The Rising Tide of State-of-the-Art Automation

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Introduction

They say *"A rising tide lifts all boats"* and this is true for advances in automation as applied to various analytical test methods. Have you checked the state of automation capabilities *recently*? There have been amazing advances in terms of (a) high throughput, (b) error reduction, (c) test organization and (d) value / analyst effort. In keeping with the desire of meeting 3R parameters (reduce, reuse, replace the need for using animals) a new solution highlighted from bioMérieux provides advantages that reduce user hands-on-time, cost per test, employs sustainable rFC reagents, and thus eliminates the need of harvesting horseshoe crabs for bleeding.

The word *"Robot"* is said to be drawn from an old Russian word, robota, for "servitude," "forced labor" or "drudgery." In many respects this view holds true, automation does what people do not want to do, however, from another vantage it also does what people are not very good at doing. In performing a task repeatedly (a.k.a. robotically) people tend to become desensitized and somewhat immune to seeing that changes or bad habits have crept into their performance activities. We view this as a "learned" behavior but from a negative vantage it is a learned "shortcut" behavior that may produce a reduction in the quality of the test. In this way robots do not have "feelings", do not get hungry, or tired, and are not besieged by a slew of co-worker related issues. Robots do not lose their place when pipetting samples into a plate and are not "late" in putting a plate into or removing it from an incubation step.

The Tecan Fluent® workstation has been paired with the bioMérieux ENDOZYME® II GO pre-coated plates to create a seamless automation solution for endotoxin testing.

(a) High Throughput

High throughput is simply the capacity to test a lot of samples in a short time. In endotoxin testing the use of recombinant Factor C reagent provides several unique benefits that make the leap in throughput possible. These include the use of an endpoint test where a beginning and end read are all that's needed to generate results thus opening up the availability of a single reader and system for multiple simultaneous tests. The process is shown in Figure 1.

1. Robotic consumable/reagent/sample/ software preparation
2. Robotic addition of samples to plates
3. INITIAL plate reading

Figure 1.

STREAMLINED ENDOTOXIN TESTING.

Cut hands-on time in half with ENDOZYME® II GO.

The GOPLATE™ reduces traditional endotoxin workflow to 3 easy steps. The innovative microplate is pre-coated with Control Standard Endotoxn (CSE), including standard curve points and positive product controls. This eliminates the need for mixing, diluting, vortexing, or adding CSE. Now, you can streamline endotoxin testing and minimize the risk of error in less than 20 minutes.

LAL WORKFLOW	ENDOZYME II GO
Standard Reconstitution	✕
Preparation of Standard Curve + Vortex + Deposit	✕
Preparation of Samples	Preparation of Samples
Adding PPCs	✕
Reagent Preparation	Reagent Preparation
Addition of Reagent	Addition of Reagent
45 minutes	20 minutes

PIONEERING DIAGNOSTICS

biomerieux-industry.com/Endotoxin

4. Incubation at 37°C
5. END plate reading

Table 1.

Plate number INITIAL read/ Time (min)						Plate number END read/ Time (min)/Σ Samples					
1	2	3	4	5	6	1	2	3	4	5	6
0	8	16	24	32	40	20	28	36	44	52	60
						20	40	60	80	100	120

In the above example each plate has 20 samples and 6 plates have been tested in a total of 60 minutes which is 120 samples (2 samples per minute on average). The 20 minute test using rFC uses the 0.05 EU/mL sensitivity which is typically adequate for purified waters that have a USP limit of 0.25 EU/mL. A more sensitive test (0.005 EU/mL) is also available by incubating each plate for 60 minutes rather than 20 minutes (incubation at 37C). While the plate is not being read, after the initial read, the robot places the plate into the incubator and proceeds with the next beginning plate read.

A second advantage of the ENDOZYME® liquid rFC reagent is that it is extremely stable at room temperature, meaning it can sit out for at least 8 hours in a transparent tube, while other recombinants and LAL products have approximately 30 minutes stability at room temperature.

