Medicines & Healthcare products
Regulatory Agency

Harmonising adalimumab anti-drug antibody assays through WHO international standards

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16 March 2026

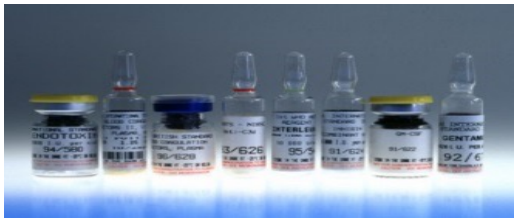
Overview

- WHO standards and application
- Workflow of development of standards
- Recent examples of potency standards for biotherapeutics
- Utility of standards in clinical application
- Adalimumab ADA standards

WHO International Standards

A core function of WHO as per its Constitution (Article 2) is *“ to develop, establish and promote international standards with respect to food, biological, pharmaceutical and similar products" as well as "to standardize diagnostic procedures as necessary’’*

- **Written Standards :** Recommendations, Guidelines etc
- **Measurement Standards** : Physical – a common language for test results from biological, immunological and other assays



Allows harmonisation, e. g. in public health applications (e.g. monitor immune status, screen for disease or susceptibility), diagnose disease, monitor therapy, or in testing for blood safety

Successful harmonisation of tests allows international consensus on interpretation of results and helps to define regulatory requirements for tests or products

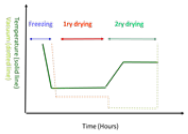
“Define an internationally agreed unit to allow comparison of biological measurements worldwide”

Facilitate transfer of laboratory science into worldwide clinical practice and development of safe and effective biological medicines

Development of a WHO International standard (IS): Key Steps

World Health Organization

Project endorsement ECBS — 0

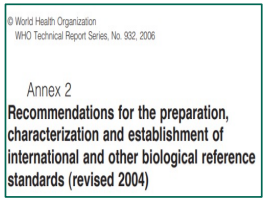


1 2

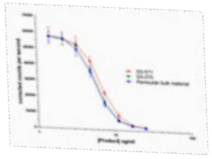
3 4

Substance X

Sourcing of materials

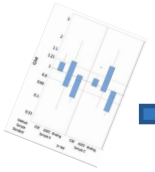


Selection of formulation for lyophilisation

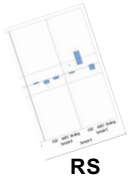


Definitive fill and production of candidates

Suitability tested in multi-centre study + Statistical analysis

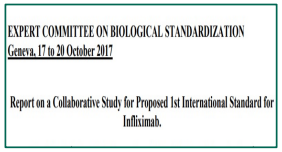


IHS



RS

Report to ECBS Proposed IS with potency in IU


EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION
Geneva, 17 to 20 October 2017

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

Establishment of IS by ECBS

World Health Organization

IS for distribution

STEPS
0 Endorsement
1 Testing of definitive fill
2 Protocol finalisation and participant recruitment, despatch
3 Stability testing
4 Report compilation and submission to ECBS

ECBS – Expert Committee Of Biological Standardisation

The process takes **pharmaceutical grade material (milligrams)** through to a **WHO international standard** –

- an assigned "**biological potency in international units (IU) to allow comparison of biological measurements worldwide**"
- **ensures harmonisation in dosage to patients globally (long history) e.g., coagulation factors, erythropoietin etc**

MHRA: WHO Collaborating Centre

- MHRA – UK regulatory Agency
- WHO Collaborating Centre
- 2 main sites – Headquarters – Canary Wharf, London
- Laboratories – South Mimms, HERTS, UK. Reference standards work undertaken (formerly NIBSC)
- Prepares and distributes >90% of WHO IS for biologicals
- Several product classes e.g., vaccines, cytokines, blood products, monoclonal antibodies, hormones diagnostics etc
- https://nibsc.org

WHO International Standards for Biosimilars (since 2013)

Protein	WHO IS Code	Year established	Critical reagent (WHO)
Peg-filgrastim	12/188	2013	-
Etanercept	13/204	2015	TNF IS (12/154)
Rituximab	14/210	2017	-
Infliximab*	16/170	2017	TNF IS (12/154)
Adalimumab*	17/236	2019	TNF IS (12/154)
Darbepoietin	17/204	2019	-
Bevacizumab	18/210	2020	VEGF IS (19/246)
Trastuzumab	19/108	2020	-
Cetuximab	21/170	2022	-
Golimumab*	22/116	2024	TNF IS (12/154)
Ranibizumab	23/124	2025	VEGF IS (19/246)
Ustekinumab	Collab study (ongoing)		

* - bioactivity & for drug monitoring. Shaded – recent/ongoing

- Potency assay (P) for lot release;
- Available from: https://nibsc.org

mAb	WHO IS Code	Activities
Rituximab	14/210	1000 IU CDC activity (P) 1000 IU ADCC activity 1000 IU CD20-cell binding activity 1000 IU apoptotic activity
Adalimumab	17/236	500 IU TNF-α neutralising activity (P) 500 IU TNF-α binding activity 500 IU ADCC activity 500 IU CDC activity
Bevacizumab	18/210	1000 IU VEGF165 neutralising activity (P) 1000 IU VEGF165 binding activity
Trastuzumab	19/108	1000 IU IOP activity (P) 1000 IU ADCC activity 1000 IU ADCP activity 1000 IU HER-2 binding activity 1000 IU FcγRIIIa binding activity
Golimumab	22/116	500 IU TNF-α neutralising activity (P) 500 IU TNF-α binding activity 500 IU Fcγ RIIIa binding 500 IU ADCC activity
Ranibizumab	23/124	1000 IU VEGF165 neutralising activity (P) 1000 IU VEGF165 binding activity

WHO IS : Bioactivity standards

- **WHO IS :** Apply to all products (originator, biosimilar)
  - Serve as a ‘tool’ :
  - To assure bioactivity data and assay performance
  - To harmonise bioactivity, to identify and control drift during product lifecycle.
  - For ‘independent testing’ when required e.g., product bioactivity over time, falsified products

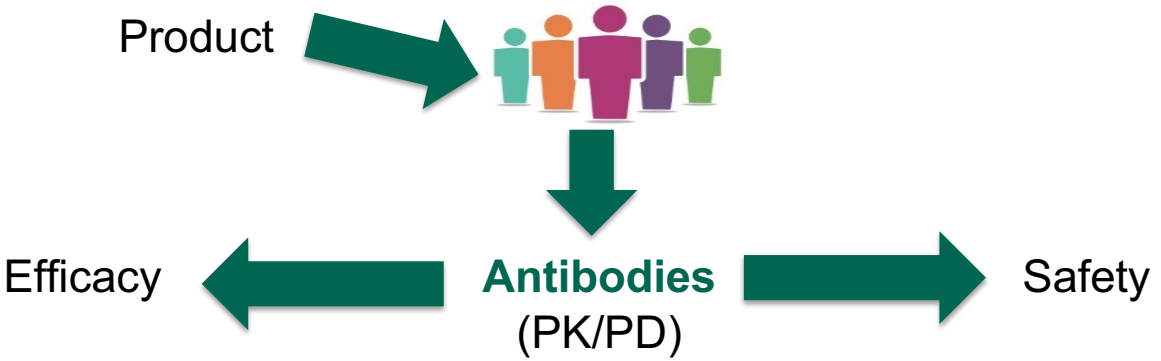
- **Regulation:** WHO IS are not mandatory unless specified in regulation.
  - Use stated in ICH6B, other GLs
  - In Europe, UK and US, a calibration of in-house standard to the IS expected (when available) (biosimilar GLs).
  - Ph Eur – product-specific monographs and supporting reference standards
  - Explicit in WHO GL so countries likely to follow ... regulatory convergence

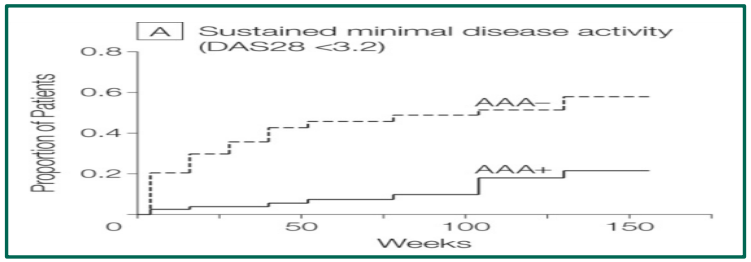
Provide assurance in availability of safe and effective medicines

Confidence in prescribing and product uptake

Supporting Clinical Application

Rationale for using IS for clinical monitoring





> N Engl J Med. 2002 Feb 14;346(7):469-75. doi: 10.1056/NEJMoa011931.

