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略号および用語の定義一覧

略号・用語	省略していない表現または用語の定義
%CV	percent coefficient of variation：パーセント変動係数
ADA	anti-drug antibodies：抗薬物抗体
ANOVA	analysis of variance：分散分析
AUC_{inf}	area under the concentration-time curve from time 0 until time infinity：0時間から無限大時間までの血清中濃度 - 時間曲線下面積
CHO	Chinese Hamster Ovary：チャイニーズハムスター卵巣
CI	confidence interval：信頼区間
CL/F	apparent clearance：見かけの全身血清クリアランス
C_{max}	peak concentration：最高血清中濃度
CSR	Clinical Study Report：治験総括報告書
CTP	carboxy-terminal-peptide：C末端ペプチド
DC	Drug control：薬物コントロール
ECL	electrochemiluminescence：電気化学発光
ELISA	enzyme linked immunosorbent assay：酵素結合免疫吸着測定法
EOW	every other week：隔週
GH	growh hormone：成長ホルモン
GHD	growth hormone deficiency：成長ホルモン分泌不全性低身長症
hCG	human Chorionic gonadotropin：ヒト絨毛性ゴナドトロピン
hGH	human Growth hormone：ヒト成長ホルモン
hGHR	human Growth hormone receptor：ヒト成長ホルモン受容体
IGF-1	insulin-like growth factor-1：インスリン様成長因子-1
IGFBP-3	insulin-like growth factor-1 binding protein 3：IGF-1結合タンパク質3
ISR	incurred sample reanalysis
LLOQ	lower limit of quantitation：定量下限値
MOD-4023	Somatrogon
MRD	minimum required dilution：リガンド結合法での分析用生体試料の最小希釈倍率
MSD	Meso Scale Discovery：Meso Scale Discovery社
NAb	neutralizing antibodies：中和抗体
NC	negative control：陰性対照
NS	normalized signal：補正シグナル
OD	optical density：光学濃度
OLE	open label extension：非盲検延長試験
PC	positive control：陽性対照
PEN	個別患者用の使い捨てプレフィルドペン製剤
PD	pharmacodynamic：薬力学
PK	pharmacokinetics：薬物動態
QC	quality control：品質管理
r-hGH	recombinant Human growth hormone：遺伝子組換えヒト成長ホルモン
RLU	relative light unit：相対光単位

略号・用語	省略していない表現または用語の定義
SD	standard deviation：標準偏差
$t_{½}$	terminal phase half-life：終末相における消失半減期
T_{max}	time to first occurrence of peak concentration：C_{max}到達時間
ULOQ	upper limit of quantitation：定量上限値
VIAL	シリンジを使用するバイアル製剤
Vz/F	apparent volume of distribution, estimated from terminal phase, for extravascular dosing：見かけの分布容積（終末相から推定，血管外投与）

1. 背景および概観

Somatrogon（MOD-4023）は，hGH と類似の受容体結合特性と作用機序を持つ長時間作用型の遺伝子組換え hGH である。本項では，MOD-4023 の生物薬剤学試験ならびに臨床試験で使用した生体試料分析法を概説する。

MOD-4023 は，遺伝子組換え技術を用いて CHO 細胞から産生される糖タンパク質であり，hGH のアミノ酸配列の N 末端に hCG の β 鎖から CTP のアミノ酸配列を 1 コピー，同じく C 末端に 2 コピー（連続）を付加したものである。MOD-4023 は，グルコシル化およびこれら CTP 部位を付加することにより半減期を延長し，週 1 回投与での治療が可能となるよう設計された新規の遺伝子組換え hGH 製剤である。MOD-4023 が GH 受容体と結合すると，IGF-1 の合成および分泌を刺激するため，IGF-1 および IGFBP-3 は GH 治療法のバイオマーカーとなる。

これまでに，健康成人男性を対象として 3 試験（CP-4-001，CP-4-007，CP-4-011），成人 GHD 患者を対象として 2 試験（CP-4-003，CP-4-005），小児 GHD 患者を対象として 3 試験（CP-4-004，CP-4-006，CP-4-009）の 8 試験が終了している。このほか，いずれも小児 GHD 患者を対象とした OLE 3 試験（CP-4-004 OLE，CP-4-006 OLE，CP-4-009 OLE）が進行中である。試験情報を Table 1 に要約し，各試験で使用した MOD-4023 の製剤およびロット番号を Table 2 にまとめた。また，上述の臨床試験では VIAL と市販を意図した PEN を使用している。PEN は第 2 相小児 GHD 患者を対象とした試験（CP-4-004）の OLE および第 3 相小児 GHD 患者を対象とした試験（CP-4-006, CP-4-009）で，その他の臨床試験では VIAL が使用されているため，本項において PEN と VIAL の生物学的同等性試験を実施した結果を示す（CP-4-011）。

Table 1. Somatrogon Studies Summary

Study	Study Design	Treatment Group Doses (volume)	Number of Subjects with PK/PD Data*	Treatment Duration
Studies in Healthy Adults Subjects				
Phase 1 Studies (Males Only)				
CP-4-001	Randomized, double-blind, placebo-controlled, single dose, dose escalation trial	somatrogon 4 mg (0.2 mL) somatrogon 7 mg (0.35 mL) somatrogon 21 mg (1.05 mL) placebo	6 6 6 6	single dose
CP-4-007	Randomized, double-blind, vehicle-controlled, single dose trial in Japanese and White subjects	somatrogon 2.5 mg (0.125 mL) somatrogon 7.5 mg (0.375 mL) somatrogon 15 mg (0.75 mL) vehicle	10 (5 Japanese, 5 White)[a] 12 (6 Japanese, 6 White) 12 (6 Japanese, 6 White) 6	single dose
CP-4-011	Randomized cross-over trial with 2 different somatrogon drug product presentations	somatrogon 12 mg (0.24 mL) pen and vial (0.3 mL)[b]	48	single dose of each presentation
Studies in Adults with GHD				
Phase 2 Study				
CP-4-003	Randomized, open-label, placebo-controlled, multicenter, parallel-group, dose and frequency finding study	*Stage 1, Period 2*[c]: somatrogon (1.23 mg) (0.25 mL) weekly somatrogon (1.29 mg) (0.26 mL) weekly somatrogon (5.62 mg) (1.14 mL) weekly somatrogon (2.98 mg) (0.60 mL) EOW *Stage 2, Period 1*[c]: Starting dose: somatrogon 2.81 mg (0.56 mL) weekly, followed by individual dose titration	 13 13 13 6 54	Stage 1, Period 2: 4 weeks Stage 2, Period 1: 16 weeks
Phase 3 Study				
CP-4-005 and OLE	Randomized, double-blind, placebo controlled, multicenter, parallel-group, efficacy and safety study	somatrogon starting dose male: ≤50 years: 2.5 mg/week >50 years: 2 mg/week female not on oral estrogen (transdermal estrogen was acceptable) ≤50 years: 3 mg/week >50 years: 2.5 mg/week	133 (main)	Main: 52 wk

Table 1. Somatrogon Studies Summary

Study	Study Design	Treatment Group Doses (volume)	Number of Subjects with PK/PD Data*	Treatment Duration
		female on oral estrogens		
		≤50 years: 4 mg/week		
		>50 years: 3.5 mg/week		
Studies in Children with GHD				
Phase 2 Study				
CP-4-004 and OLE	Randomized, open-label, active-controlled, multicenter, parallel-group dose finding study	*Periods I and II*: somatrogon		Periods 1 and 2*:* 12 months in total
		0.25 mg/kg/week[d]	13	
		0.48 mg/kg/week[d]	15	
		0.66 mg/kg/week[d]	14	
		Genotropin® 0.034 mg/kg daily	11	
		Period III (OLE) somatrogon		Period 3: 12 months
		0.25 mg/kg/week	16	
		0.48 mg/kg/week	17	
		0.66 mg/kg/week	15	
		Period IV (long term OLE) 0.66 mg/kg/week	44	
		Period V (long term OLE on pen)	NA	
Phase 3 Study				
CP-4-006 and OLE	Randomized, open-label, active-controlled, multicenter, parallel-group, safety and efficacy study	somatrogon 0.66 mg/kg/week	109	12 months (main)
		Genotropin 0.034 mg/kg daily	115	
CP-4-009 and OLE	Randomized, open-label, active-controlled, multicenter, parallel-group, safety and efficacy study in Japanese pre-pubertal GHD patients	somatrogon 3 stepwise escalating doses (0.25 mg/kg/week, 0.48 mg/kg/week and 0.66 mg/kg/week)	22	12 months (main)
		Genotropin 0.025 mg/kg daily		

a. Two (2) subjects in the 2.5 mg cohort, 1 White and 1 Japanese, had pre-dose somatrogon serum concentrations that were >5% of the corresponding C_{max} and were, therefore, excluded from the descriptive statistics for PK and PD evaluations;
b. Somatrogon pen is a single-patient-use disposable prefilled pen and is the Test Product; vial administered with needle and syringe is the Reference Product;
c. For CP-4-003, Stage 1 Period 1 was for the optimization of r-hGH Dose for 3 – 9 Weeks, Stage 2 Period 2 was a 2-week washout period;
d. The range of weights for the children in this study was ~10 kg to 25 kg, with the mean in each dosing group close to 16 kg. Assuming weight of 16 kg, the dose would be 4 mg (0.25 mg/kg/wk) to 10.6 mg (0.66 mg/kg/wk), which would correspond to injection volumes of 0.1 to 0.2 mL (lowest dose), to 0.27 to 0.53 mL (highest dose).
*PK/PD data for somatrogon treated subjects only.
Source: M5.3 CSR CP-4-001, CSR CP-4-007, CSR CP-4-011, CSR CP-4-003, CSR CP-4-005 & OLE, CSR CP-4-004 & OLE, CSR CP-4-006 and CSR CP-4-009.

1.1. 製剤開発

1.1.1. 原薬

MOD-4023 は遺伝子組換え技術を用いて CHO 細胞から産生される糖タンパク質である。MOD-4023 は，hGH のアミノ酸配列の N 末端に hCG の β 鎖から CTP のアミノ酸配列を 1 コピー，同じく C 末端に 2 コピー（連続）を付加したものである。MOD-4023 はグリコシル化およびこれら CTP 部位を付加することにより半減期が延長し，週 1 回投与が可能である。MOD-4023 の構造の詳細は M2.3.S.1.2「構造」を参照のこと。

開発中における原薬の製造工程および施設の変更については，M2.3.S.2.6「製造工程の開発の経緯」を参照のこと。

1.2. 製剤

MOD-4023 の臨床試験では皮下投与のために VIAL および PEN を使用した。

第 2 相までの早期試験（CP-4-001，CP-4-003，CP-4-004 および CP-4-005）で使用した 2 mL 凍結 VIAL は，薬剤濃度が 5 mg/mL，10 mg/mL および 20 mg/mL の 3 種類であり 0.9 mL を投与した。小児 GHD 患者の治療で使用する際，さらに複雑な投与法が可能となるように CP-4-004 試験では，濃度 40 mg/mL の VIAL が供給された。VIAL 中の溶液は 10 mmol/L クエン酸ナトリウムおよび 147 mmol/L 塩化ナトリウムを含有した。VIAL は−20±5 °C で保存し，投与前に一晩冷蔵庫で解凍して使用した。

投与をさらに簡便にするため，保存薬物溶液をあらかじめ充填した PEN を開発した。CP-4-004 の OLE および小児 GHD 患者を対象とした第 3 相試験（CP-4-006）を開始するため，24 mg/1.2 mL（20 mg/mL）および 60 mg/1.2 mL（50 mg/mL）の薬物溶液をあらかじめ充填した，PEN を開発した。本試験で使用した PEN は市販を意図したものである。PEN 中の溶液はクエン酸ナトリウム，L-ヒスチジン，塩化ナトリウム，ポロクサマー188（界面活性剤）および m-クレゾール（保存剤）を含有し，2～8°C で保存する。

製剤の処方，製造工程および施設に関する開発中の変更については，M2.3.P.2.3「製造工程の開発経緯」を参照のこと。

Table 2 に示したように，CP-4-001，CP-4-003，CP-4-004（主試験および OLE），CP-4-005（主試験および OLE），CP-4-007 および CP-4-011 では VIAL を使用した。CP-4-006（主試験および OLE），CP-4-009（主試験および OLE）および CP-4-011 では PEN を使用した。CP-4-004 の OLE では，第 V 期に VIAL から PEN へ切り替えた。CP-4-011 ではこれら 2 種類の製剤処方および形態の生物学的同等性を評価した。

臨床試験で使用した MOD-4023 製剤を Table 2 に要約した。

Table 2. Somatrogon Investigational Products Used in Clinical Trials

Study	Investigational Presentation (Concentration)	Lot Number
Adult Healthy Volunteer Studies		
CP-4-001	vial (20 mg/mL)	4-09-X003B-S1
CP-4-007	vial (20 mg/mL)	1022412
CP-4-011	vial (40 mg/mL) pen (60 mg/1.2 mL)	Vial: 1046941 Pen: W96066
Adult Growth Hormone Deficiency Studies		
CP-4-003	vial (5 mg/mL)	4-09-X003B-S2
CP-4-005 & OLE	vial (5, 10, 20 mg/mL)	5 mg/mL:1015026, 1027898, 1028380, 1031958 10 mg/mL: 1014936, 1013202, 1028382-1, 1028815, 1035372, 1034574 20 mg/mL: 1019257, 1022412, 1030991, 1031956, 1040409
Pediatric Growth Hormone Deficiency Studies		
CP-4-004 & OLE	vial (20, 40 mg/mL)	20 mg/mL: 1019257, 1022412, 1030991, 1031956, 4-11-X003-S1 40 mg/mL: 1019394, 1028421, 1046941, 4-11-X003A-S1
CP-4-004 OLE	pen (60 mg/1.2 mL)	R40667, W03522, W96066
CP-4-006 & OLE	pen (24 mg/1.2mL) pen (60 mg/1.2mL)	24 mg pen: R40666 60 mg pen: R40667, W03522, X35718, W47488, W96066
CP-4-009 & OLE	pen (24 mg/1.2mL) pen (60 mg/1.2mL)	24 mg pen: R40666, X02787 60 mg pen: R40667, W96066, CJ7323

Note: Somatrogon pen is a single-patient-use disposable prefilled pen; doses from vials are administered with needle and syringe.
Source: M2.3.P.2.3

1.3. 生体試料分析法の概要

MOD-4023 濃度の測定ならびに ADA（抗 MOD-4023 抗体，抗 hGH 抗体）および NAb（抗 MOD-4023 中和抗体，抗 hGH 中和抗体）検出のための生体試料の分析は，フルバリデーション済みの分析法を使用し，規制ガイダンスに従って実施した[1,2,3,4,5,6,7,8,9,10,11,12,13]。

MOD-4023 濃度測定のための分析法を 1.3.2 項，免疫原性（ADA および NAb）評価のための分析法を 1.3.4 項，PD 評価のための分析法を 1.3.5 項にそれぞれ要約した。PK 解析および免疫原性評価に関連するすべてのバリデーション，臨床試験，生体試料分析プロジェクト番号／報告日および分析法 ID を Table 7 に示した。各分析法のライフサイクル情報を Table 8～Table 21 に示した。また，各分析法の性能概要を Table 22～Table 37 に示した。分析法の性能の詳細は，生体試料分析報告書（各臨床試験の総括報告書に添付）ならびにバリデーション報告書を参照のこと。バリデーション報告書（改訂および補遺を含む）は，M5.3.1.4 に掲載している。

1.3.1. PK，PD および免疫原性評価のための試料採取

PK，免疫原性および PD 解析に寄与した健康成人または成人・小児 GHD 患者を対象とした全試験の一覧を Table 1 に示した。PK，PD および免疫原性評価のための試料は，それぞれの試験の

実施計画書（M5.3 の CSR CP-4-001，CSR CP-4-007，CSR CP-4-011，CSR CP-4-003，CSR CP-4-005 & OLE，CSR CP-4-004 & OLE，CSR CP-4-006，および CSR CP-4-009）に示した方法に従い，採取，処理および保存した。

1.3.2. MOD-4023 の PK 評価のための生体試料分析法

MOD-4023 の臨床試験で使用した PK 試料分析法は，感度および頑健性の向上のため，臨床開発の過程で何度か更新した。最初の 2 つの PK 試料分析法（ELISA 法および ECL 法）は [redacted]（[redacted]，[redacted]，USA，旧 [redacted]，以下，[redacted]と表記）が開発およびフルバリデーションを行った。第 3 の PK 試料分析法（ECL 法）は [redacted]（[redacted]，[redacted]，UK，以下，[redacted]と表記）が開発およびバリデーションを行った。この分析法は [redacted]が開発およびバリデーションを行った第 2 の分析法と同じ方法で，同等性確認／クロスバリデーション（OX-19/512-015）の結果，これら 2 つの ECL 法（[redacted] IC-P-0954 および [redacted] BALAR 19/014）は同等であることを確認した。第 4 の PK 試料分析法は第 2 および第 3 の分析法と同じ ECL 法で，[redacted]（[redacted], USA，以下，[redacted]と表記）で開発およびバリデーションを実施した。

最初の試験（CP-4-001）では [redacted]がバリデートした ELISA 法を使用して PK 試料を分析した。その後のすべての試験では ECL 法を使用した。CP-4-003，CP-4-004，CP-4-005，CP-4-007，CP-4-009 および CP-4-011 試験の PK 試料は [redacted]がバリデートした ECL 法で，CP-4-006 試験の PK 試料は [redacted]がバリデートした ECL 法で分析した。また，CP-4-009 試験の主試験の PK 試料は [redacted]がバリデートした ECL 法でクロスバリデーションを実施した。その結果，1.3.2.4 に詳細を記載したとおり [redacted]での分析結果は棄却するのが妥当と考え，[redacted]の結果を採用した。

上記 4 種類の PK 試料分析法のライフサイクル情報を Table 8，Table 9，Table 10 および Table 11 に示した。各分析法の性能概要およびすべての PK バリデーション報告書，改訂および補遺を Table 22，Table 23，Table 24 および Table 25 に示した。本項記載のすべてのバリデーション報告書（改訂および補遺を含む）は，M5.3.1.4 に掲載した。以下の項では，MOD-4023 の臨床試験実施のために開発したこれら 4 種類すべての PK 試料分析法を詳述する。

1.3.2.1. ELISA 法による第 1 の PK 試料分析法

ヒト血清中 MOD-4023 を測定するため，定量的サンドイッチ ELISA 法を開発し，バリデートした。この方法では，標準試料，対照試料および被験試料を 10 倍希釈し，抗 hGH モノクローナル抗体を固相化したマイクロタイタープレート上でインキュベートする。インキュベート後，結合しなかった余分な材料を洗い流し，ビオチン標識したウサギ抗 CTP ポリクローナル抗体および引き続いてストレプトアビジン—西洋ワサビペルオキシダーゼを用いて MOD-4023 を検出し，テトラメチルベンチジンにより発色させる。発色停止後，波長 450 nm（副波長：650 nm）で色の濃度を測定する。4 パラメータロジスティック曲線フィッティングプログラムを用いて OD—濃度曲線をプロットすることにより得られた検量線上で MOD-4023 濃度を決定する。各分析単位に 10 種類のゼロ濃度でない検量線用標準試料を含めたが，LLOQ と ULOQ に該当する検量線用標準試料の設定はなかった。代わりに，試料分析のための LLOQ を低濃度 QC の下位 25%範囲（150 pg/mL，無希釈 100%ヒト血清中で 1500 pg/mL と等価）として定義し，ULOQ を

高濃度 QC の上位 20%範囲（12,000 pg/mL，無希釈 100%ヒト血清中で 120,000 pg/mL と等価）として定義した。MRD が 10 倍のとき，本分析法の定量範囲は 1500～120,000 pg/mL である。カーブフィッティングを容易にするため，定量範囲外の検量線用標準試料をアンカーポイントとして含めた。試料分析では，100%血清中濃度が低濃度（2000 pg/mL），中濃度（12,000 pg/mL），高濃度（100,000 pg/mL）の QC 試料を使用した。試薬や装置を含む本分析法の詳細は，AR2665 の添付資料（分析法 ID：IC-P-0590）に記載している。

この ELISA 法は精度，真度，選択性および希釈直線性に関してフルバリデーションし，さらに安定性試験および ISR を実施した。マトリックス中の MOD-4023 は−70°C で約 6 ヵ月安定であることを確認した（AR2665 補遺 1）。本分析法はこれまで，選択性の真度基準を一貫して満たすことがなかった。これは少量の MOD-4023 を個々の血清ロットに添加し，複数回の大きな希釈を行うことで，回収率が不正確になったことに起因していた。AR2665 では，MOD-4023 無添加の個別のヒト血清 20 ロット中 20 ロットが基準（LLOQ 未満）を満たした。低濃度（2000 pg/mL）および高濃度（60,000 pg/mL）の添加ロットは，それぞれ 10 試料中 8 試料および 10 試料中 9 試料で真度が±30%であった（AR2665）。選択性の真度が基準（±20%）を一貫して満たすことはなかったが，CP-4-001 試験で判断するために適切と考えられる 30%以内の許容可能な真度が認められた。さらに，CP-4-001 試験で使用した試料の約 8%（28/336）を対象に ISR を実施した。再分析したこれら 28 試料のうち，25 試料（89%）が事前に規定した基準を満たした（AR2665 補遺 2）。

PK試料分析のためのELISA法AR2665のライフサイクル情報をTable 8に，各分析法の性能概要およびすべてのバリデーション報告書，改訂および補遺をTable 22に示した。

1.3.2.2. ECL 法による第 2 の PK 試料分析法

第 2 の PK 試料分析法では，健康成人血清および小児（3～13 歳）GHD 患者の血清中で MOD-4023 濃度を測定するため，サンドイッチ ECL 法を適用した。この方法では，MOD-4023 を含有する検量線用標準試料，QC 試料および被験試料を 5 倍に希釈し，MSD ストレプトアビジンコート 96 穴プレートの表面上に固定したビオチン標識抗 hGH モノクローナル抗体とともにインキュベートする。インキュベート後，結合しなかった余分な材料を洗い流し，Sulfo-Tag 標識した抗 CTP ポリクローナル抗体により MOD-4023 を検出する。MOD-4023 結合複合体を MSD Read Buffer T（2X）を用いて検出し，MSD の装置を用いて読み取る。4 パラメータロジスティック曲線フィッティングを用いて RLU-濃度曲線をプロットすることにより得られた検量線上で MOD-4023 濃度を決定する。

当初の分析法（IC-P-0954，version -01）では各分析単位に 12 種類のゼロ濃度でない検量線用標準試料を含めたが，LLOQ および ULOQ に該当する検量線用標準試料はなかった。代わりに，試料分析のための LLOQ を低濃度 QC の下位 25%範囲（30.0 pg/mL，無希釈 100%ヒト血清中で 150 pg/mL と等価）として定義し，ULOQ を高濃度 QC の上位 20%範囲（7200 pg/mL，無希釈 100%ヒト血清中で 36,000 pg/mL と等価）として定義した。LLOQ または ULOQ に該当する検量線用標準試料は検量線に含めなかったものの，アンカーポイントを含めた十分な数（12 種）の検量線用標準試料を含めることで，定量範囲を適切に定義した。定量範囲は検量線の再現性ならびに QC 試料の分析内・分析間の精度および真度により確認した。MRD が 5 倍のとき，本分析法の定量範囲は 150～36,000 pg/mL である。カーブフィッティングを容易にするため，定量範囲外の検量線用標準試料をアンカーポイントとして含めた。試料分析では，100%血清中濃度が

低濃度（200 pg/mL），中濃度（2000 pg/mL），高濃度（30,000 pg/mL）の QC 試料を使用した。試薬や装置を含む本分析法の詳細は，バリデーション報告書 AR3835 の添付資料（分析法 ID：IC-P-0954）に記載している。

本 ECL 法は精度，真度，選択性，特異性および希釈直線性に関してフルバリデーションし，さらに安定性試験を実施した。保存温度−70°C および−20°C における 6 ヵ月間および 40 ヵ月間の長期安定性をバリデーション報告書に追加した（AR3835 補遺 2 および補遺 3）。

本分析法（IC-P-0954，version-02）は，検量線および QC 濃度を変更するために，パーシャルバリデーションを実施し，また，健康成人血清，小児 GHD 血清，高脂血症性および溶血性ヒト血清に対する選択性を追加で評価した。この更新した分析法は各分析単位に 11 種類の検量線用標準試料を含む。定量範囲は LLOQ および ULOQ の QC 試料に基づいて定義した。試料分析における LLOQ は 31.25 pg/mL，ULOQ は 8000 pg/mL と決定した。MRD が 5 倍のとき，本分析法の定量範囲は 100%無希釈血清中で 156.25～40,000 pg/mL である。カーブフィッティングを容易にするため，定量範囲外の検量線用標準試料をアンカーポイントとして含めた。試薬や装置を含む本分析法の詳細は，AR3835 補遺 4 の添付資料（分析法 ID：IC-P-0954-02）に記載している。また，新たに 3～13 歳の小児 GHD 患者 20 例の個別血清を対象に選択性を評価し，バリデーション報告書に追加した（AR3835 補遺 5）。

本分析法はさらに検量線および QC 濃度を変更するために更新し（IC-P-0954，version -06～-07）2 回目のパーシャルバリデーションを実施した。この更新した分析法は各分析単位に 10 種類の検量線用標準試料を含む。定量範囲は LLOQ および ULOQ の QC 試料に基づいて定義した。試料分析における LLOQ は 0.2 ng/mL，ULOQ は 25.6 ng/mL と決定した。MRD が 5 倍のとき，本分析法の定量範囲は 100%無希釈血清中で 1.00～128 ng/mL である。カーブフィッティングを容易にするため，定量範囲外の検量線用標準試料をアンカーポイントとして含めた。試薬や装置を含む本分析法の詳細は，AR3835 補遺 6 の添付資料（分析法 ID：IC-P-0954-05 および-06）に記載している。また，新たなビオチン標識抗 hGH コーティング抗体の適格性を確認するため，別のパーシャルバリデーションを実施した（AR3835 補遺 7）。

PK 試料分析のための ECL 法 AR3835 のライフサイクル情報を Table 9 に，各分析法の性能概要およびすべてのバリデーション報告書，改訂および補遺を Table 23 に示した。

1.3.2.3. ECL 法による第 3 の PK 試料分析法

第 3 の PK 試料分析法（BALAR 19/014）は [redacted]が開発しバリデートした第 2 の分析法（IC-P-0954，version -05）と同一の方法を [redacted]に移管したサンドイッチ ECL 法であり，健康成人血清および 3～13 歳の小児 GHD 血清中の MOD-4023 濃度を測定した。この方法では，MOD-4023 を含有する標準試料，対照試料および被験試料を 5 倍に希釈し，MSD ストレプトアビジンコート 96 穴プレートの表面上に固定したビオチン標識抗 hGH モノクローナル抗体とともにインキュベートする。以下 1.3.2.2 項に記載と同一の手順により，MOD-4023 濃度を決定する。定量範囲は MRD を 5 倍として，LLOQ および ULOQ の QC 試料に基づいて確立した。確立した定量範囲は 100%無希釈血清中で 156.3～40,000 pg/mL である。カーブフィッティングを容易にするため，定量範囲外の検量線用標準試料をアンカーポイントとして含めた。試薬や装置を含む本分析法の詳細は，バリデーション報告書 OX-19/512-016 の添付資料（BALAR 19/014 revision 2）に記載している。

[redacted]はこの ECL 法を導入後，精度，真度，選択性，特異性および希釈直線性に関してフルバリデーションを実施した。長期および短期安定性（凍結融解および卓上安定性）の評価はすでに[redacted]が行っていたため（AR3835 ならびに補遺 2 および補遺 3），[redacted]は行わなかった。また，[redacted]の ECL 法との同等性（クロスバリデーション）を評価し，[redacted]分析法確立サマリーレポート（OX-19/512-015）として報告した。CP-4-004 試験で採取した小児 GHD 試料 10 試料を[redacted]が試験し，9 試料について[redacted]との差が±30%以内であったことから，同等性が確認された。

PK 試料分析のための ECL 法 OX-19/512-016 のライフサイクル情報を Table 10 に，分析法の性能概要およびすべてのバリデーション報告書，改訂および補遺を Table 24 に示した。

1.3.2.4. ECL 法による第 4 の PK 試料分析法

第 4 の PK 試料分析法（C0319002）は[redacted]が開発しバリデートした第 2 の分析法（IC-P-0954）と同一の方法を[redacted]に移管したサンドイッチ ECL 法であり，3～13 歳の小児 GHD 患者から得られた血清中の MOD-4023 濃度を測定した。この方法では，MOD-4023 を含有する標準試料，対照試料および被験試料を 5 倍に希釈し，MSD ストレプトアビジンコート 96 穴プレートの表面上に固定したビオチン標識抗 hGH モノクローナル抗体とともにインキュベートする。以下 1.3.2.2 項に記載と同一の手順で，5 パラメータロジスティック曲線フィッティングを用いて RLU-濃度曲線をプロットすることにより得られた検量線により，MOD-4023 濃度を決定する。定量範囲は MRD を 5 倍として，LLOQ および ULOQ の QC 試料に基づいて確立した。確立した定量範囲は 100%無希釈血清中で 1.00～128 ng/mL である。カーブフィッティングを容易にするため，定量範囲外の検量線用標準試料をアンカーポイントとして含めた。試薬や装置を含む本分析法の詳細は，バリデーション報告書 C0319002 の添付資料（Appendix 3）に記載している。

[redacted]はこの ECL 法を導入後，精度，真度，感度，選択性，特異性，希釈直線性および短期安定性（凍結融解および卓上安定性）に関してフルバリデーションを実施した。長期安定性はすでに[redacted]が評価を行っていたため（AR3835 補遺 3），[redacted]では行わなかった。また，[redacted]の ECL 法との同等性を CP-4-009 試験の主試験全試料を用いたクロスバリデーションで評価した。[redacted]の結果と[redacted]の分析結果の乖離度はクロスバリデーションの許容基準を満たさなかった一方で，[redacted]の結果は[redacted]の ISR 評価用に再分析した結果とは同等であった。[redacted]における CP-4-009 試験の主試験試料の分析結果に疑義があると判断したため同結果を棄却し，[redacted]のクロスバリデーションの結果を分析結果として採用した。疑義の詳細および評価結果は付録：説明資料（令和[redacted]年[redacted]月[redacted]日医薬品医療機器総合機構へ提出）参照

PK 試料分析のための ECL 法 C0319002 のライフサイクル情報を Table 11 に，分析法の性能概要およびすべてのバリデーション報告書，改訂および補遺を Table 25 に示した。

1.3.3. hGH の PK 評価のための生体試料分析法

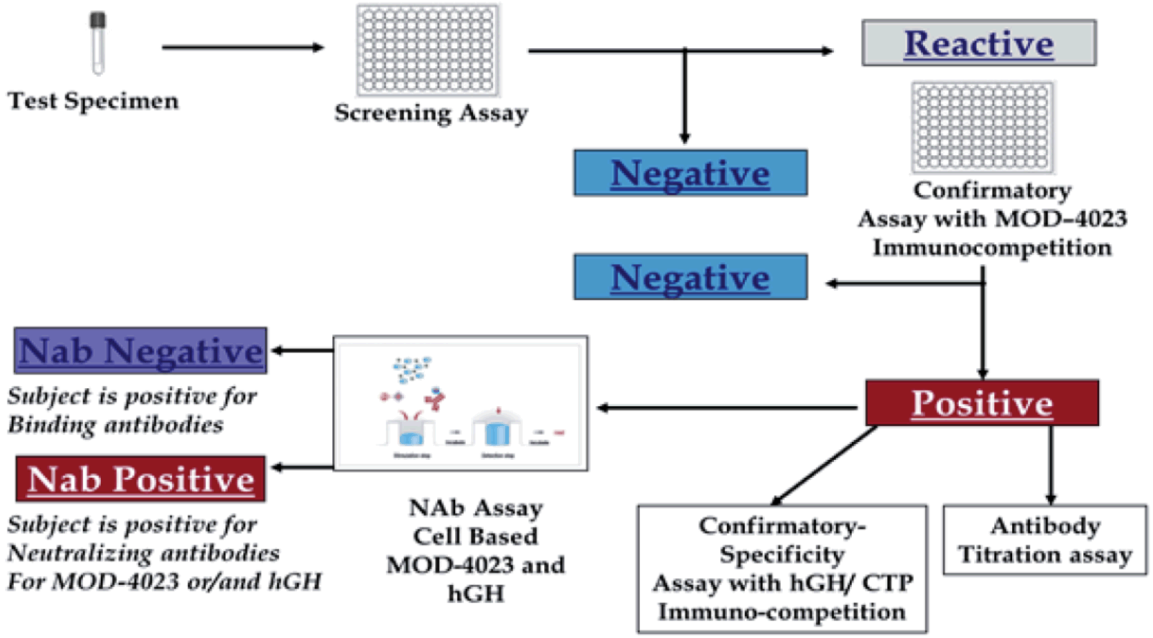
血清中 hGH 濃度は，hGH 濃度測定用分析キット IDS-iSYS（IS-3700）を用いて[redacted]（[redacted]，ドイツ）でバリデーションを実施し，CP-4-004 試験の主試験の試料分析に使用した。本分析法は化学発光を利用したサンドイッチイムノアッセイで，hGH 分子をビオチン化モノクローナル抗 GH 抗体によって捕捉し，ストレプトアビジン固定化磁性ビーズ

に結合させる。インキュベーションと洗浄の後，アクリジニウム標識抗 GH 抗体を加え，過酸化水素および水酸化ナトリウム添加により開始される化学発光シグナルを検出する。本分析法の真度，日内および日間再現精度，直線性および安定性（凍結融解，全血中）に関してバリデーションを実施した。定量範囲は 0.05 ng/mL～100 ng/mL，定量下限値は 0.05 ng/mL であった。本分析法のバリデーション報告書（IDS-iSYS IS-3700）は M5.3.1.4 に添付する。

1.3.4. 免疫原性（ADA および NAb）評価のための生体試料分析法

MOD-4023 および hGH に対する ADA および NAb の存在を検討するための試料を各臨床試験で採取した。MOD-4023 投与例から採取した試料について，段階的アプローチで試験を実施した。すなわち，最初に抗 MOD-4023 抗体についてスクリーニング検査を実施し，陽性の試料については，MOD-4023 を用いて確認試験を実施した。抗 MOD-4023 抗体陽性が確認された試料については，hGH および CTP を用いて特異性を評価後，抗体価を測定し，さらに MOD-4023 および hGH に対する NAb を評価した（Figure 1）。ジェノトロピン投与例から採取した試料についても段階的に試験を実施した。ただし，スクリーニング検査は抗 hGH 抗体について実施し，陽性であった場合には，次に hGH を用いて確認試験を実施した。抗 hGH 抗体が確認された試料は次に抗体価を測定し，さらに hGH に対する NAb のみを評価した。

