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医薬品製造販売承認申請書添付資料

第 **2** 部（モジュール **2**）

2.7.1　生物薬剤学及び関連する分析法の概要

アルジェニクスジャパン株式会社

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略語及び用語の定義

略語	略していない表現（英語）	略していない表現（日本語）
ACE	affinity capture elution	---
AChR	acetylcholine receptor	アセチルコリン受容体
ADA	anti-drug antibody	抗薬物抗体
ARGX-113	Efgartigimod Alfa (Genetical Recombination)	エフガルチギモド　アルファ（遺伝子組換え）
BEAD	biotin-drug extraction and acid dissociation	---
CCP	confirmatory cut-point	確認カットポイント
CPF	cut-point factor	カットポイント係数
ECLIA	electrochemiluminescence immunoassay	電気化学発光免疫測定法
ELISA	enzyme-linked immunosorbent assay	酵素結合免疫吸着法
Fc	crystallized fragment	---
FcRn	neonatal Fc receptor	胎児性 Fc 受容体
gMG	generalized myasthenia gravis	全身型重症筋無力症
HRP	horseradish peroxidase	ホースラディッシュペルオキシダーゼ
Ig	immunoglobulin	免疫グロブリン
MSD	Meso Scale Discovery	---
MuSK	muscle-specific receptor tyrosine kinase	筋特異的受容体型チロシンキナーゼ
PD	pharmacodynamic(s)	薬力学
PK	pharmacokinetic(s)	薬物動態
QC	quality control	---
SCP	screening cut-point	スクリーニングカットポイント

2.7.1.　生物薬剤学及び関連する分析法の概要

2.7.1.1.　背景及び概観

エフガルチギモド　アルファ（遺伝子組換え）（以下、エフガルチギモド）はヒト胎児性 Fc 受容体（FcRn）の天然リガンドであるヒト免疫グロブリン（Ig）G1 の Fc フラグメントである。ABDEG™テクノロジーにより Fc フラグメントの CH2 又は CH3 ドメインを 5 つのアミノ酸残基で改変（M252Y／S254T／T256E／H433K／N434F）し、中性及び酸性両 pH 条件下での FcRn との親和性が顕著に増大するように設計された（Module 2.3, Section 2.3.S.1.2）。エフガルチギモドは内因性 IgG と FcRn の結合を拮抗阻害することで、FcRn を介した IgG のリサイクルを妨げ、IgG の分解を促進する。エフガルチギモドは、アルブミン結合部位とは異なる部位で FcRn に結合するため、その作用機序的にはアルブミン濃度に影響を及ぼすことはないと考えられる。さらに、FcRn によるリサイクルは IgG に特異的であるため、エフガルチギモドが FcRn をブロックすることで、他の免疫グロブリンの濃度に影響を及ぼすことはないと考えられる。

エフガルチギモドは静脈内投与による全身型重症筋無力症（gMG）治療薬として開発中であり、推奨用法・用量は「成人には、エフガルチギモド　アルファ（遺伝子組換え）として 1 回 10 mg/kg（体重）を 1 週間間隔で 4 回 1 時間かけて点滴静注する。これを 1 サイクルとして、症状に応じて、次の治療サイクルを行う」である。健康被験者を対象とした第 I 相試験（Module 5.3.3.1, ARGX-113-1501 CSR 及び ARGX-113-1702 CSR）、gMG 患者を対象とした第 II 相試験（Module 5.3.5.1, ARGX-113-1602 CSR）、gMG 患者を対象とした第 III 相試験及びその非盲検継続投与試験（Module 5.3.5.1, ARGX-113-1704 CSR 及び Module 5.3.5.2, ARGX-113-1705 CSR-IA 1）で静脈内投与後の薬物動態（PK）、薬力学（PD）及び免疫原性を評価した。なお、本申請では、進行中の ARGX-113-1705 試験については、20[redacted] 年[redacted] 月[redacted] 日以前に本継続投与試験に移行した被験者のデータカットオフ日（2020 年 4 月 6 日）までのデータのみが含まれることから ARGX-113-1705 試験での生体試料分析性能は記載していない。

本申請では特別な生物薬剤学試験は実施していない。なお、細胞株[redacted] から製造されたエフガルチギモドを用いた ARGX-113-1501 試験及び細胞株[redacted] から製造されたエフガルチギモドを用いた ARGX-113-1702 試験の PK データを比較することにより、物理化学的試験により示された両株から製造されたエフガルチギモドの同等性が裏づけられた（2.7.1.3 項）。

本文書では、製剤開発の概要及び臨床開発中に使用された PK、PD 及び免疫原性の生体試料分析法の概要を記載する。

2.7.1.1.1.　物理化学的性質と製剤開発の概要

エフガルチギモドは[redacted] 社独自の細胞株[redacted] を用いて発現させ、フェドバッチ培養法により製造される。このタンパク質は一連のクロマトグラフィー及びウイルス除去／不活化工程を経て精製され、最終的な製剤化緩衝液への透析ろ過及び濃縮を経て、エフガルチギモド製剤を製造する。エフガルチギモドの分子量は約 54,000 であり、95% Fc 以上の純度及び 2 mPa·s 未満の粘度を有する。

初期臨床試験（ARGX-113-1501 試験及び ARGX-113-1602 試験）用のエフガルチギモド原薬は細胞株[redacted] を用いて製造された。その後、発現物質の不均一性を改善することを目的として、新しい細胞株[redacted] が作製された（Module 2.3, Section 2.3.S.2.6）。細胞株[redacted] と細胞株[redacted] から製造された原薬の同等性が示された後、細胞株[redacted] から製造さ

れたエフガルチギモド原薬は gMG 患者を対象とした第 III 相試験（ARGX-113-1704 試験及び ARGX-113-1705 試験）で使用されている（Module 2.3, Section 2.3.P.5.4）。

エフガルチギモド静脈内投与製剤は、25 mmol/L リン酸ナトリウム、100 mmol/L 塩化ナトリウム、150 mmol/L L-アルギニン塩酸塩、0.02 w/v%ポリソルベート 80 緩衝液を溶媒とし、有効成分を 20 mg/mL の濃度で含有するよう製剤化した静脈内投与用の無菌液剤で、pH は 6.7 である。エフガルチギモド製剤は静脈内投与前に生理食塩液で最終液量 125 mL となるように希釈する。エフガルチギモド製剤の必要液量は、投与量（10 mg/kg）及び患者の体重に基づいて患者ごとに算出する。体重が 120 kg 以上の被験者には、1 回あたりの投与量の上限を 1200 mg とする。なお、第 III 相試験（ARGX-113-1704 試験及び ARGX-113-1705 試験）で使用した製剤と市販予定の製剤は同一である。

2.7.1.1.2.　生体試料分析法の概要とバリデーション

2.7.1.1.2.1. 薬物動態評価に用いた分析法

血清及び尿中のエフガルチギモド濃度の定量には定量的酵素結合免疫吸着法（ELISA）を用いた。分析法の概略を以下に記載する。

エフガルチギモド特異的エピトープ（ABDEG™テクノロジーにより変異を導入した領域）に直接結合する 2D11-mFc（ラマ重鎖抗体クローン 2D11 の可変領域とマウス Fc フラグメントからなるキメラ分子）を固相化したプレートに血清又は尿試料を添加する。プレートを洗浄し、非結合物質を取り除いた後、酵素標識抗体であるホースラディッシュペルオキシダーゼ（HRP）標識ヤギ $F(ab')_2$ 抗ヒト Fc 抗体を添加し、エフガルチギモドを捕捉する。プレートを洗浄後、発色基質を添加し、標準試料又は測定試料中のエフガルチギモドの量を比色定量する。

開発したエフガルチギモド濃度の分析法はガイドラインに従ってバリデートされ、ガイドラインに規定されているすべての判定基準を満たした。また、試料の処理、分析及び保存の各種条件下でヒト血清及び尿中のエフガルチギモドの安定性が確認された。血清及び尿中のエフガルチギモド濃度を定量するための分析法の遷移の概要を表 2.7.1-2 に示す。

2.7.1.1.2.1.1.　血清中エフガルチギモド濃度測定

argenx 社により開発され、[redacted] 社に移管後バリデートされた分析法（Module 5.3.1.4, CP155054）を用いて ARGX-113-1501 試験、ARGX-113-1602 試験及び ARGX-113-1702 試験から得られた血清試料中エフガルチギモド濃度を定量した。

ARGX-113-1702 試験での静脈内投与に使用した細胞株[redacted]由来の新規の製剤バッチ（[redacted]）を現在の参照バッチである細胞株[redacted]由来の[redacted]と比較し、その適格性を確認した。この pre-study qualification に基づき、低濃度及び高濃度 Quality Control（QC）試料に用いた濃度範囲を定量範囲に設定した。その後、細胞株[redacted]由来の新しい参照バッチ（[redacted]）を用いた分析方法について[redacted]社で再度フルバリデーションを実施した（Module 5.3.1.4, CP185065）。初期にバリデートされた分析法と比較して、健康被験者の溶血及び脂質を多く含む試料並びに日本人健康被験者血清による測定への影響は認められなかった。この分析方法は、第 III 相試験（ARGX-113-1704 試験）から得られた試料中濃度の定量に用いられた。

血清中エフガルチギモド濃度測定のための各分析法バリデーション及び個々の臨床試験での分析性能パラメータの詳細を表 2.7.1-3 及び表 2.7.1-4 に示す。

2.7.1.1.2.1.2.　尿中エフガルチギモド濃度測定

argenx 社により開発され、[redacted] 社に移管後バリデートされた分析法（Module 5.3.1.4, CP155240）を用いて、ARGX-113-1501 試験の単回漸増投与パートで採取した尿試料中のエフガルチギモド濃度を定量した。

尿中エフガルチギモド濃度定量のための分析法バリデーション及び臨床試験での分析性能パラメータの詳細を表 2.7.1-5 に示す。

2.7.1.1.2.2.　薬力学評価に用いた分析法

総 IgG 及び IgG サブタイプ（IgG1、IgG2、IgG3 及び IgG4）濃度は、すべての臨床試験で評価されている。さらに、エフガルチギモドの効果が IgG 選択的であるかを評価するために、いくつかの試験では、他のアイソタイプである IgA、IgD、IgE 及び IgM 濃度も測定した。gMG 患者を対象とした試験では、自己抗体［抗アセチルコリン受容体（AChR）抗体及び抗筋特異的受容体型チロシンキナーゼ（MuSK）抗体を含む］を定量した。なお、本申請に含まれる ARGX-113-1705 試験の中間解析には IgG サブタイプ（IgG1、IgG2、IgG3 及び IgG4）及び自己抗体（抗 AChR 抗体及び抗 MuSK 抗体）濃度測定結果は含まれない。

2.7.1.1.2.2.1.　総 IgG

ヒト血清中総 IgG の分析法は [redacted] 社で開発、バリデートされ（Module 5.3.1.4, CP155244）、ARGX-113-1501 試験及び ARGX-113-1602 試験で得られた試料中の濃度定量に用いられた。

ヒト血清中総 IgG 濃度は定量的 ELISA により測定した。抗ヒト IgG マウスモノクローナル抗体を固相化したプレートを用い、標準試料、コントロール試料及び測定試料中のヒト IgG を捕捉する。プレートを洗浄し、非結合物質を取り除いた後、酵素標識抗体である HRP 標識ヤギ抗ヒト IgG Fc 抗体を添加し、IgG と結合させる。プレート洗浄後、発色基質を添加し、試料中のヒト IgG の量を比色定量した。

IgG は内因性物質であるため、未希釈ヒト血清試料中の内因性総 IgG 濃度を算出し、QC 試料調製に適した血清試料を選択した。

この分析方法は再現性の観点から更に改良され、[redacted] 社によりバリデートされた（Module 5.3.1.4, CP185073）。初期にバリデートされた分析法と異なり、酵素標識抗体として HRP 標識マウス抗ヒト IgG Fc 抗体を用いた。この方法を ARGX-113-1702 試験、ARGX-113-1704 試験及び ARGX-113-1705 試験から得られた試料の分析に用いた。

本 IgG 分析法はガイドラインに従ってバリデートした。さらに、試料の処理、分析及び保存の各種条件下でヒト血清中の総 IgG の安定性が示された。用いた分析法の遷移の概要を表 2.7.1-6 に示す。また、各分析法のバリデーション及び個々の臨床試験での分析性能パラメータの詳細を表 2.7.1-7 及び表 2.7.1-8 に示す。

2.7.1.1.2.2.2.　IgG サブタイプ、IgA 及び IgM

血清中の IgG1、IgG2、IgG3、IgG4、IgA 及び IgM 濃度を定量するために、市販キットを [redacted]（[redacted]）社から購入し、[redacted] 社での実測定前に使用可否を検討、バリデートした（Module 5.3.1.4, CP155243）。これらの分析法は磁気ビーズを用いた免疫測定に基づいており、測定原理はサンドイッチ ELISA に近い。

血清中の IgG1、IgG2、IgG3、IgG4、IgA 及び IgM 濃度は ARGX-113-1501 試験、ARGX-113-1602 試験及び ARGX-113-1702 試験で評価した。ARGX-113-1704 試験及び ARGX-113-1705 試験では IgG サブタイプのみを評価した。用いた分析法の遷移の概要を表 2.7.1-9 に示す。

また、分析法のバリデーション及び個々の臨床試験での分析性能パラメータの詳細を表 2.7.1-10 に示す。

ARGX-113-1501 試験中に採取した試料を用いてヒト血清中の IgG1、IgG2、IgG3、IgG4、IgA 及び IgM の長期安定性を評価した。これらの−75°C±10°C でのヒト血清中での安定性は、最長 1317 日間であった（Module 5.3.1.4, CP190133）。

2.7.1.1.2.2.3.　IgD

血清中の IgD 濃度は定量的 ELISA を用いて定量した。定量には [redacted] 社から購入した市販キットが用いられ、[redacted] 社でバリデートした（Module 5.3.1.4, CP155245）。

ARGX-113-1501 試験及び ARGX-113-1602 試験で血清中 IgD 濃度を定量した。分析法の遷移の概要を表 2.7.1-11 に示す。また、用いた分析法のバリデーション及び個々の臨床試験での分析性能パラメータの詳細を表 2.7.1-12 に示す。

2.7.1.1.2.2.4.　IgE

血清中の IgE 濃度は電気化学発光免疫測定法（ECLIA）を用いて定量した。定量には [redacted] 社から購入した市販のキットを用いた。バリデーションは実施していないものの、pre-assay qualification を [redacted] 社が実施し、分析性能を確認した（Module 5.3.1.4, CP175027）。

ARGX-113-1501 試験及び ARGX-113-1602 試験で血清中 IgE 濃度を測定した。用いた分析法の遷移の概要を表 2.7.1-13 に示す。また、分析法のバリデーション及び個々の臨床試験での分析性能パラメータの詳細を表 2.7.1-14 に示す。

2.7.1.1.2.2.5.　抗アセチルコリン受容体抗体

AChR に対する自己抗体が gMG の病因であることを踏まえ、抗 AChR 抗体の有無を gMG 患者で評価した。

バインディング抗 AChR 抗体は AChR に結合することが可能なすべての抗体を表す。gMG 患者血清中のバインディング抗 AChR 抗体濃度はラジオイムノアッセイを用いて定量した。定量には [redacted] 社から購入した市販キットが用いられ、[redacted] 社でバリデートした（Module 5.3.1.4, CP155456）。

バインディング抗 AChR 抗体濃度は、ARGX-113-1602 試験及び ARGX-113-1704 試験で評価した。用いた分析法の遷移の概要を表 2.7.1-15 に示す。また、分析法のバリデーション及び個々の臨床試験での分析性能パラメータの詳細を表 2.7.1-16 に示す。

ブロッキング抗 AChR 抗体は AChR とその競合的アンタゴニストである α-bungarotoxin との結合を阻害する抗体であり、AChR に結合した抗体を表す。血清試料中のブロッキング抗 AChR 抗体濃度は α-bungarotoxin の AChR に対する結合阻害活性としてラジオイムノアッセイを用いて測定した。測定には [redacted] 社から購入した市販キットが用いられ、[redacted] 社で部分的にバリデートした（Module 5.3.1.4, CP165306）。

ブロッキング抗 AChR 抗体濃度は ARGX-113-1602 試験で評価した。用いた分析法の遷移の概要を表 2.7.1-17 に示す。また、分析法のバリデーション及び臨床試験での分析性能パラメータの詳細を表 2.7.1-18 に示す。

2.7.1.1.2.2.6.　筋特異的受容体型チロシンキナーゼに対する自己抗体

血清中の MuSK に対する自己抗体濃度は定量的 ELISA を用いて定量した。定量には[redacted]社から購入した市販キットが用いられ、[redacted]社で部分的にバリデートした（Module 5.3.1.4, CP185285）。

ARGX-113-1704 試験で抗 MuSK 抗体の結合を評価した。用いた分析法の遷移の概要を表 2.7.1-19 に示す。また、分析法のバリデーション及び臨床試験での分析性能パラメータに関する詳細を表 2.7.1-20 に示す。

2.7.1.1.2.3.　免疫原性

すべての臨床試験でエフガルチギモドの免疫原性の評価を目的の 1 つに設定した。ヒトでのエフガルチギモドの免疫原性プロファイルを十分に評価するために、ベースライン試料及びエフガルチギモド投与後の試料を採取した。

2.7.1.1.2.3.1.　エフガルチギモドの抗薬物抗体アッセイ

免疫原性は 3 段階の段階的方法で評価した。免疫原性評価用に採取したすべての試料はまずスクリーニングアッセイで評価し、次に陽性試料について抗薬物抗体（ADA）反応特異性確認アッセイを実施した。最後にエフガルチギモドへの特異性が確認された試料について、その ADA の抗体価を半定量的に測定した。さらに、第 III 相試験（ARGX-113-1704 試験及び ARGX-113-1705 試験）では、ADA 陽性が確認されたすべての試料についてエフガルチギモドの安全性及び有効性に対する ADA の影響及びその相関を検討するためにその中和能を評価した（Module 5.3.5.3, ARGX-113-9003-ISI）。

第 I 相試験である ARGX-113-1501 試験で用いた初期の ADA アッセイでは、エフガルチギモドを固相化したプレートで ADA を捕捉し、ビオチン標識エフガルチギモドとインキュベートすることにより ADA 反応を測定した（ブリッジング ELISA、Module 5.3.1.4, CP155242）。この方法は、相対的に低い薬物干渉耐性［[redacted] ng/mL の陽性コントロールに対し、薬物干渉耐性濃度（ADA 検出の妨害が認められない最小エフガルチギモド濃度）は[redacted] µg/mL］であり、ADA の検出が妨げられた。

そこで、より高い薬物干渉耐性をもつ Affinity Capture Elution（ACE）ブリッジング ELISA が[redacted]社で開発され、バリデートされた（Module 5.3.1.4, CP165082 及び CP165083）。このアッセイでは ACE 前処理により ADA を抽出し、ビオチン標識エフガルチギモドで ADA を捕捉し、ジゴキシゲニン標識エフガルチギモドで ADA を検出した。この分析法は ARGX-113-1602 試験及び ARGX-113-1702 試験で用いられた。

