Diagnostik von Alzheimer als Begleitung aktuell möglicher Therapien

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Dr. Ingolf Lachmann, CEO & Shareholder Roboscreen GmbH

What are the future biomarkers for diagnostic and follow-up of neurodegenerative diseases?

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- Where we start from? What is currently used in Routine Diagnostics
- What future means? Which biomarkers?

Dr. Ingolf Lachmann, CEO & Shareholder Roboscreen GmbH

Biomarkers of neurodegenerative diseases in CSF

Biomarker	Routine use	Potential	
β-Amyloid 40	**Ratio 40/42**		Amyloide Plaque
β-Amyloid 42			Amyloide Plaque
hTAU total	**Cut off**		Tangles, Injury
phosphoTAU	**Cut off**	p217, p231, p50, non-pTAU …	Tangles, Injury
Synuclein	rtQuIC	Total αSyn, phospho-αSyn, β-Synuclein	Inclusions, Lewy Bodies
TDP43		x	Inclusions FTD
Neurofilament light (NFL)		x	Neuronal Injury
Prion Protein		x	CJD
GFAP		x	Neuronal Injury
14-3-3	x		CJD

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- **Only some protein biomarkers established in routine diagnostic analysis**
- Studies show potential of others
- Up to 20 different in total (incl. Albumin quotient)
- CSF disadvantage in sampling, not all patients receive lumbar punction (e. g. PD/LBD)
- Genetical: e. g. **APOE**, APP, PSEN1, PSEN2

TDP43 = Transactive response DNA binding protein 43 kDa
GFAP = glial fibrillary acidic protein
14-3-3 = family of conserved regulatory molecules

Same CSF biomarkers in blood plasma are future biomarkers

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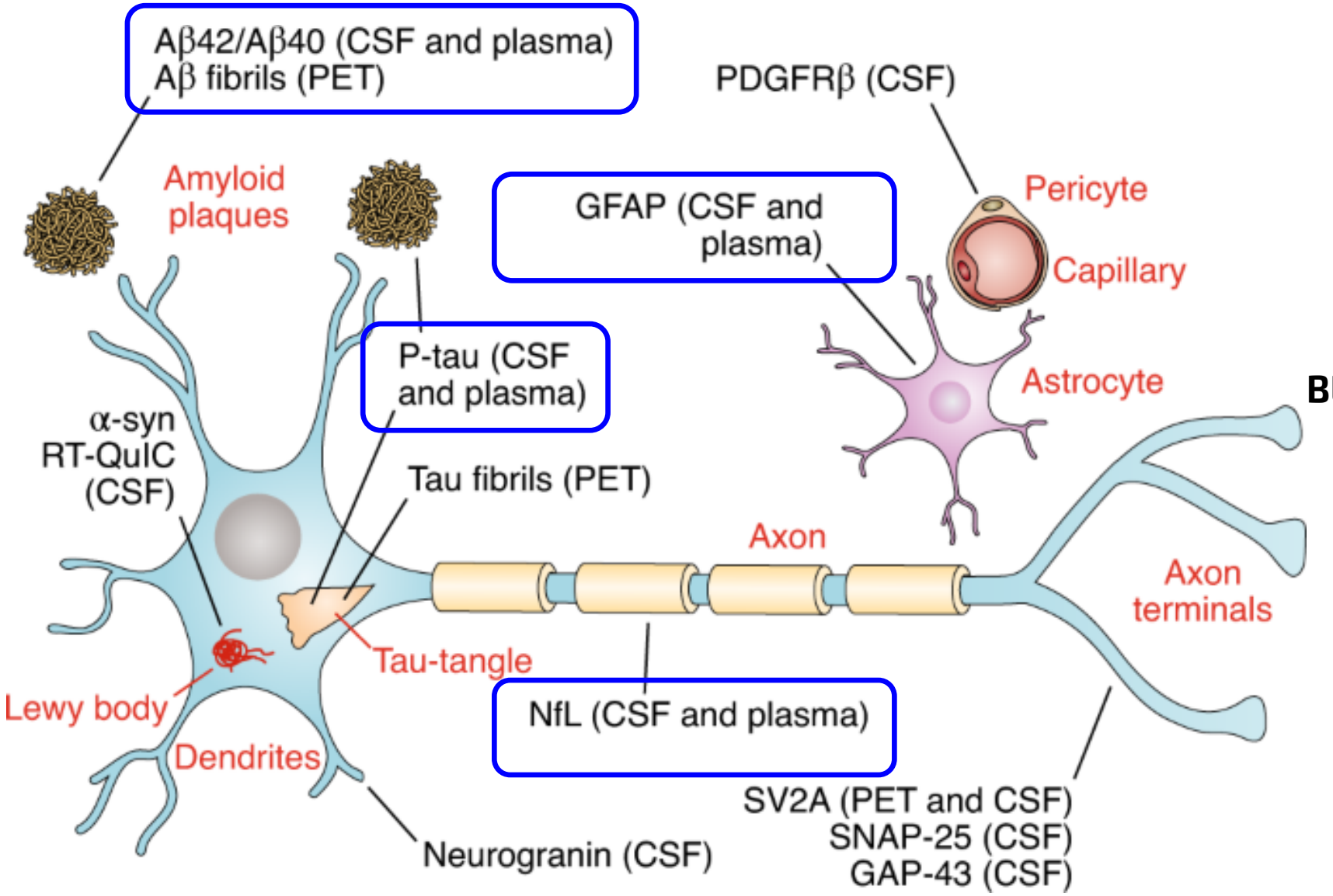

FUTURE

BLOOD based diagnostic of dementia

Hansson, O. Biomarkers for neurodegenerative diseases. *Nat Med* **27**, 954–963 (2021). https://doi.org/10.1038/s41591-021-01382-x

Current status of blood-based early diagnosis and clinical relevance for preventive therapy approaches in D-AD

