Research Article
Volume 9:2, 2021

Neurological Disorders

ISSN: 2329-6895

Open Access

Using Immunomagnetic Reduction to Assay Reagent Stability of Biomarkers Associated with Alzheimer's Disease

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Abstract

Plasma biomarker assays have become a trend for risk evaluation in Alzheimer's disease. Several studies have been performed to explore their preclinical performance. However, there are very few studies on the storage stability of the reagents used in these assays. The determination of the storage stability of reagents is important because reagents may be stored for a few months prior to end use. In this work, the stability of the reagents used for assaying plasma amyloid β $_{1-40}$ ($Aβ_{1-40}$), $Aβ_{1-42}$ and total tau protein (Tau) was assessed using immunomagnetic reduction. Reagents immediately after synthesis and reagents in opened vials were used to assay the concentrations of $Aβ_{1-40}$, $Aβ_{1-42}$ and Tau in human plasma samples. The recovery rates of the concentrations of biomarkers at different times after synthesis of open vial were calculated to determine the period of stability of reagents. The results showed that the reagents stored at 2°C-8°C were stable for at least 52 weeks. The reagents in open vials were stable for at least six weeks. These stabilities indicate that the reagents used to assay plasma $Aβ_{1-40}$, $Aβ_{1-42}$ and Tau levels are verifiably qualified for clinical use.

Keywords: Reagent stability • Plasma • Alzheimer's disease • Immunomagnetic reduction

Introduction

Biofluid biomarker assays associated with Alzheimer's disease (AD) have become common in risk evaluation [1-3]. As pathological hallmarks of Alzheimer's disease, the quantitative detection of amyloid β 1-40 ($Aβ_{1-40}$), $Aβ_{1-42}$ and total tau protein (Tau) in cerebrospinal fluid (CSF) has attracted the interest of researchers and neurologists. Many studies have validated that the levels of these CSF biomarkers significantly correlate with the clinical diagnosis of AD [4-9]. For example, the level of CSF $Aβ_{1-42}$ decreases in AD, and the level of CSF tau increases in AD compared with their levels in normal controls [10-12]. Additionally, the concentration ratio of CSF $Aβ_{1-42}$ to $Aβ_{1-40}$ shows good consistency with the standard uptake value ratio from positron emission tomography (PET) [13-15]. Although CSF biomarker assays have clinical significance, CSF sampling by lumbar puncture remains a significant burden in practice.

Successes in developing ultrasensitive technologies for immunoassays have enabled feasible assays for extremely low plasma concentrations of $Aβ_{1-40}$, $Aβ_{1-42}$ and Tau [16-19]. Relationships between CSF biomarkers and plasma biomarkers have been demonstrated in AD [20]. The levels of composited $Aβ_{1-42}$ and Tau are promising indices for use in distinguishing amnesic mild cognitive impairment (aMCI) and early-stage AD compared to normal controls [21-24]. Additionally, the baseline levels of composited plasma $Aβ_{1-42}$ and Tau in aMCI predict cognitive decline in 1-1.5 years [25]. The level of plasma Tau is elevated in subjects showing brain atrophy in images created with magnetic resonance imaging [26]. The plasma $Aβ_{1-42}$-to-$Aβ_{1-40}$ ratio significantly differs between amyloid PET-negative and amyloid PET-positive AD [27,28]. Clinical evidence has revealed the promising utility of plasma biomarkers in assisting AD diagnoses.

Among the ultrasensitive technologies for assaying plasma $Aβ_{1-40}$, $Aβ_{1-42}$ and Tau, immunomagnetic reduction (IMR) reagent kits have been documented with CE data for *in vitro* diagnosis (IVD) and approved by the Taiwan Food and Drug Administration (TFDA). The IMR reagents for $Aβ_{1-40}$, $Aβ_{1-42}$ and Tau have been applied in research and clinical uses in Europe, the Middle East, Southeast Asia, and Taiwan. In the past, preclinical assays, such as the hook effect, assay detection limit, assay linearity, precision and repeatability, spike recovery rate, dilution recovery rate, and interference, of IMR reagents for $Aβ_{1-40}$, $Aβ_{1-42}$ and Tau were investigated by following guidelines issued by Clinical and Laboratory Standards Institute and ICH Q2(R1) [20,29]. In this work, the stability of these reagents was examined based on their typical prolonged delivery and storage periods after synthesis. Reagent stability is an important issue in the clinic and includes storage stability and open-vial stability [30,31].