2019 年 1 月に最終化された FDA ガイダンスへの準拠及び新しいバッチの陽性コントロール抗体の適格性確認を目的に、ガイダンスに追加されたバリデーションパラメータに対する評価を含む本 ACE ブリッジング ELISA の補完的バリデーションを実施した（Module 5.3.1.4, CP185295）。この分析法は、第 III 相試験 ARGX-113-1704 試験及びその継続投与試験である ARGX-113-1705 試験で用いられた。

用いた分析法の遷移の概要を表 2.7.1-21 に示す。また、各分析法のバリデーション及び個々の臨床試験での分析性能パラメータに関する詳細を表 2.7.1-22～表 2.7.1-24 に示す。

2.7.1.1.2.3.1.1.　スクリーニング ADA アッセイ

本アッセイは ACE ブリッジング ELISA に基づいている。すなわち、血清試料を酸で前処理して ADA と非結合型エフガルチギモド複合体を解離させ、ストレプトアビジン固相化プレート上のビオチン標識エフガルチギモドで ADA を捕捉する。次に、プレートを洗浄し、

過剰の非結合型エフガルチギモドを除去する。続いて、酸でストレプトアビジン固相化プレートから ADA を抽出し、ビオチン標識エフガルチギモド及びジゴキシゲニン標識エフガルチギモドを含む緩衝液中で中和する。ビオチン標識エフガルチギモド及びジゴキシゲニン標識エフガルチギモドと架橋を形成した ADA 複合体を別のストレプトアビジン固相化プレート上に捕捉し、HRP 標識抗ジゴキシゲニン抗体で検出する。

60 名のエフガルチギモド投与歴のない健康被験者の血清を 6 回分析し、カットポイント係数（CPF）を [redacted] と算出し、偽陽性率が 5%に抑えられることを確認した。スクリーニングカットポイント（SCP：SCP＝CPF × 陰性コントロール反応の平均値）より高い値が検出された試料を次の確認アッセイで更に評価した。また、ARGX-113-1704 試験から得られたベースライン血清試料を用いた評価から、この CPF は gMG 患者血清の評価にも適切であることが確認された（Module 5.3.1.4, CP185211）。

2.7.1.1.2.3.1.2.　確認 ADA アッセイ

本アッセイは、試料を過剰量の非標識エフガルチギモド（1000 µg/mL）とインキュベートした以外は、スクリーニングアッセイと同様の方法で実施した。エフガルチギモド投与歴のない 60 名の血清を分析し、エフガルチギモド添加による阻害シグナルに基づきバリデーション確認カットポイント（CCP）を [redacted]%と設定し、偽陽性率が 1%に抑えられることを確認した。CCP を上回った試料について抗体価測定を実施した（2.7.1.1.2.3.1.3 項）。

2.7.1.1.2.3.1.3.　抗体価アッセイ

本アッセイは、様々な希釈系列で試料を希釈する以外は、スクリーニングアッセイと同様の方法で実施した。抗体価は SCP を挟む 2 点の希釈倍率より回帰直線を用いて算出した。

2.7.1.1.2.3.2.　中和抗体アッセイ

第 III 相試験である ARGX-113-1704 試験で得られた ADA 陽性が確認されたすべての試料について、中和抗体アッセイを用いて中和される度合いを評価し、ADA の中和能を決定した。

本アッセイは、in vitro で pH 6.0 の条件下でエフガルチギモドと組換え野生型ヒト IgG3 のヒト FcRn に対する結合が競合することに基づいた Meso Scale Discovery（MSD）をプラットフォームとした間接競合 ECLIA である。この評価系は IgG のヒト FcRn への結合を阻害することにより内因性 IgG の分解を促進させるというエフガルチギモドの作用機序をモニターするための適切な in vitro モデルである。

本分析法では試料中に含まれる過剰なエフガルチギモドの影響を避けるために、biotin-drug extraction and acid dissociation（BEAD）法により ADA を抽出する前処理を行う（Xu, 2015）。すなわち、まず ADA とエフガルチギモドの複合体を酸処理によって解離させ、過剰のビオチン標識エフガルチギモド存在下で中和する。ADA とビオチン標識エフガルチギモド複合体をストレプトアビジンビーズ上に捕捉し、酸処理により ADA を抽出する。抽出した ADA はエフガルチギモド溶液中でプレインキュベートする。この混合物を [redacted] 標識ヒト IgG3 とプレインキュベートしたビオチン標識ヒト FcRn の混合物に添加し、MSD ストレプトアビジンプレートに添加する。

エフガルチギモドが FcRn と [redacted] 標識野生型ヒト IgG3 との結合を阻害することから、エフガルチギモドに対する中和抗体がエフガルチギモドにより減弱するアッセイシグナルを回復させることを利用している。この分析法で観察されるアッセイシグナルの増加は、中和抗体の中和能に比例する。

用いた分析法の遷移の概要を表 2.7.1-25 に示す。また、分析法のバリデーション及び個々の臨床試験での分析性能パラメータの詳細を表 2.7.1-26 に示す。

2.7.1.2.　個々の試験結果の要約

特別な生物薬剤学試験は実施していないが、異なった細胞株から製造されたエフガルチギモドの同等性を裏付けるため、ARGX-113-1501 試験及び ARGX-113-1702 試験の PK データを比較した。比較結果は 2.7.1.3 項に示す。

2.7.1.3.　全試験を通しての結果の比較と解析

細胞株[redacted]及び細胞株[redacted]いずれかを用いて製造されたエフガルチギモドのヒトでの同等性を裏付けるため、ARGX-113-1501 試験及び ARGX-113-1702 試験の PK データを評価した。ARGX-113-1501 試験（コホート 3）（Module 2.7.2, Section 2.7.2.1.1.1）では細胞株[redacted]由来のエフガルチギモドを、ARGX-113-1702 試験（治療群 A）（Module 2.7.2, Section 2.7.2.1.1.2）では細胞株[redacted]由来のエフガルチギモドを 10 mg/kg の用量で健康被験者に単回静脈内投与（投与時間 2 時間）した。両試験で算出された PK パラメータは、表 2.7.1-1 に示したように、これらの異なる細胞株から製造されたエフガルチギモドの同等性を裏付ける結果であった。

表 2.7.1-1　細胞株[redacted]及び細胞株[redacted]由来エフガルチギモドを 2 時間かけて単回静脈内投与したときの薬物動態パラメータ

	10 mg/kg Efgartigimod IV Infused over 2 Hours	
	Cell Line [redacted] Batch No. [redacted] (ARGX-113-1501 - Cohort 3) (n=4)	**Cell Line [redacted] Batch No. [redacted] (ARGX-113-1702 - Treatment A) (n=8)**
C_{max} (µg/mL)	209 (27.9)	206 (55.5)
t_{max} (h)	2.1 (2.0-2.3)	2.03 (2.00-4.00)
AUC_{0-t} (µg·h/mL)	6770 (1520)	6210 (1050)
AUC_{0-inf} (µg·h/mL)	6818 (1523)	6340 (1060)
$t_{1/2}$ (h)	85.1 (7.50)	78.7 (16.9)

Source:　Module 5.3.3.1, ARGX-113-1501 CSR, Table 14.2.2.2 and Module 5.3.3.1, ARGX-113-1702 CSR, Table 14-13

Note:　Arithmetic mean and standard deviation (SD) are presented, except for t_{max} where median (range) is presented. AUC_{0-inf}=area under the concentration-time curve (AUC) from time zero to infinity; AUC_{0-t}=AUC from time zero up to last quantifiable concentration; C_{max}=maximum observed concentration; IV=intravenous; n=number of subjects; $t_{1/2}$=apparent elimination half-life; t_{max}=time of C_{max}

2.7.1.4.　付録

表 2.7.1-2　血清及び尿中エフガルチギモド濃度分析法の遷移

	Method Validation #1	Clinical Study ARGX-113-1501 (Part 1)	Clinical Study ARGX-113-1501 (Part 2)	Clinical Study ARGX-113-1602	Clinical Study ARGX-113-1702
Analyte	Efgartigimod	Efgartigimod	Efgartigimod	Efgartigimod	Efgartigimod
eCTD reference number	CP155054	CP155247	CP155248	CP165307	CP175324
Method ID	[redacted] Version [redacted] to [redacted]	[redacted] Version [redacted] to [redacted]	[redacted] Version [redacted] to [redacted]	[redacted] Version [redacted]	[redacted] Version [redacted] and [redacted]
Duration of time method is in use	[redacted] to [redacted]	[redacted] to [redacted]	[redacted] to [redacted]	[redacted] to [redacted]	[redacted] to [redacted]
Bioanalytical site	[redacted], France				
Matrix	Serum				
Platform	Enzyme-linked immunosorbent assay (ELISA)				
Format	Sandwich ELISA using 2D11-mFc as capture and goat F(ab)2 anti-human IgG Fc coupled with HRP as detection reagent.				
Stock reference, lot number, expiration date	Efgartigimod, Batch/Lot No.: [redacted], Exp. date: [redacted]	Efgartigimod, Batch/Lot No.: [redacted], End of use date: [redacted]	Efgartigimod, Batch/Lot No.: [redacted], Exp. date: [redacted]	Efgartigimod, Batch/Lot No.: [redacted], Exp. date: [redacted]	Efgartigimod, Batch/Lot No.: [redacted], Exp. date: [redacted] Batch/Lot No.: [redacted], Exp. date: [redacted]
Calibration range from the LLOQ to the ULOQ	300 to 5000 ng/mL in 100% matrix	300 to 5000 ng/mL in 100% matrix	300 to 5000 ng/mL in 100% matrix	300 to 5000 ng/mL in 100% matrix	600 to 4000 ng/mL in 100% matrix
Matrix study population	Healthy, MG, ITP, PV	Healthy	Healthy	MG	Healthy
Link to reports and applicable amendments	Module 5.3.1.4, CP155054	Module 5.3.1.4, CP155247	Module 5.3.1.4, CP155248	Module 5.3.1.4, CP165307	Module 5.3.1.4, CP175324
Synopsis of amendment history	*NA*	*NA*	*NA*	*NA*	Incurred sample reanalysis. Cross validation of efgartigimod Batch/Lot No. [redacted] and [redacted]

	Method Validation #2	Clinical Study ARGX-113-1704	Method Validation #3	Clinical Study ARGX-113-1501 (Part 1)
Analyte	Efgartigimod	Efgartigimod	Efgartigimod	Efgartigimod
eCTD reference number	CP185065	CP185211	CP155240	CP155247
Method ID	[redacted] Version [redacted]	[redacted] Version [redacted]	[redacted] Version [redacted]	[redacted] Version [redacted]
Duration of time method is in use	[redacted]-20[redacted] to Present	[redacted]-20[redacted] to [redacted]-20[redacted]	[redacted]-20[redacted] to Present	[redacted]-20[redacted] to [redacted]-20[redacted]
Bioanalytical site	[redacted], France			
Matrix	Serum		Urine	
Platform	Enzyme-linked immunosorbent assay (ELISA)			
Format	Sandwich ELISA using 2D11-mFc as capture and goat F(ab)2 anti-human IgG Fc coupled with HRP as detection reagent.			
Stock reference, lot number, expiration date	Efgartigimod, Batch/Lot No.: [redacted], Exp. date: [redacted]-20[redacted]	Efgartigimod, Batch/Lot No.: [redacted], Exp. date: [redacted]-20[redacted] Batch/Lot No.: [redacted] Exp. date: [redacted]-20[redacted]	Efgartigimod, Batch/Lot No.: [redacted], Exp. date: [redacted]-20[redacted]	Efgartigimod, Batch/Lot No.: [redacted], Exp. date: [redacted]-20[redacted]
Calibration range from the LLOQ to the ULOQ	300 to 4000 ng/mL in 100% matrix	300 to 4000 ng/mL in 100% matrix	50.0 to 1000 ng/mL in 100% matrix	50.0 to 1000 ng/mL in 100% matrix
Matrix study population	Healthy, Healthy Japanese, MG	MG	Healthy	Healthy
Link to reports and applicable amendments	Module 5.3.1.4, CP185065	Module 5.3.1.4, CP185211	Module 5.3.1.4, CP155240	Module 5.3.1.4, CP155247
Synopsis of amendment history	*NA*	Cross validation of efgartigimod Batch/Lot No. [redacted] and [redacted]. Qualification new technician and new HRP batch.	*NA*	*NA*

2D11-mFc=chimeric molecule consisting of efgartigimod-specific binding variable domain of a llama heavy chain-only antibody clone 2D11 and a mouse Fc; ELISA=enzyme-linked immunosorbent assay; Exp.=expiration; F(ab)=fragment antibody; Fc=crystallized fragment; HRP=horseradish peroxidase; Ig=immunoglobulin; ITP=idiopathic thrombocytopenic purpura; LLOQ=lower limit of quantitation; MG=myasthenia gravis; NA=not applicable; No.=number; PV=pemphigus vulgaris; ULOQ=upper limit of quantitation

表 2.7.1-3　血清中エフガルチギモド濃度分析法の概要－Validation Method #1

Bioanalytical method validation report name	**Transfer, Completion of Development and Validation of an ELISA Method for the Determination of Efgartigimod in Human Serum** (Module 5.3.1.4, CP155054)		
Method description	The method is a quantitative ELISA. 2D11-mFc is coated onto an ELISA plate and after the capture of efgartigimod a detection step is performed by using HRP labelled goat $F(ab')_2$ anti-hFc.		
Materials used for standard calibration curve and concentration	Efgartigimod Batch/Lot No.: [redacted] [redacted] mg/mL Exp. date: [redacted]-20[redacted]		
Validated assay range	300 to 5000 ng/mL in 100% matrix		
Material used for QCs and concentration	Efgartigimod Batch/Lot No.: [redacted] [redacted] mg/mL Exp. date: [redacted]-20[redacted]		
MRD	1:100		
Source and lot of reagents	Goat $F(ab)_2$ anti-human IgG-Fc (HRP), Ref [redacted], [redacted] 2D11-mFc, Batch/Lot [redacted], argenx		
Regression model and weighting	4 parameter logistical model with weighting factor of $1/Y^2$		
Validation parameters	**Method Validation Summary**		**Source Location**
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LLOQ to ULOQ	[redacted] calibrators	CP155054, Table 15.2
	Inter assay accuracy (%bias) from LLOQ to ULOQ. Data presented is from all validation runs.	[redacted]% to [redacted]%	CP155054, Table 15.2
	Cumulative precision (%CV) from LLOQ to ULOQ. Data presented is from all validation runs.	[redacted]%	CP155054, Table 15.2
Performance of QCs during accuracy and precision runs	**Intra assay accuracy (%bias) in 5 QCs**	LLOQ [redacted]% to [redacted]% Low [redacted]% to [redacted]% Mid [redacted]% to [redacted]% High [redacted]% to [redacted]% ULOQ [redacted]% to [redacted]%	CP155054, Table 15.3
	Inter batch precision %CV	LLOQ [redacted]% Low [redacted]% Mid [redacted]% High [redacted]% ULOQ [redacted]%	CP155054, Table 15.3
	Total error (TE)	LLOQ [redacted]% Low [redacted]% Mid [redacted]% High [redacted]% ULOQ [redacted]%	CP155054, Table 15.3

Selectivity & matrix effect	[redacted] lots from healthy subjects, observed bias: [redacted]% to [redacted]%. All unspiked sera were determined below LLOQ of the assay, [redacted]% of the spiked matrices were determined within [redacted]% of the LLOQ concentration. [redacted] lots from MG patients, observed bias: [redacted]% to [redacted]%. All unspiked sera were determined below LLOQ of the assay, [redacted]% of the spiked matrices were determined within [redacted]% of the LLOQ concentration. [redacted] lots from PV patients, observed bias: [redacted]% to [redacted]%. All unspiked sera were determined below LLOQ of the assay, [redacted]% of the spiked matrices were determined within [redacted]% of the LLOQ concentration. [redacted] lots from ITP patients, observed bias: [redacted]% to [redacted]%. All unspiked sera were determined below LLOQ of the assay, [redacted]% of the spiked matrices were determined within [redacted]% of the LLOQ concentration. Selectivity passed acceptance criteria as more than [redacted]% of the investigated matrices were within [redacted]% of the nominal concentration at LLOQ level and all unspiked samples demonstrated assay signals below the assay LLOQ.	CP155054, Table 15.11 to Table 15.14
Interference & specificity	Specificity and interference was not tested during validation.	*NA*
Hemolysis effect	Hemolysis effect was not tested during validation.	*NA*
Lipemic effect	Lipemic effect was not tested during validation.	*NA*
Dilution linearity & hook effect	Dilution linearity was tested with 4 dilution factors up to 1/500 (in addition to the 1/100 MRD dilution). Bias observed at a dilution of 1/500 was [redacted]% to [redacted]%. No hook effect was observed during the dilution linearity assessments.	CP155054, Table 15.4 to Table 15.10
Bench-top/process stability	Bench stability and refrigerator stability was investigated by storing initially frozen ([redacted]°C±[redacted]°C and [redacted]°C±[redacted]°C) stability samples for [redacted] hours and [redacted] minutes (after storage at [redacted]°C±[redacted]°C) and for [redacted] hours and [redacted] minutes (after storage at [redacted]°C±[redacted]°C) at [redacted] and refrigerator ([redacted]°C±[redacted]°C) temperature. All stability results were within the acceptance criteria.	CP155054, Table 15.20 to Table 15.22
Freeze-thaw stability	Freeze-thaw stability was investigated by submitting stability samples stored at [redacted]°C±[redacted]°C and [redacted]°C±[redacted]°C to [redacted] freeze-thaw cycles. All stability results were within the acceptance criteria.	CP155054, Table 15.19
Long-term storage	Long term stability was investigated by storing stability samples stored at [redacted]°C±[redacted]°C and [redacted]°C±[redacted]°C for [redacted] days. Based on the results, efgartigimod in serum is considered stable at tested temperatures for up to [redacted] days.	CP155054, Table 15.23 and Table 15.24
Robustness	Coated plates were determined stable for [redacted] days at+[redacted]°C±[redacted]°C. Inter-analyst precision was [redacted]%.	CP155054, Table 15.17 and Table 15.15
Qualification of critical reagents	HRP reagents Batch/Lotes. [redacted] and [redacted] were qualified and accepted for further use.	CP155054, Table 15.28 and Table 15.29
Parallelism	*NA*	*NA*
Carry over	*NA*	*NA*

Method Performance in Study ARGX-113-1501 (Part 1) (Module 5.3.1.4, CP155247)		
Assay passing rate	[redacted] of the [redacted] analytical runs passed ([redacted]% passing rate).	CP155247, Table 20.1
Standard curve performance	Inter assay bias range: [redacted]% to [redacted]% Inter assay precision: [redacted]%CV Results presented are of accepted runs.	CP155247, Table 20.2
QC performance	Inter assay bias range: [redacted]% to [redacted]% Inter assay precision: [redacted]%CV	CP155247, Table 20.4
Method reproducibility	Incurred sample re-analysis was not performed in this study.	*NA*
Study sample analysis/ stability	Stability of efgartigimod in human serum at [redacted]°C±[redacted]°C was demonstrated for up to [redacted] days (CP155054). First sample was collected on [redacted]-[redacted]-20[redacted] and last sample was analyzed on [redacted]-[redacted]-20[redacted] (ie, [redacted] days).	CP155247, Section 9.5
Standard calibration curve performance during accuracy and precision runs	Calibration curve contains [redacted] standards from LLOQ to ULOQ.	
Method Performance in Study ARGX-113-1501 (Part 2) (Module 5.3.1.4, CP155248)		
Assay passing rate	[redacted] of the [redacted] analytical runs passed ([redacted]% passing rate).	CP155248, Table 19.1
Standard curve performance	Inter assay bias range: [redacted]% to [redacted]% Inter assay precision: [redacted]%CV Results presented are of accepted runs.	CP155248, Table 19.2
QC performance	Inter assay bias range: [redacted]% to [redacted]% Inter assay precision: [redacted]%CV	CP155248, Table 19.4
Method reproducibility	Incurred sample re-analysis was not performed in this study.	*NA*
Study sample analysis/ stability	Stability of efgartigimod in human serum at [redacted]°C±[redacted]°C was demonstrated for up to [redacted] days (CP155054). First sample was collected on [redacted]-[redacted]-20[redacted] and last sample was analyzed on [redacted]-[redacted]-20[redacted] (ie, [redacted] days).	CP155248, Section 9.5
Standard calibration curve performance during accuracy and precision runs	Calibration curve contains [redacted] standards from LLOQ to ULOQ.	

