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Supplementary information

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A minimally invasive dried blood spot biomarker test for the detection of Alzheimer's disease pathology

In the format provided by the authors and unedited

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Supplementary Table 1. Cohorts characteristics

Study side	Inclusion criteria	Exclusion criteria	Sampling period	Shipping range; temperature	Cognitive testing available	CSF data available	Ethical approval
The "Barcelona cohort" Ace Alzheimer Center Barcelona, Spain	Participants under investigation for cognitive complaints	None	09/2022 - 04/2024	1-3 days after collection; RT	Yes (CDR)	Yes (sub group)	Ethics Committees of the Hospital Universitari de Bellvitge, Barcelona (Ref. PR148/22)
The "Gothenburg cohort" Memory clinic, Sahlgrenska University Hospital Gothenburg, Sweden	Participants under investigation for cognitive complaints	None	06/2023 - 04/2024	1-7 days after collection; RT	Yes (MMSE and CDR)	Yes	The Swedish Ethical Review Authority (Etikprövningsmyndigheten; EPM: 2023-06137-02)
The "Malmö cohort" *BioFINDER Primary Care* and BioFINDER Preclinical AD studies Malmö, Sweden	Asymptomatic AD, healthy controls, patients with cognitive symptoms undergoing diagnostic evaluation in primary care	No CSF/ blood sampling/ cognitive testing as part of clinical practice	12/2023 - 11/2024	1-7 days after collection; RT	Yes (MMSE)	Yes	Swedish Ethical Review Authority (Dnr. 2021-05724-01 and 2019-04320)
The "Brescia cohort" Center for Neurodegenerative Disorders, University of Brescia, Italy	Individuals with FTD or AD, and healthy spouses/ family members	None	10/2024 - 06/2024	1-30 days after collection; RT	Yes (MMSE and CDR)	Yes (sub group)	Local Ethics Committee University of Bresica (NP2189 and NP1965)
The "Exeter cohort" University of Exeter Medical School, United Kingdome	Participants taking part in the DailyColors polyphenol supplement study: ≥ 50 years of age, BMI ≥ 25 kg/m²	Dementia diagnosis; participation in a clinical trial; diet-related factors described previously	01/2024	1-10 days after collection; RT	No	No	Ethics Committee of the University of Exeter, Faculty of Health & Life Sciences REC (Ref. 529634)
The "Copenhagen" cohort Memory clinic, Rigshospitalet, Copenhagen University Hospital, Denmark	Participants under investigation of a neurodegenerative disease	No consent to the Danish Dementia Biobank; no lumbar puncture; clinically incapability to participate	05/2024 - 07/2024	7-40 days after collection; RT	Yes (MMSE)	Yes	Danish Research Ethics Committee (Ref. H-23078392)
The "Sant Pau cohort" Sant Pau Memory Unit, Barcelona, Spain	Participants with DS with AD or without AD-related cognitive impairment taking part in the DABNI study	None	05/2024 - 11/2024	1-14 days after collection; RT	No	Yes (sub group)	Sant Pau Ethics Committee

AD = Alzheimer's disease; BMI = Body mass index; CDR = Clinical dementia rating; CSF = Cerebrospinal fluid; DABNI = Down Alzheimer Barcelona Neuroimaging Initiativ; DS = Down Syndrome; FTD = Frontotemporal dementia; MMSE = Minimental state examination; RT = Room temperature

Supplementary Table 2. Multivariable regression models of capillary p-tau217 with plasma p-tau217 and covariates

	Model 1: Plasma only		**Model 2: + Demographics**		**Model 3: + Diagnosis**		**Model 4: + CSF status**	
Predictors	*Std. Beta*	*p*	*Std. Beta*	*p*	*Std. Beta*	*p*	*Std. Beta*	*p*
(Intercept)	0.00	0.975	0.01	0.611	0.13	0.576	0.19	0.460
Plasma p-tau217	0.69	**<0.001**	0.68	**<0.001**	0.70	**<0.001**	0.75	**<0.001**
Age			0.03	0.589	0.05	0.451	0.06	0.354
Sex (Female)			-0.01	0.908	0.01	0.953	0.00	0.983
Diagnosis (MCI)					-0.15	0.515	-0.11	0.631
Diagnosis (AD)					-0.24	0.366	-0.18	0.511
Diagnosis (Non-AD)					-0.01	0.979	-0.00	0.990
CSF (Pos)							-0.15	0.395
Observations	159		159		159		159	
R^2 / R^2 adjusted	0.475 / 0.471		0.476 / 0.465		0.481 / 0.460		0.483 / 0.459	

Standardized β-estimates and p-values from regression models predicting capillary p-tau217 levels. Model 1 includes plasma p-tau217 only; Model 2 additionally adjusts for age and sex; Model 3 additionally adjusts for diagnosis (MCI, AD, non-AD); Model 4 additionally adjusts for CSF Aβ42/P-tau181 status. Plasma p-tau217 remained the only significant predictor across all models. Aβ = Amyloid beta: AD = Alzheimer's disease; CSF = Cerebrospinal fluid; MCI = Mild cognitive impairment; p-tau217 = Phosphorylated tau 217; R^2 = Coefficient of determination; Std. Beta = Standardized beta coefficient

Supplementary Table 3. Assay repeatability and intermediate precision.

	DPS iQCS				Plasma iQCs			
	ID	Mean (pg/mL)	Intra-assay CV (%)	Inter-assay CV (%)	ID	Mean (pg/mL)	Intra-assay CV (%)	Inter-assay CV (%)
p-tau217 low	iQC1	0.022	7.3	13.1	iQC1 iQC2 iQC3 iQC4	0.6 0.4 0.4 0.1	5.7 5.2 4 6.8	7.7 10.3 12.8 7.2
p-tau217 high	iQC1	0.087	14.8	17.3	iQC1 iQC2 iQC3	2.0 1.8 0.6	2.8 5.3 2.3	7.5 11.8 14.1
GFAP low	iQC1	11.6	7.7	22.2	iQC1 iQC2 iQC3	96.2 82.7 119.3	6.0 5.7 7.8	11.5 9.7 7.8
GFAP high	iQC1	64.9	3.0	16.8	iQC1 iQC2 iQC3 iQC4	279.5 680.8 378.1 630.2	5.8 4.3 5.6 5.0	8.8 11.2 11.0 5.0
NfL low	iQC1	2.7	10.7	11.9	iQC1 iQC2 iQC3	16.7 14.6 15.8	5.8 4.3 9.7	10.0 7.5 9.7
NfL high	iQC1	11.3	8.4	13.6	iQC1 iQC2 iQC3 iQC4	87.5 96.3 184.0 92.5	5.9 5.6 6.1 6.8	9.3 7.9 14.5 7.9

For each analyte, two plasma and DPS iQC levels (high and low) were run in duplicates in the beginning and end of each plate. Reported is the analyte-specific mean of low and high iQC samples and the respective intra- and assay-CV. During the evolution of the DROP-AD project, up to four different iQC levels have been used for quality control purposes. CV = coefficient of variance; DPS = Dried plasma spots; GFAP = Glial fibrillary acidic protein; iQC = In-house quality controls; NfL = Neurofilament light; p-tau217 = Phosphorylated tau 217