APPLICATION NOTE

AUTOMATED WORKFLOW: BD-TAU AND BD-P217 TAU ON FLUENT® 780 WORKSTATION.

Integrated enrichment and luminescence measurement from blood samples.

TECAN

ABSTRACT.

Background.
Accurate measurement of brain-derived Tau (BD-Tau) and its phosphorylated form (BD-p217Tau) in blood is essential for Alzheimer's disease research and diagnosis. Conventional total Tau assays lack specificity for central nervous system (CNS) isoforms, as most blood Tau originates from peripheral tissues, limiting their diagnostic value.

Methods.
A fully automated workflow was developed using the Tecan Fluent® 780 Workstation to enrich and quantify BD-Tau from blood samples. The process integrates immunoprecipitation with CNS-specific antibodies and luminescence-based immunoassays. The automated protocol was compared to the manual workflow in terms of hands-on time and precision and checked for a potential plate drift, using pooled patient samples and standardized reagents.

Results.
The automated workflow reduced hands-on time from over 4 hours to 20 minutes per 96-well plate. Precision improved, with a coefficient of variation (CV) of 5.2% for the automated process compared to 7.1% for the manual method. No significant plate drift was observed and results between manual and automated workflows were highly concordant.

Conclusion.
Automation of BD-Tau enrichment and quantification using the Fluent® 780 Workstation enhances assay precision, reproducibility and efficiency, while minimizing user intervention. This standardized workflow supports reliable blood-based BD-Tau and BD-p217Tau measurement out of one eluate, facilitating its application in neurodegenerative disease research and clinical diagnostics.

Introduction.
The AT(N) (amyloid, tau, neurodegeneration) research framework provides a unified scheme that emphasizes on the pathophysiological evidence of amyloid beta (A), tau (T) and neurodegeneration (N) for the definition and staging of Alzheimer's disease.[1-3]
Total tau has long been recognized as a marker of neurodegeneration in CSF, but its utility in blood is limited. The majority of blood total Tau originates from peripheral tissue, not the CNS and is elevated in a range of conditions, including Creutzfeldt-Jakob disease (CJD), head trauma and anoxia, among others.
The rationale to develop brain-derived Tau specific assays lies in the properties of the Tau protein. Tau exists in multiple isoforms. Whereas CNS tau is characterized by low molecular weight forms and peripheral nervous system (PNS) tau by a larger isoform known as "big tau."[4] This main form of Tau in the PNS can be distinguished from the different CNS isoforms by the presence of a large peptide insert from the transcription of an extra exon (exon 4a).[5] Traditional total Tau assays use antibodies that target regions shared by CNS and PNS tau. To address this, our BD-Tau Luminescence immunoassay as well as our Neuro Immunoprecipitation Kit use a Tau junction antibody (2B8) that will not bind if the "big tau" insert is present (see figure below).

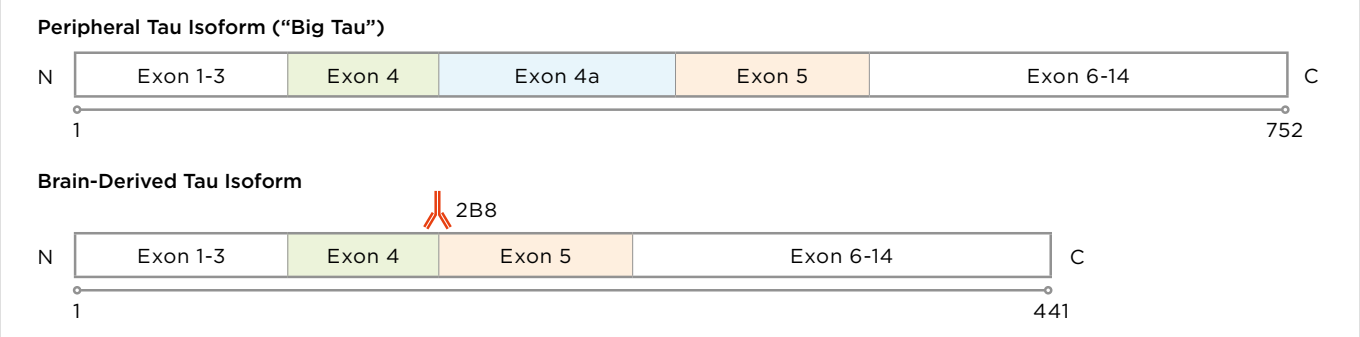

Figure 1 Illustration of sequences of peripheral in contrast to brain-derived Tau.