Materials and Methods

Test of storage stability of IMR reagents

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Received date: 03 February, 2021; Accepted date: 17 February, 2021; Published date: 24 February, 2021

Each IMR reagent, e.g., for $A\beta_{1-40}$ (MF-AB0-0060, MagQu), was immediately aliquoted after synthesis and sealed. The storage period of the IMR reagent after synthesis is referred to as the zero point. One aliquot was used for assaying $A\beta_{1-40}$ in PBS samples spiked with 100 pg/ml $A\beta_{1-40}$ (A1075, Sigma-Aldrich). The aliquots of the other PBS samples were stored at 2°C-8°C. Then, 80 µl of the IMR $A\beta_{1-40}$ reagent was extensively mixed with 40 µl of the 100-pg/ml-$A\beta_{1-40}$-PBS sample in a sample assay tube. Each aliquoted reagent was not opened until the IMR measurement was taken. An IMR analyzer (XacPro-S, MagQu) was used to assay $A\beta_{1-40}$. At different times after synthesis, one aliquot and the 100-pg/ml $A\beta_{1-40}$-PBS sample were placed at room temperature from 5 to 10 minutes, followed by mixing reagent and sample in a test tube for assaying $A\beta_{1-40}$. Similar processes were performed for assaying $A\beta_{1-42}$ in a 100-pg/ml-$A\beta_{1-42}$-PBS sample (A9810, Sigma-Aldrich) and Tau in a 100-pg/ml-Tau-PBS sample (T7951, Sigma-Aldrich). The reagent volumes for assaying $A\beta_{1-42}$ and Tau were 60 and 80 µl, respectively. The sample volumes for assaying $A\beta_{1-42}$ and Tau were 60 and 40 µl, respectively. For each biomarker assay, duplicated measurements were performed. Averaged values of the measured concentrations are reported.

Test of open-vial stability of IMR reagents

Before the IMR assay was performed, the reagent for $A\beta_{1-42}$ (MF-AB2-0060, MagQu) originally stored at 4°C was moved to room temperature. After 10 minutes, the reagent bottle was opened, and the reagent was left at room temperature for an additional 15 minutes. A portion of the reagent was used for IMR measurements of human plasma (HP1051PK3, Valley Biomedical), and the remainder was stored at 4°C. Then, 60 µl of the IMR $A\beta_{1-42}$ reagent was thoroughly mixed with 60 µl of human plasma in the sample assaying tube. An IMR analyzer (XacPro-S, MagQu) was used to assay $A\beta_{1-42}$. Measurements of each sample were repeated. The averaged value was calculated and is reported. IMR measurements of the reagent were performed every two weeks for six weeks. Similar processes were performed for assaying $A\beta_{1-40}$ and Tau in human plasma. The reagent volumes for assaying $A\beta_{1-40}$ and Tau were 80 µl. The sample volumes for assaying $A\beta_{1-40}$ and Tau were 40 µl. For each biomarker assay, duplicated measurements were performed. Averaged values of the measured concentrations are reported.

Evaluation of reagent stability

Reagent stability was evaluated by calculating the recovery rate of the measured concentration at a given time with respect to the baseline concentration:

"Recovery rate=" "Measured concentration at a given time" /"Measured concentration at baseline" x100% (1)

Once the recovery rate exceeded 110% or decreased below 90%, the measured concentrations at a given time and baseline was considered significantly distinct. Thus, reagents were considered stable at recovery rates between 110% and 90%.

Results

Storage stability of IMR reagents

The measured concentrations of $A\beta_{1-40}$, $A\beta_{1-42}$ and Tau at different times after synthesis are listed in Table 1. For $A\beta_{1-40}$, aliquoted $A\beta_{1-40}$ reagents were assayed for 53 weeks to determine $A\beta_{1-40}$ levels in 100-pg/ml-$A\beta_{1-40}$-PBS samples. The baseline (week 0) concentration was 94.52 pg/ml, which was close to the spiked concentration, i.e., 100 pg/ml. The concentrations from week 1 to week 53 were between 93.76 and 99.89 pg/ml. The recovery rates during the 53 weeks were calculated via Eq. (1) and are shown in Table 1. The highest and lowest recovery rates were 105.7% and 99.2%, respectively. These recovery rates were between 90% and 110% for the $A\beta_{1-40}$ reagent stored at 2°C-8°C for 53 weeks.

