2.7.1.4.2 ヒト CSF 中レカネマブ濃度測定法要約

	Method Validation #1	Method Validation #2	Method Validation #3
Analyte	Lecanemab	Lecanemab	Lecanemab
Validation type	Full	Full	Partial
eCTD reference number	5.3.1.4	5.3.1.4	5.3.1.4
Method ID	tcam-052.03 (Report No. tcar10-050; tcar10-050 addendum1)	btm-1416-r0 (Report No. eis-r1663; eis-r1663a1r1)	btm-1416-r1 (Report No. eis-r1663a2, eis-r1663a3, eis-r1663a4)
Duration of time method is in use	[redacted] 20[redacted] to [redacted] 20[redacted]	[redacted] 20[redacted] to [redacted] 20[redacted]	[redacted] 20[redacted] to [redacted] 20[redacted]
Bioanalytical site	[redacted] USA	[redacted]	[redacted]
Matrix	Human CSF		
Platform	ECL-MSD	LC-MS/MS	
Format	In the method, the amount of exogenously administered BAN2401 in human CSF samples was measured using a quantitative ECL assay.	An immunopurification and LC-MS/MS method for the determination of BAN2401 in human CSF. The tryptic digest peptide of BAN2401, HT9 was monitored as a surrogate for BAN2401. [redacted] was used as the IS and the tryptic digest peptide of [redacted], [redacted] (amino acid sequence) was monitored as a surrogate.	
Stock reference, lot number, expiration date	Biotinylated protofibrils (Lot# E090407 [expiry date not available]) from Eisai Inc. BAN2401 (Lot# [redacted] [[redacted] 20[redacted]]) from Eisai, Inc. anti-BAN2401 antibody (Lot# TB1039 No.1 [redacted] 20[redacted]]) from [redacted] Goat anti-rabbit Sulfo-Tag (Lot# W00133881 [redacted] 20[redacted]]) from [redacted] human CSF pool (Lot# BRH371118, BRH469184 [both [redacted] 20[redacted]]) from [redacted] human CSF AD Individuals (Lot# 8045, 8062, 8091, 8092, 8200 [all [redacted] 20[redacted]] from [redacted]) and human CSF	BAN2401 by Eisai, Lot# [redacted] ([redacted] 20[redacted]); [redacted] by Eisai, Lot# W0005265 ([redacted] 20[redacted])	BAN2401 by Eisai, Lot# [redacted] ([redacted] 20[redacted]); [redacted] by Eisai, Lot# 13-0092-D-ARS ([redacted] 20[redacted])

2.7.1.4.2 ヒト CSF 中レカネマブ濃度測定法要約

	Method Validation #1	Method Validation #2	Method Validation #3
	matched age elderly individuals (Lot# 7090003, 7090007, 709022, 709033, 709055 [all [redacted] 20[redacted]]) from [redacted]		
Calibration range from the lower limit of quantitation (LLOQ) to the upper limit of quantitation (ULOQ)	3.00 to 75.0 ng/mL	5.00 to 500.0 ng/mL	5.00 to 500.0 ng/mL
Matrix study population	Normal or AD CSF	Normal or AD CSF	Normal CSF
Link to reports and applicable amendments	tcar10-050 ([redacted] 20[redacted]) tcar10-050 addendum1 ([redacted] 20[redacted])	eis-r1663 ([redacted] 20[redacted]) eis-r1663a1r1 ([redacted] 20[redacted])	eis-r1663a2 ([redacted] 20[redacted]) eis-r1663a3 ([redacted] 20[redacted]) eis-r1663a4 ([redacted] 20[redacted])
Synopsis of amendment history	tcar10-050 addendum1 ([redacted] 20[redacted]): long-term stability for ban2401 in human CSF at -70°C nominal for 94 days was accepted. tcar10-050 ([redacted] 20[redacted]): initial report	NA	NA

AD = Alzheimer's disease, ID = identification, CSF = cerebrospinal fluid, ECL = electrochemiluminescence, eCTD = electronic common technical document, IS = internal standard, LC-MS/MS = liquid chromatography with tandem mass spectrometry, LLOQ = lower limit of quantitation, MSD = meso scale discovery, NA = not applicable, PK = pharmacokinetic, ULOQ = upper limit of quantitation.
Source: tcar10-050, tcar10-050 addendum1, eis-r1663, eis-r1663a1r1, eis-r1663a2, eis-r1663a3, eis-r1663a4.

2.7.1.4.2.1 ヒト CSF 中レカネマブ濃度測定法要約

	Clinical Study #1 (BAN2401-A001-101)	Clinical Study #2 (BAN2401-J081-104)	Clinical Study #3 (BAN2401-G000-201 Core only)
Analyte	Lecanemab	Lecanemab	Lecanemab
Validation type	In-study	In-study	In-study
eCTD reference number	5.3.5.1	5.3.5.1	5.3.5.1
Method ID	tcam-052	btm-1416-r0	btm-1416-r0 btm-1416-r1
Duration of time method is in use	[redacted] 20[redacted] to [redacted] 20[redacted]	[redacted] 20[redacted] to [redacted] 20[redacted]	[redacted] 20[redacted] to [redacted] 20[redacted]
Bioanalytical site	[redacted] USA	[redacted]	[redacted]
Matrix	Human CSF		
Platform	ECL-MSD	LC-MS/MS	
Format	In the method, the amount of exogenously administered BAN2401 in human CSF samples was measured using a quantitative ECL assay.	An immunopurification and LC-MS/MS method for the determination of BAN2401 in human CSF. The tryptic digest peptide of BAN2401, HT9 was monitored as a surrogate for BAN2401. [redacted] was used as the IS and the tryptic digest peptide of [redacted], [redacted] (amino acid sequence) was monitored as a surrogate.	
Stock reference, lot number, expiration date	BAN2401 ([redacted] [redacted] 20[redacted]) from Eisai Inc; Anti-BAN2401 (TB1039 No. 1 [redacted] 20[redacted]) from Eisai Inc; biotinylated protofibril (E090407 [NA]) from Eisai Inc; human CSF (BRH484882 [redacted] 20[redacted]) from [redacted]	BAN2401 ([redacted] [redacted] 20[redacted]) from Eisai Inc; [redacted] (W0005265 [redacted] 20[redacted]).	BAN2401 ([redacted] [redacted]) from Eisai [redacted] (W0005265 [redacted] 20[redacted]).
Calibration range from the lower limit of quantitation (LLOQ) to the upper limit of quantitation (ULOQ)	3.00 ng/mL to 75.0 ng/mL	5.00 ng/mL to 500.0 ng/mL	5.00 ng/mL to 500.0 ng/mL
Matrix study population	Subjects with mild to moderate AD	Subjects with mild cognitive impairment due to AD and mild AD	Subjects with early AD
Link to reports and applicable amendments	tcar10-051 ([redacted] 20[redacted])	eis-r2740 ([redacted] 20[redacted])	eis-r3653r1 ([redacted] 20[redacted])

2.7.1.4.2.1 ヒト CSF 中レカネマブ濃度測定法要約

	Clinical Study #1 (BAN2401-A001-101)	Clinical Study #2 (BAN2401-J081-104)	Clinical Study #3 (BAN2401-G000-201 Core only)
Synopsis of amendment history	NA	NA	eis-r3653r1 ([redacted] 20[redacted]): update to number of samples analyzed and samples not reported in Table 7 eis-r3653 ([redacted] 20[redacted]): initial report

AD = Alzheimer's disease, ID = identification, CSF = cerebrospinal fluid, ECL = electrochemiluminescence, eCTD = electronic Common Technical Document, HPLC = high performance liquid chromatography, IS = internal standard, LC-MS/MS = liquid chromatography with tandem mass spectrometry, LLOQ = lower limit of quantitation, MSD = meso scale discovery, NA = not applicable, PK = pharmacokinetic, ULOQ = upper limit of quantitation.
Source: tcar10-051, eis-r2740, eis-r3653r1.

2.7.1.4.2.2 ヒト CSF 中レカネマブ濃度測定法要約

	Clinical Study #4 BAN2401-G001-301 Core and OLE Phase
Analyte	Lecanemab
Validation type	NA
eCTD reference number	5.3.5.1
Method ID	btm-1416-r1
Duration of time method is in use	[redacted] 20[redacted] to [redacted] 20[redacted]
Bioanalytical site	[redacted]
Matrix	CSF
Platform	LC-MS/MS
Format	An immunopurification and LC-MS/MS method for the determination of BAN2401 in human CSF. The tryptic digest peptide of BAN2401, HT9 was monitored as a surrogate for BAN2401. [redacted] was used as the IS and the tryptic digest peptide of [redacted], [redacted] (amino acid sequence) was monitored as a surrogate.
Stock reference, lot number, expiration date	BAN2401 by Eisai Co., Ltd., Lot# [redacted], [redacted] 20[redacted] [redacted] by 13-0092-D-ARS, [redacted] 20[redacted]
Calibration range from the lower limit of quantitation (LLOQ) to the upper limit of quantitation (ULOQ)	5.00 to 500.0 ng/mL
Matrix study population	Subjects with early AD
Link to reports and applicable amendments	110-r10680 ([redacted] 20[redacted])
Synopsis of amendment history	NA

AD = Alzheimer's disease, ID = identification, CSF = cerebrospinal fluid, eCTD = electronic common technical document, LC-MS/MS = liquid chromatography with tandem mass spectrometry, LLOQ = lower limit of quantitation, NA = not applicable, PK = pharmacokinetic, ULOQ = upper limit of quantitation.
Source: 110-r10680.

2.7.1.4.3 ヒト血清中 ADA 測定法要約

	Method Validation #1	Method Validation #2	Method Validation #3	Method Validation #4	Method Validation #5
Analyte	Anti-BAN2401 antibodies	Anti-BAN2401 antibodies	Anti-BAN2401 antibodies	Anti-BAN2401 antibodies	Anti-BAN2401 antibodies
Validation type	Full	Full	Partial	Full	Full
eCTD reference number	5.3.1.4	5.3.1.4	5.3.1.4	5.3.1.4	5.3.1.4
Method ID	tcam-022 (Report No. tcar10-069)	tcam-082 (Report No. tcar12-038)	tliam-14225 (Report No. tnjr14-225)	bam-0039 (Report No. bav-0039-r v00)	45-1807 transfer to [redacted] (Report No. 45-1807)
Duration of time method is in use	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Bioanalytical site	[redacted] USA	[redacted] USA	[redacted]	[redacted]	[redacted]
Matrix	Human Serum				
Platform	ECL on Meso Scale Discovery platform				
Format	Detection of BAN2401 ADA in human serum was accomplished by first pretreating neat serum samples with acetic acid to preemptively disrupt ADA-BAN2401 antibody-antigen complexes at low pH, followed by pH neutralization and detection of free ADA using an immunoassay bridging format based on the formation of soluble immuno-complexes composed of biotinylated BAN2401, anti-BAN2401, and reporter labeled BAN2401 antibody in solution. The ADA complexes were subsequently captured on streptavidin-coated standard surface 96-well MULTI-ARRAY® (1-spot) SECTOR plates MSD and detected in a quasi-quantitative manner via ECL signal generation.			

2.7.1.4.3 ヒト血清中 ADA 測定法要約

	Method Validation #1	Method Validation #2	Method Validation #3	Method Validation #4	Method Validation #5
Stock reference, lot number, expiration date	Rabbit Anti-BAN2401 IgG (TB1039 No.1 [redacted]); BAN2401 [redacted] [Retest: [redacted]]); Human Serum (BRH294075-089, BRH296697-706 BRH294090-104, BRH296707-716 BRH362230-BRH362279 [redacted])	Rabbit Anti-BAN2401 IgG (TB1039 No.1 – [redacted]); BAN2401 [redacted]); Human Serum (BRH734639-734688 [redacted])	BAN2401 (316_2401) [redacted]); Anti-BAN2401 (Purified Rabbit IgG) 316_a_2401 (TB1039[redacted]); BAN2401-Biotin (N013-139B [redacted]); BAN2401-SulfoTag (N013-139S [redacted])	Rabbit polyclonal anti-BAN2401 antibody (20[redacted]-0214, Lot# TB1039 [redacted]); BAN2401 Drug Product (20[redacted]-0006, Lot #[redacted]); Biotin labeled BAN2401 (20[redacted]-0022[redacted]); Sulfo-Tag labeled BAN2401 (20[redacted]-0023[redacted]); MSD 96-well streptavidin plates (20[redacted]-0038 [redacted]); Pooled normal human serum (20[redacted]-0019, 20[redacted]-0020 [redacted])	Anti-BAN2401 (NB 3260-24A [redacted]); BAN2401 [redacted] mg/mL [redacted]); Biotinylated-BAN2401 (DJB190712-01 or equivalent [redacted]); SulfoTag-BAN2401 (DJB190712-02 or equivalent [redacted]); Human Serum (HMN81175 [redacted])
Calibration range from the lower limit of quantitation (LLOQ) to the upper limit of quantitation (ULOQ)	NA	NA	NA	NA	NA
Matrix study population	Subjects with mild cognitive impairment due to AD and mild AD	Subjects with early AD	Normal serum	Normal or AD serum	Subjects with early AD
Link to reports and applicable amendments	tcar10-069 ([redacted])	tcar12-038 ([redacted])	tnjr14-225 ([redacted])	bav-0039-r v00 ([redacted])	45-1807 ([redacted])
Synopsis of amendment history	NA	NA	NA	NA	NA

2.7.1.4.3 ヒト血清中 ADA 測定法要約

	Method Validation #1	Method Validation #2	Method Validation #3	Method Validation #4	Method Validation #5

AD = Alzheimer's disease, ADA = anti-drug antibodies, ECL = electrochemiluminescence, eCTD = electronic common technical document, ID = identification, IgG = immunoglobulin G, LLOQ = lower limit of quantitation, MSD = meso scale discovery, NA = not applicable, ULOQ = upper limit of quantitation.
Source: tcar10-069, tcar12-038, tnjr14-225, bav-0039-r v00, 45-1807.

2.7.1.4.3.1 ヒト血清中 ADA 測定法要約

	Method Validation #6
Analyte	Anti-BAN2401 antibodies
Validation type	partial
eCTD reference number	5.3.1.4
Method ID	45-1807 addendum1
Duration of time method is in use	[redacted] 20[redacted] - [redacted] 20[redacted]
Bioanalytical site	[redacted]
Matrix	Human Serum
Platform	ECL on Meso Scale Discovery platform
Format	Detection of BAN2401 ADA in human serum was accomplished by first pretreating neat serum samples with acetic acid to preemptively disrupt ADA-BAN2401 antibody-antigen complexes at low pH, followed by pH neutralization and detection of free ADA using an immunoassay bridging format based on the formation of soluble immuno-complexes composed of biotinylated BAN2401, anti-BAN2401, and reporter labeled BAN2401 antibody in solution. The ADA complexes were subsequently captured on streptavidin-coated standard surface 96-well MULTI-ARRAY (1-spot) SECTOR plates MSD and detected in a quasi-quantitative manner via ECL signal generation.
Stock reference, lot number, expiration date	Biotin-BAN2401 Lot# DJB191008-01 (Expiry date: NA) SulfoTag-BAN2401 Lot# RJW220120-02 (Expiry date: NA) Biotin-BAN2401 Lot# KLS220427-02 (Expiry date: NA) SulfoTag-BAN2401 Lot# KLS220502-03 (Expiry date: NA) Biotin-BAN2401 RJW220427-02 (Expiry date: NA) SulfoTag-BAN2401 Lot# RJW220502-03 (Expiry date: NA) Anti-BAN2401 Antibody (Purified Rabbit IgG) Lot# TB1039 (Expiry date: NA)
Calibration range from the lower limit of quantitation (LLOQ) to the upper limit of quantitation (ULOQ)	NA

2.7.1.4.3.1 ヒト血清中 ADA 測定法要約

	Method Validation #6
Matrix study population	Subjects with early AD
Link to reports and applicable amendments	45-1807 addendum1 ([redacted] 20[redacted])
Synopsis of amendment history	NA

AD = Alzheimer's disease, ADA = anti-drug antibodies, ECL = electrochemiluminescence, eCTD = electronic common technical document, ID = identification, IgG = immunoglobulin G, LLOQ = lower limit of quantitation, MSD = meso scale discovery, NA = not applicable, ULOQ = upper limit of quantitation.
Source: 45-1807 addendum1.

