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Saudi Food & Drug Authority

Guideline on Allergenic Products

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Saudi Food & Drug Authority

Drug Sector

For Inquiries & Comments *Drug.Comments@sfda.gov.sa*

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Saudi Food and Drug Authority

Vision and Mission

Vision

To be a leading international science-based regulator to protect and promote public health

Mission

Protecting the community through regulations and effective controls to ensure the safety of food, drugs, medical devices, cosmetics, pesticides and feed

Document Control

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Legal Basis

The information in this guideline is adopted from the EMA guidelines in particular the Guideline on Allergen Products: Production and Quality Issues, with some adaptations for Saudi application.

1. Introduction

Allergy is a disease that is a consequence of Type I hypersensitivity reactions which are vigorous responses of the immune system triggered by the interaction of allergens with specific immunoglobulin E (IgE) antibodies leading to the release of inflammatory mediators including histamine, cytokines and lipid mediators. While test allergens are an important part of clinical allergy diagnosis, SIT with allergen products containing the same antigens is an immuno-modulatory treatment option which is intended to generate persistent relief from allergy symptoms.

The European monograph on Allergen Products (1063) address the technical quality of allergen products that are based on allergen extracts. In recent years, more and more allergens have been generated by using recombinant DNA technology. Such recombinant proteins have been evaluated as novel therapeutic products in clinical trials on SIT.

2. Scope

This guideline provides principles and guidance for the manufacturing and quality control of allergen products of biological origin, including allergen extracts from natural source materials and allergens produced through recombinant DNA technology, used for SIT or in vivo diagnosis of IgE mediated allergic diseases. It applies to all allergen products and their intermediates manufactured by a method involving an industrial process as defined by current International Standards.

Allergen products are obtained from allergen extracts, allergoids, conjugates or allergens manufactured using recombinant DNA technology. This guideline does not cover allergenic preparations consisting of synthetic peptides, DNA or RNA constructs and/or cell preparations or low molecular weight chemical allergens. This document also provides guidance on the establishment and use of in-house reference preparations (IHRP) for quality control including the analysis of batch-to-batch consistency. Moreover, criteria for the preparation of the serum pools used for potency measurements are defined.

3. Definitions

- An **allergen** is a molecule capable of inducing an IgE response and/or a Type I allergic reaction.
- **Recombinant allergens** are proteins obtained by recombinant DNA technology. The coding sequence may represent the complete sequence of individual allergens or only parts of it. Recombinant allergens may have an allergenic activity comparable to the natural allergen but the preparations may also have low IgE-binding capacity due to the selection of natural hypoallergenic variants or induced by sequence alterations or physicochemical modifications.
- **Allergen extracts** are extracts from **natural** biological source materials containing a mixture of allergenic and non-allergenic molecules.
- **Allergen products** are medicinal products containing allergens or derivatives of allergens for the purpose of in vivo diagnosis or treatment of allergic diseases.
- **Major/minor allergens** are allergens, against which at least 50% (major allergens) or less than 50% (minor allergens) of the patients tested have allergen-specific immunoglobulin E (IgE) antibodies.
- **Relevant allergens** are allergens causing a clinically relevant effect in a significant proportion of the allergic patients.
- **Allergoids** are allergens which are chemically modified to reduce IgE reactivity.
- **Conjugates** are allergens, which are covalently coupled to other molecules to modulate their immunological properties.
- **Homologous groups:** Allergen extracts prepared from different species, different genera or different families and finished products derived from these allergen extracts may be grouped in homologous groups based on the composition and the physiochemical as well as biological properties of the source material, the cross-reactivity/structural homology of allergens, the formulation of the finished product and the production process of the allergen extract and of the finished product.
- **Total allergenic activity** is defined as the capacity to bind specific IgE antibodies from allergic subjects measured by a competitive IgE-binding test.
- A **competitive IgE-binding test** is used to determine the total allergenic activity. The assays involved comprise for example IgE-inhibition assays with human IgE being inhibited from

binding to reference allergens at the solid phase by the allergen sample (dilution series) in the liquid phase, as well as assays with a constant amount of labelled allergens and the allergen sample (dilution series) competing for specific binding to IgE- antibodies bound to a solid phase.

- The **potency** as defined in ICH Q6B guideline is the "quantitative measure of the biological activity based on the attribute of the product which is linked to the relevant biological properties", using a suitable quantitative biological assay (also called potency assay or bioassay). For unmodified allergens or allergen extracts, total allergenic activity may serve as indicator of potency.

4. Main Guideline Text

4.1. General concepts

4.1.1. Homologous groups

Due to the high number of allergens in an allergen extract or in an allergen extract mixture and the cross-reactivity of the individual components, it is impossible to determine all relevant parameters for the allergens within a given extract or a defined allergen extract mixture. Therefore, extrapolation of stability data among members of taxonomic families were defined in a very broad sense and used by applicants. The concept of homologous groups introduced here replaces the concept of taxonomic families. This new concept limits the extrapolation to groups defined and justified by scientific criteria, restricts extrapolation to a few parameters while at the same time it retains the flexibility needed.

