

Automation of the In Vitro Allergy Laboratory

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The number of potential combinations of specific allergens and patient samples for in vitro allergy testing necessitates the use of varying degrees of automation for efficient, large-volume testing. New reagent technology allows specific IgE testing to be performed without the need for a solid phase. This advance, together with the increasing acceptance of microplates in the laboratory, makes automation of allergy testing both possible and desirable. The use of computers to control a number of open system pipetting instruments reduces labor and minimizes processing and pipetting errors economically. Similarly, computer data handling methods reduce time and error when processing patient information and test results. Desirable features such as high precision, low carryover, high safety, and user-friendly software are presented together with a range of systems currently available. Directions for future developments are discussed. Correlation between manual pipetting and an automated system is shown.

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Introduction

Automation has been accepted in clinical chemistry laboratories for many years, where devices for delivering liquid volumes accurately and precisely are essential tools of the trade. More recently, automated immunoassay procedures and devices that offer the ability to mechanize some or even all of the steps have become available.

Allergy laboratories, however, face a number of challenges not presented to other laboratories. Some of the main issues are as follows:

1. The number of potential combinations of allergens and patient samples that may be requested for in vitro allergy testing is almost limitless, and cannot generally be predicted on a day-to-day basis. In addition, the selection and placing of allergen reagents has been a difficult step to automate when using solid-phase allergosorbent carriers.

2. When ordering specific allergy tests, clinicians may also request IgE tests that employ different methods, reagents, and reporting formats, e.g., multi-allergen tests for atopy and total IgE determinations for both children and adults.
3. While software packages may exist to control individual instruments (robotic sample processor [RSP], gamma counter, plate reader, etc.), there may be no straightforward way of linking these together without a considerable degree of cooperation from the various parties involved. There is often little software interfacing experience in laboratories, and the various combinations of equipment to create a system for allergy testing are numerous.
4. Tube assays are difficult to automate completely and economically; for example, tube washing requires powerful liquid delivery and suction mechanisms that are both costly and large. Such items of equipment tend to stand alone in the laboratory, because their expense often necessitates a far wider use than for allergy testing alone.
5. The variety of laboratory testing procedures that must be accommodated is wide, arising in many instances from differing regulations governing the funding of public in vitro allergy testing from country to country.

In light of these challenges, recent software and instrumentation developments, coupled with the availability of liquid-phase allergens (from Diagnostic Products Corporation—DPC) and miniaturization to the microplate format have made the use of automation a desirable reality, providing efficient, low-error, large-volume testing for the allergologist.

DPC has some five years' experience of automating the sample and allergen distribution stages of the AlaSTAT® assay with a RSP. This has resulted in the development of specifically conceived software and state-of-the-art hardware for processing all stages of the assay in its microplate format (AlaSTAT Milenia®).

General issues affecting sample handling

Blood samples for in vitro allergy testing will be

sent to laboratories of different sizes which may be engaged in different specialities. The specimens may be sent directly from doctors' offices or clinics to centralized laboratories where economies of scale will reduce the cost per test. Alternatively, in vitro testing performed in doctors' offices and clinics may offer a faster turnaround that may offset the reduced cost offered by the larger laboratory. The sample pipetting system must be flexible enough to cope with the variety of vials used and the volumes available to the laboratory.

Patient samples need to be identified to ensure that the correct one is pipetted and that the test results correspond to those of the original vial. This can be achieved by incorporating a barcode reader to correctly identify suitable labelled samples. The sample's barcode is read immediately before pipetting.

Immunoassays of relevance to the allergy laboratory

IgE tests: A wide variety of in vitro tests is available for testing patients with suspected allergic disorders. Total IgE is used for an overall measurement, allergen-specific IgE for measuring the antibodies to individual allergens, and multiallergen IgE tests (e.g., a single test for grass, mite, and cat allergens) for IgE antibodies against a grouping of either horizontal or vertical allergens (1). Specific IgE assays are complicated to pipette, and the reporting of results for each test is also difficult. More than 400 single allergens can be tested, and each individual referral may request any combination of these. The number of potential combinations of samples and allergens makes specific IgE testing unique in the laboratory and necessitates the use of automation for efficient, large-volume testing. The other assays available to the clinician are more user-friendly because only a single analyte is involved.

Allergy assays are now available in a variety of formats from different manufacturers. The allergen reagents can be in either the solid phase or the liquid phase and the assays can be conducted in either microplates or tubes. A choice of different detection methods such as radioactivity (RIA), colorimetry (EIA), or fluorimetry (FEIA) is available. Each of these has a direct bearing on the type of automation that is economically viable. The evolution of miniaturization using microplates has reduced space requirements, while taking advantage of new developments in microplate instrumentation.