Figure 1. Somatrogon Immunogenicity Testing Paradigm (All Study Periods)



1.3.4.1. 抗 MOD-4023 抗体分析法

MOD-4023 の臨床開発中，抗 MOD-4023 抗体の分析法として，2 種類の類似の ECL 法を（一方は健康成人マトリックス，他方は小児 GHD マトリックスを対象に）開発し，3 ヵ所の分析機関でバリデーションを行った。これらのブリッジング ECL 法では，被験試料，NC および PC を 10 倍に希釈後，ビオチン標識した MOD-4023 とルテニウム（Sulfo-Tag）標識した MOD-4023 の混合物に加える。この混合液をストレプトアビジンコートしたプレート中でインキュベートす

ると，ADA と試薬（標識 MOD-4023）の複合体が捕捉される。これを ECL 検出し，測定する。測定されたシグナルは PC または被験試料中の ADA 量に比例する。

MOD-4023 の開発中，これらの ADA 分析法［IC-P-0751（健康成人および成人 GHD 血清）および IC-P-1145（3～13 歳の小児 GHD 血清）］を更新し，精度，安定性，選択性，特異性，薬剤耐性，カットポイントおよび頑健性を向上させた。

抗 MOD-4023 抗体分析法 IC-P-0751 は，[redacted]がプロジェクト AR3165 としてバリデーションを行った［説明を明瞭にするため，バリデーション報告書 AR3165（改訂および補遺を含む）に記載されたすべての修正点および変更内容は，更新版の報告書であるバリデーション報告書 AR5374 に記載している］。その後，MOD-4023，hGH および CTP に対する特異性のカットポイントを，阻害率の対数変換値に基づき，偽陽性率 0.1%から 1%に変更した（AR5374 補遺 1）。CP-4-001 試験，CP-4-003 試験，CP-4-005 試験，CP-4-007 試験および CP-4-011 試験の臨床試料は，分析法 IC-P-0751 を用いて分析した（AR3165/AR5374）。

小児 GHD 血清を対象とした抗 MOD-4023 抗体分析法 IC-P-1145 は，[redacted]がプロジェクト AR4263 としてバリデーションを行った［説明を明瞭にするため，バリデーション報告書 AR4263（補遺 1 を含む）に記載されたすべての修正点および変更内容は，更新版の報告書であるバリデーション報告書 AR5303 に記載している］。その後，FDA の推奨を踏まえ，また偽陰性リスク低減のため，すべてのカットポイント基準値において偽陽性率を 0.1%から 1.0%に変更した（AR5303 改訂 1）。CP-4-004 試験の小児血清試料は分析法 IC-P-1145 を用いて分析した（AR4263/AR5303）。

この ECL 法はその後，[redacted]に移管し，バリデーションを行った（OX-18/512-011）。同社に移管したこの ECL 法（BALAR 18/031）は，移管前の ECL 法（IC-P-1145）と同一の方法である。同等性確認試験／クロスバリデーション（報告書 RIM-QUAL-000749）により，2 つの方法の同等性を確認した。分析法 BALAR 18/031 で陽性を確認した CP-4-006 試験の小児試料 55 試料を，[redacted]で分析した結果 100%一致で陽性が確認された。これらの試料はすべてスクリーニング試験と確認試験で陽性で，阻害率が確認試験のカットポイント（[redacted] 13.2%，[redacted] 17%）以上であった。本分析法は CP-4-006 試験に用いた。さらに，[redacted]の分析施設移管に伴い，本分析法を[redacted] UK から[redacted] USA（以下[redacted] US と表記）へ移管したため，[redacted] US にてパーシャルバリデーションを行い（SC-20/512-024），CP-4-009 試験に用いた。CP-4-006 試験の小児試料 30 試料を用いたクロスバリデーションの結果，一致率は 97%であった（SC-20/512-024）。

これらの抗MOD-4023抗体分析法のライフサイクル情報をTable 12（AR3165，AR5374，AR4263およびAR5303），Table 13（OX-18/512-011）およびTable 14（SC-20/512-024）に示した。各分析法の性能概要およびすべてのバリデーション報告書，改訂および補遺をTable 26（AR3165），Table 27（AR5374），Table 28（AR4263およびAR5303），Table 29（OX-18/512-011）およびTable 30（SC-20/512-024）に示した。本項に記載のすべてのバリデーション報告書はM5.3.1.4に掲載した。

1.3.4.2. 抗 hGH 抗体分析法

MOD-4023 の臨床開発中，抗 hGH 抗体の分析法として，1 種類の ECL 法を開発し，3 ヵ所の分析施設でバリデーションを行った。このブリッジング ECL 法では，被験試料，NC および PC を 10 倍に希釈後，ビオチン標識した hGH とルテニウム（Sulfo-Tag）標識した hGH の混合物に加える。この混合液をストレプトアビジンコートしたプレート中でインキュベートすると，ADA と試薬（標識 hGH）の複合体が捕捉される。これを ECL 検出し，測定する。測定されたシグナルは PC または被験試料中の抗 hGH 抗体量に比例する。

この抗 hGH 抗体分析法は，当初，3～13 歳の小児 GHD 血清を対象として，[redacted]が開発しフルバリデーションを行った（分析法 ID：IC-P-1243，報告書 ID：AR4575）。

偽陰性リスク低減のため，hGH の特異性に関する偽陽性率の値を 0.1%から 1.0%に変更した（AR4575 改訂 2）。CP-4-004 試験の小児血清試料は分析法 IC-P-1243 を用いて分析した。

この ECL 法はその後，[redacted]に移管し，バリデーションを行った（分析法 ID：BALAR 18/038，報告書 ID：OX-18/512-012）。[redacted]に移管した ECL 法は，[redacted]がバリデーションした方法と同一である。CP-4-006 試験の小児血清試料はこの分析法 BALAR 18/038 を用いて分析した。さらに，[redacted]の分析施設移管に伴い，本分析法を [redacted] UK から [redacted] US へ移管したため，[redacted] US にてパーシャルバリデーションを行い（SC-20/512-025），CP-4-009 試験の試料分析に用いた。CP-4-006 試験の小児試料 30 試料を用いたクロスバリデーションの結果，一致率は 90%であった（SC-20/512-025）。

この抗hGH抗体分析法のライフサイクル情報をTable 15（AR4575），Table 16（OX-18/512-012）およびTable 17（SC-20/512-025）に示した。各分析法の性能概要，およびバリデーション報告書，改訂および補遺をTable 31（AR4575），Table 32（OX-18/512-012）およびTable 33（SC-20/512-025）に示した。本項に記載のすべてのバリデーション報告書はM5.3.1.4に掲載した。

1.3.4.3. 抗 MOD-4023 中和抗体分析法

MOD-4023 の臨床開発中，抗 MOD-4023 中和抗体の分析法として 1 種類のセルベースアッセイ法を開発し，2 ヵ所の分析施設でバリデーションを行った。本分析法は，長時間作用型 hGH である MOD-4023 が細胞表面上の hGHR と結合すると，細胞増殖が誘導されることを利用している。MOD-4023 誘導性細胞増殖がどの程度阻害されるかを測定することにより，健康成人および小児 GHD 血清試料中で抗 MOD-4023 中和抗体の存在量を測定する。

マウスリンパ細胞株 BaFB2B2（安定な遺伝子導入により hGHR を高度に発現する BAF-3 細胞）をウシ胎児血清と（刺激性）hGH を添加した培養液中で複数回継代培養する。その後，分析日に細胞を回収し，0.5%血清培地中で数時間飢餓状態にし，希釈したヒト血清と固定濃度の MOD-4023 を含む 96 穴プレートに添加する。このプレートをインキュベートし，比色法により生存細胞数を求める。すなわち，MTS/PES CellTiter 96® AQueous One Solution をウェルに直接添加後，インキュベートし，96 穴プレートリーダーを用いて波長 490/650 nm で OD を測定し，求めた生存細胞数から細胞増殖の程度を推定する。なお，OD に関する分析間変動を相殺するため，NC で補正する。NS は，試料の平均 OD を同じプレートの NC の平均 OD で除した値とする。NS がカットポイント超の試料は NAb 陰性，カットポイント以下の試料は陽性と判定す

る。なお，この補正法は CP-4-004 試験，CP-4-005 試験および CP-4-006 試験で導入し，CP-4-003 試験および CP-4-007 試験では使用しなかった。

MOD-4023 の臨床開発中，この抗 MOD-4023 中和抗体分析法（分析法 ID：IC-P-0653）を更新し，精度，安定性，選択性，特異性，薬剤耐性，カットポイントおよび頑健性を向上させた。

抗 MOD-4023 中和抗体分析法 IC-P-0653 は，[redacted]がプロジェクト AR2862 として健康成人血清を対象にバリデーションを行った［説明を明瞭にするため，バリデーション報告書 AR2862（改訂および補遺を含む）に記載されたすべての修正点および変更内容は，更新版の報告書であるバリデーション報告書 AR5265 に記載している］。その後プロジェクト AR5265 として新たに健康成人血清 50 例を評価し，カットポイントを新たに確立した。さらに，精度，PC の各範囲（再設定），健康成人血清試料の選択性，溶血性および高脂血症性血清試料における選択性，薬剤耐性，72 時間の卓上安定性など，新たなパラメータについて試験し，AR5265 補遺 1 に報告したほか，投与前後の OD 差（%）を算出し，30.0%の陽性基準を確認した。薬剤耐性について追加で評価を行い，AR5265 補遺 2 に報告した。試料分析中に未治療小児 GHD 患者からの投与前試料を用いて CP-4-004 試験の試験内カットポイント評価を実施し，AR5265 補遺 3 に報告した。また，試料分析中に未治療成人 GHD 患者からの投与前試料を用いて CP-4-005 試験の試験内カットポイント評価を実施し，AR5265 補遺 4 に報告した。

小児 GHD 血清を対象とした抗 MOD-4023 中和抗体分析法 IC-P-0653-03 は，最初，パーシャルバリデーションを実施し（報告書 AR4268），その後，追加の分析精度検討，第 3 相 CP-4-006 試験の投与前小児 GHD 血清試料を用いた新たな試験内カットポイントの決定，ならびに対象疾患の溶血性，高脂血症性および黄疸性血清マトリックスにおける感度および選択性の決定を行い，パーシャルバリデーション報告書 AR4268 補遺 1 として報告した。

本分析法は，その後[redacted]に移管し，バリデーション（C0317007）を行い，CP-4-009 試験の試料分析に用いた。

抗MOD-4023中和抗体分析法のライフサイクル情報をTable 18（AR2862，AR5265およびAR4268）およびTable 19（C0317007）に，各分析法の性能概要およびバリデーション報告書，改訂および補遺をTable 34（AR2862，AR5265およびAR4268）およびTable 35（C0317007）に示した。本項に記載のすべてのバリデーション報告書はM5.3.1.4に掲載した。

1.3.4.4. 抗 hGH 中和抗体分析法

MOD-4023 の臨床開発中，抗 hGH 中和抗体の分析法として，2 つの類似のセルベースアッセイ法を（一方は健康成人マトリックス，他方は小児 GHD マトリックスを対象として）開発およびバリデーションした。この定性的中和抗体分析法は，hGH が細胞表面上の hGHR と結合すると細胞増殖が誘導されることを利用している。この分析法では，この hGH 誘導性細胞増殖がどの程度阻害されるかを測定することにより，健康成人および小児 GHD 血清試料中で抗 hGH 中和抗体の存在量を測定する。

この分析法では，マウスリンパ細胞株 BaFB2B2（安定な遺伝子導入により hGHR を高度に発現する BAF-3 細胞）をウシ胎児血清と（刺激性）hGH を添加した RPMI-1640 培養液中で複数回継代培養する［注：刺激性 hGH 濃度は当初，10 ng/mL であったが，最適化のためおよび抗 MOD-4023 中和抗体分析法の細胞維持手順と合わせるため，20[redacted]年のパーシャルバリデーション

（AR4266 改訂 1 補遺 3）後，100 ng/mL に変更した］。その後，分析日に細胞を回収し，0.5%血清培地中で数時間飢餓状態にした後，正常血清試料および成人 GHD 血清試料については 50 倍（2%）の MRD，小児 GHD 血清試料については 20 倍（5%）の MRD で希釈したヒト血清と固定濃度の hGH を含む 96 穴プレートに添加する。このプレートをインキュベートし，比色法により生存細胞数を求める。すなわち，MTS/PES CellTiter 96® AQueous One Solution をウェルに直接添加後，インキュベートし，96 穴プレートリーダーを用いて波長 490/650 nm で OD を測定することで，求めた生存細胞数から細胞増殖の程度を推定する。なお，OD に関する分析間変動を相殺するため，NC で補正する。NS は複製試料の平均 OD を同じプレートの NC の平均 OD で除した値とする。NS がカットポイント超の試料は NAb 陰性，カットポイント以下の試料は陽性と判定する。この補正法は CP-4-004 試験，CP-4-005 試験および CP-4-006 試験で導入し，CP-4-003 試験および CP-4-007 試験では使用しなかった。

MOD-4023 の臨床開発中，この抗 hGH 中和抗体分析法を更新し，選択性，特異性，薬剤耐性，カットポイントおよび頑健性を向上させた。

抗 hGH 中和抗体分析法 IC-P-0654 は，[redacted]が AR2919 として健康成人血清を対象にバリデーションを行った［説明を明瞭にするため，バリデーション報告書 AR2919（改訂および補遺を含む）に記載されたすべての修正点および変更内容は，更新版の報告書であるバリデーション報告書 AR5266 に記載している］。その後，精度，PC の各範囲（再設定），正常血清試料における選択性，溶血性および高脂血症性血清試料における選択性，薬剤耐性，72 時間卓上安定性など，新たなパラメータについて試験し，AR5266 補遺 1 に報告したほか，投与前後の OD 差（%）を算出し，30.0%の陽性基準を確認した。薬剤耐性について追加で評価を行い，AR5266 補遺 2 として報告した。試料分析中に検討した未治療成人 GHD 患者からの投与前試料を用いて CP-4-005 試験の試験内カットポイント評価を実施し，AR5266 補遺 3 に報告した。

小児 GHD 血清を対象とした抗 hGH 中和抗体分析法 IC-P-1133 は，フルバリデーション実施後（バリデーション報告書 AR4266），hGH と MOD-4023 に対する追加の薬剤耐性評価を行い，AR4266 改訂 1 補遺 1 として報告した。試料分析中に未治療小児 GHD 患者からの投与前試料を用いて CP-4-004 試験の試験内カットポイント評価を実施し，AR4266 改訂 1 補遺 2 として報告した。また，追加の分析精度検討，第 3 相 CP-4-006 試験の投与前小児 GHD 血清試料を用いた新たな試験内カットポイントの決定，および対象疾患の溶血性，高脂血症性および黄疸性血清マトリックスにおける感度および選択性の決定を行い，AR4266 改訂 1 補遺 3 として報告した。

本分析法は，その後[redacted]に移管し，バリデーション（C0317008）を行い，CP-4-009 試験の試料分析に用いた。

この抗 hGH 中和抗体分析法のライフサイクル情報を Table 20（AR2919，AR4266 および AR5266）および Table 21（C0317008）に，各分析法の性能概要およびバリデーション報告書，改訂および補遺を Table 36（AR2919，AR4266 および AR5266）および Table 37（C0317008）に示した。本項に記載のすべてのバリデーション報告書は M5.3.1.4 に掲載した。

1.3.5. PD 評価のための生体試料分析法

IGF-1 は 3 つのジスルフィド結合を持つ 1 本鎖ポリペプチドである。循環血中では，主に，IGFBP-3 および酸に不安定なサブユニットと結合して高分子量の三次複合体として存在する[14]。

IGF-1 の合成は GH および栄養摂取により惹起される。血漿中または血清中で IGF-1 濃度が正常であれば GH 分泌不全がないことが強く裏付けられ，一方，低値は GH 分泌不全を意味する。正確な定量には酸処理が必要である [14]。

IGF は細胞増殖の調節に関わるペプチドファミリーであり，IGFBP と結合することでその作用が媒介される。IGFBP-3 は最も多い IGFBP であり，上述のように IGF-1 と結合して糖タンパク質複合体を形成する[15]。IGFBP-3 の機能は循環中の成長因子の半減期を延長することである。IGFBP-3 は GH に依存するので，GH 分泌の評価に役立つ [15]。

IGF-1 および IGFBP-3 測定のための試料を各臨床試験で測定した。

健康成人を対象とした試験（CP-4-001 試験，CP-4-007 試験および CP-4-011 試験）で採取した IGF-1 および IGFBP-3 測定のための試料は，それぞれ IGF-1 用および IGFBP-3 用の分析キット Immulite® 2000（Siemens Healthcare Diagnostics）を用いて分析した。Immulite 2000 IGF-1 および Immulite 2000 IGFBP-3 は，化学発光を利用した固相化酵素標識イムノアッセイである。試料の前処理は専用分析装置内での希釈処理により実施した [14]。

GHD 患者を対象とした試験［CP-4-003 試験，CP-4-004 試験（OLE 含む），CP-4-005 試験（OLE 含む）および CP-4-006 試験（OLE 含む）］で採取された IGF-1 および IGFBP-3 測定のための試料は，それぞれ IGF-1 用および IGFBP-3 用の分析キット IDS-iSYS（Immunodiagnostic Systems Holdings PLC）を用いて分析した。CP-4-009 試験では，IGF-1 をロシュ・ダイアグノスティック社コバス®システムの分析キット（エクルーシス®試薬 IGF-1）を，IGFBP-3 は他の GHD 患者対象試験と同じ IDS-iSYS の分析キットを使用した。IDS-iSYS およびエクルーシスを用いたこれらの IGF-1 分析法は，最近開発されたモノクローナル抗体を用いた分析法であり，推奨される英・NIBSC（National Institute for Biological Standards and Control）の標準品（02/254）に対して較正されている[16]。また，全年齢を対象とした IGF-1 の基準範囲も研究者によって得られている[17]。

IGF-1 値と IGFBP-3 値の測定は，すべての試験で正確かつ精密な推定値が得られるよう，中央検査機関を使用し，試験内で一貫した分析法を使用することにより，注意深く実施した。IGF-1 および IGFBP-3 に関する分析法と分析機関の要約表を Table 38 および Table 39 に示した。

2. 個々の試験結果の要約

MOD-4023 の VIAL と PEN の生物学的同等性を検討した CP-4-011 試験結果を記述する。

2.1. CP-4-011 試験

CP-4-011 試験は，健康成人男性被験者に 2 つの異なる MOD-4023 製剤を投与し，MOD-4023 の PK および PD を比較する第 1 相，単一施設，無作為化，クロスオーバーで実施した生物学的同等性試験であった。

試験製剤としては投与液があらかじめ充填された PEN を使用した。これは 60 mg/1.2 mL 入りで，市販を意図した製剤である。製剤は 3 mL のガラスカートリッジであり，2～8°C で保存された。

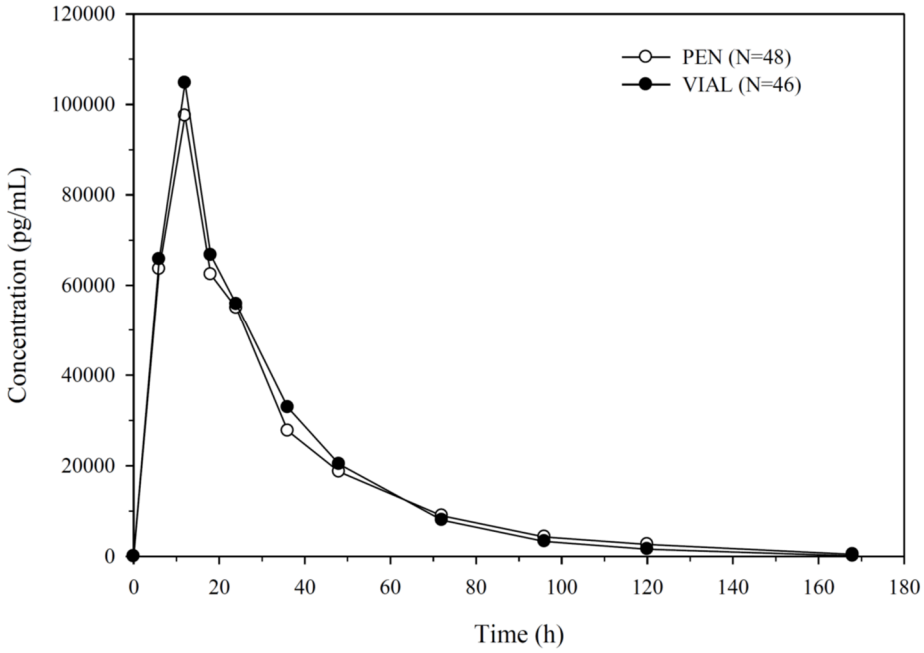
対照製剤としては凍結 VIAL を使用した。本製剤は使用前まで−20 ± 5°C で保存し，投与前に冷凍庫から取り出し，一晩冷蔵庫（2〜8°C）で解凍してから使用した。

各被験者に MOD-4023 を VIAL と PEN を用いて，それぞれ 12 mg を各 1 回（計 2 回）上腕に単回皮下投与した。投与後 0，6，12，18，24，36，48，72，96，120 および 168 時間に血清試料を採取し，MOD-4023 濃度を測定した。また，投与後 0，6，12，24，48，72，96，168 および 240 時間に血清試料を採取し，IGF-1 および IGFBP-3 濃度を測定した。測定用のスクリーニング時，Day 14 の投与前および Day 30 に血清試料を採取し，ADA 濃度を測定した。49 例を組み入れたが，1 例は初回投与後に同意を撤回し，他の 3 例は試験を完了しなかった。

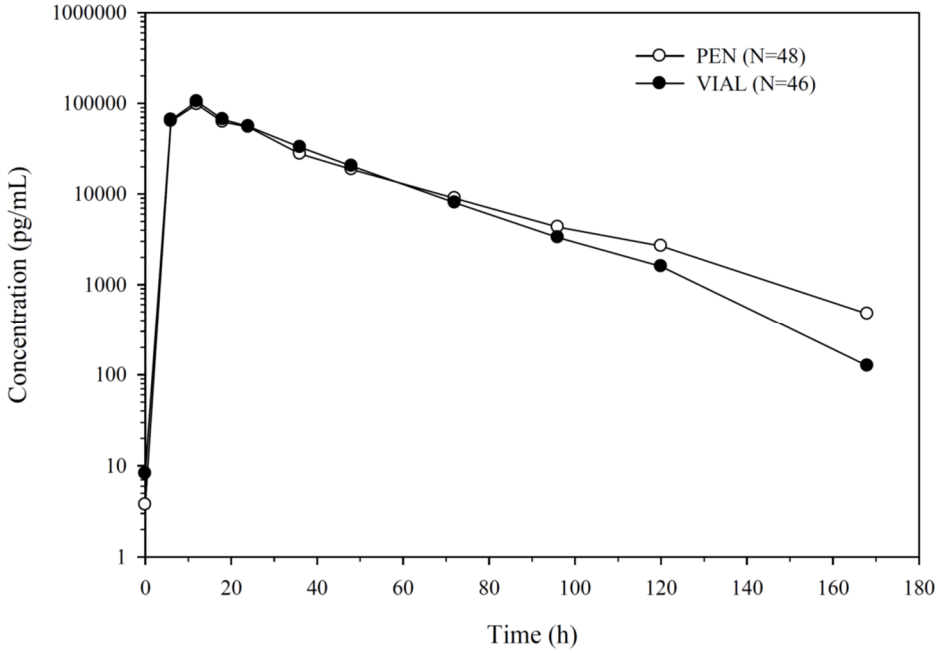
平均 MOD-4023 濃度の推移を Figure 2 に示した。

Figure 2. Mean Serum Somatrogon Concentrations-Time Profiles Following Single 12 mg Subcutaneous Injections in Healthy Male Subjects

Linear-Linear Scale



Log-Linear Scale



Source: M5.3 CSR CP-4-011 Figure 2

ノンコンパートメント法により算出した PK パラメータを Table 3 に示した。また，生物学的同等性に関する比較を Table 4 に示した。ADA 陽性例はなかった。

Table 3. Somatrogon Pharmacokinetic Parameters Following Single 12 mg Subcutaneous Injection in Healthy Male Subjects

PK Parameters	Summary Statistics	Treatment	
		PEN (Test)	VIAL (Reference)
C_{max} (pg/mL)	N	48	46
	AM	101439.55	106497.55
	SD	78274.34	85810.04
	MIN	9401.86	15602.88
	Median	77197.51	81626.44
	MAX	326753.81	393020.38
	CV%	77.2	80.6
	GM	72265.26	76879.77
T_{max} (h)	N	48	46
	AM	13.75	15.42
	SD	5.53	9.56
	MIN	6.00	6.00
	Median	12.00	12.00
	MAX	36.00	54.75
	CV%	40.2	62.0
	GM	12.84	13.89
AUC_t (h*pg/mL)	N	46[a]	46
	AM	2925723.97	2983399.52
	SD	1676670.62	1858597.38
	MIN	561668.45	816475.42
	Median	2609836.12	2408414.29
	MAX	7485504.46	7397608.72
	CV%	57.3	62.3
	GM	2461715.26	2486007.17
AUC_∞ (h*pg/mL)	N	37[a,b]	42[c]
	AM	3238519.10	3044747.51
	SD	1704068.26	1769295.60
	MIN	625402.23	869424.96
	Median	2974563.29	2545208.64
	MAX	7552530.67	7061500.36
	CV%	52.6	58.1
	GM	2798048.22	2588547.65
MRT (h)	N	37[a,b]	42[c]
	AM	36.73	33.03
	SD	13.60	9.37
	MIN	22.09	17.50
	Median	31.36	31.89
	MAX	86.68	63.71
	CV%	37.0	28.4
	GM	34.76	31.81
λz (1/h)	N	37[a,b]	42[c]
	AM	0.03	0.04
	SD	0.01	0.01

Table 3. Somatrogon Pharmacokinetic Parameters Following Single 12 mg Subcutaneous Injection in Healthy Male Subjects

PK Parameters	Summary Statistics	Treatment	
		PEN (Test)	VIAL (Reference)
	MIN	0.01	0.02
	Median	0.03	0.03
	MAX	0.05	0.06
	CV%	28.3	22.8
	GM	0.03	0.03
$t_{1/2}$ (h)	N	37[a,b]	42[c]
	AM	25.49	20.34
	SD	8.26	4.38
	MIN	13.36	11.06
	Median	23.20	20.05
	MAX	54.19	31.46
	CV%	32.4	21.5
	GM	24.37	19.88
CL/F (L/h)	N	37[a,b]	42[c]
	AM	5.07	5.45
	SD	3.36	3.14
	MIN	1.59	1.70
	Median	4.03	4.71
	MAX	19.19	13.80
	CV%	66.3	57.6
	GM	4.29	4.64
Vz/F (L)	N	37[a,b]	42[c]
	AM	193.02	165.74
	SD	173.19	111.86
	MIN	60.88	32.22
	Median	142.58	131.50
	MAX	909.80	442.05
	CV%	89.7	67.5
	GM	150.81	132.94

a. Two (2) subjects' AUC_t, AUC_∞, MRT, $t_{½}$, λz, CL/F, and Vz/F values in the test (PEN) treatment group were not evaluable due to lost to follow up;
b. Nine (9) subjects' AUC_∞, MRT, $t_{½}$, λz, CL/F, and Vz/F values in the test (PEN) treatment group were not reported because λz_period <2;
c. Four (4) subjects' AUC_∞, MRT, $t_{½}$, λz, CL/F, and Vz/F values in the reference (VIAL) treatment group were not reported because λz_period <2.
Source: M5.3 CSR CP-4-011 Table 6

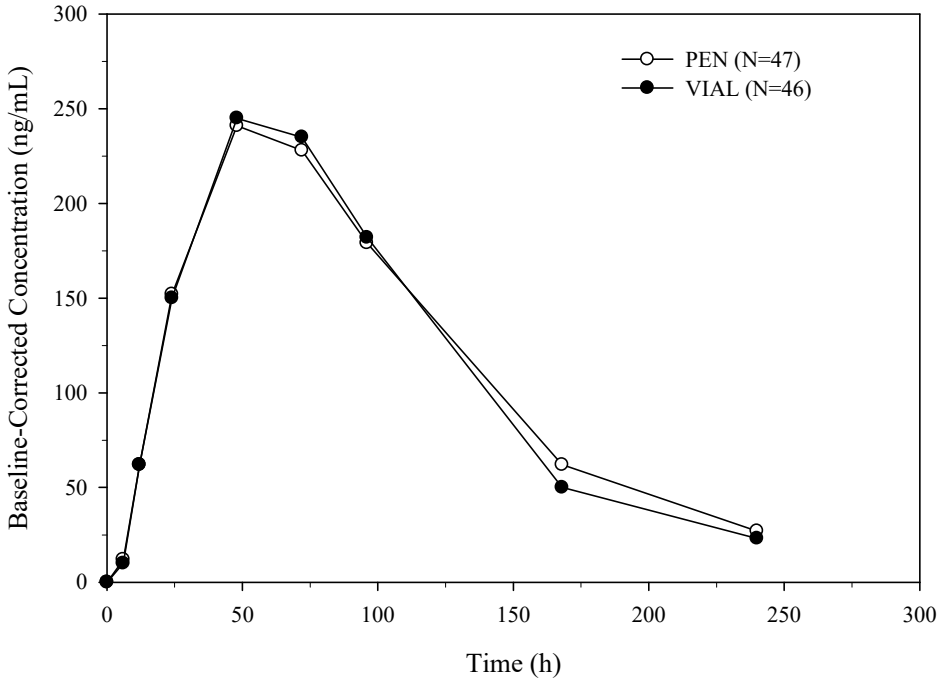
Table 4. PK Bioequivalence Comparisons

PK Parameter†	PEN (Test)		VIAL (Reference)		PEN/VIAL
	N	GLSM	N	GLSM	GMR (90% CI)
C_{max} (pg/mL)	48	71150	46	78791	90.30 (74.24, 109.84)
AUC_t (h*pg/mL)	46[a]	2385782	46	2517373	94.77 (87.05, 103.18)
AUC_∞ (h*pg/mL)	37[a,b]	2544288	42[c]	2642923	96.27 (87.64, 105.75)

† Back-transformed least squares mean and CI from ANOVA model performed on natural log-transformed values.
a. AUC_t and AUC_∞ values in two (2) subjects in the test (PEN) treatment group were not evaluable due to lost to follow up and then not used in the statistical analysis;
b. Nine (9) subjects' AUC_∞ values in the test (PEN) treatment group were not reported because λz_period <2;
c. Four (4) subjects' AUC_∞ values in the reference (VIAL) treatment group were not reported because λz_period <2;
GMR and 90% CI: Reported as percentage.
Source: M5.3 CSR CP-4-011 Table 7

IGF-1 濃度のベースライン補正した平均値の推移を Figure 3 に示した。

Figure 3. Mean Baseline-Corrected IGF-1 Serum Concentration-Time Profiles Following Single 12 mg Subcutaneous Injections of Somatrogon in Healthy Male Subjects



Linear-Linear Scale
Source: M5.3 CSR CP-4-011 Figure 3

ノンコンパートメント法により算出した IGF-1 と IGFBP-3 パラメータを用いて実施した生物学的同等性に関する比較を Table 5 および Table 6 に示した。

Table 5. IGF-1 Bioequivalence Comparisons

PD Parameter†	PEN (Test)		VIAL (Reference)		PEN/VIAL
	N	GLSM	N	GLSM	GMR (90% CI)
C_{max} (ng/mL)	47[a]	247	46	250	98.72 (95.10, 102.47)
AUC_t (h*ng/mL)	46[b]	28003	46	26537	105.52 (98.37, 113.20)
AUC_{inf} (h*ng/mL)	30[b,c]	30173	32[d]	30656	98.42 (89.88, 107.79)

† Back-transformed least squares mean and CI from ANOVA model performed on natural log-transformed values.
[a] C_{max} value in one (1) subject in the test (PEN) treatment group could not be well captured due to lost to follow up (after 24 hrs sample collection) and then were not used in the statistical analysis.
[b] AUC_t and AUC_∞ values in two (2) subjects in the test (PEN) treatment group were not evaluable due to lost to follow up (after 96 hrs sample collection) and then were not used in the statistical analysis.
[c] Thirteen (13) subjects' AUC_∞ values in the test (PEN) treatment group were not reported because λ_z_period <2 or the r^2 value for λ_z estimation < 0.9000, and three (3) subjects' AUC_∞ values in the test (PEN) treatment group could not be estimated due to fewer than 3 data points after C_{max} or positive slope.
[d] Ten (10) subjects' AUC_∞ values in the reference (VIAL) treatment group were not reported because λ_z_period <2 or the r^2 value for λz estimation < 0.9000, and four (4) subjects' AUC_∞ values in the reference (VIAL) treatment group could not be estimated due to fewer than 3 data points after C_{max} or positive slope.
GLSM=Geometric least-squares mean; GMR=Geometric least-squares mean ratio; CI=Confidence interval;
GMR and 90% CI: Reported as percentage.
Source：M5.3 CP-4-011 CSR Table 9

Table 6. IGFBP-3 Bioequivalence Comparisons

PD Parameter†	PEN (Test)		VIAL (Reference)		PEN/VIAL
	N	GLSM	N	GLSM	GMR (90% CI)
C_{max} (µg/mL)	47[a]	2.0	46	2.0	100.38 (92.29, 109.17)
AUC_t (h*µg/mL)	46[b]	197.2	46	190.1	103.74 (87.41, 123.11)
AUC_{inf} (h*µg/mL)	5[b, c]	NA[e]	5[d]	NA[e]	NA[e]

