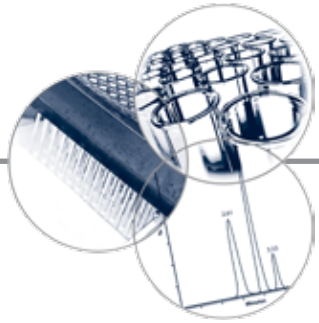

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Automated immunoassay equipment platforms for analytical support of pharmaceutical and biopharmaceutical development

Laboratory automation is not new, but few scientists have been exposed to the wide range of analytical equipment platforms, which have been available from diagnostic and research companies, with many workers focusing on one or the other disciplines throughout their career. However, many such instrument platforms play an important role in drug-development in laboratories around the world. This review covers some of the experiences I have had in what is nearly 40 years in laboratory analysis – the last 18 years being in CROs supporting pharmaceutical development. There are many platforms that I have used, which are not included here since the focus of the article is on immunoassay techniques. I think it is worthy to note that many of the capabilities within modern platforms, from a wide range of manufacturers, would appear to me to have a 'genetic' link back to the first automated analyzers launched over 50 years ago. It has been interesting to take a walk down the development road of these platforms over that timeframe and, no doubt, will continue to be at least equally of interest in the future.

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Analytical support in drug-development today covers a wide range of techniques and equipment platforms. Whilst areas in analytical sciences such as diagnostics have continually made progress and developed an increasing number of automatic utilities into a wide range of platforms, instrumentation used in research laboratories, it could be argued, by comparison, has developed little over the last 15–20 years.

Furthermore, many laboratories involved in bioanalysis in drug-development (whether pharmaceutical companies, biotechnology companies or CROs) often do not get exposed to instrumentation used in diagnostics and so do not learn of the capabilities already present in these platforms that can enhance their use today. Consequently, feedback to manufacturers of **robotics** used in research seems to have been spasmodic at best and thus the manufacturers would appear to have had little impetus to introduce new developments and enhancements to the capabilities of their systems. Notable efforts have been made such as the discussion groups within the American Association of Pharmaceutical Scientists; for example, 'The Twenty-First Century Laboratory', where a 'wish-list' of equipment capabilities was proposed. Having attended at least one of those meetings, the point made above was very relevant, since virtually everything that was on that list was already available on a number of diagnostic platforms, and had been for some time (see list of capabilities later). A very good chapter called 'The Application of Automation in Ligand-Binding Assays' that addresses research platforms can be found in the excellent book edited by Khan and Findlay [1].

Today, we see 'new' models of many xyz robotics that still lack some of the enhancements – particularly in user-interface programming – that have been present in some open diagnostic platforms for over 15 years.

The purpose of this article is to look at how we can use **automation** in bioanalytical immunoassay services today to improve and enhance both the quality of the science as well as assist in the management of the laboratory – including increasing analytical capacity and throughput.

The history of automation

Automation in laboratories is by no means a new concept. It has – not surprisingly – been lead by clinical diagnostic services, owing to the very large workload they endure and also seeking quicker and quicker turnaround times to better serve patient healthcare due to the critical clinical situations that often present themselves to hospital emergency rooms.

Nearly 40 years ago, some of the methods used for the measurement of certain analytes in blood, for which results were needed 'urgently,' could take from 30 min to several hours. They were done separately using different instruments

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Key Terms

Robotics: The application of automated machinery to tasks traditionally done by hand.

Automation: The techniques and equipment used to achieve automatic operation.

and turnaround time did not meet clinical demand requirements. Therefore, many systems have been developed over the years to improve upon this situation. These started in the areas that had the largest routine workload – mainly 'wet' chemistry methods, and saw the first fully automated analyzers launched in 1957 (Figure 1) – some 54 years ago. These were 'continuous flow' systems – basically a single stream of air-segmented fluid continually added to or modified – that allowed the reactions of the assays to take place, have the final spectrophotometric reading using a flow cell and then discard to waste. A test-tube assay automated start to finish. It revolutionized clinical laboratories worldwide.

These early systems – and their more computerized second and third generations – were seen to be unnecessarily wasteful of reagents and samples and R&D groups with manufacturers looked for ways to improve this. What developed were known as 'random access' analyzers. Here, the continuous flow system was replaced by discrete reaction vessels reducing waste almost to zero, but also allowing a specific selection of tests rather than having to conduct a panel of tests on all samples. Since this time, other areas of analysis have attracted attention for automation and immunoassay is one of those key areas – translating technology developed in standard 'wet' chemistry to the requirements of immunoassay.

Automation development in immunoassay

Immunoassay originated as radioisotopic methods – good old RIA (radio-immunoassay). However, these methods left little room for automation of the tube techniques and most development focused on improving throughput on the radioactive gamma counters used – moving from single- to multi-well detectors. With the development of 96-well microplate technology, the first 'xyz' robotics were seen and used in the early 1980s (e.g., Tecan [101], Hamilton [102]). At this stage, they were simply programmable on a protocol-by-protocol basis and were unsophisticated in being unable to organize batches of different assays simultaneously in an automated and efficient way.

Here, we saw the same systems entering both research and diagnostics laboratories but whereas little really developed to dramatically change the field of robotics in research, much was happening in the diagnostic industry.

In 1979, Abbott diagnostics introduced the Quantum II – an automated immunoassay system for enzyme immunoassays. In 1981, Abbott again launched the TDx system, which incorporated the first commercial application of fluorescence polarization, and followed it in 1988 with the IMx system. The TDx and IMx were widely used in many labs. These were systems designed to conduct one batch of a specific method at a time – typically 20–30 samples. What followed these 'first-generation' platforms were systems that would be able to have the same random-access capabilities from a large assay repertoire, which was a feature of the earlier wet chemistry analyzers.

The early 1990s saw a number of these launched including the Immulite (1993) [103] by DPC (now Siemens) and the Axsym (1994) by Abbott (Figure 2) [104].

These latest systems saw some additional capabilities that enhanced laboratory management and sample processing of immunoassays. Again, many of these had been developed on previous clinical chemistry systems and were simply incorporated into these multi-analyte capability systems. This included:

- Barcode reading of primary sample tubes;
- Liquid-level sensing of both samples and reagents;
- Clot detection;
- Volume checks of reagents on board;
- Checks on authenticity, placement and expiry dates of reagents via barcodes;
- Calibration protocols;
- On-board QC programs;
- Data reduction and direct output of final concentration results;
- Uni- and bi-directional interfaces with laboratory information management systems.

Figure 1. The Technician AutoAnalyzer 1. From right to left: reagents, autosampler (rear), peristaltic pump (fluidics), dialyser (protein removal), incubator (rear), double-beam spectrophotometer (front), chart record.
Images courtesy of manufacturers.