Allergen extracts prepared from different species, different genera or different families, and finished products which are derived from these allergen extracts and for which clinical experience already exists may be grouped into homologous groups.

The grouping should be based on following criteria:

- Comparable physicochemical and biological properties of the source material;
- Cross-reactivity/structural homology of the allergens;
- Identical formulation of the finished product;
- Identical production process of the allergen extract and of the finished product.

For group formation all four criteria have to be fulfilled. One member of a homologous group is selected as the representative species. This choice should be justified, taking into consideration for example geographical differences in the sensitisation patterns and other relevant factors.

To a limited extent, data on quality, safety and efficacy can be extrapolated from the representative source to other members of the homologous group. For allergens that cannot be included into one homologous group, the data for quality, safety and efficacy have to be provided on a single-product basis.

Detailed safety studies are only requested for the representative allergen, while post- marketing safety reports will be requested for non-representative allergens of the same group. Extrapolation of clinical data is addressed separately in the separate EWP guideline[1].

Proposed homologous groups are listed in Annex I. If justified, the applicant may define other groups or introduce new members into an existing group provided the criteria mentioned above are fulfilled. The Annex I will be updated according to the state of the art and the scientific knowledge.

4.1.2. Allergen mixtures

Allergen extract mixtures should be prepared from individual extracts from single source materials. Therefore, different source materials should not be mixed prior to extraction. Since extracts are considered as active substances (see section 4.2), each individual extract should be considered as an active substance of its own. Potency testing should be performed for each individual active substance prior to mixing. Total allergenic activity has to be determined at the finished product level or, if this is not possible, on the first homogeneous mixture. If the testing of the individual active substances in the finished product is not possible due to cross reactivity of the constituents, the total allergenic activity of the finished product should be determined by a competitive IgE-binding test.

The number of allergen extracts in a mixture should be kept to a minimum regardless of homology and cross-reactivity of the individual allergens. The number and the relative proportion of the individual active substances should be justified. If in a mixture the allergens do not belong to the same homologous group, the combination of the components has to be justified.

The following issues should be taken into consideration for allergen extract mixtures and mixtures

of recombinant allergens:

- Allergens with proteolytic activities should not be used in mixtures unless justified.
- Perennial and seasonal allergens should not be mixed.
- Hymenoptera venoms should not be mixed with any other allergens. Venoms from different genera should not be mixed.

4.1.3. Comparability

The development of an allergen product might involve changes in the manufacturing process which have impact on the finished product. Given its complex nature, it is particularly important that all stages of the development process are fully evaluated and tracked within the dossier where applicable. Applicants should take into consideration the step-by-step approach according to CHMP/ICH/FDA guidance 2, 3,4, considering not only the characterisation studies at the level of the active substance, but also the validation of the manufacturing process as well as in-process controls and stability data.

4.2. Active Substance

The following information should be referenced to a Drug Master File.

4.2.1. General information

The active substance can be an allergen extract, as well as a purified natural or recombinant protein, all of which can be unmodified or modified (e.g. physically and/or chemically as allergoid or conjugate). Preferably, the active substance is a stable preparation at the latest step before mixing or formulation. In general, adsorption and addition of excipients are considered as formulation steps.

Allergen extracts mainly consist of proteins and glycoproteins and contain various major and minor allergens as well as non-allergenic components. Because of the intrinsic variability of the natural source material, concentrations of individual allergens in such extracts may vary and standardisation is therefore very important. Active substances obtained by recombinant DNA technology consist of predefined allergenic polypeptides, for example a major allergen, or a mixture of defined polypeptides. The quantity and structure of these polypeptides can be determined and these products should be characterised as defined in the guidelines relevant for biotechnological products.

4.2.2. Manufacture

4.2.2.1. *Manufacture of the active substance derived from natural source materials of biological origin*

The employed production process steps including e.g. pre-treatment, extraction, filtration, dialysis, concentration or freeze-drying steps should be described in detail and validated. Data can be extrapolated from the representative species of the same homologous group provided that the manufacturing process for the active substance and finished product are identical (see also section 4.4.2). The in-process control methods including the corresponding acceptance criteria should be reported. For better illustration, a step-by-step diagram (flow- chart) indicating all process steps, including the relevant in-process controls, should be presented. If aseptic precautions are introduced, these should also be indicated in the flow- chart. In case of modified allergen extracts such as allergoids or conjugates, the modification processes should be described. Intermediates in the manufacturing process should be identified and controlled.

4.2.2.2. *Manufacture of the active substance derived from recombinant DNA technology*

In contrast to allergen preparations obtained from natural source materials of biological origin, the quality of individual allergen batches obtained by recombinant DNA technology does not vary according to the properties and quality of the individual source materials, but depends on the cell systems used, fermentation processes and purification procedures. Therefore, a detailed characterisation of the cell lines used and the manufacturing process is required as described in the relevant guidance documents.

For the production of recombinant allergens, all guidelines for products derived from recombinant DNA technology have to be taken into consideration (e.g.[5-8]).

