INNOTEST β-AMYLOID$_{(1-40)}$

IVD CE 0459

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Symbols used

	Manufacturer
IVD	*In vitro* diagnostic medical device
LOT	Batch code
REF	Catalogue number
	Use-by date
	Consult instructions for use
	Temperature limit
	Contains sufficient for <n> tests
EIA	Enzyme immunoassay
MT PLATE	Microtiter plate
CONJ 1 100x	Conjugate 1 100x
CONJ DIL 1	Conjugate diluent 1
CONJ 2 100x	Conjugate 2 100x

CONJ DIL 2	Conjugate diluent 2
SAMP DIL	Sample diluent
STOP SOLN	Stop solution
SUBS TMB 100x	Substrate TMB 100x
SUBS BUF	Substrate buffer
WASH SOLN 25x	Wash solution 25x
WARNING	Warning

Intended purpose

The INNOTEST β-AMYLOID$_{(1-40)}$ is a solid-phase enzyme immunoassay for the quantitative determination of β-amyloid$_{1-40}$ in human cerebrospinal fluid (CSF) in the clinical setting of dementia diagnosis. This assay is designed to detect the amyloid peptide called Aβ$_{1-40}$ resulting from the β-secretase pathway of APP (amyloid precursor protein) maturation and starting at the 1st amino acid (AA) of N-terminal extremity and finishing at position 40. This peptide, hydrophobic and neurotoxic, is a component of amyloid deposits in the brain. This manual assay is intended to be used in conjunction with INNOTEST β-AMYLOID$_{(1-42)}$ assay to determine the amyloid ratio (β-amyloid$_{1-42}$/β-amyloid$_{1-40}$ ratio) in the clinical setting of dementia diagnosis. This CSF amyloid ratio (β-amyloid$_{1-42}$/β-amyloid$_{1-40}$ ratio) is intended to be used as an aid for diagnosis in patients with cognitive impairment who are being evaluated for Alzheimer's disease (AD) and other causes of cognitive decline. The CSF amyloid ratio supports the probability of AD pathology only as an adjunct to other diagnostic evaluations.

Intended user

Laboratory technicians or researchers in clinical or research laboratories with appropriate laboratory skills and well-trained in enzyme immunoassay techniques.

Background

The most common form of dementia is Alzheimer’s disease, which is a neurodegenerative disorder histologically characterized by the accumulation of intracellular neurofibrillary tangles and extracellular amyloid plaques throughout the cortical and limbic brain regions. The ultrastructure of neurofibrillary tangles is made up of paired helical filaments composed mainly of abnormally hyperphosphorylated Tau protein (pTau). The major components of the amyloid deposits are the 40- and 42-amino acid-long β-amyloid peptides, which are derived from integral membrane-bound amyloid precursor protein(1). Values for the β-amyloid$_{1-42}$ and β-amyloid$_{1-40}$ can be expressed as a β-amyloid ratio, which is of particular use in patients in which an indeterminate CSF biomarker profile (based on β-amyloid$_{1-42}$, total Tau and pTau 181) is observed (2, 3, 4).

The combination of decreased concentrations of β-amyloid$_{1-42}$ or the β-amyloid$_{1-42}$/β-amyloid$_{1-40}$ ratio and increased concentrations of total Tau and pTau are considered to be a pathological CSF biomarker signature that is diagnostic for AD(5). Low concentrations of β-amyloid$_{1-42}$ or the β-amyloid$_{1-42}$/β-amyloid$_{1-40}$ ratio in CSF have been shown to predict the transition of mild cognitive impairment to AD and parallel brain Aβ deposition (6).

Test principle

The INNOTEST β-AMYLOID$_{(1-40)}$ is a solid-phase enzyme immunoassay in which the amyloid peptide is captured by a first monoclonal antibody, 2G3 (IgG1).

Human CSF samples, calibrators (CAL) and Run Validation Controls (RVC) are added and incubated with a biotinylated antibody, 3D6 (IgG2b). This antigen-antibody complex is then detected by a peroxidase-labeled streptavidin. After addition of substrate working solution, samples, CAL and RVC will develop a color. The color intensity is a measure for the amount of human β-amyloid$_{1-40}$ protein in the sample, CAL or RVC.