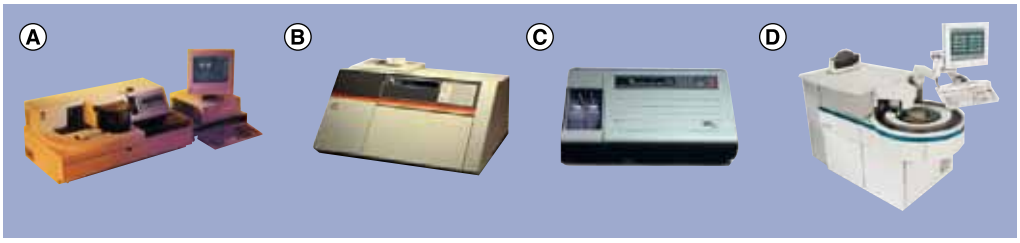

Figure 2. Automated immunoassay platforms. (A) Immulite 1000 (now Siemens). **(B)** Abbott TDx. **(C)** Abbot IMx. **(D)** Abbott Axsym.
Images courtesy of manufacturers.

The drawback of these systems versus the xyz robotics and 96-well microplate assays were two-fold. Firstly, they were (and still are) 'closed' systems, meaning that you could only use reagents from the same manufacturer as the platform itself and existing methods could not be reoptimized on these systems. Secondly, new assays for different analytes to those on the equipment's repertoire could not be developed, so that they had little place to play in large parts of the drug-development pathway – especially for pharmacokinetic (PK) and immunogenicity assays.

During the same period, whilst advances in xyz robotics were being made, and some of the components above added to their capabilities, what was largely lacking was a user-friendly interface to program the instruments for new assay protocols. Most systems today still suffer from this problem with new protocols often taking hours or even days to program. Thereafter, some require significant checking to ensure that the platform actually executes the program as expected and that there are no conflicting instructions to the robot that could cause problems.

Some manufacturers of 96-well microplate reagent kits in diagnostics looked upon this as an opportunity and sought to develop xyz robotics that could automate a microplate assay – including in-house ones – but also overcome the programming issue for new protocols. Others looked to developing everything, but the robotics, coming up with what was basically a specific plate washer and reader for their assays (e.g., Amersham Amerlite). Moreover, there have been a number of platforms that have developed to run a specific part of an assay protocol such as SPE or liquid extraction. Some of these moved quickly into 96-pipette head processing, allowing simultaneous processing of 96 samples, which dramatically improved throughput and capacity over manual methods. The most common of these that I have come across are those manufactured by Tomtec (Figure 3) [105]. It should be noted that more recent models discussed later also offer this as part of a larger platform that can process the other parts of the analytical method in addition to the extraction.

From my experience, the first truly 'open' system that I used that could claim full automation from start to finish for these methods and also demonstrate user-friendly software as its front-end interface with the user was launched in the UK in 1998 by Grifols – the Triturus (Figure 3) [106]. Typically, we trained

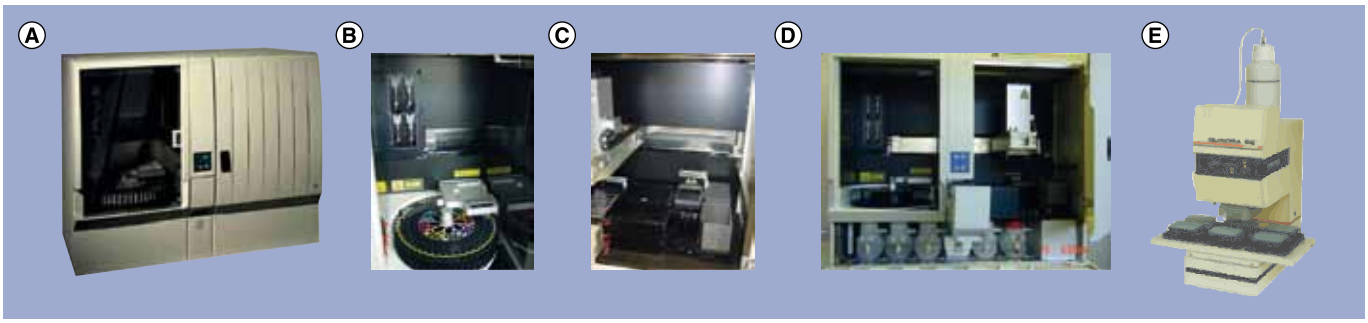

Figure 3. The Grifols Triturus. (A) Triturus. **(B)** Sample compartment with carousel for samples, QCs and calibrators. **(C)** Reagent, shaker/incubator, washer and reader compartment. **(D)** Front of instrument showing all compartments including shared fluidics and waste collection/disposal. **(E)** TomtecQuadra 96 – one of the earliest 96-pipette head processing robots.
Images courtesy of manufacturers.

Key Term

Multiplex: Multiple analytical methods that are run simultaneously within the same reaction vessel.

new operators on the instrument in less than half a day and, thereafter, new assay protocols would take about 30 min or so to program. The system has multiple self checks in the software that assist the operator and numerous automatic checks that are performed when the 'go' button is pressed. These typically ensure that all the samples and reagents are in the right positions and that there are adequate volumes on board for the programmed batches.

For those of us that really evolved immunoassay services for our laboratories throughout this period, it is interesting to look back upon how it has changed in its application from the period immediately prior to development of these platforms. Whilst outside the scope of this article, for those with an interest to review the history of immunoassays more fully, I recommend 'Immunoassays for the 80s' by Voller, Bartlett and Bidwell [2].

Throughout this period of development, it was clear that as well as looking at automation of manual processes, manufacturers were also investigating the use of new techniques to improve the performance of the analytical methods. For example, improving analyte specificity was (and still is) a driving force for some analytes and different detection and separation technologies became common in some platforms. These included fluorescence (e.g., delayed enhanced lanthanide fluorescence immunoassay [DELFIA] and time-resolved fluorescence), chemiluminescence (e.g., Amerlite) and magnetic bead separation. Additionally we saw the introduction of electrochemiluminescence to the assay repertoire.

