

Allergen Source Materials: State-of-the-Art

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Introduction

Allergen source materials are the active ingredients in a variety of diagnostic and therapeutic products. They are derived from a variety of natural sources including pollen, fungi, animal dander and pelts, and insect venoms. More recently, allergenic proteins have been produced using recombinant DNA technology.

From a regulatory perspective, it is extremely cumbersome to address hundreds of allergens individually. For this reason, the regulations tend to be general and place the burden onto the respective biologics license or marketing authorization applications of each manufacturer. In the U.S. and Europe, there has been a trend by regulatory authorities in recent years to utilize “guidance documents” to help address these types of issues. Although they are not legally binding, they provide recommendations for specific allergenic products or product groups. In particular, the CBER’s Guidance for Industry, “On the Content and Format of Chemistry, Manufacturing and Controls Information and Establishment Description Information for an Allergenic Extract or Allergen Patch Test” [1] and the European Medicines Agency’s Guideline on “Allergen Products: Production and Quality Issues” [2] are two notable examples. It is clear that a significant amount of justification for product specifications and validations for processes and test methods is required for specific manufacturers’ products. It is also clear that the work needed to satisfy these requirements continues to grow as new diagnostic and therapeutic approaches are introduced, as new standardization initiatives are pursued, and as the knowledge base in the field of allergy grows.

General Provisions: Identity, Purity and Potency

The identity, purity and potency of allergen source materials are critical for the safety and efficacy of allergen products. Thus, it should be expected that the regula-

tions as well as development plans for new allergenic products include provisions that focus on controls that impact their purity, identity, potency [3,4].

The identity of allergen source materials is critical for products intended for the diagnosis and treatment of specific allergies. Variability in the criteria used to establish the identity of similarly-labeled products exists and this can lead to some confusion by the practicing allergist as to the specificity of the products they use. Pollen extracts may be produced from single or multiple species; animal extracts may be produced using dander, hair or pelt; fungal extracts may be derived from mycelia and/or culture filtrates; and mite extracts may be produced from source materials containing various proportions of whole bodies, fecal pellets and eggs. The terminology used to define the exact source of the allergens can lead to some confusion as well. The definition of hair, dander, skin, pelt, skin scrapings, washes, fur and hide is not always clear; but they can have a significant impact on the quality of the final product [5,6]. The scientific names are generally used in conjunction with the common names, but they do not always match across different manufacturers' products. Even more confusion is generated with fungal products because individual strains of the same species can be qualitatively very different depending on the growth conditions used for their cultivation or the procedures used for their harvesting [7]. For these reasons, a variety of tests have been developed to confirm the identity of source materials and to distinguish closely related sources. Morphological evaluations at both the microscopic and macroscopic levels are routinely used to identify pollens and the plants from which allergenic pollens are collected [8]. Protein profiling based on electrophoretic fractionation, e.g. SDS-PAGE or IEF, have been used for qualitative evaluation of extracts, including identity testing [9,10]. For purified protein allergens, amino acid sequence analysis can provide definitive proof of their identity at the molecular level [11].

The ideal allergen source material would contain all of the potentially allergenic components attributed to a single source and be free of extraneous materials. For example, it has been recommended that pollen source materials contain no more than 1% foreign pollen (less than 0.5% of one individual pollen) and 1% fungal spores as demonstrated by microscopic particle count [2]. A maximum contamination limit for plant parts other than pollen must be separately justified. For fungal and mite source materials that are cultivated in the laboratory, extraneous materials such as residual culture medium and residual solvents must be controlled; and limits need to be based on results of appropriate tests and process validation studies.

Standardization efforts continue to focus on the development and validation of potency assays, which has led to increased confidence in the safety and efficacy of allergen products [12,13]. The active allergenic components of an increasing number of allergen products are being identified and quantitative assays have been developed. It is generally agreed that the widespread availability and use of specific allergen assays by manufacturers have led to more consistent final products and improves their clinical utility. However, their application in source material evaluations, process validations, in-process testing and stability testing may not be widely recognized.