Doing an immunoprecipitation step upfront the actual assay has proven to enhance the discriminatory power of the subsequently measured biomarkers especially in blood samples.[6-8]

MATERIALS AND METHODS.

Manual assay.

The combined workflow consists of two procedures, namely the enrichment of BD-Tau (BD-Tau Neuro-IP Kit Order No. 30260912) followed by the actual luminescence immunoassays (Brain-Derived Tau (BD-Tau) Luminescence Immunoassay (LUM) Order No. 30260913 and p217 TAU Luminescence immunoassay (LUM) Order No. 30270882). The BD-Tau Neuro IP Kit describes the immunoprecipitation procedure for enriching brain-derived Tau protein from peripheral cell-free body fluids, such as plasma or serum. The process begins by transferring magnetic immunobeads into a low protein binding microcentrifuge tube and immobilizing them using a magnetic separation rack. The supernatant is carefully removed to avoid bead loss and the beads are washed with buffer solution. After washing, a mixture of buffer with protease inhibitor and the sample is added to the beads and the tube is mixed and incubated under continuous agitation to ensure optimal contact between the beads and the sample. Following incubation, the tube is briefly centrifuged and the beads are again immobilized with the magnetic rack before the supernatant is removed. The beads are then subjected to several wash steps with buffer solution to remove any non-specific material. After the final wash, the beads are washed once with elution buffer. Any remaining liquid is removed and the beads are resuspended in elution buffer. The resuspended beads are heated with open caps while shaking to facilitate the release of the target protein. After heating, the tube is quickly cooled, briefly centrifuged and the beads are immobilized once more. Finally, the eluate containing the enriched Tau protein is carefully removed from the beads for subsequent analysis.

The BD-Tau LUM outlines the test procedure for quantifying brain-derived Tau protein in the obtained eluate. The process begins by adding the HRP-conjugate and standards, controls, or pre-diluted eluates to each well of the microtiter plate. The contents are mixed thoroughly and the plate is covered before being incubated on a plate shaker at room temperature for three hours. After incubation, the plate is washed five times with wash solution to remove unbound material. Next, the chemiluminescence working solution is added to each well and the plate is read using a microtiter plate reader set to luminescence mode. The reader measures the maximal luminescence according to specified settings, which include a waiting time, automatic attenuation and integration time. The resulting luminescence values are used to determine the concentration of BD-Tau in the samples, based on the standard curve generated from the included standards. The procedure for the p217 Tau LUM follows the same principle.

