World Health Organization

WHO/BS/2019.2365
ENGLISH ONLY

EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION
Geneva, 21 to 25 October 2019

Report on a Collaborative Study for Proposed 1st International Standard for Adalimumab.

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NOTE:
This document has been prepared for the purpose of inviting comments and suggestions on the proposals contained therein, which will then be considered by the Expert Committee on Biological Standardization (ECBS). Comments MUST be received by **27 September 2019** and should be addressed to the World Health Organization, 1211 Geneva 27, Switzerland, attention: Technologies, Standards and Norms (TSN). Comments may also be submitted electronically to the Responsible Officer: **Dr Ivana Knezevic** at email: knezevici@who.int.

Summary

This report describes the development of an adalimumab International Standard (IS) and evaluation of its suitability to serve as a 'reference standard' for the *in vitro* biological activity of adalimumab and for use in the clinical setting for measuring adalimumab concentrations in samples from adalimumab treated patients.

For the bioassay study, two candidate preparations of adalimumab were formulated and lyophilized at NIBSC prior to evaluation by twenty-six participants using a range of *in vitro* cell-based bioassays and binding assays. An additional lyophilised preparation to assess assay sensitivity was also included. Study results and stability evaluation indicate that the candidate preparation, coded 17/236, is stable and suitable to serve as an International Standard for bioactivity of adalimumab. Use of this standard reduced the variability in potency estimates for the tested preparations when compared to estimates expressed relative to the in-house reference standards.

The adalimumab International Standard is intended to support *in vitro* bioassay characterisation, calibration and validation based on its defined international units of bioactivity. In addition to its role in calibration of secondary standards (manufacturer's, regional), it will serve as an 'anchor' for harmonizing bioactivity across products ensuring patient access to products which are consistent in quality and effectiveness. Importantly, it does not define the specific activity of the product and neither does it serve as the reference product for biosimilarity determination. Furthermore, it is not intended to revise product labelling or change therapeutic dosing.

A separate study evaluated the applicability of the standard coded 17/236 in methods currently used for therapeutic monitoring in clinical laboratories. For this, the candidate standard and a panel of human serum samples, spiked with different amounts of the two adalimumab candidates, were assessed by sixteen participants in a range of methods. There was excellent agreement in estimates for adalimumab content in the spiked samples regardless of the standard or the method used. Inter-laboratory variability was also similar regardless of the standard, 17/236 or in-house/kit standard used. Therefore, the candidate coded 17/236 is suitable for use as a standard for calibrating in-house/kit standards for therapeutic drug monitoring tests and can facilitate harmonization in clinical practice.

On the basis of the study results, it is proposed to the ECBS that the candidate standard, coded 17/236 be established as the first International Standard for Adalimumab with assigned values for in vitro activities per ampoule as follows : 500 IU of TNF-α neutralising activity, 500 IU of ADCC activity, 500 IU of CDC activity and 500 IU of binding activity.

Additionally, it is proposed that 17/236 with its assumed mass content of 50 μg per ampoule be also accepted as the IS for therapeutic monitoring of adalimumab. This will help to standardise and harmonize clinical monitoring assays for informing clinical decisions and treatment strategies.

Responses from study participants

Study participants were requested to comment on the proposal based on the study in which they participated -

i. establishment of the proposed candidate 17/236 as the 1st International Standard for Adalimumab for the different in vitro activities assessed in this study and independent assignment of 500 IU for each of the different bioactivities attributed to Adalimumab i.e., TNF-α neutralization, binding, antibody dependent cellular cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC).

   Responses were received from all participants. One participant requested general information on standard development, six requested clarification on analysis used and acceptance criteria, derivation of ED50 values and potency estimates. Another participant sought information on practice regarding in-house standards in various laboratories while another requested re-examination of CDC and ADCC raw assay data from participants for any potential matrix effects relating to excipients in the formulation of the candidates. All comments have been addressed in the report.

   Importantly, all responding participants agreed with the establishment of the proposed candidate 17/236 as the 1st International standard for Adalimumab and with the proposed unitage for TNF-α neutralization. There was also general agreement for the proposed unitage for binding (n=25), for ADCC (n=25) and CDC (n=25). While two participants expressed concerns with assigning unitages for ADCC and CDC due to the limited data available from the study, only one disagreed with assigning unitages for ADCC, CDC and binding. Since the majority of the participants agreed with assigning of independent unitages for the different activities studied, the proposal has not been amended.

ii. establishment of the proposed candidate 17/236 with an assumed content of 50 μg per ampoule as the reference standard for use in therapeutic monitoring of adalimumab.

   Responses were received from thirteen of the sixteen participants. There was unanimous agreement among responding participants on the use of 17/236 as the International Standard for qualification of internal reference standards for clinical monitoring assays using the mass content value as the basis of measurement. One participant proposed use of the term ‘assigned’ instead of ‘assumed’ but since the term ‘assigned’ would lead to inappropriate use of the International Standard, the proposal has not been revised.

Minor comments relating to typographical errors, the names of participants and their address details were also received. All minor amendments have been addressed in the report.

Introduction

Nearly three decades ago, the recognition that tumour necrosis factor-alpha (TNF-α), a pleiotropic cytokine involved in the regulation of immune and inflammatory responses is one of the key mediators in the pathogenesis of autoimmune and inflammatory disorders prompted intense development of molecules targeting TNF-α. Five different TNF-α antagonists - infliximab (Remicade®, Janssen), etanercept (Enbrel®, Amgen/Pfizer), adalimumab (Humira®, AbbVie), certolizumab pegol (Cimzia®, UCB) and golimumab (Simponi®, Janssen) have since been approved by the US Food and Drug Administration, FDA and the European Medicines Agency, EMA (Table 1A). These medicines have revolutionised treatment with significant impact on the quality of life in patients with inflammatory, dermatology and autoimmune diseases.

Adalimumab (Humira®), the world's first fully human therapeutic mAb, derived using phage display technology, is structurally an IgG1 antibody consisting of 2 identical heavy chains (451 amino acids and 51 kDa each) and 2 identical light chains (214 amino acids and 23 kDa each) of the kappa subclass, four inter-chain and twelve intra-chain disulfide bridges, a single N-linked glycosylation site and a total molecular weight of approximately 148 kDa when glycosylated (1,2). Adalimumab binds to both soluble and transmembrane TNF-α and prevents its interaction with TNF-R1 (p55) and -R2 (p75) receptors modulating TNF-α biological activity. Initially approved for treatment of moderate-to-severe forms of rheumatoid arthritis, Humira® is currently indicated for use in moderate to severe polyarticular juvenile idiopathic arthritis (JIA), active psoriatic arthritis (PsA), active ankylosing spondylitis (AS), moderate to severe active adult Crohn's disease (CD), moderate to severe active ulcerative colitis (UC), moderate to severe plaque psoriasis, hidradenitis suppurativa, uveitis etc (2).

The clinical use of Humira®, partly attributed to its expanding list of indications and its subcutaneous route of administration has translated into considerable commercial success. Humira® achieved the highest ranking in the top 10 list of bestselling biotechnology products for the last two years with global sales for 2017 in excess of $18bn (3). Such high sales along with the unmet medical need and prospective patent expiration stimulated fierce biosimilar development with a view to increasing competition, reducing costs and widening patient access globally. Consequently, in USA and Europe, several biosimilar products are now approved (Table 1B; 4-8). Unlike USA, where patents are still in place until 2023, several biosimilars have been marketed in Europe following loss of exclusivity for Humira® in October '18, with other products either in clinical development or under regulatory review. In England, savings of up to 150 million GBP a year are expected by 2021 with implementation of biosimilars in the national health service (9) against the cost of > 500 million GBP for Humira® in 2017/2018.

In emerging markets, several versions of anti-TNF-α products (intended copies, biocopies) including biosimilars are either available or in the development pipeline (10, 11). Unfortunately even today, many biosimilars manufactured in these regions and approved using local regulatory pathways may not strictly adhere with the biosimilarity principles and the rigorous comparability exercise required by stringent regulatory agencies (7,12,13), or those defined by the WHO in its guidance on similar biotherapeutic products (14, 15). WHO has therefore recognized a global need

for standardizing monoclonal antibodies as a control measure to ensure quality, efficacy and safety (16-18).

Despite its acclaimed clinical success, some safety and efficacy issues are evident with adalimumab (19,20). For example, in Crohn's disease, 10–30% of patients do not respond to the initial treatment (primary failure) with anti-TNF-α mAbs and up to 46 % of patients lose response over time (secondary failure), potentially due to formation of anti-drug antibodies, ADA (21). To rationalise treatment strategies, therefore, implementation of therapeutic drug monitoring (TDM) which comprises measurement of the anti-TNF drug and anti-drug antibodies is being actively considered in clinical practice (22,23). The American Gastroenterology Association has provided recommendations on TDM in inflammatory bowel disease, IBD (24) while in Europe, a generalised therapeutic algorithm for treatment of inflammatory diseases has been proposed (25,26). For efficient treatment, trough levels of adalimumab need to be within a certain therapeutic window (27,28). However, despite increasing debate in favour of implementing TDM in various indications including RA, guidance on TDM (except in IBD) is lacking. Only limited evidence exists on TDM use in clinical practice in the absence of large prospective studies comparing TDM versus usual care (29,30). Furthermore, challenges have been reported with access to timely and accurate TDM results because of different analytical testing methods in use in health care settings and the recognised lack of standardisation of these methods (31). In the UK, the National Institute for Health and Care Excellence (NICE) TDM guidelines have also advocated the need for standardization of assays (NICE diagnostics guidance [DG22] (32). Such monitoring would allow for a personalised dose optimisation or for treatment withdrawal/switch with better patient outcomes (26).

Based on the rationale above, we have developed an adalimumab International Standard (IS) and assessed its suitability to serve as a 'reference standard' for bioactivity determination (Study A) and for therapeutic drug monitoring purposes (Study B) in two independent collaborative studies using a range of methods in current use. This report describes the strategy adopted for IS development, the results of the two studies along with data on the stability of the IS and the units assigned to the bioactivity of the IS. It is anticipated that the IS, when established, will serve as a primary standard and calibrant for qualifying in-house reference standards for bioassays and drug monitoring assays and aid global harmonization where possible (e.g., bioactivity of different adalimumab products, commercial assays for drug levels).

Based on its categorization as a TNF-α antagonist, this project was endorsed by the WHO Expert Committee on Biological Standardization in October 2012.

Study A: Aims and Study Design

The purpose of this study was to characterize a candidate WHO 1st International Standard (IS) for its suitability in cell-based bioassays and binding assays for adalimumab and assign a unitage for activity. The study therefore sought

- To evaluate two candidate lyophilised adalimumab preparations and assess their suitability to serve as the 1st IS for adalimumab bioassays by assaying their biological activity in a range of routine, 'in-house' bioassays,
- To assess the relative activity of the candidates in assays in current use (e.g. bioassays, immunoassays) and determine, where possible, the concentrations of adalimumab required to neutralise specific amounts of TNF-α IS.
- To compare the candidates with characterised 'in-house' laboratory standards where these are available.

An additional lyophilised preparation to assess assay sensitivity or ability of the assays to detect differences was also included in the study.

Participants

Twenty-eight participants from 14 different countries were dispatched study samples. Twenty-six participants from 13 countries returned data which contributed to the study (Table 2). The participants included 12 pharmaceutical companies, 2 contract research organisations, 9 regulatory/control laboratories, 2 pharmacopoeias and 1 commercial reagent supplier. Participating laboratories have been assigned code numbers allocated at random, and not representing the order of listing in Table 2 to retain confidentiality in the report.

Materials and Processing

Two bulk drug substance preparations of recombinant adalimumab, each from a single batch were kindly donated to WHO by two manufacturers (see Acknowledgement).

Trial fills were conducted using two different formulation buffers: A) 25mM Sodium citrate tribasic dihydrate, 150mM Sodium chloride, 1% Human serum albumin, pH 6.5 and B) 10mM L-Histidine, 10mM L-Histidine hydrochloride monohydrate, 1% D-trehalose dihydrate, 0.01% Polysorbate-20, 1% Human serum albumin, pH6.2. The biological activity of the lyophilized preparations was compared with the bulk material in two different laboratories in cytotoxicity assays using WEHI-164 and L929 cells respectively. Although both formulations proved to be suitable, Formulation A was selected for the final production fills as this retained marginally more biological activity relative to the bulk material in comparison with Formulation B in both assays (not shown).

The final lyophilization for the two candidates was performed at NIBSC using ECBS guidelines (33). For this, buffers and excipients (final compositions as shown in Table 3), were prepared using nonpyrogenic water and depyrogenated glassware and solutions filtered using sterile nonpyrogenic filters (0.22μM Stericup filter system, Millipore, USA) where appropriate.

Table 3 provides information on the preparations, the protein content, the number of ampoules and the study codes. The approximate mass content of the protein in the ampoules, given as ‘predicted μg’ in Table 3, is calculated from the dilution of the bulk material of known protein mass content as provided by the manufacturer.

As indicated in Table 3, for both preparations, a solution of adalimumab at a theoretical protein concentration predicted to be 50μg/ml was distributed in 1 ml aliquots into 5 ml ampoules. In addition, a solution of one of the candidates with a slightly lower content predicted to be 40μg/ml was also dispensed in 1ml volumes. All preparations were lyophilised under optimised and controlled conditions, the glass ampoules sealed under dry nitrogen by heat fusion and stored at -20°C in the dark until shipment at room temperature.

Characterisation of the lyophilised preparations

For each fill, a percentage of ampoules were weighed, residual moisture of each preparation was measured by the coulometric Karl-Fischer method (Mitsubishi CA100) and the headspace oxygen content was determined by frequency modulated spectroscopy using the Lighthouse FMS-760 Instrument (Lighthouse Instruments, LLC). The mean fill weights, moisture content and headspace oxygen content, which is a measure of ampoule integrity, are reported in Table 4. Testing for microbial contamination using the total viable count method did not show any evidence of microbial contamination.

Study Design

Participating laboratories were provided with a sample pack, which consisted of 5 ampoules each of the study samples A-C, for each different type of assay they were undertaking. Some laboratories were also sent sample D which contained approximately 40 μg as opposed to 50 μg for assessing the ability of the assays to detect differences. In order to reduce assay variability arising from use of human TNF-α from different suppliers, participants undertaking TNF-α neutralization assays were provided with 5 ampoules of the 3rd TNF-α IS (coded 12/154) for the bioassays.

Prior to performing the assays for the study, participants were advised to perform a pilot assay using the study samples for each of the assay types they intended to undertake to ensure appropriate assay conditions and optimal dose response curves. For TNF-α neutralization bioassays, participants were also advised to select a suitable dose of TNF-α.

Following establishment of suitable conditions, participants were asked to assay all samples concurrently on a minimum of three separate occasions using their own routine methods, within a specified layout which allocated the samples across 3 plates and allowed testing of replicates as per the study protocol (Appendix Protocol Study A). It was requested that participants test samples, A-C and their in-house (IH) standard where available on each plate and perform at least 8 dilutions for each sample using freshly reconstituted ampoules for each assay. Participants were requested to return their raw assay data, using spreadsheet templates provided, and also their own calculations of potency of the study samples relative to preparation A or their own in-house standard.

For binding assays, participants were requested to use their proprietary assay kits or in-house assays to assess the binding to human TNF-α of the three candidate preparations and their in-house

standard using serial dilutions. Participants were requested to perform three independent assays on three separate occasions and return raw data in a format that was appropriate for the assay technique used.

Statistical analysis

An independent statistical analysis of all bioassay data was performed at NIBSC. Analysis of dose-response curve data was performed using a four-parameter logistic (sigmoid curve) model (except for assays from three laboratories as specified below where a parallel line model was used)

$$y = \alpha - \frac{\delta}{1 + 10^{\beta(\log_{10}x - \log_{10}\gamma)}}$$

where y denotes the assay response, x is the concentration, α is the upper asymptote, δ is the difference between upper and lower asymptotes, β is the slope factor and γ is the EC_{50} (50% effective concentration). Assay responses (absorbance, luminescence etc.) were log transformed for this analysis and it was therefore considered reasonable to combine data from all different readout formats to then derive assay validity (parallelism) criteria. Models were fitted using the R package 'drc' (34,35). Parallelism (similarity) for a pair of dose-response curves was concluded by demonstrating equivalence of the parameters α, β and δ. Equivalence bound values and the methods for determining them are described in the Results section of this report.

Due to testing the samples at fewer dilutions than other laboratories, analysis of data from laboratories 4a (neutralization), 7 and 8 (both binding) was performed using a parallel line model. Equivalence criteria applied to the β parameter in the sigmoid curve model analysis were used to confirm parallelism of the samples tested.

Relative potency estimates were calculated as the ratio of EC_{50} estimates in all cases where acceptable parallelism was concluded. All relative potency estimates were combined to generate unweighted geometric mean (GM) potencies for each laboratory and these laboratory means were used to calculate overall unweighted geometric mean potencies. Variability between assays and laboratories has been expressed using geometric coefficients of variation (GCV = $\{10^s\text{-}1\} \times 100\%$ where s is the standard deviation of the $\log_{10}$ transformed potencies).

Stability studies

All stability srtudies were undertaken at NIBSC. Accelerated degradation studies were performed to predict the long-term stability of the candidate standard. Ampoules of the lyophilised preparation were stored at different temperatures, namely 45 °C, 37 °C, 20 °C and 4 °C and tested at indicated time points together with ampoules stored at the recommended temperature of -20 °C and -70 °C as baseline reference temperature. There was no observed loss in potency so no attempt to predict degradation rate for the proposed standard onstorage at -20 ° C has been undertaken as per the Arrhenius equation relating degradation rate to absolute temperature assuming first-order decay (36). Real time monitoring of stability is ongoing.

Results

Data returned for analysis

Results were received from 26 laboratories. All participants provided raw data from the assays so a global analysis of assay validity could be applied to allow data to be treated equally and to allow data from different laboratories to be compared to each other. Participants also reported ED50 and relative potency estimates based upon their own calculations.

Assay Methods

A summary of the assay methods used in the study is given in Tables 5, 6 and 7. All study participants used their own qualified bioassays using their own assay acceptance and validity criteria, in-house standards (majority used Humira® or their proprietary in-house standard representing therapeutic adalimumab, one laboratory used a research grade adalimumab while another used a different TNF antagonist).

For TNF-α neutralization, three different types of bioassays previously employed for ISs for infliximab and etanercept were used (37-39) . These included cytotoxicity assays based on the cytotoxic effect of TNF on murine fibroblast, L929 (40) or fibrosarcoma, WEHI-164 or the WEHI-13 variant (41) cell-lines; apoptosis assays using the human histiocytic lymphoma cell-line, U937 (42) and reporter gene assays using the human embryonic kidney cell-line, HEK-293 transfected with the TNF-α responsive NFκB regulated Firefly luciferase (FL) or secreted embryonic alkaline phosphatase (SEAP) reporter-gene constructs.

As the mechanism of action of adalimumab involves Fc-effector functions, both complement dependent cytotoxicity (CDC) and antibody dependent cytotoxicity assays (ADCC) were included in the study. However, these were performed by only a few laboratories. CDC assays in which adalimumab induces lysis of Jurkat cells expressing membrane-bound TNF-α (43) in the presence of complement were conducted by four participants while five participants performed ADCC assays. All used engineered cells expressing a non-cleavable mutant of membrane bound TNF-α as targets while effectors were either the natural killer cell line (NK92 or NK3.3) which lyses target cells upon activation (43,44) or reporter gene containing cells which luminesce in response to crosslinking of CD16 by adalimumab (45) in the presence of cells presenting surface-bound TNF-α antigen.

Binding assays were performed by eight participants. A majority employed (n=5) the direct ELISA format (TNF-α immobilised onto the plate) and used either HRP-conjugated anti-IgG (Fc specific), - anti-IgG1 or - anti-kappa chain for detection. Other assay platforms included electrochemiluminescence (ECL), fluorescence resonance energy transfer (FRET), bio-layer interferometry and surface plasmon resonance (SPR) platforms. Two participants also conducted cell-based binding assays using flow cytometry.

Assay Validity

Equivalence bounds for each model parameter (α, β and δ) were set using data returned from the neutralization assays performed by twenty-six participating laboratories. As these parameters are expected to be equivalent when testing the same sample against itself, absolute differences in α, $\log_{10}\beta$ and δ parameters for the coded duplicate samples A & C were calculated for each plate and upper equivalence bounds set as the 95th percentile of these values. This gave upper bounds 0.078, 0.140 and 0.190 for the absolute difference in α, $\log_{10}\beta$ and δ parameters respectively. The upper bound for $\log_{10}\beta$ corresponds to a slope factor ratio of 1.38. For two dose-response curves to be concluded as parallel, equivalence had to be demonstrated for all three parameters (α, β and δ). It should be noted that the equivalence bounds were intended for use in the analysis of data from this study only, in order to apply consistent criteria to all laboratories and assess their relative performance. The bounds should not be interpreted as suitable values for routine use in the assessment of assay validity within the collaborating laboratories and may be overly stringent or lenient in the case of some laboratories. Observed parameter differences are shown in Figure 1. The percentage of invalid assays per lab is shown in Appendix Table 1 illustrating the range in relative performance of the participating laboratories using the defined equivalence criteria. In several laboratories (20/51 cases) no invalid assays were noted and in several others (23/51 cases) total invalidity rates were ≤25%. The majority of invalid assays were observed in the remaining laboratories (8/51 cases), in particular for participant 04 where the high invalidity rates (>50%) were due to non-parallelism with the in-house standard (irrelevant in-house standard), and not between coded study samples A-D. In a minority of cases (a single participant for binding, CDC and ADCC assays), an inhibitory effect was noted at the very high concentrations of the samples (possibly attributed to the excipients in the formulation), these data points were excluded from the analysis.

Potency estimates relative to sample A or in-house reference standards

Potency estimates calculated relative to candidate standard sample A or relative to in-house reference standards where available are summarized for each laboratory performing neutralization assays in Table 8 and other assays in Tables 9 and 10. An overall summary for each assay type is shown in Table 11 and individual assay estimates are shown in Appendix Table 2. Boxplots of laboratory GM relative potencies are shown in Figure 2.

Intra-laboratory GCV values for the potencies of samples B and C relative to A ranged from 2.27 % to 32.02% in neutralisation assays, with a median value of 7.83% and the majority (63%) of values were less than 10% (87% less than 20%), demonstrating generally good intermediate precision in participating laboratories (n=26). Overall, the levels of variability in neutralization assays were comparable to those seen in binding assays regardless of the standard used. For binding assays, intra-laboratory GCV values ranged from 0.61% to 32.32% relative to sample A and 4.48% to 28.08% in comparison with in-house standards.

Inter-laboratory GCV values for samples B and C relative to A were 6.43% and 5.61% in neutralization assays, with 7.36% and 6.91% respectively in binding assays. Inter-laboratory GCV values for samples B and C in neutralization assays when the in-house standards were used were

17.3% and 13.6% indicating a higher level of inter-laboratory variability than for potencies relative to sample A. All neutralization assays were fairly comparable in terms of their GCVs when a common standard, A is used. However, the lowest inter-laboratory variability was observed using the L929 cytotoxicity assay – GCV was 4.33% and 4.67% for B and C relative to sample A and 12.59% and 14.83% when the in-house standards were used. Unfortunately, for laboratories undertaking WEHI-164 and U937 assays, there was insufficient data for GCV determination using in-house standards.

The potency estimates using CDC and ADCC were consistent with values seen with neutralization and binding assays. Intra-laboratory variability was noted to be similar for CDC assays (2.71% to 36.07% with a narrower range of %GCV for ADCC assays (6.91% to 23.84%) and a wider range for binding assays (0.61% to 32.32%) when assessed relative to sample A. However, for ADCC assays, the intra-lab variability range was wider when in-house standards were used.

To conclude, the study data show that the use of sample A as a reference standard to calculate the relative potency of sample B allows a close agreement between laboratories for each of the bioactivities tested in comparison with in-house standards

Potency estimates of D relative to sample A

Among laboratories which tested Sample D (n=11) in the neutralization assays, the geometric mean potency relative to sample A was less than 0.90 in all but two cases (0.94 in laboratory 06 and 1.07 in laboratory 24), giving an overall geometric mean potency of 0.86 with a GCV of 9.28% and 95% confidence limits 0.81 to 0.91. Similar observations were noted in binding assays (range 0.77 to 0.94 in four laboratories that tested sample D). The value for the mean relative potency of D was broadly consistent with the expected theoretical value suggesting that the assays where this sample was tested were adequately sensitive in detecting lower activity associated with reduced adalimumab content.