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- Blood-based preclinical dementia diagnostics for "subjective cognitive deficits due to AD" (SCD-AD) and "mild cognitive impairment due to AD" (MCI-AD) is already established in therapy research.
- Blood biomarkers can already indicate the process dynamics of neurodegeneration in AD in SCD-AD and MCI-AD
- Two monoclonal antibodies have now been approved in the USA for the secondary preventive therapy of D-AD: aducanumab (Biogen, with conditions) and lecanemab (Eisai/Biogen).
- Third – Donanemab showed on1,700 patients in the early stages of Alzheimer's a 35% slower decline in memory, thinking and their ability to perform daily activities at 76 and patients at the earliest stage of the disease had a greater benefit after taking donanemab, demonstrating a 60% slower decline in cognitive function.
- Clinical relevance: **Identification of high-risk patients, therapy monitoring, "responder prediction" and development of innovative study designs for preventive therapy approaches**

From AD/PD2024 Lissabon

Two pivotal clinical studies in the last 2 years (AD focused)

Blood based testing already started!

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nature medicine

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Article | Published: 24 May 2021

Prediction of future Alzheimer's disease dementia using plasma phospho-tau combined with other accessible measures

Sebastian Palmqvist, Pontus Tideman, Nicholas Cullen, Henrik Zetterberg, Kaj Blennow, the Alzheimer's Disease Neuroimaging Initiative, Jeffery L. Dage, Erik Stomrud, Shorena Janelidze, Niklas Mattsson-Carlgren & Oskar Hansson

Nature Medicine **27**, 1034–1042 (2021) | Cite this article

16k Accesses | **144** Citations | **497** Altmetric | Metrics

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Article | Open Access | Published: 01 December 2022

Differential roles of Aβ42/40, p-tau231 and p-tau217 for Alzheimer's trial selection and disease monitoring

Nicholas J. Ashton, Shorena Janelidze, Niklas Mattsson-Carlgren, Alexa Pichet Binette, Olof Strandberg, Wagner S. Brum, Thomas K. Karikari, Fernándo González-Ortiz, Guglielmo Di Molfetta, Francisco J. Meda, Erin M. Jonaitis, Rebecca Langhough Koscik, Karly Cody, Tobey J. Betthauser, Yan Li, Eugeen Vanmechelen, Sebastian Palmqvist, Erik Stomrud, Randall J. Bateman, Henrik Zetterberg, Sterling C. Johnson, Kaj Blennow & Oskar Hansson

Nature Medicine **28**, 2555–2562 (2022) | Cite this article

10k Accesses | **20** Citations | **260** Altmetric | Metrics

Palmqvist et al. - Blood-based prediction of D-AD in the prodromal stage MCI-AD

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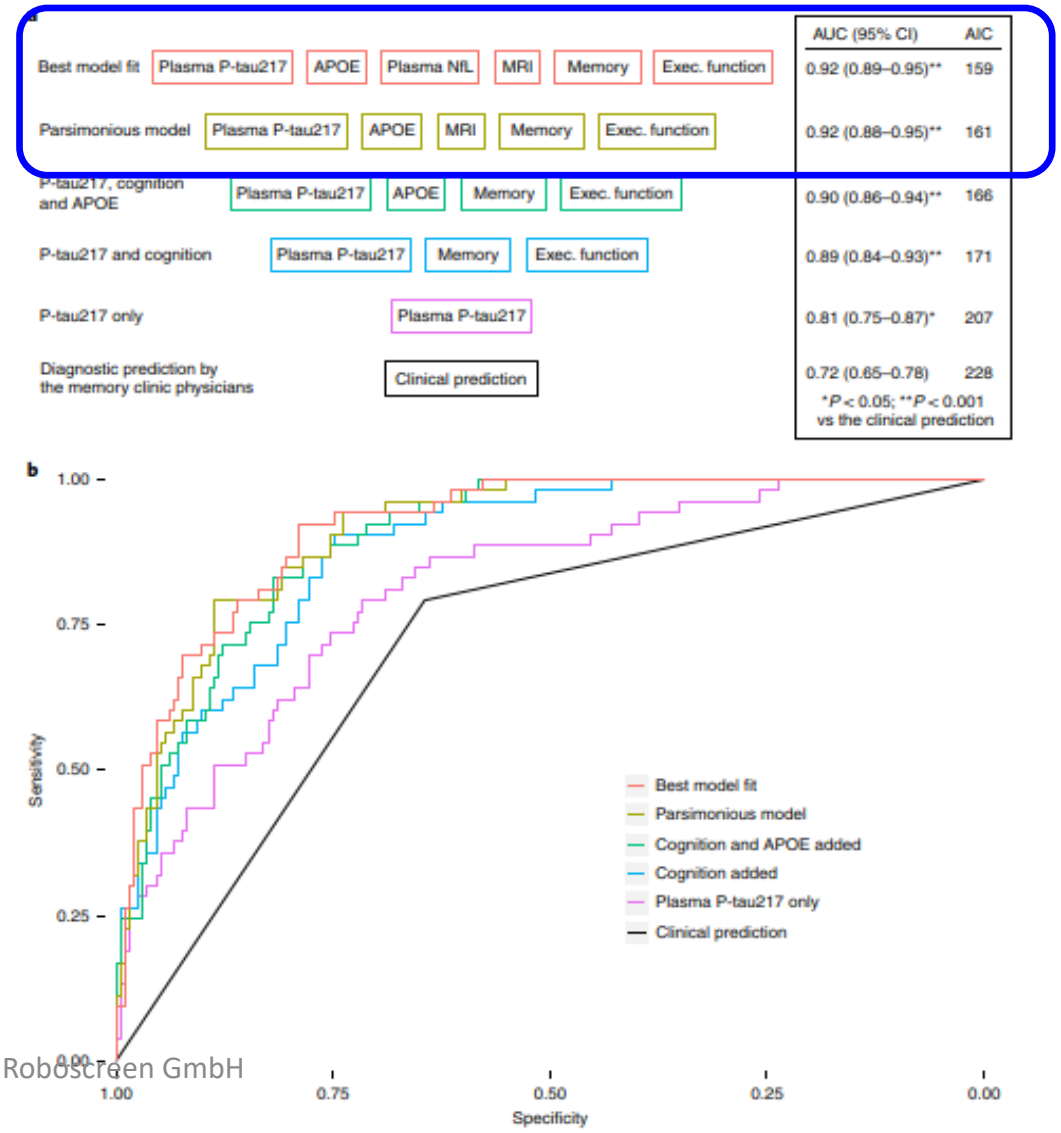
- present a blood-based diagnostic algorithm that uses phospho-tau in combination with other inexpensive and minimally invasive biomarkers to predict the impending D-AD with high diagnostic reliability in the prodromal stage of MCI-AD.
- The differential diagnosis of other forms of dementia was also examined, and how well the biomarker-based prediction performed in comparison to the clinical expert judgment of colleagues with high dementia expertise. The dementia experts were allowed to take into account structural imaging (MRI) and psychometric testing at the initial examination in their prognosis