Method Performance in Study ARGX-113-1602 (Module 5.3.1.4, CP165307)		
Assay passing rate	[redacted] of the [redacted] analytical runs passed ([redacted]% passing rate).	CP165307, Table 19.1
Standard curve performance	Inter assay bias range: [redacted]% to [redacted]% Inter assay precision: [redacted]% CV Results presented are of accepted runs.	CP165307, Table 19.2
QC performance	Inter assay bias range: [redacted]% to [redacted]% Inter assay precision: [redacted]% CV	CP165307, Table 19.3
Method reproducibility	Incurred sample re-analysis was not performed in this study.	*NA*
Study sample analysis/ stability	Stability of efgartigimod in human serum at [redacted]°C±[redacted]°C was demonstrated for up to [redacted] days (CP155054). First sample was collected on [redacted]-[redacted]-20[redacted] and last sample was analyzed on [redacted]-[redacted]-20[redacted] (ie, [redacted] days).	CP165307, Section 9.5
Standard calibration curve performance during accuracy and precision runs	Calibration curve contains [redacted] standards from LLOQ to ULOQ.	
Method Performance in Study ARGX-113-1702 (Module 5.3.1.4, CP175324)		
Assay passing rate	[redacted] of the [redacted] analytical runs passed ([redacted]% passing rate).	CP175324, Table 21.1
Standard curve performance	Inter assay bias range: [redacted]% to [redacted]% Inter assay precision: [redacted]% CV Results presented are of accepted runs.	CP175324, Table 21.12
QC performance	Inter assay bias range: [redacted]% to [redacted]% Inter assay precision: [redacted]% CV	CP175324, Table 21.13
Method reproducibility	Incurred sample re-analysis was performed in [redacted]% of study samples, and [redacted]% of the samples met the pre-specified criteria.	CP175324, Table 21.14
Bridging drug product batches	During the study, drug product ([redacted]) and drug substance ([redacted]) batches were used for the subcutaneous (SC) formulation and were bridged with the original validation batch ([redacted] – used for the intravenous formulation). The SC formulation of efgartigimod is not subject of this application. Based on the results it was decided to narrow the range of quantitation from 300 to 5000 ng/mL to 600 to 4000 ng/mL. This range demonstrated an acceptable accuracy profile and was in-study controlled by both Low QC ([redacted] ng/mL) and the High QC ([redacted] ng/mL).	CP175324, Table 21.2 to Table 21.11
Study sample analysis/ stability	Stability of efgartigimod in human serum at [redacted]°C±[redacted]°C was demonstrated for up to [redacted] days (CP155054). First sample was collected on [redacted]-[redacted]-20[redacted] and last sample was analyzed on [redacted]-[redacted]-20[redacted] (ie, [redacted] days).	CP175324, Section 9
Standard calibration curve performance during accuracy and precision runs	Calibration curve contains [redacted] standards from LLOQ to ULOQ.	

2D11-mFc=chimeric molecule consisting of efgartigimod-specific binding variable domain of a llama heavy chain-only antibody clone 2D11 and a mouse Fc; CV=coefficient of variation; ELISA=enzyme-linked immunosorbent assay; Exp.=expiration; F(ab)=fragment antibody; Fc=crystallized fragment; HRP=horseradish peroxidase; Ig=immunoglobulin; ITP=idiopathic thrombocytopenic purpura; LLOQ=lower limit of quantitation; MG=myasthenia gravis; MRD=minimum required dilution; NA=not applicable; No.=number; PV=pemphigus vulgaris; QC=quality control; SC=subcutaneous; TE=total error; ULOQ=upper limit of quantitation

表 2.7.1-4　血清中エフガルチギモド濃度分析法の概要－Validation Method #2

Bioanalytical method validation report name	**Method Full Re-validation for the Determination of Efgartigimod in Hunan Serum (with Interference Standard from New Cell Line)** (Module 5.3.1.4, CP185065)		
Method description	The method is a quantitative ELISA. 2D11-mFc is coated on an ELISA plate and after the capture of efgartigimod a detection step is performed by using HRP labelled goat F(ab')2 anti-hFc.		
Materials used for standard calibration curve and concentration	Efgartigimod Batch/Lot No.: [redacted] [redacted] mg/mL Exp. date: [redacted]-20[redacted]		
Validated assay range	300 to 4000 ng/mL in 100% matrix		
Material used for QCs and concentration	Efgartigimod Batch/Lot No.: [redacted] [redacted] mg/mL Exp. date: [redacted]-20[redacted]		
MRD	1:100		
Source and lot of reagents	Goat F(ab)2 anti-human IgG-Fc (HRP), Ref [redacted], [redacted] 2D11-mFc, Batch/Lot [redacted], argenx		
Regression model and weighting	4 parameter logistical model with weighting factor of $1/Y^2$		
Validation parameters	**Method Validation Summary**		**Source Location**
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LLOQ to ULOQ.	[redacted] calibrators	CP185065, Table 20.7
	Inter assay accuracy (%bias) from LLOQ to ULOQ. Data presented is from all validation runs.	[redacted]% to [redacted]%	CP185065, Table 20.7
	Cumulative precision (%CV) from LLOQ to ULOQ. Data presented is from all validation runs.	[redacted]%	CP185065, Table 20.7
Performance of QCs during accuracy and precision runs	**Intra run accuracy (%bias) in 5 QCs**	LLOQ [redacted]% to [redacted]% Low [redacted]% to [redacted]% Mid [redacted]% to [redacted]% High [redacted]% to [redacted]% ULOQ [redacted]% to [redacted]%	CP185065, Table 20.9
	Inter batch precision %CV	LLOQ [redacted]% Low [redacted]% Mid [redacted]% High [redacted]% ULOQ [redacted]%	CP185065, Table 20.9
	Total error (TE)	LLOQ [redacted]% Low [redacted]% Mid [redacted]% High [redacted]% ULOQ [redacted]%	CP185065, Table 20.9

Selectivity & matrix effect	[redacted] lots from healthy subjects, observed bias: [redacted]% to [redacted]%. All unspiked sera were determined below LLOQ of the assay, [redacted]% of the spiked matrices were determined within [redacted]% of the LLOQ concentration. [redacted] lots from MG patients, observed bias: [redacted]% to [redacted]7%. All unspiked sera were determined below LLOQ of the assay, [redacted]% of the spiked matrices were determined within [redacted]% of the LLOQ concentration. [redacted] lots from Japanese healthy subjects, observed bias: [redacted]% to [redacted]%. All unspiked sera were determined below LLOQ of the assay, [redacted]% of the spiked matrices were determined within [redacted]% of the LLOQ concentration.	CP185065, Table 20.2, Table 20.3 and Table 20.6
Interference & specificity	Specificity and interference was not tested during this validation.	*NA*
Hemolysis effect	[redacted] hemolytic lots from healthy subjects – observed bias: [redacted]% to [redacted]%. All unspiked sera were determined below LLOQ of the assay, [redacted]% of the spiked matrices were determined within [redacted]% of the LLOQ concentration.	CP185065, Table 20.4
Lipemic effect	[redacted] lipemic lots from healthy subjects – observed bias: [redacted]% to [redacted]%. All unspiked sera were determined below LLOQ of the assay, [redacted]% of the spiked matrices were determined within [redacted]% of the LLOQ concentration.	CP185065, Table 20.5
Dilution linearity & hook effect	Dilution linearity was tested with 2 dilution factors up to 1/500. Bias observed at a dilution of 1/500 was [redacted]%. No hook effect was observed.	CP185065, Table 20.11 and Table 20.12
Bench-top/process stability	Bench-top and refrigerator stability was investigated by storing initially frozen ([redacted]°C±[redacted]°C and [redacted]°C±[redacted]°C) stability samples for [redacted] hours at room temperature or in the refrigerator ([redacted]°C±[redacted]°C). All stability results were within the preset acceptance criteria.	CP185065, Table 20.18
Freeze-thaw stability	Freeze-thaw stability was investigated by submitting stability samples stored at ([redacted]°C±[redacted]°C and [redacted]°C±[redacted]°C to [redacted] freeze-thaw cycles. All stability results were within the preset acceptance criteria.	CP185065, Table 20.17
Long-term storage	Long term stability was investigated by storing stability samples at [redacted]°C±[redacted]°C and [redacted]°C±[redacted]°C for [redacted] days. Based on the results, efgartigimod in serum is considered stable for up to 182 days.	CP185065, Table 20.19 and Table 20.20
Robustness	Coated plates were determined stable for [redacted] days at [redacted]°C±[redacted]°C. Coating antibody was retested and expiry date was extended till [redacted]-[redacted]-20[redacted]. Inter-analyst precision was [redacted]% at the LLOQ and ULOQ level and within ±[redacted]% at the other QC levels.	CP185065, Table 20.10, Table 20.14, and Table 20.21
Parallelism	*NA*	*NA*
Carry over	*NA*	*NA*
Reference material bridging	Analytical comparability was shown between batches [redacted] and [redacted].	CP185065, Table 20.15

Method Performance in Study ARGX-113-1704 (Module 5.3.1.4, CP185211)		
Assay passing rate	[redacted] of the [redacted] analytical runs passed ([redacted]% passing rate)	CP185211, Table 23.1
Standard curve performance	Inter assay bias range: [redacted]% to [redacted]% Inter assay precision: [redacted]%CV Results presented are of accepted runs.	CP185211, Table 23.10
QC performance	Inter assay bias range: [redacted]% to [redacted]% Inter assay precision: [redacted]%CV	CP185211, Table 23.11
Method reproducibility	Incurred sample re-analysis was performed in [redacted]% of study samples, and [redacted]% of the samples met the pre-specified criteria.	CP185211, Table 23.12
Cross validation of efgartigimod batches	A new reference substance (Batch/Lot No. [redacted]) was cross validated and successfully compared with the old reference substance (Batch/Lot No. [redacted]).	CP185211, Table 23.8 and Table 23.9
Qualification runs	Qualification of new HRP batch, Batch/Lot No. [redacted] (new batch) was qualified compared to [redacted] (old batch) and accepted for further use. Qualification of a technician.	CP185211, Table 23.2 to Table 23.7
Study sample analysis/ stability	Stability of efgartigimod in human serum at [redacted]°C±[redacted]°C was demonstrated for up to [redacted] days (CP185065). This stability period covers the maximum length of time from specimen collection to analysis i.e., [redacted] days.	CP185211, Section 9
Standard calibration curve performance during accuracy and precision runs	Calibration curve contains [redacted] standards from LLOQ to ULOQ	

2D11-mFc=chimeric molecule consisting of efgartigimod-specific binding variable domain of a llama heavy chain-only antibody clone 2D11 and a mouse Fc; CV=coefficient of variation; ELISA=enzyme-linked immunosorbent assay; Exp.=expiration; F(ab)=fragment antibody; Fc=crystallized fragment; HRP=horseradish peroxidase; Ig=immunoglobulin; LLOQ=lower limit of quantitation; MG=myasthenia gravis; MRD=minimum required dilution; NA=not applicable; No.=number; QC=quality control; TE=total error; ULOQ=upper limit of quantitation

表 2.7.1-5　尿中エフガルチギモド濃度分析法の概要

Bioanalytical method validation report name	**Implementation, Optimization and Validation of an ELISA Method for the Determination of Efgartigimod in Human Urine** (Module 5.3.1.4, CP155240)		
Method description	The method is a quantitative ELISA. 2D11-mFc is coated onto an ELISA plate and after the capture of efgartigimod a detection step is performed by using HRP labelled goat F(ab')2 anti-hFc.		
Materials used for standard calibration curve and concentration	Efgartigimod Batch/Lot No.: [redacted] [redacted] mg/mL Exp. date: [redacted]-20[redacted]		
Validated assay range	50.0 to 1000 ng/mL in 100% matrix		
Material used for QCs and concentration	Efgartigimod Batch/Lot No.: [redacted] [redacted] mg/mL Exp. date: [redacted]-20[redacted]		
MRD	1:20		
Source and lot of reagents	Goat F(ab)2 anti-human IgG-FC (HRP), Ref [redacted], [redacted] 2D11-mFc, Batch/Lot [redacted], argenx		
Regression model and weighting	5 parameter logistic model with weighting factor of $1/Y^2$		
Validation parameters	**Method Validation Summary**		**Source Location**
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LLOQ to ULOQ.	[redacted] calibrators	CP155240, Table 15.2
	Intra assay accuracy (%bias) from LLOQ to ULOQ. Data presented is from all validation runs.	[redacted]% to [redacted]	CP155240, Table 15.2
	Cumulative precision (%CV) from LLOQ to ULOQ. Data presented is from all validation runs.	[redacted]%	CP155240, Table 15.2
Performance of QCs during accuracy and precision runs	**Intra assay accuracy (%bias) in 5 QCs**	LLOQ [redacted]% to [redacted]% Low [redacted]% to [redacted]% Mid [redacted]% to [redacted]% High [redacted]% to [redacted]% ULOQ [redacted]% to [redacted]%	CP155240, Table 15.3
	Inter batch %CV	LLOQ [redacted]% Low [redacted]% Mid [redacted]% High [redacted]% ULOQ [redacted]%	CP155240, Table 15.3
	Total error (TE)	LLOQ [redacted]% Low [redacted]% Mid [redacted]% High [redacted]% ULOQ [redacted]%	CP155240, Table 15.3

Selectivity & matrix effect	[redacted] lots of urine from healthy subjects were included in the selectivity assessment, observed bias: [redacted]% to [redacted]%. All non-spiked matrices were determined below LLOQ of the assay, [redacted]% of the spiked matrices were determined within [redacted]% of the LLOQ concentration and selectivity passed the acceptance criteria.	CP155240, Table 15.6
Interference & specificity	Specificity and interference was not tested during validation.	*NA*
Dilution linearity & hook effect	A dilution factor of 1/50 and 1/100 was tested giving a bias of [redacted]% and [redacted]%, respectively. Therefore samples can be diluted up to 50 times. No hook effect was observed in the dilution series.	CP155240, Table 15.4 and Table 15.5
Bench-top/process stability	Bench top stability and refrigerator stability was investigated by storing initially frozen ([redacted]°C±[redacted]°C and [redacted]°C±[redacted]°C) stability samples for [redacted] hours and [redacted] minutes at room and refrigerator temperature. All stability results were within the preset acceptance criteria.	CP155240, Table 15.12 and Table 15.13
Freeze-thaw stability	Freeze-thaw stability was investigated by submitting stability samples stored at [redacted]°C±[redacted]°C and [redacted]°C±[redacted]°C concentration to [redacted] freeze-thaw cycles. All stability results were within the preset acceptance criteria.	CP155240, Table 15.11
Long-term storage	Long term stability was investigated by storing stability samples stored at [redacted]°C±[redacted]°C and [redacted]°C±[redacted]°C for [redacted] days. Based on the results, efgartigimod in urine is considered stable at tested temperatures for up to [redacted] days.	CP155240, Table 15.14 and Table 15.15
Parallelism	*NA*	*NA*
Carry over	*NA*	*NA*
Robustness	Coated plates were determined stable for up to [redacted] days at [redacted]°C±[redacted]°C. Inter-analyst precision was [redacted]%.	CP155240, Table 15.9 and Table 15.7
Method Performance in Study ARGX-113-1501 (Part 1) (Module 5.3.1.4, CP155247)		
Assay passing rate	[redacted] of the [redacted] analytical runs passed ([redacted]% passing rate).	CP155247, Table 20.1
Standard curve performance	Inter assay bias range: [redacted]% to [redacted]% Inter assay precision: [redacted]%CV	CP155247, Table 20.5
QC performance	Inter assay bias range: [redacted]% to [redacted]% Inter assay precision: [redacted]%CV	CP155247, Table 20.7
Method reproducibility	Incurred sample re-analysis was not performed in this study.	*NA*
Study sample analysis/stability	Stability of efgartigimod in human urine at [redacted]°C±[redacted]°C was demonstrated for up to [redacted] days (CP155240). First sample was collected on [redacted]-[redacted]-20[redacted] and last sample was analyzed on [redacted]-[redacted]-20[redacted] (ie, [redacted] days).	CP155247, Section 9.5
Standard calibration curve performance during accuracy and precision runs	Calibration curve contains [redacted] standards from LLOQ to ULOQ.	

2D11-mFc=chimeric molecule consisting of efgartigimod-specific binding variable domain of a llama heavy chain-only antibody clone 2D11 and a mouse Fc; CV=coefficient of variation; ELISA=enzyme-linked immunosorbent assay; Exp.=expiration; F(ab)=fragment antibody; Fc=crystallized fragment; HRP=horseradish peroxidase; Ig=immunoglobulin; LLOQ=lower limit of quantitation; MRD=minimum required dilution; NA=not applicable; No.=number; QC=quality control; TE=total error; ULOQ=upper limit of quantitation

表 2.7.1-6　血清中総 IgG 濃度分析法の遷移

	Method Validation #1	Clinical Study ARGX-113-1501 (Part 1)	Clinical Study ARGX-113-1501 (Part 2)	Clinical Study ARGX-113-1602
Analyte	Total IgG	Total IgG	Total IgG	Total IgG
eCTD reference number	CP155244	CP155360	CP155361	CP165308
Method ID	[redacted] Version [redacted]	[redacted] Version [redacted]	[redacted] Version [redacted]	[redacted] Version [redacted]
Duration of time method is in use	[redacted] to [redacted]	[redacted] to [redacted]	[redacted] to [redacted]	[redacted] to [redacted]
Bioanalytical site	[redacted], France			
Matrix	Serum			
Platform	Enzyme-linked immunosorbent assay (ELISA)			
Format	Human IgG is captured by an anti-human IgG mouse monoclonal antibody and detected with a HRP labelled anti-human IgG Fc goat polyclonal antibody.			
Stock reference, lot number, expiration date	Purified human IgG, Ref [redacted], Batch/Lot No.: [redacted] End of use date: [redacted]	Purified human IgG, Ref [redacted], Batch/Lot No.: [redacted] End of use date: [redacted]	Purified human IgG, Ref [redacted], Batch/Lot No.: [redacted] End of use date: [redacted]	Purified human IgG Ref [redacted], Batch/Lot No.: [redacted] End of use date: [redacted]
Calibration range from the LCS to the HCS	3.13 to 100 ng/mL	3.13 to 100 ng/mL	3.13 to 100 ng/mL	3.13 to 100 ng/mL
Matrix study population	Healthy, MG, ITP, PV	Healthy	Healthy	MG
Link to reports and applicable amendments	Module 5.3.1.4, CP155244	Module 5.3.1.4, CP155360	Module 5.3.1.4, CP155361	Module 5.3.1.4, CP165308
Synopsis of amendment history	*NA*	*NA*	Qualification new batch coating reagent.	Pre-study assay qualification, including the introduction of new QC samples and verification of refence standard stability.