† Back-transformed least squares mean and CI from ANOVA model performed on natural log-transformed values.
[a] C_{max} value in one (1) subject in the test (PEN) treatment group could not be well captured due to lost to follow up and then were not used in the statistical analysis.
[b] AUC_t and AUC_∞ values in two (2) subjects in the test (PEN) treatment group were not evaluable due to lost to follow up and then were not used in the statistical analysis.
[c] Eleven (11) subjects' AUC_∞ values in the test (PEN) treatment group were not reported because λ_z_period <2 or the r^2 value for λ_z estimation < 0.9000, and thirty (30) subjects' AUC_∞ values in the test (PEN) treatment group could not be estimated due to fewer than 3 data points after C_{max} or positive slope.
[d] Nine (9) subjects' AUC_∞ values in the reference (VIAL) treatment group were not reported because λ_z_period <2 or the r^2 value for λ_z estimation < 0.9000 and thirty-two (32) subjects' AUC_∞ values in the reference (VIAL) treatment group could not be estimated due to fewer than 3 data points after C_{max} or positive slope.
[e] The GLSM, GMR, and 90% CI could not be estimated due to insufficient evaluable data.
GLSM=Geometric least-squares mean; GMR=Geometric least-squares mean ratio; CI=Confidence interval;
GMR and 90% CI: Reported as percentage; NA: Not Applicable.
Source: M5.3 CP-4-011 CSR Table 11

本試験の結論

PEN（試験製剤）と VIAL（対照製剤）の間で PK による生物学的同等性試験を実施した結果，C_{max} に関しては CI の下限値（0.74）が生物学的同等性の許容範囲（0.80）を下回っていた。しかし，AUC では生物学的同等性が示された。また，IGF-1 と IGFBP-3 で評価した生物学的同等

性試験の結果では，いずれも生物学的同等性の範囲内であった。これらのことを考え合わせると，PEN（試験製剤）と VIAL（対照製剤）の間で臨床結果に影響を及ぼす差はないと考える。

3. 全試験を通しての結果の比較と解析

- 市販を意図した PEN と VIAL は生物学的に同等であると判断した。この PEN は第 2 相小児 GHD 患者を対象とした CP-4-004 試験の OLE 試験および第 3 相小児 GHD 患者を対象とした CP-4-006 および CP-4-009 試験で使用され，その他の臨床試験では VIAL が使用された。

- 臨床試験で使用した分析法は，MOD-4023 の PK 評価，MOD-4023 の免疫原性の評価（MOD-4023 と GH に対する ADA および MOD-4023 と GH に対する NAb の評価）ならびに抗 MOD-4023 抗体の抗 GH 抗体または抗 CTP 抗体としての特異性の評価において適切である。

4. 付録

Table 7. Summary of Somatrogon Assay Methods

Method Validation	Method Life Cycle	Method Performance	Clinical Study	Bioanalytical Project (Report Date)	Method ID
Somatrogon PK					
[redacted] AR2665 – ELISA	Table 8	Table 22	CP-4-001	[redacted] 2863 ([redacted])	IC-P-0590-02
[redacted] AR3835 – ECL	Table 9	Table 23	CP-4-003 CP-4-004 CP-4-004 OLE (Years 1&2) CP-4-004 OLE (Years 3&4) CP-4-004 PEN CP-4-005 CP-4-007 CP-4-011 CP-4-009	[redacted] 3912 ([redacted]) [redacted] 5182 ([redacted]) [redacted] 5182 ([redacted]) [redacted] 106-C1087-18-0082 ([redacted]) [redacted] 106-C1087-19-0065 ([redacted]) [redacted] 6436 ([redacted]) [redacted] 6045 ([redacted]) [redacted] 106-C1087-19-0063 ([redacted]) [redacted] 106-C1087-18-0122 ([redacted])	IC-P-0954 IC-P-0954-01 IC-P-0954-01, -02 & -03 IC-P-0954-02 & -03 IC-P-0954-07 IC-P-0954-02 IC-P-0954-01 IC-P-0954-04, -06 & -07 IC-P-0954-03 & -07
[redacted] OX-19/512-016 – ECL	Table 10	Table 24	CP-4-006	[redacted] OX-19-512-017 ([redacted])	BALAR 19/014 v2
[redacted] C0319002 - ECL	Table 11	Table 25	CP-4-009	[redacted] 15-2054 ([redacted])	15-2026-02
Somatrogon ADA					
[redacted] AR3165 [redacted] AR3165 [redacted] AR4263 [redacted] AR4263/AR5303 [redacted] AR4263/AR5303 [redacted] AR4263/AR5303 [redacted] AR3165/AR5374 [redacted] AR3165/AR5374 [redacted] AR5374	Table 12	Table 26 Table 27 Table 28	CP-4-001 CP-4-003 CP-4-004 CP-4-004 OLE (Years 1&2) CP-4-004 OLE (Years 3&4) CP-4-004 PEN CP-4-005 CP-4-007 CP-4-011	[redacted] 2975 ([redacted]) [redacted] 3479 ([redacted]) [redacted] 5184 ([redacted]) [redacted] 5184 ([redacted]) [redacted] 106-C1087-18-0083 ([redacted]) [redacted] 106-C1087-19-0066 ([redacted]) [redacted] 6437 ([redacted]) [redacted] 6028 ([redacted]) [redacted] 106-C1087-19-0064 ([redacted])	IC-P-0751 IC-P-0751, -01, -02 &-03 IC-P-1145-02 & -03 IC-P-1145-03 & -04 IC-P-1145-03 & -04 IC-P-1145-05 IC-P-0751-06 IC-P-0751-05 IC-P-0751-09
[redacted] OX-18/512-011	Table 13	Table 29	CP-4-006	[redacted] OX-19/512-008 ([redacted])	BALAR 18/031

Table 7. Summary of Somatrogon Assay Methods

Method Validation	Method Life Cycle	Method Performance	Clinical Study	Bioanalytical Project (Report Date)	Method ID
[redacted] SC-20/512-024	Table 14	Table 30	CP-4-009	[redacted] SC-20/512-026 (GCL-665) ([redacted])	BALAR 18/031
hGH ADA					
[redacted] AR4575	Table 15	Table 31	CP-4-001 CP-4-003 CP-4-004 CP-4-004 OLE (Years 1&2) CP-4-004 OLE (Years 3&4) CP-4-004 PEN CP-4-005 CP-4-007 CP-4-011	N/A N/A [redacted] 5184 ([redacted]) N/A N/A N/A N/A N/A N/A	N/A N/A IC-P-1243-01 & -02 N/A N/A N/A N/A N/A N/A
[redacted] OX-18/512-012	Table 16	Table 32	CP-4-006	[redacted] OX-19/512-008 ([redacted])	BALAR 18/038
[redacted] SC-20/512-025	Table 17	Table 33	CP-4-009	[redacted] SC-20/512-026 (GCL-666) ([redacted])	BALAR 18/038
Somatrogon Nab					
N/A [redacted] AR2862 AR2862/AR4268/AR5265 [redacted] 5265 [redacted] AR4268/5265 [redacted] 5265 [redacted] 4268/5265 N/A [redacted] 4268/5265	Table 18	Table 34	CP-4-001 CP-4-003 CP-4-004 CP-4-004 OLE (Years 1-4) CP-4-004 PEN CP-4-005 CP-4-007 CP-4-011 CP-04-006	N/A [redacted] 3026 ([redacted]) [redacted] 5176 Amendment 1 ([redacted]) [redacted] 5176 MOD-4023 NAb ([redacted]) [redacted] 106-C1087-19-0068 ([redacted] 20[redacted]) [redacted] 5175 Amendment 2 ([redacted]) [redacted] 6188 ([redacted]) N/A [redacted] 106-C1087-19-0107 ([redacted] 20[redacted])	N/A IC-P-0653 and -02 IC-P-0653-05 and -06 IC-P-0653-08 and -09 IC-P-0653-09 IC-P-0653-04 IC-P-0653-06 N/A IC-P-0653-10 and -11
C0317007	Table 19	Table 35	CP-4-009	[redacted] 1953-2001A ([redacted] 20[redacted])	15-2029

Table 7. Summary of Somatrogon Assay Methods

Method Validation	Method Life Cycle	Method Performance	Clinical Study	Bioanalytical Project (Report Date)	Method ID
hGH Nab					
N/A [redacted] AR2919 [redacted] AR4266 [redacted] AR4266 [redacted] AR4266 [redacted] AR5266 [redacted] AR5266 N/A [redacted] AR4266	Table 20	Table 36	CP-4-001 CP-4-003 CP-4-004 CP-4-004 OLE (Years 1-4) CP-4-004 PEN CP-4-005 CP-4-007 CP-4-011 CP-4-006	N/A [redacted] 3025 ([redacted]) [redacted] 5176 Amendment 1 ([redacted]) [redacted] 5176 OLE ([redacted]) [redacted] 106-C1087-19-0068 ([redacted] 20[redacted]) [redacted] 5175 Amendment 2 ([redacted]) [redacted] 6188 ([redacted]) N/A [redacted] 106-C1087-19-0107 ([redacted] 20[redacted])	N/A IC-P-0654-02 IC-P-1133-01 and -02 IC-P-1133-02 IC-P-1133-02 IC-P-0654-07 IC-P-0654-06 N/A IC-P-1133-3 and -04
C0317008	Table 21	Table 37	CP-4-009	[redacted] 1953-2001B ([redacted] 20[redacted])	15-2030

Table 8. Bioanalytical Method Life Cycle Information: Somatrogon ELISA PK ([redacted] Project AR2665)

	Method validation #1
Analyte	Somatrogon (MOD-4023)
Validation type	Full validation
Method ID	IC-P-0590 ([redacted])
Duration of time method is in use	[redacted]/20[redacted]-[redacted]/20[redacted]
Bioanalytical site	[redacted]
Matrix	Human Serum
Platform	Enzyme-linked immunosorbent assay (ELISA)
Format	A validated sandwich format using anti-hGH monoclonal antibody as capture and biotinylated rabbit anti-CTP polyclonal antibody followed by streptavidin-HRP as detection
Stock reference, lot number, expiration date	Somatrogon (MOD-4023), lot EN648-01-[redacted]-005, expiration date [redacted]20[redacted]
Calibration range from the lower limit of quantitation (LLOQ) to the upper limit of quantitation (ULOQ)	1500 pg/mL to 120,000 pg/mL
Matrix study population	Normal Healthy
Link to reports and applicable amendments	AR2665 – Original Method Validation Report ([redacted]) AR2665 – Addendum 1 ([redacted]) AR2665 – Addendum 2 ([redacted])
Synopsis of amendment history	Addendum 1: Long Term Stability for 6 months at -70°C Addendum 2: Incurred sample reanalysis

Table 9. Bioanalytical Method Life Cycle Information: Somatrogon ECL PK ([redacted] Project AR3835)

	Method validation #1	Method validation #2	Method validation #3
Analyte	Somatrogon (MOD-4023)	Somatrogon (MOD-4023)	Somatrogon (MOD-4023)
Validation type	Full validation, including Addenda 1-3	Partial Validation Addenda 4-5	Partial Validation Addenda 6-7
Method ID	IC-P-0954 Original and Addenda 2 (Date [redacted]/20[redacted]) IC-P-0954-01 Addenda 1 and 3 (Date [redacted]/20[redacted])	IC-P-0954-02 Addenda 4-5 (Date: [redacted]/20[redacted])	IC-P-0954-03 (Date: [redacted]/20[redacted]) IC-P-0954-04 (Date: [redacted]/20[redacted]) IC-P-0954-05 (Date: [redacted]/20[redacted]) IC-P-0954-06 (Date: [redacted]/20[redacted]) IC-P-0954-07 (Date: [redacted]/20[redacted])
Duration of time method is in use	[redacted]/20[redacted] - [redacted]/20[redacted]	[redacted]/20[redacted] – [redacted]/20[redacted]	[redacted]/20[redacted]-present
Bioanalytical site	**Original, Addenda 1&2:** [redacted] **Addenda 3** [redacted] *New location	[redacted]	[redacted]
Matrix	Human Serum		
Platform	ECL		
Format	A validated sandwich format using biotinylated anti-hGH monoclonal antibody as capture and Sulfo-Tag anti-CTP polyclonal antibody as detection.		
Stock reference, lot number, expiration date	Somatrogon (MOD-4023), lot 4-09-X003B-S2, expiration date [redacted]20[redacted]	Somatrogon (MOD-4023), lot 1019394, expiration date [redacted]20[redacted]	Somatrogon (MOD-4023), lot 1039252, expiration date [redacted]20[redacted]

Table 9. Bioanalytical Method Life Cycle Information: Somatrogon ECL PK ([redacted] Project AR3835)

	Method validation #1	Method validation #2	Method validation #3
Calibration range from the lower limit of quantitation (LLOQ) to the upper limit of quantitation (ULOQ)	150 pg/mL to 36,000 pg/mL The range of quantitation established in assay validation was defined as the low end of the Low QC range (LLOQ) to the high end of the High QC range (ULOQ).	156.25 pg/mL to 40,000 pg/mL	1.0 ng/mL to 128 ng/mL
Matrix study population	Normal and pediatric (Ages 3-13) growth hormone deficient serum	Normal and pediatric (Ages 3-13) growth hormone deficient serum	Normal and pediatric (Ages 3-13) growth hormone deficient serum
Link to reports and applicable amendments	AR3835 Original Method Validation Report ([redacted]) Addendum 1 ([redacted]) Addendum 2 ([redacted]) Addendum 3 ([redacted])	Report AR3835 Addendum 4 ([redacted]) Addendum 5 ([redacted])	Report 106-C1087-19-0099 Addendum 6: ([redacted]) Addendum 7: ([redacted])
Synopsis of amendment history	**Addendum 1**: Human pediatric (Ages 3-13) growth hormone deficient serum matrix evaluation **Addendum 2**: Long Term Stability for 6 months at -70°C and -20°C **Addendum 3:** Long Term Stability for 40 months at -70°C and -20°C	**Addendum 4:** Partial validation to modify the standard curve and quality control concentrations. Additionally, selectivity in normal human, pediatric growth hormone deficient, lipemic and hemolyzed human serum was evaluated. **Addendum 5:** Additional set of 20 pediatric (ages 3-13) growth hormone deficient human serum individuals evaluated for selectivity	**Addendum 6:** Partial validation to modify standard curve and QC concentrations (Precision and Accuracy, Selectivity, Ruggedness and Robustness and Range of Quantitation). **Addendum 7:** Partial validation to qualify a new biotinylated anti-hGH coating antibody for bioanalytical procedure.

Table 10. Bioanalytical Method Life Cycle Information: Somatrogon ECL PK ([redacted]) Project OX-19/512-016 and OX-19/512-015)

	Method validation #1 OX-19/512-016	Method validation #2 OX-19/512-015
Analyte	Somatrogon (MOD-4023)	Somatrogon (MOD-4023)
Validation type	Full validation	Establishment, Method Transfer and Combarability (Cross validation)
Method ID	BALAR 19/014 Version 02 ([redacted])	BALAR 19/014 Version 1 ([redacted])
Duration of time method is in use	[redacted]/20[redacted] – present	[redacted]/20[redacted] - [redacted]/20[redacted]
Bioanalytical site	[redacted] UK	
Matrix	Human Serum	
Platform	ECL	
Format	A validated sandwich format using biotinylated anti-hGH monoclonal antibody as capture and Sulfo-Tag anti-CTP polyclonal antibody as detection.	
Stock reference, lot number, expiration date	Somatrogon (MOD-4023), lot 1039252, expiration date [redacted]20[redacted]	Somatrogon (MOD-4023), lot 1039252, expiration date [redacted]20[redacted]
Calibration range from the lower limit of quantitation (LLOQ) to the upper limit of quantitation (ULOQ)	156.3 pg/mL to 40,000 pg/mL	156.3 pg/mL to 40,000 pg/mL
Matrix study population	Normal and Individual Serum (GH deficient pediatric) for selectivity only	Normal and Individual Serum (GH deficient pediatric) for selectivity only
Link to reports and applicable amendments	[redacted] OX-19/512-016 – Original Method Validation Report ([redacted])	[redacted] OX-19/512-015 ([redacted])
Synopsis of amendment history	None	None

Table 11. Bioanalytical Method Life Cycle Information: Somatrogon ECL PK (C0319002)

	Method validation #1 C0319002 - Original, [redacted] 15-2026	Method validation #2 C0319002 – Cross-validation, [redacted] 15-2054[a]
Analyte	Somatrogon (PF-06836922, MOD-4023)	Somatrogon (PF-06836922, MOD-4023)
Validation type	Full validation	Cross-validation
Method ID	15-2026-00 ([redacted])	15-2026-02 ([redacted])
Duration of time method is in use	[redacted]/20[redacted]	[redacted]/20[redacted] – [redacted]/20[redacted]
Bioanalytical site	[redacted] U.S.A.	
Matrix	Human Serum	
Platform	ECL	
Format	A validated sandwich format using biotinylated anti-hGH monoclonal antibody as capture and Sulfo-Tag anti-CTP polyclonal antibody as detection.	
Stock reference, lot number, expiration date	Somatrogon (PF-06836922, MOD-4023), lot 00711666-0021-WRM, expiration date [redacted]20[redacted]	Somatrogon (PF-06836922, MOD-4023), lot 00711666-0021-WRM, expiration date [redacted]20[redacted]
Calibration range from the lower limit of quantitation (LLOQ) to the upper limit of quantitation (ULOQ)	1.00 ng/mL to 128 ng/mL	1.00 ng/mL to 128 ng/mL
Matrix study population	Normal and Individual Serum (GH deficient pediatric) for selectivity only	Normal and Individual Serum (GH deficient pediatric)
Link to reports and applicable amendments	[redacted] 15-2026 – Original Method Validation Report ([redacted])	[redacted] 15-2054 – Cross-validation Report ([redacted])
Synopsis of amendment history	None	None

a Source: Module5.3.5.1.2, Appendix16.2.5.10

Table 12. Bioanalytical Method Life Cycle Information: Somatrogon ECL ADA ([redacted] Projects AR3165, AR5374, AR4263 and AR5303)

	Method validation #1 AR3165	Method validation #2 AR5374	Method validation #3 AR4263	Method validation #4 AR5303
Analyte	Anti-Somatrogon (MOD-4023) Antibodies	Anti-Somatrogon (MOD-4023) Antibodies	Anti-Somatrogon (MOD-4023) Antibodies	Anti-Somatrogon (MOD-4023) Antibodies
Validation type	Full validation	Full validation	Full validation	Full validation
Method ID	IC-P-0751 Original ([redacted]) IC-P-0751-01 Addendum 1 ([redacted]) IC-P-0751-02 Addendum 2 ([redacted]) IC-P-0751-03 Addendum 3 ([redacted]) IC-P-0751-04 Addendum 4 ([redacted]) IC-P-0751-05 Addendum 5 ([redacted])	IC-P-0751-05 Original [redacted]) IC-P-0751-06 Addendum 1 ([redacted])	IC-P-1145 Original ([redacted]) IC-P-1145-01 Addendum 1 ([redacted])	IC-P-1145-02 Original ([redacted]) IC-P-1145-03 Amendment 1 ([redacted])
Duration of time method is in use	[redacted]/20[redacted] – [redacted]/20[redacted]	[redacted]/20[redacted] – present	[redacted]/20[redacted]-[redacted]/20[redacted]	[redacted]/20[redacted] – present
Bioanalytical site	**AR3165** [redacted]	**AR5374** [redacted] **AR5374 (Addendum 1)** [redacted] *New location	**AR4263** [redacted]	**AR5303** [redacted] **Amendment 1** [redacted] *New location
Matrix	Human Serum			
Platform	ECL			

Table 12. Bioanalytical Method Life Cycle Information: Somatrogon ECL ADA ([redacted] Projects AR3165, AR5374, AR4263 and AR5303)

	Method validation #1 AR3165	Method validation #2 AR5374	Method validation #3 AR4263	Method validation #4 AR5303
Format	A validated bridging format using biotinylated MOD-4023 as capture and Sulfo-Tag MOD-4023 as detection.			
Stock reference, lot number, expiration date	**Positive control (PC):** Rabbit affinity purified anti-MOD-4023, Lot#10325, expiration date [redacted]20[redacted] Anti-poly CTP, Lot#21114, expiration date [redacted]20[redacted] (Addendum 4) **Drug**: MOD-4023, Lot# 4-09-X003B-S1, expiration date [redacted]20[redacted] (Original) MOD-4023, Lot# 4-09-X003B-S2, expiration date [redacted]20[redacted] (Addenda 1-3) MOD-4023, Lot#1013202, expiration date [redacted]20[redacted] (Addenda 4-5) WHO International Standard, Growth Hormone, NIBSC Code 80/505	**Positive control (PC):** Rabbit affinity purified anti-MOD-4023, Lot#10325, expiration date [redacted]20[redacted] (Original) Rabbit affinity purified anti-MOD-4023, Lot#270415, expiration date [redacted]20[redacted] (Addendum 1) Anti-poly CTP, Lot#21114, expiration date [redacted]20[redacted] **Drug**: MOD-4023, Lot#1013202, expiration date [redacted]20[redacted] (Original) MOD-4023, Lot#1019394, expiration date [redacted]20[redacted] (Addendum 1) WHO International Standard, Growth Hormone, NIBSC Code 80/505	**Positive control (PC):** Rabbit affinity purified anti-MOD-4023, Lot#10325, expiration date [redacted]20[redacted] (Original) Anti-poly CTP, Lot#21114, expiration date [redacted]20[redacted] **Drug**: MOD-4023, Lot# 4-11-X003-S1, expiration date [redacted]20[redacted] (Original) WHO International Standard, Growth Hormone, NIBSC Code 80/505	**Positive control (PC):** Rabbit affinity purified anti-MOD-4023, Lot#10325, expiration date [redacted]20[redacted] (Original) Anti-poly CTP, Lot#21114, expiration date [redacted]20[redacted] **Drug**: MOD-4023, Lot# 4-11-X003-S1, expiration date [redacted]20[redacted] (Original) WHO International Standard, Growth Hormone, NIBSC Code 80/505
Calibration range from the lower limit of quantitation (LLOQ) to the upper limit of quantitation (ULOQ)	N/A, qualitative assay	N/A, qualitative assay	N/A, qualitative assay	N/A, qualitative assay

Table 12. Bioanalytical Method Life Cycle Information: Somatrogon ECL ADA ([redacted] Projects AR3165, AR5374, AR4263 and AR5303)

	Method validation #1 AR3165	Method validation #2 AR5374	Method validation #3 AR4263	Method validation #4 AR5303
Matrix study population	Normal and growth hormone deficient (GHD)	Normal	Pediatric (3-13 years) human growth hormone deficient	Pediatric (3-13 years) human growth hormone deficient
Link to reports and applicable amendments	AR3165 Original Method Validation Report ([redacted]) Addendum 1 ([redacted]) Addendum 1, Amendment 1 ([redacted]) Addendum 2 ([redacted]) Addendum 2, Amendment 1 ([redacted]) Addendum 3 ([redacted]) Addendum 3, Amendment 1 ([redacted]) Addendum 4 ([redacted]) Addendum 5 ([redacted]) Amendment 1 ([redacted]) Amendment 2 ([redacted])	AR5374 Original Method Validation Report ([redacted]) Addendum 1 ([redacted])	AR4263 Original Method Validation Report ([redacted]) Addendum 1 ([redacted]) AR4263 Method Evaluation Report ([redacted])	AR5303 Original Method Validation Report ([redacted]) Amendment 1 ([redacted])
Synopsis of amendment history	**Addendum 1**: Requalification to determine new acceptance ranges for PC, cut point RLU and non-specific binding (NSB). **Addendum 1, Amendment 1** Replace %CV with Percent Relative Difference (RPD). **Addendum 2**:	**Addendum 1**: • Partial validation due to new lots of rabbit affinity purified anti MOD-4023, Biotinylated MOD-4023, Sulfo Tag MOD-4023, and a new lot of pooled normal human serum. • A new panel of fifty drug naïve human serum individuals was	**Addendum 1**: • This addendum report presents the data from the original validation report (AR4263) with the updated cut point factor (normal panel outlier removal) and RPD.	**Amendment 1** In view of the FDA recommendation and in order to reduce the risk of false positives, the false positive rate was changed from 0.1% to 1.0% for all specification cut points.

Table 12. Bioanalytical Method Life Cycle Information: Somatrogon ECL ADA ([redacted] Projects AR3165, AR5374, AR4263 and AR5303)

	Method validation #1 AR3165	Method validation #2 AR5374	Method validation #3 AR4263	Method validation #4 AR5303
	Requalification to determine new acceptance ranges for PC. **Addendum 2, Amendment 1** Replace %CV with Percent Relative Difference (RPD). **Addendum 3**: Additional validation runs to address assay robustness **Addendum 3, Amendment 1** Replace %CV with Percent Relative Difference (RPD). **Addendum 4**: Additional CTP specificity cut point. **Addendum 5:** Requalification to determine new acceptance ranges for PC, cut point RLU and NSB. **Amendment 1** Correct the number of freeze /thaw cycles and replace %CV with Percent Relative Difference (RPD) **Amendment 2** For clarification purposes all corrections and modifications presented in Validation Report No.	evaluated to determine a new screening cut point (from dynamic to floating). • Per FDA request, Specificity cut points to MOD-4023, hGH, and CTP are being updated from a 0.1% false to a 1% false positive rate based on the log transformed % inhibition values. Data supporting these modifications can be found in original AR5374 report. • Method IC-P-0751-06, reflects the new acceptance ranges for the positive and negative controls, the cut point factor, and the specificity cut point.		

Table 12. Bioanalytical Method Life Cycle Information: Somatrogon ECL ADA ([redacted] Projects AR3165, AR5374, AR4263 and AR5303)

	Method validation #1 AR3165	Method validation #2 AR5374	Method validation #3 AR4263	Method validation #4 AR5303
	AR3165 (including all amendments and addendum reports) were captured in an updated version, Validation Report No. AR5374.			

Table 13. Bioanalytical Method Life Cycle Information: Somatrogon ADA ECL ([redacted] Project OX-18/512-011)

	Method validation #1 OX-18/512-011
Analyte	Anti-Somatrogon (MOD-4023) Antibodies
Validation type	Full Validation
Method ID	BALAR 18/031, Initial Release ([redacted]) BALAR 18/031, Revision 2 ([redacted])
Duration of time method is in use	[redacted]/20[redacted] - present
Bioanalytical site	[redacted] UK
Matrix	Human Serum
Platform	ECL
Format	A validated bridging format using biotinylated MOD-4023 as capture and Sulfo-Tag MOD-4023 as detection.
Stock reference, lot number, expiration date	Positive controls (PCs): Rabbit affinity purified Anti-MOD-4023, lot# PAb4023-001, expiration date [redacted]20[redacted] Anti-poly CTP, Lot#150117/Covance, expiration date [redacted]20[redacted] Drug/Specificity Reagents: MOD-4023, Lot 1039252, expiration date [redacted]20[redacted] CTP, Lot GCP1011A107, expiration date [redacted]20[redacted] WHO International Standard, Human Growth Hormone, NIBSC Code 80/505
Calibration range from the lower limit of quantitation (LLOQ) to the upper limit of quantitation (ULOQ)	N/A, qualitative assay
Matrix study population	Pediatric (3-13 years) human growth hormone deficient
Link to reports and applicable amendments	OX-18/512-011 Original Method Validation Report ([redacted]) OX-18/512-011 Amendment 1, Method Validation Repor ([redacted])
Synopsis of amendment history	**Amendment 01** Correct duplication of subject IDs on page 71 (formerly page 70) in Appendix 1.

Table 14. Bioanalytical Method Life Cycle Information: Somatrogon ADA ECL ([redacted] Project SC-20/512-024)

	Method validation #1 SC-20/512-024
Analyte	Anti-Somatrogon (MOD-4023) Antibodies
Validation type	Partial Validation
Method ID	GCL-665 / BALAR 18/031, Initial Release ([redacted])
Duration of time method is in use	[redacted]/20[redacted] - present
Bioanalytical site	[redacted] USA
Matrix	Human Serum
Platform	ECL
Format	A validated bridging format using biotinylated MOD-4023 as capture and Sulfo-Tag MOD-4023 as detection.
Stock reference, lot number, expiration date	Positive controls (PCs): Rabbit affinity purified Anti-MOD-4023, lot# PAb4023-001, expiration date [redacted]20[redacted] Anti-poly CTP, Lot#150117, expiration date [redacted]20[redacted] Drug/Specificity Reagents**:** MOD-4023, Lot 1039252, expiration date [redacted]20[redacted] CTP, Lot GCP1011A107, expiration date [redacted]20[redacted] WHO International Standard, Human Growth Hormone, NIBSC Code 80/505
Calibration range from the lower limit of quantitation (LLOQ) to the upper limit of quantitation (ULOQ)	N/A, qualitative assay
Matrix study population	Pediatric (3-13 years) human growth hormone deficient
Link to reports and applicable amendments	SC-20/512-024 Original Method Partial Validation Report ([redacted]) SC-20/512-024 Amendment 1, Method Partial Validation Report ([redacted])
Synopsis of amendment history	**Amendment 1** Updated Note-to-File 3 with amendments from [redacted]20[redacted]. Updated Quality Controls table cumulative statistics.

Table 15. Bioanalytical Method Life Cycle Information: hGH ADA ECL ([redacted] Project AR4575)

	Method validation #1 AR4575
Analyte	Anti-hGH Antibodies
Validation type	Full validation
Method ID	IC-P-1243 Original ([redacted]) IC-P-1243-02 Amendment 2 ([redacted])
Duration of time method is in use	[redacted]/20[redacted] - present
Bioanalytical site	[redacted]
Matrix	Human Serum
Platform	ECL
Format	A validated bridging format using biotinylated pituitary hGH as capture and Sulfo-Tag hGH as detection.
Stock reference, lot number, expiration date	Positive control (PC): Anti-hGH monoclonal antibody, Lot # 25.3.11, expiration date [redacted]20[redacted] Drug: WHO International Standard, Growth Hormone, NIBSC Code 80/505
Calibration range from the lower limit of quantitation (LLOQ) to the upper limit of quantitation (ULOQ)	N/A, qualitative assay
Matrix study population	Pediatric (3-13 years) human growth hormone deficient
Link to reports and applicable amendments	AR4575 Original Method Validation Report ([redacted]) Amendment 1 ([redacted]) Amendment 2 ([redacted])
Synopsis of amendment history	Amendment 1 In "Table 2: Cut point Calculations" (page 14 of Appendix 2 Statistical Methods and Results) of the original report, Mean NSB was corrected to Mean NC. Amendment 2 In view of the FDA recommendation and in order to reduce the risk of false positives, the hGH specificity false positive rate was changed from 0.1% to 1.0%.

Table 16. Bioanalytical Method Life Cycle Information: hGH ADA ECL ([redacted] Project OX-18/512-012)

	Method validation #1 OX-18/512-012
Analyte	Anti-hGH Antibodies
Validation type	Full validation
Method ID	BALAR18/038 Initial Release ([redacted]) BALAR18/038 Revision 2 ([redacted])
Duration of time method is in use	[redacted]/20[redacted] - present
Bioanalytical site	[redacted] UK
Matrix	Human Serum
Platform	ECL
Format	A validated bridging format using biotinylated pituitary hGH as capture and Sulfo-Tag hGH as detection.
Stock reference, lot number, expiration date	Positive control (PC): Anti-hGH monoclonal antibody, Lot # 120116/DS518, expiration date [redacted] Drug: WHO International Standard, Growth Hormone, NIBSC Code 80/505
Calibration range from the lower limit of quantitation (LLOQ) to the upper limit of quantitation (ULOQ)	N/A, qualitative assay
Matrix study population	Pediatric (3-13 years) human growth hormone deficient
Link to reports and applicable amendments	OX-18/512-012 Original Method Validation Report ([redacted])
Synopsis of amendment history	None

Table 17. Bioanalytical Method Life Cycle Information: hGH ADA ECL ([redacted] Project SC-20/512-025)

	Method validation #1 SC-20/512-025
Analyte	Anti-hGH Antibodies
Validation type	Partial validation
Method ID	GCL-666, BALAR18/038 Initial Release ([redacted])
Duration of time method is in use	[redacted]/20[redacted] - present
Bioanalytical site	[redacted] USA
Matrix	Human Serum
Platform	ECL
Format	A validated bridging format using biotinylated pituitary hGH as capture and Sulfo-Tag hGH as detection.
Stock reference, lot number, expiration date	Positive control (PC): Anti-hGH monoclonal antibody, Lot # 120116/DS518, expiration date [redacted] Drug: WHO International Standard, Growth Hormone, NIBSC Code 80/505
Calibration range from the lower limit of quantitation (LLOQ) to the upper limit of quantitation (ULOQ)	N/A, qualitative assay
Matrix study population	Pediatric (3-13 years) human growth hormone deficient
Link to reports and applicable amendments	SC-20/512-025 Original Method Validation Report ([redacted])
Synopsis of amendment history	None

Table 18. Bioanalytical Method Life Cycle Information: Somatrogon Neutralizing Cell-based NAb Assay ([redacted] Projects AR2862, AR4268 and AR5265)

	Method validation #1 AR2862	Method validation #2 AR4268	Method validation #3 AR5265
Analyte	Somatrogon Neutralizing Antibodies (NAb)	Somatrogon Neutralizing Antibodies (NAb)	Somatrogon Neutralizing Antibodies (NAb)
Validation type	Full validation	Partial validation	Full Requalification
Method ID	IC-P-0653 ([redacted]) IC-P-0653-03 Amendment 1 ([redacted])	IC-P-0653-03 ([redacted]) IC-P-0653-05 Amendment 1 ([redacted]) IC-P-0653-09 Addendum 1 ([redacted])	IC-P-0653 revision 01-05 ([redacted]) IC-P-0653-07 Addendum 1 ([redacted]) IC-P-0653-08 Addenda 2-4 ([redacted])
Duration of time method is in use	[redacted]/20[redacted] - [redacted]/20[redacted]	[redacted]/20[redacted] - Present	[redacted]/20[redacted] - Present
Bioanalytical site	**AR2862** [redacted]	**AR4268** [redacted] **Addendum 1** [redacted] USA	**AR5265** [redacted] **Addenda 1-4** [redacted] USA
Matrix	Human Serum		
Platform	Cell-based NAb assay		
Format	A validated cell-based NAb assay based on the ability of hGH/MOD-4023 to induce cell proliferation by binding to the human growth hormone receptor (hGHR) which is expressed on the cell surface of the BaFB2B2 murine cell line. Neutralizing anti-MOD-4023 (Somatrogon) antibodies in human serum will inhibit the GH-proliferative effect.		
Stock reference, lot number, expiration date	**Positive control (PC):** Rabbit affinity purified anti-MOD-4023, Lot# 90630, expiration date [redacted]20[redacted] Rabbit affinity purified anti-MOD-4023, Lot# 10325, expiration date [redacted]20[redacted]	**Positive control (PC):** Rabbit affinity purified anti-MOD-4023, Lot# 10325, expiration date [redacted]20[redacted] **Drug**:	**Positive control (PC):** Rabbit affinity purified anti-MOD-4023, Lot# 90630, expiration date [redacted]20[redacted] Rabbit affinity purified anti-MOD-4023, Lot# 10325, expiration date [redacted]20[redacted]

Table 18. Bioanalytical Method Life Cycle Information: Somatrogon Neutralizing Cell-based NAb Assay ([redacted] Projects AR2862, AR4268 and AR5265)

	Method validation #1 AR2862	Method validation #2 AR4268	Method validation #3 AR5265
	Drug: MOD-4023, Lot# EN648-01-[redacted]-005, expiration date [redacted]20[redacted] (Original)	MOD-4023, Lot# 4-11-X003-S1, expiration date [redacted]20[redacted] (Original) MOD-4023, Lot# 4-09-X003B-S2, expiration date [redacted]20[redacted] (Original) MOD-4023, Lot # 1039252, expiration: [redacted]20[redacted] (Addendum 1)	Rabbit affinity purified anti-MOD-4023, Lot# 101223, [redacted]20[redacted] (Addenda 1-4) **Drug**: MOD-4023, Lot# EN648-01-[redacted]-005, expiration date [redacted]20[redacted] (Original) MOD-4023, Lot# 1013202, expiration date [redacted]20[redacted] (Original) MOD-4023, Lot# 1019394, expiration date [redacted] 20[redacted] (Addenda 1-4)
Calibration range from the lower limit of quantitation (LLOQ) to the upper limit of quantitation (ULOQ)	N/A, qualitative assay	N/A, qualitative assay	N/A, qualitative assay
Matrix study population	Adult Normal and Pediatric GHD (3-13 years)	Adult Normal and Pediatric (3-13 years) human growth hormone deficient	Adult Normal and Pediatric (3-13 years) human growth hormone deficient
Link to reports and applicable amendments	AR2862 Original Method Validation Report ([redacted]) Amendment 1 ([redacted]) Amendment 2 ([redacted])	AR4268 Original Partial Validation Report ([redacted]) Amendment 1 ([redacted]) Addendum 1 ([redacted])	AR5265 Original Partial Validation Report ([redacted]) Addendum 1 ([redacted]) Addendum 2 ([redacted]) Addendum 3 ([redacted]) Addendum 4 ([redacted])
Synopsis of amendment history	**Amendment 1** • Replace %CV with Percent Relative Difference (RPD). • Estimated sensitivity was determined by comparing the mean OD of each level of the Positive Control to the cut point.	**Amendment 1** • Replaced referenced validation report AR2862 with the updated version AR5625, which summarized the validation work performed to validate the method for qualitative determination of	**Addendum 1** Additional parameters, some of them missing in the original validation, were tested: precision and re-establishment of PC ranges, selectivity in normal individuals, selectivity in hemolyzed and lipemic

Table 18. Bioanalytical Method Life Cycle Information: Somatrogon Neutralizing Cell-based NAb Assay ([redacted] Projects AR2862, AR4268 and AR5265)

	Method validation #1 AR2862	Method validation #2 AR4268	Method validation #3 AR5265
	The relative sensitivity information was added for reference. • Added Reference to the Qualification of MOD-4023 in Pediatric Growth Hormone Deficient (GHD) Serum ([redacted] #4268) • Other mirror corrections **Amendment 2** Note: For clarification purposes all corrections and modifications presented in the Amendment 1 Validation Report No. AR2862 were captured in an updated version, Validation Report No. AR5265. The original and amended validation reports (#s AR2862 and AR2862 Amendment 1, respectively) will be referenced in report # AR5265 for tracking. In addition, A panel of new 50 normal adult human serum individuals were re-evaluated and cut point was re-established under AR5265	neutralizing antibodies to MOD-4023 in human serum using a cell-based assay. • Updated the Bioanalytical Procedure listed in the original report (IC-P-0653-03) to the latest version, IC-P-0653-05. • Other mirror corrections. **Addendum 1** Partial validation addendum to [redacted] report AR4268, and includes an evaluation of assay Precision (across N=6 runs), establishment of new Cut Points using clinical pre-dose GHD pediatric serum samples from Phase 3 study CP-4-006, and determination of Sensitivity and Selectivity in diseased, hemolyzed, lipemic and icteric serum matrix.	individual samples, drug tolerance and bench top stability extended to 72 hours. Finally, Pre/Post% difference was calculated and used to confirm the 30.0% Difference criterion for positivity. **Addendum 2** Additional Drug Tolerance experiment. **Addendum 3** In-study cut point evaluation for the study CP-4-004 using drug-naïve pre-dose individual samples tested during the sample analysis. **Addendum 4** In-study cut point evaluation for the study CP-4-005 using drug-naïve pre-dose individual samples tested during the sample analysis.