Pure red-cell aplasia and antierythropoietin antibodies in patients treated with recombinant erythropoietin

Nicole Casadevall [1], Joelle Nataf, Beatrice Viron, Amir Kolta, Jean-Jacques Kiladjian, Philippe Martin-Dupont, Patrick Michaud, Thomas Papo, Valerie Ugo, Irene Teyssandier, Bruno Varet, Patrick Mayeux

- Monitoring for anti-drug antibodies (ADA)/ therapeutic is important
- Clinical efficacy dependent on relative amounts of drug and ADA.
- For ADA, both binding and neutralizing assays used. Several methods in use, sensitivity varies
- No universally accepted method or reference reagents

Bartelds et al (2011) JAMA, 305 (14) : 1460-1468; Cludts I et al (2017) Cytokine, 96, 16-23

EPO Antibody Reference Panel

			Binding		Nab		
Original Antibody	Target	Isotype	ECL	SPR	Cell-based Assay	Affinity	Study Code
3F5.1	High affinity receptor site 1	IgG1	+	+++	++	Low	B
9F7.1	Non-receptor	IgG2	++	+++	-	Low	C
		IgM	++	+++	-	Low	G
3A4.1	Low affinity receptor site 2	IgG4	+++	+++	++	High	F
8C10.2	High affinity receptor site 1	IgG1	+++	+++	+++	High	J
		IgG2	+++	+++	+++	High	E
		IgG4	+++	+++	+++	High	H
11D12	Unknown	IgG2	+++	++	+	Mod	D
		IgM	+++	++	+	Mod	I

Panel (B-I) represents a) non-neutralizing, usually pre-existing, b) early onset, typically non-neutralizing, IgM and IgG1, and c) those characteristic of a neutralizing antibody-mediated PRCA - IgG1, IgG2 and IgG4 isotypes

Established in Oct 2015

Intended for assay selection, characterization and performance. No units assigned

Binding assays – Platform based disparity; varied recognition based on assay and mAb characteristics. Direct ELISAs gave spurious data.

Where recognised, huge variation (not attributed to variability in data) in titres between assays/labs.

Low affinity & IgM antibodies mostly missed except in SPR/RIPAs

Neutralization assays – More consistent data

B – missed in some binding assays was neutralizing.

EPO: Selection of an assay which detects early onset (& mature ADA) important to avert PRCA

J Immunol Methods. 2016 Aug;435:32-42. doi: 10.1016/j.jim.2016.05.005. Epub 2016 May 10.

Establishment of the first WHO Erythropoietin antibody reference panel: Report of an international collaborative study

Meenu Wadhwa [1], Daniel T Mytych [2], Chris Bird [3], Troy Barger [4], Thomas Dougall [5], Hong Han [4], Peter Rigsby [5], Arno Kromminga [6], Robin Thorpe [3]; Participants of the Study

Immunogenicity Standards

- EPO antibody panel paved the way towards extension to ADA standards for other molecules
- Call from clinicians and industry
- EMA : Workshop on immunogenicity of biotherapeutic products, Mar'16
- Dialog with ABIRISK consortium and clinical labs -
- **ABIRISK : Create a legacy**; a resource that will continue to move the field forward in the improvement of our understanding of biopharmaceutical immunogenicity.
- **Open access to the data generated by ABIRISK**.
- **Open provision of positive controls & Analytical Protocols (APs) from a central suppository.**
- **Aid implementation of standardised assays globally, providing robust data. Potential to serve the clinician on treatment decisions.**
- Expert Committee on Biological Standardisation (WHO committee) – endorsed proposal on initiation of this project (Oct'16); NIBSC as custodians of PCs.

Positive Controls: Scale-up production

WWW.ABIRISK.EU

Create a sustained legacy :

Targeted Biopharmaceutical	Priority	Monoclonal Antibody	Batch Name	Type (Bab/Nab*)	Activity EC80 (ng/mL)	Affinity EC50 (ng/ml)	Binding-KD (M) SPR	Isotype	
Infliximab	1	***INA29***	VA2-17-221-1	Nab	66	9	3 E-10	IgG1	κ
		INA79	VA2-17-198-1	Nab	489	12	1.7 E-10	IgG4	
Adalimumab	1	***ADA44***	VA2-17-199-1	Nab	155	14	2.5 E-10	IgG1	κ
		ADA39	VA2-17-200-1	Nab	169	12	4.9 E-11		
Natalizumab	2	***NAA80***	VA2-17-218-1	Nab	-	(46.02)	-	IgG1	λ
		NAA96	VA2-17-222-1	Bab	4946	9	1.5 E10		
Rituximab	2	***RXA3***	VA2-17-196-1	Nab	105	11	6.1 E-12	IgG1	κ
Interferon-β	3	***sa01.54***	VA2-17-216-1	Nab	69	31	-	IgG4	K
		sa01.71	VA2-17-217-1	Nab	86	420	-	IgG1	λ

Purified human mAbs, isolated & cloned from patient's PBMC and characterized

Slide Courtesy : L. Christodolou – ADA Positive Controls: An ABIRISK legacy, EIP, Nov' 2017

The research leading to these results has received support from the Innovative Medicines Initiative Joint Undertaking under grant agreement n° [115303], resources of which are composed of financial contribution from the European Union's Seventh Framework Programme (FP7/2007-2013) and EFPIA companies' in kind contribution.
www.imi.europa.eu

Development of anti-Adalimumab Reference Standards

WHO/BS/2025.2487

World Health Organization

WHO/BS/2025.2487
ENGLISH ONLY

ESTABLISHED

EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION
Geneva, 13 - 17 October 2025

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

Meenu Wadhwa[1], Isabelle Cludts, Eleanor Atkinson, Peter Rigsby on behalf of study participants

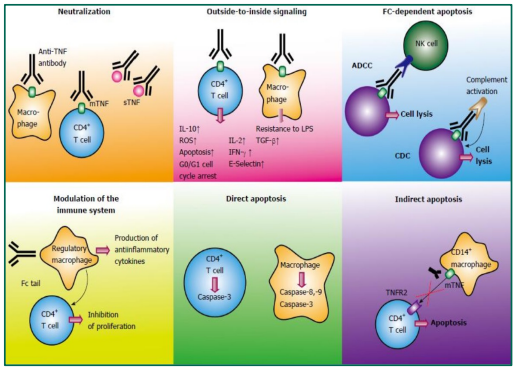
WHO ECBS

- Four standards established:
  - Preparation A (coded 19/264) with an assigned value of **50,000 IU/ampoule** as the **1st WHO International Standard** for Adalimumab Anti-Drug Antibodies for calibration of **neutralisation assays** and for assay harmonization
  - Preparation B (coded 19/266) with an assigned value of **50,000 IU/ampoule** for binding activity as the **1st WHO International Standard** for Adalimumab Anti-Drug Antibodies for use in calibration, characterization and harmonization of **binding assays**
  - Preparation C (coded FS-007) as the **WHO International Reference Reagent** for detecting **low activity** Adalimumab Anti-Drug Antibodies (**no assigned value**)
  - Preparation D (coded FS-008) as the **WHO International Reference Reagent** for detecting **low affinity** Adalimumab Anti-Drug Antibodies (**no assigned value**)
- All labelled and available for distribution

Adalimumab: Background

Fully human IgG1 monoclonal antibody targeting TNF-α

- Humira ® (Abbvie) approved by FDA 2002, EMA 2003
  - Highly successful clinically (blockbuster product); global sales exceeding $21bn in 2022
  - Biosimilars approved in several countries incl EU, US
- Listed in WHO's List of Essential Medicines
- TNF-α - mediated inflammatory diseases - rheumatoid arthritis, Crohn's disease, psoriatic arthritis, ankylosing spondylitis, uveitis etc



Mechanism of action

- Binds to both soluble and transmembrane forms of TNF-α
- Neutralizes TNF-α bioactivity by preventing binding of TNF-α to its receptors (predominant)
- Elicits Fc-mediated effector functions

Concern

- Anti-drug antibodies (ADA) in recipients resulting in secondary failure & adverse events.
- ADA vary from 5% to over 80% depending on various factors incl study, assays.
- Clinical efficacy dependent on relative amounts of drug and ADA

Billmeier et al (2016) World J Gastroenterol. 22(42): 9300-9313

Therapeutic Drug Monitoring: A powerful tool

Multicenter Study > J Crohns Colitis. 2019 Aug 14;13(8):976-981. doi: 10.1093/ecco-jcc/jjz018.

