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Automation in clinical bacteriology: what system to choose?

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Abstract

With increased activity and reduced financial and human resources, there is a need for automation in clinical bacteriology. Initial processing of clinical samples includes repetitive and fastidious steps. These tasks are suitable for automation, and several instruments are now available on the market, including the WASP (Copan), Previ-Isola (BioMerieux), Innova (Becton-Dickinson) and Inoqula (KIESTRA) systems. These new instruments allow efficient and accurate inoculation of samples, including four main steps: (i) selecting the appropriate Petri dish; (ii) inoculating the sample; (iii) spreading the inoculum on agar plates to obtain, upon incubation, well-separated bacterial colonies; and (iv) accurate labelling and sorting of each inoculated media. The challenge for clinical bacteriologists is to determine what is the ideal automated system for their own laboratory. Indeed, different solutions will be preferred, according to the number and variety of samples, and to the types of sample that will be processed with the automated system. The final choice is troublesome, because audits proposed by industrials risk being biased towards the solution proposed by their company, and because these automated systems may not be easily tested on site prior to the final decision, owing to the complexity of computer connections between the laboratory information system and the instrument. This article thus summarizes the main parameters that need to be taken into account for choosing the optimal system, and provides some clues to help clinical bacteriologists to make their choice.

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Introduction

Most clinical bacteriology laboratories are experiencing an increase in the number of samples to be processed on a daily basis. As an example, in our clinical bacteriology laboratory in Lausanne's university hospital, the number of samples to be investigated has steadily increased by about 4% per year, and this does not take into account the increased need for culture for epidemiological investigations owing to methicillin-resistant *Staphylococcus aureus* or to outbreaks of vancomycin-resistant *Enterococcus* [1]. However, human resources are not following this trend of increased number of samples, mainly because of strong financial pressure and resource shortages. Laboratory automation thus represents an appealing solution, especially for sample inoculation, which is a fastidious, repetitive process.

Pre-analytical handling of samples has been greatly improved in recent years by improved laboratory information systems (LISs) and increased use of bar-coding to trace samples and downstream processes, such as subculture, identification steps, and aliquoting [2–4]. However, despite improved LISs, the time spent on pre-analytical handling of samples and inoculation remains important, and in our own laboratory, which processes a mean of 300 samples per day and employs ten full-time laboratory technicians, we observed that about 24% of all technician activities related to sample reception, inoculation and Gram staining are dedicated to the inoculation of agar plates and broth (Fig. 1). Thus, 50–70% of the time spent by full-time technicians may be saved by automation of this task.

A first generation of automated plate streakers was developed more than 20 years ago [5]. However, the level of

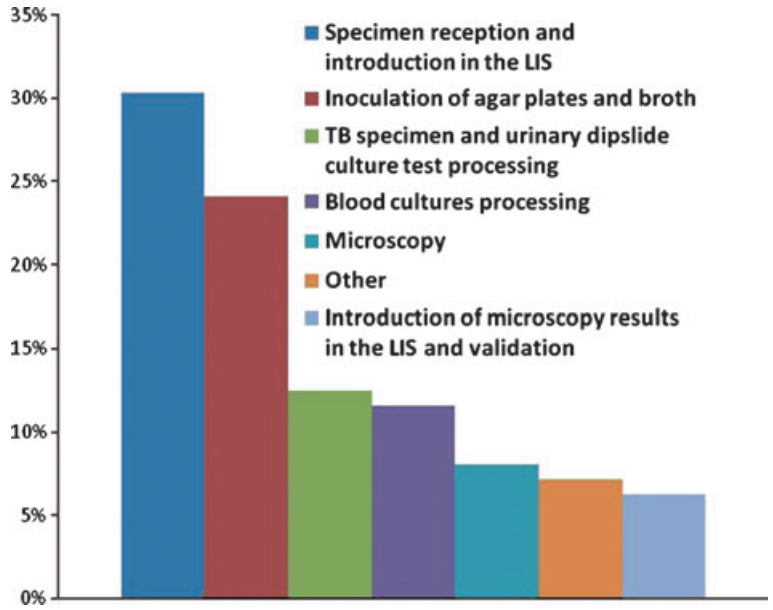

FIG. 1. Partition (%) of the workload in our bacteriology laboratory at the sample reception unit. Time was assessed by self-reporting ongoing activity at 15-min intervals; activities had to be assigned to one of the seven categories presented on the graph. Note that as much as 24% of technician time is devoted to the inoculation of agar plates and broths. LIS, laboratory information system; TB, tuberculosis.