4.2.3. Control of materials

This section includes starting (source) materials (for example natural source materials from biological origin for allergen extracts and cell substrates for the production of recombinant proteins) and raw materials (for example solvents and diluents for extraction, media for the cultivation of mites or moulds and media and reagents for production of recombinant proteins). When substances of animal or human origin are used as source materials or as raw materials, viral

safety[9, 10] and compliance with TSE requirements[11] should be demonstrated to avoid the risk of transmission of infectious diseases.

4.2.3.1. Control of source materials for allergen extracts

The name(s) and address of the supplier(s) of the allergenic source material should be stated. The description of the allergenic source materials should contain all relevant details. The name (scientific name, for example genus and species as well as any common name), and type (e.g. pollen and other plant-derived material, insect venoms, pelt, dander, saliva or foods) of the allergenic source material(s) should be stated. Details concerning the cultivation, collection, pre-treatment (e.g. irradiation steps) and storage should be supplied for each separate source material. Whenever purification steps (for example defatting) or other treatments are performed by the supplier of the source material, these activities have to be mentioned and justified; more over acceptance limits have to be defined. The quality control of source materials should be documented. Acceptance criteria and control methods for the source material(s) should be included. They should encompass requirements and control methods relating to identity and purity. The acceptance criteria should ensure the consistency of the allergenic source material from a qualitative and quantitative point of view. The source materials should be stored and transported under controlled conditions justified by stability data. If source materials from different suppliers and deliveries are mixed to achieve uniform source material batches, the underlying concept should be described. Uniformity of the source material from different origins should be justified.

Each individual source material has to be qualified regardless of whether it belongs to the same homologous group.

<u>Additional requirements for specific source materials:</u>

- *Pollens*

  Geographic location and nature of the collection areas, field characteristics, treatments, visual control, way of collection and random sampling procedures should be described. The variety of the plants used should be given including transgenic plants if used. The use of transgenic plants has to be justified. The principles of the Guideline on Good Agricultural and Collection Practice (GACP) for Starting Material of Herbal Origin[12] and other relevant guidelines for the

source material of plant origin should be followed where applicable[13,14]. Tests methods and acceptance criteria for the identification and determination of the content of impurities such as foreign pollen, mould spores, extraneous plant material from the same species and non-related contaminations should be included.

The content of relevant pesticides, heavy metals and solvents should be monitored in order to demonstrate that their levels are kept to a minimum in the allergenic source material.

The content of pollen from other species should be limited to 1% of mixed pollens and 0.5% of one individual pollen as determined by a microscopic particle count. Detectable mould spores should not exceed 1%[15]. The contamination with particles of plant origin, other than pollen, should be kept to a minimum. The maximum allowed contamination should be justified.

- *Moulds*

The strain(s) of moulds used should be specified. Morphology and other parameters for characterisation and identification (for example biochemical or genetic properties) as well as the cultivation method and the kind of source material harvested (mycelium and/or spores) should be specified. The cultivation method should be described in detail and key parameters (e.g. temperature) justified, and evidence should be provided that no detectable amounts of mycotoxins are produced by the moulds. Details on the composition of the cultivation medium and the media components should be submitted. Synthetic media i.e. media free of animal-derived material or allergen-free media should be preferably used.

Strains which produce mycotoxins such as aflatoxins or ochratoxins should not be used unless justified and their mutagenic potential should be evaluated. In this case, the amount of relevant mycotoxins should be quantified before processing and their removal through processing should be implemented and validated. Appropriate measures have to be implemented to avoid contamination by other mould strains.

- *Mites*

The mite species should be specified. Morphology and other parameters (for example biochemical or genetic properties) for the identification of the mites should be specified.

The cultivation method and the composition of the cultivation medium as well as the media components should be described. Details on the composition of the cultivation medium and the

media components should be submitted. Synthetic and consequently free of animal-derived material and allergen-free media should be preferably used. The conditions of culture and the time of harvest should be described and the corresponding key parameters defined. It should be indicated which part of the culture is used for further processing, e.g. mites, mite faeces only or the whole mite culture or mixes thereof.

- *Animal Allergens*

Only healthy animals should be used and certificates from the collector concerning their health status should be provided where possible. When killed animals are used, the source materials should be collected within a few hours after death and the dead animals should be stored under conditions that maintain the quality of the source material. Morphology and other parameters used for the identification of the allergenic source material should be described.

The composition of the source material (for example hair, pelt, epithelium, saliva or urinary fluid) should be indicated. Any possible contamination with mites and moulds for example should be addressed and avoided as far as possible. The collector should certify that the animals used have not recently been treated with antiparasitics or other medicines.

The collection of hair and dander must be performed without injuring the skin of the animal. Methods employing the grinding of whole skin and /or pelts must not be used.

The storage conditions should be described.

- *Hymenoptera Venoms*

The morphological characteristics of the animals and other parameters of characterisation should be specified. The collection method of venom from hymenoptera species should be described. Evidence should be provided that the amount of relevant pesticides is kept to a minimum. This may include a certificate that no pesticides were used in the hymenoptera culture.

4.2.3.2. *Control of source materials used for the manufacture of recombinant allergens*

For recombinant allergens, all relevant guidelines have to be considered.

4.2.3.3. Control of raw materials

For each raw material, the specifications, information on its source and justification for its use should be provided.

