A QUICK SUMMARY OF THE HIGHLIGHTS OF PARTS 1 AND 2 OF THE FIRST ROUND ROBIN ENDOTOXIN ASSAY STUDY AND A BRIEF PREVIEW OF THE NEXT ROUND

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see Table 1 below

Abstract

The two part interlaboratory round robin endotoxin assay study has been completed. In both parts of the study, filter membranes with the same approximate amount and type of cotton dust were sent for analysis to laboratories that 'routinely' perform endotoxin analyses. Each of these laboratories performed the analysis using the methodology common to their laboratory. In the second part of the study, filter membranes with cotton dust were again sent to the same laboratories where the analyses were performed as before but with a common extraction protocol. The highlights from the two-part study will be summarized. The results stimulated interest in extending the study to include cotton dust with three levels of endotoxin on two different types of filter membranes.

Introduction

The problem of cotton dust and its relationship to byssinosis has been a topic of study at the USDA, ARS, Cotton Quality Research Station (CQRS) in Clemson, SC, for over two decades. In cooperative work done here and at other laboratories world wide, endotoxin (lipopolysaccharides, LPS) in cotton dust has been implicated as the most likely etiological agent of byssinosis (Castellan, 1997; Castellan, et al. 1984, 1987; Rylander, et al., 1984; Rylander, et al., 1985). Many methods have been used to determine the level of endotoxin and in no small way, the assay for endotoxin has garnered in importance in the study of respiratory dysfunctions (Jacobs, 1997; Godby, et al., 1995; Laitinen, et al., 1992; Michel, et al., 1996; Rylander, 1997).

What has been a sticking problem for researchers and others concerned with endotoxin levels, in cotton lint or dust and now in agricultural and other organic dusts, is that when identical samples are assayed for endotoxin content that level differences, often in the orders of magnitude, may be reported between different laboratories. This was an early observation made at CQRS when identical samples were sent to different laboratories for assay. Usually the quantitative levels returned were different, but the ranking of the samples was nearly always the same between the different laboratories. While not an ideal situation, this has permitted comparisons to be made and accredits the endotoxin assay for providing useful information. Still the differences in levels has always been a nagging concern since this meant that results reported and read in the literature must be interpreted with caution with due consideration of the extraction methods and the laboratory conducting the analysis (Chun and Perkins, Jr. 1994; Jacobs and Pietrowski, 1995; Milton, et al., 1992; Walters, et al., 1994; Wood and Jacobs 1997).

To get a handle on the problem, about 6 or 7 years ago, an interlaboratory study using uniform cotton dust was discussed among scientists, most notably: Henry H. Perkins, Jr., USDA, ARS, Clemson, SC (retired); Stephen A. Olenchock, NIOSH, Morgantown, WV; Ragnar Rylander, University of Gothenburg, Sweden; and Robert R. Jacobs, University of Alabama, Birmingham, AL. Even so, very little was done except for planning, discussions and further delays, until 1995 when a study between 10 laboratories was planned and uniform cotton dust samples were collected at CQRS (Perkins, et al., 1996). Still, further delays due to the make up of the interested parties occurred. But finally in 1997, the study was conducted as a two part interlaboratory round robin endotoxin assay study. In both parts of the study, filter membranes with the same approximate amount and type of cotton dust were sent for analysis to research laboratories that 'routinely' perform endotoxin analyses. Each of these laboratories performed the analysis using the methodology common to their laboratory. In the second part of the study, filter membranes with cotton dust were again sent to the same laboratories where the analyses were performed as before but with a common extraction protocol. At last year's Beltwide Cotton Conference, the results of the first phase of this round robin endotoxin assay study was presented which was followed by a report on the preliminary results of the second phase of the same study at an ACGIH (American Conference of Governmental Industrial Hygienists) workshop held at Chapel Hill, NC (Chun et al. 1998; 1999). This paper presents a summary of the highlights of the first phase and the complete results of the second phase.

Methods and Materials

Endotoxin Assay Committee

Participants in the round robin endotoxin assay study are listed in Table 1. Originally 14 laboratories were to participate in the first part of the study but two of the interested parties dropped out (not listed) and an additional laboratory asked to take part in the study. In the second part of the study, 13 laboratories participated.

