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Proposed WHO International Biological Reference Preparations for Adalimumab anti-drug antibodies

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NOTE:
This document has been prepared for the purpose of inviting comments and suggestions on the proposal(s) contained therein, Written comments on the proposal(s) MUST be received in English by 19 September 2025 and should be addressed to:

Technical Standards and Specifications
Department of Health Products Policy and Standards
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Comments may also be submitted electronically to **Dr Ivana Knezevic** at email: knezevici@who.int.

The distribution of this document is intended to provide information to a broad audience of potential stakeholders and to improve the transparency of the consultation process. Following consideration of all comments received, the proposal(s) will then be considered by the WHO Expert Committee on Biological Standardization (ECBS) prior to a final decision being made and published in the WHO Technical Report Series.

Summary

A panel of two human monoclonal antibodies and two chimeric antibodies against adalimumab with defined characteristics (varied isotypes, different binding characteristics but all neutralising) were formulated, lyophilized and assessed for their suitability as reference standard(s) in adalimumab antibody assays in an international study. Twenty-two laboratories using different assay platforms and a diverse range of adalimumab anti-drug antibody (ADA) binding and neutralisation assays participated in the multi-centre collaborative study. In addition to the lyophilized panel, several anti-adalimumab antibodies and serum samples were also tested by the participants. Study data demonstrated the suitability and utility of all the antibodies in the reference panel. A reduction in inter laboratory variability and consistency in estimates of ADA activity was achieved when antibody B was used in binding assays as a common standard for quantitating ADA activity for the different samples in comparison with in-house standards. For neutralizing activity, use of antibody A rather than B or the in-house standards was associated with less variability and more consistent estimates. The degree of harmonization, however, was largely dependent on the assay type, the sample and the laboratory.

Since results show that the tested lyophilized antibodies would be useful for adalimumab ADA assays, it is proposed that antibody B coded 19/266, with an arbitrarily assigned unitage of 50,000 IU/ampoule for binding activity serve as the 1st international standard for use in calibration, characterization and harmonization of binding assays while antibody A coded 19/264, with an arbitrarily assigned unitage of 50,000 IU/ampoule, serve as the 1st international standard for calibration of neutralizing antibody assays and for assay harmonization. It should be noted that the assigned IU for each standard is independent and should only be used to calibrate assays for the specific activity (binding or neutralization) it is intended for. The availability of these antibodies, in particular, antibody B, would facilitate comparison and harmonization of results across the different adalimumab ADA assays/platforms and, if implemented in practice along with testing of drug levels, support patient monitoring in the clinical setting for a better therapeutic outcome. In addition, it is proposed that both antibody C coded FS-007 and antibody D coded FS-008 serve as international reference reagents for use as performance indicators for detecting antibodies with fast dissociation. Antibody C would have utility in detecting low activity ADAs while D would serve to assess the ability of the assay to detect the bivalent IgG4 only and also help in assessing the sensitivity of the neutralizing antibody assays. However, no unitage is assigned to both antibodies C and D.

Responses from study participants

Responses were received from 91% (20/22) of the study participants. Feedback was generally positive with participants appreciating the significant amount of work and effort in developing the reference preparations. Eight participants had no comments on the report. Two participants requested a slight modification of text relating to the clinical relevance of drug-sensitive assays, another requested clarification on the characteristics of the assays, particularly surface plasmon resonance data and inclusion of text specifying that the IgG4 antibody D may not be suitable for testing the ability of assays to detect the monovalent IgG4 form (Fab arm exchanged), often found

in clinical samples. One participant was interested in data from a CombiStats file for an example dataset. All these comments have been addressed along with minor comments relating to typos and participant names.

Regarding the proposal, as expected there was a variation in response. Seventeen participants agreed with the proposal for the antibody preparations. One participant stated that antibodies C and D were not applicable; another felt that antibody D may not be suitable for testing the ability of assays to detect the monovalent IgG4 form but should be included based on its lower affinity while another participant did not comment on use of antibody A for neutralizing antibody assays since this assay had not been performed in their respective laboratory. In addition, this participant did not comment on the utility of antibody D. Overall, there was unanimous agreement for the use of 19/266 as a calibrant for binding assays and the units assigned. Nearly all (19/20) agreed with the use of 19/264 as a calibrant for neutralizing antibody assays and the units assigned. All responders supported the use of FS-007 while 95% (19/20) agreed with the use of FS-008, both with no units assigned to them.

Introduction

Targeted therapy with tumor necrosis factor-alpha (TNF-α) inhibitors has achieved success in the treatment of chronic immune conditions and inflammatory diseases. One of the concerns with TNF inhibitors, however, is the treatment failure seen in some patients due to primary non-response or the inability in initial responders to maintain a response (secondary failure) and/or infusion-related adverse events, including hypersensitivity reactions. In Crohn's disease, 10–30% of patients are non-responsive and up to 60% of patients lose response to anti-TNF therapy over time. The latter is attributed to the induction of anti-drug antibodies (ADA) and the complex inter-relationship with drug concentrations and disease activity (Roda et al 2016; Chanchlani et al 2024). Therapeutic drug monitoring (TDM) which measures ADA and drug levels is therefore considered valuable for optimal therapy of patients receiving chronic treatment.

Adalimumab (marketed as Humira®, Abbvie), a fully human IgG1 monoclonal antibody which binds to TNF with high affinity was approved initially for rheumatoid arthritis (2002 – FDA; 2003 - EMA) and later for other indications. Despite being the third TNF inhibitor to be approved, it has been highly successful in the clinic. In 2022, Humira® was the most frequently prescribed drug with global sales exceeding $21bn (https://www.globaldata.com). Until recently, the high costs of long-term treatment along with constrained health care budgets has restricted patient access to therapy. However, with the approval of multiple biosimilars (Kaur et al 2017; Lu et al 2021), there is a significant opportunity for increasing the uptake of adalimumab and transforming disease in patients with inflammatory disorders worldwide.

The problem of immunogenicity also occurs with adalimumab. In adalimumab-treated patients, ADA development is associated with low drug levels and loss of clinical efficacy depending on the magnitude of the immune response (Bartelds et al 2011; van Schouwenberg et al 2013; Jyssum et al 2024). ADAs primarily target the TNF binding region and neutralise bioactivity by preventing binding of the drug to its target thus reducing the drug's efficacy (van Schouwenberg et al 2013; van Schouwenberg et al 2014; van Schie et al 2015). Formation of immune complexes also leads

to drug clearance and potentially suboptimal dosing in patients (van Schie et al 2018; Murdaca et al 2019).

The reported frequency of ADAs varies between studies, from less than 5% to over 80% of treated patients (Gorovits et al 2018). Such variation is likely due to differences in the patient population - the genetic composition (presence of HLA DQ-A1*05), disease state/type, concomitant medication, dosing schedule, the follow-up period and sampling times (Sazanovs et al 2020; Adler et al 2024). Importantly, the type of ADA assay used and whether it measures free or total ADA also influences ADA detection (Gorovits et al 2018; Ruwaard et al 2019; van Strien et al 2023; Berger et al 2022; Pedersen et al 2022).

Increasingly, evidence showing the utility of ADA assays in treatment decisions is accumulating Jyssum et al 2024). Several professional societies (e.g., gastroenterology associations, EULAR) have recommended the use of TDM in clinical algorithms (Feuerstein et al 2017; Lamb et al 2019; Krieckaert et al 2022) but implementation varies based on the indication, the clinician(s) perspective and the economic costs related to the testing. Conflicting or non-comparable results due to differences in assays and/or reporting (e.g., mass units/arbitrary units) along with the lack of standardization has been noted (Kalden and Schulze-Koops 2017; Samaan et al 2018; Mehta and Manson 2020). Attempts towards harmonizing ADA assays by generating reference standards have achieved limited success (Gils et al 2014; van Schouwenburg et al 2016; Suzuki et al 2020). At the WHO level, progress was made with the establishment of the WHO international reference panel for Infliximab ADA (Wadhwa et al 2025) in 2022. In addition, WHO international standards (IS) for Infliximab, Adalimumab and Golimumab have been established (Metcalfe et al 2019; Wadhwa et al 2021; WHO (2024) BS/2024.2467) to facilitate the harmonization of drug monitoring assays used in clinical practice. Nevertheless, there still exists a large gap in standardizing TDM and ADA testing for use in clinical practice for other biotherapeutics.

Following on from previous work on the infliximab and erythropoietin ADA reference panels (Wadhwa et al 2016), we initiated developmental work towards standardization of adalimumab ADA assays. For this, a pair of human monoclonal antibodies (mAbs) against adalimumab with defined characteristics (both IgG1, neutralising and similar affinities) were obtained from ABIRISK (a consortium of academic institutions, EFPIA member companies and small and medium-sized enterprises funded by the Innovative Medicines Initiative program, EU). Another pair of mAbs, chimeric in nature (human-rat) were made available through a collaboration with National Institute of Health Sciences, NIHS (Kangawa, Japan). All four antibodies were lyophilized and tested in a multi-centre international collaborative study involving different laboratories using different assays to assess their suitability as potential reference standards/positive controls for adalimumab ADA assays.

This report describes the development of these reference standards, the study design and tests used, participant data, the applicability and the recommendations. It is anticipated that the panel will have utility in ADA monitoring assays in the healthcare setting as well as facilitate the immunogenicity assessment of emerging biosimilar medicines and support access to safe and effective medicines (Resolution WHA67.21, 2014).

This project was endorsed by the WHO Expert Committee on Biological Standardization in October 2016 as the proposed WHO International Standards (or reference panels) for antibodies for use in immunogenicity assessment of biotherapeutic products (WHO TRS, 2016).

Study Aim

The specific aims of this international collaborative study were to evaluate the panel of antibodies against adalimumab together with serum samples to:

1) Compare the antibodies across available methods and assess their suitability for use as performance indicators

2) Assign arbitrary unitage, if feasible, for each of the lyophilized preparations to enable calibration of local standards and for assay harmonization.

Materials and Processing

Two mAbs against adalimumab, ADA39 and ADA44, were kindly donated by the ABIRISK consortium, funded by the Innovative Medicines Initiative, EU (2012-2017). The characteristics of these human mAbs (expressed in CHO cells), originally isolated and cloned from memory B cells of an adalimumab treated patient using previously described procedure (Traggiai E et al 2004) are provided in Table 1. Additionally, two human and four chimeric mAbs, generously donated by collaborators at Sanquin (Amsterdam, Netherlands) and NIHS (Kangawa, Japan) respectively, and generated as described (van Schouwenburg et al 2014, 2016; Suzuki et al 2020) were also included in the study. A brief outline of the production method and methods used for characterization of the ABIRISK and NIHS antibodies is given in Appendix 1.

As per the procedures used for biological standards (WHO TRS 2006), pilot fills using different formulations were performed and materials tested in binding and neutralisation assays in-house for selection of a suitable formulation for lyophilization.

Formulations tested were:
A) 25mM Sodium citrate tribasic dihydrate, 150mM Sodium Chloride and 2% sucrose at pH 5.2
B) 10mM L-Glutamic acid, 4% Mannitol and 2% Sucrose with or without 0.01% Tween 20, both at pH 5.2

Results showed that both glutamic acid-based formulations showed similar binding profile and neutralisation activity as the bulk antibodies as opposed to the citrate-based formulations. However, since the formulation containing 0.01% Tween-20 was previously used for ADAs directed against infliximab and erythropoietin, it was also selected for lyophilization of the adalimumab mAbs

The final lyophilization of both antibodies was performed in-house using ECBS guidelines (WHO TRS, 2006). For this, buffers and excipients (final compositions as shown in Table 2), were prepared using nonpyrogenic water and depyrogenated glassware and were filtered using sterile nonpyrogenic filters (0.22μM Stericup filter system, Millipore, USA) where appropriate.

Characteristics of the lyophilised preparations

Table 2 provides information on the mAbs, 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 2, is calculated from the dilution of the bulk material of known protein mass content as provided by the donor. For all four preparations, a solution containing the mAb at a concentration of ~ 50μg/ml in the selected formulation was distributed in 1 ml aliquots into 5 ml ampoules. 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.

For each fill, a percentage of ampoules were assessed for certain parameters. The mean fill weights, the moisture content, measured by the coulometric Karl-Fischer method (Mitsubishi CA100) and the headspace oxygen content, determined by frequency modulated spectroscopy using the Lighthouse FMS-760 Instrument (Lighthouse Instruments, LLC) which is a measure of ampoule integrity, are reported in Table 3. Testing for microbial contamination using Total viable count method did not show any evidence of microbial contamination.

Other preparations and samples

In addition to the lyophilised preparations, coded A to D, 4 liquid mAb preparations coded R to U, each containing 10 μg/ml of mAb in 20% normal healthy serum (First Link Ltd, UK) were included in the study. Characteristics of these antibodies are briefly summarised in Table 4. A panel of 6 human serum samples (pools) from healthy control subjects (First Link Ltd, UK) and adalimumab-treated patients (with none or low levels of adalimumab), sourced from a UK hospital were also included (Appendix 2, Table 1). Appropriate ethical approval was sought and materials anonymised for use. The samples were stored at -40°C until despatch or use.

Participants

Twenty-four participants from fourteen countries (Canada, UK, Germany, Sweden, Belgium, France, Netherlands, Spain, Switzerland, USA, India, Japan, South Korea, New Zealand) were recruited to the study with samples dispatched in April'24. Two participants from India and Germany withdrew from the study. Participants represented 3 therapeutic product manufacturers, 1 contract research organisation, 3 national control agencies, 1 academic laboratory, 7 commercial kit manufacturers, 6 clinical diagnostic centres/hospital laboratories and 1 reagent supplier (Table 5).

Study Design and Assay Methods

Pre-study in-house testing: All materials were evaluated for binding activity using surface plasmon resonance (SPR) as well as bridging ELISA and ECL assays. Neutralizing activity was

also assessed in-house in different assay types using both cell-based and competitive ligand binding formats. Brief description of the methods used is given in Appendix 1.

Study Design: A survey prior to the study launch informed on the study design and indicated that a variety of assays (e.g., in-house assays, commercial kits) using different formats were in use. Most assays were 'free' ADA assays which detected ADAs not complexed with drug while others were 'total' ADA assays which incorporated a dissociation step to measure all ADA present. As expected, differences were also noted in terms of the sensitivity, assay range, positive control/standard (polyclonal, monoclonal, human/animal species) used, its unitage (mass units, arbitrary units etc), quality control samples used, sample treatment (e.g., complex dissociation step, dilution), diluent used and the number of samples that could be positioned on a single plate. Based on the participant feedback, a study protocol was designed to provide the flexibility required to accommodate laboratory differences in routine ADA testing.

Participating laboratories were sent 1 sample pack, consisting of 5 ampoules of samples A to D and adequate amounts of the liquid preparations as well as unknown serum samples for each assay type they intended to perform. Since only limited amounts of sera from treated patients could be procured, sera were pooled for the purposes of the study. Instructions on sample handling and storage were given in the study protocol and participants urged to use a freshly reconstituted lyophilised sample or a freshly thawed aliquot for each assay to ensure that samples were treated consistently for the study.

Participants were requested to test for binding and/or neutralising activity of the samples using their own in-house methods e.g., own proprietary kits, commercially purchased kits or methods developed in-house. Use of the matrix employed routinely for diluting in-house/kit standard or serum samples was also recommended. Participants were advised to conduct a pilot assay and test all samples in parallel with in-house standard(s) and quality control (QC) samples (to ensure optimal dose response curves and sample dilutions could be achieved) prior to performing assay runs for the study. Following selection of a suitable dose range and/or dilution(s), all participants were requested to:

1. Perform three independent assays.
2. In each assay, create and test dilution series of the lyophilized preparations (A-D) until the activity reaches the lower detection limit of the assay (see Appendix 1 for example).
3. Include in-house/kit standard dilution series along with QC samples as used in routine test, if available.
4. Create and test dilution series of each liquid preparation R-U, ideally including not less than six dilutions of each preparation in the linear portion of the dose-response curve.
5. Create and test dilution series of serum samples 1 to 6, ideally including at least five dilutions of each sample, bracketing the assay endpoint.

Participants were requested to report data for each tested sample based on their reporting practice for ADA data e.g., qualitative (antibody +ve/-ve) or quantitative (e.g., titer or ADA concentration in mass/ml or arbitrary units/ml) relative to in-house/kit standards and, if possible, relative to candidate preparations A and B (using their own analytical method) for each assay. Information on a) the in-house standard, b) QC samples, c) the method used to define the positive cut-off and d) the assay method and analytical method for determining ADA concentration was also requested.

Collaborative study testing: Participants tested study samples in a range of binding and neutralizing assays which are briefly described in Tables 6 and 7.

Statistical Methods

The estimated activities of coded study samples were calculated relative to sample A, sample B and/or in-house reference standard (IH). For the estimates calculated relative to samples A or B these samples were assigned a nominal content of 50 µg/ml. Estimates calculated relative to IH standards are reported by the participants in a variety of different units (µg/ml, AU/ml, titer etc). Data were analysed using a sigmoidal curve model or parallel line analysis with log transformed responses. All calculations were performed using the software program CombiStats (CombiStats v.1.1.1, EDQM). Model fit was assessed visually, and non-parallelism was assessed by calculation of the ratio of fitted slopes for the test and reference samples under consideration. The samples were concluded to be non-parallel when the slope ratio was outside of the range 0.67 – 1.50. Results from valid individual assays were combined to generate unweighted geometric means (GM) for each laboratory and these laboratory means were used to calculate overall unweighted geometric mean estimates. Variability between assays and laboratories has been expressed using geometric coefficients of variation (GCV = $\{10^{s}-1\}\times100\%$ where *s* is the standard deviation of the $\log_{10}$ transformed estimates). Due the likelihood of outliers or non-normality in the distributions of estimates within groups, a non-parametric quantification of inter-laboratory variability was also calculated as the Median Absolute Deviation (MAD) of log transformed estimates, which was then anti-logged (i.e. 10^{MAD}) in order to show the 'average' fold change in laboratory GM estimates from the overall median estimate for each sample.

Stability Analyses

All stability studies were performed at MHRA.

Accelerated thermal degradation (ATD) study

Samples of the lyophilised preparations 19/264 and 19/266 were stored at elevated temperatures (4°C, 20°C, 37°C and 45°C) for over 3 and 4 years (38 months for 19/264; 54 months for 19/266) while those for FS-007 and FS-008 were stored for 14 months. All these stored samples were tested at MHRA with those stored at -70°C and at the recommended storage temperature of -20°C using an ECL assay for binding and neutralization activity. The assays were analysed as described for the main collaborative study, except the potencies of the samples stored at different temperatures were calculated relative to the -20°C samples.

Stability after reconstitution

Samples of the lyophilised preparations 19/264 and 19/266 were reconstituted and stored at 4°C and at 20°C for 24 hours or over 1 week. The reconstitutions were timed to allow all samples to be assayed concurrently with a freshly reconstituted sample using an ECL assay for binding activity.

The assays were analysed as described for the main collaborative study data. The potencies of all samples were calculated relative to fresh samples.

Stability on freeze-thaw

Samples of the lyophilised preparations 19/264 and 19/266 were reconstituted and subjected to a series of freeze-thaw cycles (up to 4). They were then assayed concurrently with a freshly reconstituted ampoule in an ECL for binding activity. The potencies of each freeze-thaw cycle were calculated relative to fresh samples.

Results

Antibody Characteristics

Information on characteristics of the different mAbs included in the study was provided by the different collaborators (Tables 1, 4). It was noted that the antibody characterization methods varied among the different laboratories precluding a direct comparison of the characteristics of the different mAbs. Therefore, the characteristics of the different mAbs were further evaluated at the MHRA for the purpose of guiding our selection of the antibodies for the study.

The results of a typical binding experiment using SPR on Biacore T200 instrument are shown in Figure 1a. As evident, mAb A shows the highest binding followed by B and C while other mAbs display moderate binding with mAb D exhibiting the lowest binding of all.

Table 8 provides association and dissociation data of the different mAbs generated from single cycle kinetic experiments using SPR and indicates the ranking of the different mAbs. The sensorgrams in Figure 1b illustrate the binding and dissociation profile of the different antibodies and provide a comparison of the behaviour of the antibodies. It should be noted that the results are generated from solid-phase binding and do not reflect the dynamics of the antigen-antibody interaction in solution phase which would be more representative of the affinity *in vivo* as solid-phase binding can be influenced by avidity effects and surface immobilization chemistry.

Both mAbs A and B show high binding to adalimumab and slow dissociation with slight differences noted. mAb A tends to associate and dissociate slightly faster than B as evident from Figure 1b and the kinetics data (Table 8). The mAbs C and D show moderate binding as displayed in the sensorgram, but their fast dissociation is distinctly different from other mAbs in particular A and B. In essence, this binding profile of C and D results in a low affinity overall in comparison with other mAbs assessed. In contrast, mAb B appears to have the highest affinity for adalimumab of all the mAbs tested followed by mAb A (Table 8). Other mAbs coded R-U can be categorised as moderate based on their affinities and binding pattern. It is clear that mAbs A, B and R have similar affinity (even though the association and dissociation rates for R are more akin to mAb B than A) but the binding profile of R appears to be fairly different to both A and B as illustrated in Figure 1a. mAb U has similar ka as B, R, S, T and D but unlike the slowly dissociating B and R, it dissociates fast akin to A resulting in a lower affinity than A, B and R. Both mAbs S and T show

similar ka (which is also seen for other mAbs) and kd values and overall affinity but their kd is distinctly different from the other mAbs tested.

For all the mAbs, binding using a bridging ELISA and ECL as well as neutralization by the competitive ligand binding (non-cell-based) assay was also assessed. A and B showed the highest binding in the ELISA (Figure 2) but this was not the case in the ECL assay (data not shown) where B showed higher binding than A. In both binding assays, mAb D was comparable in activity to A and B while C showed the lowest binding. A and B were most potent in terms of neutralization while D was the least potent (Figure 2).

In addition, for antibodies A and B, reactivity with three different biosimilar products, Hulio, Hyrimoz and Amgevita was evaluated in an ECL assay. The binding profile of the biosimilars tested was comparable to that seen with the innovator product, Humira (Figure 3).

Participant Data

Twenty-two laboratories contributed data to the study. Each participating laboratory has been assigned a code number allocated at random, and not necessarily representing the order of listing in Table 5 to retain confidentiality in the report. In cases where the same laboratory has returned two sets of data from two different methods, data has been analysed separately for each method as if from different laboratories and given a numerical code followed by a suffix such as 1a, 1b, 1c.

Each participant performed at least one assay method and contributed to a total of 28 laboratory/method combinations (23 binding assays and 5 neutralization assays). All samples were tested using serial dilutions in different laboratories/assays; the only exception was laboratory 14b which tested all serum samples at a single dilution in the neutralization assay. All samples (mAbs, sera) were tested in a minimum of 3 assays, except for laboratory 12 which did not test the serum samples.

A summary of the assay methods used in the study along with a brief overview of the procedure is given in Tables 6 and 7. Study participants mainly performed binding assays (n=23) which are commonly used for screening for antibodies although neutralisation assays were also conducted by a few participants (n=5). Most assays measured ‘free’ antibodies but assays in labs 1, 11, 13, 14, 17 and 19 measured ‘total’ antibodies. In clinical laboratories, commercially available kits were mainly used although in rare instances, in-house assays were also employed. As expected, the types of binding assays, varied from the simple ELISA (n=11) and the commonly used electrochemiluminescence (ECL, n=7) or the bead-based chemiluminescence assay (n=2), all using a bridging format to the radioimmunoassay type approach, which is rarely used now and performed in a single laboratory. In addition, the homogeneous mobility shift assay (HMSA) was also employed along with the lateral flow (LF) assay which is offered as point-of-care, both only in a single laboratory. For neutralisation, four laboratories performed non-cell-based competitive ligand binding (CLBA) ELISA or ECL assays while one laboratory performed a cell-based reporter-gene bioassay (commercial assay).

Reporting practice varied among laboratories. In rare cases, titers were reported (as per the norm for regulatory approval) but in most instances, arbitrary units (AU/ml) or mass units (ng/ml) were used for reporting results relative to the in-house standard.

Lyophilised preparations, A - D

All participants tested the mAb preparations A-D on each plate. Dose-response curves in different assays were achieved in all assays but A and B showed good and consistent sigmoidal curves across all assay platforms in comparison with C and D. Consequently, statistical analysis and calculation of estimates have been performed relative to A and B.

Parallelism

Prior to estimating concentrations of activity in the samples, the parallelism of two samples tested at serial dilutions was assessed using the ratio of their fitted slopes as calculated by CombiStats with a value of 1.0 indicating perfect parallelism. For analyses using mAbs A and B as reference, the proportions of slope-ratios within various ranges are summarised in Table 9 for the different samples and assay types while those for C, D and IH standard are in Appendix 3 Table 1. Taking all assay types into account, sample B generally demonstrated a higher degree of parallelism with the other study samples, with 72% of assay type / sample combinations shown in Table 9 having a higher percentage in the 0.80-1.25 range for analyses relative to sample B when compared to analyses relative to sample A. However, the degree of parallelism is clearly dependent on assay type and sample. In general, ECL assays demonstrated good parallelism across all samples and laboratories, as indicated by the high percentage (~87%) of values in the 0.80-1.25 range, regardless of the standard used. For the ELISA assays parallelism was more sample dependent with, for example, sample C and serum 3 generally showing poor parallelism with both samples A and B.

Based on varying degree of parallelism, for further analysis (comparative analysis of the calculated concentrations and determination of the GM, %GCV), all estimates were calculated from cases where the slope-ratio was within 0.67-1.50. All other cases, even those concluded as ADA +ve, were excluded based on an unacceptable level of non-parallelism.

Reactivity and estimates of activity calculated relative to A, B and in-house standard

While ADA assays used for regulatory approval of biotherapeutics are semi-quantitative and estimates of ADA levels not derived from use of a standard, the ones used in the clinical setting are either qualitative and assess whether sample is ADA positive/ADA negative or estimate ADA levels based on an in-house standard included in the assay. So, based on the study aims, we evaluated the ADA positivity of the study samples and then quantified ADA levels using a ‘common’ standard or the participant’s in-house (IH) standard.

Data from the analysis of results for binding (n=23) and neutralisation assays (n=5) for the mAbs and serum samples shown in Tables 10-15 and Figures 4-7 is summarized briefly below. The mAbs are ranked from high to low based on the geometric mean estimates from ELISAs calculated versus IH/kit standard. Detailed results from the individual laboratories are in Appendix 3, Table 2. Most

assays measured ‘free’ antibodies but assays in labs 1, 11, 13, 14, 17 and 19 measured ‘total’ antibodies. A few laboratories reported activity in AU/ml and in rare cases even in titers relative to the in-house/kit standard but comparisons were only possible for data generated from assays where laboratories reported their results in µg/ml.

mAb preparations:

All mAb preparations were ADA positive in six of the seven binding assay platforms employed in the study, however, differences in reactivity were noted between assay platforms and even among assays using the same assay platform. All bridging ELISAs (n=11), ECL assays (n=7), the CLIA (n=2), the LF (n=1) and the HMSA (n=1) used by different participants demonstrated positivity for the presence of ADA in the different mAbs. However, the radioimmunoassay used by a single laboratory failed to detect mAb coded S. In total, 22 of the 23 assays contributed to the study detected all mAb samples.

As shown in Appendix 3, Table 2, the immunoreactivity of the mAb preparations varied among different assay platforms. For laboratories reporting results in µg/ml, such variation resulted in wide differences in estimates for ADA levels for some mAbs among different platforms when calculated relative to the in-house/kit standard. For example, the range for mAb B varied from 28.28-973.57 in ELISAs (n=7) and 10.87-210.79 in ECL assays (n=7) to 301.20-421.43 in other binding assays (n=3), Table 11. mAb S showed the highest activity in the ECL assay with range of estimates relative to in-house standards of 3.87-114.84 in ECL (n=6) as opposed to 0.75-10.13 and 0.99-3.38 in ELISAs (n=7) and other binding assays respectively, Tables 11-13. Differences were also observed among assays using the same assay platform. This was particularly evident for ELISAs where extremely high values were noted for mAbs A and B in some assays (e.g., those in laboratories 5a and 10).

Use of A as a common standard only marginally reduced the range in calculated ADA levels for some mAbs in comparison with the IH standard. For ELISAs, with the exception of mAbs B and D (B: 28.28-973.57 and 46.25-462.36; D: 2.60-74.52 and 1.46-43.12 for estimates calculated relative to IH and A respectively), the disparity between estimates remained the same as seen with the IH standard or worsened as seen for mAbs U and R. For ECL assays, calculated estimates were generally higher with A compared with IH for all mAbs. For other binding assays, estimates were lower for all mAbs relative to A compared to those using IH and the range of estimates was narrowed for 6 mAbs (B, C, D, S, T, U) with A when compared to IH in the limited number of assays (n=3 labs reporting in µg/ml) Tables 10-14.