Proactive Therapeutic Drug Monitoring of Adalimumab Is Associated With Better Long-term Outcomes Compared With Standard of Care in Patients With Inflammatory Bowel Disease

Konstantinos Papamichael [1], Anna Juncadella [1], Danny Wong [1], Shana Rakowsky [1], Lindsey A Sattler [1], James P Campbell [2], Byron P Vaughn [2], Adam S Cheifetz [1]

Editorial > Ann Rheum Dis. 2018 Apr;77(4):473-475. doi: 10.1136/annrheumdis-2017-212376. Epub 2018 Jan 6.

Adalimumab concentration-based tapering strategy: as good as the recommended dosage

Denis Mulleman [1], Alejandro Balsa [2]

Review > Dig Dis. 2019;37(6):444-450. doi: 10.1159/000499870. Epub 2019 Apr 30.

Relationship between Serum Adalimumab Levels and Clinical Outcome in the Treatment of Inflammatory Bowel Disease

Joaquín Hinojosa [1], Fernando Muñoz [2], Gregorio Juan Martínez-Romero [3]

> Ann Rheum Dis. 2023 Jan;82(1):65-73. doi: 10.1136/annrheumdis-2022-222155. Epub 2022 May 12.

EULAR points to consider for therapeutic drug monitoring of biopharmaceuticals in inflammatory rheumatic and musculoskeletal diseases

Charlotte Lm Krieckaert [1], Astrid van Tubergen [2 3], Johanna Elin Gehin [# 4 5], Borja Hernández-Breijo [# 6], Guillaume Le Mélédo [# 7], Alejandro Balsa [6 8], Peter Böhm [9], Sasa Cucnik [10 11], Ori Elkayam [12], Guro L Goll [13], Femke Hooijberg [14], Meghna Jani [15 16], Patrick Dw Kiely [17 18], Neil McCarthy [19], Denis Mulleman [20], Victoria Navarro-Compán [8], Katherine Payne [21 22], Martin E Perry [23], Chamaida Plasencia-Rodriguez [8], Simon R Stones [24], Silje Watterdal Syversen [25], Annick de Vries [26], Katherine M Ward [27], Gertjan Wolbink [1 28], John D Isaacs [29 30]

> Clin Gastroenterol Hepatol. 2019 Aug;17(9):1655-1668.e3. doi: 10.1016/j.cgh.2019.03.037. Epub 2019 Mar 27.

Appropriate Therapeutic Drug Monitoring of Biologic Agents for Patients With Inflammatory Bowel Diseases

Konstantinos Papamichael [1], Adam S Cheifetz [2], Gil Y Melmed [3], Peter M Irving [4], Niels Vande Casteele [5], Patricia L Kozuch [6], Laura E Raffals [7], Leonard Baidoo [8], Brian Bressler [9], Shane M Devlin [10], Jennifer Jones [11], Gilaad G Kaplan [10], Miles P Sparrow [12], Fernando S Velayos [13], Thomas Ullman [14], Corey A Siegel [15]

> Inflamm Bowel Dis. 2018 Jun 8;24(7):1531-1538. doi: 10.1093/ibd/izy044.

Therapeutic Drug Monitoring Guides the Management of Crohn's Patients with Secondary Loss of Response to Adalimumab

Sophie Restellini [1 2], Che-Yung Chao [1 3], Peter L Lakatos [1 4], Achuthan Aruljothy [5], Haya Aziz [5], Omar Kherad [6], Alain Bitton [1], Gary Wild [1], Waqqas Afif [1], Talat Bessissow [1]

> Ann Rheum Dis. 2015 Feb;74(2):361-8. doi: 10.1136/annrheumdis-2013-204101. Epub 2013 Nov 21.

Personalised treatment using serum drug levels of adalimumab in patients with rheumatoid arthritis: an evaluation of costs and effects

C L M Krieckaert [1], S C Nair [2], M T Nurmohamed [3], C J J van Dongen [1], W F Lems [4], F P J G Lafeber [2], J W J Bijlsma [2], H Koffijberg [5], G Wolbink [6], P M J Welsing [7]

Review > Biologics. 2019 Jul 5:13:127-132. doi: 10.2147/BTT.S188286. eCollection 2019.

Therapeutic drug monitoring of biologics in psoriasis

MeiQi May Liau [1], Hazel H Oon [2]

Review > Gastroenterology. 2017 Sep;153(3):827-834. doi: 10.1053/j.gastro.2017.07.032. Epub 2017 Aug 3.

American Gastroenterological Association Institute Guideline on Therapeutic Drug Monitoring in Inflammatory Bowel Disease

Joseph D Feuerstein [1], Geoffrey C Nguyen [2], Sonia S Kupfer [3], Yngve Falck-Ytter [4], Siddharth Singh [5]; American Gastroenterological Association Institute Clinical Guidelines Committee

TNF inhibitors and Therapeutic Drug Monitoring (TDM)

- TDM : comprises mAb and ADA levels;
- Advocated to facilitate clinical decisions
  - drug levels optimised; treatment cessation; switch to another product/class
- Implementation ??
- Several methods, commercial kits
- Problems in interpreting results; assays not interchangeable
- Lack of assay standardization a concern
- NICE-UK; clinicians – call for reference standards;
- WHO IS for Adalimumab (17/236)– Harmonise results from different methods (mass content)
- Gap – No public standard for ADA
- Proprietary controls for ADA (neg, pos) - 'discriminating' ADA+ve & ADA-ve; assay development, characterization, validation e.g., sensitivity, specificity, drug tolerance etc

Review › Dig Dis. 2017;35(1-2):61-68. doi: 10.1159/000449085. Epub 2017 Feb 1.

Combining Therapeutic Drug Monitoring with Biosimilars, a Strategy to Improve the Efficacy of Biologicals for Treating Inflammatory Bowel Diseases at an Affordable Cost

Ann Gils [1]

› Front Immunol. 2021 Apr 15;12:636420. doi: 10.3389/fimmu.2021.636420. eCollection 2021.

The First WHO International Standard for Adalimumab: Dual Role in Bioactivity and Therapeutic Drug Monitoring

Meenu Wadhwa [1], Chris Bird [1], Eleanor Atkinson [2], Isabelle Cludts [1], Peter Rigsby [2]

› Biomedicines. 2024 Dec 6;12(12):2782. doi: 10.3390/biomedicines12122782.

Adalimumab Autoantibodies in Uveitis Patients: Do We Need Routine Drug Monitoring?

Lynn S Zur Bonsen [1], Vitus A Knecht [1], Anne Rübsam [1], Dominika Pohlmann [1 2], Uwe Pleyer [1 2]

Conclusions: Higher AAA concentrations are associated with lower adalimumab serum levels in uveitis patients. Routine clinical testing is essential for optimal therapeutic drug monitoring to prevent early loss of effectiveness.

Adalimumab treatment and reported antibody incidence

Frequencies of ADA detection, ADA titers vary based on assay

Summary of incidence of ADA detection in adalimumab-treated patients across indications and assay cut-points by assay method

	Adalimumab studies	
Immunogenicity assay	ADA-positive patients, % (no. of studies)	Assay cut-points for ADA-positive status
ELISA 15,21,24,27,28,34,35,41,45, 48,50,52,54,55,58,63,64,66, 67,69,74,77,78,80–151	0–40·0 (38)	0·1–35·0 AU/ml; 0·02–4·9 µg/ml; 0·5–20 ng/ml; OD, 0·2–1·0
Bridging ELISA 16,18,37,38,40,51,59,68,70, 71,75,79,152–181	0–54·2 (18)	≤ 1–10 AU/ml; 0·5–20 ng/ml; OD, 0·02; mean ± 6 s.d.
Sandwich ELISA [13,169,171]	87 (1)	OD, 0·02
Acid dissociation ELISA [26,47,182,183]	9·9–35·0 (4)	1·12 µg/ml; 10 ng/ml; OD, 0·14
RIA 2,4,14,17,19,22,23,28,30,46, 53,57,60,62,180,184–205	0–61·5 (22)	10–48 AU/ml; 0·02 µg/ml; or 2× level in ADA⁻ samples
ECL [32,42,206–208]	–	–
HMSA [25,39,43,44,180,209–226]	4·3–27·0 (6)	1·0–50·0 AU/ml; 0·33 µg/ml
HPLC [227,228]	–	–

- ADA incidence varied widely (0–87%)
- Different measurement units & cut-points (non-comparable)

Lack of standardized assays & interpretation

Gorovits B et al. Clin Exp Immunol. 2018

Development of Adalimumab ADA reference panel

Materials : Provided by
- ABIRISK : 2 mAbs
- Sanquin (NL) : 3 mAbs

} Human, isolated & cloned from PBMCs (treated patients)

- NIHS (Japan) : 10 chimeric mAbs
- Commercial mAbs
- All tested in a range of assays

Review › J Biol Chem. 2014 Dec 12;289(50):34482-8. doi: 10.1074/jbc.M114.615500. Epub 2014 Oct 17.

