Allergy testing on the NOVEOS™ Immunoassay System:

Moving beyond "Bucket Chemistry"

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"Life is like riding a bicycle. To keep your balance, you must keep moving."

– Albert Einstein

A HYCOR® BIOMEDICAL WHITE PAPER

EXECUTIVE SUMMARY

During the late 1990's and early 2000's, researchers in the clinical laboratory routinely referred to serology and allergy testing as "bucket chemistries". As this label suggests, these chemistries lacked sophistication and instead utilized manufacturing methods without robust biochemical techniques, characterizations or analyses.

For specific IgE assays, the source materials were mysterious, allergen extractions were proprietary and the results obtained in the clinical lab on these extracts were ambiguous due to many false positive and false negative results that did not match the clinical status of the patient.

"Bucket chemistry" was an appropriate term because allergens were and continue to be passively adsorbed to the fibers of cellulose sheets by literally dipping them in manufacturing vats of diluted extracts and/or components. A circular cellulose disc was then punched from these dried sheets for each individual test. Similarly, the ¼ inch beads used for one technology were soaked in an allergen coating solution before being dried and packaged for use. In today's modern clinical world, it's hard to envision that some manufacturers in allergy blood testing still do not follow state-of-the-art practices recommended by most biochemical researchers and public agencies; instead some manufacturers still use chemical processes that are relatively low-tech with uncertain control.

The key to moving beyond "bucket chemistry" in allergy testing is to tightly control the sourcing, analysis and performance testing of the extracts used. It is imperative each allergen be scrutinized using today's biochemical techniques as an active part of the qualification process rather than the ones developed almost 30 years ago for the technologies of the 1990's. Full characterization of allergens de-mystifies the source material and provides results in the clinical lab that are less ambiguous and are more closely related with the clinical status of the patient.

This paper provides a technical overview of the how HYCOR® Biomedical characterizes, analyzes and qualifies the allergen extracts, mixes and components used on the NOVEOS™ platform. It is intended to help laboratorians and clinicians understand the systematic approach that HYCOR utilizes to ensure each extract meets the stringent performance requirements needed for robust allergen specific IgE testing on the NOVEOS platform.

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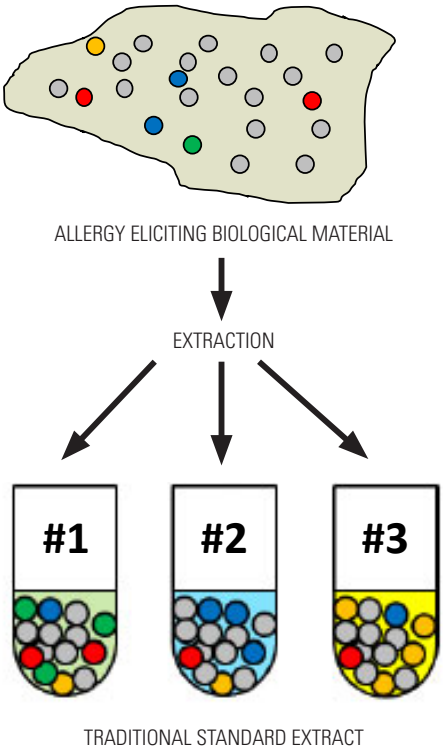
INTRODUCTION

Allergen extracts contain complex mixtures of allergenic and other non-allergenic substances that include proteins, glycoproteins, polysaccharides, lipids, nucleic acids, low molecular weight metabolites, salts, and pigments. Most allergens are proteins or glycoproteins, but in certain rare circumstances, carbohydrates or low molecular weight chemicals can act as allergens. All foreign proteins are potential allergens in theory, although only a limited number of proteins are confirmed to be allergenic in humans. No structural properties have been identified that distinguish allergenic from these other passenger proteins within the extract.