Multiplex & other specialist platforms

Not long after the Triturus was launched, we also saw the arrival of new immunoassays called '**multiplex**' methods. This seemed, and was, a big technological step forward in the science itself. Luminex was the first in the field, introducing its xMap technology in 1999 [107]. We were fortunate to buy one of the first instruments in the UK and have gained some really interesting knowledge and experiences using it and the many methods that have now been developed for it. One of the benefits of this is the fact that it is really a flow cytometer and reagents and commercial methods using the technology have

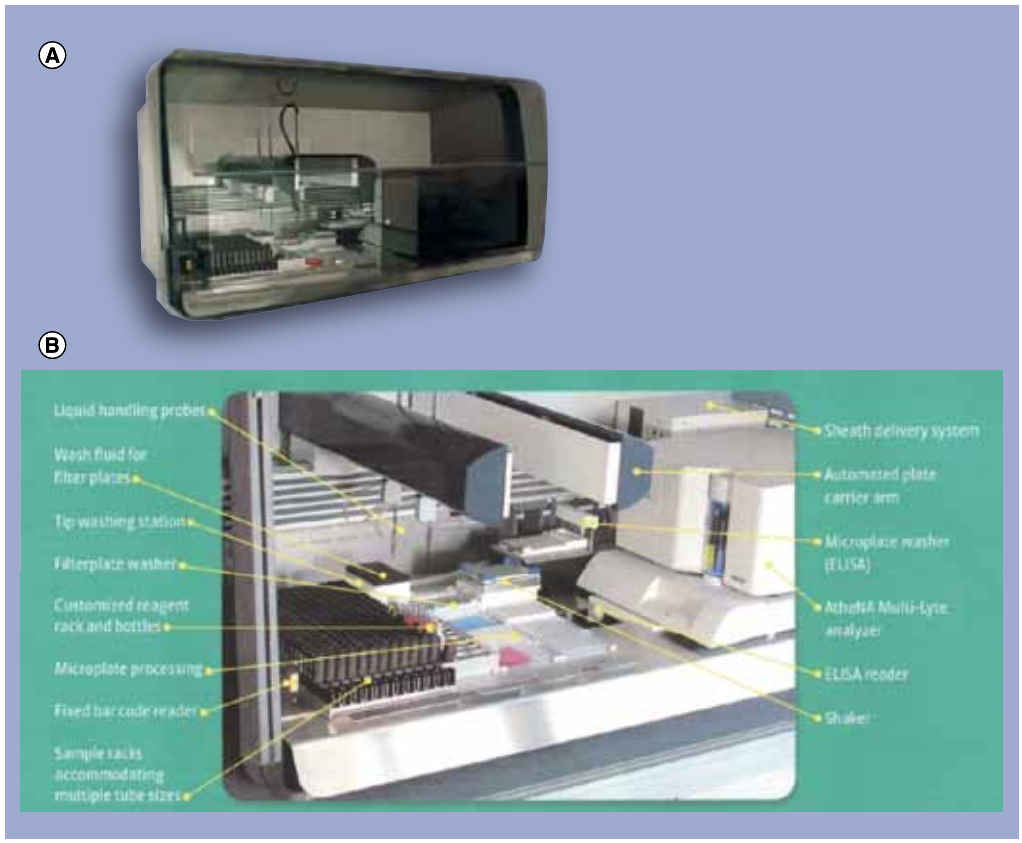

Figure 4. XYZ Robots with integrated specialist detectors. (A) Automated Multiplex Platforms (AIMS). **(B)** AIMS incorporating a Luminex xMAP platform (AtheNA Multi-Lyte® version). Images courtesy of manufacturers.

been developed by a wide range of manufacturers – so it is an 'open' system. That is, we are not restricted to the manufacturer of the instrument also being the sole supplier of reagents.

xMap technology platforms are not automated systems but really are the detectors for the end point of the methods. However, I have included them here since recently some progress has been made with robotics that allows instruments, such as the Luminex, to be incorporated into the robotic system itself and supply a fully automated 'hands-off' approach from start to finish of the assay method. One such platform is the Automated Immunoassay Multiplex System (AIMS®) from ZEUS Scientific (Figure 4) [108].

Whilst AIMS was one of the earliest to integrate these machines, other manufacturers now have platforms that can integrate a fairly wide variety of analytical equipment – not only detectors but instruments such as centrifuges, decappers, dryers, thermocyclers, cryostats and others. Hamilton and TECAN, for example, are two companies that claim this type of capability.

One of the benefits that we have seen with xMAP technology is that it has been outlicensed to multiple companies (e.g., Bio-Rad [Bioplex], ZEUS Scientific [AtheNA Multi-Lyte], Millipore [Milliplex] and so on), and all of these companies have developed a range of assays that are commercially available. Some companies have made physical hardware changes to the instrument or developed their own software (e.g., Bio-Rad). This gives a tremendous advantage in terms of variety of possible uses and availability of assay methods that are not restricted to just the instrument manufacturer itself – a potential problem with other platforms.

In addition to having less flexibility if a platform is restricted to a single provider of kits/reagents, there is also a potentially higher business risk to companies using such platforms depending upon the manufacturer's size and financial stability. Many organizations are very risk-averse in this particular area following the issues raised by the Bioveris demise a few years ago.

As well as xMAP technology, other examples of multiplex systems include the Mesoscale Discovery [109] and Aushon Searchlight systems [110]. xMAP platforms use microspheres as the solid-phase upon which to build an immunoassay. Spheres can be specifically labelled with dye combinations and antibodies to allow analyte coding and detection. The end point is measured using a flow cytometer that both identifies the sphere by the dye (and hence identifies the analyte) and also the end point of the immunoassay by the intensity of the final signal. Mesoscale Discovery uses electrochemiluminescence with proprietary microplates that have multiple detection electrodes located in the bottom of each well of the plate – each with a specific label for a particular analyte. The Aushen Searchlight uses chemiluminescence and also has multiple analytes per well by utilizing antibody array spotting. Here, the brightness of each luminescent end point is captured using a charge-coupled device camera in a standalone detector.

However, innovation continues to thrive in analytical sciences and manufacturers continue to invest in R&D of equipment platforms – some of these making radical moves in terms of technology. For many years we have seen that the most widespread technique has been the microplate – for various functions – not only the standard 96-well plate we see in many immunoassays, but also deep-well plates used for sample storage and sampling or extraction processes. Other microplates with larger numbers of smaller wells have been used with automated platforms, however most of these techniques have been in the discovery and high-throughput screening arena and rarely do these assays make their way through to 'production' assays that can be used in many laboratories or a more routine environment.

Randox evidence

In 2004, I presented at the Bioval Conference in London, UK, on the topic of 'Biomarkers in Drug Development,' where I covered a range of analytical platforms available. In that presentation, I put a slide about the Randox Evidence – the world's first protein Biochip Array Technology system.

My slide questioned whether this system may be the future of biomarker analysis, since some of the metrics being claimed were highly impressive:

- >1500 tests/h;
- 25 markers per chip;
- As little as 7 µl of sample required;
- Multiple matrices;
- Other automated capabilities as discussed above for diagnostic systems.

I believe that Randox concentrated in the early days on routine diagnostic assay arrays (cardiac, endocrine, fertility, metabolic, thyroid and tumor markers). In addition, they developed

Key Term

Microfluidics: The behavior, precise control and manipulation of fluids that are geometrically constrained to a small, typically submillimeter scale.

a number of separate immunoassays useful in diagnostics and therapeutic drug monitoring; molecular arrays related to microbiological diseases, toxicology arrays for drugs of abuse and arrays for drug residues have now been developed on this analyzer. Furthermore, and of more interest to this article, is their range of research arrays – comparable to many of the multiplex arrays available from manufacturers of assays for xMAP, MSD and Aushon technology.

Performance claims are impressive and there are a number of articles in the public domain illustrating its performance over long periods of time in independent laboratories [3].

