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New trends for automation in immunoassays

A. Truchaud[1], B. Capolaghi[2], J.P. Yvert[3], Y. Gourmelin[1], G. Glikmanas[1] and M. Bogard[1]

[1]Biochimie, Hôpital de Meaux, BP 218, 77108 Meaux, France
[2]Biochimie, Hôpital Bel Air, 57100 Thionville, France
[3]Biochimie, Hôpital Begin, 94160 St. Mandé, France

Abstract - Immunoassays are now an important part of the daily activity of the clinical laboratory, concerning various parameters as hormones, tumor markers, drugs, antibodies, viruses and bacterias.
The evolution of the instrumentation in this domain is similar to the one in biochemistry. The requirements may be summarized as follows : non isotopic immunoassays, high throughput, large panel of analytes with a low detection limit, stabilized reagents, full automation with easy calibration and even random access.
To fulfill these specifications, many analytical and technological problems have to be solved :

- Sample identification, detection, and eventual dilution.
- "Reagents" may be liquid solutions, suspensions (latex, magnetic particles, liposomes), fibrous materials, and coated tubes or wells. Each type of reagent induces a specific processing.
- Reaction processing needs an incubation step and, in the case of heterogeneous immunoassays, washing and separation steps.
  The delays of the immunological reaction and of the final signal development will determine the size of the incubator, i.e. the size of the instrument, and the throughput of the system.
- Signal measurement of low energy light was improved by reducing artefacts due to matrix and optical cuvets, but also by using photon counting detectors.
- Calibration is simplified by automated calculation, memorization and in some case replaced by a "master curve" made by the manufacturer for each reagent lot.

Future systems should better take in account the biosafety, help the operator to validate calibration, controls and results, and help to clinical interpretation by associated expert systems.

INTRODUCTION

The field of immunoassay has shown enormous growth in the last two decades and has led to a need of automation for different reasons :
- Third party payers have pressed hospitals to reduce costs dramatically and place a premium on rapid diagnosis and discharge. Testing must be done quickly, with a time horizon of hours rather than day.
- Low cost reagents systems with high labor content were often more economical than other methods. As pay levels rise, the trade-offs begin to favor higher cost reagents with less high-cost labor.

Automated systems allow the avoidance of tedious and repetitive manual procedures but above all a strict control of reaction conditions which also improves performance significantly. Beside the general targets of modern automation, immunoassays have specific requirements in specimen and reagent sampling, development of the immunological reaction, signal detection, result validation and help to diagnosis.

1. GENERAL TARGETS OF AUTOMATION

The general targets of modern automation are :
- Selective multitesting, which should replace the term "Random Access", with the ability to perform test panels on a single specimen in the same run.

- Walkaway operation by self checking and feedback system controls.
- Flexibility, which should be increased and not decreased by full automation.
- High throughput is a classical criteria of choice.
- Limitation of biohazard is and will be included in the basic specifications of analytical systems.
- Improvement of analytical performance.

All these items lead to the development of automated systems with an instrument and reagents optimized to fit with each other.

2. IMMUNOASSAY PANELS

About immunoassay panels, we would like to emphasize that it is not relevant to compare the immunoassay panels to biochemistry panels with 15 to 25 parameters.
Immunoassay panels may be listed as following :
- THYROID : T4, T3, T-UPTAKE, TSH, FT4
- ANEMIA : B12, FOLATE, FERRITIN
- FERTILITY : HCG, FSH, LH, PROLACTIN, OESTRADIOL, PROGESTERONE, TESTOSTERONE
- PREGNANCY : HCG, AFP, PHL, ESTRIOL
- OTHER HORMONES : CORTISOL, STH, PTH
- HEPATITIS : HBsAg, HBeAg, ANTI-HBs, ANTI-HBe, ANTI-HBc, ANTI-HBc IgM, ANTI-HAV, ANTI-HAV IgM, ANTI-HCV
- HIV : ANTI HIV 1+2, ANTI P25, P25 Ag...
- OTHER INFECTIOUS DISEASES : CHLAMYDIA,
- TUMOR MARKERS : CEA, AFP, PSA, CA 19-9, CA 125...

In most of the cases, a panel of 6 parameters on a single specimen is a maximum.

3. SPECIMEN AND REAGENT SAMPLING

About sampling function, beside classical requests, some specific points may be emphasized :

- Carryover between reagents may produce non specific binding and crossed reactions ; some recent systems use disposable tips placed and removed automatically.
- In immunoassays, analytes concentration range is often very wide, so carryover between specimens must be minimal ; the washing program should be programmable depending of parameters ; furthermore, an automatic dilution system in case of result exceeding the limit of the analytical range is very useful.

Sampling function is performed by dedicated of opened robots.

4. AUTOMATION OF THE IMMUNOLOGICAL REACTION

4.1 Homogeneous versus heterogeneous immunoassays

In homogeneous immunoassays, antibody binding leads to a change in the signal measurable from a label or matter in competition for binding first the substance being measured.