Estimates of ED50 derived from neutralization assays

Geometric mean estimates of ED50 for each laboratory and sample are shown in Table 12. There was no clear relationship between ED50 values and the dose of TNF-α used by participants. As expected, the geometric mean values for ED50 varied between different laboratories and assay methods with GCV for candidate samples ranging from 1.94% to 32.76 % in L929 assays (except for laboratory 5), 9.38% to 36.45% in U937 apoptosis assays and from 2.28% to 37.08% for reporter-gene assays. For the WEHI-164 assays, the GCVs were generally high in a majority of cases (except laboratory 2a), and ranged from 11.46% to 74.85%. A summary of ED50 estimates for L929 assays using a fixed TNF-α concentration of 20 IU is given in Table 13.

Stability studies

Accelerated Degradation Studies

Samples of the candidate standard 17/236 were stored at elevated temperatures (4°C, 20°C, 37°C and 45°C) for up to 15 months and assayed at NIBSC using the L929 cytotoxicity assay. Samples were tested concurrently with those stored at the recommended storage temperature of -20°C, and baseline samples stored at -70°C. The potencies of all samples were expressed relative to the appropriate -70°C baseline samples and the results are summarised in Table 14. Since no loss in activity was evident following storage at any of the elevated temperatures for 17/236, no predicted loss in activity can be calculated at the present time.

Stability after reconstitution and on freeze-thaw

Samples of the candidate standard 17/236 were reconstituted and left at 4°C or room temperature for either 1 day or 1 week. The reconstitutions were timed to allow all samples to be assayed concurrently against a freshly reconstituted ampoule. The potencies of all samples were expressed relative to the freshly reconstituted samples and the results are summarised in Table 15. Results indicate that the potency of the reconstituted candidate standard is not diminished after a week of storage at either 4°C or room temperature.

Samples of the candidate standard 17/236 were reconstituted and subjected to a series of freeze-thaw cycles (1 up to 4). They were then assayed concurrently with a freshly reconstituted ampoule. The potencies of all samples were expressed relative to the freshly reconstituted samples and the results are summarised in Table 16. The results indicate that the potency of this preparation is not diminished with repeated freeze-thaw cycles (up to 4).

Study B: Aims and Study Design

This study was aimed to assess the suitability of a candidate adalimumab preparation to serve as the 1st WHO IS for assays routinely used in measuring adalimumab levels in the clinical setting. To achieve this, the study assessed the candidate adalimumab preparation and compared it with kit standards or in-house standards using different assays/platforms. A panel of serum samples spiked with adalimumab were also assessed versus the candidate adalimumab preparation and different kit or in-house standards.

Participants

16 participants from 8 different countries were dispatched samples and all returned data for the study (Table 17). These included 1 contract research organisation, 2 control laboratories, 1

academic laboratory, 6 commercial kit manufacturers, 2 hospital laboratories and 4 clinical diagnostic centres.

Materials and Processing

The lyophilised candidate adalimumab preparation, 17/236 coded Sample A described in Study A (Materials and Processing Section, Tables 3, 4) and a panel of human serum samples containing different amounts of adalimumab were evaluated. For this, twenty-four samples were prepared by spiking two pools of normal human sera (First Link and Sigma-Aldrich respectively) with either reconstituted candidate A or the two adalimumab preparations supplied (for use as candidates – Study A) in amounts shown in Table 18. The samples were stored at -40°C until despatch or use.

Study Design

Prior to the study, a survey was conducted and based on the responses received, it was clear that participants were using a variety of assays (e.g., in-house assays, commercial kits). While differences were noted in the doses of the standard, assay range, sample treatment, use of quality control samples and the number of samples that could be accommodated on a single plate, all assays measured 'free' adalimumab. Consequently, the study was organised by taking account of these responses for the amounts of adalimumab to be used for spiking of the samples and the number of samples that could be analysed.

Participating laboratories were provided with 1 sample pack, consisting of 4 ampoules of study sample A, and adequate amounts for the serum samples for each assay type they were intending to perform. Participants were requested to use their own in house assays e.g., own proprietary kits, commercially purchased kits or methods developed in-house (Table 19) and conduct the assays as indicated in the study protocol (Appendix Protocol - Study B). Prior to performing the assays for the study, participants were advised to perform a pilot assay using the candidate A in each of the assay types they intended to undertake to ensure appropriate assay conditions and optimal dose response curves for the kit/in-house standard and candidate A.

Following establishment of suitable conditions, participants were asked to perform three independent assays and to assess all samples concurrently in each assay (Appendix Protocol - Study B). It was requested that participants perform dilutions of freshly reconstituted ampoule A for each independent assay and include their own in-house (IH) standard where available and/or the kit standards on each plate. Participants were requested to return their raw data for each assay and their own calculations of adalimumab concentrations in study samples relative to preparation A, to their own in-house standard and/or to the kit standards.

Statistical analysis

Adalimumab levels (µg/ml) in spiked serum samples were calculated relative to sample A and kit standards or in-house standards using four-parameter logistic (sigmoid curve) models. Results determined relative to sample A assumed a concentration of 50µg per ampoule for this standard.

Estimates were combined as unweighted geometric means (GM) for each laboratory and these laboratory means were used to calculate overall unweighted geometric mean estimates. Variability between laboratories has been expressed using geometric coefficients of variation (GCV = $\{10^{s}-1\} \times 100\%$ where s is the standard deviation of the $\log_{10}$ transformed estimates).

Assessment of agreement in mean estimates for each pair of laboratories was performed by calculating Lin's concordance correlation coefficient (46,47) with log transformed data. Calculations for this were performed using the R package 'DescTools' (34). A value of 1 for this coefficient indicates perfect agreement between the two laboratories.

Results

Data were received from all 16 participants. These are listed in Table 17 and assigned code numbers allocated at random, and not necessarily in the order of listing to maintain confidentiality.

Assay Methods

A summary of the assay methods used in the study is given in Table 19. Of the sixteen participants, twelve used ELISAs while four used other platforms, namely ECL assays (n=2) and lateral flow immunoassays (n=2). A majority of ELISAs were commercial kits (n=10) although in house assays were also employed (n=2). ELISAs were based on direct (TNF-α or anti-adalimumab to capture) or sandwich (anti-TNF-α immobilized to capture soluble TNF-α) formats and used different approaches for detection. The lateral flow immunochromatography (LFI) assays detected complexes formed between adalimumab, TNF-α conjugated to gold colloids and a mAb specific for adalimumab immobilized on a test membrane while the ECL assays were either performed in solution phase, (where samples were incubated simultaneously with biotin- and sulfotag-labelled TNF-α) or using the stepwise approach (using immobilised TNF-α and sulfotag-labelled anti human IgG kappa light chain for detection) like an ELISA. Most assays are specific for adalimumab, however, in some cases, other anti-TNF-α therapeutics may be detected.

All participants incorporated a kit standard and/or an in-house standard in their assay(s) as indicated in Table 19. For these standards, commercially available adalimumab (Humira®) is diluted in appropriate matrix. For assay, further dilutions of kit and/or in house standards as well as dilutions of sample A and spiked serum samples were performed using assay diluent as per individual assay protocols.

Evaluation of spiked serum samples

Data from laboratories 5T (only performed one assay with dilutions of sample A), 11T (performed dilutions of sample A in assays independently of spiked serum samples) and 13T (only one assay performed) were excluded from the analysis for this report. Additionally, data from laboratories 10T and 15T obtained using dilutions in serum were also excluded.

Adalimumab levels (µg/ml) in spiked serum samples were calculated relative to sample A and kit standards or in-house standards (see Tables 20, 21). Results for the spiked serum samples were determined relative to kit standards in laboratories 1Ta, 1Tb, 2T, 3T, 4T, 10T, 12T, 14T and relative to in-house standards (IH) in laboratories 6T, 7T, 8T, 9T, 15T, 16T.

Laboratory geometric mean estimates (µg/ml) for samples S1-S24 spiked with theoretical amounts of adalimumab (Table 17), calculated relative to kit/in-house standards and sample A as standard are summarised in Tables 20 and 21 respectively and illustrated in Figure 3. Of note, the sample with the lower amount of adalimumab (2µg/ml) spiked with anti-drug antibody (ADA) showed a lower adalimumab content relative to its counterpart samples devoid of ADA. Comparable levels of inter-laboratory variability were observed regardless of the standard used (median GCV in Table 20 is 15.40% with range 11.70% to 19.27%; median GCV in Table 21 is 15.36% with range 10.82% to 24.05%).

Concordance in log transformed laboratory geometric mean estimates (µg/ml) for samples S1-S24 calculated relative to kit/in-house standards and sample A as standard is summarised in Table 22 (values equal to or exceeding 0.90 are shaded). There is generally excellent concordance between laboratories for estimates in spiked serum samples relative to either Sample A, the kit standard or in-house reference standards regardless of the method employed.

Discussion

The expiry of patent exclusivity has led to the approval of several adalimumab products. Just like the reference product, Humira®, these products are dosed in mass units with no information on bioactivity provided on their label. However, as per regulatory principles, determination of the product's bioactivity/potency relative to a reference standard using *in vitro* bioassays, constitutes an important aspect of the routine lot release performed by the manufacturer. This assessment requires proprietary well characterised product specific in-house reference standards to ensure that the product meets its specifications (48). Moreover, potency is highly relevant for the comparability exercise during biosimilar development and for certain post-approval manufacturing changes. The latter occur frequently with monoclonal antibodies and have only rarely resulted in a shift in quality with no reported clinical impact (49-51). This is also the case with the two anti-TNF mAbs, Remicade® (infliximab) and Humira® which have undergone a significant number of post-approval changes (including site transfers and scale changes) (50,52). Unexpected changes, although seldom can also occur (53,54). In the current therapeutic landscape with many adalimumab products (and their own reference standard), each undergoing manufacturing changes, there is a potential for a shift in bioactivity and product divergence. The availability of an International Standard (IS) with defined international units of bioactivity will allow bioactivity evaluation of different products through their life-cycle, facilitate calibration of secondary standards (manufacturer's, regional) where feasible and serve as a 'bench mark' for harmonizing bioactivity across products over time.

As for infliximab, the mechanism of action of adalimumab in different inflammatory diseases may vary (55). Adalimumab binds specifically to both transmembrane and soluble forms of TNF-α, the

latter with relatively high affinity, preventing its interaction with its receptors, TNF-R1 (p55) and -R2 (p75), modulating TNF-α biological activity. In rheumatoid indications, adalimumab acts primarily by neutralization of soluble TNF-α, while in IBD, its binding to the membrane-bound form can trigger other important activities such as reverse signaling, suppression of cytokine secretion and induction of apoptosis. Additionally, Fc-effector functions such as ADCC and CDC may also contribute to the mode of action in IBD. This study, therefore, included a range of assays representative of the bioactivities of this antibody (5,55), even though TNF-α neutralization is the current lot release assay for adalimumab products. With this in mind, the suitability of adalimumab candidate preparations to serve as the 1st WHO IS was assessed in a multi-centre collaborative study.

All participant data were assessed for assay validity by setting of equivalence bounds based on a detailed analysis of all dose-response curve parameters in order to determine parallelism. This approach was then applied to all assays. It should be noted that the equivalence bounds applied here are intended for use in the analysis of data from this study only, in order to apply consistent criteria to all laboratories and assess their relative performance. The bounds should not be interpreted as suitable values for routine use in the assessment of assay validity within the collaborating laboratories. Applying the global analysis to neutralization assays meant that the majority of laboratories (18 out of 23) had less than 25% invalid assays, indicating this global analysis worked well and assays were of high quality, even with stringent validity parameters applied.

Combining the data from all TNF-α neutralization bioassays provided relative potencies which were similar to those found for each of the individual types of bioassays. Geometric mean relative potencies were 1.04 for candidate B and 1.01 for coded duplicate C when compared to candidate A for all the bioassays with a range of 0.92-1.24 in the individual laboratories for candidate B and 0.88-1.17 for coded duplicate C. Overall, the inter-laboratory GCVs were good for the neutralization bioassays when the lyophilised preparations were compared relative to the participants' in-house standards (17.3% and 13.6%) but this was further improved when the preparations were compared relative to candidate A (6.43% and 5.61%). For the cytotoxicity assays, the L929 cell-line gave better inter-laboratory GCVs than WEHI-164 (<5% for L929 and 10.08% and 6.08% for B and C respectively using WEHI-164) when relative potencies were determined relative to candidate A. In contrast to participants IH standards, expressing the potencies of B and C relative to candidate A improved the inter-laboratory GCVs associated with L929 assays (<5% vs 12.59% and 14.83% for B and C respectively). In reporter gene assays, potencies were the same for B and C (1.04) relative to A in comparison with the mean potency of 1.13 and 0.99 for B and C respectively relative to the in-house standards. Similar to other assays, the inter-laboratory GCV for B and C relative to A was considerably reduced in comparison with the in-house standards (6.58% and 5.85% vs 23.30% and 12.65%). Since there were fewer valid assays using in-house standards for laboratories undertaking WEHI-164 and U937 assays, improvement of inter-laboratory GCV with use of sample A coud not be determined.

To assess the inhibitory effect of adalimumab, ED50 estimates were derived for each laboratory performing neutralization assays. While no correlation was seen between the IU of TNF-α used in the assay and the ED50 value for the L929 assays, a positive correlation was observed for the other

TNF-α neutralization bioassays. For the proposed IS, the inhibitory activity was determined by taking the ED50 values derived for the L929 cytotoxicity assays from selected laboratories using 20 IU of TNF-α IS and by using the following equation:

$$\text{Amount of adalimumab (IU) inhibiting a fixed amount of TNF-}\alpha\text{ (IU)} = \frac{\text{potency of preparation (IU) x ED50 (ng)}}{\text{Assumed mass content (ng)}}$$

Therefore, based on data from five laboratories (Table 12), 0.085 IU of adalimumab candidate A, (code 17/236) inhibits the cytotoxic effect of 20 IU of TNF-α IS (code 12/154) in an L929 cytotoxicity assay. For deriving the inhibitory activity, the proposed arbitrary unitage of 500 IU for the adalimumab candidate A coded 17/236 has been used.

ADCC assays tend to be generally variable and are highly influenced by the target, the effector cell type, the expression of FcγRIII receptors, receptor polymorphism, the assay conditions and the readout employed (43,45,56). The glycosylation pattern of the mAb, particularly the degree of afucosylation can also impact Fc-mediated ADCC activity (53,57). In this study, engineered Jurkat T cell effectors which provide a 'surrogate assay' based on effector cell activation and NK cell-lines which promote lysis and therefore better reflect the physiologically relevant mechanism of action for ADCC were used (58,59). However, despite differences in both the target (transfected) and effector cells (some transfected) employed in the ADCC assays, the individual potencies were quite consistent among the different laboratories with values of 0.98 - 0.99 for B and 1.04 - 1.07 for C relative to A. For laboratory 26, however, the ADCC activity of the samples was slightly lower relative to A - a value of 0.88 for B with a GCV of 18.07%. However, the potency of B compared with the in-house standard was 1.10 with a GCV of 33.86% compared with the overall value of 0.98 and a GCV of 13.03%. Generally, the inter-laboratory variability for ADCC assays was excellent relative to the common standard 'sample A' and ranged from 5.44% for B to 9.32% for C, this increased to 18.10% for A, 13.03% for B and 10.84% for C when in-house standards were used. The geometric mean potency estimates relative to A (0.97 for B and 1.02 for C) or to in-house standards (0.98 for B and 1.00 for C) were very similar to those derived using TNF-α neutralization assays which are based on the antigen binding function of adalimumab.

For CDC assays, a similar trend as noted for neutralization assays was also seen with the limited number of assays that were performed. For these assays, geometric mean potencies relative to A were 1.02 and 1.06 (with a GCV of 10.34% and 9.05%) for B and C respectively but were slightly higher than those seen with the in-house standards at 0.83 for B and 0.87 for C (GCV of 27.56% and 19.11% with the in-house standards). The latter can be explained by the low values obtained in laboratories 25 (0.66, 0.67, 0.79, n=1) and 26 (0.76, 0.68, 0.71) for all samples relative to the in-house standard but the underlying reason for the low values is not clear.

In addition to bioactivity, binding assays are often used for assesssing the interaction of the Fab regions of adalimumab with TNF-α (5). Overall, the combined data from the different types of binding assays in the study (ELISAs, FRET) showed good agreement in potency values (0.89 – 1.19) relative to A with low inter-laboratory GCVs (6.91-7.36%). However, some variability in potencies (0.64 -1.05) between the laboratories for candidate samples was seen when potency was expressed relative to participants' IH standards (15.2-16.03%). For cell-based binding assays, the

potency estimates were consistent with those seen with all binding assays and also with TNF-α neutralization bioassays when expressed relative to candidate A. A single laboratory (laboratory 12) measured the affinities of the samples to TNF-α by surface plasmon resonance (SPR) using a multi-cycle kinetics approach. Comparable results for all samples including the in-house standard were obtained (data not shown).

The use of candidate A (17/236) reduces the inter-laboratory variability across a range of *in vitro* bioassays and binding assays. For TNF-α neutralization bioassays employing the 3rd WHO IS for TNF-α (12/154) as the source of TNF-α, inter-laboratory GCVs of less than 7% are easily achievable with slightly larger GCVs of less than 10% in all other assays. Furthermore, Sample D which contained 20% less adalimumab showed lower potency in a majority of assays where tested (Tables 8-11).

As stated earlier, the utility of TDM as a tool for drug optimisation and patient management is actively being considered by clinicians. Despite availability of several commercial kits, new approaches are being explored for determining trough levels of adalimumab (60-66). Each of these methods have their own advantages and limitations. As an example, the lateral flow immunoassay is a rapid point-of-care test offering a distinct advantage over other methods (61-64, 66). In this study, some of these methods were used to evaluate the suitability of the candidate, Sample A. We spiked serum samples with candidate preparations A and B to quantify the levels of adalimumab using some of the commercial kits and other methods and explored whether harmonization of the serum determinations with candidate A would improve inter-laboratory variability. The geometric mean of the estimates provided by thirteen laboratories (fourteen assays) for each of the spiked samples using their kit or in-house standards gave estimates which are in good agreement with each other, with a GCV of between 11.70 -19.27%. Estimates for the samples relative to candidate A also showed good agreement with a GCV of between 10.82-24.05%. In each case, the sample mass content was found to be comparable and consistent with the theoretical content. Inter-laboratory assay variability was also comparable regardless of the standard used. Evaluation of correlation coefficients showed excellent inter-laboratory concordance for the spiked samples (equal to or in > 0.90 in most laboratories) for estimates calibrated using kit or in-house standards and candidate A respectively, regardless of the standard or the method employed. Such excellent concordance was also seen when the same assay was used in different laboratories (3T, 12T and 14T). Although only a limited number of assay systems were evaluated here, it is evident from the study data that the candidate preparation 17/236 is suitable for use in tested assay systems and, therefore, can be used for qualification of in house standards based on the assumed mass content of the ampoule. Adoption of a common standard will facilitate assay standardisation and harmonization of clinical monitoring as per demands from clinicians and healthcare organisations (31,32).

International reference standards are intended to be long-lasting stable preparations suitable for global distribution, thus formulation and process development is optimised to fulfil this requirement whilst preserving bioactivity required for the standard's intended use (18, 33). Studies undertaken post-reconstitution showed that the potency is not diminished after 1 week of storage at either 4˚C or 20˚C nor after repeated freeze-thaw cycles. Stability studies over 15 months indicated that the bioactivity of the candidate preparation 17/236 has not deteriorated despite

storage at elevated temperatures supporting its utility as an international standard. As no loss in activity was detected at any of the elevated temperatures, no predicted loss in activity could be calculated. Further accelerated degradation and real time stability studies will be undertaken to monitor and predict potential loss of activity over time.

As this study included multiple methods for any assay type, the definition of unit is not related to any specific method of determination. Bearing in mind that different products may vary in terms of their different bioactivities, independent arbitrary unitage values are assigned for the individual activities of adalimumab. This approach will in future allow assignment of independent unitage for each activity (when calibrated against the 1st IS) when a replacement of the 1st adalimumab IS is being considered (study data shows sample B can serve as a replacement standard). Therefore, the candidate preparation (NIBSC code 17/236) with arbitrary values per ampoule of 500 IU of TNF-α neutralising activity, 500 IU of binding activity, 500 IU of ADCC activity and 500 IU of CDC activity is proposed as the 1st International Standard for adalimumab.

The IS for adalimumab with its unique characteristics and features, in common with other mAb IS is a distinct entity with a different role from the reference product which defines biosimilarity (18) and cannot be used interchangeably or as a substitute for the reference product (37,67). The proposed adalimumab IS (unitage) is not intended to define the specific activity of products for regulatory purposes nor revise product labelling or change therapeutic dosing. Nevertheless, as a higher order standard, it has an essential role in potency evaluation by supporting calibration of secondary (e.g., national, pharmacopoieal, in-house reference standards) standards where feasible, facilitating method development and qualification/validation, supporting product life-cycle management and surveillance. The IS will also support the calibration and validation of adalimumab assays in the clinical setting and enable progress towards personalised treatment and better disease/patient management.

Conclusions and Proposal

Data from the multi-centre collaborative study demonstrated the suitability of both adalimumab candidate preparations in a range of in vitro bioactivity assays that were performed. Given that stability data also confimed the suitability of the adalimumab candidate preparation NIBSC code 17/236 to serve as the 1st WHO International Standard for the *in vitro* biological activities of adalimumab, it is proposed to the WHO ECBS that the candidate preparation 17/236 be accepted as the WHO 1st IS for adalimumab with assigned values per ampoule of 500 IU of TNF-α neutralising activity, 500 IU of binding activity, 500 IU of ADCC activity and 500 IU of CDC activity.

Additionally, the study showed the suitability of the proposed 1st IS for adalimumab coded 17/236 for assays currently in use for clinical monitoring. Therefore, it is proposed that this IS with its assumed mass content of 50 μg per ampoule be also accepted as the IS for therapeutic monitoring

of adalimumab. This will facilitate standardisation of assays that are being used to inform critical clinical decisions and treatment strategies for effective patient outcomes.

Acknowledgements

We are very grateful to Abbvie, USA and Samsung Bioepis, Korea for their kind donations of adalimumab for use as candidate materials and to the participating laboratories for their support and willingness in performing the laboratory tests within the strict study timelines. We are grateful to Paul Matejtschuk and Kiran Malik for their enormous contribution in identifying suitable formulation and lyophilization conditions, the excellent support and service provided by staff in the standards processing division in lyophilizing and dispatching the study materials.