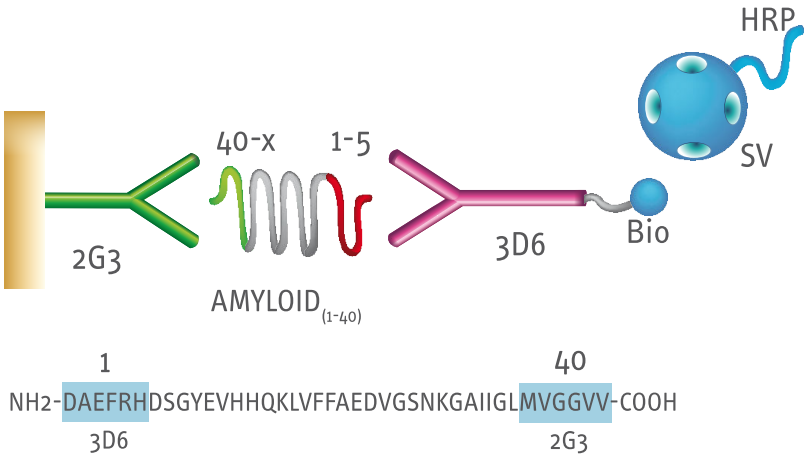

Figure 1: Schematic overview of the test principle of the INNOTEST β-AMYLOID$_{(1-40)}$ and sequence of the β-amyloid$_{1-40}$ peptide with indication of the epitopes reacting with the mAbs used (SV = streptavidin; HRP = horseradish peroxidase).

Reagents

Description, preparation for use, and recommended storage conditions

- If kept at 2°C to 8°C, opened or unopened, and stored in the original vials, the reagents are stable until the expiry date of the kit. Do not freeze reagents. Do not use the reagents beyond the expiry date. When correctly handled in-use the product's expiration date can be maintained.
- After opening the aluminum foil bag containing the strips, any unused test strips will be stable for 16 weeks if stored at 2°C to 8°C in the closed plastic minigrip bag with the silicagel.
- The ready-to-use CAL and RVC need to be stored at -20°C or lower upon arrival. The CAL and RVC are packed separately from the assay kit.
- Diluted wash solution is stable for 4 weeks if stored at 2°C to 8°C.
- Conjugate working solution 1 and 2 are stable at room temperature for 24 hours.

- Substrate working solution is stable at room temperature for 24 hours if kept in the dark.
- All reagents and the test strips should be brought to room temperature (18°C to 30°C) approximately 60 minutes before use and should be returned to the refrigerator (2°C to 8°C) immediately after use, except for the CAL and RVC as they are only for single use.
- Alterations in the physical appearance of kit components may indicate instability or deterioration.

Reagents supplied

Component	Quantity	Ref.	Description
Microtiter plate	1x 96	61338	1 sealed bag containing a strip holder with 12 x 8 coated test wells and a silicagel bag as desiccant.
Sample diluent	1x 30 mL	55706	Phosphate buffer with stabilizing proteins and 0.01% MIT/<0.1% CAA as preservative.
Conjugate 1 100x	1x 0.2 mL	61343	Mouse anti-β-amyloid$_{(1-40)}$ IgG labeled with biotin in phosphate buffer with stabilizing proteins and 0.04% MIT/<0.1% CAA as preservative, to be diluted 100x with conjugate diluent 1 before use.
Conjugate 2 100x	1x 0.3 mL	55528	Peroxidase-labeled streptavidin containing 0.02% MIT and 0.02% 5-bromo-5-nitro-1,3-dioxane as preservative, to be diluted 1/100 in conjugate diluent 2 before use.
Conjugate diluent 1	1x 30 mL	61305	Color-coded (red) phosphate buffer with stabilizing proteins and 0.01% MIT/<0.1% CAA as preservative, used to dilute conjugate 1.
Conjugate diluent 2	1x 20 mL	55825	Color-coded (green) phosphate buffer containing 0.05% Proclin 300 as preservative, bovine casein as stabilizer and bovine aprotinin as protease inhibitor, used to dilute conjugate 2.
Substrate TMB 100x	1x 0.3 mL	55733	Tetramethyl benzidine (TMB) dissolved in dimethyl sulfoxide (DMSO). Dilute 100x in substrate buffer before use. Concentrated TMB can crystallise and should therefore be melted completely before use (melting point 18°C).
Substrate buffer	1x 30 mL	55727	Phosphate-citrate buffer containing 0.02% hydrogen peroxide, used to dilute the substrate TMB.
Stop solution	1x 30 mL	55523	0.9 N sulfuric acid.

