INNOTEST β-AMYLOID(1-42)

IVD CE

Note changes highlighted

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8:00 – 17:00 GMT+1

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Symbols used

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

Intended use

The INNOTEST β-AMYLOID(1-42) is a solid-phase enzyme immunoassay for the quantitative determination of β-amyloid(1-42) in human cerebrospinal fluid (CSF). This assay is designed to detect the amyloid peptide called Aβ resulting from β-secretase way of APP (amyloid precursor protein) maturation and starting at the 1st amino acid (AA) of N-terminal extremity and finishing at position 42. This peptide, hydrophobic and neurotoxic, is a major component of amyloid deposits.

The combined use of CSF-β-amyloid(1-42) and CSF-TAU marker concentrations allows differentiation between Alzheimer's disease (AD) and normal aging or other neurological diseases such as depression (1, 2). As AD diagnosis marker, the results obtained with this assay should be interpreted in combination with other clinical and diagnostic information (eg, using results obtained with the INNOTEST hTAU Ag and the INNOTEST PHOSPHO-TAU(181P)). The discrimination of AD from non-AD types of dementia such as dementia with Lewy Bodies may further be improved using the quantification of CSF-Phospho-TAU$_{181}$ (3).

Background

AD, the most common form of dementia, 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 (Phospho-TAU). 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 (4).

Test principle

The INNOTEST β-AMYLOID(1-42) is a solid-phase enzyme immunoassay in which the amyloid peptide is captured by a first monoclonal antibody, 21F12 (IgG2a).

CSF samples 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 will develop a color. The color intensity is a measure for the amount of human β-amyloid(1-42) protein in the sample.

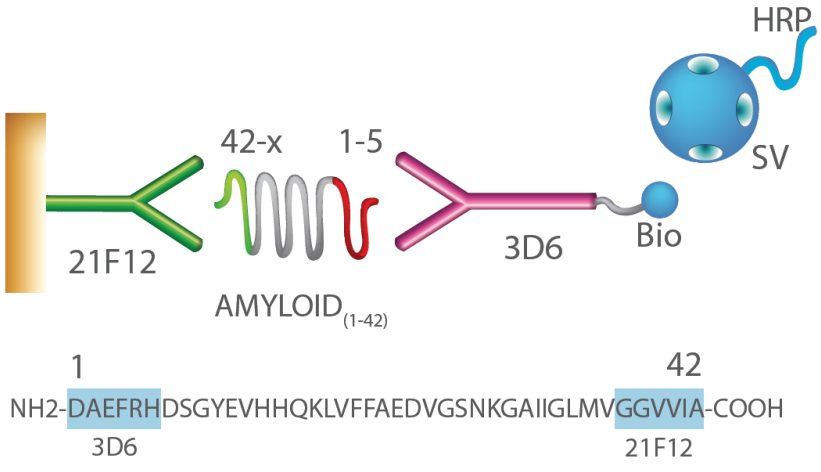

Figure 1 Test principle INNOTEST β-AMYLOID(1-42); sequence of the synthetic peptide included (SV= streptavidin; HRP= horseradish peroxidase)

Materials provided

Reagents supplied:

Component	Quantity	Ref.	Description
Microtiter plate	1x 96	57781	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	57782	Mouse anti-β-amyloid$_{(1-42)}$ 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% bromonitrodioxane as preservative, to be diluted 100x with 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 (see preparation of reagents). 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	0.9N sulfuric acid
Wash solution 25x	1x 60 mL	55742	Phosphate buffer containing 0.01% MIT/<0.1% CAA, to be diluted 25x with distilled or deionized water before use. Prepare at least 40 mL of diluted wash solution for each test strip. Salt crystals may be formed in the concentrated wash solution after storage at 2-8°C. These crystals must be completely redissolved.
Uncoated polypropylene plate	1	-	For dispensing of the samples before transferring to the coated plate to prevent reactivity shift.
Plate sealers	4	-	-
Minigrip bag	1	-	For storage of unused strips.

In separate box because of different storage conditions:
Aβ(1-42) CAL-RVC pack REF 81577 (to be used with corresponding kit lot number).

Aβ(1-42) Calibrators	2x 6 vials (0.2 mL)	12 Calibrators (CAL) vials, containing analyte spiked in diluent. Use and discard.
Aβ(1-42) Run Validation Controls	2x 2 vials (0.2 mL)	4 Run Validation Controls (RVC) vials, containing analyte spiked in diluent. Use and discard.