If any allergenic components are used in the culture medium, their removal in the manufacturing process should be demonstrated.

4.2.4. Characterisation and control of the active substance

4.2.4.1. Characterisation and control of allergen extracts

Characterisation and quality control of allergen extracts should be performed at the active substance level. If certain control tests cannot be applied to the active substance, for example allergoids, testing at intermediate stages rather than at the active substance stages may be appropriate and acceptable if justified. In such circumstances, quality specifications should be defined for the product just prior to the modification or dilution step and these acceptance criteria should be considered as in-process acceptance criteria and included in the release specifications of the active substance. Generally, the following tests and acceptance criteria are applicable: appearance and description, identity, purity and impurities, total allergenic activity and major allergen determination.

The allergens relevant for the product have to be defined by the manufacturer. It should be demonstrated that the manufacturing process is able to maintain these allergens by proving their presence using appropriate methods such as antibody-based techniques or mass spectrometry. The content of relevant allergens should be measured by validated assays using certified reference standards or biological reference preparations and assays validated in international standardisation programmes whenever possible. The protein profile should correspond to that of the in-house reference preparation (IHRP) and the presence of the relevant allergen components be verified whenever possible. The choice of the relevant allergen components subject to determination must be justified. If a significant part of the total allergenic activity or safety concerns arise from other (for example minor) allergens, these have to be measured as well.

The manufacturer should demonstrate its capability to obtain batch-to-batch consistency and provide a justification for the selected and validated test procedures.

Each lot of source material should be assayed for identity, purity, and potency using validated in-house analytical procedures, **if applicable**. Analytical procedures may include, but not be limited to, RAST, ELISA, IEF, RID, SDS-PAGE, immunoblotting, microscopy, spectroscopy, chromatography, titrimetry, reagent colorimetry. This information should be provided along with specifications and acceptable limits. All test methods should be fully described. The application should include representative data along with chromatograms, spectra, etc. Validation data should include results of studies establishing parameters for linearity, intra-assay precision (repeatability), interassay precision (intermediate precision), and recovery.

4.2.4.2. Characterisation and control of recombinant allergens

Emphasis should be put on the structural integrity and the consistency of folding since these factors may influence the immunogenic properties and safety in SIT. Investigation of post-translational modifications such as glycosylation should be considered where appropriate. The intact biological function (for example physiological function as plant enzyme) of an allergenic protein derived from recombinant DNA technology may serve as an indirect indicator of structural integrity but is not an essential property determining allergenicity or immuno-modulating activity. Therefore, the demonstration of biological function may not be necessary for recombinant allergens.

Attention should be given to potential impurities from the media or host cell components. These impurities should be identified and quantified and their potential to give rise to undesirable and potentially allergic reactions should be estimated.

Recombinant allergens should be characterised and quantified by techniques appropriate for recombinant proteins. The content should be expressed in weight per volume whenever possible. The correlation between the quantity of the individual recombinant allergens and the corresponding biological (for example allergenic) activity should be shown in validation studies. For recombinant allergen molecules, ELISA methods with specific animal antibodies may be used as potency assays as long as a correlation with the IgE-binding has been demonstrated. For recombinant allergens with a reduced IgE reactivity, potency tests should preferably consist of a discriminatory test to distinguish between molecules with high and low IgE-binding capacities, for example by quantification in ELISA systems, and an assay to verify the reduction in IgE reactivity.

For mixtures of different recombinant allergens, the content of the individual allergens should be determined by adequate quantification methods, for example ELISA just prior to mixing and in the mixture, unless otherwise justified. The general rules given in section 4.1.2 (Allergen mixtures) should be considered, where applicable.

4.2.4.3. Characterisation and control of modified allergen preparations

For modified allergens (for example denatured or chemically-modified allergoids or conjugates), antibody-based assays or other appropriate test methods have to be established to identify the relevant allergens in the modified form. Other assays should be used to analyse the expected modification of the allergens and for the characterisation of the modified allergens, and to demonstrate consistency of the modification process, for example by peptide mapping by mass spectrometry, or size-exclusion chromatography to determine the degree of polymerisation or other methods to determine the degree of polymerization (e.g. presence of amine groups).

4.2.4.4. Potency assays

The following potency tests should be performed for the different kinds of active substances:

- For allergen extracts and purified allergens without structural modification, the total allergenic activity should be determined by a competitive IgE-binding test. If the product is defined on the basis of relevant allergens, correlation with the total allergenic activity has to be demonstrated.
- Relevant individual allergens may be determined by immunological methods (for example ELISA) with specific animal antibodies.
- For allergoids, potency tests should consist of a discriminatory test or a combination of immunological tests to distinguish between native and modified molecules, for example by quantification in ELISA systems or mediator release assay, and an assay to determine the lack of IgE reactivity. As an alternative to a discriminatory immunoassay, other techniques (for example mass spectrometry) may be used to demonstrate the presence of the relevant allergens.
- For conjugates, the potency testing should consider the immuno-modulating properties of the specific modifications.

The relevant individual allergens should be identified and their content should be measured whenever possible using certified reference standards or biological reference preparations and approved assays. If activities or safety concerns arise from other (for example minor) allergens, these have to be measured as well. Potency testing of recombinant allergens is described in section 4.2.4.2.