automation was still limited, and the automated inoculation instruments initially available, such as the Inoculab (Dynacon), had only unidirectional informatic interfaces. Although updated with bi-directional connections with the LIS, the second-generation Inoculab LQH system (Dynacon) is only able to plate specimens starting from a single type of container (i.e. sterile urine container), and has a limited capacity of 38 inoculated plates, all loaded in a single silo [6]. Thus, these first-generation and second-generation systems were not developed enough to allow efficient, high-throughput and accurate inoculation of samples, including the following four main steps:

1. selecting the appropriate Petri dish
2. inoculating the sample efficiently
3. spreading the inoculum on agar plates to obtain, upon incubation, well-separated bacterial colonies
4. accurate labelling and sorting of each inoculated medium.

Very recently, third-generation instruments have become available on the market; these fulfil all of these prerequisites for automated handling of specimens in bacteriology laboratories. These new instruments include the WASP (Copan), Previ-Isola (BioMerieux), Innova (Becton-Dickinson) and Inoqula (KIESTRA) systems. The current challenge for each clinical bacteriologist is now to determine the ideal automated system for his or her own laboratory. Indeed, different solutions will be preferred, according to the types of sample, and to the variety and amount of samples that will be processed with a given automated instrument. However, this choice is difficult, owing to the limited data available in the scientific literature, partially because of the very recent availability of these systems in clinical laboratories, and partially because of the excessive level of confidentiality surrounding results obtained with prototypes. This review thus provides clues to guide the choice of clinical bacteriologists and provides a list of instrument characteristics that need to be considered (Table 1). This list should: (i) help for comparison between systems; and (ii) pinpoint the parameters that are important to choose an ideal automated system.

How to Choose the Ideal Equipment?

Instrument characteristics

The third-generation systems currently available on the market differ in a variety of respects (Table 2). One of the main differences between WASP, Previ-Isola, Innova and Inoqula is the solution used to inoculate the sample. Thus, sterile loops, combs or beads are, respectively, used with WASP, Previ-Isola, and Inoqula. Innova also uses sterile loops. The use of different applicators implies that the streaking is also different, and the comb used by Previ-Isola does not allow inoculation of plates in classical semiquantitative or quantitative ways, but rather provides circular semiquantitative inoculation (Fig. 2a). It is expected that the reproducibility of the inoculation process will be higher with

TABLE 1. Instrument characteristics that should be taken into account for choosing the ideal automated inoculation system

Instrument characteristics
General
Size, weight
Electrical requirement (V/W, etc.)
Noise (decibels)
Technical characteristics
Productivity
Number of agar plates/h
Number of broths/h
Time spent in inoculating an agar plate
Inoculation
Semiquantitative, quantitative, circular, custom
Use of re-usable or dispensable devices
Type of inoculation device (bead, calibrated loop, etc.)
Consumables only provided by the manufacturer (captive product)
Samples
Various containers
Liquid and/or semi-liquid samples
Capacity of samples pre-processing loading
Automated capping/uncapping
Non-inoculated and inoculated media
Possible on agar, biplate agar, broth media, etc
Capacity of broth and agar pre-processing loading
Number of silos available for agar plates
Sorting according to samples and/or incubation atmosphere
Relationship with laboratory information system
Bi-directional
TCP/IP link, coding protocol
Need for an additional interface
Accuracy and quality control
Reproducibility
Comparison with another system
Risk of sample contamination
Traceability (stickers, etc.)
Biosecurity issues
HEPA filter
Maintenance
Cost and frequency of maintenance
Technical support from the company (availability, delay, etc.)
Options
Automated smear preparation
Automated transfer to incubator
Chain of automation including automated reading of broth and/or agar plates
Plate inoculation for antimicrobial disk susceptibility tests

TCP/IP, Transmission Control Protocol/Internet Protocol.

automated instruments than with inoculation by hand, as demonstrated for Previ-Isola and WASP [6,7]. Moreover, in these two recent studies, it has been shown that the number of isolated colonies is much higher with Previ-Isola and WASP than when inoculation is performed manually [6,7], as expected, given the wide and regular spreading of the inoculum over the entire agar plate obtained with Previ-Isola and WASP, respectively (Fig. 2a,b). Although peer-reviewed data are lacking for the KIESTRA system, it seems that the use of magnetic beads also results in a high number of isolated colonies (Fig. 2c) as compared with what is obtained manually (Fig. 2d).