Storage and stability

- If kept at 2-8°C, and stored in the original vials, the reagents, opened or unopened, are stable until the expiry date of the kit. Do not use the reagents beyond the expiry date.
- After opening the aluminium foil bag containing the strips, any unused test strips will be stable for 16 weeks if stored at 2-8°C in the closed plastic minigrip bag with the silicagel.
- The ready-to-use calibrators (CAL) and Run Validation Controls (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 2-8°C.
- Conjugate working solution 1and 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.

Description of calibrators

A set of calibrators in the targeted concentration range of 62.5-4000 pg/mL is provided.

Materials required but not provided

- Distilled or deionized water
- Calibrated precision pipettes with disposable tips to deliver volumes in the range of 10 - 1000 µL. A calibrated multichannel pipette to deliver 25 µL, 75 µL, and 100 µL is recommended for addition of samples, conjugate working solution 1 and 2, substrate working solution, and stop solution.
- Vortex mixer or equivalent.
- Microplate washer; alternatively, washing can be performed by using a repetitive pipette delivering 400 µL volumes and an aspirating device.
- 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).
- 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

Safety and environment

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-europe.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

Hazard statements

H315	Causes skin irritation.
H317	May cause an allergic skin reaction.
H319	Causes serious eye irritation.
H335	May cause respiratory irritation.

Precautionary statements

P261	Avoid breathing mist/vapours/spray.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P302+P352	IF ON SKIN: Wash with plenty of 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. All biological materials should be considered as being potentially infectious and should be handled as such. All biological materials should be disposed of in accordance with established safety procedures.
  - Autoclave for at least 15 minutes at 121°C.
  - Incinerate disposable material.
  - Mix liquid waste with sodium hypochlorite so that the final concentration is ± 1% sodium hypochlorite. Allow to stand overnight before disposal.
    CAUTION: Neutralize liquid waste that contains acid before adding sodium hypochlorite.

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

- **Inactivation of samples**
  Clinical samples, e.g. CSF, should be autoclaved or immersed in a solution of sodium hypochlorite resulting in 20000 ppm free chlorine for 1 hour before final disposal by incineration.
- **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.

See section “Reagents supplied”, for information about hazardous substances in the mixtures.

Use of personal protective equipment is necessary: gloves and safety spectacles when manipulating dangerous or infectious agents.

Waste should be handled according to the institution's waste disposal guidelines. All federal, state, and local environmental regulations should also be observed.

Analytical precautions

- The following reagents are generic (same composition) between all INNOTEST Abeta(s) and TAU(s) 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 component used.
- All vessels used to prepare conjugate and substrate working solutions must be thoroughly cleaned to avoid contamination.
- Hold the ELISA plates to 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.
- Use a new pipette tip for each specimen.
- Ensure that specimen is added to the microwell.
- Remove any air bubbles present by tapping the microtiter plate gently or by mixing on 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.

Specimen collection, handling and storage (14)

Proper sample handling is very important to obtain accurate results. Use only **polypropylene** tubes for collection, storage and treatment of the samples (5). It is recommended to standardize all steps of the sample handling procedure to obtain reproducible results. The assay is not validated for testing post-mortem CSF, ventricular CSF, blood samples (serum, plasma), brain tissue or cell culture supernatants.

CSF collection (6, 7, 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.
Avoid too much empty space in the tube.
Pre-treatment of the samples should be done preferably within 4 hours after lumbar puncture.

CSF handling (9)
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 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
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
Preferably store the samples at -80°C until testing.
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 (10).

Directions for washing

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	15	30	60	100
CONJ DIL 1	mL	1	1.5	3	6	10
CONJ 2	µL	12	20	40	80	120
CONJ DIL 2	mL	1.2	2	4	8	12
SUBS	µL	12	20	40	80	120
SUBS BUF	mL	1.2	2	4	8	12

Preparation of diluted wash solution

Prepare at least 40 mL of diluted wash solution for each test strip.

	# 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 6 ready-to-use calibrators, 2 Run Validation Controls, and CSF samples should be foreseen.
- Since sample incubation takes 60 minutes, the dispensing time should be reduced to a minimum. In order to reduce a reactivity shift, an uncoated polypropylene microplate has been added to the kit as a “waiting station”. We advise the use of this preparation plate when **more than 6 strips** need to be used. Dispense ≥ 60 µL of sample/CAL/RVC into the wells of the preparation plate.
- Bring the aluminium foil bag containing the strips and all reagents (except the wash solution) to room temperature (18-30°C) and put the wash solution in a warm wather bath or incubator at 30-40°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 (inclusive the strips) to the refrigerator immediately after use.
- Allow CAL, RVC, and CSF samples to reach room temperature (18-30°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 strip-holder with the required number of strips. Place any unused strip in the plastic minigrip bag with the silicagel desiccant.
2. Prepare **conjugate working solution 1** according to the preparations for use.
3. Add **75 µL conjugate working solution 1** to each well of the antibody-coated plate.
4. a) *In case a limited number of samples needs to be tested:* add 25 µL of each sample/CAL/RVC to duplicate wells of the antibody-coated plate.
   b) *In case a larger number of samples needs to be tested (more than 6 strips):* transfer 25 µL from each well of the polypropylene plate to duplicate wells on the antibody-coated plate, using a multichannel pipette.