Cotton Dust

Cotton dust was collected in 1995 as described by Perkins, et al. (1996) on polyvinyl chloride (PVC) filters (Perkins,

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Jr., 1975) using CQRS's model card room (Chun and Perkins, 1997). These were uniform, card generated, vertically elutriated cotton dust averaging from 0.3-0.7 mg per filter with a target of 0.5 mg per filter; and contained endotoxin levels which did not vary significantly either between vertical elutriator (VE) locations or between positions within locations. Twelve dust laden filters were produced from each vertical elutriator run. Seventeen VE runs were made. However, complete sets of 12 filters were found for only 16 of the 17 VE runs. Half of the filters were used in part 1 of the study and the remaining half were used in part 2 of the study. Each weighed dust laden membrane was transferred to a 50 ml screw-top polypropylene conical tube (Falcon® 2998; Becton Dickinson and Co., 2 Bridgewater Lane, Lincoln Park, New Jersey 07035) and stored in the dark at room temperature (~22°±1°C) until used.

General Protocol, in Part 1 of the Study

Originally 14 laboratories were involved with part 1 of the study. These laboratories were randomly assigned a laboratory identification number except for the laboratory doing GC-mass spectrophotometric analysis for total endotoxin content. This laboratory was assigned the last laboratory identification number in both parts of the study. A randomized complete block design with VE run as blocks was used. The 12 filters in each VE lot run were randomly assigned to each laboratory so that each laboratory received a total of either 7 or 6 filter samples for analysis. The dust weight was provided along with the dust samples. Control or blank filters were not sent unless the investigator requested them. Each laboratory performed sample extraction and endotoxin analysis based on their in-house protocol.

The dust samples were mailed February 25, 1997 to the participating labs. Results were received from the participating laboratories by facsimile transmission, mail, or by e-mail. Results were provided as endotoxin units per milligram (EU/mg) or were converted to EU/mg by conversion factors provided by the researcher or by assumed conversion factors (such as, 10 EU = 1 ng endotoxin). Where the data was provided in nanomoles, the MW (environmental LPS) = 8,000 was used for conversion to EU/mg (Larsson, personal communiqué).

General Protocol, in Part 2 of the Study

The general protocol was the same as in part 1 of the study except only 13 laboratories were involved. Unlike the first part of the study, where each laboratory performed sample extraction and endotoxin analysis based on their in-house protocol, each laboratory performed a common extraction protocol but did the endotoxin analysis based on their in-house protocol. The dust samples were mailed July 29, 1997 to the participating labs. Four categories of kit types were used. Three of the four kit types were endotoxin assay kits manufactured by BioWhittaker, Inc. (BioWhittaker, Inc., P.O. Box 127, 8830 Biggs Ford Road, Walkersville, MD 2193-0127): QCL-1000 (end-point chromogenic limulus amebocyte lysate assay), Kinetic-QCL (chromogenic kinetic assay), and Pyrogent-5000 (kinetic turbidimetric assay). The fourth kit type was used as a catch-all to include assay kits not manufactured by BioWhittaker, Inc. or using a BioWhittaker kinetic LAL used with conditions and standards independently referenced to EC6. The common protocol sent to each laboratory is as follows:

Common Extraction Protocol:

1. Assay should be done same day as extraction.
2. Use Pyrogen Free Water (PFW) for extraction.

   Use conditioned borosilicate tubes and PFW for making up dilution series. Conditioned borosilicate tubes to refer to clean or new tubes which had been heat treated to render the tubes pyrogen free (heat treatment as normally done in individual's lab. For example, methods used by some labs include heating tubes in an oven at 200°C for 8 hours or more; or 180°C for 3 hours or more; or heating at 250°C for 30 minutes.)
3. Add 20 ml PFW to tubes containing membranes (extract in the shipping centrifuge tubes).
4. Place on rotary/wrist shaker and shake at fastest practical rate for 60 minutes at room temperature.
5. After extraction, assay.
6. Please report results as EU/mg and give details on whole assay (If reporting as ng/mg dust, please indicate recommended conversion factor to convert to EU, otherwise 10 EU/ng will be used): problems with extraction etc. Endotoxin assay method/kit e.g. Kinetic QCL or QCL 1000 or other.