Process Validations

Validation studies are conducted to document that manufacturing processes, equipment and analytical methods are suitable to ensure the quality of products for its intended use. In addition, validation activities are conducted to satisfy cGMP requirements [14]. The formulation of a process validation strategy for a supplier who handles hundreds of different allergen source materials in a single facility can be a daunting task. We used a traditional approach to simplify this task by grouping similar products, process equipment and cleaning methods to organize the validation activities. Products can be grouped according to various attributes such as their sources, methods of processing, intended dosage form, potency, inherent stability, and process requirements (see Table 1). Critical product attributes were defined and process parameters that might affect their quality were identified. Acceptance criteria for each critical process parameter needed to be defined and verified. The variability of natural allergen source materials is more difficult to control than those derived under laboratory conditions and thus, the expected variability in quality should be known. For example, pollen collection techniques, e.g. field collection as compared to water-setting in sheds, can have a significant impact on quality. Once current acceptance criteria are met and the processes validated, monitoring programs need to be implemented throughout the life-cycle of the products. Revalidations are often necessary to accommodate new regulations, process changes, or product specifications. For example, in recent years,

Product Groups	Common Attributes
Pollen Allergen Extracts	Collected from natural plant sources, defatted prior to extraction, produced as glycerinated and aqueous bulk concentrates.
Fungal Allergen Extracts	Cultivated as laboratory cultures, processed prior to extraction, produced as glycerinated and aqueous bulk concentrates.
Dander/Epithelial Allergen Extracts	Collected from animal sources, processed prior to extraction, produced as glycerinated and aqueous bulk concentrates.
Food Allergen Extracts	Collected from natural edible sources, processed prior to extraction, produced as glycerinated bulk concentrates.
Venom Allergen Extracts	Collected from natural insect sources, processed and lyophilized raw venom intermediate.
Latex Allergen Extract	Collected from natural plant sources, fractionated to separate C-serum.
Insect Allergen Extracts	Collected from natural and laboratory-raised insect sources, processed prior to extraction, produced as glycerinated and aqueous bulk concentrates.
Mite Allergen Extracts	Cultivated as laboratory cultures, purified from cultures and processed prior to extraction, produced as glycerinated bulk concentrate.

Table 1. *Product Groups Based on Product Attributes and Process Requirements*

more attention is being given to residual pesticides in pollens, mycotoxins in fungi, and viral clearance in animal-derived source materials. New customer requirements for *in vitro* diagnostics as well as human and veterinary medicine have placed additional emphasis on special process validation studies.

Common equipment that may be used for different products or at different stages of the same product was grouped on the basis of their design and function (see Table 2). Reusable, non-dedicated process equipment that comes into direct contact with product poses the highest risk for cross-contamination. Dedicated equipment eliminates the risk for cross-contamination but a risk of product carry over remains. Finally, single-use disposable equipment and containers pose the lowest risk. As part of the pre-validation activities, process equipment, utensils and containers were grouped on this basis and evaluated whether the current designation is appropriate based on cost and risk of contamination. Priority was also be considered for those pieces of equipment that are used most frequently and those that are difficult to clean because of their shape and/or surface quality. An appropriate strategy, therefore, was to prioritize cleaning validation studies for each equipment group and choosing those pieces of equipment representing the most frequently used and most difficult to clean; and those products representing the worst case. Relative risk for each processing step was estimated using failure mode and effects analyses (FMEA) [15,16].

Cleaning validations of reusable equipment coming into contact with the product were grouped according to the cleaning procedure and agent used. Residue limits were viewed as the cumulative residue from each piece of equipment that comes into direct contact with the product. Using the specification for raw material purity of > 99% and a safety factor of 100, the total product residue allowed can be calculated using the simple formula: $(0.01 \times 0.01) \times \text{batch size} = \text{residual contamination limit}$. Thus, for the processing of a typical 1,000 gram lot, the residue limit is 100 mg. The maximum allowable carry-over would be divided among each piece of process equipment in the manufacturing train, which is specific for each product group. A reasonable initial approach was to assume that the product residue was evenly distributed as long as the specific activity of the product did not change significantly during the processing. If, for example, the total residue can be recovered in a 100 ml sample for each piece of equipment in a manufacturing train of 5 pieces, the analytical sensitivity of the test method would have to exceed 100 mg/500 ml or 0.2 mg/ml, assuming 100% recovery for the sampling method.

Equipment Classification	Equipment	Cleaning Method
Culture Vessels	Povitsky flasks	Auto/Alconox
Defatting (Fungi)	Acetone process vessels, Dryer	Manual/Alconox
Mills	Ball, Wiley, Robocoup	Manual
Blenders	V-blender	Manual
Miscellaneous Equipment	Utensils, Vacuum flasks, Holding tanks	Manual/Alconox

Table 2. *Typical Equipment Groups Evaluated based on Design and Function*

Table 3. *Suitability of Analytical Methods for “Dust Mite” Validation Studies*

Test Method	Assay Detection Limit ¹	Assay Suitability		
		Equip Clean ²	Identity/Purity	Potency/Stability
Total Organic Carbon (TOC)	0.02 AU/ml	+	-	-
ID ₅₀ EAL Skin testing	0.02 AU/ml	-	-/+	+
<i>Der f 1</i> double-bind ELISA	0.4 AU/ml	+	+	+
UV Absorbance (280)	4 AU/ml	+	-	-
ELISA Inhibition	40 AU/ml	-	-/+	+
Bradford protein	100 AU/ml	-	-	-/+