Component	Quantity	Ref.	Description
Wash solution 25x	1x 60 mL	55742	Phosphate buffer containing 0.01% MIT/<0.1% CAA, to be diluted 1/25 with distilled or deionized water before use. Salt crystals may be formed in the concentrated wash solution after storage at 2°C to 8°C. All crystals should be dissolved before use.
Plate sealers	2x 4	-	-
Minigrip bag	1	-	For storage of unused strips.

In separate box because of different storage condition (-20°C or lower):

Aβ$_{(1-40)}$ CAL-RVC pack REF 80461 (to be used with corresponding kit lot number).

A set of calibrators with a targeted concentration range of 7.8 - 1000 pg/mL is provided.

Aβ$_{(1-40)}$ Calibrators	8x 2 vials (0.4 mL)	-	8 Calibrators (CAL) x2 vials, containing analyte spiked in diluent. Use and discard.
Aβ$_{(1-40)}$ Run Validation Controls	2x 2 vials (0.4 mL)	-	2 Run Validation Controls (RVC) x2 vials, containing analyte spiked in diluent. Use and discard.

Materials required but not provided

- Distilled or deionized water.
- Disposable gloves.
- Adjustable pipettes with disposable tips capable of delivering 10 µL, 20 - 200 µL, and 200 - 1000 µL, respectively.
- A calibrated multichannel pipette to deliver 25 µL, 75 µL, 100 µL and 200 µL is recommended for addition of samples, conjugate working solution 1 and 2, substrate working solution, and stop solution.
- Graduated cylinder for dilution of wash solution (250 mL, 500 mL, 1000 mL or 2000 mL).
- Vortex mixer or equivalent.
- Centrifuge.
- Microplate washer; alternatively, washing can be performed by using a repetitive pipette delivering 400 µL volumes and a vacuum aspirating device which contains 5% sodium hypochlorite solution).
- Timer.
- Absorbent tissues.
- Microplate reader with 450 ± 5 nm filter; optionally, with 620 nm or 690 nm filter for dual wavelength analysis, and with a linear absorbency range of 0 to 3000 or higher.
- Disposable vials for preparation of working solutions (polypropylene BD Falcon tubes recommended).
- Polypropylene tubes for preparation of sample dilutions.

- Appropriate biohazard waste containers for potentially contaminated materials.
- Microplate shaker (1000 rpm); alternatively, mixing can be performed by tapping the side of the plate.
- Incubator at 25 ± 2°C.

Metrological traceability of assigned values

Calibration of the INNOTEST β-AMYLOID$_{(1-40)}$ is traceable to inhouse reference calibrators.

Warnings and precautions

Health, safety and environmental information

Please refer to the safety data sheet (SDS) and product labeling for information on potentially hazardous components. The most recent SDS version is available on the website www.fujirebio.com.

Warning

CONJ DIL 2 | CONJ 2 100x

Contains reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H-isothiazol-3-one [EC no. 220-239-6] (3:1)
H317 P261 P280 P302+P352 P333+P313 P362+P364

SUBS | TMB | 100x

Contains dimethyl sulfoxide
H315 H319 H335 P261 P280 P302+P352 P305+P351+P338 P312 P362+P364

WASH SOLN 25x | SAMP DIL | CONJ 1 100x | CONJ DIL 1 EUH208 EUH210

STOP SOLN EUH210

Hazard statements

H315	Causes skin irritation.
H317	May cause an allergic skin reaction.
H319	Causes serious eye irritation.
H335	May cause respiratory irritation.
EUH208	Contains 2-chloracetamide. May produce an allergic reaction.
EUH210	Safety data sheet available on request.