Functional analysis of the anti-adalimumab response using patient-derived monoclonal antibodies

Pauline A van Schouwenburg [1], Simone Kruithof [1], Christian Votsmeier [2], Karin van Schie [1], Margreet H Hart [1], Rob N de Jong [3], Esther E L van Buren [3], Marieke van Ham [1], Lucien Aarden [1], Gertjan Wolbink [4], Diana Wouters [1], Theo Rispens [5]

› Biologicals. 2020 Jan:63:39-47. doi: 10.1016/j.biologicals.2019.12.003. Epub 2019 Dec 27.

Development of anti-drug monoclonal antibody panels against adalimumab and infliximab

Takuo Suzuki [1], Minoru Tada [2], Akiko Ishii-Watabe [2]

Panel of antibodies assessed in various ADA assays

21 antibodies tested
- 15 (collaborators)
- 6 (commercial)

Assay platforms
- 3 binding
- 2 neutralization

	ECL binding	ELISA binding	SPR binding	CLBA neutr	bioassay	SPR KD (affinity)	
ABIRISK ADA39	high	high	high	high	high	high	A
ABIRISK ADA44	high	high	high	high	high	high	B
NIHS A27-1C-IgE	very high	moderate	moderate	moderate	low/very low	moderate	S
NIHS A27-1C-IgM	very high	very high		low/very low	moderate	moderate	
NIHS A02-5A-IgG1	high	moderate	high	moderate	moderate	moderate	
NIHS A02-5A-IgG4	high	high	moderate	moderate	moderate	low/very low	
NIHS A21-1G-IgG4	high	moderate	high	moderate	low/very low	low/very low	
NIHS A40-1F-IgG4	high	moderate	high	low/very low	negative	low/very low	D
NIHS A12-6A-IgG1	moderate	moderate	high	moderate	high	high	R
NIHS A40-1F-IgG1	low/very low	low/very low	moderate	low/very low	negative	low/very low	
NIHS A21-1G-IgG1	low/very low	low/very low	high	high	moderate	low/very low	C
NIHS A27-1G-IgG1	low/very low	negative	low/very low	low/very low	negative	low/very low	
Sanquin 2.10	high	moderate	moderate	high	high	high	U
Sanquin 1.2	high	high	high	high	high		
Sanquin 2.7	moderate	moderate	moderate	high	high	moderate	T
Biorad HCA271	very high		moderate	moderate			
Biorad HCA203	high		moderate	high			
Biorad HCA205	low/very low			negative			
Dendritics* DDX5032P	very high			negative			
Dendritics* DDX5031P	high			moderate			
Dendritics* DD5030P	high			moderate			
polycl sheep S4733	high		high	high			

very high
high
moderate
low/very low
negative

ABIRISK ADAs – high binders (used as baseline)

Order of ADAs in table is based on ranking from ECL binding assay due to its large dynamic range
Commercial ADAs were dropped (& not tested in ELISA or in bioassay) - similar binding to other mAbs.
*Dendritics (now Eurobio scientific)

Source material: Adalimumab ADA reference materials

- 4 mAbs : 2 human mAbs - isolated and cloned from PBMC of treated patient (ABIRISK*); 2 chimeric mAbs (NIHS, Japan)

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

All were lyophilised as per WHO recommendations. An international collaborative study was organised.

- All recombinant forms - CHO cells, ND – not determined. The A40 antibody is an IgG4[3] with wild type sequence.
- Antibody affinity determined by ELISA is expressed as EC50 (concentration inducing a response halfway between baseline and maximum),
- KD: dissociation constant (k_{off}/k_{on}) as determined by SPR using ProteOn (Biorad, US) or [1]BIAcore T200 system (Cytiva, USA) respectively.
- Neutralisation activity expressed as EC90 (concentration giving 90% of E_{max}) determined by competitive ligand binding assay or reporter gene assay[2] (Promega).

*ABIRISK consortium: academic institutions EFPIA member companies and SMEs; funded by the Innovative Medicines Initiative, EU; 2012-2017

Collaborative Study Design

Aim:

- Compare the lyophilized antibodies across available methods and assess their suitability for use as performance indicators in these methods.
- Assign arbitrary unitage, if feasible, to enable calibration of local standards and for assay harmonisation.

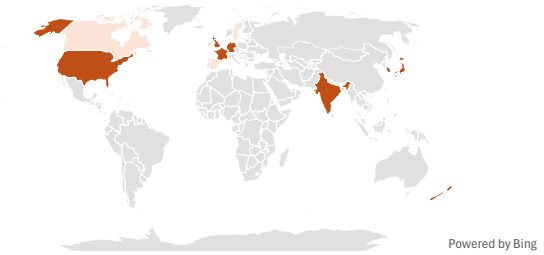
Study Materials:

- mAbs A – D (lyophilised)
- 4 mAb preparations[1], coded R-U
  - **All IgG1** except for **S (IgE**); **All binding & neutralizing**
  - 6 pre-tested pooled sera[2], 1-6
  - ADA pos (treated), neg (healthy)

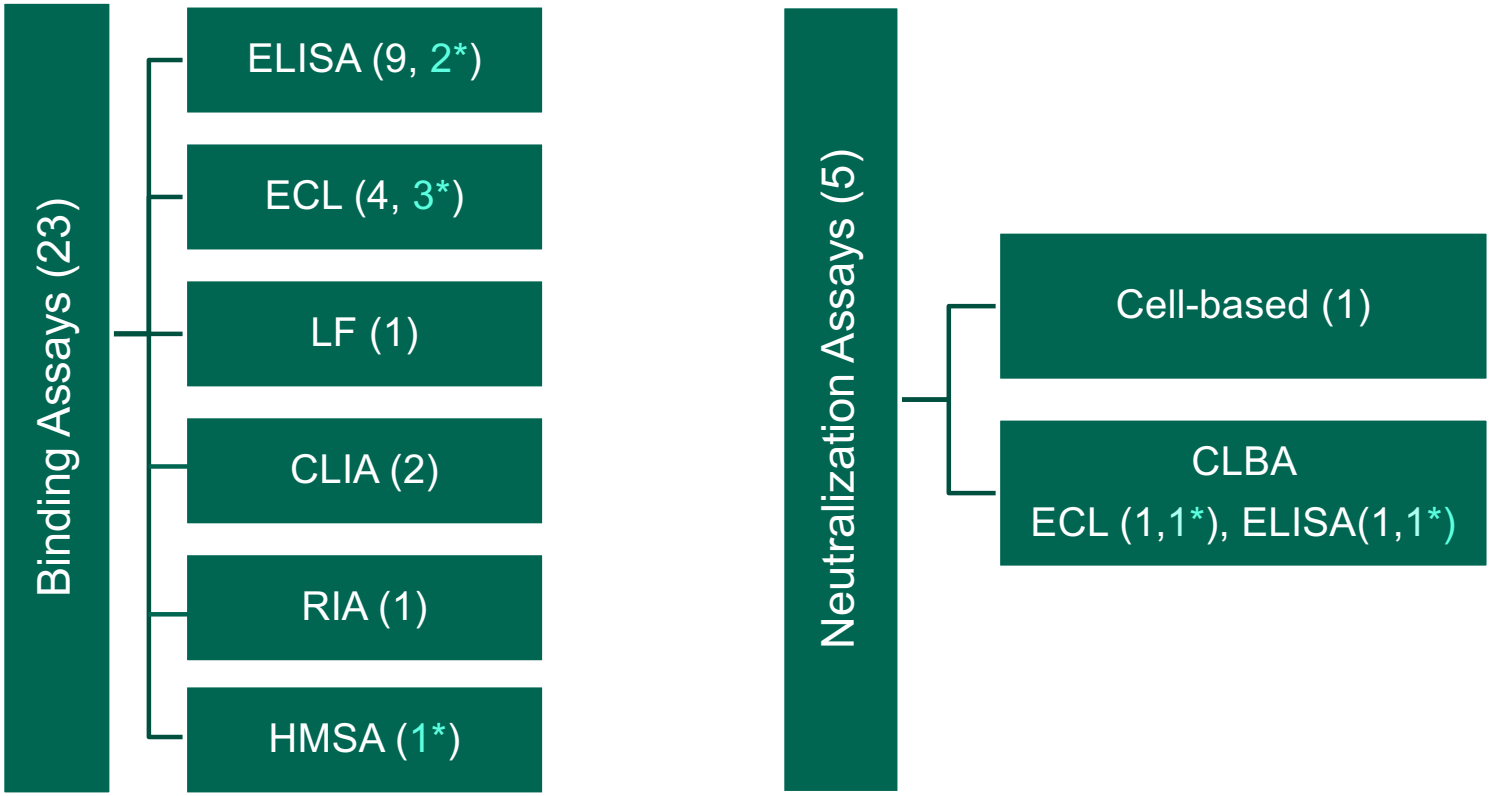
  } for ADA performance
- Determine ADA levels of all samples relative to
  - mAbs A and B
  - kit/in-house standards