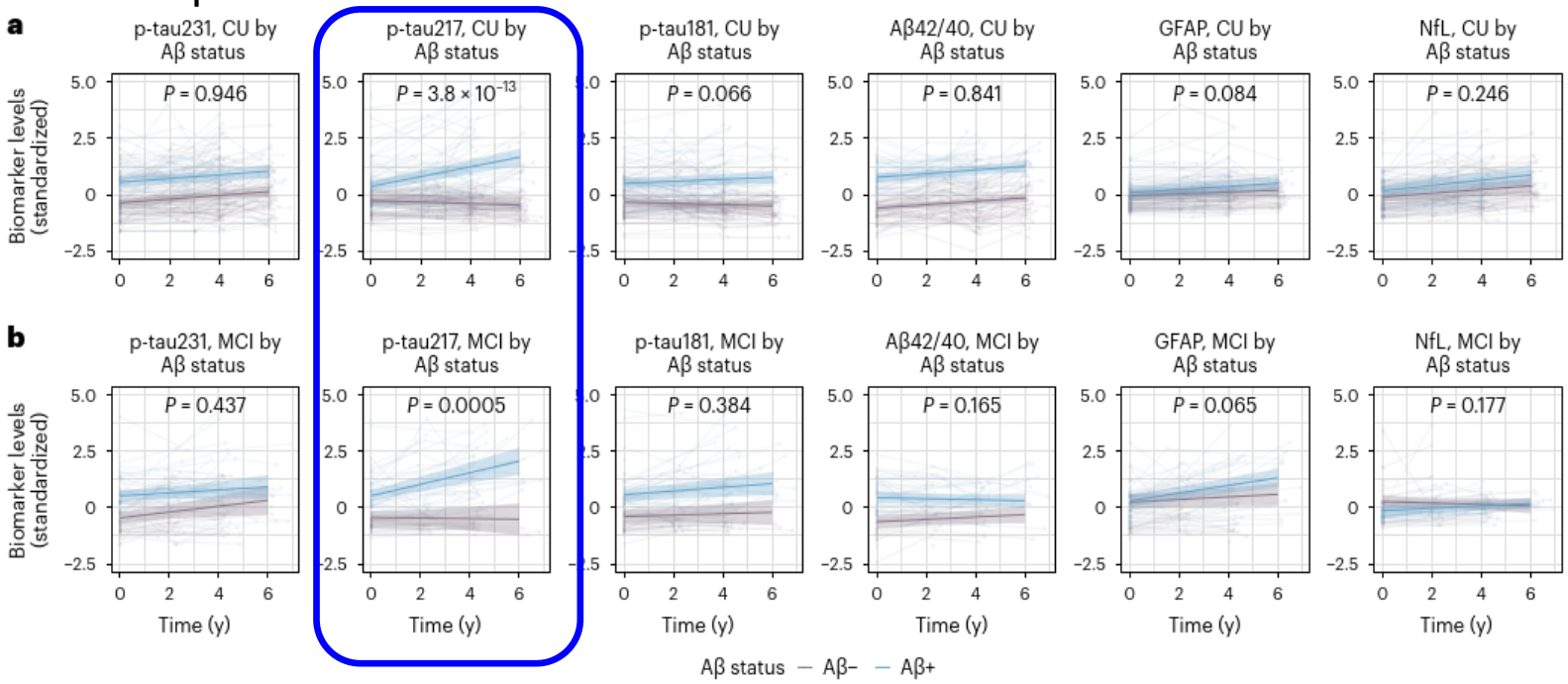
Palmqvist et al. - Blood-based prediction of D-AD in the prodromal stage MCI-AD

- Comparative presentation of the biomarker-based prediction of the development of D-AD and the quality of the prediction via the clinical expert judgment with additional consideration of structural MRI and psychometric findings at the initial examination.
- Combination of p217 Tau, ApoE, psychometric quantification of the deficits in memory and executive functions and MRI formed best.
- <u>On the basis of this prediction algorithm, a logistic regression model was then developed that can individually calculate a prediction probability for the transition to D-AD within 4 years</u>



Ashton et al. - Blood-based early identification of high-risk patients and biomarkers of neurodegenerative process dynamics

- Have compared the value of the molecular blood plasma biomarkers Aβ42/40, phospho-Tau181, phospho-Tau217, phospho-Tau231, GFAP and NfL for the identification of patients for secondary preventive therapy approaches of D-AD and their potential suitability for the monitoring of a therapeutic effectiveness examined.



Blue: β-Amyloid positive patients

Grey: β-Amyloid negative patients

Blood biomarker changes over time in cognitively unimpaired patients (a) and MCI (b) as a function of cerebral beta-amyloid pathology. Ashton et al., Nat. Med. 2022; 28(12):2555–2562.