	Method Validation #2	Clinical Study ARGX-113-1702	Clinical Study ARGX-113-1704
Analyte	Total IgG	Total IgG	Total IgG
eCTD reference number	CP185073	CP175332	CP185288
Method ID	[redacted] Version [redacted] to [redacted]	[redacted] Version [redacted] and [redacted]	[redacted] Version b and c
Duration of time method is in use	[redacted]-20[redacted] to Present	[redacted]-20[redacted] to [redacted]-20[redacted]	[redacted]-20[redacted] to [redacted]-20[redacted]
Bioanalytical site	[redacted], France		
Matrix	Serum		
Platform	Enzyme-linked immunosorbent assay (ELISA)		
Format	Human IgG is captured by an anti-human IgG mouse monoclonal antibody and detected with a HRP labelled anti-human IgG Fc mouse monoclonal antibody.		
Stock reference, lot number, expiration date	Purified human IgG, Ref [redacted], [redacted] Batch/Lot No.: [redacted], End of use date: [redacted]-20[redacted] Batch/Lot No.: [redacted] End of use date: [redacted]-20[redacted]	Purified human IgG Ref [redacted], [redacted] Batch/Lot No.: [redacted] End of use date: [redacted]-20[redacted]	Purified human IgG Ref [redacted], [redacted] Batch/Lot No.: [redacted] End of use date: [redacted]-20[redacted] Batch/Lot No.: [redacted] End of use date: [redacted]-20[redacted]
Calibration range from the LCS to the HCS	1.56 to 50 ng/mL	1.56 to 50 ng/mL	1.56 to 50 ng/mL
Matrix study population	Healthy	Healthy	MG
Link to reports and applicable amendments	Module 5.3.1.4, CP185073	Module 5.3.1.4, CP175332	Module 5.3.1.4, CP185288
Synopsis of amendment history	*NA*	Drug interference of efgartigimod was evaluated.	Qualification of Batch/Lot No.: [redacted]. Parallelism and drug interference in serum from MG patients.

ELISA=enzyme-linked immunosorbent assay; Fc=crystallized fragment; HCS=high calibration standard; HRP=horseradish peroxidase; Ig=immunoglobulin; ITP= idiopathic thrombocytopenic purpura; LCS=low calibration standard; MG=myasthenia gravis; NA=not applicable; No.=number; PV=pemphigus vulgaris; QC=quality control

表 2.7.1-7　血清中総 IgG 濃度分析法の概要–Validation Method #1

Bioanalytical method validation report name	**Feasibility and Validation of an Immunoassay for the Determination of Total IgG in Human Serum** (Module 5.3.1.4, CP155244)		
Method description	The assay to determine the total IgG concentration is a quantitative ELISA where an anti-human IgG mouse monoclonal antibody captures human IgG. Human IgG is then detected with an HRP labelled anti-Human IgG Fc goat polyclonal antibody.		
Materials used for standard calibration curve	Purified human IgG, [redacted], Ref [redacted], Batch/Lot No.: [redacted]		
Validated assay range	1300000 to 32200000 ng/mL in 100% matrix.		
Material used for QCs	Endogenous IgG concentration of human sera were used to prepare QC samples.		
MRDs	1/400000		
Source and lot of reagents	Efgartigimod, Batch/Lot No.: [redacted], [redacted] mg/mL, Exp. date: [redacted]-[redacted]-20[redacted] Mouse anti-human IgG Fd, Ref [redacted], [redacted] Goat F(ab')$_2$ anti-Human IgG-FC (HRP), Ref [redacted], [redacted]		
Regression model and weighting	4-parameter logistic model with weighting factor $1/Y^2$.		
Validation parameters	**Method Validation Summary**		**Source location**
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LCS to HCS	[redacted] calibrators	CP155244, Table 17.7
	Intra assay accuracy (%bias) from LCS to HCS	[redacted]% to [redacted]%	CP155244, Table 17.7
	Cumulative precision (%CV) from LCS to HCS	[redacted]%	CP155244, Table 17.7
Performance of QCs during accuracy and precision runs	**Intra assay accuracy (%bias) in 5 QCs**	LLOQ [redacted]% to [redacted]% Low [redacted]% to [redacted]% Mid [redacted]% to [redacted]% High [redacted]% to [redacted]% ULOQ [redacted]% to [redacted]%	CP155244, Table 17.8
	Inter batch precision (%CV)	LLOQ [redacted]% Low [redacted]% Mid [redacted]% High [redacted]% ULOQ [redacted]%	CP155244, Table 17.8
Selectivity & matrix effect	In the feasibility phase selectivity was tested by using [redacted] lots of human serum spiked at [redacted] ng/mL followed by dilution of 1/200000, 1/400000, 1/800000, 1/1600000 and 1/3200000. Observed bias range is [redacted]% to [redacted]% and based on the selectivity results a minimum required dilution of 1/400000 was established.		CP155244, Table 17.5
Interference & specificity	[redacted] lots of serum from healthy subjects were spiked with increasing concentrations of efgartigimod up to [redacted] µg/mL and diluted to the MRD. The observed bias ranged from [redacted]% to [redacted]%. [redacted] lots of serum from MG patients were spiked with increasing concentrations of efgartigimod up to [redacted] µg/mL and diluted to the MRD. The observed bias ranged from [redacted]% to [redacted]%. [redacted] lots of serum from ITP patients were spiked with increasing		CP155244, Table 17.9 and Table 17.10

	concentrations of efgartigimod up to [redacted] µg/mL and diluted to the MRD. The observed bias ranged from [redacted] % to [redacted] %. [redacted] lots of serum from PV patients were spiked with increasing concentrations of efgartigimod up to [redacted] µg/mL and diluted to the MRD. The observed bias ranged from [redacted] % to [redacted] %. No clear trend in the measurement of total IgG concentrations was observed in MG, ITP and PV sera samples. Therefore, it was concluded that efgartigimod is not interfering in the total IgG assay when testing MG, ITP and PV sera.	
Hemolysis effect	*NA*	*NA*
Lipemic effect	*NA*	*NA*
Parallelism	[redacted] lots of sera of each study population was tested at MRD and [redacted] subsequent dilutions. Parallelism was demonstrated with MG, ITP and PV patient sera.	CP155244, Table 17.11
Stock stability	Stability of purified human IgG stocks used for preparation of calibrators and QC's was tested and found stable for [redacted] days.	CP155244, Table 17.12 and Table 17.13
Bench-top/process stability	Bench-top and refrigerator ([redacted] °C±[redacted] °C) stability for [redacted] hours was shown for IgG with stability samples initially stored at [redacted] °C±[redacted] °C and [redacted] °C±[redacted] °C.	CP155244, Table 17.23 to Table 17.26
Freeze-thaw stability	Stability after [redacted] freeze-thaw cycles was shown for IgG with stability samples initially stored at [redacted] °C±[redacted] °C and [redacted] °C±[redacted] °C.	CP155244, Table 17.21 and Table 17.22
Long-term storage	Long-term stability of [redacted] days for IgG was shown for stability samples initially stored at [redacted] °C±[redacted] °C and [redacted] °C±[redacted] °C.	CP155244, Table 17.27 and Table 17.28
Carry over	*NA*	*NA*
Reagent qualification	Expiration date for detection tool goat F(ab')2 anti-human IgG Fc HRP was assessed and extended. A new batch of QC samples was qualified to be used in the total IgG sample study.	CP155244, Table 17.14 to Table 17.18
Method Performance in Study ARGX-113-1501 (Part 1) (Module 5.3.1.4, CP155360)		
Assay passing rate	[redacted] of the [redacted] analytical runs passed ([redacted] % passing rate).	CP155360, Section 6
Method Performance in Study ARGX-113-1501 (Part 2) (Module 5.3.1.4, CP155361)		
Assay passing rate	[redacted] of the [redacted] analytical runs passed ([redacted] % passing rate).	CP155361, Section 6
Method Performance in Study ARGX-113-1602 (Module 5.3.1.4, CP165308)		
Assay passing rate	[redacted] of the [redacted] analytical runs passed ([redacted] % passing rate)	CP165308, Section 6

CV=coefficient of variation; ELISA=enzyme-linked immunosorbent assay; Exp.=expiration; F(ab)=fragment antibody; Fc=crystallized fragment; HCS=high calibration standard; HRP=horseradish peroxidase; Ig=immunoglobulin; ITP=idiopathic thrombocytopenic purpura; LCS=low calibration standard; LLOQ=lower limit of quantification; MG=myasthenia gravis; MRD=minimum required dilution; NA=not applicable; No.=number; PV=pemphigus vulgaris; QC=quality control; ULOQ=upper limit of quantification

表 2.7.1-8　血清中総 IgG 濃度分析法の概要 – Validation Method #2

Bioanalytical method validation report name	**Feasibility and Validation of an Immunoassay for the Determination of Total IgG in Human Serum** (Module 5.3.1.4, CP185073)		
Method description	The assay to determine the total IgG concentration is a quantitative ELISA where an anti-human IgG mouse monoclonal antibody captures human IgG. Human IgG is then detected with an HRP labelled anti-Human IgG Fc mouse polyclonal antibody.		
Materials used for standard calibration curve	Purified human IgG, [redacted], Ref [redacted]: Batch/Lot No.: [redacted], end of use date: [redacted] Batch/Lot No.: [redacted], end of use date: [redacted]		
Validated assay range	368000 to 10000000 ng/mL in 100% matrix		
Material used for QCs	Endogenous IgG concentrations of human sera were used to prepare QC samples.		
MRD	1/200000		
Source and lot of reagents	Mouse anti-human IgG Fd, clone [redacted], Ref [redacted], Mouse anti-Human IgG Fc-HRP, Clone [redacted], Ref [redacted],		
Regression model and weighting	4-parameter logistic model with weighting factor $1/Y^2$.		
Validation parameters	**Method Validation Summary**		**Source Location**
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators LCS to HCS	[redacted] calibrators	CP185073, Table 17.8
	Inter assay accuracy (%bias) from LCS to HCS	[redacted]% to [redacted]%	CP185073, Table 17.8
	Cumulative precision (%CV) from LCS to HCS	[redacted]%	CP185073, Table 17.8
Performance of QCs during accuracy and precision runs	**Intra assay accuracy (%bias) in 6 QCs**	LLOQ [redacted]% to [redacted]% Low2 [redacted]% to [redacted]% Low1 [redacted]% to [redacted]% Mid [redacted]% to [redacted]% High [redacted]% to [redacted]% ULOQ [redacted]% to [redacted]%	CP185073, Table 17.10
	Inter batch precision (%CV)	LLOQ [redacted]% Low2 [redacted]% Low1 [redacted]% Mid [redacted]% High [redacted]% ULOQ [redacted]%	CP185073, Table 17.10
Selectivity & matrix effect	Selectivity was tested during the feasibility phase of this study. [redacted] lots of human serum were spiked with single concentration of purified IgG and serially diluted start from 1/200000 to 1/6400000. Range of observed biases was [redacted]% to [redacted]%. [redacted] out of [redacted] matrices showed that nominal value set for reported concentration at 1/200000 was consistent with the reported concentration at dilution 1/32000000.		CP185073, Table 17.5
Interference & specificity	*NA*		*NA*
Hemolysis effect	*NA*		*NA*

Lipemic effect	*NA*	*NA*
Dilution linearity & hook effect	Dilution linearity was tested in the parallelism experiment, hook effect was not specifically evaluated.	*NA*
Parallelism	Parallelism was tested during the feasibility phase of this study by serially diluting [redacted] individual human sera lots starting at a dilution of 1/200000 to 1/3200000. Range of the observed biases was [redacted] % to [redacted] %. All individual samples tested showed that nominal value set for reported concentration at 1/200000 were consistent with reported concentration at higher dilution and that parallelism was demonstrated from 1/200000 to 1/6400000.	CP185073, Table 17.4
Bench-top/process stability	Bench-top and refrigerator ([redacted] °C±[redacted] °C) stability for [redacted] hours was shown for IgG with stability samples initially stored at [redacted] °C±[redacted] °C and [redacted] °C±[redacted] °C.	CP185073, Table 17.16
Freeze-thaw stability	Stability after [redacted] freeze-thaw cycles was shown for IgG with stability samples initially stored at [redacted] °C±[redacted] °C and [redacted] °C±[redacted] °C.	CP185073, Table 17.15
Long-term storage	Long-term stability for up to [redacted] days for IgG was shown for stability samples initially stored at [redacted] °C±[redacted] °C and [redacted] °C±[redacted] °C.	CP185073, Table 17.17
Stock stability	Stability of purified human IgG stocks used for preparation of calibrators standard was tested and found stable for [redacted] days when stored at [redacted] °C±[redacted] °C.	CP185073, Table 17.13 and Table 17.14
Reagent stability	Stability of coating was tested and determined stable for up to [redacted] days at [redacted] °C±[redacted] °C.	CP185073, Table 17.12
Carry over	*NA*	*NA*
Method Performance in Study ARGX-113-1702 (Module 5.3.1.4, CP175332)		
Assay passing rate	[redacted] of the [redacted] analytical runs passed ([redacted] % passing rate).	CP175332, Section 6
Method Performance in Study ARGX-113-1704 (Module 5.3.1.4, CP185288)		
Assay passing rate	[redacted] of the [redacted] analytical runs passed ([redacted] % passing rate).	CP185288, Section 6
Interference & specificity	[redacted] lots of serum from MG patients were spiked with increasing concentrations of efgartigimod up to [redacted] µg/mL. The observed bias ranged from [redacted] % to [redacted] %.	CP185288, Table 21.3
Parallelism	[redacted] lots of sera of from MG patients was tested at MRD and [redacted] subsequent dilutions. Parallelism was demonstrated with MG patient sera.	CP185288, Table 21.11
Qualification new batch purified IgG	Batch/Lot No.: [redacted] was qualified and accepted for further use.	CP185288, Table 21.12 to Table 21.16
Qualification new HRP batch.	HRP batch number [redacted] (new batch) was qualified compared to [redacted] (old batch) and accepted for further use.	CP185288, Table 21.17 to Table 21.20

CV=coefficient of variation; ELISA=enzyme-linked immunosorbent assay; Fc=crystallized fragment; HCS=high calibration standard; HRP=horseradish peroxidase; Ig=immunoglobulin; LCS=low calibration standard; LLOQ=lower limit of quantification; MG=myasthenia gravis; MRD=minimum required dilution; NA=not applicable; No.=number; QC=quality control; ULOQ=upper limit of quantification

表 2.7.1-9　血清中 IgG サブタイプ、IgA 及び IgM 濃度分析法の遷移

	Method Validation #1	Clinical Study ARGX-113-1501 (Part 1)	Clinical Study ARGX-113-1501 (Part 2)
Analyte	IgG1, IgG2, IgG3, IgG4, IgA and IgM	IgG1, IgG2, IgG3, IgG4, IgA and IgM	IgG1, IgG2, IgG3, IgG4, IgA and IgM
eCTD reference number	CP155243	CP155360	CP155361
Method ID	[redacted] Version [redacted]	[redacted] Version [redacted]	[redacted] Version [redacted]
Duration of time method is in use	[redacted] to Present	[redacted] to [redacted]	[redacted] to [redacted]
Bioanalytical site	[redacted], France		
Matrix	Serum		
Platform	Luminex		
Format	A validated Luminex assay format using magnetic beads coupled with Anti-IgG1, 2, 3, 4, IgA and IgM as capture and streptavidin-phycoerythrin as detection.		
Stock reference, lot number, expiration date	[redacted], Cat. No.: [redacted] Batch/Lot No.: [redacted], Exp. date: [redacted] Batch/Lot No.: [redacted], Exp. date: [redacted] Batch/Lot No.: [redacted] Exp. date: [redacted]	[redacted], Cat. No.: [redacted] Batch/Lot No.: [redacted], Exp. date: [redacted]	[redacted], Cat. No.: [redacted] Batch/Lot No.: [redacted], Exp. date: [redacted] Batch/Lot No.: [redacted], Exp. date: [redacted] Batch/Lot No.: [redacted] Exp. date: [redacted]
Calibration range from the LCS to the HCS at MRD	Batch/Lot No.: [redacted], [redacted] IgG1: 0.382 to 783 ng/mL IgG2: 8.59 to 8790 ng/mL IgG3: 0.0574 to 235 ng/mL IgG4: 0.0150 to 123 ng/mL IgA: 0.114 to 468 ng/mL IgM: 0.683 to 2800 ng/mL Batch/Lot No.: [redacted] IgG1: 0.531 to 1090 ng/mL IgG2: 13.7 to 14000 ng/mL IgG3: 0.0924 to 379 ng/mL IgG4: 0.0162 to 133 ng/mL IgA: 0.213 to 874 ng/mL IgM: 0.791 to 3240 ng/mL	IgG1: 0.382 to 783 ng/mL IgG2: 8.59 to 8790 ng/mL IgG3: 0.0574 to 235 ng/mL IgG4: 0.0150 to 123 ng/mL IgA: 0.114 to 468 ng/mL IgM: 0.683 to 2800 ng/mL	Batch/Lot No.: [redacted] IgG1: 0.382 to 783 ng/mL IgG2: 8.59 to 8790 ng/mL IgG3: 0.0574 to 235 ng/mL IgG4: 0.0150 to 123 ng/mL IgA: 0.114 to 468 ng/mL IgM: 0.683 to 2800 ng/mL Batch/Lot No.: [redacted], [redacted] IgG1: 0.531 to 1090 ng/mL IgG2: 13.7 to 14000 ng/mL IgG3: 0.0924 to 379 ng/mL IgG4: 0.0162 to 133 ng/mL IgA: 0.213 to 874 ng/mL IgM: 0.791 to 3240 ng/mL
Matrix study population	Healthy, MG, ITP, PV	Healthy	Healthy
Link to reports and applicable amendments	Module 5.3.1.4, CP155243	Module 5.3.1.4, CP155360	Module 5.3.1.4, CP155361
Synopsis of amendment history	Long term stability based on samples of ARGX-113-1501 (Module 5.3.1.4, CP190133)	*NA*	*NA*