Interestingly, they have developed different size models to fit different lab requirements – from a small bench-top detector/reader, where the chip assays are processed manually, through full automation in both random-access and batch-based models.

Randox appears to be one of the few major diagnostic companies to get heavily involved in the development of research assays for use on their accredited diagnostic platforms. Their longevity and experience over their 30 years existence of manufacturing research and diagnostic products for use over a wide range of analytical platforms gives a lot of confidence for future support and financial stability. Moreover, their attitude to developing and manufacturing research assays in exactly the same way as they do their diagnostic products gives confidence in the quality of their products. The range of platforms can be seen below in Figure 5.

Gyros gyrolab

Early in 2000, we started hearing about a new nanotechnology workstation, manufactured by Gyros of Sweden – the Gyrolab [111]. This was a true innovation whereby immunoassays were developed in a microvessel within what appears to be very similar to a compact disc – indeed most users today call them CDs.

The Gyros evolved from early microfluidic system research at Pharmacia Biotech (which later became Amersham Biosciences) in Uppsala, Sweden, which began in 1989 and ended with the forming of Gyros AB as an independent company in 2000. At this stage, Gyros owned an extensive portfolio of over 40 patents related to **microfluidics**, CD manufacture, system components, surface chemistry and specific application areas.

Gyros had a wide range of challenges to overcome in entering the microfludic world, which are beyond the scope of this article, but one very interesting point is that when working at the nanoliter scale, scaling laws become very significant, in that surface tension becomes a more dominant force than gravity [4].

Unlike microplate technology where both manual and automated methods rely on very precise volume control pipettes or fluidics, respectively, here all the volume control was contained within the prefabricated CD. Everything is overfilled slightly with excess removed by gentle centrifugation and critical volumes maintained within the microvessel by hydrophobic barriers. Once the excess is spun to waste in this way, more rigorous centrifugation forces the accurate volume of remaining fluids through the hydrophobic barriers and over the solid-phase contained in the bottom of the vessel (Figure 6).

The Gyrolab brings a number of significant benefits to the laboratory with regard to immunoassay methods in biological fluids that can be challenging:

- Reagents: the nanotechnology significantly reduces the volume of reagents used and this means that assays developed to support large projects (e.g., a PK assay in a Phase III study) or large numbers of samples, will require a much

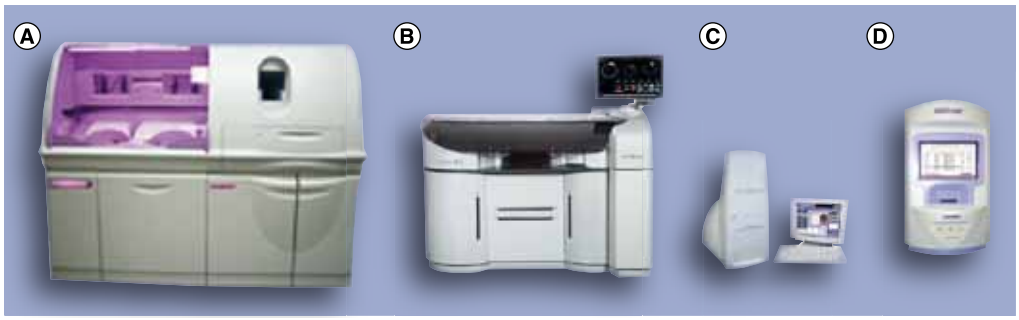

Figure 5. Range of Randox Evidence Platform. (A) Evidence, **(B)** Evidence Evolution **(C)** Evidence Investigator and **(D)** Evidence Multistat.
Images courtesy of manufacturers.

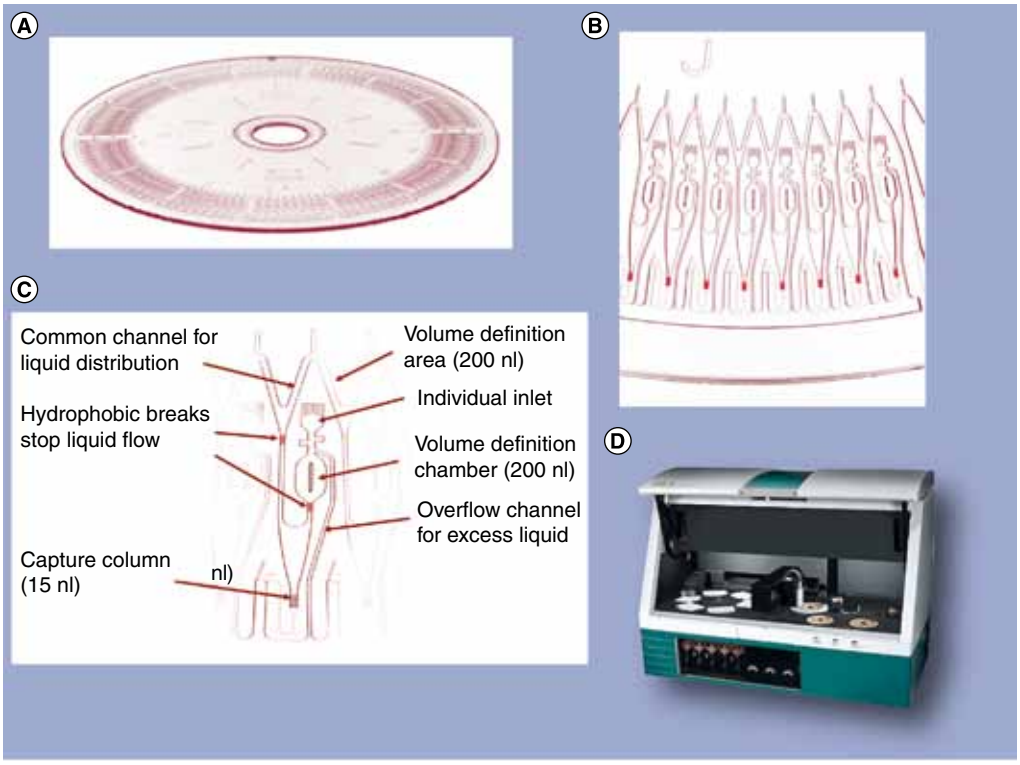

Figure 6. The Gyros Gyrolab. (A) CD. **(B)** Eight microvessel section of CD. **(C)** Microvessel with description of components. **(D)** Gyrolab instrument.
Images courtesy of manufacturers.

smaller amount of antibody to support the continued use of the method. Antibody production is not an insignificant cost and so this has considerable cost-saving implications over the course of the study or drug-development program;

- The reaction times of the antigen–antibody interactions are vastly reduced – it takes 5–7 s for the sample to flow through the 15 nl column, thereby minimizing incubation and contact time. This has three major effects. It reduces the overall time of the analytical method (each method takes around 1 h to complete). Due to this, new methods can be developed much more quickly, since multiple runs can be conducted in a single day (there is also a very good method development software package onboard that further assists this). The shortness of the antigen–antibody interaction times means that there is very little chance for nonspecific binding and so potential matrix effects seen in some methods can be eliminated in many cases; and is also why the system can tolerate 50% matrix. A further effect of this, of course, is that antibodies typically need to be have a reasonable degree of, or be high affinity;
- Samples: as with reagent volumes, the same is true for sample volume. Even given minimum dead volume requirements, the volume of sample required equates to around 5–10 µl. Since most biological fluids are diluted a minimum of 1:2; this volume allows for multiple sampling, which makes it a very useful tool where biological fluid matrix is rare – whether due to the patient (e.g., pediatrics) or the matrix (e.g., cerebrospinal fluid or tears).