Statistical Analysis

Data were analyzed using release 6.12 or earlier releases of SAS (SAS, Statistical Analysis System; SAS system for Windows version 4.0950; SAS Institute Inc., Cary, NC, USA) for making mean comparisons. Otherwise additional testing and data manipulation was done with Microsoft EXCEL 97 SR-1 for Windows 95 or earlier releases of EXCEL (Microsoft Corporation, USA) and plotted using SigmaPlot for Windows or DeltaGraph 4.0 Version 4.01 (SPSS, Inc., USA).

Results and Discussion

In part 1 of the study, the time period for results to be returned from the participating laboratories ranged from less than a month to almost four months after the samples were mailed (Table 2). In part 2 of the study, the time lag from when the samples were shipped out and when results were received was much greater, possibly because of the

time of year or that the 'common' extraction protocol caused logistic delays. The time for results to be returned ranged from less than a months to almost eight months! In both cases, the time period did not seem unusually long and no significant differences in results due to delays in assay between the laboratories were expected and so no correction was taken into account. The time for results to be returned is included here since it has value in representing real world 'wait' time and should give the uninitiated a feel for how long it can take to get results back from research orientated laboratories rather than from commercial laboratories.

In part 1 of the study, the results from different laboratories made on almost 'identical' dust samples, were significantly different from one another and it is these differences that underscores the problem of comparing results made from one laboratory with those made by another laboratory (Table 3). This in itself is valuable information in that I have met people involved with endotoxin and its consequences who were unaware of these interlaboratory discrepancies. Fortunately, variation within laboratories appear to be small so that results within a laboratory can be usefully employed to rank samples having different endotoxin contents (Figure 1).

In this study, a GC-mass spectrophotometric analysis was used by one laboratory to measure total endotoxin in a sample. The other laboratories used an extraction protocol and one of the limulus amebocyte lysate (LAL) type assays which accounted for about a tenth to a hundredth of the total endotoxin present (Table 3 and Figure 1; Sonesson et al., 1990). Practically, the results can be separated into two groups, the result from the laboratory that measured total endotoxin and the results obtained by the other laboratories. Argumentatively, one might suggest adopting analysis for total endotoxin as the standard method of analysis. However, the method is not readily available to most laboratories currently involved with endotoxin analysis and requires greater resources to obtain and to maintain. In both part 1 and part 2 of the study, the dust laden filters are the same. Yet the results obtained using the purely analytical method for total endotoxin resulted in significantly different concentrations of endotoxin between the two parts of the study (Table 5 and Figure 1). While one may argue that the samples had aged since part 1 of the study was carried out, this would not explain why the overall concentrations by the other laboratories did not show a decrease but actually tended to be higher in the second part of the study (Table 5 and Figure 1). However, more crucial is the question of whether total endotoxin relates best to the biological availability of endotoxin and hence it's biological activity. Current feeling is that the limulus-type of assays which involves aqueous extraction better reflect the biological active endotoxin since total endotoxin may also include inactive and inaccessible endotoxin (Sonesson et. al, 1990). Whether this is true or not would have to be determined elsewhere. Still, total endotoxin may be very useful as an upper base line or upper limit for comparisons and in determining a practical extraction and assay protocol.

Ideally, the second part of the study would have been conducted with all the participating laboratories using the same extraction protocol, the same lot of the same assay kit, and all using identical assay protocol including the same plate reader and analysis software. However, this study is not being supported by any external funding other than the generosity of the on hand resources of the participating laboratories. In planning for part 2 of the study, more weight was placed on the methodology used by the laboratories whose assays yielded the higher levels of endotoxin (Table 3 and Figure 1). For the second part of the study, a common extraction protocol seemed to be the best approach to reduce the variation between laboratories since changing to a common LAL assay kit, plate reader and analysis software, was an unrealistic request to be made of the participating laboratories; and these factors will remain as unexplained systematic error.

