**Allergen Products**

This monograph does not apply to: chemicals that are used solely for diagnosis of contact dermatitis; chemically synthesised products; allergens derived by recombinant DNA. It does not necessarily apply to allergen products for veterinary use.

**Definition**

Allergen products are pharmaceutical preparations derived from extracts of naturally occurring source materials containing allergens, which are substances that lead to and/ or provoke allergic reactions. The allergenic components are most often of a proteinaceous nature. Allergen products are intended for *in vivo* diagnosis or treatment of allergic diseases attributed to these allergens.

Allergen products are available as finished products and as finished products used as customized therapeutic preparations. Allergen products are generally presented as parenteral preparations, eye preparations, preparations for inhalation, preparations for oral use, sublingual preparations or preparations for skin tests.

For diagnostic use, allergen products are usually prepared as unmodified extracts in a 50 per cent V/V solution of glycerol for skin-prick testing. For intradermal diagnosis,or for provocation tests by nasal, ocular or bronchial administration, suitable dilutions of allergen products may be prepared by dilution of aqueous or glycerinated extracts, or by reconstitution of unmodified freeze-dried extracts.

For *specific immunotherapy*, allergen products are usually unmodified extracts.

**Production**

Source Materials

Source materials for the preparation of allergen products are products of animal or vegetable origin, mostly pollens, moulds, mites, animal epithelia.and outgrowths (such as hair and feathers) and/or dander, hymenoptera venoms, insects

The source materials are defined by their origin, nature, method of collection or production and pretreatment. Control methods and acceptafice criteria relating to identity and purity are established. The acceptance criteria must ensure the consistency of the allergenic source material from qualitative and quantitative point of view. The source materials are stored under defined conditions justified by stability data.

The collection or production, as well as the handling of the source materials are such that uniform composition is ensured as far as possible from batch to batch.

**Pollens.** Potential chemical contaminants, such as pesticides, heavy metals, must be minimised. The content of foreign pollen must be limited to 1 per cent of total mixed pollens and 0.5 per cent of any individual pollen as determined by a microscopic particle count. Detectable mould spores must not exceed 1 per cent. The contamination with particles of plant origin other than pollen must be kept to a minimum. The maximum allowed contamination must be justified.

**Moulds.** Biologically active contaminants such as mycotoxins in moulds must be minimised and any presence justified. Appropriate measures, have to be implemented to avoid contamination by foreign mould strains. Care must be taken to minimize any allergenic constituents of the media used for the cultivation of moulds as source materials. Culture media that contain substances of human or animal origin must be justified and, when required, must be suitably treated to ensure the inactivation or elimination of possible transmissible agents of disease,

The production method is validated to demonstrate that allergen products obtained from moulds and intended for parenteral administration, if tested, would comply with the test for abnormal toxicity for immunosera and vaccines for human use (2.2.1).

**Mites.** Appropriate measures have to be implemented to avoid contamination by foreign mite strains. Care must be taken to minimise any allergenic constituents of the media used for the cultivation of mites as source materials. Culture media that contain substances of human or animal origin must be justified and, when required, must be suitably treated to ensure the inactivation or elimination of possible transmissible agents of disease.

**Animal epithelia and outgrowths and/or dander.** They are obtained from healthy animals selected to avoid possible transmissible agents of disease.

**Hymenoptera venoms.** The species of hymenoptera from which the venom is extracted is identified and specified. The methods of insect collection and venom extraction are described and must ensure that the source material is of proper quality.

Allergen products are generally obtained by extraction, and may be purified, from the source materials using appropriate methods shown to preserve the allergenic properties of the components. Allergens for which there are not enough patients to determine the total allergenic activity in vivo or in vitro, the extraction ratio indicating the relative proportions (m/V) of allergenic source materials and solvents is a minimum requirement. Allergen products presented as parenteral preparations, eye preparations, preparations for inhalation and preparations for skin testing are manufactured under aseptic conditions.

In the manufacture, packaging, storage and distribution of allergen products intended for administration by other routes, suitable measures are taken to ensure their microbial quality; recommendations on this aspect are provided in chapter 2.2.9. Microbial contamination in non-sterile product.

All allergen preparations are manufactured under conditions designed to minimise exogenous and endogenous enzymatic degradation.

Any purification procedure is designed to minimise the content of any potential irritant low molecular mass components and non-allergenic components.

Allergen products may contain suitable antimicrobial preservative, the nature and concentration of which have to be justified.

