Standardized Extract Testing

at HYCOR® Biomedical for the NOVEOS™ Immunoassay System:

Elevating a Common "Broth" to a More Refined "Consommé"

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"Only the pure in heart can make a good soup"

– Ludwig Van Beethoven

A HYCOR® BIOMEDICAL
WHITE PAPER

EXECUTIVE SUMMARY

There are many similarities between the culinary world of soup preparation and the biochemical world of allergen extract formulation. In the culinary world, many believe that "broth", "stock" and "consommé" are interchangeable terms for the same thing. Although the initial processes for making each of these liquids are quite similar, there are significant differences in their preparation (ingredients, cook time, seasoning and clarification of the final product) that set these liquids/soups apart as completely different things[1]. Most seasoned French chefs would never use a common broth or stock when the powerful flavor of a consommé is demanded in a recipe. Similarly, most chefs would not go to the expense of cooking a consommé when a simple broth is sufficient as the backbone for the soup.

In the biochemical preparation of extracts, many laboratorians assume that every specific IgE assay in the allergy world, just like various "broths", "stocks" and "consommé", are interchangeable. Customers believe this because manufacturers broadcast their "extensive experience in allergen production" to try to distinguish that manufacturer's product as more consistent, advanced or unique. This ignores the fact that almost all major allergen manufacturers for both skin prick testing (SPT) and *in vitro* Diagnostics (IVD) use the same traditional techniques that were developed almost 100 years ago to generate extracts[2]. Although there have been advances in the processes to manufacture better allergenic materials, most large diagnostic manufacturers have been resistant to switch to these new techniques since they are more expensive, require updated processes/procedures, but more significantly may require a change from the allergen used in original regulatory filings, which would result in the need for additional submissions and costs.

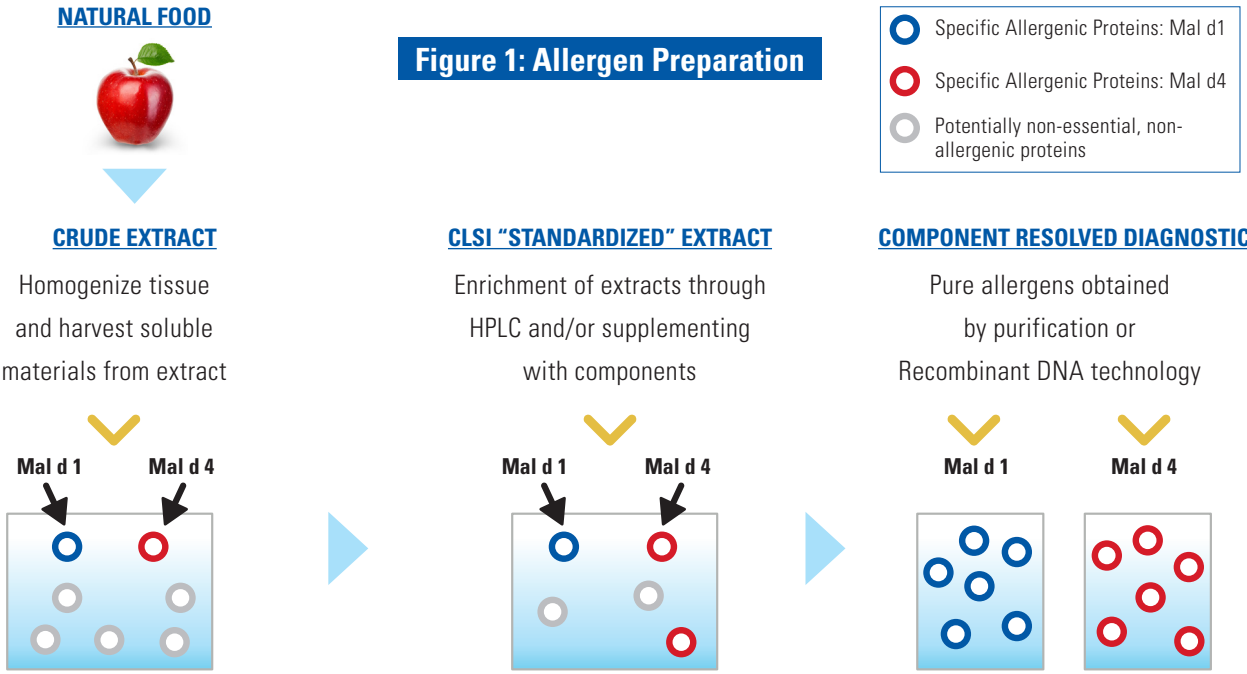
This paper provides a technical overview of differences in prepared allergens across vendors and lots for both standardized and crude allergenic materials. It reviews the analytical and performance characteristics of each extract identified as part of the selection process of appropriate extracts for the NOVEOS™ Specific IgE tests. It is also intended to help laboratorians and clinicians understand the systematic approach that HYCOR® Biomedical utilizes to identify vendors with consistent processes.

