Annual report of the Quality Assessment and Standardization (QAS) Committee – 2020/2021

Luis Andrade

Composition of the QAS Committee

Allergy Subcommittee (Stefan Vieths, Germany, Chair)

Autoantibody Standardization Subcommittee (Edward Chan, USA, Chair)

Cytokine Subcommittee (in negotiation) (Meenu Wadhwa, United Kingdom)

Big Data for Immunology Subcommittee (Jamie Scott, USA, Chair)

Complement Subcommittee (Michael Kirschfink, Germany, Chair)

Leukocyte Subcommittee (Pablo Engel, Spain, Chair)

I. Edition of the Research Topic in Frontiers in Immunology designated "Contemporary Challenges in Immunologic Testing in Clinical and Research Laboratories"

In the year 2020, the QAS Committee submitted a proposal of a research topic in Frontiers in Immunology and this proposal was accepted. The Chairs of the QAS subcommittees were invited to participate as Editors. Over the last year, we have been successful in obtaining several contributions for this Research Topic. As of October 2021, there are 39 expected manuscripts, 25 of which have already been submitted, and 16 of them are already published (https://www.frontiersin.org/research-topics/16482/contemporary-challenges-in-immunologic-testing-in-clinical-and-research-laboratories). There have been 53,783 views and 12, 982 downloads. We expect to complete 25 manuscripts within the next couple of months.

II. Autoantibody Standardization Sub-committee (ASC) of the QAS Committee - Report of Activities - 2020/2021

Edward Chan (Chair and Treasurer)

Current Members

Luis E.C. Andrade, M.D., Ph.D. (2018) – São Paulo, Brazil

Kathleen A. Arntsen (2016) – Verona, NY, USA – President & CEO, Lupus and Allied Diseases Association, Inc.

Elaine Y.L. Au, M.D. (Associate Member 2018-2020) – Hong Kong

Donald B. Bloch, M.D. (2017) – Cambridge, MA, USA
Edward K.L. Chan, Ph.D. (2018) – **ASC Chair & Treasurer** – Gainesville, FL, USA
Marvin J. Fritzler, Ph.D., M.D. (2017) – Calgary, Alberta, Canada
Ignacio García de la Torre, M.D. (2018) – Guadalajara, Mexico
Ranjan Gupta, M.D. (Associate Member 2019-2021) – New Delhi, India
Falk Hiepe, M.D. – Berlin, Germany
Takao Koike, M.D. – Sapporo, Japan
Konstantin Konstantinov, M.D., Ph.D. (2014) – Albuquerque, NM, USA
Robert Lahita, M.D., Ph.D. (2014) – Newark, NJ, USA
Pier Luigi Meroni, M.D. (2014) – Milan, Italy
Westley H. Reeves, M.D. (2014) – Gainesville, FL, USA
Johan Rönnelid, M.D., Ph.D. (2014) – Uppsala, Sweden
Minoru Satoh, M.D., Ph.D. (2019) – **ASC Vice-Chair** – Kitakyushu, Japan
Carlo Selmi, M.D. – Milan, Italy
Joanna Sheldon, Ph.D. (2018) – London, UK
Yehuda Shoenfeld, M.D. (2017) – Tel-Hashomer, Israel
Günter Steiner, Ph.D. – Wien, Austria
Yoshinari Takasaki, M.D., Ph.D. (2017) – Tokyo, Japan
Angela Tincani, M.D. (2017) – Brescia, Italy
Robert F. Vogt, Jr., Ph.D. (2017) – Centers for Disease Control and Prevention – Division of Laboratory Sciences – Atlanta, GA, USA
Mark Wener, M.D. (2019) – **ASC Secretary** – Seattle, WA, USA

