EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

6 December 2018
EMA/CVMP/IWP/170689/2016
Committee for Medicinal Products for Veterinary Use (CVMP)

Guideline on requirements for the quality (production and control), safety and efficacy of allergen products for use in horses, dogs and cats

Draft

Draft agreed by Immunologicals Working Party	October 2018
Adopted by CVMP for release for consultation	6 December 2018
Start of public consultation	19 December 2018
End of consultation (deadline for comments)	31 August 2019

This guideline will replace the existing NtA guideline on 'Specific Requirements for the Production and Control of Allergen products (7BIm11a)', adopted prior to September 1994.

Comments should be provided using this template. The completed comments form should be sent to vet-guidelines@ema.europa.eu

Keywords	Allergen products, requirements, cats, dogs, horses

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Guideline on requirements for the quality (production and control), safety and efficacy of allergen products for use in horses, dogs and cats

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Executive summary

This Guideline lays down the quality recommendations for allergen products of biological origin, including allergen extracts derived from natural source material and allergens produced through recombinant DNA technology, used for treatment or specific immunotherapy (SIT) or *in vivo* diagnosis of immunoglobulin E (IgE)-mediated allergic diseases in horses, dogs and cats.

In addition, guidance is given for the clinical testing regarding safety and efficacy of the products.

1. Introduction (background)

Allergic diseases in animals are gaining importance in veterinary practice. Allergies are various conditions brought on by the hypersensitivity of the immune system to exogenous substances. The major allergic diseases of dogs, cats and horses affect the skin (e.g. canine and feline atopic dermatitis, insect bite in horses), respiratory tract (e.g. feline asthma, equine recurrent airway obstruction) and intestinal tract (dietary hypersensitivity).

Immunologically, the majority of these allergic diseases are suggested to have a Type I hypersensitivity pathogenesis. These type I (immediate) reactions are defined as 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.

The diagnosis of allergies is aided by the use of allergens for intradermal testing and some allergies can be treated with allergen immunotherapy. A good knowledge of relevant allergens for the individual species is therefore of great importance. Currently, the knowledge about relevant veterinary allergens is based on sensitisation rates identified by intradermal testing or serum testing for allergen-specific IgE; crude allergen extracts are the basis for most evaluations.

Recent years have seen the development of novel reagents and technologies which have been applied to the field of veterinary allergology, resulting in newly identified clinically relevant allergens and a deeper understanding of animal-specific pathogenesis of allergic diseases. This availability of methodologies for diagnosis and therapy, together with an increasing awareness of allergic diseases in veterinary practice as well as in the general population of companion animal owners, facilitated this development.

In recent years, in the human field 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. In the veterinary field these recombinant proteins are used for *in vitro* testing and their potential therapeutic use is also under research. It is expected that in the near future these could be used for commercial *in vivo* tests and/or SIT.

With the advance of knowledge on veterinary allergies and allergens, there is a need to provide an updated guidance on the data requirements for quality, safety and efficacy of conventional and novel allergen products for *in vivo* diagnosis and SIT.

The Ph. Eur. Monograph on Allergen Products (1063) addresses the technical quality of allergen products that are based on allergen extracts. Although it is recognised that the Monograph 1063 does not necessarily apply to allergen products for veterinary use, it is considered that the manufacturing process is similar if not the same for human and veterinary allergens and the general principle included in this monograph can also be applied to veterinary allergens. Additionally, it is to be taken on board that five monographs on source materials for allergen products have been elaborated (Animal epithelia

and outgrowths -Ph. Eur. 2621, Hymenoptera venoms- Ph. Eur. 2623, Mites- Ph. Eur. 2625, Moulds- Ph. Eur. 2626 and Pollens- Ph. Eur. 2627).

The previous veterinary allergen guideline (1994) included in its scope bacterial and parasite allergens (tuberculin, brucellin, toxoplasma, echinococcus, etc.). As these agents cause infectious diseases and are now covered by other guidelines and monographs, they are not included in the scope of the current guideline. The current guideline applies only to allergens that cause allergic diseases.

Another reason to revise the previous guideline is to define and apply (wherever possible) the general concept of “homologous groups” as described in the human allergen guideline.