The measured concentration of $A\beta_{1-42}$ in the 100 pg/ml-$A\beta_{1-42}$-PBS sample using IMR $A\beta_{1-42}$ reagent at baseline (week 0) was 100.35 pg/ml, as listed in Table 1. The measured concentrations of $A\beta_{1-42}$ during a storage period of 68 weeks were between 96.41 and 100.80 pg/ml, which corresponded with recovery rates from 96.1% to 100.4%. The results showed that the IMR $A\beta_{1-42}$ reagent stored at 2°C-8°C was stable for at least 68 weeks.

Biomarker	Storage period after synthesis (week)	Measured concentration (pg/ml)	Recovery rate (%)
$A\beta_{1-40}$	0	94.52	-
	4	99.89	105.7
	8	94.9	100.4
	12	98.95	104.7
	16	98.41	104.1
	20	96.86	102.5
	24	97.74	103.4
	28	94.61	100.1
	32	93.76	99.2
	36	94.64	100.1
	53	99.81	105.6
$A\beta_{1-42}$	0	100.35	-
	4	99.38	99
	8	96.41	96.1
	12	100.55	100.2
	16	100.56	100.2
	20	100.54	100.2
	24	100.28	99.9
	32	100.51	100.2
	36	100.8	100.4
	40	100.47	100.1
	44	100.26	99.9
	48	100.25	99.9
	52	100.13	99.8
	68	100.01	99.7
Tau	0	98.61	-
	4	93.76	95.1
	8	93.98	95.3
	12	102.6	104
	16	100.86	102.3
	20	101.98	103.4
	24	100.62	102
	28	100.06	101.5
	32	100.71	102.1
	36	100.52	101.9
	44	100.25	101.7
	48	99.83	101.2
	52	99.94	101.3
	54	100.37	101.8

Table 1. Variation in the measured $A\beta_{1-40}$, $A\beta_{1-42}$, and Tau concentrations in PBS samples measured at different times after synthesis.

For the Tau reagent, the baseline concentration of the 100-pg/ml-Tau-PBS sample was 98.61 pg/ml when the IMR Tau reagent was used. The monitoring period of the Tau reagent was 54 weeks. The measured concentrations of Tau during the storage period ranged from 93.76 to 102.6 pg/ml. The recovery rates obtained via Eq. (1) ranged from 95.1% to 104%, which was within the acceptable range of 90% – 110%. Hence, the IMR Tau reagent was stable when stored at 2°C-8°C for 54 weeks.

Open-vial stability of IMR reagents

IMR measurements using IMR $A\beta_{1-40}$, $A\beta_{1-42}$ and Tau reagents were performed every two weeks after vial opening. Between successive measurements, the reagents were stored at 2°C-8°C, thereby subjecting the reagents to cool/thaw cycles between tests.

Immediately after the vial was opened, the baseline human plasma IMR measurement of $A\beta_{1-40}$ was 52.07 pg/ml, as listed in Table 2. The measured concentrations at weeks 2, 4, and 6 after vial opening were 53.41, 51.99, and 53.54 pg/ml, respectively, with corresponding recovery rates of 102.6%, 99.8% and 102.8%. This implied no significant differences in the measured $A\beta_{1-40}$ concentrations in human plasma of open vial IMR $A\beta_{1-40}$ reagents stored at 2°C-8°C for 6 weeks.

Biomarker	Storage period after open vial (week)	Measured concentration (pg/ml)	Recovery rate (%)
$A\beta_{1-40}$	0	52.07	-
	2	53.41	102.6
	4	51.99	99.8
	6	53.54	102.8
$A\beta_{1-42}$	0	16.22	-
	2	16.12	99.4
	4	16.39	101
	6	16.26	100.9
Tau	0	20.79	-
	2	20.54	98.8
	4	20.55	98.8
	6	21.31	102.5

Table 2. Variations in the measured $A\beta_{1-40}$, $A\beta_{1-42}$ and Tau concentrations in the human plasma sample measured at different times after opening the vial.

Measured $A\beta_{1-42}$ concentrations in human plasma using the IMR $A\beta_{1-42}$ reagent immediately after vial opening and 2, 4, and 6 weeks after vial opening were 16.22, 16.12, 16.39, and 16.26 pg/ml, respectively, with corresponding recovery rates (as calculated via Eq. (1)) of 99.4% at week 2, 101.0% at week 4, and 100.9% at week 6. Similar to the stability of the IMR $A\beta_{1-40}$ reagent, the open-vial IMR $A\beta_{1-42}$ reagent was stable for no less than 6 weeks.