Precautionary statements

P261	Avoid breathing mist/vapors/spray.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P302+P352	IF ON SKIN: Wash with plenty of soap and water.

P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P312	Call a POISON CENTER/doctor if you feel unwell.
P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
P362+P364	Take off contaminated clothing and wash it before reuse.

- Only adequately trained personnel should be permitted to perform the test procedure.
- Specimens should always be handled as potentially infectious. Therefore, all biological materials and any other contaminated disposable materials or reagents should be considered as being potentially infectious and should be handled as such. All (potentially) infectious materials should be treated and disposed of in accordance with established safety procedures and applicable regulations.

NOTE: Special precautions for Transmissible Spongiform Encephalopathy (TSE)/Prion contaminated materials:

- Inactivation of samples

  Clinical samples, e.g. CSF, should be autoclaved for at least 18 minutes at 134°C or immersed in a solution of sodium hypochlorite resulting in 20000 ppm free chlorine for 1 hour before final disposal as routine hospital waste (e.g. by incineration or autoclavation).

  CAUTION: Neutralize liquid waste that contains acid before adding sodium hypochlorite.
- Waste disposal

  All material classified as clinical waste should be disposed of by incineration at an authorized incineration site. For the safe handling of clinical waste, use secure leak-proof containers, e.g. double bagging, where appropriate. Avoid external contamination of the container.
- Reference:
  - Advisory Committee on Dangerous Pathogens (UK) - Spongiform Encephalopathy Advisory Committee - Transmissible Spongiform Encephalopathy Agents: Safe Working and the Prevention of Infection
  - World Health Organization (WHO): WHO Infection Control Guidelines for Transmissible Spongiform Encephalopathies.
- Use of personal protective equipment is necessary: wear lab coat, gloves and safety spectacles when manipulating dangerous or infectious agents.
- Dispose of all used or residual reagents and other contaminated disposal materials according to the institution's waste disposal guidelines. It is the responsibility of each laboratory to handle solid and liquid waste according to their nature and degree of hazardousness and to treat and dispose of them in accordance with any applicable (inter)national and local regulations.

- Please contact Fujirebio Europe N.V. and the regulatory authority of the relevant country within 24h in case a serious incident has occurred.

Analytical precautions

- The following reagents are generic (same composition) between all INNOTEST β-AMYLOID and TAU assays: sample diluent, wash solution, substrate, substrate buffer, and stop solution. To ensure a correct traceability of used reagents, it is recommended to use a set of these generic reagents from the same box and to record the batch number for each reagent used.
- Do not mix reagents from different kits unless the components have identical lot numbers.
- Do not reuse disposable lab material.
- Pipette tips with cotton plugs are recommended. Use only disposable lab materials.
- Use a new pipette tip for each specimen.
- All vessels used to prepare conjugate and substrate working solutions should be thoroughly cleaned and rinsed with distilled water.
- Hold the ELISA plates at the sides to avoid contamination of the wells.
- Avoid microbial contamination of reagents.
- To ensure that samples, RVC, and CAL are homogeneous, vortex them for 10 seconds before use.
- Ensure that specimen is added to the microwell.
- Remove any air bubbles by tapping the microtiter plate gently or by mixing on a plate shaker for 1 minute at 1000 rpm.
- Do not expose substrate working solution to strong light during incubation or storage. Substrate working solution must be colorless when used. If not colorless, new substrate working solution must be prepared.
- Stop solution, substrate working solution, or conjugate working solutions should not come into contact with metals or metal ions to avoid unwanted color formation.
- If the wells cannot be filled with conjugate or substrate working solution immediately after washing, place the strips upside down on an absorbent tissue soaked in wash solution for no longer than 15 minutes.
- Do not use blood collection tubes for the preparation of the reagent working solutions.
- If a sample has a concentration above the ULOQ, a new dilution (e.g. 1/200 or 1/400) should be prepared starting from the neat CSF sample, do not further dilute the initial 1/100 sample dilution.