(b) Error reduction

The new robotic offering employs the use of ENDOZYME® II GO plates that provide pre-spiked product control wells (PPCs) and pre-loaded standard curve wells which can be reconstituted by simply adding endotoxin free water. In this way, plates are used that already have the expectation of standard curve and PPCs that are always in range. This method, automation within automation (nested testing), streamline's user performed activities to the bare minimum. This reduction of user inputs translates into an error-free, hands off, seamless performance expectation. In this way, the slogan "just add samples" or "just add water" realizes the true potential of automation.

The complete solution includes the following capabilities:

- Full (walk away) automation
- Uses rFC – Animal/Horseshoe Crab free
- High throughput up to 120 samples perhour (water)
- Any dilution level with water or any buffer (with finished products)
- Fully traceable 21CFR part11 with LIMS connectivity - Single system
- Rapid IOQ – roughly1 week
- Pre-coated plates no dilution, vortexing or addition of RSE/CSE necessary
- Flexible open system allows, LAL, ELISA or other automation
- HEPA hood to prevent false positives

(c) Test Organization

Organizational improvements apply to both, (i) the physical organization and manipulation of consumables, reagents and samples but also to (ii) the data and meta-data gathering and organization capabilities. These organizational improvements largely fulfill the ALCOA expectations built around CFR Part 11 compliance expectations.

ALCOA is an acronym for *Accurate, Legible, Contemporaneously created, Original and Attributable* electronic records for review and approval. In short, a robot doesn't know how to be disingenuous in an effort to either short cut assay tasks or to try to make a failing assay pass.

In this way it is clearly superior in excluding the possibility of injecting human feelings and/or failings.

From the scheme in Figure 2 the entire process becomes an interface for human interaction in a more organized and efficient way and in a manner that facilitates regulatory compliance.

The scheme in Figure 3 shows that specific activities are *integrated* in a way that human and material interactions alone cannot be. For example, the PC includes the reader software as well as the robot controlling software which scripts include the consumables placement and movement as well as the liquid handling capabilities. Furthermore, it allows automatic, pre-programmed control by integrating external reader and incubator loading and unloading that contains an exacting need for timing which the robot can automatically monitor, execute, and document the performance. The tracking of all these activities, as documented in real time

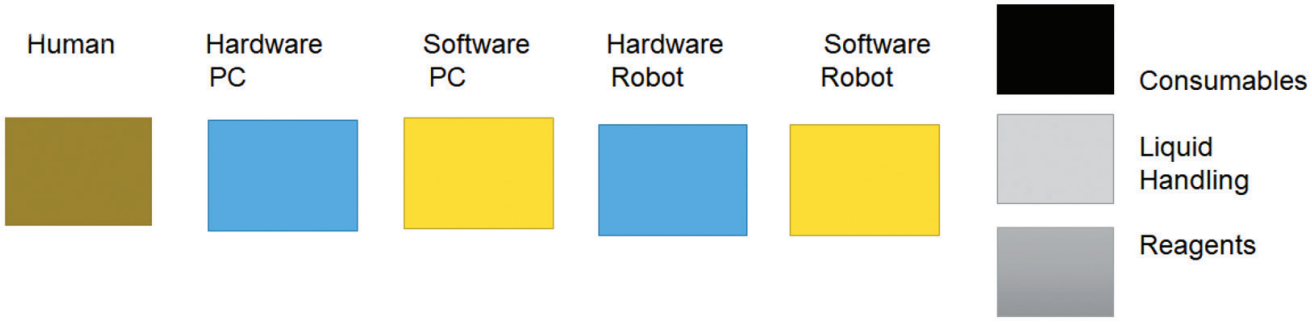

Figure 2.

Figure 3.

(contemporaneous in ALCOA), contrasts an analyst's performance which cannot be readily tracked (short of a constant video of her activities). These activities can be viewed as "meta-data", which stand between the script and the actual test data and is certainly relevant for to the test performance consequently. Moreover, the full automation enables retesting and/or investigation activities from the beginning to the end, what allows to speed up processes. This saves valuable resources like time (hands on time) and money.