2.7.1.4.3.2 ヒト血清中 ADA 測定法要約

	Clinical Study #1 (BAN2401-A001-101)	Clinical Study # 2 (BAN2401-J081-104)	Clinical Study #3 (BAN2401-G000-201 Core only)	Clinical Study #4 (BAN2401-G000-201 Core only)	Clinical Study #5 (BAN2401-G000-201 OLE Phase)
Analyte	Anti-BAN2401 antibodies	Anti-BAN2401 antibodies	Anti-BAN2401 antibodies	Anti-BAN2401 antibodies	Anti-BAN2401 antibodies
Validation type	In-study	In-study	In-study	In-study	In-study
eCTD reference number	5.3.3.1	5.3.3.1	5.3.3.1	5.3.3.1	5.3.3.1
Method ID	tcam-022	tliam-14225	bam-0039	tcam-082	45-1807
Duration of time method is in use	[redacted] 20[redacted] – [redacted] 20[redacted]	[redacted] 20[redacted] – [redacted] 20[redacted]	[redacted] 20[redacted] – [redacted] 20[redacted]	[redacted] 20[redacted] – [redacted] 20[redacted]	[redacted] 20[redacted] to [redacted] 20[redacted] (data cutoff for interim CSR)
Bioanalytical site	[redacted]	[redacted] USA	[redacted]	[redacted] USA	[redacted]
Matrix	Human Serum				
Platform	ECL on Meso Scale Discovery platform				
Format	Detection of BAN2401-ADA in human serum was accomplished by first pretreating neat serum samples with acetic acid to preemptively disrupt ADA-BAN2401 antibody-antigen complexes at low pH, followed by pH neutralization and detection of free ADA using an immunoassay bridging format based on the formation of soluble immuno-complexes composed of biotinylated BAN2401, anti-BAN2401, and reporter labeled BAN2401 antibody in solution. The ADA complexes were subsequently captured on streptavidin-coated standard surface 96-well MULTI-ARRAY® (1-spot) SECTOR plates MSD and detected in a quasi-quantitative manner via ECL signal generation.				
Stock reference, lot number, expiration date	BAN2401 ([redacted], [redacted] mg/mL [Retest date: [redacted] 20[redacted]]) Anti-BAN2401 (TB1039 No.1, 3.5 mg/mL [Retest date: [redacted] 20[redacted]]) Human Serum (BRH362230-33, 35-60, 62-71, and 73-79 [redacted] 20[redacted]])	BAN2401 (316_2401) ([redacted] 20[redacted]]); Anti-BAN2401 (Purified Rabbit IgG) 316_a_2401 (TB1039[redacted] 20[redacted]]); BAN2401-Biotin (N013-139B [redacted] 20[redacted]]); BAN2401-SulfoTag (N013-139S [redacted] 20[redacted]]) Pooled normal human serum (N013-153 [redacted] 20[redacted]])	Rabbit polyclonal anti-BAN2401 antibody (20[redacted]-0214, Source Lot# TB1039[redacted] 20[redacted]] BAN2401 Drug Product (20[redacted]-0006, Source Lot# [redacted] [redacted] 20[redacted]]) Biotin labeled BAN2401 (20[redacted]-0022, 2018-0141 [redacted] 20[redacted]]) Sulfo-Tag labeled BAN2401 (20[redacted]-0023[redacted] 20[redacted]])	BAN2401 - 316_2401 (N013-073 {INV089}, [redacted] {INV3010} [redacted] 20[redacted]]); Anti-BAN2401Antibody – Purified Rabbit IgG - 316_a_2401 (TB1039 {INV026}, TB1039 {INV3009}; [redacted] 20[redacted]]); BAN2401 Biotin (N013-075 [redacted] 20[redacted]], N013-139B [redacted] 20[redacted]], N393-005, 008, 011, 015, 016, 018, 021, 033	BAN2401 (Lot# [redacted] [redacted] 20[redacted]]) from Eisai; Monoclonal Anti-BAN2401 (Clone [redacted] 29817), Lot# NB 3260-24A (20[redacted]-0230) ([redacted] 20[redacted]) from Eisai; BiotinBAN2401 (Lot# DJB191008-01 or equivalent [redacted] 20[redacted]] labeled at [redacted]; SulfoTag-BAN2401 (Lot# DJB191008-02 or equivalent

2.7.1.4.3.2 ヒト血清中 ADA 測定法要約

	Clinical Study #1 (BAN2401-A001-101)	Clinical Study # 2 (BAN2401-J081-104)	Clinical Study #3 (BAN2401-G000-201 Core only)	Clinical Study #4 (BAN2401-G000-201 Core only)	Clinical Study #5 (BAN2401-G000-201 OLE Phase)
		Normal human serum (BRH978991[redacted])	MSD 96-well streptavidin plates (20[redacted]-0038 [redacted]) Pooled normal human serum (20[redacted]-0020 [redacted])	[redacted], N393-51A, 052,057, 060, 061, 064 [redacted]; N393-095 [redacted], N393-084 [redacted] BAN2401 Sulfo Tag (N013-139S [redacted], N013-076 [redacted], N393-002, 006, 009, 012, 017, 019, 022, 034 [redacted], N39351B, 053, 056, 059, 062, 065 [redacted], N393-084, 090, 095, 100, 101, 103, 105, 107 [redacted]	[redacted] from labeled at [redacted]
Calibration range from the lower limit of quantitation (LLOQ) to the upper limit of quantitation (ULOQ)	NA	NA	NA	NA	NA
Matrix study population	Subjects with mild to moderate AD	Subjects with mild cognitive impairment due to AD and mild AD	Subjects with early AD	Subjects with early AD	Subjects with early AD
Link to reports and applicable amendments	tcar10-070 ([redacted])	tnjr14-227 ([redacted])	bas-0051-r v00 ([redacted])	tnjr14-226 ([redacted])	45-1809 ([redacted]) 45-1809 interim2 ([redacted])
Synopsis of amendment history	NA	NA	NA	NA	NA

AD = Alzheimer's Diseas, ADA = anti-drug antibodies, ECL = electrochemiluminescence, eCTD = electronic common technical document, ID = identification, IgG =immunoglobulin G, LLOQ = lower limit of quantitation, NA = not applicable, OLE = open label extension, ULOQ = upper limit of quantitation.
Source: tcar10-070, tnjr14-227, bas-0051-r v00, tnjr14-226, 45-1809, 45-1809 interim2.

2.7.1.4.3.3 ヒト血清中 ADA 測定法要約

	Clinical Study #6 BAN2401-A001-004	Clinical Study #7 BAN2401-G001-301 Core and OLE Phase
Analyte	Anti-BAN2401 antibodies	Anti-BAN2401 antibodies
Validation type	In-study	In-study
eCTD reference number	5.3.1.4	5.3.1.4
Method ID	45-1807	45-1807 addendum1
Duration of time method is in use	[redacted] 20[redacted] to [redacted] 20[redacted]	[redacted] 20[redacted] to [redacted] 20[redacted]
Bioanalytical site	[redacted]	[redacted]
Matrix	Human Serum	Human Serum
Platform	ECL on Meso Scale Discovery platform	ECL on Meso Scale Discovery platform
Format	Detection of BAN2401-ADA in human serum was accomplished by first pretreating neat serum samples with acetic acid to preemptively disrupt ADA-BAN2401 antibody-antigen complexes at low pH, followed by pH neutralization and detection of free ADA using an immunoassay bridging format based on the formation of soluble immuno-complexes composed of biotinylated BAN2401, anti-BAN2401, and reporter labeled BAN2401 antibody in solution. The ADA complexes were subsequently captured on streptavidin-coated standard surface 96-well MULTI-ARRAY® (1-spot) SECTOR plates MSD and detected in a quasi-quantitative manner via ECL signal generation.	
Stock reference, lot number, expiration date	BAN2401, Lot#[redacted] Expiry date: [redacted] 20[redacted] Monoclonal Anti-BAN2401 (Clone [redacted] 29817) Lot# NB 3260-24A (20[redacted]-0230) ([redacted] 20[redacted]) Biotin-BAN2401 Lot# DJB191008-01 or equivalent ([redacted] 20[redacted]) SulfoTag-BAN2401 DJB191008-02 or equivalent ([redacted] 20[redacted])	BAN2401, Lot#[redacted], ([redacted] 20[redacted]) Biotin-BAN2401, Lot# DJB191008-01, ([redacted] 20[redacted]) SulfoTag-BAN2401, Lot# RJW220120-03, ([redacted] 20[redacted]) Biotin-BAN2401, Lot# KLS220427-02, ([redacted] 20[redacted]) SulfoTag-BAN2401, Lot# KLS220502-03, ([redacted] 20[redacted]) Biotin-BAN2401, Lot# RJW220427-02, ([redacted] 20[redacted]) SulfoTag-BAN2401, Lot# RJW220502-03, ([redacted] 20[redacted]) Biotin-BAN2401, Lot# KLS220726-01, ([redacted] 20[redacted]) SulfoTag-BAN2401, Lot# KLS220726-02, ([redacted] 20[redacted]) Biotin-BAN2401, Lot# KLS220810-01, ([redacted] 20[redacted]) SulfoTag-BAN2401, Lot# KLS220810-01, ([redacted] 20[redacted])
Calibration range from the lower limit of quantitation (LLOQ) to the upper limit of quantitation (ULOQ)	NA	NA
Matrix study population	Healthy subjects	Subjects with early AD
Link to reports and applicable amendments	45-2103a ([redacted] 20[redacted])	45-1908 ([redacted] 20[redacted])
Synopsis of amendment history	NA	NA

2.7.1.4.3.3 ヒト血清中 ADA 測定法要約

	Clinical Study #6 BAN2401-A001-004	Clinical Study #7 BAN2401-G001-301 Core and OLE Phase

AD = Alzheimer's disease, ADA = anti-drug antibodies, ECL = electrochemiluminescence, eCTD = electronic common technical document, ID = identification, IgG = immunoglobulin G, LLOQ = lower limit of quantitation, MSD = meso scale discovery, NA = not applicable, OLE = open-label extension, ULOQ = upper limit of quantitation.
Source: 45-2103a, 45-1908.

2.7.1.4.4 ヒト血清中 NAb 測定法要約

	Method Validation #1	Method Validation #2	Clinical Study #1 (BAN2401-J081-104)	Clinical Study #2 (BAN2401-G000-201 Core)	Clinical Study #3 (BAN2401-G000-201 OLE Phase)
Analyte	Anti-BAN2401 NAb	Anti-BAN2401 NAb	Anti-BAN2401 NAb	Anti-BAN2401 NAb	Anti-BAN2401 NAb
Validation type	Full	Full	In-study	In-study	In-study
eCTD reference number	5.3.1.4	5.3.1.4	5.3.5.1	5.3.5.1	5.3.5.1
Method ID	bam-0031 (Report No. bav-0031-r v02)	bam-0031 transferred to [redacted] (Report No. 45-1808)	bam-0031	bam-0031	45-1808
Duration of time method is in use	[redacted]	[redacted]	[redacted]	[redacted]	OLE Study: [redacted] (data cutoff for interim CSR)
Bioanalytical site	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Matrix	Human serum				
Platform	ECL immunoassay				
Format	The NAb assay was an immunoassay, which was based on the capture of biotin labeled BAN2401 on streptavidin coated microtiter plates, followed by detection with a ruthenium labeled [redacted] and subsequent electrochemiluminescence (ECL) signal detection. Neutralizing ADA activity in ADA positive serum samples was detected by measuring the inhibition of BAN2401/[redacted] binding interaction using a threshold response value, the neutralization cut point, in which signal values above (antibody-antigen complex formation) or below (inhibition of complex formation) the cut point indicated negative or positive results, respectively.				
Stock reference, lot number, expiration date	Neutralizing murine anti-BAN2401 monoclonal IgG antibody (clone 3G7G6 ; [redacted]) from [redacted] BAN2401 drug product ([redacted]) from Eisai; pooled normal human serum ([redacted]) from [redacted]; individual male normal human serum ([redacted]	Neutralizing murine anti-BAN2401 monoclonal IgG antibody (clone 3G7G6; [redacted]) from Eisai, Inc.; BAN2401 [redacted] mg/mL [redacted] from Eisai, Inc.; biotin-BAN2401 1000 µg/mL (DJB190712-01 or equivalent [redacted]) labeled at [redacted];	Neutralizing murine anti-BAN2401 monoclonal IgG antibody (clone 3G7G6; [redacted]) from [redacted]; pooled normal human serum ([redacted]) from [redacted]; biotinylated BAN2401 ([redacted]:1 challenge ratio]; [redacted]) from [redacted];	Neutralizing murine anti-BAN2401 monoclonal IgG antibody (clone 3G7G6; [redacted]) from [redacted]; pooled normal human serum ([redacted]) from Bioreclamation; biotinylated BAN2401 ([redacted]:1 challenge ratio]; [redacted]) from [redacted];	anti-BAN2401 ([redacted]) from Eisai; biotin-labeled BAN2401 1000 µg/mL (DJB191008-01 or equivalent [redacted]) labeled at [redacted]; Sulfo Tag-[redacted] 500 µg/mL ([redacted]) from Eisai

2.7.1.4.4 ヒト血清中 NAb 測定法要約

	Method Validation #1	Method Validation #2	Clinical Study #1 (BAN2401-J081-104)	Clinical Study #2 (BAN2401-G000-201 Core)	Clinical Study #3 (BAN2401-G000-201 OLE Phase)
	[redacted] from [redacted]; individual female normal human serum ([redacted]) from [redacted]; individual AD human serum ([redacted]) from [redacted]; biotinylated BAN2401 ([redacted]); sulfo-tag labeled [redacted]; MSD 96-well streptavidin plate ([redacted]) from MSD	Sulfo Tag-[redacted] 500 µg/mL ([redacted]) from Eisai, Inc.; pooled human serum (HMN81175 [redacted]) from [redacted]	Sulfo-tag labeled [redacted]; MSD 96-well streptavidin plate ([redacted]) from MSD	Sulfo-tag labeled [redacted]; MSD 96-well streptavidin plate ([redacted]) from MSD	
Calibration range from the LLOQ to the ULOQ	NA				
Matrix study population	Normal or AD serum	Normal or AD serum	Subjects with mild cognitive impairment due to AD and mild AD	Subjects with early AD	Subjects with early AD
Link to reports and applicable amendments	bav-0031-r v02 ([redacted])	45-1808 ([redacted])	bas-0044-r ([redacted])	bas-0052-r v00 ([redacted])	45-1810 ([redacted]) 45-1810 interim2 ([redacted])
Synopsis of amendment history	bav-0031-r v02 (Amended II; [redacted]): Long-term -80°C and freeze/thaw stability data added.	NA	NA	NA	NA

2.7.1.4.4 ヒト血清中 NAb 測定法要約

	Method Validation #1	Method Validation #2	Clinical Study #1 (BAN2401-J081-104)	Clinical Study #2 (BAN2401-G000-201 Core)	Clinical Study #3 (BAN2401-G000-201 OLE Phase)
	bam-0031-p v01 (Amendment I; [redacted] 20[redacted]): HQC concentration revised from 50 µg/mL to 20 µg/mL. Six and 12 months stability data added. bam-0031-p v00 ([redacted] 20[redacted]): new report				

AD = Alzheimer's disease, ADA = anti-lecanemab antibodies, CSR = clinical study report, ID = identification, ECL = electrochemiluminescence, eCTD = electronic Common Technical Document, HQC = high quality control, IgG = immunoglobulin G, LLOQ = lower limit of quantitation, NA = not applicable, NAb = neutralizing antibody, OLE = open-label extension, ULOQ = upper limit of quantitation.
Source: bav-0031-r v02, 45-1808, bas-0052-r v00, bas-0044-r, 45-1810, 45-1810 interim2.

2.7.1.4.4.1 ヒト血清中 NAb 測定法要約

	Clinical Study #4 BAN2401-A001-004	Clinical Study #5 BAN2401-G000-301 Core and OLE
Analyte	Anti-BAN2401 NAb	Anti-BAN2401 NAb
Validation type	In-study	In-study
eCTD reference number	5.3.1.4	5.3.1.4
Method ID	45-1808	45-1808
Duration of time method is in use	[redacted] 20[redacted] to [redacted] 20[redacted]	[redacted] 20[redacted] to [redacted] 20[redacted]
Bioanalytical site	[redacted]	[redacted]
Matrix	Human Serum	
Platform	ECL immunoassay	
Format	The NAb assay was an immunoassay, which was based on the capture of biotin labeled BAN2401 on streptavidin coated microtiter plates, followed by detection with a ruthenium labeled [redacted] and subsequent electrochemiluminescence (ECL) signal detection. Neutralizing ADA activity in ADA positive serum samples was detected by measuring the inhibition of BAN2401/[redacted] binding interaction using a threshold response value, the neutralization cut point, in which signal values above (antibody-antigen complex formation) or below (inhibition of complex formation) the cut point indicated negative or positive results, respectively.	
Stock reference, lot number, expiration date	Anti-BAN2401 (Clone 3G7G6), Lot# 20[redacted]-0009, Expiry date: [redacted] 20[redacted]	Anti-BAN2401 Clone 3G7G6, Lot # 20[redacted]-0009, Expiry date: [redacted] 20[redacted] Pooled normal human serum, Lot# HMN81175, Expiry date: [redacted] 20[redacted] Biotin-BAN2401, Lot# DJB190712-01, Expiry date: [redacted] 20[redacted] Biotin-BAN2401, Lot# KLS220331-01, Expiry date: [redacted] 20[redacted] SulfoTag-[redacted], Lot# 20[redacted]-0047, Expiry date: [redacted] 20[redacted]
Calibration range from the LLOQ to the ULOQ	NA	NA
Matrix study population	Healthy subjects	Subjects with early AD
Link to reports and applicable amendments	45-2103b ([redacted] 20[redacted])	45-1909 ([redacted] 20[redacted])
Synopsis of amendment history	NA	NA

AD = Alzheimer's disease, ADA = anti-lecanemab antibodies, ID = identification, ECL = electrochemiluminescence, eCTD = electronic Common Technical Document, [redacted], LLOQ = lower limit of quantitation, NA = not applicable, NAb = neutralizing antibody, OLE = open-label extension, [redacted],
ULOQ = upper limit of quantitation.
Source: 45-2103b, 45-1909.

