Draft for comments of stakeholders

Draft Monograph: Allergen Products

ALLERGEN PRODUCTS

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

DEFINITION

Allergen products arepharmaceutical/biologicalpreparationsderivedfrom extracts of naturallyoccurring sourcematerialscontainingallergens,whicharesubstancesthat leadtoand/or provoke allergic reactions.Theallergenic componentsaremostoften of aproteinaceous nature. Allergenproducts are intended for *in vivo* diagnosis or treatment of allergic diseases attributed to these allergens.

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

For *specific immunotherapy*, allergen productsare 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 and certain foods.

The source materials are defined by their origin, nature, method of collection or production and pretreatment. Control methods and acceptance criteria relating to identity and purity are established. The acceptance criteria must ensure the consistency of the allergenic source material from a qualitative and quantitative point of view. The source materials are stored under controlled conditions justifiedby 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 and solvents, 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 determinedby 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 minimise 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 fromwhich 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 ofthe components. Allergens for which there are not enough patients to determine the total allergenic activity *in vivo orin vitro*, the extraction ratio indicating the relativeproportions (*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 preservatives, the nature and concentration of which have to be justified.

The manufacturing process comprises various stages:

- source material;
- active substance: it is generally an unmodified allergen extract; where applicable it is

stored under conditions ensuring its stability, for example freeze-dried;

- finishedproduct.

All otherstages of the manufacturingprocess are consideredasintermediates.

IDENTIFICATION

Identity is confirmedby sodiumdodecyl sulfatepolyacrylamidegelelectrophoresis (2.4.12),

TESTS

Various biochemical and immunological tests have been developed in order to characterise allergens qualitatively and quantitatively. In those cases where such methods cannot be applied, particularly for the determination of allergenicactivity and allergen and/or protein profile, justification must be provided.

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

Protein content (Modified Lowry's Method): 80 per cent to 120 per cent of the stated content, unless otherwise justifiedand authorised.

The **Modified Lowry's**procedure: The procedure is based on Peterson's modification of the Lowry method and utilizes sodium dodecylsulfate, to facilitate the dissolution of relatively insoluble lipoproteins. For many proteins, the Lowry reaction 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 peptide bonds of the protein resulting in formation of tetradentate copper-protein complexes. This is followed by the reduction of the Folin&Ciocalteu's phenol reagent, producing a water-soluble product which yields a purple color. Absorbance of the colored solution is read at a 750 nm. The protein concentration is determined from a calibration curve.

Prepare all the following reagents/ buffers for protein estimation.

PhosphateBuffered Saline (PBS)(1000 ml): Dissolve 8.0 gm of Sodium chloride, 0.2 g of Potassium chloride, 0.2g of Potassium dihydrogen phosphate, 1.15 mg of Disodium hydrogen phosphate, 2.0 mg of Sodium azide in 1000 ml of Purified Water. Adjust the pH to 7.2

Solution A (25 ml): Dissolve 50 mg of Copper sulphate, 100 mg of Potassium-Sodium 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 B to yield 50 ml of Solution C.

Sodium dodecyl sulphate (SDS)(100 ml): Dissolve 10 g of SDS in 100 ml of Purified Water.

0.8N Sodium hydroxide(100 ml): Dissolve 3.2 g of 100 ml of Purified Water.

FolinCiocalteau (FC) reagent (60 ml): Mix 10 ml of FC reagent with 50 ml ofPurified Water.

Alkaline Copper sulphate (40 ml): Mix 10 ml of Solution C, 10 ml of 0.8 N NaOH, 10 ml of 10% SDS and 10 ml of Purified water.

Prepare the stock concentration of protein standard (Bovine Serum Albumin (BSA)) containing1mg per 2ml of PBSand test products (1mg/ml)in PBS. Prepare different dilutions of BSA standard (0 µg/ml (duplicates), 10 µg/ml, 20 µg/ml, 30 µg/ml, 40µg/ml & 60µg/ml) make the final volume upto 1 ml usingpurified water.Add 0.1ml of Sodium deoxycholate (0.15%) to each test tube containing blank, standard and s and mix well.After 10 minutes, add 0.1ml of Tri-cholro acetic acid (72%) to each sample, mix immediately and incubate at RT for 10 minutes.Centrifuge the test tubes at 3000g for 15 minutes.Discard the supernatant and dissolve the pellets in 1ml of purified water.Add 1ml of alkaline Copper sulphate solution in each test tube, mix them well and incubate at Room Temperature for 10 minutes. Add 0.5ml of Folin-Ciocalteu reagent in each test tube mix them well and incubate at Room Temperature for 30 minutes. Read in spectrophotometer at wavelength of 750nm.Estimate the amount of protein present in the given sample from the standard curve.

Proteinprofile.Theband pattern in the protein profileof the Allergen product is to be determined by SDS- PAGE *(2.4.12).*

Potency: Potency estimation of Allergen products by Skin Prick Testing (SPT) is an *in vivo* test to be done directly on suitable number of consecutive patients till at least five histamine equivalent positive patients (with not more than 1+ variation) to that allergen have been tested. These tests are to be performed under **strict medical supervision** done in clinical set up as per latest guidelines of Indian College of Allergy, Asthma and Applied Immunology.

STORAGE

Adsorbed allergenproducts are nottobe frozen, unlessotherwise justifiedandauthorised.

LABELLING OF DIGNOSTIC ALLERGENS

Thelabel states:

- Name of the allergen product
- Total volume and w/v concentration
- Protein content
- For Intradermal / Prick Test
- Storage conditions
- Dispensing date
- Expiry date

LABELLING OF THERAPEUTIC ALLERGENS

Thelabel states:

- Name of the patient
- Name of prescribing Physician
- Name of the allergen product
- Total volume and w/v concentration
- Protein content
- Route of administration
- Storage conditions
- Dispensing date
- Expiry date
- Prescription / Identification number

Note:
The diagnostic / therapeutic allergens should be used on patients under the supervision of medical professional trainedin Allergy and Immunotherapy. The manufacturer should dispense therapeutic vaccines only after obtaining skin test results of the patients.