The part 2 results where the participating laboratories used a common extraction protocol with their own in-house assay is shown in Table 4 and Figure 1. Again, the variations found within the different laboratories were very small. Still the differences between the different laboratories were significantly different; however, this time there were fewer Duncan grouping differences — seven groupings in part 1 vs. only four groupings in part 2 of the study (Tables 3 & 4). The range of average EU/mg (Log_{10}EU/mg) was large and ranged from 0.84 to 3.98 in the first part of the study; and even when omitting the one anomalous low value, the range of average Log_{10}EU/mg was still large, ranging from 2.84 to 3.98. In this second part of the study, the range of the average Log_{10}EU/mg was much reduced and ranged within the same order of magnitude, 3.20 to 3.97 (Table 4). Using the common extraction protocol, tended to increase the overall concentration of endotoxin reported (Tables 3-5) and within individual laboratories, the average concentrations reported tended to be higher (Table 5). Of some interest was the curious observation that the laboratories showing no or little difference between results reported in part 1 and part 2 of the study, the kit type used was the Kinetic-QCL assay kit; while the laboratories reporting higher concentrations with the common extraction protocol were those using the QCL-1000, Pyrogent-5000 or an in-house assay type kit.

Still no strong distribution pattern was observed between the average Log_{10}EU/mg and the endotoxin assay kit type used (Table 5). Even so, when the average Log_{10}EU/mg obtained was sorted by endotoxin assay kit type, significant differences were observed between the assay kit type (Table 6 and Figure 2) and that the in-house and Kinetic-QCL kit types favored higher concentrations. The variation within assay kit type was small. The range of concentration was from 3.39 to 3.72. Still the number of laboratories using

each of the endotoxin kit types were small and no strong conclusions should be drawn at this time that one endotoxin kit type favored production of higher results over others.

One important issue resolved by this study and of considerable interest to CQRS and very likely others, was to see how wide the gap was between results from different laboratories. Was this simply the difference between the few laboratories that provided results to CQRS in the past or was this widespread? The results from this study that included research laboratories over a worldwide geographical and national range clearly indicate that significant differences in results can be expected when the same sample is assayed. The study is far from the most comprehensive since many factors are not addressed. Some of the factors have been explored elsewhere and dealt mostly with extraction, bioaerosol source and filter media (Chun and Perkins, 1994; Jacobs and Pietrowski, 1995; Thorne, et al., 1997; Wood and Jacobs, 1997); among those factors not addressed here is that possibly a major source of variability in results had derived from differences between lots of LAL used in the analyses (Milton, personal communiqué).

A secondary goal of this study was that once an idea of how wide the gap was between results from different laboratories, could the gap between different laboratories be reduced. The results from the second part of this study were encouraging since by just adopting a common extraction protocol, the gap was reduced considerably. This suggests strongly that further standardization might reduce the differences even more to the point that interlaboratory results might become directly comparable.

This study came about partially because uniform vertically elutriated cotton dust samples were made available for study (Perkins et al., 1996). When the dust samples were originally made, testing beyond what has been described was not foretold. However, since these results have become public, the need for additional samples developed and again the initiative was taken to collect the cotton dust samples at the USDA, Cotton Quality Research Station, Clemson, SC. One is reluctant to produce uniform dust laden membranes again because of the material and resources required (Perkins, et al., 1996), but dust laden membranes can be more easily collected in lots without regard to uniformity between lots so that different laboratories can perform the analysis on the same cotton dust from the same lot. Thusly, in 1998, cotton dust samples were collected again (Chun, et al., 1999). To anticipate potential future studies, three different endotoxin concentrations of cotton dust were collected on glass filters or PVC filters. A weight range of 0.3 – 0.7 mg for a target weight of 0.5 mg was set and over 3,000 dust samples were collected; however, the range of dust weights was larger so that different populations of dust weights for a study might be possible.