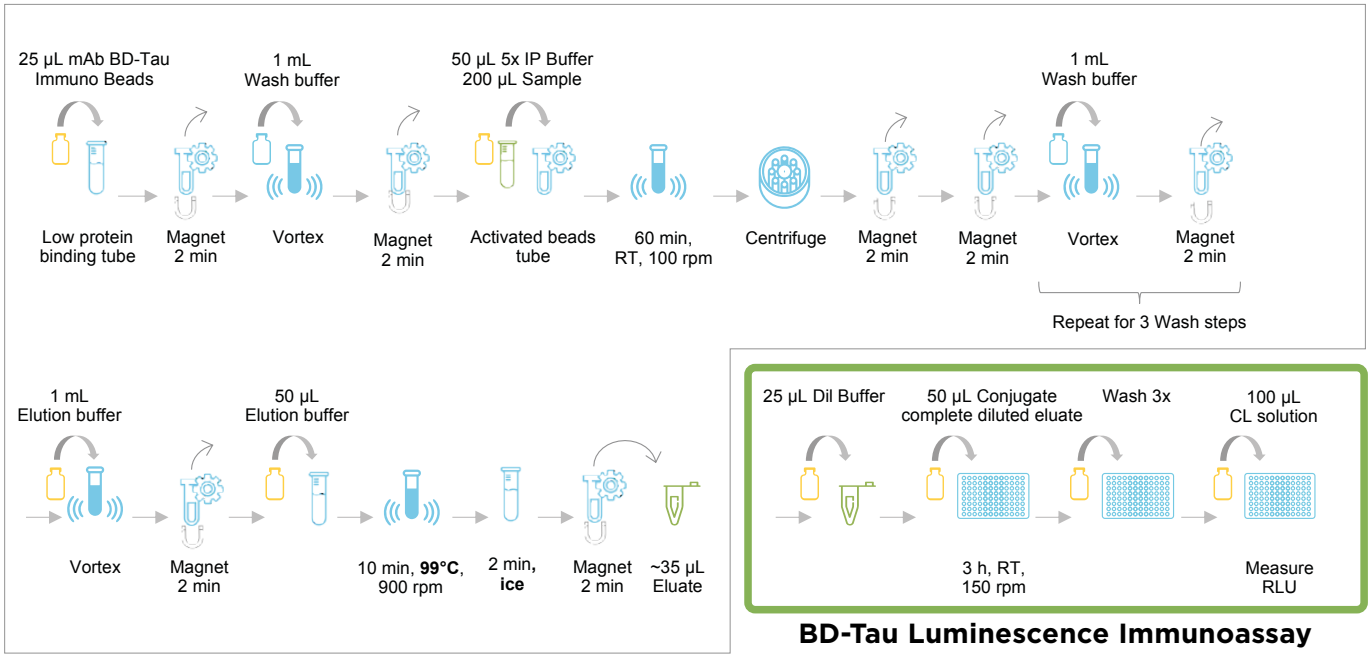

Figure 2 Manual pipetting steps for both, the BD-Tau Neuro IP Kit and Human BD-Tau Luminescence Immunoassay.

Automation plattform.

The entire BD-Tau beads enrichment as well as the luminescence assay workflow was performed on a Fluent 780 system equipped with a liquid displacement Flexible Channel Arm™ (FCA) with a mixed tip configuration consisting of four standard fixed tips and four disposable tip adapters, a Robotic Gripper Arm™ (RGA), a Fluent ID barcode reader, an integrated HydroFlex washer, an Infinite® M200 plate reader, two BioShake 3000-T ELM as well as an Alpaqua Magnum FLX magnetic plate nest (Figures 3-5).

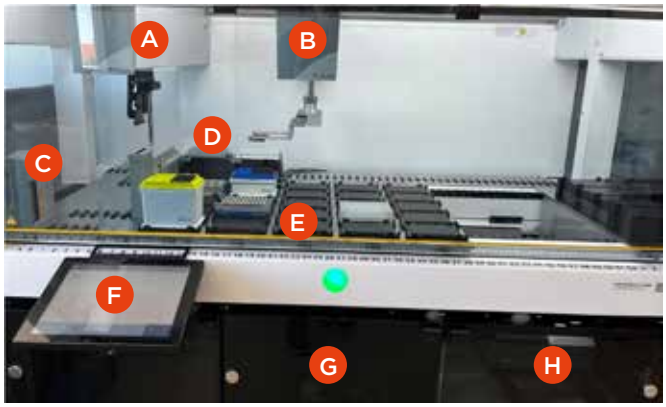

Figure 3 Fluent 780 workstation equipped with a flexible channel arm (A), a robotic gripper arm (B), a Fluent ID barcode scanner (C), two BioShakes (D), an Alpaqua magnetic plate (E), a TouchTools User interface (F), an Infinite M200 plate reader (F) and a Hydroflex plate washer (H).

The utilized system is designed for maximum flexibility to ensure the ideal translation of the manual into the automated workflow of the underlying assays. With its mixed configuration, the FCA can handle both, disposable as well as fixed tips in order to optimize speed, limit carryover, as well as save resources. Likewise, the automation protocol was scripted to cater for both clinical studies as well as for research needs offering user prompts for developers to individualize the workflow to an unprecedented extent. This was established using the Tecan Touch Tools tablet interface, which allows users to control the instrumentation, carry out maintenance tasks as well as protocols without needing any scripting skills themselves.

Automated assay.