The organizational aspect of robotic testing is shown in Figure 4 as it applies to endotoxin testing using FLUENT®.

(d) Value / Analyst efforts

Testing 30 to 50 samples a day, much less >100, is a significant investment of analyst time and effort. The time part is an easy robotic fix facilitated by adding the capability of performing 120 samples in an hour. **Value** is gained in several ways including the use of all liquid reagents that allows for exacting use of exact volumes with having only a small dead volume (few µL), as opposed to LAL that must be combined from discrete units (2.6 or 5.2 mL vials) that invariably will leave some significant reagent waste. The performance of all activities by a single analyst also reduces the need to focus an entire QC team on daily testing.

Let's look at the **effort** part: Manual testing requires reader software setup, sample preparation as per protocol, CSE preparation (serial mixing and diluting), sample preparation and dispensation, completing worksheets, documentation, analyst verification, and result reporting. The following robotic attributes greatly contribute to streamlining this entire process including:

- User-focused daily operation
- Workflow specific guidance
- Intuitive Touch Screen interface
- Active stop and resume
- Drag and drop assay setup
- 3-D Simulator to program without complexity

It is often said that the most expensive test is a test that must be repeated. Reducing the error rate by handling errors is only one of the advantages of **using a pre-spiked plate:**

- No reconstitution of CSE vial, dilution and mixing (reduce false positives due to lab exposure to CSE aerosol)
- the standard curve is already pre-coated on each plate (no manually prepared serial dilution necessary)
- No addition of tiny sample spikes (already added to wells)
- Concentrates the analyst's attention to
    - properly preparing the samples for test
    - completing necessary documentation steps

In conclusion, the robotic application fulfills the requirements of high throughput, high quality, and high integrity testing in a way that hasn't been done before. One hundred and twenty samples in an hour is a leap of sample processing power unparalleled in the industry to date. Being able to process one hundred and twenty samples in one hour offers a new, powerful high throughput solution for the endotoxin market.

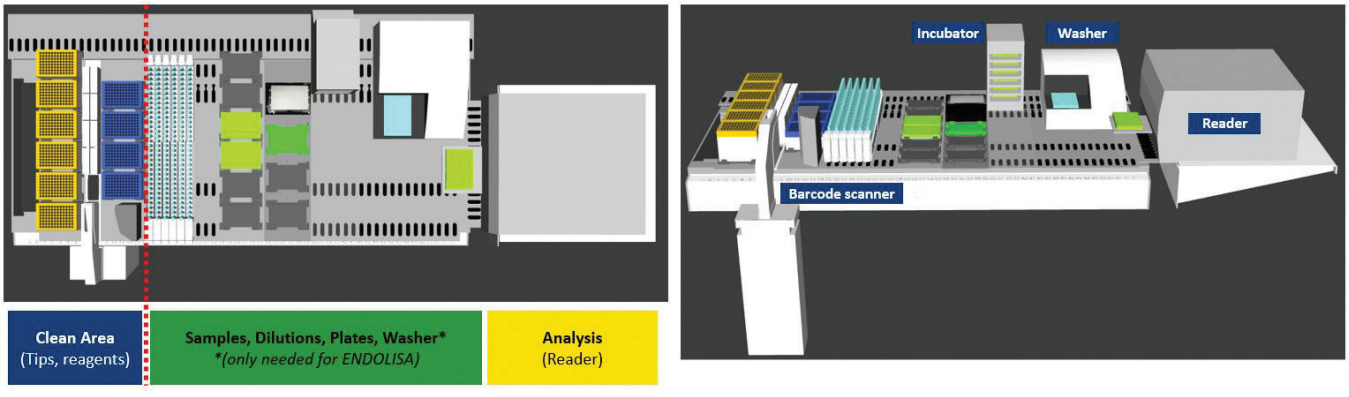

Figure 4.