Other tests of interest to the allergy laboratory: An analyte of growing importance to the allergologist is cotinine as a marker for nicotine and its intake. Studies have shown that 90% of sufferers of occupational allergies are smokers (Dr. C. McSharry, personal communications), and testing for this metabolite provides an objective indication of smoking status and

intake. This is important because a subject's recall and reporting of smoking status are often inaccurate and unquantifiable.

Cotinine has become the preferred marker of both smoking status and cigarette smoke intake for both smokers and nonsmokers. It is more sensitive and specific than carboxyhemoglobin or thiocyanate as a marker of smoking status, it can be measured reliably at low concentrations, and its excretion appears to be unaffected by physiological changes in urinary pH (2). A double antibody radioimmunoassay procedure is available for this analyte as a liquid-phase competitive assay (DPC Nicotine Metabolite) that can be used with either urine or serum.

Manual operation

Manual processing of specific IgE testing is cumbersome. When using a tube format, the operator must manually generate a worklist and label each reaction tube with both the sample and the allergen identity, and pipette both the samples and the allergens into the reaction tubes. After the incubation, washing, reagent addition, and reading steps, the operator must then reconcile the result with the original test request. In the smaller microplate format, labelling of specific reaction positions is difficult and the operator has to pipette by row and column. The microplate does, however, offer easier reagent addition, washing, incubation, and reading because all the wells in a strip can be processed simultaneously. It is therefore a good format for automation.

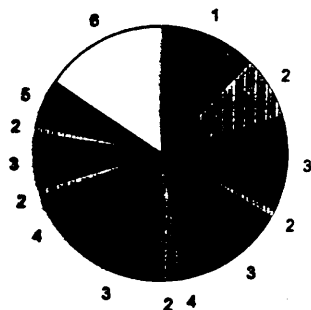
Why automate?

A study comparing the manual versus automated versions of DPC's specific IgE tests was presented at the 1993 Behring Symposium (3). The authors reported a significant saving in technician time (Fig. 1). Automated systems offer repeatability both in terms of precision and accuracy as well as in preparation time. It is the considerable reduction in preparation time that leads to a more reproducible assay because any drift is reduced to a minimum. Consequently, more tests can be made in a single assay, and this in turn reduces the average turnaround time of the laboratory. This allows the operator to leave the instrument and perform other tasks. The automated system is integrated through efficient software that transfers data through the system, eliminating the possibility of transcription errors. The software deals with issues ranging from downloading test requests from a mainframe computer to printing patient reports.

What are the desirable features of an allergy pipetting system?

An ideal system for allergy testing has many attributes, not all of which are essential. It should be

Manual AlaSTAT Tube (85 tests)



Automated AlaSTAT Milenia (85 Tests)

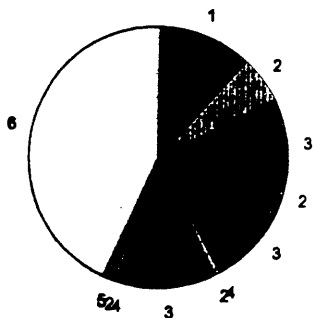


Fig. 1 Times taken to perform an AlaSTAT tube assay (by hand) and an AlaSTAT Milenia assay (automated) as a proportion of the technician's 8-hour day. An overall saving of more than 2 hours is made using the automated system, and the technician's time is reduced by about 5 hours.

- 1) Preparation
- 2) Reagent addition
- 3) Incubation
- 4) Washing
- 5) Reading
- 6) Happy hours

easy to maintain and should reduce operator contact with biohazardous materials. The RSP for pipetting sample and allergen should perform precisely and accurately without carryover from one reaction position to another. An open system approach is ideal for the allergy laboratory because of its flexibility for running assays in a wide variety of formats including specific IgE testing. To reduce drift and turnaround time, this system should provide a rapid distribution of sample and allergen. The RSP should be able to handle pediatric tubes without major procedural change as well as barcoded primary tubes. Microplate processors should offer reagent addition, shaking, efficient washing, and reading. Reagent addition systems should operate with

a minimum of waste and be able to add reagents to reaction positions rapidly and precisely.

These requirements are complex and can be controlled most effectively with computer software that should handle the data communication between the instruments to eliminate transcription errors. Test requests for other assays should be integrated with the data even if they were performed manually. Other assays that can be performed on the system, such as total IgE, should have the test requests entered on the same worksheet. Interfaces with laboratory information management systems (LIMS) enable test requests to be downloaded from hospital computers. This information, coupled with barcoded sample tubes, can help reduce operator error. The software should be able to create a wide variety of reports, maintain doctor and laboratory information, and allow for data archiving and retrieval.