The Tau concentration was measured as 20.79 pg/ml at baseline in human plasma. Concentrations measured 2, 4, and 6 weeks after vial opening were 20.54, 20.55, and 21.31 pg/ml, respectively. The recovery rates ranged from 98.8% to 102.5%, which confirmed that the stability of this IMR Tau reagent in an open vial can persist for at least than 6 weeks.

Discussion

In testing the storage stability of the reagents, the IMR measurements of $A\beta_{1-40}$, $A\beta_{1-42}$, and Tau in spiked PBS samples were terminated at the 53rd, 68th, and 54th weeks, respectively. These endpoints did not indicate that the stability of the $A\beta_{1-40}$, $A\beta_{1-42}$, or Tau reagents decreased after 53, 68, and 54 weeks. Clinical practice does not necessitate the storage of these reagents at 2°C-8°C beyond one year; therefore, monitoring the storage stability of the $A\beta_{1-40}$, $A\beta_{1-42}$, and Tau reagents is not necessary after weeks 53, 68, and 54. Overall, the results indicated that the stability of the $A\beta_{1-40}$, $A\beta_{1-42}$, and Tau reagents stored for 53, 68, and 54 weeks at 2°C-8°C was sufficient for clinical use.

In addition to IMR measurements, the reagents were investigated by visual inspection to observe the precipitation of magnetic nanoparticles. The IMR reagents consisted of antibody-functionalized magnetic Fe_3O_4 nanoparticles dispersed in PBS solution. The mean diameter of the nanoparticles was approximately 55 nm. In reagent preparation, the surfactant, i.e., dextran, of the Fe_3O_4 nanoparticles was oxidized to add aldehyde groups (i.e.,-CHO) [30]. Dextran covalently associates with antibodies via-CH=N-. Unbound aldehyde groups on dextran were then reduced. Without dextran, the nanoparticles would easily aggregate and precipitate in the reagents. Unbound antibodies were separated from reagent through magnetic separation. If these antibody immobilization steps are not followed, then the magnetic nanoparticles aggregate and precipitate. Thus, the total binding area covered by the antibody and antigen is reduced. The reagent characterizations were changed. During the storage stability tests of the IMR $A\beta_{1-40}$, $A\beta_{1-42}$, and Tau reagents, no nanoparticle precipitation was observed. This indicated that the antibody-functionalized magnetic nanoparticles were stably and well-dispersed in the reagents. Reagent preparation was well controlled and resulted in highly stable IMR reagents. As shown in Table 1, the suspensions of magnetic nanoparticles biofunctionalized with antibodies against $A\beta_{1-40}$, $A\beta_{1-42}$, and Tau in the reagents were stable for 53, 68, and 54 weeks, respectively. The long-term stability of antibody-functionalized magnetic nanoparticle suspensions in the IMR reagents has also been observed for c-reactive protein, carcinoembryonic antigen, vascular endothelial growth factor, human hemoglobin (Hb), human HbA1c, and des-gamma-carboxy prothrombin as previously reported.

In the open-vial stability tests, the reagent was stored at 2°C-8°C after synthesis. To perform the test at week 0, the reagent was moved from a refrigerator to a bench, i.e., from 2°C-8°C to room temperature. Reagent (40 or 60 µl) was used for IMR measurement. The remainder of the reagent was returned to the refrigerator at 2°C-8°C. Thus, the reagent experienced cool-down/thaw cycles. The reagent was subjected to a cool-down/thaw cycle before each IMR test. The reagent was tested 0, 2, 4, and 6 weeks after synthesis and experienced 1, 2, 3 and 4 cool-down/thaw cycles, respectively. Table 2 shows no significant differences in the measured concentrations of plasma $A\beta_{1-40}$, $A\beta_{1-42}$, and Tau using the IMR $A\beta_{1-40}$, $A\beta_{1-42}$, and Tau reagents at weeks 0, 2, 4, and 6. These results implied that the reagent quality did not significantly change or degrade over 4 cool-down/thaw cycles.

Conclusion

The stability of reagents used for assaying $A\beta_{1-40}$, $A\beta_{1-42}$, and Tau via immunomagnetic reduction persisted for at least 53, 68, and 54 weeks at 2°C-8°C. The reagents in open vials were stable for at least 6 weeks. Moreover, the quality of these reagents did not significantly change or degrade when subjected to 4 cool-down/thaw cycles. These results showed that these IMR reagents remained sufficiently stable for clinical use.