Miniaturize and integrate sandwich immunoassays

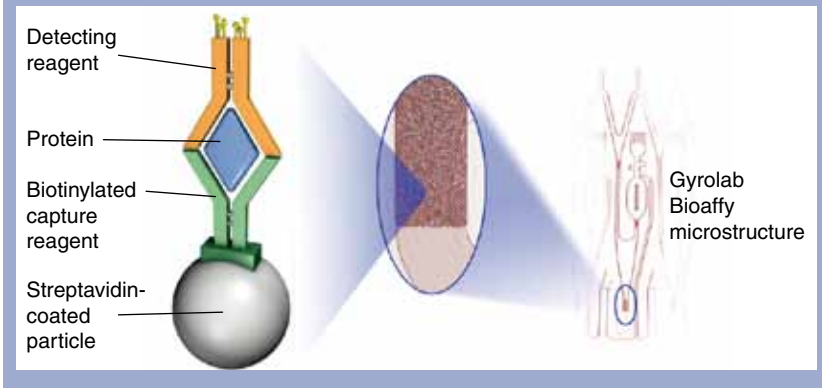

Figure 7. Illustration of sandwich immunoassay.
Image courtesy of manufacturers.

The immunoassay reactions take place using this solid-phase and the final signal is detected using laser-induced fluorescence (FIGURE 7).

Personally, I am very interested in the potential future developments of this platform – and understand that fully automated antidrug antibody (ADA) assays, which require an acid dissociation step, may be available soon.

Practical use of instrument platforms in drug-development

Clearly, these different instrument platforms have many utilities to help laboratories support the analytical requirements of drug-development. Depending upon assay methods used, they can have considerable impact upon processing samples for various requirements.

In small-molecule development, immunoassays have largely been limited to analysis of biomarkers for various purposes. Here, as demonstrated above, we have a potentially wide selection of platforms from both research and diagnostics to assist us in the support of this work. However, in the larger-molecule development of biological therapies, the scope for immunoassay widens into both PK and ADA/immunogenicity assays, in addition to biomarker requirements.

In certain circumstances, where trial design and analyte choice has allowed, we have found it possible to use a fully automated platform to measure multiple analytes (in separate methods – i.e., not multiplexed) to produce PK and biomarker pharmacodynamic (PD) data simultaneously on the same sample aliquot. Obviously, when such opportunities arise, there are tremendous efficiencies and other benefits to the management and conduct of the study. Typically, smaller volumes of biological fluid and fewer number of aliquots are required to be produced. When you consider the organization of producing these, labeling appropriately, the cost of consumables, storage post collection at investigator sites and laboratories and the logistics costs of fewer shipments, financial savings can be considerable and the practical aspects of conducting the collection, storage and so on is much simpler.

Automated versus manual methods

It is relatively easy to see how automation can improve performance characteristics of methods when compared with the same technique conducted manually. If we take a look at a 'standard' 96-well microplate ELISA method, it can be broken down into several different steps:

- Dilution of samples;
- Pipetting calibrators, QCs and samples into the plate;
- Pipetting reagents into the plate;
- Incubation(s) (with or without shaking) – possibly at different temperatures;
- Washing the plate;
- Reading the end point of the reaction.

All these steps have intrinsic errors to them and some of those errors can be reduced by automation, notably:

- Accurate and precise fluidics that usually have better precision than manual pipetting. This actually has a cumulative reduction in the overall error of the method, since there are always several pipetting steps in each assay – each one with a potential improvement. So, even if the difference in the precision of the automated versus manual pipetting is only marginally improved, the cumulative effect has a real impact on the overall system performance;
- Incubation times that are always accurately controlled. How many times does a manual assay overrun its incubation due to the operator being unavailable to conduct the next step at the exact time required? Whilst this will not necessarily affect the results of the individual plate significantly, it can contribute to increased variability on an interbatch basis, since prolonged incubation will have an impact upon final raw data response;
- Temperature control for incubation – in our experience, fewer problems occur within automated systems than moving plates manually between separate instruments for each of the steps in an assay. Platform-to-platform variation is also overcome;
- Plate washing – some robotics allow for different and specific wash programs for different types of plates (e.g., flat, rounded or conical-bottomed). This degree of sophistication may not be available in some standalone plate washers. Problems with plate washing often causes problems in immunoassays and having this process standardized can improve a method's performance when viewed over multiple batches dramatically;
- Reading the final end point of the method – the real advantage of automation here is that the time of the reading is always the same.

Once again, the major impact here is across multiple batches when looking at assay performance.

The overall impact of all of these together is seen in the full system response. Firstly, the raw data tends to be more reproducible from batch-to-batch. FIGURE 8 shows comparison of the raw data responses of several batches for three assays for the same biomarker (Amyloid AB 1-42 in cerebrospinal fluid) being conducted in three different ways – two manual assays (a Luminex and DELFIA method) with different end points and a sandwich ELISA, which was fully automated.

What is clear from these charts is the improved precision of the overall system response on the automated platform. This will nearly always translate into better interassay precision and accuracy.

One interesting point – though not really the subject of this article – which I feel it is worth mentioning here, is that many workers routinely tabulate and evaluate calibration data