2.7.1.4.5 ヒト血清中レカネマブ濃度測定法（EIS-R1696）

Bioanalytical method validation report name, amendments, and hyperlinks	Validation Report for Method BTM-1425-R0: Determination of BAN2401 in Human Serum by LC-MS/MS – eis-r1696 [redacted] 20[redacted]) and eis-r1696a1 ([redacted] 20[redacted])		
Method description	Method btm-1425-r0 was an immunopurification and LC-MS/MS method for the determination of BAN2401 in human serum. The tryptic digest peptide of BAN2401, HT9 was monitored as a surrogate for BAN2401.		
Materials used for standard calibration curve and concentration	BAN2401 (Lot #[redacted]) and [redacted] (internal standard) (Lot # W0005265) Concentrations: 0.5, 1.0, 3.0, 6.0, 20.0, 40.0, 120.0, and 150.0 µg/mL		
Validated assay range	0.5 to 150 µg/mL		
Materials used for quality controls (QCs) and concentration	BAN2401 (Lot #[redacted]) Concentrations: 1.5 µg/mL (LQC), 12 µg/mL (MQC), and 112.5 µg/mL (HQC)		
Minimum required dilutions	NA		
Source and lot of reagents	BAN2401 by Eisai, Lot#[redacted] ([redacted] 20[redacted]); [redacted] by Eisai, Lot# W0005265 ([redacted] 20[redacted])		
Regressing model and weighting	Linear regression with a weighting factor of $1/x^2$		
Validation parameters	**Method Validation Summary**		**Source Location**
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LLOQ to ULOQ	8	eis-r1696; Table 9
	%Nominal from LLOQ to ULOQ	95.2 to 104.9%	eis-r1696; Table 9
	Cumulative precision (%CV) from LLOQ to ULOQ	≤10.5	eis-r1696; Table 9
Performance of QCs during accuracy and precision runs	<table><tr><th></th><th>Intra-run</th><th>Inter-run</th></tr><tr><td>Accuracy (%Nominal)</td><td>87.2% to 105.4%</td><td>94.9% to 98.7%</td></tr><tr><td>Precision (%CV)</td><td>≤12.9</td><td>≤9.2</td></tr></table>		eis-r1696 Table 11
Selectivity & matrix effect	No interfering peaks were detected at the retention times of the tryptic peptide HT9 of BAN2401 and the internal standard in blank human serum. In addition, 3 lots of blank selectivity serum from donors with AD and 3 lots of blank serum from age-matched healthy donors were evaluated and no interfering peaks were detected at the retention times of the tryptic peptide HT9 of BAN2401 and the internal standard. The matrix effect was evaluated at the Low QC concentration level (1.5 µg/mL for BAN2401), in which six lots of human serum were used and a single replicate for each lot was prepared and processed. The matrix factor was 1.35 ± 0.13 at 1.5 µg/mL and the %CV was 9.6%. The results were acceptable. In addition, serum from 3 donors with AD and 3-age matched healthy individuals were also evaluated for matrix effect. The matrix factor was 0.99 ± 0.08 at 1.5 µg/mL and the %CV was 8.1%.		eis-r1696; Section Selectivity eis-r1696; Table 5 eis-r1696; Table 6
Interference & specificity	NA		NA
Hemolysis effect	NA		NA
Lipemic effect	NA		NA

2.7.1.4.5 ヒト血清中レカネマブ濃度測定法（EIS-R1696）

Dilution linearity & hook effect	450 µg/mL diluted 20-fold for BAN2401 The method met the acceptance criteria for dilution integrity.	eis-r1696; Validation summary
Bench-top/process stability	BAN2401 was stable at room temperature in human serum for at least 19 hours.	eis-r1696; Table 15
Freeze-Thaw stability	BAN2401 was stable for at least three freeze/thaw cycles in human serum at -70 °C.	eis-r1696; Table 16
Long-term storage	BAN2401 was stable for at least 34 days when stored at a nominal freezer temperature of -70 °C. BAN2401 was stable for at least 371 days when stored at a nominal freezer temperature of -70 °C.	eis-r1696; Table 22 eis-r1696A1; Table 1
Parallelism	NA	NA
Carry over	The samples met the criteria.	eis-r1696; Section 5.4
Method performance in study: LC/MS/MS Analysis for the Determination of BAN2401 in Human Serum and CSF Samples: A Randomized, Double-blind, Placebo-controlled Study to Assess Safety, Tolerability, Pharmacokinetics, Immunogenicity, and Pharmacodynamic Response of Repeated Intravenous Infusions of BAN2401 in Subjects with Mild Cognitive Impairment Due to Alzheimer's Disease and Mild Alzheimer's Disease (eis-r2740)		
Assay passing rate	Pass: 17; Fail: 0	eis-r2740; Table 8
Standard curve performance	● Cumulative bias range: -4.7 to 3.0% ● Cumulative precision: ≤10.4%CV	eis-r2740; Table 5
QC performance	● Cumulative bias range: -3.5% to 5.3% ● Cumulative precision: ≤8.6%CV	eis-r2740; Table 3
Method reproducibility	Incurred sample re-analysis was performed and 95% of the samples met the pre-specified criteria.	eis-r2740; Table 12
Study sample analysis/stability	The established stability sufficiently covers the storage of all the study samples from collection date until last day of analysis.	eis-r2740; Section 5.1
Standard calibration curve performance during accuracy and precision runs	0.500, 1.00, 3.00, 6.00, 20.00, 40.00, 120, and 150 µg/mL	
Method performance in study: LC/MS/MS Analysis for the Determination of BAN2401 in Human Serum by LC-MS/MS: A Randomized, Double-blind, Placebo-controlled, Combined Single Ascending Dose and Multiple Ascending Dose Study to Assess Safety, Tolerability, Immunogenicity, Pharmacodynamic Response and Pharmacokinetics of Intravenous Infusions of BAN2401 in Subjects with Mild to Moderate Alzheimer's Disease (eis-r1760)		
Assay passing rate	Pass: 19; Fail: 0	eis-r1760; Table 7
Standard curve performance	● Cumulative %Nominal: 98.6 to 101.4% ● Cumulative precision: ≤9.3% CV	eis-r1760; Table 5
QC performance	● Cumulative %theoretical: 97.1% to 102.0% ● Cumulative precision: ≤14.94% CV	eis-r1760; Table 4
Method reproducibility	Incurred sample re-analysis was performed and 87.69% of the samples met the pre-specified criteria.	eis-r1760; Table 13
Study sample analysis/stability	NA	NA

2.7.1.4.5 ヒト血清中レカネマブ濃度測定法（EIS-R1696）

Standard calibration curve performance during accuracy and precision runs	0.5, 1, 3, 6, 20, 40, 120, and 150 µg/mL	
Method performance in study: LC/MS/MS Analysis for the Determination of BAN2401 in Human Serum and Human Cerebrospinal Fluid Human Samples Originating from Eisai Study: A Placebo-controlled, Double-Blind, Parallel-Group, Bayesian Adaptive Randomization Design and Dose Regimen-Finding Study to Evaluate the Safety, Tolerability and Efficacy of BAN2401 in Subjects with Early Alzheimer's Disease (eis-r3653r1)		
Assay passing rate	Pass: 144; Fail: 7	eis-r3653r1; Table 14
Standard curve performance	● Cumulative %Bias: -2.3 to 1.7% ● Cumulative precision: ≤8.8% CV	eis-r3653r1; Table 10
QC performance	● Cumulative %Bias: -5.6 to -0.8% ● Cumulative precision: ≤8.7% CV	eis-r3653r1; Table 19
Method reproducibility	Incurred sample re-analysis was performed and 88.6% of the samples met the pre-specified criteria.	eis-r3653r1; Table 17
Study sample analysis/stability	The maximum storage period between the day of sample collection to the day of last analysis was 1636 days. 2% samples were analyzed outside of the established stability period (371 days at -70 °C). BAN2401 was stable for at least 866 days when stored at a nominal freezer temperature of -70 °C.	eis-r3653r1; Table 1 eis-r3653r1; Section 6.4 eis-r1696a4; Table 1
Standard calibration curve performance during accuracy and precision runs	0.5, 1, 3, 6, 20, 40, 120, and 150 µg/mL	

AD = Alzheimer's disease, CSF = cerebrospinal fluid, CV = coefficient of variation, ELISA = enzyme-linked immunosorbent assay, HQC = high QC, LC-MS/MS = liquid chromatography and tandem mass spectrometry, LQC = low QC, LLOQ = lower limit of quantitation, MQC = mid QC, NA = not applicable, PK = pharmacokinetic, ULOQ = upper limit of quantitation.

a: Stability will be established at the maximum storage period of 1636 days.

Source: eis-r1696, eis-r1696a1, eis-r2740, eis-r1760, eis-r3653r1, eis-r1696a4.

2.7.1.4.6 ヒト血清中レカネマブ濃度測定法（TCAR10-120）

<table>
<tr><td>Bioanalytical method validation report name, amendments, and hyperlinks</td><td colspan="3">Validation of an Enzyme-Linked Immunosorbent Assay (ELISA) Method for the Quantification of BAN2401 in Human Serum – tcam-040 ([redacted] 20[redacted]), tcar10-120 addendum1 ([redacted] 20[redacted])</td></tr>
<tr><td>Method Description</td><td colspan="3">This method determined the concentration of BAN2401 in human serum using a quantitative ELISA.</td></tr>
<tr><td>Materials used for standard calibration curve and concentration</td><td colspan="3">BAN2401 ([redacted] [redacted] 20[redacted])
Concentrations: 3.00, 6.00 (LLOQ), 10.0, 15.0, 20.0, 30.0, 45.0, 60.0, 75.0 (ULOQ), and 100 µg/mL</td></tr>
<tr><td>Validated assay range</td><td colspan="3">6.00 to 75.0 µg/mL</td></tr>
<tr><td>Materials used for quality controls (QCs) and concentration</td><td colspan="3">BAN2401 ([redacted] [redacted] 20[redacted])
Concentrations: 18.0 (LQC), 40.0 (MQC), 65.0 (HQC) µg/mL</td></tr>
<tr><td>Minimum required dilutions (MRDs)</td><td colspan="3">1:1000</td></tr>
<tr><td>Source and lot of reagents</td><td colspan="3">Biotinylated protofibrils by Eisai, Inc., Lot# E090407 (expiry date not available); BAN2401 by Eisai, Inc., Lot#[redacted] ([redacted] 20[redacted]); Rabbit anti-BAN2401 antibody by Medical &[redacted] Lot# TB1039 No.1 (expiry date not available); Goat anti rabbit IgG-HRP conjugate by[redacted], Lot# 790305 ([redacted] 20[redacted]); Human Serum Pool by[redacted] Lot# BRH362230-BRH362279 ([redacted] 20[redacted]); Human Serum AD Individuals by[redacted] Lot# 8011, 8029, 8062, 8007, 8058, 8093 ([redacted] 20[redacted])</td></tr>
<tr><td>Regressing model and weighting</td><td colspan="3">Four Parameter Curve Fit with weighting $1/Y^2$</td></tr>
<tr><td>Validation parameters</td><td colspan="2">Method Validation Summary</td><td>Source Location</td></tr>
<tr><td rowspan="3">Standard calibration curve performance during accuracy and precision runs</td><td>Number of standard calibrators from LLOQ to ULOQ</td><td>10</td><td rowspan="3">tcar10-120; Table 1</td></tr>
<tr><td>Cumulative accuracy (%bias) from LLOQ to ULOQ</td><td>-0.1 to 1.6</td></tr>
<tr><td>Cumulative precision (%CV) from LLOQ to ULOQ</td><td>≤5.1</td></tr>
<tr><td>Performance of QCs during accuracy and precision runs</td><td colspan="2"><table><tr><td></td><td>Intra-assay</td><td>Inter-assay</td></tr><tr><td>Accuracy (%AR)</td><td>75.7% to 114.3%</td><td>84.6% to 95.6%</td></tr><tr><td>Precision (%CV)</td><td>≤13.3</td><td>≤15.5</td></tr></table></td><td>tcar 10-120; Table 3
tcar 10-120; Table 4</td></tr>
<tr><td>Selectivity & matrix effect</td><td colspan="2">Twelve individual human serums were analyzed un-spiked and spiked at 6.00 µg/mL in duplicate: Six males (3 AD and 3 Normal) and six females (3 AD and 3 Normal).
Of the twelve spiked individual human serum samples evaluated, eleven met acceptance criteria (91.7%) for %AR and %CV. For the accepted individuals, the %ARs ranged from 79.7 to 97.2 and %CVs from 0.1 to 4.6.</td><td>tcar 10-120; Section 5.5
tcar 10-120; Table 7</td></tr>
<tr><td>Interference & specificity</td><td colspan="2">NA</td><td>NA</td></tr>
<tr><td>Hemolysis effect</td><td colspan="2">NA</td><td>NA</td></tr>
<tr><td>Lipemic effect</td><td colspan="2">NA</td><td>NA</td></tr>
<tr><td>Dilution linearity & hook effect</td><td colspan="2">Dilutional Linearity (hook effect): A high spike sample at 5,000 µg/mL was diluted 1:5 (1,000 µg/mL), 1:10 (500 µg/mL), 1:50 (100 µg/mL), 1:100 (50.0 µg/mL), 1:200 (25.0 µg/mL), 1:300 (16.7 µg/mL), and 1:500 (10.0 µg/mL) into pooled human serum.</td><td>tcar10-120; Section 5.4
tcar10-120; Table 6</td></tr>
</table>

2.7.1.4.6 ヒト血清中レカネマブ濃度測定法（TCAR10-120）

	Dilutional linearity was demonstrated up to 1:500. There was no evidence of a hook effect within the range of the standard curve.	
Bench-top/process stability	Low and high QC samples were stored at -70 °C and removed and stored at room temperature between 4-6 hours and 16-24 hours. Stability for both LQC and HQC samples met acceptance criteria and room temperature stability for BAN2401 was established for up to 21 hours, 39 minutes. The %AR ranged from 89.1 to 105.0 and the %CV ranged from 0.0 to 12.1.	tcar10-120; Section 5.6.2 tcar10-120; Table 9
Freeze-Thaw stability	Three sets of low and high QC samples stored at -70 °C nominal were thawed on ice ≤ 2 hours and then frozen at -70°C with at least 12 hours freeze time between consecutive freeze thaw cycles for two, four and six cycles. Three sets of LQCs and HQCs underwent two, four, and six freeze/thaw cycles and met acceptance criteria for all cycles. The %AR ranged from 88.3 to 101.8 with %CVs ranging from 0.4 to 7.4.	tcar10-120; Section 5.6.1 tcar10-120; Table 8
Long-term storage	Low and high QC samples (3 sets each) were stored at -70°C for approximately 1 month (30 days) and thawed on ice ≤2 hours prior to analysis. Long-Term Stability for BAN2401 in human serum at -70°C nominal for 199 days was accepted.	tcar10-120 addendum1; Section 2 tcar10-120 addendum1; Table 4
Parallelism	NA	- NA
Carry over	NA	- NA
Method performance in study: Quantification of Samples for Analysis of BAN2401 in Human Serum in Support of Eisai Protocol BAN2401-A001-101 (tcar10-048)		
Assay passing rate	Pass: 23; Fail: 1	tcar10-048; Table 1
Standard curve performance	• Cumulative bias range: -1.9 to 2.3 • Cumulative precision: ≤4.6	tcar10-048; Table 8
QC performance	• %AR: 97.5 to 107.8 • %CV: ≤ 15.9	tcar10-048; Table 10
Method reproducibility	Incurred sample re-analysis was performed and 93.2% of the samples met the pre-specified criteria.	tcar10-048; Table 13
Study sample analysis/stability	The maximum storage period from collection to analysis was 210 days[a].	tcar10-048; Sample storage stability
Standard calibration curve performance during accuracy and precision runs	NA	

AD = Alzheimer's disease, CV = coefficient of variation, HQC = high QC, LQC = low QC, LLOQ = lower limit of quantitation, MQC = mid QC, NA = not applicable, PK = pharmacokinetic, TE = total error, ULOQ = upper limit of quantitation.
a: Eight samples were outside of stability.
Source: tcar10-120 addendum1, tcar10-048.

