Differential Dementia Diagnostics
ELISA based Diagnosis of Neurodegeneration
ELISA based Diagnosis of Neurodegeneration

These easy-to-handle and ultrasensitive immunoassays provide improved diagnosis for Alzheimer’s, Parkinson’s, and Creutzfeldt-Jakob disease. They use recombinant proteins and rapidly generating monoclonal antibodies unique to a variety of biomarkers.

- **Offers multiparameter analysis:** expanded diagnostic output
- **Certified for in vitro diagnostics:** proven reliability in this application
- **Uses lyophilized standards and controls:** convenient and precise quantitative results
- **Comes with fast, easy handling procedures:** clinically relevant results achieved using daily protocols
- **Makes processing quicker than ever:** easy automation of routine diagnostics with the corresponding platforms
- **Has flexible applications depending on sample throughput:** standardized ELISA format on 12x8 immuno strips
ELISA based Diagnosis of Neurodegeneration
Differential Dementia Diagnostics
Alzheimer’s Disease

The most common form of dementia, Alzheimer’s disease (AD) is a progressive neurodegenerative disorder. AD affects more than 36 million people worldwide and occurs most commonly in Western Europe.

The prevalence of AD may triple by 2050 due to increasing life expectancies. AD is associated with a progressive loss of neuronal functioning and the subsequent gradual deterioration in cognition and behavior, which finally leads to death. Abnormal protein deposits are the hallmark of AD brain pathology.

There are two types of these. Amyloid plaques, made of β-amyloid protein peptides, build up between the brain’s nerve cells. Additionally, neurofibrillary tangles (NFT) accumulate inside brain cells; they are formed by the tau protein. Both biomarkers are generally accepted as early indicators of AD in humans.

With Analytik Jena’s hTAU total, phosphoTAU, and pTAU rel ELISA, the company offers high-quality diagnostics that achieve exceptional results. These assays promise specificity and sensitivity in measuring various tau fractions in the CSF of patients suspected to have Alzheimer’s disease.

The hTAU total ELISA detects all isoforms of tau protein and estimates total tau content. Additionally, the phosphoTAU ELISA identifies phosphorylated tau proteins.

The pTAU rel ELISA utilizes a monoclonal antibody specific to the non-phosphorylated TPP sequences of the tau protein (positions T175 and T181). After binding the antigens in the CSF, a HRP-conjugated secondary antibody specific to the tau protein performs detection using the chromogenic substrate tetramethylbenzidine (TMB). The lyophilized standards and controls included ensure clinically relevant, highly precise, and accurate results. All ELISAs are CE-IVD certified and particularly suitable for automation using the corresponding platforms. Automation allows for convenient routine diagnostics of CSF samples. Analytik Jena recommends using the standardized protocols in conjunction with Amyloid-β CSF ELISAs produced by IBL Internationals (Hamburg, Germany).
hTAU total ELISA

Product specifications

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<th>Target</th>
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Performance assessment

hTAU total ELISA was used to assess the total tau protein in CSF from patients with Alzheimer’s disease (AD), mild cognitive impairments (MCI) and from a control group. The level of total tau was significantly higher in the patient group compared to the control population (p<0.001). This speaks for reasonable sensitivities and specificities.

References

The analyses were performed in the Lab for Clinical Neurochemistry and Neurochemical Dementia Diagnostics, Department of Psychiatry and Psychotherapy, Universitätsklinikum Erlangen, Erlangen, Germany. (Head: Piotr Lewczuk)

Verification of results: significant differences in tau protein concentration in CSF samples from patients with AD and MCI (summarized as AD, n=72) and from the control group (n=41).