Allergens used in both skin prick and *in vitro* testing are usually prepared by extracting proteins directly from natural source material. The extraction can involve grinding, chopping, homogenizing, clarifying, dissolving, dialyzing and/or lyophilizing biologic tissue into a number of buffers, mediums, salt solutions or detergents. Almost all allergens used in extracts for this type testing are soluble in water-based solutions and therefore must be separated from non-aqueous matter (fats, complex carbohydrates, particulate material) before storage.

Each extract, when sourced from even the same biologic species, may vary since there are many factors that can influence the material's composition, potency and properties. These include:

WHERE IT CAME FROM

- Growth & Nutrition
- Environment/Climate
- Cooked vs. raw
- Young vs. old material
- Other genetics

HOW IT WAS EXTRACTED

- Procedure
- Timing
- Temperature
- Buffers
- Detergents

HOW IT WAS STORED

- Humidified
- Frozen
- Lyophilized
- Ambient temperature

For batch-to-batch consistency, it is sufficient to consider the proteins as active ingredients; it is the active ingredient's potency, longevity and consistency that assures reliable results in allergy testing.

Figure 1: *Most source material for typical extracts uses conventional procedures involving homogenization in an appropriate buffer. Not all source material is created equal! For example, apples from two different trees of the same species can give diverse allergenic profiles. Other factors (age, nutrition, stability, etc.) can also impact consistency of the resulting extract.*

DEMONSTRATING EXTRACT CONSISTENCY

HYCOR® Biomedical is committed to providing consistent allergens that meet the stringent requirements of the clinical laboratory. To make that assurance, HYCOR has dedicated significant resources to carry out extensive testing during raw material (RM) sourcing, RM qualification, in-process testing and final quality control (QC) release. During these stages, HYCOR conducts rigorous analytical testing to clearly define the nature of the extract and to set rigid potency, purity, reproducibility, concentration and reactivity criterion. Upon acceptable analytical testing, HYCOR then conducts extensive performance evaluations that define how the allergen will perform in the clinical laboratory. Every lot is evaluated against the published performance including studies that repeat the method comparison, Limit of Blank, Precision and clinical concordance for a given allergen.

CRUDE & STANDARDIZED EXTRACTS

ALLERGEN CHARACTERIZATION PROCESS

1 RAW MATERIAL SOURCING

- Multiple vendors
- Supplier controls
- CoA[1] review

2 RAW MATERIAL CONFORMANCE

- Total Protein Analysis
- SDS-PAGE[2]
- HPLC[3] Traces
- Western Blot
- Limit of Blank
- Method Comparison
- Accelerated Stability
- Within-run precision

3 IN-PROCESS TESTING

- Method Comparison
- Specificity
- Limit of Blank

4 QUALITY CONTROL RELEASE TESTING

- Curve and Control Analysis
- Limit of Blank
- Method Comparison
- Microbial Testing
- Real Time Stability

1. Certificate of Analysis
2. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis
3. High-performance liquid chromatography

ANALYTICAL TESTING:

There are a multitude of biochemical and analytical tests that can be performed to assess the integrity of a given extract. Most common of these is to enumerate the proteins in a given extract through the use of Polyacrylamide Gel Electrophoresis (PAGE, SDS-PAGE) and/or High Performance Liquid Chromatography (HPLC). In PAGE, proteins are separated by electrophoresis through a gel matrix; smaller proteins migrate faster due to less resistance from the gel matrix. Other influences on the rate of migration through the gel matrix include the structure and charge of the proteins. Similarly, HPLC is a technique used to separate, identify, and quantify each protein in a mixture. It relies on pumps to pass a pressurized liquid solvent containing the sample mixture through a column filled with a solid adsorbent material. Each protein in the sample interacts slightly differently with the adsorbent material, causing different flow rates for the different proteins and separation as they flow out of the column. Examples of this analysis is shown in Figure 2.

In these complex mixture of proteins, additional scientific techniques can be used to detect specific allergen species. One of these procedures, Western blot analysis, is well suited for this purpose. For this technique, mixtures separated by SDS-PAGE are probed for individual allergenic species by incubating with sensitized patient sera. Bound IgE to the allergens can be visualized using enzyme-linked monoclonal antibodies direct human IgE. In this way, manufacturers can ensure that the correct complement of allergenic species is present within a preparation.

