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EDITOR'S COMMENTS

Municipal water systems supply the bulk of the water used for parenteral manufacturing and usually contain 10-to-100 EU/mL of environmental endotoxin. However, contamination within a WFI unit presents the greatest challenge to maintaining a high-quality water system. In this second part of a series addressing endotoxin testing in the production process, we will explain how water systems become contaminated and how to best monitor system performance, manage data, qualify new systems and trend results.

Dr. James F. Cooper

MONITORING WATER SYSTEMS FOR ENDOTOXIN

Dr. James F. Cooper and C. Scott Polk

Overview. Water is the principal source of endotoxin in parenteral products. The **USP** set the endotoxin limit for Water for Injection (WFI) at 0.25 EU/mL, twice as stringent as a large-volume parenteral (LVP), due to concern that there might be an additive effect of low levels of endotoxin in a drug product and its vehicle. Fortunately, twenty years of experience in the industry has failed to substantiate this concern. This discussion addresses LAL methods, sampling plans and trend analysis used in qualification and maintenance of a pharmaceutical water system.

Endotoxin Limits. Realistic alert and action limits may be set on a local level based on historical data and process capability. Representative limits adopted by parenteral firms are presented in Table 1. Ideally, endotoxin levels are measured and reviewed at suitable intervals to discover changes in endotoxin levels before they become problematic. Kinetic systems offer the greatest efficiency in managing and trending endotoxin levels.

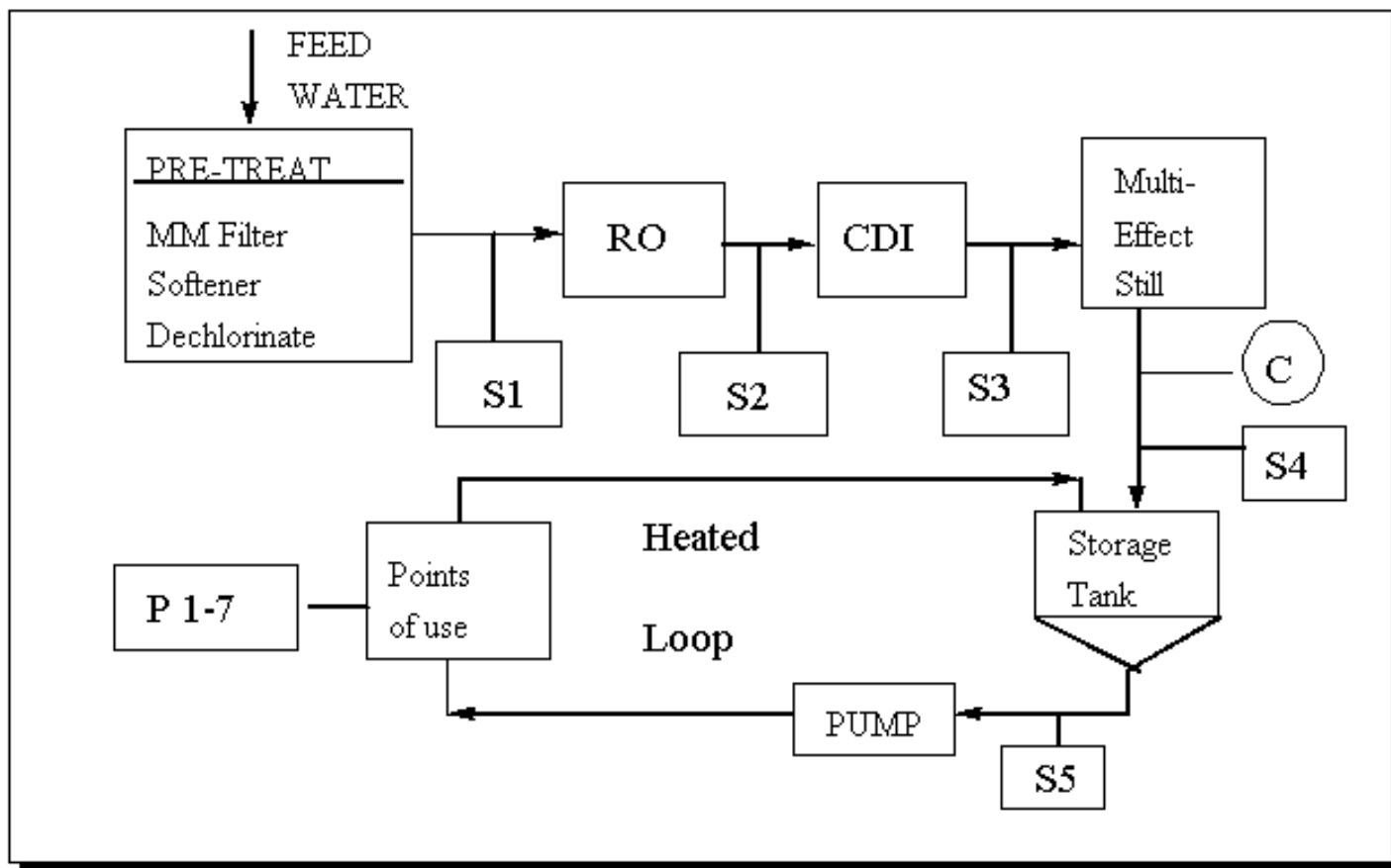
Table 1. Representative Action and Alert Limits for Endotoxin in WFI

