Variability in the Bacterial Endotoxins Test

***Karen Zink McCullough**, MMI Associates*

We've all heard the expression, "the error of the gel clot test is one two-fold dilution." This assumption describes the range of 50-200% of nominal value of label claim or PPC in a kinetic test, which is really a rough measure of the aggregate effect of different sources of variability in the assay.

We take for granted, and gladly accept the 50-200% range when we qualify gel clot reagents. If the assigned label claim is 0.125 EU/mL but our observed result is 0.25 EU/mL, we "win" and happily use the assigned label claim of 0.125 EU/mL in all subsequent calculations. With the kinetic test, if we recover 50-200% of the nominal value of the positive control spike, we "win". That's the way it works.

We're fine with the two-fold "error" until we test a sample that contains endotoxin that approaches the endotoxin limit. Now, that same error might fail a "good" product or pass a "bad" product. We become concerned with the variability of the assay and look to everything from consumables to lab error to changing lysates or test methods to demonstrate that the product is really good to release.

What causes this variability, and how can it be controlled? Remember that the LAL test, whether it be gel clot, endpoint or kinetic is not your standard analytical assay. It is a biological system at work in a tube or well, and is subject to many sources of variability including reagents, instrumentation, lot-to-lot variability in the product, raw material variability, manufacturing variability, test method variability, standard curves for quantitative tests, analyst competency and consumables. A few of these sources of variability are discussed below.

Reagents. Lysate manufacturers have proprietary manufacturing processes and formulations that may result in reagents that differ in buffering capacity, glucan reactivity and divalent cation concentration, to name but a few. Within a lysate manufacturer, gel clot, turbidimetric and chromogenic reagents are optimized to meet the requirements of the assay methodology, and may differ from each other as well. All of this reagent optimization might not affect how well endotoxin in water is detected (the BET "gold standard"), but it may affect how well the lysate detects endotoxin in product (Tsuchiya 2009, LAL Users' Group, unpublished data, McCullough and Weidner-Loven, 1991). If you have difficulty demonstrating test suitability with your current lysate, try another manufacturer or formulation before you give up.

Standard Curves. Quantitative tests (endpoint and kinetic) are inherently more susceptible to variability than gel. With quantitative tests, the quality of the standard curve is essential to the accuracy of the test result. Although linearity is the only compendial requirement for standard curves, slope and y-intercept play a large part in overall variability as well. For example, a 1% variability in the y-intercept of a perfectly linear kinetic standard curve can result in a 36% inaccuracy in reported value of an unknown. The trick to controlling the standard curve is to understand expected onset times and resulting slope and y-intercept values. Changes in onset times will affect slope and y-intercept, and may affect linearity. Changes from the norm will tip you off that something is not right, and should be investigated. As for linearity, try for $|r| \geq 0.995$ rather than 0.980.

Consumables. Plastic accessories (plates, tubes, pipettes) can be a source of interferences (Roslansky, et al, 1990). According to USP, plastic labware must be checked for inhibition and/or resident endotoxin prior to use. One way to do this is to test consumables as if they were medical devices with very low endotoxin limits. If you accept a manufacturer's certificate of analysis, be certain that you understand and accept methodology that was used to generate the certificate.

Analyst competency. The BET is hugely technique dependent, so analyst technique is most important. Analysts must not only understand what they're doing, but they need to have good aseptic technique so that they don't inadvertently contaminate the product during the course of sample preparation and testing. Although not required by the Pharmacopeia, analyst qualification per the 1987 Guideline is one way to check on technique (making dilutions and keeping the test "clean"). Such training should be clearly documented and updated as needed.

How does a laboratory know if variability is in or out of control? A baseline and trend analysis of Out of Specification (OOS) and invalid test results is essential. An OOS is an indicator that they product may be

unacceptable, and invalid tests are an indicator that the assay itself is unacceptable. Any adverse trend in OOS or invalid tests will sent up a red flag to the lab. For example, an increased number of invalid tests due to lack of PPC recovery on a sample might suggest a look into changes in manufacturing processes, changes vendors or lots of raw materials used in the manufacture and formulation of the product, changes in consumables used to take prepare, and test samples, changes in reagent lots, changes in analysts, etc.

For more information on identifying and mitigating LAL Test Variability, consider attending the LAL Users' Group/PMF Bacterial Endotoxin Summit to be held in San Francisco March 22-23, http://www.microbiologyforum.org/2010/HPA1003/index.htm

Citations

LAL Users' Group, Unpublished data on the effect of heparin on endotoxin detection in four gel clot lysates

McCullough, Karen and Cindy Wiedner-Loven. 1992. Variability in the LAL test: comparison of three kinetic methods for the testing of pharmaceutical products. J. Parent. Sci. Tech. 44:69-72

Roslansky, PF, ME Dawson, TG Novitsky. 1991. Plastics, endotoxins, and the limulus amebocyte lysate test. J. Parent Sci Tech, 45: 83-87.

Tsuchiya, Masakazu. 2009. PMFList, December 7, response to "Variation in results of BET"