Specifications, acceptable limits, and analytical methods used to insure the safety, identity, purity, potency, as well as lot-to-lot consistency should be provided. Validation of the analytical systems, including validation data, should be provided if this application is a request for the use of a new testing method or equivalent methods and processes. Materials to be submitted include (but are not limited to) chromatograms, instrumental recordings, calibration curves, linear regressions, etc. For testing that does not entail a specific analytical method, such as microscopic determination of purity and identity, specifications and acceptable limits should be provided. Certificates of analysis and analytical results for representative lots should also be included.

4.2.5. Stability

If the active substance is stored, stability data should be obtained according to the relevant guidelines on stability testing (e.g. ICH Q1A)[16] to provide information concerning the allowed maximum storage period. The general principles defined in ICH Q5C[6] guideline for biological/biotechnological products should also be considered for allergen extracts.

Regarding the homologous groups, a full set of data should be presented for the "representative" allergen of the particular homologous group. For the "non-representative" allergens, stability studies may be performed on an ongoing basis for the overall shelf life of the active substance. If these data are not available at the time of submission of a marketing authorisation application, a commitment should be made to continue the stability studies after approval. The marketing authorisation application should contain a detailed protocol of the stability studies of the "non-representative" allergens. If justified, some stability data may be extrapolated from the "representative" allergen. The extrapolation of the results from the "representative" allergen" should be discussed and justified, taking into account data concerning the activity of those enzymes (such as proteases) which might impact on the structure of the individual molecules.

4.3. Standards and Reference Materials

Reference standard materials should be established and characterised for all types of allergen products.

In-House Reference Preparations (IHRP) for allergen extracts:

Individual company's established IHRP should only be used as long as no official standards with confirmed and monitored content of the major allergens and total allergenic activity are available.

Allergen extracts are potentially different between manufacturers and, due to the variability of biological source materials, may even vary to a certain extent from one batch to another within a single manufacturer. These characteristics represent a problem with regard to any further harmonisation between products from different manufacturers. At least an appropriate batch-to-batch consistency has to be reached by a company within its production runs by introducing an IHRP, which should be used as internal reference preparation, and using a number of biological and analytical procedures. The IHRP is derived from a production run following the manufacturing process as defined in the dossier. If additional measures are taken to increase the stability of the IHRP (e.g. lyophilisation or addition of stabiliser), these should be justified by the applicant. The IHRP establishes a reference point against which extracts from all future production runs will be compared. Therefore, the qualitative and quantitative composition of regular production batches should meet predefined acceptance criteria when compared with the IHRP. These criteria have to be justified.

The IHRP should be characterised using available relevant methods and its specific allergenic activity shall be established. Data should be provided on protein and, whenever possible, carbohydrate composition. The relevance of glycoproteins for the IgE-binding should be considered. The presence of all relevant allergens in the IHRP should be demonstrated.

Some of the following methods should be applied for biochemical and structural characterisation: isoelectric focusing, determination of the distribution of molecular weight by SDS-PAGE, capillary electrophoresis, chromatographic techniques, mass spectrometry or other appropriate techniques. As far as possible, individual allergens should be identified using specific antibodies or other techniques and the internationally accepted allergen nomenclature should be used.

The concentration of the relevant individual allergens should be determined if possible using certified reference standards or biological reference preparations and assays validated in international standardisation programs.

Information regarding the allergenic properties of the proteins in the IHRP may be obtained from experiments involving immunoblotting techniques using patient pool serum. Sera from individual patients can be used to obtain an allergogram. The potency of the IHRP should be determined by immuno-assays (for example competitive IgE-binding test or cellular mediator release assays) and expressed in terms of units of biological activity. In addition, the T-cell proliferation capacity may be analysed.

The IHRP should be biologically standardised by appropriate methods on the basis of skin reactivity test using methods such as those described by Turkeltaub[17] and Nordic Council of Medicines[18]. In case not enough patients are available for *in vivo* standardisation, standardisation of the IHRP by *in vitro* methods may be justified. The stability of the IHRP and the storage conditions should be documented.

If a new batch of an IHRP for the same allergen has to be established, both the old and the new IHRP should be tested in parallel to avoid a shift of the biological activity. This standardisation should be performed using a predefined set of *in vitro* methods, but *in vivo* standardisation procedures may also be included. A detailed description of the protocol should be provided. In general, the results of the quantitative assays should be in agreement with the results of the previous batch of the IHRP. In case of deviating results, a correlation factor should be established. Trending analysis has to be performed to avoid a shifting of quality parameters.

<u>In-House Reference Preparations (IHRP) for recombinant proteins:</u>

For the IHRP used for the quality control of recombinant allergens, the criteria defined in ICH Q6B[8] guideline should be followed and potency testing according to section 4.2.4.2 should be applied. Justification for the reference material as well as the testing strategy chosen should be provided.