Tau total in CSF samples from patients with AD and MCI evaluated for sensitivity and specificity.
phosphoTAU ELISA

- Has CE-IVD certification
- Supports the diagnosis of Alzheimer’s disease through quantifying the phosphorylated tau in human CSF
- Works with a reproducible six-point standard curve using lyophilized standards and controls
- Includes daily protocol option
- Uses antibodies with outstanding specificity, well-characterized binding capabilities, and optimized stability
- Optimized clinical and analytical sensitivity

Product specifications

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<th>Target</th>
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Performance assessment

The phosphoTAU ELISA was applied to analyze the phosphorylated tau protein in CSF samples from patients with Alzheimer’s disease (n = 157) and from a control group (n=104). The level of phosphorylated tau was significantly higher (p<0.001) in the patient group compared to the control population.

The analysis of phosphorylated tau protein from patients with AD and control group performed using phosphoTAU ELISA shows a sensitivity of 82.2 % and specificity of 87.5 %.
pTAU rel ELISA

- Features CE-IVD certification
- Analyzes a novel nonphosphorylated fraction of tau protein in human CSF
- Ensures high diagnostic quality and exceptional specificity and sensitivity
- Acts as an additional measure, a companion diagnostic, that further supports the diagnosis of Alzheimer’s disease
- Has flexible applications depending on sample throughput: standardized ELISA format on 12x8 immuno strips
- Promises fast and easy handling: obtain clinically relevant results within 3.5 hours

Product specifications

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<td>Non-phosphorylated tau fraction</td>
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<td>12 months at 4 °C</td>
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Performance assessment

The relevant parameters that currently define Alzheimer’s disease are β-amyloid 1-42, total tau, and phospho-tau (P-tau181). The novel pTAU rel ELISA functions an alternative to P-tau181. The pTAU rel ELISA was used to determine the non-phosphorylated tau fraction in CSF from patients with Alzheimer’s disease (AD) and mild cognitive impairments (MCI) and from a control group. The level of non-phosphorylated tau fraction was significantly higher in the patient group compared to the control population (p<0.001).

References


Controls

Verification of results: significant differences in tau protein concentration in CSF samples from patients with AD and MCI (summarized as AD, n=58) and from control group (n=42)

Non-phosphorylated tau fraction in CSF samples from patients with AD and MCI evaluated for sensitivity and specificity
TAU aggregate ELISA

- Uses an ELISA that is unique around the world for analyzing aggregated tau (e.g., in human and mouse brain tissue)
- Works with a six-point standard curve using lyophilized standards and controls
- Handles easily using an overnight procedure
- Promises optimal storage stability of antibodies produced in house: allows for preservation of specific and exceptionally well-characterized binding capabilities
- Has flexible applications depending on sample throughput: standardized ELISA format on 12x8 immuno strips

Product specifications

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<tr>
<td></td>
<td>Biological samples from animal and cell models</td>
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Performance assessment

Tau protein is mainly expressed in neurons, where it binds and stabilizes microtubules. In Alzheimer's disease tau protein with low binding affinity to microtubule changes helical structure to β-sheets and forms aggregates as oligomers and filaments. Evidence of such aggregates related to Alzheimer's disease is detected in nerve tissue compared to control patients.

References

Kindly performed by Max Holzer, Paul-Flechsig-Institute in Leipzig, Germany.

Verification of results: significant differences in the concentration of tau aggregates as measured by the TAU aggregate ELISA. Samples examined were nerve tissue from patients with Alzheimer’s disease and from a neurological control group.

The TAU aggregate ELISA was used to find the development of tau aggregation during aging in P301L mice as an animal model for Alzheimer’s disease.
Parkinson’s Disease

The hallmark of Parkinson’s disease is the presence of Lewy bodies in subcortical brain regions. Lewy bodies are deposits made of aggregated and phosphorylated α-synuclein.

Researchers view the pathologically relevant formations of α-synuclein as promising surrogate markers for Parkinson’s disease and dementia with Lewy bodies. Running a detection procedure for it in bodily fluids (CSF and plasma) provides supportive testing for accompanying diagnostics.

