How efficient and automated can be Serology and Stool Testing?

DiaSorin scientific contribution
Euromedlab Paris, June 23, 2015

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How efficient and automated can be Serology and Stool Testing?

A laboratory has to face several challenges every day: a constant increase in the number of tests to be performed, the need to provide results quickly and the need to achieve a lower turn-around-time (TAT), while maintaining quality assurance and complete traceability of the results. In this context, in order to meet all the clinical needs efficiently without any compromise on quality, one solution could be to look towards innovation and optimize the laboratory with innovative systems (Figure 1).

Automated techniques are currently adopted for the most commonly used serological methods. The first step towards innovation is to move away from the ELISA method to embrace a new technology, chemiluminescence (CLIA), and the new fully automated analysers: LIAISON® and LIAISON® XL.

CLIA systems represent a technical improvement over the ELISA automated system. They reduce TAT, while maintaining high quality and full traceability, with the added advantage of a random access system. Ready-to-use reagents on board, a touch-screen monitor, auto-dilution, re-run and reflex testing automatically performed by the system and a STAT position for emergency results are some of the technical characteristics of the new systems. Moreover, with the LIAISON® systems it is possible to test several types of samples (e.g., serum, cerebrospinal fluid and stools) at the same time, in the absence of cross-contamination.

With LIAISON® XL, efficiency can be improved with several advantages: new infectious disease markers (e.g., HIV, hepatitis C); a larger number of reagent integrals on board (from 15 to 25); no more daily maintenance, and disposable tips. Moreover, most of the reagents are the same for the LIAISON® and LIAISON® XL systems, which means easy validation files; the system also provides a backup, if needed.

Another challenge in a microbiology laboratory is performance. Implementation of highly automated instrumentation fulfils the needs of the laboratory – i.e., TAT and logistic improvement without any compromise on quality - and enables a good response to clinical needs, including confidence in the results and flexibility (Figure 2).
The chronological evolution of the Liège laboratory
The laboratory of Infectious Serology and Antigen Detection is part of the division of Clinical Microbiology of the University Hospital of Liège and performs both infection serology and antigen detection for infectious diseases including *Clostridium difficile*.

The collaboration with DiaSorin started in 2001. Since that time, DiaSorin has been able to support the evolution of the Liège laboratory with an increasing number of infectious disease markers available on the fully-automated analyser and with the ability to consolidate the serology platform.

After that, in addition to the ETI-Max 3000 (ELISA analyzer), between 2003 and 2006 three LIAISON® systems were acquired and an increasing number of infectious disease markers were implemented over the years. In 2011, with the advent of the LIAISON® XL system, the LIAISON® was gradually phased out.

Finally, *C. difficile* diagnosis on stool specimens was introduced in 2013, first on the LIAISON® and then on the LIAISON® XL (Figure 3).

Experience with *Clostridium difficile* infection diagnosis
A fully automated solution is necessary for the diagnosis of *C. difficile* infection especially in a laboratory that performs a large number of tests per year.

The advantages of a random access system are flexibility and the ability to provide results quickly and several times a day, as with the rapid tests but with the full traceability of an automated system. Moreover, the LIAISON® can run toxin tests on stools but also on bacterial colonies of *C. difficile*.

The picture shows the workflow in the Liège laboratory. Blood and cerebrospinal fluid samples are aliquoted (if needed) and then tested either manually or by the ELISA automated system or by CLIA. For *C. difficile* diagnosis, after a short extraction step, stools go directly to the LIAISON® system for antigen and toxin detection. At the same time, they are also incubated for 24-48 hours in the bacteriology laboratory, and the colonies obtained after incubation can be tested for toxins on the LIAISON®, if needed (Figure 4).

Future perspectives
A large panel of fully automated parameters has been implemented on the LIAISON® system over the years, and new parameters will be available in the near future, e.g., for Chlamydia, Bordetella pertussis toxins, and, for stool testing, for adenovirus and rotavirus antigens.
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To conclude, clinical needs have to be met efficiently and without any compromise on quality. Moving from ELISA to CLIA allows us to consolidate a serology platform and successfully tackle all the challenges faced by laboratories. Indeed quality and innovation are key success factors for the evolution of the laboratory and contribute to improving performance with a good response to clinical needs.
To understand the possible application of fully automated methods in microbiology, it is first necessary to focus on why, where and how these tests, and in particular immunological stool testing, should be used. The main reasons to run a test are to identify the cause of symptoms, to treat the patient appropriately and to avoid complications.

Sometimes there are no standardized reasons to request a test. A study from the Netherlands (Van Den Berg RJ et al) tested a number of stool samples for *C. difficile*; the stool samples were divided into two groups, in one group *C. difficile* testing was requested and in the other it was not. The results showed the same *C. difficile* positivity rate (8%) in the two groups. So, why was this test requested or why was it not?

Immunological tests using blood are very well established in that they have good sensitivity and specificity. By contrast, stool testing is challenging as there could be different concentrations of the target in each sample and many endogenous materials can interfere with the test. However, when an infection (e.g., *C. difficile*) is local it must be tested on the spot.

Concerning methodology, there is a choice between classic culture microscopy and immunological methods. Classic microbiology is tricky, slow and needs experienced personnel; on the other hand, immunological tests are highly automated, quick to run and require no special experience of the staff.