Other important differences between the different systems are the plate throughput per hour, the robustness of the robotic system, the level of automation (including capping/uncapping and selection of plates), and the connections of the instruments with the LIS. Moreover, some 'automated' systems may require significant technician time to feed the machine, and others are not fully automated (i.e. manual interactive version of the KIESTRA system). Thus, some automated inoculation systems include slide spreading (Innova, Inoqula, and WASP) and/or slide staining (Innova). This may be a semi-automated task (Innova and Inoqula) or be fully automated as part of a supplementary module (WASP). It is noteworthy that, when agar and broth inoculation are tightly coupled with microscopy slide preparation, the overall performance of the automated system may decrease with a lower number of agar plates/h.

Characteristics of the laboratory

The choice of the ideal instrument is also highly dependent on the characteristics of the laboratory and on the expected use of automation. Thus, as an example, some large laboratories intend to use automation only for urines. In this specific situation, the cost of consumables and performance (number of plates per hour) will represent the main parameters for the decision. In contrast, when a laboratory intends to automate the inoculation of most samples, the decision complexity greatly increases. If the laboratory receives samples of low diversity and uses a low number of different inoculation protocols, the constraints on the choice of instrument will be lower than in a university hospital such as ours, where very diverse samples are handled. This diversity of samples introduces a constraint on the instrument, which needs to accommodate a large variety of containers. However, a parameter that is even more important than the diversity of samples is the proportion of samples obtained in a liquid format, which are easily processed with all automated instruments, and the proportion of swabs, stools, and tissues, for which solutions clearly differ from one manufacturer to another. In our laboratory, as swab samples represent about 34% of received samples (Table 3), the use of swabs such as those commercialized by Copan will be considered, because these swabs allow accurate and easy release of the sample, and its straightforward transformation in a liquid format, which is amenable to automated culture with most systems. In addition, one should remain aware that not all samples will be suitable for automation, and in our laboratory we will continue to inoculate a large variety of samples manually, such as cerebrospinal fluids, catheters (Macki method [8]), port-a-cath needles, joint prostheses, prosthetic valves, and vascular prostheses. Moreover, for some very low-volume specimens and/or small biopsy tissue samples, manual handling of the specimen will be preferred.

The variety of inoculation protocols used daily in a given laboratory will also greatly impact on the final choice of an automated system, as some automated systems, such as WASP, provide as many as nine different agar plate input

TABLE 2. Comparison of the four different third-generation automated systems currently available on the market; information was mainly deduced or derived from company websites, as available in March 2011 [http://www.copanusa.com/index.php/products/wasp/; http://www.biomerieux-usa.com/servlet/srt/bio/usa/dynPage?doc=USA_PRD_LST_G_PRD_USA_17; http://www.dynacon.ca/en/solutions/innova.html; http://www.kiestra.nl/pageid=19/Total_Lab_Automation.html]

	WASP	Previ-Isola	Innova	Inoqula-FLA
Company (country)	Copan (Italy)	BioMerieux (France)	Becton-Dickinson (USA)	KIESTRA (The Netherlands)
Inoculation device	Calibrated loop[a]	Combs	Calibrated loop[a]	Beads
Type of inoculation	Four quadrants, single streaking, bi-plate, etc.	Circular inoculation (semicircular for bi-plate)	Four quadrants, single streaking, bi-plate, etc.	Four quadrants, single streaking, bi-plate, etc.
Use of dispensable devices	Re-usable metal loops	Disposable combs[b] and disposable pipette tips[c]	Re-usable loops, disposable pipette tips[c]	Re-usable beads, disposable pipette tips[c]
Agar plate loading capacity	Nine silos (~350 agar plates)	Five silos (270 agar plates)	Six silos (270 agar plates)	Six buffers[d] (720 agar plates)
Sample loading capacity	72 e-swab tubes	114 samples	200 containers (on 5 drawers)	NA
Productivity (plates/h)	180 plates/h	180 plates/h	180 plates/h	400 plates/h
Decapping/recapping	Automated	Manual[e]	Automated	Automated
Agitation/centrifugation	Automated agitation and centrifugation, per specimen[e]	No automated agitation/centrifugation	Automated agitation, per rack[f]	Automated agitation, per specimen[g]

[a]Calibrated tri-loop device (Triquetra) and bi-loop device for WASP and Innova, respectively, which allows inoculation of 1, 10 and 30 μL.
[b]One comb per agar plate.
[c]One pipette tip per liquid sample.
[d]Each KIESTRA buffer may receive up to 120 agar plates; the number of buffers might be further increased if needed.
[e]Containers are placed in racks already decapped (biosecurity issue and need for new caps when unloading the samples).
[f]Any type of container may be agitated; however, all samples loaded on a given rack will all be agitated; Inoqula-FLA, Inoqula full laboratory automation.
[g]Automated pre-analytical handling of the specimen (agitation/centrifugation) may be specified for each specimen (different protocols for each specimen); however, centrifugation device only for Copan Uriswab and not for any type of container.
NA, information not available.