Variability between laboratories has been quantified using both geometric coefficients of variation (%GCV) and non-parametric Median Absolute Deviation (MAD) values. The analysis of the GCV by assay type showed that inter-laboratory was high for laboratories reporting results relative to IH standards in µg/ml, with median values of 298% and 196% respectively for estimates of ADA levels calculated using ELISA and ECL assays respectively (Tables 11-12). For ELISAs, these high GCV values increased for most mAbs (except C, T) when calculated relative to A, although

a modest reduction in overall median GCV to 225% was seen, as shown in Table 11b. In ECL assays, a reduction in GCV was seen for all mAbs (except R) and the overall median GCV reduced to 94% (Table 12b). Corresponding overall reductions in 10^{MAD} when using A as standard were from 1.72 to 1.45 for ELISA and from 1.95 to 1.35 for ECL assays (Tables 11b, 12b). For the other binding assays, inter-laboratory variability was lower for estimates relative to the IH standards for the majority of mAbs when compared to estimates relative to A (Table 13).

When B was used as the common standard (as opposed to A), the disparity in ADA estimates observed with the IH standard was generally more reduced for the different mAbs and also led to more consistent values across different assays/platforms in several cases. As shown in Appendix 3 Table 2, generally, estimates from ELISAs of all laboratories (laboratory 7 excluded) are quite consistent for all mAbs (except for sample D in laboratories 8a and 9) indicating harmonization with use of B. Such consistency in values with B as the standard was also observed across different assays/platforms. For example, in ELISAs, sample A relative to IH standard gave a range of 3.06-1036.53 which was reduced to 5.41-54.05 relative to B and concurs to some extent with the range of 3.98-23.49 and 36.51-62.09 obtained with B in ECL and other binding assays respectively Tables 10-13. Reductions in GCV values were observed for all samples for both ELISA and ECL assays, except for samples R and U by ELISA, with overall median GCV values reducing from 298% to 133% and 196% to 73% for ELISA and ECL assays respectively, a larger reduction than observed when using A as standard. Corresponding overall reductions in 10^{MAD} when using B as standard were from 1.72 to 1.53 for ELISA and from 1.95 to 1.14 for ECL assays. Values of 10^{MAD} were <2 for all mAbs when using sample B as standard in ELISA and ECL assays (Table 12a). Furthermore, for mAbs A, R, S, T and U, a similar GM range was seen when using B as standard across assays of different platforms – ELISAs and other binding assays (CLIAs - laboratories 5b, 20; HMSA -19a; LF- 18; RIA-21) albeit there was a tendency in the HMSA assay to provide higher values for the different mAbs than other assays within the same category.

Taking all data into account, the use of B reduces the spread of estimates for the mAbs across different ECL and ELISAs assays and indicates the advantage of using a common standard, while for the other binding assays, A and B appear to perform similarly. Overall, data from binding assays shows that B is a better choice for harmonizing estimates and for reducing variability across different assays/platforms.

For neutralizing activity, samples are often reported as positive or negative with titers determined in some instances. This approach was used by participants to report data relative to their IH standard. All mAbs tested positive for neutralizing activity across the three assay types, namely the CLBA-ECL (n=2), CLBA-ELISA (n=2) and the cell-based reporter gene assay (n=1). Notably, only one CLBA-ELISA detected neutralizing activity in all mAbs while another laboratory's assay did not detect neutralization in mAb coded D in all three tests conducted (Table 15). Estimates of activity were fairly similar for all mAbs (except B, C) across the different assays relative to A but this was not the case when using B as the standard as slightly higher values were calculated for a single assay (laboratory 13b) compared with other assays for all mAbs. GCV and 10^{MAD} values were lower with A than B in most instances (Tables 10c, 15). Overall inter-laboratory variability was lower with A than B, so A appears to perform better than B with regards to harmonizing estimates from these assays.

Serum samples:

Of the six sera, serum coded 1 was identified as ADA negative in all 22 assays where tested. All assays were also able to distinguish samples coded 4, 5 and 6 as ADA positive except the assay laboratory 2 which missed the moderately positive sample 5 (due to use of inappropriate dilutions). For the residual ADA positive samples, coded 2 and 3, discrepancies were noted. All ELISAs did not detect the low positive sample coded 2 except for those in laboratories 1 and 11 (which incidentally used the same ELISA). along with both CLIA assays, LF, HMSA and one of the seven ECL assays (Appendix 3, Table 2, Figures 4-5, Tables 11, 12, 14). Eight ELISAs and the LF assay also failed to detect ADA in sample coded 3. Binding activity in the positive serum samples was fairly low, with not much evidence of variation among different assays/platforms. This was also true for sample 6 which had slightly higher activity compared with other samples. In these assays, estimates relative to A and B were fairly similar – the highest values for samples 4, 5 and 6 relative to A or B are from the same laboratory (7), which had no valid estimates relative to IH (all non-parallel) (Appendix 3, Table 2, Figure 4), Tables 11,14. In ECL assays, all sera were found to be positive, with estimates relative to A higher than those seen with the IH standard in most laboratories - assay in laboratory 15 gave highest estimates for all sera (Appendix 3, Table 2). The only exception was laboratory14a which did not detect serum 2 as ADA positive and showed lower values with A relative to IH standards (Appendix 3, Table 2). In all cases, there was improved agreement in estimates with use of B (and not A) as shown in Table 12.

Based on laboratories reporting results in µg/ml relative to IH standards the inter-laboratory %GCV values for ELISAs were high but were reduced by use of A or B as standard, for all positive samples. The overall median GCV was 312%, reducing to 166% or 77% for A or B respectively. Corresponding overall reductions in 10^{MAD} when using A or B as standard were from 2.63 to 1.96 or 1.30 respectively (Table 11b). Similar reductions were observed for ECL assays, with overall median GCV of 233%, reducing to 141% or 65% with the use of A or B as standard respectively. In the majority of cases, and overall, the use of B as standard led to the best harmonization of estimates across different laboratories. Notably, values of 10^{MAD} were <2 in all cases for serum samples 4, 5 and 6 when using B as standard (Table 11b). Only limited data was available for other binding assays (and none for HMSA assays relative to IH), with high inter-laboratory variability for the sera (due to the high ADA estimates from HMSA) relative to either A or B.

All ADA positive samples had neutralizing activity - sample 2 was neutralizing in one of the five assays (laboratory 8c) and sample 3 in four assays. With IH standards, only positivity or negativity or in some cases, titers were determined. Estimates of neutralising activity determined relative to A generally showed greater consistency among the different assays/labs despite limited data, and A appeared to demonstrate a superior performance compared to B (10^{MAD} values for estimates relative to A were <2 in all cases where calculated) (Table 10c).

Stability Testing

Accelerated thermal degradation study

Samples of the lyophilised preparations A (19/264) and B (19/266) were stored for 38 and 54 months respectively while those for C (FS-007) and D (FS-008) were stored for 14 months at elevated temperatures (4°C, 20°C, 37°C and 45°C) and tested in-house with those stored at -70°C and at the recommended storage temperature of -20°C using an ECL for both binding and neutralisation activity. The potencies of all samples were expressed relative to the appropriate -70°C baseline samples and the results are summarised in Table 16. No loss in activity was evident following storage at elevated temperatures up to 20°C.

Stability after reconstitution and on freeze-thaw

Samples of all lyophilised preparations A-D 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 freshly reconstituted ampoules. The potencies of all samples were expressed relative to the freshly reconstituted samples and the results are summarised in Table 17a. Results suggest that while the activity of the reconstituted candidate standards, A and B is not diminished after a week of storage at either 4°C or room temperature, a slight loss of activity is noted for D post-storage for 7 days at room temperature. For C, however, loss of binding activity is evident when the reconstituted preparation is stored even for a day at either 4°C or room temperature. So, C should be used immediately after reconstitution.

Samples of the lyophilised preparations A-D were reconstituted and subjected to a series of freeze-thaw cycles (1 up to 4). They were then assayed concurrently with freshly reconstituted ampoules. The potencies of all samples were expressed relative to the freshly reconstituted samples and the results are summarised in Table 17b. The results suggest that the potency of preparations A, B and D is unaffected with repeated freeze-thaw cycles (up to 4) but the potency of C is diminished after a single freeze-thaw event. Therefore, C cannot be frozen and should be used immediately after reconstitution.

Discussion

Immunogenicity testing is mandatory for regulatory approval of a biotherapeutic. Such testing involves a multi-tiered strategy comprising a screening assay followed by a confirmatory step to confirm antibody-positive samples and subsequent analysis of positives for titer determination and assessment of neutralising ability as per regulatory guidance (EMA 2017; FDA 2019).

It is generally recognized that immunogenicity data is highly dependent on the ADA assays used. Selection of the most appropriate assay platform/format is critical for generating reliable ADA data from testing of clinical samples (Wadhwa et al 2015). The choice of suitable ADA assay controls (positive, negative) is also important. Reference standards/positive controls (PC) have a critical role throughout the ADA assay life-cycle (development, validation, post-approval) for testing different assay parameters (e.g., sensitivity, selectivity, specificity, drug interference etc) and for formulating quality control samples which allows for assay performance monitoring. Therefore, long-term provision of PC with attributes that support different assays (e.g., binding, neutralising activity) is essential for ADA assays. Typically, a “surrogate” positive control (generated from hyperimmunized animals or hybridoma) is employed although in rare instances, ADA positive purified human serum may also be used (e.g., post-licensure clinical studies). Based

on their heterogeneous nature, polyclonal antibodies are ideal for representing the immune response but reproducibility issues with potential replacement batches can impact assay performance and preclude long-term use. Therefore, monoclonal antibody (mAb) based PCs are often the preferred option for long term use and for life-cycle management of ADA assays.

A diverse range of assay platforms including novel procedures are available for ADA detection of biotherapeutics including adalimumab. For regulatory purposes, the high through-put bridging format assays using enzyme-based (Mikulskis et al 2011) or electrochemiluminescence detection (Moxness et al 2005; Lu et al 2021) are often the assays of 'choice' for screening clinical samples. For diagnostic use, commercial ELISAs or chemiluminescence assays (Montaillier 2020; Berger et al 2022) have become the norm. In some clinical laboratories, however, RIAs remain the preferred option (Ruwaard et al 2019) due to special assay characteristics (e.g., sensitivity, detection of Fab arm exchanged IgG4 isotype, less target and drug interference) which enable better ADA detection than bridging assays which have some limitations e.g., potential to miss IgG4 antibodies, suffer from interference from target, drug. To overcome drug-related interference, drug-tolerant ADA assays (measuring total ADA) are adopted (Ding et al 2024), however, they are not necessarily more useful in clinical practice. Drug-sensitive assays are commonly used as highlighted here due to their ability to detect clinically relevant ADA (Atiqi et al 2020). Increasingly, lateral flow assays which are point-of-care and offer timely and rapid results for treatment optimization are gaining momentum in the clinic (Ricken et al 2019). However, other approaches such as HMSA (Wang et al 2013,), flow-induced dispersion analysis (Pedersen et al 2022) and SPR (Beeg et al 2019) have also been explored; the latter detecting ADA positivity in patient sera considered ADA-negative by ELISA (Beeg et al 2021). The hybrid LBA-LC-MS/MS platform is also being used especially when ADA isotyping is needed (Schalk et al 2025). For evaluating neutralising capacity, reporter gene assays (Lallemand et al 2011) and CLBA (Finco et al 2011; Hock et al 2016) continue to be the most favoured although LC-MS/MS (El Amrani et al 2019) has also been investigated for ADAs directed against another TNF mAb.

Unwanted immunogenicity is a concern in adalimumab responsive patients who lose efficacy and develop treatment resistance. Clinical guidance from professional associations e.g., gastroenterology and the European Alliance of Associations for Rheumatology, EULAR recommends the use of reactive TDM in specific clinical situations for management of inflammatory disease (Feuerstein et al 2017; Lamb et al 2019; Krieckaert et al 2022). Indeed, evidence supporting the benefits of TDM in predicting response to anti-TNF for effective patient treatment continues to accumulate (Papamichael et al 2019; Chanchlani et al 2024; Jyssum et al 2024). Widespread implementation of TDM, however, remains scarce due to several challenges, one of which is the inability to interpret disparate ADA data generated from the heterogeneity of ADA assays (including commercial kits) in use. Differences in assay formats, sensitivity, cut-off criteria (for ADA positives), reporting units and importantly, the lack of standardization have contributed to the observed non-comparability and variability of results between assays.

In this study, we have therefore made attempts towards standardising ADA assays by developing a reference panel for adalimumab anti-drug antibodies. The expectation is that this would fulfil the clinical need for standardization of ADA assays for better patient diagnosis and management (van Schouwenburg et al 2016; Kalden and Schulze-Koops 2017; Samaan et al 2018, Mehta and Manson 2020; National Institute for Clinical excellence, NICE, UK). Towards this objective, two

monoclonal antibodies (A, B) which are representative of the antibody repertoire in adalimumab treated patients along with two chimeric human-rat antibodies (C, D) were tested for their suitability to serve as positive controls for adalimumab ADA assays that are currently being used routinely for ADA testing. Based on the characteristics (Table 1) and the behaviour of the two antibody pairs (Table 8, Figure 1b), it is evident that the antibody pairs are distinct in terms of their binding and dissociation profile. The human mAb pair comprising A and B, both high affinity IgG1 antibodies demonstrated strong binding by SPR and also in other assay types, for example, the ELISA and the ECL assay used in the study. In comparison, the chimeric C and D mAb pair displayed moderate to low binding activity by SPR and a fast dissociation which resulted in an overall low affinity for these mAbs. C, an IgG1 mAb elicited low binding in different assays except for SPR (moderate) and weakly neutralized the activity of adalimumab while D, an IgG4 (wild-type sequence) showed moderate binding except by SPR (low) but was weakest of all four mAbs in neutralization activity.

While the expectation is that using a 'common' ADA standard would improve the comparability between assays, it must be borne in mind that assay formats and/or platforms differ in terms of their inherent characteristics which can nevertheless lead to dissimilar assay results. This is clearly illustrated by the study data. Among all assays which recognized all mAbs as ADA positive, the RIA alone was unable to detect mAb S which has the IgE isotype. This is not unexpected given that the properties of the RIA are dependent on protein-A which preferentially binds with high affinity and specificity to the Fc region of certain isotypes, in particular IgGs, and to the Fab-regions of antibodies that contain the abundantly used VH3 family segments, which may not be the case for this particular mAb.

For the two main assays in the study namely, the ELISAs and the ECL assays, differences in immunoreactivity were evident. Both employ the bridging format - the ELISA is a step-wise assay while the ECL, which is usually an 'in-solution' assay has a wider dynamic range than the ELISA. Other factors which can influence results include the dynamics of complex formation ADA-drug (drug being the detection reagent) which differs between assays/platforms, the affinity of the ADA and the absence/presence of residual drug (adalimumab) in the samples. Any residual drug, whether free or complexed with the ADA in the samples, will affect the formation of ADA-reagent complex in the assay, thereby affecting the assay signal. Consequently, as highlighted in the study, ECL assays in general, tend to have an increased sensitivity than ELISAs and are able to detect ADAs missed by ELISAs and, in some instances, even in the presence of drug. In contrast, ELISAs are more susceptible to drug interference and fail to detect ADAs. For example, the ADA positivity of serum samples 2 and 3 was largely missed in laboratories performing ELISAs which measured only 'free' ADAs (laboratories 2-9). In such instances, an increased sample dilution or prolonged incubation period with the detection reagents (in the absence of an acid dissociation step for disruption of ADA immune complexes) or even both can help to improve the drug tolerance of assays. However, both samples 2 and 3 were detected in ELISAs of two laboratories (laboratories 1, 11) which measured 'total' ADA. A similar situation as noted for the free ELISAs was also seen with other clinical monitoring assays. Both CLIAs (laboratories 5b, 20), the LF (laboratory 18), the RIA (laboratory 21) and the HMSA (laboratory 19a) missed the presence of ADAs, at least in sample 2 containing around 1μg/ml of adalimumab, however, 3/5 assays (HMSA, RIA, CLIA from one laboratory) recognized ADA in sample 3 despite the presence of a lower amount of adalimumab. The ECL assays, often used in the regulatory context, detected ADAs in samples 2

and 3 except for a single laboratory (laboratory 14a) which missed sample 2. The reason for the lack of recognition is not obvious but likely contributed by the multitude of factors that can vary between assays of different laboratories and ultimately affect the assay signal.

Indeed, as shown by the data, individual assays of the same platform, in particular ELISAs differ markedly when in-house standards are used for estimating binding activity. For example, for sample A, the range of GM estimates for binding activity varied between 3.06 - 1036.53 µg/ml while a narrower range of 5.41 - 54.05 µg/ml was observed when estimates were determined relative to B. This variability between results of individual assays is not unexpected. Any single factor or possibly a combination of multiple interlinked factors relating to differences in assay protocols (e.g., minimum required dilution, incubation times, drug tolerance, sensitivity, affinity and stability of the ADA), the choice of critical reagents, labelling procedures where relevant, the nature and properties of the positive control, the assay execution procedure and finally data analysis (e.g., cut-point as per industry practice or other method for defining the cut-off value for discriminating ADA +ve from ADA-ve sample) can influence results. A thorough assay optimization and validation exercise is imperative prior to testing of clinical samples to ensure that all ADA positive samples are recognized. Of the 27 binding assays where sera were tested, only a single laboratory's ELISA (laboratory code 2), failed to detect ADA in sample 5 (possibly due to use of inappropriately high sample dilutions). Importantly, our findings showing discrepancies in estimates for ADA activity in assays which are widely used for clinical monitoring emphasize the need for assay harmonization.

Of all the lyophilized antibodies tested, B demonstrated a higher degree of parallelism with other samples and gave more consistent estimates for the study samples in binding assays when used as a standard compared to estimates obtained with A. Undoubtedly, the study results illustrate that use of B as a common standard for reporting results was associated with less inter-laboratory variation, potentially harmonizing results across binding assays/platforms. This was not the case with neutralizing antibody assays (n=5). For these, A was found to be better than B in providing consistency and harmonizing estimates across the limited number of assays performed. Data relative to in-house standards was, in most instances, defined as positive/negative or given in titers.

It is interesting to note that while some laboratories using the same commercial kit for measuring anti-adalimumab antibodies reported similar results for the samples, there were also instances where quite the opposite was seen. In all cases, better assay harmonization was seen relative to a common standard rather than in-house standards. These results emphasize the importance of consistency between baches of the same kits/reagents, the use of independent in-house standards (in clinical laboratories), strict adherence to protocol for assay execution, analysis and data interpretation. Furthermore, it has important implications in the clinic. Published evidence for adalimumab ADAs as well as for infliximab ADAs indicates that the same assay should be used for longitudinal follow-up of a patient, as the kits are not interchangeable (Rissel et al 2023) notably because of the lack of standardization. The same situation was also noted previously with respect to Infliximab ADA assays (Bertin et al 2020, Bader et al 2017). Our study suggests that B has the potential to generate greater consistency in estimates between different ADA assays and platforms.

Treatment with a biotherapeutic induces ADAs which at onset typically have low titer, affinity and avidity and with progressive treatment, mature into strong binding high titer ADAs with varied isotype (including IgG4) - all such ADAs must be detectable in ADA assays. Although calibrants are not used for ADA assays for regulatory approval of a biotherapeutic, calibrants are valuable for clinical harmonization. The characteristics of the lyophilised mAbs and the collaborative study data demonstrate the suitability of the reference panel mAbs for adalimumab ADA assays. Therefore, a panel comprising mAb B (rather than A) as a standard for use in calibration (assay performance, validation) and harmonization of binding assays, mAb A as a calibrant for neutralizing antibody assays and for assay harmonization while mAbs C and D as performance indicators for detecting antibodies with fast dissociation are considered suitable for users. In essence, mAb C would be useful for detecting low activity ADAs while D would serve to assess the ability of the assay to detect the bivalent IgG4 isotype and for assessing the sensitivity of the neutralizing antibody assays (if needed). However, it should be noted that antibody D (recombinant bivalent) does not serve the purpose of testing the specific challenges of detecting Fab arm exchanged/monovalent IgG4 often found in the clinical scenario (Lighaam and Rispens, 2016). mAbs B (for binding activity) and A (for neutralizing activity) can also be used as additional positive controls for life-cycle management of adalimumab ADA assays where needed.

Importantly, this study is associated with some caveats which must be noted. Firstly, there were only a limited number of serum samples including those containing adalimumab which is not fully representative of the clinical scenario. Secondly, for the overall analysis, data from both free (n=17) and total assays were combined since total assays are limited (n=6). Nonetheless, for all binding assays, there was no obvious difference in estimates between the two assay types for the mAb samples. For serum samples, total assays were evidently better and gave higher estimates than the free assays as they detected ADA in samples despite the presence of residual drug (code 2, 3).

Accelerated thermal degradation studies at 38 and 54 months for 19/264 (A) and 19/266 (B) and for 14 months for FS-007 (C) and FS-008 (D) indicates no loss of stability when stored at -20^{o}C. As the stability could not be predicted using the Arrhenius equation, further studies may be needed over subsequent time-points to confirm the stability and to predict degradation rate (% potency loss per year) of both preparations.

Stability studies post-reconstitution indicate that while the activity of the reconstituted preparations, A and B remains unaffected on storage up to 1 week at either 4°C or room temperature or after repeated freeze-thaw (up to 4 cycles), a slight loss is seen with D after 7 days of storage at room temperature. Notably, C loses binding activity post-reconstitution under the conditions tested. Therefore, C should be used immediately after reconstitution. This will be explicitly stated in the Instructions for Use leaflet accompanying this material.

These results indicate that the lyophilized preparations 19/264 (A), 19/266 (B), FS-007 (C) and FS-008 (D) are stable and suitable for use as WHO International Biological Reference Preparations for Adalimumab anti-drug antibodies.

Conclusions and Proposal

Use of a common antibody reference standard has the potential to align results and enable harmonisation of assays as shown in this study compared with the existing situation where reporting units are not comparable (even if stated as ng/ml or µg/ml). Although there are many kits in use for clinical monitoring, reporting practice (positive/negative, arbitrary units, titers) varied for only 30% of the participating laboratories in this study. Data showed that the degree of harmonization is dependent on the assay type, the sample and the laboratory. The establishment of WHO International Standards would facilitate comparison of results across immunogenicity assays, if implemented in practice and aid TDM for better patient outcome globally.

Our proposal to WHO is to establish:

- Preparation, A (coded 19/264) as the 1st WHO International Standard for Adalimumab Anti-Drug Antibodies for neutralisation assays with an assigned value of 50,000 IU/ampoule
- Preparation, B (coded 19/266) as the 1st WHO International Standard for Adalimumab Anti-Drug Antibodies for binding assays with an assigned value of 50,000 IU/ampoule

In addition, it is proposed that mAb preparations C and D serve as International Reference Reagents as they are suitable for use as performance indicators for detecting antibodies with fast dissociation. We propose:

- Preparation C (coded FS-007) as the WHO International Reference Reagent for detecting low activity Adalimumab Anti-Drug Antibodies with fast dissociation (no assigned value)
- Preparation D (coded FS-008) as the WHO International Reference Reagent for detecting low affinity, bivalent IgG4 Adalimumab Anti-Drug Antibodies (no assigned value)

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Table 1: Characteristics of the antibodies in the adalimumab ADA reference panel

Antibody	Origin	Clone	Isotype	Light chain	Binding		Neutralisation	
					Affinity EC50 (ng/ml)	KD (M) SPR	Status	Activity EC90 (ng/ml)
ADA39	human PBMC	VA2-17-476-1	IgG1	κ	12	4.9 E-11	+ve	169
ADA44	human PBMC	VA2-17-477-1	IgG1	κ	14	2.5 E-10	+ve	155
A21	chimeric human-rat	cl A21-1G-IgG1	IgG1	κ	ND	1.4 E-10[1]	+ve[2]	ND
A40[3]	chimeric human-rat	cl A40-1C-IgG4[3]	IgG4	κ	ND	4.4 E-10[1]	+ve[2]	ND

All recombinant forms of the mAbs were produced in CHO cells.
Affinity of the antibodies is expressed as EC50: the concentration inducing a response halfway between baseline and maximum as determined by ELISA or as KD: dissociation constant (k_{off}/k_{on}) determined by surface plasmon resonance (SPR) using either the ProteOn (Biorad, US) or the [1]BIAcore T200 system (Cytiva, USA).
Neutralisation activity is expressed as EC90: the concentration giving 90% of $E_{max)}$ and determined by competitive ligand binding assay or reporter gene assay[2] employing the GloResponse™ NF-κB-RE-luc2P HEK293 cell line (Promega), ND – not determined.
Brief description of the characterization methods used are given in Appendix 1.
The A40 antibody is an IgG4[3] with wild type sequence.

Table 2: Information on lyophilized antibody preparations

Antibody code	Ampoule code	Study code	Fill date	No ampoules in stock	Protein (predicted mass μg)	Excipients
ADA39	19/264	A	6/03/2020	1399	50	10mM L-Glutamic acid, 4% Mannitol, 2% Sucrose, 0.01% Tween20; pH 5.2
ADA44	19/266	B	6/03/2020	1247	50	

A21	FS-007	C	23/02/2024	360	50	
A40	FS-008	D	1/03/2024	360	50	

All mAbs were expressed in CHO cells. All ampoules for each of the preparations is available for WHO use. Storage will be at -20ºC at MHRA as the custodian laboratory

Table 3: Mean fill weights and residual moisture content of adalimumab ADA preparations

Ampoule code	Study code	Fill Weight		Residual moisture		Headspace Oxygen	
		Mean g (n)	CV%	Mean % (n)	CV%	Mean % (n)	CV%
19/264	A	1.0075 (69)	0.19	1.11 (12)	17.65	0.31 (12)	53.60
19/266	B	1.0080 (70)	0.24	0.89 (12)	24.53	0.24 (11)	66.04
FS-007	C	1.0221 (6)	0.10	0.60 (3)	26.6	0.78(3)	24.2
FS-008	D	1.0230 (6)	0.15	0.66 (3)	19.8	0.65(3)	10.2

CV: Coefficient of Variation;g: gram; n : indicates number of determinations. Residual moisture of each preparation was measured by the coulometric Karl-Fischer method (Mitsubishi CA100). Headspace oxygen content was determined by frequency modulated spectroscopy (Lighthouse FMS).

Table 4: Details of liquid monoclonal antibody preparations

Sample code	Antibody origin	Clone/Other identifier	Isotype	Light chain	Binding Affinity KD (M)	Neutralising Antibody Status	Reference
R	chimeric human-rat	cl A12-6A-IgG1[1,3]	IgG1	κ	5.7 E-11[1]	+ve	Suzuki et al 2020
S	chimeric human-rat	cl A27-1C-IgE[1,3]	IgE	κ	5.7 E-11[1]	+ve	
T	human B Cells	cl 2.7	IgG1	κ	1.95 E-10	+ve	van Schouwenberg et al 2014
U	human B Cells	cl 2.10	IgG1[2]	κ	1.15 E-10	+ve	

Binding assessed by SPR using BIAcore T200 system for mAbs[1] sourced from [3]NIHS, Japan and using fluorescence assisted HPLC for mAbs for those sourced from Sanquin, Netherlands; recombinant form of the mAbs was produced in [1]CHO or HEK293 cells. This antibody[2] was isolated as an IgG4 but produced as an IgG1 construct for studies. Neutralisation activity for chimeric mAbs[1] determined using the reporter gene assay employing the GloResponse™ NF-κB-RE-luc2P HEK293 cell line (Promega) while others tested by use of the IL-8 producing TNF-responsive ECRF cell-line as described in the cited publications.

Table 5: List of Participants

Participant Details	Country	Organisation
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KM and PM denote kit and product manufacturers respectively, CD – indicates clinical/diagnostic lab, CS- cell-lines/reagents supplier, CRO- contract research organization, C – control lab and A – academic lab.