Table 19. Bioanalytical Method Life Cycle Information: Somatrogon Neutralizing Cell-based NAb Assay (C0317007)

	Method validation C0317007
Analyte	Somatrogon Neutralizing Antibodies (NAb)
Validation type	Full validation
Method ID	C0317007 ([redacted] 15-2029)
Duration of time method is in use	[redacted]/20[redacted] - Present
Bioanalytical site	[redacted] U.S.A.
Matrix	Human Serum
Platform	Cell-Based NAb Assay
Format	A validated cell-based NAb assay based on the ability of hGH/MOD-4023 to induce cell proliferation by binding to the human growth hormone receptor (hGHR) which is expressed on the cell surface of the BaFB2B2 murine cell line. Neutralizing anti-MOD-4023 (Somatrogon) antibodies in human serum will inhibit the GH-proliferative effect.
Stock reference, lot number, expiration date	**Positive control (PC)**: Rabbit affinity purified anti-MOD-4023, Lot# PAb4023-001, expiration date [redacted]20[redacted] **Drug:** MOD-4023, Lot# AG8262, expiration date [redacted]20[redacted]
Calibration range from the lower limit of quantitation (LLOQ) to the upper limit of quantitation (ULOQ)	N/A, qualitative assay
Matrix study population	Adult Normal and Pediatric (3-13 years) human growth hormone deficient
Link to reports and applicable amendments	C0317007 Original Method Validtion Report ([redacted]20[redacted]) Amendments and Addendums: N/A
Synopsis of amendment history	N/A

Table 20. Bioanalytical Method Life Cycle Information: hGH Neutralizing Cell-based NAb Assay ([redacted] Projects AR2919, AR4266 and AR5266)

	Method validation #1 AR2919	Method validation #2 AR4266	Method validation #3 AR5266
Analyte	hGH Neutralizing Antibodies (NAb)	hGH Neutralizing Antibodies (NAb)	hGH Neutralizing Antibodies (NAb)
Validation type	Full validation	Full validation	Full validation
Method ID	IC-P-0654 ([redacted]) IC-P-0654-03 Amendment 1 ([redacted])	IC-P-1133 ([redacted]) Original IC-P-1133-01 ([redacted]) Amendment 1 IC-P-113-02 ([redacted]) Addenda 1-3	IC-P-0654 ([redacted]) IC-P-0654-01 ([redacted]) IC-P-0654-02 ([redacted]) IC-P-0654-03 ([redacted]) IC-P-0654-04 ([redacted]) IC-P-0654-05 ([redacted]) IC-P-0654-06 ([redacted]) IC-P-0654-07 ([redacted]) Addenda 1-3
Duration of time method is in use	[redacted]20[redacted] – [redacted]	[redacted] - Present	[redacted] - present
Bioanalytical site	**AR2919** [redacted]	**AR4266** [redacted] **Addenda 1-3** [redacted] USA	**AR5266** [redacted] **Addenda 1-3** [redacted] USA
Matrix	Human Serum		
Platform	Cell-based NAb assay		
Format	A validated cell-based NAb assay based on the ability of hGH to induce cell proliferation by binding to the human growth hormone receptor (hGHR) which is expressed on the cell surface of the BaFB2B2 murine cell. Neutralizing anti-hGH antibodies in human serum will inhibit the GH-proliferative effect.		

Table 20. Bioanalytical Method Life Cycle Information: hGH Neutralizing Cell-based NAb Assay ([redacted] Projects AR2919, AR4266 and AR5266)

	Method validation #1 AR2919	Method validation #2 AR4266	Method validation #3 AR5266
Stock reference, lot number, expiration date	**Positive control (PC):** Anti-hGH Polyclonal antibody, Lot# HRY0207051 **Drug**: WHO International Standard, Growth Hormone, NIBSC Code 80/505	**Positive control (PC):** Anti-hGH Polyclonal antibody, Lot# HRY0811121 (Original and Amendment 1) Anti-hGH Polyclonal antibody, Lot# HRY0816101, expiration date [redacted] (Addenda 1-3), [redacted] Anti-hGH Polyclonal antibody, Lot# 073019LBQ1, expiration date [redacted]20[redacted] (Addenda 3) [redacted] **Drug**: WHO International Standard, Growth Hormone, NIBSC Code 80/505	**Positive control (PC):** Anti-hGH Polyclonal antibody, Lot# HRY0207051 Anti-hGH Polyclonal antibody, Lot# HRY0811121 Anti-hGH Polyclonal antibody, Lot# HRY0815082 (Addendum 1) HRY0816101, expiration date [redacted] (Addenda 2-3), [redacted] **Drug**: WHO International Standard, Growth Hormone, NIBSC Code 80/505 MOD-4023, Lot# 1019394, expiration date [redacted]20[redacted]
Calibration range from the lower limit of quantitation (LLOQ) to the upper limit of quantitation (ULOQ)	N/A, qualitative assay	N/A, qualitative assay	N/A, qualitative assay
Matrix study population	Normal Adult	Pediatric (3-13 years) human growth hormone deficient	Normal Adult and Adult human growth hormone deficient

Table 20. Bioanalytical Method Life Cycle Information: hGH Neutralizing Cell-based NAb Assay ([redacted] Projects AR2919, AR4266 and AR5266)

	Method validation #1 AR2919	Method validation #2 AR4266	Method validation #3 AR5266
Link to reports and applicable amendments	AR2919 Original Method Validation Report ([redacted]) Amendment 1 ([redacted]) Amendment 2 ([redacted])	AR4266 Original Method Validation Report ([redacted]) Amendment 1 ([redacted]) Amendment 1, Addendum 1 ([redacted]) Amendment 1, Addendum 2 ([redacted]) Amendment 1, Addendum 3 ([redacted])	AR5266 Original Method Validation Report ([redacted]) Addendum 1 ([redacted]) Addendum 1, Amendment 1 ([redacted]) Addendum 1, Amendment 2 ([redacted]) Addendum 2 ([redacted]) Addendum 3 ([redacted])
Synopsis of amendment history	**Amendment 1** • Replace %CV with Percent Relative Difference (RPD). • Included relative sensitivity information. • Updated usable cell passage numbers from 2-14. • Other mirror corrections **Amendment 2** For clarification purposes all corrections and modifications presented in the Amendment 1 Validation Report No. AR2919 will be captured in an updated version, Validation Report No. AR5266. The original and amended validation reports (#s AR2919 and AR2919 Amendment 1, respectively) will be referenced in report # AR5266 for tracking.	**Amendment 1** • Updated the Bioanalytical Procedure listed in the original report (IC-P-1133) to the latest version, IC-P-1133-01. • Other mirror corrections **Amendment 1, Addendum 1** • Added Drug Tolerance for hGH and MOD-4023 **Amendment 1, Addendum 2** • In-study Cut Point evaluation for the study CP-4-004 pediatric GHD **Amendment 1, Addendum 3** • Evaluation of assay Precision (across N=6 runs), • Establishment of new Cut	**Addendum 1** • Additional parameters, some of them missing in the original validation, were tested: precision and re-establishment of PC ranges due to a new lot of anti-hGH Ab, selectivity in normal individuals, selectivity in hemolyzed and lipemic individual samples, drug tolerance and bench top stability extended to 72 hours. Finally, Pre/Post% difference was calculated and used to confirm the 30.0% difference criterion for positivity. **Addendum 1, Amendment 1** • Correct Inter-Assay %CV and nominal concentrations in Table D, which were entered incorrectly. • Conclusion: acceptance criteria for lower limit mean OD in was corrected for 0.391 µg/mL for each plate from 0.077 to 0.075

Table 20. Bioanalytical Method Life Cycle Information: hGH Neutralizing Cell-based NAb Assay ([redacted] Projects AR2919, AR4266 and AR5266)

	Method validation #1 AR2919	Method validation #2 AR4266	Method validation #3 AR5266
		Points using clinical pre-dose GHD pediatric serum samples from Phase 3 study CP-4-006 • Determination of sensitivity and selectivity in diseased, hemolysed, lipemic and icteric serum matrix.	**Addendum 1, Amendment 2** • Corrected typos in the PC ranges to be consistent with the correct values reported in Table D. **Addendum 2** • Added Drug Tolerance for MOD-4023 **Addendum 3** • In-study Cut Point evaluation for the study CP-4-005 Adult GHD

Table 21. Bioanalytical Method Life Cycle Information: hGH Neutralizing Cell-based NAb Assay (C0317008)

	Method validation C0317008
Analyte	hGH Neutralizing Antibodies (NAb)
Validation type	Full validation
Method ID	C0317008 ([redacted] 15-2030)
Duration of time method is in use	[redacted]/20[redacted] – Present
Bioanalytical site	[redacted] U.S.A.
Matrix	Human Serum
Platform	Cell-Based NAb Assay
Format	A validated cell-based NAb assay based on the ability of hGH to induce cell proliferation by binding to the human growth hormone receptor (hGHR) which is expressed on the cell surface of the BaFB2B2 murine cell. Neutralizing anti-hGH antibodies in human serum will inhibit the GH-proliferative effect.
Stock reference, lot number, expiration date	**Positive control (PC):** hGH Affinity Purified Polyclonal antibody, Lot# HRY0819041 or equivalent, expiration date [redacted]20[redacted] **Drug:** hGH, Lot# Not assigned by manufacturer, expiration date [redacted]20[redacted] MOD-4023, Lot# AG8262, expiration date [redacted]20[redacted]
Calibration range from the lower limit of quantitation (LLOQ) to the upper limit of quantitation (ULOQ)	N/A, qualitative assay
Matrix study population	Adult Normal and Pediatric (3-13 years) human growth hormone deficient
Link to reports and applicable amendments	C0317008 Original Method Validtion Report ([redacted]20[redacted]) Amendments and Addendums: N/A
Synopsis of amendment history	None

Table 22. Summary of Method Performance: Somatrogon ELISA PK ([redacted] Project AR2665)

Bioanalytical method validation report name, amendments and hyperlinks	Original [redacted] Report AR2665: Quantitative Determination of MOD-4023 in Human Serum using an Enzyme Linked Immunosorbent Assay (ELISA) AR2665 Addendum 1: Long Term Stability Studies for the Quantitative Determination of MOD-4023 in Human Serum using an Enzyme Linked Immunosorbent Assay (ELISA) AR2665 Addendum 2: Incurred Sample Reanalysis for the Quantitative Determination of MOD-4023 in Human Serum using an Enzyme Linked Immunosorbent Assay (ELISA)		
Method description	A validated sandwich ELISA format using anti-hGH monoclonal antibody as capture and biotinylated rabbit anti-CTP polyclonal antibody followed by streptavidin-HRP as detection		
Materials used for standard calibration curve and concentration	Somatrogon (MOD-4023), lot EN648-01-[redacted]-005, 20.54 mg/mL		
Validated assay range	1500 pg/mL to 120,000 pg/mL in undiluted human serum		
Material used for QCs and concentration	Somatrogon (MOD-4023), lot EN648-01-[redacted]-005, 20.54 mg/mL		
Minimum required dilutions (MRDs)	1:10		
Source and lot of reagents	Coat, monoclonal anti-hGH, 1.0 mg/mL, Lot# DS 2.1.08, [redacted], [redacted], Israel Rabbit anti-CTP polyclonal antibody, 3 mg/mL, Lot# 906021ctp, [redacted], [redacted], Israel		
Regression model and weighting	4 parameter logistic (4PL), no weighting		
Validation parameters	**Method validation summary**		**Source location**
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LLOQ to ULOQ	10[a]	Table 1 of Original Report AR2665
	Cumulative accuracy (%bias)[b] from LLOQ to ULOQ	Not Calculated	Table 1 of Original Report AR2665
	Cumulative precision (%CV) from LLOQ to ULOQ	1.3 to 10.6%	Table 1 of Original Report AR2665
Performance of QCs during accuracy and precision runs	**Cumulative accuracy (%bias)[b] in 5 QCs** (Low2, Low, Medium, High, and High2)	Not Calculated	Table 2 of Original Report AR2665
	Inter-batch %CV	10.0 to 25.6%	Table 2 of Original Report AR2665
	Total Error (TE)	Not Calculated	
Selectivity and matrix effect	Unspiked: 20/20 individual lots passed (BQL) Low (2000 pg/mL) spike: 5/10 individual lots were ±25% accuracy (8/10 were within ±30% accuracy) High (60000 pg/mL) spike: 3/10 individual lots were ±20% accuracy (8/10 were within ±25% accuracy)		Tables 5A of Original Report AR2665

Table 22. Summary of Method Performance: Somatrogon ELISA PK ([redacted] Project AR2665)

	Repeat High (60000 pg/mL) spike: 3/10 individual lots were ±20% accuracy (5/10 were within ±25% accuracy and 9/10 within ±30% accuracy)	Table 5B of Original Report AR2665
Interference and specificity	No additional interference tests were conducted because the method was only used in this first in human healthy volunteer study	N/A
Hemolysis effect	Not Assessed	N/A
Lipemic effect	Not Assessed	N/A
Dilution Linearity and hook effect	Dilution linearity was tested by spiking MOD-4023 stock standard into normal human serum at a concentration of 100 µg/mL. The first dilution was made in assay Diluent to yield 10,000,000 pg/mL. Three 1:10 dilutions were made in matrix diluent (10% human serum in Assay Diluent) to yield 1,000,000 pg/mL, 100,000 pg/mL and 10,000 pg/mL. Then, serial 2-fold dilutions were made in Matrix Dilution down to 19.5 pg/mL. No hook effect was seen in any of the above-range concentrations. The % accuracy was within 77.7% - 91.8% for all of the concentrations that fell within the quantitation limits.	Figure 3 of Original Report AR2665
Bench-top/process stability	24 hours in human serum at ambient temperature	Table 4 of Original Report AR2665
Freeze-Thaw stability	5 Freeze-Thaw cycles stability established at -70°C	Table 4 of Original Report AR2665
Long-term storage stability	6 Months at -70°C days established	Table 3 of Addendum 1 Report AR2665
Parallelism	N/A	
Carryover	N/A	

a. A total of 10 standard calibrators were included in each run as well as cumulative accuracy and precision statistics. However, there we no set standard calibrators at LLOQ and ULOQ. Rather, the lower limit of quantitation (LLOQ) for sample analysis was defined to be the lower 25% range of the Low QC (150 pg/mL, equivalent to 1500 pg/mL in undiluted sample); the upper limit of quantitation (ULOQ) was identified as the upper 20% range of the High QC (12,000 pg/mL, equivalent to 120,000 pg/mL in undiluted sample). With a MRD of 1:10, the assay range of quantitation is 1500 pg/mL to 120,000 pg/mL.
b. Bias refers to %RE as listed in the report.

Table 23. Summary of Method Performance: Somatrogon ECL PK ([redacted] Project AR3835)

Bioanalytical method validation report name, amendments and hyperlinks	Original [redacted] Report AR3835: Quantitative Determination of MOD-4023 in Human Serum using an Electrochemiluminescent (ECL) Assay AR3835 Addendum 1: Human Pediatric (Ages 3-13) Growth Hormone Deficient Serum Matrix Evaluation for the Quantitative Determination of MOD-4023 in Human Serum using an Electrochemiluminescent (ECL) Assay AR3835 Addendum 2: Long Term Stability Studies for the Quantitative Determination of MOD-4023 in Human Serum using an Electrochemiluminescent (ECL) Assay AR3835 Addendum 3: Long Term Stability Studies for the Quantitative Determination of MOD-4023 in Human Serum using an Electrochemiluminescent (ECL) Assay AR3835 Addendum 4: Partial validation to modify the standard curve and quality control concentrations. Additionally, selectivity in normal human, pediatric growth hormone deficient, lipemic and hemolyzed human serum was evaluated. AR3835 Addendum 5: Additional set of 20 pediatric (ages 3-13) growth hormone deficient human serum individuals evaluated for selectivity. AR3835 Addendum 6: Partial validation to modify standard curve and QC concentrations (Precision and Accuracy, Selectivity, Ruggedness and Robustness and Range of Quantitation). AR3835 Addendum 7: Partial validation to qualify a new biotinylated anti-hGH coating antibody for bioanalytical procedure
Method description	A validated sandwich ECL format using biotinylated anti-hGH monoclonal antibody as capture and Sulfo-Tag anti-CTP polyclonal antibody as detection.
Materials used for standard calibration curve and concentration	Somatrogon (MOD-4023), lot 4-09-X003B-S2, 5 mg/mL (Original, Addenda 1-3) Somatrogon (MOD-4023), lot 1019394, 40 mg/mL (Addenda 4-5) Somatrogon (MOD-4023), lot 1039252, 5 mg/mL (Addenda 6-7)
Validated assay range	150 pg/mL to 36,000 pg/mL in undiluted human serum (Original, Addenda 1-3) 156.25 pg/mL to 40,000 pg/mL in undiluted human serum (Addenda 4-5) 1.0 ng/mL to 128 ng/mL in undiluted human serum (Addenda 6-7)
Material used for QCs and concentration	Somatrogon (MOD-4023), lot 4-09-X003B-S2 (Original, Addenda 1-3) Somatrogon (MOD-4023), lot 1019394 (Addenda 4-5) Somatrogon (MOD-4023), lot 1039252, 5 mg/mL (Addenda 6-7)
Minimum required dilutions (MRDs)	1:5

Table 23. Summary of Method Performance: Somatrogon ECL PK ([redacted] Project AR3835)

Source and lot of reagents	Coat, monoclonal anti-hGH antibody, 2.46 mg/mL, Lot# DS 10.10.06, OPKO (formerly [redacted], [redacted], Israel) (Original, Addenda 2-3) Coat, monoclonal anti-hGH antibody, Lot # 25.3.11, 0.80 mg/mL, OPKO ([redacted]) (Addenda 1, 4-5) Coat, monoclonal anti-hGH antibody, Lot # MabGH-022, 2.2 mg/mL, OPKO (Addenda 6) Coat, Biotinylated Goat anti-hGH polyclonal antibody, Lot # HXA0914071, OPKO (Addenda 7) Rabbit anti-CTP polyclonal antibody, 2.24 mg/mL, Lot# 251209, OPKO (formerly [redacted], [redacted], Israel) (Original, Addenda 2-3) Rabbit anti-CTP polyclonal, Lot # 21114, 0.42 mg/mL, OPKO (formerly [redacted]) (Addenda 1, 4-5) Rabbit anti-CTP polyclonal, Lot # 150117, 1.9 mg/mL, OPKO (Addenda 6-7)		
Regression model and weighting	4 parameter logistic (4PL), no weighting (Original, Addenda 1-5) 4 parameter logistic (4PL), $1/Y^2$ (Addenda 6-7)		
Validation parameters	**Method validation summary**		**Source location**
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LLOQ to ULOQ[a]	12 9 8 8	Table 1 of Original Report AR3835 Table 1 of Addendum 4 Report AR3835 Table B of Addendum 6 Report AR3835 Table B of Addendum 7 Report AR3835
	Cumulative accuracy (%bias)[b] from LLOQ to ULOQ	Not Calculated -9.0 to 10.4% -2.5% to 10.4% -3.1 to 4.5%	Table 1 of Original Report AR3835 Table 1&A of Addendum 4 Report AR3835 Table B of Addendum 6 Report AR3835 Table B of Addendum 7 Report AR3835
	Cumulative precision (%CV) from LLOQ to ULOQ	≤ 7.6% ≤ 3.8% ≤ 8.8% ≤ 3.5%	Table 1 of Original Report AR3835 Table 1 of Addendum 4 Report AR3835 Table B of Addendum 6 Report AR3835 Table B of Addendum 7 Report AR3835
Performance of QCs during accuracy and precision runs	**Cumulative accuracy (%bias)[b] in 5 QCs** (Low2, Low, Medium, High, and High2) (LLOQ, LQC, MQC, HQC, ULOQ) (LLOQ, LQC, MQC, HQC, ULOQ)	-15.4 to 0.3% -2.2 to 0.6% 0.3 to 15.0%	Table 2 of Original Report AR3835 Table 2&B of Addendum 4 Report AR3835 Table C of Addendum 6 Report AR3835 Table C of Addendum 7 Report AR3835
	Inter-batch %CV	≤ 11.1% ≤ 9.1% ≤ 15.3% ≤ 8.7%	Table 2 of Original Report AR3835 Table 2&B of Addendum 4 Report AR3835 Table C of Addendum 6 Report AR3835 Table C of Addendum 7 Report AR3835

Table 23. Summary of Method Performance: Somatrogon ECL PK ([redacted] Project AR3835)

	Total Error (TE)	Not Calculated ≤ 24.2% ≤16.7 (ULOQ) ≤ 23.7 (LLOQ)	Table 2 of Original Report AR3835 Table 2 of Addendum 4 Report AR3835 Not reported. Calculated from Table C of Addendum 6 Report AR3835 Not reported. Calculated from Table C of Addendum 7 Report AR3835
Selectivity and matrix effect	Unspiked normal individual serum lots: 18/20 individual lots passed (BQL)		Tables 3 of Original Report AR3835
	Low (200 pg/mL): 10/10 normal individual lots were ±25% Recovery Medium (2000 pg/mL): 10/10 normal individual lots were ±20% Recovery High (15000 pg/mL): 0/10 normal individual lots were ±20% Recovery[C]		Table 5A of Original Report AR3835
	Repeat High (15000 pg/mL): 10/10 individual lots were ±20% Recovery		Table 5B of Original Report AR3835
	Unspiked normal individual serum lots: 10/10 individual lots passed (BQL)		Table 3 of Addendum 4 Report AR3835
	LLOQ (156.25 pg/mL): 10/10 normal individual lots were ±25% Recovery High (30000 pg/mL): 10/10 normal individual lots were ±20% Recovery		Table 4 of Addendum 4 Report AR3835
	Unspiked normal individual serum lots: 10/10 individual lots passed (BQL) LLOQ (1.0 ng/mL): 8/10 normal individual lots were ±25% Recovery High (48.0 ng/mL): 2/10 normal individual lots were ±20% Recovery		Table 5 of Addendum 6 Report AR3835
	Unspiked normal individual serum lots: 10/10 individual lots passed (BQL) LLOQ (1.0 ng/mL): 10/10 normal individual lots were ±25% Recovery High (48.0 ng/mL): 10/10 normal individual lots were ±20% Recovery		Table 6 of Addendum 7 Report AR3835
Interference and specificity	Unspiked Pediatric (Ages 3-13) Growth Hormone Deficient Human Serum 8/10 individual lots passed (BQL)		Table 5 of Addendum 1 Report AR3835
	Low (200 pg/mL): 10/10 Pediatric individual lots were ±25% Recovery High (30000 pg/mL): 10/10 Pediatric individual lots were ±20% Recovery		Table 4 of Addendum 1 Report AR3835
	Unspiked Pediatric (Ages 3-13) Growth Hormone Deficient Human Serum 10/10 individual lots passed (BQL)		Table 7A of Addendum 4 Report AR3835
	LLOQ (156.25 pg/mL): 5/10 Pediatric individual lots were ±25% Recovery High (30000 pg/mL): 7/10 Pediatric individual lots were ±20% Recovery		Table 7A of Addendum 4 Report AR3835
	Unspiked Pediatric (Ages 3-13) Growth Hormone Deficient Human Serum Repeat 10/10 individual lots passed (BQL)		Table 7B of Addendum 4 Report AR3835

Table 23. Summary of Method Performance: Somatrogon ECL PK ([redacted] Project AR3835)

	Repeat LLOQ (156.25 pg/mL): 9/10 Pediatric individual lots were ±25% Recovery Repeat High (30000 pg/mL): 9/10 Pediatric individual lots were ±20% Recovery	Table 7B of Addendum 4 Report AR3835
	Unspiked Pediatric (Ages 3-13) Growth Hormone Deficient Human Serum 28/30 individual lots passed (BQL) LLOQ (156.25 pg/mL): 27/30 (90%) Pediatric individual lots were ±25% Recovery High (30000 pg/mL): 21/30 (70%) Pediatric individual lots were ±20% Recovery (Note: 28/30 individual lots were within ±25% Recovery)	Table 3 of Addendum 5 Report AR3835
	Unspiked Pediatric (Ages 3-13) Growth Hormone Deficient Human Serum 10/10 individual lots passed (BQL) LLOQ (1.0 ng/mL): 9/10 Pediatric individual lots were ±25% Recovery High (48.0 pg/mL):6/10 Pediatric individual lots were ±20% Recovery	Table 5 of Addendum 6 Report AR3835
Hemolysis effect	Unspiked hemolysis individual serum lots: 6/6 individual lots passed (BQL) LLOQ (156.25 pg/mL): 6/6 hemolysis individual lots were ±25% Recovery High (30000 pg/mL): 2/6 hemolysis individual lots were ±20% Recovery Repeat High (30000 pg/mL): 6/6 hemolysis individual lots were ±20% Recovery	Table 6A of Addendum 4 Report AR3835 Table 6A of Addendum 4 Report AR3835 Table 6A of Addendum 4 Report AR3835 Table 6B of Addendum 4 Report AR3835
	Unspiked hemolysis individual serum lots: 2/2 individual lots passed (BQL) LLOQ (1.0 ng/mL): 0/2 hemolysis individual lots were ±25% Recovery High (48.0 ng/mL): 1/2 hemolysis individual lots were ±20% Recovery	Table 5 of Addendum 6 Report AR3835
Lipemic effect	Unspiked lipemic individual serum lots: 5/6 individual lots passed (BQL) LLOQ (156.25 pg/mL): 6/6 lipemic individual lots were ±25% Recovery High (30000 pg/mL): 6/6 lipemic individual lots were ±20% Recovery	Table 5 of Addendum 4 Report AR3835
	Unspiked lipemic individual serum lots: 2/2 individual lots passed (BQL) LLOQ (1.0 ng/mL): 0/2 lipemic individual lots were ±25% Recovery High (48.0 ng/mL): 0/2 lipemic individual lots were ±20% Recovery	Table 5 of Addendum 6 Report AR3835
Icteric effect	Unspiked icteric individual serum lots: 2/2 individual lots passed (BQL) LLOQ (1.0 ng/mL): 2/2 icteric individual lots were ±25% Recovery High (48.0 ng/mL): 2/2 icteric individual lots were ±20% Recovery	Table 5 of Addendum 6 Report AR3835

Table 23. Summary of Method Performance: Somatrogon ECL PK ([redacted] Project AR3835)

Dilution Linearity and hook effect	Dilution linearity was tested by spiking MOD-4023 stock standard into normal human serum at a concentration of 25 µg/mL. The first dilution was made in assay Diluent to yield 5,000,000 pg/mL. Three subsequent 1:10 dilutions were made in matrix diluent (20% human serum in Assay Diluent) to yield 1,000,000 pg/mL, 100,000 pg/mL and 10,000 pg/mL. Then, serial 2-fold dilutions were made in Matrix Dilution down to 9.77 pg/mL. No hook effect was seen in any of the above-range concentrations. The % accuracy was within 81% - 107% for all of the concentrations that fell within the quantitation limits.	Figure 3 of Original Report AR3835
Bench-top/process stability	24 hours in human serum at ambient temperature	Table 4 of Original Report AR3835
Freeze-Thaw stability	7 Freeze-Thaw cycles stability established at -70°C	Table 4 of Original Report AR3835
Long-term storage stability	6 Months at -70°C days established 6 Months at -20°C days established	Table 3 of Addendum 2 Report AR3835 Table 4 of Addendum 2 Report AR3835
	40 Months at -70°C days established 40 Months at -20°C days established	Table 3 of Addendum 3 Report AR3835 Table 4 of Addendum 3 Report AR3835
Parallelism	Not Assessed	
Carryover	N/A	

a. Original method validation included total 12 standard calibrators in each run as well as cumulative accuracy and precision statistics. However, there we no set standard calibrators at LLOQ and ULOQ. Rather, the lower limit of quantitation (LLOQ) for sample analysis was defined to be the lower 25% range of the Low QC (30.0 pg/mL, equivalent to 150 pg/mL in undiluted sample); the upper limit of quantitation (ULOQ) was identified as the upper 20% range of the High QC (7200 pg/mL, equivalent to 36,000 pg/mL in undiluted sample). With a MRD of 1:5 (20% normal human serum), the assay range of quantitation is 150-36,000 pg/mL. Addendum 4 Partial validation to modify the standard curve and quality control concentrations included standard calibrators and QCs at the set LLOQ and ULOQ concentration. The lower limit of quantitation (LLOQ) for sample analysis was identified as 31.25 pg/mL; the upper limit of quantitation (ULOQ) was identified 8000.00 pg/mL. With a MRD of 1:5, the assay range of quantitation is 156.25 – 40000.00 pg/mL.

b. Bias refers to %RE as listed in the report.

c. The failures were attributed to spiking small volumes of somatrogon drug into individual serum lots and performing multiple large dilutions leading to inaccuracies in recovery.