   RECOMMENDATION**:** Vortex each sample, RVC, and CAL again for 3 seconds exactly before pipetting them into the plate.
5. Make sure that the calibrators, Run Validation Controls, 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** 60 ± 3 minutes in an incubator at 25 ± 2°C.
6. Prepare **conjugate working solution 2** – according to the preparation for use - just before the end of step 5.
7. **Wash** each well **5 times** (see “Directions for washing”).
8. 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.
9. Prepare **substrate working solution** just before the end of step 8.
10. **Wash** each well **5 times** (see “Directions for washing”).
11. Add **100 µL** of **substrate working solution** to each well. **Incubate** for 30 ± 3 minutes at 25 ± 2°C in the dark.
12. 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.
13. **Read** (within 15 minutes after step 12) the absorbance at 450 nm (single wavelength). For dual wavelength analysis 620 nm or 690nm can be used as the reference wavelength.

Control procedure

Internal control

Accredited labs should include an internal control in each run.
Please follow the applicable quality standard of your accreditation.

Results

Validation

- The absorbance at 450 nm of CAL 6 should be lower than 0.300.
- The absorbance at 450 nm of CAL 1 should be higher than 1.700.
- The RVC should be within the defined concentration range mentioned on the inserted label of the Aβ(1-42) 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 inserted label are determined using a sigmoidal 4 parameter (no weighting) curve fitting.

Calculation of results

Calculate the mean absorbance for the CAL, RVC, and the unknown CSF samples. Repeat the test if the % difference (**((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$_{(1-42)}$ calibrators on the vertical (Y) axis versus the corresponding β-amyloid$_{(1-42)}$ 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 unknown CSF sample to determine the corresponding concentration of β-amyloid$_{(1-42)}$ in pg/mL from the calibration curve.

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 incorrect results.

Performance characteristics

Analytical performance characteristics

Selectivity and specificity

Epitope mapping of the N-terminal–specific mAb 3D6 against the Aβ_{42} peptide was done with sandwich immunoassays using biotinylated 21F12 as detection antibody. No antigen reactivity was found when the 3D6 antibody was incubated with Aβ peptides blocked at the N-terminus by acetylations or with Aβ peptides having the mouse sequence (modified at positions 5, 10, and 13). 3D6 does not recognize soluble amyloid precursor protein or full length β-APP (10).

Antibody specificity of the combined use of 21F12 and 3D6 was further confirmed using different synthetic human Aβ peptides and rat A$\beta_{(1-42)}$. The 21F12/3D6 ELISA is highly specific for amyloid peptides starting at amino acid 1 and ending at the carboxyterminus at amino acid 42 or 43. A 50x higher affinity is obtained for Aβ_{42} as compared to Aβ_{43}. There was no cross reactivity in the assay format with A$\beta_{(1-38)}$, A$\beta_{(1-39)}$, A$\beta_{(1-40)}$, A$\beta_{(2-42)}$, and A$\beta_{(8-42)}$. No antigen reactivity was found when the 3D6 antibody was incubated with Aβ peptides having the mouse sequence (modified at positions 5, 10, and 13).