<u>Sera Pools:</u>

A sera pool should be established for batch control and for the qualification of individual IHRP. The problem of geographically different sensitisation patterns should be taken into consideration

in the preparation of the pools. For the used sera, the frequency of IgE-recognition of different allergens as well as the content of allergen-specific IgE antibodies and the clinical relevance of sensitisation should be taken into account when preparing the pool. The pool should be composed of sera from 10 to 15 individuals. Sera recognising carbohydrate epitopes and sera from patients who had a previous SIT treatment with the respective or cross-reactive allergen should not be included in the pool. In addition, sera containing IgE antibodies against bovine serum albumin, milk proteins or gelatin could cause experimental problems and should therefore be avoided in the pool unless otherwise justified.

Specifications should be set for the sera pool, including criteria for the reactivity profile of the pool. Prior to use, the adequate quality of the pooled sera should be demonstrated by appropriate control experiments. This should include the demonstration that the relevant allergens are recognised by the pools.

4.4. Finished Product

This section should contain information on the final biological drug product including all active ingredients and excipients in the final product. If any proprietary preparations or mixtures are used as components, the information should include a complete statement of composition and other information that will properly describe and identify these materials. For all ingredients of human or animal origin, testing results or certificates of analysis demonstrating their freedom from adventitious agents should be provided. Appropriate information may be cross referenced to those under the section on the drug substance.

4.4.1. Description and composition of the finished product

A detailed description of the finished product should be given. If the finished product consists of a mixture of active substances, a complete list of all the active substances used should be given. In general, adsorption and addition of excipients are regarded as formulation, and these steps should be described in the manufacturing process of the finished product.

1. Drug Substance

A list of each drug substance should be provided.

2. Excipient

This section should contain a list of all inactive components with the rationale for inclusion of each in the final product. The information should include certificates of analysis, a list and description of tests performed, results of analytical testing or other information that will describe or identify each excipient. If compendial excipients are used, citations may be included in lieu of analytical testing. Excipients may include, but not be limited to, the following:

- diluents;
- bulking agents;
- adsorbents (other than adjuvants); and
- stabilizers.

3. Desiccants

Any agent used to promote dryness should be included in this section.

4. Adjuvant

This section should contain a list of the chemical formula and precise quantity of each adjuvant. The method for quantity determination should also be specified.

5. Preservative

Each preservative should be identified by chemical as well as any trade name. The results of the preservative effectiveness validation should be included in the Microbiology section of this document. Reference may be made to other files or compendial sources.

6. Ancillary Components

For the Allergen Patch Test, a description of the ancillary components used to apply the drug product, such as syringes, or to hold the product in place, such as the support and surgical tape, should be provided.

4.4.2. Manufacture

The manufacturing process should be described in detail, including process scale. A step-by- step diagram (flow-chart) should be presented, indicating all process steps and including the relevant in-process controls. If aseptic precautions are introduced, these should also be described and

indicated in the flow chart. Any allowed process holding times should be identified and justified. Description, documentation and results of the validation of the manufacturing process should be provided. If justified, a reduced validation program can be applied for the non-representative allergen products of the same homologous group provided that the manufacturing process is identical to that of the representative allergen product and for which full validation data should be available. For the nonrepresentative allergens, the critical steps and key parameters should be identified and integrated in the reduced validation program.

If further adsorption or modification steps are performed, these manufacturing steps have to be described in detail and reported in the flow chart. The purpose of these steps should be explained. In addition, tests should be carried out to demonstrate the success of these activities and the consistency of production.

In case human plasma derived materials are used during the manufacturing process or as excipients/stabilizers in the formulation of allergen finished products, the quality and safety with respect to transmissible agents have to comply with the current SFDA guideline on plasma-derived medicinal products[19].

4.4.3. Control of the finished product

Appropriate specifications should be set for the finished product. If any of the control tests (e.g. potency tests) cannot be performed on the finished product, specifications should be defined for the intermediate at the latest stage prior to the modification step. In such cases, the test result should comply with the acceptance criteria defined in the finished product specification. The characteristics of the finished product should be documented for all strengths (dilutions). Where appropriate testing is not possible due to methodological limitations, this should be justified. Guidance provided in previous parts of this guideline that are also relevant to the control of the finished product should be taken into account.

<u>Control of non-modified allergen preparations:</u>

Total allergenic activity determined by a competitive IgE-binding test is required for the standardisation and batch control of finished products containing non-modified allergens. Consequently, the labelling should be indicated in potency units. If test systems validated in international standardisation programs are available for the quantification of individual allergens,

these should be applied. In that case, the content in weight per volume of the individual allergens should be included in the specifications of the finished product and should be indicated in the Summary of Product Characteristics in addition to potency. If safety concerns arise from individual minor allergens, these have to be measured as well.

Control of allergen mixtures:

For allergen mixtures, potency testing should be performed for each individual allergen active substance in the mixture. If the testing of the individual active substances in the finished product is not possible due to cross reactivity of the constituents, the total potency of the finished product should be determined by a competitive IgE-binding test.

Control of adsorbed products:

For adsorbed products, the efficacy and stability of the adsorption has to be determined by measuring the amount of total soluble protein and/or the presence of IgE-binding components in the supernatant or by using other relevant methods at least at release and at the end of the shelf life period. These parameters should be followed during the stability studies performed for adsorbed products.

