PDA Journal
of Pharmaceutical Science and Technology

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PDA J Pharm Sci and Tech **2010**, *64* 211-221

RESEARCH

Evaluation of the Endosafe® Portable Testing System™ for the Rapid Analysis of Biopharmaceutical Samples

LUIS JIMENEZ, NARENDRA RANA, KASEY TRAVERS, VERCE TOLOMANOSKA, and KIMBERLY WALKER

Microbiology Laboratory, Immunomedics, Inc., 300 American Road, Morris Plains, New Jersey 07950

ABSTRACT: The Endosafe® Portable Testing System™ (PTS™) portable system for endotoxin testing was evaluated to analyze biopharmaceutical samples such as raw materials and finished products. The installation, operational, and performance qualification procedures were successfully implemented and verified to determine the system functionality under good manufacturing practices. During the validation stages the PTS™ was compared to the gel-clot test method in terms of presence or absence of endotoxin substances, ease of use, completion time, resource optimization, and sample volume. Water for injection and product samples were analyzed with both methods. All water for injection and product samples were negative for the presence of endotoxin by both methods. However, PTS™ results were obtained after 15 min while the gel-clot completion time was 1 h. Miniaturization of endotoxin testing by the PTS™ allowed optimization of testing procedures by reducing sample volume, analyst manipulations, accessory materials, and turnover time, and by minimizing the risk of exogenous contamination of the reaction.

Introduction

Endotoxins are lipopolysaccharides located in the outer membrane of gram-negative bacteria (1). Gram-negative bacteria are one of the major causes of microbial contamination in sterile products (2, 3). Water, a common raw material and widely used to rinse equipment and parts, is a major source of bacterial and endotoxin contamination in pharmaceutical manufacturing. Other potential sources of endotoxin contamination are excipients such as solvents, thickening agents, chelating agents, antioxidants, reducing agents, preservatives, buffers, bulking agents, and special additives. The absence of endotoxins in biopharmaceutical raw materials and finished product samples is a crucial safety control parameter because if introduced into the human body, endotoxins can cause inflammation, fever, and in some cases mortality. Endotoxins can also contaminate manufacturing equipment, packaging materials, containers, vials, and stoppers. Depyrogenation of materials used during manufacturing of biopharmaceuticals is of critical importance because product contamination from materials can also compromise integrity, toxicity, and potency.

Current bacterial endotoxin testing (BET) in pharmaceutical environments is performed by three basic technologies, which are based upon the highly sensitive reagent limulus amoebocyte lysate (LAL). These technologies are the gel-clot, endpoint analysis, and kinetic assays. In the gel-clot a sample loaded into a tube containing a lysate of specific sensitivity is incubated for 60 min and the results are based upon the formation of clot in the tubes upon inversion (4, 5). The endpoint method is based upon the linear relationship between endotoxin concentration and absorbance values (6). Regression analysis and endotoxin quantification can be performed using Beer's law. Even though the endpoint assay is quick, its range is limited to one log. Kinetic methods showed a wider range, for example, 4 logs, but are based upon the linear relationship between the log endotoxin concentration and the log reaction time (7, 8). Basically, the reaction time is the time needed for the standard sample to change by a specified optical density.

Because of industry's increasing awareness of the process analytical technology (PAT) initiative, rapid methods are currently being pursued to promote real-time or faster measurements during manufacturing

Send all correspondence to: Luis Jimenez, Microbiology Laboratory, Immunomedics, Inc., 300 American Road, Morris Plains, New Jersey 07950, Telephone: 973-605-8200, Email: papojbmicro@gmail.com

Figure 1

Spectrophotometer and reader. Courtesy of Charles River Laboratories.