Specimen collection, handling, and storage

Proper CSF sample handling is very important to obtain accurate results (7). Use only **polypropylene** tubes for collection, storage and treatment of the samples. It is recommended to standardize all steps of the sample handling procedure to obtain reproducible results. Use with post-mortem CSF, ventricular CSF, blood samples (serum, plasma), brain tissue, or cell culture supernatants, might give erroneous results.

CSF collection (8)

- The lumbar puncture should be executed preferably in the morning.
- The lumbar puncture should be performed at level L3/L4 or L4/L5.
- Discard the 20 first drops of CSF, then collect CSF in a **polypropylene** collection tube. (9, 10)
- Avoid too much empty space in the tube.
- Processing of the samples should be done preferably within 4 hours after lumbar puncture.

CSF handling (11, 12)

- Exclude hemorrhagic CSF samples.
- Centrifuge CSF 10 minutes at ± 2000 *g* at controlled room temperature to exclude cells and other insoluble material.
- Aliquot the CSF into polypropylene tubes.
- Avoid too much empty space in the tube (13) e.g. preferably dispense 400 µL CSF in a 500 µL tube.
- Handle the aliquots in line with shipment conditions.

Shipment to distant testing laboratory (12, 14)

- **Not frozen**: ship the samples within 48 hours at ambient temperature.
- **Frozen**: ship the samples on dry ice; avoid thawing of samples.

Storage of CSF aliquots

- In case of fresh samples, store no more than 48 hours at ambient temperature until testing.
- Preferably store the samples at -80°C until testing (15).
- Samples can be stored at -20°C if tested within 2 months.
- Avoid thawing of the samples; repeated freeze/thaw cycles will result in incorrect concentrations (11, 12, 13, 14, 15, 16).

Directions for washing

NOTE: Please follow the guidance on waste handling described in section "Health, safety and environmental information".

For automatic washing, a proposed protocol is as follows:

Pre-rinse the washer with the diluted wash solution.

Perform 5 wash cycles ensuring that:

- the fill volume is 400 µL/well.
- the dispensing height is set to completely fill the well.
- the time taken to complete one aspiration/wash/soak cycle is approximately 30 seconds.
- after the washing procedure, the plate is inverted and tapped dry on absorbent tissue.

Manual wash can be performed as follows:

1. Remove the plate sealer.
2. Discard the solution, invert the plate on an absorbent tissue and tap dry.
3. Dispense 400 µL washing solution into each well, soak approximately 30 seconds.

4. Discard the solution, invert the plate on an absorbent tissue and tap dry.
5. Repeat steps 3) and 4) 4 times.

Incomplete washing will adversely affect the test outcome. Contamination of wash solution and washer can cause extensive problems. In case problems occur, disinfect the wash bottles and washer overnight with an appropriate disinfectant solution and rinse with water.

Preparation of the reagents

Preparation of conjugate working solution 1 and 2 and substrate working solution

# wells		8	16	32	64	96
CONJ 1	µL	10	10	15	20	40
CONJ DIL 1	mL	1	1	1.5	2	4
CONJ 2	µL	20	30	40	80	120
CONJ DIL 2	mL	2	3	4	8	12
SUBS	µL	20	30	40	80	120
SUBS BUF	mL	2	3	4	8	12

Preparation of diluted wash solution

Prepare at least 40 mL of diluted wash solution for each test strip (8 wells).

# wells		8	16	32	64	96
WASH SOLN 25x	mL	5	10	20	40	60
H_2O	mL	120	240	480	960	1440

Test procedure

Please read “Analytical precautions” before performing the test.

NOTE:

- Determine the total number of wells required, considering that for each test run, duplicate wells of the ready-to-use CAL (n = 8 or n = 6), 2 RVC, and CSF samples should be foreseen. In case of a 6-point CAL curve, exclude CAL1 and CAL3 from the plate set-up.
- Bring the aluminum foil bag containing the strips and all reagents (except the wash solution) to room temperature (18°C to 30°C) and put the wash solution in a warm water bath or incubator at 30°C to 40°C, approximately 60 minutes before use. To avoid water condensation into the wells, the aluminum foil bag must be kept closed until the strips have reached room temperature. Return all reagents (including the unused strips) to the refrigerator immediately after use.
- Allow CAL, RVC, and CSF samples to reach room temperature (18°C to 30°C) approximately 60 minutes before use.
- Make sure that all reagents, CAL, RVC, and CSF samples are ready before starting the assay. Once the test has started, it must be performed without any interruption in order to achieve the most reliable and consistent results. CSF samples, RVC, and CAL must be vortexed 10 seconds before testing.