Table 6a: Brief details of binding assays contributed to the study

Format	Brief Method	Reference	Lab Codes
ELISA (Bridging)	Various formats as listed below were used. Some assays, however included an immune-complex dissociation step for detecting total ADAs which has not been described. • Adalimumab is immobilised on the surface of 96-well plate and the ADA present in the positive control/test sample binds to adalimumab (solid-phase) and to biotinylated adalimumab during incubation. The bound immunocomplexes are detected using horse radish peroxidase (HRP) - labelled streptavidin followed by addition of enzyme substrate TMB, and measured spectrophotometrically in a plate reader. Alternatively, for detection, adalimumab is directly conjugated to HRP and ADA detected spectrophotometrically following TMB addition. The colour is directly proportional to the amount of anti-adalimumab antibodies. • Instead of the approach stated above, an assay wherein the complexes of biotinylated adalimumab, ADA and HRP-labelled adalimumab in a reaction tube are captured onto streptavidin coated plates via the biotin,and detected by addition of TMB substrate.	Mikulskis et al 2011; Chen et al 2015	3,4,5a,9,10 2,6,7,8a 1,11
ECL (Bridging)[1]	• Samples/positive control are incubated with biotin-conjugated adalimumab and Sulfo-Tag conjugated adalimumab, and the mixture transferred to specific streptavidin coated carbon electrode plates (Meso Scale Discovery, MSD, US). The complexes are detected by addition of the read buffer (tripropylamine) which stimulates an oxidation–reduction reaction when voltage is applied and generates electrochemiluminescence, which is measured using a vendor-specific plate reader (MSD, US). The ECL counts are proportional to the amount of ADA present in the sample. Some assays incorporated an acid-dissociation step for quantifying total ADAs.	Kaur et al 2017; Cludts et al 2017; Suzuki et al 2020; Lu et al 2021	8b, 12, 13a,14a, 15,16,17
CLIA (Bridging)[1]	• Adalimumab coupled magnetic microparticles are mixed with human serum/plasma allowing binding of ADA to the microparticles on incubation. Adalimumab conjugated to acridinium ester is then added to the microparticle-antigen-ADA immunocomplex and the light emitted detected by a reagent which triggers chemical events resulting in chemiluminescence which is measured in a dedicated analyzer. The intensity of light emission is proportional to the amount of anti-adalimumab antibodies in the sample.	Montailler 2020; Berger et al 2022	5b,20
RIA[1]	• Protein A-Sepharose is incubated with human serum/plasma followed by incubation with ^{125}I-labeled adalimumab F(ab')$_2$. Total IgG in the samples binds to Protein A-Sepharose through its Fc domain while the ^{125}I-labeled adalimumab F(ab')$_2$ binds to the adalimumab-specific IgG in the samples. The quantity of Protein A-Sepharose-bound I^{125} correlates with the amount of adalimumab-specific-IgG and is measured using a gamma counter.	Wolbink et al 2006	21
LF	• This rapid immunochromatography test uses the principle of a sandwich immunoassay. An adalimumab reactive molecule conjugated to gold binds to ADA in the sample to form a complex that migrates until it is selectively captured on the Test zone (T), causing a pink/purple line to appear on the strip. If the sample is ADA negative, no visible T line is seen. A control gold conjugate reagent continues to flow to the end of the strip where it binds to the control zone (C) and shows a pink/purple line indicating that the test has worked.	Ricken et al 2019	18
HMSA[1]	• This method detects the formation of drug-ADA complexes in solution phase in sera spiked with fluorescent labelled drug. Besides a semi-quantitative measure of ADA concentration, the method allows determination of the size of the ADA-drug complexes formed *in vitro*. The sample is injected onto a HPLC system, and any complexes formed are separated by size exclusion chromatography (SEC-HPLC) and detected by a fluorescent detector. While a qualitative assay, it allows semi-quantification of the ADA amount using area under relevant peaks. Dimer complexes of ADA and drug (150kDa each) appear in the 300kDa region with immune complexes 400-700kDa representing multimers of ADA and drug. A standardised ratio of area under the ADA peak relative to total area in arbitrary units (AU) is used. One arbitrary unit (AU) means there is perfect alignment of the area under the sample chromatogram over the negative control chromatogram indicating undetectable ADA.A discriminating cut-value of 2 AU was obtained after testing multiple adalimumab free serum samples including those with rheumatoid factor activity.	Wang et al 2013; Hock et al 2018. Keating et al 2024	19a

Table 6b: Brief details of neutralising assays contributed to the study

Format	Brief Method	Assay Reference	Lab Codes
CLBA - ECL	• A non-cell-based assay in which NAbs, if present in the sample(s), prevent the binding of TNF-α to adalimumab which results in none or reduced assay signal. For assay, controls and samples are incubated with biotinylated adalimumab and sulfo-tag labelled TNF-α (or biotinylated TNF and sulfo-tag labelled adalimumab), the mixtures transferred to pre-blocked MSD streptavidin-coated plates followed by addition of read buffer. The plates are read by a dedicated instrument (MSD, US). The signal intensity is inversely proportional to the neutralising activity of the antibodies.	Finco et al 2011 Ding et al 2024	8c, 13b
CLBA- ELISA	• Principle is the same as for ECL above. • For assay, controls and samples are incubated with biotinylated adalimumab, the mixtures transferred to pre-blocked TNF-α-coated plates followed by addition of streptavidin-HRP and TMB substrate prior to reading plates in an ELISA reader. The signal intensity is inversely proportional to the neutralising activity of the antibodies. Here acid-dissociation step which involved use of adalimumab-F(ab')$_2$-fragment coated plates was incorporated. • The presence of NAbs is assessed by spiking samples with adalimumab and measuring TNF-α levels in an ELISA. The relative difference in free TNF-α levels between a spiked and unspiked sample is used to determine the presence of ADA i.e. competitive ligand binding assay. As adalimumab elicits specific antibodies, spiking with adalimumab is indicative of the agent inducing the functional antibodies. It is only possible to detect ADA in patient sera with low/undetectable adalimumab.	Finco et al 2011 Ogric et al 2019 Hock et al 2016	14b 19b
Cell-based RGA	• In this assay, adalimumab blocks the activation of TNF-α signalling pathway and impacts a downstream signalling event (i.e. NF-κB activation). A fixed amount of adalimumab is incubated with the sample prior to exposure to TNF-α (at a fixed amount) followed by an incubation step with cells (e.g., human lymphoblast K-562 transfected with NFκB regulated firefly luciferase reporter-gene construct). NAbs, if present, will prevent adalimumab from inhibiting TNF induced signalling and result in binding of free TNF-α to its receptor followed by NF-κB activation which increases luciferase levels which can be detected by an appropriate substrate (luciferase). The amount of hydrolysed substrate is proportional to the neutralizing activity of the ADA and is measured in a luminometer.	Lallemand et al 2011	22

ECL - Electrochemiluminescence, CLIA – Chemiluminescence based immunoassay, RIA – Radioimmunoassay, LF – Lateral flow, HMSA - Homogeneous mobility shift assay, solution phase assay[1] , CLBA - Competitive-ligand binding assay, RGA – reporter gene assay

Table 7a: Details of binding assays performed by study participants

Assay type: ELISA								
Lab Code	ADA	[1]C/IH	Positive Control/In-house standard				Quality controls (QCs)	Cut-off/Cut-point (CP) information
	Free/Total		Nature	Use[2]	Assay range	Units		
1	Total	C	mAb human[3]	Cut-off	10-500	AU/ml	Neg (human sera), Pos (patient sera)	Cut-off :10 AU/ml based on >100 determinations using negative samples
2	Free	C	mAb human[4]	Cal	18-640	ng/ml	Pos – low, mid (IH) Neg (human sera)	Cut-off: mean of blank (neg) x 3 times standard deviation of blank
3	Free	C	mAb murine[5]	Cal	[9]2.5-125; 20-1000	ng/ml	Pos - low, mid (IH) Neg (buffer)	Cut-off: Pos >2.5 ng/ml (1:25 dilution) and >20 ng/ml (1:200 dilution) by extrapolation from a 6-point standard curve.
4	Free	C	mAb murine[5]	Cal	1.23-125	ng/ml	Pos - low, mid (IH) Neg (buffer)	Cut-off: 1.23 ng/ml (1:25 dilution)
5a	Free	C	pAb (rabbit)	Cal	10 -160	ng/ml	Pos – low, high (IH), Kit QC – mid, Neg (buffer)	Cut-off: 10 ng/ml derived using the percentile method (>99th percentile). Tested >100 sera from untreated patients or healthy donors
6	Free	C	human serum with ADA[3]	Cal	12 – 495[10]	AU/ml	Pos (IH), Neg (human sera)	Cut-off: Mean value of ADA plus 12 SD using sera from naïve patients (different pathologies)
7	Free	C	human serum with ADA[3]	Cal	10 - 400	AU/ml	Pos (IH), Neg (human sera)	Cut-off: Mean ADA value plus 12 SD derived using samples from untreated patients
8a	Free	IH	pAb (sheep)	Cal	3.9 - 500	ng/ml	Neg (buffer)	Cut-off: Not determined; dose response curves relative to pos controls
9	Free	IH	mAb human[4]	Cal	7.8 - 4000	ng/ml	Neg (buffer)	Cutoff = mean x 3(SD)
10	Free	C	pAb (rabbit)	Cal	10-160	ng/ml	Pos - low & high (IH); Kit QC – mid, ADA spiked sample in human serum, Neg (buffer)).	Cut-off: 10 ng/ml based on data from >100 sera from healthy donors or naive patients
11	Total	C	mAb human[3]	Cut-off	10-500	AU/ml	Pos - low & high (patient sera), Kit QCs, Neg (human sera)	Cut-off: 14 AU/mL, based on manufacturer's recommended cut-off and internal studies
Assay type: ECL								

8b	Free	IH	pAb (sheep)	Cal	0.5 - 500	ng/ml	Neg (buffer)	Cut-off: Not determined; Pos by analysis of dose response curves relative to neg and pos controls
12	Free	IH	mAb human[4]	AP	1-1000	ng/ml	Pos (IH), Neg (human sera)	CP (Floating): Plate specific - average of responses of neg samples multiplied by the N-factor (set at 5% false-positive rate using naïve human sera during validation)
13a	Total	IH	mAb human[4]	AP	6.3-25000	ng/ml	Pos – low, mid, high (IH), Neg (human sera)	CP: 1.260 (Floating); Titer CP: 1.390. Titer reported as the reciprocal of the highest dilution with a response > the CP inclusive of the assay minimal required dilution
14a	Total	IH	pAb (rabbit)	Cut-off, AP	60-9600	ng/ml	Pos - low, mid, high (IH), Neg (human sera)	CP (Floating): Pos is sample with [raw signal] ≥ [blank signal] multiplied by N-factor of 1.30; N-factor determined using 300 data points from a set of negative individual sera.
15	Free	IH	pAb (rabbit)	AP, Cal	1.67-1215	ng/ml; results S/N	Pos – low, high (IH), Neg (human sera)	CP: S/N >/= 1.22; statistically determined using the Shen method
16	Free	IH	cocktail of 4 mAbs human	Cut-off, Cal,AP	10-500	ng/ml;	Pos – low, high (IH), Neg (human sera)	CP: S/N >1,4033
17	Total	IH	mAb (rabbit IgG)[6]	AP	2-1000	ng/ml; titer	Pos – low, high (IH); Neg (human sera)	CP: used Titer CP: 1.31; Plate specific – calculated by multiplying the respective average neg control value by the study titer cut-point determined using 300 data points.
Assay type: CLIA								
5b	Free	C, Cal	pAb (rabbit)	Cal, Cut-off	10-2000	ng/ml	Pos: low, high (IH)	Cut-off: 10 ng/ml derived using the percentile method (>99th percentile). Tested >100 sera from untreated patients or healthy donors
20	Free	C, Cal	pAb (rabbit)	Cal	10-3000	ng/ml	Pos – low, high (IH), Neg (human sera)	Cut-off: 10 ng/ml based on data from >100 sera from healthy donors or naive patients
Assay type: LF								

18	Free	C	mAb human[3]	Cal	N/A	ng/ml	Pos - low, high (IH), Neg (buffer)	Cut-off: Pos if equal to and above 0.2 μg_{eq}/mL
Assay type: RIA								
21	Free	IH	ADA pos human sera (pooled)	Cal	12-975	AU/ml	Pos - mid (IH), Neg (human sera)	Cut-off: S/N ratio >2 (representing samples spiked with 12 AU/mL). S/N ratio determined by dividing mean values from spiked samples by corresponding blank samples.
Assay type: HMSA								
19a	Total	IH	ADA pos human sera	AP	N/A	AU	Pos (IH), Neg (normal sera).	Cut-off: 2 AU obtained by testing multiple adalimumab free sera including those with rheumatoid factor activity. ADA weak: Samples with HMSA > 2AU; ADA positive: 3-8 AU; Strong ADA positive: >8 AU. A standardised ratio of area under the ADA peak relative to total area in AU is used here. In addition, the size of immune complexes formed is visually assessed relative to a negative control using retention times.

[1]C or IH – commercial kit or in-house developed assay; Use[2]: Cut-off - pos/neg, Cal - calibration, AP - assay suitability/performance; [3]further information not disclosed; antibody sourced from [4]Biorad, HCA 204 or [5] Gils et al (2014), MA-ADM6A10 or [6] R&D Systems, #MAB9616 or [7]Merck SILu™Lite MSQC16 or [8]Svar life Science #BM3159 ; [9]Assay range dependent on the sample dilution so 2.5-125ng/ml (for 1:25 dilution) and 20-1000 (for 1:200 dilution); [10] Assay range can be extended up to 1,475 with additional recommended dilutions; QCs denoted by IH refers to the nature of the QC being the same as the PC or IH std; ND - not determined; N/A – not available. Total ADA is defined by incorporation of an immune complex disruption step in the procedure (except for HMSA). However, some bound ADA can be detected in 'free' assays as a result of the chosen dilutions and/or characteristics of the critical reagents/assays.

Table 7b: Details of neutralisation assays performed by study participants

Lab Code	Assay	ADA Free/total	[1]C/IH	Positive Control/ In-house standard			Results	QCs	Cut-off/ Cut-point (CP) information
	CLBA/ Bioassay			Nature	Use[2]	Assay range/units			
8c	CLBA (ECL)	Free	IH	pAb (sheep)	AP	Qualitative; ng/ml	pos/neg	Neg (buffer)	Cut-off: Not determined; dose response curves relative to pos controls
13b	CLBA (ECL)	Total	IH	mAb human[4]	AP	Qualitative; ng/ml	pos/neg	Pos – high, low (IH); Neg (normal sera)	Cut-off: Pos if S/N < 0.942
14b	CLBA (ELISA)	Total	IH	mAb human[3]	AP	Qualitative; ng/ml	pos/neg; titer	Pos – low, mid, high (IH); Neg (normal sera)	CP: 11.2% neutralization; determined using 300 data points from a set of negative individual sera using a false positive rate of 1%.
19b	CLBA (ELISA)	Free	IH	mAb[7]	AP	Qualitative; ng/ml	pos/neg; titer	4 Pos - 1patient sera (at ~ 4 mg/L); 3 serum samples spiked with 5, 10 and 20 mg/L adalimumab. Neg (human sera).	CP: 20% Neutralisation; determined using negative samples
22	Bioassay (RGA)	Free	IH	pAb (rabbit)[8]	AP, cut-off	Qualitative; ng/ml	ng/ml; titer	Pos (IH), Neg (heat-inactivated normal sera).	CP (floating): based on variance evaluation of naïve patient samples – threshold factor for Adalimumab = 1.33

[1]C or IH – commercial kit or in-house developed assay; Use[2]: Cut-off - pos/neg, Cal - calibration, AP - assay suitability/performance; [3]further information not disclosed; antibody sourced from [4]Biorad, HCA 204 or [5] Gils et al (2014), MA-ADM6A10 or [6] R&D Systems, #MAB9616 or [7]Merck SILu™Lite MSQC16 or [8]Svar life Science #BM3159 ; [9]Assay range dependent on the sample dilution so 2.5-125ng/ml (for 1:25 dilution) and 20-1000 (for 1:200 dilution); QCs denoted by IH refers to the nature of the QC being the same as the PC or IH std; ND - not determined; N/A – not available. Total ADA is defined by incorporation of an immune complex disruption step in the procedure (except for HMSA). However, some bound ADA can be detected in 'free' assays as a result of the chosen dilutions and/or characteristics of the critical reagents/assays.

Table 8: SPR binding data for the different anti-adalimumab antibodies

Antibody Code	ka (1/Ms)	kd (1/s)	KD (M)	Ranking Order
A	2.035E+06	5.129E-05	2.520E-11	2
B	6.857E+05	6.035E-06	8.801E-12	1
C	3.082E+06*	6.186E-02*	2.007E-08*	-
D	1.450E+05	1.063E-02	7.327E-08	7
R	1.253E+05	5.478E-06	4.371E-11	3
S	1.222E+05	1.629E-04	1.333E-09	5
T	9.997E+04	1.655E-04	1.656E-09	6
U	1.368E+05	5.565E-05	4.068E-10	4

Results shown above were generated from a representative single cycle kinetic (SCK) experiment using the Biacore T200 in which all the different mAbs (lyophilized A to D - bulk material; solution R-U) were comparatively assessed in a single run allowing for ranking of the mAbs based on their KD. For C, kinetic constants are difficult to determine (due to limits of instrument)

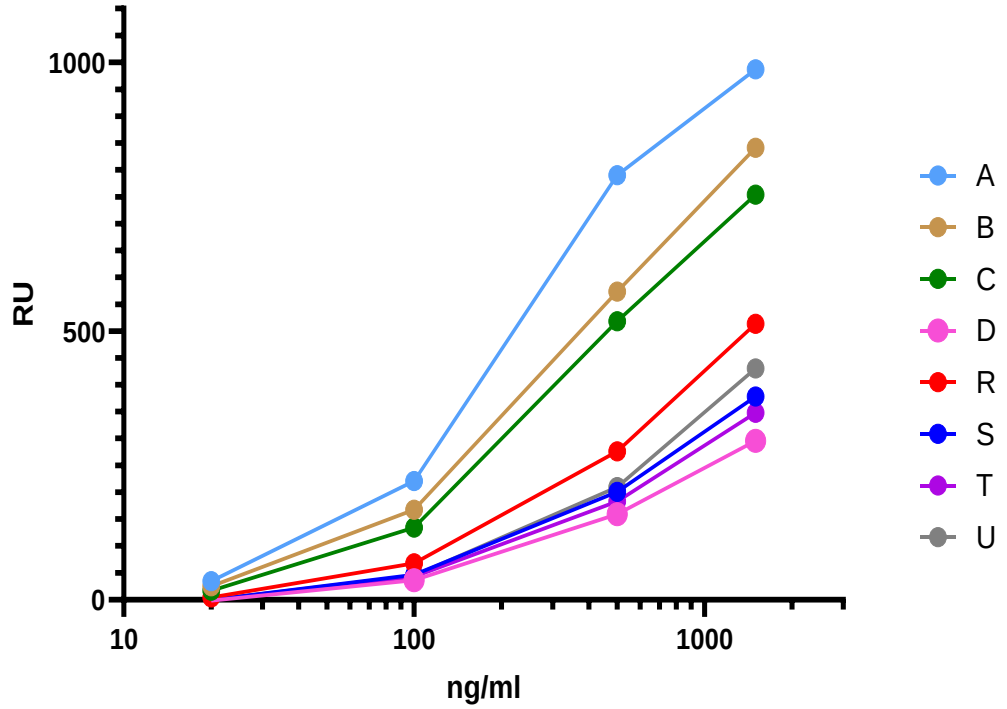

Figure 1a: Binding profile of the adalimumab ADA mAbs A, B, C & D (bulk material) and the liquid mAbs as demonstrated by SPR using Biacore T200.

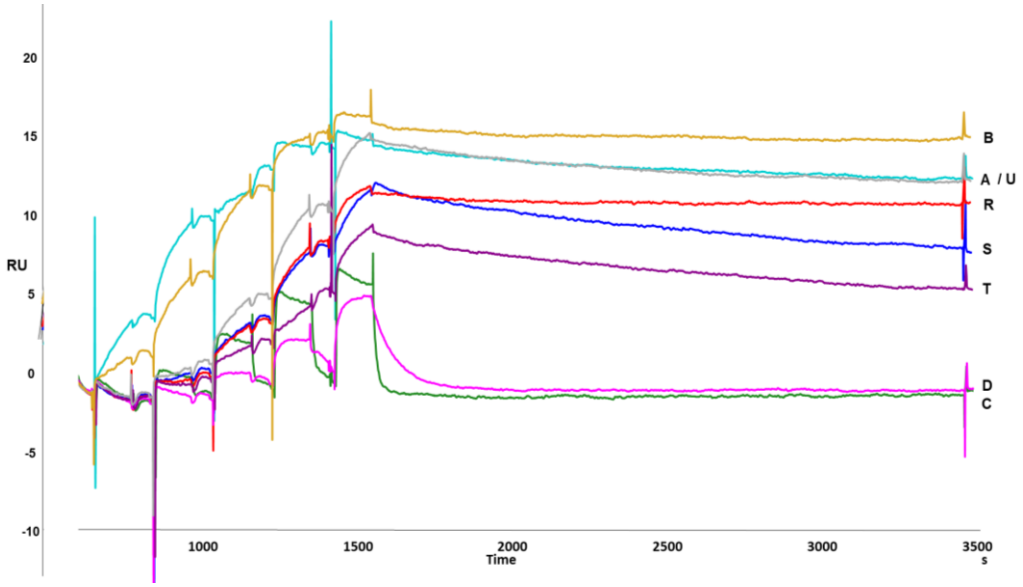

Figure 1b : Sensorgrams of the adalimumab mAbs generated from a representative single cycle kinetic experiment (SCK) using the Biacore T200 in which all the different mAbs (lyophilized A to D - bulk material; solution R-U) were comparatively assessed in a single run for ranking of the mAbs.

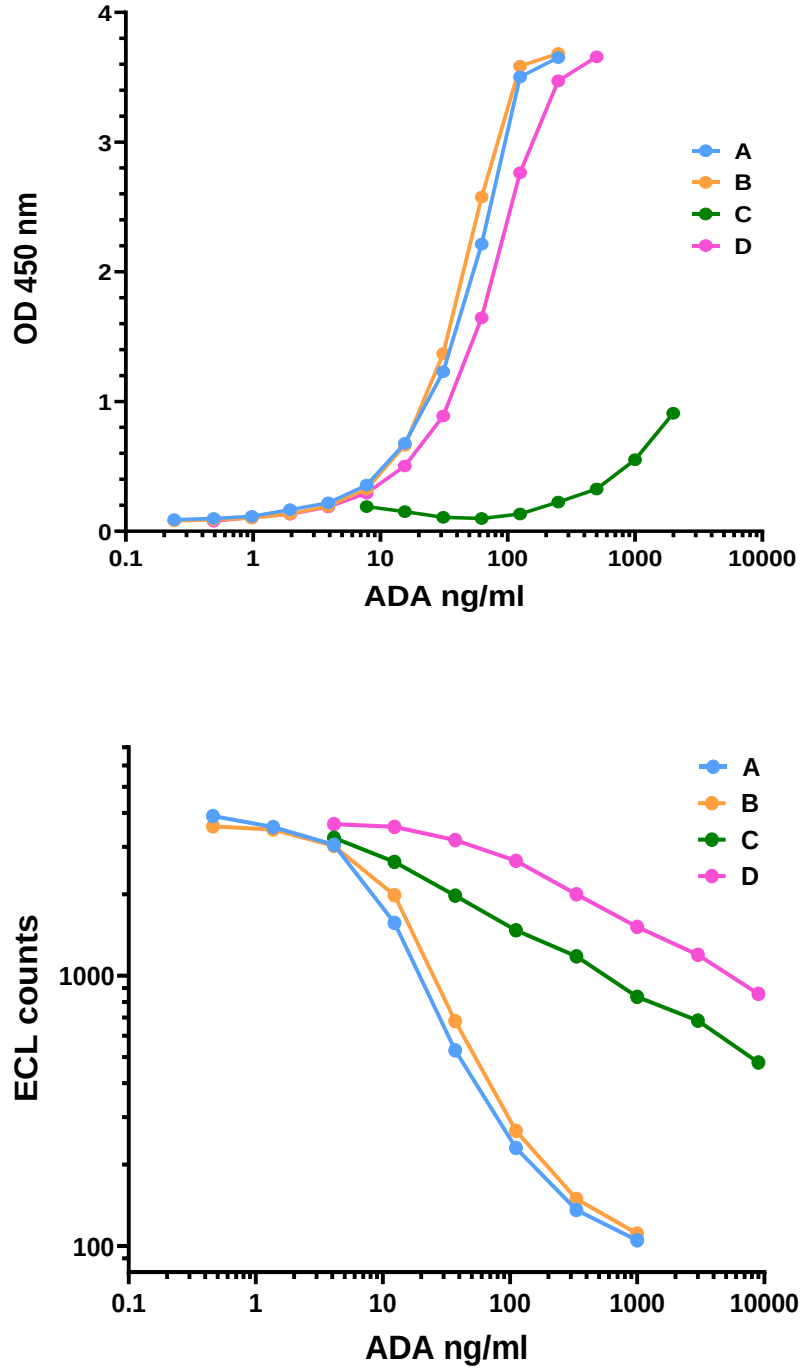

Figure 2: Binding (Top Panel) and neutralizing activity (Bottom Panel) of the adalimumab ADA mAbs A – D. Representative data from a bridging ELISA (Top Panel) and a non-cell-based competitive ligand binding assay using ECL are shown

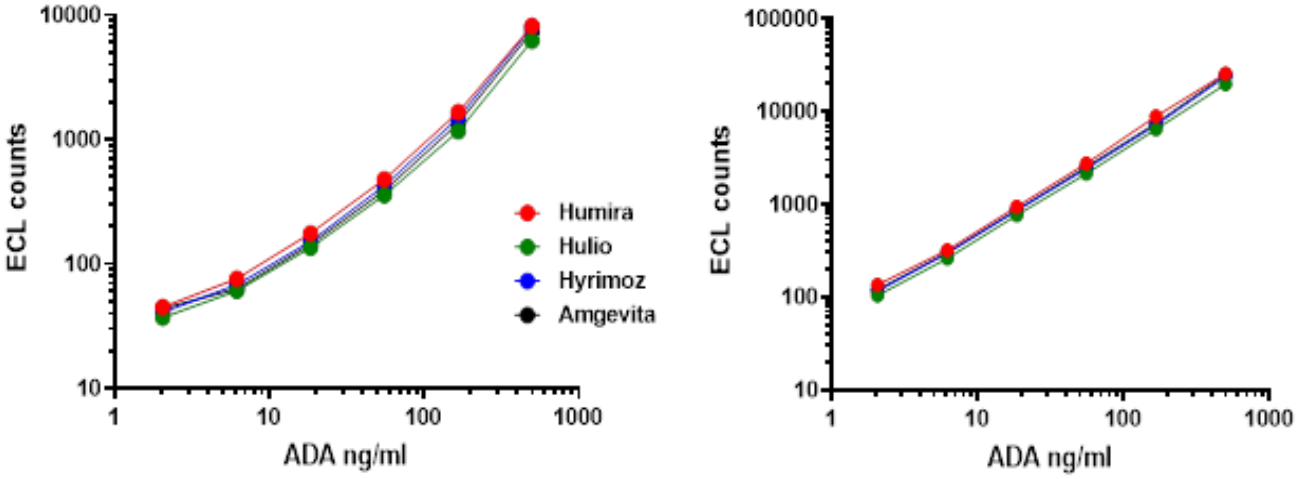

Figure 3: Binding of mAb A (left panel) and mAb B (right panel) to labelled adalimumab products (originator and biosimilars) in an ECL assay.