Figure 2: Analytical Testing

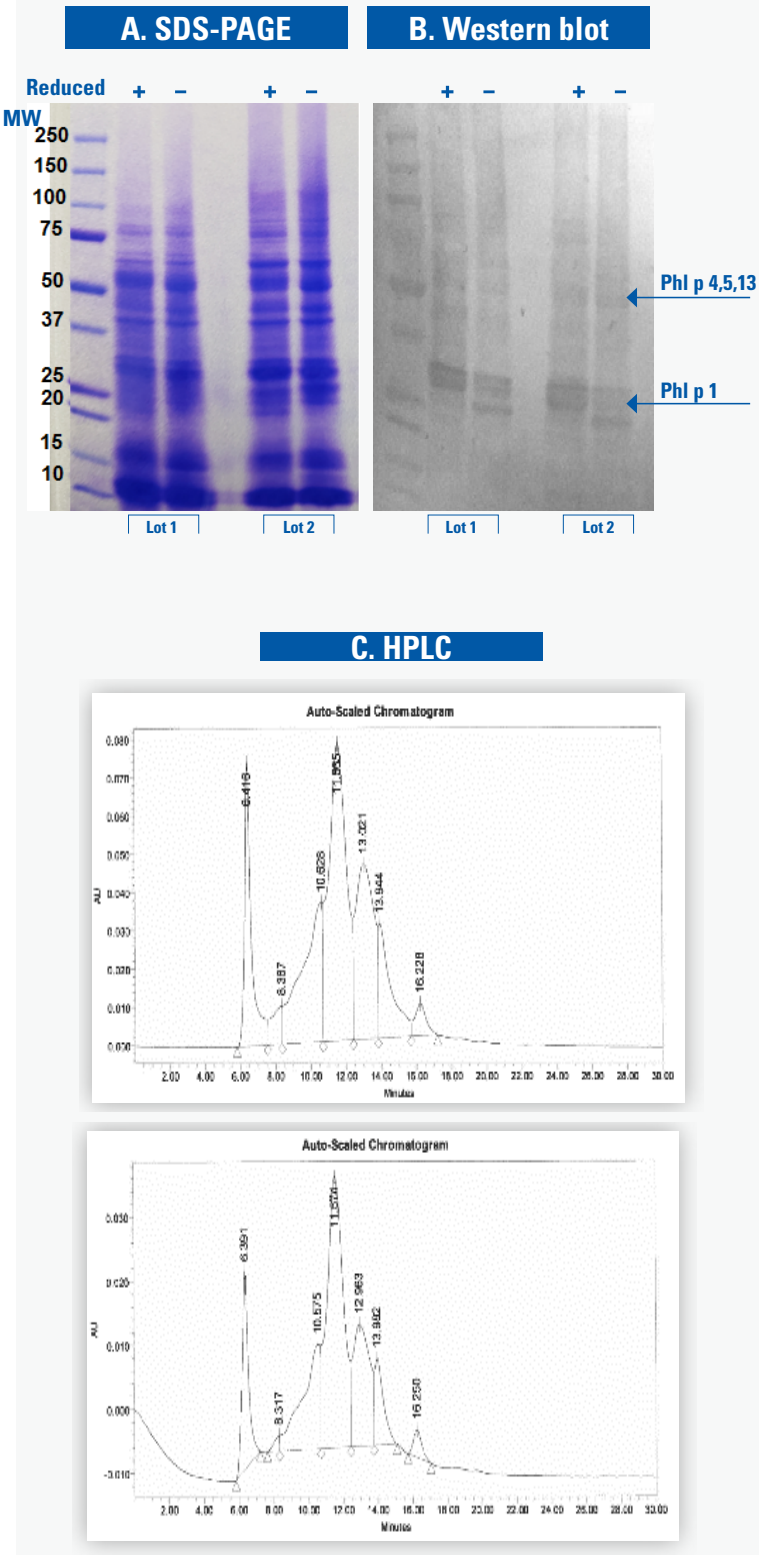


Figure 2: *Examples of analytical testing conducted on every allergen lot. A) Samples are evaluated by SDS-PAGE in the presence/absence of reducing agents to reduce protein folding. B) Samples are evaluated by Western blot to observe reactivity and potency of extract. C) Samples are quantified by HPLC to establish a biochemical "fingerprint" for the material.*