1. Take the stripholder with the required number of strips. Place any unused strips in the plastic minigrip bag with the silicagel desiccant.
2. Prepare conjugate working solution 1 according to the preparations for use (see "Preparation of the reagents").
3. Dilute CSF samples 100x in sample diluent and mix properly. Only use polypropylene tubes. CAL and RVC are ready-to-use.
4. Add 25 µL of conjugate working solution 1 to each well of the antibody-coated plate. Add 75 µL of each diluted sample/CAL/RVC to duplicate wells of the antibody-coated plate.

   NOTE: Vortex each sample, RVC, and CAL again for 3 seconds exactly before pipetting them into the plate.
5. Make sure that the CAL, RVC, and CSF samples are adequately mixed by carefully tapping the stripholder or by shaking 1 minute at 1000 rpm. Cover the strips with an adhesive sealer. Incubate overnight 14 to 18 hours in the fridge at 2° to 8°C.
6. The next day, bring all reagents needed at day 2, to room temperature.
7. Prepare conjugate working solution 2 according to the preparations for use (see "Preparation of the reagents").
8. Wash each well 5 times (see "Directions for washing").
9. Add 100 µL of conjugate working solution 2 to each well. Cover the strips with a new adhesive sealer and incubate for 30 ± 3 minutes in an incubator at 25 ± 2°C.
10. Prepare substrate working solution just before end of step 9 (see "Preparation of the reagents").
11. Wash each well 5 times (see "Directions for washing").
12. Add 100 µL of substrate working solution to each well. Incubate for 30 ± 3 minutes at 25 ± 2°C in the dark.

13. To stop the reaction, add 50 µL of stop solution to each well in the same sequence and at the same time intervals as the substrate solution. Tap the stripholder carefully to ensure optimal mixing.
14. Read (within 15 minutes after step 13) the absorbance at 450 nm (single wavelength). For dual wavelength analysis 620 nm or 690 nm can be used as the reference wavelength.

NOTE: For automation options, please contact your local distributor.

Quality control

Internal control

Accredited labs should include an internal control in each run.

Please follow the applicable quality standard of your accreditation.

Results

Test run validation

- The absorbance at 450 nm of CAL 8 should be lower than 0.300.
- The absorbance at 450 nm of CAL 1 should be higher than 1.700 (in case of an 8-point CAL curve).
- The absorbance at 450 nm of CAL 2 should be higher than 1.500 (in case of a 6-point CAL curve).
- The RVC should be within the defined concentration range mentioned on the included concentrations label of the Aβ$_{(1-40)}$ CAL-RVC pack.

NOTE:

- Absorbance values for dual wavelength (450 nm and 620 nm or 690 nm) analysis differ about 50 mOD from the single wavelength values. This does not affect the final outcome of the test.
- RVC concentrations on the included concentrations label are determined using a sigmoidal 4 parameter (no weighting) curve fitting.

Calculation of the results

Calculate the mean absorbance for the CAL, RVC, and the tested CSF samples. Repeat the test if the % difference ($\mathbf{((abs (V_1 - V_2)) / ((V_1 + V_2)/2)) * 100}$ with v = value) between individual OD values is more than 20%.

Construct the calibration curve by plotting the mean absorbance values obtained for each of the β-amyloid$_{1-40}$ CAL on the vertical (Y) axis versus the corresponding β-amyloid$_{1-40}$ concentrations on the horizontal (X) axis. Draw the best fitting curve through these points.

NOTE: A sigmoidal 4 parameter (no weighting) curve fitting is recommended. If a blank was included in the run, do not use it in the curve fitting.

Use the mean absorbance value of each tested CSF sample to determine the corresponding concentration of β-amyloid$_{1-40}$ in pg/mL from the calibration curve, taking into account the dilution factor.