What systems are available?

Semi-Automated systems: The volume of tests performed will dictate the degree of automation desirable for the allergy laboratory. Semi-automated systems exist for the smaller laboratory where parts of the assay are processed by instruments, but there is still some degree of manual intervention. These systems generally use the microplate format to enable rapid transfer from one system component to another. Computerized worklist generation, coupled with a pipetting guide that uses light arrays to direct the technician in dispensing each allergen and patient sample to microplate wells, helps to minimize errors in assay setup and pipetting. The microplates are then incubated, and reagent addition is performed manually, while washing and reading steps are automated. Raw data generated by a reader can then be processed by system software so as to produce patient and statistical reports.

The following companies offer light-guided systems for distributing samples and allergen reagents into microplates: DPC (Milenia MicroLite 3), Kabi-Pharmacia (ImmunoCAP pipetting guide 96), and Sanofi (Allergy Microplate 384).

The semi-automated DPC system is centered on MAXsoft, a software package to control a number of small instruments in the allergy laboratory for microplate automation. The Milenia MicroLite 3 is a pipetting guide that uses multicolored light-emitting diodes (LED) to illuminate the microplate wells in order to indicate where to pipette sample or allergen. The Milenia MicroMix 4 is a microplate shaker offering consistent electronically controlled shaking for room temperature incubations. The Milenia MicroWash is a flexible microplate washer that can perform four wash cycles on a 96-well plate in approximately 5 minutes. Plate reading is accomplished by the Milenia Analyzer which passes raw data to MAXsoft for data reduction

and reporting. MAXsoft optimizes the pipetting sequence so that samples are pipetted to random positions in the plate and allergens are always pipetted into vertically adjacent wells. This reduces the time taken to pipette allergens and thus increases the number of tests a technician can perform in a single assay.

Automated systems: By far the biggest savings in labor are offered by the automated systems. MAXsoft, with the same user interface as the semi-automated system, can control the RSP (DPC MARK 5) and the microplate processor (MileniaMAX). After sample and allergen pipetting has been completed on the MARK 5, the technician need only move the microplate to the MileniaMAX for automated shaking, reagent addition, washing, and reading. The MARK 5 eliminates the possibility of incorrect sample and allergen combinations being pipetted and is optimized to pipette the allergens very quickly. The MileniaMAX offers reproducible timing for all the assay steps without the need for intervention. Such a system gives results comparable to manually pipetted assays (Fig. 2). This is an open system and can be used with all the different microplate format assays in the laboratory, not only the specific IgE determinations.

MARK 5 features: The MARK 5 is a high-speed four-probe RSP. Its 1.25-mL syringes and teflon-coated probes are able to offer high precision on all four channels and good accuracy. Some recent carry-over studies with elevated morphine samples have demonstrated figures lower than 1 in 10^5 . The level-sensing system employed is a conductive concentric probe that is able to handle most shapes and sizes of sample tube, including pediatric samples batched together with adult ones with no special rack requirements. The four channels are independently switched and therefore can accommodate wide differences in serum levels. The probes are free-fall and maintain a high degree of safety in that they are unable to puncture skin.

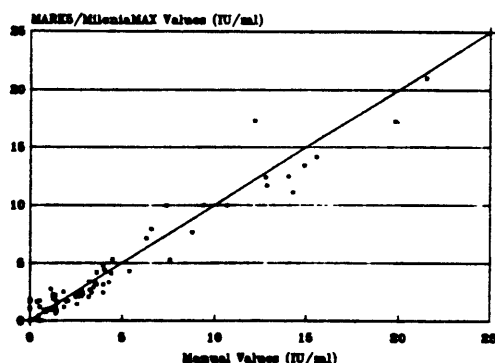


Fig. 2 Correlation of manual and automated AlaSTAT Milenia. 83 sample-allergen combinations were pipetted both manually and automatically. The graph's equation is $y = 0.93x + 0.21$.

Samples and calibrator distribution are optimized for precision. The instrument first aspirates an airgap, followed by sensing of the serum surface using the MARK 5 conductive probes. Sufficient serum for one test is then aspirated plus a small excess volume (both of which may be user-defined), and following a tip wash, the patient volume is dispensed into the reaction position. For liquid allergen distribution, the instrument is optimized for speed. The MARK 5 aspirates enough allergen for several tests and rapidly dispenses them to the reaction positions with a degree of precision suitable for this step in the assay (Table 1). This reduces assay drift, which in turn increases the maximum number of tests that can be practically run in one batch.