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Table 1A: TNF-α antagonists and their year of approval

Trade name	INN	Manufacturer	Product Type	US approval	EU approval
Remicade®	Infliximab	Janssen	Chimeric mAb	1998	1999
Enbrel®	Etanercept	Amgen/Pfizer	Soluble TNF receptor II Fc-fusion protein	1998	2000
Humira®	Adalimumab	AbbVie	Fully human mAb	2002	2003
Cimzia®	Certolizumab pegol	UCB	Humanized antibody Fab' fragment conjugated to polyethylene glycol	2009	2009
Simponi®	Golimumab	Janssen	Fully human mAb	2009	2009

Table 1B : Adalimumab biosimilars and their year of approval in Europe and USA

EU Biosimilar Product				USA Biosimilar Product		
Tradename	**INN**	**Approval Year**	**Manufacturer**	**Tradename**	**INN**	**Approval Year**
Amgevita Solymbic	Adalimumab	2017	Amgen	Amjevita	Adalimumab-atto	2016
Imraldi	Adalimumab	2017	Samsung Bioepis	-	-	-
Cyltezo[1]	Adalimumab	2017	Boehringer Ingelheim	Cyltezo	Adalimumab-adbm	2017
Halimatoz Hefiya Hyrimoz	Adalimumab	2018	Sandoz	Hyrimoz	Adalimumab-adaz	2018
Hulio	Adalimumab	2018	Mylan/ Fujifilm Kyowa Kirin Biologics	-	-	-
Idacio Kromeya	Adalimumab	2019	Fresenius Kabi	-	-	-

Withdrawn in Europe in January 2019[1]

Table 2 : List of Participants (Study A)

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Table 3: Materials used in final adalimumab candidate preparations[1]

Ampoule code	Fill date	Study code	No of Ampoules in Stock	Protein (Predicted Mass - µg)	Excipients
17/236[1]	25/01/18	A, C	~9755[3]	50	25mM Sodium citrate tribasic dihydrate, 150mM Sodium chloride pH 6.5, 1% Human serum albumin
18/124[1]	25/05/18	B	~7859	50	
SS711[2]	08/08/18	D	150	40	

[1] The candidate preparations were expressed in CHO cells; they will be stored at -20°C at NIBSC as the custodian laboratory; [2] This preparation was produced from the same bulk drug substance as used for 17/236 - this was included for assessing assay sensitivity or ability of the assays to detect differences but is not a candidate standard.[3]All ampoules are intended for use as WHO International standard

Table 4: Mean fill weights and residual moisture content of Adalimumab preparations

Ampoule	Fill Weight		Residual Moisture		Headspace Oxygen	
Code	Mean g (n)	CV[1] %	Mean % (n)	CV[1] %	Mean % (n)	CV[1] %
17/236	1.0082 (402)	0.175	0.195 (12)	23.03	0.36 (12)	36.00
18/124	1.0082 (270)	0.180	0.402 (12)	14.41	0.28 (12)	46.02
SS711	1.0011 (3)	0.080	0.150 (3)	34.78	0.38 (3)	7.06

[1]CV : Coefficient of Variation; n : number of estimates

Table 5: Brief details of TNF-α neutralisation assays contributed to the study

Lab code	Cell line	Assay Type	Final TNF-α concentration IU/ml	Actinomycin D concentration µg/ml	In House standard (IH)	Incubation time (hrs)	Assay readout	Readout reagent
1	WEHI-164	Cytotoxicity	40	1	No	24	Absorbance	MTS (CellTiter 96® AQueous One)
2	WEHI-164	Cytotoxicity	60	0.5	No	23	Absorbance	WST-8
3	WEHI-164	Cytotoxicity	80	2	Yes (H)	24	Absorbance	MTS/PMS
4a	WEHI-164	Cytotoxicity	40	2	Yes ($O^{£}$)	18-20	Absorbance	MTT
5a	WEHI-164	Cytotoxicity	10	1	No	20	Absorbance	MTT
6	WEHI-164	Cytotoxicity	5	0.5	Yes (IH)	19-24	Absorbance	CCK-8
7	WEHI-13VAR	Cytotoxicity	15	1	No	20	Absorbance	MTS (CellTiter 96® AQueous One)
8a/b	L929	Cytotoxicity	20	1	No	20 - 24	Absorbance[$]	Alamar Blue
9	L929	Cytotoxicity	134	N/A	No	48	Absorbance	Crystal violet
10	L929	Cytotoxicity	~12	1	No	18-20	Absorbance	MTS
11	L929	Cytotoxicity	20	1	Yes (IH^{+})	22	Fluorescence	Resazurin
12	L929	Cytotoxicity	20	5	Yes (IH)	18-22	Absorbance	MTS (CellTiter 96® powder)
13	L929	Cytotoxicity	15	4	Yes (IH)	14-18	Absorbance	MTS (CellTiter 96® AQueous One)
14	L929	Cytotoxicity	15	1	Yes (IH)	16-18	Absorbance	CCK-8
15	L929	Cytotoxicity	20	4	No	18-22	Fluorescence	Alamar blue
16	L929	Cytotoxicity	~ 4	20	Yes (IH)	16	Absorbance	CCK-8
17	L929	Cytotoxicity	5	2	Yes (H)	20-22	Absorbance	MTT
18	L929	Cytotoxicity	10	1	No	20-24	Luminescence	ATP-Lite
19	L929	Cytotoxicity	~ 134	N/A	Yes (H)	48	Absorbance	Crystal violet
20	L929	Cytotoxicity	10	0.1	Yes (H)	23-26	Absorbance	CCK-8
5b	L929	Cytotoxicity	10	1	No	20	Absorbance	MTT
4b	HEK293 NF-κB-SEAP	Reporter gene	40	N/A	Yes ($O^{£}$)	22	Absorbance	Quanti-Blue
8c	HEK293 NF-κB-SEAP	Reporter gene	40	N/A	No	20 - 24	Absorbance	Quanti-Blue
21	HEK293 NF-κB-Luc	Reporter gene	172	N/A	Yes (H)	5	Luminescence	ONE-GloTM Luciferase Assay
22	HEK293 NF-κB-Luc	Reporter gene	80	N/A	Yes (H)	4	Luminescence	ONE-GloTM Luciferase Assay
23	HEK293 NF-κB-Luc	Reporter gene	50	N/A	Yes (IH)	24	Luminescence	Steady-Glo® Luciferase Assay
24	HEK293 NF-κB-Luc	Reporter gene	100	N/A	Yes (H)	5	Luminescence	DualGlo Luciferase assay
26a	HEK293 NF-κB-Luc	Reporter gene	172	N/A	Yes (IH)	16-24	Luminescence	Steady Glo Luciferase assay
2	U937	Apoptosis	40	N/A	No	2.5	Luminescence	Caspase-Glo 3/7
25	U937	Apoptosis	2000	N/A	Yes (IH)	3.5	Luminescence	Caspase-Glo 3/7
26b	U937	Apoptosis	172	N/A	Yes (IH)	4	Luminescence	Caspase-Glo 3/7

H : Humira®; IH : in-house/proprietary therapeutic adalimumab; IH^{+} : in-house research grade adalimumab; $O^{£}$: another TNF antagonist (non adalimumab) ; [$]both absorbance and fluorescence measured

Table 6: Brief details of ADCC, CDC and cell binding assays contributed to the study

Lab code	Bioactivity	Source of complement	Effector cells (E)	Target cells (T)	Ratio E:T	Assay Type	In house standard (IH)	Assay duration (hrs)	Assay readout	Readout reagent
12	ADCC	N/A	Jurkat-NFAT-luc-FcγRIlla	CHO-mTNFα	1:10	Reporter gene	Yes (IH)	4 – 6	Luminescence	Bright-Glo
16	ADCC	N/A	NK92- FcγRIlla	CHO-mTNFα	5:1	NK cell killing	Yes (IH)	4	Absorbance	Cytotoxicity detection kit PLUS (LDH)
23	ADCC	N/A	NK92- FcγRIlla	3T3-mTNFα	1:1	NK cell killing	Yes (IH)	4	Luminescence	CytoTox-Glo
25	ADCC	N/A	Jurkat-NFAT-luc-FcγRIlla	CHO-mTNFα	1:1	Reporter gene	No	20	Luminescence	Bio-Glo
26	ADCC	N/A	NK 3.3	HEK-mTNFα	10:1	NK cell killing	Yes (IH)	1	Fluorescence	Calcein-AM
6	CDC	human	N/A	Jurkat-mTNFα	N/A	Viability	Yes (IH)	2	Absorbance	CCK-8
16	CDC	rabbit	N/A	CHO-mTNFα	N/A	Viability	Yes (IH)	4	Luminescence	CellTiter-Glo
25	CDC	human	N/A	CHO-mTNFα	N/A	Viability	Yes (IH)	4	Luminescence	CellTiter-Glo
26	CDC	human	N/A	Jurkat-mTNFα	N/A	Viability	Yes (IH)	2	Luminescence	CellTiter-Glo
12b	Cell binding	N/A	N/A	CHO-mTNFα	N/A	Flow cytometry	Yes (IH)	1 – 1.5	Fluorescence	Anti-human IgG (H+L)-FITC
25b	Cell binding	N/A	N/A	CHO-mTNFα	N/A	Flow cytometry	Yes (IH)	1	Fluorescence	Anti-Human IgG Fc-PE

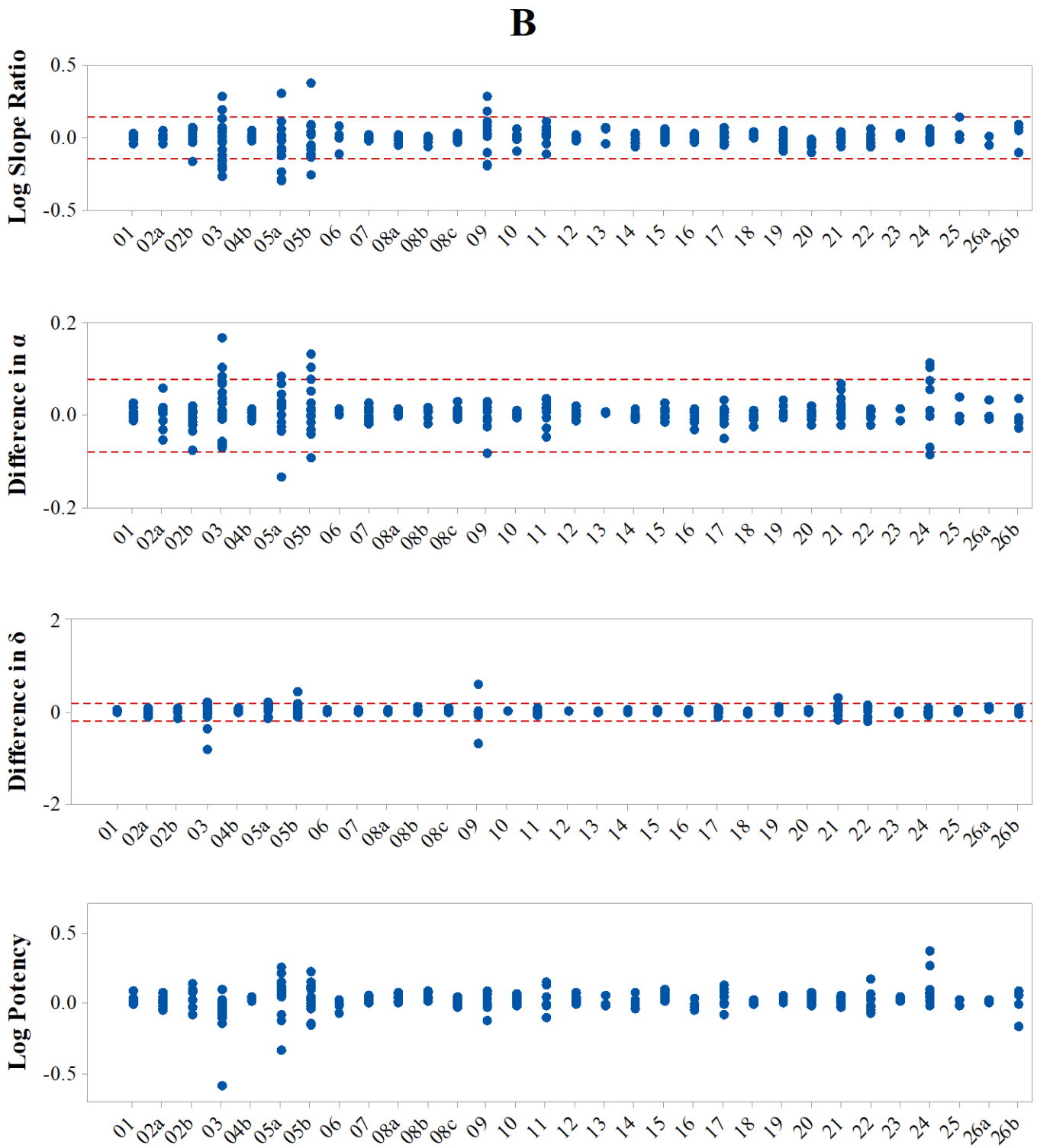
IH : proprietary adalimumab

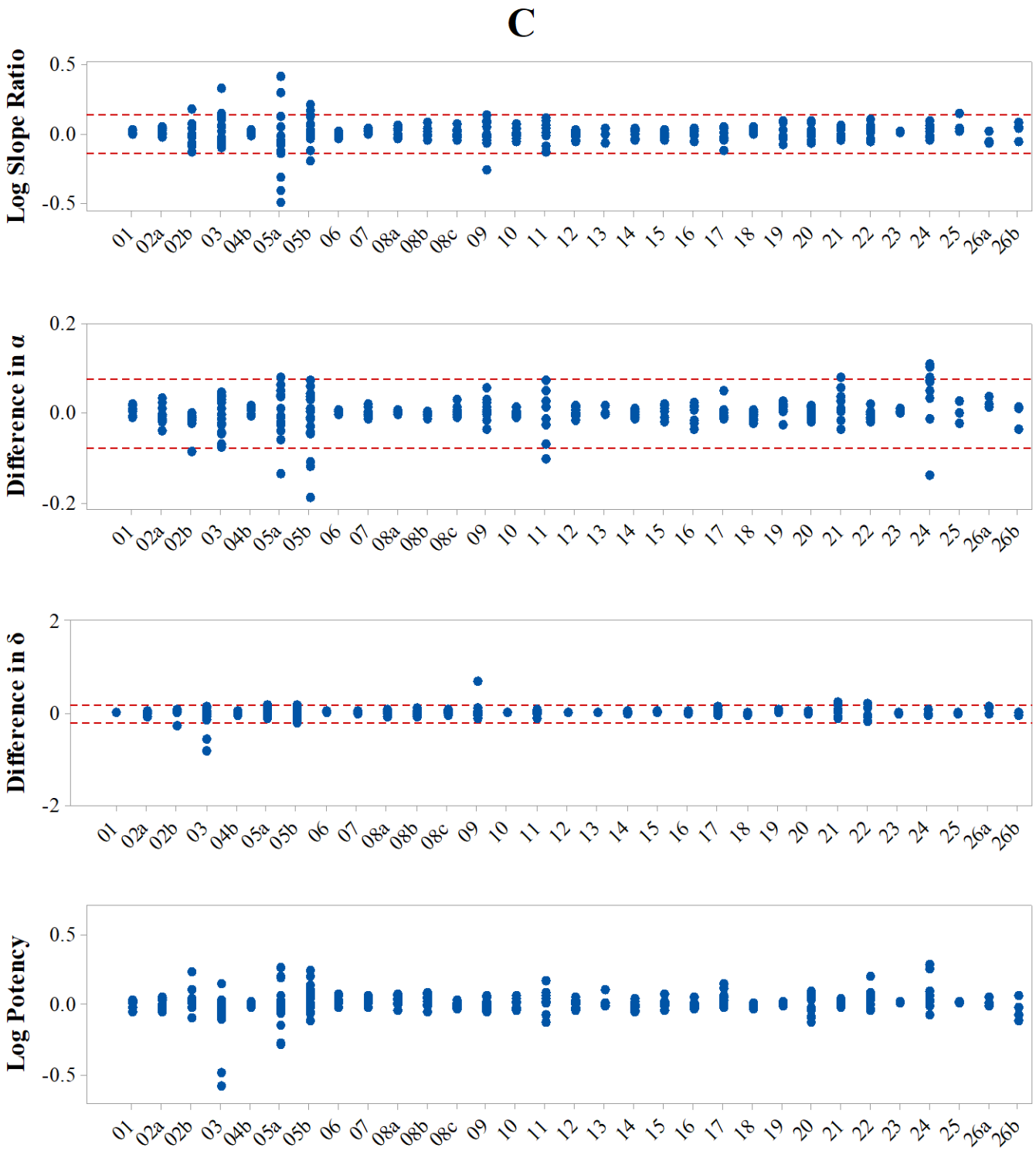
Table 7: Brief details of binding assays contributed to the study

Lab code	Assay Type	In house standard (IH)	Assay description	Detection reagent	Assay readout	Readout reagent
7	ELISA kit	Yes (IH[1])	Adalimumab binds to TNF-α coated plates.	Anti-human IgG Fc-HRP	Absorbance	TMB
8	Bridging ECL	Yes (H)	Adalimumab binds to Biotinylated and Sulfo-Tag labelled TNF-α, captured onto streptavidin coated plates.	Biotinylated + Sulfo Tag TNF-α	Electrochemiluminescence	MSD Read buffer
10	ELISA	No	Adalimumab binds to TNF-α coated plates.	Anti-human IgG-HRP	Absorbance	TMB
12a	ELISA	Yes (IH)	Adalimumab binds to TNF-α coated plates.	Anti-human Kappa-HRP	Absorbance	TMB
20	ELISA	Yes (H)	Adalimumab binds to TNF-α coated plates.	Anti-human IgG-HRP	Absorbance	TMB
23	TR-FRET	Yes (IH)	Europium labelled adalimumab and Cy5 labelled TNF-α form fluorescent complex which is competitively inhibited by unlabelled adalimumab	Europium labelled adalimumab + Cy5 labelled TNF-α	Fluorescence	N/A
25a	ELISA	Yes (IH)	Adalimumab binds to TNF-α coated plates.	Anti-human IgG Fc-HRP	Absorbance	TMB
3	Biolayer Interferometry	Yes (H)	Adalimumab binds to biotinylated TNF-α captured onto streptavidin biosensor.	N/A	Response binding rate (nm/s)	N/A
12	SPR	Yes (IH)	Adalimumab captured onto sensor chip immobilised with Anti Human IgG Fc, followed by concentrations of TNF-α	N/A	Response units: expressed as Equilibrium affinity constant KD (M)	N/A

H = Humira; IH = proprietary adalimumab; IH[1] – kit standard

Figure 1a: Differences in fitted model parameters (α, $\log_{10}\beta$, δ, $\log_{10}\gamma$) for neutralisation assays

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Log Potency
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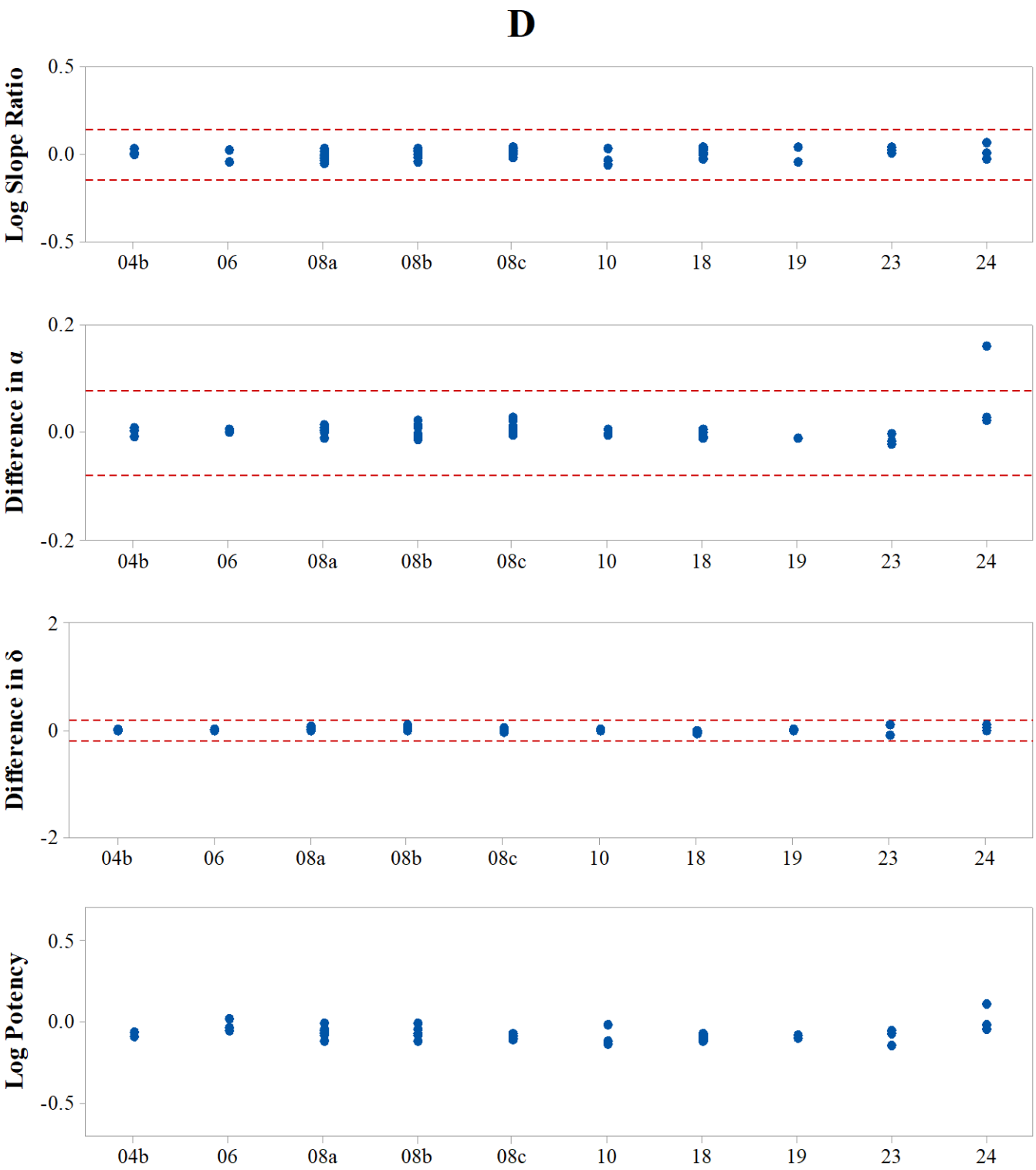
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08c
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24