¹ Assay detection limits based on *D. farinae* dust mite extract used as standard

² Equipment cleaning validations based on >50% recovery (0.2 mg source material = 40 AU)

Sampling and Analytical Methods

Rinses, swabbing and wipes, coupons, placebo or product sampling can be used to sample the presence of residues and microbial contaminants that may adulterate source materials. Control points were identified that could be used to test the integrity of the product during processing, packaging or transferring. The choice of the sampling technique used in validation studies were selected after considering the manufacturing equipment to be sampled as well as the product characteristics. The sampling efficiency, recovery and reproducibility were established in conjunction with the analytical method used. The typical validation characteristics that were considered for analytical methods used included (1) specificity and sensitivity, (2) linearity or dilution recovery, (3) dynamic range and quantitation limit, (4) accuracy and precision, and (5) robustness, including assay interference [17]. Direct visual examination, conductivity, total organic carbon, specific allergen or contaminant, e.g. detergents, enzymatic analysis, UV absorbance, and HPLC were used, as appropriate, to detect the presence of residues or to establish product integrity or stability during processing (see Table 3). Method selection was based on overall performance and suitability of the assay method for each validation study [18].

Risk Assessments

Risk identification and assessments sometimes require evaluating the suitability of established specifications. An evaluation of the allergen source material purity limits for detectable foreign allergens from other sources or species suggests that the limits may vary depending on the intended use of the product. For a therapeutic product, the maximum limit for a foreign allergen can be calculated using a simple proportion based on the minimal immunogenic dose of the contaminant and the maximum therapeutic dose of the product. Assuming a minimal immunogenic dose of 10 BAU for a contaminating allergen and a maximum (maintenance) dose of 1,000 BAU for the product, then the limit is 10 BAU/1,000 BAU, or 1%. This limit

is consistent with the current regulations. However, for a diagnostic product, a similar calculation using corresponding values of 10 BAU/ml for the minimum effective diagnostic concentration and 10,000 BAU/ml for the maximum prick test concentration, the limit is 10 BAU/10,000 BAU, or 0.1%. In some cases, allergists routinely employ 100,000 BAU/ml prick test concentrations, which can further increase the risk for false positive reactions by ten-times in selected patients.

Summary

A variety of positive outcomes can be realized from validation and risk management activities (see Table 4). They are dependent on the participation of multiple functional groups including the quality unit, regulatory and legal affairs, engineering and production operations, research and development, and sales and marketing.

Quality risk management is receiving increased attention in the area of public health, pharmacovigilance, and pharmaceutical manufacturing. Recent examples of its regulatory use in our industry include the assessment of the potential risks of transmissible spongiform encephalopathies (TSE) agents through contaminated products [19], the risks of precipitates in allergenic extracts [20], and the revision of the potency limits for standardized dust mite and grass allergen vaccines [21].

Its application to allergen source material process validation activities allowed for a practical strategy, especially in a complex manufacturing environment involving hundreds of products with multiple intended uses. In addition, the use of tools such as FMEA was useful in evaluating proposed changes made to manufacturing procedures and product specifications, new regulatory actions, and customer feedback or complaints.

The success of such a quality assurance programs will ultimately be reflected in the elimination or reduction of product failures, improvement in the detection and prediction of potential product failures, and increased confidence in product quality.

Studies	Outcomes
Cleaning Validations	Replaced difficult to clean equipment; moved toward disposable containers and dedicated equipment; optimized facilities and equipment cleaning agents.
Process Validations	Streamlined manufacturing steps; identified critical process parameters; reduced risk and cost; attention toward consistency in upstream processes such as collection and cultivation techniques.
Assay Validations	Defined appropriate use of assays; applications in source material evaluations, in-process testing, stability testing, and process improvements.
Risk Assessments (FMEA)	Increased confidence in processes in product quality; facilitated appropriate corrective/preventive actions to address product failures and risks of failure.

Table 4. *Outcomes of Validation and Risk Management Activities*

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Discussion

Vieths: I have a question regarding your slide on food allergens. You mentioned that you extract them after processing. Does that mean that, e.g., you are preparing a wheat extract after heating the wheat or baking bread?

Esch: For foods, this varies from manufacturer to manufacturer, but for our starting materials, e. g. edible products that we get from farmers or the groceries, processing mainly means defatting and powdering them to make them easily extractable.