and/or quality control analysis, integrating data generation with process control (9, 10). Advances in analytical technologies and detection systems have allowed the miniaturization of genetic and immunological testing (11, 12). DNA microchips and immunoassays have been developed to optimize quality control testing and manufacturing of pharmaceutical products (11, 13). Furthermore, several studies have demonstrated the use of molecular analysis such as adenosine triphosphate (ATP) and polymerase chain reaction (PCR) analyses for quality control testing of pharmaceutical samples and environmental monitoring (14–17). These studies demonstrated that sample analyses could be completed in a shorter period of time with less sample volume leading to higher resolution analysis of product quality and environmental conditions. Following recent trends in the miniaturization of analytical testing, a portable endotoxin testing system (PTS™) has been developed where samples are loaded onto cartridges and read in a portable reader at the point of sampling. A previous study has demonstrated that the Endosafe® PTS™ was capable of rapid release of endotoxin testing for time-sensitive cell therapy products (18). However, no studies have been reported on the application of the system to biopharmaceutical samples. Nevertheless, the system is not intended to test sterile end-products at the point of sampling.

The major objective of this study was to document and verify the installation, operation, and performance qualification of the PTS™ reader and disposable cartridges and evaluate the system side-by-side with the "traditional" BET, gel-clot, for the analysis of biopharmaceutical water for injection (WFI) and product samples.

Materials and Methods

The Endosafe® Portable Testing System (PTS™)

The PTS™ (Charles River, Charleston, SC) is based upon kinetic chromogenic detection of pyrogens by measuring color intensity related to endotoxin concentrations in a sample. The system is composed of two parts. The hand-held spectrophotometer reader (Figure 1) and test cartridges (Figure 2). Polystyrene cartridges contain precise amounts of LAL reagents, chromogenic substrates, and control standard endotoxin (CSE) loaded into wells (Figure 2). The cartridges contain 2 sample wells and 2 spiked wells. The analyst loaded 0.025-mL samples into the cartridge wells, and the reader draws, mixes, and incubates the samples at different time intervals. Once the incubation is completed, the samples are transferred to the optical chambers where the spectrophotometer monitors the changes in optical density and determines the endotoxin concentration based upon the obtained kinetic values. The PTS™ reader was connected to an Epson TM-U220d printer through a power cord to print the test results (Figure 3).

Installation Qualification

A list of specifications, manuals, standard operating procedures, reports, and certifications associated with

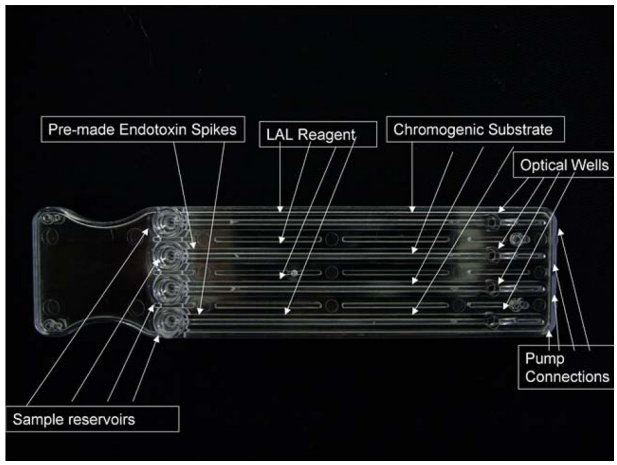

Figure 2

Test cartridge. Courtesy of Charles River Laboratories.

Figure 3

Epson TM-U220d printer.

the system was completed. An equipment component list based upon information from nameplate, manufacturer name, model number, calibration date, purchase orders, serial numbers and descriptions of the PTS™ reader and Epson printer was completed. A calibrated multimeter (Fluke 177 True RMS, Everett, WA) was used to verify that the required utilities such as power outlets were within specifications as defined by the operating manuals. A power supply was connected to the PTS™ reader and the reader was turned on by pressing the MENU key. The installed software program version and serial number of the reader were recorded. After this, the printer was plugged into the power outlet and the PTS™ reader. A verification procedure was performed to determine the efficiency of the installation.

Operation Qualification

Plugging the PTS™ reader to the power supply and turning it on by pressing the MENU key results in the performance of the self-test check. The reader will display SELF TEST OK when/if the temperature reaches 37 ± 1 °C. The MENU key and the number 5 were pressed to turn the system off. A minimum of three consecutive tests were performed.