Sample code	Antibody origin	Source	Clone	Isotype	Light chain	Binding Affinity KD (M)[1]	Neutralising Antibody Status[5]
R	chimeric human-rat	NIHS	cl A12-6A-IgG1[1,3]	IgG1	k	5.7 E-11	+ve
S	chimeric human-rat	NIHS	cl A27-1C-IgE[1]	IgE	k	5.7 E-11	+ve
T	human B Cells	Sanquin	cl 2.7[2,4]	IgG1	k	1.95 E-10	+ve
U	human B Cells	Sanquin	cl 2.10[2]	IgG1[5]	k	1.15 E-10	+ve

- **Participants :**
- **22 laboratories; 14 countries**; 4 WHO regions
- 8 reagents/kits manufacturers, 3 product manufacturers
- 6 clinical/diagnostic laboratories , 1 CRO
- 3 control & 1 academic laboratory



Collaborative Study: ADA Assays



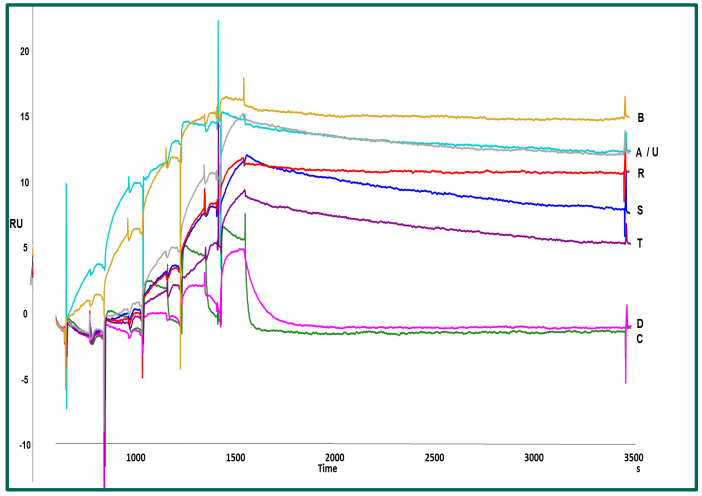
Binding : ECL – regulatory submissions, Others – clinical monitoring.
Majority – free ADA, some *- total ADA (free ADA + ADA complexed with drug).

Results: In-house binding data of mAbs using SPR

Antibody Code	ka (1/Ms)	kd (1/s)	KD (M)	Ranking Order (affinity)
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

Lyo

Liq



B, A

D,C

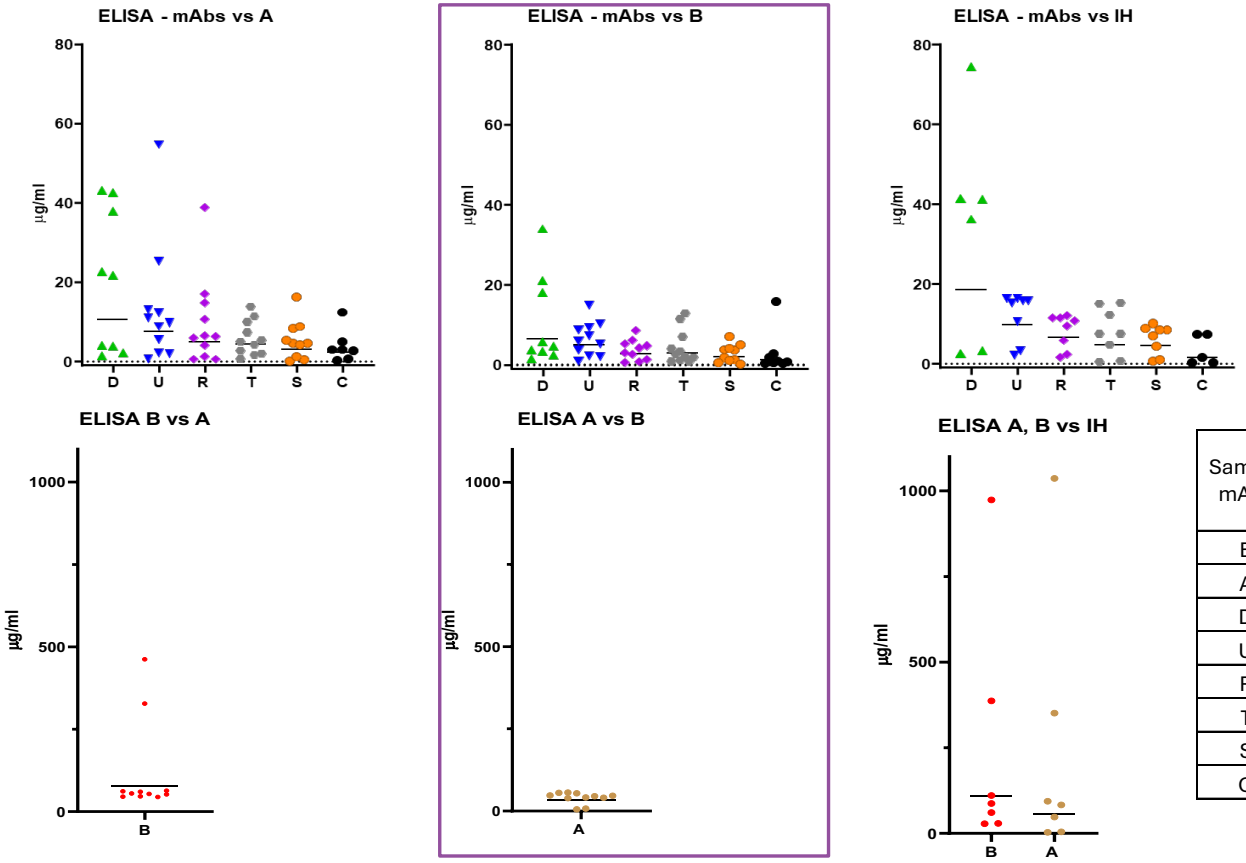
Data from a representative kinetic experiment using the Biacore T200. For C, kinetic constants* are difficult to determine (due to limits of instrument)

Results: Participants

- All labs : Followed study protocol
- 3 assays (min), tested serial diln except 1 (neut); 1 lab – no sera tested;
- ADA results reported as per routine practice as **pos/neg**, **μg/ml or AU/ml relative to IH/kit standards or titers**. Therefore, a comparison of all results was precluded in terms of the **IH/kit standards**.