Table 24. Summary of Method Performance: Somatrogon ECL PK ([redacted] Project OX-19/512-016 and OX-19/512-015)

Bioanalytical method validation report name, amendments and hyperlinks	[redacted] Validation Report OX-19/512-016: Pre-Study Validation of a Test Method for the Measurement of MOD-4023 In Human Serum using an Electrochemiluminescence Method. Effective Date: [redacted]20[redacted] [redacted] Validation Report OX-19/512-015: Establishment of an Analytical Method for the Measurement of MOD4023 In Human Serum using an Electrochemiluminescence Method. Effective Date: [redacted]20[redacted]		
Method description	A validated sandwich ECL format using biotinylated anti-hGH monoclonal antibody as capture and Sulfo-Tag anti-CTP polyclonal antibody as detection.		
Materials used for standard calibration curve and concentration	Somatrogon (MOD-4023), lot 1039252, 5 mg/mL		
Validated assay range	156.25 pg/mL to 40,000 pg/mL in undiluted human serum		
Material used for QCs and concentration	Somatrogon (MOD-4023), lot 1039252		
Minimum required dilutions (MRDs)	1:5		
Source and lot of reagents	Coat, monoclonal anti-hGH monoclonal antibody, Lot# MAbGH-027, 1 mg/mL, OPKO Biologics Ltd, Israel Rabbit anti-CTP polyclonal antibody, Lot# 150117, 1.9 mg/mL OPKO Biologics Ltd, Israel		
Regression model and weighting	4 parameter logistic (4PL), $1/Y^2$		
Validation parameters	**Method validation summary**		**Source location**
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LLOQ to ULOQ	9	Table 1 of [redacted] Report OX-19/512-016
	Cumulative accuracy (%bias)[a] from LLOQ to ULOQ	-2.6 to 2.1%	Table 1 of [redacted] Report OX-19/512-016
	Cumulative precision (%CV) from LLOQ to ULOQ	≤ 5.8%	Table 1 of [redacted] Report OX-19/512-016
Performance of QCs during accuracy and precision runs	**Cumulative accuracy (%bias)[a] in 5 QCs** (LLOQ, LQC, MQC, HQC, ULOQ)	-1.1 to 6.5%	Table 4 of [redacted] Report OX-19/512-016
	Inter-batch %CV	≤ 9.4%	Table 4 of [redacted] Report OX-19/512-016
	Total Error (TE)	≤ 15.5	Table 4 of [redacted] Report OX-19/512-016
Selectivity and matrix effect	Unspiked Pediatric (Ages 3-13) Growth Hormone Deficient Human Serum 10/10 individual lots passed (BQL) Repeat LLOQ (156.25 pg/mL): 9/10 Pediatric individual lots were ±25% Recovery Repeat High (30000 pg/mL): 7/10 Pediatric individual lots were ±20% Recovery		Table 11 of [redacted] Report OX-19/512-016

Table 24. Summary of Method Performance: Somatrogon ECL PK ([redacted] Project OX-19/512-016 and OX-19/512-015)

	Unspiked Pediatric (Ages 3-13) Growth Hormone Deficient Human Serum 10/10 individual lots passed (BQL) Repeat LLOQ (156.25 pg/mL): 8/10 Pediatric individual lots were ±25% Recovery Repeat High (30000 pg/mL): 9/10 Pediatric individual lots were ±20% Recovery	Table 8 and 9 of [redacted] Report OX-19/512-015
Interference and specificity	Unspiked icteric individual serum lots: 3/3 individual lots passed (BQL) LLOQ (156.25 ng/mL): 3/3 icteric individual lots were ±25% Recovery High (30000 ng/mL): 3/3 icteric individual lots were ±20% Recovery	Table 8 of [redacted] Report OX-19/512-016
Hemolysis effect	Unspiked hemolysis individual serum lots: 3/3 individual lots passed (BQL) LLOQ (156.25 pg/mL): 2/3 hemolysis individual lots were ±25% Recovery High (30000 pg/mL): 2/3 hemolysis individual lots were ±20% Recovery	Table 6 of [redacted] Report OX-19/512-016
Lipemic effect	Unspiked lipemic individual serum lots: 3/3 individual lots passed (BQL) LLOQ (156.25 pg/mL): 3/3 lipemic individual lots were ±25% Recovery High (30000 pg/mL): 2/3 lipemic individual lots were ±20% Recovery	Table 7 of [redacted] Report OX-19/512-016
Dilution Linearity and hook effect	Prozone samples were prepared by spiking pooled serum to 2,000,000 pg/mL MOD-4023 and then serially diluting 1 in 2 seven times using neat serum to yield a total of 6 samples above the range of the assay and 2 samples within range. No hook effect was seen in any of the above-range concentrations.	Table 12 of [redacted] Report OX-19-512-016
	Dilution linearity was tested by spiking MOD-4023 into normal human serum at a concentration of 3125 pg/mL, 60,000 pg/mL and 30,00,000 pg/mL and diluting 1:10, 1:100 and 1:1000, respectively by two analysts. Cumulative accuracy (%bias)[a] range: 5.2 to 25.0%. Cumulative precision (%CV): ≤ 12.0%	Table 13 of [redacted] Report OX-19-512-016
Comparability (Cross-validation) between [redacted] and [redacted]	Comparability was demonstrated at [redacted] as 9 of 10 samples were ≤30% from original values obtained by [redacted] in study CP-4-004.	Table 11 of [redacted] Report OX-19-512-015
Bench-top/process stability	Not Reassessed	N/A
Freeze-Thaw stability	Not Reassessed	N/A
Long-term storage stability	Not Reassessed	N/A
Parallelism	Not Assessed	N/A
Carryover	N/A	N/A

a. Bias refers to %RE as listed in the report.

Table 25. Summary of Method Performance: Somatrogon ECL PK (C0319002)

Bioanalytical method validation report name, amendments, and hyperlinks	Pfizer Validation# C0319002 / [redacted] Project# 15-2026 The Validation of an ECL Method for the Determination of Somatrogon in Human Serum, Effective date: [redacted]20[redacted] The Cross Validation of an ECL Method for the Determination of Somatrogon in Human Serum, [redacted] Project 15-2054, Effective date: [redacted]20[redacted] [a]	
Method Description	An ECL assay was validated for quantifying Somatrogon (PF-06836922, MOD-4023) in human serum. Briefly, standards, controls, and test samples containing somatrogon were diluted 1:5 in assay buffer and incubated with biotinylated anti-hGH monoclonal antibody that was captured on the surface of a MSD Streptavidin Coated 96-Well Plate. After incubation, unbound material was washed away and somatrogon was detected with SulfoTag anti-CTP polyclonal antibody. The bound somatrogon complex was detected with MSD Read Buffer T (1X) and read on the MSD instrument. Somatrogon concentrations were determined on a standard curve obtained by plotting RLUs versus concentration using a 5-parameter logistic curve-fitting weighted $1/Y^2$. The range of quantitation established were based on the LLOQ and ULOQ QC samples with a MRD of 1:5, the assay range of quantitation is 1.00 – 128 ng/mL in 100% undiluted serum. Calibration standards outside the qualified range of the assay were included to serve as anchor points to facilitate curve fitting. Full details of the method including reagents and instruments are documented in the Method ID: 15-2026, which is appended to the method validation report. (Appendix 3, C0319002)	
	Matrix	Human Serum
	Anticoagulant	N/A
	Matrix Modification	None
	Sample Volume Required for Analysis	25 µL
	Method Type	ECL
	Instrumentation	MSD Sector Imager S600 or equivalent
	Data Acquisition and Processing Systems (Software and Version)	Watson™ LIMS Version 7.4.1 or equivalent
	Assay Signal (Units)	RLU
Materials used for standard calibration curve and concentration	PF-06836922 (Somatrogon) Lot #: 00711666-0021-WRM See Section 5.1 and Section 6.1 of [redacted] Report 15-2026 and 15-2054, respectively for additional details.	
Validated assay range	1.00 ng/mL to 128 ng/mL	
	LLOQ	1.00 ng/mL
	ULOQ	128 ng/mL
	Anchor points	0.5 ng/mL and 160 ng/mL

Table 25. Summary of Method Performance: Somatrogon ECL PK (C0319002)

Material used for quality controls (QCs) and concentration	PF-06836922 (Somatrogon) Lot #: 00711666-0021-WRM See Section 5.1 and Section 6.1 of [redacted] Report 15-2026 and 15-2054, respectively for additional details.		
QC Concentrations (in undiluted matrix, prior to MRD)	3.00, 12.0, 100, 2000 ng/mL (LQC, MQC, HQC, DQC)		
Minimum Required Dilution (MRD)	1:5		
Source and lot of reagents	See Section 5 and 6 of [redacted] Report 15-2026 and 15-2054, respectively for details		
Regression model and weighting	5-PL (Auto Estimate), $1/Y^2$		
Validation parameters	**Method validation summary**		**Source location**
Standard calibration curve performance during all validation runs	Number of standard calibrators from LLOQ to ULOQ	9	Table 3 of [redacted] Report 15-2026
	Cumulative accuracy (%bias) from LLOQ to ULOQ	-1.2 to 2.7 %RE	Table 3 of [redacted] Report 15-2026
	Cumulative precision (%CV) from LLOQ to ULOQ	≤ 3.3% CV	Table 3 of [redacted] Report 15-2026
Performance of QCs during accuracy and precision runs	**Cumulative accuracy (%bias) in 5 QCs**		Table 4 of [redacted] Report 15-2026
	QCLLOQ	2.0 %RE	
	QCL	2.0 %RE	
	QCM	0.0 %RE	
	QCH	5.0 %RE	
	QCULOQ	4.7 %RE	
	Inter-batch %CV		Table 4 of [redacted] Report 15-2026
	QCLLOQ	5.3 %CV	
	QCL	5.3 %CV	
	QCM	5.1 %CV	
	QCH	3.4 %CV	
	QCULOQ	4.3 %CV	
	Total Error (TE)		Table 4 of [redacted] Report 15-2026
	QCLLOQ	≤ 7.3%	
	QCL	≤ 7.3%	
	QCM	≤ 5.1%	
	QCH	≤ 8.4%	
	QCULOQ	≤ 9.0%	
Performance of QCs during all validation runs	**Cumulative accuracy and precision in QCL, QCM, QCH**		Table 5 of [redacted] Report 15-2026
	Accuracy (%RE)	0.0 to 6.0 %RE	
	Precision (%CV)	≤ 6.7 %CV	

Table 25. Summary of Method Performance: Somatrogon ECL PK (C0319002)

Selectivity & matrix effect	**Disease (Pediatric Human Growth Hormone Deficiency)** Unspiked QCLLOQ QCULOQ 10/10 sources (100%) of the unspiked matrix were BQL, 9/10 sources (90.0%) at LLOQ, and 9/10 sources (90.0%) at ULOQ were within ±25.0% RE	BQL -69.3 to 9.0 %RE -52.9 to 18.8 %RE		Table 6 of [redacted] Report 15-2026
Hemolysis effect	**Hemolysis Level Tested** Unspiked QCLLOQ QCULOQ 6/6 sources (100%) of the unspiked matrix were BQL, 6/6 sources (100%) at LLOQ, and 6/6 sources (100%) at ULOQ were within ±25.0% RE	BQL -14.5 to 2.0 % RE 2.3 to 21.9 % RE		Table 7 of [redacted] Report 15-2026
Lipemic effect	**Lipemic Level Tested** Unspiked QCLLOQ QCULOQ 6/6 sources (100%) of the unspiked matrix were BQL, 5/6 sources (83.3%) at LLOQ, and 5/6 sources (83.3%) at ULOQ were within ±25.0% RE	BQL -49.3 to -0.1 % RE -10.2 to 27.3 % RE		Table 8 of [redacted] Report 15-2026
Dilution linearity & hook effect	**DQC (2000 ng/mL)** 1-Fold (MRD) 50-Fold 100-Fold 200-Fold	%RE AQL 18.5% 8.5% 14.0%	%CV NA 3.0% 5.1% 10.5%	Table 10 of [redacted] Report 15-2026
	DQC (200ng/mL) 1-Fold (MRD) 2-Fold 5-Fold 10-Fold 20 Fold	%RE AQL 9.5% 9.0% 7.0% 7.0%	%CV NA 3.8% 4.4% 3.8% 4.0%	Table 9 of [redacted] Report 15-2026
Bench-top /process stability	24 hours at AmbientTemp°C			Table 11 of [redacted] Report 15-2026
Freeze-thaw stability	7 F/T Cycles at -20°C 7 F/T Cycles at -70°C			Table 12 of [redacted] Report 15-2026 Table 13 of [redacted] Report 15-2026

Table 25. Summary of Method Performance: Somatrogon ECL PK (C0319002)

Long-term storage stability	Established 40 months at -20°C and -70°C ([redacted] AR3835 Addendum 3)		N/A
Parallelism	TBD		N/A
Cross validation (Original [redacted] Report Results)	Total number of cross validation samples analyzed	285	Table 5 Figure 2 and Figure 3 of [redacted] Report 15-2054[a]
	Total number of evaluable cross validation sample results (excluding BLQ/Not Reportable results)	254	
	Total number of evaluable cross validation samples within ±30% difference	108/254 (43%)	
	Correlation (r)	0.843	
	Slope	>2	
Cross validation ([redacted] Repeat ISR-1)	Total number of cross validation samples analyzed	59	Table 6 Figure 4 of [redacted] Report 15-2054[a]
	Total number of evaluable cross validation sample results (excluding BLQ/ALQ samples)	55	
	Total number of evaluable cross validation samples within ±30% difference	51/55 (93%)	
Cross validation ([redacted] Repeat ISR-2)	Total number of cross validation samples analyzed	59	Table 6 Figure 4 of [redacted] Report 15-2054[a]
	Total number of evaluable cross validation sample results (excluding BLQ/ALQ samples)	54	
	Total number of evaluable cross validation samples within ±30% difference	53/54 (98%)	
Repeat Sample Analysis			Table 7 of [redacted] Report 15-2054[a]

a Source: Module5.3.5.1.2, Appendix16.2.5.10

Table 26. Summary of Method Performance: Somatrogon ECL ADA ([redacted] Project AR3165)

Bioanalytical method validation report name, amendments and hyperlinks	[redacted] Original Method Validation Report AR3165: Qualitative Determination of Antibodies to MOD-4023 in Human Serum Using an Electrochemiluminescence (ECL) Assay. Effective Date: [redacted] Report AR3165 Amendment 1 Effective Date: [redacted] Report AR3165 Amendment 2 Effective Date: [redacted] Report AR3165 Addendum 1 Effective Date: [redacted] Report AR3165 Addendum 1, Amendment 1 Effective Date: [redacted] Report AR3165 Addendum 2 Effective Date: [redacted] Report AR3165 Addendum 2, Amendment 1 Effective Date: [redacted] Report AR3165 Addendum 3 Effective Date: [redacted] Report AR3165 Addendum 3, Amendment 1 Effective Date: [redacted] Report AR3165 Addendum 4 Effective Date: [redacted] Report AR3165 Addendum 5 Effective Date: [redacted]
Method description	A validated bridging format using biotinylated MOD-4023 as capture and Sulfo-Tag MOD-4023 as detection.
Minimum required dilutions (MRDs)	1:10
Source and lot of reagents	Source: OPKO (formerly [redacted], [redacted], Israel) **Positive control (PC):** Rabbit affinity purified anti-MOD-4023, Lot#10325 Anti-poly CTP, Lot#21114, (Addendum 4) **Drug:** MOD-4023, Lot# 4-09-X003B-S1, (Original) MOD-4023, Lot# 4-09-X003B-S2, (Addenda 1-3) MOD-4023, Lot#1013202, (Addenda 4-5) **Specificity :** WHO International Standard, Growth Hormone, NIBSC Code 80/505 CTP, Lot # 11-03-0005, 20 mg/mL (Addendum 5)

Table 26. Summary of Method Performance: Somatrogon ECL ADA ([redacted] Project AR3165)

Validation parameters	Method validation summary				Source location
Positive Control (PC) Precision	**PC Concentration (100% serum)**	**Intra-Assay (%CV)**	**Inter-Assay (%CV)**	**Acceptance Range (RLU)**	Table 1 of Original Report AR3165
	2000 ng/mL	5.3%	30.2%	3001 – 7002	
	1000 ng/mL	6.8%	29.9%	1492 – 3481	
	250 ng/mL	5.3%	27.3%	397 - 927	
	PC Concentration (100% serum)	**Intra-Assay (%CV)**	**Inter-Assay (%CV)**	**Acceptance Range (RLU)**	Table 1 of Report AR3165 Addendum 1
	2000 ng/mL	5.7%	16.8%	2018 – 4708	
	1000 ng/mL	3.8%	11.2%	967 – 2257	
	250 ng/mL	11.6%	9.7%	281 – 655	
	PC Concentration (100% serum)	**Intra-Assay (%CV)**	**Inter-Assay (%CV)**	**Acceptance Range (RLU)**	Table 1 of Report AR3165 Addendum 2
	2000 ng/mL	2.9%	11.0%	5616 - 13105	
	1000 ng/mL	1.8%	8.5%	2516 – 5871	
	250 ng/mL	8.2%	9.9%	616 - 1437	
	PC Concentration (100% serum)	**Intra-Assay (%CV)**	**Inter-Assay (%CV)**	**Acceptance Range (RLU)**	Table 1 of Report AR3165 Addendum 3
	2000 ng/mL	10.6%	47.9%	6767 - 15789	
	1000 ng/mL	7.1%	47.0%	3250 - 7583	
	250 ng/mL	8.2%	44.1%	813 - 1897	
	PC Concentration (100% serum)	**Intra-Assay (%CV)**	**Inter-Assay (%CV)**	**Acceptance Range (RLU)**	Table 1 of Report AR3165 Addendum 5
	2000 ng/mL	9.2%	16.6%	2008 -4684	
	1000 ng/mL	3.9%	15.0%	1045 – 2439	
	250 ng/mL	5.7%	12.7%	278 - 650	
Negative Control (NC) Precision	**Cumulative precision (%CV)**		8.9%	42 - 127	Table 2 of Original Report AR3165
			14.1%	65 - 196	Table 2 of Report AR3165 Addendum 1
			15.2%	65 - 196	Table 2 of Report AR3165 Addendum 3
			3.5%	45 - 135	Table 2 of Report AR3165 Addendum 5
Screening Cut Point	85 RLU based on a 5% false positive rate				Table 2 of Original Report AR3165
	130 RLU based on a 5% false positive rate				Table 2 of Report AR3165 Addendum 1
	90 RLU based on a 5% false positive rate				Table 2 of Report AR3165 Addendum 5
Confirmatory (Specificity Cut points)	24% for 100 ng/mL MOD-4023 based on a 0.1% false positive rate 27% for 100 ng/mL hGH based on a 0.1% false positive rate				Table 9A of Original Report AR3165 Table 9B of Original Report AR3165

Table 26. Summary of Method Performance: Somatrogon ECL ADA ([redacted] Project AR3165)

	35% for 100 ng/mL CTP based on a 0.1% false positive rate			Table 5 of Report AR3165 Addendum 4
Sensitivity	1.6 ng/mL (16 ng/mL in 100% matrix)			Table 5 of Original Report AR3165
Drug Tolerance	**PC Concentration (100% serum)**	**MOD-4023 Concentration**	**Acceptance Criteria**	Table 6A of Original Report AR3165
	2000 ng/mL	1000 ng/mL	Response remained > cut point (85 RLUs)	
	1000 ng/mL	1000 ng/mL		
	250 ng/mL	100 ng/mL		
	PC Concentration (100% serum)	**hGH Concentration**	**Acceptance Criteria**	Table 6C of Original Report AR3165
	1820 ng/mL	1000 ng/mL	Response remained > cut point (85 RLUs)	
	910 ng/mL	1000 ng/mL		
	230 ng/mL	1000 ng/mL		
Selectivity and matrix effect	10 growth hormone deficiency (GHD) samples were diluted to MRD (1:10) and analyzed. All 10 samples were below the cut point (85 RLUs)			Table 10 of Original Report AR3165
Interference and specificity	1000 ng/mL and 28 ng/mL of human serum albumin (HAS) showed no interference			Table 7 of Original Report AR3165
	100, 50, 10, 1 and 0.1 ng/mL of human IgG showed no interference			Table 8 of Original Report AR3165
Hemolysis effect	Not assessed			N/A
Lipemic effect	Not assessed			N/A
Dilution Linearity and hook effect	No hook effect observed starting at 4000 ng/mL			Table 5 of Original Report AR3165
Bench-top/process stability	24 hours in human serum at ambient temperature			Table 4 of Original Report AR3165
Freeze-Thaw stability	5 Freeze-Thaw cycles stability established at -70°C			Table 4 of Report AR3165 Amendment 1

Table 27. Summary of Method Performance: Somatrogon ECL ADA ([redacted] Project AR5374)

Bioanalytical method validation report name, amendments and hyperlinks	For clarification purposes all corrections and modifications presented in Validation Report No. AR3165 (including all amendments and addendum reports) were captured in an updated version, Validation Report No. AR5374. [redacted] Original Method Validation Report AR5374: Qualitative Determination of Antibodies to MOD-4023 in Human Serum Using an Electrochemiluminescence (ECL) Assay. Effective Date: [redacted]20[redacted] [redacted] Report AR5374 Addendum 1 Effective Date: [redacted]20[redacted]				
Method description	A validated bridging format using biotinylated MOD-4023 as capture and Sulfo-Tag MOD-4023 as detection.				
Minimum required dilutions (MRDs)	1:10				
Source and lot of reagents	Source: OPKO (formerly [redacted], [redacted], Israel) **Positive control (PC):** Rabbit affinity purified anti-MOD-4023, Lot#10325, (Original) Rabbit affinity purified anti-MOD-4023, Lot#270415 (Addendum 1) Anti-poly CTP, Lot#21114 **Drug:** MOD-4023, Lot#1013202 (Original) MOD-4023, Lot#1019394 (Addendum 1) **Specificity**: CTP, Lot # 11-03-0005 WHO International Standard, Growth Hormone, NIBSC Code 80/505				
Validation parameters	**Method validation summary**				**Source location**
Positive Control (PC) Precision	**PC Concentration (100% serum)**	**Intra-Assay (%CV)**	**Inter-Assay (%CV)**	**Acceptance Range (RLU)**	Table 1 of Original [redacted] Report AR5374
	2000 ng/mL	9.2%	16.6%	2008 -4684	
	1000 ng/mL	3.9%	15.0%	1045 – 2439	
	250 ng/mL	5.7%	12.7%	278 - 650	
	PC Concentration (100% serum)	**Intra-Assay (%CV)**	**Inter-Assay (%CV)**	**Acceptance Range (RLU)**	Table A and Table 1 of [redacted] Report AR5374 Addendum 1
	2000 ng/mL	4.7%	13.7%	1574 – 2758	
	1000 ng/mL	5.2%	12.9%	777 – 1315	
	125 ng/mL	4.0%	10.8%	133 - 207	
Negative Control (NC) Precision	N/A	N/A	3.5%	45 - 135	Table 2 of Original [redacted] Report AR5374
		4.5%	13.9%	≤85	Table 2 of [redacted] Report AR5374 Addendum 1
Screening Cut Point	Dynamic Cut Point: 90 RLU based on a 5% false positive rate				Table 2 of Original [redacted] Report AR5374
	Floating Cut Point: 8.5 (cut point factor) Mean RLU of Negative Control (NC) + Cut Point Factor				Appendix 1 of [redacted] Report AR5374 Addendum 1

Table 27. Summary of Method Performance: Somatrogon ECL ADA ([redacted] Project AR5374)

Confirmatory (Specificity Cut points)	24% for 100 ng/mL MOD-4023 based on a 0.1% false positive rate 27% for 100 ng/mL hGH based on a 0.1% false positive rate 35% for 100 ng/mL CTP based on a 0.1% false positive rate			Table 6 of Original [redacted] Report AR5374 Table 7 of Original [redacted] Report AR5374 Table 8 of Original [redacted] Report AR5374
Confirmatory (Specificity Cut points)	19% for 100 ng/mL MOD-4023 based on a 1% false positive rate 22% for 100 ng/mL hGH based on a 1% false positive rate 27% for 100 ng/mL CTP based on a 1% false positive rate			Attachment 1 – Bioanalytical Procedure IC-P-0751-06 of [redacted] Report AR5374 Addendum 1 Supporting Data located in Original [redacted] Report AR5374
Sensitivity	16 ng/mL in 100% matrix			Table 5 of Original [redacted] Report AR5374 Table 4 of [redacted] Report AR5374 Addendum 1
Drug Tolerance	**PC Concentration (100% serum)**	**MOD-4023 Concentration**	**Acceptance Criteria**	Table 9A of Original [redacted] Report AR5374
	2000 ng/mL	1000 ng/mL	Response remained > cut point (85 RLUs)	
	1000 ng/mL	1000 ng/mL		
	250 ng/mL	100 ng/mL		
	PC Concentration (100% serum)	**hGH Concentration**	**Acceptance Criteria**	Table 10A of Original [redacted] Report AR5374
	1820 ng/mL	1000 ng/mL	Response remained > cut point (85 RLUs)	
	910 ng/mL	1000 ng/mL		
	230 ng/mL	1000 ng/mL		
Selectivity and matrix effect	10 growth hormone deficiency (GHD) samples were diluted to MRD (1:10) and analyzed. All 10 samples were below the cut point (85 RLUs)			Table 13 of Original [redacted] Report AR5374
	No matrix effect observed in unspiked and spiked PC at 2000 ng/mL and 125 ng/mL into 20 (10 male/10 female) normal human serum individuals			Table 6C of [redacted] Report AR5374 Addendum 1
Interference and specificity	1000 ng/mL and 28 ng/mL of human serum albumin (HAS) showed no interference			Table 11 of Original [redacted] Report AR5374
	100, 50, 10, 1 and 0.1 ng/mL of human IgG showed no interference			Table 12 of Original [redacted] Report AR5374
Hemolysis effect	No hemolysis effect observed in the 2000 ng/mL and 125 ng/mL PC			Table 6A of Report [redacted] AR5374 Addendum 1
Lipemic effect	No lipemic effect observed in the 2000 ng/mL and 125 ng/mL PC			Table 6B of Report [redacted] AR5374 Addendum 1
Dilution Linearity and hook effect	No hook effect observed starting at 4000 ng/mL			Table 5 of Original [redacted] Report AR5374
Bench-top/process stability	24 hours in human serum at ambient temperature			Table 4 of Original [redacted] Report AR5374
	24 hours in human serum at ambient temperature 72 hours in human serum at 2-8°C			Table 5A of [redacted] Report AR5374 Addendum 1
Freeze-Thaw stability	5 Freeze-Thaw cycles stability established at -70°C 6 Freeze-Thaw cycles stability established at -70°C			Table 4 of Original [redacted] Report AR5374 Table 5B of [redacted] Report AR5374 Addendum 1

Table 27. Summary of Method Performance: Somatrogon ECL ADA ([redacted] Project AR5374)

Robustness	**PC Concentration (100% serum)**	**Intra-Assay (%CV)**	**Inter-Assay (%CV)**	**Acceptance Range (RLU)**	Table 14A of Original [redacted] Report AR5374
	2000 ng/mL	10.6%	47.9%	6767 - 15789	
	1000 ng/mL	7.1%	47.0%	3250 - 7583	
	250 ng/mL	8.2%	44.1%	813 - 1897	
	Negative Control		15.2%	65 - 196	Table 14B of Original [redacted] Report AR5374

Table 28. Summary of Method Performance: Somatrogon ECL ADA ([redacted] Project AR4263 and AR5303)

Bioanalytical method validation report name, amendments and hyperlinks	[redacted] Original Method Validation Report AR4263: Qualitative Determination of Antibodies to MOD-4023 in Pediatric Human Growth Hormone Deficient Human Serum Using an Electrochemiluminescence (ECL) Assay. Effective Date: [redacted]20[redacted] Report AR4263 Addendum 1 Effective Date: [redacted]20[redacted]. Updated screening cut point factor from cut point constant. Report AR5303 Effective Date: [redacted]20[redacted]. AR5303 is just rereporting of AR4263 original and addendum 1. Report AR5303 Amendment 1 Effective Date: [redacted]20[redacted]. Updated specificity cut points to 1% false positive rate. Report AR4263 Evaluation Report Effective Date: [redacted]20[redacted]. Evaluation of drug tolerance and selectivity with pediatric GHD individuals, as well as hemolyzed and lipemic samples.				
Method description	A validated bridging format using biotinylated MOD-4023 as capture and Sulfo-Tag MOD-4023 as detection.				
Minimum required dilutions (MRDs)	1:10				
Source and lot of reagents	Source: [redacted], [redacted], Israel **Positive control (PC):** Rabbit affinity purified anti-MOD-4023, Lot#10325 (Original) Anti-poly CTP, Lot# 21114 **Drug**: MOD-4023, Lot# 4-11-X003-S1 (Original) **Specificity**: WHO International Standard, Growth Hormone, NIBSC Code 80/505				
Validation parameters	**Method validation summary**				**Source location**
Positive Control (PC) Precision	**PC Concentration (100% serum)**	**Intra-Assay (%CV)**	**Inter-Assay (%CV)**	**Acceptance Range (RLU)**	Table 3 & A of Original Report AR4263
	2000 ng/mL	7.2%	11.9%	5877 – 17631	
	500 ng/mL	5.4%	9.5%	1344 – 4032	
	125 ng/mL	6.1%	6.8%	336 - 1009	
Negative Control (NC) Precision	N/A	5.1%	8.8%	≤80	Table 5 & B of Original Report AR4263
Screening Cut Point (GHD pediatric)	Cut Point Constant: 40 (based on a 5% false positive rate) Mean RLU of Negative Control (NC) + Cut Point Constant				Table 4 &C of Original Report AR4263
	Cut Point Factor: 1.096 (based on a 5% false positive rate) Mean RLU of Negative Control (NC) x Cut Point Factor				Table C and Appendix 3 of Report AR4263 Addendum 1

Table 28. Summary of Method Performance: Somatrogon ECL ADA ([redacted] Project AR4263 and AR5303)

Confirmatory (Specificity Cut points)	22% for 1000 ng/mL MOD-4023 based on a 0.1% false positive rate 24% for 1000 ng/mL CTP based on a 0.1% false positive rate 22% for 1000 ng/mL hGH based on a 0.1% false positive rate			Table 8 of Original Report AR4263 Table 9 of Original Report AR4263 Table 10 of Original Report AR4263
	17% for 1000 ng/mL MOD-4023 based on a 1% false positive rate 17% for 1000 ng/mL CTP based on a 1% false positive rate 17% for 1000 ng/mL hGH based on a 1% false positive rate			Table 8 of Report AR5303 Amendment 1 Table 9 of Report AR5303 Amendment 1 Table 10 of Report AR5303 Amendment 1
Sensitivity	8.0 ng/mL in 100% matrix 0.5 ng/mL in 100% matrix			Table 7 of Original Report AR4263 Table 7 of Report AR4263 Addendum 1
Drug Tolerance	**PC Concentration (Anti-MOD-4023)**	**MOD-4023 Concentration**	**Acceptance Criteria**	Table 11 of Original Report AR4263
	2000 ng/mL	1000 ng/mL	Response remained > cut point (110 RLUs)	
	500 ng/mL	1000 ng/mL		
	125 ng/mL	100 ng/mL		
	PC Concentration (Anti-MOD-4023)	**hGH Concentration**	**Acceptance Criteria**	Table 12 of Original Report AR4263
	2000 ng/mL	1000 ng/mL	Response remained > cut point (110 RLUs)	
	500 ng/mL	1000 ng/mL		
	125 ng/mL	1000 ng/mL		
	PC Concentration (Anti-CTP)	**MOD-4023 Concentration**	**Acceptance Criteria**	Table 13 of Original Report AR4263
	5000 ng/mL	1000 ng/mL	Response remained > cut point (110 RLUs)	
	2000 ng/mL	1000 ng/mL		
	PC Concentration (Anti-MOD-4023)	**MOD-4023 Concentration**	**Acceptance Criteria**	Table 2 of AR4263 Evaluation Report
	2000 ng/mL	25 µg/mL	Response remained > cut point (64 RLUs)	
	500 ng/mL	25 µg/mL		
	125 ng/mL	25 µg/mL		
	100 ng/mL	2.5 µg/mL		
	50 ng/mL	0 ng/mL		
Selectivity and matrix effect	No matrix effect observed in unspiked and spiked LPC at 125 ng/mL into 10 pediatric GHD human serum individuals			Table 3 of AR4263 Evaluation Report
Interference and specificity	1000 ng/mL and 28 ng/mL of human serum albumin (HAS) showed no interference			Table 14 of Original Report AR4263
	100, 50, 10, 1 and 0.1 ng/mL of human IgG showed no interference			Table 15 of Original Report AR4263
Hemolysis effect	No hemolysis effect observed in unspiked and spiked LPC at 125 ng/mL			Table 3 of AR4263 Evaluation Report

Table 28. Summary of Method Performance: Somatrogon ECL ADA ([redacted] Project AR4263 and AR5303)

Lipemic effect	No lipemic effect observed in unspiked and spiked LPC at 125 ng/mL	Table 3 of AR4263 Evaluation Report
Dilution Linearity and hook effect	No hook effect observed starting at 2000 ng/mL	Table 7 of Original Report AR4263
Bench-top/process stability	24 hours in human serum at ambient temperature	Table E and 6 of Original Report AR4263
Freeze-Thaw stability	5 Freeze-Thaw cycles stability established at -70°C	Table E and 6 of Original Report AR4263
Robustness	Inter- and intra-assay variability was assessed under the following parameters: 3 analysts performed the assay, 2 lots of blocking buffers were used, 6 lots of wash buffer, 2 positive control lots, and blocking time ranged from 1 hour 30 minutes to 2 hours 49 minutes	Table 16 of Original Report AR4263

Table 29. Summary of Method Performance: Somatrogon ECL ADA ([redacted] Project OX-18-512-011)

Bioanalytical method validation report name, amendments and hyperlinks	[redacted] Method Validation Report OX-18-512-011: Validation of an Analytical Method to Screen and Confirm Antibodies to MOD-4023 in GHD Paediatric Serum using Electrochemiluminescence. Effective Date [redacted] 20[redacted] Report OX-18-512-011, Amendment 01, Effective Date [redacted] 20[redacted]: Correct duplication of subject IDs on page 71 (formerly page 70) in Appendix 1.				
Method description	A validated bridging format using biotinylated MOD-4023 as capture and Sulfo-Tag MOD-4023 as detection.				
Minimum required dilutions (MRDs)	1:10				
Source and lot of reagents	Source: OPKO Biologics **Positive controls (PCs):** Rabbit affinity purified Anti-MOD-4023, lot# PAb4023-001 Anti-poly CTP, Lot#150117/Covance **Drug:** MOD-4023, Lot 1039252 **Specificity:** CTP, Lot GCP1011A107 WHO International Standard, Human Growth Hormone, NIBSC Code 80/505				
Validation parameters	**Method validation summary**				**Source location**
Positive Control (PC) Precision	**PC Concentration (100% serum)**	**Intra-Assay (%CV)**	**Inter-Assay (%CV)**	**Acceptance Range (RLU)**	
	2000 ng/mL (HPC)	<23.1%	17.3%	3143 – 8653	Table 12 of [redacted] Report OX-18-512-011
	100 ng/mL (MPC)	<19.6%	13.9%	141 – 310	Table 11 of [redacted] Report OX-18-512-011
	30 ng/mL (LPC)	<9.5%	9.6%	68 – 115	Table 9 of [redacted] Report OX-18-512-011
Negative Control (NC) Precision	N/A	<7.4%	6.4%	≤65	Table 8 of [redacted] Report OX-18-512-011
Screening Cut Point (GHD pediatric)	1.07 (cut point factor) based on 5% false positive rate Mean RLU of Negative Control (NC) x Cut Point Factor				Table 1 and ANNEX of [redacted] Report OX-18-512-011
Titer cut point	1.18 (cut point factor) based on 0.1% false positive rate Mean RLU of Negative Control (NC) x Cut Point Factor				Table 1 and ANNEX of [redacted] Report OX-18-512-011
Confirmatory (Specificity Cut points)	13.2% for 1000 ng/mL MOD-4023 based on a 1% false positive rate 9.0% for 1000 ng/mL CTP based on a 1% false positive rate 8.7% for 1000 ng/mL hGH based on a 1% false positive rate				Table 2 of [redacted] Report OX-18-512-011 Table 4 of [redacted] Report OX-18-512-011 Table 5 of [redacted] Report OX-18-512-011
Sensitivity	5.6 ng/mL in 100% matrix				Table 3 of [redacted] Report OX-18-512-011