The concentration of the samples can only be determined if the absorbance is within the limits of the calibration curve. Extrapolation of results from OD values which are

higher than the highest calibrator or lower than the lowest calibrator can lead to inaccurate results.

Performance characteristics

Analytical performance characteristics

Analytical specificity

Levels of cross-reactivity between -1.3% and +0.6% were achieved in spiking experiments using the peptides Aβ $_{(1-37)}$, Aβ $_{(1-38)}$, Aβ $_{(1-42)}$, and Aβ $_{(1-43)}$ at levels up to 1000 pg/mL.

The following performance characteristics apply to calculations performed with an 8- and 6-point CAL curve, unless otherwise specified.

Analytical sensitivity

The Limit of Detection (LoD) and Limit of Quantification (LoQ) for β-amyloid$_{1-40}$ in this assay were found to be 2.8 pg/mL and 3.4 pg/mL, respectively, as determined following the CLSI protocol EP17-A2 as guidance.

Accuracy

Accuracy determination or demonstration of traceability towards certified reference material is not possible as there is no internationally approved reference standard available.

Precision

Repeatability and intermediate precision (inter-lot, inter-operator and inter-run variability) was evaluated. A total of 3 CSF samples (Low-Mid-High), 3 buffer samples and 2 RVC were tested by 3 operators over different days on 1 lot in triplicate (3 reported values). One operator performed this experiment on a second lot.

The precision of the CSF, buffer- and the RVC samples is presented in Table 1 (8-point CAL curve) and Table 2 (6-point CAL curve).

Table 1: Overview of precision (%CV) on CSF, buffer samples and RVC samples using a 8-point CAL curve

Overview Repeatability			
RVC	RVC1: RVC2:	**2.8** (0.6 - 5.8) **2.7** (0.4 - 4.7)	Average RVC: 2.8 (0.4 - 5.8)
Buffer Samples	QC1: QC2: QC3:	**3.8** (1.4 - 6.6) **2.7** (0.9 - 4.6) **3.0** (1.0 - 6.2)	Average buffer samples: 3.2 (0.9 - 6.6)
CSF	CSF-L: CSF-M: CSF-H:	**2.4** (0.1 - 5.8) **3.3** (0.8 - 6.3) **6.0** (0.7 - 14.7)	Average CSF: 3.9 (0.1 - 14.7)

Intermediate precision				
	Lot	operator	Run	Total Intermediate

RVC	RVC1	n.a.	3.4	3.0	4.6	Average RVC: 3.9 (3.2 - 4.6)
	RVC2	n.a.	2.3	2.2	3.2	
Buffer Samples	QC1	2.3	4.1	3.2	5.8	Average buffer samples: 4.3 (3.3 - 5.8)
	QC2	0.4	3.1	2.2	3.8	
	QC3	0.03	1.0	3.2	3.3	
CSF	CSF-L	0.2	1.6	2.3	2.8	Average CSF: 5.5 (2.8 - 8.2)
	CSF-M	3.3	2.9	3.0	5.3	
	CSF-H	3.7	5.9	4.3	8.2	

Table 2: Overview of precision (%CV) on CSF, buffer samples and RVC samples using a 6-point CAL curve

Overview Repeatability			
RVC	RVC1:	**2.9** (0.6 - 5.7)	Average RVC: 2.8 (0.4 - 5.7)
	RVC2:	**2.7** (0.4 - 4.6)	
Buffer Samples	QC1:	**3.9** (1.4 - 6.6)	Average buffer samples: 3.2 (0.9 - 6.6)
	QC2:	**2.7** (0.9 - 4.5)	
	QC3:	**3.0** (0.9 - 6.2)	
CSF	CSF-L:	**2.4** (0.1 - 5.8)	Average CSF: 4.0 (0.1 - 15.3)
	CSF-M:	**3.4** (0.8 - 6.5)	
	CSF-H:	**6.2** (0.7 - 15.3)	