2.7.1.4.7 ヒト血清中レカネマブ濃度測定法（EIS-R1696A2）

Bioanalytical method validation report name, amendments, and hyperlinks	Protocol Addendum No. 2 for the Partial Validation of Method BTM-1425-R3: Determination of BAN2401 in Human Serum by LC-MS/MS – eis-r1696a2 ([redacted] 20[redacted]), eis-r1696a3 ([redacted] 20[redacted]), eis-r1696a4 ([redacted] 20[redacted])		
Method Description	Method btm-1425-r3 was an LC-MS/MS method for the determination of BAN2401 in human serum. The tryptic digest peptide of BAN2401, HT9 was monitored as a surrogate for BAN2401.		
Materials used for standard calibration curve and concentration	BAN2401 ([redacted] [redacted] 20[redacted]) Concentrations: 0.500, 1.00, 3.00, 6.00, 20.00, 40.00, 120.00, and 150 µg/mL		
Validated assay range	0.500 to 150 µg/mL		
Materials used for quality controls (QCs) and concentration	BAN2401 ([redacted] [redacted] 20[redacted]) Concentrations: 0.500 µg/mL (LLOQ), 1.50 µg/mL (LQC), 12.0 µg/mL (Mid1 QC), 60.0 µg/mL (Mid2 QC), 113 µg/mL (HQC), 450 µg/mL (DQC), and 1130 µg/mL (DQC)		
Minimum required dilutions (MRDs)	NA		
Source and lot of reagents	BAN2401 by Eisai, Lot# [redacted] ([redacted] 20[redacted]); [redacted] by Eisai, Lot# 13-0092-D-ARS ([redacted] 20[redacted])		
Regressing model and weighting	Linear regression with weighting $1/X^2$		
Validation parameters	**Method validation summary**		**Source location**
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LLOQ to ULOQ	8	eis-r1696a2; Table 10
	Cumulative accuracy (%bias) from LLOQ to ULOQ	-4.7 to 3.5%	
	Cumulative SD from LLOQ to ULOQ	≤6.87	
Performance of QCs during accuracy and precision runs	Intra-run: Accuracy (%Bias) -15.0 to 9.2; Precision (%CV) ≤19.6 Inter-run: Accuracy (%Bias) -6.7 to 5.6; Precision (%CV) ≤14.8		eis-r1696a2; Section 4 eis-r1696a2; Table 12
Selectivity & matrix effect	The selectivity evaluation met the acceptance criteria: no significant baseline interference (larger than 20.0% of the lower limit of quantitation, LLOQ for BAN2401 or larger than 5.0% of the IS peak area of the accepted calibration standards and QC samples for the IS) was detected at the retention times of the analyte or the IS in any of the serum lots. Internal standard-normalized matrix factor = 1.34 at 1.50 µg/mL with %CV = 11.3% Internal standard-normalized matrix factor = 1.31 at 113 µg/mL with %CV = 4.6%		eis-r1696a2; Table 4 eis-r1696a2; Table 7
Interference & specificity	There was no significant interference detected from the analyte onto its internal standards.		eis-r1696a2; Section 4 eis-r1696a2; Table 19
Hemolysis effect	Six replicates of the Low and High QCs in hemolyzed human serum were extracted and analyzed with standards and bracketing QC samples prepared in non-hemolyzed human serum. The mean concentration should be within ± 20.0% of the nominal concentration and the %CV should be no more than 20.0%. %CV: ≤13%		eis-r1696a2; Section 9.4 eis-r1696a2; Table 8

2.7.1.4.7 ヒト血清中レカネマブ濃度測定法（EIS-R1696A2）

	%Bias: 0.0% to ≤5.3%	
Lipemic effect	Six replicates of the Low and High QCs in lipemic human serum were extracted and analyzed with standards and bracketing QC samples prepared in normal human serum. The mean concentration should be within ± 20.0% of the nominal concentration and the %CV should be no more than 20.0%. %CV: ≤16.8 %Bias: 3.5 to 10%	eis-r1696a2; Section 9.5 eis-r1696a2; Table 9
Dilution linearity & hook effect	For dilution linearity, pooled serum was fortified with BAN2401 at a concentration approximately 4 times above the High QC sample concentration (450 µg/mL) of the method. Six replicate samples were prepared by diluting the samples 4-fold with blank pooled serum. A dilution factor up to 4-fold was considered validated for this method. %Bias: -11.1 Pooled serum was fortified with BAN2401 at a concentration approximately 10 times above the High QC sample concentration (=1130 µg/mL) of the method. Six replicate samples were prepared by diluting the samples 10fold with blank pooled serum. A dilution factor up to 10fold was considered validated for this method. %Bias: 2.7	eis-r1696a2; Section 9.11 eis-r1696a2; Table 14 eis-r1696a3; Section 7.1 eis-r1696a3; Table 1
Bench-top/process stability	QC samples at two concentration levels, which were maintained un-extracted at 6.0 hours at room temperature, were assayed in six replicates (from three separate tubes for each concentration level) against freshly prepared calibration standards. The results demonstrated that BAN2401 in human serum were stable at 6.0 hours at room temperature. %Bias: 6.2 to 6.7	eis-r1696a2; Section 9.12.1 eis-r1696a2; Table 15
Freeze-Thaw stability	The stability of BAN2401 in human serum through five freeze/thaw cycles was assessed at two QC concentration levels: 1.50 µg/mL and 113 µg/mL for BAN2401, with six replicates at each concentration level. QC samples stored at -70 °C were subjected to five freeze/thaw (room temperature) cycles and the concentrations were measured against freshly prepared calibration standards. The results demonstrated that BAN2401 was stable for at least five freeze (-70 °C)/thaw (room temperature) cycles. %Bias: 1.8 to 8.0	eis-r1696a2; Section 9.12.2 eis-r1696a2; Table 16
Long-term storage	371 days at -70 °C 866 days at -70 °C	eis-r1696a2; Section 4 eis-r1696a4; Section 7.1
Parallelism	NA	- NA
Carry over	All of the double blank samples that were evaluated for carryover met the acceptance criteria (analyte peak areas were <20.0% of the lower limit of quantitation, LLOQ for BAN2401 and IS peak areas were <5.0% of the mean IS peak area of the accepted calibration standards and QC samples).	eis-r1696a2; Section 4 eis-r1696a2; Table 6

2.7.1.4.7 ヒト血清中レカネマブ濃度測定法（EIS-R1696A2）

Method performance in study: LC-MS/MS Determination of BAN2401 in Human Serum Samples Originating from Eisai Study: A Placebo-Controlled, Double–Blind, Parallel-Group, Bayesian Adaptive Randomization Design and Dose Regimen–Finding Study with an Open-Label Extension Phase to Evaluate Safety, Tolerability and Efficacy of BAN2401 in Subjects With Early Alzheimer's Disease (110-r10679)		
Assay passing rate	Pass: 33; Fail: 4	110-r10679; Table 9
Standard curve performance	• Cumulative % bias range: -2.8 to 1.0 • Cumulative precision: ≤9.4 %CV	110-r10679; Table 5
QC performance	• Cumulative bias range: -6.2 to 5.3 % • Cumulative precision: ≤17.9 %CV	110-r10679; Table 3
Method reproducibility	Incurred sample re-analysis was performed and 92.5% of the samples met the pre-specified criteria.	110-r10679; Section 6.6
Study sample analysis/stability	The maximum storage period from sample collection to last sample analysis was 948 days.[a] BAN2401 was stable for at least 866 days when stored at a nominal freezer temperature of -70 °C.	110-r10679; Section 5.3.2 eis-r1696a4; Table 1
Standard calibration curve performance during accuracy and precision runs	NA	

CV = coefficient of variation, DQC = dilution quality control, HQC = high QC, IS = internal standard, , LC-MS/MS = liquid chromatography and tandem mass spectrometry, LLOQ = lower limit of quantitation, LQC = low QC, NA = not applicable, PK = pharmacokinetic, SD = standard deviation, ULOQ = upper limit of quantitation.
a: QC long term will be conducted and reported in an addendum report to support this data integrity.
Soure: eis-r1696a2, eis-r1696a3, eis-r1696a4, 110-r10679.

2.7.1.4.8 ヒト CSF 中レカネマブ濃度測定法（TCAR10-050）

Bioanalytical method validation report name, amendments, and hyperlinks	Validation of an Electrochemiluminescence (ECL) Method for the Quantification of BAN2401 in Human Cerebral Spinal Fluid (CSF) - tcar10-050 ([redacted] 20[redacted]), tcar10-050 addendum1 ([redacted] 20[redacted])	
Method Description	Assay platform: ECL-MSD In the method, the amount of exogenously administered BAN2401 in human CSF samples was measured by using a quantitative ECL assay. MSD® streptavidin microtiter plates were coated with biotinylated protofibril and non-specific binding was blocked by incubation with 3% skim milk in PBS. The blocked plate was washed and BAN2401 standards, quality controls, and unknown samples were added to the plate at an MRD of 1:2 and incubated for ~1 hour. The plate was washed and anti-BAN2401 antibody (rabbit antiserum: IgG purified) was added to the plate and incubated for ~1 hour. After washing away unbound material, goat anti-rabbit IgG-Sulfo-tag was added to the plate and incubated for ~1 hour. After washing away unbound material, 2X read buffer was added to the plate to provide the appropriate chemical environment for ECL. The plate was then loaded into an MSD® SECTOR™ Imager for analysis. Inside the MSD® SECTOR™ Imager, a voltage applied to the plate electrodes caused the labels bound to the electrode surface to emit light. The instrument measured intensity of the emitted light to afford quantitative measurement of the amount of BAN2401 in the samples, and the data were analyzed using Watson LIMS™.	
Materials used for standard calibration curve and concentration	BAN2401 ([redacted] [redacted] 20[redacted]); Anti-BAN2401 antibody (TB1039 No.1 [redacted] 20[redacted]) Concentrations: 1.00, 3.00, 6.00, 10.0, 15.0, 20.0, 30.0, 45.0, 65.0, 75.0, and 100 ng/mL	
Validated assay range	3.00 ng/mL (LLOQ) to 75.0 ng/mL (ULOQ).	
Materials used for QCs and concentration	BAN2401 ([redacted] [redacted] 20[redacted]); Anti-BAN2401 antibody (TB1039 No.1 [redacted] 20[redacted]) Concentrations: 9.00 (LQC), 40.0 (MQC), 60.0 (HQC) ng/mL	
MRD	1:2	
Source and lot of reagents	Biotinylated protofibrils (E090407 [expiry date not available]) from Eisai, Inc; BAN2401 ([redacted] [redacted] 20[redacted]) from Eisai, Inc anti-BAN2401 antibody (TB1039 No.1 [redacted] 20[redacted]) from [redacted]; Goat anti-rabbit Sulfo-Tag (W00133881 [redacted] 20[redacted]) from MSD®; human CSF pool (BRH371118, BRH469184 [both [redacted] 20[redacted]) from [redacted]; human CSF AD Individuals (8045, 8062, 8091, 8092, 8200 [all [redacted] 20[redacted]] from [redacted]) and human CSF matched age elderly individuals (7090003, 7090007, 709022, 709033, 709055 [all [redacted] 20[redacted]]) from [redacted]	
Regressing model and weighting	Four parameter curve fit with weighting 1/Y	
Validation parameters	**Method Validation Summary**	**Source Location**
Standard calibration curve performance during accuracy and precision runs	In all runs, the anchor points at 1.00 ng/mL and 100 ng/mL did not quantitate in Watson LIMS® since they were outside the quantifiable range and are noted as NR. The standard curve dynamic range was 1.00, 3.00, 6.00, 10.0, 15.0, 20.0, 30.0, 45.0, 65.0, 75.0, and 100 ng/mL. The quantifiable range determined was from 3.00 ng/mL (LLOQ) to 75.0 ng/mL (ULOQ). %bias (for quantifiable range): -2.0% to 7.4% %CV (for quantifiable range): ≤ 7.6%	tcar10-050; Section 2 tcar10-050; Section 5.1 tcar10-050; Table 2.

2.7.1.4.8 ヒト CSF 中レカネマブ濃度測定法（TCAR10-050）

<table>
<tr><td>Performance of QCs during accuracy and precision runs</td><td>For intra- and inter-assay accuracy (AR) and precision (CV), seven accuracy and precision runs were performed over 4 days by two analysts.
Accuracy and precision evaluations were performed at eight nominal VS concentrations: 3.00 (VS1LLOQ1), 6.00 (VS2LLOQ2), 9.00 (VS3LQC1), 18.0 (VS4LQC2), 40.0 (VS5MQC), 60.0 (VS6HQC), 75.0 (VS7ULOQ) and 300 (VS8DQC @ 1:5 and 1:10) ng/mL. The overall inter-assay target acceptance criteria for accuracy and precision was met for all eight levels of validation samples
<table>
<tr><th></th><th>Intra-assay</th><th>Inter-assay</th></tr>
<tr><td>Accuracy (%AR)</td><td>87.2% to 131.5%</td><td>102.4% to 115.3%</td></tr>
<tr><td>Precision (%CV)</td><td>0.8% to 6.1%</td><td>5.8% to 11.3%</td></tr>
</table>
</td><td>tcar10-050; Section 2
tcar10-050; Section 5.2
tcar10-050; Table 3
tcar10-050; Table 4</td></tr>
<tr><td>Selectivity & matrix effect</td><td>Ten individual human CSF samples were assayed in duplicate both unspiked and spiked at a concentration of 6 ng/mL. Samples included 5 AD and 5 aged matched normal elderly controls.
Unspiked Samples: All samples below the limit of quantitation.
Spiked Samples at 6.00 ng/mL: 10 of 10 individual samples (100%) were within acceptable %AR and %CV limits (±25.0%).
Accuracy (%AR): 100.5% to 119.5%
Precision (%CV): 0.1% to 4.2%</td><td>tcar10-050; Section 2
tcar10-050; Section 5.5
tcar10-050; Table 10</td></tr>
<tr><td>Interference & specificity</td><td>NA</td><td>NA</td></tr>
<tr><td>Hemolysis effect</td><td>NA</td><td>NA</td></tr>
<tr><td>Lipemic effect</td><td>NA</td><td>NA</td></tr>
<tr><td>Dilution linearity & hook effect</td><td>A high spike sample at 5000 ng/mL was diluted 1:5 (1000 ng/mL), 1:10 (500 ng/mL), 1:50 (100 ng/mL), 1:100 (50.0 ng/mL), 1:200 (25.0 ng/mL), 1:300 (16.7 ng/mL) and 1:500 (10.0 ng/mL) into pooled human CSF.
Dilutional linearity was demonstrated up to 1:500. There was no evidence of a hook effect within the range of the standard curve.</td><td>tcar10-050; Section 2
tcar10-050; Table 9</td></tr>
<tr><td>Bench-top/process stability</td><td>No ambient RT stability established.
For refrigerated stability, LQC and HQC samples stored at -70 °C were removed and stored at 1 to 8 °C for 4 to 6 hours and 16 to 24 hours. Refrigerated stability for BAN2401 was established for up to 20 hours and 27 minutes.
%AR: 90.9 to 106.8%
%CV: 0.1 to 10.2%</td><td>tcar10-050; Section 2
tcar10-050; Table 12</td></tr>
</table>

2.7.1.4.8 ヒト CSF 中レカネマブ濃度測定法（TCAR10-050）

Freeze-Thaw stability	Three sets of LQC and HQC samples stored at -70 °C nominal were thawed ≤ 2 hours and then frozen at -70 °C with at least 12 hours freeze time between consecutive freeze thaw cycles for two, four and six cycles. Freeze/thaw stability of BAN2401 was established for up to six freeze/thaws on ice and at RT. %AR: 95.0% to 112.3% (ice); 90.9% to 116.7% (RT) %CV: 0.3% to 11.6% (ice); 0.2% to 8.5% (RT)	tcar10-050; Section 2 tcar10-050; Table 11
Long-term storage	Long-term stability was assessed using three sets of LQC and three sets of HQC in CSF stored at -70 °C nominal for 94 days. Long-term stability for BAN2401 in human CSF at -70 °C nominal for 94 days was accepted. %AR: 98.7% to 111.0% %CV: 1.2 to 5.5%	tcar10-050 addendum1; Table 3
Parallelism	NA	NA
Carry over	NA	NA
Method performance in study: Quantification of Samples for Analysis of BAN2401 in Human Cerebrospinal Fluid in Support of Protocol BAN2401-A001-101 (Report Number: tcar10-051)		
Assay passing rate	NA	-
Assay performance	Accepted runs: 7, rejected runs: 1	tcar10-051; Table 1
Standard curve performance	• Cumulative bias range: -2.1 to 9.4% • Cumulative precision: ≤ 4.3 % CV	tcar10-051; Table 2
QC performance	• Cumulative %AR range: 94.9 to 105.6% • Cumulative precision: ≤ 8.3% CV	tcar10-051; Table 4
Method reproducibility	NA	NA
Study sample analysis/stability	The maximum storage period between sample collection and analysis was 229 days. 33 samples were outside storage stability[a].	tcar10-051; sample storage stability
Standard calibration curve performance during accuracy and precision runs	1.00, 3.00, 6.00, 10.0, 15.0, 20.0, 30.0, 45.0, 65.0, 75.0, and 100 ng/mL[b]	

AD = Alzheimer's disease, CV = coefficient of variation, DQC = dilutional QC, HQC = high QC, IgG = immunoglobulin G, LIMS = laboratory information management systems, LLOQ = lower limit of quantitation, LQC = Low QC, MQC = mid QC, MSD = meso scale discovery, MRD = minimum required dilution, NA = not applicable, NR = not reportable, PBS = phosphate-buffered saline, PK = pharmacokinetic, RT = room temperature, ULOQ = upper limit of quantitation, VS = validation sample.
a: Per sponsor, stability up to 7.5 months at -70°C and was to be established at an alternate site.
b: 1.00 and 100 ng/mL were anchor points, and their results were reported as NR in all runs.
Source: tcar10-050, tcar10-050 addendum1, tcar10-051.