	Clinical Study ARGX-113-1602	Clinical Study ARGX-113-1702	Clinical Study ARGX-113-1704
Analyte	IgG1, IgG2, IgG3, IgG4, IgA and IgM	IgG1, IgG2, IgG3, IgG4, IgA and IgM	IgG1, IgG2, IgG3 and IgG4
eCTD reference number	CP165308	CP175332	CP185288
Method ID	[redacted] Version [redacted]	[redacted] Version [redacted]	[redacted] Version [redacted] and [redacted]
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], France		
Matrix	Serum		
Platform	Luminex		
Format	A validated Luminex assay format using magnetic beads coupled with Anti IgG1, 2, 3, 4, IgA and IgM as capture and streptavidin-phycoerythrin as detection.		
Stock reference, lot number, expiration date	[redacted], Cat. No.: [redacted] Batch/Lot No.: [redacted] Exp. date: [redacted]-20[redacted]	[redacted], Cat. No.: [redacted] Batch/Lot No.: [redacted] Exp. date: [redacted]-20[redacted] Batch/Lot No.: [redacted], Exp. date: [redacted]-20[redacted] Batch/Lot No.: [redacted], Exp. date: [redacted]-20[redacted]	[redacted], Cat. No.: [redacted] Batch/Lot No.: [redacted] Exp. date: [redacted]-20[redacted] Batch/Lot No.: [redacted] Exp. date: [redacted]-20[redacted] Batch/Lot No.: [redacted] Exp. date: [redacted]-20[redacted] Batch/Lot No.: [redacted] Exp. date [redacted]-20[redacted]
Calibration range from the LCS to the HCS at MRD	IgG1: 0.531 to 1090 ng/mL IgG2: 13.7 to 14000 ng/mL IgG3: 0.0924 to 379 ng/mL IgG4: 0.0162 to 133 ng/mL IgA: 0.213 to 874 ng/mL IgM : 0.791 to 3240 ng/mL	Batch/Lot No.: [redacted] IgG1: 0.531 to 1090 ng/mL IgG2: 13.7 to 14000 ng/mL IgG3: 0.0924 to 379 ng/mL IgG4: 0.0162 to 133 ng/mL IgA: 0.213 to 874 ng/mL IgM: 0.791 to 3240 ng/mL Batch/Lot No.: [redacted], [redacted] IgG1: 0.785 to 1610 ng/mL IgG2: 4.24 to 4340 ng/mL IgG3: 0.125 to 511 ng/mL IgG4: 0.0239 to 196 ng/mL IgA: 0.220 to 226 ng/mL IgM: 1.18 to 4840 ng/mL	Batch/Lot No.: [redacted] IgG1: 0.785 to 1610 ng/mL IgG2: 4.24 to 4340 ng/mL IgG3: 0.125 to 511 ng/mL IgG4: 0.0239 to 196 ng/mL Batch/Lot No.: [redacted] IgG1: 0.336 to 751 ng/mL IgG2: 9.00 to 9210 ng/mL IgG3: 0.0937 to 384 ng/mL IgG4: 0.0231 to 190 ng/mL Batch/Lot No.: [redacted] IgG1: 1.06 to 2160 ng/mL IgG2: 2.84 to 2910 ng/mL IgG3: 0.0553 to 227 ng/mL IgG4: 0.0228 to 187 ng/mL Batch/Lot No.: [redacted] IgG2: 2.90 to 2970 ng/mL IgG3: 0.0657 to 269 ng/mL IgG4: 0.0255 to 209 ng/mL
Matrix study population	MG	Healthy	MG
Link to reports and applicable amendments	Module 5.3.1.4, CP165308	Module 5.3.1.4, CP175332	Module 5.3.1.4, CP185288
Synopsis of amendment history	Qualification of new QC samples	Qualification of Batch/Lot No. [redacted]	Qualification of Batch/Lot No. [redacted], [redacted] and [redacted]. Qualification of technician. Qualification of new QC samples

Exp.=expiration; HCS=high calibration standard; Ig=immunoglobulin; ITP= idiopathic thrombocytopenic purpura; LCS=low calibration standard; MG=myasthenia gravis; MRD=minimum required dilution; NA=not applicable; No.=number; PV=pemphigus vulgaris; QC=quality control.

表 2.7.1-10　血清中 IgG サブタイプ、IgA 及び IgM 濃度分析法の概要

Bioanalytical method validation report name	**Feasibility and Validation of a Multiplex Immunoassay for the Determination of IgG1, IgG2, IgG3, IgG4, IgA and IgM in Human Serum** (Module 5.3.1.4, CP155243)		
Method description	[redacted] assays are solid phase immunoassays where magnetic beads are coated with capture antibodies against the IgG subtypes and IgA and IgM that bind these analytes in serum sample. Samples are analyzed in a Luminex system which quantifies the different markers.		
Materials used for standard calibration curve	Calibration curve materials were included in the [redacted] kits.		
Validated assay range (LLOQ to ULOQ)	IgG1 11.9 to 8560 µg/mL IgG2 304 to 55200 µg/mL IgG3 2.74 to 1900 µg/mL IgG4 0.884 to 1470 µg/mL IgA 29.9 to 12600 µg/mL IgM 20.0 to 22400 µg/mL (in 100% matrix)		
Material used for QCs	Quality controls were prepared using endogenous concentration analytes and standard [redacted] of the [redacted] kit.		
MRD	1/20000 for IgG1, IgG2, IgG3, IgG4 and IgM and 1/80000 for IgA		
Source and lot of reagents	Batch/Lot No.: [redacted] Expiry date: [redacted]		
Regression model and weighting	5-parameter logistic model fitting curve.		
Validation parameters	**Method Validation Summary**		**Source Location**
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LCS to HCS	IgG1 [redacted] calibrators IgG2 calibrators IgG3 calibrators IgG4 calibrators IgA calibrators IgM calibrators	CP155243, Table 18.32 to Table 18.43
	Inter assay accuracy (%bias) from LCS to HCS over the batches ([redacted], [redacted] and [redacted])	IgG1 [redacted] to [redacted] % IgG2 [redacted] to [redacted] % IgG3 [redacted] to [redacted] % IgG4 [redacted] to [redacted] % IgA [redacted] to [redacted] % IgM [redacted] to [redacted] %	CP155243, Table 18.32 to Table 18.43
	Cumulative precision (%CV) from LLOQ to ULOQ over the batches ([redacted], [redacted] and [redacted])	IgG1 [redacted] % IgG2 [redacted] % IgG3 [redacted] % IgG4 [redacted] % IgA [redacted] % IgM [redacted] %	CP155243, Table 18.32 to Table 18.43

<table>
<tr><td rowspan="12">Performance of QCs during accuracy and precision runs</td><td colspan="7">Intra assay accuracy (%bias) in 5 QCs</td><td rowspan="7">CP155243, Table 18.44 to Table 18.49</td></tr>
<tr><td></td><td>IgG1</td><td>IgG2</td><td>IgG3</td><td>IgG4</td><td>IgA</td><td>IgM</td></tr>
<tr><td>LLOQ</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td></tr>
<tr><td>Low</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td></tr>
<tr><td>Mid</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td></tr>
<tr><td>High</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td></tr>
<tr><td>ULOQ</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td></tr>
<tr><td colspan="7">Inter batch %CV</td><td rowspan="6">CP155243, Table 18.44 to Table 18.49</td></tr>
<tr><td></td><td>IgG1</td><td>IgG2</td><td>IgG3</td><td>IgG4</td><td>IgA</td><td>IgM</td></tr>
<tr><td>LLOQ</td><td>[redacted]%</td><td>[redacted]%</td><td>[redacted]%</td><td>[redacted]%</td><td>[redacted]%</td><td>[redacted]%</td></tr>
<tr><td>Low</td><td>[redacted]%</td><td>[redacted]%</td><td>[redacted]%</td><td>[redacted]%</td><td>[redacted]%</td><td>[redacted]%</td></tr>
<tr><td>Mid</td><td>[redacted]%</td><td>[redacted]%</td><td>[redacted]%</td><td>[redacted]%</td><td>[redacted]%</td><td>[redacted]%</td></tr>
<tr><td>High</td><td>[redacted]%</td><td>[redacted]%</td><td>[redacted]%</td><td>[redacted]%</td><td>[redacted]%</td><td>[redacted]%</td></tr>
<tr><td>ULOQ</td><td>[redacted]%</td><td>[redacted]%</td><td>[redacted]%</td><td>[redacted]%</td><td>[redacted]%</td><td>[redacted]%</td></tr>
<tr><td>Selectivity & matrix effect</td><td colspan="7">In the feasibility phase selectivity was investigated by spiking[redacted] lots from healthy subjects at a single concentration level and diluted with the following dilution factors: 1/20000, 1/40000, 1/80000, 1/160000 and 1/320000. Observed bias per analyte at MRD:
IgG1 [redacted] to [redacted]%
IgG2 [redacted] to [redacted]%
IgG3 [redacted] to [redacted]%
IgG4 [redacted] to [redacted]%
IgA [redacted] to [redacted]%
IgM [redacted] to [redacted]%
Based on the results of parallelism and selectivity, a MRD was defined (1/20000 for IgG1, IgG2, IgG3, IgG4 and IgM and 1/80000 for IgA).</td><td>CP155243, Table 18.20 to Table 18.25</td></tr>
</table>

<table>
<tr><td>Interference & specificity</td><td>No interference was observed from efgartigimod. Interference of efgartigimod was investigated by spiking different lots of sera from healthy volunteer (HV) and the target population with increasing concentrations of efgartigimod up to [redacted] µg/mL. Observed bias range:
<table>
<tr><td></td><td>HV ([redacted] lots)</td><td>MG ([redacted] lots)</td><td>ITP ([redacted] lots)</td><td>PV ([redacted] lots)</td></tr>
<tr><td>IgG1</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td></tr>
<tr><td>IgG2</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td></tr>
<tr><td>IgG3</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td></tr>
<tr><td>IgG4</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td></tr>
<tr><td>IgA</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td></tr>
<tr><td>IgM</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td></tr>
</table></td><td>CP155243, Table 18.50 to Table 18.61</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>Dilution linearity was investigated during parallelism experiment and acceptable from 1/20000 of IgG1, IgG2, IgG3, IgG4 and IgM, and from 1/80000 for IgA.</td><td>CP155243, Table 18.14 to Table 18.19.</td></tr>
<tr><td>Bench-top/process stability</td><td>Bench-top and refrigerator stability for [redacted] hours and [redacted] minutes was shown for all analytes.</td><td>CP155243, Table 18.86 to Table 18.91</td></tr>
<tr><td>Freeze-thaw stability</td><td>Stability after [redacted] freeze-thaw cycles was shown for all analytes with stability samples stored at [redacted] °C± [redacted] °C and [redacted] °C± [redacted] °C.</td><td>CP155243, Table 18.74 to Table 18.85</td></tr>
<tr><td>Long-term storage</td><td>Long-term stability of [redacted] days for all analytes was shown for stability samples stored at [redacted] °C± [redacted] °C and [redacted] °C± [redacted] °C.</td><td>CP155243, Table 18.92 to Table 18.103</td></tr>
<tr><td>Parallelism</td><td>Parallelism was investigated during the feasibility phase with [redacted] lots of healthy subject (HV) sera which were diluted from 1/20000 to 1/320000 (5 dilutions), resulting in a MRD of 1/20000. The parallelism experiment was extended to diseased matrixes during validation where [redacted] individual sera of each study population were tested at MRD and [redacted] subsequent dilutions. Observed bias is presented below.
<table>
<tr><td></td><td>HV ([redacted] lots)</td><td>MG ([redacted] lots)</td><td>ITP ([redacted] lots)</td><td>PV ([redacted] lots)</td></tr>
<tr><td>IgG1</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td></tr>
<tr><td>IgG2</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td></tr>
<tr><td>IgG3</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td></tr>
<tr><td>IgG4</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td></tr>
<tr><td>IgA</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td></tr>
<tr><td>IgM</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td><td>[redacted] to [redacted]%</td></tr>
</table>
Parallelism was demonstrated in MG, IPT and PV patient sera and MRD established with healthy sera can be applied.</td><td>CP155243, Table 18.14 to Table 18.19 and Table 18.62 to Table 18.67</td></tr>
</table>

Qualification new Kit Lot No.	Kit Lot No.: [redacted] and [redacted] were qualified and accepted for further use	CP155243, Table 18.104 to Table 18.127
Carry over	*NA*	*NA*
Long term stability	Stabilities were assessed using samples collected during the clinical study ARGX 113-1501. Long-term stability for up to [redacted] 7 days was demonstrated for all analytes stored at [redacted] °C± [redacted] °C.	CP190133 Section 6
Method Performance in Study ARGX-113-1501 (Part 1) (Module 5.3.1.4, CP155360)		
Assay passing rate	[redacted] of the [redacted] analytical runs passed ([redacted] % passing rate).	CP155360, Section 6
Method Performance in Study ARGX-113-1501 (Part 2) (Module 5.3.1.4, CP155361)		
Assay passing rate	IgG1 [redacted] of the [redacted] analytical runs passed ([redacted] % passing rate). IgG2 [redacted] of the [redacted] analytical runs passed ([redacted] % passing rate). IgG3 [redacted] of the [redacted] analytical runs passed ([redacted] % passing rate). IgG4 [redacted] of the [redacted] analytical runs passed ([redacted] % passing rate). IgA [redacted] of the [redacted] analytical runs passed ([redacted] % passing rate). IgM [redacted] of the [redacted] analytical runs passed ([redacted] % passing rate).	CP155361, Section 6
Method Performance in Study ARGX-113-1602 (Module 5.3.1.4, CP165308)		
Assay passing rate	IgG1 [redacted] of the [redacted] analytical runs passed ([redacted] % passing rate). IgG2 [redacted] of the [redacted] analytical runs passed ([redacted] % passing rate). IgG3 [redacted] of the [redacted] analytical runs passed ([redacted] % passing rate). IgG4 [redacted] of the [redacted] analytical runs passed ([redacted] % passing rate). IgA [redacted] of the [redacted] analytical runs passed ([redacted] % passing rate). IgM [redacted] of the [redacted] analytical runs passed ([redacted] % passing rate).	CP165308, Section 6
Method Performance in Study ARGX-113-1702 (Module 5.3.1.4, CP175332)		
Assay passing rate	IgG1 [redacted] of the [redacted] analytical runs passed ([redacted] % passing rate). IgG2 [redacted] of the [redacted] analytical runs passed ([redacted] % passing rate). IgG3 [redacted] of the [redacted] analytical runs passed ([redacted] % passing rate). IgG4 [redacted] of the [redacted] analytical runs passed ([redacted] % passing rate). IgA [redacted] of the [redacted] analytical runs passed ([redacted] % passing rate). IgM [redacted] of the [redacted] analytical runs passed ([redacted] % passing rate).	CP175332, Section 6
Qualification new Kit Lot No.	Kit Lot No.: [redacted] was qualified and accepted for further use.	CP175332, Table 19.2 to Table 19.13
Method Performance in Study ARGX-113-1704 (Module 5.3.1.4, CP185288)		
Assay passing rate	IgG1 [redacted] of the [redacted] analytical runs passed ([redacted] % passing rate). IgG2 [redacted] of the [redacted] analytical runs passed ([redacted] % passing rate). IgG3 [redacted] of the [redacted] analytical runs passed ([redacted] % passing rate). IgG4 [redacted] of the [redacted] analytical runs passed ([redacted] % passing rate).	CP185288, Section 6
Qualification runs	Kit Lot No.: [redacted], [redacted] and [redacted] were qualified and accepted for further use. Qualification of a technician.	CP185288, Table 21.23 to Table 21.102, Table 21.103 to Table 21.118

CV=coefficient of variation; HCS=high calibration standard; HV=healthy volunteer; Ig=immunoglobulin; ITP=idiopathic thrombocytopenic purpura; LCS=low calibration standard; LLOQ=lower limit of quantification; MG=myasthenia gravis; MRD=minimum required dilution; NA=not applicable; No.=number; PV=pemphigus vulgaris; QC=quality control; ULOQ=upper limit of quantification

表 2.7.1-11　　血清中 IgD 濃度分析法の遷移

	Method validation #1	Clinical Study ARGX-113-1501 (Part 1)	Clinical Study ARGX-113-1501 (Part 2)	Clinical Study ARGX-113-1602
Analyte	IgD	IgD	IgD	IgD
eCTD reference number	CP155245	CP155360	CP155361	CP165308
Method ID	[redacted] Version [redacted] to [redacted]	[redacted] Versions [redacted] to [redacted]	[redacted] Versions [redacted] to [redacted]	[redacted] Version [redacted] and [redacted]
Duration of time method is in use	[redacted]-20[redacted] to Present	[redacted]-20[redacted] to [redacted]-20[redacted]	[redacted]-20[redacted] to [redacted]-20[redacted]	[redacted]-20[redacted] to [redacted]-20[redacted]
Bioanalytical site	[redacted], France			
Matrix	Serum			
Platform	Enzyme-linked immunosorbent assay (ELISA)			
Format	Human IgD is captured by an anti-human IgD goat polyclonal antibody and detected with a HRP labelled anti-human IgD goat polyclonal antibody.			
Stock reference, lot number, expiration date	Human IgD ELISA Quantitation Set, Cat No.: [redacted] Batch/Lot No.: [redacted] Exp. date: [redacted]-20[redacted] Batch/Lot No.: [redacted] Exp. date: [redacted]-20[redacted] Batch/Lot No.: [redacted] Exp. date: [redacted]-20[redacted]	Human IgD ELISA Quantitation Set, Cat No.: [redacted] Batch/Lot No.: [redacted] Exp. date: [redacted]-20[redacted]	Human IgD ELISA Quantitation Set, Cat No.: [redacted] Batch/Lot No.: [redacted] Exp. date: [redacted]-20[redacted] Batch/Lot No.: [redacted] Exp. date: [redacted]-20[redacted]	Human IgD ELISA Quantitation Set, Cat No.: [redacted] Batch/Lot No.: [redacted] Exp. date: [redacted]-20[redacted]
Calibration range from the LCS to the HCS	3.13 to 200 ng/mL	3.13 to 200 ng/mL	3.13 to 200 ng/mL	3.13 to 200 ng/mL
Matrix study population	Healthy, MG, ITP, PV	Healthy	Healthy	MG
Link to reports and applicable amendments	Module 5.3.1.4, CP155245	Module 5.3.1.4, CP155360	Module 5.3.1.4, CP155361	Module 5.3.1.4, CP165308
Synopsis of amendment history	*NA*	*NA*	Qualification Kit Lot No. [redacted].	Qualification Kit Lot No. [redacted]. Adjustment of incubation time.