Technique	Alert Limit	Action Limit
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Gel-Clot	0.03 EU/mL	0.125 EU/mL
Kinetic	0.01 EU/mL	0.1 EU/mL

Review of Water Systems: Many combinations of components may be joined to form a water system, depending on local needs, as discussed by Meltzer. (1) Figure 1 presents a typical WFI system for a clinical production setting; it assumes that heat facilitates microbial control in the critical loop.

Fig. 1 WFI SYSTEM



Major components include the following:

1. pretreatment to remove particulates, oxidants and hardening ions
2. reverse osmosis to remove dissolved matter
3. ion-exchange to remove salts
4. distillation to complete the purification process
5. storage for monitoring and distribution
6. heated circulating loop for final use.

System components, such as deionization and water-softener units, are subject to fouling and bacterial colonization, and thus, become the primary source of endotoxin. These colonies are gram-negative rods which become a constant source of bioburden and endotoxin, requiring sanitization or replacement of the contaminated unit. Development of biofilm in water pipes and tanks is another common source of endotoxin. Biofilm is an accumulation of microbes (dead and live) on pipes and surfaces, particularly near joints, elbows or dead legs. **(2)** The purpose of endotoxin and bioburden monitoring is to detect the development of these kinds of problems.

A sampling protocol must be designed to detect sources of endotoxin caused by some type of system failure or bacterial growth. The principal depyrogenating treatments for water systems are distillation, reverse osmosis, ultrafiltration and ozonation. Distillation or reverse osmosis (RO) is required for WFI production by **USP 23. (3)** A properly functioning still or ultrafilter will reduce endotoxin levels by at least 4 logs. Therefore, sampling sites should include all endotoxin-removal steps, water storage and points of use. The sampling ports (S or P) marked in Figure 1 represent important sites to monitor endotoxin levels at appropriate intervals.

Validating Storage. At least two types of containers should be qualified as collection devices. Depyrogenated glass is the best choice, but there are new types of heat-stable plastic containers that can be depyrogenated. The qualification procedure must prove that collection devices will store endotoxin for as long as water samples are held prior to testing. Suitable endotoxin samples for this qualification include LPS solutions and mixed endotoxins, such as sterilized tap water.

Validation of a New Water System

Most regulatory agencies require an operational qualification for microbiological control to persist for 3 to 10 weeks. **(1)** During this time all sampling ports are tested daily to show that operational and maintenance protocols are adequate, and that the system will consistently produce water that meets specifications. The final phase of validation or performance qualification may persist for at least 6 months to confirm that the water system and protocols accommodate seasonal variations. During this phase critical points are monitored daily and all points-of-use are tested at least weekly. Annual re-qualification of a system requires 1-to-3 weeks of a daily sampling protocol. An increased sampling rate should follow any corrective action, repair or system upgrade.