Finally the safety and efficacy sections have been updated in line with the current legislation as previous guidance on clinical aspects was very limited.

2. Scope

This document provides principles and guidance for the manufacturing and quality control, safety and efficacy 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 for horses, dogs and cats. It applies to all allergen products and their intermediates prepared industrially by a method involving an industrial process as defined by Directive 2001/82/EC.

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.

Further, this document provides guidance on the design of studies to be performed to demonstrate safety and efficacy of allergen products.

3. Legal basis

This guideline has to be read in conjunction with the introduction, general principles and Title II of the Annex I to Directive 2001/82 as amended.

Additionally, the following monographs may be taken into account: General Ph. Eur. monograph 1063 for Allergen products and specific Ph. Eur. monographs on source materials for allergen products (Animal epithelia and outgrowths – Ph. Eur. monograph 2621, Hymenoptera venoms - Ph. Eur. monograph 2623, Mites - Ph. Eur. monograph 2625, Moulds- Ph. Eur. monograph 2626 and Pollens - Ph. Eur. monograph 2627).

4. Quality

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, in the previous guideline on “Specific requirements for the production and control of allergen products (7BIm11a, September 1994) extrapolation of stability, safety and efficacy data among members of taxonomic families was defined in a very broad sense.

The concept of homologous groups introduced in the guideline for human allergens EMEA/CHMP/BWP/304831/2007 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 required flexibility.

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 (for the different animal species in which they are intended to be used).

The grouping of allergens should be based on following criteria:

- For the same animal species (horse, dog or cat);
- Comparable physicochemical and biological properties of the source material;
- Cross-reactivity (between the homologous group)/structural homology of the allergens;
- Identical formulation of the finished product;
- Identical production process of the allergen extract and of the finished product.

All five criteria must be fulfilled to define a homologous group.

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

As a general statement, data on quality (stability), safety and efficacy can be extrapolated from the representative allergen to other members of the homologous group. For allergens that cannot be included into a homologous group, data for quality, safety and efficacy have to be provided on an individual basis.

Within a homologous group, safety and efficacy studies are only requested for the representative allergen. Post-marketing safety and/or efficacy data could be requested for non-representative allergens of the same homologous group.

At the moment, no homologous groups have been defined for horse, dog and cat allergens but some major allergens have been identified. The proposed homologous groups should be specifically justified by the applicant.

Annex I includes a list of allergens of documented importance in horses, dogs and cats and allergens that have been characterised at the molecular level (with bibliographic references).

Proposed homologous groups for human allergens are also listed in Annex II for reference.

Annexes I and II reflect the current situation and may change over time.

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 on 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 or by a suitable equivalent *in vitro* method.

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 (batch-to batch consistency)

During the development of an allergen product, changes may be introduced in the manufacturing process which would have an impact on the finished product. Given its complex nature, it is particularly important that all stages of the development process are fully evaluated and all the changes identified within the dossier where applicable.

Applicants should take into consideration the step-by-step manufacturing approach according to veterinary medicines EU legislation, European pharmacopoeia and CVMP/VICH guidance applicable to Immunological veterinary medicinal products-IVMP (for example, Guideline on requirements for the production and control of immunological veterinary medicinal products EMA/CVMP/IWP/206555/2010), 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

4.2.1. General information

The principles laid down in the section “source materials” of Ph. Eur. monograph 1063 should be applied.

The active substance can be an allergen extract, a purified natural or recombinant protein, all of which can be unmodified or modified (e.g. physically and/or chemically as an allergoid or conjugate). The

active substance should be a stable preparation at the latest step before mixing or formulation of the final product. 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 (for each animal species those are intended for).

Active substances obtained by recombinant DNA technology consist of pre-defined 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 Ph. Eur. monographs and the EMA/VICH guidelines relevant for biotechnological products for veterinary use.

4.2.2. Manufacture

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

The principles laid down in the section “source materials” of Ph. Eur. monograph 1063 should be applied.