PERFORMANCE EVALUATIONS

Many companies simply do not conduct sufficient performance testing on each new raw material because it has an impact on the manufacturability of allergens; i.e., it is expensive, requires a multitude of patient samples and delays the release of that extract to the field. Instead, the release process for many manufacturers involves evaluating control material to ensure conformance rather than carrying out systematic evaluations to reproduce the performance of previous lots with the new material. Subsequently, much of the weight of validating the comparability of new allergen lots falls on the clinical laboratory. This becomes a "black hole" that wastes reagents, time and money in a time when laboratorians need to be conscientious of how their resources are being used.

HYCOR does not subscribe to the idea to limit the testing of allergens. Instead, every lot is evaluated against the published performance including studies that repeat the method comparison, limit of blank, precision and clinical concordance for a given allergen. Some of this type of analyses are shown in figures 3, 4, 5, 6 and 7.

HYCOR is able to incorporate this level of testing primarily due to the NOVEOS micro particle-based chemistry. This foundation of the NOVEOS system allows for larger batch sizes that are limited by the size of the container rather than by the number of dipped and/or soaked cellulose sheets or ¼ inch beads being processed. Further, there is essentially no waste in using the allergens for the NOVEOS assays since all of the extract goes into the capture reagent; for emersion technologies, most of the allergen stays in the coating solution which is subsequently discarded.

This technology selection and manufacturing process allows HYCOR to manufacture larger batch sizes for stock material that is used across multiple lots and larger individual lots that can be available to the clinical laboratory for extended periods. The large batches reduce the incidence of variation in assay performance in a laboratory that stem