I. Publications in the period 2020-2021

1. Chan, E.K.L., von Mühlen, C.A., Fritzler, M.J., Damoiseaux, J., Infantino, M., Klotz, W., Satoh, M., Musset, L., Garcia-De La Torre, I., Carballo, O.G., Herold, M., de Melo Cruvinel, W., Mimori, T., and Andrade, L.E.C. (2022) The International Consensus on ANA Patterns (ICAP) in 2021 – the 6th Workshop and current perspectives. J. Appl. Lab. Med., in press.
2. von Mühlen, C.A., Garcia-De La Torre, I., Infantino, M., Damoiseaux, J., Andrade, L.E.C., Carballo, O.G., Conrad, K., Francescantonio, P.L.C., Fritzler, M.J., Herold, M., Klotz, W., de Melo Cruvinel, W., Mimori, T., Satoh, M., Musset, L., and Chan, E.K.L. (2021) How to report the Antinuclear Antibodies (Anti-Cell Antibodies) test in HEp-2 cells: guidelines from the ICAP initiative. Immunol. Res., in press.
3. Röber, N., Dellavance, A., Ingenito, F., Reimer, M.-L., Carballo, O.G., Conrad, K., Chan, E.K.L., and Andrade, L.E.C. (2021) Strong association of the myriad discrete speckled nuclear pattern with anti-SS-A/Ro60 antibodies: consensus experience of four international expert centers. Front. Immunol., in press.
4. Tebo, A.E., Schmidt, R.L., Kadkhoda, K., Peterson, L.K., Chan, E.K.L., Fritzler, M.J., and Werner, M.H. (2021) The antinuclear antibody HEp-2 indirect immunofluorescence assay: a survey of laboratory performance, pattern recognition and interpretation. Auto Immun Highlights 12:4.
5. Van Hoovels, L., Broeders, S., Chan, E.K.L., Andrade, L., de Melo Cruvinel, W., Damoiseaux, J., Viander, M., Herold, M., Coucke, W., Heijnen, I., Bogdanos, D., Calvo-Alén, J., Eriksson,

C., Kozmar, A., Kuhi, L., Bonroy, C., Lauwerys, B., Schouwers, S., Lutteri, L., Vercammen, M., Mayer, M., Patel, D., Egner, W., Puolakka, K., Tesija-Kuna, A., Shoenfeld, Y., José Rego de Sousa, M., Hoyos, M.L., Radice, A., and Bossuyt, X. (2020) Current laboratory and clinical practices in reporting and interpreting anti-nuclear antibody indirect immunofluorescence (ANA IIF) patterns: results of an international survey. Auto Immun Highlights 11:17.

6. Zheng, B., Mora, R.A., Fritzler, M.J., Satoh, M., Bloch, D.B., Garcia-De La Torre, I., Boylan, K., Kohl, K., Wener, M.H., Andrade, L.E.C., and Chan, E.K.L. (2020) Establishment of international autoantibody reference standards for the detection of autoantibodies directed against multiple nuclear dots, GW bodies, and NuMA. Clin. Chem. Lab. Med. 59:197-207.

II. Activities of the International Consensus on ANA Patterns (ICAP)

ICAP was established in 2014 as an initiative from the Autoantibody Standardization Committee, with the goal of promoting the harmonization and standardization in the indirect immunofluorescence assay on HEp-2 cells (HEp-2 IFA), historically known as the antinuclear antibody (ANA) test. Over the last seven years, ICAP has promoted several publications and workshops in Europe, North America, South America, and Asia. In addition, ICAP holds an interactive website (www.anapatterns.org) that offers instructive and didactic content, including the classification algorithm of HEp-2 IFA patterns, illustrative images, clinical and immunological relevance, training modules, and frequently asked questions (FAQ).

Activities of ICAP in the period 2020/2021 include:

- Over 3,200 registered users from over 100 countries in the five continents.
- First ICAP training module released free of charge at the website; second, third, and fourth modules are in preparation.
- Website translated into 13 languages with 2 more ready-to-go in late 2021.
- FAQ – committee members address questions raised from users on a regular basis
- The ICAP patterns classification algorithm was edited and updated in 2021.

III. Reference sera established by the ASC

For several decades the ASC has offered free of charge a collection of reference sera for clinically relevant autoantibodies in systemic autoimmune diseases. This reference material is useful for the harmonization and setting up of specific immunoassays in research and clinical laboratories, as well as for kit developers in the in vitro diagnostics industry. The total number of reference sera available from ASC now is 23 (https://asc.dental.ufl.edu/reference-sera/).

In the year 2020, three new reference sera were added containing autoantibodies directed against multiple nuclear dots, GW bodies, and NuMA protein, respectively (Zheng, B., Mora, R.A., Fritzler, M.J., Satoh, M., Bloch, D.B., Garcia-De La Torre, I., Boylan, K., Kohl, K., Wener, M.H., Andrade, L.E.C., and Chan, E.K.L. (2020) Establishment of international autoantibody reference standards for the detection of autoantibodies directed against multiple nuclear dots, GW bodies, and NuMA. Clin. Chem. Lab. Med. 59:197-207.).