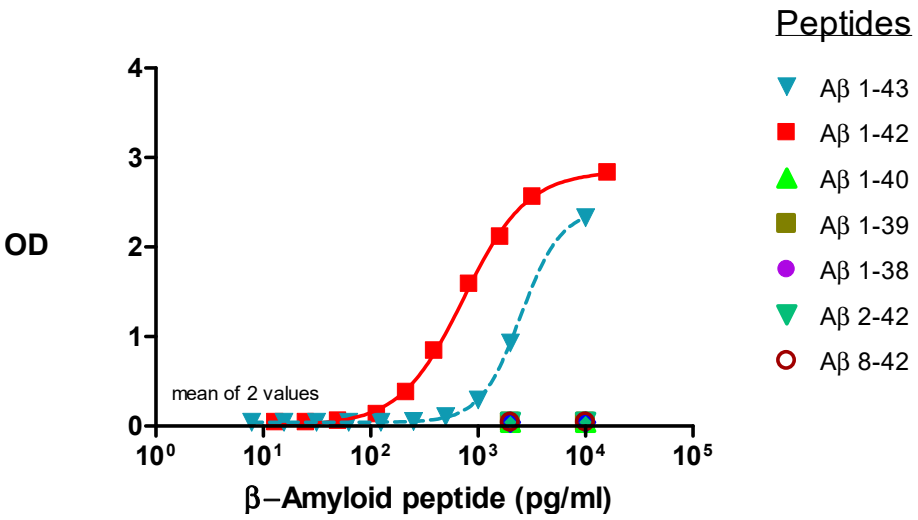

Figure 2 Specificity of the coated plate for detection of different Aβ peptides.

Analytical sensitivity

A blank sample (= sample diluent) was tested in 28-fold over different runs to test the Limit of Detection (LoD). The mean + 2*Standard Deviation (SD) of the blank sample was calculated and subsequently converted, via the inverse sigmoid relation, into a concentration. The obtained LoD was 65 pg/mL.

Assay range

The assay range is determined by the Lower Limit of Quantitation (LLoQ) and the Upper Limit of Quantitation (ULoQ), which is defined as the lower and upper points where the precision and the estimated bias does not exceed 20%.

A total of 15 CSF samples and 2 RVC samples were tested by 2 operators over different days, and in duplicate (2 reported values).

The obtained assay range using this sample set is 225 pg/mL (LLoQ) (i.e. the CSF with the lowest concentration) to 1452 pg/mL (ULoQ) (i.e. the RVC1 sample, sample evaluated with highest concentration).

Accuracy

At present, the accuracy for detection of the AD biomarkers is difficult to investigate since no reference material (internationally recognized gold standard) is currently available. Proteins from different sources can vary in their immunoreactivity due to the production and/or purification processes. In addition, the reference material may not be truly representative for the native protein due to differences in post-translational modifications (e.g. phosphorylation, dimerization, glycosylation, deamidation, isomerization). No standard has currently been deposited with any organization, such as the World Health Organization (WHO) or United States Pharmacopoeia (USP).

Precision

Precision (intra-assay and inter-assay variability) was evaluated. A total of 16 CSF samples, 2 RVC and 6 RTU calibrators were tested by 2 operators over different days, and in duplicate (2 reported values). The precision of the CSF samples and the RVC samples is presented in Table 1.

Table 1: Overview of precision on CSF and RVC samples

	CSF samples (n=15*)	RVC 1 (high concentration)	RVC 2 (low concentration)
Intra-assay variation	4.6% (range 0.8% - 11.0%)	4.7% (range 3.0% - 6.6%)	2.5% (range 1.0% - 4.9%)
Inter-assay variation	7.8% (range 1.4% - 18.0%)	3.8% n.a.	4.3% n.a.

*One sample was excluded because it was a blank sample

The variability on the 6 RTU calibrators is schematically represented in a precision profile (Figure 3). The individual reactivity levels of 40 RTU calibration curves were analyzed towards their average calibration curve. The variability over their back calculated concentrations for each of the calibrators was determined.
The % CV (combined inter- and intra-assay variation) at each level of RTU calibrators does not exceed 15% except for the highest and lowest calibrator point (= anchor points).

	conc. pg/mL	%CV
CAL 1	4317	>25%
CAL 2	1770	8.2
CAL 3	1063	5,3
CAL 4	552	4.9
CAL 5	138	7.8
CAL 6	62.5	21.9