Table 9: Distribution of slope-ratios (relative to sample A or sample B) for the different samples and assay types

Sample A

ELISA

Sample	Slope ratios vs A					
	% <0.67	% 0.67-0.80	% 0.80-1.25	% 1.25-1.50	% >1.50	n
B	0.0	6.9	92.0	1.1	0.0	87
C	62.1	17.2	18.4	2.3	0.0	87
D	23.0	24.1	44.8	6.9	1.1	87
R	9.2	25.3	63.2	1.1	1.1	87
S	14.9	18.4	63.2	2.3	1.1	87
T	3.5	7.1	85.9	3.5	0.0	85
U	0.0	26.4	70.1	3.4	0.0	87
1	Neg	Neg	Neg	Neg	Neg	0
2	7.7	7.7	76.9	7.7	0.0	13
3	39.3	0.0	39.3	7.1	14.3	28
4	4.8	19.3	61.4	4.8	9.6	83
5	1.4	14.9	79.7	4.1	0.0	74
6	3.5	8.2	76.5	3.5	8.2	85

ECL

Sample	Slope ratios vs A					
	% <0.67	% 0.67-0.80	% 0.80-1.25	% 1.25-1.50	% >1.50	n
B	0.0	0.0	87.3	6.3	6.3	63
C	4.8	6.3	85.7	3.2	0.0	63
D	0.0	3.2	85.7	9.5	1.6	63
R	0.0	1.6	87.3	1.6	9.5	63
S	1.6	1.6	84.1	1.6	11.1	63
T	1.6	0.0	87.3	9.5	1.6	63
U	0.0	3.2	85.7	4.8	6.3	63
1	Neg	Neg	Neg	Neg	Neg	0
2	2.2	11.1	84.4	2.2	0.0	45
3	0.0	7.4	81.5	7.4	3.7	54
4	5.6	1.9	81.5	5.6	5.6	54
5	3.7	3.7	85.2	5.6	1.9	54
6	3.9	5.9	66.7	11.8	11.8	51

Other

Sample	Slope ratios vs A					
	% <0.67	% 0.67-0.80	% 0.80-1.25	% 1.25-1.50	% >1.50	n
B	0.0	4.8	95.2	0.0	0.0	21
C	28.6	9.5	9.5	42.9	9.5	21
D	10.0	20.0	55.0	0.0	15.0	20
R	5.0	25.0	70.0	0.0	0.0	20
S	5.6	27.8	50.0	0.0	16.7	18
T	28.6	0.0	71.4	0.0	0.0	21
U	0.0	28.6	66.7	4.8	0.0	21
1	Neg	Neg	Neg	Neg	Neg	0
2	Neg	Neg	Neg	Neg	Neg	0
3	25.0	0.0	75.0	0.0	0.0	8
4	0.0	41.7	58.3	0.0	0.0	12
5	16.7	25.0	58.3	0.0	0.0	12
6	0.0	0.0	57.1	28.6	14.3	21

Binding (all)

Sample	Slope ratios vs A					
	% <0.67	% 0.67-0.80	% 0.80-1.25	% 1.25-1.50	% >1.50	n
B	0.0	4.1	90.6	2.9	2.3	171
C	36.8	12.3	42.1	7.6	1.2	171
D	12.9	15.9	61.2	7.1	2.9	170
R	5.3	16.5	72.9	1.2	4.1	170
S	8.9	13.1	69.6	1.8	6.5	168
T	5.9	3.6	84.6	5.3	0.6	169
U	0.0	18.1	75.4	4.1	2.3	171
1	Neg	Neg	Neg	Neg	Neg	0
2	3.4	10.3	82.8	3.4	0.0	58
3	14.4	4.4	67.8	6.7	6.7	90
4	4.7	14.8	68.5	4.7	7.4	149
5	3.6	11.4	80.0	4.3	0.7	140
6	3.2	6.4	70.7	9.6	10.2	157

n indicates the total number of assays included for determination of parallelism

Sample B

ELISA

Sample	Slope ratios vs B					
	% <0.67	% 0.67-0.80	% 0.80-1.25	% 1.25-1.50	% >1.50	n
A	0.0	1.1	92.0	6.9	0.0	87
C	59.8	10.3	27.6	2.3	0.0	87
D	25.3	9.2	55.2	8.0	2.3	87
R	11.5	12.6	70.1	4.6	1.1	87
S	12.6	13.8	67.8	3.4	2.3	87
T	2.4	7.1	85.9	4.7	0.0	85
U	2.3	10.3	82.8	4.6	0.0	87
1	Neg	Neg	Neg	Neg	Neg	0
2	0.0	15.4	76.9	7.7	0.0	13
3	39.3	0.0	39.3	7.1	14.3	28
4	9.6	6.0	66.3	7.2	10.8	83
5	1.4	2.7	87.8	8.1	0.0	74
6	1.2	2.4	76.5	11.8	8.2	85

ECL

Sample	Slope ratios vs B					
	% <0.67	% 0.67-0.80	% 0.80-1.25	% 1.25-1.50	% >1.50	n
A	6.3	6.3	87.3	0.0	0.0	63
C	4.8	15.9	79.4	0.0	0.0	63
D	0.0	3.2	96.8	0.0	0.0	63
R	3.2	0.0	95.2	0.0	1.6	63
S	1.6	0.0	98.4	0.0	0.0	63
T	0.0	4.8	93.7	1.6	0.0	63
U	0.0	4.8	93.7	1.6	0.0	63
1	Neg	Neg	Neg	Neg	Neg	0
2	2.2	4.4	88.9	4.4	0.0	45
3	5.6	7.4	81.5	5.6	0.0	54
4	5.6	1.9	92.6	0.0	0.0	54
5	5.6	5.6	88.9	0.0	0.0	54
6	2.0	5.9	90.2	2.0	0.0	51

Other

Sample	Slope ratios vs B					
	% <0.67	% 0.67-0.80	% 0.80-1.25	% 1.25-1.50	% >1.50	n
A	0.0	0.0	95.2	4.8	0.0	21
C	28.6	9.5	14.3	33.3	14.3	21
D	5.0	25.0	55.0	0.0	15.0	20
R	0.0	25.0	70.0	5.0	0.0	20
S	0.0	16.7	66.7	0.0	16.7	18
T	19.0	9.5	66.7	4.8	0.0	21
U	0.0	23.8	71.4	0.0	4.8	21
1	Neg	Neg	Neg	Neg	Neg	0
2	Neg	Neg	Neg	Neg	Neg	0
3	25.0	0.0	75.0	0.0	0.0	8
4	0.0	16.7	83.3	0.0	0.0	12
5	16.7	8.3	75.0	0.0	0.0	12
6	0.0	0.0	52.4	33.3	14.3	21

Binding (all)

Sample	Slope ratios vs B					
	% <0.67	% 0.67-0.80	% 0.80-1.25	% 1.25-1.50	% >1.50	n
A	2.3	2.9	90.6	4.1	0.0	171
C	35.7	12.3	45.0	5.3	1.8	171
D	13.5	8.8	70.6	4.1	2.9	170
R	7.1	9.4	79.4	2.9	1.2	170
S	7.1	8.9	79.2	1.8	3.0	168
T	3.6	6.5	86.4	3.6	0.0	169
U	1.2	9.9	85.4	2.9	0.6	171
1	Neg	Neg	Neg	Neg	Neg	0
2	1.7	6.9	86.2	5.2	0.0	58
3	17.8	4.4	67.8	5.6	4.4	90
4	7.4	5.4	77.2	4.0	6.0	149
5	4.3	4.3	87.1	4.3	0.0	140
6	1.3	3.2	77.7	11.5	6.4	157

n indicates the total number of assays included for determination of parallelism

Table 10a: Summary of geometric mean (GM) estimates and geometric coefficients of variation (GCV) for Binding assays. Results versus in-house standards (IH) based on laboratories where results are reported in µg/ml. Results versus A and B are for all laboratories.

Sample	Binding (all)																	
	Estimates vs A (µg/ml)						Estimates vs B (µg/ml)						Estimates vs IH (µg/ml)					
	Range	GM	GCV	Median	10^MAD	N	Range	GM	GCV	Median	10^MAD	N	Range	GM	GCV	Median	10^MAD	N
mAbs																		
B	40.26 - 628.28	98.11	134%	62.50	1.38	23							10.87 - 973.57	102.21	253%	98.36	3.05	16
A							3.98 - 62.09	25.48	134%	40.00	1.38	23	2.52 - 1036.53	42.50	623%	52.73	6.54	16
D	1.46 - 249.57	17.91	276%	22.22	2.13	20	1.54 - 57.78	8.23	150%	7.56	1.94	20	2.60 - 74.52	19.57	247%	36.25	2.01	15
U	0.74 - 235.63	13.25	356%	11.09	2.98	23	0.85 - 20.10	6.83	151%	8.75	1.76	23	2.24 - 67.87	12.50	147%	15.22	1.47	16
R	0.58 - 38.75	5.10	277%	6.43	2.49	23	0.46 - 12.67	2.47	150%	2.80	2.02	22	0.50 - 12.05	4.05	152%	5.27	2.11	16
T	0.77 - 54.72	7.70	187%	10.40	1.94	21	0.82 - 12.86	3.56	134%	3.83	1.73	22	0.47 - 24.79	5.17	220%	6.62	2.06	16
S	0.23 - 211.51	9.07	611%	6.70	5.18	20	0.26 - 30.51	4.11	296%	3.94	3.36	20	0.75 - 114.84	8.27	239%	8.70	1.56	16
C	0.36 - 28.06	3.68	200%	3.08	1.66	17	0.33 - 29.06	1.51	234%	1.19	1.76	18	0.24 - 44.51	2.20	443%	1.66	4.44	13
Median			***276%***		***2.13***				***150%***		***1.76***				***243%***		***2.09***	
Sera																		
2	0.01 - 0.55	0.05	314%	0.05	2.40	7	0.003 - 0.04	0.02	183%	0.02	1.78	7	0.001 - 0.04	0.01	359%	0.01	3.43	4
3	0.01 - 1.65	0.21	342%	0.22	2.44	11	0.01 - 1.46	0.09	326%	0.12	2.05	11	0.01 - 0.25	0.04	291%	0.05	2.69	6
4	0.02 - 3.38	0.20	299%	0.26	3.02	21	0.02 - 3.69	0.10	276%	0.07	1.90	21	0.01 - 0.62	0.11	284%	0.13	3.11	14
5	0.04 - 4.39	0.30	273%	0.45	3.62	21	0.04 - 5.06	0.16	225%	0.11	1.74	21	0.03 - 1.17	0.18	240%	0.21	2.60	14
6	1.14 - 53.31	4.62	173%	5.00	2.30	22	0.78 - 17.58	2.47	130%	1.83	1.41	22	0.38 - 68.20	3.81	354%	4.06	2.73	15
Median			***276%***		***2.40***				***225%***		***1.76***				***291%***		***2.73***	

n/a = not calculated as N < 3; Neg = Sample reported as negative or below assay lower quantitation limit; NP = non-parallel to standard; MAD = median absolute deviation; N = number of estimates used in calculation. All mAbs have been ranked from high to low based on the geometric mean estimates from ELISAs calculated versus IH/kit standard.

Table 10b: Summary of geometric mean (GM) estimates and geometric coefficients of variation (GCV) for Binding assays. Results based only on laboratories whose results versus IH standards are reported in µg/ml

Sample	Binding (all)																	
	Estimates vs A (µg/ml)						Estimates vs B (µg/ml)						Estimates vs IH (µg/ml)					
	Range	GM	GCV	Median	10^MAD	N	Range	GM	GCV	Median	10^MAD	N	Range	GM	GCV	Median	10^MAD	N
mAbs																		
B	40.26 - 628.28	120.57	145%	85.37	1.92	16							10.87 - 973.57	102.21	253%	98.36	3.05	16
A							3.98 - 62.09	20.73	145%	29.29	1.92	16	2.52 - 1036.53	42.50	623%	52.73	6.54	16
D	1.46 - 249.57	27.26	249%	36.23	1.94	14	1.54 - 57.78	10.14	156%	9.42	1.82	14	2.60 - 74.52	19.57	247%	36.25	2.01	15
U	0.74 - 235.63	15.18	455%	16.75	3.64	16	0.85 - 20.10	6.36	180%	7.95	1.99	16	2.24 - 67.87	12.50	147%	15.22	1.47	16
R	0.58 - 38.75	4.66	311%	6.41	3.43	16	0.46 - 6.16	2.03	125%	2.47	1.97	16	0.50 - 12.05	4.05	152%	5.27	2.11	16
T	0.77 - 54.72	7.76	220%	8.58	2.03	14	0.82 - 7.01	2.74	113%	3.41	1.72	15	0.47 - 24.79	5.17	220%	6.62	2.06	16
S	0.47 - 211.51	10.00	545%	6.27	4.86	16	0.51 - 27.24	4.12	254%	3.75	3.26	16	0.75 - 114.84	8.27	239%	8.70	1.56	16
C	1.24 - 14.72	4.00	107%	3.53	1.42	10	0.41 - 2.21	1.02	77%	1.18	1.31	11	0.24 - 44.51	2.20	443%	1.66	4.44	13
Median			***249%***		***2.03***				***145%***		***1.92***				***243%***		***2.09***	
Sera																		
2	0.02	0.06	375%	0.04	1.80	4	0.003 - 0.04	0.01	224%	0.01	2.65	4	0.001 - 0.04	0.01	359%	0.01	3.43	4
3	0.01	0.14	448%	0.16	1.66	6	0.01 - 0.13	0.04	215%	0.04	2.46	6	0.01 - 0.25	0.04	291%	0.05	2.69	6
4	0.02	0.14	225%	0.13	2.00	14	0.02 - 0.16	0.06	84%	0.06	1.65	14	0.01 - 0.62	0.11	284%	0.13	3.11	14
5	0.04	0.22	245%	0.20	2.43	14	0.04 - 0.28	0.09	75%	0.08	1.42	14	0.03 - 1.17	0.18	240%	0.21	2.60	14
6	1.14	3.95	170%	3.31	2.02	15	0.78 - 4.39	1.73	54%	1.66	1.34	15	0.38 - 68.20	3.81	354%	4.06	2.73	15
Median			***245%***		***2.00***				***84%***		***1.65***				***291%***		***2.73***	

n/a = not calculated as N < 3; Neg = Sample reported as negative or below assay lower quantitation limit; NP = non-parallel to standard; MAD = median absolute deviation; N = number of estimates used in calculation.

Table 10c: Summary of geometric mean (GM) estimates and geometric coefficients of variation (GCV) for Neutralisation assays. Results versus A and B are for all laboratories.

Sample	Neut (all)											
	Estimates vs A (µg/ml)						Estimates vs B (µg/ml)					
	Range	GM	GCV	Median	10^MAD	N	Range	GM	GCV	Median	10^MAD	N
mAbs												
B	5.22 - 56.11	29.27	165%	41.84	1.11	5						
A							44.56 - 478.64	85.41	165%	59.75	1.11	5
D	1.03 - 2.01	1.44	n/a	1.44	n/a	2	3.57 - 22.32	8.92	n/a	8.92	n/a	2
U	0.39 - 7.37	1.59	234%	1.50	2.05	4	1.85 - 7.94	3.28	96%	2.80	1.44	4
R	1.38 - 8.39	2.69	120%	2.13	1.29	4	1.74 - 21.34	5.51	212%	4.98	2.28	4
T	1.76 - 7.19	4.69	85%	6.71	1.07	5	2.56 - 69.67	8.84	326%	5.85	1.74	4
S	0.41 - 4.12	1.21	235%	1.13	2.63	4	0.56 - 25.39	3.97	576%	4.43	5.73	3
C	5.26 - 30.85	12.74	n/a	12.74	n/a	2	1.08 - 47.96	11.97	712%	33.21	1.44	3
Sera												
2	0.04 - 0.04	0.04	n/a	0.04	n/a	1	0.06 - 0.06	0.06	n/a	0.06	n/a	1
3	0.06 - 0.30	0.16	137%	0.22	1.36	3	0.08 - 2.33	0.35	463%	0.24	3.08	3
4	0.09 - 0.47	0.24	143%	0.32	1.50	3	0.12 - 4.55	0.54	381%	0.40	2.28	4
5	0.15 - 0.34	0.23	n/a	0.23	n/a	2	0.21 - 4.59	0.64	455%	0.28	1.33	3
6	1.17 - 7.60	3.56	109%	3.40	1.84	5	0.95 - 66.94	6.14	374%	4.47	1.90	5

n/a = not calculated as N < 3; Neg = Sample reported as negative or below assay lower quantitation limit; NP = non-parallel to standard; MAD = median absolute deviation; N = number of estimates used in calculation. Results versus IH standards were reported as pos/neg or in titers (not shown)

Table 11a: Summary of geometric mean (GM) estimates and geometric coefficients of variation (GCV) for ELISAs. Results versus in-house standards (IH) based on laboratories where results are reported in µg/ml. Results versus A and B are for all laboratories.

Sample	ELISA																	
	Estimates vs A (µg/ml)						Estimates vs B (µg/ml)						Estimates vs IH (µg/ml)					
	Range	GM	GCV	Median	10^MAD	N	Range	GM	GCV	Median	10^MAD	N	Range	GM	GCV	Median	10^MAD	N
mAbs																		
B	44.23 - 462.36	76.37	127%	55.09	1.15	11							28.28 - 973.57	108.91	272%	87.36	3.01	7
A							5.41 - 56.52	32.73	127%	45.38	1.15	11	3.06 - 1036.53	56.49	741%	83.10	4.22	7
D	1.46 - 43.12	10.64	292%	21.81	1.98	9	1.54 - 33.99	6.34	189%	4.60	1.88	9	2.60 - 74.52	18.52	324%	38.68	1.44	6
U	0.77 - 54.70	7.50	236%	9.81	1.74	11	0.85 - 14.85	4.92	135%	5.92	1.58	11	2.24 - 16.37	9.10	132%	15.28	1.07	7
R	0.58 - 38.75	4.89	293%	6.43	2.30	11	0.64 - 8.63	2.72	152%	3.50	1.62	10	1.51 - 12.05	6.04	131%	9.49	1.27	7
T	0.77 - 13.85	4.33	144%	5.10	1.96	11	0.82 - 12.86	2.84	165%	2.62	2.43	11	0.47 - 15.23	4.36	342%	7.52	2.00	7
S	0.23 - 16.28	3.10	289%	4.64	1.86	10	0.26 - 7.17	1.92	192%	2.55	1.97	10	0.75 - 10.13	4.05	198%	7.01	1.44	7
C	0.36 - 12.44	2.31	237%	3.02	1.70	7	0.33 - 15.77	1.20	255%	0.82	2.08	8	0.24 - 7.35	0.98	392%	0.72	2.63	4
Median			***237%***		***1.86***				***165%***		***1.88***				***298%***		***1.72***	
Sera																		
2	0.01 - 0.05	0.02	n/a	0.02	n/a	2	0.01 - 0.04	0.02	n/a	0.02	n/a	2	Neg	Neg	n/a	Neg	n/a	0
3	0.07 - 0.40	0.17	n/a	0.17	n/a	2	0.06 - 0.33	0.15	n/a	0.15	n/a	2	Neg	Neg	n/a	Neg	n/a	0
4	0.02 - 3.38	0.13	337%	0.09	2.40	10	0.02 - 3.69	0.08	378%	0.05	1.81	10	0.02 - 0.62	0.08	312%	0.10	2.97	6
5	0.04 - 4.39	0.21	305%	0.12	2.36	10	0.04 - 5.06	0.13	332%	0.09	1.50	10	0.03 - 1.17	0.14	303%	0.14	2.90	6
6	1.14 - 12.22	3.12	133%	2.53	1.96	11	0.78 - 13.81	2.06	135%	1.58	1.53	11	0.45 - 22.86	3.24	336%	4.06	2.23	7
Median			***237%***		***1.96***				***255%***		***1.81***				***312%***		***2.63***	

MAD = median absolute deviation; N = number of estimates used in calculation

Table 11b: Summary of geometric mean (GM) estimates and geometric coefficients of variation (GCV) for ELISAs.
Results based only on laboratories whose results versus IH standards are reported in µg/ml

Sample	ELISA																	
	Estimates vs A (µg/ml)						Estimates vs B (µg/ml)						Estimates vs IH (µg/ml)					
	Range	GM	GCV	Median	10^MAD	N	Range	GM	GCV	Median	10^MAD	N	Range	GM	GCV	Median	10^MAD	N
mAbs																		
B	46.25 - 462.36	96.75	162%	61.62	1.17	7							28.28 - 973.57	108.91	272%	87.36	3.01	7
A							5.41 - 54.05	25.84	162%	40.57	1.17	7	3.06 - 1036.53	56.49	741%	83.10	4.22	7
D	1.46 - 43.12	18.59	326%	37.91	1.14	5	1.54 - 33.99	7.82	246%	5.78	3.63	5	2.60 - 74.52	18.52	324%	38.68	1.44	6
U	0.77 - 54.70	8.08	319%	9.81	2.58	7	0.85 - 9.34	4.19	143%	5.92	1.53	7	2.24 - 16.37	9.10	132%	15.28	1.07	7
R	0.58 - 38.75	5.38	316%	6.43	2.65	7	0.64 - 6.16	2.79	133%	4.19	1.47	7	1.51 - 12.05	6.04	131%	9.49	1.27	7
T	0.77 - 9.98	3.90	137%	5.10	1.45	7	0.82 - 7.01	2.02	132%	1.95	2.06	7	0.47 - 15.23	4.36	342%	7.52	2.00	7
S	0.47 - 16.28	3.58	225%	4.60	1.83	7	0.51 - 4.08	1.86	118%	1.76	2.10	7	0.75 - 10.13	4.05	198%	7.01	1.44	7
C	2.71 - 5.13	3.48	41%	3.02	1.12	3	0.41 - 1.79	0.79	91%	0.73	1.52	4	0.24 - 7.35	0.98	392%	0.72	2.63	4
Median			***225%***		***1.45***				***133%***		***1.53***				***298%***		***1.72***	
Sera																		
2	Neg	Neg		Neg			Neg	Neg		Neg			Neg	Neg		Neg		
3	Neg	Neg		Neg			Neg	Neg		Neg			Neg	Neg		Neg		
4	0.02 - 0.26	0.08	166%	0.09	2.40	6	0.02 - 0.09	0.04	77%	0.03	1.30	6	0.02 - 0.62	0.08	312%	0.10	2.97	6
5	0.04 - 0.54	0.13	168%	0.11	2.33	6	0.04 - 0.12	0.06	50%	0.06	1.28	6	0.03 - 1.17	0.14	303%	0.14	2.90	6
6	1.14 - 9.31	2.75	111%	2.53	1.96	7	0.78 - 2.87	1.45	60%	1.24	1.30	7	0.45 - 22.86	3.24	336%	4.06	2.23	7
Median			***166%***		***1.96***				***77%***		***1.30***				***312%***		***2.63***	

MAD = median absolute deviation; N = number of estimates used in calculation

Table 12a: Summary of geometric mean (GM) estimates and geometric coefficients of variation (GCV) for ECL assays.
Results versus in-house standards (IH) based on laboratories where results are reported in µg/ml. Results versus A and B are for all laboratories.

Sample	ECL																	
	Estimates vs A (µg/ml)						Estimates vs B (µg/ml)						Estimates vs IH (µg/ml)					
	Range	GM	GCV	Median	10^MAD	N	Range	GM	GCV	Median	10^MAD	N	Range	GM	GCV	Median	10^MAD	N
mAbs																		
B	106.42 - 628.28	224.97	71%	215.97	1.18	7							10.87 - 210.79	51.71	205%	38.94	2.08	6
A							3.98 - 23.49	11.11	71%	11.58	1.18	7	2.52 - 58.12	11.15	251%	11.07	3.22	6
D	15.30 - 249.57	51.78	159%	44.99	1.95	7	3.58 - 57.78	11.41	152%	9.59	1.66	7	2.90 - 72.86	13.22	243%	11.60	2.95	6
U	33.09 - 235.63	70.48	87%	71.43	1.45	7	12.36 - 20.10	15.98	17%	15.55	1.05	7	4.37 - 67.87	17.25	182%	13.22	1.99	6
R	0.74 - 29.36	9.18	234%	13.74	1.39	7	0.46 - 4.02	2.27	110%	2.69	1.17	7	0.50 - 9.15	2.10	155%	1.91	1.28	6
T	11.59 - 54.72	20.81	65%	17.44	1.22	7	3.41 - 6.30	4.61	27%	4.35	1.25	7	1.37 - 24.79	4.88	187%	3.52	1.81	6
S	35.05 - 211.51	84.19	78%	76.93	1.31	7	12.38 - 30.51	18.32	40%	16.83	1.23	7	3.87 - 114.84	17.40	232%	12.04	1.91	6
C	2.15 - 14.72	4.83	101%	4.08	1.34	7	0.42 - 2.21	1.06	69%	1.18	1.21	7	0.25 - 4.98	1.17	165%	1.17	1.33	6
Median			***87%***		***1.34***				***69%***		***1.21***				***196%***		***1.95***	
Sera																		
2	0.02 - 0.55	0.07	301%	0.05	2.34	5	0.003 - 0.04	0.01	196%	0.02	1.78	5	0.003 - 0.04	0.01	359%	0.01	3.43	4
3	0.09 - 1.65	0.29	193%	0.24	2.23	6	0.02 - 0.15	0.06	137%	0.08	1.68	6	0.01 - 0.25	0.04	358%	0.08	3.20	5
4	0.26 - 2.06	0.44	125%	0.30	1.15	6	0.06 - 0.16	0.10	54%	0.11	1.32	6	0.01 - 0.41	0.08	259%	0.08	1.89	5
5	0.45 - 3.46	0.76	117%	0.56	1.22	6	0.11 - 0.28	0.17	40%	0.17	1.24	6	0.03 - 0.56	0.13	194%	0.14	1.64	5
6	4.90 - 53.31	10.34	135%	7.62	1.33	6	1.44 - 4.39	2.33	47%	2.24	1.25	6	0.38 - 9.16	1.82	235%	1.49	2.38	5
Median			***121%***		***1.33***				***61%***		***1.25***				***233%***		***1.93***	

MAD = median absolute deviation; N = number of estimates used in calculation

Table 12b: Summary of geometric mean (GM) estimates and geometric coefficients of variation (GCV) for ECL assays.
Results based only on laboratories whose results versus IH standards are reported in µg/ml

Sample	ECL																	
	Estimates vs A (µg/ml)						Estimates vs B (µg/ml)						Estimates vs IH (µg/ml)					
	Range	GM	GCV	Median	10^MAD	N	Range	GM	GCV	Median	10^MAD	N	Range	GM	GCV	Median	10^MAD	N
mAbs																		
B	106.42 - 628.28	229.45	79%	223.99	1.23	6							10.87 - 210.79	51.71	205%	38.94	2.08	6
A							3.98 - 23.49	10.90	79%	11.16	1.23	6	2.52 - 58.12	11.15	251%	11.07	3.22	6
D	15.30 - 249.57	59.25	162%	54.66	1.78	6	3.58 - 57.78	12.78	161%	11.71	1.73	6	2.90 - 72.86	13.22	243%	11.60	2.95	6
U	33.09 - 235.63	74.78	94%	73.71	1.35	6	14.87 - 20.10	16.68	13%	15.82	1.05	6	4.37 - 67.87	17.25	182%	13.22	1.99	6
R	0.74 - 29.36	8.36	265%	11.65	1.68	6	0.46 - 3.89	2.06	114%	2.51	1.14	6	0.50 - 9.15	2.10	155%	1.91	1.28	6
T	11.59 - 54.72	21.88	70%	19.30	1.26	6	3.41 - 6.30	4.72	29%	4.87	1.25	6	1.37 - 24.79	4.88	187%	3.52	1.81	6
S	35.05 - 211.51	78.32	82%	69.85	1.26	6	12.38 - 27.24	16.83	31%	16.65	1.14	6	3.87 - 114.84	17.40	232%	12.04	1.91	6
C	2.15 - 14.72	5.21	108%	4.49	1.75	6	0.42 - 2.21	1.13	73%	1.19	1.12	6	0.25 - 4.98	1.17	165%	1.17	1.33	6
Median			***94%***		***1.35***				***73%***		***1.14***				***196%***		***1.95***	
Sera																		
2	0.02 - 0.55	0.06	375%	0.04	1.80	4	0.003 - 0.04	0.01	224%	0.01	2.65	4	0.003 - 0.04	0.01	359%	0.01	3.43	4
3	0.09 - 1.65	0.25	211%	0.22	1.90	5	0.02 - 0.13	0.05	135%	0.06	2.13	5	0.01 - 0.25	0.04	358%	0.08	3.20	5
4	0.26 - 2.06	0.42	145%	0.27	1.01	5	0.06 - 0.16	0.09	58%	0.10	1.60	5	0.01 - 0.41	0.08	259%	0.08	1.89	5
5	0.45 - 3.46	0.75	138%	0.51	1.15	5	0.11 - 0.28	0.17	44%	0.14	1.31	5	0.03 - 0.56	0.13	194%	0.14	1.64	5
6	4.90 - 53.31	10.03	159%	7.41	1.10	5	1.44 - 4.39	2.24	52%	2.17	1.21	5	0.38 - 9.16	1.82	235%	1.49	2.38	5
Median			***141%***		***1.31***				***65%***		***1.26***				***233%***		***1.93***	

MAD = median absolute deviation; N = number of estimates used in calculation

Table 13a: Summary of geometric mean (GM) estimates and geometric coefficients of variation (GCV) for other binding assays.
Results versus in-house standards (IH) based on laboratories where results are reported in µg/ml. Results versus A and B are for all laboratories.