Revised criteria for diagnosis and staging of Alzheimer's disease: Alzheimer's AssociationWorkgroup Clifford R. Jack Jr. in Alzheimer & Dementia 2024

Intension: To present objective criteria for diagnosis and stagingAD, incorporating recent advances in biomarkers, to serve as a bridge between research and clinical care. These criteria are intended to serve as general principles to inform diagnosis and staging of AD that reflect current science.

Revised criteria for diagnosis and staging of Alzheimer's disease: Alzheimer's AssociationWorkgroup Clifford R. Jack Jr. in Alzheimer & Dementia 2024

Fundamental principles

AD is defined by its unique neuropathologic findings; therefore, detection of AD neuropathologic change by biomarkers is equivalent to diagnosing the disease.

AD exists on a continuum. The disease is first evident in vivo with the appearance of disease-specific Core biomarkers while people are asymptomatic.

Unimpaired individuals with abnormal biomarker test results are at risk for symptoms due to AD. They are not at risk for a disease they already have.

Clinical syndromes commonly seen with AD may also be caused by disorders other than AD, and therefore clinical presentation alone is not diagnostic of AD.

The same AD biology may result in different phenotypic presentations.

Revised criteria for diagnosis and staging of Alzheimer's disease: Alzheimer's AssociationWorkgroup Clifford R. Jack Jr. in Alzheimer & Dementia 2024

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Biomarker category	CSF or plasma analytes	Imaging
Core Biomarkers		
Core 1		
A (Aβ proteinopathy)	Aβ 42	Amyloid PET
T_1: (phosphorylated and secreted AD tau)	p-tau217, p-tau181, p-tau231	
Core 2		
T_2 (AD tau proteinopathy)	MTBR-tau243, other phosphorylated tau forms (e.g., p-tau205), non-phosphorylated mid-region tau fragments[a]	Tau PET
Biomarkers of non-specific processes involved in AD pathophysiology		
N (injury, dysfunction, or degeneration of neuropil)	NfL	Anatomic MRI, FDG PET
I (inflammation) Astrocytic activation	GFAP	
Biomarkers of non-AD copathology		
V vascular brain injury		Infarction on MRI or CT, WMH
S α-synuclein	αSyn-SAA[a]	

Revised criteria for diagnosis and staging of Alzheimer's disease: Alzheimer's AssociationWorkgroup Clifford R. Jack Jr. in Alzheimer & Dementia 2024

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Intended use	CSF	Plasma	Imaging
Diagnosis			
A: (Aβ proteinopathy)	—	—	Amyloid PET
T_1: (phosphorylated and secreted AD tau)	—	p-tau217	—
Hybrid ratios	p-tau181/Aβ42, t-tau/Aβ42, Aβ42/40	%p-tau217	—
Staging, prognosis, as an indicator of biological treatment effect			
A: (Aβ proteinopathy)	—	—	Amyloid PET
T_1: (phosphorylated and secreted AD tau)	—	p-tau217	—
Hybrid ratios	p-tau181/Aβ42, t-tau/Aβ42, Aβ42/40	%p-tau217	—
T_2: (AD tau proteinopathy)	MTBR-tau243, other p-tau forms (e.g., p-tau205), non-phosphorylated mid-region tau fragments	MTBR-tau243, other p-tau forms (e.g., p-tau205)	Tau PET
N (injury, dysfunction, or degeneration of neuropil)	NfL	NfL	Anatomic MRI, FDG PET
I (inflammation) Astrocytic activation	GFAP	GFAP	—
Identification of copathology			
N (injury, dysfunction, or degeneration of neuropil)	NfL	NfL	Anatomic MRI, FDG PET
V vascular brain injury	—	—	Infarction on MRI or CT, WMH
S α-synuclein	αSyn-SAA	—	

Summary

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- Blood biomarkers such as phospho-tau (p217, p231, p181) identify the impending Alzheimer's dementia even in cognitively asymptomatic test subjects **10-15 years before the dementia stage**!

- Blood biomarkers together with ApoE-ε genotyping and simple psychometric tests realize surprisingly precise predictive dementia diagnostics.

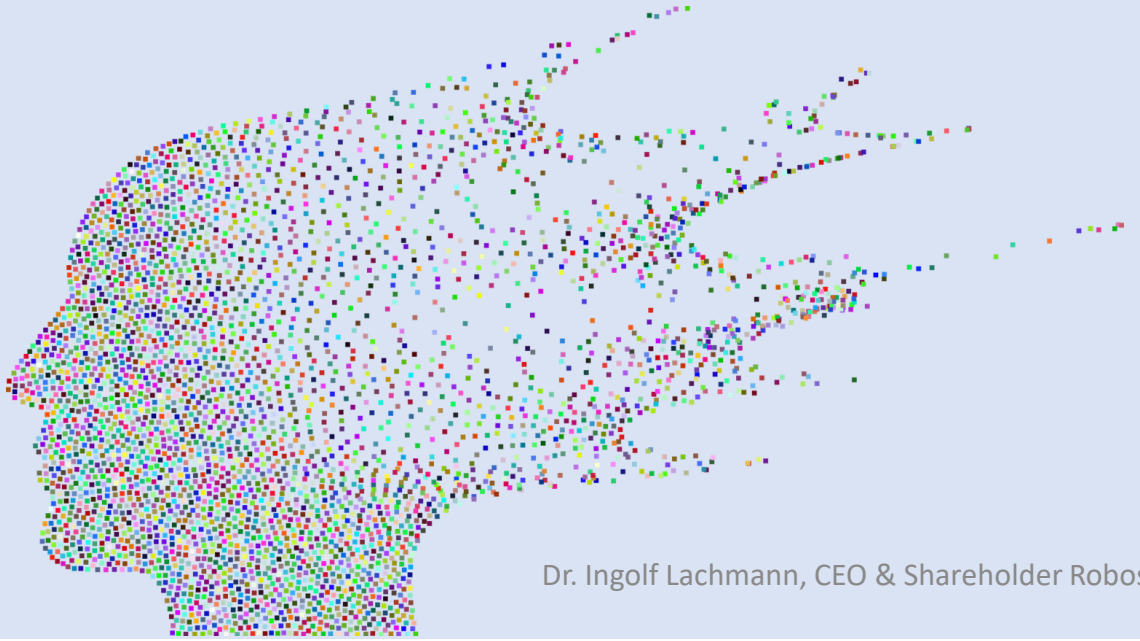
- Blood biomarkers such as phospho-tau and tau-forms, NfL and GFAPcan be used as "state markers" of the process dynamics of neurodegeneration and promise to map the therapeutic effectiveness of innovative preventive therapy approaches in the blood early and with high sensitivity to change.