Cat.=catalogue; ELISA=enzyme-linked immunosorbent assay; Exp.=expiration; HCS=high calibration standard; HRP=horseradish peroxidase; Ig=immunoglobulin; ITP=idiopathic thrombocytopenic purpura; LCS=low calibration standard; MG=myasthenia gravis; NA=not applicable; No.=number; PV=pemphigus vulgaris

表 2.7.1-12　血清中 IgD 濃度分析法の概要

Bioanalytical method validation report name	**Feasibility and Validation of an Immunoassay for the Determination of IgD in Human Serum** (Module 5.3.1.4, CP155245)		
Method description	The assay is a quantitative ELISA where IgD is captured with anti-human IgD goat polyclonal antibody and detected with a HRP conjugated anti-human IgD goat polyclonal antibody.		
Materials used for standard calibration curve	Calibration curve reagents were included in the human IgD ELISA Quantification Set.		
Validated assay range	Batch/Lot No.: [redacted]: 1645 to 84000 ng/mL Batch/Lot No.: [redacted] and [redacted]: 1725 to 100000 ng/mL (in 100% matrix)		
Material used for QCs	Quality control reagents were included in the human IgD ELISA Quantification Set.		
MRD	1/500		
Source and lot of reagents	Batch/Lot No., Exp. date: [redacted] Efgartigimod, Batch/Lot No.: [redacted], end of use dates: [redacted] and [redacted]		
Regression model and weighting	5-parameter logistic model with weighting factor $1/Y^2$.		
Validation parameters	**Method Validation Summary**		**Source Location**
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LCS to HCS	[redacted] calibrators	CP155245, Table 15.8
	Inter assay accuracy (%bias) from LCS to HCS	[redacted]% to [redacted]%	CP155245, Table 15.8
	Cumulative precision (%CV) from LCS to HCS	[redacted]%	CP155245, Table 15.8
Performance of QCs during accuracy and precision runs	**Intra assay accuracy (%bias) in 5 QCs**	LLOQ [redacted]% to [redacted]% Low [redacted]% to [redacted]% Mid [redacted]% to [redacted]% High [redacted]% to [redacted]% ULOQ [redacted]% to [redacted]%	CP155245, Table 15.10
	Inter batch precision (%CV)	LLOQ [redacted]% Low [redacted]% Mid [redacted]% High [redacted]% ULOQ [redacted]%	CP155245, Table 15.10
Selectivity & matrix effect	During the feasibility phase selectivity was assessed by analyzing [redacted] lots of human serum at MRD unspiked and spiked with IgD demonstrating a bias of [redacted]% to [redacted]%.		CP155245, Table 15.6

Interference & specificity	[redacted] lots of serum from healthy subjects were spiked with increasing concentrations of efgartigimod up to [redacted] µg/mL and diluted to the MRD. The observed bias ranged from [redacted] % to [redacted] %. [redacted] lots of serum from MG patients were spiked with increasing concentrations of efgartigimod up to [redacted] µg/mL and diluted to the MRD. The observed bias ranged from [redacted] % to [redacted] %. [redacted] lots of serum from ITP patients were spiked with increasing concentrations of efgartigimod up to [redacted] µg/mL and diluted to the MRD. The observed bias ranged from [redacted] % to [redacted] %. [redacted] lots of serum from PV patients were spiked with increasing concentrations of efgartigimod up to [redacted] µg/mL and diluted to the MRD. The observed bias ranged from [redacted] % to [redacted] %.	CP155245, Table 15.11 to Table 15.14
Hemolysis effect	*NA*	*NA*
Lipemic effect	*NA*	*NA*
Parallelism	[redacted] lots of serum from healthy subjects were analyzed undiluted and diluted: 1/250, 1/500, 1/1000 and 1/2000. Bias compared to the concentration obtained at 1/500 was within [redacted] % for [redacted] out of [redacted] samples and MRD was determined at 1/500. The MRD at 1/500 was confirmed for human sera in MG, ITP and PV patients,	CP155245, Table 15.5 and Table 15.15
Bench-top/process stability	Bench-top and refrigerator stability was shown up to [redacted] hours at [redacted] and [redacted] °C± [redacted] °C for samples initially frozen at [redacted] °C± [redacted] °C and [redacted] °C± [redacted] °C.	CP155245, Table 15.20 to Table 15.23
Freeze-thaw stability	Freeze-thaw stability was shown up to [redacted] freeze-thaw cycles for samples initially frozen at ([redacted] °C± [redacted] °C and [redacted] °C± [redacted] °C).	CP155245, Table 15.18 and Table 15.19
Long-term storage	Long term storage stability was shown up to [redacted] days for samples initially frozen at [redacted] °C± [redacted] °C and [redacted] °C± [redacted] °C.	CP155245, Table 15.27 and Table 15.31
Carry over	*NA*	*NA*
Qualification runs	Qualification of a new Kit Lot No.: [redacted]. Qualification of a technician.	CP155245, Table 15.32 to Table 15.33
Method Performance in Study ARGX-113-1501 (Part 1) (Module 5.3.1.4, CP155360)		
Assay passing rate	[redacted] of the [redacted] analytical runs passed ([redacted] % passing rate).	CP155360, Section 6
Method Performance in Study ARGX-113-1501 (Part 2) (Module 5.3.1.4, CP155361)		
Assay passing rate	[redacted] of the [redacted] analytical runs passed ([redacted] % passing rate).	CP155361, Section 6
Method Performance in Study ARGX-113-1602 (Module 5.3.1.4, CP165308)		
Assay passing rate	[redacted] of the [redacted] analytical runs passed ([redacted] % passing rate).	CP165308, Section 6

CV=coefficient of variation; ELISA=enzyme-linked immunosorbent assay; Exp.=expiration; HCS=high calibration standard; HRP=horseradish peroxidase; Ig=immunoglobulin; ITP=idiopathic thrombocytopenic purpura; LCS=low calibration standard; LLOQ=lower limit of quantitation; MG=myasthenia gravis; MRD=minimum required dilution; NA=not applicable; No.=number; PV=pemphigus vulgaris; QC=quality control; ULOQ=upper limit of quantitation

表 2.7.1-13　血清中 IgE 濃度分析法の遷移

	Method Validation #1	Clinical Study ARGX-113-1501 (Part 1)	Clinical Study ARGX-113-1501 (Part 2)	Clinical Study ARGX-113-1602
Analyte	IgE	IgE	IgE	IgE
eCTD reference number	CP175027	CP155360	CP155361	CP165308
Method ID	[redacted] Version [redacted] and [redacted]	[redacted] Version [redacted]	[redacted] Version [redacted]	[redacted] Version [redacted] and [redacted]
Duration of time method is in use	[redacted]-20[redacted] to Present	[redacted]-20[redacted] to [redacted]-20[redacted]	[redacted]-20[redacted] to [redacted]-20[redacted]	[redacted]-20[redacted] to [redacted]-20[redacted]
Bioanalytical site	[redacted], France			
Matrix	Serum			
Platform	Electrochemiluminescence immunoassay (ECLIA)			
Format	Samples were incubated with mixture of biotinylated and ruthenium labelled anti-IgE monoclonal antibody. A sandwich complex was formed with the human IgE in the sample. Complexes were captured by the addition of streptavidin-coated microparticles and the IgE antibodies were detected by electrochemiluminescence.			
Stock reference, lot number, expiration date	[redacted], Cat. No.: [redacted], Batch/Lot No.: [redacted], Exp. date: [redacted]-20[redacted], [redacted], [redacted], Cat. No.: [redacted], Batch/Lot No.: [redacted], Exp. date: [redacted]-20[redacted], [redacted], Cat. No.: [redacted], Batch/Lot No.: [redacted], Exp. date: [redacted]-20[redacted]	[redacted], Cat. No.: [redacted], Batch/Lot No.: [redacted], Exp. date: [redacted]-20[redacted], [redacted], [redacted], Cat. No.: [redacted], Batch/Lot No.: [redacted], Exp. date: [redacted]-20[redacted], [redacted], Cat. No.: [redacted], Batch/Lot No.: [redacted], Exp. date: [redacted]-20[redacted]	[redacted], Cat. No.: [redacted], Batch/Lot No.: [redacted], Exp. date: [redacted]-20[redacted], [redacted], [redacted], Cat. No.: [redacted], Batch/Lot No.: [redacted], Exp. date: [redacted]-20[redacted], [redacted], Cat. No.: [redacted], Batch/Lot No.: [redacted], Exp. date: [redacted]-20[redacted], Batch/Lot No.: [redacted], Exp. date: [redacted]-20[redacted]	[redacted], Cat. No.: [redacted], Batch/Lot No.: [redacted], Exp. date: [redacted]-20[redacted], [redacted], [redacted], Cat. No.: [redacted], Batch/Lot No.: [redacted], Exp. date: [redacted]-20[redacted], [redacted], Cat. No.: [redacted], Batch/Lot No.: [redacted] and [redacted], Exp. date: [redacted]-20[redacted] and [redacted]-20[redacted]

	Method Validation #1	Clinical Study ARGX-113-1501 (Part 1)	Clinical Study ARGX-113-1501 (Part 2)	Clinical Study ARGX-113-1602
Calibration range from the LCS to the HCS	0.240 to 6000 ng/mL	0.240 to 6000 ng/mL	0.240 to 6000 ng/mL	0.240 to 6000 ng/mL
Matrix study population	Healthy, MG, ITP, PV	Healthy	Healthy	MG
Link to reports and applicable amendments	Module 5.3.1.4, CP175027	Module 5.3.1.4, CP155360	Module 5.3.1.4, CP155361	Module 5.3.1.4, CP165308
Synopsis of amendment history	*NA*	Qualification of QC samples. Stability assessment of IgE in human serum (ie, bench-top stability, freeze-thaw stability and long term stability)	*NA*	*NA*

Cat.=catalogue; ECLIA=electrochemiluminescence immunoassay; Exp.=expiration; HCS=high calibration standard; Ig=immunoglobulin; ITP=idiopathic thrombocytopenic purpura; LCS=low calibration standard; MG=myasthenia gravis; NA=not applicable; No.=number; PV=pemphigus vulgaris; QC=quality control

表 2.7.1-14　血清中 IgE 濃度分析法の概要

Bioanalytical method validation report name	**Parallelism and Efgartigimod Drug Interference Assessments for the Determination of IgE in Human Serum from Healthy Volunteers and Diseases Patients by ECLIA on the [redacted] and Stability of IgE in Human Serum to Storage at [redacted] °C± [redacted] °C for Approximately [redacted] Months** (Module 5.3.1.4, CP175027)		
Method description	IgE was detected by using [redacted] ECLIA kit of [redacted] analyzed with a [redacted].		
Materials used for standard calibration curve	[redacted], catalogue No.: [redacted], Batch/Lot No.: [redacted], Exp. date: [redacted], [redacted]		
Validated assay range	LLOQ: 1.20 ng/mL (functional sensitivity)		
Material used for QCs	Control samples for Calibration curve: [redacted], Cat. No.: [redacted], Batch/Lot No.: [redacted], Exp. date: [redacted], [redacted] Quality control samples: Serum samples with endogenous concentrations IgE		
MRD	*NA*		
Source and lot of reagents	Efgartigimod, Batch/Lot No.: [redacted], Exp. date: [redacted]		
Regression 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 LCS to HCS	[redacted] standards	CP175027, Table 9.1
	Cumulative accuracy (%bias) from LCS to HCS	*NA*	*NA*
	Cumulative precision (%CV) from LCS to HCS	*NA*	*NA*
Performance of QCs during accuracy and precision runs	**Intra assay accuracy (%bias) in QCs**	QC1: [redacted] % QC2: [redacted] % QC3: [redacted] %	CP175027, Table 15.2
	Intra assay batch precision (%CV)	QC1: [redacted] % QC2: [redacted] % QC3: [redacted] %	CP175027, Table 15.2
Selectivity & matrix effect	*NA*		*NA*
Interference & specificity	[redacted] lots of serum from healthy volunteers were spiked with increasing concentrations of efgartigimod up to [redacted] µg/mL. The observed bias ranged from [redacted] % to [redacted] %. [redacted] lots of serum from MG patients were spiked with increasing concentrations of efgartigimod up to [redacted] µg/mL. The observed bias ranged from [redacted] % to [redacted] %. [redacted] lots of serum from ITP patients were spiked with increasing concentrations of efgartigimod up to [redacted] µg/mL. The observed bias ranged from [redacted] % to [redacted] %. [redacted] lots of serum from PV patients were spiked with increasing concentrations of efgartigimod up to [redacted] µg/mL. The observed bias ranged from [redacted] % to [redacted] %.		CP175027, Table 15.7 to Table 15.10

Hemolysis effect	*NA*	*NA*
Lipemic effect	*NA*	*NA*
Dilution linearity	*NA*	*NA*
Parallelism	Parallelism was evaluated by testing [redacted] lots of healthy subjects serum, [redacted] lots MG patient serum, [redacted] lots ITP patient serum and [redacted] lots of PV patient serum. Sera were analyzed undiluted and with the dilution factors: 1/2, 1/4 and 1/8. The following bias range for undiluted samples was observed: Healthy subjects: [redacted]% to [redacted]% MG: [redacted]% to [redacted]% ITP: [redacted]% to [redacted]% PV: [redacted]% to [redacted]% [redacted] samples, [redacted] healthy subject sample and [redacted] ITP sample demonstrated in the highest dilution an bias [redacted]%. No apparent effect of dilution was observed with dilution.	CP175027, Table 15.3 to Table 15.6
Bench-top/process stability	*NA*	*NA*
Freeze-thaw stability	*NA*	*NA*
Long-term storage	The stability of IgE in human serum stored at [redacted]°C±[redacted]°C was demonstrated for up to [redacted] days.	CP175027, Table 15.11
Carry over	*NA*	*NA*
Method Performance in Study ARGX-113-1501 (Part 1) (Module 5.3.1.4, CP155360)		
Assay passing rate	[redacted] of the [redacted] analytical runs passed ([redacted]% passing rate).	CP155360, Table 21.1
Study sample analysis/ stability	Stability of IgE in human serum was demonstrated at room temperature for [redacted] hours, at [redacted]°C±[redacted]°C for up to [redacted] days under this study and for [redacted] freeze/thaw cycles ([redacted]°C±[redacted]°C). This stability period covers the length of time from specimen collection to analysis, ie, [redacted] days.	CP155360, Section 16.5
Method Performance in Study ARGX-113-1501 (Part 2) (Module 5.3.1.4, CP155361)		
Assay passing rate	[redacted] of the [redacted] analytical runs passed ([redacted]% passing rate).	CP155361, Section 6
Method Performance in Study ARGX-113-1602 (Module 5.3.1.4, CP165308)		
Assay passing rate	[redacted] of the [redacted] analytical runs passed ([redacted]% passing rate)	CP165308, Table 23.1

CV=coefficient of variation; ECLIA=electrochemiluminescence immunoassay; Exp.=expiration; HCS=high calibration standard; Ig=immunoglobulin; ITP=idiopathic thrombocytopenic purpura; LCS=low calibration standard; LLOQ=lower limit of quantitation; MG=myasthenia gravis; MRD=minimum required dilution; NA=not applicable; No.=number; PV=pemphigus vulgaris; QC=quality control

表 2.7.1-15　血清中抗 AChR 抗体分析法の遷移

	Method Validation #1	Clinical Study ARGX-113-1602	Clinical Study ARGX-113-1704
Analyte	AChR-Ab	AChR-Ab	AChR-Ab
eCTD reference number	CP155456	CP165308	CP185288
Method ID	[redacted] Version [redacted]	[redacted] Version [redacted]	[redacted] Version [redacted]
Duration of time method is in use	[redacted]-20[redacted] to Present	[redacted]-20[redacted] to [redacted]-20[redacted]	[redacted]-20[redacted] to [redacted]-20[redacted]
Bioanalytical site	[redacted], France		
Matrix	Serum		
Platform	Radioimmunoassay (RIA)		
Format	Acetylcholine receptor from human muscle is labelled with ^{125}I-alpha-bungarotoxin. Autoantibodies present in the patient's serum bind to the radioactive labelled receptors and are precipitated with anti-human IgG.		
Kit reference, lot number, expiration date	[redacted] Cat. No.: [redacted]; Batch/Lot No.: [redacted], Exp. date: [redacted]-20[redacted]	[redacted] Cat. No.: [redacted]; Batch/Lot No.: [redacted], Exp. date: [redacted]-20[redacted] Batch/Lot No.: [redacted], Exp. date: [redacted]-20[redacted] Batch/Lot No.: [redacted], Exp. date: [redacted]-20[redacted] Batch/Lot No.: [redacted], Exp. date: [redacted]-20[redacted]	[redacted] Cat. No.: [redacted]; Batch/Lot No.: [redacted], Exp. date: [redacted]-20[redacted] Batch/Lot No.: [redacted] Exp. date: [redacted]-20[redacted] Batch/ Lot No.: [redacted] Exp. date: [redacted]-20[redacted] Batch/ Lot No.: [redacted] Exp. date: [redacted]-20[redacted] Batch/ Lot No.: [redacted] Exp. date: [redacted]-20[redacted]
Calibration range from the LCS to the HCS	0.200 to 1.50 nmol/L	0.200 to 1.50 nmol/L	0.200 to 3.00 nmol/L
Matrix study population	Healthy, MG	MG	MG
Link to reports and applicable amendments	Module 5.3.1.4, CP155456	Module 5.3.1.4, CP165308	Module 5.3.1.4, CP185288
Synopsis of amendment history	*NA*	*NA*	Qualification of technician

AChR=acetylcholine receptor; AChR-Ab=anti-acetylcholine receptor antibodies; Cat.=catalogue; Exp.=expiration; HCS=high calibration standard; Ig=immunoglobulin; LCS=low calibration standard; MG=myasthenia gravis; NA=not applicable; No.=number; RIA=radioimmunoassay; [redacted]=[redacted]

表 2.7.1-16　血清中抗 AChR 抗体濃度分析法の概要

Bioanalytical method validation report name	**Implementation and Validation of a Method for the Determination of AChR-Ab in Human Serum by RIA** (Module 5.3.1.4, CP155456)		
Method description	Acetylcholine receptor from human muscle is labelled with ^{125}I-alpha-bungarotoxin. Autoantibodies present in the patient's serum bind to the radioactive labelled receptors and are precipitated with anti-human IgG.		
Materials used for standard calibration curve	Calibration curve material was included in the [redacted] kits.		
Validated quantitative range	0.265 to 1.49 nmol/L in 100% matrix		
Material used for QCs	QC material was included in the [redacted] kits.		
MRD	No dilution required.		
Source and lot of reagents	[redacted], Cat. No.: [redacted], Batch/Lot No.: [redacted], Exp. date: [redacted] Efgartigimod, Batch/Lot No.: [redacted]9, Exp. date: [redacted]		
Regression model and weighting	4 parameter logistical model.		
Validation parameters	**Method Validation Summary**		**Source Location**
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LCS to HCS	[redacted] calibrators	CP155456, Table 16.8
	Inter assay accuracy (%bias) from LCS Standard to HCS	[redacted] % to [redacted] %	CP155456, Table 16.8
	Inter assay precision (%CV) from LCS to HCS	[redacted] %	CP155456, Table 16.8
Performance of QCs during accuracy and precision runs	**Intra assay accuracy (%bias) in 5 QCs**	LLOQ [redacted] % to [redacted] % Low [redacted] % to [redacted] % Mid [redacted] % to [redacted] % High [redacted] % to [redacted] % ULOQ [redacted] % to [redacted] %	CP155456, Table 16.9
	Inter batch precision %CV	LLOQ [redacted] % Low [redacted] % Mid [redacted] % High [redacted] % ULOQ [redacted] %	CP155456, Table 16.9
Selectivity & matrix effect	During the implementation phase of the validation [redacted] lots of healthy subjects sera were analyzed unspiked and spiked with [redacted] and [redacted] nmol/L. All unspiked samples were determined below LLOQ. Bias: [redacted] nmol/L [redacted] %, [redacted] nmol/L [redacted] %.		CP155456, Table 16.6
Interference and specificity	[redacted] lots of MG serum with different ARAb concentrations were spiked with increasing concentrations of efgartigimod, no drug interference was observed up to [redacted] µg/mL efgartigimod.		CP155456, Table 16.10
Hemolysis effect	*NA*		*NA*
Lipemic effect	*NA*		*NA*