Summary

The results from a two-part interlaboratory endotoxin assay study used filter membranes with the same approximate amount and type of cotton dust. Endotoxin assays were done by laboratories that 'routinely' perform endotoxin analysis. Each of these laboratories performed the analysis using the methodology common to their laboratory. The results from the first part of the study showed that when different laboratories assay almost identical samples for endotoxin that the results can vary by as much as one or more orders of magnitude. However, the intralaboratory variations were very small and ranking of samples to different endotoxin levels is valid. The LAL assays only measured soluble endotoxin and the concentrations reported were a tenth to a hundredth of the total sample endotoxin. In the second part of the study, the results were encouraging since by just adopting a common extraction protocol, the gap between laboratories was reduced considerably. This suggests strongly that further standardization might reduce the differences even more to the point that interlaboratory results might become directly comparable.

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Table I. Principal laboratory investigators participating in the two-part round robin endotoxin assay study – 'Endotoxin Assay Committee'[1].

Principal Participant/ Contact Person	Affiliation	Location
Bartlett, Karen[2]	University of British Columbia, Occupational Hygiene Programme	Vancouver, Canada
Chew, Victor[3]	USDA, ARS, South Atlantic Area (SAA) Biometrical Services	Gainesville, FL, USA
Chun, David T.W.	USDA, ARS, CQRS	Clemson, SC, USA
Gordon, Terry	New York University Medical Center, Nelson Institute of Environmental Medicine	Tuxedo, NY, USA
Jacobs, Robert R.	University of Alabama-Birmingham, Environmental Health Sciences	Birmingham, AL, USA
Larsson, Britt-Marie	National Institute for Working Life, Dept. of Occupational Medicine	Sweden
Larsson, Lennart	Dept. of Medical Microbiology	Sweden
Lewis, Daniel M.	NIOSH, Division of Respiratory Disease Studies (DRDS)	Morgantown, WV, USA
Liesivuori, Jyrki	Kuopio Regional Institute of Occupational Health, Occupational Hygiene and Toxicology Section	Finland
Michel, Olivier	Hopital Universitaire Saint-Pierre, Clinique de Pneumologie et D'Allergologie	Belgium
Milton, Donald K.	Harvard School of Public Health, Dept. of Environmental Health	Boston, MA, USA
Rylander, Ragnar	University of Gothenburg, Dept. of Environmental Health	Gothenburg, Sweden
Thorne, Peter S.	University of Iowa, Dept. of Preventive Medicine and Environmental Health	Iowa City, IA, USA
White, Eugene M. & Brown, Mary E.	NIOSH, Division of Physical Sciences and Engineering Methods Research Branch (DPSEMRB)	Cincinnati, OH, USA

[1] Two laboratories dropped out of the first part of the study (not listed) and were not participants in the second part of the study.
[2] Joined the study too late to participate in the first part of the study.
[3] Biometrician.

Table 2. Approximate date results from participating laboratories were received by facsimile transmission, mail, or e-mail.

PART 1[1]		PART 2[2]	
Lab ID	Approx. Date	Lab ID	Approx. Date
1	March 27, 1997	1	September 15, 1997
2	March 20, 1997	2	February 29, 1998
3	May 30, 1997	3	August 23, 1997
4	April 21, 1997	4	December 22, 1997
5	May 28, 1997	5	August 15, 1997
6	April 1, 1997	6	November 12, 1997
7	March 12, 1997	7	December 12, 1997
8	May 5, 1997	8	August 6, 1997
9	Dropped out of study	9	November 18, 1997
10	March 21, 1997	10	January 26, 1998
11	June 2, 1997	11	February 9, 1998
12	April 30, 1997	12	November 10, 1997
13	Dropped out of study	13	March 3, 1998
14	June 5, 1997		

[1] Dust samples were mailed February 25, 1997 to the participating labs
[2] Dust samples were mailed July 29, 1997 to the participating labs

Table 3. Average assay results as EU/mg of the participating laboratories, from Part 1 of the Round Robin Endotoxin Assay Study.