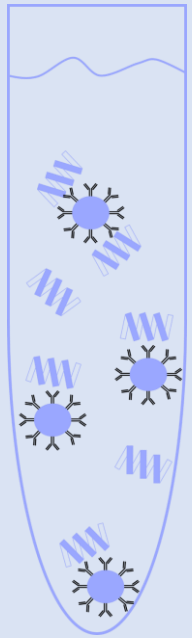
- As a strategy exists for Clinical staging for individuals on the Alzheimer's disease continuum using a 6-stages concept.

NEURO – IP Kits

IP Kit
Enrichment of $_{phospho}$tau, tau, abeta from fluid samples e.g. blood plasma

AD/PD 2024





Dr. Ingolf Lachmann, CEO & Shareholder Roboscreen GmbH

Semi-automated two step immuno assay platform combining initial robotic immunoprecipitation (IP) with commercially high throughput capable immunoassays (IP-IA).

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Alzheimers Res Ther. 2022; 14: 127.
Published online 2022 Sep 7. doi: 10.1186/s13195-022-01071-y
PMCID: PMC9450259
PMID: 36071505

Diagnostic performance of automated plasma amyloid-β assays combined with pre-analytical immunoprecipitation

Hans-W. Klafki,[✉1] Jonathan Vogelgsang,[1,2] Ekaterina Manuilova,[3] Chris Bauer,[4] Alexander Jethwa,[3] Hermann Esselmann,[1] Anke Jahn-Brodmann,[1] Dirk Osterloh,[5] Ingolf Lachmann,[5] Benedict Breitling,[1] Carolin Rauter,[1] Niels Hansen,[1] Caroline Bouter,[6] Stefan Palme,[3] Johannes Schuchhardt,[4] and Jens Wiltfang[✉1,7,8]

Int J Mol Sci. 2020 Sep; 21(18): 6564.
Published online 2020 Sep 8. doi: 10.3390/ijms21186564
PMCID: PMC7555726
PMID: 32911706

Development and Technical Validation of an Immunoassay for the Detection of $APP_{669-711}$ ($Aβ_{-3-40}$) in Biological Samples

Hans W. Klafki,[1,*] Petra Rieper,[1] Anja Matzen,[2] Silvia Zampar,[1] Oliver Wirths,[1] Jonathan Vogelgsang,[1] Dirk Osterloh,[3] Lara Rohdenburg,[1] Timo J. Oberstein,[4] Olaf Jahn,[5] Isaak Beyer,[6] Ingolf Lachmann,[3] Hans-Joachim Knölker,[6] and Jens Wiltfang[1,7,8]

Semi-automated two step immuno assay platform combining initial robotic immunoprecipitation (IP) with commercially high throughput capable immunoassays (IP-IA).

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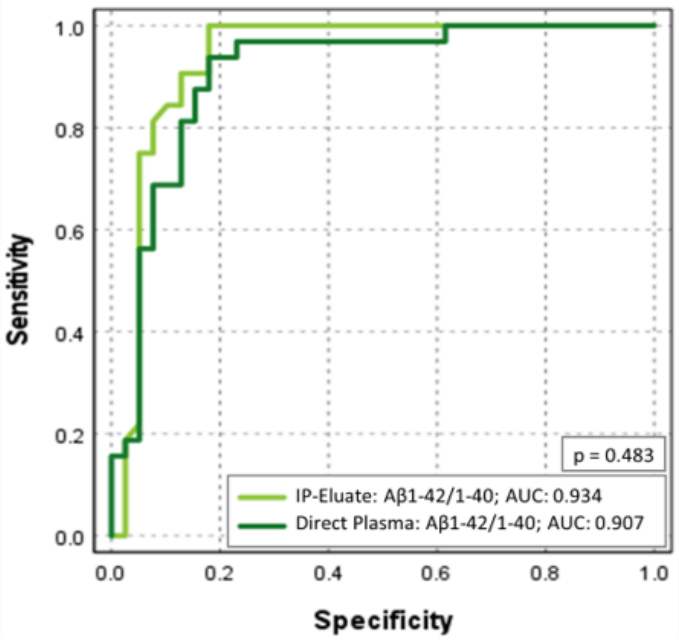
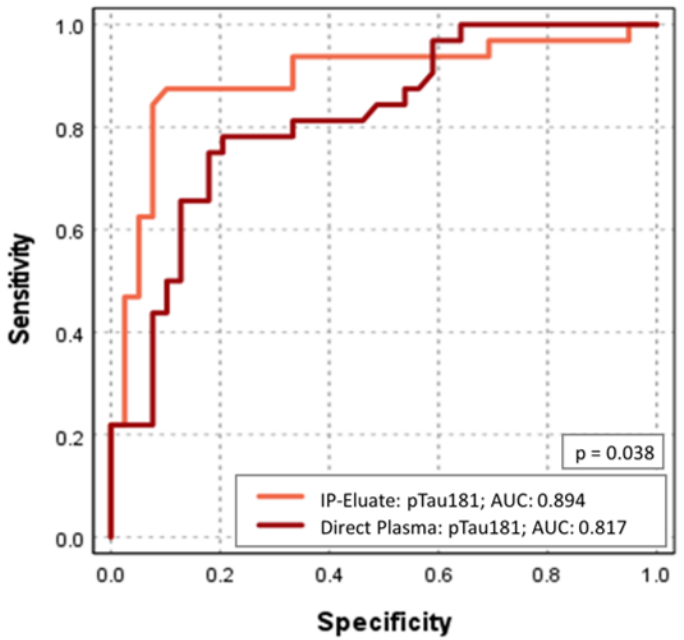
Measurement of plasma pTau181 and Aß42/40 on the Lumipulse G System with and without pre-analytical sample workup by magnetic bead immunoprecipitation showed great compatibility of the Neuro IP Kit with the Lumipulse platform. Pre-analytical Tau-IP improved the diagnostic contrast significantly.
Morgado et al. ADPD2023.