Table 29. Summary of Method Performance: Somatrogon ECL ADA ([redacted] Project OX-18-512-011)

Drug Tolerance	PC Concentration (Anti-MOD-4023)	MOD-4023 Concentration	Acceptance Criteria	
	2000 ng/mL	>5000 ng/mL	Response remained > screening cut point (65 RLU)	Table 14 of [redacted] Report OX-18-512-011
	100 ng/mL	2500 ng/mL		
	30 ng/mL	100 ng/mL		
Selectivity and matrix effect	10/10 individuals at the LPC and HPC screened and confirmed positive. Unspiked samples all confirmed negative. % differences between pooled serum and individuals were within the acceptance criteria.			Table 6 of [redacted] Report OX-18-512-011
Interference and specificity	**Haemolysed, icteric and lipaemic samples** The selectivity matrices at the LoPC and HPC screened and confirmed positive. The % difference between pooled serum and individuals for unspiked and spiked with HPC were within acceptance criteria. The % difference between pooled serum and individuals spiked with the LoPC was >20% for individuals. This parameter is considered a conditional pass since for ADA analysis, which is qualitative in nature, the 20% difference criteria are too strict. The positive / negative status of all the haemolysed, icteric and lipaemic selectivity samples were within acceptance criteria.			Table 7 of [redacted] Report OX-18-512-011
Dilution Linearity and hook effect	No hook effect observed starting at 2000 ng/mL			Table 3 of [redacted] Report OX-18-512-011
Bench-top/process stability	24 hours in human serum at ambient temperature			Table 15 of [redacted] Report OX-18-512-011
	72 hours in human serum at 4°C			Table 16 of [redacted] Report OX-18-512-011
Freeze-Thaw stability	6 Freeze-Thaw cycles stability established at -70°C			Table 17 of [redacted] Report OX-18-512-011
Robustness	Assay robustness was investigated by making deliberate variations in assay parameters to provide an indication of reliability during normal use. The effect on the assay was assessed using the NCS, LoPC, MePC and HiPC samples. Sample incubation times were varied by approximately ±20% of the normal assay time, for the following parameters: confirmatory sample incubation, Master mix incubation and online plate incubation.			Table 18 of [redacted] Report OX-18-512-011

Table 30. Summary of Method Performance: Somatrogon ECL ADA ([redacted] Project SC-20/512-024)

Bioanalytical method validation report name, amendments and hyperlinks	[redacted] Method Validation Report SC-20/512-024: GCL665 Partial Validation of an Analytical Method to Screen and Confirm Antibodies to MOD-4023 in GHD Pediatric Serum using Electrochemiluminescence. Effective Date: [redacted]20[redacted] Report SC-20/512-024, Amendment 1, Effective Date [redacted]20[redacted]: Updated Note-to-File 3 with amendments from [redacted]20[redacted], and updated Quality Controls table cumulative statistics for correctness of record.				
Method description	A validated bridging format using biotinylated MOD-4023 as capture and Sulfo-Tag MOD-4023 as detection.				
Minimum required dilutions (MRDs)	1:10				
Source and lot of reagents	Source: OPKO Biologics **Positive controls (PCs):** Rabbit affinity purified Anti-MOD-4023, lot# PAb4023-001 Anti-poly CTP, Lot#150117 **Drug:** MOD-4023, Lot 1039252 **Specificity:** CTP, Lot GCP1011A107 WHO International Standard, Human Growth Hormone, NIBSC Code 80/505				
Validation parameters	**Method validation summary**				**Source location**
Positive Control (PC) Precision	**PC Concentration (100% serum)**	**Intra-Assay (%CV)**	**Inter-Assay (%CV)**	**Acceptance Range (RLU)**	
	2000 ng/mL (HPC)	<20.0%	16.7%	5632 – 12810	Table 2 and Table 8 of [redacted] Report SC-20/512-024
	100 ng/mL (MPC)	<15.8%	12.0%	191 – 417	Table 2 and Table 8 of [redacted] Report SC-20/512-024
	30 ng/mL (LPC)	<9.7%	10.1%	76 – 131	Table 2 and Table 8 of [redacted] Report SC-20/512-024
Negative Control (NC) Precision	N/A	<7.7%	8.6%	≤69	Table 2 and Table 8 of [redacted] Report SC-20/512-024
Screening Cut Point (GHD pediatric)	1.07 (cut point factor) based on 5% false positive rate Mean RLU of Negative Control (NC) x Cut Point Factor				Table 3 and Table 4 of [redacted] Report SC-20/512-024
Titer cut point	1.18 (cut point factor) based on 0.1% false positive rate Mean RLU of Negative Control (NC) x Cut Point Factor				Table 1 and ANNEX of [redacted] Report OX-18-512-011
Confirmatory (Specificity Cut points)	13.2% for 1111 ng/mL MOD-4023 based on a 1% false positive rate 21.4% for 1111 ng/mL CTP based on a 1% false positive rate 22.6% for 1111 ng/mL hGH based on a 1% false positive rate				Table 3 and Table 4 of [redacted] Report SC-20/512-024 Table 5 of [redacted] Report SC-20/512-024 Table 5 of [redacted] Report SC-20/512-024
Sensitivity	1.8 ng/mL in 100% matrix				Table 7 of [redacted] Report SC-20/512-024

Table 30. Summary of Method Performance: Somatrogon ECL ADA ([redacted] Project SC-20/512-024)

Selectivity and matrix effect	10/10 individuals at the LPC screened and confirmed positive. 9/10 unspiked samples confirmed negative. % differences between pooled serum and individuals were within the acceptance criteria.	Table 9 of [redacted] Report SC-20/512-024
Cross-validation	Cross validation of samples run in [redacted] for Sponsor Study CP-4-006 (Table 10) showed 97% concordance in ADA results between [redacted] and [redacted].	Table 10 of [redacted] Report SC-20/512-024

Table 31. Summary of Method Performance: hGH ECL ADA ([redacted] Project AR4575)

Bioanalytical method validation report name, amendments and hyperlinks	[redacted] Original Method Validation Report AR4575: Qualitative Determination of Antibodies to Human Growth Hormone (hGH) in Pediatric Human Growth Hormone Deficient Human Serum Using an Electrochemiluminescence (ECL) Assay. Effective Date: [redacted]20[redacted] Report AR4575 Amendment 1 Effective Date: [redacted]20[redacted]. In "Table 2: Cut point Calculations" (page 14 of Appendix 2 Statistical Methods and Results) of the original report, Mean NSB was corrected to Mean NC. Report AR4575 Amendment 2 Effective Date: [redacted]20[redacted]. Updated hGH specificity cut point to 1% false positive rate				
Method description	A validated bridging format using biotinylated pituitary hGH as capture and Sulfo-Tag hGH as detection.				
Minimum required dilutions (MRDs)	1:10				
Source and lot of reagents	Source: [redacted], [redacted] Israel **Positive control (PC)**: Anti-hGH monoclonal antibody, Lot # 25.3.11 **Drug**: WHO International Standard, Growth Hormone, NIBSC Code 80/505				
Validation parameters	**Method validation summary**				**Source location**
Positive Control (PC) Precision	**PC Concentration (100% serum)**	**Intra-Assay (%CV)**	**Inter-Assay (%CV)**	**Acceptance Range (RLU)**	Table A & 3 of Original Report AR4575
	2000 ng/mL (HPC)	4.7%	26.4%	1733 – 5200	
	500 ng/mL (MPC)	4.1%	24.7%	367 – 1101	
	125 ng/mL (LPC)	2.8%	19.6%	103 – 310	
Negative Control (NC) Precision	N/A	3.5%	11.6%	≤104	Table B & 5 of Original Report AR4575
Screening Cut Point (GHD pediatric)	1.358 (cut point factor) based on 5% false positive rate Mean RLU of Negative Control (NC) x Cut Point Factor				Appendix 2 of Original Report AR4575
Titer cut point	1.358 (same as screening cut point)				N/A
Confirmatory (Specificity Cut points)	17% for 1000 ng/mL hGH based on a 0.1% false positive rate 12% for 1000 ng/mL hGH based on a 1% false positive rate				Table 8 of Original Report AR4575 Table 8 of Report AR4575 Amendment 2
Sensitivity	15.6 ng/mL (in neat serum)				Table 7 of Original Report AR4575
Drug Tolerance	**PC Concentration (Anti-hGH)**	**hGH Concentration**	**Acceptance Criteria**		Table 9 of Original Report AR4575
	2000 ng/mL (HPC)	100 ng/mL	Response remained > screening cut point		
	500 ng/mL (MPC)	100 ng/mL			
	125 ng/mL (LPC)	1 ng/mL			
Selectivity and matrix effect	Not assessed				

Table 31. Summary of Method Performance: hGH ECL ADA ([redacted] Project AR4575)

Interference and specificity	1000 ng/mL and 28 ng/mL of human serum albumin (HSA) showed no interference	Table 10 of Original Report AR4575
	100, 50, 10, 1 and 0.1 ng/mL of human IgG showed no interference	Table 11 of Original Report AR4575
Dilution Linearity and hook effect	No hook effect observed starting at 2000 ng/mL	Table 7 of Original Report AR4575
Bench-top/process stability	24 hours in human serum at ambient temperature	Table 6 of Original Report AR4575
Freeze-Thaw stability	5 Freeze-Thaw cycles stability established at -70°C	Table 6 of Original Report AR4575
Robustness	In order to address assay robustness and assay consistency, changes (reagent lot, analyst, instruments) were evaluated as part of assay validation. Inter- and intra-assay variability of the positive control samples was assessed following changes of the following parameters: 2 analysts performed the assay, 5 lots of wash buffer, 2 positive control lots, 2 shakers and blocking time ranged from 1 hour 5 minutes to 2 hours 40 minutes	Table 11 of Original Report AR4575

Table 32. Summary of Method Performance: hGH ECL ADA ([redacted] Project OX-18-512-012)

Bioanalytical method validation report name, amendments and hyperlinks	[redacted] Method Validation Report OX-18-512-012: Validation of an Analytical Method to Screen and Confirm Antibodies to hGH in GHD Paediatric Serum using Electrochemiluminescence. Effective Date: [redacted]20[redacted]				
Method description	A validated bridging format using biotinylated pituitary hGH as capture and Sulfo-Tag hGH as detection.				
Minimum required dilutions (MRDs)	1:10				
Source and lot of reagents	Source: OPKO Biologics **Positive controls (PCs)**: Anti-hGH monoclonal antibody, Lot # 120116/DS518 **Drug**: WHO International Standard, Growth Hormone, NIBSC Code 80/505				
Validation parameters	**Method validation summary**				**Source location**
Positive Control (PC) Precision	**PC Concentration (100% serum)**	**Intra-Assay (%CV)**	**Inter-Assay (%CV)**	**Acceptance Range (RLU)**	
	2000 ng/mL (HPC)	<13.7%	15.3%	8695 – 21046	Table 10 of [redacted] Report OX-18-512-012
	100 ng/mL (MPC)	<20.5%	11.4%	473 – 902	Table 9 of [redacted] Report OX-18-512-012
	20 ng/mL (LPC)	<7.9%	13.1%	78 – 164	Table 8 of [redacted] Report OX-18-512-012
Negative Control (NC) Precision	N/A	<6.8%	6.6%	≤68	Table 6 of [redacted] Report OX-18-512-012
Screening Cut Point (GHD pediatric)	1.07 (cut point factor) based on 5% false positive rate Mean RLU of Negative Control (NC) x Cut Point Factor				Table 1 and ANNEX of [redacted] Report OX-18-512-012
Titer cut point	1.19 (cut point factor) based on 0.1% false positive rate Mean RLU of Negative Control (NC) x Cut Point Factor				Table 1 and ANNEX of [redacted] Report OX-18-512-012
Confirmatory (Specificity Cut points)	12.6% for 1000 ng/mL hGH based on a 1% false positive rate				Table 2 and ANNEX of [redacted] Report OX-18-512-012
Sensitivity	4.2 ng/mL in 100% matrix				Table 3 of [redacted] Report OX-18-512-012
Drug Tolerance	**PC Concentration (Anti-hGH)**	**hGH Concentration**	**Acceptance Criteria**		
	2000 ng/mL	500 ng/mL	Response remained > screening cut point (73 RLU)		Table 11 of [redacted] Report OX-18-512-012
	100 ng/mL	100 ng/mL			
	20 ng/mL	10 ng/mL			

Table 32. Summary of Method Performance: hGH ECL ADA ([redacted] Project OX-18-512-012)

Selectivity and matrix effect	All individual sera spiked to the LoPC and HPC concentrations gave a response above the screening cut point. In the confirmatory assay, all individual sera spiked to the LoPC and HPC concentrations were above the confirmatory cut point (% depletion). The % difference between spiked responses in pooled serum and individuals, were not ≤20% for 8 out of 10 individuals at the LoPC. This parameter is considered a conditional pass since for ADA analysis, which is qualitative in nature, the 20% difference criteria are too strict.	Table 4 of [redacted] Report OX-18-512-012
Interference and specificity	**Haemolysed, icteric and lipaemic samples** The selectivity matrices at the LoPC and HPC screened and confirmed positive. The % difference between pooled serum and individuals for unspiked and spiked with HPC were within acceptance criteria. The % difference between pooled serum and individuals spiked with the LoPC was >20% for individuals. However, the overall positive / negative status of the haemolysed, icteric and lipaemic selectivity samples were the same as the baseline samples (one unspiked lipaemic individual screened positive at the SCP but confirmed negative).	Table 5 of [redacted] Report OX-18-512-012
Dilution Linearity and hook effect	No hook effect observed starting at 2000 ng/mL	Table 3 of [redacted] Report OX-18-512-012
Bench-top/process stability	72 hours in human serum at ambient temperature	Table 12 of [redacted] Report OX-18-512-012
	72 hours in human serum at 4°C	Table 13 of [redacted] Report OX-18-512-012
Freeze-Thaw stability	6 Freeze-Thaw cycles stability established at -70°C	Table 14 of [redacted] Report OX-18-512-012
Robustness	Assay robustness was investigated by making deliberate variations in assay parameters to provide an indication of reliability during normal use. The effect on the assay was assessed using the NCS, LoPC, MePC and HiPC samples. Sample incubation times were varied by approximately ±20% of the normal assay time, for the following parameters: confirmatory sample incubation, Master mix incubation and online plate incubation.	Table 15 of [redacted] Report OX-18-512-012

Table 33. Summary of Method Performance: hGH ECL ADA ([redacted] Project SC-20/512-025)

Bioanalytical method validation report name, amendments and hyperlinks	[redacted] Method Validation Report SC-20/512-025: Partial Validation of an Analytical Method to Screen and Confirm Antibodies to hGH in GHD Pediatric Serum using Electrochemiluminescence. Effective Date: [redacted]20[redacted]				
Method description	A validated bridging format using biotinylated pituitary hGH as capture and Sulfo-Tag hGH as detection.				
Minimum required dilutions (MRDs)	1:10				
Source and lot of reagents	Source: OPKO Biologics **Positive controls (PCs)**: Anti-hGH monoclonal antibody, Lot # 120116/DS518 **Drug**: WHO International Standard, Growth Hormone, NIBSC Code 80/505				
Validation parameters	**Method validation summary**				**Source location**
Positive Control (PC) Precision	**PC Concentration (100% serum)**	**Intra-Assay (%CV)**	**Inter-Assay (%CV)**	**Acceptance Range (RLU)**	
	2000 ng/mL (HPC)	<10.2%	9.5%	8043 – 16658	Table 2 and Table 6 of [redacted] Report SC-20/512-025
	100 ng/mL (MPC)	<14.1%	10.6%	264 – 691	Table 2 and Table 6 of [redacted] Report SC-20/512-025
	20 ng/mL (LPC)	<8.9%	6.9%	80 – 125	Table 2 and Table 6 of [redacted] Report SC-20/512-025
Negative Control (NC) Precision	N/A	<7.4%	12.7%	≤71	Table 2 and Table 6 of [redacted] Report SC-20/512-025
Screening Cut Point (GHD pediatric)	1.07 (cut point factor) based on 5% false positive rate Mean RLU of Negative Control (NC) x Cut Point Factor				Table 3, Table 4 and Appendix E of [redacted] Report SC-20/512-025
Titer cut point	1.19 (cut point factor) based on 0.1% false positive rate Mean RLU of Negative Control (NC) x Cut Point Factor				Table 3 and Table 4 of [redacted] Report SC-20/512-025
Confirmatory (Specificity Cut points)	12.6% for 1000 ng/mL hGH based on a 1% false positive rate				Table 3 and Table 4 of [redacted] Report SC-20/512-025
Sensitivity	1.7 ng/mL in 100% matrix				Table 5 of [redacted] Report SC-20/512-025
Selectivity and matrix effect	Selectivity was assessed in a total of 5 assessments, as shown in Table 7. Assessments 1, 2, 4, and 5 used the same 10 individual lots and consisted of 3 independent preparations. Assessment 3 used 10 new individual lots. As a result of Assessment 2 to 5 compiliation for original 10 and new 10 individual lots, 100% passed for spiked samples screened positive and 80% of unspikied samples that screened negative. Details are in Section 8 and Table 7.				Table 7 of [redacted] Report SC-20/512-025

Table 33. Summary of Method Performance: hGH ECL ADA ([redacted] Project SC-20/512-025)

Cross-validation	Cross validation of samples run in [redacted] for Sponsor Study CP-4-006 (Table 10) showed 90% concordance in ADA results between [redacted] and [redacted].	Table 8 of [redacted] Report SC-20/512-025

Table 34. Summary of Method Performance: Somatrogon Neutralizing Cell-based NAb Assay ([redacted] Projects AR2862, AR4268 and AR5265)

Bioanalytical method validation report name, amendments and hyperlinks	[redacted] Original Method Validation Report AR2862: Qualitative Determination of Neutralizing Antibodies to MOD-4023 in Human Serum Using a Cell-Based Assay. Effective Date: [redacted]20[redacted] Report AR2862 Amendment 1 Effective Date: [redacted]20[redacted]. Added estimated sensitivity Report AR2862 Amendment 2 Effective Date: [redacted]20[redacted]. Note: for clarification purposes all corrections and modifications presented in the Amendment 1 Validation Report No. AR2862 will be captured in an updated version, Validation Report No. AR5265. The original and amended validation reports (#s AR2862 and AR2862 Amendment 1, respectively) will be referenced in report # AR5265 for tracking. [redacted] Original Method Validation Report AR4268: Qualitative Determination of Neutralizing Antibodies to MOD-4023 in Growth Hormone Deficient Child Serum Using a Cell-Based Assay. Effective Date: [redacted]20[redacted] Report AR4268 Amendment 1 Effective Date: [redacted]20[redacted] Report AR4268 Addendum 1 Effective Date: [redacted]20[redacted] [redacted] Original Method Validation Report AR5265: Method Validation Report for the Qualitative Determination of Neutralizing Antibodies to MOD-4023 in Human Serum Using a Cell-Based Assay. Effective Date: [redacted]20[redacted] Report AR5265 Addendum 1 Effective Date: [redacted]20[redacted] Report AR5265 Addendum 2 Effective Date: [redacted]20[redacted] Report AR5265 Addendum 3 Effective Date: [redacted]20[redacted] Report AR5265 Addendum 4 In study cut point evaluation for the qualitative determination of neutralizing antibodies to MOD-4023 in adult human serum using a cell-based assay. Effective Date: [redacted]20[redacted]
Method description	A validated cell-based NAb assay based on the ability of hGH/MOD-4023 to induce cell proliferation by binding to the human growth hormone receptor (hGHR) which is expressed on the cell surface of the BaFB2B2 murine cell line. Neutralizing anti-MOD-4023 (Somatrogon) antibodies in human serum will inhibit the GH-proliferative effect.
Minimum required dilutions (MRDs)	1:50

Table 34. Summary of Method Performance: Somatrogon Neutralizing Cell-based NAb Assay ([redacted] Projects AR2862, AR4268 and AR5265)

Source and lot of reagents	Source: OPKO Biologics (formerly [redacted], [redacted], Israel) **Positive control (PC):** Rabbit affinity purified anti-MOD-4023, Lot# 90630 (AR2862) Rabbit affinity purified anti-MOD-4023, Lot# 10325 (AR2862, AR4268 and AR5265) Rabbit affinity purified anti-MOD-4023, Lot# PAb4023-001 (AR4268 Addendum 1) Rabbit affinity purified anti-MOD-4023, Lot# 101223 (AR5265 Addenda 1-4) **Drug:** MOD-4023, Lot# EN648-01-[redacted]-005 (AR2862) MOD-4023, Lot # 4-11-X003-S1 (AR4268) MOD-4023, Lot# 4-11-X003B-S2 (AR4268) MOD-4023, Lot # 1039252 (AR4268 Addendum 1) MOD-4023, Lot# 1019394 (AR5265 Addenda 1-4) **Cell Line:** B2B2 hGHR BAF (BaFB2B2) murine lymphocyte cell line, Mus musculus origin				
Validation parameters	**Method validation summary**				**Source location**
Precision Positive Control (PC) (Lot #s 90630 and 10325) in 100% neat human serum	**PC Concentration (100% serum)**	**Intra-Assay (%CV)[a]**	**Inter-Assay (%CV)[b]**	**Acceptance Range (OD)**	Tables 1 and 2 of Original Report AR2862
	2500 µg/mL	4.2%	29.9%	0.198 – 0.594	
	1250 µg/mL	6.6%	22.0%	0.193 – 0.578	
	625 µg/mL	11.8%	20.2%	0.199 – 0.596	
	313 µg/mL	11.4%	21.4%	0.216 – 0.647	
	156 µg/mL	13.1%	19.7%	0.218 – 0.655	
	78.1 µg/mL	16.5%	19.8%	0.213 – 0.638	
	39.1 µg/mL	15.3%	24.4%	0.257 – 0.770	
	19.5 µg/mL	6.5%	22.9%	0.525 – 1.575	
	9.77 µg/mL	9.5%	21.3%	0.678 – 2.035	
	4.88 µg/mL	15.5%	18.5%	0.722 – 2.166	
	2.44 µg/mL	5.7%	19.2%	0.791 – 2.372	
	0	2.6%	19.2%	0.840 – 2.521	
	a. All passages b. Passages 2-14				

Table 34. Summary of Method Performance: Somatrogon Neutralizing Cell-based NAb Assay ([redacted] Projects AR2862, AR4268 and AR5265)

Precision Positive Control (PC) (Lot # 10325) in 100% neat human serum	**PC Concentration (100% serum)**	**Intra-Assay (RPD)[a]**	**Inter-Assay (%CV) [b]**	**Acceptance Range (OD)**	Tables 1 and 2 of Original Report AR4268
	313 µg/mL	5.8%	48.7%	0.216 – 0.647	
	19.6 µg/mL	11.0%	26.0%	0.525 – 1.575	
	9.75 µg/mL	6.5%	23.6%	0.678 – 2.035	
	4.90 µg/mL	5.7%	24.0%	0.722 – 2.166	
	0	5.9%	22.1%	0.840 – 2.521	
	a. Relative Percent Difference = ([R1-R2]/R) x100); where R1-R2 = absolute difference between replicate OD values and R = absolute mean of the 2 values b. Passages 7-23				
Precision Positive Control (PC) (Lot # 10325) in 100% neat human serum	**PC Concentration (100% serum)**	**Intra-Assay (RPD)[a]**	**Inter-Assay (%CV) [b]**	**Acceptance Range (OD)**	Table 6 of Report AR5265
	313 µg/mL	6.7%	12.1%	0.216 – 0.647	
	19.6 µg/mL	6.1%	22.4%	0.525 – 1.575	
	9.75 µg/mL	4.8%	16.6%	0.678 – 2.035	
	4.90 µg/mL	5.3%	15.7%	0.722 – 2.166	
	0	4.4%	17.3%	0.840 – 2.521	
	a. Relative Percent Difference = ([R1-R2]/R) x100); where R1-R2 = absolute difference between replicate OD values and R = absolute mean of the 2 values b. Passages 7-23				
Precision Positive Control (PC) (Lot # 1039252) in 100% neat human serum	**PC Concentration (100% serum)**	**Intra-Assay (%CV)**	**Inter-Assay (%CV)**	**Mean OD**	Tables F and 2 of Report AR4268 Addendum 1
	500 µg/mL	1.4%	12.4%	0.187	
	166.7 µg/mL	1.9%	11.8%	0.182	
	55.6 µg/mL	2.9%	12.0%	0.184	
	18.5 µg/mL	5.7%	32.5%	0.428	
	6.2 µg/mL	8.5%	20.8%	1.705	
	2.1 µg/mL	9.1%	21.6%	1.917	
	0.7 µg/mL	9.2%	18.4%	1.999	

Table 34. Summary of Method Performance: Somatrogon Neutralizing Cell-based NAb Assay ([redacted] Projects AR2862, AR4268 and AR5265)

Precision Positive Control (PC) (Lot # 101223) in 100% neat human serum	**PC Concentration (100% serum)**	**Intra-Assay (RPD)**	**Inter-Assay (%CV)**	**Acceptance Range (OD)**	Tables 1 and C of Report AR5265 Addendum 1
	625 µg/mL	14.9%	7.8%	0.058 – 0.173	
	313 µg/mL	5.2%	5.5%	0.064 – 0.192	
	156 µg/mL	14.0%	5.3%	0.071 – 0.213	
	78.1 µg/mL	13.0%	5.8%	0.076 – 0.229	
	39.1 µg/mL	23.2%	43.1%	0.253 – 0.760	
	19.5 µg/mL	2.9%	7.7%	0.663 – 1.990	
	9.77 µg/mL	7.1%	11.4%	0.740 – 2.220	
	4.88 µg/mL	2.6%	11.4%	0.770 – 2.311	
	2.44 µg/mL	0.9%	7.1%	0.768 – 2.304	
	1.22 µg/mL	2.6%	6.2%	0.767 – 2.301	
	0.610 µg/mL	4.0%	6.7%	0.746 – 2.237	
	0	3.0%	7.3%	0.773 – 2.318	
Precision Positive Control (PC) (Lot # 10325) in 100% neat human serum to be used for sample analysis	**PC Concentration (100% serum)**	**Intra-Assay (RPD)**	**Inter-Assay (%CV)**	**Acceptance Range (OD)**	Tables 2 and D of Report AR5265 Addendum 1
	250 µg/mL	4.8%	19.5%	0.072 – 0.215	
	62.5 µg/mL	7.7%	18.4%	0.077 – 0.232	
	31.3 µg/mL	13.1%	33.5%	0.228 – 0.685	
	15.7 µg/mL	7.2%	11.7%	0.580 – 1.741	
Precision Blank Control	**2% Human Serum + 25 ng/mL of MOD-4023**	6.3%	17.3%	1.700	Table 3 of Original Report AR2862
Precision Negative Control	**Negative Control**	**Intra-Assay (RPD)**	**Inter-Assay (%CV)**	**Mean OD**	Table 3 of Original Report AR4268
	NC-1: Cells + Assay Medium	6.0%	38.7%	0.286	
	NC-2: Cells + 2% GHD Pediatric serum	12.8%	37.5%	0.448	
	NC-3: Cells + Drug + Assay Medium	3.7%	23.9%	1.438	

Table 34. Summary of Method Performance: Somatrogon Neutralizing Cell-based NAb Assay ([redacted] Projects AR2862, AR4268 and AR5265)

	NC-4: Cells + Drug + 2% GHD Pediatric serum	3.0%	23.7%	1.577	
Precision Negative Control	**Negative Control**	**Intra-Assay (%CV)**	**Inter-Assay (%CV)**	**Mean OD**	Tables E and 1 of Report AR4268 Addendum 1
	NC-1: Cells + Assay Medium	1.4%	13.0%	0.144	
	NC-2: Cells + 2% GHD Pediatric serum	3.7%	18.0%	0.223	
	NC-3: Cells + Drug + Assay Medium	1.3%	15.7%	1.599	
	NC-4: Cells + Drug + 2% GHD Pediatric serum	3.2%	15.7%	2.024	
Precision Negative Control	**Negative Control**	**Intra-Assay (%RPD)**	**Inter-Assay (%CV)**	**Mean OD**	Table 6 of Report AR5265
	NC-1: Cells + Assay Medium	2.5%	7.8%	0.247	
	NC-2: Cells + 2% Normal serum	7.0%	15.1%	0.304	
	NC-3: Cells + Drug + Assay Medium	3.6%	15.0%	1.108	
	NC-4: Cells + Drug + 2% Normal serum	3.7%	16.4%	1.168	
Precision Blank Control	**NC-0: 2% Human Serum + 25 ng/mL of MOD-4023**	4.3%	7.9%	1.486	Table 3 of Report AR5265 Addendum 1

Table 34. Summary of Method Performance: Somatrogon Neutralizing Cell-based NAb Assay ([redacted] Projects AR2862, AR4268 and AR5265)

Precision Negative Control	**Negative Control**	**Intra-Assay (%RPD)**	**Inter-Assay (%CV)**	**Mean OD**	Table 4 of Report AR5265 Addendum 1
	NC-1: Cells + Assay Medium	4.3%	15.1%	0.153	
	NC-2: Cells + 2% Normal serum	5.2%	15.4%	0.160	
	NC-3: Cells + Drug + Assay Medium	2.2%	8.0%	1.44	
	NC-4: Cells + Drug + 2% Normal serum	6.0%	9.5%	1.48	
Cut Points	Normal Human serum: 1.222 OD (Mean OD of 50 individuals – (2 x SD of the same individuals)				Table 4 of Original Report AR2862
	GHD Serum Samples: 0.787 OD, 5% False Positive Rate (Mean OD of individuals - (1.645 x SD of the same individuals)				Table 4 of Original Report AR4268
	Normal Adult Human Serum: 0.826 OD, 5% False Positive Rate				Table 6 and Appendix 1 of Report AR5265
	0.694 Normalized Signal (NS), Floating, 1% False Positive Rate Cut point for controls normalized signal (NS) responses				Table 4 of Report AR4268 Addendum 1
	Normal Human serum: 1.204 OD, 1% False Positive Rate (Mean OD of 50 individuals – (2.326 x SD of the same individuals)				Table 5 of Report AR5265 Addendum 1
	0.263 Normalized Signal (NS), In-study, 1% False Positive Rate Individuals pediatric GHD Pre-dose serum samples from study CP-4-004				Table 1 of Report AR5265 Addendum 3
	0.287 Normalized Signal (NS), In-study, 1% False Positive Rate Individuals Adult GHD Pre-dose serum samples from study CP-4-005				Table 1 of Report AR5265 Addendum 4
	0.538 Normalized Signal (NS), 1% False Positive Rate Individuals pediatric GHD Pre-dose serum samples (Visit 2/baseline) from study CP-4-006				Table 4 of Report AR4268 Addendum 1
Sensitivity	19.5 µg/mL in 100% neat serum				Table 1 of Report AR2862 Amendment 1
	8.18 µg/mL in 100% neat serum				Tables H and 5a, 5b and 5c of Report AR4268 Addendum 1
Drug Tolerance	**PC Concentration (Anti-MOD-4023)**	**MOD-4023 Concentration**	**Acceptance Criteria**		Table 10 of Report AR5265 Addendum 1
	250 µg/mL	5.00 µg/mL			

Table 34. Summary of Method Performance: Somatrogon Neutralizing Cell-based NAb Assay ([redacted] Projects AR2862, AR4268 and AR5265)

	62.5 µg/mL	1.00 µg/mL	Response remained < plate cut point (1.190 OD)	
	31.25 µg/mL	0.05 µg/mL		
Drug Tolerance	**PC Concentration (Anti-MOD-4023)**	**MOD-4023 Concentration**	**Acceptance Criteria**	Table 2 of Report AR5265 Addendum 2
	250 µg/mL	>800 ng/mL	Response remained < plate cut point (1.157 OD)	
	62.5 µg/mL	>800 ng/mL		
	31.25 µg/mL	351 ng/mL		
	25.0 µg/mL	220 ng/mL		
	15.7 µg/mL	< 50 ng/mL		
Selectivity and matrix effect	Anti-MOD-4023 antibody was spiked into twenty (20) individual growth hormone deficient pediatric serum samples at High and Low levels (78.1 µg/mL and 19.5 µg/mL in 100% neat matrix, respectively) treated with 25 ng/mL MOD4023 and incubated with BaFB2B2 cells overnight. The % inhibition was calculated for each individual. 16/20 unspiked individuals were negative. 20/20 HPC individuals were positive (below cut point 1.222 OD). 16/20 LPC individuals were positive (below cut point 1.222 OD)			Table 6 of Original Report AR2862
	Anti-MOD-4023 antibody was spiked into twenty (20) individual normal human serum samples at High, Medium and Low PC levels (78.1 µg/mL, 39.1 µg/mL and 19.5 µg/mL in 100% neat matrix, respectively) treated with 25 ng/mL MOD4023 and incubated with BaFB2B2 cells overnight. All 20 non-spiked samples were negative, and 20 of Low, Medium and High PC spiked individual samples were positive.			Table 5 of Original Report AR4268
	All 12/12 non-spiked samples were negative, and all 12/12 of LPC-spiked individual samples were positive.			Tables J and 7 of Report AR4268 Addendum 1
	Anti-MOD-4023 antibody was spiked into 10 normal and 10 GHD pediatric individual human samples, 5 male and 5 female from each cohort, at High and Low levels (final concentrations 62.5 µg/mL and 31.3 µg/mL, respectively), treated with 25 ng/mL MOD-4023 and incubated with BaFB2B2 cells overnight. All non-spiked samples were negative, and 10/10 of both Low and High PC spiked normal individual samples were positive. 8/10 of Low and 10/10 High PC spiked GHD pediatric individual samples were positive.			Table 7 of Report AR5265 Addendum 1

Table 34. Summary of Method Performance: Somatrogon Neutralizing Cell-based NAb Assay ([redacted] Projects AR2862, AR4268 and AR5265)