IH

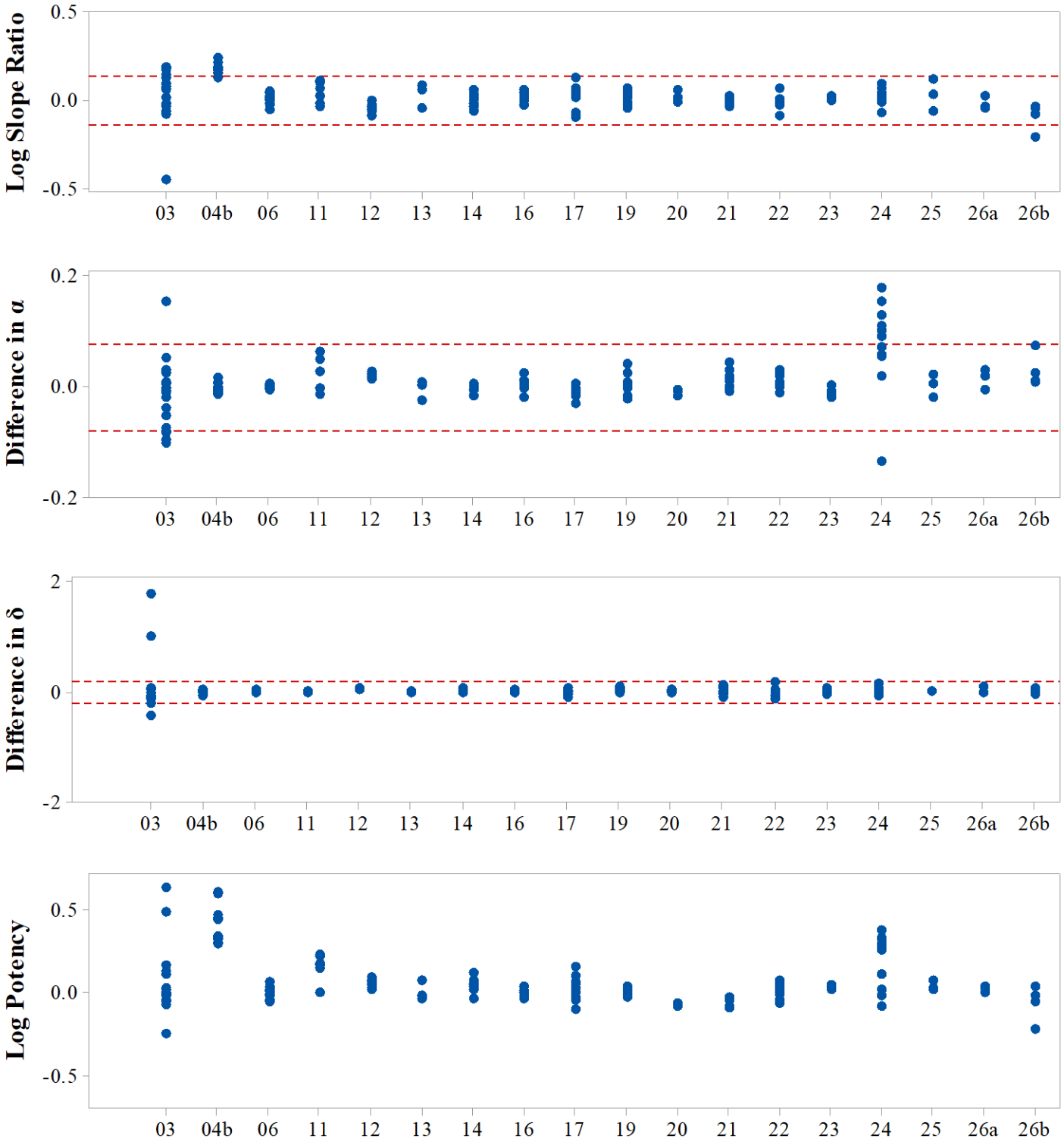
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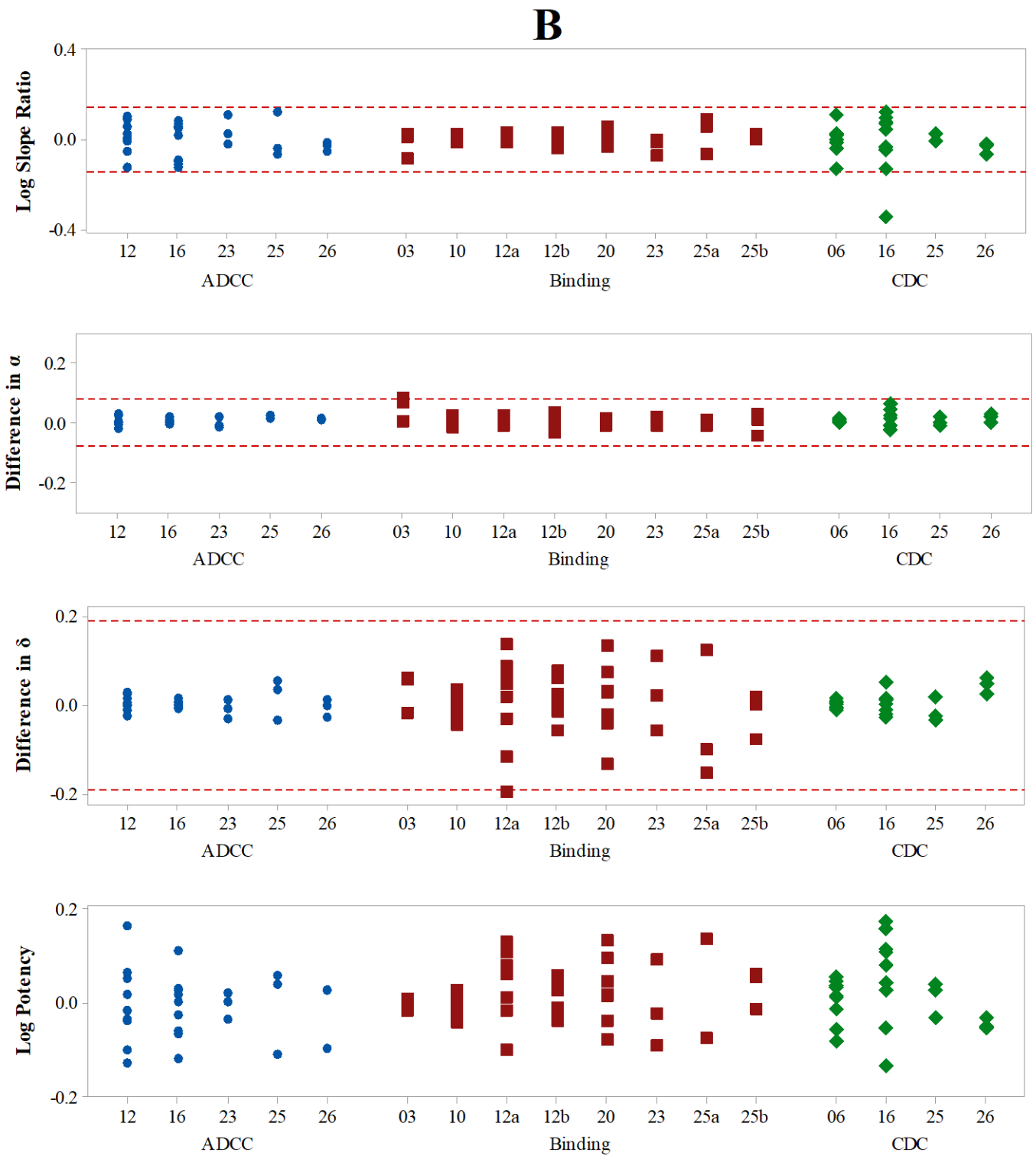
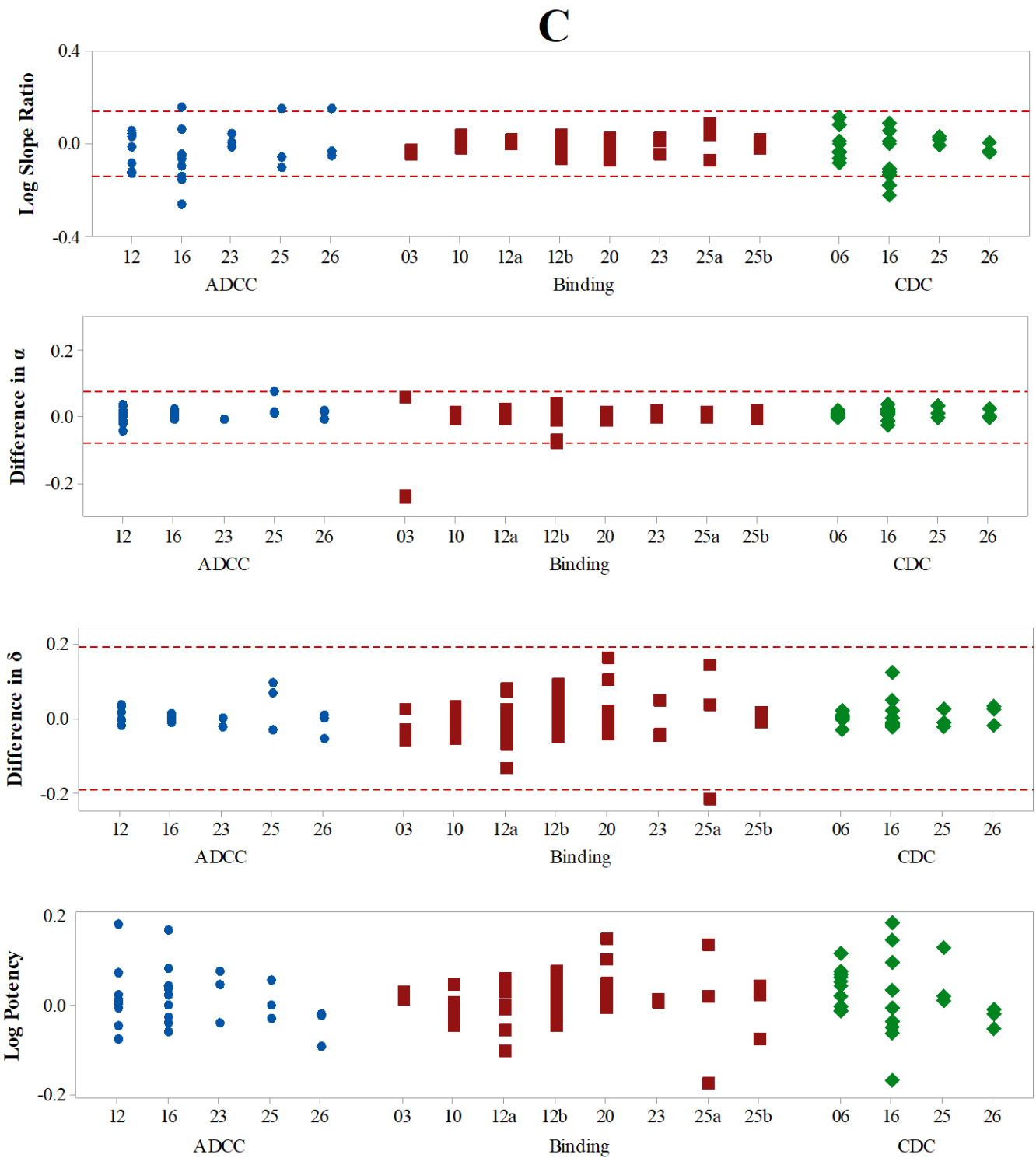
Figure 1b: Differences in fitted model parameters (α, $\log_{10}$β, δ, $\log_{10}$γ) for other assays

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ADCC
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Difference in δ
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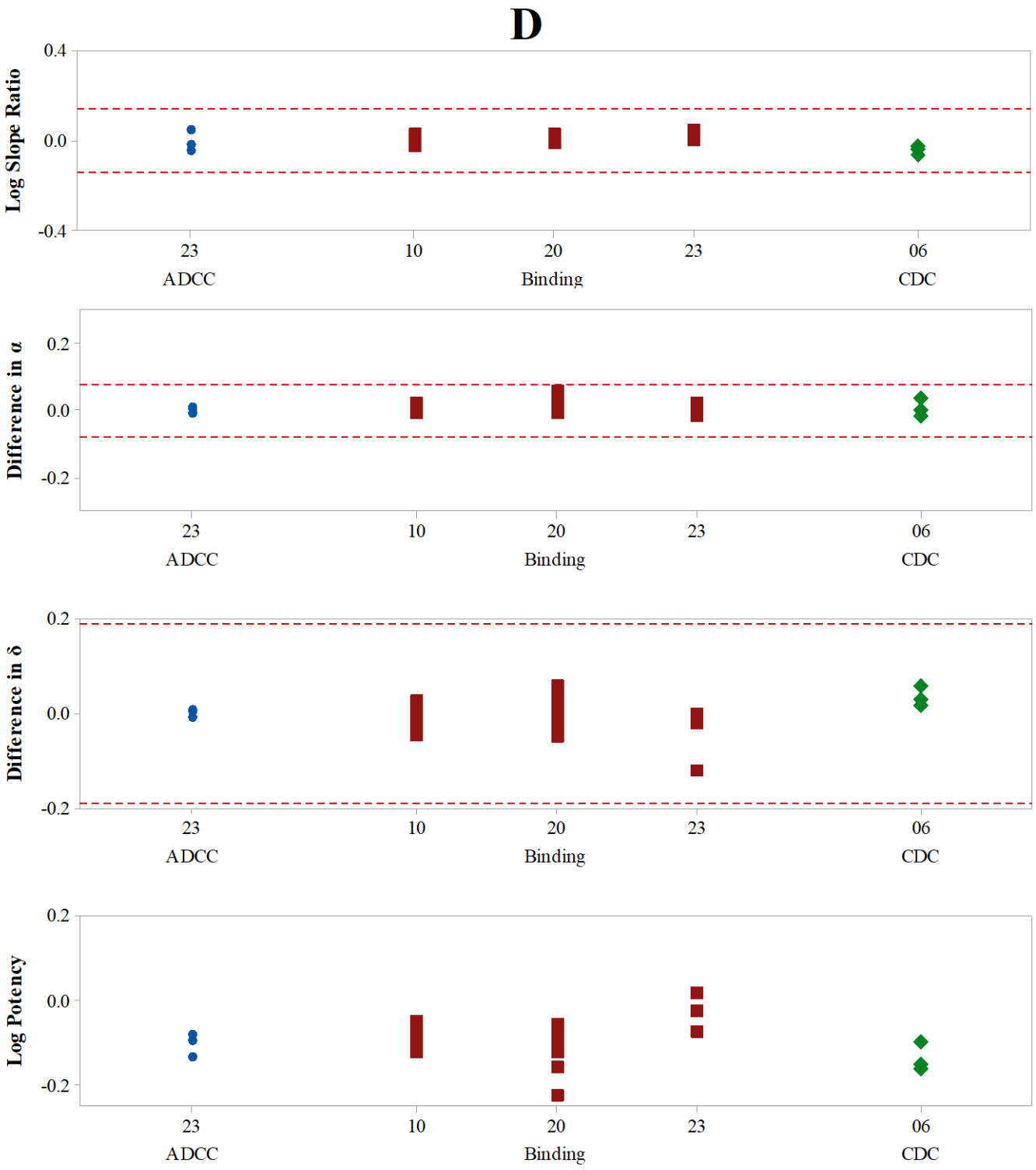
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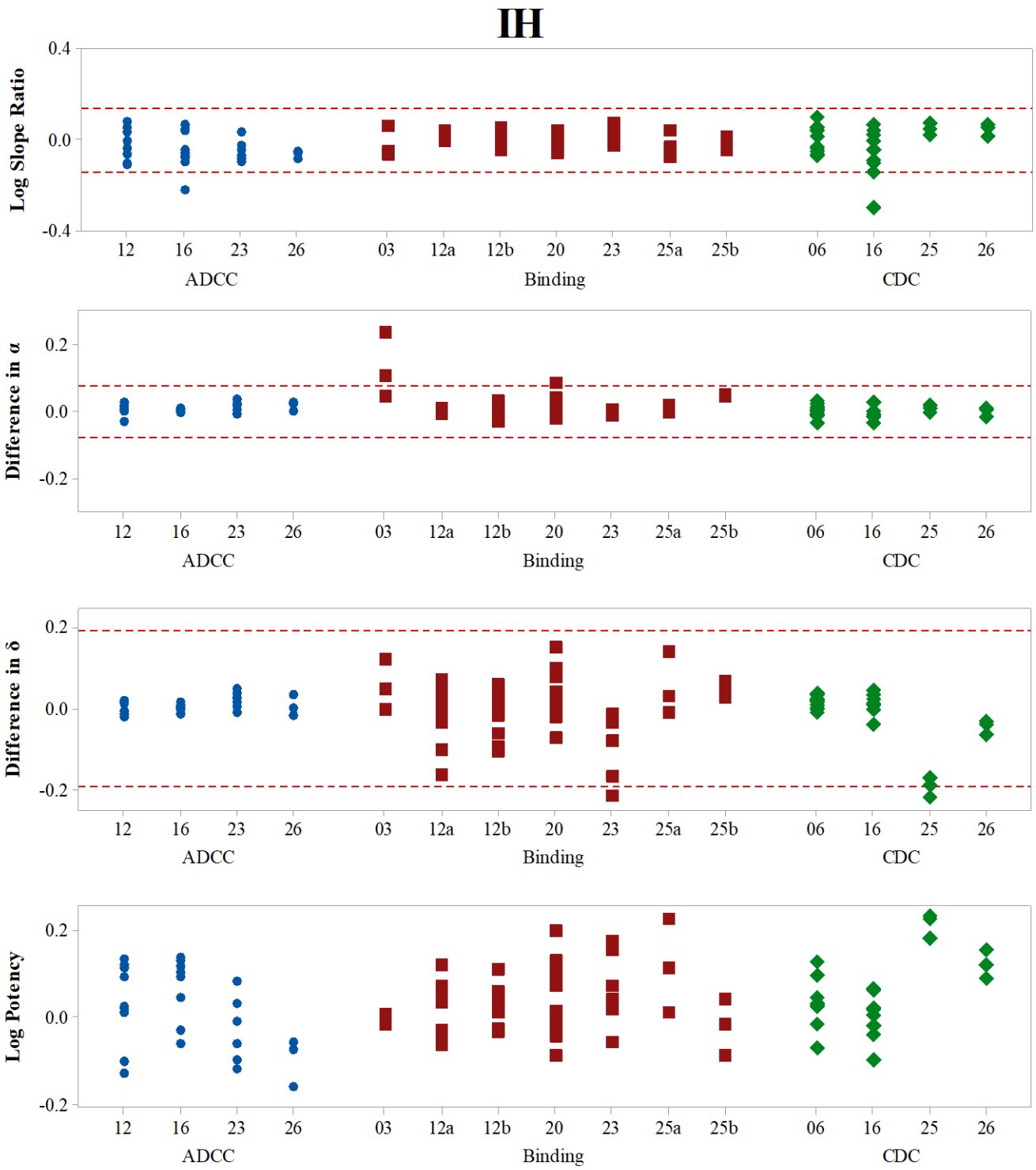
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ADCC
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Table 8: Laboratory geometric mean relative potency estimates for neutralisation assays

Cell line	Lab	Potencies relative to sample A									Potencies relative to in-house reference											
		Sample B			Sample C			Sample D			Sample A			Sample B			Sample C			Sample D		
		GM	GCV	N	GM	GCV	N	GM	GCV	N	GM	GCV	N	GM	GCV	N	GM	GCV	N	GM	GCV	N
WEHI-164	01	1.04	6.16	9	1.00	6.08	9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
WEHI-164	02a	1.00	9.51	9	0.99	8.28	9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
WEHI-164	03	0.92	7.84	12	0.94	14.47	17	-	-	-	0.97	17.73	10	0.94	15.31	10	0.90	19.28	12	-	-	-
WEHI-164	04a[1,2]	1.04	10.61	9	0.97	12.40	9	0.82	5.77	7	-	-	-	-	-	-	-	-	-	-	-	-
WEHI-164	05a	1.24	26.59	10	0.88	25.54	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
WEHI-164	06	0.96	8.21	6	1.05	8.71	6	0.94	8.49	3	0.99	8.08	15	1.01	7.69	12	0.99	8.40	12	0.92	7.51	3
WEHI-13VAR	07	1.06	4.35	9	1.02	6.91	9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L929	05b	1.03	25.38	11	1.11	20.76	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L929	08a	1.07	5.62	9	1.05	9.17	9	0.85	7.11	9	-	-	-	-	-	-	-	-	-	-	-	-
L929	08b	1.08	7.09	9	1.04	10.23	9	0.86	7.31	9	-	-	-	-	-	-	-	-	-	-	-	-
L929	09	0.97	5.83	8	0.97	7.75	11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L929	10	1.02	6.44	9	1.01	8.27	9	0.81	15.41	3	-	-	-	-	-	-	-	-	-	-	-	-
L929	11	1.04	19.69	9	1.02	22.61	8	-	-	-	0.71	20.89	6	0.74	17.02	6	0.71	20.39	6	-	-	-
L929	12	1.04	5.86	9	0.99	7.72	9	-	-	-	0.89	6.10	9	0.93	7.00	9	0.89	5.60	9	-	-	-
L929	13	1.01	10.11	3	1.06	14.99	3	-	-	-	0.99	14.59	3	1.00	4.20	3	1.05	5.39	3	-	-	-
L929	14	1.01	7.46	9	0.98	7.78	9	-	-	-	0.91	10.16	9	0.92	10.01	9	0.89	4.85	9	-	-	-
L929	15	1.13	6.54	9	1.01	7.81	9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L929	16	0.97	7.10	9	0.97	5.82	9	-	-	-	1.01	5.46	9	0.98	8.14	9	0.98	5.09	9	-	-	-
L929	17	1.09	16.60	9	1.10	13.25	9	-	-	-	0.95	19.68	9	1.04	20.60	9	0.98	14.00	7	-	-	-
L929	18	1.00	2.74	9	0.97	2.73	9	0.80	3.27	9	-	-	-	-	-	-	-	-	-	-	-	-
L929	19	1.05	4.03	8	1.00	2.47	8	0.81	n/a	2	1.01	4.90	10	1.06	3.48	8	1.01	3.52	8	0.81	n/a	2
L929	20	1.06	8.32	9	0.96	18.06	12	-	-	-	1.19	2.23	3	-	-	-	1.10	2.55	3	-	-	-
NF-κB-SEAP	04b[1]	1.03	2.76	9	1.00	3.18	9	0.85	3.75	3	-	-	-	-	-	-	-	-	-	-	-	-
NF-κB-SEAP	08c	1.02	4.65	15	0.98	4.88	12	0.81	2.80	12	-	-	-	-	-	-	-	-	-	-	-	-

NF-κB-Luc	21	1.06	4.05	7	1.03	4.84	7	-	-	-	1.15	6.00	9	1.20	2.78	8	1.16	3.44	8	-	-	-
NF-κB-Luc	22	0.96	11.25	8	1.04	11.22	7	-	-	-	0.97	11.64	9	1.02	12.15	8	1.06	15.36	8	-	-	-
NF-κB-Luc	23	1.05	3.86	3	1.02	2.27	3	0.81	11.92	3	0.93	2.00	6	0.98	3.58	3	0.95	1.50	3	0.76	9.42	3
NF-κB-Luc	24	1.18	26.35	6	1.17	32.02	6	1.07	n/a	2	0.80	38.12	4	1.59	52.03	3	0.84	81.03	8	0.50	n/a	2
NF-κB-Luc	26a	1.00	2.90	3	1.03	7.81	3	-	-	-	0.96	3.85	3	0.96	4.92	3	0.98	9.19	3	-	-	-
U937	02b	1.13	13.73	7	1.01	14.11	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
U937	25	0.99	n/a	2	1.02	n/a	2	-	-	-	0.92	7.18	3	0.92	13.35	3	0.93	6.31	3	-	-	-
U937	26b	0.96	29.98	4	0.91	19.84	4	-	-	-	1.21	35.54	3	1.16	69.92	3	1.22	n/a	2	-	-	-

GM: Geometric Mean; GCV: Geometric Coefficient of Variation (%); N: Number of estimates used in calculation of GM and GCV; [1] : potencies relative to IH standard excluded as a different TNF antagonist used as IH standard; [2] : potencies calculated using a parallel line model

Table 9: Laboratory geometric mean relative potency estimates for ADCC and CDC assays

Assay	Lab	Potencies relative to sample A									Potencies relative to in-house reference											
		Sample B			Sample C			Sample D			Sample A			Sample B			Sample C			Sample D		
		GM	GCV	N	GM	GCV	N	GM	GCV	N	GM	GCV	N	GM	GCV	N	GM	GCV	N	GM	GCV	N
ADCC	12	0.99	22.85	9	1.04	18.75	9	-	-	-	0.93	24.48	9	0.94	23.10	8	0.93	22.27	8	-	-	-
ADCC	16	0.98	16.78	9	1.07	20.81	5	-	-	-	0.86	19.00	8	0.84	22.41	9	0.90	18.26	8	-	-	-
ADCC	23	0.99	6.91	3	1.06	15.10	3	0.79	6.33	3	1.07	19.69	6	1.05	n/a	2	1.05	7.73	3	0.91	9.61	3
ADCC	25	0.99	23.84	3	1.06	n/a	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ADCC	26	0.88	18.07	3	0.87	n/a	2	-	-	-	1.26	13.48	3	1.10	33.86	3	1.12	n/a	2	-	-	-
CDC	06	1.01	11.46	9	1.11	9.81	9	0.73	8.07	3	0.92	12.71	12	0.90	13.46	9	0.99	7.79	9	0.69	n/a	2
CDC	16	1.14	27.92	8	1.08	36.07	6	-	-	-	0.97	14.73	7	1.12	16.88	9	1.01	17.35	9	-	-	-
CDC	25	1.03	9.06	3	1.13	16.27	3	-	-	-	0.66	n/a	1	0.67	n/a	2	0.79	n/a	1	-	-	-
CDC	26	0.90	2.71	3	0.93	5.30	3	-	-	-	0.76	7.61	3	0.68	10.05	3	0.71	10.10	3	-	-	-

GM: Geometric Mean; GCV: Geometric Coefficient of Variation (%); N: Number of estimates used in calculation of GM and GCV

Table 10: Laboratory geometric mean relative potency estimates for binding assays