Laboratory[1] ID	Average EU/mg, Log_{10}EU/mg[2]	Average EU/mg, Log_{10}EU/mg[2,3]
14	4.941[A]	—
8	3.982[B]	3.982[A]
4	3.669[C]	3.669[B]
6	3.525[D]	3.525[C]
2	3.452[DE]	3.452[CD]
11	3.401[E]	3.401[D]
7	3.260[F]	3.260[E]
10	3.247[F]	3.247[E]
3	3.080[G]	3.080[F]
12	2.848[H]	2.848[G]
1	2.838[H]	2.838[G]
5[4]	0.840[I]	0.840[H]

[1] Two laboratories dropped out of the first part of the study (not listed) andwere not participants in the second part of the study.

[2] Mean separation within columns by Duncan's multiple range test, 5% level. Means with the same letter are not significantly different.

[3] Average assay results as EU/mg of the participating laboratories, excluding Lab 14 (which assayed for total Endotoxin).

[4] Assay was later redone on a second set of filters, which indicated that an error had probably occurred in the first assay resulting in the unusually low results reported here.

Table 4. Average assay results as EU/mg and endotoxin assay kit type of the participating laboratories, from Part 2 of the Round Robin Endotoxin Assay Study.

Laboratory ID	Average EU/mg, Log_{10}EU/mg[1]	Average EU/mg, Log_{10}EU/mg[1,2]	Endotoxin Assay Kit Type[3]
13	4.755[A]		
8	3.968[B]	3.968[A]	Kinetic-QCL
11	3.819[C]	3.819[B]	in-house
7	3.758[CD]	3.758[BC]	Kinetic-QCL
9	3.685[DE]	3.685[CD]	in-house
2	3.636[DE]	3.636[CD]	in-house
1	3.566[EF]	3.566[DE]	QCL-1000
3	3.558[EF]	3.558[DEF]	Kinetic-QCL
5	3.443[FG]	3.443[EFG]	Pyrogent-5000
10	3.429[G]	3.429[FG]	Kinetic-QCL
12	3.404[G]	3.404[G]	QCL-1000
4	3.223[H]	3.223[H]	Kinetic-QCL
6	3.202[H]	3.202[H]	QCL-1000

[1] Mean separation within columns by Duncan's multiple range test, 5% level. Means with the same letter are not significantly different.

[2] Average assay results as EU/mg of the participating laboratories, excluding Lab 13 (which assayed for total Endotoxin).

[3] Four categories of kit types: three of the four kit types were endotoxin assay kits manufactured by BioWhittaker, Inc. (QCL-1000, Kinetic-QCL, and Pyrogent-5000) and the fourth kit type was a catch-all to include assay kits not manufactured by BioWhittaker, Inc. or using a BioWhittaker kinetic LAL used with conditions and standards independently referenced to EC6.

Table 5. Comparison of average assay results as EU/mg and endotoxin assay kit type of the participating laboratories, from Part 1 and Part 2 of the Round Robin Endotoxin Assay Study.

	PART 2[1]	**PART 1[1]**	
Laboratory ID[1,2]	Average EU/mg, Log_{10}EU/mg	Average EU/mg, Log_{10}EU/mg	Endotoxin Assay Kit Type[3]
1***	3.566	3.080	QCL-1000
2***	3.636	2.848	in-house
3	3.558	3.525	Kinetic-QCL
4	3.223	3.260	Kinetic-QCL
5[4]	3.443	3.316	Pyrogent-5000
6***	3.202	2.838	QCL-1000
7	3.758	3.669	Kinetic-QCL
8	3.968	3.982	Kinetic-QCL
9***	3.685	3.401	in-house
10	3.429	3.452	Kinetic-QCL
11[4]***	3.819	3.398	in-house
12***	3.404	3.247	QCL-1000
13***	4.754	4.941	(GC-Mass Spec.)

[1] Laboratory Identification number refers to Lab ID numbers used in Part 2, corresponding laboratory results from Part 1 is compared with results from Part 2.

[2] t-test, average EU/mg, Log_{10}EU/mg, difference between assay done in Part 2 and Part 1 of the study is equal to zero: *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$.

[3] Four categories of kit types: three of the four kit types were endotoxin assay kits manufactured by BioWhittaker, Inc. (QCL-1000, Kinetic-QCL, and Pyrogent-5000) and the fourth kit type was a catch-all to include assay kits not manufactured by BioWhittaker, Inc. or using a BioWhittaker kinetic LAL used with conditions and standards independently referenced to EC6

[4] Either joined study too late to participate in Part 1 of study or an error was found in performance of assay in Part 1; a new set of samples were assayed using in-house extraction and assay protocols and results compared with results from Part 2 of study.

