



IMMULITE® 2000 Allergy: A Third-Generation System

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While the past two decades have seen many technological advances among in vitro allergy assays, the recent release of IMMULITE® 2000 Allergy ushered in a brand new era of allergen-specific IgE testing. The system, which has been fully validated according to NCCLS I/LA20-A performance guidelines, represents a dramatic improvement over “first-generation” RIAs and “second-generation” EIA batch processors, with respect to performance, turnaround time, throughput, and automation.¹ As the first and only in vitro allergy system of its caliber on the market, IMMULITE 2000 Allergy has essentially defined the functional criteria for any “third-generation” specific-IgE assay that may follow.¹

IMMULITE 2000 Allergy combines third-generation sensitivity (Figures 2 – 4), factory calibration, full random access automation, a large menu of allergens and panels, and the ability to run allergy assays alongside any IMMULITE 2000 immunoassay DPC offers. The resulting benefits include

- Unparalleled accuracy and precision
- Faster turnaround
- High throughput
- Elimination of batch testing
- Decreased labor costs
- Ability to consolidate platforms
- Cost-effective allergy testing for even the smallest laboratory.

The following summary describes the assay characteristics and performance of IMMULITE 2000 Allergy.

Full Random Access Automation

IMMULITE 2000 Allergy offers full automation, including continuous random access sample-processing capability. Continuous random access gives laboratories the option of processing allergy assays alongside routine assays. Consequently, laboratories can conduct patient-specific testing according to physician-directed clinical priorities, without sacrificing productivity. Automation also enhances turnaround time. IMMULITE 2000 Allergy features a time-to-first-result of 65 minutes, compared to 3 – 5 hours for second-generation assays. Throughput is 100 tests per hour.

¹The “generational” characterization of allergen-specific IgE assays is widespread in the literature. See, for example, M. Plebani et al.² and RG Hamilton et al.³

Calibration and Sensitivity

Unlike allergy assays of previous generations, there is no need to run a calibration curve with every IMMULITE 2000 Allergy assay. Calibration takes place at the DPC production facility and is transmitted to the system via barcodes, which are scanned into the system software with each new kit lot. Factory precalibration is traceable to WHO standard reference material.

During generation of the stored curve, a zero calibrator is used, allowing a calibration range of 0.1 to 100 kU/L. Compared to second-generation assays with calibration ranges of 0.35 to 100 kU/L (IU/mL), IMMULITE 2000 Allergy offers a significant increase in analytical sensitivity. Clearly, a detection limit of 0.1 kU/L will ensure much greater reliability than a detection limit of 0.35 kU/L when measuring low values, particularly near the cutoff level of 0.35 kU/L.

The exceptional sensitivity of IMMULITE 2000 Allergy is enhanced through enzyme-amplified chemiluminescence, the most sensitive detection method available. Compared to direct label chemiluminescence, which produces a momentary, single flash of light, enzyme-amplified chemiluminescence produces thousands of light signals per IgE-binding event, generating a sustained light signal and facilitating accurate measurement.

Performance Validation

IMMULITE 2000 Allergy has been validated according to NCCLS document I/LA20-A, an approved guideline for specific-IgE assay performance evaluation.⁴ The following results were obtained from data generated during this validation procedure.

- **Analytical sensitivity:** 0.1 kU/L

A conservative lower limit was determined by running replicates of the zero calibrator (N = 20), plotting two standard deviations above the mean counts per second (kcps) for that calibrator, and then reading the corresponding concentration from a graph of the calibration curve.

- **Calibration range:** 0.1 – 100 kU/L

- **Calibration:** IMMULITE 2000 Allergy is calibrated to the WHO 2nd IRP 75/502 for total IgE.

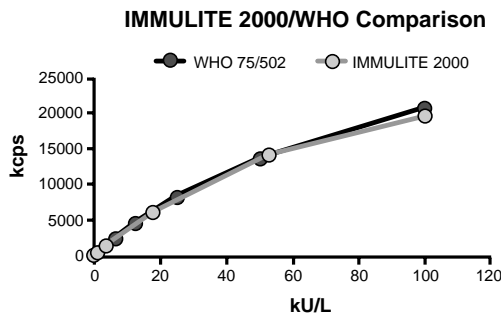


Figure 1.

- **Functional sensitivity:** 0.2 kU/L

IMMULITE 2000 Allergy offers a functional sensitivity of 0.2 kU/L, with a total CV well below 20% at that concentration, as indicated by a precision profile (Figure 2) based on data points generated from an NCCLS EP5 study performed with a variety of allergens and samples.

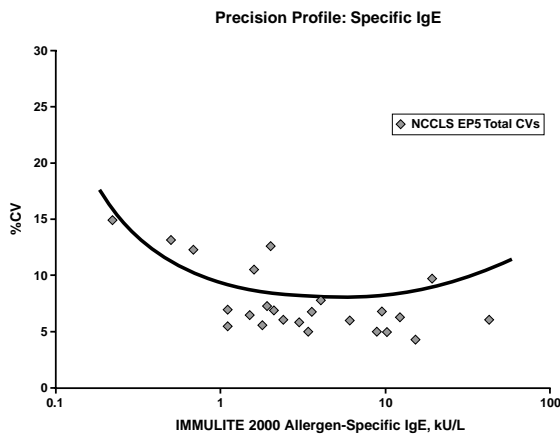


Figure 2.