The production process steps including e.g. pre-treatment, extraction, filtration, dialysis, concentration or freeze-drying should be described in detail and validated. Data can be extrapolated from the representative allergen 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. A 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 flowchart. 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, relevant Ph. Eur. monographs for veterinary medicinal products derived from recombinant DNA technology (0784 Products of recombinant DNA technology) have to be taken into consideration, and any relevant guidelines (e.g. VICH GL40 Test procedures and acceptance criteria for new biotechnological/biological veterinary medicinal products) – could be considered, even if those are not directly applicable for allergenic extracts.

4.2.3. Control of starting materials

This section includes starting (source) materials (for example, natural source materials of 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 (Ph. Eur. 5.1.7) and compliance with TSE requirements (Note for guidance EMA/410/01 rev.3) should be demonstrated to avoid the risk of transmission of infectious diseases. Source materials should be shown to be free from extraneous agents, in line with Ph. Eur. texts, monographs and EU guidelines applicable to IVMPs.

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, as indicated below. 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; moreover, 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 are mixed to achieve uniform source material batches, the underlying concept should be described and the uniformity of the mixture should be justified.

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

Requirements for specific source materials:

Pollens: The Ph. Eur. monograph 2627 is considered applicable to veterinary allergens.

Moulds: The Ph. Eur. monograph 2626 is considered applicable to veterinary allergens.

Strains which produce mycotoxins such as aflatoxins or ochratoxins should not be used unless justified and, if used, 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 Ph. Eur. monograph 2625 is considered as applicable to veterinary allergens.

Insects: Insects such as *Culicoides spp.* for horses and fleas for dogs which are important allergens, do not have specific Ph. Eur. monographs, however for these source materials the principles laid down in the section “source materials” of Ph. Eur. monograph 1063 should be applied.

Animal epithelia and outgrowths, human epithelia:

Ph. Eur. monograph 2621 is considered applicable to veterinary allergens and for human epithelia the principles laid down in the section “source materials” of Ph. Eur. monograph 1063 should be applied.

All substances of human and/or animal origin should be either sterilised or subject to an inactivation procedure by a suitable validated method. These materials should be shown to be free from extraneous agents in line with Ph. Eur. texts, monographs and EU guidelines applicable to IVMPs.

Hymenoptera venoms: Ph. Eur. monograph 2623 is considered as applicable to veterinary allergens.

Food allergens: The principles laid down in the section “source materials” of the Ph. Eur. 1063 should be applied.

Food should be of quality for animal consumption.

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

For recombinant allergens, all relevant Ph. Eur. monographs and guidelines indicated above and included in 4.2.2.2 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

The principles laid down in Ph. Eur. monograph 1063 should be followed, with the following specific points for allergens for veterinary use:

IDENTIFICATION (by comparison with in-house reference materials (IHRP))

TESTS

- water or loss on drying;
- sterility;
- microbial contamination (non-sterile allergen products)
- protein content
- protein profile: that should correspond to that of the IHRP
- aluminium (when aluminium hydroxide or aluminium phosphate is used as absorbent)
- calcium
- allergen profile: relevant individual allergens may be determined by immunochemical methods (for example ELISA) using allergen-specific animal antibodies for the target species.

Major allergen content for the target species: This should be determined by immunochemical methods (for example ELISA) using allergen-specific monoclonal/ polyclonal antisera.

Total allergenic activity for the target species: assayed by inhibition of the binding capacity of specific IgE antibodies or by a suitable equivalent *in vitro* method.

Individual allergens as indicated in the Ph. Eur. monograph 1063.

The allergens relevant for the product have to be defined by the manufacturer. During the manufacturing process the presence of the allergens should be confirmed 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 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 provide batch-to-batch consistency data and provide a justification for the selected and validated test procedures.

4.2.4.2. Characterisation and control of recombinant allergens

Emphasis should be put on the structural integrity and the consistency of protein 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.

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 (taking into account all applicable legislation and guidelines for recombinant IVMPs). 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, the potency should be measured by testing inhibition of the binding capacity of specific IgE antibodies or by a suitable equivalent *in vitro* method.

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 polymerisation (e.g. presence of amine groups).

4.2.4.4. Potency assays

As stated in Ph. Eur. monograph 1063, the potency assay should be performed as late as possible in the manufacturing process, preferable at the finished product. If justified, potency control can be performed on the active substance and /or at the intermediate stage between the active substance and the finished product prior to mixing single allergens.