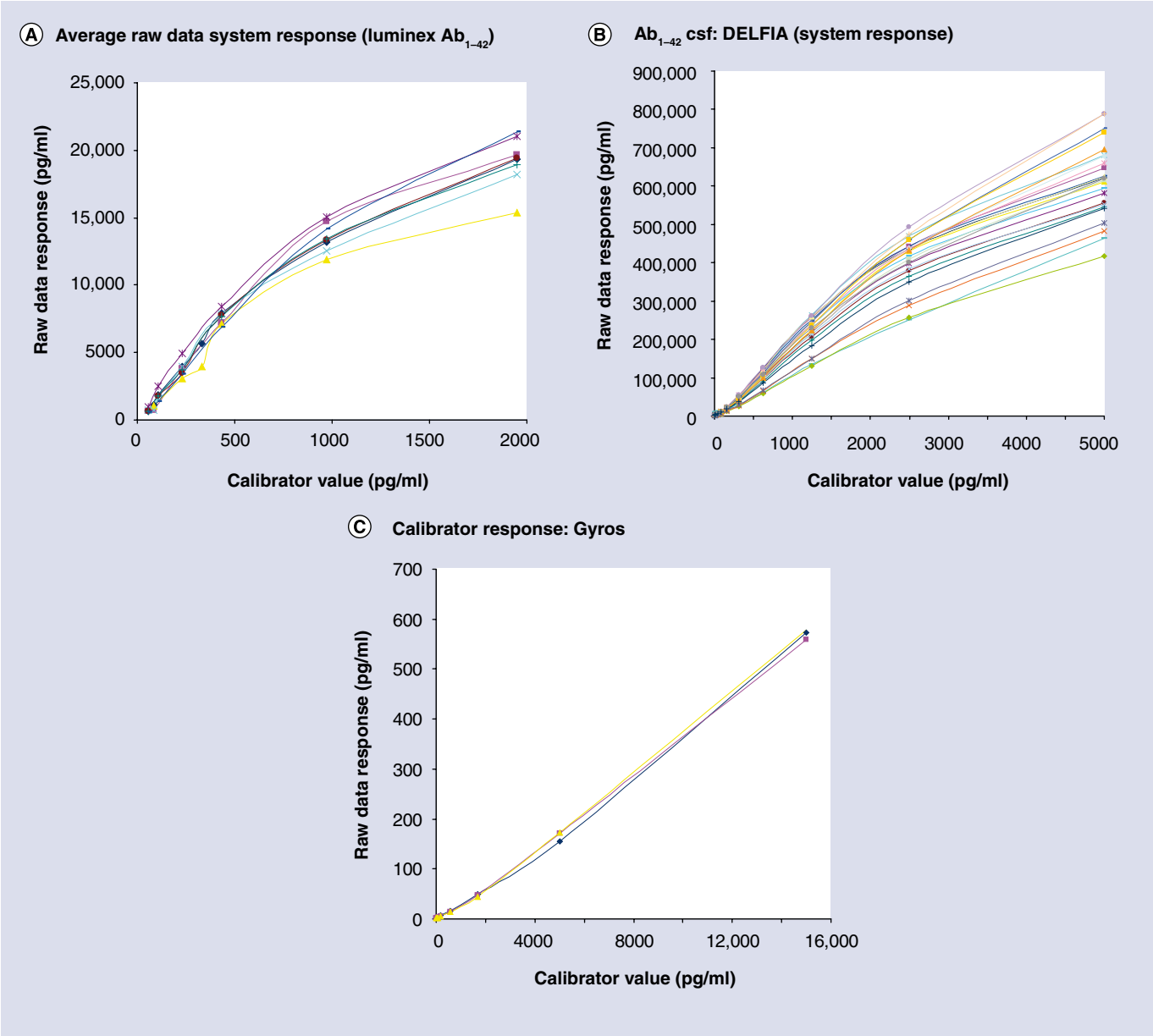

Figure 8. Comparison of raw data responses from manual and automated systems. (A) Luminex xMAP method. **(B)** DELFIA method with PE Victor reader. **(C)** Fully automated – Gyros system.
Images courtesy of manufacturers.

when validating assays – often including those tables in reports. However, in most instances that I have seen, these tables have included back-calculated results as opposed to raw data. This will often not demonstrate what is happening in the system responses that are shown in Figure 8. Indeed, if we graphically represent the same data as back-calculated results, we see the graphs shown in Figure 9.

Since these assays are nonlinear and use algorhithms, such as four- and five-parameter logistic fits, it is not surprising that the observed versus expected results always show relatively good data. The actual overall method performance is better evaluated by looking at raw data as shown in Figure 8 and demonstrates the different performance of the automated versus manual techniques.

As a further example of how automation may improve overall performance, Figure 10 represents the interassay precision (CV %) of a PK immunoassay method for a biological molecule.

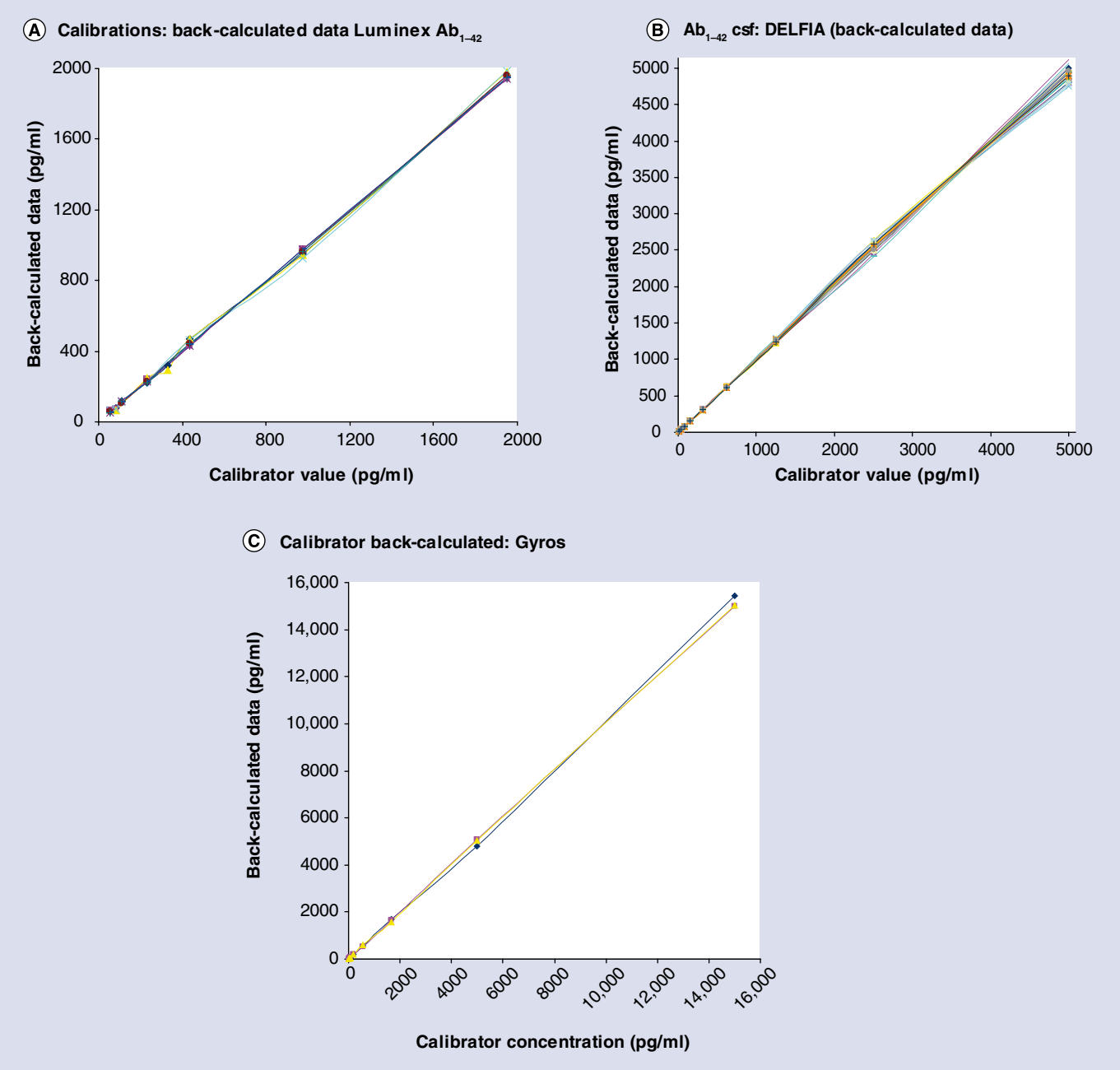

Figure 9. Back-calculated results of the data represented in Figure 8.
Images courtesy of manufacturers.