Control of recombinant allergens:

Finished products containing recombinant allergens have to comply with the ICH Q6B guideline[8]. The content of the purified protein (for example major allergen) and the potency as described in chapter 4.2.4.2 and 4.2.4.4 should be determined.

Non-standardised allergen extracts:

Certain allergens cannot be fully standardised because a sufficient number of patients is not available for biological standardisation and to create an appropriate sera pool, such as in rare allergies. In this case, a range of *in vitro* methods such as determination of an antigen profile, protein profile and the content of total protein and individual allergens may be applied for the control of the finished product. If one of the above-mentioned parameters is not tested, a justification has to be given.

4.4.4. Container closure system

The container closure system(s) used for the various strengths should be described in detail. Additionally, all other parts of the final medicinal product including for example solvents for reconstitution or syringes have to be described.

4.4.5. Stability

Stability testing should be performed as real-time stability studies as indicated in the relevant guidance documents (e.g.[6,16] where applicable), using stability-indicating assays. Sterility testing should be performed for all parenteral preparations, eye preparations, preparations for inhalation or preparations intended for skin prick testing. If preservatives are used e.g. in multi-use containers, the efficacy of the antimicrobial preservation should be tested according to the relevant Ph. Eur. Monograph (5.1.3. Efficacy of Antimicrobial Preservation). Products not required to be sterile (e.g. for oral route) have to comply with the requirements defined in the Ph Eur. Monograph 5.1.4. (Microbiological Quality of Pharmaceutical Preparations). For allergen extracts belonging to the same homologous group, a full set of stability data has to be provided for the representative allergen. For the non-representative allergens some stability data may be extrapolated from the "representative" allergen. Therefore, only a limited number of parameters may be tested in these studies. The applicant should justify the choice of these parameters. The extrapolation of the results from the "representative allergen" should be discussed and justified. Extrapolation may not be possible for all allergen products, e.g. differences of enzymatic activities between the representative and the non-representative allergens have to be considered if relevant for the stability of the product.

The data for the non-representative allergens may be obtained in ongoing real-time stability studies after granting of a marketing authorisation. If the data are not available at the time of submission of a marketing authorisation, a commitment should be made to continue the stability studies after approval. The marketing authorisation application should contain a detailed protocol of the stability studies of the "non-representative" allergens.

If the finished product consists of a mixture of allergen extracts not belonging to the same homologous group, stability studies have to be performed for the mixture considering each individual active substance. If the individual extracts in a mixture belong to the same homologous group and therefore cross-reactivity occurs between the relevant allergens, it may not be possible to determine the activity of the individual active substances. In such cases (for example a mixture of grass pollen extracts), an overall potency determined by a competitive IgE-binding test may be appropriate. The selected testing strategy should be described in detail and justified by the applicant.

For allergen extracts, stability studies of finished products manufactured with active substance at the end of its shelf life should be considered. If performed, the study should be initiated once during development or a commitment should be given to initiate such a study after marketing approval.

For adsorbed products, the stability of the adsorption and /or modification has to be proven at the end of the shelf life by testing the total amount of soluble protein in the supernatant and/or by determining the presence of IgE-binding components in the supernatant or by using other relevant methods. In order to prove the stability of products containing native and modified allergens, mediator release assays (for example with mouse IgE and rat basophil leukaemia cells) may be considered as potency tests.

If it is not possible to perform potency tests, for example in case of adsorbed material, *in vivo* immunogenicity tests or validated alternative *in vitro* tests should be performed in the stability studies at the beginning and end of the proposed shelf-life period. The stability study should be initiated during development, to provide evidence on the stability of the finished product.

5. References

1. Guideline on the Clinical Development of Products for Specific Immunotherapy for the Treatment of Allergic Diseases (CHMP/EWP/18504/2006)
2. Note for Guidance on Biotechnological/Biological Products Subject to Changes in their Manufacturing Process (ICH Q5E; CPMP/ICH/5721/03)
3. Guideline on Comparability of Medicinal Products containing Biotechnology-derived Proteins as Active Substance -Quality Issue (EMEA/CPWP/BWP/3207/00/Rev1)
4. Guidance for Industry On the Content and Format of Chemistry, Manufacturing and Controls Information and Establishment Description Information for an AllergenicExtract or Allergen Patch Test (FDA/ April 1999)
5. (5) Note for Guidance on Quality of Biotechnological Products: Analysis of the Expression Construct in Cell Lines Used for Production of r-DNA Derived Protein Products (ICH Q5B; PMP/ICH/139/95)
6. Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products (ICH Q5C; CPMP/ICH/138/95)
7. Derivation and Characterisation of Cell Substrates Used for Production of Biotechnological / Biological Products (ICH Q5D; CPMP/ICH/294/95)
8. Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products (ICH Q6B; CPMP/ICH/365/96)
9. Note for Guidance on Quality of Biotechnological Products: Viral Safety Evaluation of Biotechnology Products derived from Cell Lines of Human or Animal Origin (ICH Q5A; CPMP/ICH295/95)
10. Note for Guidance on Virus Validation Studies: The Design, Contribution and Interpretation of Studies validating the Inactivation and Removal of Viruses (CPMP/BWP/268/95)
11. Note for Guidance on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Human and Veterinary Medicinal Products (EMEA/410/01)
12. Guideline on Good Agricultural and Collection Practice (GACP) for Starting Materials of Herbal Origin (EMEA/HMPC/246816/2005)
13. Guideline on Specifications: Test Procedures and Acceptance Criteria for Herbal Substances, Herbal Preparations and Herbal Medicinal Products/Traditional Herbal Medicinal Products (CPMP/QWP/2820/00 Rev 1)
14. Guideline on Quality of Herbal Medicinal Products/Traditional Herbal Medicinal Products (CPMP/QWP/2819/00 Rev 1)
15. Ph. Eur. monograph on Allergen Products (1063)

