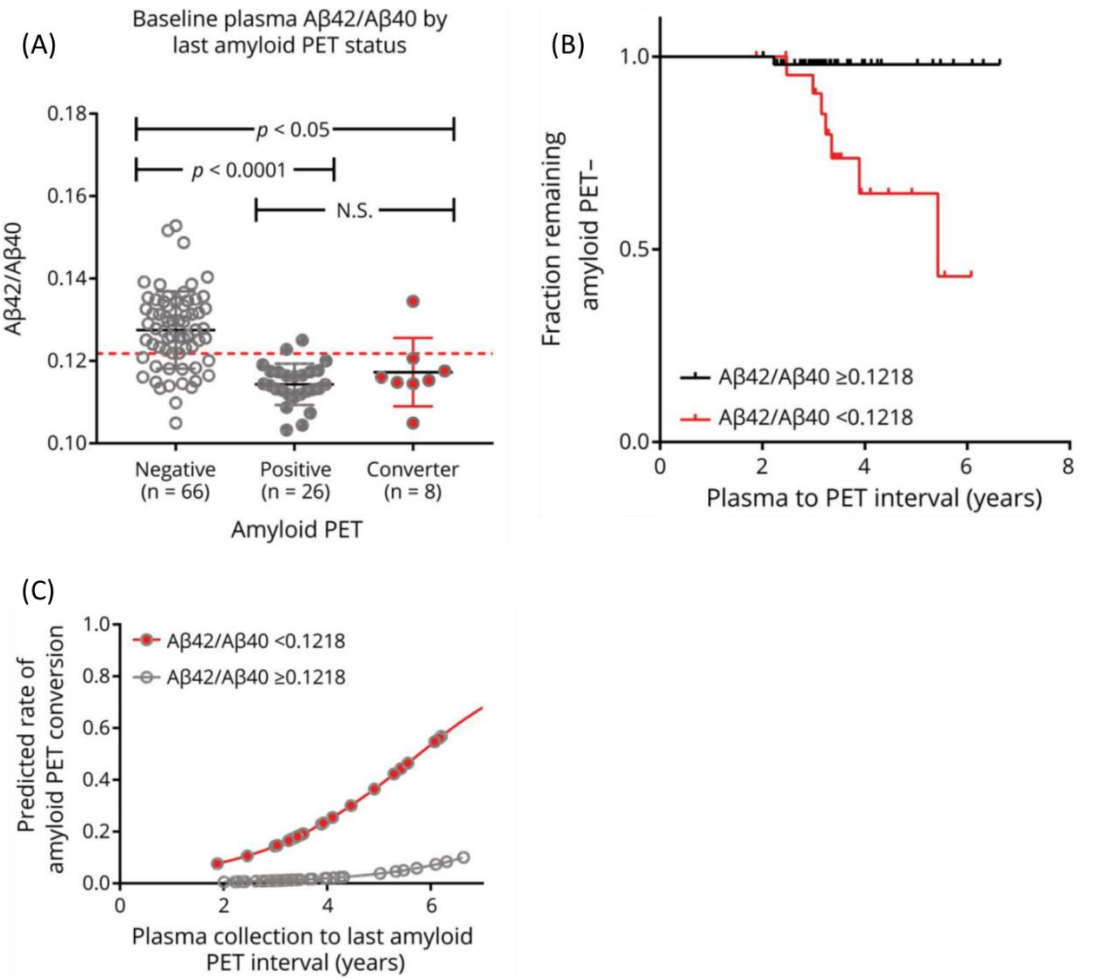

Figure B4: Plasma Aβ42/40 detects brain amyloidosis earlier than Aβ-PET. (A) Individuals who were Aβ-PET negative at baseline and converted to Aβ-PET positive over the follow-up period had significantly lower baseline plasma Aβ42/40 than individuals who remained Aβ-PET negative (*P*<0.05). (B) Aβ-PET negative individuals with a positive plasma Aβ42/40 ratio (<0.1218) had a 15-fold increased risk of conversion to Aβ-PET positive compared to individuals with a negative plasma Aβ42/40 (≥0.1218). (C) Prediction model to indicate the Aβ-PET conversion of individuals with a positive plasma Aβ42/40 ratio (<0.1218) or negative plasma Aβ42/40 (≥0.1218) over time. Adapted from Schindler et al. (2019)[16].