- **Estimates of activity calculated relative to A, B and IH**
- **Parallelism** of samples was assessed using the **ratio of their fitted slopes** by CombiStats for the different assay types.
- Value of 1.0 – perfect parallelism;
- All estimates were calculated in cases where the slope-ratio was **within 0.67-1.50**.
- All **other cases were excluded due to unacceptable level of non-parallelism.**

mAbs: ELISA data from individual laboratories



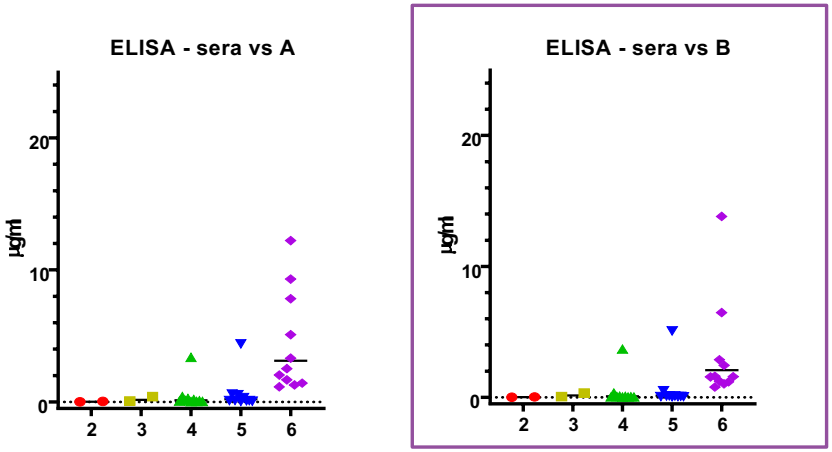
- All mAbs positive – all assays/ labs
- Range of estimates narrowed relative to A (except for R, S, U);
- Extreme values disappear when B as standard; estimates narrowed for all
- **Data consistent and harmonised relative to B for all**

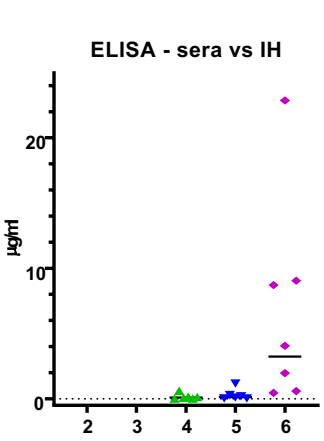
Sample mAbs	ELISA					
	A (µg/ml)		B (µg/ml)		IH (µg/ml)	
	Range	GM	Range	GM	Range	GM
B	46.25 - 462.36	96.75			28.28 - 973.57	108.91
A			5.41 - 54.05	25.84	3.06 - 1036.53	56.49
D	1.46 - 43.12	18.59	1.54 - 33.99	7.82	2.60 - 74.52	18.52
U	0.77 - 54.70	8.08	0.85 - 9.34	4.19	2.24 - 16.37	9.10
R	0.58 - 38.75	5.38	0.64 - 6.16	2.79	1.51 - 12.05	6.04
T	0.77 - 9.98	3.90	0.82 - 7.01	2.02	0.47 - 15.23	4.36
S	0.47 - 16.28	3.58	0.51 - 4.08	1.86	0.75 - 10.13	4.05
C	2.71 - 5.13	3.48	0.41 - 1.79	0.79	0.24 - 7.35	0.98

Results based on labs which reported results in µg/ml

Different scales (top & bottom panels) : mAbs, calibrators

Serum samples: ELISA data from individual laboratories





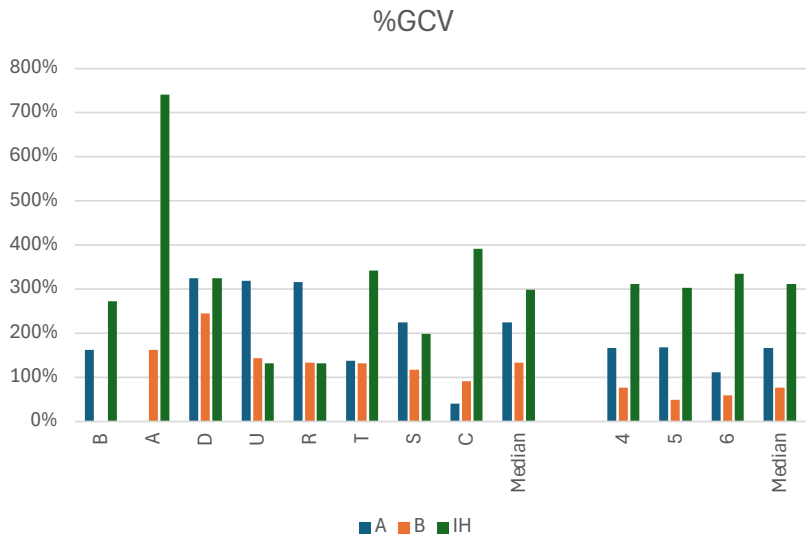
- S1: negative in all
- S4,6 : positive in all
- S2 : missed in 9/11 } have adalimumab
- S3 : missed in 8/11 }
- S5 : missed in 1/11

- No effect of standards on sample positivity
- ADA positivity was assay dependent – free/total.
- Highest values for sera 4, 5 and 6 vs A/B are from the same lab (7), which had no acceptable values vs IH (all NP)

Sample	A (µg/ml)		B (µg/ml)		IH (µg/ml)	
Sera	Range	GM	Range	GM	Range	GM
2	Neg	Neg	Neg	Neg	Neg	Neg
3	Neg	Neg	Neg	Neg	Neg	Neg
4	0.02 - 0.26	0.08	0.02 - 0.09	0.04	0.02 - 0.62	0.08
5	0.04 - 0.54	0.13	0.04 - 0.12	0.06	0.03 - 1.17	0.14
6	1.14 - 9.31	2.75	0.78 - 2.87	1.45	0.45 - 22.86	3.24

B better than A to reduce spread of data

ELISA Data : Variability



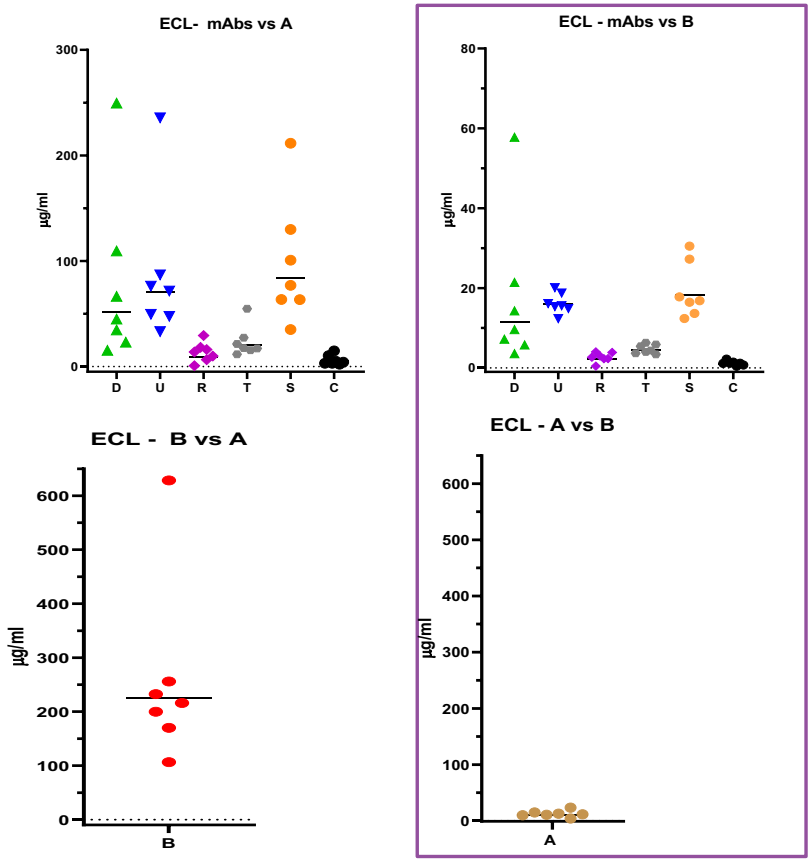
mAbs	A	B	IH
B	162%		272%
A		162%	741%
D	326%	246%	324%
U	319%	143%	132%
R	316%	133%	131%
T	137%	132%	342%
S	225%	118%	198%
C	41%	91%	392%
Median	225%	133%	298%

Sera	A	B	IH
2	n/a	n/a	n/a
3	n/a	n/a	n/a
4	166%	77%	312%
5	168%	50%	303%
6	111%	60%	336%
Median	166%	77%	312%

- Relative to B, %GCV reduced in all samples except for R and U;
- mAbs: median GCV value reduced to 133% from 298% with IH std.
- Sera : median GCV much reduced with B
- **B as a common standard a better option than A**