Assay	Lab	Potencies relative to sample A									Potencies relative to in-house reference											
		Sample B			Sample C			Sample D			Sample A			Sample B			Sample C			Sample D		
		GM	GCV	N	GM	GCV	N	GM	GCV	N	GM	GCV	N	GM	GCV	N	GM	GCV	N	GM	GCV	N
Binding	03	1.00	3.23	3	1.02	n/a	1	-	-	-	1.04	n/a	1	1.01	n/a	2	1.02	n/a	1	-	-	-
Binding	07[1]	0.89	n/a	2	0.96	n/a	2	-	-	-	0.67	n/a	2	0.70	n/a	1	0.64	n/a	2	-	-	-
Binding	08[1]	0.98	6.78	9	0.98	9.00	9	0.80	9.09	9	0.93	7.00	9	0.91	8.76	9	0.91	9.85	9	0.74	8.38	9
Binding	10	0.99	5.67	9	0.96	7.11	9	0.80	6.18	9	-	-	-	-	-	-	-	-	-	-	-	-
Binding	12a	1.16	11.54	8	1.01	13.62	9	-	-	-	0.94	15.26	9	1.05	15.83	9	0.96	11.44	9	-	-	-
Binding	20	1.04	16.47	9	1.09	13.06	9	0.77	12.96	9	0.90	19.78	17	0.91	23.21	9	0.96	15.14	9	0.73	26.42	8
Binding	23	0.98	24.01	3	1.02	0.61	3	0.94	11.18	3	0.90	19.41	5	0.94	18.45	3	0.98	16.99	3	0.77	n/a	2
Binding	25a	0.99	32.32	3	1.19	n/a	2	-	-	-	0.77	28.08	3	0.76	14.32	3	0.92	n/a	2	-	-	-
Binding (cell based)	12b	1.06	8.72	9	1.03	10.57	8	-	-	-	0.93	10.83	9	0.98	16.36	9	0.99	9.80	7	-	-	-
Binding (cell based)	25b	1.09	9.98	3	0.99	15.60	3	-	-	-	1.05	16.16	3	1.12	n/a	2	1.04	4.48	3	-	-	-

GM: Geometric Mean; GCV: Geometric Coefficient of Variation (%); N: Number of estimates used in calculation of GM and GCV; [1] : potencies calculated using a parallel line model

Table 11: Overall geometric mean relative potency estimates for all assays contributed to the study

Method	Sample	Potencies relative to sample A					Potencies relative to in-house reference*				
		GM	LCL	UCL	GCV	N	GM	LCL	UCL	GCV	N
Neutralisation (all)	A	-	-	-	-	-	0.97	0.90	1.03	14.03	17
	B	1.04	1.01	1.06	6.43	32	1.01	0.93	1.10	17.30	16
	C	1.01	0.99	1.03	5.61	32	0.97	0.91	1.04	13.60	17
	D	0.86	0.81	0.91	9.28	11	0.73	0.48	1.11	30.55	4
Neutralisation (WEHI)	A	-	-	-	-	-	0.98	n/a	n/a	n/a	2
	B	1.03	0.94	1.13	10.08	7	0.97	n/a	n/a	n/a	2
	C	0.98	0.93	1.03	6.08	7	0.94	n/a	n/a	n/a	2
	D	0.88	n/a	n/a	n/a	2	0.92	n/a	n/a	n/a	1
Neutralisation (L929)	A	-	-	-	-	-	0.95	0.84	1.07	15.88	8
	B	1.04	1.01	1.06	4.33	15	0.95	0.85	1.06	12.59	7
	C	1.02	0.99	1.04	4.67	15	0.94	0.84	1.06	14.83	8
	D	0.83	0.79	0.86	3.26	5	0.81	n/a	n/a	n/a	1
Neutralisation (Reporter Gene)	A	-	-	-	-	-	0.96	0.81	1.12	13.83	5
	B	1.04	0.98	1.11	6.58	7	1.13	0.87	1.46	23.30	5
	C	1.04	0.98	1.09	5.85	7	0.99	0.86	1.15	12.65	5
	D	0.88	0.71	1.09	14.48	4	0.62	n/a	n/a	n/a	2
Neutralisation (U937)	A	-	-	-	-	-	1.05	n/a	n/a	n/a	2
	B	1.02	0.83	1.26	8.88	3	1.03	n/a	n/a	n/a	2
	C	0.98	0.84	1.14	6.35	3	1.07	n/a	n/a	n/a	2
	D	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
ADCC	A	-	-	-	-	-	1.02	0.78	1.34	18.10	4
	B	0.97	0.90	1.03	5.44	5	0.98	0.80	1.19	13.03	4
	C	1.02	0.91	1.14	9.32	5	1.00	0.85	1.17	10.84	4
	D	0.79	n/a	n/a	n/a	1	0.91	n/a	n/a	n/a	1
Binding	A	-	-	-	-	-	0.90	0.80	1.00	15.22	9
	B	1.01	0.96	1.07	7.36	10	0.92	0.82	1.03	16.03	9
	C	1.02	0.98	1.07	6.91	10	0.93	0.83	1.03	15.53	9
	D	0.83	0.72	0.95	9.10	4	0.75	0.71	0.80	2.46	3
CDC	A	-	-	-	-	-	0.82	0.62	1.08	19.13	4
	B	1.02	0.87	1.19	10.34	4	0.83	0.56	1.22	27.56	4
	C	1.06	0.92	1.22	9.05	4	0.87	0.66	1.14	19.11	4
	D	0.73	n/a	n/a	n/a	1	0.69	n/a	n/a	n/a	1

GM: Geometric Mean; LCL: Lower 95% Confidence Limit; UCL: Upper 95% Confidence Limit; GCV: Between-laboratory Geometric Coefficient of Variation (%); N: Number of laboratories used in calculation of GM and GCV; *Lab 4b excluded (used a different TNF antagonist as IH reference standard

Figure 2 : Laboratory geometric mean relative potency estimates for all different assay types (Top panel) as well as for the different TNF-α cell-based neutralization assays (Bottom Panel). Outliers are denoted by an asterisk

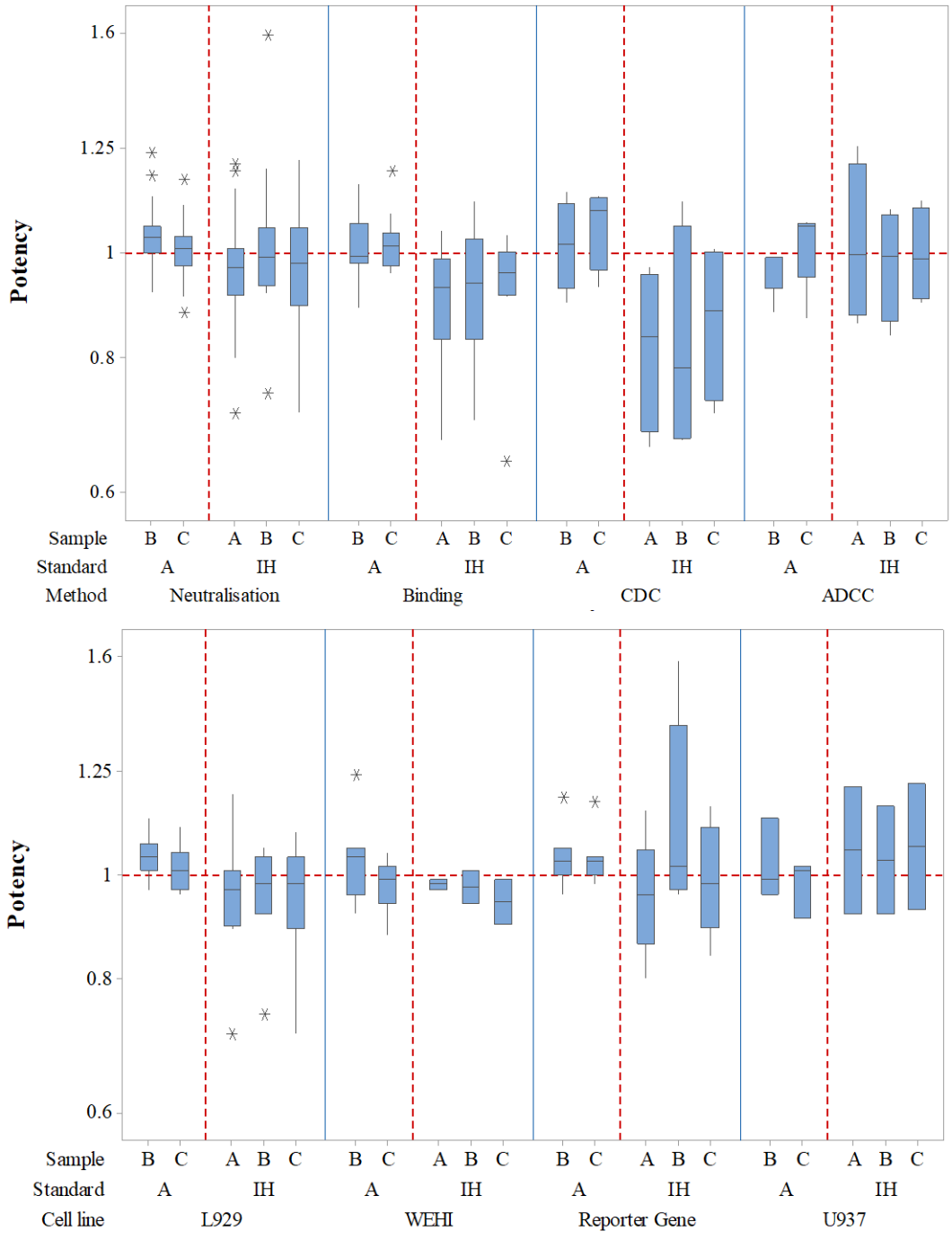

Table 12: Laboratory geometric mean ED50 estimates (ng) and final TNF-α concentrations for neutralisation assays

Cell line	Lab	Final TNF-α conc in assay IU/ml	GM					GCV				
			A	B	C	D	IH	A	B	C	D	IH
WEHI-164	01	40	12.31	11.84	12.28	-	-	18.98	16.72	21.44	-	-
WEHI-164	02a	60	32.54	32.57	32.97	-	-	11.74	11.74	11.46	-	-
WEHI-164	03	80	11.00	13.14	13.21	-	10.48	74.85	51.47	56.43	-	76.83
WEHI-164	04a[1]	40	-	-	-	-	-	-	-	-	-	-
WEHI-164	05a	10	17.06	15.25	17.60	-	-	32.05	27.84	34.10	-	-
WEHI-164	06	5	1.70	1.67	1.77	1.88	1.71	33.54	37.34	35.39	3.66	32.74
WEHI-13VAR	07	15	19.30	18.28	18.87	-	-	24.35	22.14	17.19	-	-
L929	05b	10	12.74	11.96	11.63	-	-	64.38	71.41	60.54	-	-
L929	08a	20	8.53	7.94	8.13	9.98	-	4.92	6.93	6.96	5.44	-
L929	08b	20	8.04	7.45	7.71	9.37	-	4.77	7.20	7.37	4.27	-
L929	09	134	7.36	7.55	7.48	-	-	14.70	13.98	13.57	-	-
L929	10	11.625	15.99	15.15	15.41	21.57	-	13.90	14.94	15.78	20.85	-
L929	11	20	5.39	5.20	5.19	-	3.49	31.35	30.47	32.76	-	17.24
L929	12	20	11.30	10.83	11.37	-	10.09	6.00	6.22	6.33	-	7.90
L929	13	15	5.03	4.98	4.73	-	4.99	7.85	4.49	10.43	-	7.39
L929	14	15	4.62	4.59	4.73	-	4.21	12.94	13.50	8.80	-	8.71
L929	15	20	10.43	9.25	10.34	-	-	20.74	15.64	28.40	-	-
L929	16	3.58	46.01	47.42	47.36	-	46.55	8.31	7.86	7.35	-	4.29
L929	17	5	6.85	6.28	6.20	-	6.51	19.16	5.87	27.84	-	19.54
L929	18	10	10.13	10.16	10.42	12.64	-	8.58	9.92	9.64	9.10	-
L929	19	134.4	8.85	8.45	8.83	10.91	8.90	1.94	4.51	3.01	n/a	4.86
L929	20	10	5.40	5.26	5.60	-	5.89	16.84	22.43	17.01	-	10.00
HEK293 NF-κB-SEAP	04b	40	16.23	15.69	16.24	19.25	-	7.00	8.68	6.86	3.00	-
HEK293 NF-κB-SEAP	08c	40	11.36	11.25	11.56	14.04	-	10.02	9.74	11.00	10.55	-

HEK293 NF-κB-Luc	21	172	36.22	35.27	35.53	-	41.74	10.35	10.09	9.25	-	7.02
HEK293 NF-κB-Luc	22	80	45.68	45.27	41.83	-	44.40	37.08	22.68	23.90	-	30.35
HEK293 NF-κB-Luc	23	50	8.52	7.98	8.25	10.64	7.95	9.00	13.19	12.37	9.25	7.85
HEK293 NF-κB-Luc	24	100	58.89	46.61	49.29	58.64	36.88	9.97	31.68	32.56	6.40	38.78
HEK293 NF-κB-Luc	26a	172	20.60	20.51	20.07	-	19.73	5.22	2.28	2.46	-	6.92
U937	02b	40	12.79	11.69	11.97	-	-	34.99	36.45	32.44	-	-
U937	25	2000	178.61	178.24	175.03	-	163.47	12.63	19.10	11.73	-	5.11
U937	26b	172	187.30	195.17	205.19	-	218.67	33.96	9.38	32.90	-	62.49

GM: Geometric Mean; GCV: Geometric Coefficient of Variation (%); IH: In-house reference; [1] : ED50 values not calculated as parallel line model used for analysis (see statistical analysis section)

Table 13: Summary of ED50 estimates (ng) for selected L929 neutralization assays using a fixed amount of TNF-α (20IU)

Sample	**GM**	LCL	UCL	**GCV**	**N**
A	8.47	5.92	12.12	33.49	5
B	7.90	5.62	11.12	31.63	5
C	8.25	5.64	12.06	35.76	5
D	9.67	n/a	n/a	n/a	2
IH	5.94	n/a	n/a	n/a	2

GM: Geometric Mean; LCL and UCL: Lower and Upper 95% confidence limits; GCV: Geometric Coefficient of Variation (%); N: Number of estimates used in calculation of GM and GCV

Table 14: Summary of results from accelerated temperature degradation studies of candidate preparation 17/236 assayed using an L929 cell cytotoxicity assay

Time stored (years)	Storage Temperature (°C)	LCL	Relative Potency[1] to -70°C	UCL
1.208	-20	0.93	0.96	0.99
1.208	+4	0.96	1.00	1.05
1.208	+20	0.97	1.00	1.03
1.208	+37	0.97	1.02	1.06
1.208	+45	1.03	1.09	1.17

[1] **:** Geometric Mean potency derived from 9 estimates in all cases; LCL and UCL: Lower and Upper 95% confidence limits

Table 15: Summary of results from reconstitution stability studies of candidate preparation 17/236 assayed using an L929 cell cytotoxicity assay

Temperature (°C)	Time (Days)	LCL	Relative Potency to a freshly reconstituted ampoule	UCL
+4	1	0.90	0.98	1.07
+4	7	0.83	0.94	1.06
Room temperature	1	0.81	0.91	1.02
Room temperature	7	0.88	0.94	1.01

[1] **:** Geometric Mean potency derived from 6 estimates in all cases; LCL and UCL: Lower and Upper 95% confidence limits

Table 16: Summary of results from freeze-thaw studies of candidate preparation 17/236 using an L929 cell cytotoxicity assay

Number of freeze/thaw cycles	LCL	Relative Potency to a freshly reconstituted ampoule	UCL
1x	0.90	0.97	1.05
2x	0.96	1.04	1.14
3x	0.98	1.07	1.16
4x	0.93	0.99	1.06

[1] : Geometric Mean potency derived from 9 estimates in all cases; LCL and UCL: Lower and Upper 95% confidence limits

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Table 18: Details of spiked serum samples

Serum 1	First Link: product 20-00-810 (batch HSS8350)						
Serum 2	Sigma: product H6914 (batch SLBW5405)						
Amount µg/ml	**Serum 1 + Preparation A**	**Serum 1 + Preparation B**	**Serum 2 + Preparation A**	**Serum 2 + Preparation B**	**Serum 1 + 17/236[1]**	**Serum 2 + 17/236[1]**	**Serum 1 + Preparation A**
0	S1	S5	S9	S13			S21*
2	S2	S6	S10	S14	S17	S19	S22*
5					S18	S20	
6	S3	S7	S11	S15			S23*
12	S4	S8	S12	S16			S24*

* samples also spiked with an IgG1 anti-adalimumab at 0.5 µg/ml; [1] Amounts of 17/236 are diluted from a reconstituted ampoule, assumed to be 50 µg/ml

Table 19: Brief details of assays contributed to the study

Lab Code	Assay Platform	Assay description	Assay Standard(s)	Read-out	Specific
1T, 10T, 11T	ELISA (C)	Plates coated with TNF-α, adalimumab captured, detected with biotin anti-human IgG followed by HRP-streptavidin	Kit & IH (1T) Kit (10T, 11T)	OD	no
1T	ELISA (C)	Plates coated with TNF-α, adalimumab captured, detected with biotin anti-adalimumab followed by HRP-streptavidin	Kit & IH	OD	yes
2T	ELISA (C)	Plates coated with anti-adalimumab, adalimumab captured, detected with HRP anti-adalimumab.	Kit	OD	yes
3T, 12T-14T	ELISA (C)	Plates coated with anti-adalimumab, adalimumab captured, detected with HRP antibody.	Kit & IH (3T) Kit (12T-14T)	OD	yes
4T	ELISA (C)	Plates coated with TNF-α, adalimumab captured, detected with HRP-anti-adalimumab	Kit & IH	OD	yes
5T	ELISA (C)	Plates coated with anti-adalimumab, adalimumab captured, detected with HRP conjugate.	kit	OD	yes
6T	ELISA (IH)	Plates coated with TNF-α, adalimumab captured, detected with HRP anti-human IgG	IH	OD	no
9T	ELISA (IH)	Plates coated with anti-TNF-α, followed by capturing of adalimumab using TNF-α and detection with biotin anti-adalimumab and HRP streptavidin.	IH	OD	yes
7T	ECL (IH)	Plates coated with TNFα, adalimumab captured, then addition of sulfotag anti-human kappa light chain.	IH	counts	no
8T	ECL (IH)	Samples incubated with biotinylated TNFα and sulfotag TNFα, transferred to streptavidin plate.	IH	counts	no
15T	LFI (C)	Adalimumab is detected via the formation of a ‘sandwich’ with TNF-α and an anti-adalimumab	Kit (pre-defined) & IH	OD	yes
16T	LFI (C)	Adalimumab detected via the formation of a ‘sandwich’ with TNF-α and an anti-adalimumab	Kit (pre-defined) & IH	OD	yes

All standards are Humira® based; Kit standards only: participants using a purchased commercial kit; IH-In-house standards only: participants using an in-house method; Kit & IH: commercial kit manufacturers: LFI:lateral flow immunoassay; ECL: electochemiluminescence, Parentheses indicates commercial C or in-house IH assay.

Table 20. Laboratory geometric mean content estimates (µg/ml) and inter-laboratory GCV values for spiked samples S1-S24 calculated relative to kit or in-house standards

Sample	Laboratory														Overall GM	Theoretical amount[1]	Inter-lab GCV%
	1Ta	1Tb	2T	3T	4T	6T	7T	8T	9T	10T	12T	14T	15T	16T			
S1	bl	bl	bl	bl	bl	bl	bl	bl	bl	bl	bl	bl	bl	bl		0	
S2	1.86	1.93	1.67	2.45	2.09	1.88	2.22	1.91	1.83	1.63	2.43	2.63	2.17	1.74	2.01	2	16.04
S3	5.43	5.20	5.18	6.47	5.84	6.03	5.61	5.98	5.59	4.74	7.07	7.57	5.87	5.70	5.83	6	13.08
S4	12.3	10.8	10.5	12.3	12.2	10.8	10.6	12.2	11.2	9.12	12.7	13.7	12.0	9.89	11.40	12	11.70
S5	bl	bl	bl	bl	bl	bl	bl	bl	bl	bl	bl	bl	bl	bl		0	
S6	2.06	1.87	1.80	2.57	2.08	1.57	1.67	1.73	1.76	1.61	2.41	2.70	2.16	1.86	1.96	2	18.76
S7	6.36	5.97	5.20	7.25	5.58	4.34	5.77	5.85	5.43	4.58	6.66	7.60	6.29	5.49	5.82	6	16.93
S8	11.2	12.2	11.7	13.8	13.2	8.66	11.8	11.3	10.4	8.90	12.5	14.0	12.5	11.9	11.61	12	15.34
S9	bl	bl	bl	bl	bl	bl	bl	bl	bl	bl	bl	bl	bl	bl		0	
S10	1.96	1.86	2.09	2.39	2.06	2.24	1.96	1.64	1.67	1.65	2.21	2.50	2.14	1.74	1.99	2	14.82
S11	6.30	5.72	5.86	6.83	5.91	6.32	5.57	5.93	5.14	4.76	6.76	7.46	6.47	5.28	5.98	6	12.91
S12	12.3	11.2	9.71	12.3	12.7	10.9	11.1	11.2	9.73	9.74	12.5	14.9	13.2	7.75	11.22	12	17.75
S13	bl	bl	bl	bl	bl	bl	bl	bl	bl	bl	bl	bl	bl	bl		0	
S14	1.99	2.06	1.94	2.46	2.07	1.59	1.96	1.68	1.66	1.53	2.33	2.58	1.97	1.95	1.96	2	16.97
S15	6.21	6.07	5.47	7.03	5.87	4.46	5.78	5.73	5.20	4.79	6.78	7.51	5.94	5.24	5.81	6	15.40
S16	11.7	12.6	10.6	13.6	12.0	8.76	11.9	10.4	11.3	9.50	12.1	14.1	11.3	9.59	11.29	12	14.57
S17	1.83	2.03	1.96	2.47	2.09	2.16	2.07	1.66	1.67	1.55	2.30	2.88	2.20	1.97	2.03	2	17.93
S18	4.72	5.43	4.51	5.82	4.44	4.39	4.97	4.24	4.16	4.23	5.56	6.19	5.06	4.61	4.84	5	13.65
S19	1.86	2.16	1.78	2.38	1.97	2.10	2.24	1.91	1.67	1.55	2.37	2.51	2.46	1.95	2.04	2	16.06
S20	4.84	5.33	4.98	5.96	5.17	4.73	4.89	4.30	4.25	3.96	5.70	6.16	4.78	5.01	4.97	5	13.41
S21	bl	bl	bl	bl	bl	bl	bl	bl	bl	bl	bl	bl	bl	bl		0 (+ADA)	
S22	1.76	1.76	1.38	2.04	1.49	1.39	1.69	1.30	1.46	1.35	2.06	2.28	1.52	1.48	1.62	2 (+ADA)	19.27
S23	5.28	6.09	5.04	6.80	4.60	4.25	5.48	5.14	5.59	4.70	6.55	7.18	5.62	5.17	5.48	6 (+ADA)	16.36
S24	11.7	12.0	10.3	13.0	11.7	8.93	10.3	10.8	9.93	9.75	12.6	13.5	11.7	11.3	11.16	12 (+ADA)	12.51

GM: Geometric Mean; GCV: Geometric Coefficient of Variation (%); bl : below limit of quantitation; ADA : anti-drug antibodies; shaded boxes : unspiked samples or spiked but containing ADA; [1] : adalimumab

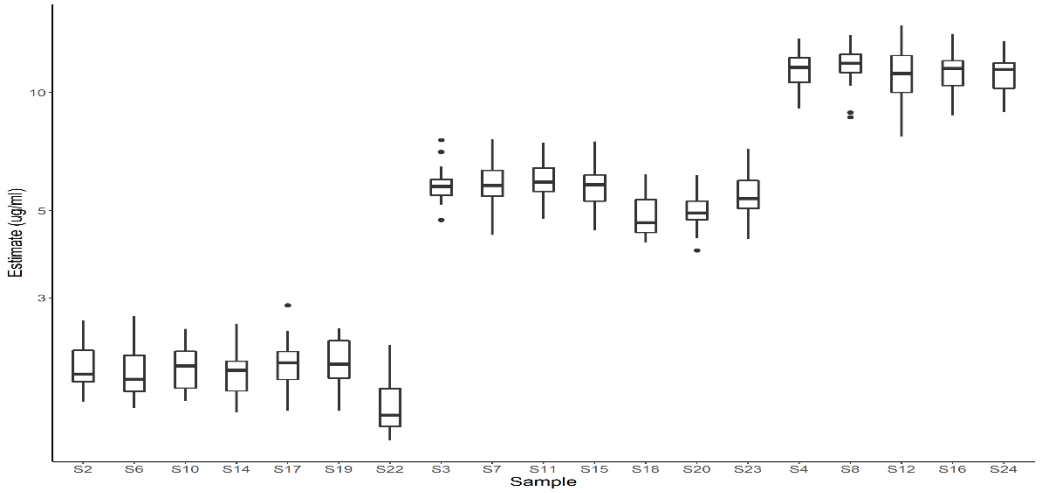
Table 21. Laboratory geometric mean content estimates (µg/ml) and inter-laboratory GCV values for spiked samples S1-S24 calculated relative to sample A