In combination of both biomarkers + ApoE Status ROC curve (AUC) has shifted significant to 0,953.



Pre-analytical Aß-IP increased the area under the ROC curve (AUC) for plasma Aß42/40 from 0.907 to 0.934 (p=0.48)



Pre-analytical Tau-IP increased the AUC for pTau181 significantly from 0.817 to 0.894 (p=0.038)

NEURO – IP Kits

Easily clear off inhibiting matrix effects & enrich your target with ROBOSCREEN's NEURO IP for total TAU, p50TAU, BRAIN-DERIVED TAU and beta-AMYLOID and improve downstream analysis of your choice.

ELECSIS

Results: Pre-analytical immunoprecipitation resulted in a significant increase in the area under the ROC curve (AUC) from 0.73 to 0.88 (p = 0.01547) for identifying subjects with abnormal CSF Aβ42/40. A similar improvement in the diagnostic performance by pre-analytical immunoprecipitation was also observed when a biomarker-supported clinical diagnosis was used as a second endpoint (AUC increase from 0.77 to 0.92, p = 0.01576). [5]

MSD

In summary, we have developed a novel, highly selective sandwich immunoassay for measuring Aβ–3–40 in biological samples. **In combination with pre-analytical Aβ enrichment by magnetic bead IP, the assay can serve to measure the relative levels of Aβ–3–40 in human blood plasma.** Thus, the methodological groundwork has been set for future studies addressing the diagnostic potential of the Aβ42/Aβ–3–40 ratio (or reverse) as a novel surrogate biomarker candidate of cerebral amyloid deposition. [6]

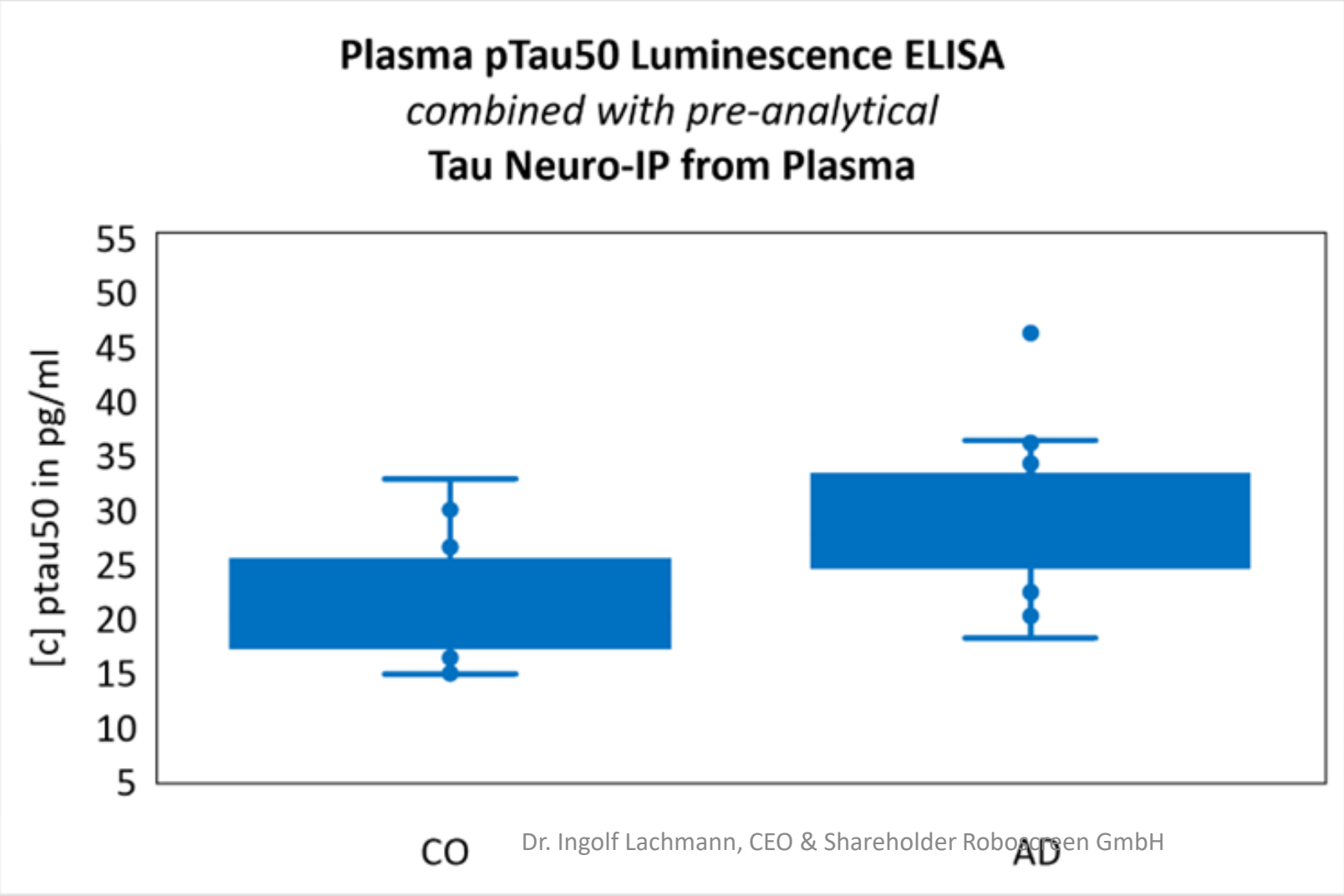
SIMOA

Pre-analytical Tau-IP (IP-Eluates) **allows differentiation between AD and Control pools using an in-house SIMOA assay for threonine-50 phosphorylated, brain derived Tau (p50-BD-Tau).** For direct measurement without Neuro IP, only 8 of the 19 plasma samples can be measured at all, with an over-
lap of AD and Control pools.