INTRODUCTION

Since the early 1900's, extracts have been used to aid in the diagnosis of allergen sensitization, and for allergen-specific immunotherapy of type 1 hypersensitivity. Unfortunately, the variability of the quality of commercial allergen extracts can limit their utility for immunotherapy[3,4]. This prompted significant efforts to improve the quality of extracts beyond simple homogenization of tissue to create various types of allergens and extracts (crude extract, standardized extract, biochemically purified allergen, recombinant allergens) that differ in the level of preparation needed to produce the material. These are denoted in Figure 1.

Figure 1: *Types of available allergens: crude extracts, standardized extracts and purified proteins.*

Figure 1: Allergen Preparation



CRUDE EXTRACTS:

As denoted above, tissue is homogenized to generate a non-standardized (or crude) extract. The extract contains the necessary allergenic proteins along with non-allergenic materials. The level and complexity of the other materials can reduce the overall potency or specific activity of the extract and increase the variability in a given allergen lot.

CLINICAL AND LABORATORY STANDARDS INSTITUTE (CLSI) STANDARDIZED EXTRACTS:

To improve the overall potency of allergen extracts, manufacturers have begun processing some crude extracts to enrich the allergenic material. This additional processing requires a series of chromatographic columns that effectively separates non-allergic proteinaceous matter from desirable allergenic protiens in the sample.

In conjunction with CLSI, the Center for Biologics Evaluation and Research (CBER) in the US Food and Drug Administration (FDA) established 19 allergenic reference standards including six Hymenoptera venoms, two house dust mites, two cat dander extracts, one short ragweed pollen, and eight grass pollens[5]. "For standardized allergen extracts, manufacturers must compare the extract to a U.S. reference standard to ensure potency. More specifically, CBER established a biochemical enrichment of grass and mite allergen extracts that can be monitored for purity using pooled serum in a standard ELISA. Enriched extracts for cat and ragweed allergens can be evaluated using immunodiffusion. Finally, the allergenicity of Hymenoptera venoms can be assessed after enrichment by using total protein and enzymatic activities.

COMPONENT RESOLVED DIAGNOSTICS (CRD):

The use of allergen components as opposed to whole extracts allows for the precise identification of clinically significant proteins to create a detailed molecular profile of the allergic patient[6]. Components can be generated using either traditional biochemical techniques (affinity chromatography, electrophoresis, etc.) or direct molecular cloning of DNA of an allergen into an appropriate expression vector (bacterial, mammalian, insect, etc.). Components are pure proteins, therefore the use of CRD is advantageous both in the manufacturing of the allergy product and to obtain consistency of the preparation itself. However, there are also key disadvantages including lack of availability of clinically relevant components, increased cost of materials, and additional manufacturing labor. Also, components could be less potent compared to corresponding whole extracts due to incorrect protein folding, protein degradation, and improper transport conditions.

THE "BROTH":
Biochemistry of Crude Extracts

In 1911, British physicians Leonard Noon and John Freeman discovered the benefit of exposing patients to allergenic crude extracts for immunotherapy[7]. Since then, the same extract preparation procedure has remained mostly unchanged in the laboratories of every major allergen manufacturers with only minor modifications to improve stability, reduce non-specific hypersensitivity, and adapt to various clinical uses (Skin Prick Testing, Oral Food Challenge, in vitro diagnostics, etc.).

However, manufacturing procedures across major allergen vendors may vary. Differences in growth, harvest, and storage of the source material can contribute to differences in allergenic protein composition, leading to extract variability between vendors or within lots produced by an individual vendor. Modifications in the extraction procedure to improve stability, allergen-yield or reduction of impurities must be closely scrutinized to ensure consistent product. For this reason, HYCOR® Biomedical conducts extensive biochemical and performance testing on every allergen extract lot to identify potential issues before each lot is introduced into the NOVEOS™ product. Typical biochemical testing on key performance criterion is shown in Figure 2.