The manufacturing process comprises various stages:

— source material;

active substance: it is generally a unmodified allergen extract; where applicable it is stored under conditions ensuring its stability, for example freeze-dried;

- finished product.
- **IN-HOUSE REFERENCE PREPARATION**
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- An appropriate representative preparation is selected as the In-House Reference Preparation (IHRP), characterised and used to verify batch-to-batch consistency. The IHRP is stored in suitably sized aliquots under conditions ensuring its stability, for example freeze-dried.
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- **Characterisation of the In-House Reference Preparation**.*The extent of characterisation of the IHRP depends on the nature of the allergenic source material, knowledge of the allergenic components and availability of suitable reagents, as well as the intended use. The characterised IHRP is used as reference in the batch control of active allergen substances or intermediate and, if possible, in the batch control of finished products*.
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The In -House Reference Preparation (IHRP) is characterised by the protein content determination and a protein profile using appropriate methods (such as sodium dodecyl sulphate- polyacrylamide gel

electrophoresis). Allergenic componentsmay be detected by appropriate methods (for example, immunoblotting or crossed radio-immunoelectrophoresis). Characterisation of the allergenic components may include identification of relevant allergens based on serological or other techniques using a pooled or individual sera from allergic patients, or allergen-specific polyclonal or monoclonal antibodies.

Determination of the content of relevant allergens is performed wherever possible. This determination may be made using individual allergen specific reference standards, when available. The choice of the relevant allergen components subjected to the determination must be justified. Individual allergens are identified and named according to internationally established nomenclature whenever possible.

- The biological potency of the first IHRP is determined in patients by in-vivo techniques such as skin testing, and expressed in units of biological activity except when not enough patients are available. In this case, the potency of the first IHRP is determined by an in-vitro method. Subsequently, the biological activity of future IHRPs is demonstrated by in-vitro methods by comparison with the results obtained with the first IHRP.The in-vitro potency may be measured by a suitable immunoassay (for example, an essay based on the inhibition of the binding capacity of specific immunoglobulin E antibodies).

Identification

The tests for identification are performed as late as possible in the manufacturing process. In the case of products used on a named patient basis, the control is performed on the active substance and/ or at the intermediate stage between the active substance and the finished product.

Identity is confirmed by comparison with the IHRP using protein profiling by appropriate methods (for example, sodium dodecyl sulphate-polyacrylamide gel electrophoresis (2.4.12).

In exceptional cases if no IHRP is available, a representative batch may be used to confirm identity.

Identity may also be confirmed by comparison with individual allergen specific reference standards, when available.

Tests

The tests are performed as late as possible in the manufacturing process. In the case of products used on a named patient basis, the control is performed on the active substance and/ or at the intermediate stage between the active substance and the finished product.

**Water**(2.3.43)Not more than 5 per cent for freeze-driedproducts.In the case of oral lyophilizates the water content may be higher than 5 per cent where, justified and authorised.

Sterility (2.2.11). Allergen products presented as parenteral preparations, eye preparations, preparations for inhalation or preparations for skin testing comply with the test for sterility.

Protein content 80 per cent to 120 per cent of the statedcontent, unless otherwise justified and authorised.

Modified Lowry's procedure. The procedure is based on Peterson's modification of the Lowry method and utilizes sodium dodecyl sulphate, to facilitate the dissolution of relatively insoluble lipoproteins. For many proteins, the Lowry réaction can be run directly on the protein solution. However, interference in the direct Lowry procedure is commonly caused by other chemicals in the

protein solution, such as tris, ammonium sulfate, EDTA, sucrose, citrate, amino acid and peptide buffers, and phenols. The procedure with protein precipitation, which uses DOC (deoxycholate) and TCA (trichloroacetic acid), eliminates all these interferences with the exception of phenols. However, the amount of various proteins recovered through the precipitation step may vary depending on the particular proteins assayed.

The procedure is based on two chemical reactions. The first is the biuret reaction, in which the alkaline cupric tartrate reagent complexes with the peptidé bonds of the protein resulting in formation of tetradentate Copper-protein complexes. This is followed by the reduction of the Folin and Ciocalteu's phenol reagent, producing a'water-soluble product which yields a purple color. Absorbance of the colored solution is read at 750 nm. The protein concentration is determined from a calibration curve.

Prepare all the following reagents/ buffers for protein estimation.

Phosphate Buffered Saline (1000 ml). Dissolve 8.0 g of sodiun chloride, 0.2 g of potassium chloride, 0.2 g of potassium dihydrogen phosphate, 1.15 g of disodium hydrogen phosphate, 2.0 mg of sodium azide in 1000 ml of purified water, Adjust to pH 7.2.

Solution A (25 ml). Dissolve 50 mg of copper sulphate, 100 mg of potassium sodiun tartrate in 25 ml of purified water. (Prepare fresh for every use).

Solution B (100 ml). Dissolve 20 g of sodium carbonate in 100 ml of purified water.

Solution C (50 ml). Mix 25 ml of Solution A and 25 ml of Solution to yield 50 ml of Solution C.

10 per cent sodium dodecyl sulphate (100 ml). Dissolve 10g of sodium dodecyl sulphate in 100 ml of purified water.

0.8N sodium hydroxide (100 ml). Dissolve 3.2 g of sodium hydroxide in 100 ml of purified water.

Folin-ciocalieau (FC) reagent (60 ml). Mix 10 ml of Folin- ciocalteau reagent with 50 ml of purified water.

Alkaline copper sulphate solution (40 ml). Mix 10 ml of Solution C, 10 ml of 0.8 N sodium hydroxide, 10 ml of 10 per cent sodium dodecyl sulphate and 10 ml of purified water.