LUMI-PULSE

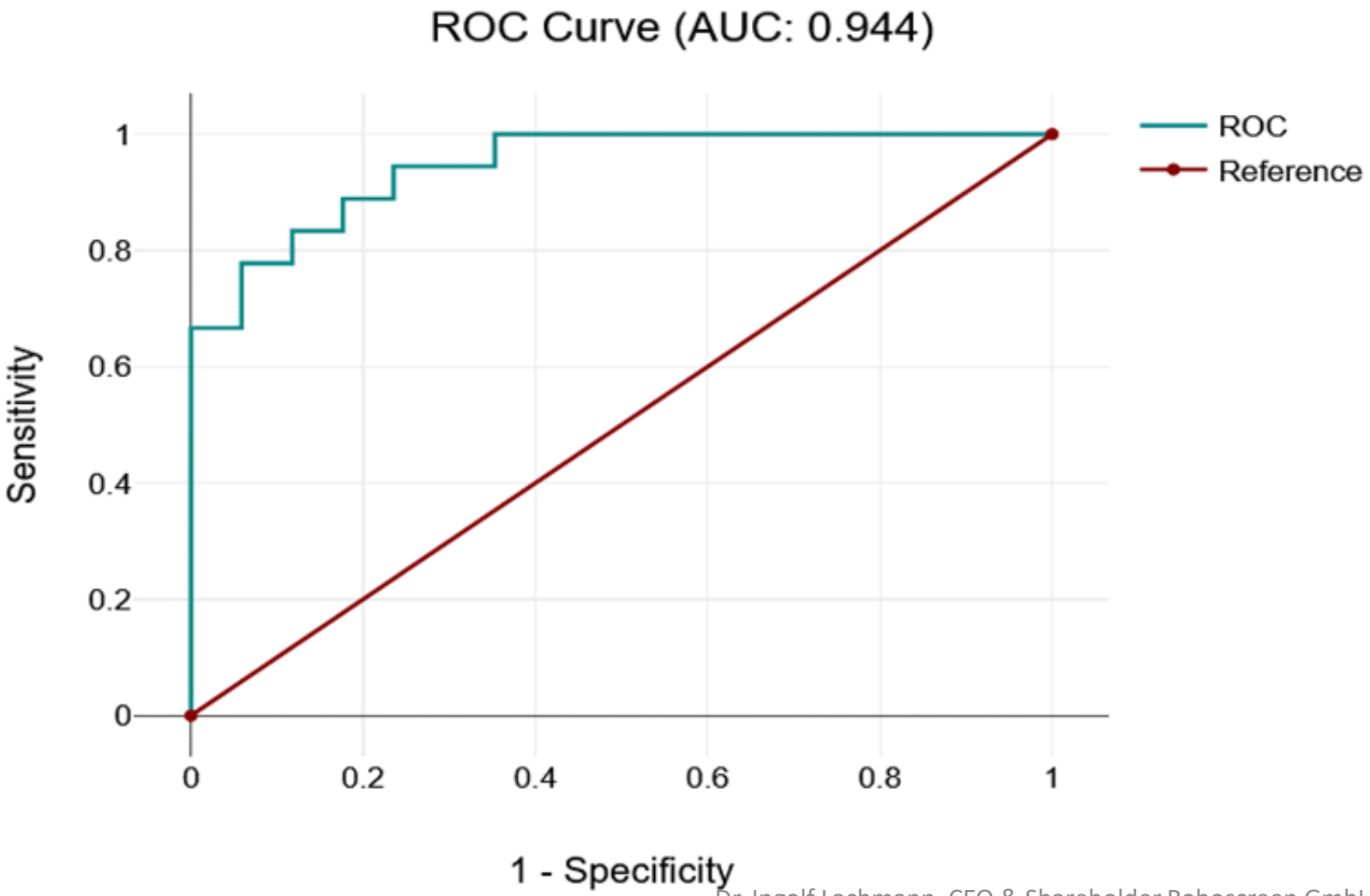
Pre-analytical immunoprecipitation of Tau resulted in increased differences between the diagnostic groups in terms of median and mean phosphorylated Tau 181 levels. Furthermore, we observed a greater Cohen's d ($p < 0.001$) and a larger area under the ROC curve (p = 0.038) after Tau-IP. [7]

p50TAU in plasma samples



Tau Neuro IP from 400 µl Plasma of AD (n=18) and Control patients (n=17) with subsequent measure-ment of eluates with Plasma pTau50 Luminescence ELISA.

p50TAU in plasma samples



ROC analysis of

Aβ 1-40/ x pTau50
Aβ 1-42
of AD (n=18) and

Control patients (n=17).