While the automated assay proceeds to carry out the same steps as outlined in the respective instructions for use for the enrichment as well as for the immunoassay protocols, it is designed to reduce hands-on time of lab personnel to a minimum. With the FCA doing all the pipetting (see Figure 6) and

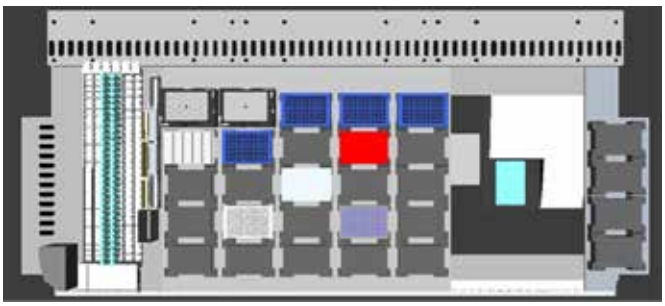

Figure 4 Top View of simulated Fluent Worktable

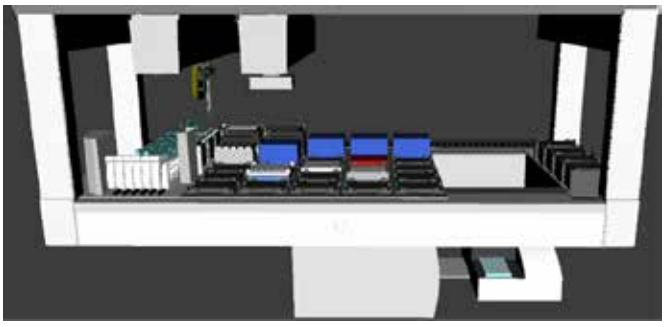

Figure 5 Front view of simulated Fluent work station

Figure 6 Pipetting by FCA with Disposable Tips

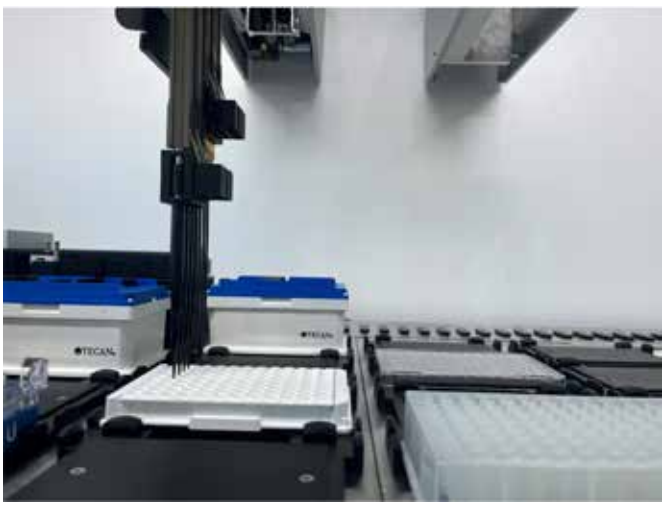

Figure 7 Plate Transfer by RGA.

the RGA doing all the plate transfers (see Figure 7), all that's left to do for the operator is to load the worktable in order to provide the respective samples, reagents and labware as well as to give some initial user prompts e.g. regarding the desired placement of control samples on the 96-well plate. Therefore, the user workflow is rather simple and displayed in Figure 8, while the actual protocol steps are significantly more complex in order to facilitate the ideal handling of samples (see Figure 9 and 10).