Currently, three other reference sera are in the process of validation, containing antibodies to sp100, mitotic spindles, and nuclear envelope protein gp210, respectively. Our aim is to establish reference materials for all AC patterns classified by the International Consensus on ANA Patterns (www.anapatterns.org).

III. Big Data for Immunology Sub-committee of the QAS Committee - Report of Activities - 2020/2021

Jamie Scott (Chair)

Co-chairs: Jamie Scott (Canada) & Christian Busse (Germany)
Members: Felix Breden (Canada), Dharshan De Silva (Sri Lanka), Helder Nakaya (Brazil), Bjoern Peters (USA)

Meetings and Progress

- Six meetings were held over the past year;
- Found appropriate members, approved the proposal for this subcommittee, and completed its Terms of Reference, which will be added to a new website for the Bid Data for Immunology (BDI) subcommittee that is under planning.
- Overall goal: To standardize (and/or make interoperable) metadata, following well-defined clinical and experimental ontologies to support sharing of all types of big data for immunology. This will support work with specific types of BDI, as well as systems analyses that involve multidimensional BDI.
- From the wide variety of BDI, we chose to first focus on three types of data:
    - Flow cytometry/CYTOF data
    - RNA-seq data
    - Adaptive immune receptor repertoire sequencing (**AIRR-seq**) data.
- We have been investigating each type of the above BDI in the literature, particularly their use in systems-based analyses;
- We plan to identify and engage collaborators with expertise in each of the three types of BDI and knowledge of their standardization. This will involve engaging societies and interest groups on their terms. Pre-existing metadata, ontologies, and experimental standards will be employed insofar as possible.
- Further ahead, we will work to combine overlapping metadata, clinical and experimental standards, and ontologies and where there are differences, create interoperability for these three data types.
- Once clear standards have been envisioned, we will work with partners to communicate them to relevant societies and institutions for review and acceptance.
- Other types of BDI (e.g., metabolomics, microbiome, structural, microscopic data) will eventually be added following the same overall approaches of investigation and engagement, to grow shared BDI standards.

IV. International Cytokine and Interferon Society (ICIS) Standards Committee - Report of Activities - 2020/2021

Meenu Wadhwa

The International Cytokine and Interferon Society (ICIS) Standards Committee was established to make recommendations regarding interferon and cytokine standards and standardization to the ICIS membership, and thereby to the international cytokine scientific community. The Committee works closely with the World Health Organization (WHO), the National Institute for Biological Standards and Control (NIBSC), the U.S. National Institutes of Health (NIH), the Biodefense and Emerging Infections Resources Repository (BEI Resources), pharmaceutical manufacturers, and regulatory agencies.

The ICIS Standards Committee is not an official subcommittee of IUIS QAS Committee. We have been in negotiation to have the ICIS Committee join the IUIS QAS Committee since 2018. In 2019, the Cytokine Committee collaborated with the QAS by providing a comprehensive report on WHO International Standards (IS), WHO reference reagents (WRR), and other cytokine and growth factor standards available from the NIBSC. Earlier this year, Meenu Wadhwa and colleagues contributed the manuscript “The First WHO International Standard for Adalimumab: Dual Role in Bioactivity and Therapeutic Drug Monitoring” to the Frontiers in Immunology Research Topic “Contemporary Challenges in Immunologic Testing in Clinical and Research Laboratories” edited by the IUIS Quality Assessment and Standardization Committee. As of today, this manuscript has obtained 1,911 views.

Lately, the ICIS Standards Committee has expanded its activities to embrace the standardization of therapeutic monoclonal antibodies against cytokines and cytokine receptors. In addition to the publication in the Frontiers in Immunology Research Topic, International standards have been established for Rituximab, Adalimumab, Bevacizumab, and Trastuzumab. The pipeline includes new standards for Ustekinumab, Golimumab, and Ranibizumab. The committee also promotes initiatives to establish renewed standards for those that are close to exhaustion. This is currently the case for the standards for Interferon alpha-2b and for IL-6.

In addition to the establishment of international standards, the ICIS Standards Committee is coordinating a multicenter study on anti-drug antibodies to Infliximab, including 21 laboratories in 11 countries.