Sample	Laboratory														Overall GM	Theoretical amount[1]	Inter-lab GCV
	1Ta	1Tb	2T	3T	4T	6T	7T	8T	9T	10T	12T	14T	15T	16T			
S1	bl	loq	bl	bl	bl	bl	bl	bl	bl	bl	bl	bl	bl	bl		0	
S2	2.26	2.51	1.64	2.43	2.14	2.30	2.87	2.17	2.02	1.96	3.30	2.63	2.19	2.38	2.31	2	18.62
S3	6.28	6.34	4.91	6.36	5.62	6.83	6.81	6.65	5.79	5.25	7.50	7.01	5.79	6.83	6.24	6	12.69
S4	15.1	12.7	9.63	12.3	11.8	13.0	12.3	13.3	11.5	10.4	12.9	12.5	11.8	10.8	12.08	12	11.97
S5	bl	loq	bl	bl	bl	bl	bl	bl	bl	bl	bl	bl	bl	bl		0	
S6	2.51	2.46	1.76	2.55	2.13	1.93	2.20	1.97	1.91	1.92	3.25	2.70	2.19	2.23	2.23	2	18.11
S7	7.31	7.20	4.92	7.13	5.38	5.06	6.98	6.52	5.61	5.10	7.18	7.04	6.22	6.61	6.24	6	16.07
S8	13.5	14.3	11.0	13.9	12.7	10.6	13.7	12.3	11.6	10.1	12.6	12.8	12.4	13.2	12.41	12	11.04
S9	bl	bl	bl	bl	bl	bl	bl	bl	bl	bl	bl	bl	bl	bl		0	
S10	2.38	2.48	2.04	2.37	2.12	2.67	2.55	1.87	1.90	1.96	3.09	2.52	2.19	2.31	2.30	2	15.36
S11	7.28	6.91	5.52	6.71	5.68	7.05	6.75	6.60	5.55	5.28	7.31	6.92	6.35	6.51	6.42	6	11.55
S12	15.2	13.1	9.06	12.3	12.2	13.0	12.8	12.2	10.7	11.2	12.8	13.7	13.0	9.36	12.08	12	15.28
S13	bl	loq	bl	bl	bl	bl	bl	bl	bl	bl	bl	bl	bl	bl		0	
S14	2.43	2.67	1.90	2.44	2.13	1.93	2.55	1.91	1.93	1.86	3.17	2.59	1.99	2.60	2.26	2	18.47
S15	7.13	7.29	5.16	6.91	5.65	5.09	6.99	6.38	5.46	5.29	7.29	6.97	5.85	6.44	6.23	6	14.55
S16	14.3	14.8	9.44	13.7	11.6	10.6	13.8	11.3	11.7	10.9	12.3	12.9	11.1	10.9	11.99	12	13.91
S17	2.26	2.67	1.92	2.45	2.15	2.78	2.69	1.89	1.71	1.89	3.20	2.86	2.24	2.56	2.34	2	20.53
S18	5.50	6.59	4.30	5.71	4.33	5.40	6.07	4.76	4.30	4.71	6.26	5.82	5.05	5.82	5.28	5	15.63
S19	2.27	2.79	1.74	2.37	2.03	2.71	2.89	2.17	1.79	1.84	3.24	2.53	2.48	2.74	2.36	2	21.15
S20	5.63	6.48	4.73	5.84	5.00	5.78	5.99	4.83	4.38	4.40	6.38	5.79	4.79	6.20	5.40	5	14.70
S21	bl	bl	bl	bl	bl	bl	bl	bl	bl	bl	bl	bl	bl	bl		0 (+ADA)	
S22	2.14	2.30	1.36	2.03	1.54	1.83	2.22	1.49	1.63	1.64	2.89	2.30	1.52	1.86	1.87	2 (+ADA)	24.05
S23	6.14	7.32	4.78	6.67	4.47	5.24	6.65	5.75	6.00	5.15	7.11	6.46	5.58	6.36	5.92	6 (+ADA)	15.93
S24	14.2	14.0	9.57	13.1	11.3	11.6	12.0	11.8	10.9	11.2	12.7	12.3	11.5	12.4	11.99	12 (+ADA)	10.82

GM: Geometric Mean; GCV: Geometric Coefficient of Variation (%); bl : below limit of quantitation; loq : level at limit of quantitation; ADA : anti-drug antibodies; shaded boxes : unspiked samples or spiked but containing ADA; [1] : adalimumab

Figure 3. Boxplot of laboratory geometric mean content estimates (µg/ml) for spiked samples S1-S24 calculated relative to kit or in-house standards (A) and Sample A (B)

A



B

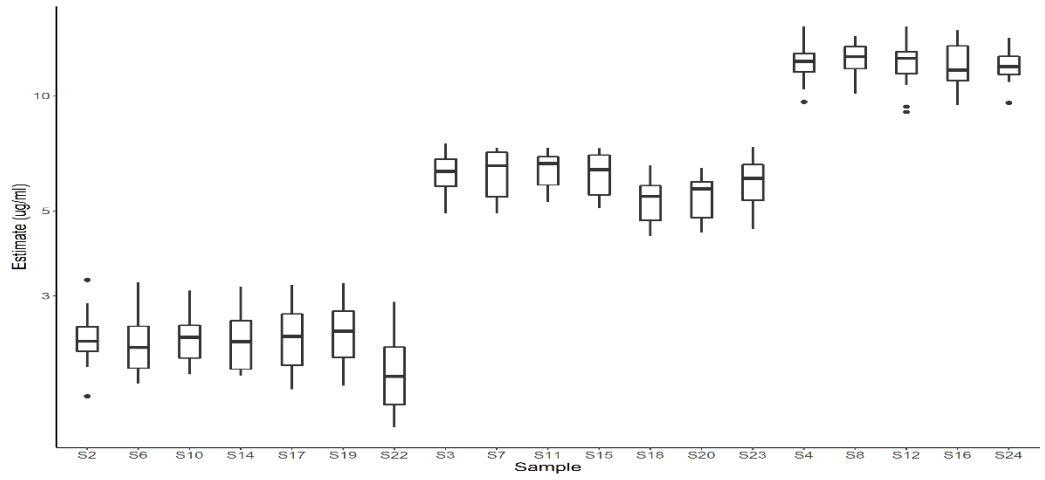


Table 22. Concordance correlation coefficients for log transformed laboratory geometric mean content estimates (µg/ml) of spiked samples S1-S24 calculated relative to kit or in-house standards (A) and Sample A (B)

A

Lab	1Ta	1Tb	2T	3T	4T	6T	7T	8T	9T	10T	12T	14T	15T	16T
1Ta														
1Tb	0.99													
2T	0.99	0.98												
3T	0.97	0.97	0.93											
4T	0.99	0.99	0.99	0.96										
6T	0.96	0.95	0.98	0.89	0.96									
7T	0.99	0.99	0.99	0.96	0.99	0.97								
8T	0.99	0.98	0.99	0.93	0.98	0.97	0.98							
9T	0.98	0.98	0.99	0.92	0.98	0.97	0.98	0.99						
10T	0.96	0.95	0.98	0.87	0.95	0.97	0.96	0.98	0.99					
12T	0.98	0.98	0.95	1.00	0.97	0.91	0.97	0.95	0.94	0.89				
14T	0.94	0.94	0.90	0.99	0.94	0.86	0.93	0.90	0.88	0.82	0.99			
15T	0.99	0.99	0.98	0.98	0.99	0.96	0.99	0.98	0.97	0.93	0.98	0.95		
16T	0.98	0.98	0.99	0.93	0.98	0.97	0.98	0.98	0.99	0.97	0.94	0.88	0.97	

B

Lab	1Ta	1Tb	2T	3T	4T	6T	7T	8T	9T	10T	12T	14T	15T	16T
1Ta														
1Tb	0.99													
2T	0.91	0.88												
3T	0.99	0.99	0.91											
4T	0.96	0.93	0.98	0.96										
6T	0.96	0.95	0.94	0.96	0.97									
7T	0.98	0.99	0.89	0.99	0.95	0.96								
8T	0.97	0.95	0.96	0.97	0.99	0.97	0.96							
9T	0.95	0.92	0.98	0.95	0.99	0.95	0.93	0.99						
10T	0.94	0.91	0.99	0.94	0.99	0.96	0.92	0.98	0.99					
12T	0.95	0.97	0.79	0.96	0.87	0.91	0.97	0.88	0.85	0.84				
14T	0.99	0.99	0.89	0.99	0.95	0.96	0.99	0.96	0.93	0.92	0.98			
15T	0.97	0.96	0.96	0.98	0.99	0.98	0.97	0.99	0.98	0.98	0.90	0.97		
16T	0.97	0.98	0.93	0.99	0.96	0.97	0.98	0.97	0.95	0.94	0.94	0.98	0.98	

Shaded boxes represent > or equal to 90% concordance

Appendix

Appendix Table 1: Percentage of invalid assays per laboratory (Study A)

Assay	Lab	% of assays invalid *vs* sample A			% of assays invalid vs in-house reference			
		Sample B	Sample C	Sample D	Sample A	Sample B	Sample C	Sample D
Neut	01	0	0	-	-	-	-	-
Neut	02a	0	0	-	-	-	-	-
Neut	02b	22.2	11.1	-	-	-	-	-
Neut	03	40	15	-	50	50	40	-
Neut	04a	0	0	22.2	100	100	100	100
Neut	04b	0	0	0	91.7	100	88.9	100
Neut	05a	33.3	46.7	-	-	-	-	-
Neut	05b	26.7	33.3	-	-	-	-	-
Neut	06	0	0	0	0	0	0	0
Neut	07	0	0	-	-	-	-	-
Neut	08a	0	0	0	-	-	-	-
Neut	08b	0	0	0	-	-	-	-
Neut	08c	0	0	0	-	-	-	-
Neut	09	33.3	8.3	-	-	-	-	-
Neut	10	0	0	0	-	-	-	-
Neut	11	0	11.1	-	0	0	0	-
Neut	12	0	0	-	0	0	0	-
Neut	13	0	0	-	0	0	0	-
Neut	14	0	0	-	0	0	0	-
Neut	15	0	0	-	-	-	-	-
Neut	16	0	0	-	0	0	0	-
Neut	17	0	0	-	0	0	22.2	-
Neut	18	0	0	0	-	-	-	-
Neut	19	11.1	11.1	33.3	16.7	11.1	11.1	33.3
Neut	20	0	0	-	0	-	0	-
Neut	21	22.2	22.2	-	0	11.1	11.1	-
Neut	22	11.1	22.2	-	0	11.1	11.1	-
Neut	23	0	0	0	0	0	0	0
Neut	24	33.3	33.3	33.3	66.7	66.7	11.1	33.3
Neut	25	33.3	33.3	-	0	0	0	-
Neut	26a	0	0	-	0	0	0	-
Neut	26b	0	0	-	25	25	50	-
ADCC	12	0	0	-	0	11.1	11.1	-
ADCC	16	0	44.4	-	11.1	0	11.1	-
ADCC	23	0	0	0	0	33.3	0	0
ADCC	25	0	33.3	-	-	-	-	-
ADCC	26	0	33.3	-	0	0	33.3	-

Binding	03	0	66.7	-	66.7	33.3	66.7	-
Binding	07	0	0	-	0	50	0	-
Binding	08	0	0	0	0	0	0	0
Binding	10	0	0	0	-	-	-	-
Binding	12a	11.1	0	-	0	0	0	-
Binding	12b	0	11.1	-	0	0	22.2	-
Binding	20	0	0	0	5.6	0	0	11.1
Binding	23	0	0	0	16.7	0	0	33.3
Binding	25a	0	33.3	-	0	0	33.3	-
Binding	25b	0	0	-	0	33.3	0	-
CDC	06	0	0	0	0	0	0	33.3
CDC	16	11.1	33.3	-	22.2	0	0	-
CDC	25	0	0	-	66.7	33.3	66.7	-
CDC	26	0	0	-	0	0	0	-

- Denotes no data

Appendix Table 2: Individual assay relative potency estimates (Study A)

Lab	Assay	Day	Plate	Sample B	Sample C	Sample D	In-house reference
01	Neut	1	1	1.01	1.02	-	-
01	Neut	1	2	1.04	0.94	-	-
01	Neut	1	3	1.04	1.04	-	-
01	Neut	2	1	1.19	1.04	-	-
01	Neut	2	2	0.96	0.88	-	-
01	Neut	2	3	1.05	1.02	-	-
01	Neut	3	1	1.05	1.03	-	-
01	Neut	3	2	1.00	1.06	-	-
01	Neut	3	3	1.04	1.01	-	-
02a	Neut	1	1	1.09	1.07	-	-
02a	Neut	1	2	0.91	0.96	-	-
02a	Neut	1	3	1.01	0.94	-	-
02a	Neut	2	1	0.99	1.08	-	-
02a	Neut	2	2	0.95	0.99	-	-
02a	Neut	2	3	0.99	0.96	-	-
02a	Neut	3	1	1.18	1.11	-	-
02a	Neut	3	2	0.87	0.92	-	-
02a	Neut	3	3	1.05	0.88	-	-
02b	Neut	1	1	1.36	1.09	-	-
02b	Neut	1	2	1.03	1.07	-	-
02b	Neut	1	3	0.93	0.95	-	-
02b	Neut	2	1	1.18	1.02	-	-
02b	Neut	2	2	NP	1.02	-	-
02b	Neut	2	3	1.23	0.95	-	-
02b	Neut	3	1	1.18	1.25	-	-
02b	Neut	3	2	1.04	0.79	-	-
02b	Neut	3	3	NP	NP	-	-
03	Neut	1	2	NP	NP	-	NP
03	Neut	1	3	NP	0.77	-	NP
03	Neut	2	1	0.80	0.90	-	NP
03	Neut	2	2	NP	0.92	-	NP
03	Neut	2	3	0.90	0.90	-	NP
03	Neut	3	1	0.91	0.85	-	0.95
03	Neut	3	2	1.00	0.92	-	0.90
03	Neut	3	3	0.91	0.87	-	0.83

03	Neut	4	1	0.93	1.05	-	0.99
03	Neut	4	2	1.04	1.04	-	1.05
03	Neut	4	3	0.95	1.02	-	1.04
03	Neut	5	1	0.98	NP	-	0.97
03	Neut	5	2	0.93	0.97	-	0.98
03	Neut	5	3	NP	1.40	-	NP
03	Neut	6	1	NP	NP	-	NP
03	Neut	6	2	NP	0.81	-	NP
03	Neut	6	3	0.81	0.91	-	NP
03	Neut	7	1	0.88	0.93	-	1.44
03	Neut	7	2	NP	0.99	-	1.28
03	Neut	7	3	NP	0.83	-	NP
04a	Neut	1	1	1.06	0.98	0.78	NP
04a	Neut	1	2	0.85	0.89	0.78	NP
04a	Neut	1	3	1.06	0.85	0.90	NP
04a	Neut	2	1	1.07	1.04	0.84	NP
04a	Neut	2	2	1.05	1.05	0.86	NP
04a	Neut	2	3	1.06	0.97	0.84	NP
04a	Neut	3	1	1.20	1.22	NP	NP
04a	Neut	3	2	0.91	0.96	0.78	NP
04a	Neut	3	3	1.11	0.84	NP	NP
04b	Neut	1	1	1.01	0.97	-	NP
04b	Neut	1	2	1.02	1.03	-	NP
04b	Neut	1	3	1.04	1.03	-	NP
04b	Neut	1	4	-	-	0.87	NP
04b	Neut	2	1	1.06	0.97	-	NP
04b	Neut	2	2	1.09	1.05	-	NP
04b	Neut	2	3	1.03	0.99	-	NP
04b	Neut	2	4	-	-	0.86	NP
04b	Neut	3	1	1.01	0.99	-	2.74
04b	Neut	3	2	1.00	1.00	-	NP
04b	Neut	3	3	1.0-2	0.95	-	NP
04b	Neut	3	4		-	0.81	NP
05a	Neut	1	1	NP	NP	-	-
05a	Neut	1	2	1.23	1.14	-	-
05a	Neut	1	3	NP	NP	-	-
05a	Neut	2	1	NP	0.90	-	-
05a	Neut	2	2	0.74	0.52	-	-
05a	Neut	2	3	1.60	NP	-	-
05a	Neut	3	1	1.79	1.80	-	-

05a	Neut	3	2	1.38	NP	-	-
05a	Neut	3	3	NP	NP	-	-
05a	Neut	4	1	1.08	0.90	-	-
05a	Neut	4	2	1.28	0.91	-	-
05a	Neut	4	3	NP	0.98	-	-
05a	Neut	5	1	1.30	0.97	-	-
05a	Neut	5	2	1.20	NP	-	-
05a	Neut	5	3	1.15	0.86	-	-
05b	Neut	1	1	1.24	1.55	-	-
05b	Neut	1	2	NP	NP	-	-
05b	Neut	1	3	0.69	NP	-	-
05b	Neut	2	1	1.28	NP	-	-
05b	Neut	2	2	0.71	0.97	-	-
05b	Neut	2	3	0.89	1.34	-	-
05b	Neut	3	1	NP	0.86	-	-
05b	Neut	3	2	NP	1.27	-	-
05b	Neut	3	3	1.07	0.87	-	-
05b	Neut	4	1	1.27	1.15	-	-
05b	Neut	4	2	1.29	NP	-	-
05b	Neut	4	3	0.98	1.01	-	-
05b	Neut	5	1	1.09	NP	-	-
05b	Neut	5	2	NP	1.14	-	-
05b	Neut	5	3	1.05	1.08	-	-
06	Neut	1	1	0.99	-	-	1.03
06	Neut	1	2	-	1.01	-	1.14
06	Neut	1	4	1.03	-	-	1.01
06	Neut	1	5	-	1.01	-	1.02
06	Neut	1	7	-	-	0.89	0.94
06	Neut	2	1	0.94	-	-	0.98
06	Neut	2	2	-	1.14	-	1.01
06	Neut	2	4	1.03	-	-	0.88
06	Neut	2	5	-	1.05	-	1.01
06	Neut	2	7	-	-	1.03	1.06
06	Neut	3	1	0.83	-	-	0.95
06	Neut	3	2	-	1.17	-	1.16
06	Neut	3	4	0.97	-	-	0.89
06	Neut	3	5	-	0.93	-	1.02
06	Neut	3	7	-	-	0.92	1.08
07	Neut	1	1	1.02	1.04	-	-
07	Neut	1	2	1.05	1.05	-	-

07	Neut	1	3	1.08	1.07	-	-
07	Neut	2	1	1.12	1.02	-	-
07	Neut	2	2	1.04	1.08	-	-
07	Neut	2	3	1.12	1.13	-	-
07	Neut	3	1	1.02	0.94	-	-
07	Neut	3	2	1.08	0.95	-	-
07	Neut	3	3	0.99	0.94	-	-
08a	Neut	1	1	1.14	1.16	0.86	-
08a	Neut	1	2	1.07	1.02	0.84	-
08a	Neut	1	3	1.02	1.06	0.84	-
08a	Neut	2	1	1.18	1.18	0.97	-
08a	Neut	2	2	1.00	1.09	0.88	-
08a	Neut	2	3	1.07	1.00	0.85	-
08a	Neut	3	1	1.02	0.89	0.82	-
08a	Neut	3	2	1.08	0.99	0.75	-
08a	Neut	3	3	1.10	1.10	0.89	-
08b	Neut	1	1	1.18	1.16	0.85	-
08b	Neut	1	2	1.05	0.99	0.83	-
08b	Neut	1	3	1.00	1.03	0.84	-
08b	Neut	2	1	1.21	1.19	0.98	-
08b	Neut	2	2	1.01	1.11	0.90	-
08b	Neut	2	3	1.10	0.99	0.85	-
08b	Neut	3	1	1.00	0.87	0.82	-
08b	Neut	3	2	1.09	0.98	0.76	-
08b	Neut	3	3	1.08	1.10	0.90	-
08c	Neut	1	1	0.91	0.95	-	-
08c	Neut	1	2	1.06	-	0.83	-
08c	Neut	1	3	-	0.93	0.79	-
08c	Neut	1	4	1.03	1.07	-	-
08c	Neut	1	5	1.04	-	0.79	-
08c	Neut	2	1	1.04	0.99	-	-
08c	Neut	2	2	-	0.96	0.80	-
08c	Neut	2	3	1.03	-	0.85	-
08c	Neut	2	4	1.05	1.07	-	-
08c	Neut	2	5	0.99	-	0.81	-
08c	Neut	3	1	0.99	0.93	-	-
08c	Neut	3	2	-	0.99	0.83	-
08c	Neut	3	3	1.03	-	0.79	-
08c	Neut	3	4	1.03	0.97	-	-
08c	Neut	3	5	1.09	-	0.83	-

08c	Neut	4	1	0.95	0.95	-	-
08c	Neut	4	2	-	0.95	0.81	-
08c	Neut	4	3	1.07	-	0.81	-
08c	Neut	4	4	1.00	1.01	-	-
08c	Neut	4	5	-	-	0.77	-
09	Neut	1	1	0.96	0.94	-	-
09	Neut	1	2	0.94	1.02	-	-
09	Neut	1	3	0.94	0.93	-	-
09	Neut	2	1	NP	1.02	-	-
09	Neut	2	2	0.99	1.12	-	-
09	Neut	2	3	NP	0.87	-	-
09	Neut	3	1	1.05	0.98	-	-
09	Neut	3	2	0.93	0.97	-	-
09	Neut	3	3	0.91	0.91	-	-
09	Neut	4	1	NP	NP	-	-
09	Neut	4	2	NP	0.90	-	-
09	Neut	4	3	1.06	1.04	-	-
10	Neut	1	1	1.07	1.04	-	-
10	Neut	1	2	1.04	1.00	-	-
10	Neut	1	3	1.01	0.95	-	-
10	Neut	1	4	-	-	0.96	-
10	Neut	2	1	0.94	1.01	-	-
10	Neut	2	2	0.99	0.89	-	-
10	Neut	2	3	1.08	1.10	-	-
10	Neut	2	4	-	-	0.73	-
10	Neut	3	1	1.00	0.92	-	-
10	Neut	3	2	1.14	1.14	-	-
10	Neut	3	3	0.96	1.04	-	-
10	Neut	3	4	-	-	0.76	-
11	Neut	1	1	0.77	0.83	0.99	-
11	Neut	1	2	1.32	1.03	1.47	-
11	Neut	1	3	0.95	1.01	1.65	-
11	Neut	2	1	1.38	1.46	1.67	-
11	Neut	2	2	1.09	1.09	1.46	-
11	Neut	2	3	0.93	0.75	1.39	-
11	Neut	3	1	0.96	NP	NP	-
11	Neut	3	2	1.08	1.15	NP	-
11	Neut	3	3	0.97	1.01	NP	-
12	Neut	1	1	1.05	0.98	-	1.05
12	Neut	1	2	1.16	0.99	-	1.15

12	Neut	1	3	1.10	1.04	-	1.19
12	Neut	2	1	0.97	1.02	-	1.22
12	Neut	2	2	1.03	1.07	-	1.12
12	Neut	2	3	1.01	0.92	-	1.05
12	Neut	3	1	1.10	1.12	-	1.17
12	Neut	3	2	1.00	0.89	-	1.09
12	Neut	3	3	1.00	0.93	-	1.04
13	Neut	1	1	1.13	1.25	-	1.18
13	Neut	1	2	0.96	0.96	-	0.96
13	Neut	1	3	0.95	1.01	-	0.91
14	Neut	1	1	1.04	0.90	-	1.03
14	Neut	1	2	1.17	1.08	-	1.30
14	Neut	1	3	0.89	0.94	-	1.11
14	Neut	2	1	1.01	0.87	-	0.91
14	Neut	2	2	0.99	0.99	-	1.11
14	Neut	2	3	0.97	0.97	-	1.09
14	Neut	3	1	0.99	0.94	-	1.07
14	Neut	3	2	1.04	1.04	-	1.12
14	Neut	3	3	0.99	1.08	-	1.16
15	Neut	1	1	1.15	0.90	-	-
15	Neut	1	2	1.12	0.96	-	-
15	Neut	1	3	1.13	0.96	-	-
15	Neut	2	1	1.17	1.03	-	-
15	Neut	2	2	1.22	1.04	-	-
15	Neut	2	3	1.23	0.98	-	-
15	Neut	3	1	1.06	1.18	-	-
15	Neut	3	2	1.08	1.02	-	-
15	Neut	3	3	1.01	1.04	-	-
16	Neut	1	1	0.88	0.98	-	1.02
16	Neut	1	2	0.93	0.95	-	0.94
16	Neut	1	3	0.96	0.96	-	0.91
16	Neut	2	1	1.06	1.11	-	1.08
16	Neut	2	2	0.93	0.93	-	1.02
16	Neut	2	3	0.93	0.92	-	0.99
16	Neut	3	1	0.97	1.00	-	0.97
16	Neut	3	2	1.06	0.96	-	1.02
16	Neut	3	3	1.05	0.94	-	0.95
17	Neut	1	1	1.09	0.95	-	0.94
17	Neut	1	2	1.18	1.11	-	1.12
17	Neut	1	3	1.33	1.07	-	0.99