The manual method results in this chart, generated in our laboratory matched closely to the data from the original published method for this molecule. Transferring the exact method to an automated platform, we saw two major improvements. Firstly, the performance in terms of precision improved dramatically (as did accuracy [relative error] and total error) and, secondly, we were able to extend the analytical range of the method.

It is often surprising – to me at least – to hear that immunoassay methods can never be as precise as other methods, such as LC–MS/MS, due to the inherent variability that is generally expected within immunoassays. However, when we interrogate performance data of many immunoassay methods available on these platforms, we often find that this is not true at all.

As an example, Figure 11 shows Levey-Jennings QC charts from a project our laboratory was once involved with about 14 years ago. This is a peptide hormone assay, analyzed on a fully automated platform and used for PD analysis (it could equally be used for PK analysis if this peptide hormone was developed as a drug). The validation of the assay showed that the method had interassay precision (CV) of 4% across the whole analytical range.

Now, to use this as a PK assay under latest guidelines would require QC results in sample batches to pass '4–6–20' rules (4 out of 6 QCs within ±20% of its target value [25% at the LLOQ] with at least 50% of QCs passing at each level). It is clear that this method is far better than the criteria demand, and indeed accepting results up to five-times CV of the method we would argue is inappropriate as, statistically speaking, results with that level of performance would actually demonstrate that the method is 'out-of-control' according to its performance criteria at validation. However, that is probably another argument for another article.

Using it as a PD assay, we would more appropriately use acceptance limits linked to the method performance itself. These could be 95% confidence limits (±2 SD) as 'warning' limits on method problems, and ±3 SD as batch failure limits. When we look at the validation performance, this would translate to failing a batch if even a single QC result in a sample batch was outside ±12% of its target value.

Hence, looking back on immunoassay data, the expectations of them always being poorer than other method types is clearly not correct. In fact, we have a number of immunoassays with CVs similar to the peptide hormone assay quoted above.

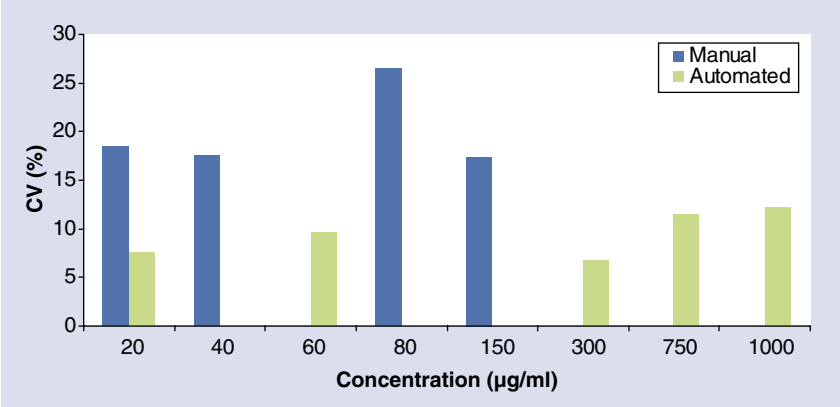

Figure 10. Method performance of a manual immunoassay compared with the fully automated version of the same method.
Image courtesy of manufacturers.

PK assays

Virtually all immunoassay methods for PK analysis can be automated. The degree of automation

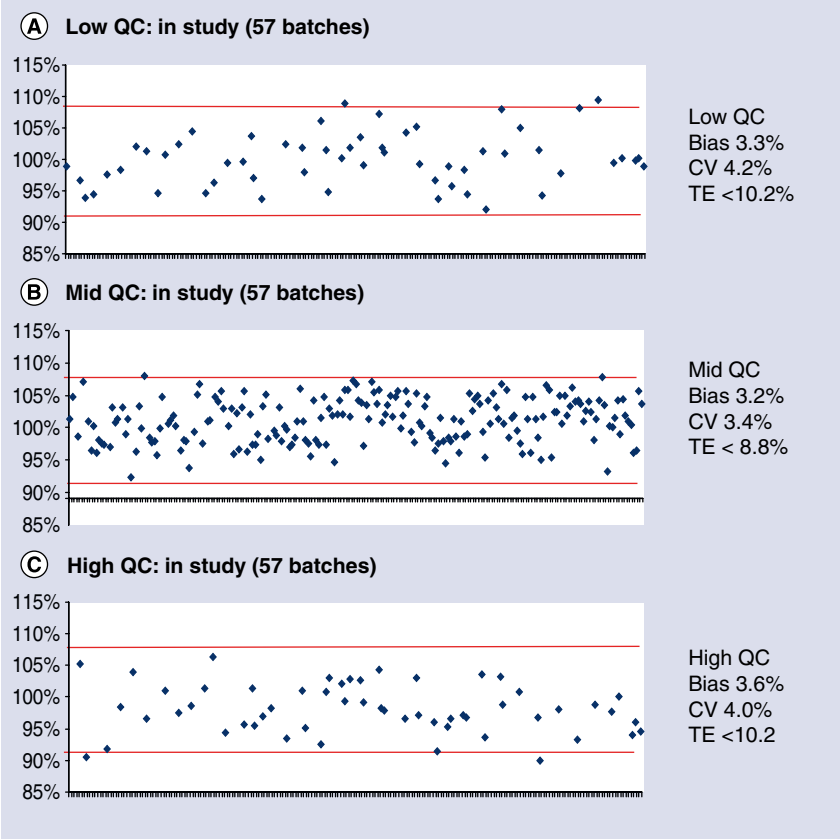

Figure 11. Levey–Jennings charts of low, medium and high quality controls over study timeline with performance metrics of the method over 57 analytical sample analysis batches covering several months.
Images courtesy of manufacturers.