We expect to have the ICIS Standards Committee officially join the QAS Committee in the near future.

V. Complement Sub-committee of the QAS Committee - Report of Activities - 2020/2021

Michael Kirschfink (Chair) and Zoltán Prohászka (Vice-Chair)

At the 2008 International Complement Workshop in Basel, Switzerland, a group of interested members of the International Complement Society (ICS) met to discuss the formation of a standardization committee and to define the major aims of this quality management initiative (https://www.complement.org/committee).

In 2010 the International Union of Immunological Societies (IUIS, http://www.iuisonline.org/) established the initiative officially as *Subcommittee for the Standardization of Complement Analysis.* Michael Kirschfink (Heidelberg, Germany) is Chairman, Zoltán Prohászka (Budapest, Hungary), Bo Nilsson (Uppsala, Sweden) and Ashley Frazer-Abel (Denver, USA) serve as Co-Chairs.

The subcommittee with its associated laboratories aims at
- initiating and organizing measures to improve the quality of complement analysis
- making various standard materials available to the scientific and clinical communities that apply.
- defining standardized methods of modern complement analysis
- organizing national and international workshops and training courses on modern complement analysis
- developing guidelines for a high-quality standardized complement analysis

In the past 12 months, the 13th and 14th complement external quality assessment programs (EQA) have been conducted with the help of INSTAND e.V. (Düsseldorf, Germany), a WHO accredited institution for quality control in laboratory analysis. Prof. Zoltán Prohászka has provided the EQA samples and now chairs the EQA evaluation, with Prof. Michael Kirschfink serving as co-chair.
In October, 2020, 207 laboratories from >30 countries participated in the full program (determination of 20 complement parameter including multiple autoantibodies and activation products).
Due to the Corona pandemic a detailed analysis of the data could not be presented on the occasion of the 14th European Complement Meeting, scheduled first for July 2020, then for September 2021 as this meeting has now been further postponed to December as an online meeting. For March 2021, 186 Labs (majority from Europe, mainly Germany) enrolled for the 13th EQA, 11 parameters are included (restricted to the widely used complement determinations and anti-C1q) and for the 14th EQA (October 2021) 222 labs have now participated. The evaluation process will take place later this year. Data of the EQA programs 2020/2021 will then be reported early in 2022 on a separate online meeting of the standardization group.

Compelling evidence accumulated over the past 10 years shows that there is a high degree of variation among labs in the methods used, reference ranges, and reported results. Since the EMCHD meeting in Madrid in September 2019 the committee has continued to take active steps to further improve the standardization of the complement assays. The work is split among working groups dedicated to functional assays, complement factors, complement activation biomarkers, and autoantibodies. Special attention is given to the development of the “golden” quantification standards that will be produced via dedicated protocols within certified facilities and made available to the complement laboratories.

After the first calibrator for anti-C1-Inhibitor testing, developed in Prof. Prohászka's lab (Budapest) has been and still is distributed upon request, various working groups continue working on the preparation and characterization of standards for C3, C4, C1-Inhibitor, and sC5b-9. We are currently in contact with the FDA and the IFCC to evaluate the required steps for official calibrator recognition.

Due to the fact that with the increase of participating labs in the EQA program (from 12 to 222 Labs in fall 2021), the standardization committee, founded in 2008, needs to be reorganized and redefined. The official committee has been restricted to those members included in the strategy group of Budapest 2016 who since then is active in several working groups. There is, however, a possibility to join the committee. With recent mails and newsletters, we invited colleagues who are actively involved in complement diagnostics and would like to make a contribution to the work of the committee to send a short statement of interest (max 1 A4) introducing themselves and their work in complement diagnostics to Prof. Kirschfink (kirschfink@uni-hd.de) or Prof. Prohászka (prohaszka.zoltan@med.semmelweis-univ.hu). Furthermore, a call went out to members of the International Complement Society to send nominations for the elections of new chairs and vice-chairs for the complement standardization committee. Elections will then be made online during the upcoming International Complement Workshop in early December 2021.

To better enable collaborations with the industry aiming at improving the quality of commercial complement tests, recently a *Policy for Interactions* with *Industry/Vendors* has been developed by the board of the International Complement Society (ICS). On the occasion of the next International Complement Workshop in Berlin (now postponed to December 2021), the standardization group plans the following activities: 14th Meeting of the C-standardization group and a meeting with international companies providing complement assays to initiate future collaborations (e.g. to prepare a comparative analysis of commercial assays for defined complement parameters).