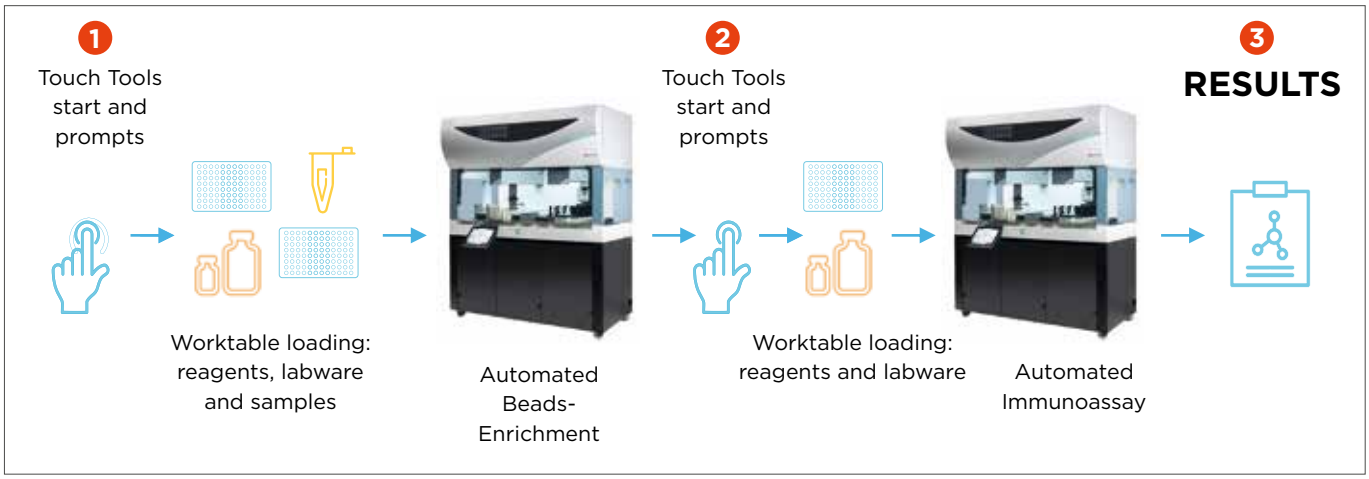

Figure 8 Automated workflow of BD-Tau Neuro IP Kit and BD-Tau and/or p217 Tau Luminescence immunoassay

DATA ANALYSIS.

The maximum luminescence of samples, calibrators and controls was measured by an Infinite® M200 plate reader controlled by the software Magellan™. In case of the automated workflow, Fluent Control™ had a Magellan interface which was integrated in the automation protocol. The waiting time was set to 1 min with automatic attenuation. The integration time was set to 100 ms with a rest time of 0 ms. For the determination of Tau concentration in controls and samples, a linear regression based on the six calibrators ranging from 1-100 pg/ml was utilized. This regression curve was based on a duplicate set of calibrators, one at the beginning and one at the end of the plate. In both cases Magellan created the results automatically with its underlying method settings.

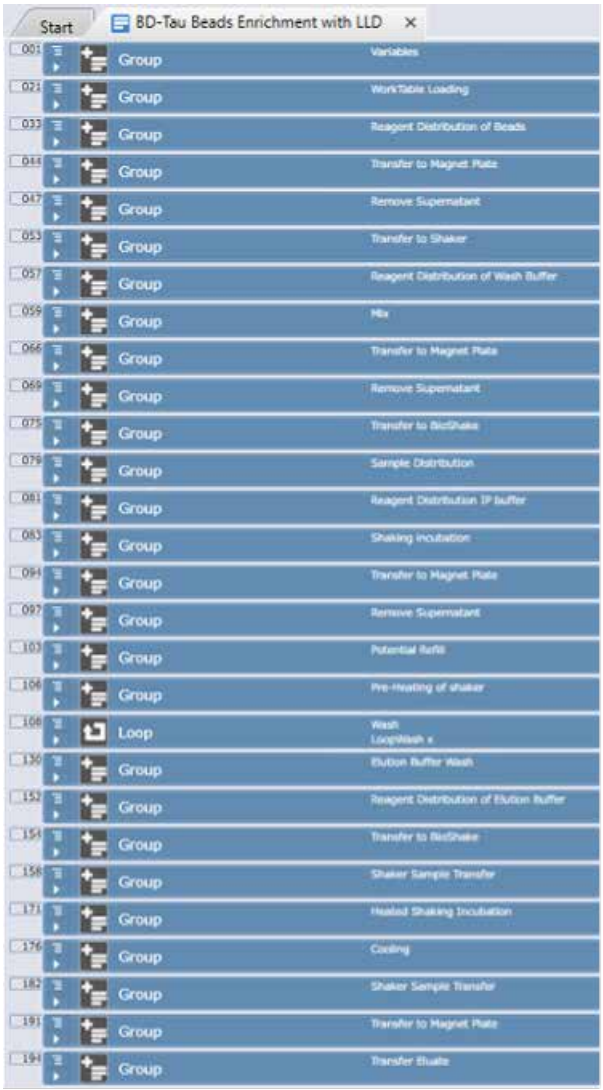

Figure 9 Protocol for the automation of BD Tau Neuro IP Kit.