Sample	Other																	
	Estimates vs A (µg/ml)						Estimates vs B (µg/ml)						Estimates vs IH (µg/ml)					
	Range	GM	GCV	Median	10^MAD	N	Range	GM	GCV	Median	10^MAD	N	Range	GM	GCV	Median	10^MAD	N
mAbs																		
B	40.26-68.48	53.26	23%	50.64	1.23	5							301.20 - 421.43	344.27	19%	321.45	1.15	3
A							36.51 - 62.09	46.94	23%	49.37	1.23	5	236.11- 393.55	317.86	30%	345.61	1.07	3
D	5.06 - 17.72	9.02	69%	8.58	1.40	4	5.19 - 12.94	8.38	46%	8.57	1.28	4	30.14 - 61.86	47.86	49%	58.79	1.03	3
U	0.74 - 10.21	4.46	189%	6.59	1.55	5	0.92 - 11.40	4.29	169%	4.81	1.82	5	5.67 - 27.74	13.79	125%	16.67	1.29	3
R	0.64 - 12.83	2.45	247%	1.54	2.41	5	0.79 - 12.67	2.29	257%	1.12	1.42	5	5.23 - 7.27	5.86	20%	5.30	1.17	3
T	1.93 - 12.08	6.24	177%	10.40	1.16	3	1.03 - 12.29	4.20	229%	4.97	2.26	4	6.03 - 14.94	8.68	61%	7.28	1.43	3
S	1.30 - 2.72	1.79	46%	1.62	1.25	3	0.99 - 3.38	1.58	94%	1.19	1.20	3	5.60 - 21.67	9.90	102%	7.99	1.43	3
C	1.24 - 28.08	5.79	377%	5.59	4.52	3	1.55 - 29.06	6.35	335%	5.68	3.67	3	15.11 - 44.51	22.60	80%	17.15	1.61	3
Sera																		
2	Neg	Neg	n/a	Neg	n/a	0	Neg	Neg	n/a	Neg	n/a	0	Neg	Neg	n/a	Neg	n/a	0
3	0.01 - 1.53	0.13	1220%	0.15	10.12	3	0.01 - 1.46	0.11	1398%	0.15	9.48	3	0.04 - 0.04	0.04	n/a	0.04	n/a	1
4	0.05 - 1.84	0.18	375%	0.08	1.48	5	0.04 - 1.82	0.17	402%	0.07	1.67	5	0.41 - 0.47	0.44	7%	0.42	1.06	3
5	0.05 - 1.15	0.21	269%	0.11	2.09	5	0.07 - 1.22	0.20	290%	0.08	1.23	5	0.46 - 0.72	0.58	25%	0.59	1.10	3
6	1.54 - 17.80	4.19	196%	2.28	1.47	5	1.66 - 17.58	3.91	212%	1.86	1.12	5	7.72 - 68.20	18.95	212%	12.93	2.30	3

MAD = median absolute deviation; N = number of estimates used in calculation

Table 13b: Summary of geometric mean (GM) estimates and geometric coefficients of variation (GCV) for other binding assays. Results based only on laboratories whose results versus IH standards are reported in µg/ml

Sample	Other																	
	Estimates vs A (µg/ml)						Estimates vs B (µg/ml)						Estimates vs IH (µg/ml)					
	Range	GM	GCV	Median	10^MAD	N	Range	GM	GCV	Median	10^MAD	N	Range	GM	GCV	Median	10^MAD	N
mAbs																		
B	40.26 - 68.48	55.65	33%	62.50	1.10	3							301.20 - 421.43	344.27	19%	321.45	1.15	3
A							36.51 - 62.09	44.93	33%	40.00	1.10	3	236.11- 393.55	317.86	30%	345.61	1.07	3
D	7.44 - 17.72	10.93	56%	9.90	1.33	3	7.95 - 12.94	9.83	28%	9.24	1.16	3	30.14 - 61.86	47.86	49%	58.79	1.03	3
U	0.74 - 6.59	2.72	216%	4.12	1.60	3	0.92 - 4.81	2.44	138%	3.29	1.46	3	5.67 - 27.74	13.79	125%	16.67	1.29	3
R	0.64 - 1.54	1.04	56%	1.14	1.35	3	0.79 - 1.12	0.92	19%	0.89	1.12	3	5.23 - 7.27	5.86	20%	5.30	1.17	3
T	1.93 - 1.93	1.93	n/a	1.93	n/a	1	1.03 - 2.40	1.57	n/a	1.57	n/a	2	6.03 - 14.94	8.68	61%	7.28	1.43	3
S	1.30 - 2.72	1.79	46%	1.62	1.25	3	0.99 - 3.38	1.58	94%	1.19	1.20	3	5.60 - 21.67	9.90	102%	7.99	1.43	3
C	1.24 - 1.24	1.24	n/a	1.24	n/a	1	1.55 - 1.55	1.55	n/a	1.55	n/a	1	15.11 - 44.51	22.60	80%	17.15	1.61	3
Sera																		
2	Neg	Neg	n/a	Neg	n/a	0	Neg	Neg	n/a	Neg	n/a	0	Neg	Neg	n/a	Neg	n/a	0
3	0.01 - 0.01	0.01	n/a	0.01	n/a	1	0.01 - 0.01	0.01	n/a	0.01	n/a	1	0.04 - 0.04	0.04	n/a	0.04	n/a	1
4	0.05 - 0.08	0.06	23%	0.06	1.08	3	0.04 - 0.07	0.06	29%	0.06	1.24	3	0.41 - 0.47	0.44	7%	0.42	1.06	3
5	0.05 - 0.11	0.08	46%	0.09	1.25	3	0.07 - 0.08	0.07	11%	0.07	1.08	3	0.46 - 0.72	0.58	25%	0.59	1.10	3
6	1.54 - 2.28	1.94	22%	2.08	1.10	3	1.66 - 1.86	1.72	7%	1.66	1.00	3	7.72 - 68.20	18.95	212%	12.93	2.30	3

MAD = median absolute deviation; N = number of estimates used in calculation

Table 14a: Summary of geometric mean (GM) estimates and geometric coefficients of variation (GCV) obtained for the different binding assays (except for assays where data was limited, shaded blue) when A is used as the common standard

Sample	ELISA		ECL		CLIA		LF	RIA	HMSA
	GM	GCV	GM	GCV	Lab 5b	Lab 20	Lab 18	Lab 21	19a
mAbs									
B	76.37	127%	224.97	71%	62.50	68.48	40.26	49.13	50.64
D	10.64	292%	51.78	159%	9.90	17.72	7.44	5.06	NP
U	7.50	236%	70.48	87%	4.12	6.59	0.74	8.60	10.21
R	4.89	293%	9.18	234%	1.14	1.54	0.64	6.13	12.83
T	4.33	144%	20.81	65%	NP	NP	1.93	12.08	10.40
S	3.10	289%	84.19	78%	1.30	1.62	2.72	Neg	NP
C	2.31	237%	4.83	101%	NP	NP	1.24	5.59	28.06
Sera									
2	0.02	n/a	0.07	301%	Neg	Neg	Neg	Neg	Neg
3	0.17	n/a	0.29	193%	Neg	0.01	Neg	0.15	1.53
4	0.13	337%	0.44	125%	0.05	0.08	0.06	0.42	1.84
5	0.21	305%	0.76	117%	0.09	0.11	0.05	0.59	1.15
6	3.12	133%	10.34	135%	2.08	2.28	1.54	9.92	17.80

n/a = not calculated as N < 3; Neg = Sample reported as negative or below assay lower quantitation limit; NP = non-parallel to standard. Results for ELISAs and ECLs relative to A are for all laboratories

Table 14b: Summary of geometric mean (GM) estimates and geometric coefficients of variation (GCV) obtained for the different binding assays when B is used as the common standard

Sample	ELISA		ECL		CLIA		LF	RIA	HMSA
	GM	GCV	GM	GCV	Lab 5b	Lab 20	Lab 18	Lab 21	19a
mAbs									
A	32.73	127%	11.11	71%	40.00	36.51	62.09	50.88	49.37
D	6.34	189%	11.41	152%	7.95	12.94	9.24	5.19	NP
U	4.92	135%	15.98	17%	3.29	4.81	0.92	8.75	11.40
R	2.72	152%	2.27	110%	0.89	1.12	0.79	6.28	12.67
T	2.84	165%	4.61	27%	1.03	NP	2.40	12.29	10.27
S	1.92	192%	18.32	40%	0.99	1.19	3.38	Neg	NP
C	1.20	255%	1.06	69%	NP	NP	1.55	5.68	29.06
Sera									
2	0.02	n/a	0.01	196%	Neg	Neg	Neg	Neg	Neg
3	0.15	n/a	0.06	137%	Neg	0.01	Neg	0.15	1.46
4	0.08	378%	0.10	54%	0.04	0.06	0.07	0.43	1.82
5	0.13	332%	0.17	40%	0.07	0.08	0.07	0.60	1.22
6	2.06	135%	2.33	47%	1.66	1.66	1.86	10.10	17.58

n/a = not calculated as N < 3; Neg = Sample reported as negative or below assay lower quantitation limit; NP = non-parallel to standard. Results for ELISAs and ECLs relative to B are for all laboratories

Table 15a: Summary of geometric mean (GM) estimates obtained for the different neutralisation assays when A is used as the common standard. Results based on all laboratories

Sample	ECL		ELISA		RGA
	Lab 8c	Lab 13b	Lab 14b	Lab 19b	Lab 22
mAbs					
B	37.72	5.22	56.11	41.84	46.45
D	1.03	2.01	Neg	NP	NP
U	1.39	0.39	NP	1.62	7.37
R	1.98	2.28	NP	1.38	8.39
T	1.76	7.19	6.71	3.72	7.16
S	0.45	2.84	NP	0.41	4.12
C	NP	5.26	NP	NP	30.85
Sera					
2	0.04	Neg	Neg	Neg	Neg
3	0.06	0.22	0.30	Neg	NP
4	0.09	0.47	0.32	NP	NP
5	0.15	NP	0.34	NP	NP
6	3.40	6.27	1.17	3.03	7.60

Table 15b: Summary of geometric mean (GM) estimates obtained for the different neutralisation assays when B is used as the common standard. Results based on all laboratories

Sample	ECL		ELISA		RGA
	Lab 8c	Lab 13b	Lab 14b	Lab 19b	Lab 22
mAbs					
A	66.28	478.64	44.56	59.75	53.82
D	NP	22.32	Neg	3.57	NP
U	1.85	3.82	NP	2.05	7.94
R	2.74	21.34	NP	1.74	9.03
T	2.56	69.67	NP	4.44	7.71
S	0.56	25.39	NP	NP	4.43
C	NP	47.96	NP	1.08	33.21
Sera					
2	0.06	Neg	Neg	Neg	Neg
3	0.08	2.33	0.24	Neg	NP
4	0.12	4.55	0.26	NP	0.62
5	0.21	4.59	0.28	NP	NP
6	4.47	66.94	0.95	3.62	8.52

Neg = Sample reported as negative or below assay lower quantitation limit; NP = non-parallel to standard.

Figure 4: Geometric mean estimates obtained in ELISAs of different laboratories for monoclonal antibodies (Top panel) and serum samples (Bottom panel) relative to A, B and in-house standard (IH). For IH, estimates from labs which provided data in 'µg' included

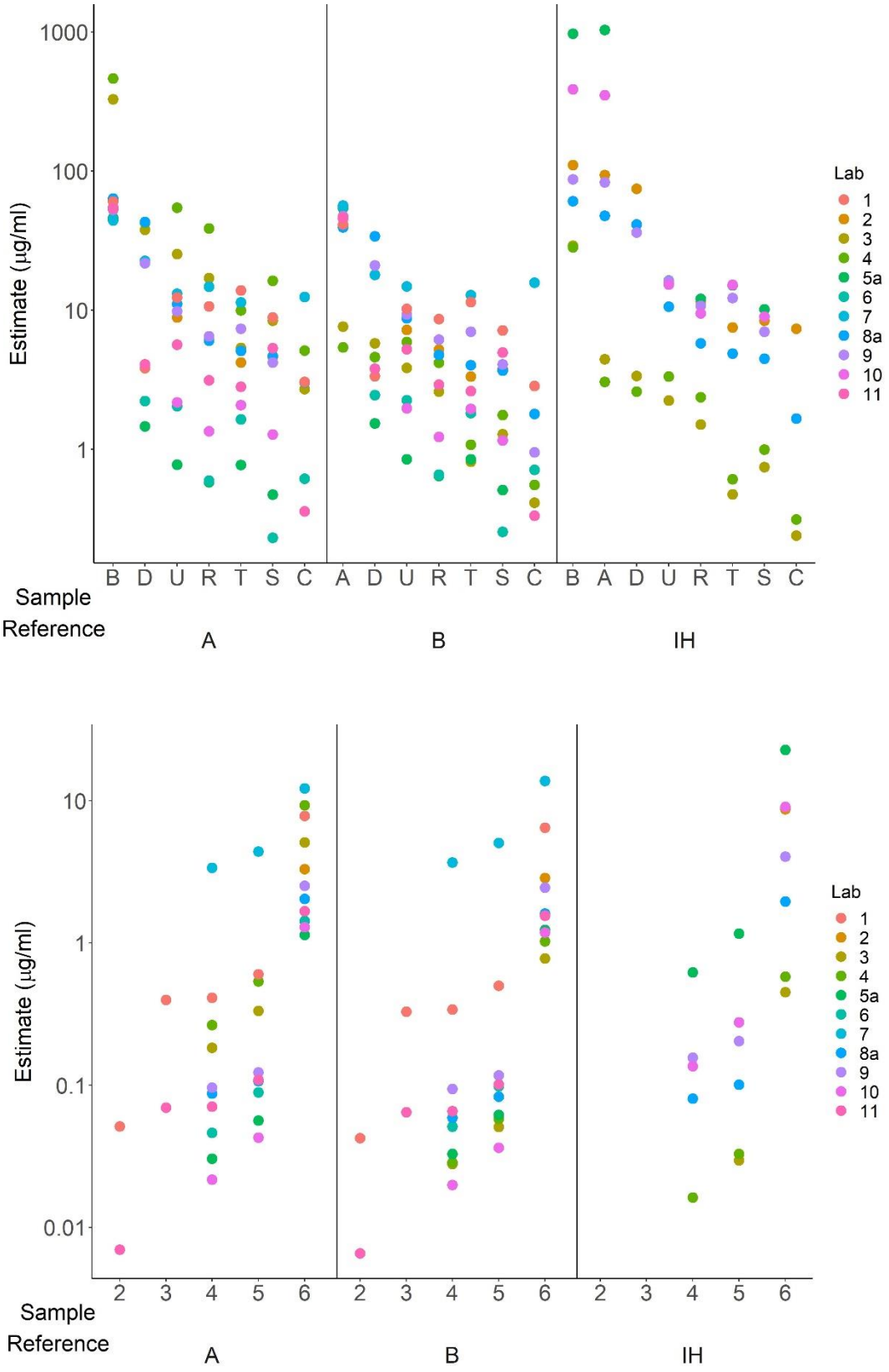

Figure 5: Geometric mean estimates obtained in ECLs of different laboratories for monoclonal antibodies (Top panel) and serum samples (Bottom panel) relative to A, B and in-house standard (IH). For IH, estimates from labs which provided data in ‘µg’ included

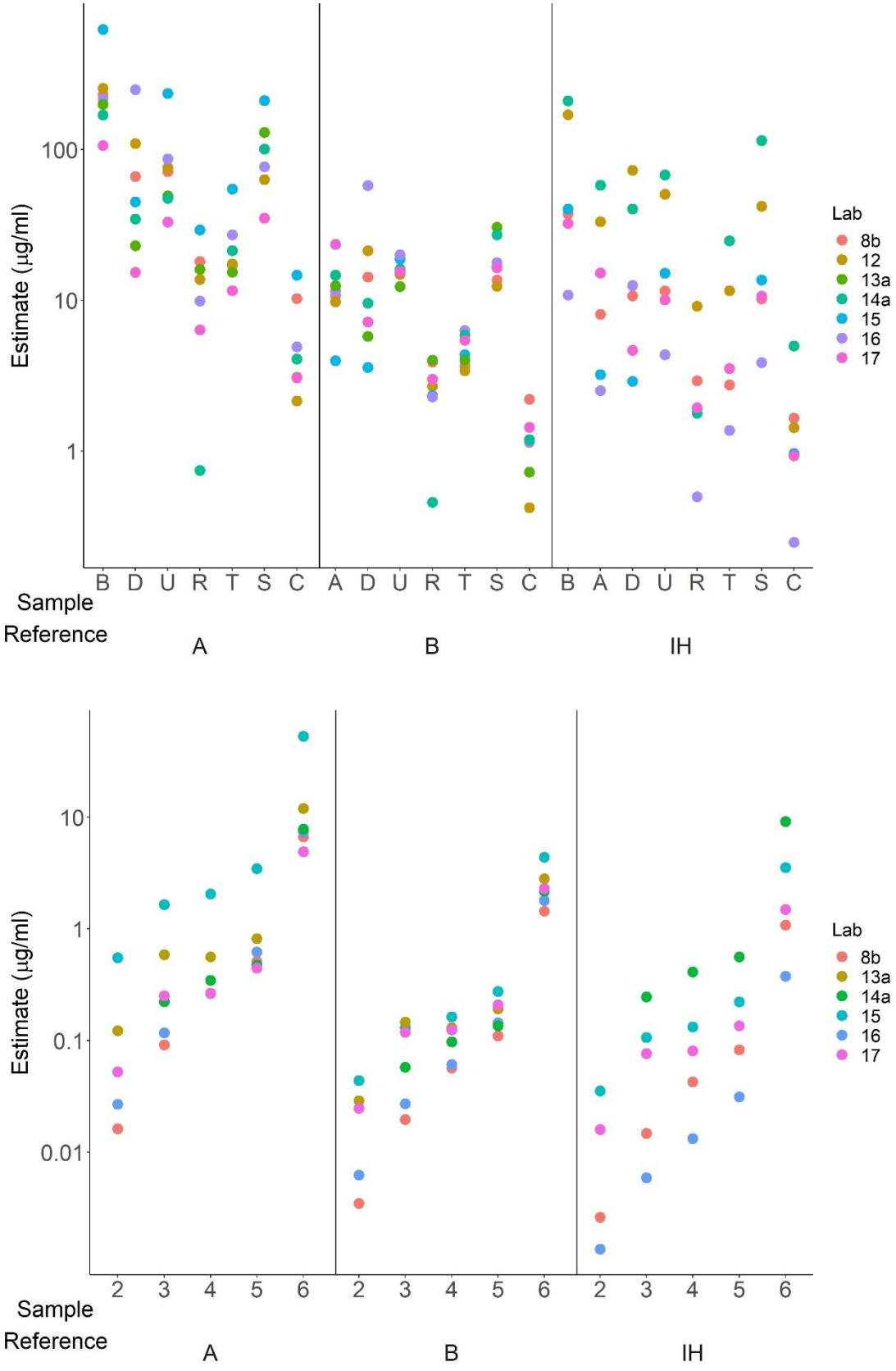

Figure 6: Geometric mean estimates obtained in different assays for monoclonal antibodies (Top panel) and serum samples (Bottom panel) relative to A, B and in-house standard (IH). For IH, estimates from labs which provided data in 'µg' included

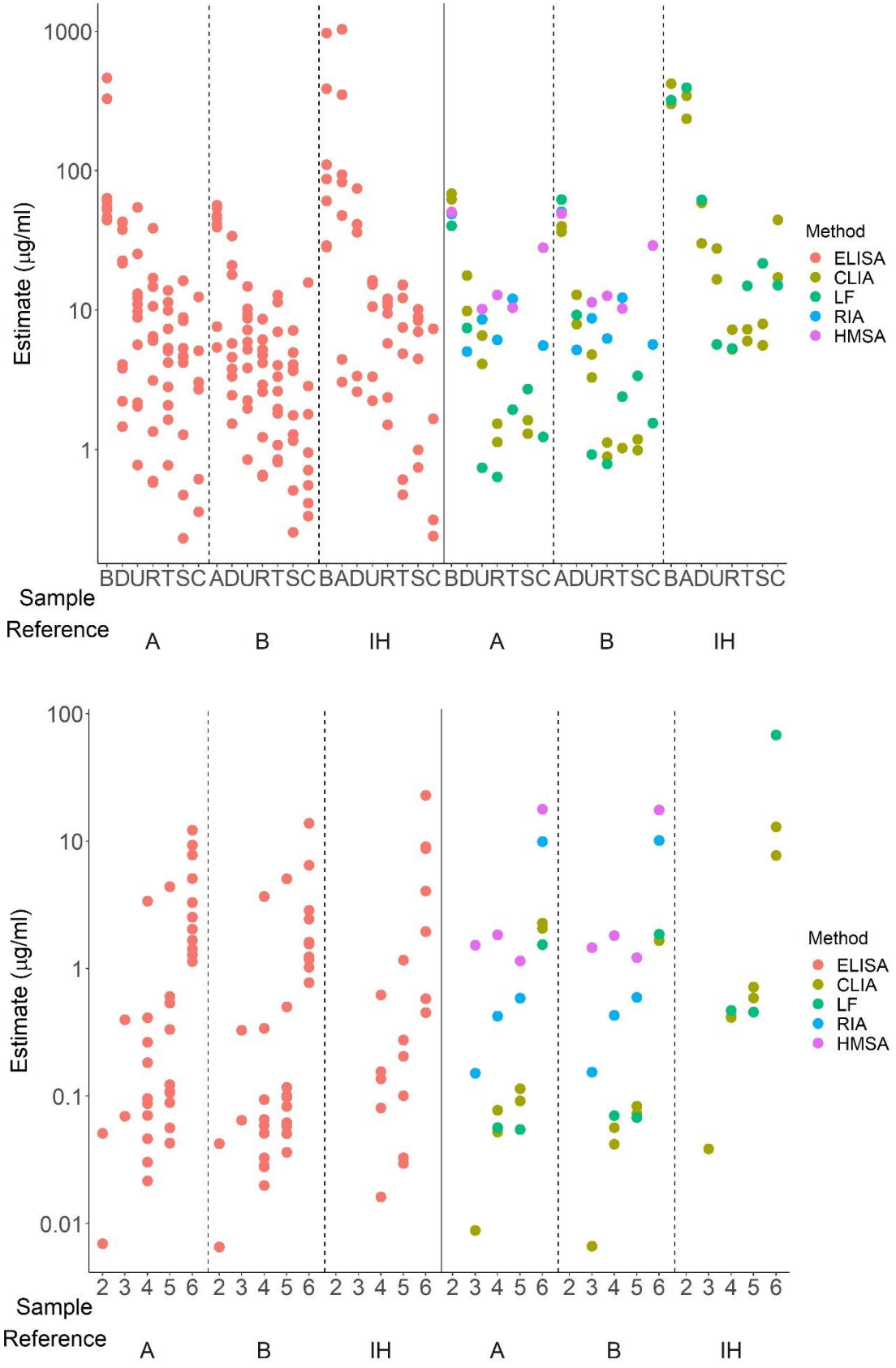

Figure 7: Geometric mean estimates obtained in different ELISAs and ECL assays for monoclonal antibodies (Top panel) and serum samples (Bottom panel) relative to A, B and in-house standard (IH). For IH, estimates from labs which provided data in 'µg' included

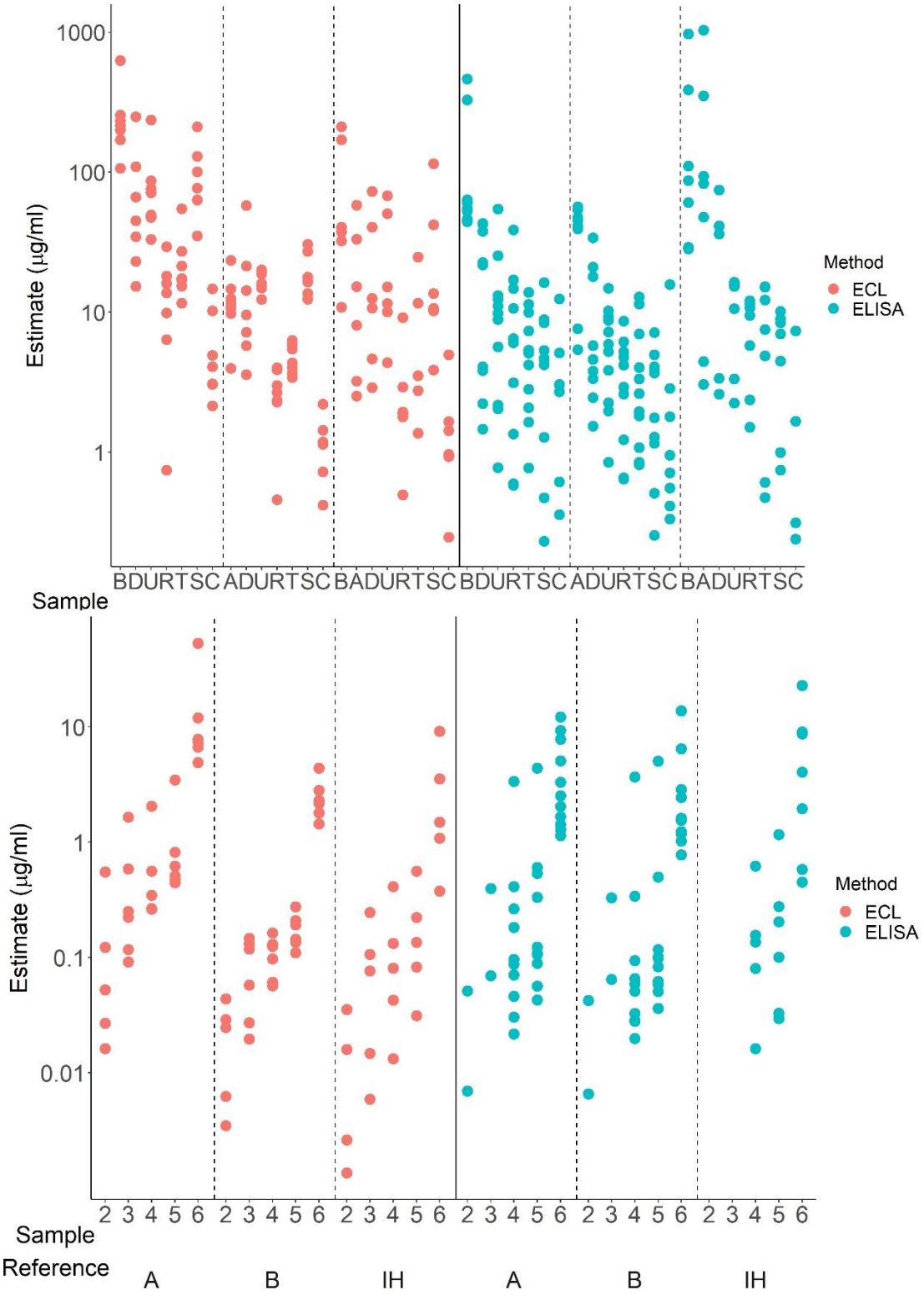

Table 16: Summary of results from accelerated temperature degradation studies of candidate preparations

Method	Sample	Time stored (years)	Storage Temperature (°C)	LCL	Relative Potency to -70°C	UCL
Binding	19/264	3.167	-20	0.96	0.97	0.98
Binding	19/264	3.167	+4	1.00	1.00	1.01
Binding	19/264	3.167	+20	0.97	0.98	0.99
Binding	19/264	3.167	+37	0.98	0.99	0.99
Binding	19/264	3.167	+45	0.40	0.60	0.90
Binding	19/266	4.500	-20	0.94	0.99	1.04
Binding	19/266	4.500	+4	0.95	0.99	1.05
Binding	19/266	4.500	+20	0.92	0.97	1.02
Binding	19/266	4.500	+37	0.87	0.92	0.96
Binding	19/266	4.500	+45	0.61	0.64	0.67
Binding	C	1.167	-20	1.07	1.10	1.14
Binding	C	1.167	+4	0.97	1.03	1.08
Binding	C	1.167	+20	1.11	1.21	1.31
Binding	C	1.167	+37	0.94	0.98	1.02
Binding	C	1.167	+45	0.84	0.88	0.93
Binding	D	1.167	-20	0.97	1.00	1.04
Binding	D	1.167	+4	0.99	1.02	1.04
Binding	D	1.167	+20	0.97	1.00	1.02
Binding	D	1.167	+37	0.90	0.96	1.02
Binding	D	1.167	+45	0.31	0.47	0.71
Neutralisation	19/264	3.167	-20	0.95	1.00	1.06
Neutralisation	19/264	3.167	+4	0.90	1.01	1.13
Neutralisation	19/264	3.167	+20	0.90	1.04	1.21
Neutralisation	19/264	3.167	+37	0.96	1.09	1.24
Neutralisation	19/264	3.167	+45	0.38	0.61	0.98
Neutralisation	19/266	4.500	-20	1.01	1.05	1.10
Neutralisation	19/266	4.500	+4	1.01	1.07	1.15
Neutralisation	19/266	4.500	+20	1.02	1.09	1.16
Neutralisation	19/266	4.500	+37	0.96	1.06	1.16
Neutralisation	19/266	4.500	+45	0.67	0.74	0.81
Neutralisation	C	1.167	-20	0.96	1.00	1.04
Neutralisation	C	1.167	+4	0.81	0.94	1.09
Neutralisation	C	1.167	+20	0.86	0.98	1.11
Neutralisation	C	1.167	+37	0.94	1.02	1.10
Neutralisation	C	1.167	+45	0.84	0.98	1.15
Neutralisation	D	1.167	-20	0.91	1.01	1.12
Neutralisation	D	1.167	+4	0.92	1.05	1.20
Neutralisation	D	1.167	+20	0.88	1.08	1.31
Neutralisation	D	1.167	+37	0.88	1.05	1.26
Neutralisation	D	1.167	+45	0.32	0.57	0.99

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

Table 17a: Summary of results from reconstitution stability studies of candidate preparations

Sample	Temperature (°C)	Time (Days)	95% lower confidence limit	Relative Potency to a freshly reconstituted ampoule	95% upper confidence limit
A	+4	1	0.89	0.96	1.04
A	+4	7	0.95	0.99	1.03
A	Room temperature	1	0.92	0.95	0.99
A	Room temperature	7	0.93	0.96	1.00
B	+4	1	0.93	1.01	1.10
B	+4	7	0.90	0.98	1.06
B	Room temperature	1	0.93	1.01	1.10
B	Room temperature	7	0.92	1.00	1.08
C	+4	1	0.46	0.65	0.93
C	+4	7	0.56	0.59	0.63
C	Room temperature	1	0.41	0.52	0.67
C	Room temperature	7	0.32	0.38	0.44
D	+4	1	0.92	0.95	0.98
D	+4	7	0.90	0.93	0.96
D	Room temperature	1	0.91	0.94	0.97
D	Room temperature	7	0.83	0.86	0.89

Geometric Mean potency derived from 6 estimates for Sample C and 4 estimates in all other cases; LCL and UCL: Lower and Upper 95% confidence limits

Table 17b: Summary of results from freeze-thaw studies of candidate preparations

Sample	Number of freeze/thaw cycles	LCL	Relative Potency	UCL
A	1x	1.01	1.08	1.15
A	2x	0.99	1.05	1.12
A	3x	0.98	1.05	1.11
A	4x	0.98	1.05	1.12
B	1x	0.83	0.95	1.07
B	2x	0.82	0.93	1.05
B	3x	0.82	0.93	1.06
B	4x	0.80	0.91	1.03
C	1x	0.64	0.78	0.96
C	2x	0.62	0.73	0.86
C	3x	0.74	0.78	0.81
C	4x	0.72	0.75	0.79
D	1x	0.88	0.94	0.99
D	2x	0.86	0.91	0.97
D	3x	0.87	0.92	0.97
D	4x	0.87	0.92	0.97

Geometric Mean potency derived from 4 estimates in all cases; LCL and UCL: Lower and Upper 95% confidence limits

References:

1. Roda, G, Jharap B, Neeraj N, Colombel JF (2016) Loss of Response to Anti-TNFs: Definition, Epidemiology, and Management. *Clin. Transl. Gastroenterol;* 7: e135.