Acknowledgment

None.

Statement of Ethics

No human sample is used in this study.

Conflict of Interest Statement

H.C. Liu, C.Y. Lin, H.H. Chen, C.S. Ho and S.Y. are employees of MagQu Co., Ltd. H.H. Chen and S.Y. Yang are shareholders of MagQu Co., Ltd.

Funding Sources

The authors have no funding sources to declare.

Author Contributions

H.C. Liu and C.J. Hu designed the study. C.Y. Lin and H.H. Chen performed the IMR measurements. Chia-Shin Ho prepared reagents. Shieh-Yueh Yang conducted statistics and prepared the manuscript.

References

1. Carlyle, BC, Trombetta BA and Arnold SE. "Proteomic approaches for the discovery of biofluid biomarkers of neurodegenerative dementias." *Proteomes* (2018) 6: 32.
2. Kaipainen, A, Jääskeläinen O, Liu Y and Haapalinna F, et.al. "Cerebrospinal fluid and MRI biomarkers in neurodegenerative diseases: A retrospective memory clinic-based study" *J Alz Dise* (2020) 75: 751-65.
3. Irwin, DJ, Trojanowski JQ and Grossman M. "Cerebrospinal fluid biomarkers for differentiation of frontotemporal lobar degeneration from Alzheimer's disease." *Front Aging Neurosci* (2013) 5: 6-1-11.
4. Boumenir, A, Cognat E, Sabia S, and Hourregue C, et.al. "CSF level of βamyloid peptide predicts mortality in Alzheimer's disease" *Alz Res Ther* (2019) 11: 29-1-9.
5. Bibl, M, Mollenhauer B, Esselmann H, and Lewczuk P, et.al. "CSF amyloid-beta-peptides in Alzheimer's disease, dementia with Lewy bodies and Parkinson's disease dementia" *Brain* (2016) 129: 1177-1187.
6. Andreasen, N, Hesse C, Davidsson P, and Minthon L, et.al. "Cerebrospinal fluid β-amyloid(1-42) in Alzheimer disease: Differences between early and late-onset Alzheimer disease and stability during the course of disease." *Arch Neurol* (1999) 56: 673-680.
7. Tapiola, T, Alafuzoff I, Herukka SK, and Parkkinen L, et.al. "Cerebrospinal fluid β-amyloid 42 and tau proteins as biomarkers of Alzheimer-type pathologic changes in the brain." *Arch Neurol* (2009) 66: 382-389.
8. Ritchie, C, Smailagic N, Noel-Storr AH, and Ukoumunne O, et.al. "CSF tau and the CSF tau/Aβratio for the diagnosis of Alzheimer's disease dementia and other dementias in people with mild cognitive impairment (MCI)" *Cochrane Database Syst Rev* (2017) 22: 3.
9. de Souzaa, LC, Chupina M, Lamarie F, and Jardel C, et.al. "Cerebrospinalfluid biomarkers for differentiation of frontotemporal lobar degeneration from Alzheimer's disease" *Front Aging Neurosci* (2013) 5: 6-1-11.
10. Dayon, L, Galindo AN, Wojcik J, and Cominetti O, et.al. "Alzheimer disease pathology and the cerebrospinal fluid proteome." *Alz Res Ther* (2018) 10: 66-1-12.
11. Galasko, D, Chang L, Motter R, and Clark CM, et.al. "High cerebrospinal fluid Tau and low amyloid β42 levels in the clinical diagnosis of Alzheimer disease and relation to Apolipoprotein E genotype" *Arch Neurol* (1998) 55:937–945.
12. Sunderland, T, Linker G, Mirza N, and Putnam KT, et.al. "Decreased β-Amyloid1-42 and increased Tau levels in cerebrospinal fluid of patients with Alzheimer disease." *J American Medi Asso* (2003) 289: 2094-2103.
13. Riemenschneider, M, Schmolke M, Lautenschlager N, and Guder WG, et.al. "Cerebrospinal βamyloid (1–42) in early Alzheimer's disease: association with apolipoprotein E genotype and cognitive decline." *Neurosci Lett* (2000) 284: 85-88,
14. Spallazzi, M, Barocco F, Michelini G, and Immovilli P, et.al. "CSF biomarkers and amyloid PET: concordance and diagnostic accuracy in a MCI cohort." *Acta Neurol Belg* (2019) 119: 445-452.