Due to the frequent co-occurrence of Lewy body pathology with Alzheimer’s disease (AD) pathology, it is crucial to differentiate it from pure AD forms. To do so, it’s best to make use of it in early clinical diagnosis and not only to use it for autopsies. This way, the patient can still potentially benefit from the results. Via the hSYN total ELISA, Analytik Jena provides an improved ELISA for detection of total human α-synuclein.

The HUMAN α-Synuclein PATHO ELISA detects its pathological relevant aggregates. Initial clinical trials have provided evidence that the combined detection of total α-synuclein and disease-specific α-synuclein makes up a promising diagnostic tool for Parkinson’s disease (Unterberger et al. 2014). The high specificity of the HUMAN α-Synuclein PATHO ELISA comes from the unique characteristics of anti-human α-synuclein 5G4, a monoclonal antibody that has set new standards in diagnosing conditions such as Parkinson’s disease (Kovacs et al. 2012 and 2014). These novel assays have an impressively easy and fast handling procedure. They also help in differentiation of patients with neurodegenerative diseases by quantification of level of α-synuclein as well as its pathological related molecular forms detected by 5G4 antibody, due to the extremely stable standards and controls that have been implemented. No matter which sample input material is used, results are guaranteed.
Anti-human α-Synuclein 5G4, MONOCLONAL ANTIBODY

- 5G4, worldwide unique antibody that clearly recognizes β-sheet-dependent epitopes by binding to pathological relevant α-synuclein structures in neuropathology of Lewy body/Parkinson's disease
- Promises optimal storage stability: allows for reservation of specific and exceptionally well-characterized binding capabilities
- Is a highly purified monoclonal antibody (>95 %)

Product specifications

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<th>Clone</th>
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Performance assessment

The anti-human α-synuclein 5G4 shows high specificity for disease-specific forms of α-synuclein. This includes a high molecular weight fraction of β-sheet–rich oligomers. Additionally, no binding has been observed to primarily disordered oligomers or monomers.

References


Light microscopic immunostaining patterns of α-synuclein using antibody 5G4. Tiny dots, thin neurites in the subependymal area, as well as tiny dots between ependymal cells and amorphous plaques.
hSYN total ELISA

- Optimized for total α-synuclein measurement from human CSF (CE-IVD pending)
- Works with a six-point standard curve using lyophilized standards and controls
- Promises optimal storage stability of antibodies produced in house: allows for preservation of specific and exceptionally well-characterized binding capabilities
- Has flexible applications depending on sample throughput: standardized ELISA format on 12x8 immuno strips

Product specifications

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<tr>
<td>Detection time</td>
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</table>

Performance assessment

By quantifying α-synuclein, a presynaptic protein, in human CSF, differentiations can be made between Lewy body pathology and other neurodegenerative pathologies (left). Furthermore, quantification of α-synuclein was performed using an in-house assay based on the Mesoscale platform (UMG) in comparison to the hSYN total ELISA (AJ) to differentiate forms of Lewy body pathologies including DLB, PD and PDD versus a control group (right).

References


Feasibility study: total α-synuclein in the CSF from patients with Lewy body pathology, AD, and from a control group. The level in LBP patients dropped in comparison to AD patients and was significantly lower than in control groups (p=0.016).

Means for α-synuclein concentration with standard deviation of the measurements per group and method (AJ and UMG) is shown. Further statistically comparison showed significant lower means for the hSYN total ELISA than for the in-house assay (p-value < 0.05), but data proved the hSYN total ELISA to be a reasonable diagnostic tool enabling sharp distinction of DLB and PD (PD*: exclusion of one sample showing an extraordinary high value of α-synuclein level).
HUMAN α-Synuclein PATHO ELISA

- Functions as an ELISA for analyzing disease associated α-synuclein from human CSF, plasma, or serum
- Works with six-point standard curve using lyophilized standards and controls
- Offers detection using either absorption (TMB) or chemiluminescence (ECL)
- Application of worldwide unique, patented antibodies
- Uses the standardized ELISA format on 12x8 immuno strips for flexible application of various sample throughput

Product specifications

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<th>Target</th>
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| Detection time | 24 hours |

Performance assessment

By quantifying α-synuclein, a presynaptic protein, in human CSF, differentiations can be made between Lewy body pathology (LBP) and other neurodegenerative pathologies. The detection of pathologically relevant formations of α-synuclein can also provide additional manifestations for accompanying diagnostics and prognosis.