Dilution linearity	[redacted] lots of MG serum with ARAb concentrations above ULOQ were tested. Depending on the endogenous concentration ARAb the dilution steps 1/3, 1/4, 1/8, 1/10, 1/16, 1/20 or 1/40 were tested. A bias range of [redacted]% to [redacted]% was observed and the highest dilution within acceptance criteria was 1/40.	CP155456, Table 16.11
Bench-top/process stability	*NA*	*NA*
Freeze-Thaw stability	*NA*	*NA*
Long-term storage	*NA*	*NA*
Parallelism	During the implementation phase [redacted] lots of MG sera were used to investigate parallelism. Parallelism was accepted for dilution 1/1 and 1/2 where a bias within [redacted]% was observed.	CP155456, Table 16.5
Carry over	*NA*	*NA*
Method Performance in Study ARGX-113-1602 (Module 5.3.1.4, CP165308)		
Assay passing rate	[redacted] of the [redacted] analytical runs passed ([redacted]% passing rate).	CP165308, Section 6
Method Performance in Study ARGX-113-1704 (Module 5.3.1.4, CP185288)		
Assay passing rate	[redacted] of the [redacted] analytical runs passed ([redacted]% passing rate).	CP185288, Section 6
Qualification runs	Qualification of a technician.	CP185288, Table 21.142 and Table 21.143

AChR=acetylcholine receptor; AChR-Ab=anti-acetylcholine receptor antibodies; [redacted]=[redacted]; Cat.=catalogue; CV=coefficient of variation; Exp.=expiration; HCS=high calibration standard; Ig=immunoglobulin; LCS=low calibration standard; LLOQ=lower limit of quantitation; MG=myasthenia gravis; MRD=minimum required dilution; NA=not applicable; No.=number; QC=quality control; RIA=radioimmunoassay; [redacted]=[redacted]; ULOQ=upper limit of quantitation

表 2.7.1-17　血清中ブロッキング抗 AChR 抗体濃度分析法の遷移

	Method Validation #1	Clinical Study ARGX-113-1602
Analyte	AChR-Ab blocking	AChR-Ab blocking
eCTD reference number	CP165306	CP165308
Method ID	[redacted] Version [redacted]	[redacted] Version [redacted]
Duration of time method is in use	[redacted]-20[redacted] to Present	[redacted]-20[redacted] to [redacted]-20[redacted]
Bioanalytical site	[redacted], France	
Matrix	Serum	
Platform	Radioimmunoassay (RIA)	
Format	^{125}I-alpha-bungarotoxin labelled AChR is added to the serum samples. Blocking antibodies will compete with ^{125}I-alpha-bungarotoxin and percentage inhibition is determined after a pull down with Con-A Sepharose by using a gamma counter.	
Kit reference, lot number, expiration date	[redacted] kit Cat. No. [redacted]; Batch/Lot No.: [redacted], Exp. date: [redacted]-20[redacted]	[redacted] kit Cat. No. [redacted]; Batch/Lot No.: [redacted], Exp. date: [redacted]-20[redacted] Batch/Lot No.: [redacted], Exp. date: [redacted]-20[redacted] Batch/Lot No.: [redacted], Exp. date: [redacted]-20[redacted]
Calibration range from the LLOQ to the ULOQ	*NA*	*NA*
Matrix study population	Healthy, MG	MG
Link to reports and applicable amendments	Module 5.3.1.4, CP165306	Module 5.3.1.4, CP165308
Synopsis of amendment history	*NA*	*NA*

AChR=acetylcholine receptor; AChR-Ab=anti-acetylcholine receptor antibodies; Cat.=catalogue; Con-A=Concanavalin A; Exp.=expiration; LLOQ=lower limit of quantitation; MG=myasthenia gravis; NA=not applicable; No.=number; RIA=radioimmunoassay; ULOQ=upper limit of quantitation

表 2.7.1-18　　血清中ブロッキング抗 AChR 抗体濃度分析法の概要

Bioanalytical method validation report name	**Implementation and Partial Validation of a Method for the Determination of AChR-Ab Blocking in Human Serum by RIA** (Module 5.3.1.4, CP165306)		
Method description	Concentration of blocking AChR-Ab in serum was determined with ^{125}I-alpha-bungarotoxin labelled AChR which was added to the serum samples. Blocking antibodies compete with ^{125}I-alpha-bungarotoxin and percentage inhibition was determined.		
Upper limit of the normal range	29.0%		
Material used for QCs and concentration	QC material was included in the [redacted] kits.		
MRD	No dilution required		
Source and lot of reagents	[redacted], Cat. No.: [redacted], Batch/Lot No.: [redacted], Exp. date: [redacted] Efgartigimod, Batch/Lot No.: [redacted], Exp. date: [redacted]		
Validation parameters	**Method Validation Summary**		**Source Location**
Upper limit determination	The upper limit of 29.0% was determined by testing the %inhibition of 100 individual serum batches of healthy subjects.		CP165306, Table 17.6
Performance of QCs during accuracy and precision runs	**Intra assay precision %CV**	High PC [redacted] % Low PC [redacted] %	CP165306, Table 17.7 and Table 17.8
	Inter assay precision %CV	High PC [redacted] % Low PC [redacted] %	CP165306, Table 17.7 and Table 17.8
Drug interference	[redacted] lots of MG serum with different %inhibition values did not demonstrate drug interference up to [redacted] mg/mL efgartigimod.		CP165306, Table 17.9
Hemolysis effect	*NA*		*NA*
Lipemic effect	*NA*		*NA*
Dilution linearity	*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 ARGX-113-1602 (Module 5.3.1.4, CP165308)			
Assay passing rate	[redacted] of the [redacted] analytical runs passed ([redacted]% passing rate).		CP165308, Section 6

AChR=acetylcholine receptor; AChR-Ab=anti-acetylcholine receptor antibodies; ARAb=acetylcholine receptor antibodies; CV=coefficient of variation; Exp.=expiration; MG=myasthenia gravis; MRD=minimum required dilution; NA=not applicable; No.=number; PC=positive control; QC=quality control; RIA=radioimmunoassay

表 2.7.1-19　血清中抗 MuSK 抗体濃度分析法の遷移

	Method Validation #1	Clinical Study ARGX-113-1704
Analyte	Anti-MuSK antibodies	Anti-MuSK antibodies
eCTD reference number	CP185285	CP185288
Method ID	[redacted] Version [redacted]	[redacted] Version [redacted]
Duration of time method is in use	[redacted]-20[redacted] to Present	[redacted]-20[redacted] to [redacted]-20[redacted]
Bioanalytical site	[redacted], France	
Matrix	Serum	
Platform	Enzyme-linked immunosorbent assay (ELISA)	
Format	Anti-MuSK antibodies bind to the antigen pre-coated wells and were detected via an alkaline phosphatase coupled detection antibody on an ELISA platform.	
Kit reference, lot number, expiration date	[redacted], Cat. No.: [redacted], Batch/Lot No.: [redacted], Exp. date: [redacted]-20[redacted]	[redacted], Cat. No.: [redacted], Batch/Lot No.: [redacted], Exp. date: [redacted]-20[redacted] Batch/Lot No.: [redacted], Exp. date: [redacted]-20[redacted] Batch/Lot No.: [redacted], Exp. date: [redacted]-20[redacted]
Calibration range from the LCS to the HCS	0.400 to 12.0 U/mL	0.400 to 12.0 U/mL
Matrix study population	Healthy, MG	MG
Link to reports and applicable amendments	Module 5.3.1.4, CP185285	Module 5.3.1.4, CP185288
Synopsis of amendment history	*NA*	Qualification new kit Lot No. [redacted] and [redacted].

Ab=antibody; Cat.=catalogue; ELISA=enzyme-linked immunosorbent assay; Exp.=expiration; HCS=high calibration standard; LCS=low calibration standard; MG=myasthenia gravis; MuSK=muscle-specific receptor tyrosine kinase; NA=not applicable; No.=number

表 2.7.1-20　血清中抗 MuSK 抗体濃度分析法の概要

Bioanalytical method validation report name	**Method Implementation and Partial Validation for the Determination of anti-MuSK Antibodies in Human Serum** (Module 5.3.1.4, CP185285)		
Method description	Anti-MuSK antibodies bind to the antigen pre-coated wells and were detected via an alkaline phosphatase coupled detection antibody on an ELISA platform.		
Materials used for standard calibration curve	Calibration curve material was included in the [redacted] kits.		
Validated assay range	99.4 and 1110 U/mL in 100% matrix		
Material used for QCs	QC material was included in the MuSK-Ab ELISA kits.		
MRD	1/100		
Source and lot of reagents	[redacted], Cat. No.: [redacted]1, Batch/Lot No.: [redacted], Exp. date: [redacted] Efgartigimod, Batch/Lot No.: [redacted] ([redacted]), Exp. date: [redacted]		
Regression model and weighting	4 parameter logistical model with weighting factor $1/Y^2$.		
Validation parameters	**Method Validation Summary**		**Source Location**
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LCS to HCS	[redacted] calibrators	CP185285, Table 16.8
	Inter assay accuracy (%bias) from LCS to HCS	[redacted]% to [redacted]%	CP185285, Table 16.8
	Inter assay precision (%CV) from LCS to HCS	[redacted]%	CP185285, Table 16.8
Performance of QCs during accuracy and precision runs	**Intra assay accuracy (%bias) in 5 QCs**	LLOQ [redacted]% to [redacted]% Low [redacted]% to [redacted]% Mid [redacted]% to [redacted]% High [redacted]% to [redacted]% ULOQ [redacted]% to [redacted]%	CP185285, Table 16.9
	Inter batch precision %CV	LLOQ [redacted]% Low [redacted]% Mid [redacted]% High [redacted]% ULOQ [redacted]%	CP185285, Table 16.9
	Total error (TE)	LLOQ [redacted]% Low [redacted]% Mid [redacted]% High [redacted]% ULOQ [redacted]%	CP185285, Table 16.9
Selectivity & matrix effect	During the implementation phase selectivity was investigated. [redacted] lots of sera from healthy subjects were analyzed spiked and unspiked at LLOQ level ([redacted] U/mL). Observed bias in spiked concentration was [redacted]%.		CP185285, Table 16.6
Drug interference	Human serum pool was spiked with anti-MuSK antibodies at the Low QC level ([redacted] U/mL) and increasing concentrations of efgartigimod up to [redacted] ng/mL was added. It was concluded that efgartigimod does not interfere in the anti-MuSK assay.		CP185285, Table 16.10
Hemolysis effect	*NA*		*NA*
Lipemic effect	*NA*		*NA*

Dilution linearity	During the implementation phase dilution linearity was investigated. [redacted] lots of healthy subject sera were spiked at high QC level ([redacted] U/mL). Spiked samples were after MRD further diluted 1/2, 1/4 and 1/8. Observed bias [redacted]% to [redacted]%. [redacted] lots of MG patient sera were analyzed spiked at high QC level ([redacted]0 U/mL). Spiked samples were after MRD further diluted 1/2, 1/4, 1/8 and 1/16. Observed bias [redacted]% to [redacted]%.	CP185285, Table 16.4 and Table 16.5
Bench-top/process stability	*NA*	*NA*
Freeze-thaw stability	*NA*	*NA*
Long-term storage	*NA*	*NA*
Parallelism	Based on the results of the dilutional linearity, a MRD was defined (1/10).	CP185285, Section 11.4
Carry over	*NA*	*NA*
Method Performance in Study ARGX-113-1704 (Module 5.3.1.4, CP185288)		
Assay passing rate	[redacted] of the [redacted] analytical runs passed ([redacted]% passing rate).	CP185288, Section 6
Qualification run	Qualification new kit Lot No. [redacted] and [redacted] were qualified and accepted for further use.	CP185288, Table 21.149 to Table 21.152

Ab=antibody; Cat.=catalogue; CV=coefficient of variation; ELISA=enzyme-linked immunosorbent assay; Exp.=expiration; HCS=high calibration standard; LCS=low calibration standard; LLOQ=lower limit of quantitation; MG=myasthenia gravis; MRD=minimum required dilution; MuSK=muscle-specific receptor tyrosine kinase; NA=not applicable; No.=number; QC=quality control; TE=total error; ULOQ=upper limit of quantitation

表 2.7.1-21　エフガルチギモドに対する薬物抗体分析法の遷移

	Validation Method #1	Clinical Study ARGX-113-1501 (Part 1)	Clinical Study ARGX-113-1501 (Part 2)	Validation Method #2	Clinical Study ARGX-113-1602
Analyte	ADA against efgartigimod	ADA against efgartigimod	ADA against efgartigimod	ADA against efgartigimod	ADA against efgartigimod
eCTD reference number	CP155242	CP155247	CP155248	CP165083	CP165307
Method ID	[redacted] Version [redacted]	[redacted] Version [redacted]	[redacted] Version [redacted]	[redacted] Version [redacted] and [redacted]	[redacted] Version [redacted] and [redacted]
Duration of time method is in use	[redacted] to [redacted]	[redacted] to [redacted]	[redacted] to [redacted]	[redacted] to [redacted]	[redacted] to [redacted]
Bioanalytical site	[redacted], [redacted], France				
Matrix	Serum				
Platform	Enzyme-linked immunosorbent assay (ELISA)				
Format	Efgartigimod ADA is detected by applying a bridging format using efgartigimod as capture and biotinylated efgartigimod as detection tool.			Affinity captured and eluted anti-efgartigimod ADAs (ACE methodology) are detected by applying a bridging format using biotinylated efgartigimod as capture and digoxigenylated efgartigimod as detection tool.	
Stock reference, lot number, expiration date	**Efgartigimod:** Batch/Lot No.: [redacted], End of use date: [redacted] **Positive control:** 2D11hFc Batch/Lot No.: [redacted] End of use date: [redacted]	**Efgartigimod:** Batch/Lot No.: [redacted], End of use date: [redacted] **Positive control:** 2D11hFc Batch/Lot No.: [redacted] End of use date: [redacted]	**Efgartigimod:** Batch/Lot No.: [redacted], End of use date: [redacted] **Positive control:** 2D11hFc Batch/Lot No.: [redacted] End of use date: [redacted]	**Efgartigimod:** Batch/Lot No.: [redacted], Exp. date: [redacted] **Positive control:** 2D11hFc Batch/Lot No.: [redacted] Batch/Lot No.: [redacted]	**Efgartigimod:** Batch/Lot No.: [redacted], Exp. date: [redacted] **Positive control:** 2D11hFc Batch/Lot No.: [redacted] End of use date: [redacted]
Matrix study population	Healthy	Healthy	Healthy	Healthy, MG, ITP, PV	MG
Link to reports and applicable amendments	Module 5.3.1.4, CP155242	Module 5.3.1.4, CP155247	Module 5.3.1.4, CP155248	Module 5.3.1.4, CP165083	Module 5.3.1.4, CP165307
Synopsis of amendment history	*NA*	*NA*	*NA*	*NA*	*NA*

	Clinical Study ARGX-113-1702	Supplemental Validation Method #2	Clinical Study ARGX-113-1704
Analyte	ADA against efgartigimod	ADA against efgartigimod	ADA against efgartigimod
eCTD reference number	CP175324	CP185295	CP185211
Method ID	[redacted] Version [redacted] and [redacted]	[redacted] Version [redacted] and [redacted]	[redacted] Version [redacted] and [redacted]
Duration of time method is in use	[redacted]-20[redacted] to [redacted]-20[redacted]	[redacted]-Jan-20[redacted] to Present	[redacted]-20[redacted] to [redacted]-20[redacted]
Bioanalytical site	[redacted] France		
Matrix	Human serum		
Platform	Enzyme-linked immunosorbent assay (ELISA)		
Format	Affinity captured and eluted anti-efgartigimod ADAs are detected by applying a bridging format using biotinylated efgartigimod as capture and digoxigenylated efgartigimod as detection tool.		
Stock reference, lot number, expiration date	**Efgartigimod:** Batch/Lot No.: [redacted], Exp. date: [redacted]-20[redacted] Batch/Lot No.: [redacted] Exp. date: [redacted]-20[redacted] Batch/Lot No. [redacted] Exp. date: [redacted]-20[redacted] **Positive control:** 2D11hFc Batch/Lot No.: [redacted] End of use date: [redacted]-20[redacted]	**Efgartigimod:** Batch/Lot No.: [redacted], Exp. date: [redacted]-20[redacted] **Positive control:** 2D11hFc Batch/Lot No.: [redacted] Exp. date: [redacted]-20[redacted] Batch/Lot No.: [redacted] Exp. date: [redacted]-20[redacted]	**Efgartigimod:** Batch/Lot No.: [redacted] Exp. date: [redacted]-20[redacted] Batch/Lot No.: [redacted] Exp. date: [redacted]-20[redacted] **Positive control:** 2D11hFc Batch/Lot No.: [redacted] Exp. date: [redacted]-20[redacted]
Matrix study population	Healthy	Healthy, MG, ITP	MG
Link to reports and applicable amendments	Module 5.3.1.4, CP175324	Module 5.3.1.4, CP185295	Module 5.3.1.4, CP185211
Synopsis of amendment history	Qualification critical reagents batches: [redacted] and [redacted]	*NA*	Amendment No. 2: Adaptation of titer ranges

ACE= affinity capture elution; ADA=anti-drug antibody; ELISA=enzyme-linked immunosorbent assay; Exp.=expiration; Fc=crystallized fragment; ITP=idiopathic thrombocytopenic purpura; MG=myasthenia gravis; NA=not applicable; No.=number; PV=pemphigus vulgaris

表 2.7.1-22　エフガルチギモドに対する薬物抗体分析法の概要–Validation Method #1

Bioanalytical method validation report name	**Validation of an Immunogenicity Method for the Detection of Anti-Efgartigimod Antibodies in Human Serum by ELISA** (Module 5.3.1.4, CP155242)		
Method description	Bridging format using efgartigimod as capture tool and biotinylated efgartigimod as detection tool using an ELISA platform		
Material used for quality controls (QCs)	2D11hFc (chimeric molecule consisting of efgartigimod-specific binding variable domain of a llama heavy chain-only antibody clone 2D11 and a human crystallized fragment (Fc)) Batch/Lot No.: [redacted] Conc. [redacted] mg/mL		
Source and lot of reagents	Biotinylated efgartigimod, Batch/Lot [redacted] Efgartigimod, Batch/Lot No.: [redacted]		
MRD	1/10		
Validation parameters	**Method Validation Summary**		**Source Location**
Screening cut-point (SCP) factor determination	A multiplicative cut-point factor of [redacted] was determined with sera from healthy subjects, assuring a 5% false positive error rate.		CP155242, Table 15.3
Confirmatory cut-point (CCP) determination	Confirmatory cut-point of [redacted]% inhibition was determined with sera from healthy subjects, assuring a 1% false positive error rate. The drug concentration in the confirmatory tier is [redacted] µg/mL efgartigimod.		CP155242, Table 15.5 and Table 15.6
Performance of QCs during precision runs	**Intra batch %CV (OD values)**	HSP ([redacted] µg/mL): [redacted]% LSP ([redacted]0 µg/mL): [redacted]% NC: [redacted]%	CP155242, Table 15.9
	Inter batch %CV (OD values)	HSP ([redacted] µg/mL): [redacted]% LSP ([redacted] µg/mL): [redacted]% NC: [redacted]%	CP155242, Table 15.9
	Inter analyst precision	[redacted]%	CP155242, Table 15.10
Sensitivity	[redacted] µg/mL ADA		CP155242, Table 15.8
Drug Tolerance	[redacted] ng/mL ADA is detectable in presence of [redacted] µg/mL efgartigimod. [redacted] ng/mL ADA is detectable in presence of [redacted] µg/mL efgartigimod.		CP155242, Table 15.7
Expiry date extension	Biotinylated efgartigimod at [redacted]°C±[redacted]°C expiry date was extended for approximately [redacted] months. ADA positive control (2D11hFc) expiry date was extended for approximately [redacted] months.		CP155242, Table 15.12 and Table 15.11
Method Performance in Study ARGX-113-1501 (Part 1) (Module 5.3.1.4, CP155247)			
Assay passing rate	[redacted] of the [redacted] analytical runs passed ([redacted]% passing rate).		CP155247, Table 20.1
Method Performance in Study ARGX-113-1501 (Part 2) (Module 5.3.1.4, CP155248)			
Assay passing rate	[redacted] of the [redacted] analytical runs passed ([redacted]% passing rate).		CP155248, Table 19.1

