

PERSPECTIVE

This issue of the Endosafe Times addresses 2 topics that have significant impact on day-to-day LAL laboratory activities. The first topic is an important update to an FDA regulatory issue that has captured the attention of the entire pharmaceutical industry. It pertains to the issuance of a new Draft Guidance Document on Electronic Records and Electronic Signatures.

The FDA is considering significant changes in its approach to 21 CFR Part 11 compliance. The new Draft Guidance promotes a risk based approach to compliance. The nature of software development does not allow it to be reactive. Programmers and vendors must be proactive. The software program we use, EndoScan-V, takes this approach. As the regulations change, EndoScan-V complies with the most stringent and the more relaxed regulations. EndoScan-V's audit trail not only meets the regulatory requirements but exceeds them through an unprecedented level of detail. EndoScan-V gives users the most scalability in using either data reduction or data reduction and electronic signatures. EndoScan-V allows users with either a single installation or multiple installations to comply with regulations without adding layers of complexity.

The second topic presents the summary of a study conducted by the Endosafe Technical Service staff. This study was initiated in an attempt to address one of the most frequent concerns presented to us, namely the question of endotoxin stability of various samples in different storage conditions.

Storage of samples can have an impact on reportable endotoxin levels. To determine the impact that storage may have on endotoxin values, a validation study should be performed. The validation study should consider temperature, container type, duration of storage, as well as differences in sample (product) formulations. As our short study illustrates, the stability pattern varies, depending on conditions, which make it imperative to perform the analysis in individual facilities.

21 CFR PART 11 DEVELOPMENTS

Complying with 21 CFR Part 11 has been a significant undertaking for the pharmaceutical industry. One of the major points of contention is the scope of 21 CFR Part 11. The definition provided by the FDA is very broad. Significant capital and effort have been devoted to meeting Part 11 requirements, even while questioning the practicality in some cases.

As part of the FDA's CGMP initiative, the Agency is re-examining Part 11 as it applies to FDA regulated products. They have released a Guidance for Industry Part 11, Electronic Records: Electronic Signatures-Scope and Application. The full text of the draft document may be found at www.fda.gov/cber/gdlns/prt11elect.htm. To avoid confusion during the re-examination period, all previous Guidance for Industry documents relating to Part 11 and the Compliance Policy Guide for Electronic Records and Electronic Signatures have been withdrawn. Of particular interest throughout the newly issued document is the Agency language that repeatedly states that predicate rules, such as GMP's and GLP's must be followed, and that the Agency will use "enforcement discretion" in any enforcement actions. This statement is encouraging, in that it indicates the FDA's willingness to look at the "big picture" when deciding on enforcement action.

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We should keep in mind the Agency is not repealing Part 11, rather they are simply re-thinking their enforcement of the items listed below. The Agency will continue to enforce the remaining portions of Part 11. It is clear from the document that the Agency does not expect the industry to discard positive changes brought about by Part 11.

It may also be understood from the statements about “business practices” and “enforcement discretion” that if you currently comply with Part 11, you cannot choose to stop complying because of this guidance.

As with any Draft Guidance, this represents the Agency’s current thinking and contains non-binding recommendations. It is prudent that companies do not stop 21 CFR Part 11 programs that are in currently place. In light of the scope of this guidance, it is also prudent that these programs be re-examined and fine tuned until a final guidance is released. While the re-examination is in process, the FDA has stated the following:

- The Agency will narrowly interpret the scope of Part 11.
- The Agency will not normally take enforcement action involving validation, audit trail, record retention and record copying requirements of Part 11 as stated in this guidance.
- The Agency will exercise discretion with enforcement of systems in place prior to Part 11.

In this document, the Agency has acknowledged some of the key concerns of the Industry. The voices of many individuals and industry coalitions helped influence the decision. The industry strongly voiced several key concerns. One such concern was statements from individuals being interpreted as Agency policy. The regulations also unnecessarily placed restrictions on the use of technology. Additionally, there were significant increases in the cost of compliance that were not contemplated at the time 21 CFR Part 11 was issued. The guidelines also had the effect of discouraging innovation and technological advances

without providing significant public health benefit.