In addition, the MARK 5 can be readily configured, using a simple keypad, for standard assays in batch mode, or a Windows®-based interface can provide random access handling for routine assays.

MileniaMAX automated plate processor: Dynatech Medical Products Ltd. (Guernsey) is the original equipment manufacturer of the MileniaMAX automated plate processor. The instrument manages all the reagent addition steps other than allergen addition, and provides washing, shaking, high-speed substrate addition, and reading capability. Raw data are returned to the software for data reduction and reporting. There is no need for manual intervention, except to transfer the plates to the instrument after sample and allergen pipetting. When under the control of MAXsoft, the instrument is dedicated to allergy, but it can also be used for a wide variety of other applications with a flexible Windows®-based software package (Dynatech).

The MileniaMAX offers a high-precision reagent addition head and multiway heads for rapid washing and substrate addition. A pressure system controls the dispensing of reagent and washing liquid. The integral colorimetric microplate reader supports a kinetic read process for the color development cycle, after which the raw data are collected by MAXsoft for results reporting.

MAXsoft offers flexible reporting of results as a further step toward reducing laboratory time and errors in transcription. Reports can be generated in a user-definable manner for all the tests performed on a

TABLE 1 Precision of sample and allergen dispensing on the MARK 5 using tracer. The instrument was optimized for maximum speed rather than precision.

	Samples at 25 μ L	Allergens at 50 μ L
Mean (counts per minute)	16,403	42,290
SD	398	1,477
CV%	2.4	3.5
n	88	74

patient sample. This also includes any total IgE tests, and the technician can also type in results from any additional test that may not even have been performed in the same laboratory. The report can include the requesting clinician's name and address, rather than the laboratories, so that this information can be given directly to patients without any transcription. MAX-software can also give statistical reports on the quantity of each allergen ordered and the results. This can be used both for analysis and for optimizing the MARK 5's pipetting by placing the most commonly used allergens at the start of the allergen rack on the instrument.

In larger laboratories, further savings in attendance time and reduction in technician error can be gained by the use of barcoded sample tubes, an interface to a LIMS, and an instrument for reading barcoded sample tubes. The technician does not need to concentrate on placing sample tubes in the correct location for pipetting by the MARK 5. The technician (a) downloads the test requests from the LIMS, (b) places the samples on the barcode reader, allergens, controls, and standards on the MARK 5, (c) starts the MARK 5 pipetting samples and allergens, (d) puts reagents and wash solutions on the MileniaMAX, and (e) transfers the plates to the MileniaMAX. After the assay is completed, the reduced data are uploaded to the LIMS for centralized reporting.

To our knowledge, none of the currently available fully automated closed systems offers allergy among their range of assays (4). Kabi-Pharmacia offers an automated system that is integrated using the MasterCAP software. Samples and IgE-specific allergen CAPs are dispensed into the microplate. The plate then has to be transferred to the AutoCAP for reagent addition, incubation, and washing. The plate is then read on a fluorimeter after a second plate transfer. Results are available for printing patient reports and archiving.

Conclusion

Automation provides a method both for reducing the average turnaround time to produce accurate results and for simplifying the process for technicians. At present, there is no fully automated system capable of performing allergy tests, but combinations of currently available instruments, integrated with software, offer systems that require a minimum of operator intervention. Assays using the microplate format are easier to automate than the older tube-based assays and generally require less time to complete. The use of

liquid-phase allergens rather than solid-phase techniques enables a system to be created using only liquid handling components because all the allergens can be delivered using the same system that is used to deliver samples. Open systems offer the allergy laboratory the opportunity to automate parts of a variety of other tests that may not even be in microplate format, as well as the allergen-specific IgE test. At present, these attributes are best satisfied by the AlaSTAT Milenia system.

There is much scope for further automation in the allergy laboratory. None of the systems currently available is fully automated because the operator is required to move microplates during the assay process. The systems currently available do, however, free the technician for a much greater time than when using manual techniques. Barcoding of reagents and allergens would further reduce the possibility of operator error, especially in view of the potentially large number of allergens in use in the laboratory. Computer software could be further enhanced to help with the interpretation of results by being able to report on testing histories and any patient questionnaires that may have been used.

The past few years have seen rapid advances in the automation of the allergy laboratory, and all the advances in the assay format, the instrumentation, and the software are leading toward a totally automated solution.

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From EURO/DPC Ltd., Llanberis, Gwynedd, UK (Goodman, Meredith) and Diagnostic Products Corporation, Los Angeles, CA (Weiss).

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