Total allergenic activity for the target species should be measured by testing inhibition of the binding capacity of specific IgE antibodies from a sera pool (c.f. standard & reference materials) or by a suitable equivalent *in vitro* method.

For allergoids, potency tests should consist of a discriminatory test or a combination of immunological tests to distinguish between native and modified molecules (e.g. 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 (e.g. 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.

4.2.5. Stability of the active substance

For all allergens, if the active substance is stored, stability data should be obtained according to the relevant guidelines on stability testing (EMA/CVMP/IWP/206555/2010) to support the maximum storage period. The general principles defined in VICH GL 17 (CVMP/VICH/501/99-FINAL) guideline for biological/biotechnological products should also be considered for allergen extracts.

Regarding homologous groups, full stability data should be presented for the “representative” allergen of each homologous group. For the “non-representative” allergens within a homologous group, stability studies may be performed on an ongoing basis for the 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 and for each target species.

In-House Reference Preparations for allergen extracts:

Follow the guidance in Ph. Eur. monograph 1063, taking into account the following:

The extent of characterisation of the IHRP depends on the source material, knowledge of the allergenic components and availability of suitable reagents, as well as the intended use including target animal species. The proposed and characterised IHRP is used as the reference in the batch control of active substances and intermediates and if possible in the batch control of finished product.

The biological potency of the first IHRP is determined in the target species (by *in vivo* method such as skin reactivity and/or by an *in vitro* suitable method). Subsequently the biological activity of the future IHRPs is determined by *in vitro* methods by comparison with the results in the first IHRP.

In-House Reference Preparations for recombinant proteins:

For the IHRP used for the quality control of recombinant allergens, in general the criteria defined in VICH GL17 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.

Sera Pools:

A sera pool could 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 at least 10 animals of the target species, unless justified. Sera recognising carbohydrate epitopes and sera from animals that 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 gelatine should 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, 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. Manufacturing and control of finished product

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.

4.4.2. Manufacture

The manufacturing process should be described in detail, including process scale. A step-by-step diagram (flowchart) 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. 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 non-representative 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.

4.4.3. Control of the finished product

Appropriate specifications should be set for the finished product, in line with Ph. Eur. monograph 1063. As stated in the monograph, control tests should be performed as late as possible in the manufacturing process. If justified, defined control tests can be performed on the active substance and/or at the

intermediate stage between the active substance and the finished product prior to mixing single allergens.

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.

Appropriate tests are as follows and as indicated in point 4.2.4.1.:

Identification (by comparison with IHRP), Water, Sterility, Microbial contamination, Protein content, Protein profile, Aluminium, Calcium, Allergen profile, Total allergenic activity, Individual allergens.

Control of non-modified allergen preparations

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 include an indication of the strength in potency units. If test systems validated in international standardisation programmes 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 Ph. Eur. monograph 0784 “Products of recombinant DNA technology”, and also the VICH GL40 “Test procedures and acceptance criteria for new biotechnological/biological veterinary medicinal products” could be considered even if not directly applicable for allergenic extracts.

The content of the purified protein (for example major allergen) and the potency, as described in chapters 4.2.4.2 and 4.2.4.4, should be determined.

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 of the finished product

Stability testing should be performed as real-time stability studies as indicated in the relevant guidance documents (e.g. EMA/CVMP/IWP/206555/2010 where applicable), using stability-indicating assays (including potency). Sterility testing (Ph. Eur. 2.6.1 monograph) 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 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 (e.g. 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. Safety and Efficacy testing

The mechanism of action of allergens/specific immunotherapies (SITs) is not fully understood in the three animal species which are the subject of this guideline. Therefore only the minimum requirements for safety and efficacy studies to be performed with veterinary allergens are described.

For safety and efficacy testing of IVMPs, Annex1 of Dir. 2001/82/EC and Ph. Eur. monographs 5.2.6 and 5.2.7 require laboratory studies on the final product in each category of each target animal species, by each recommended administration route**.** Laboratory studies according to these requirements may not be possible for allergens/SITs due to the unique nature of allergic diseases in sensitised animals. Furthermore, GLP safety studies in unsensitised animals have little relevance for the safety profile of the SIT in the target group of sensitised animals and in the interests of the 3Rs such studies should only be conducted if there are specific concerns related to the use of the SIT in non-allergic animals.