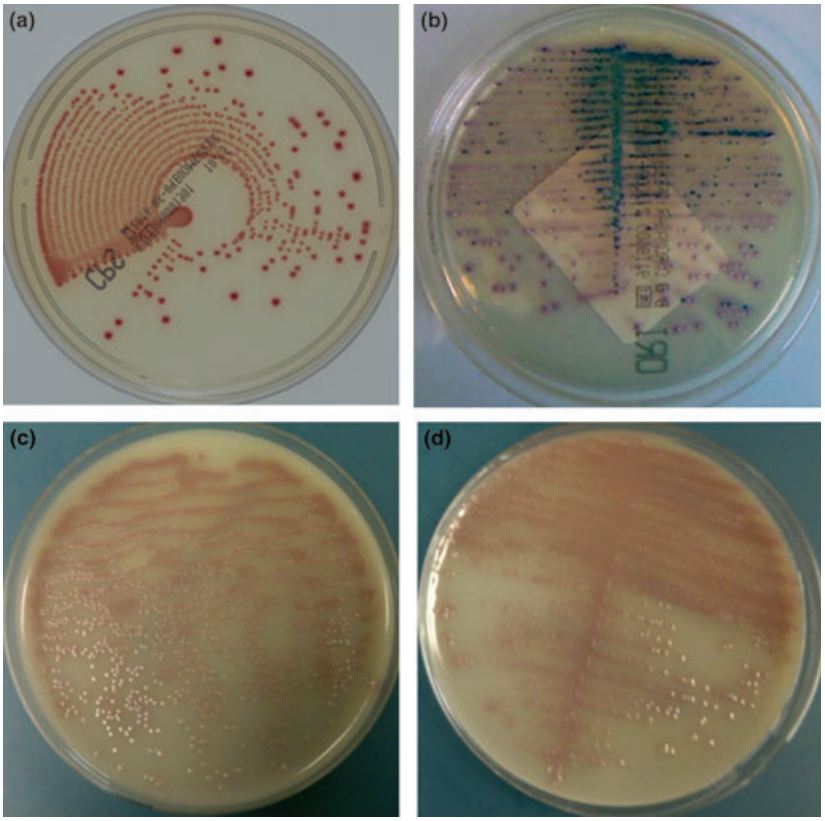

FIG. 2. Agar plates inoculated (a) with Previ-Isola, (b) with WASP, (c) withInoqula, and (d) manually. Note that isolated colonies were generally obtained, although high inocula were tested (about 10^6 bacteria/mL). The use of the Previ-Isola comb leads to circular semiquantitative inoculation (a), whereas, with WASP and Inoqula, both single-streaking (b, d) and four-quadrant inoculation (not shown) may be performed. Only 1 μL of urine was inoculated with the Inoqula bead (c), whereas 10 μL of the same urine was inoculated manually (d). Note that automated inoculation allow us to obtain more isolated colonies than manual inoculation, at least with Previ-Isola and Inoqula (Rice and Baruch, 109th ASM, 2009, Poster C064; Sturm *et al.*, 20th ECCMID, 2010, Poster 1766).

silos, whereas there are currently only five silos in Previ-Isola. As an example, in our diagnostic bacteriology laboratory, where more than 160 different plating protocols are used, 20–30 of which are performed on a regular basis, i.e. at least once a day, and where a large variety of agar plates are inoculated (Table 4), we will prefer systems with a high

TABLE 3. **Proportions of different main types of specimen received in our diagnostic laboratory; note that most specimens received are liquid, and that swabs also represent a very high number of specimens**

Type of specimen	Specimens per week	Percentages of specimens (%)	Condition before specimen processing
Swabs	304	34	Liquid[a]
Stools	94	10	Solid
Tissue specimen	44	5	Solid
Liquid specimen	463	51	Liquid

[a]Using Copan swabs.