Interference and specificity	Hemolysis (1% and 2% hemoglobin) Lipemia (3 mg/mL triglycerides) Icterus (30 µg/mL bilirubin) Although slight differences in sensitivity curves were observed, still able to detect positive NAb in the presence of potential matrix interferents.	Tables K and 8 of Report AR4268 Addendum 1
	Three (3) of each Hemolyzed and Lipemic Individuals, unspiked and spiked with the anti-MOD-4023 positive control antibody at the final concentrations of 250 µg/mL (High Spiked) and 15.7 µg/mL (Low Spiked), were treated with 25 ng/mL MOD-4023 and incubated with BaFB2B2 cells overnight. All High Spiked Hemolyzed and Lipemic samples tested positive. All the Low Spiked samples were spiked with 15.7 µg/mL of anti-MOD-4023 antibody, which is below the sensitivity level of the assay; therefore, they were not detected as positive.	Table 8 of Report AR5265 Addendum 1
Dilution Linearity and hook effect	No hook effect observed starting at 500 µg/mL	Tables F and 2 of Report AR4268 Addendum 1
Bench-top/process stability	24 hours in human serum at ambient temperature	Table 5 of Original Report AR2862
	72 hours in human serum at ambient temperature	Table 9 of Report AR5265 Addendum 1
Freeze-Thaw stability	5 Freeze-Thaw cycles established	Table 5 of Original Report AR2862
Robustness	Two analysts assessed precision and reliability of the assay over 20 runs. The inter-assay variability of the positive control was within 30%, which is acceptable for a cell-based assay. Also cell passages 2-14 were confirmed.	Table 1 of Original Report AR2862

Table 35. Summary of Method Performance: Somatrogon Neutralizing Cell-based NAb Assay (C0317007)

Bioanalytical method validation report name, amendments and hyperlinks	Pfizer Validation# C0317007 / [redacted] Project# 15-2029 The Validation of a Cell-Based Method for the Determination of Neutralizing Anti-Somatrogon Antibodies in Human Serum, Effective Date: [redacted]20[redacted]				
Method description	A validated cell-based NAb assay based on the ability of hGH/MOD-4023 to induce cell proliferation by binding to the human growth hormone receptor (hGHR) which is expressed on the cell surface of the BaFB2B2 murine cell line. Neutralizing anti-MOD-4023 (Somatrogon) antibodies in human serum will inhibit the GH-proliferative effect.				
Minimum required dilutions (MRDs)	1:50				
Source and lot of reagents	Source: OPKO Biologics (PC1), [redacted] Catalog# AF1067 (PC2), Pfizer (MOD-4023), NIBSC Catalog# 80/505 (hGH) **Positive control (PC):** PC1: Rabbit affinity purified polyclonal anti-MOD-4023, Lot# PAb4023-001 PC2: Goat hGH affinity purified pAB, Lot# HRY0819041 or equivalent **Drug:** MOD-4023, Lot# AG8262 hGH, Lot# Not assigned by manufacturer Cell Line: B2B2 hGHR BAF (BaFB2B2) murine lymphocyte cell line, Mus musculus origin				
Validation parameters	**Method validation summary**				**Source location**
Assay Cut Point(ACP) Patient Human Serum	0.724 for Pediatric GHD Human Serum				Table 2 and Appendix 5, Statistical Analysis Report
Mass-based Sensitivity[a]	PC1: 13.6 µg/mL PC2: 11.5 µg/mL				Table 3 and Table 4 Table 5 and Table 6
Final LPC Level[b]	LPC: 12.5 µg/mL (based on historical data and assay performance in normal human serum)				
Precision Positive Control (PC1) in 100% neat human serum	**PC1 Concentration (100% serum)**	**Intra-Assay (%CV)**	**Inter-Assay (%CV)**	**Acceptance Range (OD)**	Table 7 and Table 8
	50 µg/mL (HPC)	3.5%	37.5%	0.004 – 0.204	
	20 µg/mL (MPC)	15.3%	27.1%	NA	
	12.5 µg/mL (LPC)	11.2%	12.6%	0.414 – 0.810[a]	
	0 (DC Signal)	3.7%	22.6%	> 0.8114	

Table 35. Summary of Method Performance: Somatrogon Neutralizing Cell-based NAb Assay (C0317007)

Selectivity and matrix effect	**Matrix Recovery PC1 in Patient Human Serum** 4/10 reportable spiked samples HPC (S/DC) < LPC (S/DC) ≤ ACP, where LPC = 12.5 µg/mL, based on historical assay performance in normal human serum 9/10 reportable spiked samples HPC (S/DC) < LPC (S/DC) ≤ ACP, where LPC = 16.6 µg/mL, based on calculated assay sensitivity in Table 3 10/10 reportable blank sample results were negative (> ACP)			Table 9
	Hemolysis (PC in Hemolyzed Serum) 6/6 reportable hemolyzed samples HPC (S/DC) < LPC (S/DC) ≤ACP, where LPC = 16.6 µg/mL 2/6 reportable blank samples results were negative (> ACP) Hemolysis may cause false positive results. All samples will be assessed for visual hemolysis and potential hemolytic interference will be noted in the sample analysis report			Table 10
	Lipemic (PC in Lipemic Serum) 6/6 reportable lipemic samples HPC (S/DC) < LPC (S/DC) ≤ ACP, where LPC = 16.6 µg/mL 5/5 reportable blank sample results > ACP			Table 11
Specificity Drug Tolerance (PC1)	**Control**	**MOD-4023 Conc. (µg/mL)**	**Comment**	Table 12
	HPC (50 µg/mL)	2 µg/mL	≤ ACP	
	MPC (20 µg/mL)	1 µg/mL	≤ ACP	
	LPC (12.5 µg/mL)	<0.05 µg/mL	≤ ACP	
	NC	≥5 µg/mL	> ACP	
Prozone Effect	No Prozone Effect observed			Table 13 and Figure 1
Plate Heomogeneity	5.4 %CV (N = 94 individual wells) at LPC in Normal Human Serum			Table 14
Bench-top/process stability	24 hours in human serum at ambient temperature			Table 15
Freeze-Thaw stability	8 Freeze-Thaw cycles established at -20°C			Table 16
	8 Freeze-Thaw cycles established at -70°C			Table 17

Table 35. Summary of Method Performance: Somatrogon Neutralizing Cell-based NAb Assay (C0317007)

Robustness	Assay robustness was demonstrated by 28 validation runs on 7 different days performed by 3 different analysts using multiple reagent lots, incubation times and equipment. Also cell passages 12-22 were confirmed.	Table 18 to 23

a Mass-based Sensitivity (95% Upper Limit) = Antilog [Mean + t0.05, df * SD]
b LPC Concentration (99% Upper Limit) = Antilog [Mean + t0.01, df * SD]

Table 36. Summary of Method Performance: hGH Neutralizing Cell-based NAb Assay (Projects AR2919, AR4266 and AR5266)

Bioanalytical method validation report name, amendments and hyperlinks	Original Method Validation Report AR2919: Qualitative Determination of Neutralizing Antibodies to Human Growth Hormone (hGH) in Human Serum Using a Cell-Based Assay. Effective Date: 20 Report AR2919 Amendment 1 Effective Date: 20. Added estimated sensitivity and updated usable cell passage to 2-24 Report AR2919 Amendment 2 Effective Date: 20. Note: for clarification purposes all corrections and modifications presented in the Amendment 1 Validation Report No. AR2919 will be captured in an updated version, Validation Report No. AR5266. The original and amended validation reports (#s AR2919 and AR2919 Amendment 1, respectively) will be referenced in report # AR5266 for tracking. Original Method Validation Report AR4266: Qualitative determination of neutralizing antibodies to human growth hormone (hGH) in growth hormone deficient child serum using a cell-based assay. Effective Date: 20 Report AR4266 Amendment 1 Effective Date: 20 Report AR4266 Amendment 1, Addendum 1 Effective Date: 20. Additional drug tolerance. Report AR4266 Amendment 1, Addendum 2 Effective Date: 20. In-study Cut Point for the study CP-4-004. Report AR4266 Amendment 1, Addendum 3: Partial validation report of cell based assay for the detection of neutralizing antibodies to human growth hormone (HGH) in growth hormone deficient child serum. Effective Date: 20. Original Method Validation Report AR5266: Method validation report for the qualitative determination of neutralizing antibodies to human growth hormone (hgh) in human serum using a cell-based assay. Effective Date: 20 Report AR5266 Addendum 1, Amendment 2: Method requalification report for the qualitative determination of neutralizing antibodies to human growth hormone (hgh) in human serum using a cell-based assay. Effective Date: 20 Report AR5266 Addendum 2: Drug tolerance investigation for the qualitative determination of neutralizing antibodies to human growth hormone (hgh) in human serum using a cell-based assay. Effective Date: 20 Report AR5266 Addendum 3: In study cut point evaluation for the qualitative determination of neutralizing antibodies to human growth hormone (hgh) in adult human serum using a cell-based assay. CP-04-005. Effective Date: 20
Method description	A validated cell-based NAb assay based on the ability of hGH to induce cell proliferation by binding to the human growth hormone receptor (hGHR) which is expressed on the cell surface of the BaFB2B2 murine cell line. Neutralizing anti-hGH antibodies in human serum will inhibit the GH-proliferative effect.
Minimum required dilutions (MRDs)	1:50 (AR2919 and AR5266) Normal and Adult GHD 1:20 (AR4266) Pediatric GHD

Table 36. Summary of Method Performance: hGH Neutralizing Cell-based NAb Assay ([redacted] Projects AR2919, AR4266 and AR5266)

Source and lot of reagents	Source: OPKO Biologics (formerly [redacted], [redacted], Israel) **Positive control (PC):** Anti-hGH Polyclonal antibody, Lot# HRY0207051, (AR2919 and AR5266 Original) Anti-hGH Polyclonal antibody, Lot# HRY0811121, (AR4266 Original and Amendment 1 and AR5266) Anti-hGH Polyclonal antibody, Lot# HRY0816101, (AR4266 Addenda 1-3) Source: [redacted] Anti-hGH Polyclonal antibody, Lot# 073019LBQ1, (AR4266 Addenda 1-3) Source: [redacted] Anti-hGH Polyclonal antibody, Lot# HRY0815082, (AR5266 Addendum 1) Source: [redacted] Anti-hGH Polyclonal antibody, Lot# HRY0816101, (AR5266 Addenda 2-3) Source: [redacted] **Drug:** WHO International Standard, Growth Hormone, NIBSC Code 80/505 **Cell Line:** B2B2 hGHR BAF (BaFB2B2) murine lymphocyte cell line, Mus musculus origin				
Validation parameters	**Method validation summary**				**Source location**
Precision Positive Control (PC) (Lot # HRY0207051) in 100% neat human serum	**PC Concentration (100% serum)**	**Intra-Assay (%CV)[a]**	**Inter-Assay (%CV)[a]**	**Acceptance Range (OD)**	Tables 1 and 2 of Original Report AR2919
	78.2 µg/mL	10.4 %	20.8 %	0.254 – 0.763	
	39.1 µg/mL	11.0 %	22.9 %	0.237 – 0.710	
	19.5 µg/mL	14.1 %	24.8 %	0.237 – 0.712	
	9.77 µg/mL	12.0 %	29.5 %	0.279 – 0.838	
	4.88 µg/mL	11.4 %	23.1 %	0.299 – 0.898	
	2.44 µg/mL	14.8 %	19.7 %	0.481 – 1.442	
	1.22 µg/mL	12.8 %	14.6 %	0.584 – 1.751	
	0.611 µg/mL	9.8 %	16.0 %	0.634 – 1.901	
	0.305 µg/mL	12.9 %	16.4 %	0.648 – 1.944	
	0.153 µg/mL	10.2 %	13.1 %	0.676 – 2.027	
	0.076 µg/mL	11.7 %	14.6 %	0.695 – 2.086	
	0 µg/mL	8.6 %	16.7 %	0.778 – 2.334	
	a. All passages (1-18)				

Table 36. Summary of Method Performance: hGH Neutralizing Cell-based NAb Assay ([redacted] Projects AR2919, AR4266 and AR5266)

Precision Positive Control (PC) (Lot # HRY0811121) in 100% neat human serum	**PC Concentration (100% serum)**	**Intra-Assay (RPD)[a]**	**Inter-Assay (%CV)[a]**	**Acceptance Range (OD)**	Tables 1 and 4 of Original Report AR4266
	31.3 µg/mL	9.2 %	21.4 %	0.159 – 0.476	
	15.6 µg/mL	8.2 %	30.7 %	0.158 – 0.473	
	7.82 µg/mL	6.9 %	23.6 %	0.155 – 0.464	
	3.91 µg/mL	7.6 %	22.7 %	0.156 – 0.467	
	1.95 µg/mL	8.4 %	24.0 %	0.161 – 0.482	
	0.977 µg/mL	14.7 %	27.7 %	0.237 – 0.710	
	0.488 µg/mL	11.2 %	18.5 %	0.418 – 1.252	
	0.244 µg/mL	8.0 %	13.8 %	0.542 – 1.625	
	0.122 µg/mL	4.2 %	13.6 %	0.609 – 1.826	
	0.0611 µg/mL	5.6 %	15.2 %	0.563 – 1.689	
	0.0305 µg/mL	6.7 %	16.2 %	0.552 – 1.656	
	0 µg/mL	6.7 %	18.8 %	0.546 – 1.637	
	a. All passages (6-24)				
Precision Positive Control (PC) (Lot # HRY0816101 and 073019LBQ1) in 100% neat pediatric GHD human serum	**PC Concentration (100% serum)**	**Intra-Assay (%CV)**	**Inter-Assay (%CV)**	**Mean Normalized Signal**	Tables F and 2 of Report AR4266 Amendment 1, Addendum 3
	20.0 µg/mL	1.6 %	18.5 %	0.106	
	6.66 µg/mL	1.7 %	18.7 %	0.106	
	2.22 µg/mL	3.0 %	17.6 %	0.107	
	0.74 µg/mL	1.9 %	33.3 %	0.178	
	0.25 µg/mL	3.4 %	17.1 %	0.701	
	0.08 µg/mL	6.1 %	11.8 %	0.894	
	0.03 µg/mL	3.2 %	11.2 %	0.969	
Precision Positive Control (PC) (Lot #) in 100% neat pediatric GHD human serum	**PC Concentration (100% serum)**	**Run %CV**	**Mean OD**	**Mean Normalized Signal**	Tables I and 6 of Report AR4266 Amendment 1, Addendum 3
	0.5 µg/mL (LPC)	1.2 %	0.694	0.416	
	0.5 µg/mL (LPC)	1.9 %	0.780	0.517	
	0.8 µg/mL (MPC)	9.0 %	0.400	0.242	
	0.8 µg/mL (MPC)	0.1 %	0.506	0.301	
	2 µg/mL (HPC)	1.9 %	0.152	0.115	
	2 µg/mL (HPC)	1.9 %	0.197	0.105	

Table 36. Summary of Method Performance: hGH Neutralizing Cell-based NAb Assay ([redacted] Projects AR2919, AR4266 and AR5266)

Precision Positive Control (PC) (Lot # HRY0815082) in 100% neat human serum	**PC Concentration (100% serum)**	**Intra-Assay (RPD)**	**Inter-Assay (%CV)**	**Acceptance Range (OD)**	Tables C and 1 of Report AR5266 Addendum 1, Amendment 2
	78.2 µg/mL	1.6 %	2.5 %	0.071 – 0.217	
	39.1 µg/mL	6.9 %	3.9 %	0.069 – 0.208	
	19.5 µg/mL	6.6 %	7.0 %	0.069 – 0.207	
	9.77 µg/mL	4.9 %	7.4 %	0.069 – 0.208	
	4.88 µg/mL	10.1 %	17.9 %	0.083 – 0.259	
	2.44 µg/mL	20.0 %	20.1 %	0.349 – 1.046	
	1.22 µg/mL	2.9 %	5.1 %	0.566 – 1.698	
	0.611 µg/mL	2.1 %	9.3 %	0.676 – 2.029	
	0.305 µg/mL	1.9 %	3.6 %	0.704 – 2.112	
	0.153 µg/mL	0.4 %	4.0 %	0.709 – 2.128	
	0.076 µg/mL	3.6 %	2.4 %	0.706 – 2.118	
	0 µg/mL	4.2 %	1.7 %	0.697 – 2.092	
Precision Positive Control (PC) (Lot #HRY0815082) in 100% neat human serum to be used for sample analysis	**PC Concentration (100% serum)**	**Intra-Assay (RPD)**	**Inter-Assay (%CV)**	**Acceptance Range (OD)**	Tables D and 2 of Report AR5266 Addendum 1, Amendment 2
	19.55 µg/mL	8.7 %	15.9 %	0.075 – 0.226	
	2.45 µg/mL	14.8 %	61.5 %	0.260 – 0.781	
	1.2 µg/mL	9.4 %	16.3 %	0.550 – 1.649	
	0.15 µg/mL	5.0 %	6.8 %	0.731 – 2.193	
Precision Blank Control	**2% Human Serum + 1 ng/mL of hGH**	6.8% (%CV)	24.0%		Table 3 of Original Report AR2919
Precision Negative Controls	**Negative Control**	**Intra-Assay (RPD)**	**Inter-Assay (%CV)**	**Mean OD**	Table 5 of Report AR4266 Amendment 1
	NC-1: Cells + Assay Medium	5.9%	31.0%	0.220	
	NC-2: Cells + 5% GHD Pediatric serum	5.5%	22.0%	0.494	
	NC-3: Cells + hGH + Assay Medium	2.9%	14.9%	1.204	
	NC-4: Cells + hGH +5% GHD Pediatric serum	3.4%	14.1%	1.177	

Table 36. Summary of Method Performance: hGH Neutralizing Cell-based NAb Assay ([redacted] Projects AR2919, AR4266 and AR5266)

Precision Negative Control	**Negative Control**	**Intra-Assay (%CV)**	**Inter-Assay (%CV)**	**Mean OD**	
	NC-1: Cells + Assay Medium	1.9%	14.1%	0.156	Tables E and 1 of Report AR4266 Amendment 1, Addendum 3
	NC-2: Cells + 5% GHD Pediatric serum	2.6%	23.0%	0.500	
	NC-3: Cells + hGH + Assay Medium	2.0%	12.8%	1.614	
	NC-4: Cells + hGH + 5% GHD Pediatric serum	3.7%	12.2%	1.653	
Precision Negative Control	**Negative Control**	**Intra-Assay (%RPD)**	**Inter-Assay (%CV)**	**Mean OD**	
	NC-1: Cells + Assay Medium	3.7%	16.0%	0.145	Table 4 of Report AR5266, Addendum 1, Amendment 2
	NC-2: Cells + 2% Normal serum	5.8%	8.6%	0.168	
	NC-3: Cells + hGH + Assay Medium	4.5%	14.3%	1.318	
	NC-4: Cells + hGH + 2% Normal serum	5.8%	8.2%	1.498	
Cut Points	Normal Human serum: 0.810 OD (Mean OD of 50 individuals – (2 x SD of the same individuals)				Table 4 of Original Report AR2919
	GDH Pediatric (3-13) Serum Samples: 0.856 OD (Mean OD of individuals – (1.645 x SD of the same individuals) Cut Point Factor: 0.275 During sample analysis, the run cut point will be calculated as the mean negative control signal minus the cut point factor. Samples ≤ run cut point are considered NAb positive				Table 2 and Appendix 1 of Report AR4266 Amendment 1
	Normal Human serum: 1.008 OD, 1% False Positive Rate (Mean OD of 50 individuals – (2.326 x SD of the same individuals)				Tables E and 5 of Report AR5266 Addendum 1, Amendment 2
	0.126 Normalized Signal (NS), In-study, 1% False Positive Rate Individuals pediatric GHD Pre-dose serum samples from study CP-4-004				Table 1 of Report AR4266 Amendment 1, Addendum 2

Table 36. Summary of Method Performance: hGH Neutralizing Cell-based NAb Assay ([redacted] Projects AR2919, AR4266 and AR5266)

	0.400 Normalized Signal (NS), In-study, 1% False Positive Rate Individuals Adult GHD Pre-dose serum samples from study CP-4-005			Table 1 of Report AR5266 Addendum 3
	0.395 Normalized Signal (NS), Floating, 1% False Positive Rate Individuals pediatric GHD Pre-dose serum samples (Visit 2/baseline) from study CP-4-006 (CP-3) 0.574 Normalized Signal (NS), Floating, 1% False Positive Rate Used for Controls (CP-4)			Table 3 of Report AR4266 Amendment 1, Addendum 3
Sensitivity	4.90 µg/mL in 100% neat serum			Table 1 of Report AR2919 Amendment 1
	0.48 µg/mL in 100% neat pediatric GHD serum			Tables 10 of Report AR4266 Amendment 1
	0.31 µg/mL in 100% neat pediatric GHD serum			Table 4 of Report AR4266 Amendment 1, Addendum 3
Drug Tolerance 100% neat Pediatric GHD human serum	**PC Concentration (Anti-hGH)**	**hGH Concentration**	**Acceptance Criteria**	Table 8 of Report AR4266 Amendment 1
	3.90 µg/mL	20.0 ng/mL	Response remained < plate cut point (0.856 OD)	
	1.96 µg/mL	20.0 ng/mL		
	0.98 µg/mL	20.0 ng/mL		
	PC Concentration (Anti-hGH)	**hGH Concentration**	**Acceptance Criteria**	Table B and 1 of Report AR4266 Amendment 1 Addendum 1
	3.90 µg/mL	94.9 ng/mL	Response remained < plate cut point (1.433 OD)	
	0.98 µg/mL	22.4 ng/mL		
	0.48 µg/mL	9.0 ng/mL		
	PC Concentration (Anti-hGH)	**MOD-4023 Concentration**	**Acceptance Criteria**	Table C and 2 of Report AR4266 Amendment 1 Addendum 1
	3.90 µg/mL	245.6 ng/mL	Response remained < plate cut point (1.433 OD)	
	0.98 µg/mL	87.5 ng/mL		
	0.48 µg/mL	4.2 ng/mL		
Drug Tolerance 100% neat Normal human serum	**PC Concentration (Anti-hGH)**	**MOD-4023 Concentration**	**Acceptance Criteria**	Table 10 of Report AR5266 Addendum 1, Amendment 2 Addition of anti-hGH antibodies at 1.20 µg/mL and 0.15 µg/mL in neat serum is on the borderline of assay sensitivity, thus cannot be tolerated in the presence of any amount of MOD-4023 in the samples.
	19.55 µg/mL	1.00 µg/mL	Response remained < plate cut point (0.972 OD)	
	2.45 µg/mL	0.05 µg/mL		
	1.20 µg/mL	0.00 µg/mL	Response remained > plate cut point (0.972 OD)	
	0.15 µg/mL	0.00 µg/mL		

Table 36. Summary of Method Performance: hGH Neutralizing Cell-based NAb Assay ([redacted] Projects AR2919, AR4266 and AR5266)

Selectivity and matrix effect	Anti-hGH antibody was spiked into twenty (20) individual growth hormone deficient pediatric serum samples at High and Low levels (19.5 µg/mL and 1.20 µg/mL in 100% neat matrix, respectively) treated with 1 ng/mL hGH and incubated with BaFB2B2 cells overnight. All non-spiked samples and Low PC were negative, and all 20 High PC spiked individual samples were positive.	Table 6 of Original Report AR2919
	Anti-hGH antibody was spiked into twenty (20) individual pediatric GHD human serum samples at High, Medium and Low PC levels (3.9 µg/mL, 0.98 µg/mL and 0.48 µg/mL in 100% neat pediatric GHD matrix, respectively) treated with 1 ng/mL hGH and incubated with BaFB2B2 cells overnight. 19/20 non-spiked samples were negative. all 20 High and Medium PC spiked individual samples were positive. 14/20 Low PC spiked individual samples were positive. Cut Point: 0.856	Table 9 of Report AR4266 Amendment 1
	Twelve (12) drug naïve pediatric GHD Individual Serum Samples were spiked with anti-hGH antibody at the LPC level (0.5 µg/mL 100% neat serum). All non-spiked samples were negative, and all LPC spiked individual samples were positive relative to CP-3 (0.395).	Tables J and 7 of Report AR4266 Amendment 1, Addendum 3
	Anti-hGH antibody was spiked into ten (10), 5 male and 5 female individual normal human serum samples at High and Low levels (final concentrations 19.55 µg/mL and 1.2 µg/mL in 100% serum, respectively), treated with 1 ng/mL hGH and incubated with BaFB2B2 cells overnight. All non-spiked samples were negative, and all HPC and LPC spiked individual samples were positive relative to plate cut point (1.198 OD).	Table 7 of Report AR5266 Addendum 1, Amendment 2
Interference and specificity	Hemolysis (1% and 2% hemoglobin) Lipemia (3 mg/mL triglycerides) Icterus (30 µg/mL bilirubin) Although slight differences in sensitivity curves were observed, still able to detect positive NAb in the presence of potential matrix interferents.	Tables K and 8 of Report AR4266 Amendment 1, Addendum 3
	Three (3) of each Hemolyzed and Lipemic Individuals, unspiked and spiked with the anti-hGH positive control antibody at the final concentrations of 19.55 µg/mL (High Spiked) and 1.2 µg/mL (Low Spiked), were treated with 25 ng/mL MOD-4023 and incubated with BaFB2B2 cells overnight. All un-spiked Hemolyzed and Lipemic samples tested negative and all high spiked Hemolyzed and Lipemic samples tested positive. Two out of three the Low Spiked samples spiked Hemolyzed and Lipemic tested positive.	Table 8 of Report AR5266 Addendum 1, Amendment 2

Table 36. Summary of Method Performance: hGH Neutralizing Cell-based NAb Assay ([redacted] Projects AR2919, AR4266 and AR5266)

Dilution Linearity and hook effect	No hook effect observed starting at 78.2 µg/mL	Tables 1 and 2 of Original Report AR2919
Bench-top/process stability	24 hours in normal human serum at ambient temperature	Table 5 of Original Report AR2919
	24 hours in pediatric GHD human serum at ambient temperature	Table 7 of Report AR4266 Amendment 1
	72 hours in human serum at ambient temperature	Table 9 of Report AR5266 Addendum 1, Amendment 2
Freeze-Thaw stability	5 Freeze-Thaw cycles established in normal human serum	Table 5 of Original Report AR2919
	5 Freeze-Thaw cycles established in pediatric GHD human serum	Table 7 of Report AR4266 Amendment 1
Robustness	Two analysts assessed precision and reliability of the assay over 17 runs. The inter-assay variability of the positive control was within 30%, which is acceptable for a cell-based assay. Also cell passages 1-18 were confirmed.	Table 1 of Original Report AR2919
	Two analysts assessed precision and reliability of the assay over 23 sets of positive control. Inter- and intra-assay variability of the positive control samples was assessed following these parameters: 8 passages of cells, 3 plate shakers, 3 plate readers, and one lot of each of the 1X PBS buffer, Starve Medium, Assay Medium, pooled GHD serum, anti-hGH antibody, hGH, CellTiter 96® AQueous One Solution Reagent. The inter-assay variability of the positive control was within 31%, which is acceptable for a cell-based assay. This indicates there was no significant difference between analysts on different days across validation runs.	Report AR4266 Amendment 1

Table 37. Summary of Method Performance: hGH Neutralizing Cell-based NAb Assay (C0317008)

Bioanalytical method validation report name, amendments and hyperlinks	Pfizer Validation# C0317008 / [redacted] Project# 15-2030 The Validation of a Cell-Based Method for the Determination of Neutralizing Anti-hGH Antibodies in Human Serum, Effective Date: [redacted]20[redacted]				
Method description	A validated cell-based NAb assay based on the ability of hGH to induce cell proliferation by binding to the human growth hormone receptor (hGHR) which is expressed on the cell surface of the BaFB2B2 murine cell line. Neutralizing anti-hGH antibodies in human serum will inhibit the GH-proliferative effect.				
Minimum required dilutions (MRDs)	1:50				
Source and lot of reagents	Source: [redacted] Catalog# AF1067(PC1), OPKO Biologics(PC2), NIBSC Catalog# 80/505(hGH), Pfizer(MOD-4023) **Positive control (PC):** PC1: Goat hGH affinity purified pAB, Lot# HRY0819041 or equivalent PC2: Rabbit affinity purified polyclonal anti-MOD-4023, Lot# PAb4023-001 **Drug:** hGH, Lot# Not assigned by manufacturer MOD-4023, Lot# AG8262 **Cell Line:** B2B2 hGHR BAF (BaFB2B2) murine lymphocyte cell line, Mus musculus origin				
Validation parameters	**Method validation summary**				**Source location**
Assay Cut Point(ACP) Patient Human Serum	0.636 for Pediatric GHD Human Serum				Table 2 and Appendix 5, Statistical Analysis Report
Mass-based Sensitivity[a]	PC1: 0.52 µg/mL PC2: 0.57 µg/mL				Table 3 and Table 4 Table 5 and Table 6
Final LPC Level[b]	LPC1: 1.2 µg/mL				
Precision Positive Control (PC1) in 100% neat human serum	**PC1 Concentration (100% serum)**	**Intra-Assay (%CV)**	**Inter-Assay (%CV)**	**Acceptance Range (OD)**	Tables 7 to 9
	19.55 µg/mL (HPC)	9.7%	31.6%	0.027 – 0.261	
	2.45 µg/mL (MPC)	10.5%	29.5%	NA	
	1.2 µg/mL (LPC1)	10.0%	30.3%	0.046 – 0.636(ACP)	
	0.69 µg/mL (LPC2)	9.1%	12.4%	NA	
	0 (DC Signal)	5.5%	20.6%	> 0.7257	

Table 37. Summary of Method Performance: hGH Neutralizing Cell-based NAb Assay (C0317008)

Selectivity and matrix effect	Matrix Recovery PC1 in Patient Human Serum 9/10 reportable spiked samples HPC (S/DC) < LPC (S/DC) ≤ ACP, where LPC = 0.69 µg/mL 10/10 reportable blank sample results were negative (> ACP)			Table 10 and Table 11
	Hemolysis (PC in Hemolyzed Serum) 5/5 reportable hemolyzed samples HPC (S/DC) < LPC (S/DC) ≤ACP, where LPC = 0.69 µg/mL 6/6 reportable blank samples results > ACP			Table 12
	Lipemic (PC in Lipemic Serum) 6/6 reportable lipemic samples HPC (S/DC) < LPC (S/DC) ≤ ACP, where LPC = 0.69 µg/mL 6/6 reportable blank sample results were negative (> ACP)			Table 13
Specificity Drug Tolerance (PC1) with hGH	**Control**	**hGH Conc. (µg/mL)**	**Comment**	Table 14
	HPC (19.55 µg/mL)	0.05 µg/mL	≤ ACP	
	MPC (2.45 µg/mL)	0.05µg/mL	≤ ACP	
	LPC1 (1.2 µg/mL)	<0.01 µg/mL	≤ ACP	
	LPC2 (0.69 µg/mL)	<0.01 µg/mL	≤ ACP	
	NC	≥2 µg/mL	> ACP	
Specificity Drug Tolerance (PC1) with MOD-4023	**Control**	**MOD-4023 Conc. (µg/mL)**	**Comment**	Table 15
	HPC (19.55 µg/mL)	1.0 µg/mL	≤ ACP	
	MPC (2.45 µg/mL)	0.05 µg/mL	≤ ACP	
	LPC2 (0.69 µg/mL)	<0.01 µg/mL	≤ ACP	
	NC	≥2 µg/mL	> ACP	
Prozone Effect	No Prozone Effect observed			Table 16 and Figure 1

Table 37. Summary of Method Performance: hGH Neutralizing Cell-based NAb Assay (C0317008)

Plate Heomogeneity	11.4 %CV (N = 94 individual wells) at LPC in Normal Human Serum	Table 17
Bench-top/process stability	24 hours in human serum at ambient temperature	Table 18
Freeze-Thaw stability	8 Freeze-Thaw cycles established at -20°C	Table 19
	8 Freeze-Thaw cycles established at -70°C	Table 20
Cell Passage Stability	From Passage 13 to Passage 23 of the working cell bank	Table 21
Robustness	Assay robustness was demonstrated by 30 validation runs on 8 different days performed by 3 different analysts using multiple reagent lots, incubation times, and equipment	Tables 22 to 26

a Mass-based Sensitivity (95% Upper Limit) = Antilog [Mean + t0.05, df * SD]
b LPC Concentration (99% Upper Limit) = Antilog [Mean + t0.01, df * SD]

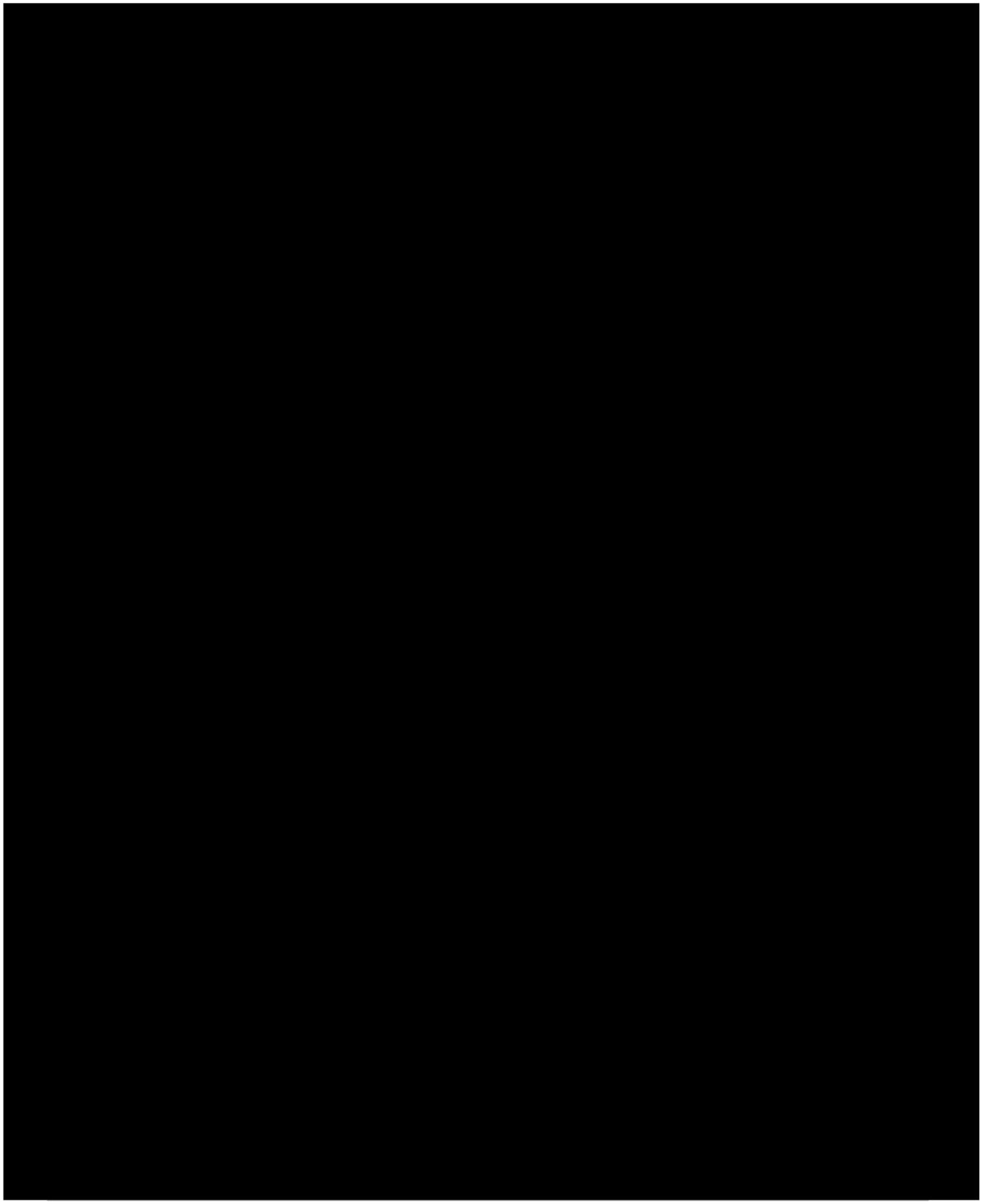
Table 38. Bioanalytical Method Life Cycle Information: IGF-1