Green shaded boxes – values reduced compared with IH
Inter-lab variability – % geometric coefficient of variation (GCV)

mAbs: ECL data from individual laboratories

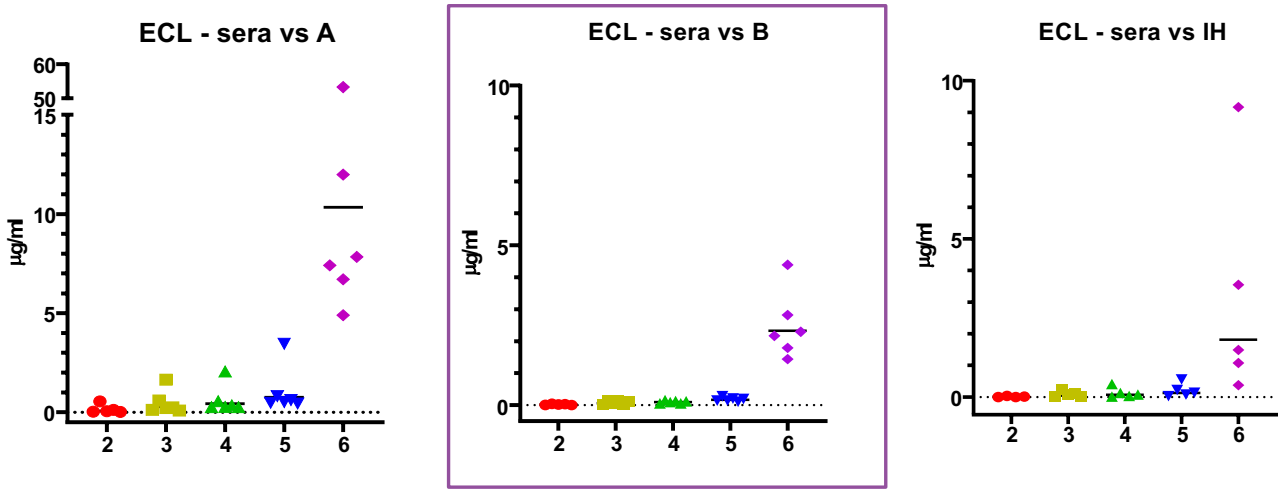


- All mAbs positive – all assays/labs
- IH : Wide estimates for A/B
- A : Estimates dispersed – worse than IH; high values
- B : Estimates better harmonised in all cases compared with IH or A
- **B better than A in reducing spread of data**.

Sample mAbs	ECL					
	A (µg/ml)		B (µg/ml)		IH (µg/ml)	
	Range	GM	Range	GM	Range	GM
B	106.42 - 628.28	229.45			10.87 - 210.79	51.71
A			3.98 - 23.49	10.90	2.52 - 58.12	11.15
D	15.30 - 249.57	59.25	3.58 - 57.78	12.78	2.90 - 72.86	13.22
U	33.09 - 235.63	74.78	14.87 - 20.10	16.68	4.37 - 67.87	17.25
R	0.74 - 29.36	8.36	0.46 - 3.89	2.06	0.50 - 9.15	2.10
T	11.59 - 54.72	21.88	3.41 - 6.30	4.72	1.37 - 24.79	4.88
S	35.05 - 211.51	78.32	12.38 - 27.24	16.83	3.87 - 114.84	17.40
C	2.15 - 14.72	5.21	0.42 - 2.21	1.13	0.25 - 4.98	1.17

Different scales (top & bottom panels) : mAbs, calibrators NOTE : Wider scale relative to A versus B & IH in top panels

Serum samples : ECL data from individual laboratories



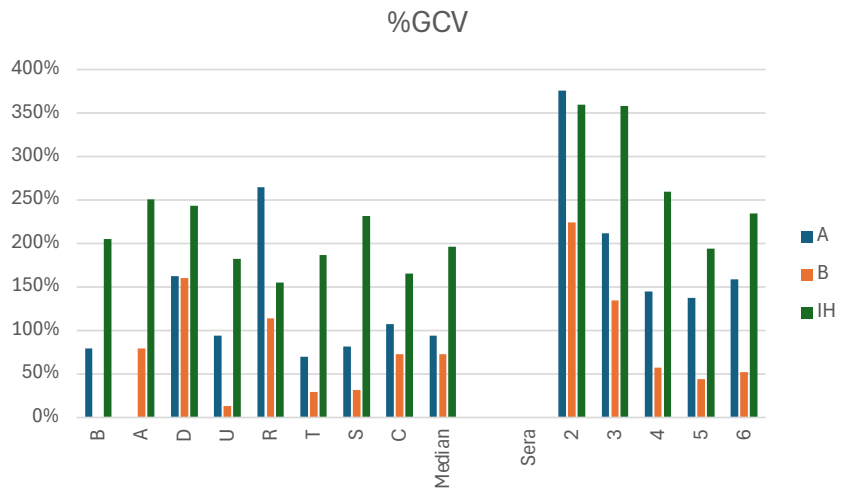
- S1: negative in all
- S3-6 : positive in all
- S2 : negative in 1/7 (total ADA, sensitivity?)

	A (µg/ml)		B (µg/ml)		IH (µg/ml)	
Sera	Range	GM	Range	GM	Range	GM
2	0.02 - 0.55	0.06	0.003 - 0.04	0.01	0.003 - 0.04	0.01
3	0.09 - 1.65	0.25	0.02 - 0.13	0.05	0.01 - 0.25	0.04
4	0.26 - 2.06	0.42	0.06 - 0.16	0.09	0.01 - 0.41	0.08
5	0.45 - 3.46	0.75	0.11 - 0.28	0.17	0.03 - 0.56	0.13
6	4.90 - 53.31	10.03	1.44 - 4.39	2.24	0.38 - 9.16	1.82

B better than A in harmonizing estimates

NOTE : Wider scale relative to A versus B & IH

ECL Data : Variability



mAbs	A	B	IH
B	79%		205%
A		79%	251%
D	162%	161%	243%
U	94%	13%	182%
R	265%	114%	155%
T	70%	29%	187%
S	82%	31%	232%
C	108%	73%	165%
Median	94%	73%	196%

Sera	A	B	IH
2	375%	224%	359%
3	211%	135%	358%
4	145%	58%	259%
5	138%	44%	194%
6	159%	52%	235%
Median	141%	65%	233%

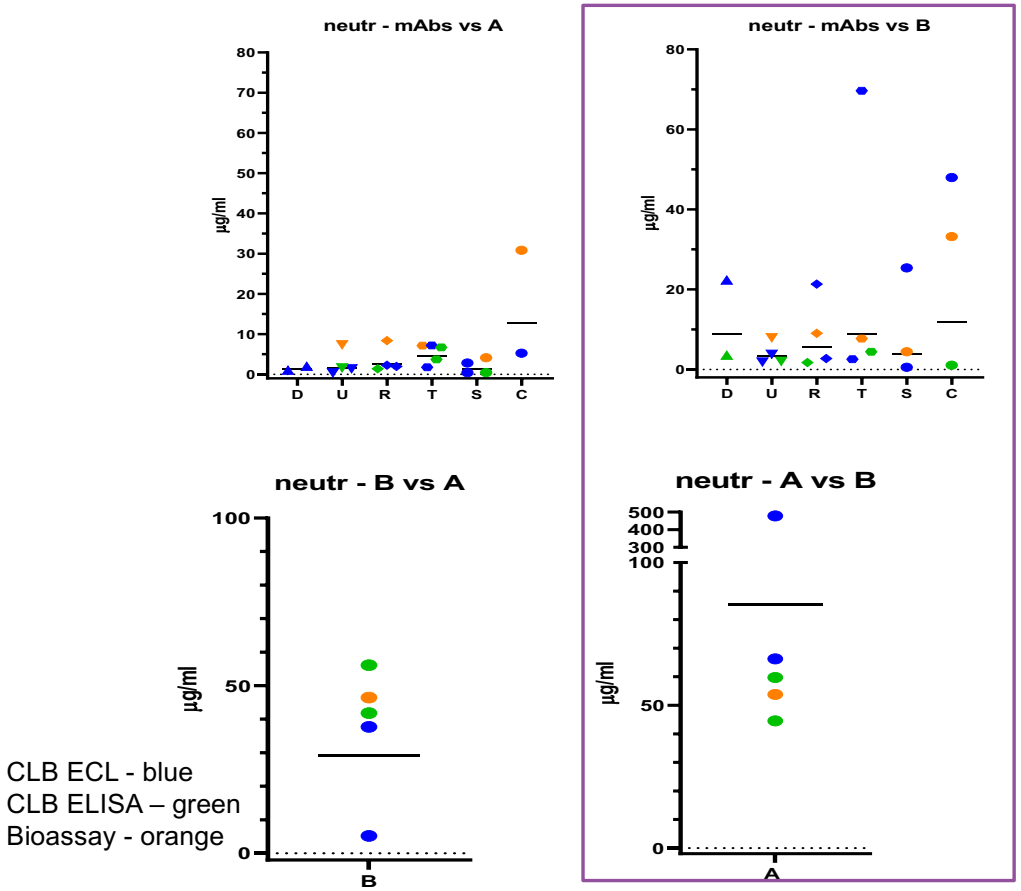
- Relative to B, %GCV much reduced in all cases as opposed to IH.
- mAbs - Median GCV 73% from 196% with IH std
- Sera - median GCV reduced with B
- **B as a common standard a better option than A**