2. Chanchlani N, Lin S, Bewshea C, Hamilton B, Thomas A, Smith R, et al (2024). Mechanisms and management of loss of response to anti-TNF therapy for patients with Crohn's disease: 3-year data from the prospective, multicentre PANTS cohort study. *Lancet Gastroenterol Hepatol.* 9:521-38. doi: 10.1016/S2468-1253(24)00044-X.

3. Kaur P, Chow V, Zhang N, Moxness M, Kaliyaperumal A, Markus R (2017) A randomised, single-blind, single-dose, three-arm, parallel-group study in healthy subjects to demonstrate pharmacokinetic equivalence of ABP 501 and adalimumab *Ann. Rheum. Dis*. 76:526–33. doi: 10.1136/annrheumdis-2015-208914

4. Lu X, Hu R, Peng L, Liu M, Sun Z (2021) Efficacy and Safety of Adalimumab Biosimilars: Current Critical Clinical Data in Rheumatoid Arthritis. *Front Immunol.* 6;12:638444. doi: 10.3389/fimmu.2021.638444.

5. Bartelds GM, Krieckaert CL, Nurmohamed MT, van Schouwenburg PA, Lems WF, Twisk JW, et al (2011). Development of antidrug antibodies against adalimumab and association with disease activity and treatment failure during long-term follow-up. *JAMA.* 305:1460–8. doi: 10.1001/jama.2011.406

6. van Schouwenburg P, van de Stadt LA, de Jong RN, van Buren EEL, Kruithof S, de Groot E, Hart M, van Ham SM, Rispens T, Aarden L, Wolbink GJ, Wouters D (2013) Adalimumab elicits a restricted anti-idiotypic antibody response in autoimmune patients resulting in functional neutralization. *Ann. Rheum. Dis*. 72: 104-109 https://doi.org/10.1136/annrheumdis-2012-201445

7. Jyssum I, Gehin JE, Sexton J, Kristianslund EK, Hu Y, Warren DJ, Kvien TK, Haavardsholm EA, Syversen SW, Bolstad N, Goll GL (2024). Adalimumab serum levels and anti-drug antibodies: associations to treatment response and drug survival in inflammatory joint diseases. *Rheumatology.*63(6):1746-1755.doi: 10.1093/rheumatology/kead525.

8. van Schouwenburg PA, Kruithof S, Votsmeier C, van Schie K, Hart MH, de Jong RN, van Buren EE, van Ham M, Aarden L, Wolbink G, Wouters D, Rispens T (2014) Functional of the anti-adalimumab response using patient-derived monoclonal antibodies. *J Biol Chem.* 289(50):34482-8. doi: 10.1074/jbc.M114.615500.

9. van Schie KA, Hart MH, de Groot ER, Kruithof S, Aarden LA, Wolbink GJ, Rispens T (2015) The antibody response against human and chimeric anti-TNF therapeutic antibodies primarily targets the TNF binding region. *Ann Rheum Dis.* 74(1):311-4

10. van Schie KA, Kruithof S, Ooijevaar-de Heer P, L Derksen NI, van de Bovenkamp FS, Saris A et al (2018) Restricted immune activation and internalisation of anti-idiotype complexes between drug and antidrug antibodies. *Ann Rheum Dis*.77(10):1471-1479

11. Murdaca G, Negrini S, Greco M, Schiavi C, Giusti F, Borro M, Puppo F (2019) Immunogenicity of infliximab and adalimumab. *Exp Opin Drug Safety.* 18, 343-345.

12. Gorovits B, Baltrukonis DJ, Bhattacharya I, Birchler MA, Finco D, Sikkema D, Vincent MS, Lula S, Marshall L, Hickling TP (2018). Immunoassay methods used in clinical studies for the detection of anti-drug antibodies to adalimumab and infliximab. *Clin Exp Immunol.* 192(3):348-365

13. Sazonovs A, Kennedy NA, Moutsianas L, Heap GA, Rice DL, Reppell M, et al (2020) HLA-DQA1*05 carriage associated with development of anti-drug antibodies to infliximab and adalimumab in patients with Crohn's disease. *Gastroenterology.* 158:189-99

14. Adler J, Galanko JA, Ammoury R, Benkov KJ, Bousvaros A, Boyle B, et al (2025) HLA DQA1*05 and Risk of Antitumor Necrosis Factor Treatment Failure and Anti-Drug Antibody Development in Children With Crohn's Disease. Am J Gastroenterol. 120(5):1076-1086. doi: 10.14309/ajg.0000000000003135. Epub 2024

15. Ruwaard J, Marsman AF, Nurmohamed MT, van der Horst-Bruinsma IE, Te Velthuis H, Bloem K, de Vries A, Rispens T, Wolbink G (2019) . Antidrug antibody detection for adalimumab depends on the type of assay used: an experimental approach to help clinicians interpret diagnostic data. *Clin Exp Rheumatol*. 37(5):756-761

16. van Strien J, Dijk L, Atiqi S, Schouten R, Bloem K, Wolbink GJ et al (2023). Drug-tolerant detection of anti-drug antibodies in an antigen-binding assay using europium chelate fluorescence. *J Immunol Methods*. 514:113436. doi: 10.1016/j.jim.2023.113436

17. Berger AE, Gleizes A, Waeckel L, Roblin X, Krzysiek R, Hacein-Bey-Abina S, Soriano A, Paul S (2022). Validation Study of a New Random-Access Chemiluminescence Immunoassay Analyzer i-TRACK10® to Monitor Infliximab and Adalimumab Serum trough Levels and Anti-Drug Antibodies. *Int. J. Mol. Sci.* 23, 9561. https://doi.org/ 10.3390/ijms23179561

18. Pedersen ME, Østergaard J, Glintborg B et al (2022) Assessment of immunogenicity and drug activity in patient sera by flow-induced dispersion analysis. *Sci Rep.* 12, 4670 https://doi.org/10.1038/s41598-022-08682-3

19. Feuerstein JD, Nguyen GC, Kupfer SS, Falck-Ytter Y, Singh S (2017) American Gastroenterological Association Institute Clinical Guidelines Committee. American Gastroenterological Association Institute Guideline on Therapeutic Drug Monitoring in Inflammatory Bowel Disease. *Gastroenterology*. 153(3):827–34

20. Lamb CA, Kennedy NA, Raine T, Hendy PA, Smith PJ, Limdi JK (2019) British Society of Gastroenterology consensus guidelines on the management of inflammatory bowel disease in adults. *Gut*. 68(Suppl 3):s1-s106. doi: 10.1136/gutjnl-2019-318484.

21. Krieckaert CL, van Tubergen A, Gehin JE, Hernández-Breijo B, Le Mélédo G, Balsa A, Böhm P, Cucnik S, Elkayam O, Goll GL, Hooijberg F, Jani M, Kiely PD, McCarthy N, Mulleman D, Navarro-Compán V, Payne K, Perry ME, Plasencia-Rodriguez C, Stones SR, Syversen SW, de Vries A, Ward KM, Wolbink G, Isaacs JD (2022). EULAR points to consider for therapeutic drug monitoring of biopharmaceuticals in inflammatory

rheumatic and musculoskeletal diseases. *Ann Rheum Dis.* doi: 10.1136/annrheumdis-2022-222155. Epub ahead of print. PMID: 35551063.

22. Kalden JR, Schulze-Koops H (2017). Immunogenicity and loss of response to TNF inhibitors: implications for rheumatoid arthritis treatment. *Nat Rev Rheumatol.* 13(12):707-718.

23. Samaan MA, Arkir Z, Ahmad T, Irving PM (2018) Wide variation in the use and understanding of therapeutic drug monitoring for anti-TNF agents in inflammatory bowel disease: an inexact science? *Expert Opin Biol Ther.* 18(12):1271-1279.

24. Mehta P, Manson JJ (2020) What is the clinical relevance of TNF Inhibitor immunogenicity in the management of patients with rheumatoid arthritis? *Front Immunol*. 11:589.10.3389/fimmu.2020.00589

25. Gils A, Van de Casteele N, Poppe R et al (2014). Development of a universal anti-adalimumab antibody standard for interlaboratory harmonization. *Ther. Drug Monit.* 36(5), 669–673

26. van Schouwenburg PA, Kruithof S, Wolbink G et al (2016). Using monoclonal antibodies as an international standard for the measurement of anti-adalimumab antibodies. *J. Pharm. Biomed. Anal.* 120, 198–201.

27. Suzuki T, Tada M, Ishii-Watabe A (2020) Development of anti-drug monoclonal antibody panels against adalimumab and infliximab. *Biologicals*. 63:39-47.

28. Wadhwa M, Cludts I, Atkinson E, Rigsby P and Study Participants (2025). The first WHO reference panel for Infliximab anti-drug antibodies: a step towards harmonizing therapeutic drug monitoring. *Front Immunol.* 16:1550655. doi: 10.3389/fimmu.2025.1550655. PMID: 40181987; PMCID: PMC11965635

29. Metcalfe C, Dougall T, Bird C, Rigsby P, Behr-Gross ME, Wadhwa M, Study POT (2019) The first World Health Organization International Standard for infliximab products: A step towards maintaining harmonized biological activity. *MAbs*. 11(1):13-25.

30. Wadhwa M, Bird C, Atkinson E, Cludts I, Rigsby P (2021) The First WHO International Standard for Adalimumab: Dual Role in Bioactivity and Therapeutic Drug Monitoring. *Front Immunol.* 12:636420. doi: 10.3389/fimmu.2021.636420.

31. World Health Organization. (2024). WHO/BS/2024.2467. Report on a Collaborative Study for Proposed 1st International Standard for Golimumab.

32. Wadhwa M, Mytych DT, Bird C et al (2016). Establishment of the first WHO erythropoietin antibody reference panel: report of an international collaborative study. *J. Immunol. Methods.* 435, 32–42

33. Resolution WHA67.21. Access to biotherapeutic products including similar biotherapeutic products and ensuring their quality, safety and efficacy. Sixty-seventh World Health Assembly, Geneva, 18–26 May 2014. Geneva: World Health Organization; 2014 (http://apps.who.int/gb/ebwha/pdf_files/WHA67/A67_R21-en.pdf, accessed 19 July 2022).

34. WHO Expert Committee on Biological Standardisation (2016) Sixty seventh Report. Proposed First WHO international standards (or reference panels) for antibodies for use in immunogenicity assessments of biotherapeutic products. *WHO Technical Report Series.* 1004: 53-54

35. Traggiai E, Becker S, Subbarao K, Kolesnikova L, Uematsu Y, Gismondo MR, Murphy BR, Rappuoli R, Lanzavecchia A. (2004) An efficient method to make human monoclonal antibodies from memory B cells: potent neutralisation of SARS coronavirus. *Nat Med,* 10:871-5

36. WHO Expert Committee on Biological Standardisation (2006) Fifty fifth Report. Recommendations for the preparation, characterization and establishment of international and other biological reference standards. *WHO Technical Report Series.* 932:73-130.

36. Mikulskis A, Yeung D, Subramanyam M, Amaravadi L et al (2011) Solution ELISA as a platform of choice for development of robust, drug tolerant immunogenicity assays in support of drug development. *J Immunol Methods*. 365:38-49

37. Chen DY, Chen YM, Tsai WC, Tseng JC, Chen YH, Hsieh CW, Hung WT, Lan JL (2015) Significant associations of antidrug antibody levels with serum drug trough levels and therapeutic response of adalimumab and etanercept treatment in rheumatoid arthritis. *Ann Rheum Dis*. 74(3):e16. doi: 10.1136/annrheumdis-2013-203893.

38. Cludts I, Romana Spinelli F, Morello F, Hockley J, Valesini G,Wadhwa M (2017) Anti-therapeutic antibodies and their clinical impact in patients treated with the TNF antagonist adalimumab. *Cytokine*. 96:16-22 https://doi.org/10.1016/j.cyto.2017.02.015

39. Montaillier C (2020) Development and evaluation of i-Tracker Adalimumab and i-Tracker Anti-Adalimumab kits: fast innovative chemiluminescent assays for the monitoring of patients treated with Adalimumab. *Gastroenterology.* https://doi.org/10.1016/S0016-5085(20)31857-6

40. Wolbink GJ, Vis M, Lems W, Voskouyl AE, de Groot E, Nurmohamed MT, Stapel S, Tak PP, Aarden L, Dijkmans B (2006) Development of antiinfliximab antibodies and relationship to clinical response in patients with rheumatoid arthritis. *Arth Rheum.* 54(3): 711-715 doi: 10.1016/j.jim.2011.09.011

41. Ricken B, Schneider M, Bantleon FI, Velayutham S, Trapani D, Afonso J, Magro F and Abel AP (2019). Quantum Blue® Anti-Adalimumab: Development and evaluation of a point of care rapid test for measuring anti-adalimumab antibodies in human serum *Journal of Crohn's and Colitis*. Vol 13,Supplement_1, S165–S166, https://doi.org/10.1093/ecco-jcc/jjy222.277

42. Wang S, Hauenstein S, Ohrmund L, Shringarpure R, Salbato J, Reddy R, McCowen K, Shah S, Lockton S, Chuang E, Singh S. (2013) Monitoring of adalimumab and antibodies-to- adalimumab levels in patient serum by the homogeneous mobility shift assay. *J Pharm Biomed Anal. 78-79*:39-44

43. Hock BD, McKenzie JL, Goddard L, Smith SM, McEntyre CJ and Keating PE (2018) Discrimination of anti-drug antibodies with neutralizing capacity in Infliximab- and Adalimumab-treated patients: Comparison of the Homogeneous Mobility Shift Assay and the Affinity Capture and Elution Assay. *Ther Drug Monit.* 40:705-715.

44. Keating PE, Hock BD, Chin PKL, O'Donnell JL, Barclay ML (2024). Evaluation of the Homogenous Mobility Shift Assay for Infliximab and Adalimumab Anti-drug Antibody Detection in the Clinical Laboratory. *Ther Drug Monit.* 46(5):619-626. doi: 10.1097/FTD.0000000000001200.

45. Finco D, Baltrukonis D, Clements-Egan A, Delaria K, Gunn GR, Lowe J et al (2011) Comparison of competitive ligand-binding assay and bioassay formats for the measurement of neutralising antibodies to protein therapeutics. *J Pharm Biomed Anal*. 54:351-358

46. Ding X, Xue L, Wang M, Zhu S, Zhu K, Jiang S, Wu J and Miao L (2024) Dynamics and implications of anti-drug antibodies against adalimumab using ultra-sensitive and highly drug-tolerant assays. *Front. Immunol.* 15:1429544. doi: 10.3389/fimmu.2024.1429544

47. Ogrič, M., Žigon, P., Lakota, K. et al (2019) Clinically important neutralizing anti-drug antibodies detected with an in-house competitive ELISA. *Clin Rheumatol*. 38, 361–370 https://doi.org/10.1007/s10067-018-4213-0

48. Hock BD, Stamp LK, Hayman MW, Keating PE, Helms ETJ, Barclay ML (2016) Development of an ELISA-Based Competitive Binding Assay for the Analysis of Drug Concentration and Antidrug Antibody Levels in Patients Receiving Adalimumab or Infliximab. *Ther Drug Monit.* 38 (1): 32 - 41

49. Lallemand C, Kavrochorianou N, Steenholdt C, Bendtzen K, Ainsworth MA, Meritet JF, Blanchard B, Lebon P, Taylor P, Charles P, Alzabin S, Tovey MG (2011) Reporter gene assay for the quantification of the activity and neutralising antibody response to TNFα antagonists. *J Immunol Methods.* 373(1-2):229-239.

50. CombiStats v.1.1.1, EDQM – Council of Europe. www.combistats.edqm.eu.

51. U.S. Food & Drug Administration. Immunogenicity Testing of Therapeutic Protein Products — Developing and Validating Assays for Anti-Drug Antibody Detection. January 2019, Accessed 22nd May'25

52. EMA Guideline on Immunogenicity assessment of therapeutic proteins. 18 May 2017, Accessed 20th May'25

53. Wadhwa M, Knezevic I, Kang HN, Thorpe R (2015). Immunogenicity assessment of biotherapeutic products: An overview of assays and their utility. *Biologicals*. 43(5):298-306.

54. Moxness M, Tatarewicz S, Weeraratne D, Murakami N, Wullner D, Mytych D et al (2005) Immunogenicity testing by electrochemiluminescent detection for antibodies directed against therapeutic human monoclonal antibodies. *Clin Chem*. 51:1983-1985

55. Atiqi S, Hooijberg F, Loeff FC, Rispens T, Wolbink GJ (2020). Immunogenicity of TNF-Inhibitors. *Front Immunol.* 11:312. doi: 10.3389/fimmu.2020.00312.

56. Beeg M, Nobili A, Orsini B, Rogai F, Gilardi D, Fiorino G, Danese S, Salmona M, Garattini S, Gobbi M (2019) Surface Plasmon Resonance-based assay to measure serum concentrations of therapeutic antibodies and anti-drug antibodies. *Nature*. 9:2064 https://doi.org/10.1038/s41598-018-37950-4)

57. Beeg M, Burti C, Allocati E, Ciafardini C, Banzi R, Nobili A, et al (2021) Surface plasmon resonance unveils important pitfalls of enzyme-linked immunoassay for the detection of anti-infliximab antibodies in patients' sera. *Sci Rep*.11:14976. doi: 10.1038/s41598-021-94431-x.

58. Schalk FB, Guerrieri D, Poetzl J, van de Merbel NC (2025) Simultaneous isotyping and semi-quantitation of anti-drug antibodies to an IgG1 biotherapeutic using hybrid LBA-LC-MS/MS. Bioanalysis. 17(2):87-98. doi: 10.1080/17576180.2024.2441058.

59. El Amrani M, Göbel C, Egas AC, Nierkens S, Hack CE, Huitema ADR, van Maarseveen EM (2019) Quantification of neutralising anti-drug antibodies and their neutralising capacity using competitive displacement and tandem mass spectrometry: Infliximab as proof of principle. *J Transl Autoimmun.* 1:100004. doi: 10.1016/j.jtauto.2019.100004.

60. Papamichael K, Juncadella A, Wong D, Rakowsky S, Sattler LA, Campbell JP et al (2019) Proactive therapeutic drug monitoring of adalimumab is associated with better long-term outcomes compared to standard of care in patients with inflammatory bowel disease. *J Crohn's Colitis.* 13:976-981

61. National Institute for Health and Care Excellence. Therapeutic monitoring of TNF-alpha inhibitors in rheumatoid arthritis. Diagnostics guidance: DG36. Manchester: National Institute for Health and Care Excellence, 2019

62. Rissel F, Cazaubon Y, Saffar S, Altwegg R, Artasone M, Lozano C, Vincent T, Jentzer A (2023) Comparative Evaluation of Four Commercially Available Immunoassays for Therapeutic Drug Monitoring of Infliximab and Adalimumab. *Int J Mol Sci.* 24:10379. doi: 10.3390/ijms241210379.

63. Bertin D, Serrero M, Grimaud JC, Desjeux A, Desplat-Jego S (2020) Monitoring of infliximab trough levels and anti-infliximab antibodies in inflammatory bowel diseases: A comparison of three commercially available ELISA kits. *Cytokine.* 126:154859. 10.1016/j.cyto.2019.154859

64. Bader LI, Solberg SM, Kaada SH, Bolstad N, Warren DJ, Gavasso S, Gjesdal CG, Vedeler CA (2017) Assays for Infliximab Drug Levels and Antibodies: A Matter of Scales and Categories. *Scand J Immunol*. 86(3):165-170. doi: 10.1111/sji.12572.

65. Lighaam LC, Rispens T (2016) The Immunobiology of Immunoglobulin G4. Semin Liver Dis ; 36(3):200-15. doi: 10.1055/s-0036-1584322. Epub 2016 Jul 28. PMID: 27466791

Appendix 1A: Methods for isolation and characterization of monoclonal anti-drug antibodies (IRB, Switzerland)

Biopharmaceutical products (BPs) used as antigens for different assays. Rebif (Merck Serono) was used as source of IFN-beta. Rituximab, Natalizumab, Adalimumab and Infliximab were produced recombinantly as chimeric human-IgG1(CH1)-mouse-IgG2a(CH2-CH3) to

avoid cross-reaction of secondary anti-human Fc-specific antibodies used to detect binding of human monoclonal antibodies.

Isolation and production of monoclonal antibodies from patients with serum ADAs. Peripheral blood samples were obtained from treated patient. Memory B cells were isolated from cryopreserved PBMCs using anti-FITC microbeads (Miltenyi Biotec) following staining of PBMCs with CD22-FITC (BD Phamingen), and were immortalized with Epstein-Barr virus and CpG in multiple wells as described previously [1]. Culture supernatants were tested for binding to specific BPs by ELISA. cDNA was synthesized from positive cultures and both heavy chain and light chain variable regions were sequenced. Positive cultures were expanded and supernatants were collected and purified. When positive cultures could not be expanded, monoclonal antibodies were produced recombinantly as IgG1 by transient transfection of Expi293 cells (Invitrogen) using polyethylenimine (PEI) and tested for binding to Infliximab.

Sequence analysis of antibodies.
The usage of VH and VL genes and the amount of somatic mutations were determined by analyzing the homology of VH and VL sequences of mAbs to known human V, D and J genes by the IMGT (international ImMunoGeneTics information system) database [2].

Antibody purification. Chimeric BPs and human mAbs were purified by protein A or protein G chromatography (GE Healthcare) and concentrated by Amicon Ultra filter units (100K, Millipore). Total IgGs were quantified by Pierce BCA protein assay (Thermofischer).

Scaled up Antibody production. To enable scaled up production, antibodies were cloned in a stable recombinant Chinese hamster ovary (rCHO) cell line. The antibodies were expressed from these suspension-cultured rCHO cell lines (not clonal cell line). Culture supernatants containing the secreted protein were harvested ten to fourteen days post-inoculum, centrifuged and filtered on a 0.22 µm membrane. Antibody was purified by affinity chromatography on Protein A (MabSelectsure, GE Healthcare) using acidic conditions for elution. Subsequently, the pool of fractions containing the antibody was purified by size exclusion chromatography (Superdex 200, GE Healthcare) equilibrated with DPBS. The first set of purification was done on an automated platform at 10 mg “small-scale”. All the characterization data obtained for this “small-scale” production was reviewed, along with expression yields, and a selection of antibodies was made for “large-scale” production. Ten clones were produced at 0.5-1 g scale-up. Production was similar in process to the “small-scale” however the purification, was performed on larger chromatography columns using a parallel device system. Purified antibodies were transferred to the NIBSC. rCHO cell lines are kept with Sanofi.

ELISA assays.
Binding to BPs was tested by ELISA using 384-well SpectraPlates (PerkinElmer) for primary screenings or 96-well MaxiSorp plates (Nunc) for any following test. Briefly, ELISA plates were coated with 1 µg/ml of BP, blocked with 1% BSA and incubated with titrated antibodies, followed by AP-conjugated anti-human IgG - Fc gamma specific secondary antibodies (Jackson ImmunoResearch). Plates were then washed, substrate (p-NPP, Sigma) was added and plates were read at 405 nm. EC50 (ng/ml) was calculated for every sample by nonlinear regression analysis using GraphPad Prism 5 software.

Surface plasmon resonance (SPR) assays. Monoclonal antibodies (50 nM) were stabilized in 10 mM acetate buffer, pH 4.5, and immobilized onto a EDC/NHS pre-activated ProteOn sensor chip (Biorad) through amine coupling; unreacted groups were blocked by injection of

ethanolamine HCl (1 M). HEPES buffered saline (HBS) (10 mM HEPES, pH 7.4, 150 mM NaCl, 3 mM EDTA, 0.005% surfactant Tween-20) was used as running buffer. All injections were made at flow rate of 100 µl/min. M were diluted and titrated in HBS (90-30-10-3.3-1.1 nM) and injected onto the BP coated chip; one channel of the chip was injected with HBS and used as reference for the analysis. Injection time and dissociation time were 240 s and 900 s, respectively. Each binding interaction of mAbs was assessed using a ProteON XPR36 instrument (Biorad) and data processed with ProteOn Manager Software. Ka, Kd and KD were calculated applying the Langmuir fit model.

Neutralisation assays.
ELISA plates were coated with 2 µg/ml of TNF-alpha (antibody target) and blocked with 1% BSA. Chimeric Infliximab was diluted to 60 ng/ml (final dilution) and incubated with titrated monoclonal antibodies for 1 h, 37°C. The mixes were transferred to the ELISA plates and incubated for 90 min, RT, followed by AP-conjugated anti-mouse IgG (Southern Biotech). Plates were then washed, substrate (p-NPP, Sigma) was added and plates were read at 405 nm. Neutralisation was calculated as percentage of inhibition to TNF-alpha with the following formula: [1-(OD of a single well – average OD of control cells incubated without Infliximab)/(average OD of control cells incubated with Infliximab – average OD of control cells incubated without Infliximab)] x 100. IC90 (ng/ml) was calculated for every sample by a nonlinear regression analysis using GraphPad Prism 5 software.

References.
1. Traggiai, E., *et al.* An efficient method to make human monoclonal antibodies from memory B cells: potent neutralisation of SARS coronavirus. *Nature medicine* **10**, 871-875 (2004).
2. Lefranc, M.P., *et al.* IMGT, the international ImMunoGeneTics information system. *Nucleic acids research* **37**, D1006-1012 (2009).

Appendix 1B: Methods for isolation and characterization of chimeric anti-drug antibodies (NIHS, Japan)

Generation of chimeric ADAs.
The generation of anti-adalimumab chimeric mAbs was performed as described[1]. Hybridomas expressing rat anti-adalimumab antibodies were generated by ITM Co. (Nagano, Japan). Adalimumab (Humira®; Abbott, Baar, Switzerland) F(ab')2 was crosslinked by glutaraldehyde and then immunized to WKY rats with Freund's complete adjuvant. Two weeks later, lymphocytes were obtained from the iliac lymph nodes of the immunized rats and were fused

to a myeloma. The resultant hybridomas were screened by ELISA and SPR, and hybridomas secreting rat anti-adalimumab mAbs were established. From the obtained hybridomas, total RNA was purified using the RNeasy Plus Micro Kit (Qiagen, Hilden, Germany), and cDNA synthesis and 5′-RACE PCR were performed by using a SMARTer RACE 5'/3′ Kit (Clontech, Mountain View, CA) according to the manufacturer's instructions. The sequences of the variable regions of the heavy chain and light chain were determined by Takara Bio (Shiga, Japan). For the construction of the expression vectors of the human-rat chimeric anti-adalimumab mAbs, the DNA fragments encoding the variable region of the heavy chain and light chain were synthesized (GenScript Japan, Tokyo) and subcloned into pFUSE-CHIg-hG1 or pFUSE2-CLIg-hk vector for the expression of IgG1-type antibodies (Invivogen, San Diego, CA). CHO–S cells were co-transfected with the obtained vectors expressing the heavy chain and light chain of an antibody by using FreeStyle MAX Reagent (Thermo Fisher Scientific, Waltham, MA), and were cultured for 1 week in FreeStyle CHO Expression Medium (37 °C, 8% CO2). The cell culture medium was centrifuged and filtered with a bottletop filter (Corning, Tewksbury, MA). The collected supernatant was applied to a HiTrap Protein G HP column (Cytiva, Buckinghamshire, UK) equilibrated with PBS (pH 7.2). After the column was washed with PBS (pH 7.2), the mAb was eluted with the use of 0.1 M glycine-HCl (pH 2.7) and neutralized by 1 M Tris-HCl (pH 8.0), followed by buffer exchange to PBS (pH 7.2) using a PD 10 column (Cytiva).
For expression of IgG4-type antibodies, pFUSE-CHIg-hG4 vector (Invivogen) incorporated with the variable region was used. The generation methods were the same as those of IgG1-type antibodies. For expression of IgE-type antibodies, pFUSE-CHIg-hE (Invivogen) incorporated with the variable region was used, and the expressed antibodies were purified with HiTrap protein L column (Cytiva) according to the manufacture’s instruction.