Figure 3: Various D001 Lots – NOVEOS™ Results

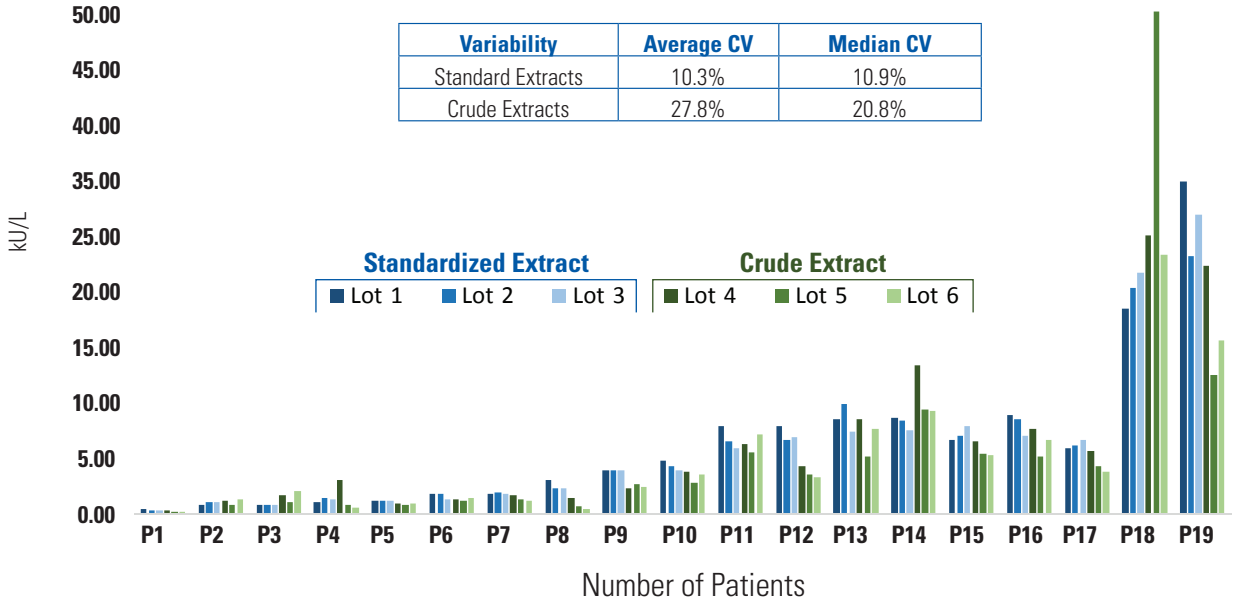
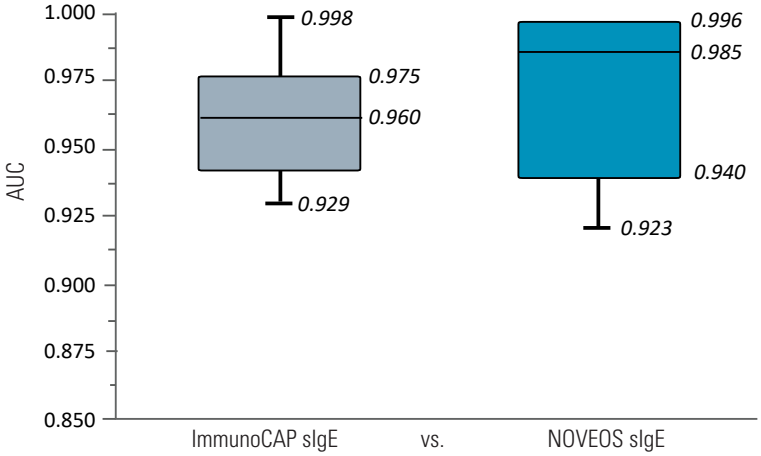


Figure 3: *Performance Variability: Multiple lots of D001 perform similarly using Production pools. Some lots would be rejected based on their performance with these pools.*

from frequent lot changes. Lot-to-lot variability is routinely seen in most "bucket chemistry" formats, and HYCOR has moved clinical allergy testing beyond the restrictions routinely seen for these archaic chemistries. Taken together, by demystifying the characteristics of the source material, and using microparticles to ensure that results are less ambiguous, more consistent, and are more closely related to the clinical status of the patient, HYCOR has moved beyond the typical "Bucket Chemistry" used by many leading manufactures!

Testing conducted at HYCOR for each allergen to ensure that new lots of material are qualified according to their reference standards.

Figure 4: Clinical Concordance



Allergen	Name	n	NOVEOS™ sIgE	ImmunoCAP sIgE
D001	D. Pteronyssinus	234	0.985	0.935
D002	D. farinae	213	0.984	0.998
E001	Cat Dander	194	0.996	0.964
G006	Timothy Grass	189	0.956	0.962
W001	Common Ragweed	181	0.923	0.929
All		1011	0.972	0.958