The classical example is EMIT, the enzyme immunoassay (ref.1) : the specimen antigen competes with labelled conjugate. CEDIA, cloned enzyme donor immunoassays, is an other example (ref.2).

Immunoprecipitation, immunoagglutination are also used in quantitation of small molecules, both directly and as inhibition processes, with turbidimetric or nephelometric detection.

In heterogeneous immunoassays, antibody-bound and free analyte must be separated. Usually, antibody is fixed on a solid phase and the other components are added sequentially ; antigens with several binding sites allow assays in "sandwich" form, i.e. a sandwich with the antigen and a conjugate with an antibody which combines with an other epitope of the antigen and the label : this is an extraction process. This technique also allows competition between a free antigen and an antigen-labelled antibody conjugate.

In the two cases, the components which are not bound are eliminated by a separation step, usually also involving washing steps.

We can summarized the main features of the two types of immunological reactions:
- The reaction mode : competition for homogeneous immunoassays, competition and extraction for heterogeneous immunoassays ; a separation step is required for heterogeneous immunoassays.
- In homogeneous mode, we measure a modulation of the signal ; in heterogeneous mode, we measure the total remaining signal.

- With homogeneous immunoassays we can determine high concentration analytes with low molecular weight; heterogeneous immunoassays can show similar sensitivity and imprecision as to radioimmunoassays, with no restriction on molecular size.

4.2 Constraints in automation of the immunological reaction

In the homogeneous mode, there are only two analytical steps, (reagents addition, incubation and measurements) similar to chemistry assays. Many of these methods can be adapted in conventional chemistry analysers.

In the heterogeneous mode, there are many analytical steps. The separation step is a specific requirement, not found in conventional chemistry analyzers.
The constraints for automation are :
- To choose, design and manufacture a solid phase which is equivalent to a reagent.
- To reduce the duration of the incubation steps because if the reaction delay is 1 hour, a very huge incubator with more than 300 places will be necessary.
- To combine and automate the different components in a compact automatic system : such systems are often based on patented components or analytical procedures.

4.3 Automated separation using different solid phases

Many different solid phases are used in immunoassays :

* Coated tubes, wells or beads : with coated tubes or wells, automation is possible through dedicated or reprogrammable robots; a recent improvement in tube technology is to use "universal" tubes coated with strepavidin and a biotinilated antibody specific to the antigen, then an enzyme conjugate. This is called the "soluble sandwich" approach (ref.3).
To accelerate the reaction by increasing the rate of immunological events, one can reduce the size and geometric space in which the components diffuse, increase antibody concentration and split the solid phase into microparticles.

* Latex particles : latex particles are now available as a commercial product. Separation process may be performed by capture in a glass fibre filter (ref.4).

* Magnetic particles : magnetic particles seem very attractive, because at first sight, the separation process appears simple. In fact, magnetic particles are "high tech" products with very precise requirements : limited non specific binding, stability of the suspension of these particles, avoidance of capturing residual liquid during the separation process ("entrapment"), short delay for resuspension, and avoidance of loss of particles during washing steps.
If one has the know-how, it is possible to meter and deliver the suspension in optical cuvets of a system very similar to a chemistry analyzer, except that magnets are located near the cuvets (5).

* Fibrous and film materials : it is also possible to fix antibodies on fibrous and film materials. The immunological events are provocated by moving the other components by diffusion through the solid support in a standardized physical process (ref.6, ref.7).

5. LABELS AND MEASUREMENTS

The first labels used in immunoassays were radioisotopic labels. Due to environment problems, they are gradually replaced by enzymatic, chemiluminescent and fluorescent labels. Specific activities of non isotopic labels may be compared in term of detectable event per molecule.

Chemiluminescent and fluorescent labels have a good score with one detectable event per molecule.

The specific activity of enzymes labels is determinated by the detectability of reaction product but the binding process to the solid phase is well established. To improve their detectability, enzymes labels must be amplified by recurrent enzymatic system or used with substrates giving a luminescent or fluorescent product (ref.8) : alkaline phosphatase with paranitrophenylphosphate produce paranitrophenol measured by photometry, but with methylumbelliferyl phosphate a fluorescent product is obtained, and with dioxetane-phosphate a luminescent product.

6. SIGNAL PROCESSING, VALIDATION

Complete automation allows a standardization of the performance of analytical steps : sampling, timing of incubation and final reaction development, temperature...
Validation of calibration curve and controls, automatic or by the operator, will be based on

different criteria : quality of the signal (level, background...), quality of calibration curve-fit, comparison with a memorized previous curve, external controls, agreement of a result with other results of an immunoassay panel, but also with the previous results of the same test, eventual performance of complementary tests to assess the first results.

All these informations and data may be processed in embedded or external expert systems.

CONCLUSION

To conclude, we can list some targets for a better automation of immunoassays : improved reagent stability in the system, faster reactions which allow compact automation, combination of homogeneous and heterogeneous immunoassays, multilabeling for simultaneous multiple assays, development of new solid phases like biosensors, multiparametric calibration and control solutions, and help to results validation by expert systems.

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