*0.15 per cent* sodium*deoxycholate* (100ml): Dissolve 150mg of *sodium deoxycholate*in 100ml of *purified water.*

*72 percentTri-chloroacetic acid:* Dissolve 2g of *Tri-chloroacetic acid* in 1.2ml of *purified water.*

Prepare the stock concentration of protein standard (Bovine Serum Albumin) containing 1 mg per 2 ml of phosphate buffered saline and test products (1 mg per ml) in phosphate buffer saline. Prepare different dilutions of bovine serum albumin standard (0 µg/ml as Blank) (duplicates), 10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml and 60 µg/ml) make the final volume upto 1 ml using purified water. Add 0.1 ml of sodium deoxycholate (0.15 per cent) to each test tube including blank, standards and test products (sample). Mix well. After 10 minutes, add 0.1 ml of Tri-cholro acetic acid (72 per cent) to each sample, mix immediately and incubate at room temperature for 10 minutes. Centrifuge the test tubes at 3000 g for 15 minutes. Discard the supernatant and dissolve the pellets in Iml of purified water. Add 1 ml of alkaline copper sulphate solution in each test tube. Mix them well and incubate at room temperature for 10 minutes. Add 0.5 ml of folin- ciocalteau reagent in each test tube, mix them well and incubate at room temperature for 30 minutes. Read in spectrophotometer at wavelength of 750 nm within 30 minutes. Estimate the amount of protein present in the given sample from the standard curve.

**Bradford method:** It is an alternate method for allergen extracts containing phenol. It is based on the absorption shift from 470 nm to

595 nm observed when the Coomassie brilliant blue G250 dye binds to protein. The Coomassie brilliant blue G250 dye binds most readily to arginine and lysine residues in the protein which can lead to variation in the response of the assay to different proteins. The protein used as reference substance must therefore be the same as the protein to be determined. There are relatively few interfering substances, but it is preferable to avoid detergents and ampholytes in the test sample. Highly alkaline samples may interfere with the acidic reagent.

Use distilled water R to prepare all buffers and reagents used for this method.

Test solution. Dissolve a suitable quantity of the substance to be examined in the prescribed buffer to obtain a solution having a concentration within the range of the standard curve.

Reference solutions. Dissolve the reference substance for the protein to be determined in the prescribed buffer (e.g. Phosphate buffer saline with 0.4 per cent Phenol). Dilute portions of this solution with the same buffer to obtain not fewer than five reference solutions having protein concentrations evenly spaced over a suitable range situated between 0.1 mg/ml and 1 mg/ml.

Blank. Use the buffer used to prepare the test solution and the reference solutions.

Bradford reagent. Dissolve 0.10 g of Coomassie brilliant blue G250 in 50 ml of ethanol. Add 100 ml of phosphoric acid R, dilute to 1000 ml with distilled water R and mix. Filter the solution and store in an amber bottle at room temperature. Slow precipitation of the dye occurs during storage. Filter the reagent before using.

Test tube Procedure. Add 5 ml of Bradford reagent to 0.100 ml of each reference solution, of the test solution and of the blank. Mix by inversion. Avoid foaming, which will lead to poor reproducibility. Determine the absorbances of the standard solutions and of the test solution at 595 nm, using the blank as compensation liquid. **Do not use quartz (silica) spectrophotometer cells because the dye binds to this material**.

Microplate Procedure. Add 0.25ml of Bradford reagent to 0.010 ml of each reference solution in triplicates, of the test solution and of the blank to the designated wells in the microplate. Mix for 10 seconds on shaker at 500 rpm. Avoid foaming, which will lead to poor reproducibility. Incubate for 10 minutes at room temperature. Determine the absorbances of the standard solutions and of the test solution at 595 nm, using the blank as compensation liquid.

The assay method described here can also be performed using kits from commercial sources.

Calculations. The relationship of absorbance to protein concentration is non -linear; however, if the range of concentrations used to prepare the standard curve is sufficiently small, the latter will approach linearity. Plot the absorbances of the reference solutions against protein concentrations and use linear regression to establish the standard curve. From the standard curve and the absorbance of the test solution, determine the concentration of protein in the test solution.

Protein profile. The band pattern in the protein profile of the Allergen product is to be determined by sodium dodecyl sulphate-

polyacrylamide gel electrophoresis (2.4.12). The protein profile determined by suitable methods corresponds to that of the IHRP.

Storage. Unless otherwise prescribed, store in a sterile, airtight, tamper-proof container, protected from light, at a temperature of 2 °C to 8 °C.

LABELLING

The label states:

— Name of the allergen product;

- —the protein content (µg/ml or mg/ml) and/or biological potency and/or the extraction concentration ;
- the route of administration and the intended use;
- the storage conditions;
- where applicable, the name and amount of added antimicrobial preservative;
- where applicable, for freeze-dried preparations:
    - the name, composition and volume of the reconstituting liquid to be added
    - the period of time within which the preparation is to be used after reconstitution;
- where applicable, that the preparation is sterile;
- Caution: It is dangerous to take this prescription except under medical supervision of professional trained in Allergy and Immunotherapy.