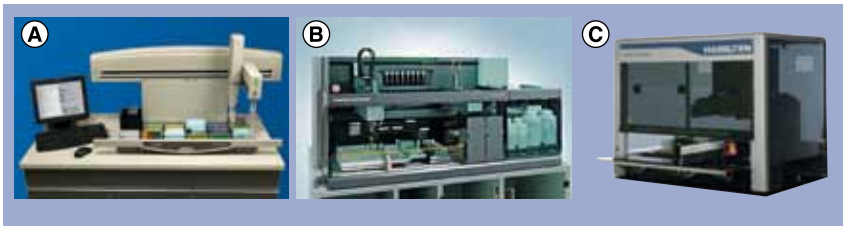

Figure 12. Some current xyz robotics platforms. (A) Beckman-Coulter's Biomek 3000 [112]. **(B)** Tecan's Freedom Evolyzer. **(C)** Hamiltons STARLET. Images courtesy of manufacturers.

depends on individual laboratories, although the benefit of automation for immunoassays has been demonstrated repeatedly in the diagnostics industry, and, certainly in our experience, the argument for automation is very strong. Clearly, there are many ways to organize an analytical laboratory and many factors come into play when choosing how to conduct each assay. Busy laboratories are regularly conducting a large number of different assay methods every day and if the batch numbers of each assay are relatively small in this scenario, full automation becomes more difficult – especially on platforms that have user interfaces, which are not that efficient. As mentioned previously, platforms like the Grifols Triturus have major benefits over many others due to the ease of programming new method protocols into the instrument. Where fully automated systems really come into their own is on projects that have large numbers of samples that require analysis for either one method or a small number of methods.

Immunogenicity assays

Whilst overall assay formats may differ in some ways for immunogenicity assays, the same basic premise exists as for other immunoassays – many will be capable of automating. There will almost certainly be some issues to overcome with integrating some platforms that are really end point detectors, which will be more difficult for some and may make that step inefficient or too costly, whilst other fully automated systems are already being used in this field. Moreover, a number of these platform manufacturers are already investing in development of their systems to enable their use in this field, where previously such platforms may not have been considered. I am certain this is an area that we will hear much more of in the future.

Figure 13. Bioscale's VIBE Workstation. Described as a 'A New Generation of Protein Analysis'.
Image courtesy of manufacturer.

Use of accredited biomarker assays on automated platforms for PK & PD analysis

One of the real benefits that the diagnostic industry can bring to drug-development is that when biological drugs are being evaluated and PK assessment is required, it is often possible to use accredited diagnostic kits with only very small modifications. Here, we can get the benefit of very robust methods – sometimes having been in use in thousands of laboratories around the world for many years. In addition, where they have been developed on fully automated platforms, such as some of those mentioned previously, we also get all of the benefits that automation brings to the method, as discussed above.

Examples of some of the drugs where we may take advantage of these methods would be molecules such as growth hormones, insulin and vitamins (e.g., D and B12), which all exist as endogenous molecules and have very good robust methods available on a wide range of platforms (e.g., Abbott Axsym/Architect, Siemens Immulite, Roche Elecsys, Beckman-Coulter Access and others).

Future perspective

It is clear that much research and development has been invested into laboratory equipment platforms used for immunoassays over many years now. In the xyz robotics field, we are seeing more instruments increasing their repertoire of capabilities. Some of those platforms, currently available, are shown below in Figure 12 [112]. Whilst this is not an exhaustive list, it will be interesting to see how these capabilities develop and how well received the platforms are – only time will tell.

Additionally, I expect that there will be other platforms that come to the forefront, which may be more critically developed around more specific methodologies (as opposed to the typically totally open and flexible approach seen in the

Figure 14. Point-of-care immunoassay platforms. (A) Alere Triage system. **(B)** LifeAssays system. **(C)** Avantra Q400 Multiplex biomarker system.
Images courtesy of manufacturers.

xyz robots discussed in this article). One such instrument is Bioscale's VIBE™ (Figure 13) [113] – others I am sure will follow – all aimed at simplifying analytical techniques in the laboratory, whilst trying to improve upon method performance criteria.

I also expect there to be more moves towards companion diagnostic assays, which developers may wish to get closer to the doctor's office or even bedside via point-of-care (POC) testing equipment. Some appear to be already there, for example, Alere's Triage system (formerly Biosite [114]), and Radiometer's AQT90 FLEX. LifeAssays also have a system based on a sandwich immunometric immunoassay principle, where the test system uses magnetic nanoparticles (Figure 14) [115].

It is also interesting that some manufacturers share this vision. I recently learned that Bioscale (VIBE, above) have already started miniaturizing the proprietary nonoptical detection capabilities of its assay into a compact device for diagnostic and POC applications. I look forward to seeing the outcome.

Moreover, having recently viewed Courtagen Life Sciences, Inc.'s Avantra Q400 Multiplex biomarker system [116], I was impressed at the capability of this POC equipment – based on a fully enclosed cartridge system using microfluidics and quoting very respectable performance criteria for a number of popular research assays in plasma/serum. It is the most advanced POC immunoassay system that I have seen to date and I am looking forward to evaluating it soon.

Many assays are already available on such platforms and I believe we will see others come along – perhaps on new platforms, such as those Bioscale have in mind – in the future. Here, microfluidics and perhaps nanotechnology will also play a part in their development, as has been witnessed with some of the platforms already discussed. Whilst these platforms are obviously directed towards and used in diagnostics, they do potentially have a major part to play in drug-development in the future. With the advent of using (or wishing to use) new biomarker assays for stratifying patient populations for study

Executive summary

- Laboratory automation is not new, but few scientists have been exposed to the wide range of analytical equipment platforms, which have been available from diagnostic and research companies, with many workers focusing on one or the other disciplines throughout their career.
- Many such instrument platforms play an important role in drug-development in laboratories around the world.
- Analytical support in drug-development today covers a wide range of techniques and equipment platforms.
- Today, we see 'new' models of many xyz Robotics that still lack some of the enhancements – particularly in user-interface programming – that has been present in some open diagnostic platforms for over 15 years.
- Throughout this period of development, it was clear that as well as looking at automation of manual processes, manufacturers were also investigating the use of new techniques to improve the performance of the analytical methods.
- However, innovation continues to thrive in analytical sciences and manufacturers continue to invest in R&D of equipment platforms – some of these making radical moves in terms of technology.
- One very interesting point is that when working at the nanoliter scale, scaling laws become very significant, in that surface tension becomes a more dominant force than gravity.
- Virtually all immunoassay methods for pharmacokinetic analysis can be automated.
- It is clear that much research and development has been invested into laboratory equipment platforms used for immunoassays over many years now.

inclusion and 'companion diagnostics', it is clear that as analytical methods for new biomarkers are developed, the opportunity to perform technology transfers to POC platforms such as those discussed here has very attractive advantages for global clinical trials in the future.

One thing is certain – this field of analytical science is not standing still. I, for one, look forward to seeing whatever new developments come our way.

Acknowledgements

All images are used at the courtesy of the companies listed in the references. The instruments are used as examples in this article and are not meant to be an exhaustive list of those available; they simply represent those that the author has either used or has learned of during the course of his career.

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