Intermediate precision						
		Lot	operator	Run	Total Intermediate	
RVC	RVC1	n.a.	3.9	3.0	5.0	Average RVC: 4.0 (3.0 - 5.0)
	RVC2	n.a.	1.6	2.5	3.0	
Buffer Samples	QC1	1.9	5.1	3.3	6.4	Average buffer samples: 4.6 (3.6 - 6.4)
	QC2	0.5	3.1	2.1	3.8	
	QC3	0.5	0.2	3.5	3.6	
CSF	CSF-L	0.7	0.9	2.7	2.9	Average CSF: 5.8 (2.9 - 9.1)
	CSF-M	2.9	3.6	2.9	5.5	
	CSF-H	3.4	6.8	4.8	9.1	

Proportional linearity

Based on the guidelines in CLSI protocol EP6-A, linearity was evaluated by mixing a high and low concentrated CSF sample in different proportions and testing these with two batches of the INNOTEST β-AMYLOID$_{(1-40)}$. Linearity was shown between 2095 pg/ml and 13480 pg/mL.

Dilutional Linearity

The accuracy of the measurement for diluted samples was assessed by diluting high concentrated samples with sample diluent. Back-calculated concentrations obtained with INNOTEST β-AMYLOID$_{(1-40)}$ were all within 20% variation of the concentration of the undiluted sample, up to 1/600 dilution.

High Dose Hook

There is no observed high dose hook effect in the INNOTEST β-AMYLOID$_{(1-40)}$ test at very high analyte concentration levels (more than ten times the highest calibrator point).

Interfering substances

There is no interference in the assay format for whole blood contamination up to 1%.

No interferences are expected for the following therapeutic agents: Acetaminophen, Acetylsalicylic Acid, Ampicillin, Ascorbic Acid, Caffeine, Chloramphenicol, Digoxin, Hydrochlorothiazide, Metoprolol, Theophylline, Warfarin, Donepezil, Rivastigmine, Galantamine, Memantine, Aripiprazole, and Quetuapine.

Method comparison

An external method comparison study was conducted in a European Neurobiology hospital lab. CSF levels of β-amyloid$_{(1-40)}$ protein were determined in residual CSF stored for research purposes of 148 subjects. Quantification of β-amyloid$_{(1-40)}$ was done using INNOTEST β-AMYLOID$_{(1-40)}$ and Human β-amyloid$_{(1-40)}$ (N) Assay Kit (IBL Japan). Regression parameters are described in the table below.

	Parameter	**Estimate**	**Lower C.I. 95%**	**Upper C.I. 95%**
8-point CAL curve	Intercept	2250	1141	3233
	Slope	0.856	0.775	0.955
	r	0.847	0.794	0.887
6-point CAL curve	Intercept	2033	1063	3073
	Slope	0.882	0.797	0.969
	r	0.853	0.801	0.891

Reference ranges

An external study was conducted with the INNOTEST β-AMYLOID$_{(1-40)}$, in which a biological reference interval was derived from 126 subjects referred to a memory clinic or neurology department under investigation for dementia.

Following references values have been determined (2.5th lower limit to 97.5th upper limit percentile):

6-point CAL curve: 4128 (90%CI:2787-5439) to 22454 (90%CI:21206-23696).

8-point CAL curve: 4347 (90%CI:2971-5596) to 22063 (90%CI:20897-23278).

Note that it is strongly advised that each laboratory assesses the acceptability of the transference of a reference interval obtained in another laboratory; the reference interval provided above should only be considered as guidance values to start the in-house validation.

Clinical performance characteristics

Using a large prospective multicenter cohort of patients with cognitive disorders (160 AD subjects and 207 non-AD subjects), optimum cut-offs for the Aβ42/40 ratio ranged from 0.050 to 0.082, with 0.055 as the mean cut-off, with 73% sensitivity and 78% specificity.

Limitations of the test procedure

The INNOTEST β-AMYLOID$_{(1-40)}$ assay procedure was designed to quantify β-amyloid$_{1-40}$ in human CSF. Insufficient data are available to interpret tests performed on other body fluids or brain tissue samples.

The possible use of the assay for therapy monitoring will largely depend on the type of therapy, which could interfere with the assay design (e.g. antibody treatments procedures).

Trademarks

- **INNOTEST** is a trademark of Fujirebio Europe N.V., registered in US and other countries.
- BD Falcon is a trademark of Corning Incorporated.

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