17	Neut	1	4	0.95	1.06	-	1.41
17	Neut	1	5	0.81	1.39	-	0.79
17	Neut	1	6	1.16	1.29	-	1.25
17	Neut	1	7	0.98	1.00	-	0.89
17	Neut	1	8	1.18	1.00	-	1.06
17	Neut	1	9	1.24	1.15	-	1.16
18	Neut	1	1	1.04	0.97	0.77	-
18	Neut	1	2	0.98	0.98	0.81	-
18	Neut	1	3	0.98	1.01	0.84	-
18	Neut	2	1	0.97	0.92	0.79	-
18	Neut	2	2	1.02	0.98	0.83	-
18	Neut	2	3	0.95	0.99	0.79	-
18	Neut	3	1	1.01	0.99	0.79	-
18	Neut	3	2	1.01	0.95	0.77	-
18	Neut	3	3	1.01	0.96	0.83	-
19	Neut	1	1	1.11	1.04	-	1.09
19	Neut	1	2	1.05	1.01	-	1.01
19	Neut	1	3	NP	NP	-	NP
19	Neut	1	4	-	-	NP	NP
19	Neut	2	1	1.06	1.04	-	1.02
19	Neut	2	2	1.03	0.99	-	1.02
19	Neut	2	3	1.10	0.99	-	0.99
19	Neut	2	4	-	-	0.83	1.04
19	Neut	3	1	0.99	1.00	-	0.94
19	Neut	3	2	1.01	0.97	-	0.95
19	Neut	3	3	1.04	0.98	-	0.94
19	Neut	3	4	-	-	0.79	0.97
20	Neut	1	1	1.07	0.80	-	-
20	Neut	1	2	1.08	0.89	-	-
20	Neut	1	3	0.97	0.73	-	-
20	Neut	1	4	-	0.90	-	0.83
20	Neut	2	1	1.02	1.16	-	-
20	Neut	2	2	0.93	1.14	-	-
20	Neut	2	3	0.99	0.81	-	-
20	Neut	2	4	-	0.94	-	0.87
20	Neut	3	1	1.15	1.06	-	-
20	Neut	3	2	1.17	1.24	-	-
20	Neut	3	3	1.15	1.09	-	-
20	Neut	3	4	-	0.95	-	0.84
21	Neut	1	1	NP	NP	-	0.80

21	Neut	1	2	1.02	0.96	-	0.82
21	Neut	1	3	NP	1.06	-	0.80
21	Neut	2	1	0.99	1.10	-	0.93
21	Neut	2	2	1.04	1.00	-	0.89
21	Neut	2	3	1.11	1.00	-	0.91
21	Neut	3	1	1.06	1.07	-	0.89
21	Neut	3	2	1.08	1.06	-	0.89
21	Neut	3	3	1.09	1.01	-	0.90
22	Neut	1	1	1.06	1.08	-	0.89
22	Neut	1	2	0.82	NP	-	1.15
22	Neut	1	3	0.89	0.92	-	0.85
22	Neut	2	1	1.15	1.19	-	1.11
22	Neut	2	2	0.92	1.07	-	0.98
22	Neut	2	3	NP	1.58	-	1.19
22	Neut	3	1	0.95	0.90	-	1.02
22	Neut	3	2	0.93	1.15	-	1.04
22	Neut	3	3	1.03	0.99	-	1.08
23	Neut	1	1	1.05	1.00	-	1.06
23	Neut	1	2	1.10	1.05	-	1.09
23	Neut	1	3	1.02	1.01	-	1.09
23	Neut	1	4	-	-	0.85	1.08
23	Neut	1	5	-	-	0.88	1.08
23	Neut	1	6	-	-	0.71	1.04
24	Neut	1	1	1.18	1.06	-	NP
24	Neut	1	2	1.09	1.07	-	NP
24	Neut	1	3	1.22	1.14	-	NP
24	Neut	1	4	-	-	1.27	NP
24	Neut	2	1	1.02	0.83	-	NP
24	Neut	2	2	NP	1.23	-	1.27
24	Neut	2	3	0.94	NP	-	0.94
24	Neut	2	4	-	-	0.91	1.96
24	Neut	3	1	NP	NP	-	NP
24	Neut	3	2	1.83	NP	-	NP
24	Neut	3	3	NP	1.92	-	1.04
24	Neut	3	4	-	-	NP	NP
25	Neut	1	1	NP	NP	-	1.05
25	Neut	1	2	1.04	1.01	-	1.05
25	Neut	1	3	0.94	1.03	-	1.18
26a	Neut	1	1	0.98	0.96	-	1.08
26a	Neut	1	2	0.99	1.01	-	1.00

26a	Neut	1	3	1.04	1.12	-	1.05
26b	Neut	1	1	1.10	1.15	-	1.08
26b	Neut	1	2	0.66	0.76	-	NP
26b	Neut	1	3	1.20	0.83	-	0.60
26b	Neut	1	4	0.97	0.95	-	0.88
12	ADCC	1	1	1.04	0.84	-	1.03
12	ADCC	1	2	1.46	1.52	-	1.23
12	ADCC	1	3	0.92	1.18	-	1.29
12	ADCC	2	1	0.92	0.98	-	0.74
12	ADCC	2	2	0.96	1.02	-	1.05
12	ADCC	2	3	1.16	1.05	-	1.31
12	ADCC	3	1	0.80	1.01	-	0.79
12	ADCC	3	2	1.13	1.00	-	1.35
12	ADCC	3	3	0.74	0.89	-	1.05
16	ADCC	1	1	1.01	NP	-	1.30
16	ADCC	1	2	1.29	0.94	-	1.11
16	ADCC	1	3	0.87	0.91	-	1.26
16	ADCC	2	1	0.86	1.47	-	1.35
16	ADCC	2	2	1.06	NP	-	1.37
16	ADCC	2	3	1.07	1.05	-	1.26
16	ADCC	3	1	1.04	1.09	-	0.93
16	ADCC	3	2	0.94	NP	-	NP
16	ADCC	3	3	0.76	NP	-	0.87
23	ADCC	1	1	0.92	0.91	-	0.79
23	ADCC	1	2	1.01	1.11	-	1.07
23	ADCC	1	3	1.05	1.19	-	1.21
23	ADCC	1	4	-	-	0.81	0.98
23	ADCC	1	5	-	-	0.83	0.87
23	ADCC	1	6	-	-	0.74	0.76
25	ADCC	1	1	1.09	NP	-	-
25	ADCC	1	2	1.15	1.14	-	-
25	ADCC	1	3	0.77	1.00	-	-
26	ADCC	1	1	0.80	NP	-	0.84
26	ADCC	1	2	1.06	0.80	-	0.69
26	ADCC	1	3	0.80	0.94	-	0.87
03	Binding	1	1	0.96	NP	-	0.96
03	Binding	1	2	1.02	NP	-	NP
03	Binding	1	3	1.01	1.02	-	NP
07	Binding	1	1	1.07	1.08	-	1.54
07	Binding	1	2	0.74	0.85	-	1.44

08	Binding	1	1	1.00	0.98	0.80	1.11
08	Binding	1	2	0.93	0.93	0.72	1.00
08	Binding	1	3	0.91	0.91	0.75	1.00
08	Binding	2	1	1.08	1.08	0.89	1.03
08	Binding	2	2	0.95	0.86	0.72	1.06
08	Binding	2	3	0.93	0.94	0.75	1.04
08	Binding	3	1	1.08	1.13	0.91	1.09
08	Binding	3	2	0.99	0.97	0.84	1.23
08	Binding	3	3	0.92	1.03	0.84	1.15
10	Binding	1	1	1.06	1.11	0.89	-
10	Binding	1	2	0.91	0.90	0.76	-
10	Binding	1	3	1.03	0.91	0.86	-
10	Binding	2	1	1.04	1.01	0.83	-
10	Binding	2	2	0.93	0.94	0.80	-
10	Binding	2	3	0.98	0.97	0.81	-
10	Binding	3	1	0.96	0.90	0.77	-
10	Binding	3	2	1.03	0.94	0.77	-
10	Binding	3	3	0.96	0.96	0.75	-
12a	Binding	1	1	1.20	1.00	-	1.32
12a	Binding	1	2	NP	0.79	-	0.89
12a	Binding	1	3	1.03	0.97	-	0.86
12a	Binding	2	1	1.15	1.10	-	1.08
12a	Binding	2	2	1.17	1.11	-	1.11
12a	Binding	2	3	0.96	1.14	-	1.16
12a	Binding	3	1	1.29	1.06	-	1.09
12a	Binding	3	2	1.17	0.88	-	0.93
12a	Binding	3	3	1.35	1.14	-	1.18
12b	Binding	1	1	0.98	1.03	-	1.28
12b	Binding	1	2	0.96	0.93	-	1.02
12b	Binding	1	3	1.14	1.10	-	1.12
12b	Binding	2	1	0.92	NP	-	1.14
12b	Binding	2	2	1.13	0.98	-	0.92
12b	Binding	2	3	1.12	1.19	-	1.12
12b	Binding	3	1	1.09	1.16	-	1.14
12b	Binding	3	2	1.15	0.90	-	1.08
12b	Binding	3	3	1.06	1.00	-	0.94
20	Binding	1	1	1.11	1.02	-	0.97
20	Binding	1	2	1.03	0.98	-	0.81
20	Binding	1	3	0.92	0.99	-	0.90
20	Binding	1	4	-	-	0.84	0.92

20	Binding	1	5	-	-	0.86	0.90
20	Binding	1	6	-	-	0.78	0.95
20	Binding	2	1	1.04	1.12	-	1.18
20	Binding	2	2	1.25	1.26	-	1.25
20	Binding	2	3	0.84	1.01	-	1.21
20	Binding	2	4	-	-	0.69	NP
20	Binding	2	5	-	-	0.87	1.03
20	Binding	2	6	-	-	0.75	1.00
20	Binding	3	1	1.03	1.04	-	1.29
20	Binding	3	2	1.35	1.41	-	1.57
20	Binding	3	3	0.92	1.08	-	1.31
20	Binding	3	4	-	-	0.60	1.21
20	Binding	3	5	-	-	0.75	1.34
20	Binding	3	6	-	-	0.84	1.22
23	Binding	1	1	0.81	1.02	-	0.88
23	Binding	1	2	1.24	1.03	-	1.10
23	Binding	1	3	0.95	1.01	-	1.18
23	Binding	1	4	-	-	0.94	NP
23	Binding	1	5	-	-	0.84	1.04
23	Binding	1	6	-	-	1.04	1.42
25a	Binding	1	1	1.36	1.37	-	1.67
25a	Binding	1	2	0.84	1.04	-	1.02
25a	Binding	1	3	0.84	NP	-	1.29
25b	Binding	1	1	1.14	1.10	-	1.10
25b	Binding	1	2	1.15	1.05	-	0.96
25b	Binding	1	3	0.97	0.84	-	0.81
06	CDC	1	1	0.97	0.96	-	1.08
06	CDC	1	2	1.11	0.99	-	1.06
06	CDC	1	3	1.13	1.13	-	1.24
06	CDC	1	4	-	-	0.79	1.11
06	CDC	2	1	1.03	1.30	-	1.34
06	CDC	2	2	1.08	1.15	-	1.07
06	CDC	2	3	1.09	1.18	-	1.24
06	CDC	2	4	-	-	0.70	1.07
06	CDC	3	1	0.87	1.17	-	1.11
06	CDC	3	2	0.83	1.10	-	1.05
06	CDC	3	3	1.03	1.05	-	0.96
06	CDC	3	4	-	-	0.69	0.85
16	CDC	1	1	1.28	1.24	-	1.15
16	CDC	1	2	0.88	0.67	-	0.79

16	CDC	1	3	1.44	1.53	-	1.16
16	CDC	2	1	NP	NP	-	NP
16	CDC	2	2	1.06	0.89	-	1.04
16	CDC	2	3	1.49	1.39	-	1.16
16	CDC	3	1	0.73	NP	-	NP
16	CDC	3	2	1.20	0.98	-	1.01
16	CDC	3	3	1.30	NP	-	0.95
25	CDC	1	1	1.10	1.34	-	NP
25	CDC	1	2	0.93	1.02	-	NP
25	CDC	1	3	1.06	1.05	-	1.51
26	CDC	1	1	0.93	0.97	-	1.22
26	CDC	1	2	0.89	0.95	-	1.42
26	CDC	1	3	0.88	0.88	-	1.31

NP – not parallel; - indicates no data.

PROTOCOL STUDY A : WHO COLLABORATIVE STUDY FOR 1ST International Standard (IS) for Adalimumab
Project leader: Meenu Wadhwa & Chris Bird

1) BACKGROUND

Recombinant therapeutic monoclonal antibodies are an expanding therapeutic product class with many monoclonal antibodies approved for use in humans and many more in clinical development. The patents on some of the earlier licensed monoclonal antibodies have expired resulting in the development of biosimilar medicines, thereby widening the market for these products and increasing patient accessibility. The WHO have recognised a global need for standardisation of biotechnology products following requests for advice on appropriate control measures to ensure safety, quality and efficacy (WHO Technical Report Series, 56th Report, 941: 12-13, 2007). In the EU eight biosimilar adalimumab products have been approved, therefore it is important to develop an IS for bioactivity to facilitate global harmonisation.

2) AIMS OF THE STUDY

a) To assess the suitability of ampouled preparations of adalimumab to serve as the 1st WHO IS for adalimumab by assaying their biological activity in a range of bioassays and binding assays.
b) To assess the relative activity of the ampouled preparations of adalimumab in different assays (e.g. bioassays, immunoassays and binding assays etc.) in current use, and to determine, if possible, the concentrations of adalimumab required to neutralise specific amounts of TNF-α IS (12/154).
c) To compare the ampouled preparations with characterised 'in-house' laboratory standards where these are available.

1) MATERIALS INCLUDED IN THE STUDY

All participants will be sent:

a) A set of samples coded by letter **A, B, C** (5 ampoules for each preparation) for testing in adalimumab bioassays and/or binding assays. Each ampoule of candidate material contains approximately 50 µg of adalimumab.

b) 5 ampoules of the current IS for TNF-α (12/154), containing 43,000 IU of TNF-α.

Some participants will also be sent:
An additional sample (in addition to A,B & C) coded **D** for testing in the assays. This ampoule contains an unknown amount of adalimumab, but essentially should be treated/diluted in a similar way to samples A, B & C.

2) RECONSTITUTION AND STORAGE OF PREPARATIONS

Prior to initiating the study, please read the Instructions for Use provided with the collaborative study. Please note the statements regarding safety and that these preparations are not for human use. Lyophilized preparations provided should be stored at -20ºC or below until used.

a) All preparations, A to D should be reconstituted with 1ml of sterile distilled water. Mix GENTLY and ensure contents are completely dissolved prior to use. Use carrier protein (e.g. assay medium containing foetal bovine serum) where extensive dilution is required.

b) Reconstitute the IS for TNF-α coded 12/154 with 1ml of sterile distilled water. Mix GENTLY and ensure contents are completely dissolved prior to use. This solution contains TNF-α at a concentration of 43,000 IU/ml. Use carrier protein (e.g. assay medium containing foetal bovine serum) where extensive dilution is required.

3) ASSAYS

Cell based TNF-α neutralisation assays

These are based on the inhibitory action of adalimumab which neutralises the biological activity of TNF-α. To achieve the aims of the study, participants are requested to use a fixed dose of the **TNF-α IS (12/154)** which has been provided. The concentration of TNF-α used should provide a biological response similar to the dose of TNF-α routinely used by the participants in their in-house cell-based assays for adalimumab.
In our hands, the following concentrations of TNF-α provide acceptable adalimumab dose response curves. Please adhere to these concentrations as much as possible, however, please confirm that these doses are suitable for your assay by conducting a pilot assay prior to the final runs*.

- For L929 assays –a final dose of 10 - 20 IU/ml of TNF- α IS.
- For U937 and WEHI 164 assays –a final dose of 40 - 80 IU/ml of TNF- α IS.
- For HEK-Blue CD40L reporter gene assays – a final dose of 40 IU/ml of TNF- α IS.

***Please contact us for further guidance if you feel the suggested doses would not provide suitable data.**
For performing these assays, follow each of the steps a) – f) listed in Assay Design below.

Use validated in-house assays where possible. Remember to include appropriate controls in the assays- blank control wells (cells with culture medium but no TNF-α) and also wells containing cells with TNF-α only, at the fixed concentration used for the assay.

Cell based ADCC/CDC assays
For performing these assays, **follow each of the steps a) – f) listed in Assay Design below.** Use validated in-house assays where possible.

Binding assays

These should be carried out using in-house methods or kits, **follow each of the steps a) – f) in Assay Design below.**

PLEASE NOTE: The IS for TNF-α 12/154, contains 0.6% Human serum albumin as an excipient and therefore is unsuitable for use in binding assays; participants should use TNF-alpha from a commercial supplier.

4) ASSAY DESIGN

Participants are requested to use their own in-house validated procedures and follow steps a) to f) as listed below:

a) Use a freshly reconstituted ampoule of each preparation A to C in each of the assays. An assay is considered independent if the assay is carried out on different days/occasions.
b) Participants are asked to perform a pilot assay for each assay method used, to ensure that all preparations (A to C) are diluted such that the concentration range falls within the working range of the assay.

c) Following suitable dose response curves and an adequate signal above background ratio in the pilot assay, perform at least 3 independent assays for each of the preparations A to C (and in-house standard where available) using the most appropriate dilutions derived from the pilot assay for the different preparations tested.

d) For each independent assay each plate should include at least 1 independent dilution series for each preparation (A, B, C) in duplicate. This should be repeated 3 times across 3 different plates for each independent assay as illustrated in the example assay plate layouts at the end of the protocol. This will provide a total of 9 estimates from 3 independent assays in total. Please try to vary the positions of the samples on different plates to ensure that estimates are not susceptible to systematic bias due to positional effects.

e) Suggested assay layouts are given in Appendix 2 of this protocol. The layouts can be amended to suit in house methods if preferred, however, it is important to ensure that each plate includes at least 1 dilution series for each of the samples (A, B, C) + in-house reference standard if available.

f) Include appropriate controls in the assays

g) **NOTE:**

Participants assaying the additional sample D, please follow steps a) to f) listed in Assay Design above. Example assay layouts are given in Appendix 2. If using example layout B, sample D may need to be included on a separate plate (Plate 4), please also include sample A and in-house reference standard if available on this separate plate 4. **Please vary the position of IH, A, D in different assays** to ensure that estimates are not susceptible to systematic bias due to positional effects. See suggested plate layouts for Plate 4 in Appendix 2.
PLEASE NOTE: All concentrations are reported as the final dilution in the assay after all components and cells have been added. We ask all participants to report concentrations and doses in this format.

5) INFORMATION TO BE SUPPLIED AND PRESENTATION OF RESULTS

We have included assay information sheets in appendix 1, and example layouts (A & B) for microtiter plate formats in appendix 2. Excel templates are provided as separate excel files for returning assay layouts, sample dilutions and bioassay results for the samples tested. For binding assays, this will need to be amended as per the plate layout used for the assay.
Please answer the questions on the assay information sheets supplied in Appendix 1 to provide all of the details requested for EACH assay AND please record all concentrations and dilutions of assay components for EACH assay replicate on the results spreadsheet:
Information required for each assay is to include:

a) Assay, and how it was performed.

b) In-house adalimumab standard.

c) TNF-α preparation.

d) Dilutions used – stock solutions and final dilutions in assay. **Report all dilutions (adalimumab candidate samples, in-house standards and TNF- α 12/154) as the final dilution in the assay after all components and cells have been added.**

e) Please PROVIDE ALL RAW DATA (microtiter plate readouts e.g. O.D. or Luminescence Units etc.) as direct analysis of the raw data provided by the assays permits data from all participants to be handled consistently, as far as possible.

f) We request participants to follow the examples provided and enter data as indicated in the Excel template (that has been provided separately).

g) Although NIBSC will calculate relative potencies from the raw data provided by the participants, participants are requested (if possible) to calculate the potencies of each preparation using their own in-house methods relative to their in-house standard. Please provide information of all methods used for calculating results.

Where participants do not have an in-house adalimumab standard, please report all potencies relative to coded sample A.

6) DATA SUBMISSION

a) Please provide all information requested as this is needed for compilation of the study report following instructions given in the relevant sections of this report by using the provided data reporting sheets and Excel file.

b) Please return all completed data spreadsheets and assay method sheets for all of the replicates of all of the assays electronically.

Please aim to submit results by the 1st week of January 2019 at the latest.

7) REPORTING OF RESULTS

A draft report will be prepared and circulated to all participants for comment prior to submission to the Expert Committee on Biological Standardization of WHO. In the report, participating laboratories will be identified by a laboratory number only and any request to treat information in confidence will be respected.
For submission of study results and for any further information please email:

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8) CONDITIONS OF PARTICIPATION

Participants in the collaborative study should note the following conditions:

a) They participate in the study with the understanding that they agree not to publish or circulate information concerning the materials sent to them without the prior consent of the NIBSC study organisers.
b) The participants results may be shared anonymously with not-for-profit public health bodies in the interests of global harmonisation.
c) It is normal practice to acknowledge participants as contributors of data rather than co-authors in publications describing the establishment of the standard.
d) Individual participants' data will be reported and coded blind to other participants during the preparation of the study report and supporting publications.
e) All participants will receive a copy of the study report and proposed conclusions for comment before final establishment of the international standard

APPENDIX 1 – WHO Collaborative Study for adalimumab

Participants Study Details

Laboratory identification……

Local standard information

1. What is the nature of your local standard?
   Please state expression system _____

2. How did you obtain the standard?
   Bought ___ Source _____
   Made in-house ____ (please give reference if available)

3. What units do you use with the standard?
   Mass _____
   Units _____

4. If units, please provide information on how they were derived

_____

5. Assay methodology information

PLEASE NOTE: If more than one assay type is performed then please provide the details for each assay type.

Please record all information on concentrations and dilutions of assay components relating to EACH assay on the results spreadsheets provided as separate Excel templates.
Briefly outline the assay methods used (provide full protocol on separate sheets if available):

6. **Cell-based neutralisation assay**

a) Please report details of the cell line, source, seeding density and passage number of cells used in each assay, if applicable:
b) any pre-treatment of the cells before the assay
c) Details on ampoule reconstitution, dilution steps and dilution buffer used:

TNF-α IS
A
B
C
D
In-house standard

Please also provide details of any blanks if applicable:

d) Dose range of adalimumab used in the assay and the plate layout (please also provide separately in the spreadsheet).
e) Report details of the readout of the assay (e.g. Absorbance, Luminescence) and the reagent and equipment used to obtain this readout:
f) Additional comments:
g) For TNF- α neutralisation assays indicate the concentration of TNF-α used in the assay: Indicate the concentration of the initial working solution of TNF-α, the concentration used in neutralization of adalimumab samples, and also the final concentration in the assay after addition of the cells as appropriate for the assay

For example in some assays - TNF-α working solution = 100 IU/ml; diluted 1:1 with adalimumab samples = 50 IU/ml; further diluted 1:1 with cells = 25 IU/ml.

h) Length of incubation of the cells with adalimumab + TNF-α:

7. **ADCC/CDC assay**

Please report/provide information as indicated in 6a – 6f above. For ADCC assay please provide information for both target and effector cells.