Previously, positive controls (PCs) were not always used for water samples because water was considered non-inhibitory. Now, PCs are prudent because we know there are several sources of inhibition in water samples, including the following:

1. trace amounts of tri-valent cations, such as ferric ions,
2. trace amounts of oxidants, disinfectants or cleaning materials,
3. extraction of inhibitors from plastic collection tubes.

Enhancement occurs in kinetic testing when low-level standards become weak or the standard range exceeds the sensitivity of a kinetic reagent, *i.e.*, the linear portion of the curve. This enhancement is avoided by using freshly-prepared standards, LAL reagent with good linearity (>0.998), and a limited range, such as 1 to 0.01 EU/mL. Glucan enhancement won't be a problem unless water is exposed to a cellulosic source somewhere in the system.

Routine Monitoring of a Water System: The purpose of endotoxin tests for water systems is to release WFI quality water for production needs and monitor the function of critical components in the system. Endotoxin data is used to assess how well individual components are performing and to compare new with historical data to identify a significant change in performance.

The SOPs should include sampling locations, frequencies, alert limits, actions limits and corrective actions. A minimum, routine sampling plan for a representative water system (Fig. 1) might require samples taken from the following sites and intervals:

1. RO permeate, tested weekly
2. CDI effluent, tested weekly
3. still condensate, tested daily or weekly
4. storage tank(s), tested daily
5. 2 or more points-of-use from each loop, tested daily
6. all point-of-use tested at least weekly, with alternating PCs.

It is industry practice to test all water storage tanks and 2 or more points-of-use from each WFI loop, daily. Other system devices could be monitored on a weekly basis to establish a data base. A significant upward trend in endotoxin levels may initiate sanitization actions. These devices should be tested immediately if an alert limit is exceeded at a critical point, such as a storage tank or point-of-use. Some production facilities test all tanks and points-of-use on a daily basis. In that case, only one Positive Control is required for each water loop. However, it is prudent to rotate the PC site so that all sampling ports are controlled within a one or two-week period.

Kinetic LAL Systems for Water Testing: Kinetic turbidimetric assay (KTA) is the most economical, informative way to collect and store data from water testing. The optimum standard curve for monitoring a water system with kinetic assays is 1-to-0.01 EU/mL. This range is wide enough to measure levels from various components in the system. This range also makes results available for production promptly because the lowest point on the curve will be recovered within 45 minutes with Endosafe® KTA with the Bio-Tek ELx880 with Biolise software.

Trend Analysis with Biotrend: Charles River Endosafe has created BioTrend, a product trending database that maximizes the benefits of collecting kinetic LAL data. Biotrend operates by importing information from Biolise endotoxin detection software. Data is exported manually or

automatically by designing a Biolise protocol to export the information to the database with point-and-click convenience. Trended data may be displayed in graphical or tabular form.

May 01, 1998
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Charles River Endosafe BioTrend
Product Endotoxin Trend Report