As for human allergen products, no pharmacokinetic / pharmacodynamic studies are required for veterinary allergens. This is also in line with the classification of these products as immunologicals. Pharmocokinetic studies are not possible for products of specific immunotherapy. During specific immunotherapy usually plasma concentrations of the active substance are not measurable, due to the nature of the product.

To show the effect of specific immunotherapy on the immune system, immunological changes (e.g. changes in allergen specific IgG levels, T-cell responses, and/or cytokine production) and/or modifications of the end organ (e.g. respiratory tract, skin) specific response (e.g. provocation tests) should be measured. These parameters can be followed in other studies on specific immunotherapy.

Clinical field studies are considered the most appropriate means of demonstrating the safety and efficacy of SITs as the complexity of studying allergic disease in sensitised animals cannot be replicated easily under laboratory conditions.

Field trials using sensitised animals should be appropriately designed and conducted with typical batches of products. Consideration should be given to the minimum and maximum number of allergens and the approach justified, the target animals and categories and recommended routes of administration, and dosing schedule.

The concept of homologous groups can be adopted for the evaluation of safety and efficacy. Data obtained on one member of the group (representative allergen) may be extrapolated to another member of that group providing that all manufacturing procedures applied are the same. In the case of mixtures of members of different homologous groups, extrapolation from one group to the other is not acceptable.

5.1. Safety studies for veterinary allergen treatment or specific immunotherapy products

Depending on the nature and variability of these final products, safety data obtained from “single allergen extracts” if adequately justified, could be also acceptable to demonstrate the safety of the final product, provided that adjuvants and/or other immunostimulants included in the final product are also included in the studies evaluating safety of the “single allergen extracts”.

Safety profile data derived from use of the allergen products in sensitised dogs are expected to be more informative than in unsensitised animals; thus, results/data from target animal field trials, from validated experimental models and if available from pharmacovigilance reports are considered suitable

when assessing target animal safety. As such, it is of high relevance to investigate the safety profile of these products in detail in the pivotal field trials in naturally sensitised animals.

Any experimental model of sensitisation needs to be appropriately validated, and its biological relevance justified as relates to extrapolating the results to spontaneous allergic disease.

If laboratory studies in sensitised animals are possible, the safety and efficacy may be demonstrated in the same laboratory studies. Standard batches may be used with no requirement to demonstrate the safety with batches formulated with maximum allergen content, but maximum allergen content should be justified.

Overdose safety studies for SIT products are not required.

As outlined above due to the unique nature of allergic diseases it is acceptable to investigate the safety of allergens only by field studies in sensitized animals. The efficacy of the product could also be demonstrated in the same field trial. Standard batches may be used but minimum and maximum allergen content should be justified.

Consideration should be given to the target animals and categories in these studies. It may be required to include a specifically sensitive category in the safety studies or provide appropriate published data to this extent. Alternatively, the specific exclusion of sensitive categories may be required.

The safety parameters to be evaluated include:

Possible adverse reactions such as local reactions (injection site, for injectables) and systemic reactions (lethargy, vomiting, diarrhoea, pruritus, anaphylaxis);

Expected allergic side effects should be distinguished into immediate or delayed effects according to the time of appearance (immediate when the onset of the reaction is during the first 30 minutes after administration and delayed when the onset is later than 30 minutes after administration) and into local and systemic effects according to the site of the appearance of the reaction (local when the reaction takes place at the administration site and systemic when the reaction takes place at a site other than the administration site);

Safety of repeated administration, following the SIT protocol recommendation, should be performed, for each target species and by each recommended route.

Extrapolation of safety results from one route to another could be accepted, if appropriately justified.

- Studies for the examination of reproductive performance and immunological functions may be omitted. If such studies are not performed, relevant warnings should be included in the SPC.

- The use of documentation based on scientific publications to suitably support safety of the allergen treatment product is acceptable, provided the data is relevant/appropriate.

5.2. Efficacy studies for veterinary allergen treatment or specific immunotherapy (SIT) products

Taking into consideration the nature of these products, efficacy of allergen treatments could be shown for each “allergen extract” alone and/ or in the final product.