The activities of the Complement standardization committee have also been presented within various lectures to online meetings: 1. DFGI Complement working group (September 2020); 2. Meeting "Primary Immunodeficiencies in Children and Adults: Infections in the Spotlight", Moscow, Russia (April 2021); and 3. The recent LASID congress, Santiago de Chile, Chile (October 2021).

The following manuscripts on or including the issue of complement standardization have been published since 2020:

Frazer-Abel A, Kirschfink M, Prohászka Z. Expanding Horizons in Complement Analysis and Quality Control. Front Immunol. 2021 Aug 9;12:697313. doi: 10.3389/fimmu.2021.697313. eCollection 2021. PMID: 34434189

Willrich MAV, Braun KMP, Moyer AM, Jeffrey DH, Frazer-Abel A. Complement testing in the clinical laboratory. Crit Rev Clin Lab Sci. 2021 Nov;58(7):447-478. doi: 10.1080/10408363.2021.1907297.

Prohászka Z, Frazer-Abel A. Complement multiplex testing: Concept, promises and pitfalls. Mol Immunol. 2021 Oct 22;140:120-126. doi: 10.1016/j.molimm.2021.10.006.

VI. Leukocyte Sub-committee of the QAS Committee - Report of Activities - 2020/2021

Chair: Pablo Engel (pengel@ub.edu) - University of Barcelona (Spain)

Website:www.HCDM.org

List of current committee members

Pablo Engel (President HCDM and IUIS Nomenclature Chair)

Laurence Boumsell (France) (Honorary President)

Robert Balderas (USA)

Armand Bensussan (France)

Georgina Clark (Australia)

Valter Gattei (Italy)

Tomas Kalina (Czech Republic)

Ana Funaro (Italy)

Frank Mortari (USA)

Hannes Stockinger (Austria)

Menno C. van Zelm (The Netherlands)

Upcoming changes: Tomas Kalina will become the new chair in 2021. Bo-Quan Jin (China) and Fabio Malavasi (Italy) will step down from the council. HCDM will actively reach for new council members.

I. Objective

To establish the standardization and validation of monoclonal antibodies against leukocyte cell-surface molecules and other cell-surface molecules of the immune system

II. Recent Accomplishments

- **The CD Maps project**.

The goal of this project was to define the expression patterns of all established CD molecules using 8 color-flow cytometry. This project has allowed the quantitative determination of the expression of these cell markers in leukocyte and lymphocyte subsets of the blood, tonsil, and thymus (44 different populations).

The publication of the preliminary results of this project, showing CDMaps pilot project, had an enormous impact since the paper has received more than 20.000 visits since its publication (Kalina T, et. Al . CD Maps-Dynamic Profiling of CD1-CD100 Surface Expression on Human Leukocyte and Lymphocyte Subsets. Front Immunol. 2019 Oct 23;10:2434).

- **HCDM web**

The HCDM/HLDA web www.hcdm.org has been improved. The contribution of the IUIS is acknowledged on the web page. This page has had more than 9 million visits since its creation in 2016.

A database with all the expression results corresponding to CD1 to CD100 has been already finished and will be open to the public and can be found on our website www.hcdm.org (http://bioinformin.cesnet.cz/CDmaps/).

III. Education

We have published a position paper about antibody validation:

Kalina T, Lundsten K, Engel P. Relevance of Antibody Validation for Flow Cytometry. Cytometry A. 2020 Feb;97(2):126-136.

The following talks have been presented in different online international meetings related to antibody validation.

- Engel P Antibody validation for flow cytometry. Reproducible Science Week (ABCAM) Cambridge (UK).1st June. 2020 (virtual meeting)
- Engel P Reproducibility Crisis and Antibody Validation for Flow Cytometry ISAC Webinar. 3rd March 2021 (virtual meeting)

III. Ongoing Projects

We are about to submit g paper about standardization of the analysis of leukocytes subsets using multiparametric flow cytometry for the Frontiers in Immunology special issue: **Contemporary Challenges in Immunologic Testing in Clinical and Research Laboratories.**