Scaled up Antibody production.
ExpiCHO expression system (Thermo Fisher Scientific) was used for the scale up production of A21-1G-IgG1 and A40-1F-IgG4 clones. ExpiCHO-S cells were co-transfected with the heavy chain and light chain vectors, and were cultured for 13-14 days with adding feed according to the manufacture’s instruction. The antibodies were purified from the collected supernatant with a HiTrap Protein G HP column as shown above and the buffer exchange to PBS (pH7.2) was performed with a centrifugal filter (Amicon® Ultra-15; Millipore).

Surface plasmon resonance (SPR) assays.
The Biotin CAPture Kit (Cytiva) was used for the kinetic assay and the ADA detection assay of IgG1 and IgG4-type antibodies. Adalimumab was biotinylated with an EZ-Link™ Sulfo–NHS–Biotinylation Kit (Thermo Fisher Scientific). The capture of biotinylated therapeutics and the regeneration of the sensor chip were performed according to the manufacturer's instructions. All measurements were performed at 25 °C, and HBS-EP+ was used as a running buffer. For the evaluation of the affinity between the therapeutics and their ADAs, biotinylated therapeutics (approx. 0.4 μg/ml of biotinylated adalimumab) were captured on the Sensor Chip CAP by 120-sec injection at the flow rate of 30 μl/min. Then, the serial dilution of anti-adalimumab mAbs (adalimumab-ADAs) was injected for 120 s, and the dissociation was observed for 600 s at the flow rate of 30 μl/min. The kinetic parameters were calculated using the 1:1 binding model of the Biacore T200 ver.3 software. In the ADA detection assay, biotinylated adalimumab was captured to the sensor chip by a 120-sec injection at the concentration of approx. 40 μg/ml, and serially diluted ADAs were injected on the sensor chip for 600 s at the flow rate of 10 μl/min. The binding response was calculated at the end of the binding phase.

For the SPR assay of IgE-type antibodies, IgG capture kit (Cytiva) was used. Anti-human IgG antibody was immobilized to sensor chip with amine coupling kit (Cytiva). Adalimumab was captured and the binding between adalimumab and IgE-type antibodies were analyzed.

Biolayer interferometry (BLI) analyses.
The BLI analyses were performed using an Octet RED 384 system (FortéBio, Fremont, CA). All experiments were performed at 30 °C and 1000 rpm in HBS-EP + buffer, and the data were analyzed using Octet Data Acquisition 9.0 software (FortéBio). The biotinylated adalimumab used in this assay was the same as that described in the description of the SPR assay using the Biotin CAPture Kit. The biotinylated therapeutic antibody was immobilized to streptavidin coated biosensor chips (FortéBio) by reacting for 60 s at the concentration of 1 μg/ml. For the analysis of the binding between therapeutic antibodies and ADAs, serial diluted ADAs were poured into wells and reacted with the biosensor chips for 10 min.

Electrochemiluminescence (ECL) assays.
Ruthenium complexes of adalimumab were prepared using MSD GOLD SULFO-TAG NHS-Ester Conjugation Packs (Meso Scale Discovery, Rockville, MD), and biotinylated adalimumab were prepared using an EZ-Link Sulfo–NHS–LC Biotinylation Kit (Thermo Fisher Scientific) according to the manufacturer's instructions. For the analysis of the binding between adalimumab and adalimumab-ADAs, 1 μg/ml biotinylated adalimumab, 1 μg/ml ruthenium-labeled adalimumab, and serially diluted adalimumab-ADAs diluted in assay diluent (1% Blocker A in PBS) were mixed and incubated at room temperature for 2 h with shaking. An MSD GOLD 96-well Streptavidin QUICKPLEX Plate (Meso Scale Discovery) was blocked with 3% Blocker A in PBS for 2 h and then washed with PBS containing 0.05% Tween 20. Next, 50 μl of the pre-incubated mixtures described above were added to each well and incubated at room temperature for 1 h with shaking. The plate was washed with PBS containing 0.05% Tween 20, and 150 μl of (2×) MSD Read Buffer T was added to each well, followed by the detection of ECL signals using MESO QuickPlex SQ120 (Meso Scale Discovery).

Neutralization assays.
TNF reporter assays were performed using the GloResponse™ NF-κB RE-luc2P HEK293 cell line (Promega, Madison, WI). The serial diluted adalimumab-ADAs and 2 μg/ml of adalimumab were mixed at the volume ratio of 1:1 in a 96-well plate and then incubated at 37 °C for >10 min. Next, 10 μl of the mixture and 10 μl of 0.1 μg/ml of TNF-α was mixed and incubated at 37 °C until the cells were prepared. NF-κB-RE-luc2P HEK293 cells were suspended in assay medium (90% DMEM, 10% FBS) at a density of 500,000 cells/ml. To the mixture of adalimumab, adalimumab-ADA, and TNF-α in the wells of the 96-well plate, 80 μl of the cell suspension was added, and the plate was incubated in a humidified 37 °C, 5% CO2 incubator for 5 h. After incubation, 100 μl of ONE-Glo™ Luciferase Assay System Reagent was added to each well according to the manufacturer's instructions, and luminescence was measured using an EnSight™ multimode plate reader (PerkinElmer, San Jose, CA).

Reference.
1. Suzuki T, *et al*. Development of anti-drug monoclonal antibody panels against adalimumab and infliximab. Biologicals 63, 39-47 (2020)

Appendix 1C: Methods for characterization of anti-drug antibodies (MHRA, UK)
Surface plasmon resonance (SPR) assays.

Binding activity: These experiments were performed on a Biacore T200 instrument, using a Biotin CAPture kit (Cytiva, US). Adalimumab was labelled with biotin at a challenge ratio of 1:1, diluted to 45 μg/ml in running buffer (HBS-EP+ buffer: Hepes buffered saline with EDTA and surfactant P20) and captured on the CAP sensor chip by a 240-sec injection at a flow rate of 30μl/min. No biotin-adalimumab was captured on the control flow cell. The mAbs were injected for 600 sec at a flow rate of 30μl/min, at concentrations of 20, 100, 500 and 1500 ng/ml.

For binding affinity determination, single cycle kinetic (SCK) experiments were conducted. Adalimumab, labelled with biotin at a challenge ratio of 1:1, was diluted to 0.4 µg/ml in running buffer) and captured on the CAP sensor chip by a 120-sec injection at a flow rate of 30µl/min. The mAbs at 5, 20 80, 320 and 1280 nM were then injected for 120 sec at a flow rate of 30µl/min, followed by a dissociation phase of 3600 sec. The kinetics parameters (association and dissociation) of the injected mAbs were calculated using the 1:1 binding model (Biacore Evaluation ver.3.1 software, Cytiva).

ELISAs

Adalimumab (1µg/ml in phosphate buffered saline, PBS, 100µl per well) was immobilized overnight at 4°C in 96-well plates (Nunc maxisorp,Thermo Fisher Scientific, UK). After washing with PBS-0.05%Tween20, plates were blocked with casein buffer (Thermo Fisher Scientific, UK) for 1h at room temperature and washed again. Samples and controls were distributed into wells (at appropriate dilutions using PBS-0.5%BSA) and incubated for 1.5h at room temperature on a plate shaker. After another wash step, horse radish peroxidase HRP labelled adalimumab (at a challenge ratio 4 HRP:1 Ab, Lynx Rapid HRP Ab conjugation kit, Biorad) at 125ng/ml (100µl per well) was added and the plates incubated for 1.5h at room temperature on a shaker. For detection, TMB peroxidase EIA substrate kit (Biorad) was used (100µl per well), the reaction stopped after color development with 1M sulfuric acid (50µl per well) and the absorbance read at 450 nm in a Spectramax M5 plate reader.

Electrochemiluminescence (ECL) assays

Ruthenium-labelled and biotin-conjugated adalimumab were prepared using MSD SULFO-TAG NHS-Ester label (Meso Scale Discovery, Gaithersburg, MD) and EZ-Link Sulfo–NHS–LC Biotin (Thermo Fisher Scientific, UK) respectively according to the manufacturer's instructions. For analysis of adalimumab-ADAs, serially diluted samples (in PBS-0.5%BSA) and controls were distributed in wells of dilution plates and a mastermix of ruthenium-labelled adalimumab and biotinylated adalimumab (each at 500 ng/ml) added. The mixture was incubated for 1.5h at room temperature on a plate shaker, transferred (50µl per well) to a pre-blocked MSD streptavidin plate and incubated for 1h at room temperature on a plate shaker. After a wash step (PBS-0.05%Tween20), MSD Read buffer T (1x, 150µl per well was added to each well and the ECL signals detected using the MSD Meso QuickPlex SQ120 instrument (Meso Scale Discovery).This method was also used for assessing the reactivity of the ADAs with different adalimumab products.

Neutralization assays

For assessing the neutralizing activity of the samples, the competitive ligand binding assay was used. Serially diluted samples (in PBS-0.5%BSA) were distributed in wells of dilution plates and a mastermix of ruthenium-labelled adalimumab and biotinylated adalimumab (each at 5 ng/ml) added. After incubation for 1.5h at room temperature on a plate shaker, the mixtures were transferred to pre-blocked MSD streptavidin plates (50µl per well) and incubated for 1h at room temperature on a plate shaker. After a wash step (PBS-0.05%Tween20), MSD Read buffer T (1x, 150µl per well was added to each well and the ECL signals detected using the MSD Meso QuickPlex SQ120 instrument (Meso Scale Discovery).

Appendix 2 - Table 1: Details of clinical serum samples

Sample code	ADA level (anticipated)	ADA level*	Drug level**

1	negative	nhs (sterile mixed pool, First Link #20-00-850, batch HSS8963)	negative
2	very low	Pooled sera with ADA titers <10 to 100	~1 µg/ml
3	low	Pooled sera with ADA titers of 100-200 and >200 diluted in1:10 (equal ratio)	~0.125 µg/ml
4	moderate	Pooled sera with ADA titers >200 AU/ml diluted 1:10	negative
5	moderate	Pooled sera with ADA titers >200 AU/ml diluted 1:5	negative
6	high	Pooled sera with ADA titers >200 AU/ml	negative

*ADA titers determined by ELISA in the hospital providing the samples; nhs – normal human serum
** drug levels estimated by ECL assay. No acid dissociation step included in the protocol so potential for adalimumab detection to be impaired by the presence of ADA.

Appendix 3 – Table 1: Distribution of slope-ratios (relative to IH standard) for the different samples and assay types

ELISA

Sample	Slope ratios vs IH					
	% <0.67	% 0.67-0.80	% 0.80-1.25	% 1.25-1.50	% >1.50	n
A	1.4	5.4	58.1	18.9	16.2	74
B	2.7	6.8	67.6	9.5	13.5	74
C	54.1	16.2	25.7	1.4	2.7	74
D	16.2	14.9	56.8	1.4	10.8	74
R	6.8	8.1	75.7	2.7	6.8	74
S	6.8	10.8	75.7	1.4	5.4	74
T	2.8	9.7	76.4	0.0	11.1	72
U	4.1	13.5	71.6	0.0	10.8	74
1	Neg	Neg	Neg	Neg	Neg	0
2	Neg	Neg	Neg	Neg	Neg	0
3	73.3	0.0	0.0	0.0	26.7	15
4	8.6	11.4	60.0	0.0	20.0	70
5	1.6	8.2	78.7	0.0	11.5	61
6	1.4	6.9	68.1	2.8	20.8	72

ECL

Sample	Slope ratios vs IH					
	% <0.67	% 0.67-0.80	% 0.80-1.25	% 1.25-1.50	% >1.50	n
A	5.6	7.4	87.0	0.0	0.0	54
B	0.0	0.0	100.0	0.0	0.0	54
C	1.9	13.0	85.2	0.0	0.0	54
D	0.0	1.9	98.1	0.0	0.0	54
R	1.9	1.9	90.7	3.7	1.9	54
S	0.0	0.0	98.1	1.9	0.0	54
T	0.0	0.0	100.0	0.0	0.0	54
U	0.0	0.0	100.0	0.0	0.0	54
1	Neg	Neg	Neg	Neg	Neg	0
2	0.0	5.6	86.1	8.3	0.0	36
3	4.4	6.7	84.4	2.2	2.2	45
4	0.0	2.2	97.8	0.0	0.0	45
5	4.4	2.2	93.3	0.0	0.0	45
6	0.0	7.1	78.6	14.3	0.0	42

Other

Sample	Slope ratios vs IH					
	% <0.67	% 0.67-0.80	% 0.80-1.25	% 1.25-1.50	% >1.50	n
A	0.0	0.0	100.0	0.0	0.0	3
B	0.0	0.0	100.0	0.0	0.0	3
C	0.0	66.7	33.3	0.0	0.0	3
D	0.0	0.0	100.0	0.0	0.0	2
R	0.0	0.0	100.0	0.0	0.0	2
S	Neg	Neg	Neg	Neg	Neg	0
T	0.0	0.0	100.0	0.0	0.0	3
U	0.0	0.0	100.0	0.0	0.0	3
1	Neg	Neg	Neg	Neg	Neg	0
2	Neg	Neg	Neg	Neg	Neg	0
3	0.0	0.0	100.0	0.0	0.0	3
4	0.0	0.0	100.0	0.0	0.0	3
5	0.0	0.0	100.0	0.0	0.0	3
6	0.0	0.0	100.0	0.0	0.0	3

Binding (all)

Sample	Slope ratios vs IH					
	% <0.67	% 0.67-0.80	% 0.80-1.25	% 1.25-1.50	% >1.50	n
A	3.1	6.1	71.0	10.7	9.2	131
B	1.5	3.8	81.7	5.3	7.6	131
C	31.3	16.0	50.4	0.8	1.5	131
D	9.2	9.2	74.6	0.8	6.2	130
R	4.6	5.4	82.3	3.1	4.6	130
S	3.9	6.3	85.2	1.6	3.1	128
T	1.6	5.4	86.8	0.0	6.2	129
U	2.3	7.6	84.0	0.0	6.1	131
1	Neg	Neg	Neg	Neg	Neg	0
2	0.0	5.6	86.1	8.3	0.0	36
3	20.6	4.8	65.1	1.6	7.9	63
4	5.1	7.6	75.4	0.0	11.9	118
5	2.8	5.5	85.3	0.0	6.4	109
6	0.9	6.8	72.6	6.8	12.8	117

n indicates the total number of assays included for determination of parallelism

Appendix 3 – Table 1: Distribution of slope-ratios (relative to sample C and D) for the different samples and assay types

Sample C

ELISA

Sample	Slope ratios vs C					
	% <0.67	% 0.67-0.80	% 0.80-1.25	% 1.25-1.50	% >1.50	n
A	2.3	2.3	18.4	19.5	57.5	87
B	2.3	2.3	27.6	10.3	57.5	87
D	2.3	2.3	42.5	11.5	41.4	87
R	3.4	0.0	41.4	5.7	49.4	87
S	3.4	2.3	39.1	9.2	46.0	87
T	2.4	0.0	32.9	10.6	54.1	85
U	2.3	3.4	29.9	17.2	47.1	87
1	Neg	Neg	Neg	Neg	Neg	0
2	30.8	23.1	7.7	0.0	38.5	13
3	42.9	0.0	25.0	0.0	32.1	28
4	1.2	1.2	38.6	13.3	45.8	83
5	1.4	0.0	33.8	20.3	44.6	74
6	2.4	1.2	30.6	11.8	54.1	85

ECL

Sample	Slope ratios vs C					
	% <0.67	% 0.67-0.80	% 0.80-1.25	% 1.25-1.50	% >1.50	n
A	0.0	3.2	85.7	6.3	4.8	63
B	0.0	0.0	79.4	15.9	4.8	63
D	0.0	0.0	87.3	7.9	4.8	63
R	0.0	1.6	79.4	9.5	9.5	63
S	0.0	0.0	84.1	6.3	9.5	63
T	0.0	1.6	90.5	3.2	4.8	63
U	0.0	0.0	84.1	12.7	3.2	63
1	Neg	Neg	Neg	Neg	Neg	0
2	0.0	6.7	80.0	6.7	6.7	45
3	0.0	5.6	61.1	24.1	9.3	54
4	1.9	1.9	75.9	14.8	5.6	54
5	1.9	1.9	79.6	14.8	1.9	54
6	0.0	2.0	68.6	21.6	7.8	51

Other

Sample	Slope ratios vs C					
	% <0.67	% 0.67-0.80	% 0.80-1.25	% 1.25-1.50	% >1.50	n
A	9.5	42.9	9.5	9.5	28.6	21
B	14.3	33.3	14.3	9.5	28.6	21
D	0.0	45.0	20.0	30.0	5.0	20
R	5.0	50.0	20.0	25.0	0.0	20
S	5.6	44.4	11.1	33.3	5.6	18
T	23.8	28.6	38.1	9.5	0.0	21
U	28.6	23.8	19.0	28.6	0.0	21
1	Neg	Neg	Neg	Neg	Neg	0
2	Neg	Neg	Neg	Neg	Neg	0
3	25.0	12.5	50.0	0.0	12.5	8
4	8.3	8.3	33.3	41.7	8.3	12
5	16.7	8.3	25.0	16.7	33.3	12
6	0.0	4.8	66.7	9.5	19.0	21

Binding (all)

Sample	Slope ratios vs C					
	% <0.67	% 0.67-0.80	% 0.80-1.25	% 1.25-1.50	% >1.50	n
A	2.3	7.6	42.1	13.5	34.5	171
B	2.9	5.3	45.0	12.3	34.5	171
D	1.2	6.5	56.5	12.4	23.5	170
R	2.4	6.5	52.9	9.4	28.8	170
S	2.4	6.0	53.0	10.7	28.0	168
T	4.1	4.1	55.0	7.7	29.0	169
U	4.7	4.7	48.5	17.0	25.1	171
1	Neg	Neg	Neg	Neg	Neg	0
2	6.9	10.3	63.8	5.2	13.8	58
3	15.6	4.4	48.9	14.4	16.7	90
4	2.0	2.0	51.7	16.1	28.2	149
5	2.9	1.4	50.7	17.9	27.1	140
6	1.3	1.9	47.8	14.6	34.4	157

Sample D

ELISA

Sample	Slope ratios vs D					
	% <0.67	% 0.67-0.80	% 0.80-1.25	% 1.25-1.50	% >1.50	n
A	1.1	6.9	44.8	25.3	21.8	87
B	3.4	6.9	55.2	9.2	25.3	87
C	43.7	11.5	42.5	2.3	0.0	87
R	3.4	4.6	71.3	5.7	14.9	87
S	4.6	5.7	71.3	11.5	6.9	87
T	1.2	2.4	71.8	14.1	10.6	85
U	2.3	4.6	67.8	8.0	17.2	87
1	Neg	Neg	Neg	Neg	Neg	0
2	38.5	15.4	7.7	15.4	23.1	13
3	42.9	0.0	28.6	7.1	21.4	28
4	3.6	7.2	60.2	10.8	18.1	83
5	1.4	4.1	70.3	13.5	10.8	74
6	2.4	1.2	58.8	17.6	20.0	85

ECL

Sample	Slope ratios vs D					
	% <0.67	% 0.67-0.80	% 0.80-1.25	% 1.25-1.50	% >1.50	n
A	1.6	9.5	85.7	3.2	0.0	63
B	0.0	0.0	96.8	3.2	0.0	63
C	4.8	7.9	87.3	0.0	0.0	63
R	1.6	1.6	90.5	3.2	3.2	63
S	0.0	1.6	92.1	4.8	1.6	63
T	0.0	1.6	96.8	1.6	0.0	63
U	0.0	3.2	95.2	1.6	0.0	63
1	Neg	Neg	Neg	Neg	Neg	0
2	2.2	20.0	73.3	4.4	0.0	45
3	3.7	5.6	81.5	5.6	3.7	54
4	5.6	1.9	90.7	1.9	0.0	54
5	5.6	0.0	90.7	3.7	0.0	54
6	2.0	7.8	78.4	11.8	0.0	51

Other

Sample	Slope ratios vs D					
	% <0.67	% 0.67-0.80	% 0.80-1.25	% 1.25-1.50	% >1.50	n
A	15.0	0.0	55.0	20.0	10.0	20
B	15.0	0.0	55.0	25.0	5.0	20
C	5.0	30.0	20.0	45.0	0.0	20
R	15.0	0.0	85.0	0.0	0.0	20
S	0.0	0.0	100.0	0.0	0.0	18
T	15.0	0.0	85.0	0.0	0.0	20
U	15.0	5.0	80.0	0.0	0.0	20
1	Neg	Neg	Neg	Neg	Neg	0
2	Neg	Neg	Neg	Neg	Neg	0
3	42.9	14.3	42.9	0.0	0.0	7
4	27.3	0.0	63.6	9.1	0.0	11
5	27.3	0.0	63.6	9.1	0.0	11
6	15.0	0.0	20.0	65.0	0.0	20

Binding (all)

Sample	Slope ratios vs D					
	% <0.67	% 0.67-0.80	% 0.80-1.25	% 1.25-1.50	% >1.50	n
A	2.9	7.1	61.2	16.5	12.4	170
B	3.5	3.5	70.6	8.8	13.5	170
C	24.7	12.4	56.5	6.5	0.0	170
R	4.1	2.9	80.0	4.1	8.8	170
S	2.4	3.6	82.1	7.7	4.2	168
T	2.4	1.8	82.7	7.7	5.4	168
U	2.9	4.1	79.4	4.7	8.8	170
1	Neg	Neg	Neg	Neg	Neg	0
2	10.3	19.0	58.6	6.9	5.2	58
3	19.1	4.5	61.8	5.6	9.0	89
4	6.1	4.7	71.6	7.4	10.1	148
5	5.0	2.2	77.7	9.4	5.8	139
6	3.8	3.2	60.3	21.8	10.9	156

n indicates the total number of assays included for determination of parallelism

Appendix 3 – Table 2: Individual laboratory data for Binding Assays

ELISA – Calculated vs A (µg/ml)

Sample	Lab										
	2	3	4	5a	6	7	8a	9	10	1	11
A	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00
B	61.62	327.71	462.36	46.25	45.26	44.23	63.44	52.57	55.09	60.40	53.77
C	NP	2.71	5.13	NP	0.62	12.44	3.02	NP	NP	3.05	0.36
D	NP	37.91	42.55	1.46	2.22	22.63	43.12	21.81	NP	3.84	4.08
R	6.43	17.05	38.75	0.58	0.60	14.77	6.05	6.48	1.35	10.67	3.14
S	4.60	8.41	16.28	0.47	0.23	NP	4.68	4.22	1.28	8.86	5.34
T	4.21	5.35	9.98	0.77	1.65	11.37	5.10	7.37	2.08	13.85	2.82
U	8.86	25.30	54.70	0.77	2.04	13.14	11.09	9.81	2.18	12.37	5.65
1	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
2	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	0.05	0.01
3	Neg	Neg	Neg	Neg	Neg	Neg	NP	Neg	Neg	0.40	0.07
4	NP	0.18	0.26	0.03	0.05	3.38	0.09	0.10	0.02	0.41	0.07
5	Neg	0.33	0.54	0.06	0.09	4.39	0.11	0.12	0.04	0.60	0.11
6	3.31	5.09	9.31	1.14	1.43	12.22	2.04	2.53	1.29	7.82	1.67
	Free									Total	

ELISA – Calculated vs B (µg/ml)

Sample	Lab										
	2	3	4	5a	6	7	8a	9	10	1	11
A	40.57	7.63	5.41	54.05	55.24	56.52	39.41	47.56	45.38	41.39	46.49
B	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00
C	NP	0.41	0.55	NP	0.71	15.77	1.79	0.95	NP	2.85	0.33
D	NP	5.78	4.60	1.54	2.45	18.00	33.99	21.02	NP	3.35	3.79
R	5.22	2.60	4.19	0.64	0.66	NP	4.77	6.16	1.23	8.63	2.92
S	3.81	1.28	1.76	0.51	0.26	NP	3.69	4.08	1.16	7.17	4.97
T	3.34	0.82	1.08	0.85	1.82	12.86	4.02	7.01	1.95	11.47	2.62
U	7.23	3.86	5.92	0.85	2.25	14.85	8.74	9.34	1.98	10.24	5.25
1	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
2	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	0.04	0.01
3	Neg	Neg	Neg	Neg	Neg	Neg	NP	Neg	Neg	0.33	0.06
4	NP	0.03	0.03	0.03	0.05	3.69	0.06	0.09	0.02	0.34	0.07
5	Neg	0.05	0.06	0.06	0.10	5.06	0.08	0.12	0.04	0.50	0.10
6	2.87	0.78	1.03	1.24	1.58	13.81	1.61	2.45	1.18	6.47	1.56
	Free									Total	

ELISA – Calculated vs IH/kit standards

Sample	Lab										
	2	3	4	5a	6	7	8a	9	10	1	11
A	93.59	4.43	3.06	1036.53	182907	NP	47.85	83.10	350.95	101691	-
B	110.74	29.06	28.28	973.57	165551	NP	60.70	87.36	386.69	111375	-
C	7.35	0.24	0.31	NP	2240	855	1.66	NP	NP	27202	-
D	74.52	3.36	2.60	41.38	8123	NP	41.26	36.25	NP	29043	-
R	11.51	1.51	2.37	12.05	2182	1206	5.79	10.77	9.49	21523	-
S	8.43	0.75	1.00	10.13	846	568	4.48	7.01	8.98	19328	-
T	7.52	0.47	0.61	15.06	6020	NP	4.88	12.25	15.23	26596	-
U	15.85	2.24	3.35	16.37	7465	NP	10.61	16.31	15.28	24652	-
1	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	-
2	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	151	-
3	Neg	Neg	Neg	Neg	Neg	Neg	NP	Neg	Neg	567	-
4	NP	0.02	0.02	0.62	169	NP	0.08	0.16	0.14	587	-
5	Neg	0.03	0.03	1.17	325	NP	0.10	0.20	0.28	821	-
6	8.72	0.45	0.58	22.86	5218	NP	1.96	4.06	9.05	11933	-
	Free									Total	
units	µg/ml	µg/ml	µg/ml	µg/ml	AU/ml	AU/ml	µg/ml	µg/ml	µg/ml	AU/ml	

ECL – Calculated vs A (µg/ml)

Sample	Lab						
	8b	12	15	16	13a	14a	17
A	50.00	50.00	50.00	50.00	50.00	50.00	50.00

B	232.32	255.73	628.28	215.97	199.86	170.10	106.42
C	10.25	2.15	14.72	4.93	3.08	4.08	3.06
D	66.41	109.49	44.99	249.57	23.07	34.61	15.30
R	18.09	13.74	29.36	9.88	16.05	0.74	6.38
S	63.43	63.30	211.51	76.93	129.86	100.78	35.05
T	17.05	17.44	54.72	27.23	15.40	21.35	11.59
U	71.43	76.06	235.63	86.81	49.39	47.57	33.09
1	Neg	n/t	Neg	Neg	Neg	Neg	Neg
2	0.02	n/t	0.55	0.03	0.12	Neg	0.05
3	0.09	n/t	1.65	0.12	0.59	0.22	0.25
4	0.26	n/t	2.06	0.26	0.56	0.35	0.27
5	0.51	n/t	3.46	0.62	0.82	0.48	0.45
6	6.71	n/t	53.31	7.41	11.99	7.84	4.90
	Free				Total		

ECL – Calculated vs B (µg/ml)