Figure 5: Precision Testing

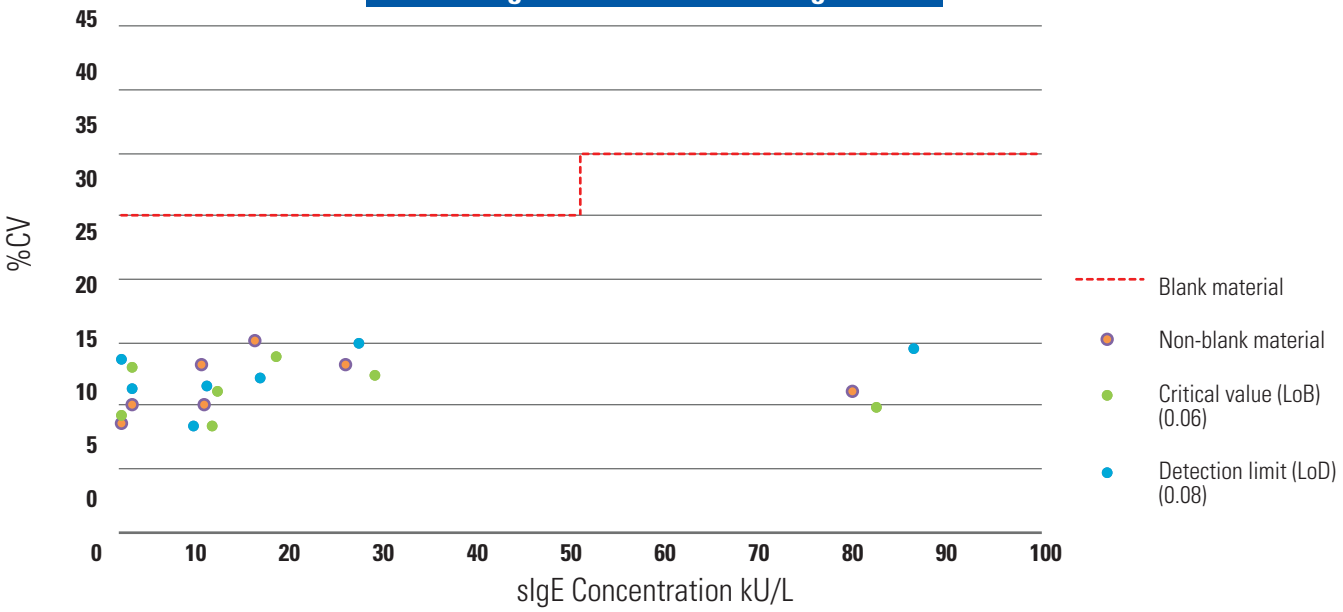


Figure 4: *Clinical concordance to either Skin Prick Testing or Oral Food Challenge. Specific IgE (sIgE) Assay Area Under Curve (AUC) vs. Skin Prick Test (SPT)* NOVEOS™: Area Under Curve (AUC) of 0.94 – 1.00 against Skin Prick Testing (SPT) – D1 (dust mite), E1 (Cat Dander), D2 (dust mite), G6 (Timothy Grass), W1 (Ragweed). ImmunoCAP: AUC of 0.93 – 0.980 on these same samples against SPT.*

Figure 5: *Precision testing showing the reproducibility of results NOVEOS™ sIgE-D001.*