15. Ben Bouallègue, F, Mariano-Goulart D and Payoux P. "Alzheimer's Disease Neuroimaging Initiative (ADNI). Comparison of CSF markers and semi-quantitative amyloid PET in Alzheimer's disease diagnosis and in cognitive impairment prognosis using the ADNI-2 database." *Alz Res Ther* (2017) 26: 32-1-13.
16. Niemantsverdriet, E, Ottoy J, Somers C, and De Roeck, et.al. "The cerebrospinal fluid Aβ1-42/$Aβ_{1-40}$ ratio improves concordance with amyloid-PET for diagnosing Alzheimer's disease in a clinical setting." J *Alz Dis* (2017) 60: 561-576.
17. Lachno, DR, Emerson JK, Vanderstichele H, and Gonzales C, et.al. "Validation of a multiplex assay for simultaneous quantification of amyloid-β peptide species in human plasma with utility for measurements in studies of Alzheimer's disease therapeutics." *J Alz Dis* (2012) 32: 905-918.
18. Baird, AL, Westwood S and Lovestone S "Blood-based proteomic biomarkers of Alzheimer's disease pathology." *Front Neurol* (2015) 6: 236.
19. Lue, LF, Guerra A and Walker DG "Amyloid beta and tau as Alzheimer's disease blood biomarkers: Promise from new technologies" *Neurol Ther* (2017) 6: 25-36.
20. Nakamura, A, Kaneko N, Villemagne VL, and Kato T, et.al. "High performance plasma amyloid-β biomarkers for Alzheimer's disease." *Nature* (2018) 554: 249-254.
21. Teunissen, CE, Chiu MJ, Yang CC and Yang SY, et al. "Plasma amyloid-β($Aβ_{42}$) correlates with cerebrospinal fluid $Aβ_{42}$ in Alzheimer's disease." *J Alz Dis 62* (2018): 1857-1863.
22. Chiu, MJ, Yang SY, Horng HE, and Yang CC, et.al. "Combined plasma biomarkers for diagnosing mild cognition impairment and Alzheimer's disease." *ACS Chem Neurosci* (2013) 4: 1530-1536.
23. Chiu, MJ, Chen TF, Hu CJ, and Yan SH, et.al. "Nannoparticle-based immunomagnetic assay of plasma biomarkers for differentiating dementia and prodromal states of Alzheimer's disease: A cross-validation study." *Nanomed Nanotechnol Biol Med* (2020) 26: 102182-1-9.
24. Jiao, F, Yi F, Wang Y, and Zhang S, et.al. "The validation of multifactor model of plasma Aβ2 and total-Tau in combination with MoCA for diagnosing probable Alzheimer disease." *Front Aging Neurosci* (2020) 12: 212-1-9.
25. Lue, LF, Sabbagh MN, Chiu MJ, and Jing N, et.al "Plasma levels of Aβ42 and Tau identified probable Alzheimer's dementia: Findings in two cohorts." *Front Aging Neurosci* (2017) 9: 226-1-9.
26. Chen, TB, Lee YJ, Lin SY, and Chen JP, et.al. "Plasma $Aβ_{42}$ and total Tau predict cognitive decline in amnestic mild cognitive impairment." *Sci Reports* (2019) 9: 13984-1-10.
27. Chiu, MJ, Chen YF, Chen TF, and Yang SY, et.al. "Plasma tau as a window to the brain-negative associations with brain volume and memory function in mild cognitive impairment and early Alzheimer's disease." *Hum Brain Mapp* (2014) 35: 3132-3142.
28. Tzen, KY, Yang SY, Chen TF, and Cheng, et.al. "Plasma Aβ but not tau related to brain PiB retention in early Alzheimer's disease." *ACS Chem Neurosci 5* (2014):830-836.
29. Lin, SY, Lin KJ, Lin PC, and Huang CC, et.al. "Plasma amyloid assay as a pre-screening tool for amyloid positron emission tomography imaging in early stage Alzheimer's disease." *Alz Res Ther* (2019) 11: 111-1-10
30. Yang, SY, Chiu MJ, Chen TF, and Lin CH, et.al. "Analytical performance of reagent for assaying tau protein in human plasma and feasibility study screening neurodegenerative diseases." *Sci Reports* (2017) 7: 9304-1-12.
31. Horng, HE, Yang SY, Huang YW, and Jiang WQ, et.al. "Nanomagnetic particles for SQUID-based magnetically labeled immunoassay." *IEEE Trans Appl Supercond* (2005) 15: 668-671.