2.7.1.4.9 ヒト CSF 中レカネマブ濃度測定法（EIS-R1663）

Bioanalytical method validation report name, amendments, and hyperlinks	Validation Report for Method BTM-1416-R0: Determination of BAN2401 in Human Cerebrospinal Fluid by LC-MS/MS - eis-r1663 ([redacted] 20[redacted]); eis-r1663a1r1 ([redacted] 20[redacted])	
Method Description	Method platform: LC-MS/MS Method btm-1416-r0 was an immunopurification and LC-MS/MS method for the determination of BAN2401 in human CSF. The tryptic digest peptide of BAN2401, HT9 was monitored as a surrogate for BAN2401. [redacted] was used as the IS and the tryptic digest peptide of [redacted], [redacted] (amino acid sequence) was monitored as a surrogate.	
Materials used for standard calibration curve and concentration	BAN2401 by Eisai, Lot# [redacted] ([redacted] 20[redacted]) Concentrations: 5, 10, 20, 50, 100, 200, 400, and 500 ng/mL	
Validated assay range	5.00 ng/mL (LLOQ) to 500.0 ng/mL (ULOQ)	
Materials used for quality controls (QCs) and concentration	BAN2401 by Eisai, Lot# [redacted] ([redacted] 20[redacted]) Concentrations: 15 ng/mL (LQC), 75 ng/mL (MQC), and 375 ng/mL (HQC)	
Minimum required dilutions	NA	
Source and lot of reagents	BAN2401 by Eisai, Lot# [redacted] ([redacted] 20[redacted]); [redacted] by Eisai, Lot# W0005265 ([redacted] 20[redacted])	
Regressing model and weighting	Linear regression with weighing factor $1/x^2$	
Validation parameters	**Method Validation Summary**	**Source Location**
Standard calibration curve performance during accuracy and precision runs	No of standard calibrators from LLOQ to ULOQ: 8 %Nominal from LLOQ to ULOQ: 97.1% to 103.5% Cumulative precision (%CV) from LLOQ to ULOQ: ≤11.4%	eis-r1663; Table 9
Performance of QCs during accuracy and precision runs	**Intra-run** / **Inter-run** **Accuracy (%Nominal)**: 94.6 to 110.4% / 100.6 to 107.8% **Precision (%CV)**: ≤13.8% / ≤11.9%	eis-r1663; Validation summary eis-r1663; Table 11
Selectivity & matrix effect	Selectivity: Selectivity was determined in six individual lots of normal human CSF with and without the IS. In addition, 3 individual lots of human CSF from donors with AD were evaluated with and without IS. The selectivity evaluation met the acceptance criteria. Matrix effect: The matrix effect was evaluated at LQC level (15 ng/mL), in which six lots of human CSF were used, and a single replicate for each lot was prepared and processed. In addition, CSF from 3 donors with AD were also evaluated for matrix effect. Matrix factor = 1.50 ± 0.26 at 15 ng/mL with %CV = 17.3% Matrix factor (3 diseased CSF samples) = 1.77 ± 0.08 at 15 ng/mL with %CV = 4.5%	eis-r1663; Validation summary eis-r1663; Section 5.1 eis-r1663; Table 3 eis-r1663; Table 4 eis-r1663; Section 5.2 eis-r1663; Table 5 eis-r1663; Table 6
Interference & specificity	NA	NA
Hemolysis effect	NA	NA
Lipemic effect	NA	NA

2.7.1.4.9 ヒト CSF 中レカネマブ濃度測定法（EIS-R1663）

Dilution linearity & hook effect	Pooled CSF was fortified with BAN2401 at a concentration 10 times above the high QC sample concentration (=3750 ng/mL). Six replicate samples were prepared by diluting the samples 10-fold with blank pooled CSF. Dilution factor up to 10fold was considered validated for this method (ie, 3750 ng/mL diluted 10fold). %Nominal: 112.2%; %CV: 10.2%	eis-r1663; Validation summary eis-r1663; Table 14
Bench-top/process stability	16 hours at RT %Nominal: 87.1 to 102.1%; %CV: 6.9 to 9.5%	eis-r1663; Validation summary eis-r1663; Table 16
Freeze-Thaw stability	3 freeze (-70 °C)/thaw cycles %Nominal: 94.2 to 96.6%; %CV: 7.6 to 11.4%	eis-r1663; Validation summary eis-r1663; Table 17
Long-term storage	594 days at -70 °C	eis-r1663a1r1; Table 1
Parallelism	NA	NA
Carry over	The samples met the criteria showing insignificant carryover for the analyte (<20% of LLOQ responses) and for the IS (<5.0% of the IS response in the control zero samples).	eis-r1663; Validation summary eis-r1663; Section 5.3
Method performance in study: LC/MS/MS Analysis for the Determination of BAN2401 in Human Serum and CSF Samples: A Randomized, Double-blind, Placebo-controlled Study to Assess Safety, Tolerability, Pharmacokinetics, Immunogenicity, and Pharmacodynamic Response of Repeated Intravenous Infusions of BAN2401 in Subjects with Mild Cognitive Impairment Due to Alzheimer's Disease and Mild Alzheimer's Disease (eis-r2740)		
Assay passing rate	Pass: 2; Fail: 0	eis-r2740; Table 8
Standard curve performance	Cumulative bias range: -13.7 to 10.2%	eis-r2740; Table 6
QC performance	Cumulative bias range: 0.0 to 9.9%	eis-r2740; Table 4
Method reproducibility	Incurred sample re-analysis was performed for 9 samples, and 100% of the samples met the pre-specified criteria.	eis-r2740; Table 13
Study sample analysis/stability	The established stability sufficiently covered the storage of all the study samples from collection date until last day of analysis.	eis-r2740; Section 5.1
Standard calibration curve performance during accuracy and precision runs	5.00, 10.0, 20.0, 50.0, 100.0, 200.0, 400.0, 500.0 ng/mL	

AD = Alzheimer's disease, CSF = cerebrospinal fluid, CV = coefficient of variation, HPLC = high performance liquid chromatography, HQC = high QC, IS = internal standard, LC-MS/MS = liquid chromatography with tandem mass spectrometry, LLOQ = lower limit of quantitation, LQC = low QC, MQC = mid QC, NA = not applicable, PK = pharmacokinetic, RT = room temperature, ULOQ = upper limit of quantitation.
Source: eis-r1663, eis-r1663a1r1, eis-r2740.

2.7.1.4.10 ヒト CSF 中レカネマブ濃度測定法（EIS-R1663A2）

Bioanalytical method validation report name, amendments, and hyperlinks	Addendum Report for the Partial Validation of Method BTM-1416-R1: Determination of BAN2401 in Human CSF by LC-MS/MS - eis-r1663a2 ([redacted] 20[redacted]); eis-r1663a3 ([redacted] 20[redacted]); eis-r1663a4 ([redacted] 20[redacted])		
Method Description	Method platform: LC-MS/MS Method BTM-1416-R1 was an immunopurification and LC-MS/MS method for the determination of BAN2401 in human CSF. The tryptic digest peptide of BAN2401, HT9 was monitored as a surrogate for BAN2401. [redacted] was used as the IS and the tryptic digest peptide of [redacted], [redacted] (amino acid sequence) was monitored as a surrogate.		
Materials used for standard calibration curve and concentration	BAN2401 by Eisai, Lot# [redacted] ([redacted] 20[redacted]); Concentrations: 5.00, 10.0, 20.0, 50.0, 100, 200, 400, 500.0 ng/mL		
Validated assay range	5.00 ng/mL (LLOQ) to 500.0 ng/mL (ULOQ)		
Materials used for quality controls (QCs) and concentration	BAN2401 by Eisai, Lot# [redacted] ([redacted] 20[redacted]); Concentrations: 5.00 (LLOQ), 15.0, 75.0, 150.0, 375, and 3750 ng/mL (Dilution QC)		
Minimum required dilutions	NA		
Source and lot of reagents	BAN2401 by Eisai, Lot# [redacted] ([redacted] 20[redacted]); [redacted] by Eisai, Lot# 13-0092-D-ARS ([redacted] 20[redacted])		
Regressing model and weighting	Linear regression with weighing factor $1/x^2$		
Validation parameters	**Method Validation Summary**		**Source Location**
Standard calibration curve performance during accuracy and precision runs	No of standard calibrators from LLOQ to ULOQ	8	eis-r1663a2; Table 6
	Cumulative accuracy (%bias) from LLOQ to ULOQ	-5% to 6.8%	
	Cumulative precision (%CV) from LLOQ to ULOQ	≤ 13.4%	
Performance of QCs during accuracy and precision runs	**Intra-run** / **Inter-run** **Accuracy (%Bias)**: -10.5% to 7.3% / -9.5% to 4.7% **Precision (%CV)**: ≤16.0% / ≤13.0%		eis-r1663a2; Section 4 eis-r1663a2; Table 10
Selectivity & matrix effect	Selectivity: Selectivity was determined in six individual lots of human CSF with and without the IS. The selectivity evaluation met the acceptance criteria: no significant baseline interference (>20.0% of the lower limit of quantitation, LLOQ for BAN2401 or larger than 5.0% of the IS peak area of the accepted calibration standards and QC samples for the IS) was detected at the retention time of the analyte and the IS in any of the CSF lots. Matrix effect: The matrix effect was evaluated by extracting single replicates of six lots of blank Human CSF. Each lot was spiked at the Low and High QC concentration levels: (15.0 ng/mL and 375 ng/mL for BAN2401) post extraction. IS-normalized matrix factor = 1.53 ± 0.252 at 15.0 ng/mL with %CV = 16.5% IS-normalized matrix factor = 1.32 ± 0.0301 at 375 ng/mL with %CV = 2.3%		eis-r1663a2; Section 4 eis-r1663a2; Section 9.1 eis-r1663a2; Table 2 eis-r1663a2; Table 3 eis-r1663a2; Section 9.3 eis-r1663a2; Table 5

2.7.1.4.10 ヒト CSF 中レカネマブ濃度測定法（EIS-R1663A2）

Interference & specificity	The interference of the analytes on the IS was also evaluated by preparing three replicates of the upper limit of quantitation (500 ng/mL for BAN2401) and processing and analyzing the samples without the addition of the IS. There was no significant interference detected from the analyte onto its internal standards.	eis-r1663a2; Section 4 eis-r1663a2; Section 9.11 eis-r1663a2; Table 22
Hemolysis effect	NA	-
Lipemic effect	NA	-
Dilution linearity & hook effect	Pooled CSF was fortified with BAN2401 at a concentration 10 times above the high QC sample concentration (= 3750 ng/mL). Six replicate samples were prepared by diluting the samples 10-fold with blank pooled CSF. Dilution factor up to 10fold was considered validated for this method (i.e, 3750 ng/mL diluted 10-fold). %Bias: -5.6% , %CV (3.7%)	eis-r1663a2; Section 4 eis-r1663a2; Section 9.9 eis-r1663a2; Table 16
Bench-top/process stability	7.0 hours at RT (%Bias: -4.3 to 2.7%)	eis-r1663a2; Section 4 eis-r1663a2; Table 17
Freeze-Thaw stability	5 cycles for freeze (-70 °C)/thaw (RT) (%Bias: -6.1 to 4.7%)	eis-r1663a2; Section 4 eis-r1663a2; Table 18
Long-term storage	115 days at -20 °C was established (%Bias: -7.5 to 4.0%) 387 days at -20 °C was established (%Bias: -4.0 to 17.1%) 792 days at -70 °C was established (%Bias: -1.6 to 6.7%)	eis-r1663a2; Table 24 eis-r1663a3; Table 1 eis-r1663a4; Table 1
Parallelism	NA	-
Carry over	All of the double blank samples that were evaluated for carryover met the acceptance criteria (analyte peak areas were < 20.0% of LLOQ for BAN2401 and IS peak areas were < 5.0% of the mean IS peak area of the accepted calibration standards and QC samples).	eis-r1663a2; Section 4 eis-r1663a2; Table 4
Method performance in study: LC/MS/MS Analysis for the Determination of BAN2401 in Human Serum and Cerebrospinal Fluid Human Samples Originating from Eisai Study: “A Placebo-Controlled, Double-Blind, Parallel-Group, Bayesian Adaptive Randomization Design and Dose Regimen-Finding Study to Evaluate the Safety, Tolerability and Efficacy of BAN2401 in Subjects With Early Alzheimer’s Disease” (eis-r3653r1)		
Assay passing rate	Pass: 10; Fail: 0	eis-r3653r1; Table 16
Standard curve performance	• Cumulative bias range: -4.0 to 4.5% • Cumulative precision: ≤ 10.9% CV	eis-r3653r1; Table 11
QC performance	• Cumulative bias range: -0.3 to 14.0% • Cumulative precision: ≤ 18.1% CV	eis-r3653r1; Table 9
Method reproducibility	Incurred sample re-analysis was performed for 30 samples, and 80% of the samples met the pre-specified criteria.	eis-r3653r1; Section 6.6 eis-r3653r1; Table 18

2.7.1.4.10 ヒト CSF 中レカネマブ濃度測定法（EIS-R1663A2）

Study sample analysis/stability	The maximum storage period between the day of sample collection to the day of last analysis was 1301 days. 14% samples were analyzed outside of the established stability period (594 days at -70 °C). 792 days at -70 °C was established, the results of the extended long-term sample storage stability at -70 °C for 792 days for method BTM1416R1 are reported in this addendum report.[a]	eis-r3653r1; Section 5.1 eis-r3653r1; Table 1 eis-r1663a4; Table 1
Standard calibration curve performance during accuracy and precision runs	5.00, 10.0, 20.0, 50.0, 100, 200, 400, 500.0 ng/mL	

CSF = cerebrospinal fluid, CV = coefficient of variation, HPLC = high performance liquid chromatography, IS = internal standard, LC-MS/MS = liquid chromatography with tandem mass spectrometry, LLOQ = lower limit of quantitation, MRD = minimum required dilution, NA = not applicable, PK = pharmacokinetic, RT = room temperature, ULOQ = upper limit of quantitation.

a: Stability data will be presented in addendum to the validation report.

Source: eis-r1663a2, eis-r1663a3, eis-r1663a4, eis-r3653r1.

2.7.1.4.11 ヒト血清中 ADA 測定法（BAV-0039-R v00）

Bioanalytical method validation report name, amendments, and hyperlinks	Validation of Electrochemiluminescent Immunoassay for the Detection of Anti-BAN2401 Antibodies in Human Serum - bav-0039-r v00 ([redacted] 20[redacted])		
Method Description	Assay platform: ECL-MSD Detection of BAN2401 ADA in human serum was accomplished by first pretreating neat serum samples with acetic acid to preemptively disrupt ADA-BAN2401 antibody-antigen complexes at low pH, followed by pH neutralization and detection of free ADA using an immunoassay bridging format based on the formation of soluble immuno-complexes composed of biotinylated BAN2401, anti-BAN2401, and reporter labeled BAN2401 antibody in solution. The ADA complexes were subsequently captured on streptavidin-coated standard surface 96-well MULTI-ARRAY® (1-spot) SECTOR plates MSD and detected in a quasi-quantitative manner via ECL signal generation.		
Biological matrix	Human Serum		
Sample pre-treatment	Acid dissociation; acetic acid to preemptively disrupt ADA-BAN2401 antibody-antigen complexes at low pH		
Positive control antibody	Rabbit polyclonal anti-BAN2401 antibody		
Negative control	Pooled normal human serum only		
Materials used for standard calibration curve and concentration	NA		
Validated assay range	NA		
Materials used for quality controls (QCs) and concentration	Rabbit polyclonal anti-BAN2401 and normal human serum HQC: 15 µg/mL rabbit polyclonal anti-BAN2401 in pooled normal human serum LQC: 0.05 µg/mL rabbit polyclonal anti-BAN2401 in pooled normal human serum NQC: pooled normal human serum only		
Minimum required dilutions (MRDs)	NA		
Source and lot of reagents	Rabbit polyclonal anti-BAN2401 antibody by [redacted] Lot# 20[redacted]-0214 (Lot # TB1039); BAN2401 Drug Product by Eisai, Inc., Lot# 20[redacted]-0006 (Lot # [redacted]); Biotin labeled BAN2401, [redacted] Lot# 20[redacted]-0022; Sulfo-Tag labeled BAN2401, [redacted] Lot# 20[redacted]-0023; HQC (15 µg/mL polyclonal anti-BAN2401 in pooled normal human serum) by [redacted] Lot# 20[redacted]-0064, 20[redacted]-0090, 20[redacted]-0114; MQC (1.0 µg/mL polyclonal anti-BAN2401 in pooled normal human serum) by [redacted] Lot# 20[redacted]-0091; LQC (0.05 µg/mL polyclonal anti-BAN2401 in pooled normal human serum) by [redacted] Lot# 20[redacted]-0066, 20[redacted]-0092, 20[redacted]-0115; NQC (pooled normal human serum) by [redacted] Lot# 20[redacted]-0067; MSD 96-well streptavidin plates by Meso Scale Discovery, Lot# 20[redacted]-0038; Pooled normal human serum by [redacted], Lot# 20[redacted]-0019, 20[redacted]-0020		
Regressing model and weighting	Four parameter curve fit with weighting $1/Y^2$		
Validation parameters	**Method Validation Summary**		**Source Location**
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LLOQ to ULOQ	NA	NA
	Cumulative accuracy (%bias) from LLOQ to ULOQ Product A	NA	NA

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	Cumulative precision (%CV) from LLOQ to ULOQ Product A	NA	NA
Performance of QCs during accuracy and precision runs	**Cumulative accuracy (%bias) in 5 QCs** QCs for product A:	NA	NA
	Inter-batch %CV QCs for Product A:	NA	NA
	Total Error (TE) QCs for Product A:	NA	NA
Selectivity	Baseline serum samples from 10 drug-naïve AD individuals along with a single lot of pooled normal human serum were spiked with either 15 or 0.05 µg/mL rabbit polyclonal antibody and analyzed in the assay. At least 80% of individual AD patient sera from study BAN2401-G000-201 spiked with a low and high levels of ADA must generate ECL values ≤±20.0% different to comparable ADA spike levels in pooled normal human serum. Low ADA: 10/10 (100%) individuals yielded results with acceptable % differences. High ADA: 10/10 (100%) individuals yielded results with acceptable % differences.		bav-0039-r v00; Table 1 bav-0039-r v00; Section 5.8, Table 14,
Specificity assay precision	Precision samples were assayed using a balanced assay design in which 6 independent assay runs performed separately by 2 analysts, each of whom completed 3 runs on different days with 2 different ECL plate readers. Pooled repeatability and intermediate %CVs must be ≤ 20.0% for the HQC+ Drug, MQC + Drug, and LQC + Drug % inhibition values. HQC + Drug Repeatability %CV = 0.0% Intermediate %CV = 0.0% MQC + Drug Repeatability %CV = 0.3% Intermediate %CV = 0.7% LQC + Drug Repeatability %CV = 5.1% Intermediate %CV = 8.0%		bav-0039-r v00; Table 1 bav-0039-r v00; Section 5.3, Table 7,
Screening assay precision	Precision samples were assayed using a balanced assay design in which 6 independent assay runs performed separately by 3 analysts, each of whom completed 2 runs on different days with 2 different ECL plate readers. Pooled repeatability and intermediate %CVs must be ≤20.0% for the HQC, MQC, and LQC ECL values. HQC Repeatability %CV = 4.6% Intermediate %CV = 7.2% MQC Repeatability %CV = 3.1% Intermediate %CV = 6.1%		bav-0039-r v00; Table 1 bav-0039-r v00; Section 5.1, Table 5