MultiDIAG - Immuno PCR for sensitive ReadOut of plasma biomarkers for Alzheimer

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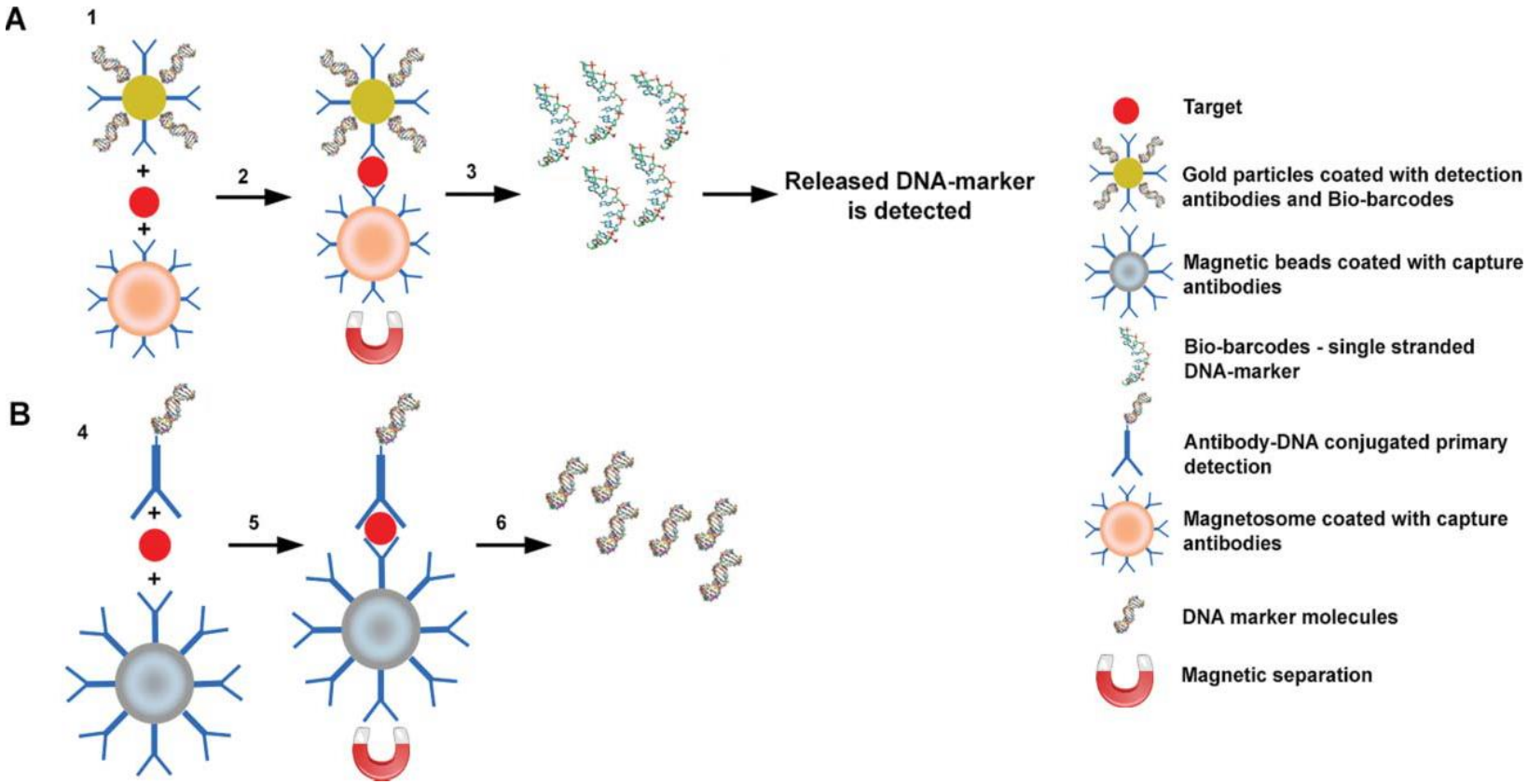

Co-funded by the European Union

Freistaat SACHSEN

This project is co-financed from tax revenues on the basis of the budget adopted by the Saxon State Parliament.

MultiDIAG - device for nucleic acid extraction + Neuro-IP + immune assay + qPCR –
Founded cooperation project of Roboscreen + IMK + EDC

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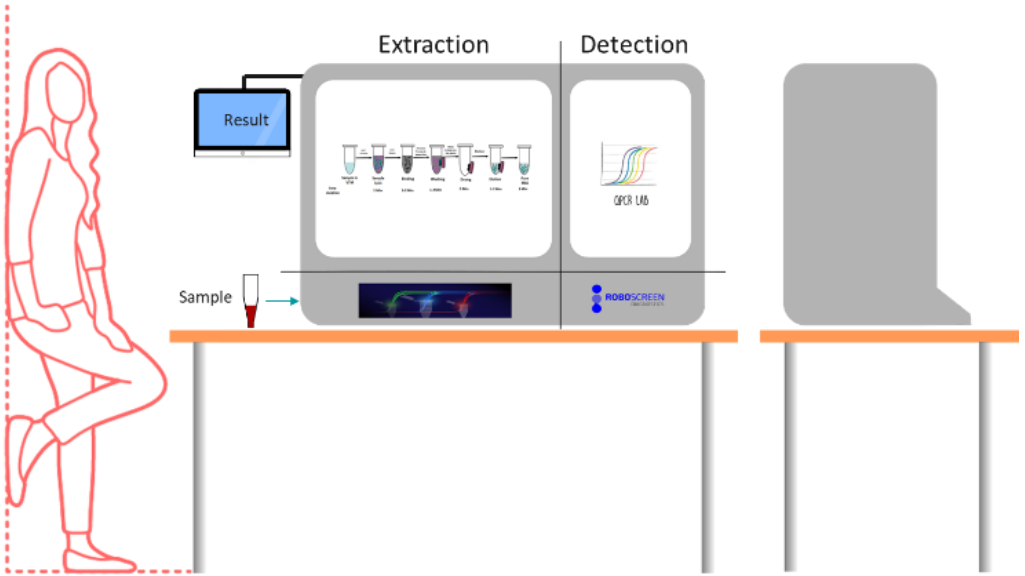

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Danke für das Interesse und die Aufmerksamkeit!

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