Sponsor: Charles River Endosafe
Product: WF1

Date Range: 2/28/98 - 4/15/98

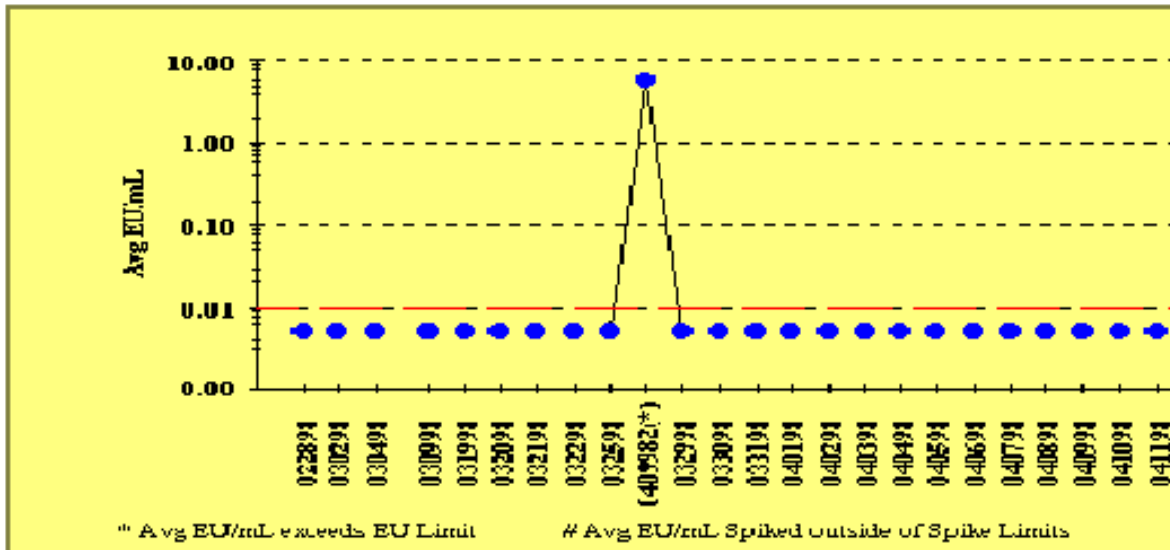


Figure 2. The graph presents endotoxin values collected over a period of time with kinetic turbidimetric methods. An endotoxin excursion occurred on day 10 which exceeded the alert limit and represented a significant departure from historical data. The out-of-specification alert triggered an investigation into the cause of the failure.

The ability to monitor the consistency of standard curve parameters represents another application of BioTrend.

In summary, Kinetic endotoxin testing is a quick, reliable way to document and monitor the quality and performance of a water system by means of collection, storage and trending of essential information. Ongoing evaluation of trended data aids recognition of underlying or developing problems, and may indicate that limits or monitoring frequencies need modification.

References

1. Meltzer, TH. *Pharmaceutical Water Systems*, Tall Oaks Publishing, Littleton, CO, 1995, pp. 739-744.
2. Schaule, G., Flemming, H.C. Pathogenic microorganisms in water system biofilms, *Ultrapure Water*, Vol 14, No. 4, 1997, pp. 21-27.

3. *The United States Pharmacopeia 23*, fifth supplement, General Information Chapter <1231> Water For Pharmaceutical Purposes, U. S. Pharmacopeial Convention Inc., Rockville, MD. (Nov. 15, 1996).

CALENDAR

LAL Users Group - June 12, 1998, West Point, PA

Speaker: Dr. James F. Cooper Topic: **LAL Technology in the Year 2000**

The discussion addresses the impact of BET harmonization, compliance with FDA's new scope for endotoxin testing, endotoxin-specific LAL reagents, and greater efficiency from equipment and software improvements that provide enhanced data management and trend analysis.

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CREL LAL Workshop, May 20-22, 1998, Stoke Rochford Hall, Nr Grantham, Lincolnshire. For more information, contact Sara Marsh: Phone (44)1843 822331; Fax (44)1843 822989.

IRELAND - LAL Workshop, May 28-29, 1998, Red Cow Conference Centre, Dublin. For more information, contact Dr. Bernie Lannon, at Medical Supply Co., Ltd, Phone:353 (01) 822 4222; Fax: 353 (01) 822 4100.

AAMI Annual Meeting, June 1-3, 1998, Philadelphia, PA. On June 2, Dr. Cooper will discuss endotoxin bioeffects and endotoxin detection methods.

Annual Charleston LAL Workshops, July 22-30, 1998, Charleston, SC - **Gel-clot workshop: July 22-25; Kinetic workshop: July 27-30**. Please register early for this 20th anniversary workshop. For workshop information, including registration forms or an expanded syllabus, contact Frances Cooper (Phone 843-795-7316; Fax: 843-795-7221). Hotel space will be held until June 21.

Fifth Conference of the International Endotoxin Society, September 12-15, 1998, Santa Fe, NM - This conference will include invited speakers and those chosen from submitted abstracts. For further information visit the Society web site at: <http://www.ies98.org>,

Email: c-c-m@worldnet.att.net; Phone: 619-299-6673; Fax: 619-299-6675.