Figure 10 Automation Protocol for the automation of BD-Tau or p217 Tau Luminescence immunoassay.

RESULTS.

In total, 20 samples were processed manually as well as with the automated workflow. While for the manual workflow 200 µl plasma, 50 µl IP, 25 µl beads, 60 min RT incubation, 2 x 1 ml wash buffer, 1 x 1 ml elution buffer and 50 µl elution solution were utilized, for the automated workflow 400 µl plasma, 100 ml IP, 50 µl beads, 60 min RT incubation, 2 x 1 ml wash buffer, 1 x 1 ml elution buffer and 100 µl of elution solution was utilized to ensure that there is sufficient eluate to be split for the following luminescence immunoassays to quantify BD-Tau and p217 Tau. Furthermore, 20 samples were processed automatically specifically to determine potential plate drifts. For those, 200 µl plasma, 50 µl IP, 25 µl beads, 60 min RT incubation, 2 x 1 ml wash buffer, 1 x 1 ml elution buffer and 100 µl of elution solution were utilized.

Reduction of hands-on time.

The most abundant difference for the user when comparing a manual to an automated workflow is the hands-on time, meaning the time during which laboratory personnel are occupied with processing a 96-well plate with 32 samples. For this case, the calculated difference in hands-on time was more than 3.5 hours (see Table 1).

Table 1: Hands-on time for processing a 96-well plate with 32 samples for both the beads enrichment as well as the immunoassay workflow.

Steps	Manual	Automated
14 x pipetting and washing steps	154 min	-
7 x short waiting times	14 min	-
24 x labware transfers	48 min	-
Sample tracking/ scanning	30 min	5 min
Worktable loading	-	5 min
Prompts	-	10 min
Total	**4 h 6 min**	**20 min**

Precision and drift of the automated workflow.

The precision of the automated as well as for the manual workflow was based on 20-fold determination of pooled patient samples. For the manual workflow the CV was 7.1 % and for 5.2 % for the automated one. Results of the same pool samples at the end as well as at the beginning of the 96-well plate showed no difference (see Table 2), showcasing a stable drift-less workup procedure.

Table 2: Drift determination comparing a sample set at the end as well as at the beginning of a plate.

	Beginning of plate	End of plate
Mean	9.14	8.98
SD	0.64	0.64
CV%	6.97	7.14

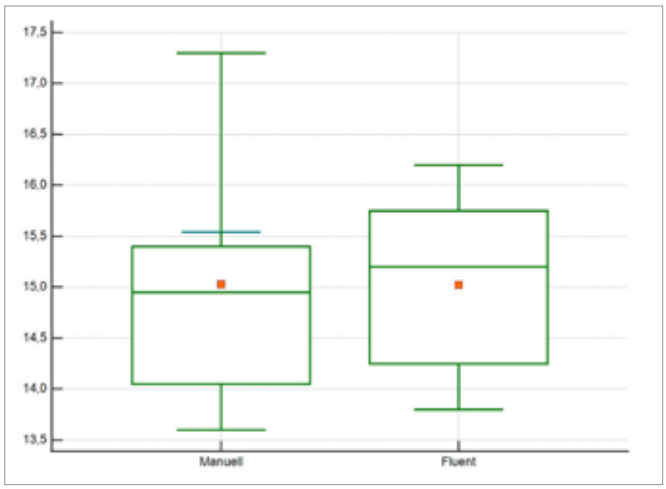

Figure 11 Boxplot illustration of sample results highlighting the variance of manual and automated workflows as well as overall result accordance between manual (Manuell) and automated (Fluent).

CONCLUSION.

The data presented in this application note clearly demonstrates the significant value of a fully automated bead-enrichment and immunoassay workflow, which not only increases precision but also maintains the accuracy of results obtained using the BD-Tau Neuro IP Kit in combination with BD-Tau Luminescence Immunoassay and p217 Tau Luminescence Immunoassay. The manual processing of the BD-Tau Neuro IP Kit is comparably complex, involving numerous intricate and labor-intensive steps such as repeated pipetting and incubation cycles and extensive sample and data management. This high level of manual complexity substantially increases the risk of errors, including pipetting inaccuracies, sample misplacement and carry-over effects, while also demanding considerable hands-on time from laboratory personnel.