Green shaded – values reduced compared with IH
Inter-lab variability – % geometric coefficient of variation (GCV)

Other ADA Assays

- LF, RIA, HMSA – one lab for each and CLIA – 2 labs
- **mAbs** : All positive in assays/labs except for mAb S (IgE) - negative in RIA
  - CLIA – 2 labs; discrepancy for C, D and U; HMSA – high values for C; D, S – NP
  - RIA , HMSA – AU/ml used so no data vs in-house standard
- **Sera**：Similar situation to ELISAs
  - S1: negative in all
  - S4,5 & 6 : positive in all
  - S2 (adalimumab ~ 1μg/ml): missed in both CLIAs, LF, RIA and HMSA
  - S3 (lower adalimumab amount than S2) : missed in 1 CLIA & LF but detected in other assays
- **A and B reduce spread of data.**
- **ADA levels more comparable between assays when B used as common standard**.

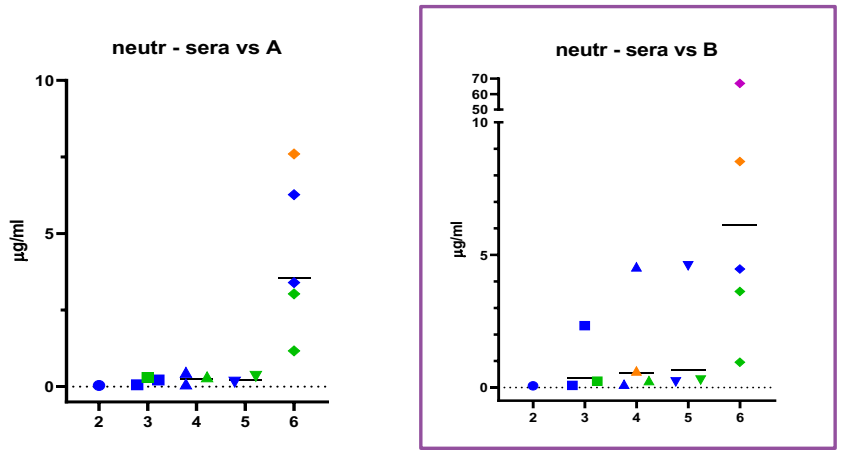
mAbs: Neutralization assay data from individual laboratories



- All labs reported in-house data as pos/neg or in some cases in titers
- All mAbs positive – all assays/labs with one exception mAb D – missed in 1 CLBA ELISA
- A : Estimates of activity were fairly similar for all mAbs (except B, C).
- B : Estimates more dispersed; slightly high values calculated for a single assay (lab 13b) versus other assays for all mAbs.
- A better than B in reducing spread of data.
- %GCV was lower with A than B in most instances (except U)
- **A appears to be superior compared with B**

Different scales (top & bottom panels) : mAbs, calibrators (different scales relative to A & B in bottom panels)

Serum Samples: Neutralization data from individual laboratories



CLB ECL - blue
CLB ELISA – green
Bioassay – orange

- S1: negative in all,
- S4,5 & 6 : positive in all
- S2 : positive in 1 CLBA-ECL only, missed in 4 assays
- S3 : positive in 4 assays but missed in 1 CLBA-ELISA

- NOTE : Wider scale relative to B versus A

- All labs reported in-house data as pos/neg or in some cases in titers
- Estimates of neutralising activity relative to A generally showed greater consistency among different assays
- %GCV were lower with A than B in all cases
- **A appears to perform better than B in harmonizing estimates and in terms of inter-laboratory variability.**

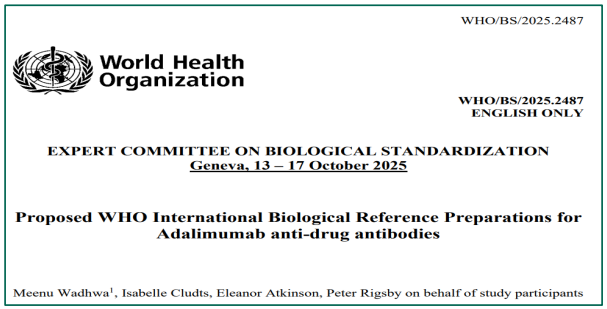
Conclusion (1):

- Degree of reactivity varied depending on assay and sample
  - Levels varied - assay platforms, assays using same platform (IH standards).
  - **Potential for ADAs to be missed in some assays (e.g. ELISAs, other assays)**
    - Low ADA/drug on board (sera 2, 3), inappropriate dilution (sera 5), format (mAb S - RIA)
  - **All missed ADA samples were neutralizing**
- 'Common' standard
  - Did not impact ADA positivity of samples (unlike the Infliximab ADA study)
  - Improved consistency in estimates and harmonised data across assays/labs/assay platforms (reduced GCV). Degree of harmonization dependent on assay type and the sample.
  - **B (code 19/266) was better than A for binding assays** (incl clinical monitoring assays)
  - **A (code 19/264) was superior than B for neutralization assays**.
- **Assigning arbitrary unitage beneficial**
  - Current : Many kits (varied reporting practice) used for clinical monitoring.
  - Labs can align/harmonize if a common reporting unit assigned e.g. **B with X units for calibrating binding assays** and same for **A for neutralization assays.**

Conclusion (2):

- **Study:** Total of 23 binding; 5 neutralization
  - Overall **~ 30%** of the assays in study reported in different units for clinical purposes
  - **Use of B** (code 19/266) with **Adalimumab IS (established**) can aid TDM and improve patient outcome globally
- **Other mAbs:**
  - C and D are different from A & B as shown by binding data & affinity (SPR); fast dissociation
  - Like B, **C has an IgG1 isotype but, unlike B, showed the lowest binding activity in assays**
  - D is an IgG4 with potential to be missed in some bridging assays.
  - This was not the case here as bivalent recombinant IgG4 tested rather than monovalent Fab exchanged IgG4 (clinical scenario). However, **D was missed in neutralization assay of 1 lab**.
  - **Both have utility as 'performance indicators'** to test if ADAs with fast dissociation detected.
  - **C** (FS-007) **for** detecting **low activity ADAs** whereas **D** (FS-008) for **low affinity** bivalent IgG4 ADAs**.**

Immunogenicity : ADA Standards



WHO/BS/2025.2487

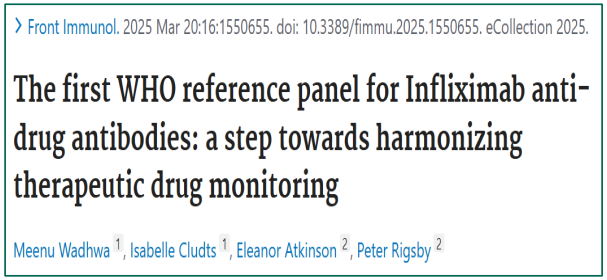
World Health Organization

WHO/BS/2025.2487
ENGLISH ONLY

EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION
Geneva, 13 – 17 October 2025

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

Meenu Wadhwa[1], Isabelle Cludts, Eleanor Atkinson, Peter Rigsby on behalf of study participants



Front Immunol. 2025 Mar 20:16:1550655. doi: 10.3389/fimmu.2025.1550655. eCollection 2025.

The first WHO reference panel for Infliximab anti-drug antibodies: a step towards harmonizing therapeutic drug monitoring

Meenu Wadhwa [1], Isabelle Cludts [1], Eleanor Atkinson [2], Peter Rigsby [2]

For standardising assays in use for clinical monitoring

- For use in assay characterisation and calibration where possible
- Monitor routine assay performance
- Increase the robustness of reported data
- Continuity and life-cycle management
- Compare assay platforms
- **Harmonise immunogenicity** reporting - access to safe and effective medicines
- **Allow clinical decisions** (e.g., dose optimisation, product/class switch) towards personalised treatments for better patient outcomes, financial savings**.**
- **Support pharmaco-vigilance where needed!**

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- Despatch Team – J Condron and colleagues
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Any questions?

Thank you

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