2.7.1.4.11 ヒト血清中 ADA 測定法（BAV-0039-R v00）

	LQC Repeatability %CV = 3.1% Intermediate %CV = 7.3%	
Specificity cut point	A specificity cut point to be determined. 11% signal inhibition was established as the cut point value for confirmation of ADA specificity to BAN2401.	bav-0039-r v00; Table 1 bav-0039-r v00; Section 5.5, Table 10
Screening cut point	A floating screening cut point normalization factor to be determined. NQC + 4 ECL units was established for use in the determination of preliminary positive and negative results.	bav-0039-r v00; Table 1 bav-0039-r v00; Table 9
Sensitivity	The sensitivity limit must be ≤100 ng/mL ADA. Assay sensitivity was estimated to be 3.9 ng/mL of rabbit polyclonal ADA.	bav-0039-r v00: Table 1 bav-0039-r v00; Table 12
Hemolysis effect	NA	NA
Lipemic effect	NA	NA
Dilution linearity & hook effect	NA	NA
Bench-top/process stability **Freeze-Thaw stability** **Long-term storage**	Method performance for stability was suitably demonstrated in the previous validations and was not repeated.	BAV-0039-R v00: Section 1
Parallelism	NA	NA
Carry over	NA	NA
Method performance in study: Detection of Anti-BAN2401 Antibodies in Human Serum Samples from Clinical Study BAN2401-G000-201 (Report Number: bas-0051-r v00, 23 Oct 20[redacted])		
Assay passing rate	NA	NA
Assay performance	Using the tiered evaluation approach for immunogenicity assessment, a total of 53 assay runs were performed with 45 passing validity criteria for a success rate of 84.9%. Of the 53 runs, 27 were screening, 16 were specificity, and 10 were titering.	bas-0051-r v00; Section 9.1 bas-0051-r v00; Table 3
Standard curve performance	• Cumulative bias range: NA • Cumulative precision: NA	NA

2.7.1.4.11 ヒト血清中 ADA 測定法（BAV-0039-R v00）

<table>
<tr><td>QC performance</td><td>The cumulative mean signal values for the HQC, LQC, and NQC from valid runs were all well within the relevant system suitability limits established for assay performance during validation and cumulative CV values for the control samples ranged from 7.3% to 9.6%.
<table><tr><th></th><th>HQC</th><th>LQC</th><th>NQC</th><th>%Inhibition Specificity Control LQC</th></tr><tr><td>Mean ECL Signal Values</td><td>23927</td><td>134</td><td>77</td><td>41</td></tr><tr><td>%CV</td><td>9.6</td><td>7.3</td><td>9.5</td><td>7.5</td></tr></table></td><td>bas-0051-r v00; Table 4</td></tr>
<tr><td>Method reproducibility</td><td colspan="3">NA</td><td>NA</td></tr>
<tr><td>Study sample analysis/stability</td><td colspan="3">All sample handling and analyses were conducted within the limits of sample stability for freeze/thaw, ambient temperature processing and 2 to 8 °C incubation established during method validation[a].</td><td>bas-0051-r v00; Section 6.0</td></tr>
<tr><td>Standard calibration curve performance during accuracy and precision runs</td><td colspan="4">NA</td></tr>
</table>

AD = Alzheimer's disease, ADA = anti-lecanemab antibodies, CV = coefficient of variation, ECL = electrochemiluminescence, HQC = high QC, LLOQ = lower limit of quantitation, LQC = low QC, MSD = meso scale discovery, MQC = mid QC, NA = not applicable, NQC = negative QC, ULOQ = upper limit of quantitation.
a: Long-term stability of frozen ADA samples has currently been confirmed for up to 645 days at -70 °C. However, 22 samples were analyzed outside of the established stability, these samples are identified with a * in Table 5 of bas-0051-r v00.
Source: bav-0039-r v00, bas-0051-r v00.

2.7.1.4.12 ヒト血清中 ADA 測定法（TCAR10-069）

Bioanalytical method validation report name, amendments, and hyperlinks	Validation of an MSD Immunogenicity Method for Detection of Antibodies to 316_2401 in Human Serum – tcar10-069 ([redacted] 20[redacted])		
Method Description	Assay platform: ECL-MSD This method was an ECL ligand-binding assay which detects antibodies to BAN2401 in human serum using the MSD technology. In the method, anti-drug antibodies were complexed between Biotinylated Drug and Ruthenylated Drug.		
Biological matrix	Human Serum		
Sample pre-treatment	NA		
Positive control antibody	Rabbit Anti-BAN2401 IgG		
Negative control	Pooled normal human serum only		
Materials used for standard calibration curve and concentration	NA		
Validated assay range	NA		
Materials used for quality controls (QCs) and concentration	Rabbit Anti-BAN2401 IgG and Human serum Low QC: 160 ng/mL Mid QC: 1000 ng/mL High QC: 15000 ng/mL		
Minimum required dilutions	1:5		
Source and lot of reagents	Rabbit Anti-BAN2401 IgG by [redacted] Lot# TB1039 No.1 ([redacted] 20[redacted]); BAN2401 by Eisai Co., Ltd., Tsukuba Research Laboratories, Lot# [redacted] (Retest: [redacted] 20[redacted]); Human Serum by [redacted] Lot# BRH294075-089, BRH296697-706, BRH294090-104, BRH296707-716, BRH362230-BRH362279 ([redacted] 20[redacted]).		
Regressing model and weighting	NA		
Validation parameters	**Method validation summary**		**Source location**
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LLOQ to ULOQ	NA	NA
	Cumulative accuracy (%bias) from LLOQ to ULOQ Product A	NA	NA
	Cumulative precision (%CV) from LLOQ to ULOQ Product A	NA	NA
Performance of QCs during accuracy and precision runs	**Cumulative accuracy (%bias) in 5 QCs** QCs for product A:	NA	NA
	Inter-batch %CV QCs for Product A:	NA	NA
	Total Error (TE) QCs for Product A:	NA	NA
Selectivity	Ten individual human serum samples, 5 males and 5 females, were tested for selectivity by analyzing unspiked and spiked with antibody at the LQC concentration (160 ng/mL). The assay was considered selective if: 80% of the unspiked samples were classified as negative		tcar10-069; Section 2 tcar10-069; Table 10

2.7.1.4.12 ヒト血清中 ADA 測定法（TCAR10-069）

<table>
<tr><td></td><td>At least 80% of the spiked samples classify as positive
Target of mean ECLU %CV≤25.
Unspiked samples-80% classified as negative
Spiked samples-All samples classified as positive
The %CVs for spiked and unspiked samples ranged from 0.5% to 7.6%.</td><td></td></tr>
<tr><td>Intra and Inter-precision for QC</td><td>The LQC, MQC and HQC were prepared by spiking human serum with Rabbit Anti-BAN2401 IgG antibody. Each plate contained NC, LQC, MQC and HQC in six duplicate sets (n=12) and the target acceptance criteria %CV for the average mean response for each duplicate was ≤ 25%.

Intra-assay precision:
<table>
<tr><th></th><th>NC</th><th>LQC</th><th>MQC</th><th>HQC</th></tr>
<tr><td>Mean ECLU</td><td>61 to 69</td><td>241 to 279</td><td>1281 to 1602</td><td>31245 to 35800</td></tr>
<tr><td>%CV</td><td>2.4% to 4.2%</td><td>1.7% to 3.5%</td><td>2.9% to 6.8%</td><td>1.7% to 8.3%</td></tr>
</table>
Inter-assay precision:
<table>
<tr><th></th><th>NC</th><th>LQC</th><th>MQC</th><th>HQC</th></tr>
<tr><td>Mean ECLU</td><td>65</td><td>263</td><td>1423</td><td>33634</td></tr>
<tr><td>%CV</td><td>4.6%</td><td>5.2%</td><td>8.5%</td><td>6.6%</td></tr>
</table></td><td>tcar10-069; Section 2
tcar10-069; Table 5</td></tr>
<tr><td>Intra and Inter-assay precision for titration controls</td><td>The acceptance criteria for the method end PC titer was set at 3125 ± 1 dilution with CV ≤25%.
Intra-assay precision: %CV: 0.0% to 9.5%
Inter-assay precision: %CV: 3.0% to 7.5%</td><td>tcar10-069; Section 2
tcar10-069; Table 4</td></tr>
<tr><td>Drug tolerance</td><td>The amount of drug required to drop the response of 500 ng/mL of antibody below the cut point provided an estimate of the drug tolerance in the assay.
The highest amount of drug that still allowed an antibody positive response from a 500 ng/mL PC was 31.3 µg/mL.</td><td>tcar10-069; Section 2
tcar10-069; Table 8</td></tr>
<tr><td>Interference & Specificity</td><td>NA</td><td>-</td></tr>
<tr><td>Screening and confirmation cut points</td><td>Screening and confirmatory assay cut points were determined.
[redacted] individual sera were analyzed on three occasions.
Screening cut point: Using the 95th percentile, the validated cut point was determined to be 69. The mean (non-outlier) NC of the individuals was determined to be 62 and the CPF was determined to be 7 (validated cut point-mean NC individuals).
Confirmation cut point: Mean response in presence of 250 µg/mL BAN2401 was -0.002 (using log spiked/unspiked) % inhibition cut point: 12.1% (100*[1-antilog])</td><td>tcar10-069; Section 2
tcar10-069; Table 2
tcar10-069; Table 11</td></tr>
</table>

2.7.1.4.12 ヒト血清中 ADA 測定法（TCAR10-069）

Sensitivity	The lowest concentration of the antibody that classified as positive (at or above cut point) was reported as the sensitivity of the method with respect to that antibody. Sensitivity was evaluated from at least 3 separate runs with means and % CV reported. Sensitivity was determined to be 9.77 ng/mL. The %CV for all sensitivity samples ranged from 0.0% to 9.6%.	tcar10-069; Section 2 tcar10-069; Table 9
Hemolysis effect	NA	-
Lipemic effect	NA	NA
Dilution linearity & hook effect	NA	NA
Bench-top/process stability	NA	NA
Freeze-Thaw stability	NA	NA
Long-term storage	NA	NA
Parallelism	NA	NA
Carry over	NA	NA
Method performance in study: Detection of Antibodies to BAN2401 in Serum by Meso Scale Discovery (MSD®) Electrochemiluminescence (ECL) Method in Support of Study: BAN2401-A001-101 (Report Number: tcar10-070)		
Assay passing rate	NA	-
Assay performance	A total of 35 runs were performed for sample analysis; 8 runs failed to meet acceptance criteria for the following reasons: 4 runs failed due to the NC being incorrect, 1 run failed due to high %CV at the endpoint titer, 1 run did not develop and 2 failed the TPC endpoint.	tcar10-070; Section Summary of Immunogenicity Assessments
Standard curve performance	• Cumulative bias range: NA • Cumulative precision: NA	-
QC performance	NA	-
Method reproducibility	NA	-
Study sample analysis/stability	The maximum storage period between blood sampling and sample preparation was 340 days, which was within the range of stability reported for this validated method.	tcar10-070; Section Summary of Sample Storage and Stability
Standard calibration curve performance during accuracy and precision runs	NA	

AD = Alzheimer's disease, ADA = anti-lecanemab antibodies, CPF = cut point factor, CV = coefficient of variation, ECL = electrochemiluminescence, ECLU = electrochemiluminescence units, HQC = high QC, IgG = immunoglobulin G, LLOQ = lower limit of quantitation, LQC = low QC, MSD = meso scale discovery, MQC = mid QC, NA = not applicable, NC = negative control, NQC = negative QC, PC = positive control, TPC = titer positive control, ULOQ = upper limit of quantitation.
Source: tcar10-069, tcar10-070.

2.7.1.4.13 ヒト血清中 ADA 測定法（45-1807）

Bioanalytical method validation report name, amendments, and hyperlinks	The Validation of an ECL method for the Detection of Anti-Drug Antibodies (ADA) Directed Against BAN2401 in Human Serum – 45-1807 ([redacted] 20[redacted])		
Method description	Assay platform: ECL-MSD A quasi-quantitative immunogenicity assay to detect the presence of anti-BAN2401 antibodies in human serum was validated.		
Biological matrix	Human Serum		
Sample pre-treatment	Assay dissociation: acetic acid		
Positive control	Anti-BAN2401 in pooled human serum		
Negative control	Pooled human serum		
Materials used for standard calibration curve and concentration	NA		
Validated assay range	NA		
Materials used for quality controls (QCs) and concentration	Monoclonal Anti-BAN2401 (Clone [redacted] 29817), BAN2401, Biotinylated-BAN2401 (biotinylated-drug), SulfoTag-BAN2401, Pooled human serum (Lot# HMN81175) Concentration Anti-BAN2401 in LPC: 12.1 ng/mL anti-BAN2401 in pooled human serum MPC: 1000 ng/mL anti-BAN2401 in pooled human serum HPC: 5000 ng/mL anti-BAN2401 in pooled human serum		
Minimum required dilutions	1:16		
Source and lot of reagents	Monoclonal Anti-BAN2401 by Eisai Inc., Lot# NB 3260-24A ([redacted] 20[redacted]); BAN2401 by Eisai Inc., Lot# [redacted] ([redacted] 20[redacted]); Biotinylated-BAN2401 by [redacted] Lot#DJB190712-01 or equivalent ([redacted] 20[redacted]); SulfoTag-BAN2401 by [redacted] Lot#DJB190712-02 or equivalent ([redacted] 20[redacted]); Pooled human serum by [redacted] Lot# HMN81175 ([redacted] 20[redacted]).		
Regressing model and weighting	NA		
Validation parameters	**Method Validation Summary**		**Source Location**
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LLOQ to ULOQ	NA	NA
	Cumulative accuracy (%bias) from LLOQ to ULOQ Product A	NA	NA
	Cumulative precision (%CV) from LLOQ to ULOQ Product A	NA	NA
Performance of QCs during accuracy and precision runs	**Cumulative accuracy (%bias) in 5 QCs** QCs for product A:	NA	NA
	Inter-batch %CV QCs for Product A:	NA	NA
	Total Error (TE) QCs for Product A:	NA	NA

2.7.1.4.13 ヒト血清中 ADA 測定法（45-1807）

<table>
<tr><td>Selectivity</td><td>The matrix selectivity was performed by analyzing 10 normal, 10 AD, 5 lipemic and 5 hemolyzed individual human serum lots and NC spiked with and without 6.06 and 5000 ng/mL of antiBAN2401 antibodies by screening and confirmatory assay.
The response of at least 80% of the unspiked individual lots should be less than the plate specific cut point.
The RLU of at least 80% of the individual lots of matrix spiked at each PC level should ≥ plate-specific cut point, and in the presence of BAN2401 should ≥ CCP.
The response of at least 80% of the individual lots of matrix spiked at each PC level should have a %Recovery within 75-125%.
The results show that 93.3% of unspiked lots had a mean RLU signal less than the plate cut point and 96.7% of unspiked lots confirmed negative. 100% of all tested lots spiked with 6.06 or 5000 ng/mL of PC screened positive and 96.7% of lots showed %Recovery between 75%%-125%. In the presence of 70.3 µg/mL of BAN2401, 100% of the lots spiked with 5000 ng/mL of HPC and 96.7% of lots spiked with LPC (6.06 ng/mL) had a %inhibition greater than the confirmatory cut point (11.7%).</td><td>45-1807; Section 4 and 11.3
45-1807; Table 5a</td></tr>
<tr><td>Intra and Inter-precision for QC</td><td>The intra-batch precision was determined from LPC, MPC and HPC (n=6) and NCs at n=3 on one plate. The results for intra-batch precision for the NC, LPC, MPC and HPC were 7.8%, 6.6%, 4.0% and 14.7%, respectively.