If the final formulation includes adjuvants and/or other immunostimulants, the efficacy studies should be performed including these components.

In the studies performed to demonstrate efficacy, as clinical response to SIT is allergen specific, the allergen to be tested should be selected on basis of the patient´s clinical history and results of intradermal testing or allergen specific serum IgE testing.

The efficacy studies should include control non-treated/placebo animals.

The efficacy of each “allergen extract” should be demonstrated by each recommended route of administration unless scientific data can be provided demonstrating that extrapolation from one recommended route to another is possible.

From the data available to date, the target animal category (e.g. age, breeds with special predispositions, etc.) used for the demonstration of efficacy of allergens appears in general not to be a crucial point. Nevertheless, efficacy should be evaluated in animals' representative of the target population and the test population should be described in detail.

Possible known negative impact on the efficacy induced by certain “allergen extracts” mixed in the same final product to be administered should be taken into account. This evaluation could be based on published scientific data.

If it is possible, efficacy could be also demonstrated under laboratory conditions in animals naturally or experimentally sensitised and treated with the allergen product. In principle, the efficacy of the allergen could be demonstrated by a challenge/provocation study in laboratory conditions (e.g. for human allergens this is done for dose-finding studies). Any experimental model of sensitisation needs to be appropriately validated, and its biological relevance justified as relates to extrapolating the results to spontaneous allergic disease.

The safety and efficacy may be demonstrated in the same laboratory studies. Standard batches may be used with no requirement to demonstrate the efficacy with batches formulated with minimum allergen content, but minimum allergen content should be justified.

As outlined above due to the unique nature of allergic diseases it is acceptable to investigate the efficacy of allergens only by field studies. The safety of the product could also be demonstrated in the same field trial. Standard batches may be used, but minimum and maximum allergen content should be justified.

- Data to be recorded: success rates could be evaluated by complete remission, and/or improvement of clinical signs and/or reduction of concomitant medication.

Clinical signs that could be measured in dogs: lesions scores, pruritus scores, medication scores.

Clinical signs that could be measured in cats: lesions scores, pruritus scores for feline eosinophilic granuloma complex, milliary dermatitis, self-inflicted alopecia, pruritus, seborrhoea.

Clinical signs that could be measured in horses: lesions scores, pruritus scores for atopy, urticaria and recurrent airway obstruction.

Further surrogate parameters for efficacy might be acceptable if a correlation can be demonstrated between the specific parameters and protection induced by the treatment. A follow up of these surrogate parameters might be considered sufficient to substantiate the efficacy claim.

(Examples for Antibody response: increase in IgG, decrease in IgE, etc.; Examples for Cellular immunity response: increase in IFN gamma, IL-10; decrease in IL-4 or others, as indicated in the publication Lowestein and Mueller, 2009)

- Time to observe the efficacy of the SIT: Any time period chosen should be justified by the applicant.

- Interaction with other medicinal treatment: The possible interaction with other treatments (e.g. antihistamines, glucocorticoids, cyclosporine and/or monoclonal antibodies) should be considered (either evaluated in the context of clinical studies or by reference to the published literature) and indicated in the product information.

- The use of documentation based on scientific publications to demonstrate the efficacy of allergen treatment products, is acceptable, provided the studies reported are relevant to the product under evaluation, and are appropriately designed and described in detail.

5.3. Safety and Efficacy studies for "in vivo" diagnosis products

To demonstrate the safety and efficacy of these products, the final formulation, the route of administration, target concentration and the different animal species should be considered.

The safety data that would be acceptable are in general the same as for immunotherapy products (for each separate allergen).

To demonstrate the efficacy of diagnosis, the specificity and sensitivity of the testing procedure should be demonstrated, with the dose indicated and the time period of observation proposed for each target species.

Taking into account the different formulation and in general different administration routes between therapy and "in vivo" diagnosis products, safety and efficacy studies already performed for SIT allergens from the same manufacturer could be appropriate to demonstrate safety and could be supportive for the efficacy of the same allergens used for *in vivo* diagnosis (skin test allergen).

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 physico-chemical 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 physio-chemical 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.