Figure 6: Limit of Blank and Detection

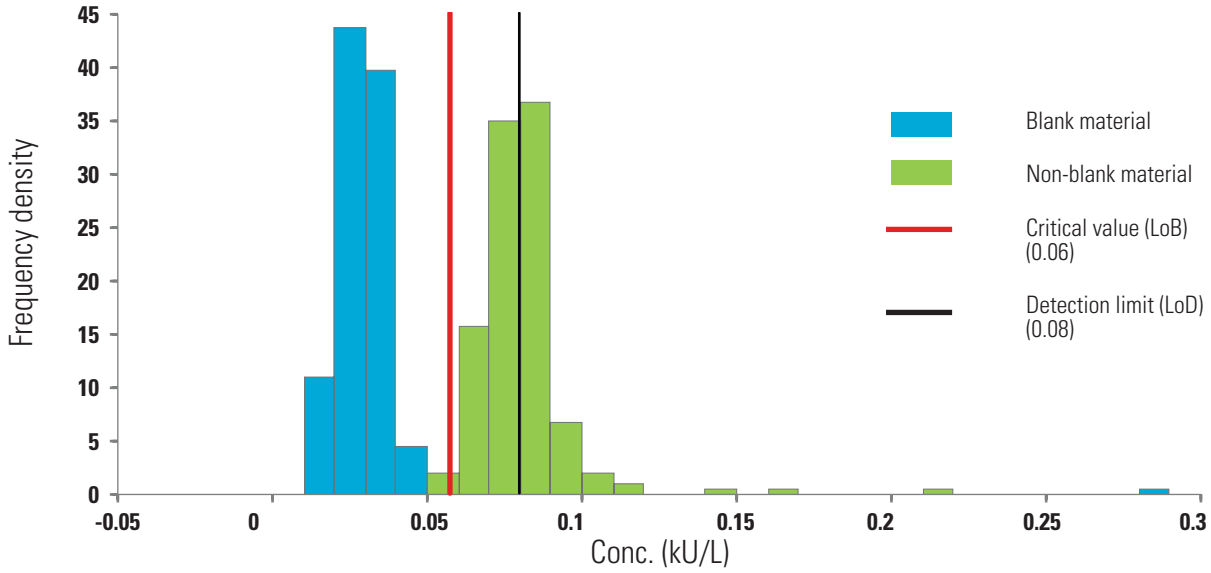


Figure 7: CE Method Comparison

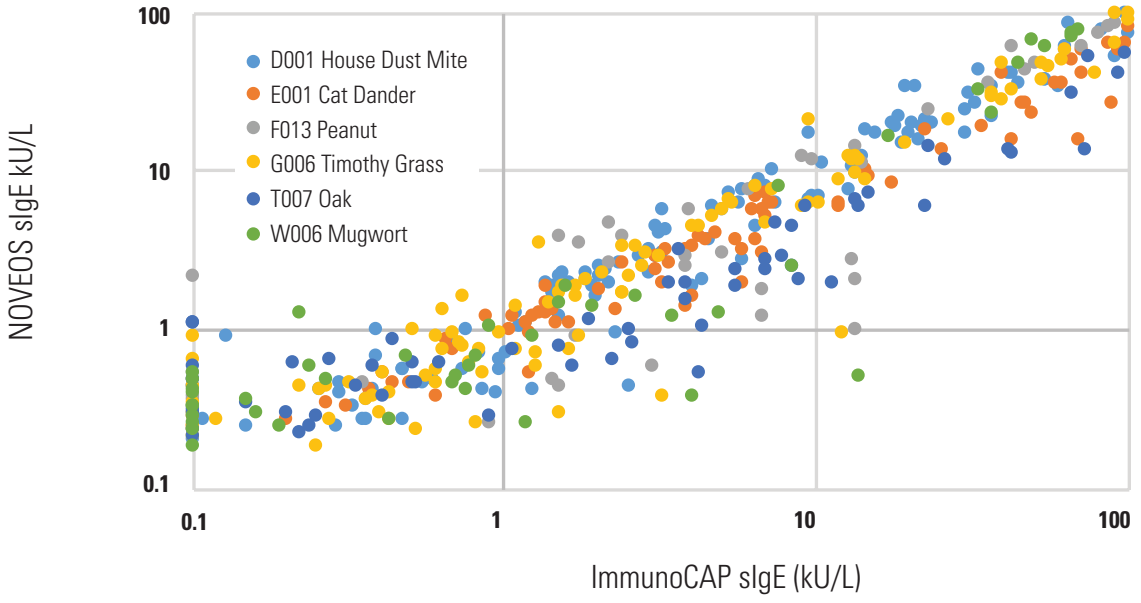


Figure 6: *Limit of Blank and detection testing showing the analytical sensitivity of the allergens. Lot 2 LoB & LoD Histogram.*

Figure 7: *CE Method Comparison; Bivariate Fit. Method comparison to ImmunoCAP Specific IgE (sIgE).*

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.

References

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4. Singh AB. Allergen Preparation and Standardization: *An Update. Glob J Oto.* 2018; 17(4): 555968. DOI: 10.19080/GJO.2018.17.555968.
5. *Data on file.
6. **The NOVEOS Immunoassay System and NOVEOS Capture Reagent D001 and D002 are CE-marked and U.S. FDA 510(k) cleared. Other allergens are CE-marked and for Investigational Use only pending U.S. FDA 510(k) submission and clearance.

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