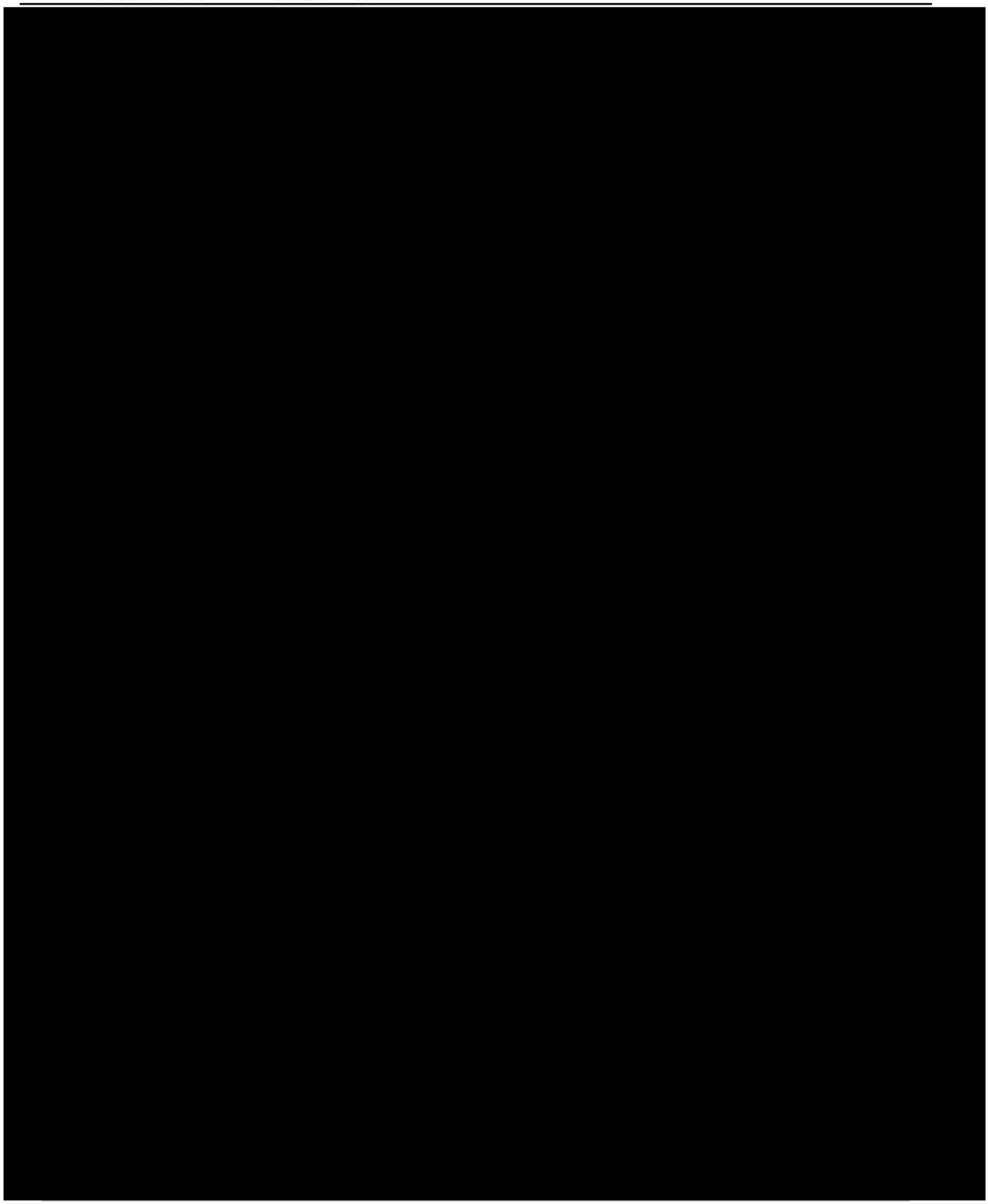
	CP-4-001	CP-4-007	CP-4-011	CP-4-003	CP-4-005	CP-4-004	CP-4-006	CP-4-009
Analyte	IGF-1	IGF-1	IGF-1	IGF-1	IGF-1	IGF-1	IGF-1	IGF-1
Method ID	Immulite 2000 IGF-1	Immulite 2000 IGF-1	Immulite 2000 IGF-1	IDS-iSYS	IDS-iSYS	IDS-iSYS	IDS-iSYS	Elecsys IGF-1
Bioanalytical site	[redacted] Israel	[redacted]	[redacted]	[redacted]				[redacted] Japan
Matrix	Human Serum	Human Serum	Human Serum	Human serum	Human serum	Human serum	Human serum	Human serum
Platform	Immulite 2000 (Siemens Healthcare Diagnostics)			IGF-1 SDS iSYS (Immunodiagnostic Systems)				cobas 8000 e801 (Roche Diagnostics)
Format	Solid phase enzyme labeled chemiluminescent immunometric			chemiluminescent				chemiluminescent
Calibration range from the LLOQ to the ULOQ	15 – 1000 ng/mL	15 – 1000 ng/mL	15 – 1000 ng/mL	10 – 1200 ng/mL	10 – 1200 ng/mL	10 – 1200 ng/mL	10 – 1200 ng/mL	15 – 1600 ng/mL
Link to reports and applicable amendments	NA – no report beyond CSR	NA – no report beyond CSR	Study trial TMF	NA – no report beyond CSR	NA – no report beyond CSR	NA – no report beyond CSR	NA – no report beyond CSR	NA – no report beyond CSR

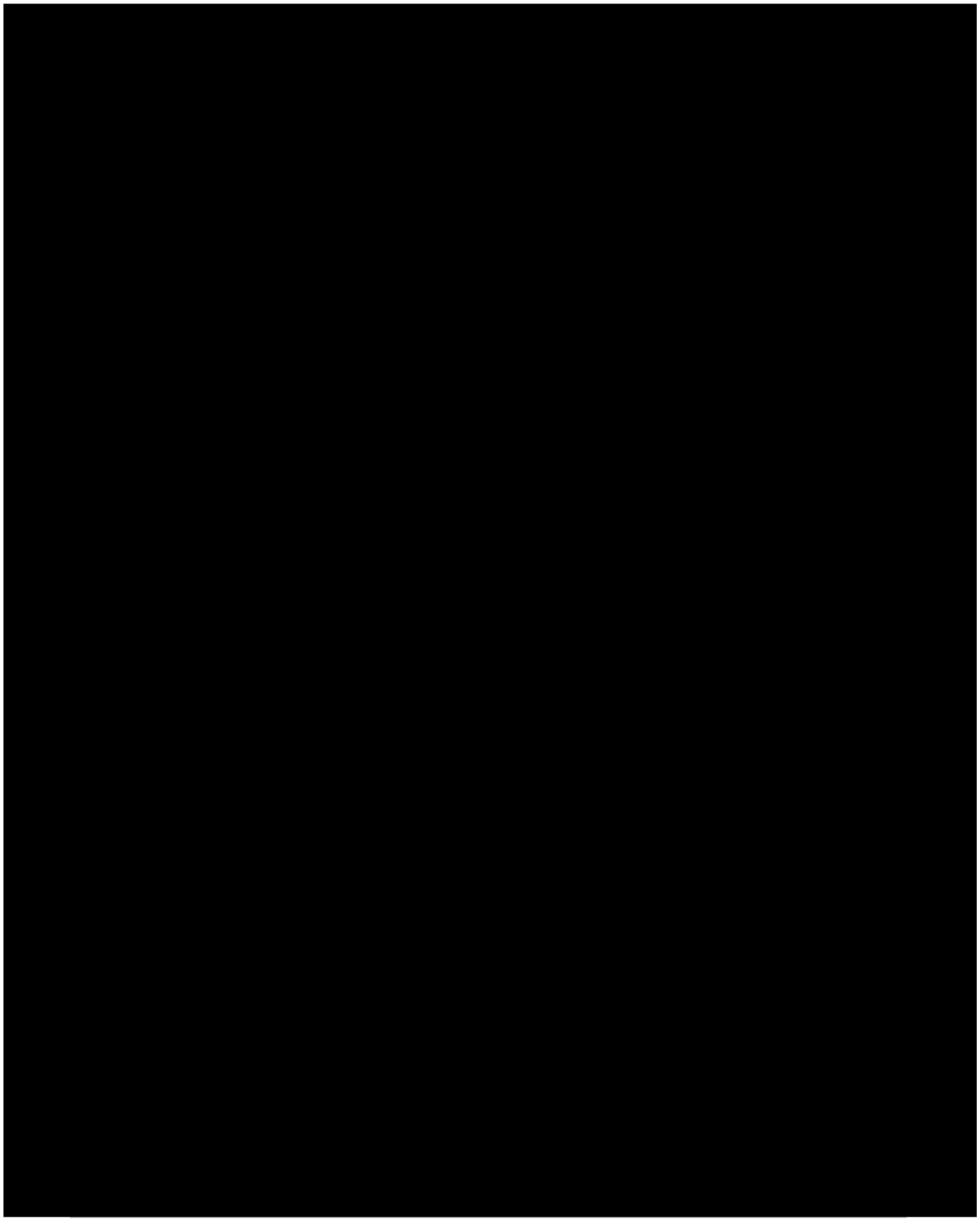
Table 39. Bioanalytical Method Life Cycle Information: IGFBP-3

	CP-4-001	CP-4-007	CP-4-011	CP-4-003	CP-4-005	CP-4-004	CP-4-006	CP-4-009
Analyte	IGFBP-3	IGFBP-3	IGFBP-3	IGFBP-3	IGFBP-3	IGFBP-3	IGFBP-3	IGFBP-3
Method ID	Immulite 2000	Immulite 2000	Immulite 2000	IDS-iSYS IS-4400	IDS-iSYS IS-4400	IDS-iSYS IS-4400	IDS-iSYS IS-4400	IDS-iSYS IS-4400
Bioanalytical site	[redacted] Israel	[redacted]	[redacted]	[redacted]				[redacted] Singapore
Matrix	Human Serum	Human Serum	Human Serum	Human serum	Human serum	Human serum	Human serum	Human serum
Platform	Immulite 2000 (Siemens Healthcare Diagnostics)			IGFBP-3 SDS iSYS (Immunodiagnostic Systems)				
Format	Automated chemiluminescent immunoassay, noncompetitive or sandwich assay format			chemiluminescent				
Calibration range from the LLOQ to the ULOQ	0.5 – 16.0 mcg/mL			80 – 10,000 ng/mL				
Link to reports and applicable amendments	NA – no report beyond CSR	NA – no report beyond CSR	Study trial TMF	NA – no report beyond CSR	NA – no report beyond CSR	NA – no report beyond CSR	NA – no report beyond CSR	NA – no report beyond CSR

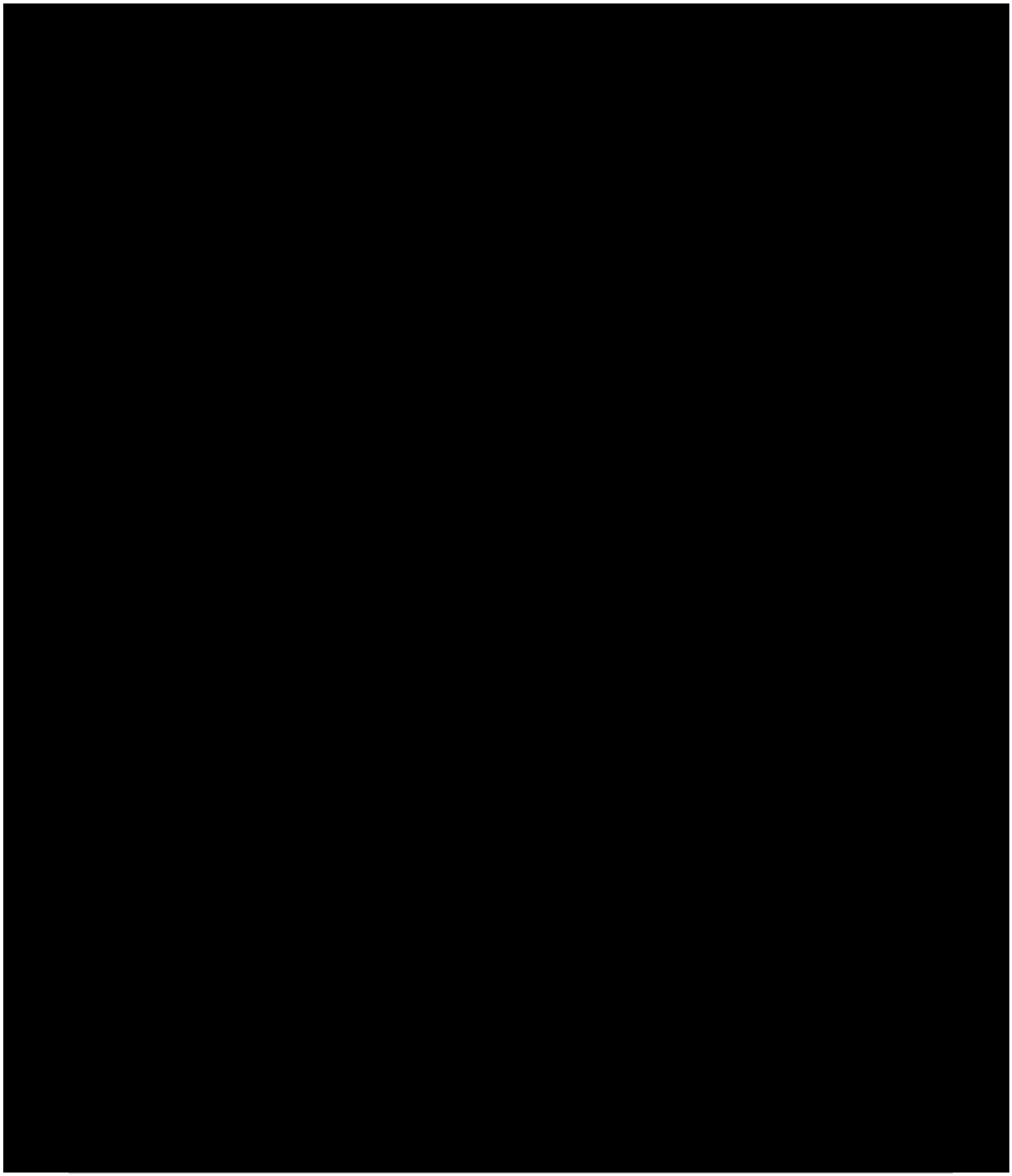
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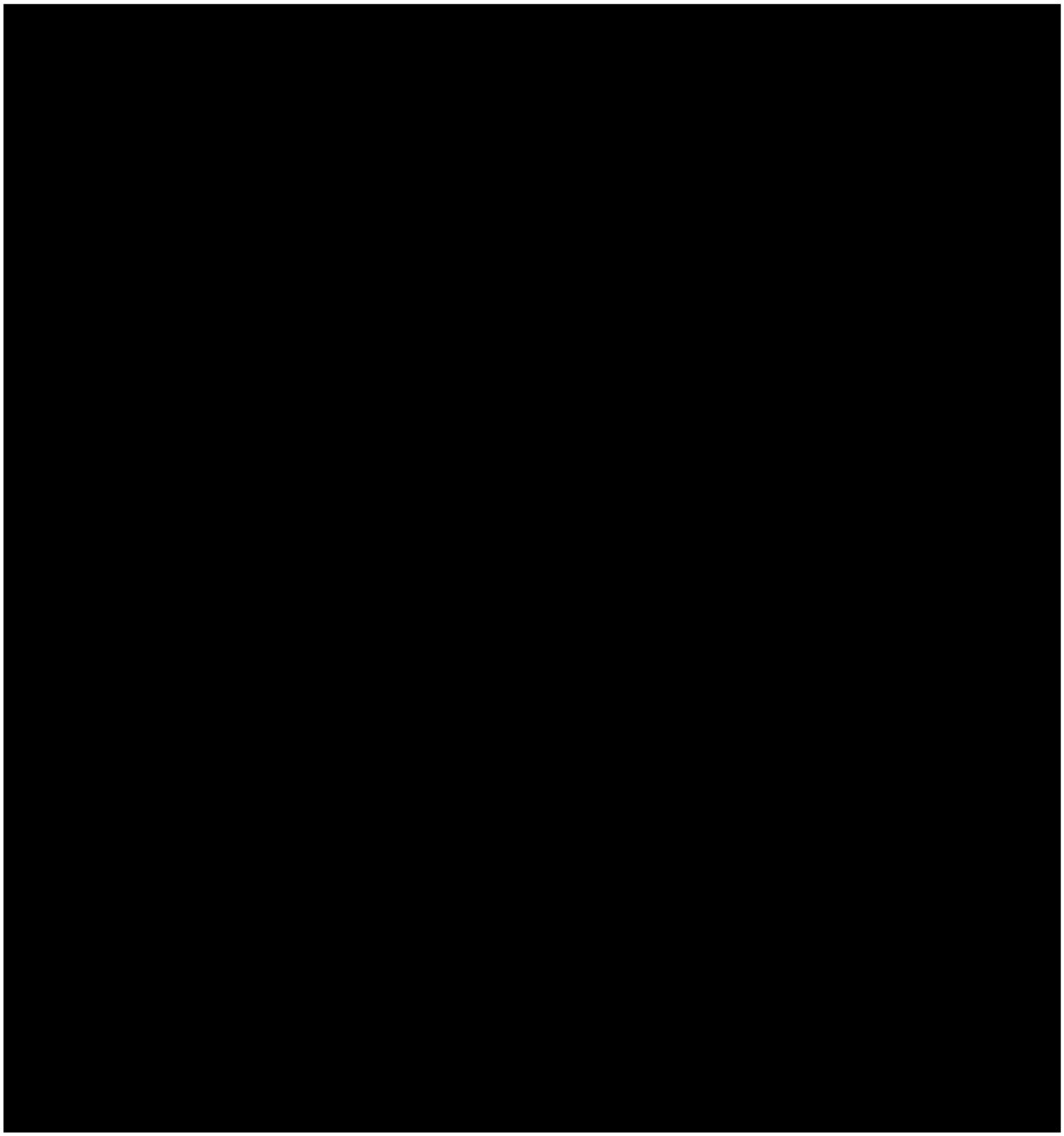


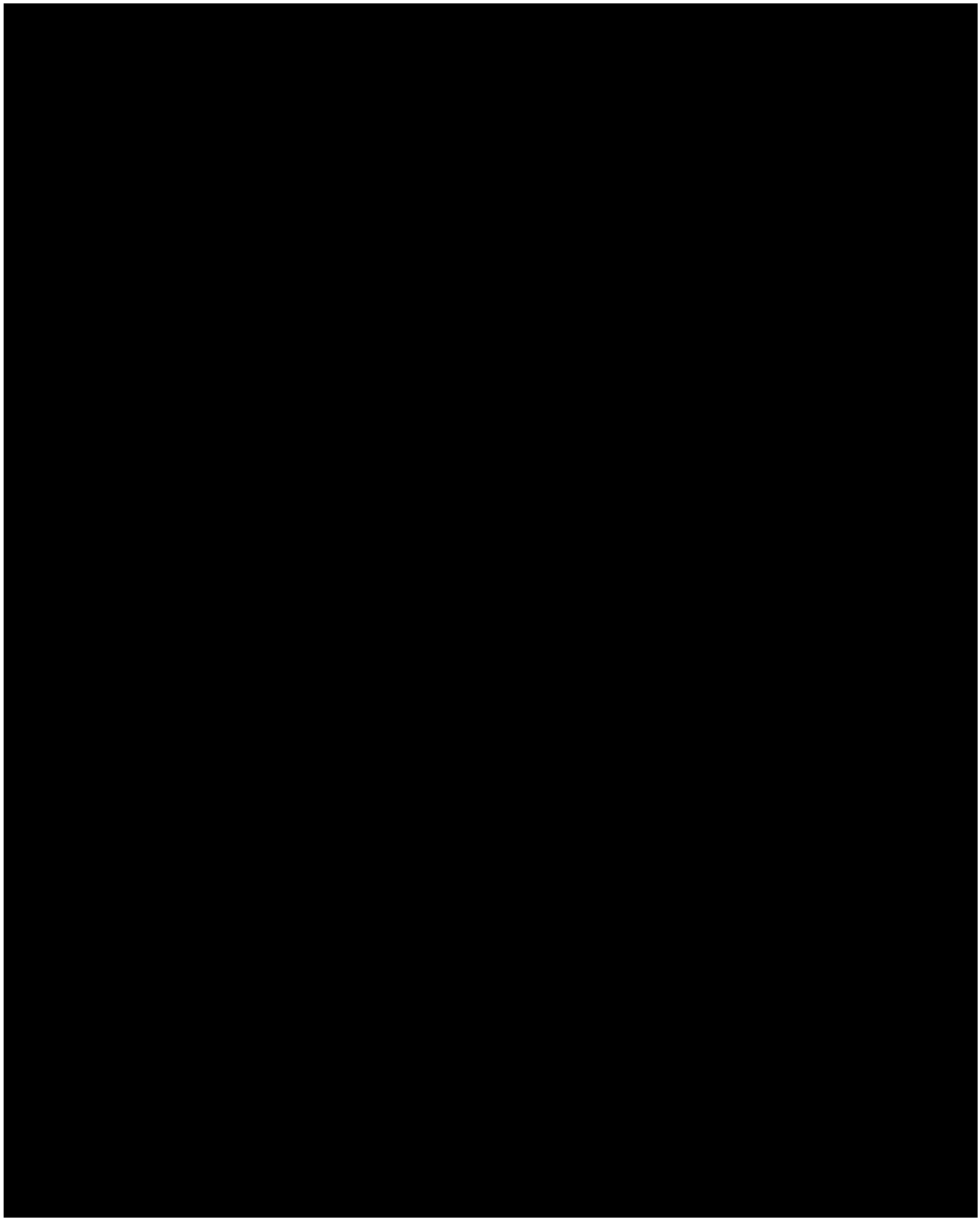


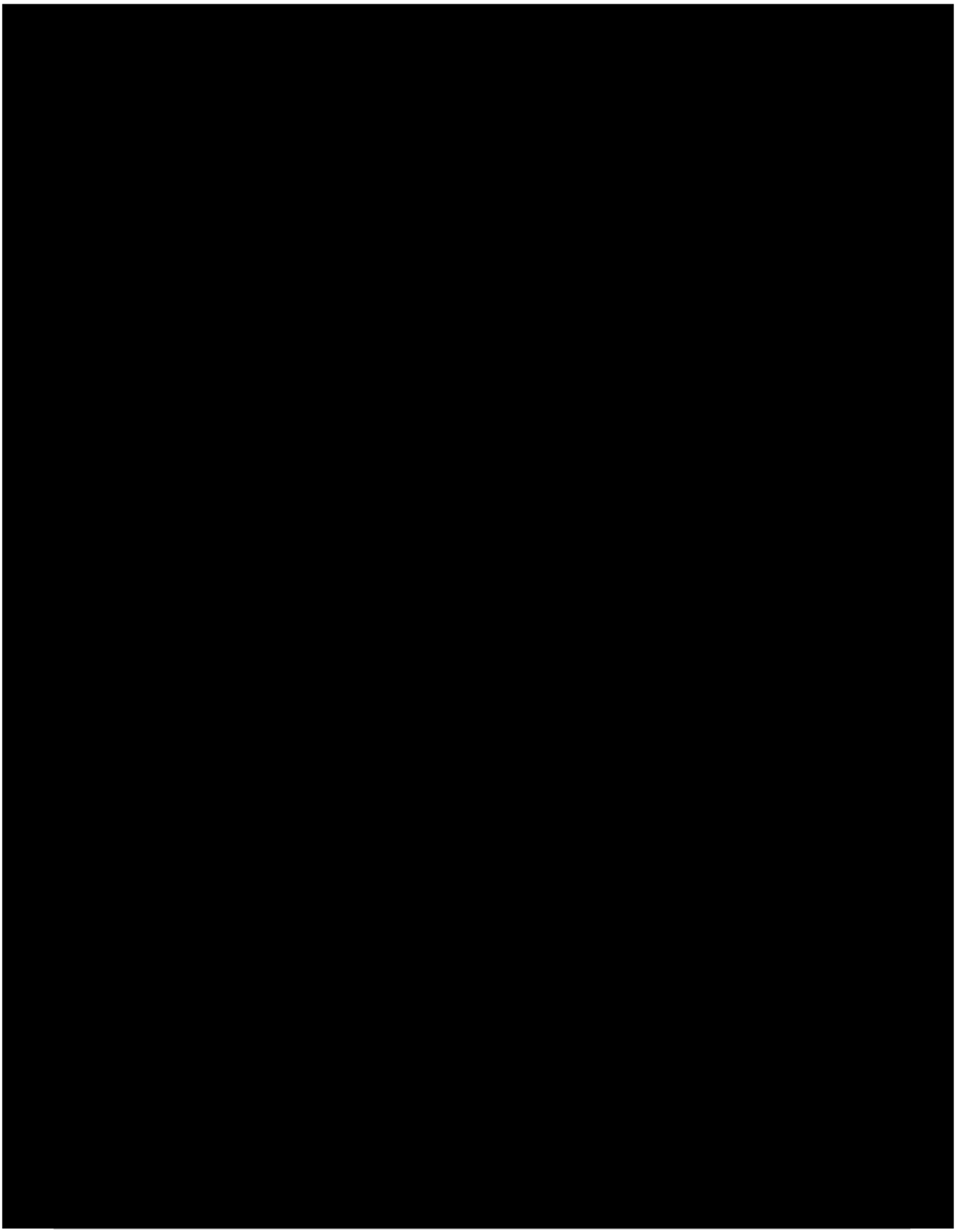




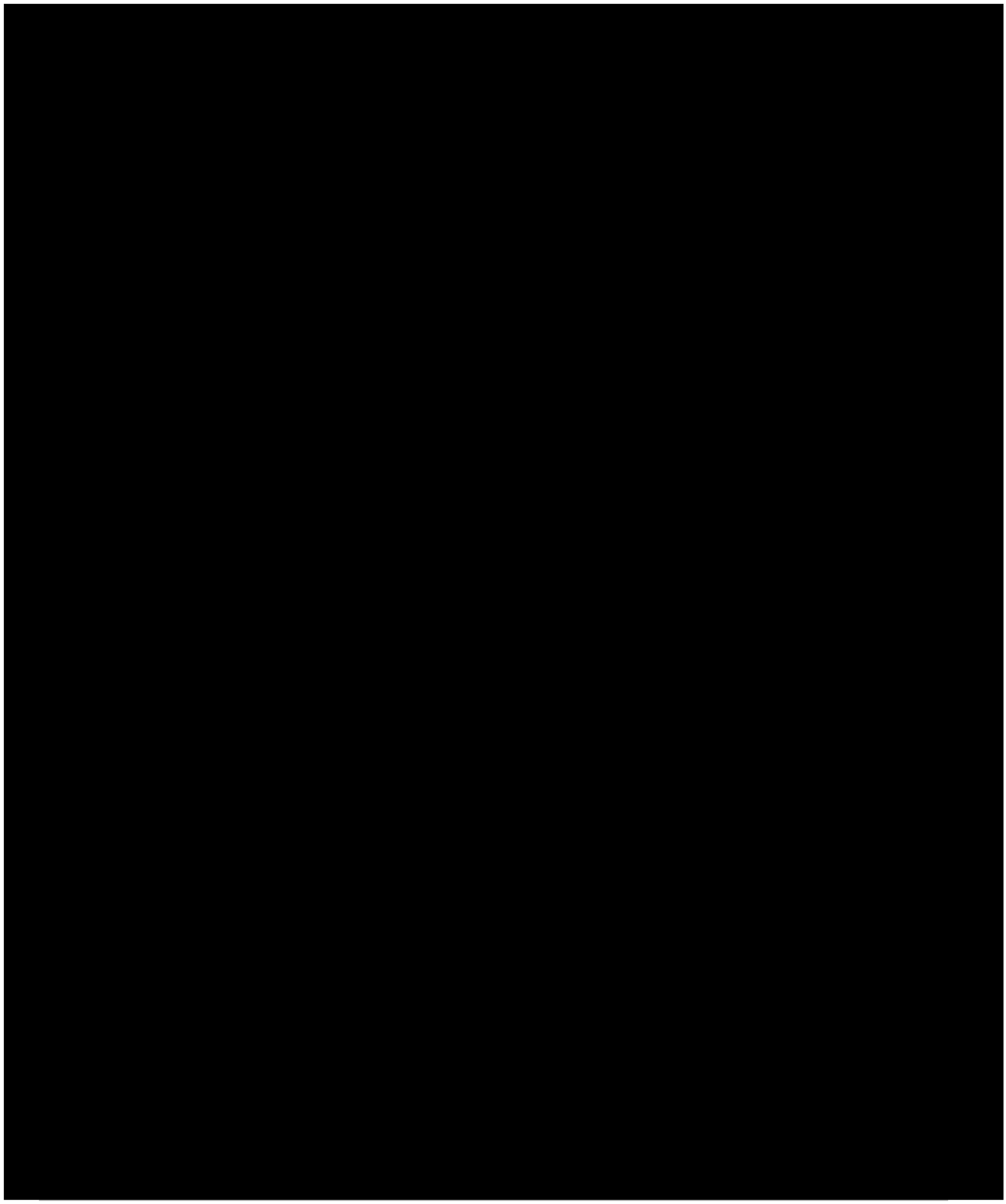


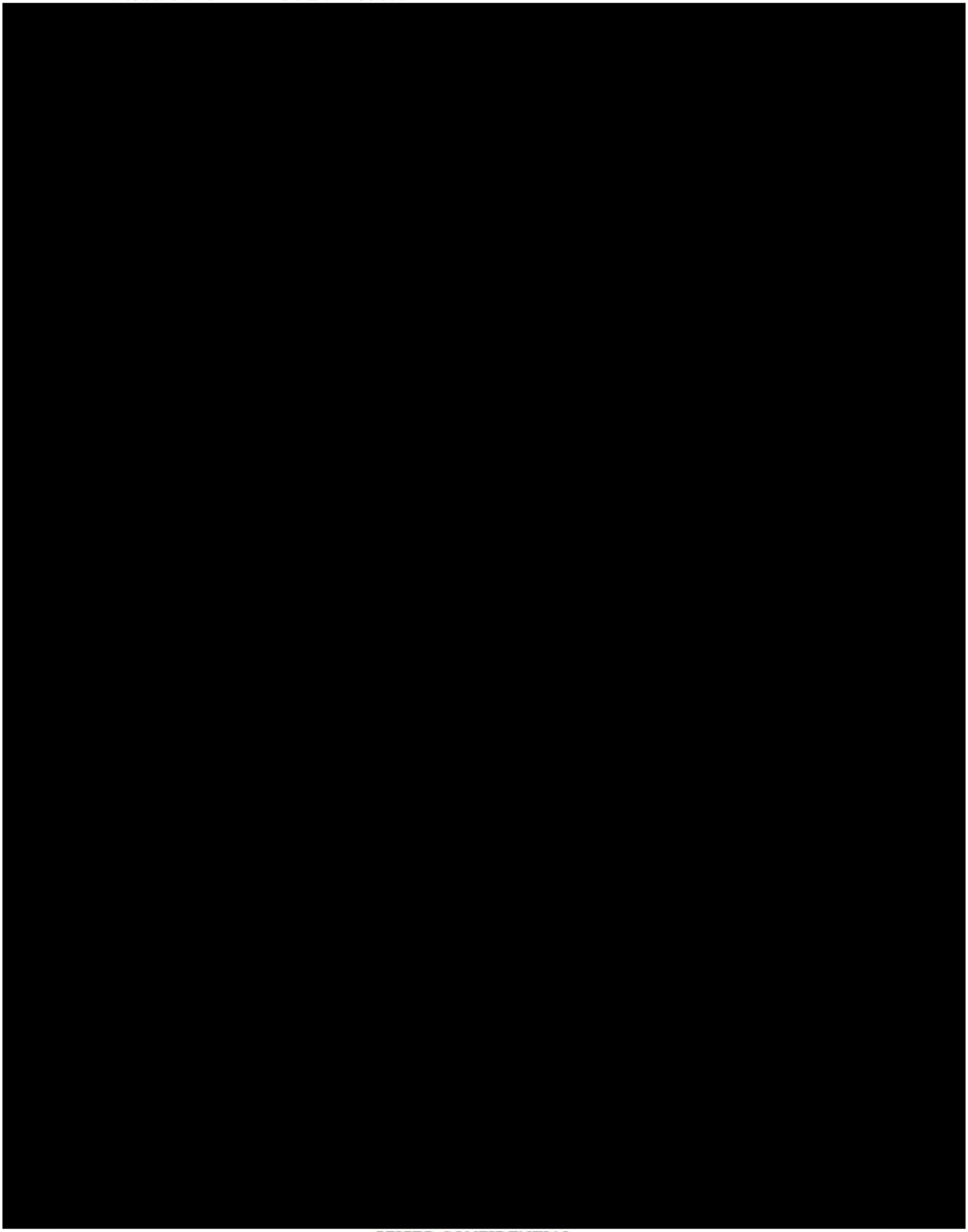












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