- **Precision:** Per NCCLS EP5-A protocol: 2 replicates per run, 2 runs per day for 20 days
 - **Intraassay precision:** CVs well below 10%
 - **Total precision:** CVs below 20% (Figure 2)

- **Linearity under dilution:** Excellent linearity down to 0.1 kU/L

Using the IMMULITE 2000 Allergy system, samples and calibrators were diluted with a negative sample down to 0.1 kU/L and below (Figures 4 and 5), compared to 0.5 kU/L for a second-generation FEIA (Figure 3[†]). The dilution factors extended from 1 to beyond 1000 for IMMULITE 2000 Allergy, instead of from 1 to 100 for the second-generation FEIA.

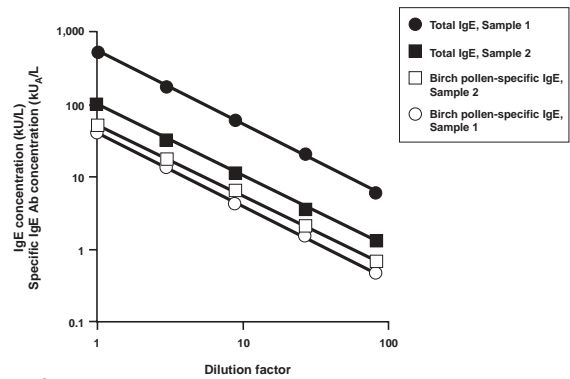


Figure 3.

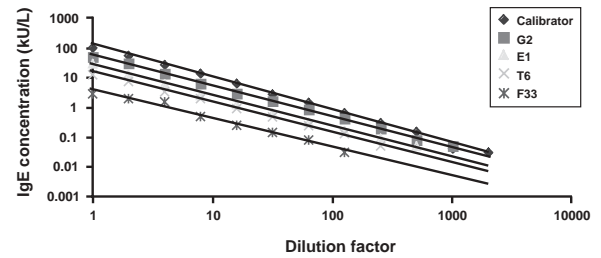


Figure 4.

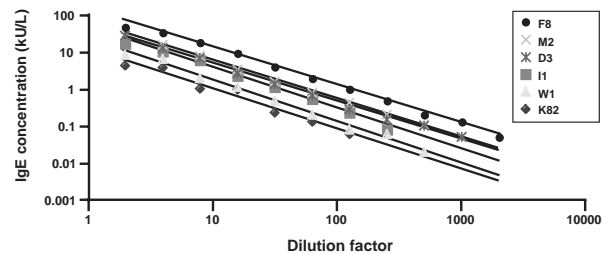


Figure 5.

Linear regression analysis of dilution curves for IMMULITE 2000 Allergy yielded slopes ranging from 0.95 – 1.1 with good correlation coefficients.

[†] Reprinted from JW Yunginger et al.³ Copyright 2000. With permission from Elsevier.

Liquid-phase Allergens

The liquid-phase allergens utilized in IMMULITE 2000 Allergy offer several advantages over solid-phase allergens.⁶ The allergenic proteins of liquid-phase allergens retain their natural conformations because of their liquid backbone, unlike the situation for solid-phase allergens.

This results in enhanced allergen recognition and rapid binding kinetics between patient antibodies and liquid allergens. Liquid-phase allergens also minimize steric hindrance, while solid-phase allergens can physically hinder accessibility of allergenic epitopes. Furthermore, liquid-phase allergens are easy to titer, enabling the optimal allergen-binding capacity to be obtained for each assay, thereby maximizing assay performance.

Another very distinctive advantage of liquid allergens is that they have facilitated development of the complete automation of DPC's allergy assays. One of the combined benefits of liquid allergens and automation is the very low nonspecific binding (NSB) found in IMMULITE 2000 Allergy assays. While NSB has been an issue in allergy assays of previous generations, IMMULITE 2000 Allergy provides efficient tracer removal through the use of both liquid allergens and a unique, proprietary centrifugal wash technique.

Quality Control

DPC conducts quality control evaluations of all extracts, allergens and reagents. Extracts undergo biochemical analysis of allergenic determinants through immunoblotting (Figure 6), while immunological analysis is determined by competitive inhibition studies. Once selected extracts are synthesized into liquid allergens, the allergens are titered for maximal performance, and immunoreactivity is tested using a multisera evaluation with a panel of positive and negative sera. Currently, the allergens receive a 2-year expiration date. The universal reagents are tested and evaluated for lot-to-lot reproducibility.

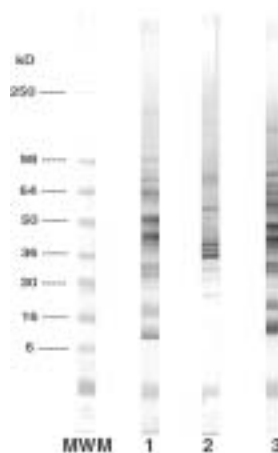


Figure 6. 1- Reference Latex Extract, 2- Evaluation Latex Extract (Rejected), 3- Evaluation Latex Extract (Accepted)

Making Allergy Testing Accessible

The third-generation performance of IMMULITE 2000 Allergy has placed it in a class by itself. Having been validated according to the NCCLS I/LA20-A guidelines, IMMULITE 2000 Allergy has demonstrated spectacular performance, including such characteristics as a 0.1 kU/L detection limit, which is 3.5 times lower than other allergen-specific IgE assays, and linearity under dilutions down to 0.1 kU/L. In addition, its fully automated features allow for a quick time to first result, high throughput, rapid turnaround and immunoassay platform consolidation. These benefits offer all laboratories the opportunity to perform allergy testing according to the medical needs of their region. Never before has allergy testing been so accessible for every laboratory and every patient.

IMMULITE 2000 Allergy currently offers 274 allergens and 34 allergen panels for specific-IgE testing, as well as AlaTOP® Allergy Screen, Total IgE and ECP*.

* Available outside the U.S.

References

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