Inter-assay precision:
<table>
<tr><th></th><th>LPC</th><th>HPC</th><th>NC</th></tr>
<tr><td>%CV</td><td>19.3%</td><td>19.8%</td><td>17.8%</td></tr>
</table>
Intra-assay precision:
<table>
<tr><th></th><th>LPC</th><th>HPC</th><th>NC</th></tr>
<tr><td>%CV</td><td>6.6%</td><td>14.7%</td><td>7.8%</td></tr>
</table></td><td>45-1807; Section 4
45-1807; Table 9
45-1807; Table 6</td></tr>
<tr><td>Drug tolerance</td><td>Drug tolerance was reported as the highest concentration of drug for which a given ADA level remains at or above the plate cut point.
Less than 1.0 µg/mL for the 6.06 ng/mL PC
At least 2.5 µg/mL BAN2401 for the 12.1 ng/mL LPC
At least 10.0 µg/mL BAN2401 for the 100 ng/mL PC
At least 50.0 µg/mL BAN2401 for the 5000 ng/mL HPC</td><td>45-1807; Section 4
45-1807; Table 12</td></tr>
<tr><td>Interference & Specificity</td><td>NA</td><td>-</td></tr>
</table>

2.7.1.4.13 ヒト血清中 ADA 測定法（45-1807）

Cut points	Screening assay cut point factor 1.07 Confirmatory assay cut point 1: 11.7% Confirmatory assay cut point 2: 14.9% Titration assay cut point factor 1: 1.07 Titration assay cut point factor 2: 1.15	45-1807; Section 4 45-1807; Table 2 45-1807; Table 3
Sensitivity	Screening assay sensitivity: 3.47 ng/mL Confirmatory assay sensitivity: 12.2 ng/mL	45-1807; Section 4 45-1807; Table 4
Hemolysis effect	NA	-
Lipemic effect	NA	-
Dilution linearity & hook effect	Hook effect tested at 150 µg/mL and observed at PC concentration above 9375 ng/mL. All values screened positive.	45-1807; Section 4
Bench-top/process stability	Room temperature (ambient) stability of positive control: The PC was considered to be stable in a biological matrix if the mean of the response at each discrete level was no more than ±25% different from the untreated sample (reported as %Diff). The %CV of the replicates must not exceed 20.0%. Bench top stability of PC in pooled HUSE was 6 hours.	45-1807; Section 4 45-1807; Table 13
Freeze-Thaw stability	Freeze/Thaw stability of positive control: The PC was considered to be stable in a biological matrix if the mean of the response at each discrete level was no more than ± 25% different from the untreated sample (reported as %Diff). The %CV of the replicates must not exceed 20.0%. Freeze/Thaw stability of PC in Pooled HUSE was 5 cycles at -20 °C and -70 °C. Critical reagent Freeze/Thaw stability: The labeled test articles were considered stable after the number of freeze/thaw cycles tested if the mean of the response at each discrete level was no more than ±25.0% different from the untreated sample (reported as %Diff). The %CV of the replicates must not exceed 20.0%. Critical reagent Freeze/Thaw stability was 2 cycles at -80 °C.	45-1807; Section 4 45-1807; Table 14 45-1807; Table 15 45-1807; Table 16
Long-term storage	NA	NA
Parallelism	NA	NA
Carry over	NA	NA
Method performance in study: Detection of Anti-BAN2401 Antibodies in Human Serum Samples by ECL: BAN2401-G000-201 (Report Number: 45-1809)		
Assay passing rate	NA	-
Assay performance	Of the 1380 primary samples received, 1378 samples were analyzed in 63 runs, of which, 60 met the acceptance criteria. Two samples had insufficient volume for the screening assay. Final results were reported for 1377 samples as one screened sample had insufficient volume for the confirmation assay.	45-1809 ; Section 5
Standard curve performance	• Cumulative bias range: NA • Cumulative precision: NA	NA
QC performance	NA	NA
Method reproducibility	NA	NA

2.7.1.4.13 ヒト血清中 ADA 測定法（45-1807）

Study sample analysis/stability	The maximum storage period between sample collection and analysis was 927 days.	45-1809; Section 10.5
Standard calibration curve performance during accuracy and precision runs	NA	

AD = Alzheimer's disease, ADA = anti-lecanemab antibodies, CCP = confirmatory cut point, CV = coefficient of variation, ECL = electrochemiluminescence, FDA = Food and Drug Administration, HPC = high positive control, HUSE = human serum, IgG = immunoglobulin G, LLOQ = lower limit of quantitation, LPC = low positive control, MPC = mid positive control, MSD = meso scale discovery, NA = not applicable, NC = negative control, PC = positive control, QC = quality control, RLU = relative light unit, ULOQ = upper limit of quantitation.
Source: 45-1807, 45-1809.

2.7.1.4.14 ヒト血清中 ADA 測定法（45-1807 Addendum1）

Bioanalytical method validation report name, amendments, and hyperlinks	The Validation of an ECL method for the Detection of Anti-Drug Antibodies (ADA) Directed Against BAN2401 in Human Serum – 45-1807 addendum1 ([redacted] 20[redacted])		
Method description	Assay platform: ECL-MSD A quasi-quantitative immunogenicity assay to detect the presence of anti-BAN2401 antibodies in human serum was validated.		
Biological matrix	Pooled Human Serum		
Sample pre-treatment	Assay dissociation: acetic acid		
Positive control	Anti-BAN2401 in pooled human serum		
Negative control	Pooled human serum		
Materials used for standard calibration curve and concentration	NA		
Validated assay range	NA		
Materials used for quality controls (QCs) and concentration	Pooled HUSE was used as negative control (NC) Anti-BAN2401 (Clone[redacted]29817) monoclonal antibodies were used as positive controls (12.1 ng/mL LPC and 5000 ng/mL HPC). Polyclonal anti-BAN2401 were also used as positive controls (100 ng/mL, 1000 ng/mL, and 5000 ng/mL).		
Minimum required dilutions	1:16		
Source and lot of reagents	Biotin-BAN2401, Lot# DJB191008-01 (Expiry date: NA) SulfoTag-BAN2401, Lot# RJW220120-02 (Expiry date: NA) Biotin-BAN2401, Lot# KLS220427-02 (Expiry date: NA) SulfoTag-BAN2401, Lot# KLS220502-03 (Expiry date: NA) Biotin-BAN2401, Lot# RJW220427-02 (Expiry date: NA) SulfoTag-BAN2401, Lot# RJW220502-03 (Expiry date: NA) Anti-BAN2401 Antibody (Purified Rabbit IgG) (Expiry date: NA)		
Regressing model and weighting	NA		
Validation parameters	**Method Validation Summary**		**Source Location**
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LLOQ to ULOQ	NA	NA
	Cumulative accuracy (%bias) from LLOQ to ULOQ Product A	NA	NA
	Cumulative precision (%CV) from LLOQ to ULOQ Product A	NA	NA

2.7.1.4.14 ヒト血清中 ADA 測定法（45-1807 Addendum1）

Performance of QCs during accuracy and precision runs	**Cumulative accuracy (%bias) in 5 QCs** QCs for product A:	NA	NA
	Inter-batch %CV QCs for Product A:	NA	NA
	Total Error (TE) QCs for Product A:	NA	NA
Selectivity	NA		NA
Intra and Inter-precision for QC	Polyclonal PC inter-assay precision: 100 ng/mL PC: 13.1% 1000 ng/mL PC: 9.5% 5000 ng/mL PC: 8.0% Polyclonal PC intra-assay precision 100 ng/mL PC: 8.2% 1000 ng/mL PC: 2.3% 5000 ng/mL PC: 2.7%		45-1807 addendum1; Table 7 45-1807 addendum1; Table 8
Drug tolerance	Drug Tolerance (Monoclonal anti-BAN2401): At least 5.0 µg/mL BAN2401 for the 12.1 ng/mL LPC At least 31.3 µg/mL BAN2401for the 50 ng/mL PC At least 31.3 µg/mL BAN2401 for the 100 ng/mL PC At least 125 µg/mL BAN2401 for the 250 ng/mL PC At least 250 µg/mL BAN2401 for the 1000 ng/mL PC At least 50 µg/mL BAN2401 (highest level tested) for the HPC Drug Tolerance (polyclonal anti-BAN2401) At least 62.5 µg/mL BAN2401for the 50 ng/mL PC At least 80.0 µg/mL BAN2401 for the 100 ng/mL PC At least 125 µg/mL BAN2401 for the 250 ng/mL PC At least 500 µg/mL BAN2401 for the 1000 ng/mL PC		45-1807 addendum1; Table 6
Interference & Specificity	NA		-
Cut points	Screening assay cut point factor: 1.07 Confirmatory assay cut point: 11.7% Confirmatory assay cut point 2: 14.9%		45-1807 addendum1; Table 5
Sensitivity	Screening assay sensitivity: Original Validation: 3.47 ng/mL (Using PC Clone [redacted]29817) Addendum 1: 1.09 ng/mL (Using PC Clone [redacted]29817) Polyclonal anti-BAN2401: 2.5 ng/mL Confirmatory assay sensitivity:		45-1807 addendum1; Table 2

2.7.1.4.14 ヒト血清中 ADA 測定法（45-1807 Addendum1）

	Original validation: 12.2 ng/mL (Using PC Clone [redacted]29817) Addendum 1: 6.84 ng/mL (Using PC Clone [redacted]29817) Polyclonal anti-BAN2401: 17.8 ng/mL	
Hemolysis effect	NA	NA
Lipemic effect	NA	NA
Dilution linearity & hook effect	NA	NA
Bench-top/process stability	NA	NA
Freeze-Thaw stability	NA	NA
Long-term storage	NA	NA
Parallelism	NA	NA
Carry over	NA	NA
Method performance in study: Detection of Anti-BAN2401 Antibodies in Human Serum Samples by ECL: BAN2401-G000-301 Core and OLE (Report Number: 45-1908)		
Assay passing rate	NA	Table 2, 3, 4 Report 45-1908
Assay performance	NA	Table 2, 4 Report 45-1908
Standard curve performance	• Cumulative bias range: NA • Cumulative precision: NA	Table 4 Report 45-1908
QC performance	NA	Table 8 Report 45-1908
Method reproducibility	NA	Table 5, 6, 7 Report 45-1908
Study sample analysis/stability	The maximum storage period between sample collection and analysis was 1213 days.	Section 11, Report 45-1908
Standard calibration curve performance during accuracy and precision runs	NA	

AD = Alzheimer's disease, ADA = anti-lecanemab antibodies, CCP = confirmatory cut point, CV = coefficient of variation, ECL = electrochemiluminescence, ECLU = electrochemiluminescence units, FDA = Food and Drug Administration, HPC = high positive control, HUSE = human serum, IgG = immunoglobulin G, LLOQ = lower limit of quantitation, LPC = low positive control, MPC = mid positive control, MSD = meso scale discovery, NA = not applicable, OLE = open-label extension, PC = positive control, QC = quality control, RLU = relative light unit, ULOQ = upper limit of quantitation.
Source: 45-1807 addendum1, 45-1908.

2.7.1.4.15 ヒト血清中 ADA 測定法（TCAR12-038）

Bioanalytical method validation report name, amendments, and hyperlinks	Validation of an Analytical Method (with Acid Dissociation) for the Detection of Antibodies to 316_2401 (BAN2401) in Human Serum by Meso-Scale Discovery (MSD®) Electrochemiluminescence (ECL) - tcar12-038 ([redacted] 20[redacted])		
Method description	Assay platform: ECL-MSD In this MSD® method, anti-BAN2401 antibodies were bridged between biotinylated BAN2401 and Sulfo-tagged BAN2401. Human serum samples, QCs and NC were first treated with acetic acid followed by acid neutralization with 1.5 M Tris and incubation with Master Mix (Biotinylated BAN2401 and Sulfo-tagged BAN2401). The resulting complexes, (biotin-BAN2401: anti-BAN2401 antibody: sulfo-tagged-BAN2401) were loaded into the wells of a blocked MSD® Streptavidin coated plate. After unbound material was removed by washing the plate, MSD® read buffer was added and the bound complexes were detected by electrochemiluminescence in the MSD® instrument.		
Biological matrix	Human Serum		
Sample pre-treatment	Acid dissociation: acetic acid		
Positive control antibody	Rabbit Anti-BAN2401 IgG		
Negative control	Human serum		
Materials used for standard calibration curve and concentration	NA		
Validated assay range	NA		
Materials used for quality controls (QCs) and concentration	Rabbit Anti-BAN2401 IgG and human Serum High QC (HQC): 15000 ng/mL rabbit Anti-BAN2401 IgG in human serum Mid QC (MQC): 1000 ng/mL rabbit Anti-BAN2401 IgG in human serum Low QC (LQC): 50.0 ng/mL rabbit Anti-BAN2401 IgG in human serum Negative QC (NQC): human serum only <u>TPC:</u> The TPC was prepared by spiking human serum (NC) with anti-BAN2401 (3.50 mg/mL). The TPC concentration was set at 20000 ng/mL and diluted 1:5 in assay diluent (20% pooled human serum in 1% Blocker A in PBS) for a total of 8 TPC titers at concentrations of 20,000, 4,000, 800, 160, 32.0, 6.40, 1.28, and 0.256 ng/mL.		
MRDs	1:5		
Source and lot of reagents	Rabbit Anti-BAN2401 IgG 3.50 mg/mL (TB1039 No.1 [redacted] 20[redacted]) from [redacted]; BAN2401 [redacted] mg/mL ([redacted] [redacted] 20[redacted]) from Eisai Co., LTD; human serum (BRH734639-734688 [redacted] 20[redacted]) from [redacted]		
Regressing model and weighting	NA		
Validation parameters	**Method Validation Summary**		**Source Location**
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LLOQ to ULOQ	NA	NA
	Cumulative accuracy (%bias) from LLOQ to ULOQ Product A	NA	NA
	Cumulative precision (%CV) from LLOQ to ULOQ Product A	NA	NA

2.7.1.4.15 ヒト血清中 ADA 測定法（TCAR12-038）

<table>
<tr><td rowspan="3">Performance of QCs during accuracy and precision runs</td><td><u>Cumulative accuracy (%bias) in 5 QCs</u>
QCs for product A:</td><td>NA</td><td>NA</td></tr>
<tr><td><u>Inter-batch %CV</u>
QCs for Product A:</td><td>NA</td><td>NA</td></tr>
<tr><td><u>Total Error (TE)</u>
QCs for Product A:</td><td>NA</td><td>NA</td></tr>
<tr><td>Cut points</td><td colspan="2"><u>Cut point determination for plate acceptance:</u>
[redacted] individual serum samples analyzed by two analysts over two days in three runs (six plates). The validated CPF was determined to be 1.11 using the 95th percentile limit.
<u>Cut point determination for sample analysis:</u>
[redacted] individual pre-dose serum samples analyzed by two analysts over two days in three runs (seven plates). The mean (non-outlier) NC of the individuals was determined to be 50, and the CPF was determined to be 6.</td><td>tcar12-038; Section 2
tcar12-038; Table 1
tcar12-038; Table 2
tcar12-038; Table 3
tcar12-038; Table 4</td></tr>
<tr><td>Sensitivity</td><td colspan="2">Sensitivity was evaluated from six separate runs. The mean interpolated sensitivity was determined to be 3.388 ng/mL ± 1.092 ng/mL (SD) resulting in a sensitivity range of 2.295 to 4.480 ng/mL.</td><td>tcar12-038; Section 6.3
tcar12-038; Table 5</td></tr>
<tr><td>Selectivity</td><td colspan="2">Ten individual human serum samples (5 male and 5 female) were analyzed spiked and unspiked at the LQC (50.0 ng/mL) level. Response of the spiked sample must be higher than the unspiked sample. For those individuals that have unspiked matrix values below cut point, 80% of the spiked response must classify as positive (above cut point).
All samples for selectivity had %CVs ≤25.0. All 10 spiked samples were higher than the unspiked samples and 9 of 10 unspiked samples were below the assay cut point. Assessment of selectivity was accepted.</td><td>tcar12-038; Section 2
tcar12-038; Table 12</td></tr>
<tr><td>Intra- and inter-assay precision of QC</td><td colspan="2">Precision assays to assess quality controls were performed by two analysts over three days for a total of six runs. The inter and intra-assay %CVs across all runs was ≤25.0%.
<table>
<tr><th></th><th>NC</th><th>LQC</th><th>MQC</th><th>HQC</th></tr>
<tr><td>Intra-assay Precision (%CV)</td><td>2.5 to 6.2%</td><td>2.0 to 4.7%</td><td>2.2 to 3.6%</td><td>1.6 to 3.5%</td></tr>
<tr><td>Inter-assay Precision (%CV)</td><td>7.8%</td><td>4.1%</td><td>5.5%</td><td>5.8%</td></tr>
</table></td><td>tcar12-038; Section 2
tcar12-038; Section 6.3
tcar12-038; Table 6</td></tr>
<tr><td>Titer positive control precision</td><td colspan="2">The TPC was prepared by spiking human serum (NC) with anti-BAN2401 (3.50 mg/mL). TPC precision assays were performed over two days for a total of three runs (n = 3 duplicate sets of TPC per plate).
The %CVs of the replicate TPC titrations across runs were < 25.0%. The acceptance criteria for the method TPC end point titer was set at 3125 ± 1 titration dilution with CV ≤25.0%.</td><td>tcar12-038; Section 2
tcar12-038; Section 6.3
tcar12-038; Table 5</td></tr>
</table>

2.7.1.4.15 ヒト血清中 ADA 測定法（TCAR12-038）

<table>
<tr><td>Hemolysis effect</td><td>NA</td><td>NA</td></tr>
<tr><td>Lipemic effect</td><td>NA</td><td>NA</td></tr>
<tr><td>Dilution linearity & hook effect</td><td>NA</td><td>NA</td></tr>
<tr><td>Bench-top/process stability</td><td>21 hours, 27 minutes</td><td>tcar12-038; Section 2</td></tr>
<tr><td>Freeze-Thaw stability</td><td>Up to 6 freeze/thaw cycles</td><td>tcar12-038; Section 2</td></tr>
<tr><td>Long-term storage</td><td>645 Days at -70 °C nominal</td><td>tcar12-038; Section 2</td></tr>
<tr><td>Parallelism</td><td>NA</td><td></td></tr>
<tr><td>Carry over</td><td>NA</td><td></td></tr>
<tr><td colspan="3">Method performance in study: Detection of Antibodies to BAN2401 in Serum by Meso Scale Discovery (MSD) Electrochemiluminescence (ECL) Method in Support of Study: BAN2401-G000-201 (tnjr14-226)</td></tr>
<tr><td>Assay passing rate</td><td>NA</td><td>NA</td></tr>
<tr><td>Assay performance</td><td>TCAS12-039 ([redacted]) & TNJS14-226 ([redacted])
<table><tr><th></th><th>Accepted Runs</th><th>Failed Runs</th></tr><tr><td>TCAS12-039</td><td>113</td><td>25</td></tr><tr><td>TNJS14-226</td><td>285</td><td>51</td></tr></table></td><td>tnjr14-226; Report Summary</td></tr>
<tr><td>Standard curve performance</td><td>NA</td><td>NA</td></tr>
<tr><td>QC performance</td><td><table><tr><th></th><th>Normal</th><th>LQC</th><th>MQC</th><th>HQC</th></tr><tr><td>Mean ECLU</td><td>82</td><td>163</td><td>1815</td><td>37901</td></tr><tr><td>%CV</td><td>40.4%</td><td>24.1%</td><td>21.2%</td><td>27.1%</td></tr></table>
TPCs:
%CV: ≤17.7%</td><td>tnjr14-226; Table 6
tnjr14-226; Table 7</td></tr>
<tr><td>Method reproducibility</td><td>NA</td><td>-</td></tr>
<tr><td>Study sample analysis/stability</td><td>Maximum time from collection to analysis was 1065 days. Eleven samples exceeded the maximum established stability of up to 645 days. Additionally, one sample exceeded the maximum established number of freeze/thaw cycles of up to 6 cycles, with a maximum of 8 freeze/thaw cycles. Samples outside of established stabilities were noted in Table 3 of report tnjr14-226.</td><td>tnjr14-226; Compliance Statement
tnjr14-226; Sample Storage and Stability
tnjr14-226; Table 3</td></tr>
<tr><td>Standard calibration curve performance during accuracy and precision runs</td><td colspan="2">NA</td></tr>
</table>

AD = Alzheimer's disease, ADA = anti-lecanemab antibodies, CV = coefficient of variation, CPF = cut point factor, ECLU = electrochemiluminescence units, HQC = high QC, IgG = immunoglobulin G, LLOQ = lower limit of quantitation, MRD = minimum required dilution, NA = not applicable, NC = negative control, PBS = phosphate-buffered saline, QC = quality control, SD = standard deviation; TPC = titer positive control, ULOQ = upper limit of quantification.
Source: tcar12-038, tnjr14-226.