References


Feasibility study using the HUMAN α-Synuclein PATHO ELISA: pathological relevant α-synuclein measurements in the CSF from patients with Lewy body pathology (LBP), Alzheimer’s diseases (AD), and from a control group. The level of disease-associated α-synuclein was - on average - higher in LBP than in other groups.

Oligomeric synuclein measurements using HUMAN α-Synuclein PATHO ELISA showed a significant correlation with TNF, IL10, and IL6 in Mexico City Metropolitan Area (MCMIA) children. This reveals that processes of inflammation may be correlated with neurodegeneration in younger population.
Creutzfeldt-Jakob Disease

The third area in this profile focuses on differential diagnostics for rapid-onset Creutzfeldt-Jakob disease (CJD). This disease is caused by the aggregation of cellular prion protein. Human prion disorders are rare and only affect one to two cases per million people.

Current diagnostic criteria related to CJD using 14-3-3 protein and due to misclassification of up to 46% of atypical AD cases indicates that additional biomarker more closely related to the pathological process would improve the accuracy of CJD testing.

A recent study showed that detecting the prion protein in CSF samples can clearly distinguish between atypical cases of Alzheimer's disease and CJD (Dorey et al. 2015). The BetaPrion® HUMAN ELISA performs the quantification of precisely this biomarker. It may also prove beneficial in clinical practice alongside the current classic biomarkers.

However, CJD, which is extremely serious for the patient, shot to international prominence in the bovine spongiform encephalopathy crisis (“mad cow disease”). Identifying this disease and distinguishing it from other forms of dementia such as Alzheimer’s disease have proven to be a major challenge in neurochemical diagnostics.

In particular, this difficulty arises from the fact that atypical AD phenotypes can mirror the intense neuronal degeneration found in CJD due to high levels of total tau protein and/or positive 14-3-3 protein in the CSF.
BetaPrion® HUMAN ELISA

- Quantitatively detects human prion protein in human CSF and in diverse biological samples
- Features an ultrasensitive immunoassay that uses TMB substrate
- Promises optimal storage stability of antibodies produced in house: allows for preservation of specific and exceptionally well-characterized binding capabilities
- Offers fast and easy handling: clinically relevant results within 3 – 5 hours
- Has flexible applications depending on sample throughput: standardized ELISA format on 12x8 immuno strips Works with six-point standard curve using lyophilized standards and controls

Product specifications

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<th>Target</th>
<th>Prion protein</th>
<th>Detection time</th>
<th>Storage/Stability</th>
</tr>
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</table>
| Starting material | - Serum, plasma and CSF  
| | - Biological samples from animal and cell models | 3–5 hours or 24 hours | 12 months at 4°C |

Performance assessment

Although typical forms of AD and CJD are clinically distinguishable, atypical AD phenotypes remain a diagnostic challenge. The current clinical diagnostic criterion for CJD, 14-3-3 protein in cerebrospinal fluid (CSF), unfortunately only has a low diagnostic specificity for atypical AD. The BetaPrion® HUMAN ELISA was used to evaluate the relevance of determining the total prion protein (t-PrP) level in CSF for a differential biological diagnosis.

References


The misclassification rate of atypical AD phenotypes decreased from 43.5% (p14-3-3 results) to only 4.3% when calculating the ratio of Tau total/(P-tau181 x t-PrP).

An analysis of total cerebrospinal fluid prion protein in controls, AD, and CJD populations. Typical AD indicated by definite AD and portable AD; CJD indicated by definite CJD and portable CJD.
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