Companies suggesting any advantage of their crude extracts over competitors grossly mislead consumers. The real key to consistency in the use of crude extracts is extensive biochemical and performance testing on every lot of allergen extract. It is also important to understand the history of the starting material, these include the environmental background, nutritional factor, and genetic composition of the source material, the allergen extraction procedure, and the storage and maintenance of the final product. If possible, work with the vendor of the source material to identify and control factors that can impact the integrity of lots of extract.

Figure 2: Analytical Testing

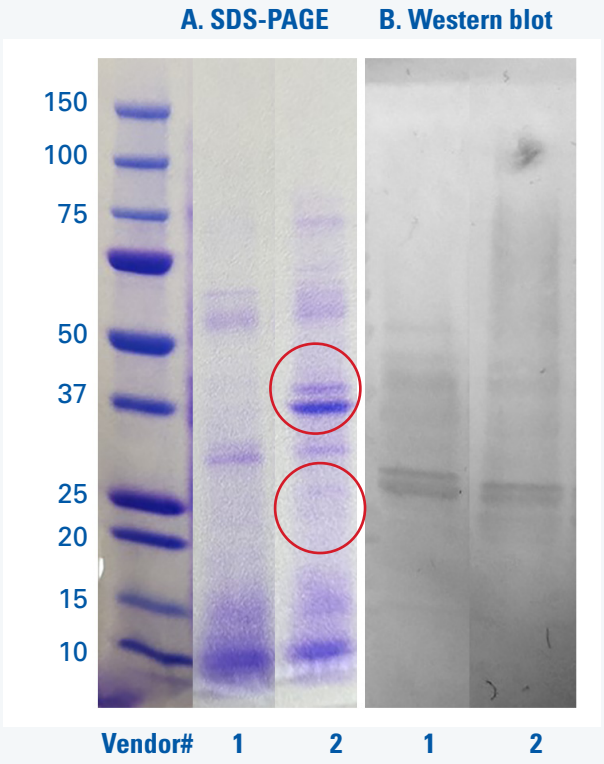


Figure 2: *A, B, & C identifying, analyzing and characterizing extracts. Vendor to vendor differences were demonstrated for timothy grass (G006) that was prepared by two vendors. The protein diversity of the extract were observed by SDS-PAGE (highlighted circles) and in the HPLC traces of the total extracts. The data suggests consistent biochemical characteristics on successive lots.*

THE "STOCK":
Standardized Extracts Improve Consistency

Several commonly used extracts have been standardized such that allergenic reactivity is consistent between manufacturers and between lots made from the same manufacturer[8]. Specifically, reference standards from the Center for Biologics Evaluation and Research (CBER) are used in Enzyme-linked immunosorbent assays (ELISA) or immunodiffusion to help control the specific activity (potency) of biochemically enriched extracts. Extracts are then standardized based on intradermal skin test responses in allergic individuals.

There has been much excitement on published reports around standardized extracts since they demonstrate improvements in the fidelity of extracts used in intradermal testing responses. The same extracts appear to also dramatically improve the fidelity of extracts when used for *in vitro* diagnostic testing (IVD)[9]. That data is shown below in Figure 3.

The NOVEOS™ platform was the first to utilize standardized extracts in its chemistry when it was introduced in 2017. HYCOR® plans to use all 19 standardized extracts on its NOVEOS platform to ensure the consistency of the allergenic material. It is not clear whether other manufacturers will take advantage of these more consistent extracts due to the exorbitant waste associated with their process of passively coating extracts onto cellulose or ¼ inch bead solid phase surfaces, therefore adoption may be cost prohibitive.

Figure 3:

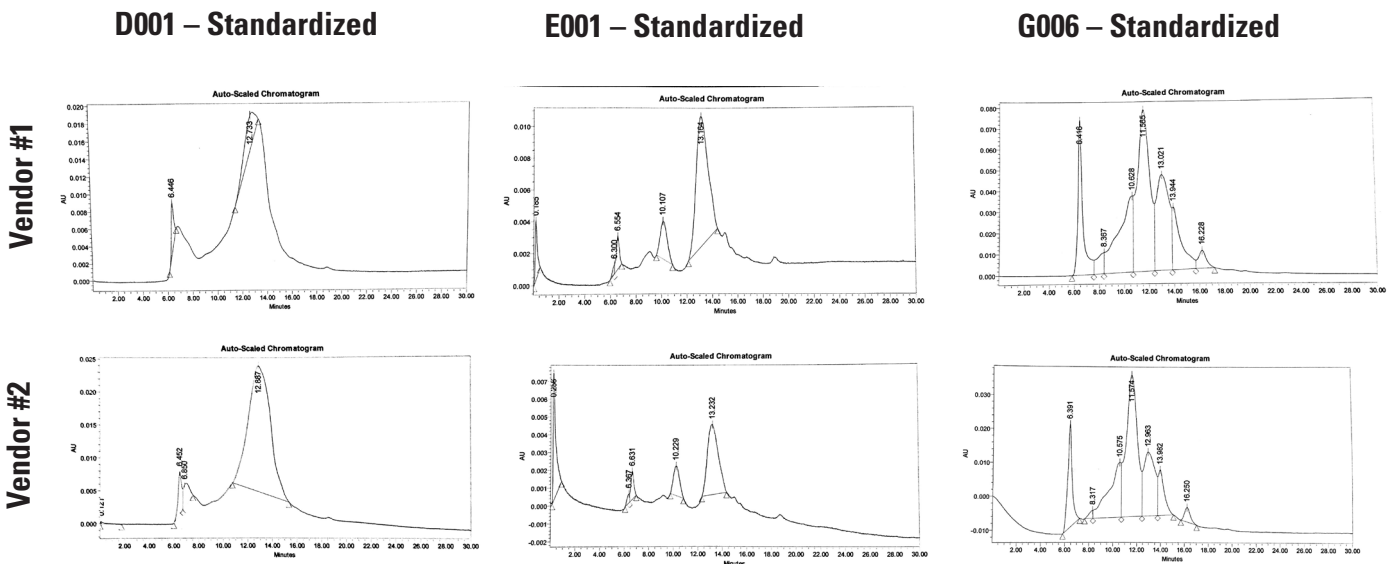


Figure 3: *A single lot of three different extracts were obtained from two vendors that manufacture standardized extracts. For each of these allergens, the material was analyzed and demonstrated consistent profiles to qualify and meet internal acceptance criteria for usage on the NOVEOS platform.*

THE "CONSOMMÉ":

Practicing CRD in the Clinical Laboratory

Progress over the last 3 decades has allowed laboratories to identify and characterize single allergens in detail at a molecular level. The use of single allergenic molecules (instead of extracts) has introduced a new area of high-resolution molecular allergy diagnostics, improved diagnostic specificity, and changed the understanding of sensitization profiles and cross-reactivity. To utilize the full potential in clinical practice, an in-depth general knowledge of molecular allergology is needed as it relates to when and how allergenic molecules can be used for diagnostic purposes[10].

The increasing number of available single allergens, particularly for peanut proteins, allow for a comprehensive review of the pattern of sensitization. Research into the structural similarity between allergens and the amino acid sequence homology between food allergens also helps to explain cross-reactivity between allergens, which may be clinically relevant. Similarly, the availability of single allergen components for peanut, cow's milk, hen's egg, soy and wheat allows for review of sensitization in those anaphylactic inducing materials. Those single allergens are shown in Figure 4.

Both allergy diagnostics and allergen immunotherapy (AIT) are still heavily dependent on extracts of the allergen sources. Molecular approaches are gaining ground rapidly in diagnostics[11], but extracts can certainly not be dismissed[12]. Thus, similar to the culinary need for broths, stocks and consommé, allergy diagnostics will continue to be highly dependent on crude and standardized extracts as well as purified or molecular components. Each provide unique value in the diagnosis of patients.

Figure 4:

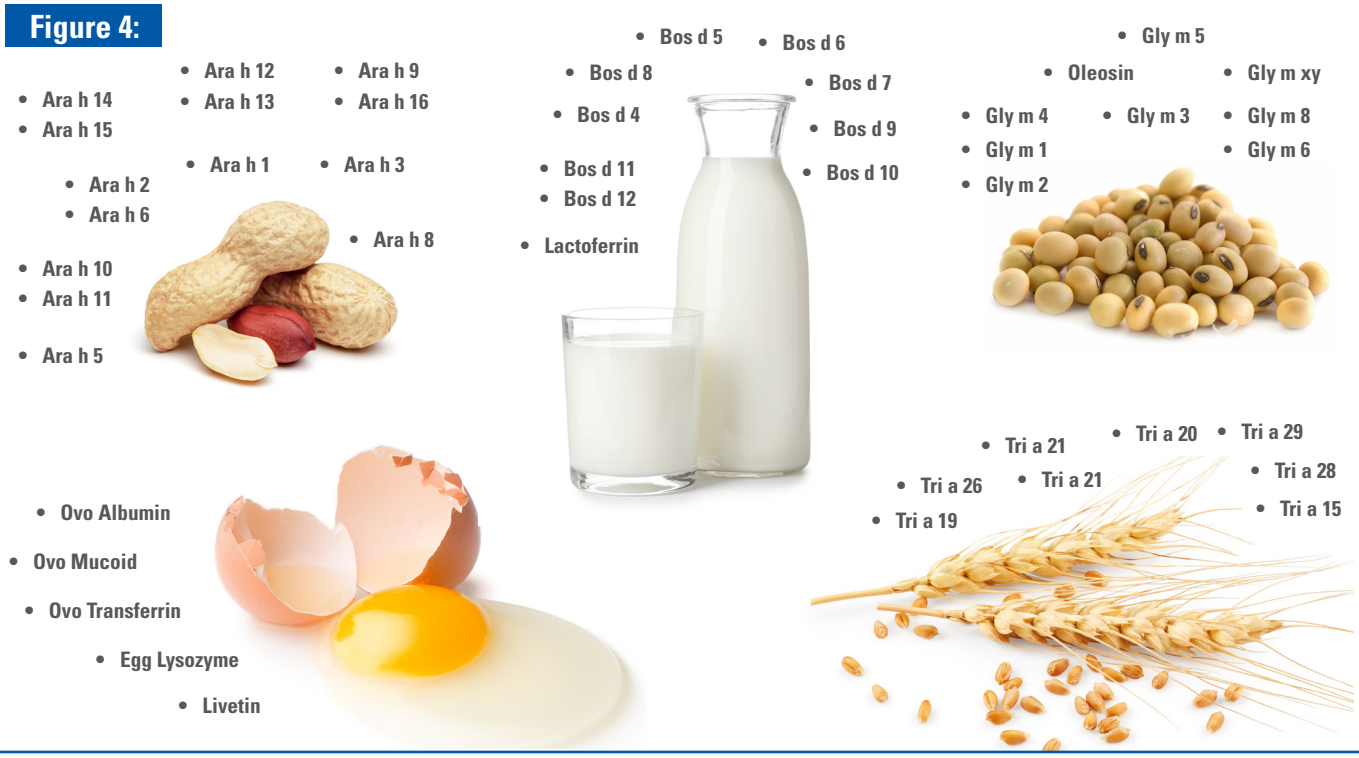


Figure 4: *The use of CRD in allergy testing for 5 primary food allergies. Components are available for Peanut (14), Cow's Milk (10), Hen's Egg (5), Soy (9) and Wheat (7).*

The NOVEOS™ Chemluminescent Method

Cost-Effective, Accurate and Precise

- Highly-automated
- Superior walk-away time/ability
- Intuitive user interface for ease of training and operation
- Liquid, ready-to-use reagents

Uses only 4µL of Specimen per Test

- Improves lab workflow and operational costs
- Reduces Quantity Not Sufficient (QNS) errors
- Reduces patient resampling due to insufficient volume
- Reduces trauma for hard-to-draw patients

Reduction of variability

- Large reagent lot sizes
- Use of standardized extracts when available
- Every allergen receives extensive biochemical characterization to ensure performance
- Assay design is unaffected by biotin or cellulose-related cross-reactive carbohydrate determinants interferences

Trusted Analytical Performance

- Cutting-edge immunochemistry technology
- Paramagnetic microparticles
- High sensitivity and excellent low-end precision

About HYCOR® Biomedical

With over 40 years of experience, HYCOR Biomedical is a global manufacturer and marketer of *in vitro* diagnostic products.

Since its founding in 1981, HYCOR has supported clinical laboratories, hospitals and doctors' offices worldwide with allergy and autoimmune instrumentation and reagents. Among its products, HYCOR markets the HYTEC™ and AUTOSTAT® instruments and most recently the NOVEOS™ Immunoassay System. Each has received CE Mark for the European Union and FDA clearance in the United States.

The company is focused on delivering innovative technology products and comprehensive services that provide the highest value to clinicians and laboratories.

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