The Agency has a simple and well-crafted approach to enforcing compliance until an official guidance is issued. Some of the key points to consider are stated in the Draft Guidance as follows:

- Narrower interpretation - “We are now clarifying that fewer records will be considered subject to Part 11.”
- For those records subject to Part 11, the Agency intends to use “enforcement discretion” regarding requirements for validation, audit trails, record retention and copying, and in applying Part 11 to legacy systems.
- Predicate rule requirements to records that are subject to Part 11 will be enforced.
- All other provisions of Part 11 not specifically noted in this document (Draft Guidance) will be enforced. For example, limiting system access to authorized individuals, device checks, validation, and protection of records.
- The following is a direct quote from the draft document: “... when persons use computers to generate paper printouts of electronic records, those paper records meet all the requirements of applicable predicate rules, and persons rely on the paper records to perform their regulated activities, the merely incidental use of computers in those instances would not trigger Part 11. In such instances, the FDA would generally not consider persons “to be using electronic records in lieu of paper records” under 11.2 (a) and 11.2 (b).”

Until another Draft Guidance (or a Final Guidance) is released, if the paper record is stated to be your official record, then Part 11 does not apply. An important distinction needs to be made here. If you print the EndoScan-V report and distribute it for your “official” record, all copies and activities must stem from this paper record. If the electronic file was used for any regulated activity, such as releasing a batch for sale, then the EndoScan-V report would be considered electronic and subject to Part 11.

Definition of Part 11 records

Part 11 will be applicable to the following records or signatures in electronic format:

- Records that are required by predicate rule and maintained in electronic format in place of paper format. By the same token, records maintained in electronic format not required by predicate rules or SOP’s are not Part 11 records.
- Records that are required by predicate rules to be maintained are maintained in electronic and paper format, and are relied upon to perform regulated activities. Interestingly, the FDA will consider your business practices for enforcing this rule (see EndoScan-V example). The FDA further recommends that you state in your SOP whether you plan to rely on paper or electronic records to perform regulated activities.
- Records submitted to the FDA under the predicate rules. However, if a record is used to generate a submission but is not included in the submission, it is not subject to Part 11 unless a predicate rule requires that it be maintained, and it is maintained in electronic format.

Validation

All predicate rule requirements for validation must be followed. It is interesting to note, however, that the FDA repeatedly and specifically points out that this requirement is necessary even if there is no predicate requirement for validation or audit trail. If it is important to guarantee the accuracy and reliability of the Part 11 records on the system, then they may use enforcement discretion to enforce Part 11 in those situations. The agency states throughout this section of the guidance, that you should use a justified and documented risk assessment based on product quality/safety/record integrity in determining the level of compliance. The specific example the Agency uses in this section is a word processor being used only to generate SOP’s, which would most likely not require validation.

Audit Trail

Here again, the Agency states they may use enforcement discretion regarding audit trails. The Agency states: "... it may nonetheless be important to have audit trails or other physical, logical, or procedural security measures to ensure the trustworthiness and reliability of records."

The Agency suggests your decision should be based on a justified and documented risk assessment. The Agency will look particularly closely at audit trails in situations where the users are expected to create, modify, or delete regulated records during normal operations.

Legacy Systems

At this time the Agency has realigned their position with that of the industry. The Agency will not normally enforce components of Part 11 on systems that were previously and are currently in compliance with predicate rules, provided they were in place prior to the release of Part 11 and are fit for their intended use. This language clearly indicates that, under most conditions, legacy systems are exempt, but the Agency will encourage movement away from older systems.

Copies of Records

The Agency has departed from the position of a universal format for review and manipulation by the Agency outside of the facility. Instead, the Agency has stated it would like a common format for computer-generated files. The Agency recognizes that proprietary formats do exist, and records may only be available in such proprietary formats.

- An investigator should be supplied with all reasonable and useful access to records.
- "You should allow inspection, review, and copying of records in a human readable form, on your site, using your hardware and software, and following your established procedures and techniques for accessing those records."

- This statement clears the way for continued use of proprietary file formats.
- Where documents are held in a common portable format, those documents should be supplied to the FDA in that particular format.
- Use established conversion methods when possible to make copies of documents in a portable format, including PDF.
- If the Part 11 records submitted have the capability to search, sort, and trend, copies provided to the Agency should have the same capabilities when technically feasible.

Record Retention

The decision on how to maintain records should be based on a justified and documented risk assessment, and a determination of the value of records over time. All predicate rule requirements must be met. The FDA does not normally intend to object to archiving required records that are in electronic format to a non-electronic format, such as microfilm, microfiche, and paper, or to a standard electronic file format such as PDF. The archiving method must preserve the records' integrity, content, and meaning. In addition, paper and electronic records and signature components can co-exist, as long as content and meaning are preserved.

The issuance of this Draft Guidance is an encouragement to the industry. Although Part 11 brought about an increase in the quality of software, it also served as an impediment to developing custom applications for end-users. This more rational approach will make new software and changes to existing software easier and more useful.