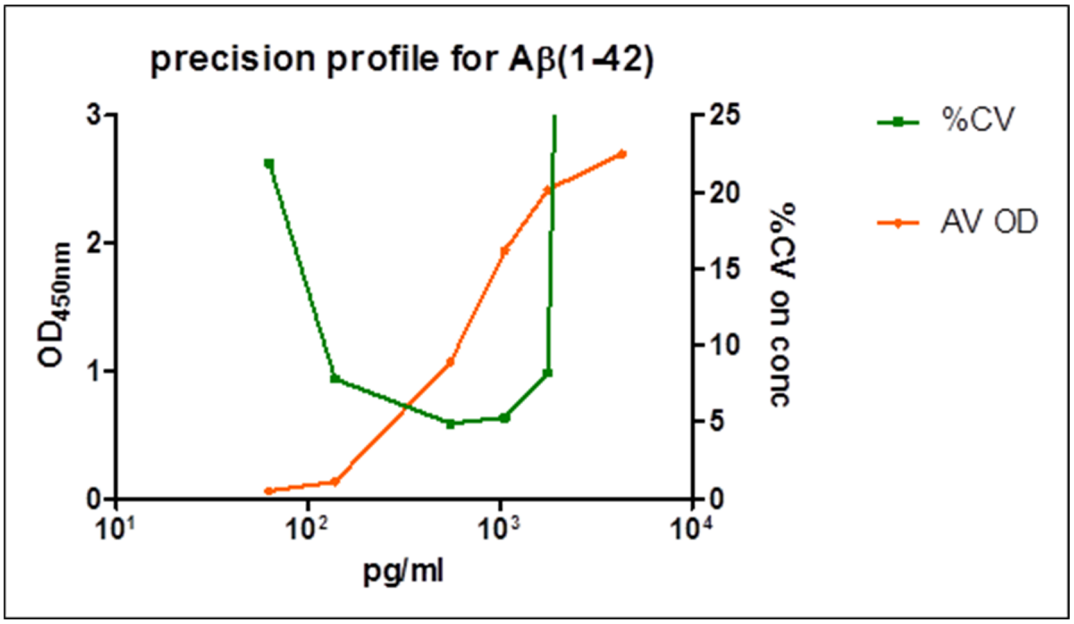

Figure 3: Variability in function of the defined calibrator concentration. Average reactivity levels of the calibrators are plotted against the left Y axis, % CV values are plotted against the right Y axis. CAL1 is not present on the figure (%CV > 25%).

Interfering substances
Experiments revealed that under the experimental conditions used, there is no interference in the assay format for heparin, EDTA, hemoglobin, globulins, vitamin C, albumins or fibrinogens (10).

Biological reference interval

An external study (13) was conducted with the INNOTEST β-AMYLOID$_{(1-42)}$ and INNOTEST hTAU Ag, in which biological reference intervals were derived from 231 neurologically and psychiatrically healthy individuals.

Following references values have been determined (mean ± SD), partitioned according to age:

Age	CSF Aβ$_{(1-42)}$ (pg/mL)
21-51 years	792 ± 182
51-70 years	790 ± 228
>71 years	797 ± 230

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 intervals provided above should only be considered as guidance values to start the in-house validation.

Clinical results

An external validation study was conducted in eight European and two US university centers involved in CSF research. CSF levels of β-amyloid$_{(1-42)}$ and TAU protein were determined in residual CSF stored for research purposes of 150 AD patients (AD), 79 patients with non-AD types of dementia (NAD), 84 patients with other neurological disorders (ND), and 100 healthy volunteers or patients with disorders not associated with pathological conditions of the brain (CON). Quantification of β-amyloid$_{(1-42)}$ was done using INNOTEST β-AMYLOID$_{(1-42)}$, TAU protein concentrations were measured using INNOTEST hTAU Ag.
Median levels of β-amyloid$_{(1-42)}$ were significantly lower in AD (487 pg/mL) than in CON (849 pg/mL; p=0.001), ND (643 pg/mL; p=0.001), and NAD (603 pg/mL; p=0.001). Discrimination of AD from CON and ND was significantly improved by the combined assessment of β-amyloid$_{(1-42)}$ and TAU protein (discrimination line $A\beta_{42}$=240 + 1.18 TAU). At 85% sensitivity, specificity of the combined test was 86% (95% CI [81%, 91%]), compared with 55% (95% CI [47%, 62%]) for β-amyloid$_{(1-42)}$ alone and 65% (95% CI [58%, 72%]) for TAU protein alone. The combined test at 85% sensitivity was 58% (95% CI [47%, 69%]) specific for NAD.

The clinical value of β-amyloid$_{(1-42)}$, TAU protein and CSF-phospho-TAU$_{(181)}$ has been demonstrated in autopsy-confirmed dementia cases, achieving sensitivity, specificity and diagnostic accuracy levels, consistently exceeding 80% (11).

In conclusion, the combined measure of β-amyloid$_{(1-42)}$ and TAU protein concentrations meets the requirements set by the Consensus guidelines for discriminating AD from normal aging and specific neurological disorders such as depression: a useful biomarker should have a sensitivity and a specificity of more than 80% (12).

Limitations of the test procedure

The INNOTEST β-AMYLOID$_{(1-42)}$ assay procedure was designed to quantify β-amyloid$_{(1-42)}$ in human cerebrospinal fluid. Insufficient data are available to interpret tests performed on other body fluids or brain tissue samples. Therefore, testing of such specimens with this test protocol is not recommended.

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 treatment procedures).

Disclaimer

A license for the use of amyloid beta monoclonal antibodies contained in this product under patents US 6114133, US 7811769, and EP 0792458 has been obtained from Eli Lilly and Company.

Trademarks

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