Table 6. Average results from Part 2 based on endotoxin assay kit type.

Endotoxin Assay Kit Type[1]	Average EU/mg, Log_{10}EU/mg[2]	Average EU/mg, Log_{10}EU/mg[2,3]
GC-Mass Spec.	4.755[A]	
In-house	3.717[B]	3.717[A]
Kinetic-QCL	3.593[C]	3.593[B]
Pyrogent-5000	3.443[D]	3.443[C]
QCL-1000	3.391[D]	3.391[C]

[1] Four categories of kit types: three of the four kit types were endotoxin assay kits manufactured by BioWhittaker, Inc. (QCL-1000, Kinetic-QCL, and Pyrogent-5000) and the fourth kit type was a catch-all to include assay kits not manufactured by BioWhittaker, Inc. or using a BioWhittaker kinetic LAL used with conditions and standards independently referenced to EC6

[2] Mean separation within columns by Duncan's multiple range test, 5% level. Means with the same letter are not significantly different.

[3] Average assay results as EU/mg of the participating laboratories, excluding Lab 13 (which assayed for total Endotoxin).

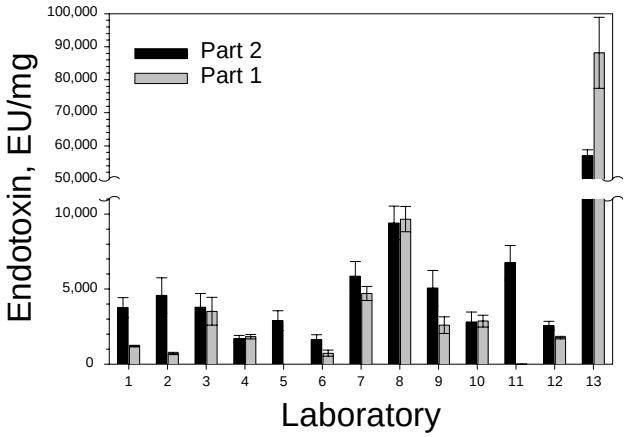

Figure 1. Average assay results from Part 2 and 1 of the Round Robin Endotoxin Assay study by the participating laboratories; the axis break represents a change in scale to accommodate results from the laboratory doing total endotoxin content, EU/mg; each half bar represents 2 s.e.

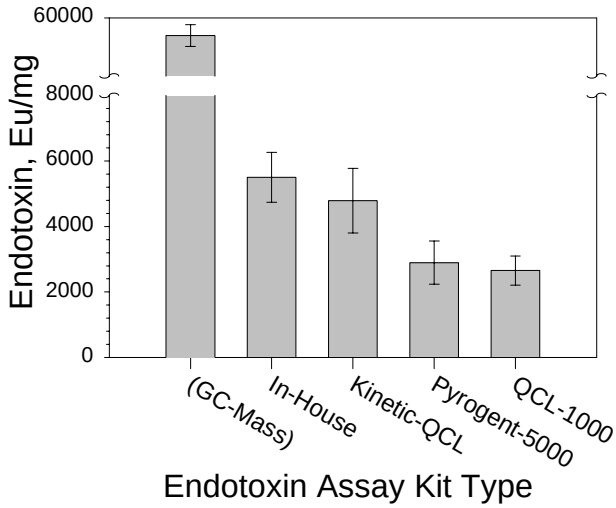

Figure 2. Average assay results from Part 2 of the study made with the method for total endotoxin content and by the four categories of kit types: three of the four kit types were endotoxin assay kits manufactured by BioWhittaker, Inc. (QCL-1000, Kinetic-QCL, and Pyrogent-5000) and the fourth kit type was a catch-all to include assay kits not manufactured by BioWhittaker, Inc. or using a BioWhittaker kinetic LAL used with conditions and standards independently referenced to EC6 (EU/mg; each half bar represents 2 s.e.).