16. Note for Guidance on Stability Testing: Stability Testing of New Drug Substances and Products (ICH Q1A (rev. 2); CPMP/ICH/2736/99)
17. Turkeltaub PC. Biological Standardization of allergen extracts. Allergol.Immunopathol., 1989; 17; 53-65
18. Nordic Council of Medicines. Registration of Allergenic Preparations. Nordic Guidelines, Vol. 23, 2nd edition. Uppsala, Sweden: NLN Publications 1988. pp 1-34
19. Guidelines For Blood Products (SFDA, 2007)
20. A.R. Lorenz, D. Lüttkopf, S. May, S. Scheurer, S. Vieths. The Principle of Homologous Groups in Regulatory Affairs of Allergen Products – A Proposal. Int ArchAllergy Immunol 2008 Aug 12;148(1):1-17

ANNEX 1: PROPOSED HOMOLOGOUS GROUPS

One member of a homologous group is selected as the representative species. This choice should be justified, taking into consideration for example geographical differences in the sensitisation patterns and other relevant factors.

Proposed homologous groups [Lorenz[20]]

1. **Tree pollen**

The 'birch group' or 'fagales group'
*Betula verrucosa = B. pendula** = *B. alba* European white birch
Alnus glutinosa Alder
Carpinus betulus Hornbeam
Corylus avellana Hazel
Quercus alba Oak
Castanea satavia
Fagus sylvatica

* Correct taxonomic name according to NCBI taxonomic database

The group of *Oleaceae*
Olea europaea Olive *Fraxinus excelsior* Ash *Ligustrum vulgare* Privet *Syringa vulgaris* Lilac

The group of *Cupressaceae Juniperus* sp. Cedar *Cupressus* sp. Cypress

Non-grouped species within tree pollen species. Justification required.

Fagus sylvatica European beech
Acer sp. Maple
Platanus sp. Plane tree
Populus sp. Poplar
Robinia pseudoacacia False acacia, Locust tree
Salix sp. Sallow / Willow
Tilia sp. Linden / Lime tree
Ulmus sp. Elm

Cryptomeria japonica Japanese Cedar

2. Grass and cereal pollen

The group of sweet grasses of the *Poaceae* (*Gramineae*) family, subfamily of *Pooideae*
Anthoxanthum odoratum Sweet vernal grass
Avena sativa Oat
Dactylis glomerata Orchard grass/Cocksfoot
Festuca sp. Meadow fescue
Holcus lanatus Velvet grass/Yorkshire fog
Hordeum vulgare Barley
Lolium perenne Perennial ryegrass
Phleum pratense Timothy grass
Poa pratensis Kentucky bluegrass
Secale cereale Cultivated rye
Triticum aestivum Cultivated wheat

Additional grass species belonging to the homologous group of *Pooideae* with reservations
Agropyron sp. Couch grass, Crested wheatgrass
Agrostis sp. Bent grass
Alopecurus pratensis Meadow foxtail
Arrhenatherum elatius False oat
Bromus sp. Brome grass

Non-grouped grass pollen species. Justification required.
Cynodon dactylon Bermuda grass
Cynosurus cristatus Dogstail

3. Weed pollen

The group of weed pollen species
Ambrosia artemisiifolia, Ambrosia trifida Ragweed
Artemisia vulgaris Mugwort
Parietaria judaica, Parietaria officinalis Pellitory

Non-grouped weed species. Justification required.
Plantago sp. Plantain

4. Mites

The group of house dust mites of the *Dermatophagoides* genus
Dermatophagoides pteronyssinus
Dermatophagoides farinae

Non-grouped mite species. Justification required.
Acarus siro flour mite
Glycyphagus domesticus house mite *Lepidoglyphus destructor* house mite *Thyreophagus entomophagus* flour mite *Tyrophagus putrescentiae* storage mite

5. Insect venoms

No homologous groups formed. Justification required.

6. Allergen extracts derived from vertebrates

Extracts such as animal epithelia, hair, dander.
No homologous group formed. Non-grouped species. Justification required.
Canis familiaris Dog
Felis domesticus Cat
Cavia porcellus Guinea pig
Cricetus cricetus Hamster
Equus caballus Horse
Mus musculus Mouse
Oryctolagus cuniculus Rabbit
Rattus sp. Rat

7. Moulds

No homologous group formed. Justification required; in case of justification of grouping of mould species, special emphasis on similar stability is necessary.