By fully automating the immunoassay process on the Tecan Fluent, all these challenging and error-prone manual steps are rendered obsolete. Automation enables a new level of standardization and reproducibility, while streamlining sample handling and tracking, absorbance measurements, data processing and data storage. This minimizes the potential for errors associated with manual workflows and virtually eliminates risks such as incorrect handling and sample carry-over. Compared to manual processing, hands-on time is dramatically reduced – from more than 4 hours to just 20 minutes – freeing laboratory staff for other critical tasks.

In conclusion, the combination of established manual BD-Tau kits with a state-of-the-art automated solution is transforming the efficiency, reliability and quality of complex immunoassay workflows.

ACKNOWLEDGEMENTS.

We would like to sincerely thank Dr. Ingolf Lachmann, CEO of Roboscreen, for carrying out the assay on the Fluent Automation Workstation and providing the data presented in this application note. We would also like to thank Dr. Marcel Debong and Dr. Maike Arndt for providing technical details and reviewing the work presented here.

REFERENCES.

1. Jack, C. R., Jr, Bennett, D. A., Blennow, K., Carrillo, M. C., Dunn, B., Haeberlein, S. B., Holtzman, D. M., Jagust, W., Jessen, F., Karlawish, J., Liu, E., Molinuevo, J. L., Montine, T., Phelps, C., Rankin, K. P., Rowe, C. C., Scheltens, P., Siemers, E., Snyder, H. M., Sperling, R., ... Contributors (2018). NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. Alzheimer's & dementia: the journal of the Alzheimer's Association, 14(4), 535–562. https://doi.org/10.1016/j.jalz.2018.02.018
2. Jack, C. R., Jr, Bennett, D. A., Blennow, K., Carrillo, M. C., Feldman, H. H., Frisoni, G. B., Hampel, H., Jagust, W. J., Johnson, K. A., Knopman, D. S., Petersen, R. C., Scheltens, P., Sperling, R. A., & Dubois, B. (2016). A/T/N: An unbiased descriptive classification scheme for Alzheimer disease biomarkers. Neurology, 87(5), 539–547. https://doi.org/10.1212/WNL.0000000000002923
3. Dubois, B., Villain, N., Frisoni, G. B., Rabinovici, G. D., Sabbagh, M., Cappa, S., Bejanin, A., Bombois, S., Epelbaum, S., Teichmann, M., Habert, M. O., Nordberg, A., Blennow, K., Galasko, D., Stern, Y., Rowe, C. C., Salloway, S., Schneider, L. S., Cummings, J. L., & Feldman, H. H. (2021). Clinical diagnosis of Alzheimer's disease: recommendations of the International Working Group. The Lancet. Neurology, 20(6), 484–496. https://doi.org/10.1016/S1474-4422(21)00066-1
4. Fischer, I., & Baas, P. W. (2020). Resurrecting the Mysteries of Big Tau. Trends in neurosciences, 43(7), 493–504. https://doi.org/10.1016/j.tins.2020.04.007
5. Couchie, D., Mavilia, C., Georgieff, I. S., Liem, R. K., Shelanski, M. L., & Nunez, J. (1992). Primary structure of high molecular weight tau present in the peripheral nervous system. Proceedings of the National Academy of Sciences of the United States of America, 89(10), 4378–4381. https://doi.org/10.1073/pnas.89.10.4378
6. Klafki, H. W., Vogelgsang, J., Manuilova, E., Bauer, C., Jethwa, A., Esselmann, H., Jahn-Brodmann, A., Osterloh, D., Lachmann, I., Breitling, B., Rauter, C., Hansen, N., Bouter, C., Palme, S., Schuchhardt, J., & Wiltfang, J. (2022). Diagnostic performance of automated plasma amyloid-β assays combined with pre-analytical immunoprecipitation. Alzheimer's research & therapy, 14(1), 127. https://doi.org/10.1186/s13195-022-01071-y
7. Klafki, H. W., Morgado, B., Wirths, O., Jahn, O., Bauer, C., Esselmann, H., Schuchhardt, J., & Wiltfang, J. (2022). Is plasma amyloid-β 1-42/1-40 a better biomarker for Alzheimer's disease than AβX-42/X-40?. Fluids and barriers of the CNS, 19(1), 96. https://doi.org/10.1186/s12987-022-00390-4
8. Morgado, B., Klafki, H. W., Bauer, C., Waniek, K., Esselmann, H., Wirths, O., Hansen, N., Lachmann, I., Osterloh, D., Schuchhardt, J., & Wiltfang, J. (2025). Assessment of immunoprecipitation with subsequent immunoassays for the blood-based diagnosis of Alzheimer's disease. European archives of psychiatry and clinical neuroscience, 275(8), 2215–2227. https://doi.org/10.1007/s00406-023-01751-2