8. **Binding assay**

a. Details on ampoule reconstitution, dilution steps and dilution buffer used:
   TNF-α IS 12/154
   A
   B
   C
   D
b) Please also provide details of any blanks if applicable:
c) Dose range of adalimumab used in the assay and the plate layout (separately).
d) Concentration and Source/Supplier of TNF-α used in the assay
e) Please report details of the readout of the assay (e.g. Absorbance, Luminescence) and the reagent and equipment used to obtain this readout:
f) Additional comments

APPENDIX 2:

Suggested plate layouts for WHO Collaborative Study for 1st International Standard for adalimumab
For reporting of data and dilutions please use the Excel spreadsheet templates provided with this protocol.
The following layouts are suggested for TNF-α neutralisation assays. Similar plate layouts should be adopted for ADCC and binding assays.

Example Layout A – Dilutions down the plate
Plate 1. Sample Layout:

	1	2	3	4	5	6	7	8	9	10	11	12
A	blank	IH	IH	A	A	B	B	C	C	D	D	TNFα
B	blank	IH	IH	A	A	B	B	C	C	D	D	TNFα
C	blank	IH	IH	A	A	B	B	C	C	D	D	TNFα
D	blank	IH	IH	A	A	B	B	C	C	D	D	TNFα
E	blank	IH	IH	A	A	B	B	C	C	D	D	TNFα
F	blank	IH	IH	A	A	B	B	C	C	D	D	TNFα
G	blank	IH	IH	A	A	B	B	C	C	D	D	TNFα
H	blank	IH	IH	A	A	B	B	C	C	D	D	TNFα

Plate 2. Sample Layout:

	1	2	3	4	5	6	7	8	9	10	11	12
A	blank	B	B	C	C	D	D	IH	IH	A	A	TNFα
B	blank	B	B	C	C	D	D	IH	IH	A	A	TNFα
C	blank	B	B	C	C	D	D	IH	IH	A	A	TNFα
D	blank	B	B	C	C	D	D	IH	IH	A	A	TNFα
E	blank	B	B	C	C	D	D	IH	IH	A	A	TNFα
F	blank	B	B	C	C	D	D	IH	IH	A	A	TNFα
G	blank	B	B	C	C	D	D	IH	IH	A	A	TNFα
H	blank	B	B	C	C	D	D	IH	IH	A	A	TNFα

Plate 3. Sample Layout:

	1	2	3	4	5	6	7	8	9	10	11	12
A	blank	C	C	IH	IH	A	A	B	B	D	D	TNFα
B	blank	C	C	IH	IH	A	A	B	B	D	D	TNFα
C	blank	C	C	IH	IH	A	A	B	B	D	D	TNFα
D	blank	C	C	IH	IH	A	A	B	B	D	D	TNFα
E	blank	C	C	IH	IH	A	A	B	B	D	D	TNFα
F	blank	C	C	IH	IH	A	A	B	B	D	D	TNFα
G	blank	C	C	IH	IH	A	A	B	B	D	D	TNFα
H	blank	C	C	IH	IH	A	A	B	B	D	D	TNFα

IH=In-house Standard; Blank=Cells only Control Wells; TNFα= cells + TNFα Control Wells

Example Layout B – Dilutions across the plate

Plate 1. Sample Layout:

	1	2	3	4	5	6	7	8	9	10	11	12
A	IH	IH	IH	IH	IH	IH	IH	IH	IH	IH	IH	blank
B	IH	IH	IH	IH	IH	IH	IH	IH	IH	IH	IH	blank
C	A	A	A	A	A	A	A	A	A	A	A	blank
D	A	A	A	A	A	A	A	A	A	A	A	blank
E	B	B	B	B	B	B	B	B	B	B	B	TNFα
F	B	B	B	B	B	B	B	B	B	B	B	TNFα
G	C	C	C	C	C	C	C	C	C	C	C	TNFα
H	C	C	C	C	C	C	C	C	C	C	C	TNFα

Plate 2. Sample Layout:

	1	2	3	4	5	6	7	8	9	10	11	12
A	B	B	B	B	B	B	B	B	B	B	B	blank
B	B	B	B	B	B	B	B	B	B	B	B	blank
C	C	C	C	C	C	C	C	C	C	C	C	blank
D	C	C	C	C	C	C	C	C	C	C	C	blank
E	IH	IH	IH	IH	IH	IH	IH	IH	IH	IH	IH	TNFα
F	IH	IH	IH	IH	IH	IH	IH	IH	IH	IH	IH	TNFα
G	A	A	A	A	A	A	A	A	A	A	A	TNFα
H	A	A	A	A	A	A	A	A	A	A	A	TNFα

Plate 3. Sample Layout:

	1	2	3	4	5	6	7	8	9	10	11	12
A	C	C	C	C	C	C	C	C	C	C	C	blank
B	C	C	C	C	C	C	C	C	C	C	C	blank
C	IH	IH	IH	IH	IH	IH	IH	IH	IH	IH	IH	blank
D	IH	IH	IH	IH	IH	IH	IH	IH	IH	IH	IH	blank
E	A	A	A	A	A	A	A	A	A	A	A	TNFα
F	A	A	A	A	A	A	A	A	A	A	A	TNFα
G	B	B	B	B	B	B	B	B	B	B	B	TNFα
H	B	B	B	B	B	B	B	B	B	B	B	TNFα

IH=In-house standard; Blank=Cells only Control Wells; TNFα= cells + TNFα Control Wells

Example Layout B (Plate 4 only) FOR PARTICIPANTS ASSAYING SAMPLE D – Dilutions across the plate

Example sample layouts (please vary the order of IH, A, D on the plate in subsequent assays as shown below):

Assay 1: Plate 4

	1	2	3	4	5	6	7	8	9	10	11	12
A												blank
B	IH	IH	IH	IH	IH	IH	IH	IH	IH	IH	IH	blank
C	IH	IH	IH	IH	IH	IH	IH	IH	IH	IH	IH	blank
D	A	A	A	A	A	A	A	A	A	A	A	blank
E	A	A	A	A	A	A	A	A	A	A	A	TNFα
F	D	D	D	D	D	D	D	D	D	D	D	TNFα
G	D	D	D	D	D	D	D	D	D	D	D	TNFα
H												TNFα

Assay 2: Plate 4

	1	2	3	4	5	6	7	8	9	10	11	12
A												blank
B	A	A	A	A	A	A	A	A	A	A	A	blank
C	A	A	A	A	A	A	A	A	A	A	A	blank
D	D	D	D	D	D	D	D	D	D	D	D	blank
E	D	D	D	D	D	D	D	D	D	D	D	TNFα
F	IH	IH	IH	IH	IH	IH	IH	IH	IH	IH	IH	TNFα
G	IH	IH	IH	IH	IH	IH	IH	IH	IH	IH	IH	TNFα
H												TNFα

Assay 3: Plate 4

	1	2	3	4	5	6	7	8	9	10	11	12
A												blank
B	D	D	D	D	D	D	D	D	D	D	D	blank
C	D	D	D	D	D	D	D	D	D	D	D	blank
D	IH	IH	IH	IH	IH	IH	IH	IH	IH	IH	IH	blank
E	IH	IH	IH	IH	IH	IH	IH	IH	IH	IH	IH	TNFα
F	A	A	A	A	A	A	A	A	A	A	A	TNFα
G	A	A	A	A	A	A	A	A	A	A	A	TNFα
H												TNFα

IH=In-house standard; Blank=Cells only Control Wells; TNFα= cells + TNFα Control Wells

PROTOCOL STUDY B : WHO COLLABORATIVE STUDY FOR THE 1st International Standard (IS) for adalimumab therapeutic drug monitoring
Determination of adalimumab concentrations spiked into normal human serum.

1) BACKGROUND

Recombinant therapeutic monoclonal antibodies are an expanding therapeutic product class with numerous monoclonal antibodies approved for use in humans and many more in clinical development. The patents on some of the earlier licensed monoclonal antibodies have expired resulting in the development/approval of biosimilar medicines, thereby widening the market for these products and increasing patient accessibility. The WHO have recognised a global need for standardisation of biotechnology products following requests for advice on appropriate control measures to ensure safety, quality and efficacy (WHO Technical Report Series, 56th Report, 941: 12-13, 2007). Consequently, WHO international standards for Rituximab (for bioactivity) and Infliximab (for bioactivity and therapeutic drug monitoring) were established in October 2017. Currently, we are developing an international standard for adalimumab for determination of biological activity. As part of this effort, we are also investigating the suitability of a candidate lyophilised adalimumab preparation in assays for therapeutic drug monitoring to facilitate global harmonisation.

2) AIMS OF THE STUDY

a) To assess the suitability of the candidate adalimumab preparation to serve as 1st WHO IS for adalimumab therapeutic drug monitoring (TDM) using different assays/platforms.

b) To compare the ampouled adalimumab preparation with kit standards or calibrators and in-house standards where these are available.

3) MATERIALS INCLUDED IN THE STUDY

Participants will be sent:

a) 4 ampoules of candidate adalimumab preparation coded by letter A for testing in adalimumab drug monitoring assays. For the purpose of this study please assume that each ampoule of candidate material contains 50 µg of adalimumab.

b) One or two sets (depending on volume needed) of 24 normal human serum samples spiked with adalimumab, labelled 1-24. Each of these samples contains adalimumab spiked at a concentration between 0 and 15 µg/ml.

4) RECONSTITUTION AND STORAGE OF PREPARATIONS

Prior to initiating the study, please read the Instructions for Use provided. Please note the statements regarding safety and that these samples are not for human use.
The lyophilized preparation and samples provided should be stored at -20ºC or below until used.

a) The adalimumab preparation A should be reconstituted before use with 1ml of sterile distilled water. Mix GENTLY and ensure contents are completely dissolved prior to use. Use 1 ampoule per independent assay/run.

b) At least 24-48 hours prior to the first assay, serum samples 1-24 should be aliquoted in equal volumes as needed for assays. All aliquots should be stored at -20ºC or below until required for inclusion in assay. Thaw and use 1 aliquot per independent assay/run. **This is important to ensure that samples are treated consistently for each independent assay/run.**

5) ASSAY DESIGN

Participants are requested to use their own procedure (in-house assay or commercial kit) for evaluating the collaborative study samples. Please follow steps a)-d) as listed below

a) Participants may perform a pilot assay to ensure that preparation A is diluted such that the concentration range falls within the working range of the assay. An extra ampoule of adalimumab preparation A has been provided for this purpose.

b) Once suitability of the assay is confirmed, perform **three** independent assays including preparation A, kit standard, in-house standard (if available) and serum samples 1-24 using the most appropriate dilutions. All 24 serum samples should be analysed on the same plate along with all the standards, preferably in duplicate. If this is a problem, please contact us as indicated in 8).

Use a freshly reconstituted ampoule of preparation A in each of the independent assays/runs.
Use a newly thawed aliquot of serum samples 1-24 in each of the independent assays/runs.
An assay/run is considered independent if carried out on different days/occasions.
The serum samples 1-24 should be diluted in the appropriate matrix diluent in accordance with the participants' assay protocols.

c) **For each independent assay each plate should contain a serial dilution (i.e. a dose-response curve) for kit standard, for in-house standard (if available) and for the candidate preparation A, preferably in duplicate.**

d) Include appropriate controls as per your protocols (e.g. QC)

e) For assays that use a 96 well plate format a suggested layout is given in appendix 2.

6) INFORMATION TO BE SUPPLIED AND PRESENTATION OF RESULTS

We have included an assay method sheet in appendix 1 and an example layout for microtiter plate format in appendix 2.
Please answer the questions on the assay method sheets supplied in appendix 1 to provide all the details requested for EACH assay AND please record all concentrations and dilutions of assay components for EACH assay replicate on the results spreadsheet.

Information required for each assay is to include:

a) Assay, and how it was performed

b) Details of the kit standards, in-house standard (if available) and QC and how it was diluted

c) Details of how Candidate sample A was diluted and titrated.

d) Details of how the serum samples 1-24 were diluted.

e) Please PROVIDE ALL RAW DATA (microtiter plate readouts e.g. O.D. or Luminescence Units etc.) as direct analysis of the raw data provided by the assays permits data from all participants to be handled consistently, as far as possible.

f) Although NIBSC will calculate adalimumab levels from the raw data provided, participants are requested (if possible) to **calculate the adalimumab levels for each of the serum samples 1-24 using their own methods, relative to kit standard, relative to in-house standard (if available) AND relative to candidate preparation A.** Please provide information of methods used for calculating results.

7) DATA SUBMISSION

Please return all raw data, assay method sheets and plate layouts electronically. **Deadline for data submission – Please aim to submit all electronic files by Monday 14th January 2019 at the latest.**

8) REPORTING OF RESULTS

A draft report will be prepared and circulated to all participants for comments prior to submission to the Expert Committee on Biological Standardization of WHO. In the report, participating laboratories will be identified by a laboratory number only and any request to treat information in confidence will be respected.
For submission of study results and for any further information please email:

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9) CONDITIONS OF PARTICIPATION

Participants in the collaborative study should note the following conditions:

a) They participate in the study with the understanding that they agree not to publish or circulate information concerning the materials sent to them without the prior consent of the NIBSC study organisers.
b) The participants results may be shared anonymously with not-for-profit public health bodies in the interests of global harmonisation.
c) It is normal practice to acknowledge participants as contributors of data rather than co-authors in publications describing the establishment of the standard.
d) Individual participants' data will be reported and coded blind to other participants during the preparation of the study report and supporting publications.
e) All participants will receive a copy of the study report and proposed conclusions for comment before final establishment of the international standard

APPENDIX 1 – Participants Study Details and Assay Method

Laboratory identification:
Laboratory standard(s) information:

What is the nature of your kit standard?
What is the nature of your in-house standard?
How did you obtain the standard?
Bought : Source:

Assay methodology information

PLEASE NOTE: If more than one assay type is performed then please provide the details for each assay type.

a) Briefly outline the assay methods used or provide full protocol:

b) Details of the ampoule reconstitution, dilution steps and dilution buffer used:

A:
Samples 1-24:
Kit standard:
In-house standard:
QC:
Details of any blanks/negative controls if applicable:

c) Dose range of adalimumab kit standard used in the assay:

d) Dose range of adalimumab in-house standard used in the assay:

e) Dose range of adalimumab candidate A used in the assay:

f) Details of QC and/or positive controls:

g) Details of the readout of the assay (e.g. Absorbance, Luminescence) and the equipment used to obtain this readout:

h) Additional comments:

APPENDIX 2: Proposed plate layout

We suggest the following plate layout to indicate the assay design and the information that we would like on each plate used in the assay

	1	2	3	4	5	6	7	8	9	10	11	12
A	IH	IH	kit std	kit std	A	A	1	1	9	9	17	17
B	IH	IH	kit std	kit std	A	A	2	2	10	10	18	18
C	IH	IH	kit std	kit std	A	A	3	3	11	11	19	19
D	IH	IH	kit std	kit std	A	A	4	4	12	12	20	20
E	IH	IH	kit std	kit std	A	A	5	5	13	13	21	21
F	IH	IH	kit std	kit std	A	A	6	6	14	14	22	22
G	QC	QC	kit std	kit std	A	A	7	7	15	15	23	23
H	QC	QC	kit std	kit std	A	A	8	8	16	16	24	24

IH: in-house standard; Kit std: kit standards or calibrators

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WHO International Standard
1st International Standard for Adalimumab
NIBSC code: 17/236
Instructions for use
(Version 2.01, Dated)

1. INTENDED USE

The World Health Organization (WHO) Expert Committee on Biological standardization (ECBS) recognised the need for a reference standard to evaluate the performance of in vitro biological assays for adalimumab.

The preparation 17/236 was assessed in an international collaborative study (described in section 3), for in vitro biological activities of adalimumab. In addition, the standard was also assessed for use in therapeutic drug monitoring.

The standard is intended to support the calibration, characterisation and validation of assays used for assessing adalimumab and to support the establishment of in-house standards.

It should be noted that the unitage or mass content of the standard should not be used to define the specific activity of adalimumab products for regulatory purposes nor to describe product labelling or dosage requirements. Furthermore, the standard and its unitage is not intended to serve any regulatory role in defining biosimilarity, and should not be inferred as serving this purpose.

2. CAUTION

This preparation is not for administration to humans or animals in the human food chain

The preparation contains material of human origin, and either the final product or the source materials, from which it is derived, have been tested and found negative for HBsAg, anti-HIV and HCV RNA. As with all materials of biological origin, this preparation should be regarded as potentially hazardous to health. It should be used and discarded according to your own laboratory's safety procedures. Such safety procedures should include the wearing of protective gloves and avoiding the generation of aerosols. Care should be exercised in opening ampoules or vials, to avoid cuts.

3. UNITAGE

The preparation has been assigned the following arbitrary unitage per ampoule:

500 international units (IU)* of tumor necrosis factor-alpha (TNF-alpha) neutralising activity.
500 IU of TNF-alpha binding activity.
500 IU of ADCC activity.
500 IU of CDC activity.

The use of a mass content of 50 micrograms of adalimumab per ampoule is recommended for therapeutic drug monitoring assays (see section 4).

*These units are independent of the amount of TNF-alpha used in various bioassays. For details regarding neutralising activity in terms of the 3rd IS for TNF-alpha (coded 12/154), see report referenced in section 9.

It should be noted that the neutralising activity may vary according to the assay format. Therefore, a relationship between the unitage of the WHO IS coded 17/236 and the activity assigned to in-house standards in the assay system in routine use should be established.

Users should also note that the biological activity of TNF-alpha is likely to vary between different suppliers and this should be controlled by use of an appropriate standard (e.g. WHO IS).

The adalimumab IS was tested in a multi-centre collaborative study involving 26 laboratories in 13 countries. Participants tested the IS using assays established in-house, and reported results for cytotoxicity, apoptosis, reporter gene, ADCC, CDC and binding assays (see reference in section 9, WHO/BS/).

4. CONTENTS

Country of origin of biological material: United Kingdom.
Each ampoule contains the residue after freeze-drying of 1.0 ml of a solution containing:

Adalimumab, approximately 50 micrograms
25mM tri-sodium citrate dihydrate
150mM sodium chloride
1.0% human serum albumin

The adalimumab protein was expressed in CHO cells.

5. STORAGE

Unopened ampoules should be stored at -20ºC.
For economy of use, it is recommended that the solution be sub divided into aliquots and stored at -40ºC or below. Avoid repeated thawing/freezing.

Please note: because of the inherent stability of lyophilized material, NIBSC may ship these materials at ambient temperature.

6. DIRECTIONS FOR OPENING

DIN ampoules have an 'easy-open' coloured stress point, where the narrow ampoule stem joins the wider ampoule body.
Tap the ampoule gently to collect the material at the bottom (labeled) end. Ensure that the disposable ampoule safety breaker provided is pushed down on the stem of the ampoule and against the shoulder of the ampoule body. Hold the body of the ampoule in one hand and the disposable ampoule breaker covering the ampoule stem between the thumb and first finger of the other hand. Apply a bending force to open the ampoule at the coloured stress point, primarily using the hand holding the plastic collar.
Care should be taken to avoid cuts and projectile glass fragments that might enter the eyes, for example, by the use of suitable gloves and an eye shield. Take care that no material is lost from the ampoule and no glass falls into the ampoule. Within the ampoule is dry nitrogen gas at slightly less than atmospheric pressure. A new disposable ampoule breaker is provided with each DIN ampoule.

7. USE OF MATERIAL

No attempt should be made to weigh out any portion of the freeze-dried material prior to reconstitution

Dissolve the total contents of the ampoule in 1.0ml of sterile distilled water. This solution will contain adalimumab at a concentration of 500 IU/ml. Use carrier protein where extensive dilution is required.

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8. STABILITY

Reference materials are held at NIBSC within assured, temperature-controlled storage facilities and they should be stored on receipt as indicated on the label. It is the policy of WHO not to assign an expiry date to their international reference materials. Accelerated degradation studies have indicated that this material is suitably stable, when stored at -20°C or below, for the assigned values to remain valid until the material is withdrawn or replaced. These studies have also shown that the material is suitably stable for shipment at ambient temperature without any effect on the assigned values. Once reconstituted, diluted or aliquoted, users should determine the stability of the material according to their own method of preparation, storage and use. Users who have data supporting any deterioration in the characteristics of any reference preparation are encouraged to contact NIBSC.

9. REFERENCES

This standard was produced under WHO guidelines cited in the WHO Technical Reports Series, No. 932, 2006, Annex 2.

Report on a Collaborative Study for Proposed 1st International Standard for Adalimumab WHO/BS/

For details of the WHO endorsement of the standard and recommendations for its use in therapeutic drug monitoring see WHO Technical Reports Series,

10. ACKNOWLEDGEMENTS

We are thankful to Abbvie and Samsung for their generous donations of the adalimumab materials used in the collaborative study. We are grateful to all participants of the collaborative study for their contribution in evaluating the candidate preparations.

11. FURTHER INFORMATION

Further information can be obtained as follows;
This material: enquiries@nibsc.org
WHO Biological Standards:
http://www.who.int/biologicals/en/
JCTLM Higher order reference materials:
http://www.bipm.org/en/committees/jc/jctlm/
Derivation of International Units:
http://www.nibsc.org/standardisation/international_standards.aspx
Ordering standards from NIBSC:
http://www.nibsc.org/products/ordering.aspx
NIBSC Terms & Conditions:
http://www.nibsc.org/terms_and_conditions.aspx

12. CUSTOMER FEEDBACK

Customers are encouraged to provide feedback on the suitability or use of the material provided or other aspects of our service. Please send any comments to enquiries@nibsc.org

13. CITATION

In all publications, including data sheets, in which this material is referenced, it is important that the preparation's title, its status, the NIBSC code number, and the name and address of NIBSC are cited and cited correctly.

14. MATERIAL SAFETY SHEET

Physical and Chemical properties		
Classification in accordance with Directive 2000/54/EC, Regulation (EC) No 1272/2008: Not applicable or no Physical appearance: Freeze dried powder	Corrosive:	No
Stable: Yes	Oxidising:	No
Hygroscopic: No	Irritant:	No
Flammable: No	Handling:	See caution, Section 2
Other (specify):	Contains material of human origin	
Toxicological properties		
Effects of inhalation:	Not established, avoid inhalation	
Effects of ingestion:	Not established, avoid ingestion	
Effects of skin absorption:	Not established, avoid contact with skin	
Suggested First Aid		
Inhalation:	Seek medical advice	
Ingestion:	Seek medical advice	
Contact with eyes: Wash with copious amounts of water. Seek medical advice		
Contact with skin: Wash thoroughly with water.		
Action on Spillage and Method of Disposal		
Spillage of ampoule contents should be taken up with absorbent material wetted with an appropriate disinfectant. Rinse area with an appropriate disinfectant followed by water. Absorbent materials used to treat spillage should be treated as biological waste.		

15. LIABILITY AND LOSS

In the event that this document is translated into another language, the English language version shall prevail in the event of any inconsistencies between the documents.

Unless expressly stated otherwise by NIBSC, NIBSC's Standard Terms and Conditions for the Supply of Materials (available at http://www.nibsc.org/About_Us/Terms_and_Conditions.aspx or upon request by the Recipient) ("Conditions") apply to the exclusion of all other terms and are hereby incorporated into this document by reference. The Recipient's attention is drawn in particular to the provisions of clause 11 of the Conditions.

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16. INFORMATION FOR CUSTOMS USE ONLY

Country of origin for customs purposes*: United Kingdom * Defined as the country where the goods have been produced and/or sufficiently processed to be classed as originating from the country of supply, for example a change of state such as freeze-drying.
Net weight: 4.6g
Toxicity Statement: Toxicity not assessed
Veterinary certificate or other statement if applicable. **Attached**: No

17. CERTIFICATE OF ANALYSIS

NIBSC does not provide a Certificate of Analysis for WHO Biological Reference Materials because they are internationally recognised primary reference materials fully described in the instructions for use. The reference materials are established according to the WHO Recommendations for the preparation, characterization and establishment of international and other biological reference standards http://www.who.int/bloodproducts/publications/TRS932Annex2_Inter_biolefstandardsrev2004.pdf (revised 2004). They are officially endorsed by the WHO Expert Committee on Biological Standardization (ECBS) based on the report of the international collaborative study which established their suitability for the intended use.

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