Appendix C: Ongoing clinical trials involving PrecivityAD as a screening test

Table C1: Ongoing clinical trials of AD management involving PrecivityAD as a screening test for trial inclusion

Study (Trial ID)	Estimated enrolment	Brief study description	Involvement of the PrecivityAD test	Estimated study completion date
Can Lifestyle Changes Reverse Early-Stage Alzheimer's Disease (NCT04606420)	100	A randomised crossover study to determine if comprehensive lifestyle changes may slow, stop, or reverse the progression of early-stage AD.	PET scans and/or C2N testing will be performed at baseline if there is any doubt about the clinical diagnosis of AD	December 2022
DISCOVER (NCT02925650)	24	A RCT to evaluate the safety and pharmacological effects of 3 different doses of Posiphen® when compared to a placebo, in adult male and female patients with early AD.	To qualify for entry, subjects will have CSF Aβ42 levels that are consistent with AD as measured via mass spectrometry by C2N.	December 2021
Development and Evaluation of Computerized Olfactory Training Program for Cognitive Decline in Early Alzheimer's Disease (NCT05122598)	200	A RCT to determine whether daily treatment with this new treatment approach, called Computerized Olfactory Training would be effective in protecting the memory and brain regions of people who are already showing signs of memory loss.	Participants to have either CSF Aβ42 levels that are consistent with AD as measured via mass spectrometry by C2N, or document elevated amyloid burden consistent with AD from PET imaging	April 2023
AHEAD 3-45 (NCT04468659)	1400	A placebo-controlled, double blind randomised controlled trial to evaluate the efficacy and safety of treatment with BAN2401 in individuals with preclinical AD and elevated amyloid (A45 Trial), and in individuals with early preclinical AD and intermediate amyloid (A3 Trial).	Plasma screening with C2N Diagnostic's mass spectrometry platform (PrecivityAD) will be used to quantitate the Aβ42/40 ratio which has been shown to be a reliable predictor of brain amyloid level.	October 2027

Abbreviations: Aβ; beta-amyloid; AD, Alzheimer's disease; CSF, cerebrospinal fluid; PET, positron emission tomography.

Appendix D: Other supporting information pertaining to the PrecivityAD test

Table D1: Comparison of PrecivityAD test with other Aβ assays in the BioFINDER cohort

Plasma Aβ42/40 assay	AUC (95% CI)	
	CSF Aβ42/40	Aβ-PET
Entire Cohort		
Aβ+, n	118	110
Aβ-, n	168	176
IP-MS-WashU (PrecivityAD)	0.855 (0.810 to 0.899)	0.833 (0.787 to 0.879)
IA-Elc	0.778 (0.725 to 0.832)[b]	0.727 (0.669 to 0.784)[c]
LC-MS-Arc	0.776 (0.721 to 0.830)[b]	0.753 (0.696 to 0.811)[b]
IA-EI	0.697 (0.635 to 0.758)[c]	0.672 (0.609 to 0.735)[c]
IA-N4PE	0.687 (0.626 to 0.748)[c]	0.655 (0.591 to 0.719)[c]
Subcohort with IP-MS-Shim Aβ42/40[d]		
Aβ+, n	86	86
Aβ-, n	114	114
IP-MS-WashU (PrecivityAD)	0.872 (0.824 to 0.920)	0.872 (0.824 to 0.920)
IP-MS-Shim	0.825 (0.767 to 0.882)	0.825 (0.767 to 0.882)
LC-MS-Arc	0.775 (0.711 to 0.839)[b]	0.775 (0.711 to 0.839)[b]
IA-Elc	0.773 (0.709 to 0.837)[b]	0.773 (0.709 to 0.837)[b]
IA-EI	0.704 (0.631 to 0.777)[c]	0.704 (0.631 to 0.777)[c]
IA-N4PE	0.679 (0.605 to 0.753)[c]	0.679 (0.605 to 0.753)[c]
Subcohort with IP-MS-UGOT and IA-Quan Aβ42/40		
Aβ+, n	91	86
Aβ-, n	136	141
IP-MS-WashU (PrecivityAD)	0.838 (0.785 to 0.891)	0.814 (0.760 to 0.868)
IA-Elc	0.795 (0.738 to 0.853)	0.728 (0.663 to 0.793)[b]
LC-MS-Arc	0.763 (0.700 to 0.827)[a]	0.742 (0.676 to 0.809)[a]
IA-N4PE	0.706 (0.639 to 0.773)[b]	0.649 (0.577 to 0.721)[c]
IA-EI	0.697 (0.628 to 0.767)[c]	0.667 (0.596 to 0.738)[c]
IP-MS-UGOT	0.678 (0.605 to 0.750)[c]	0.632 (0.557 to 0.707)[c]
IA-Quan	0.636 (0.563 to 0.709)[c]	0.600 (0.525 to 0.675)[c]

Abbreviations: AUC, area under the curve; IA-EI, immunoassay from Euroimmun; IA-Elc, Elecsys immunoassay from Roche Diagnostics; IA-N4PE, N4PE Simoa immunoassay from Quanterix; IA-Quan, Simoa immunoassay from Quanterix; IP-MS-Shim, immunoprecipitation coupled mass spectrometry method developed by Shimadzu; IP-MS-WashU, immunoprecipitation-coupled mass spectrometry method developed at Washington University (PrecivityAD); IP-MS-UGOT, immunoprecipitation-coupled mass spectrometry method developed at the University of Gothenburg; LC-MS-Arc, antibody-free liquid chromatography-mass spectrometry method developed by Araclon.