TABLE 4. **Main media used to inoculate specimen in our bacteriology laboratory; the mean number of media used per week was assessed over a 1-year period**

Media	No. per week
MacConkey agar	482
Chocolate agar	304
Columbia with human blood agar	238
Thioglycolate broth	219
Chromogenic urine agar	217
Schaedler's agar	143
Chocolate agar with bacitracine	135
Columbia agar with sheep blood	127
Columbia agar with sheep blood with optochin disk	98
Chromogenic yeast agar	77
Gardnerella agar	75
Sabouraud agar	68
Chromogenic *Campylobacter* agar	65
Selenite broth	65
Chromogenic *Salmonella* agar	65
Cycloserine–cefoxitin–fructose agar	52
Granada agar	40
Lim broth	40

number of input agar silos. However, this parameter is not crucial, as it should be combined with the number of agar plates inoculated per hour. Indeed, if the capacity of a system with many silos is insufficient, two automated plating instruments may be used, and this will double the number of input agar silos available. Thus, the number of samples processed also greatly influences the choice of a particular machine. Importantly, the number of agar plates that should be inoculated per hour is not simply the number of agar plates inoculated in a median working day (about 1000 plates/day in our laboratory) divided by 24 h (42 plates/h), or divided by 9 h for a laboratory open only from 8 a.m. to 5 p.m. (111 plates/h), but should ideally be the maximum number of plates that need to be inoculated during activity peaks, i.e. about 220 plates/h (Fig. 3).

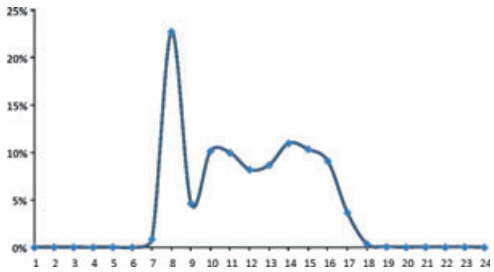

FIG. 3. Partition in percentage of the arrival of the specimen for processing (working hours 08:00–17:00).

Other specific issues

Biosecurity is another important issue that needs to be considered when a system is being chosen. Indeed, given the increasing number of multiresistant bacteria (i.e. extensively drug-resistant *Mycobacterium tuberculosis* [9]), the system should ideally cap and uncap samples and perform all processes that may generate aerosols in a confined area. The biosafety issue is not restricted to tuberculosis and respiratory tract specimens. Thus, another example is the risk of cytomegalovirus infection that may occur among our laboratory staff (especially when pregnant), owing to the occurrence of very high levels of cytomegalovirus in urines from infected newborns. This is especially important because automated inoculation systems are at least dedicated to the inoculation of urines.

To help bacteriologists in their decision, several companies selling automated instruments propose an audit by a third partner. We may wonder whether this third partner is really objective and provides an unbiased opinion, or whether the companies actually use such a third partner to influence our final choice rather than to help us in our decision. The final choice is also complicated by the fact that automated systems may not easily be tested on site, owing to the complexity of computer connections between the instruments and the LIS.

Of course, a variety of intermediate solutions may make sense, such as acquiring a progressively automated inoculation system that will initially be used only for liquid samples such as urines, before the level of automation is extended. In this perspective, the acquisition of several automated instruments may be appealing, as it allows increased flexibility. However, this advantage is counterbalanced by the possible increased maintenance costs and increased need for space.

The possibility of having a full chain of automation is also appealing [10,11]. Thus, in addition to sorting agar plates and/or slide preparation (see above), some systems, such as KIESTRA, also provide belts that allow automated transport of inoculated agar plates in incubators with different atmospheres. However, some automated systems, such as PreviIsola, which provide circular inoculation may not be compatible with automated readers (Telebacteriology) and automated colony-picking systems provided by another company, such as KIESTRA [10]. Thus, before choosing a system that provides unusual inoculation, clinical bacteriologists should

be aware that they will be captives of this system for some downstream automated applications. Overall, we may hope that companies that are now working on the solutions of tomorrow will see the importance of maintaining a high level of compatibility with other systems, rather than playing the non-productive game of delimiting their market.

Conclusion

The challenge for each clinical bacteriologist is to determine the ideal automated system suited to his or her own laboratory. Indeed, different solutions will be preferred, according to the number and variety of samples, and the types of sample that will be processed with the automated handling system.

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Transparency Declaration

The authors have no conflicts of interests to declare.

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