**Immunological methods**

Immunoassays are all based on specific antigen-antibody reactions. They can be used to test for viruses, bacteria, toxins, drugs, etc. Typical examples of these methods are: EIA (enzyme-linked immunosorbent assay), ELISA (enzymatic colour reaction), RIA (radioimmune assay) and CLIA (chemiluminescence assay).

In **ELISA (and EIA)**, an antibody (or antigen on EIA) is marked with an enzyme. The reaction catalysed by the enzyme is a representation of the presence of the antigen. The result can usually be detected by a colour change, fluorescence or chemiluminescence.

Another typically used test is the **Sandwich-ELISA**. The Sandwich-ELISA uses two different antibodies (Ab) binding to the specific antigen, but on different epitopes.

**Chemiluminescence (CLIA)** is a process in which a chemical reaction emits electromagnetic radiation in the visible light region. The maximum light emission is up to 1 sec., which allows a very high sample turnover that is important if rapid testing is needed.

**Automation in microbiology**

Automated processes are fast, turn-around-time is short and staff costs per specimen are low. On the other hand, the implementation of automated systems is complex and the cost is high.

The main opposition to automating microbiology is that microbiology is strictly separated in many laboratories. As an example, in a typical workflow for stool testing the only connection between microbiology and the laboratory is the administration (Figure 1).
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In order to automate microbiology, a new workflow has been designed to transfer microbiology tests into an automated laboratory, to overcome old boundaries and establish an interactive process. This workflow shifts the microbiology section into the automated laboratory. Entry of the specimen and registration of the patient and testing are done in the laboratory; in the processing phase there is interaction between the two sections, and validation and report printing is done in the laboratory. This way, there is dynamic registration and interactive processing. (Figure 2)

The new workflow is much faster and provides a better test quality.

Figure 1. Typical Workflow

Figure 2. New Workflow
To test the differences between classic and immunological tests, and between classic and automated workflow, a good example could be the diagnosis of *C. difficile*.

*C. difficile* is an anaerobe, Gram-positive, spore-forming bacteria. Pathogenic *C. difficile* strains produce multiple toxins; complications are toxic megacolon, colon perforation and septic shock. “Classic” tests to detect *C. difficile* in stools are: culture or PCR tests. These classic tests show a good correlation with immunological tests, such as glutamate-dehydrogenase (GDH) or toxin tests.

In order to test the new workflow using LIAISON® XL, a highly automated laboratory and a classic microbiology section were used in parallel. The comparison shows that the new workflow is much faster than the old one and the automated quality control ensures a better test quality. (Figure 3)

In conclusion, the aim of the new workflow is to achieve a fast and specific diagnosis. Immunoassays are as good in stools as in blood and allow for “on the spot” testing. Many methods are available for bacteria, viruses and other pathogens. Finally, automation makes it possible to cross laboratory boundaries and achieve goals together.
Questions and Answers

Q: How are the reports of the cultures and toxicological tests provided? Are they integrated or supplied separately?
A (Burde): We provide a single integrated report comprising all the results.

Q: How do you handle any discrepant results between a culture and an immunological test?
A (Huynen): The culture is used to dispel doubts in the event of discordant immunological tests (antigens and toxins). If these are both positive, the culture is irrelevant.

Q: How do you tackle the issue of making the laboratory and microbiology staff feel responsible with regard to the use of automatic testing?
A (Burde): We have had to make them gradually take on more responsibility. My view is that we should not bring microbiology to automation but automation to microbiology. This way, the responsibility for the preparation of specimens remains with the same people.

Q: What can you tell me about the risk of contamination? It is possible to test stool and blood at the same time?
A (Huynen): Because we too initially had doubts about this, in the early phases we validated the use of both matrices on the same system, and found that there were no problems.

Q: Dr. Huynen, how do you go about the diagnosis of H. pylori?
A (Huynen): We use both direct methods (culture and antigen detection) and the search for antibodies, as requested by the clinicians. The search for antibodies is especially useful for IgG.

Q: Glutamate-dehydrogenase (GDH) followed by toxin tests. Do you always do that or do you carry out the toxin tests only when the GDH is positive?
A (Burde): Only when the GDH is positive. In the event of a positive GDH, the LIAISON® system automatically runs the toxin test so the technologists do not even need to look at the GDH results.

Q: The recent literature suggests the possible use of PCR testing without GDH plus toxin testing. Is that possible for you?
A (Burde): The PCR test is not reimbursed in Germany, so the patient has to pay for it. Another problem is that it is too sensitive, and it detects toxins even after the infection has subsided.
Q: Given that it can take some time for the samples to reach the laboratory after collection and the antigen may degrade in the meantime, would it not be better to run the PCR test, which is not affected by this problem?

A (Huynen): We carry out the toxin tests on samples stored in a refrigerator for a maximum of three days.

Q: How do you handle the transport and extraction of the sample. Do you do these steps yourselves or send the samples to a satellite laboratory?

A (Huynen): When the stool sample arrives, one aliquot is kept for culture and another one goes to the technologist who extracts it and feeds it to the LIAISON® systems.

Q: Do you have any advice for the accreditation for stool testing?

A (Huynen): Generally, to accredit such a test you need to have a reference method carried out in parallel.

Bibliography