LABORATORY NOTEBOOK

Protocol For Sample Storage Temperatures

The Endosafe Technical Service staff conducted a study that examined

storage temperatures for samples that are delivered to the LAL laboratory for testing. This "sample stability" testing was performed using two (2) sterile ATCC bacterial cultural filtrates and a purified Control Standard Endotoxin. The ATCC bacterial cultures were adapted to grow in a diluted Nutrient Broth medium. Diluted media was used to intentionally mimic natural, water born endotoxins that could potentially contaminate a manufacturing process.

Test samples themselves were evaluated fresh, and then evaluated while stored at room temperature, refrigerated, and frozen conditions. An attempt was made to cover as wide a sample spectrum as possible. Towards that end, 3 sample types were evaluated for endotoxin stability. They were:

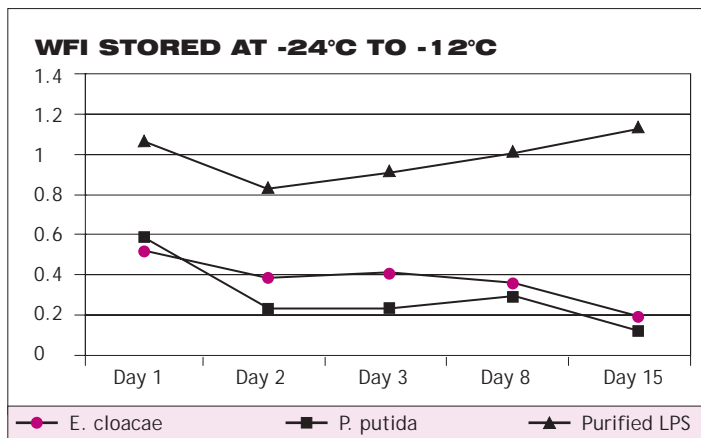
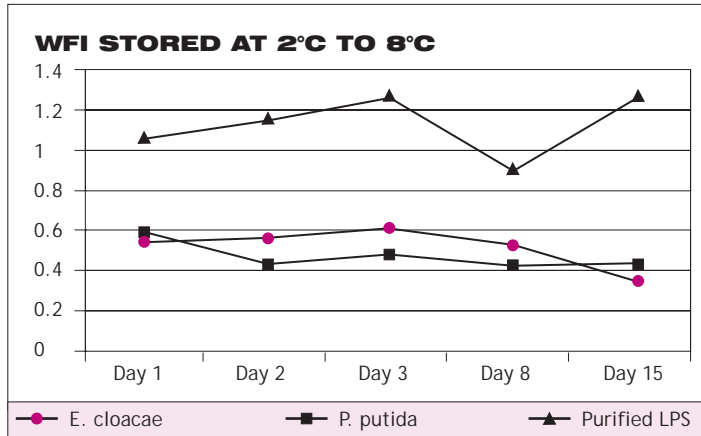
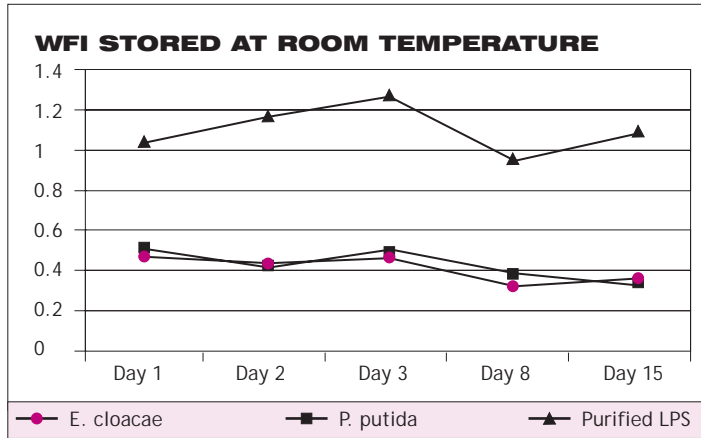
- Water For Irrigation (WFI)
- Simple Salt Solution (0.9% NaCl)
- Sterile Biological (HSA)

Each of the above sample types was inoculated with two naturally-occurring bacterial culture filtrates and a purified endotoxin. In each study, the samples were prepared in polystyrene containers, and then allocated to another set of polystyrene containers according to their respective storage condition. It is significant to note that aliquots of the materials to be frozen were distributed into five separate tubes, one tube for each day of testing. This was done in order to prevent variability that can be associated with multiple freeze and thaw cycles.

Routine kinetic turbidimetric LAL testing, using a standard curve range of 5, 0.5, and 0.05 EU/mL was used to evaluate sample endotoxin levels. All samples were tested on day one (time zero), day two, day three, day five, day eight, and day fifteen. All routine test criteria, such as linearity, negative water controls, spike recovery and coefficient of variation, were applied. During the course of testing, all suitability criteria were valid.