About the authors.

Dr. Ingolf Lachmann *is the CEO of our partner company Roboscreen in Leipzig. He studied biology at the Friedrich-Schiller University in Jena and at the University of Leipzig. During his PhD he focused on the fat metabolism in cattle. In his first industry position as Head of R&D at Roboscreen he developed a BSE post-mortem test. This brought him into the field of neurodegenerative diseases and the goal of his work is to develop tests for different, especially new, markers to be able to identify and distinguish between the various forms of neurodegenerative diseases.*

Oliver Schmidt *is product manager for research reagents, neurodegeneration and bone and mineral products at Tecan, IBL International GmbH in Hamburg with more than 20 years' experience in bringing these products to the Life Science and academic community. He studied Environmental Engineering at the Technical University of Hamburg-Harburg and joined Tecan in 2005.*

Dr. Marcel Debong *is field application specialist at Tecan, IBL International GmbH in Germany. He studied food chemistry at the Friedrich-Alexander University of Erlangen-Nuremberg (FAU) in Germany. During his PhD he focused on the analysis of human specimen at the chair of aroma and smell research at the FAU before working in the Irish food industry as an analytical scientist. He joined Tecan in May 2024 and focuses on LC-MS applications as well as on applications on the Tecan Fluent.*

LEARN MORE

For research Use Only. Not for use in diagnostic procedures.

Australia +61 3 9647 4100 **Austria** +43 62 46 89 330 **Belgium** +32 15 42 13 19 **China** +86 21 220 63 206 **France** +33 4 72 76 04 80 **Germany** +49 79 51 94 170
Italy +39 02 92 44 790 **Japan** +81 44 556 73 11 **Netherlands** +31 18 34 48 17 4 **Nordic** +46 8 750 39 40 **Singapore** +65 644 41 886 **South Korea** +82 2 818 3301 **Spain** +34 93 595 25 31
Switzerland +41 44 922 89 22 **UK** +44 118 9300 300 **USA** +1 919 361 5200 **Other countries** +41 44 922 81 11

Tecan Group Ltd. makes every effort to include accurate and up-to-date information within this publication; however, it is possible that omissions or errors might have occurred. Tecan Group Ltd. cannot, therefore, make any representations or warranties, expressed or implied, as to the accuracy or completeness of the information provided in this publication. Changes in this publication can be made at any time without notice. For technical details and detailed procedures of the specifications provided in this document please contact your Tecan representative. This brochure may contain reference to applications and products which are not available in all markets. Please check with your local sales representative.

All mentioned trademarks are protected by law. In general, the trademarks and designs referenced herein are trademarks, or registered trademarks, of Tecan Group Ltd., Mannedorf, Switzerland. A complete list may be found at www.tecan.com/trademarks. Product names and company names that are not contained in the list but are noted herein may be the trademarks of their respective owners.

Tecan Flexible Channel Arm™ (FCA), Robotic Gripper Arm™ (RGA), Magellan™ and Fluent Control™ are registered trademarks of Tecan Group Ltd., Männedorf, Switzerland.

© 2026, Tecan Trading AG, Switzerland, all rights reserved. For disclaimer and trademarks please visit www.tecan.com.

403521 V1.0, 2026-07

www.tecan.com

TECAN.