[a] $P<0.05$ compared with IP-MS-WashU (PrecivityAD).

[b] $P<0.01$ compared with IP-MS-WashU (PrecivityAD).

[c] $P<0.001$ compared with IP-MS-WashU (PrecivityAD).

[d] In this subcohort, CSF Aβ42/40 and Aβ-PET concordance was 100%.

Adapted from Janelidze et al. (2021)[13].

Table D2: Comparison of PrecivityAD test with other Aβ assays in the Alzheimer Disease Neuroimaging Initiative cohort

Plasma Aβ42/40 assay	Aβ-PET, AUC (95% CI)
Aβ+, n	118
Aβ-, n	167
IP-MS-WashU (PrecivityAD; Aβ42/40)	0.845 (0.772 to 0.917)
IP-MS-Shim (composite biomarker)	0.821 (0.747 to 0.895)
IA-Elc (Aβ42/40)	0.740 (0.651 to 0.829)[a]
IA-N4PE (Aβ42/40)	0.685 (0.590 to 0.781)[b]
IP-MS-UGOT (Aβ42/40)	0.662 (0.565 to 0.758)[c]
IA-Quan (Aβ42/40)	0.634 (0.534 to 0.734)[c]

Abbreviations: Aβ, amyloid-β; AUC, area under the curve; IA-Elc, Elecsys immunoassay from Roche Diagnostics; IA-N4PE, N4PE Simoa immunoassay from Quanterix; IA-Quan, Simoa immunoassay from Quanterix; IP-MS-Shim, immunoprecipitation coupled mass spectrometry method developed by Shimadzu; IP-MS-UGOT, immunoprecipitation-coupled mass spectrometry method developed at the University of Gothenburg; IP-MS-WashU, immunoprecipitation-coupled mass spectrometry method developed at Washington University (PrecivityAD); ROC, receiver operating characteristic; PET, positron emission tomography.

[a] $P<0.05$ compared with IP-MS-WashU (PrecivityAD).

[b] $P<0.01$ compared with IP-MS-WashU (PrecivityAD).

[c] $P<0.001$ compared with IP-MS-WashU (PrecivityAD).

Adapted from Janelidze et al. (2021)[13].

Table D3: AUC values of plasma Aβ42/40 combined with ApoE4 genotype in the BioFINDER cohort

Plasma Aβ42/40 assay + ApoE4	CSF Aβ42/40, AUC (95% CI)
Entire Cohort	
Aβ+, n	118
Aβ-, n	167
IP-MS-WashU (PrecivityAD)	0.882 (0.842 to 0.922)
LC-MS-Arc	0.841 (0.794 to 0.887)
IA-Elc	0.820 (0.771 to 0.869)[a]
IA-EI	0.794 (0.741 to 0.846)[b]
IA-N4PE	0.783 (0.729 to 0.836)[c]
Subcohort with IP-MS-Shim	
Aβ+, n	86
Aβ-, n	113
IP-MS-WashU (PrecivityAD)	0.902 (0.861 to 0.944)
IP-MS-Shim	0.868 (0.819 to 0.918)
LC-MS-Arc	0.863 (0.812 to 0.913)
IA-Elc	0.834 (0.778 to 0.889)
IA-EI	0.816 (0.757 to 0.875)
IA-N4PE	0.798 (0.736 to 0.861)
Subcohort with IP-MS-UGOT and IA-Quan	
Aβ+, n	91
Aβ-, n	136
IP-MS-WashU	0.870 (0.823 to 0.917)
LC-MS-Arc	0.841 (0.788 to 0.894)
IA-Elc	0.841 (0.790 to 0.891)
IP-MS-UGOT	0.805 (0.747 to 0.864)
IA-EI	0.805 (0.747 to 0.864)
IA-N4PE	0.794 (0.735 to 0.854)

IA-Quan	0.779 (0.717 to 0.841)

Abbreviations: Aβ, amyloid-β; AUC, area under the curve; IA-Elc, Elecsys immunoassay from Roche Diagnostics; IA-N4PE, N4PE Simoa immunoassay from Quanterix; IA-Quan, Simoa immunoassay from Quanterix; IP-MS-Shim, immunoprecipitation coupled mass spectrometry method developed by Shimadzu; IP-MS-UGOT, immunoprecipitation-coupled mass spectrometry method developed at the University of Gothenburg; IP-MS-WashU, immunoprecipitation-coupled mass spectrometry method developed at Washington University (PrecivityAD); ROC, receiver operating characteristic; PET, positron emission tomography.

[a] $P<0.05$ compared with IP-MS-WashU (PrecivityAD).

[b] $P<0.01$ compared with IP-MS-WashU (PrecivityAD).

[c] $P<0.001$ compared with IP-MS-WashU (PrecivityAD).

Adapted from Janelidze et al. (2021)[13].