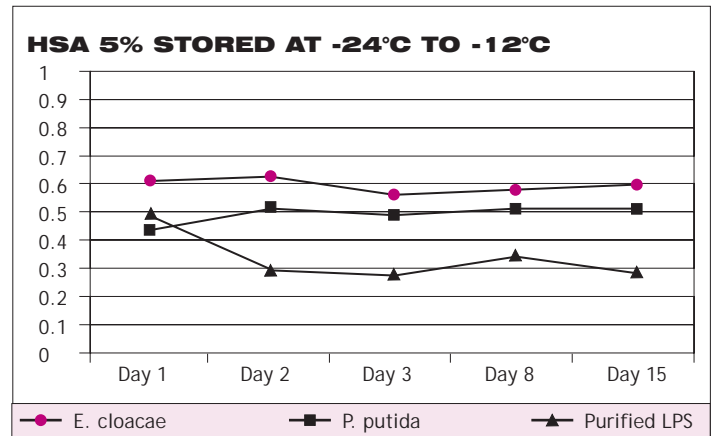
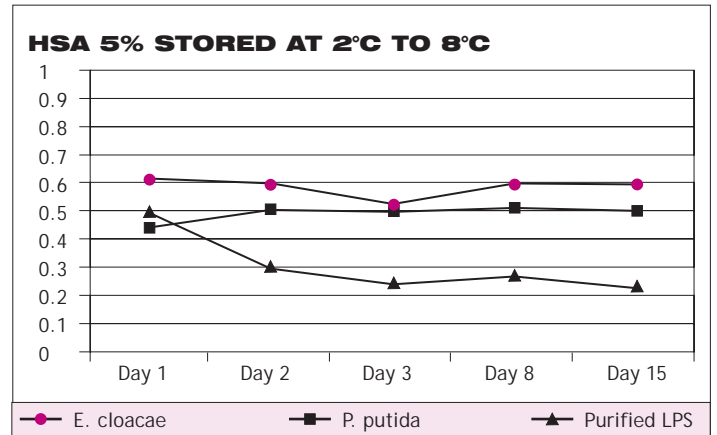
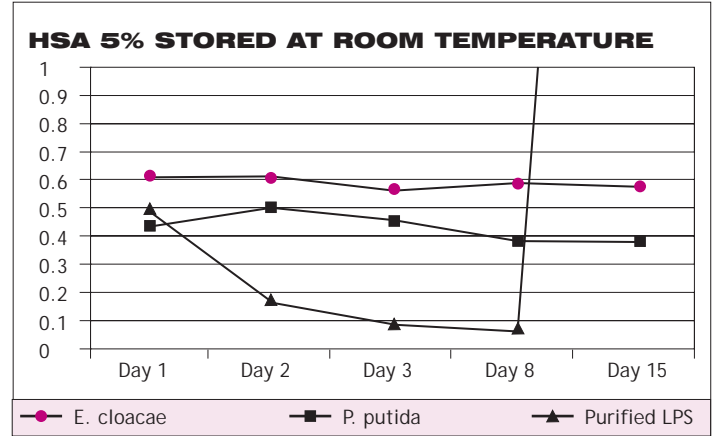
Sample A: WFI

With WFI, endotoxin samples stored at room temperature and at refrigerated temperatures appear to be more suitable over frozen conditions.