ADA=anti-drug antibody; CCP=confirmatory cut-point; CV=coefficient of variation; ELISA=enzyme-linked immunosorbent assay; Fc=crystallized fragment; HSP=high screen positive (ie, high positive control); LSP=low screen positive (ie, low positive control); MRD=minimum required dilution; NC=negative control; No.=number; OD=optical density; QC=quality control; SCP=screening cut-point

表 2.7.1-23　エフガルチギモドに対する薬物抗体分析法の概要–Validation Method #2

Bioanalytical method validation report name	**Validation of an Immunogenicity Method with Improved Drug Tolerance for the Detection of Anti-Efgartigimod Antibodies in Human Serum by ELISA** (Module 5.3.1.4, CP165083)	
Method description	Anti-drug antibodies against efgartigimod will be detected with an ACE bridging ELISA.	
Material used QCs	2D11hFc (chimeric molecule consisting of efgartigimod-specific binding variable domain of a llama heavy chain-only antibody clone 2D11 and a human crystallized fragment (Fc)) Batch/Lot No.: [redacted] Conc. [redacted] mg/mL Batch/Lot No.: [redacted] Conc. [redacted] mg/mL	
Source and lot of critical reagents	Biotinylated efgartigimod, Batch [redacted] Digoxigenylated efgartigimod, Batch [redacted] Efgartigimod, Batch/Lot [redacted]	
MRD	Samples are tested undiluted (MRD=1).	
Validation parameters	**Method Validation Summary**	**Source Location**
Screening cut-point factor (CPF) determination	A multiplicative screening cut-point factor of [redacted] was determined with sera from 60 healthy subjects, assuring a 5% false positive error rate.	CP165083, Table 15.2
Cut-point factor in diseased population	To verify whether the CPF determined with sera from healthy subjects could be applied on disease population (MG, ITP and PV), [redacted] sera from MG, ITP and PV patients were tested in the screening assay. For MG and PV the multiplicative CPF of [redacted] can be applied, for ITP a multiplicative CPF of [redacted] was determined. In study ARGX-113-1704, the representativeness of the validation CP (CPF [redacted]) for the MG study population was verified. The validation CPF of [redacted] was found to be suitable for use in the MG study population.	CP165083, Table 15.10 to Table 15.12 CP185211, Table 23.23
Confirmatory cut-point (CCP) determination	Confirmatory cut-point of [redacted] % inhibition was determined with 60 sera from healthy subjects, assuring a 1% false positive error rate. The drug concentration in the confirmatory tier is [redacted] µg/mL efgartigimod.	CP165083, Table 15.4 and Table 15.5
Titer range	Titer range: 3.73≤ titer ≤14.9 CV(%): [redacted] %	CP165083, Table 15.3
Confirmation of sensitivity (selectivity testing)	Sensitivity of [redacted] ng/mL PC was demonstrated in the ADA screening and confirmatory assay using [redacted] (for the ADA screening assay) and [redacted] (for the ADA confirmatory assay) individual sera from healthy subjects.	CP165083, Table 15.7 to Table 15.9
Drug Tolerance	[redacted] ng/mL ADA is detectable in presence of [redacted] µg/mL efgartigimod. [redacted] ng/mL ADA is detectable in presence of [redacted] µg/mL efgartigimod.	CP165083, Table 15.6
Precision of QCs (screening assay)	**Intra batch %CV (OD values)**: HSP ([redacted] ng/mL): [redacted] % LSP ([redacted] ng/mL): [redacted] % NC: [redacted] %	CP165083, Table 15.13
	Inter batch %CV (OD values): HSP ([redacted] ng/mL): [redacted] % LSP ([redacted] ng/mL): [redacted] % NC: [redacted] %	CP165083, Table 15.13
	Inter analyst precision: [redacted] %	CP165083, Table 15.16
Qualification of new positive control antibody Batch/Lot [redacted]	All criteria were met and Batch/Lot No. [redacted] was successfully qualified.	CP165083, Table 15.17
Stability	Stability of biotinylated and digoxigenylated efgartigimod was shown for	CP165083,

	[redacted] days when stored at [redacted]°C±[redacted]°C.	Table 15.18
Method Performance in Study ARGX-113-1602 (Module 5.3.1.4, CP165307)		
Assay passing rate	[redacted] of the [redacted] analytical runs passed ([redacted]% passing rate).	CP165307, Table 19.1
Method Performance in Study ARGX-113-1702 (Module 5.3.1.4, CP175324)		
Assay passing rate	[redacted] of the [redacted] analytical runs passed ([redacted]% passing rate).	CP175324, Table 21.1

ACE=affinity capture elution; ADA=anti-drug antibody; CCP=confirmatory cut-point; CPF=cut-point factor; CV=coefficient of variation; ELISA=enzyme-linked immunosorbent assay; Fc=crystallized fragment; HSP=high screen positive (ie, high positive control); ITP=idiopathic thrombocytopenic purpura; LSP=low screen positive (ie, low positive control); MG=myasthenia gravis; MRD=minimum required dilution; NC=negative control; No.=number; OD=optical density; PC=positive control; PV=pemphigus vulgaris; QC=quality control

表 2.7.1-24　エフガルチギモドに対する薬物抗体分析法の概要–Validation Method #2 に対する補完的バリデーション

Bioanalytical method validation report name	**Complement of Validation of an Immunogenicity Method with Improved Drug Tolerance for the Detection of Anti-Efgartigimod Antibodies in Human Serum by ELISA to Fully Comply with FDA Guidance 2016** (Module 5.3.1.4, CP185295)	
Method description	Anti-drug antibodies against efgartigimod will be detected with an ACE bridging ELISA.	
Material used for QCs	2D11hFc (chimeric molecule consisting of efgartigimod-specific binding variable domain of a llama heavy chain-only antibody clone 2D11 and a human crystallized fragment (Fc)) Batch/Lot No.: [redacted]; Conc. [redacted] mg/mL, Exp. date: [redacted] (used in one bridging run) Batch/Lot No.: [redacted]; Conc. [redacted] mg/mL, Exp. date: [redacted] (used throughout the method validation study)	
Source and lot of reagents	Biotinylated efgartigimod, Batch/Lot: [redacted] Digoxigenylated efgartigimod, Batch/Lot: [redacted]	
MRD	Samples are tested undiluted (MRD=1).	
Validation parameters	**Method Validation Summary**	**Source Location**
	The aim of the analytical study was to perform a complement of validation to comply with Food and Drug Administration (FDA) guidance (draft version, 2016, which was issued as a Final Guidance Document in January 2019) and to qualify a new batch of positive control antibody. Screening cut-point factor and confirmatory cut-point were determined in study CP165083.	
Sensitivity	Average relative assay sensitivity was calculated at [redacted] ng/mL at 95% CI.	CP185295, Table 15.3
Confirmation of sensitivity (selectivity in healthy subjects, MG patients and ITP patients)	In healthy subjects: [redacted] individuals scored positive at LSP level of [redacted] ng/mL. This LSP level is increased to [redacted] ng/mL during method validation since this level did not consistently score positive leading to run failures. In ITP patients: [redacted] individuals scored positive at LSP level of [redacted] ng/mL. In MG patients: [redacted] individuals scored positive at LSP level of [redacted] ng/mL. • [redacted] individuals from study ARGX-113-1704 (who consented to future research) scored positive at LSP level of [redacted] ng/mL.	CP185295, Table 15.5, Section 11.4 CP185295, Table 15.8 and Table 15.9 CP185211, Table 23.23
Titer range	Titer range: 1.67≤ titer ≤6.68 CV(%): [redacted]% A new titer range was determined in study CP185211 since new biotinylated and digoxigenylated drug batches were used in the study as compared to the method validation. Titer range: 1.00 ≤ titer ≤ 3.68.	CP185295, Table 15.2 CP185211, Amendment 2, Table 23

Precision of QCs (screening assay, titration assay)	**Intra batch %CV (OD values)**	HSP (ng/mL): % MSP (ng/mL): % LSP (ng/mL): % LSP (ng/mL): % NC: % Titer: %	CP185295, Table 15.6, Table 15.7 and Table 15.28
	Inter batch %CV (OD values)	HSP (ng/mL): % MSP (ng/mL): % LSP (ng/mL): % LSP (ng/mL): % NC: % Titer: %	CP185295, Table 15.6, Table 15.7 and Table 15.29
Precision of QCs (confirmatory assay)	**Intra batch %CV (OD values)**	IDC (ng/mL): % IDC (ng/mL): %	CP185295, Table 15.6, Table 15.7 and Table 15.28
	Inter batch %CV (OD values)	IDC (ng/mL): % IDC (ng/mL): %	CP185295, Table 15.6, Table 15.7 and Table 15.29
Qualification of new batch of biotinylated ARGX-113	Qualification of Batch/Lot No. Drug Tolerance at µg/mL of PC2: µg/mL (screening assay) and µg/mL (confirmatory assay). Drug Tolerance at µg/mL of PC2: µg/mL screening assay) and µg/mL (confirmatory assay).		CP185295, Table 15.10 to Table 15.13
Qualification of new batch of digoxigenylated ARGX-113	Qualification of Batch/Lot No. Drug Tolerance at µg/mL of PC2: µg/mL (screening assay) and µg/mL (confirmatory assay). Drug Tolerance at µg/mL of PC2: µg/mL (screening assay) and µg/mL (confirmatory assay).		CP185295, Table 15.14 to Table 15.17
Qualification of pooled batch of biotinylated ARGX-113	Qualification of Batch/Lot No. Drug Tolerance at µg/mL of PC2: µg/mL (screening assay) and µg/mL (confirmatory assay). Drug Tolerance at µg/mL of PC2: µg/mL (screening assay) and µg/mL (confirmatory assay).		CP185295, Table 15.18 to Table 15.21
Qualification of human serum pool	All acceptance criteria were met.		CP185295, Table 15.23 and Table 15.24
Hook effect	Hook effect was evaluated by testing human serum with spiked with positive control at concentration levels (ng/mL to ng/mL). No hook effect was observed up to ng/mL positive control.		CP185295, Table 15.22
Hemolysis effect	LSP (ng/mL) scores positive in / (%) hemolytic samples.		CP185295, Table 15.27
Lipemic effect	LSP (ng/mL) scores positive in / (%) lipemic samples.		CP185295, Table 15.26
Bench-top/process stability	Bench-top and refrigerator (°C± °C) stability was shown up to hours with samples initially stored at (°C± °C).		CP185295, Table 15.32 and Table 15.33

Freeze-thaw stability	Freeze-thaw stability was shown up to [redacted] freeze-thaw cycles with samples initially stored at [redacted] °C± [redacted] °C.	CP185295, Table 15.31
Long-term storage	Stability of PC samples stored at [redacted] °C± [redacted] °C was shown for [redacted] days.	CP185295, Table 15.34 to Table 15.38
Method Performance in Study ARGX-113-1704 (Module 5.3.1.4, CP185211)		
Assay passing rate	[redacted] of the [redacted] analytical runs passed ([redacted]% passing rate).	CP185211, Section 6

ACE=affinity capture elution; ARGX-113=efgartigimod; CI=confidence interval; CV=coefficient of variation; ELISA=enzyme-linked immunosorbent assay; Exp.=expiration; FDA=Food and Drug Administration; HSP=high screen positive; IDC=immuno depletion control; ITP=idiopathic thrombocytopenic purpura; LSP=low screen positive; MG=myasthenia gravis; MRD=minimum required dilution; MSP=mid screen positive (ie, mid positive control); NC=negative control; OD=optical density; PC=positive control; QC=quality controlt

表 2.7.1-25　エフガルチギモドに対する中和抗体分析法の遷移

	Method Validation #1	Clinical Study ARGX-113-1704
Analyte	Neutralizing anti-efgartigimod antibodies	Neutralizing anti-efgartigimod antibodies
eCTD reference number	CP185186	CP185211
Method ID	[redacted] Version [redacted]	[redacted] Version [redacted]
Duration of time method is in use	[redacted]-20[redacted] to Present	[redacted]-20[redacted] to [redacted]-20[redacted]
Bioanalytical site	[redacted], France	
Matrix	Serum	
Platform	Electrochemiluminescence immunoassay (ECLIA)	
Format	The NAb assay against efgartigimod is an indirect competition assay where anti-drug antibodies are extracted using the BEAD procedure.	
Stock reference, lot number, expiration date	**Positive control:** Anti-ARGX-113 polyclonal antibody purified from immunized rabbit, Batch/Lot No.: [redacted] Exp. date: [redacted]-20[redacted]	**Positive control:** Anti-ARGX-113 polyclonal antibody purified from immunized rabbit, Batch/Lot No.: [redacted] Exp. date: [redacted]-20[redacted]
Matrix study population	Healthy, MG	MG
Link to reports and applicable amendments	Module 5.3.1.4, CP185186	Module 5.3.1.4, CP185211
Synopsis of amendment history	*NA*	*NA*

ARGX-113=efgartigimod; BEAD=biotin-drug extraction and acid dissociation; ECLIA=electrochemiluminescence immunoassay; Exp.=expiration; MG=myasthenia gravis; NA=not applicable; NAb=neutralizing antibody; No.=number

表 2.7.1-26　エフガルチギモドに対する中和抗体分析法の概要

Bioanalytical method validation report name	**Validation of a Method for the Detection of a Neutralizing Anti-Efgartigimod Antibodies in Human Serum** (Module 5.3.1.4, CP185186)	
Method description	The NAb assay is an indirect ECLIA competition assay where anti-drug antibodies are extracted using the BEAD procedure.	
Material used QCs	Anti-ARGX-113 polyclonal antibody purified from immunized rabbit, Batch/Lot No.: [redacted]	
Source and lot of reagents	Efgartigimod, Batch/Lot No.: [redacted], argenx Biotinylated ARGX-113, Batch/Lot No.: [redacted] Biotinylated human FcRn, Batch/Lot No.: [redacted], [redacted] [redacted]-IgG3-SulfoTag, Batch/Lot No.: [redacted], argenx	
MRD	Samples are tested undiluted (MRD=1).	
Validation parameters	**Method Validation Summary**	**Source Location**
Determination of the cut-point factor (CPF)	A multiplicative CPF of [redacted] was determined with 50 sera from healthy subjects assuring a 1% false positive error rate.	CP185186, Table 16.2
Hook effect	Hook effect was evaluated by testing human serum with positive control concentrations at [redacted] concentration levels ([redacted] µg/mL to [redacted] µg/mL). No hook effect was observed up to [redacted] µg/mL positive control.	CP185186, Table 16.3
Assay sensitivity	Relative assay sensitivity was calculated be[redacted] µg/mL PC at 95% CI. The low positive control sample (LSP) is set at a concentration of [redacted] µg/mL.	CP185186, Table 16.4 and Table 16.5
Sensitivity confirmation (Selectivity testing)	Healthy subjects: [redacted] ([redacted]%) individuals scored positive at LSP level of [redacted] µg/mL. MG patients: [redacted] ([redacted]%) individuals scored positive at LSP level of [redacted] µg/mL. Ten baseline samples from MG patients from study ARGX-113-1704 who consented to future research were used for this assessment.	CP185186, Table 16.5 CP185211, Table 23.31
Drug Tolerance	[redacted] µg/mL PC is detectable in presence of [redacted] µg/mL efgartigimod. [redacted] µg/mL PC is detectable in presence [redacted] µg/mL efgartigimod.	CP185186, Table 16.7 and Table 16.8
Precision of QCs	**Intra batch %CV** (ECL signal) HSP ([redacted] µg/mL): [redacted]% MSP ([redacted] µg/mL): [redacted]% LSP ([redacted] µg/mL): [redacted]% NC: [redacted]%	CP185186, Table 16.9
	Inter batch %CV (normalized values (ECL signal/NC)) HSP ([redacted] µg/mL): [redacted]% MSP ([redacted] µg/mL): [redacted]% LSP ([redacted] µg/mL): [redacted]%	CP185186, Table 16.9
Hemolysis effect	LSP scores positive in [redacted] ([redacted]%) hemolytic samples.	CP185186, Table 16.6
Lipemic effect	LSP scores positive in [redacted] ([redacted]%) lipemic samples.	CP185186, Table 16.6
Bench-top/process stability	Bench-top and refrigerator [redacted]°C±[redacted]°C stability was shown up to [redacted] hours with samples initially stored at [redacted]°C±[redacted]°C.	CP185186, Table 16.13 and Table 16.14

Freeze-thaw stability	Freeze-thaw stability was shown up to [redacted] freeze-thaw cycles with samples initially stored at [redacted] °C± [redacted] °C.	CP185186, Table 16.12
Long-term storage	Stability of PC samples stored at [redacted] °C± [redacted] °C was shown for [redacted] days.	CP185186, Table 16.15
Robustness	The inter-analyst precision for each positive control level was [redacted] % on ECL signals and [redacted] % on normalized values.	CP185186, Table 16.10
Method Performance in Study ARGX-113-1704 (Module 5.3.1.4, CP185211)		
Assay passing rate	[redacted] of the [redacted] analytical runs passed ([redacted] % passing rate).	CP185211, Section 6

ARGX-113=efgartigimod; BEAD=biotin-drug extraction and acid dissociation; CI=confidence interval; CPF=cut-point factor; CV=coefficient of variation; ECL=electrogenerated chemiluminescence; ECLIA=electrochemiluminescence immunoassay; FcRn=neonatal Fc receptor; HSP=high screen positive; Ig=immunoglobulin; LSP=low screen positive; MG=myasthenia gravis; MRD=minimum required dilution; MSP=mid screen positive; NAb=neutralizing antibody; NC=negative control; No.=number; PC=positive control; QC=quality control

2.7.1.5.　参考文献

Xu W, Jiang H, Titsch C, et al. Development and characterization of a pre-treatment procedure to eliminate human monoclonal antibody therapeutic drug and matrix interference in cell-based functional neutralizing antibody assays. J Immunol Methods. 2015;416:94-104.