Representative/ non-representative allergens in an homologous group: Each homologous group is represented by one or more allergens based on scientific information about the allergens and their cross-reactivity with other members of the same homologous group. Data may be extrapolated from these representative allergens to other allergens of the same homologous group. These other allergens of the same homologous group are referred to as ‘non-representative’ allergens.

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 animal 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 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.

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ANNEX I

Allergens with documented importance (Horses/Dogs/Cats)

Summary from Mueller et al. , Allergy 2016 (71: 27-35).

Horses:

Mites: Reported, but no clear conclusions

Insects: *Culicoides spp.*

Plant derived: (tree, grass and weed pollens).

Mould: *Aspergillus fumigatus, Alternaria alternata and Penicillium notatum*

Dogs:

Mites: Dermatophagoides pteronyssinus and Dermatophagoides farinae

Less frequent: Acarus siro, Lepidoglyphus destructor and Tyrophagus putrescentiae

Plant derived: (tree, grass and weed pollens)

The most important is Cryoptomeria japonica pollen (CryJ3 is a major allergen in dogs)

Insects: Flea (Major allergen in dogs is Ctef1 of Ctenocephalides felis) and other very minor)

Moulds: Not known

Cats:

Mites: Dermatophagoides pteronyssinus and Dermatophagoides farinae

Plant derived: (pollens).

Insects: Flea, and also hymenoptera and mosquito

Moulds: Not known

Allergens of documented importance in domestic animals that have been characterised at the molecular level. (*Mueller et al 2016):*

Allergen source	**Allergen name**	**Identity/ homology**	**MW(kDa)**	**Relevant in species**
Mites:				
Dermatophygoides farinae	Der f 15	Chitinase	98/109	Dog
	Der f 18	Chitinase	60	Dog
Pollen:				
Cryptomeria japonica	Cry j 1	Pectate lyase	41	Human/ Dog
Cryptomeria japonica	Cry j 2	Polygalacturonase	56	Human/ Dog
Cryptomeria japonica	Cry j 3	Thaumatin-like protein	24	Human/ Dog
Insects:				
Ctenocephalides felis	Cte f 1	None	18	Dog
Culicoides nubeculosus	Cul n 1	Antigen-5 like	25	Horse
	Cul n 2	Hyaluronidase	46.7	Horse
	Cul n 3	Cysteine endopeptidase	44.6	Horse
	Cul n 4	None	17.5	Horse
	Cul n 5	None	45.7	Horse

	Cul n 6	None 16.9	Horse
	Cul n 7	None 20.9	Horse
	Cul n 8	Maltase 68.7	Horse
	Cul n 9	D7-related 15.5	Horse
	Cul n 10	None 47.8	Horse
	Cul n 11	Trypsin 30.1	Horse
Culicoides obsoletus	Cul o 1*	Maltase 66.8	Horse
	Cul o 2*	Hyaluronidase 42.3	Horse
	Cul o 3	Antigen-5 like 27.9	Horse
	Cul o 4	Trypsin 27.1	Horse
	Cul o 5	None 17.9	Horse
	Cul o 6	D7-related 15.2	Horse
	Cul o 7	None 15	Horse
	Cul o1*	Kunitz protease inhibitor 23.3	Horse
	Cul o 2*	D7-related 17.5	Horse
Culicoides sonorensis	Cul s 1	Maltase 66	Horse
Simulium vittatum	Sim v 1	Antigen 5 like 29.8	Horse
	Sim v 2	Kunitz protease Inhibitor 9.6	Horse
	Sim v 3	A-amylase 28	Horse
	Sim v 4	a-amylase 26	Horse
Moulds:			
Aspergillus fumigatus	Asp f 7	None 27.4	Human/ horse
	Asp f 8	Acidic P 2 ribosomal proteins 11	Human/ horse

*Nomenclature needs modification. These allergen sequences were submitted to GenBank at the same time by different groups.

ANNEX II

Proposed homologous groups- Human allergens [Lorenz 2008]

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.

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

2. Grass and cereal pollen

The group of sweet grasses of the *Poaceae* (*Gramineae*) family, subfamily of *Pooideae*

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
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 farina

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.