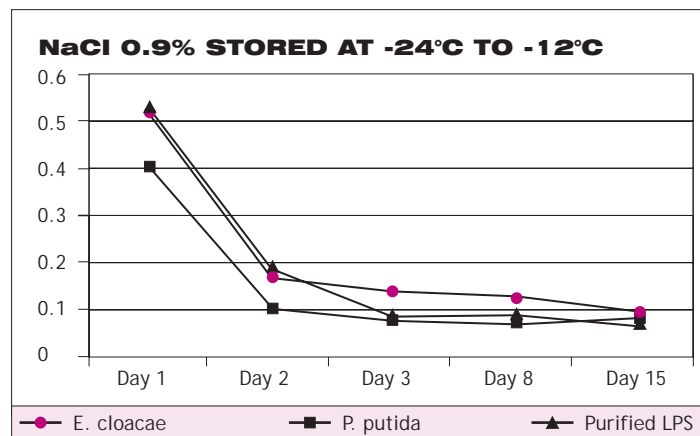
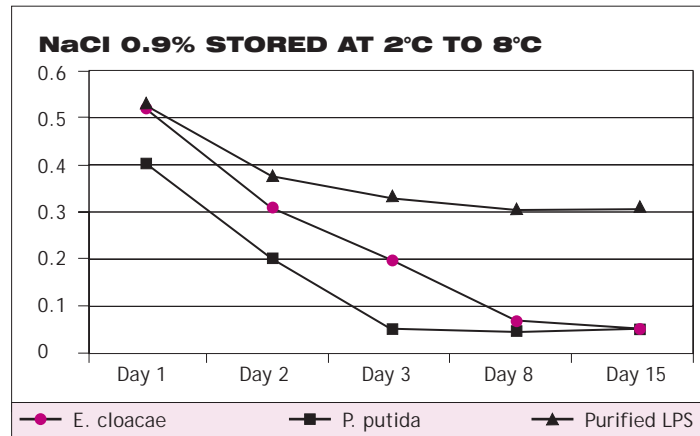
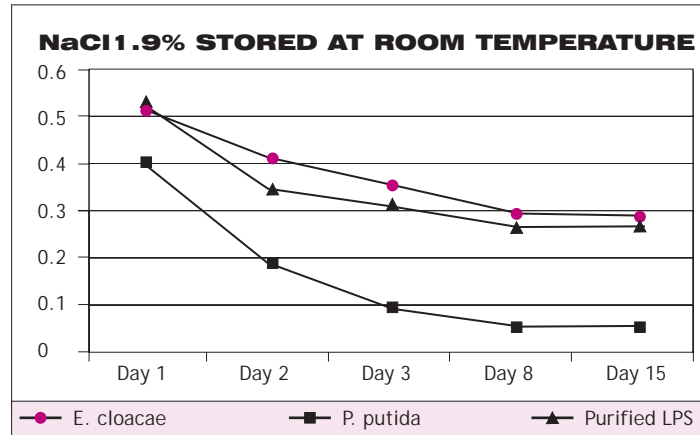
Sample B: Sterile Biologic (HSA)

As the results clearly indicate, the naturally occurring endotoxins exhibited remarkable stability under all temperature storage conditions. However, spurious data were obtained with the purified CSE. HSA does have an affinity for the Lipid A component of bacterial endotoxins. At room temperature storage, binding and masking is demonstrated. The 50-fold increase at day 15 at room temperature is attributed to bacterial contamination and outgrowth of the sample.



Sample C: Simple Salt Solution (9% NaCl)

In a simple salt solution, *Pseudomonas putida* did not demonstrate any stability for all 3 temperature storage conditions. On the other hand, the CSE and the natural endotoxin from *Enterobacter cloacae* appear to be more stable at room temperature than at refrigerated and frozen temperature conditions.



Summary of Results

The respective graphs provide a summary of the observed results of these studies. Please note that changes in endotoxin values were held significant only if a difference of plus or minus a two-fold dilution was seen from time zero.

CONCLUSION

The results from this study highlight the biological diversity of bacterial endotoxins. The data demonstrate that storage temperature, sample formulation (type), and the type of gram negative bacteria colonizing a particular environment all influence the endotoxin stability of a sample. Clearly endotoxin stability/activity cannot be predicted. As such, it is necessary that each facility validate the storage of their samples. Charles River Laboratories' Technical staff can help with the development of your protocol by providing the template we used for this study.

LAL POINTERS

Endotoxin:

Units/Volume versus Total Units

Quite often, we receive questions like: If you only take 0.1ml of an endotoxin standard, why do you not report data on a per 0.1 ml basis? The answer is twofold. The first part to this answer pertains to product endotoxin limits. All endotoxin limits are based on patient dosing (the K/M formula). The LAL endotoxin limit is based on worst case patient dosing and/or the potency of the product. LAL limits are expressed as concentrations; i.e. endotoxin units per mL or endotoxin units per mg of product.

The second part of the answer pertains to different LAL tests methods. Most methods use 0.1 mL of sample and standards. However, some methods use 0.05 or 0.4 mL. Regardless of the LAL method, standards and samples are treated the same way.

As such, each LAL assay measures the "relative" activity of a test sample against endotoxin standards whose concentrations were prepared on a per mL basis.

Note to Remember:

Endotoxin Units/mL is not necessarily going to be additive. For example, if you mix 2 EU/mL of a product with 2 EU/mL of another product (equal volumes), then the Endotoxin concentration will still be 2 EU/mL, not 4 EU/mL.

WHAT'S NEW

Charles River Laboratories Annual Summer LAL Workshop:

- August 19-22, 2003
Kinetic & Gel Clot LAL Test Methods held in Charleston, SC

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