Standardization of procedures and polychromatic flow cytometry panels for large scale quantitative expression profiling of surface antigens on blood leukocyte subsets. HCDM CD Maps initiative. Daniela Kuzílková, Joan Puñet-Ortiz, Pei M. Aui, Javier Fernández, Karel Fiše[1], Pablo Engel, Menno C. van Zelm, Tomas Kalina

- **CD Maps**. We have started the second phase of the project to complete the analysis of all the CD molecules expression profiles and add these data to the database

- **HLDA11**. We are organizing HLDA11 Workshop focused on Seven-span receptors and ion channels. We have already collected the relevant monoclonal antibodies. We expect to have created a panel of antibodies of more than 200 mAbs. We are testing these mAbs using 12-color flow cytometry and giving CD nomenclature to the newly validated monoclonal antibodies.

Unfortunately, this project presents a considerable delay due to the pandemic crisis. However, we expect to finish in 2022.

IV. Future Directions

- Continue the validation of monoclonal antibodies.

- Continue with the CDMaps project and complete the analysis of the expression of CD101 to CD371.

- Finish the HLDA11 Workshop

- Prepare new guidelines for monoclonal antibody standardization and validation.

VII. Allergen Standardization Subcommittee - Report of Activities - 2020/2021

Retrospect and major ongoing activities

The main project supervised by the subcommittee is the development of recombinant allergen standards and corresponding ELISA methods for their quantification. In the last years, the respective activities were centred in the Biological Standardization Project (BSP090), performed in collaboration with the European Directorate for the Quality of Medicines (EDQM). In the course of BSP090, two recombinant allergen standards for the major allergens of birch and Timothy grass pollen (Bet v 1 and Phl p 5) have been implemented and the respective ELISA methods have been successfully validated in international collaborative studies [1–3]. Both ELISAs are commercially available.

The General Text for the European Pharmacopoeia (Ph. Eur.) describing the Bet v 1-specific ELISA (*2.7.36 Assay of Bet v 1 allergen*) will be presented for adoption at the meeting of the European Commission in November 2021. The corresponding text for the Phl p 5-specific ELISA is currently in final reconciliation with the Allergen Working Party at the EDQM. Once these General Texts are in force, all allergen products based on birch and Timothy grass pollen to be sold in the EU must be tested for their major allergen content, enabling for the first time cross-product comparability in allergen products with respect to an active ingredient.

As the described implementation of the two allergen-specific ELISA methods in the Ph. Eur. is approaching, it can be assumed that the demand for the two allergen CRS will increase. Therefore, a new project was initiated under the auspices of the EDQM (BSP163) to prepare a second batch of the two respective CRS to meet the need. The in-depth analyses of the candidate batches of the recombinant proteins, including amino acid analysis, are currently ongoing in cooperation with the University of Salzburg and the Paul-Ehrlich-Institut.

Future activities

Unfortunately, the subcommittee meeting planned to be held at the International Paul Ehrlich Seminar 2020 could not take place as the seminar had to be cancelled due to the Corona pandemic. The subcommittee has met at the beginning of December 2020 in a digital format instead. This meeting was used to define new objectives for the Allergen Standardization Subcommittee. It was decided to start a new initiative to standardize additional allergens, covering up to six of the most important allergens from house dust mite and peanut (Der p 1, Der f 1, Der p 2, Der f 2, Ara h 1, Ara h 2). In order to accelerate the process, it was decided to place a public call for allergen-specific ELISA methods and corresponding allergen standards, followed by evaluation of the available methods and materials by a review committee. The call is currently in preparation.

References

1. Kaul S, Zimmer J, Dehus O, Costanzo A, Daas A, Buchheit K-H, et al. Validation of ELISA methods for quantification of the major birch allergen Bet v 1 (BSP090). Pharmeur Bio Sci Notes. 2017;2017:69–87.
2. Kaul S, Zimmer J, Dehus O, Costanzo A, Daas A, Buchheit K-H, et al. Standardization of allergen products: 3. Validation of candidate European Pharmacopoeia standard methods for quantification of major birch allergen bet v 1. Allergy. 2016;71:1414-1424. doi:10.1111/all.12898.
3. Zimmer J, Schmidt S, Kaul S, Costanzo A, Buchheit K-H, Brown S, et al. Standardisation of allergen products: 4. Validation of a candidate European Pharmacopoeia standard method for quantification of major grass pollen allergen Phl p 5. Allergy 2021. doi:10.1111/all.15003.