Sample	Lab						
	8b	12	15	16	13a	14a	17
A	10.76	9.78	3.98	11.58	12.51	14.70	23.49
B	50.00	50.00	50.00	50.00	50.00	50.00	50.00
C	2.21	0.42	1.19	1.14	0.72	1.18	1.44
D	14.29	21.41	3.58	57.78	5.77	9.59	7.19
R	3.89	2.69	2.34	2.29	4.02	0.46	3.00
S	13.65	12.38	16.83	17.81	30.51	27.24	16.47
T	3.67	3.41	4.35	6.30	4.01	5.88	5.44
U	15.37	14.87	18.75	20.10	12.36	16.10	15.55
1	Neg	n/t	Neg	Neg	Neg	Neg	Neg
2	0.003	n/t	0.04	0.01	0.03	Neg	0.02
3	0.02	n/t	0.13	0.03	0.15	0.06	0.12
4	0.06	n/t	0.16	0.06	0.13	0.10	0.12
5	0.11	n/t	0.28	0.14	0.19	0.14	0.21
6	1.44	n/t	4.39	1.79	2.82	2.17	2.30
	Free				Total		

ECL – Calculated vs IH/kit standards

Sample	Lab						
	8b	12	15	16	13a	14a	17
A	8.07	33.27	3.22	2.52	-	58.12	15.20
B	37.50	170.17	40.43	10.87	-	210.79	32.35
C	1.66	1.43	0.96	0.25	-	4.98	0.93
D	10.72	72.86	2.90	12.56	-	40.44	4.65
R	2.92	9.15	1.89	0.50	-	1.78	1.94
S	10.24	42.12	13.61	3.87	-	114.84	10.65
T	2.75	11.60	3.52	1.37	-	24.79	3.52
U	11.53	50.61	15.16	4.37	-	67.87	10.06
1	Neg	n/t	Neg	Neg	-	Neg	Neg
2	0.003	n/t	0.04	0.001	-	Neg	0.02
3	0.01	n/t	0.11	0.01	-	0.25	0.08
4	0.04	n/t	0.13	0.01	-	0.41	0.08
5	0.08	n/t	0.22	0.03	-	0.56	0.14
6	1.08	n/t	3.55	0.38	-	9.16	1.49
	Free				Total		
Units	µg/ml	µg/ml	µg/ml	µg/ml		µg/ml	µg/ml

Other assays – Calculated vs A (µg/ml)

Sample	Lab				
	5b	18	20	21	19a
A	50.00	50.00	50.00	50.00	50.00
B	62.50	40.26	68.48	49.13	50.64

C	NP	1.24	NP	5.59	28.06
D	9.90	7.44	17.72	5.06	NP
R	1.14	0.64	1.54	6.13	12.83
S	1.30	2.72	1.62	Neg	NP
T	NP	1.93	NP	12.08	10.40
U	4.12	0.74	6.59	8.60	10.21
1	Neg	Neg	Neg	Neg	Neg
2	Neg	Neg	Neg	Neg	Neg
3	Neg	Neg	0.01	0.15	1.53
4	0.05	0.06	0.08	0.42	1.84
5	0.09	0.05	0.11	0.59	1.15
6	2.08	1.54	2.28	9.92	17.80
	Free				Total

Other assays – Calculated vs B (µg/ml)

Sample	Lab				
	5b	18	20	21	19a
A	40.00	62.09	36.51	50.88	49.37
B	50.00	50.00	50.00	50.00	50.00
C	NP	1.55	NP	5.68	29.06
D	7.95	9.24	12.94	5.19	NP
R	0.89	0.79	1.12	6.28	12.67
S	0.99	3.38	1.19	Neg	NP
T	1.03	2.40	NP	12.29	10.27
U	3.29	0.92	4.81	8.75	11.40
1	Neg	Neg	Neg	Neg	Neg
2	Neg	Neg	Neg	Neg	Neg
3	Neg	Neg	0.01	0.15	1.46
4	0.04	0.07	0.06	0.43	1.82
5	0.07	0.07	0.08	0.60	1.22
6	1.66	1.86	1.66	10.10	17.58
	Free				Total

Other assays – Calculated vs IH/kit standards

Sample	Lab				
	5b	18	20	21	19a
A	345.61	393.55	236.11	41795	-
B	421.43	321.45	301.20	41069	-
C	44.51	15.11	17.15	4669	-
D	58.79	61.86	30.14	4453	-
R	7.27	5.30	5.23	5389	-
S	7.99	21.67	5.60	Neg	-
T	7.28	14.94	6.03	10098	-
U	27.74	5.67	16.67	7189	-
1	Neg	Neg	Neg	Neg	-
2	Neg	Neg	Neg	Neg	-
3	Neg	Neg	0.04	127	-
4	0.42	0.47	0.41	354	-
5	0.72	0.46	0.59	490	-
6	12.93	68.20	7.72	8296	-
	Free				Total
Units	µg/ml	µg/ml	µg/ml	AU/ml	

Binding – all assays – Calculated vs A (µg/ml)

Sample	Lab																						
	2	3	4	5a	5b	6	7	8a	8b	9	10	12	15	16	18	20	21	1	11	13a	14a	17	19a
A	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00
B	61.62	327.71	462.36	46.25	62.50	45.26	44.23	63.44	232.32	52.57	55.09	255.73	628.28	215.97	40.26	68.48	49.13	60.40	53.77	199.86	170.10	106.42	50.64
C	NP	2.71	5.13	NP	NP	0.62	12.44	3.02	10.25	NP	NP	2.15	14.72	4.93	1.24	NP	5.59	3.05	0.36	3.08	4.08	3.06	28.06
D	NP	37.91	42.55	1.46	9.90	2.22	22.63	43.12	66.41	21.81	NP	109.49	44.99	249.57	7.44	17.72	5.06	3.84	4.08	23.07	34.61	15.30	NP
R	6.43	17.05	38.75	0.58	1.14	0.60	14.77	6.05	18.09	6.48	1.35	13.74	29.36	9.88	0.64	1.54	6.13	10.67	3.14	16.05	0.74	6.38	12.83
S	4.60	8.41	16.28	0.47	1.30	0.23	NP	4.68	63.43	4.22	1.28	63.30	211.51	76.93	2.72	1.62	Neg	8.86	5.34	129.86	100.78	35.05	NP
T	4.21	5.35	9.98	0.77	NP	1.65	11.37	5.10	17.05	7.37	2.08	17.44	54.72	27.23	1.93	NP	12.08	13.85	2.82	15.40	21.35	11.59	10.40
U	8.86	25.30	54.70	0.77	4.12	2.04	13.14	11.09	71.43	9.81	2.18	76.06	235.63	86.81	0.74	6.59	8.60	12.37	5.65	49.39	47.57	33.09	10.21
1	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	n/t	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
2	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	0.02	Neg	Neg	n/t	0.55	0.03	Neg	Neg	Neg	0.05	0.01	0.12	Neg	0.05	Neg
3	Neg	Neg	Neg	Neg	Neg	Neg	Neg	NP	0.09	Neg	Neg	n/t	1.65	0.12	Neg	0.01	0.15	0.40	0.07	0.59	0.22	0.25	1.53
4	NP	0.18	0.26	0.03	0.05	0.05	3.38	0.09	0.26	0.10	0.02	n/t	2.06	0.26	0.06	0.08	0.42	0.41	0.07	0.56	0.35	0.27	1.84
5	Neg	0.33	0.54	0.06	0.09	0.09	4.39	0.11	0.51	0.12	0.04	n/t	3.46	0.62	0.05	0.11	0.59	0.60	0.11	0.82	0.48	0.45	1.15
6	3.31	5.09	9.31	1.14	2.08	1.43	12.22	2.04	6.71	2.53	1.29	n/t	53.31	7.41	1.54	2.28	9.92	7.82	1.67	11.99	7.84	4.90	17.80
	Free																	Total					

Binding – all assays – Calculated vs B (µg/ml)

Sample	Lab																						
	2	3	4	5a	5b	6	7	8a	8b	9	10	12	15	16	18	20	21	1	11	13a	14a	17	19a
A	40.57	7.63	5.41	54.05	40.00	55.24	56.52	39.41	10.76	47.56	45.38	9.78	3.98	11.58	62.09	36.51	50.88	41.39	46.49	12.51	14.70	23.49	49.37
B	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00
C	NP	0.41	0.55	NP	NP	0.71	15.77	1.79	2.21	0.95	NP	0.42	1.19	1.14	1.55	NP	5.68	2.85	0.33	0.72	1.18	1.44	29.06
D	NP	5.78	4.60	1.54	7.95	2.45	18.00	33.99	14.29	21.02	NP	21.41	3.58	57.78	9.24	12.94	5.19	3.35	3.79	5.77	9.59	7.19	NP
R	5.22	2.60	4.19	0.64	0.89	0.66	NP	4.77	3.89	6.16	1.23	2.69	2.34	2.29	0.79	1.12	6.28	8.63	2.92	4.02	0.46	3.00	12.67
S	3.81	1.28	1.76	0.51	0.99	0.26	NP	3.69	13.65	4.08	1.16	12.38	16.83	17.81	3.38	1.19	Neg	7.17	4.97	30.51	27.24	16.47	NP
T	3.34	0.82	1.08	0.85	1.03	1.82	12.86	4.02	3.67	7.01	1.95	3.41	4.35	6.30	2.40	NP	12.29	11.47	2.62	4.01	5.88	5.44	10.27
U	7.23	3.86	5.92	0.85	3.29	2.25	14.85	8.74	15.37	9.34	1.98	14.87	18.75	20.10	0.92	4.81	8.75	10.24	5.25	12.36	16.10	15.55	11.40
1	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	n/t	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
2	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	0.003	Neg	Neg	n/t	0.04	0.01	Neg	Neg	Neg	0.04	0.01	0.03	Neg	0.02	Neg
3	Neg	Neg	Neg	Neg	Neg	Neg	Neg	NP	0.02	Neg	Neg	n/t	0.13	0.03	Neg	0.01	0.15	0.33	0.06	0.15	0.06	0.12	1.46
4	NP	0.03	0.03	0.03	0.04	0.05	3.69	0.06	0.06	0.09	0.02	n/t	0.16	0.06	0.07	0.06	0.43	0.34	0.07	0.13	0.10	0.12	1.82
5	Neg	0.05	0.06	0.06	0.07	0.10	5.06	0.08	0.11	0.12	0.04	n/t	0.28	0.14	0.07	0.08	0.60	0.50	0.10	0.19	0.14	0.21	1.22
6	2.87	0.78	1.03	1.24	1.66	1.58	13.81	1.61	1.44	2.45	1.18	n/t	4.39	1.79	1.86	1.66	10.10	6.47	1.56	2.82	2.17	2.30	17.58
	Free																	Total					

Binding – all assays – Calculated vs IH/kit standards

Sample	Lab																						
	2	3	4	5a	5b	6	7	8a	8b	9	10	12	15	16	18	20	21	1	11	13a	14a	17	19a
A	93.59	4.43	3.06	1036.53	345.61	182907	NP	47.85	8.07	83.10	350.95	33.27	3.22	2.52	393.55	236.11	41795	101691	-	-	58.12	15.20	-
B	110.74	29.06	28.28	973.57	421.43	165551	NP	60.70	37.50	87.36	386.69	170.17	40.43	10.87	321.45	301.20	41069	111375	-	-	210.79	32.35	-
C	7.35	0.24	0.31	NP	44.51	2240	855	1.66	1.66	NP	NP	1.43	0.96	0.25	15.11	17.15	4669	27202	-	-	4.98	0.93	-
D	74.52	3.36	2.60	41.38	58.79	8123	NP	41.26	10.72	36.25	NP	72.86	2.90	12.56	61.86	30.14	4453	29043	-	-	40.44	4.65	-
R	11.51	1.51	2.37	12.05	7.27	2182	1206	5.79	2.92	10.77	9.49	9.15	1.89	0.50	5.30	5.23	5389	21523	-	-	1.78	1.94	-
S	8.43	0.75	1.00	10.13	7.99	846	568	4.48	10.24	7.01	8.98	42.12	13.61	3.87	21.67	5.60	Neg	19328	-	-	114.84	10.65	-
T	7.52	0.47	0.61	15.06	7.28	6020	NP	4.88	2.75	12.25	15.23	11.60	3.52	1.37	14.94	6.03	10098	26596	-	-	24.79	3.52	-
U	15.85	2.24	3.35	16.37	27.74	7465	NP	10.61	11.53	16.31	15.28	50.61	15.16	4.37	5.67	16.67	7189	24652	-	-	67.87	10.06	-
1	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	n/t	Neg	Neg	Neg	Neg	Neg	Neg	-	-	Neg	Neg	-
2	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	0.003	Neg	Neg	n/t	0.04	0.001	Neg	Neg	Neg	151	-	-	Neg	0.02	-
3	Neg	Neg	Neg	Neg	Neg	Neg	Neg	NP	0.01	Neg	Neg	n/t	0.11	0.01	Neg	0.04	127	567	-	-	0.25	0.08	-
4	NP	0.02	0.02	0.62	0.42	169	NP	0.08	0.04	0.16	0.14	n/t	0.13	0.01	0.47	0.41	354	587	-	-	0.41	0.08	-
5	Neg	0.03	0.03	1.17	0.72	325	NP	0.10	0.08	0.20	0.28	n/t	0.22	0.03	0.46	0.59	490	821	-	-	0.56	0.14	-
6	8.72	0.45	0.58	22.86	12.93	5218	NP	1.96	1.08	4.06	9.05	n/t	3.55	0.38	68.20	7.72	8296	11933	-	-	9.16	1.49	-
	Free																	Total					
Units	µg/ml	µg/ml	µg/ml	µg/ml	µg/ml	AU/ml	AU/ml	µg/ml	µg/ml	µg/ml	µg/ml	µg/ml	µg/ml	µg/ml	µg/ml	µg/ml	AU/ml	AU/ml			µg/ml	µg/ml	

NP	Non-parallel to standard
n/t	Sample not tested by lab
x	Sample reported as negative or below assay lower quantitation limit

Appendix 4: Study protocol

COLLABORATIVE STUDY FOR THE EVALUATION OF MONOCLONAL ANTIBODIES AGAINST ADALIMUMAB

1. BACKGROUND

It is well recognized that a proportion of patients treated with TNF antagonists develop anti-drug antibodies (ADAs) which can result in drug's loss of efficacy. Currently, several methods that differ in sensitivity and types of antibodies detected are in use by manufacturers, clinical laboratories, and hospitals to detect these anti-drug antibodies. Standardization of antibody assays is lacking and there is a need for common reference standards to evaluate assay performance and, if possible, standardize testing across different assay platforms.

A panel of antibodies directed against adalimumab has been prepared for testing in binding and neutralization assays. The panel includes two lyophilized human (ABIRISK consortium) and two lyophilized chimeric rat-human (NIHS) monoclonal antibodies as well as liquid antibodies, provided by collaborators, which differ in terms of their affinity and isotype e.g. IgG1 and IgG4.

2. AIM

The aim of this collaborative study is to evaluate the panel of antibodies against adalimumab together with samples to:

1) Compare the antibodies across available methods and assess their suitability for use as performance indicators.

2) Assign arbitrary unitage, if feasible, for each of the lyophilized preparations to enable calibration of local standards and for assay harmonization.

3. MATERIALS PROVIDED

The panel contains purified monoclonal antibodies and includes:

- 4 lyophilized preparations coded **A, B, C and D**, each ampoule containing 50 μg of antibody. 5 ampoules of each are provided.
- 4 liquid monoclonal antibody (mAb) preparations coded **R to U**, each tube containing 10 μg of antibody in 20% normal healthy serum. 1 aliquot (1.0 ml) of each preparation is provided.

Additionally, 6 serum samples, coded **1 to 6**, from adalimumab-treated patients and healthy control subjects are provided in 1 aliquot each (1.0 ml).

All materials provided are listed in the Table below.

Sample Type	Code	ADA Amount	Containers provided of each
Lyophilized	**A, B, C, D**	**50 μg**	**5**

Liquid mAbs	**R – U**	**10 μg/ml**	**1**
Serum samples	**1 – 6**	**Unknown**	**1**

Participants performing more than 1 assay method will receive 5 extra ampoules and those requiring higher sample volume for testing will receive additional aliquots along with the materials listed in Table above.

Prior to initiating the study, please read the Instructions for Use provided with the study materials and note information relating to reconstitution and storage. Please note the statements regarding safety and that these preparations are not for human use. Please note that all preparations including sera should be regarded as potentially hazardous to health. They should be used and discarded according to your own laboratory's safety procedures.

4. RECONSTITUTION AND STORAGE OF LYOPHILISED PREPARATIONS

Store ampoules at –20°C or below until reconstitution and use.

Reconstitution: Reconstitute lyophilized antibodies with 1ml of sterile distilled water and mix **gently** to dissolve ampoule contents prior to use. Allow resting ~20 min at RT to ensure complete dissolution.

5. OTHER PREPARATIONS AND SERUM SAMPLES

Store all liquid mAb preparations and serum samples at –20°C or below until aliquoting and use.

Liquid mAb preparations and serum samples: At least 24-48 hours prior to the pilot assay, thaw all samples and prepare further smaller aliquots in volumes adequate for your assays. Store all aliquots at –20°C or below until use.

For each assay run, a fresh aliquot should be used to ensure samples are treated consistently.

6. TESTS

The antibody preparations should be tested in assay platforms in use for anti-adalimumab binding and/or neutralizing activity and tested in parallel with applicable reference standards (referred to as 'in-house standards' in this document) and assay controls.

Participants are asked to carry out **three independent assays (= 3 assay runs). For this study, assays are considered independent if new ampoules and new aliquots are used, and the assays carried out on different occasions/days**. **Include three plates in each independent assay. All preparations and samples to be tested, as well as in-house standard(s), should be included in singlicate on each plate in each assay run.**

Note: Testing of the preparations and samples does **not** require a confirmatory assay and does **not** require an additional step to dissociate immune complexes (e.g. acid dissociation, SPEAD, etc.).

Participants are requested to

- **Perform three independent assays/assay runs according to the plate layout specified in example plate layout template (see Appendix 1 below). Please include three plates per independent assay as indicated in the plate layout.**
- **For each assay/run, create and test dilution series of the lyophilized preparations A to D. Continue the dilutions until the activity reaches the detection limit of the assay. The assay must include lyophilized preparations A, B, C and D in singlicate on each plate as indicated in the example plate layout.**
- **Include in-house/kit standard dilution series along with QC samples as used in routine test.**
- **Create and test dilution series of each liquid preparation R-U in singlicate, including not less than six (6) dilutions of each preparation in a linear section of the dose-response curve.**
- **Create and test dilution series of each serum samples 1-6, including not less than five (5) dilutions of each preparation, bracketing the assay endpoint (except for any identified negative sample).**

A. Pilot Assay

Test all samples (lyophilized mAbs, liquid mAbs and serum samples) in parallel with in-house standard(s) and quality control (QC) samples in the assay.

I. Lyophilized preparations: Following reconstitution of 1 ampoule of each lyophilized antibody with 1ml of sterile distilled water (as stated above), perform further dilutions (e.g., 2-, 2.5- or 3- fold dilution series) for a dose-response curve in a suitable matrix (see below). It is imperative to include these preparations in parallel with any in-house standard(s) used in the assay.
II. Liquid preparations: Thaw 1 frozen aliquot of each sample and prepare dilution series for a dose-response curve in suitable matrix (see below).
III. Serum samples: Thaw 1 frozen aliquot of each sample and prepare dilution series in suitable matrix (see below).

For matrix, it is advisable to use the same matrix as is routinely used for dilution of in-house/kit standard or serum samples. A suitable matrix can be pooled normal human sera (drug naïve i.e., negative for adalimumab and ADAs) or assay diluent which does not show any matrix effects or interference in the assay(s).

If using more than 1 assay method/platform (e.g., 1 binding assay and 1 neutralization assay), the same aliquot can be used for another pilot assay.

Based on the results obtained, **select the most appropriate dose range and suitable dilution series (e.g., 2-, 2.5- or 3- fold dilution) for evaluation in further assays. If dose range and dilution series used in pilot assay are appropriate, use these in further assays. In this case, the data obtained in the pilot assay can be submitted as part of the final data set and considered as 1 of the 3 independent assays.**

B. Assay 1

Reconstitute 1 fresh ampoule of each lyophilized antibody and test using selected dose range and appropriate dilution series (established from pilot assay). The assay must include lyophilized preparations A to D in singlicate on each plate along with dilution series of in-house standard(s) and QC samples in the assay if available.

For other samples, including serum samples, thaw aliquots and use as indicated in Steps II and III of the pilot assay; test as appropriate in singlicates based on the dilution series established from the pilot assay.

Record data for each antibody as indicated on the results sheets provided.

C. Assay 2 and Assay 3

Reconstitute 1 fresh ampoule of each lyophilized antibody, thaw aliquots of the other samples and test as for Assay 1 and report data as indicated.

For each assay method/platform, repeat the above steps from 6A to 6C. Record data for each antibody as indicated on the results sheets provided.

7. RESULTS AND DATA PRESENTATION

Participants must supply all raw data electronically, as clearly annotated as possible, using the exemplar Excel template (96-well plate format) provided.

The exemplar Excel template can be copied or modified as required based on the assay design, number of plates and/or dilutions conducted.

Please let us know, as clearly as possible, how the assay was performed, how the antibody preparations were diluted, and **the dilutions included in the assay** (and at what positions of microtiter plates).

Participants are requested to report data for each tested preparation/sample based on their reporting practice for ADA levels e.g. titer or ADA concentration relative to in-house/kit standards AND, if possible, relative to candidate preparations A and B as shown in Appendix 2.

Please provide information regarding a) your local positive control/in-house standard, b) quality control samples, c) your assay method including critical reagents, d) the method used to define the assay cut-point or ADA positive samples and e) analytical method for determining ADA concentration.

Participants in the study are advised to take note of the **Collaborative Study Terms and Conditions** attached, and disclaimers in the 'Instructions for Use' which accompany the samples, detailing the prohibitions against (i) use in humans (ii) further transfer of material (iii) use for commercial purposes, and (iv) use for any purpose other than the establishment of a reference standard.

In accordance with procedures of the Expert Committee on Biological Standardization of the WHO, please note that laboratories participating in a collaborative study are requested not to publish or circulate information on the materials included in the study. Once the final report has been agreed by participants and submitted to WHO, this reservation no longer applies.

Deadline for data submission: please return all raw data (not in pdf format), assay method sheets, plate layouts and results by **31st July 2024** to
Dr Meenu Wadhwa (Meenu.Wadhwa@mhra.gov.uk) and Dr Isabelle Cludts (Isabelle.Cludts@mhra.gov.uk)

International Collaborative Study for evaluation of anti- adalimumab antibodies

Laboratory identification:

Assay information

Please provide information regarding a) your local positive control/in-house standard, b) quality control samples, c) your step-by-step assay method including critical reagents (nature, concentrations), d) the method used to define the assay cut-point or ADA positive samples and e) analytical method for determining ADA concentration.

Appendix 1: Example of plate layout for 1 assay

3 plates per assay; 3 independent assay runs (→ data from 9 plates).

The number of plates and dilution points can be adjusted BUT please contact us for further advice prior to performing the assay.

A, B, C, D: lyophilised preparations; R-U: liquid preparations; 1-6: serum samples

Plate 1

	1	2	3	4	5	6	7	8	9	10	11	12
A	IH	A	B	C	D	R	S	T	1	2	4	5
B	IH	A	B	C	D	R	S	T	1	2	4	6
C	IH	A	B	C	D	R	S	U	1	3	4	6
D	IH	A	B	C	D	R	S	U	1	3	4	6
E	IH	A	B	C	D	R	T	U	1	3	5	6
F	IH	A	B	C	D	R	T	U	2	3	5	6
G	QC	A	B	C	D	S	T	U	2	3	5	NC
H	QC	A	B	C	D	S	T	U	2	4	5	NC

Plate 2

	1	2	3	4	5	6	7	8	9	10	11	12
A	R	S	T	IH	C	D	1	2	4	5	A	B
B	R	S	T	IH	C	D	1	2	4	6	A	B
C	R	S	U	IH	C	D	1	3	4	6	A	B
D	R	S	U	IH	C	D	1	3	4	6	A	B
E	R	T	U	IH	C	D	1	3	5	6	A	B
F	R	T	U	IH	C	D	2	3	5	6	A	B
G	S	T	U	QC	C	D	2	3	5	NC	A	B
H	S	T	U	QC	C	D	2	4	5	NC	A	B

Plate 3

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	2	4	5	A	B	IH	C	D	R	S	T
B	1	2	4	6	A	B	IH	C	D	R	S	T
C	1	3	4	6	A	B	IH	C	D	R	S	U
D	1	3	4	6	A	B	IH	C	D	R	S	U
E	1	3	5	6	A	B	IH	C	D	R	T	U
F	2	3	5	6	A	B	IH	C	D	R	T	U
G	2	3	5	NC	A	B	QC	C	D	S	T	U
H	2	4	5	NC	A	B	QC	C	D	S	T	U

Appendix 2: Reporting of results (see Excel template)

Reporting

For ADA-positive samples, if routinely reporting titer, complete titer column. If concentration determined, complete calculated concentration.

	ADA +ve/-ve	If ADA+ve, titer *	Concentration relative to in-house/kit standard	Concentration relative to A	Concentration relative to B
C					
D					
R					
S					
T					
U					
1					
2					
3					
4					
5					
6					

*** last dilution above cut-point**

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WHO International Reference Panel
1st Adalimumab Antibody Reference Panel
NIBSC code: 25/xxx
Instructions for use
(Version [Q-DOCS_Version], Dated [Q-DOCS_Date_Published])

§

1. INTENDED USE

The International Reference Panel for Adalimumab anti-drug antibodies (ADAs) is intended for the development, characterization and validation of adalimumab anti-drug antibody assays. The antibodies can be used for assay selection and for monitoring assay performance.
The panel contains:
19/264 - a high affinity, neutralizing human IgG1, intended for calibration of neutralizing antibody assays. It has been assigned an arbitrary unitage for neutralising activity.
19/266 – a high affinity, neutralizing human IgG1 intended for calibration of in-house and commercially available ADA binding assays. It has been assigned an arbitrary unitage for binding activity. This would facilitate comparison and harmonization of results across adalimumab ADA assays.
FS-007 – a low affinity, neutralizing chimeric IgG1, for utility in detecting low binding activity ADAs and those with fast dissociation. No unitage is assigned to this reference preparation.
FS-008 - a low affinity, neutralizing chimeric IgG4, for assessing the ability of the assay to detect the IgG4 isotype. No unitage is assigned to this reference preparation.
Detailed information on these antibodies can be found in the collaborative study report for the 1st WHO International reference Panel for Adalimumab anti-drug antibodies.

2. CAUTION

This preparation is not for administration to humans or animals in the human food chain.
The material is not of human or bovine origin. 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

19/264 - 50,000 IU/ampoule for neutralizing activity
19/266 - 50,000 IU/ampoule for binding activity
FS-007 - No unitage is assigned to this antibody
FS-008 - No unitage is assigned to this antibody

4. CONTENTS

Country of origin of biological material: France/Japan.
Each ampoule contains the residue after freeze-drying of 1.0 ml of a solution that contained:

50.0 µg adalimumab antibody produced in CHO cells
10mM L-Glutamic acid
4% Mannitol
2% Sucrose
0.01%Tween20

The material has not been sterilised and contains no bacteriostat.

5. STORAGE

Unopened ampoules should be stored at –20°C.
If materials are stored at 4°C or room temperature following reconstitution, it is strongly advised to use the materials within 24 hours. For longer storage post-reconstitution, please keep the materials at –20°C. Please note storage of FS-007 following reconstitution is not recommended. This material should be used directly after reconstitution.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. Various types of ampoule breaker are available commercially. To open the ampoule, tap the ampoule gently to collect material at the bottom (labelled) end and follow manufactures instructions provided with the ampoule breaker.

7. USE OF MATERIAL

No attempt should be made to weigh out any portion of the freeze-dried material prior to reconstitution
Reconstitution: dissolve the total contents in 1ml of sterile distilled water. For further dilutions, use a suitable buffer solution with carrier protein (free of peptidase), to minimise loss by surface adsorption.

8. STABILITY

Reference materials are held at NIBSC within assured, temperature-controlled storage facilities. Reference Materials should be stored on receipt as indicated on the label.

NIBSC follows the policy of WHO with respect to its reference materials.

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 WHO International Reference Panel for Adalimumab anti-drug antibodies

10. ACKNOWLEDGEMENTS

We are thankful to the ABIRISK consortium (funded by the Innovative Medicines Initiative program, EU) and the National Institute of Health Sciences (Japan) for donating the antibodies, and to the study participants for supporting the study.

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

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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

Classification in accordance with Directive 2000/54/EC, Regulation (EC) No 1272/2008: Not applicable or not classified

Physical and Chemical properties			
Physical appearance: Freeze-dried powder		Corrosive:	No
Stable:	Yes	Oxidising:	No
Hygroscopic:	No	Irritant:	No
Flammable:	No	Handling: See caution, Section 2	
Other (specify):	n/a		

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.

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: 5g
Toxicity Statement: Toxicity not assessed
Veterinary certificate or other statement if applicable. **Attached:** No

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