2.7.1.4.16 ヒト血清中 ADA 測定法（TNJR14-225）

Bioanalytical method validation report name, amendments, and hyperlinks	Validation of an Analytical Method (with Acid Dissociation) for the Detection of Antibodies to BAN2401 in Human Serum by Meso-Scale Discovery (MSD) Electrochemiluminescence (ECL) - tnjr14-225 ([redacted] 20[redacted])		
Method Description	Assay platform: ECL-MSD The Antibodies to 316_2401 were detected (with acid dissociation) in human serum by MSD ECL. In this MSD® method, anti-analyte (BAN2401) antibodies were bridged between biotinylated-analyte and sulfo-tagged analyte. Human serum samples were first treated with acetic acid followed by neutralization with 1.5 M Tris and incubation with master mix (biotinylated BAN2401 and sulfo-tagged BAN2401). The resulting complexes, (biotin-analyte: anti-analyte antibody: sulfo-tagged analyte) were loaded into the wells of a blocked MSD® streptavidin coated plate and the bound complexes were detected by ECL in the MSD® sector imager. The ECL units were measured, and the detection and/or confirmation of anti-drug antibodies in the sample was determined.		
Sample pre-treatment	Acid dissociation: acetic acid		
Biological matrix	Human serum		
Positive control antibody	Rabbit Anti-BAN2401 IgG		
Negative control	Pooled normal human serum		
Materials used for standard calibration curve and concentration	NA		
Validated assay range	NA		
Materials used for quality controls (QCs) and concentration	BAN2401 (316_2401), Anti-BAN2401 (Purified Rabbit IgG - 316_a_2401), BAN2401-Biotin, BAN2401-SulfoTag LPC (50 ng/mL), MPC (1000 ng/mL), HPC (15000 ng/mL), and TPC (20000 ng/mL)		
Minimum required dilutions (MRDs)	1:5		
Source and lot of reagents	BAN2401 (316_2401), Lot#[redacted] by Eisai Anti-BAN2401 (Purified Rabbit IgG) (316_2401), Lot# TB1039 by Eisai BAN2401-Biotin, Lot# N013-139B by [redacted] BAN2401-SulfoTag, Lot# N013-139S by [redacted] Normal Human Serum Lot# BRH978991 by [redacted]		
Regressing model and weighting	NA		
Validation parameters	**Method validation summary**		**Source location**
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LLOQ to ULOQ	NA	-
	Cumulative accuracy (%bias) from LLOQ to ULOQ Product A	NA	-
	Cumulative precision (%CV) from LLOQ to ULOQ Product A	NA	-
Performance of QCs during accuracy and precision runs	Precision of Screening (%CV of ECLU)		tnjr14-225; Table 1

	NC	LPC	MPC	HPC
Intra-assay	7.3 to 9.0%	2.6 to 8.7%	2.0 to 3.0%	2.0 to 7.5%
Inter-assay	7.6%	3.4%	1.8%	1.4%

2.7.1.4.16 ヒト血清中 ADA 測定法（TNJR14-225）

Precision of titer positive control:	The last positive dilution had %CV <25.0% for all of the sets tested. The titer value (last positive) was 3125 for 8 sets. Two sets did not cross the cut point.	tnjr14-225; Table 2
Cut points	Screening cut point factor: 1.11 multiplicative (QCs/TPCs); 6 additive (samples) Screening cut point: $NC_{Mean\ ECLU}$ x 1.11 (QCs/TPCs); $PNC_{Mean\ ECLU}$ + 6 (samples) Confirmation cut point: 25.2% inhibition (QCs/TPCs); 13.3% inhibition (samples)	tnjr14-225; Appendix B Validation summary
Sensitivity	3.388 ng/mL	tnjr14-225; Appendix B Validation summary
Selectivity & matrix effect	10 of 10 spiked samples were > cut point 9 of 10 unspiked samples were < cut point	tnjr14-225; Appendix B Validation summary
Interference & specificity	NA	-
Hemolysis effect	NA	-
Lipemic effect	NA	-
Dilution linearity & hook effect	NA	-
Bench-top/process stability	Bench top stability was established up to 30 hours.	tnjr14-225; Table 7
Freeze-Thaw stability	Freeze thaw stability was assessed in human serum at 6 freeze/thaw cycles.	tnjr14-225; Appendix B Validation summary
Long-term storage	Long-term stability was demonstrated for up to 645 days at - 70 °C.	tnjr14-225; Appendix B Validation summary
Parallelism	NA	-
Carry over	NA	-
Method performance in study: Detection of Antibodies to BAN2401 in Human Serum by Meso-Scale Discover (MSD) Electrochemiluminescence (ECL) in Support of Protocol Number BAN2401-J081-104 (Report Number: tnjr14-227)		
Assay passing rate	A total of 19 runs were performed for sample analysis; 2 runs failed to meet acceptance criteria for the following reasons: 1 LPC run did not meet acceptance and 1 TPC run did not meet acceptance.	tnjr14-227, Table 1
Standard curve performance	• Cumulative bias range: NA • Cumulative precision: NA	-
QC performance	• Cumulative bias range: NA • Cumulative precision: NA • TE: NA	-
Method reproducibility	NA	-

2.7.1.4.16 ヒト血清中 ADA 測定法（TNJR14-225）

Study sample analysis/stability	The maximum storage period between sample collection and analysis was 574 days.	tnjr14-227; Study samples: Source and storage
Standard calibration curve performance during accuracy and precision runs	NA	

CV = coefficient of variation, ECLU = electrochemiluminescence unites, HPC = high positive control, IgG = immunoglobulin G, LLOQ = lower limit of quantitation, LPC = low positive control, MPC = mid positive control, NA = not applicable, NC = negative control, PNC = pre-dose negative control, TPC = titer positive control, TE = total error, ULOQ = upper limit of quantitation.
Source: tnjr14-225, tnjr14-227.

2.7.1.4.17 ヒト血清中 NAb 測定法（BAV-0031-R v02）

Bioanalytical method validation report name, amendments, and hyperlinks	Validation of Electrochemiluminescent Immunoassay for the Measurement of Anti-BAN2401 Neutralizing Antibodies in Human Serum - bav-0031-r v02 (Amendment 2; [redacted])		
Method description	Assay Platform: ECL-MSD immunoassay The immunoassay format was based on the capture of biotin labeled BAN2401 on streptavidin coated microtiter plates, followed by detection with a ruthenium labeled [redacted] and subsequent ECL signal detection. Neutralizing ADA activity in ADA positive serum samples was detected by measuring the inhibition of BAN2401/[redacted] binding interaction using a threshold response value, the neutralization cut point, in which signal values above (antibody-antigen complex formation) or below (inhibition of complex formation) the cut point indicated negative or positive results, respectively.		
Biological matrix	Human Serum		
Sample pre-treatment	Acid dissociation; 2% acetic acid to preemptively disrupt NAb-BAN2401 antibody-antigen complexes at low pH		
PC antibody	Neutralizing murine anti-BAN2401 monoclonal IgG antibody, clone 3G7G6		
NC	Pooled human serum only		
Materials used for standard calibration curve and concentration	NA		
Validated assay range	NA		
Materials used for QCs and concentration	Anti-BAN2401 NAb; neat human serum HQC (50 µg/mL anti-BAN2401 NAb in neat human serum [20[redacted]-0089] from [redacted]), HQC (20 µg/mL anti-BAN2401 NAb in neat human serum [20[redacted]-0137]) from [redacted]], LQC (5.0 µg/mL anti-BAN2401 NAb in neat human serum [20[redacted]-0090]) from [redacted]], NQC (neat human serum [20[redacted]-0091] from [redacted]		
MRDs	MRD was not needed.		
Source and lot of reagents	Neutralizing murine anti-BAN2401 monoclonal IgG antibody (clone 3G7G6; 20[redacted]-0009 [redacted] 20[redacted]]) from [redacted]; BAN2401 drug product (20[redacted]-0064 [redacted] 20[redacted]]) from Eisai; pooled normal human serum (20[redacted]-0024, 20[redacted]-0052 [redacted] 20[redacted], [redacted] 20[redacted]]) from [redacted]; individual male normal human serum (20[redacted]-0044, 20[redacted]-0049 [redacted] 20[redacted], [redacted] 20[redacted]]) from [redacted]; individual female normal human serum (20[redacted]-0045, 20[redacted]-0050 [redacted] 20[redacted], [redacted] 20[redacted]]) from [redacted]; individual AD human serum (20[redacted]-0336 [redacted] 20[redacted]]) from [redacted]; biotinylated BAN2401 ([redacted]:1 challenge ratio]; 20[redacted]-0021, 20[redacted]-0065 [redacted] 20[redacted], [redacted] 20[redacted]]) from [redacted]; sulfo-tag labeled [redacted][[redacted]:1 challenge ratio]; 20[redacted]-0047 [redacted] 20[redacted]]) from [redacted]; MSD 96-well streptavidin plate (20[redacted]-0204 [redacted] 20[redacted]]) from MSD		
Regressing model and weighting	4-parameter logistic regression fit		
Validation parameters	**Method Validation Summary**		**Source Location**
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LLOQ to ULOQ	NA	NA
	Cumulative accuracy (%bias) from LLOQ to ULOQ Product A	NA	NA
	Cumulative precision (%CV) from LLOQ to ULOQ Product A	NA	NA

2.7.1.4.17 ヒト血清中 NAb 測定法（BAV-0031-R v02）

Performance of QCs during accuracy and precision runs	**Cumulative accuracy (%bias) in 5 QCs** QCs for product A:	NA	NA
	Inter-batch %CV QCs for Product A:	NA	NA
	TE QCs for Product A:	NA	NA
Cut point	A floating screening cut point normalization factor was determined. Neutralizing cut point: NQC ECL signal value - 431 ECL units		bav-0031-r v02; Section 5.3, Table 6
Sensitivity	Serial dilutions of the surrogate NAb were prepared in pooled human serum ranging from 90 µg/mL to 0.04 µg/mL and assayed in triplicate in 6 independent runs by 3 analysts using a balanced assay design. No acceptance criteria were defined. The mean assay sensitivity was determined to be 584 ng/mL NAb using a surrogate NAb murine monoclonal anti-BAN2401 IgG antibody in neat human serum.		bav-0031-r v02; Section 5.2 bav-0031-r v02; Table 7
Selectivity	Human serum samples from 10 normal individuals and 10 AD individuals along with a single lot of pooled human serum were spiked with either 50 µg/mL or 5.0 µg/mL murine monoclonal anti-BAN2401 IgG antibody and analyzed in the assay. At least 16/20 (80%) individual serum samples spiked with a low and high concentrations of NAb must generate ECL signal values ≤20.0% different to comparable NAb spike levels in pooled serum at each concentration level. Selectivity was established in normal and AD serum: 19/20 individuals met the acceptance criteria of ≤20.0% difference at the low spike level of 5.0 µg/mL NAb, with a range of -13.7% to 6.9% difference; and 16/20 individuals met the acceptance criteria at the high spike level of 50 µg/mL NAb, with a range of -19.8% to 5.9% difference.		bav-0031-r v02; Table 1 bav-0031-r v02; Table 8
Precision	Neutralizing Assay: 3 aliquots of HQC, LQC and unspiked pooled serum (NQC) were thawed and assayed using a balanced assay design in which 6 independent assay runs performed separately by 3 analysts, each of whom completed 2 runs on different days. Pooled repeatability and intermediate %CV must be ≤20.0% for both the HQC and LQC ECL signal values. Titration Assay: Pooled repeatability and intermediate %CVs must be ≤20.0% for Log10 titer values. MSR was determined. Neutralizing Assay[a]: The LQC samples generated a pooled repeatability %CV of 9.7% and intermediate %CV of 18.6%, which met the acceptance criteria of ≤20.0% for each precision evaluation. Titration Assay: Repeatability %CV = 10.7%		bav-0031-r v02; table 1 bav-0031-r v02; table 9 bav-0031-r v02; Table 12

2.7.1.4.17 ヒト血清中 NAb 測定法（BAV-0031-R v02）

	Intermediate %CV = 11.5% MSR = 5	
System suitability	For system suitability criteria, ECL response limits were established based on the precision analysis for the HQC, LQC, and NQC samples using a 99% confidence interval to use as acceptance criteria during in-study assay runs. QC ECL signal values must be in the relative order: HQC < LQC < NQC • HQC: 221 to 508 ECL units • LQC: 423 to 1417 ECL unit • NQC: >1352 ECL units	bav-0031-r v02; table 1 bav-0031-r v02; Table 11
Drug tolerance	The lowest drug concentration that raised the NAb response signal above the cut point was determined. Drug tolerance was determined to be ≤31.3 µg/mL BAN2401 in the presence of 5.0 µg/mL murine monoclonal anti-BAN2401 IgG antibody.	bav-0031-r v02; Table 1 bav-0031-r v02; Table 13
Interference & specificity	NA	-
Hemolysis effect	NA	-
Lipemic effect	NA	-
Dilution linearity & hook effect	NA	-
Bench-top/process stability	4 days at ambient temperature in pooled human serum. %CV: HQC, 3.1%; LQC, 1.6%; NQC, 2.6%	bav-0031-r v02; Table 1 bav-0031-r v02; Table 14
Freeze-Thaw stability	11 cycles in pooled human serum. %CV: HQC, 0.5%; LQC, 0.2%; NQC, 0.3%	bav-0031-r v02; Table 1 bav-0031-r v02; Table 14
Long-term storage	12 months at-20 °C in pooled human serum. %CV: HQC, 0.3%; LQC, 3.6%; NQC, 0.5% 3 years, 2 months, and 30 days at -80 °C in pooled human serum. %CV: HQC, 0.0%; LQC, 0.8%; NQC, 0.5%	bav-0031-r v02; Table 1 bav-0031-r v02; Table 15 bav-0031-r v02; Table 16
Parallelism	NA	-
Carry over	NA	-
Method performance in study: Detection of Neutralizing Anti-BAN2401 Antibodies in Human Serum Samples from Clinical Study BAN2401-G000-201 (bas-0052-r v00)		
Assay passing rate	NA	-
Assay performance	Total of 44 valid assay runs were performed with 38 passing validity criteria for a success rate of 86.4%. Of the 44 valid runs, 30 were screening runs and 14 were titration runs.	bas-0052-r v00; Section 9.1 bas-0052-r v00; Table 3
Standard curve performance	NA	-

2.7.1.4.17 ヒト血清中 NAb 測定法（BAV-0031-R v02）

<table>
<tr><td>QC performance</td><td>The cumulative mean signal values for the HQC, LQC and NQC from passing runs were all well within the relevant system suitability limits established for assay performance during validation and cumulative CV values for the QC samples ranged from 9.0 to 14.2%.
<table><tr><th></th><th>HQC</th><th>LQC</th><th>NQC</th></tr><tr><td>Mean ECL Signal Values</td><td>408</td><td>1002</td><td>2798</td></tr><tr><td>%CV</td><td>14.2%</td><td>11.8%</td><td>9.0%</td></tr></table></td><td>bas-0052-r v00; Section 9.1
bas-0052-r v00; Table 4</td></tr>
<tr><td>Method reproducibility</td><td>NA</td><td>-</td></tr>
<tr><td>Study sample analysis/stability</td><td>All sample handling and analyses were conducted within the limits of sample stability for freeze/thaw, ambient temperature processing and 2 to 8 °C incubation established during method validation[b].</td><td>bas-0052-r v00; Section 6.0</td></tr>
<tr><td>Standard calibration curve performance during accuracy and precision runs</td><td colspan="2">NA</td></tr>
<tr><td colspan="3">Method performance in study: Detection of Anti-BAN2401 Antibodies in Human Serum Samples from Clinical Study BAN2401-J081-104 (bas-0044-r)</td></tr>
<tr><td>Assay passing rate</td><td>NA</td><td>-</td></tr>
<tr><td>Assay Performance</td><td>A total of two assay runs were performed with both passing validity criteria for a success rate of 100%. Of the two runs, one was a neutralizing screening assay, and one was a neutralizing titration assay.</td><td>bas-0044-r; Section 9.1
bas-0044-r; Table 5</td></tr>
<tr><td>Standard curve performance</td><td>NA</td><td>-</td></tr>
<tr><td>QC performance</td><td>The cumulative mean ECL signal values for the HQC, LQC and NQC from valid runs were all well within the relevant system suitability limits established for assay performance during validation and cumulative %CV values for the control samples ranged from 13.5 to 18.1%.
<table><tr><th></th><th>HQC</th><th>LQC</th><th>NQC</th></tr><tr><td>Mean ECL Signal Values</td><td>390</td><td>820.5</td><td>2434</td></tr><tr><td>%CV</td><td>17.0%</td><td>13.5%</td><td>18.1%</td></tr></table></td><td>bas-0044-r; Section 9.1
bas-0044-r; Table 6</td></tr>
</table>