The initiation acceptability test was performed by turning the reader on as previously described, and a blank test cartridge was inserted into the front slot of the reader before it reached 37 ± 1 °C. The reader will display REMOVE CARTRIDGE and will not initiate the test. A minimum of three consecutive tests were performed.

To perform the information input acceptability test, the reader was turned on as previously described and the SYSTEM SELF TEST was completed. The reader displayed the INSERT CARTRIDGE message. A test cartridge was inserted as previously described. Once the cartridge was inserted, the user initials were entered but an invalid lot number for the test cartridges was entered. Once the invalid lot number was entered, the reader displayed an INVALID LOT NUMBER message on the screen. The test was repeated three times. After the test was completed, a valid lot number was entered. A similar procedure was performed to enter invalid calibration codes for the test cartridges. An INVALID CODE message on the screen was displayed. After the three trials were completed, then a valid calibration code was entered and the system became functional.

The battery operation test was performed when the battery was fully charged. The system was turned on as previously described and the battery was verified to be fully charged. The system was turned off as previously described. The PTS™ reader was then removed from the power supply. The reader was turned back on again. The reader will complete the self-test and display the INSERT CARTRIDGE message on the screen. The reader was turned off and the test was repeated two more times. The system was plugged into the power supply and then turned on with the battery power verified to be fully charged. After this the system was turned off again.

Performance Qualification

The internal pump performance test was performed by using blank cartridges with no LAL or endotoxin. The PTS™ reader was run as previously described during the operation qualification procedure until the self-test was completed and the INSERT CARTRIDGE message was displayed on the reader screen. A blank

cartridge was then inserted into the front slot of the reader. The user initials were entered followed by the correct lot number and calibration code of the blank cartridge as previously described. The sample ID, lot number, and dilution factor were also entered. Samples of WFI (0.025 mL) were added to the 4 wells in the blank test cartridge. The test was performed for approximately 10 min. A beep was sounded when the test was complete. All 4 wells will be empty if the sample was pulled into the optical cells.

To confirm that the baseline of the PTS™ remains stable during a kinetic test, WFI samples were added to the wells of a blank test cartridge. The reader was run as previously described during the operation qualification procedure until the self-test was completed and the INSERT CARTRIDGE message was displayed on the reader screen. A blank cartridge was then inserted into the front slot of the reader. The user initials, correct lot number, and calibration code of the blank cartridges were entered as previously described. After all the information was entered, the MENU key was pressed to go to the number 2 and then the word KINETIC TEST was entered. Before sample addition, all required sample information was entered as previously described. WFI samples (0.025 mL) were added to the 4 wells in the blank test cartridge. The test was performed for approximately 20 min. After completion, the onset times for all four channels on the test report must be greater than 1200 s.

The calibration verification/qualification test was performed by using test cartridges with endotoxin standard solutions ranging from 1 to 0.01 EU/mL. The PTS™ reader was run as previously described during the operation qualification procedure until the self-test was completed and the INSERT CARTRIDGE message was displayed on the reader screen. A test cartridge with endotoxin ranges from 1 to 0.01 EU/mL was then inserted into the front slot of the reader. The user initials, correct lot number, and calibration code of the cartridge were entered as previously described. After all the information was entered, the word QUALIFICATION TEST was entered under the sample identification. Before sample addition, all required sample information was entered as previously described. WFI samples (0.025 mL) were added to the 4 wells of the test cartridge. The test was performed for approximately 15 min. To verify the test, the onset times for the negative product control channels (channels 1 and 3) must be greater than (>) the mean onset time for the lowest point on the archived standard curve and the recovery of the spiked values (spike recovery) must be within 50%–200%. The coefficient of variation for the negative product control channels (sample CV) must be <25% and the coefficient of variation for the positive product control channels (spike CV) must be <25%.

To perform the endotoxin verification test, the PTS™ reader was run as previously described during the operation qualification procedure until the self-test was completed and the INSERT CARTRIDGE message was displayed on the reader screen. A test cartridge with endotoxin ranges from 1 to 0.01 EU/mL was inserted into the front slot of the reader. The user initials, correct lot number, and calibration code of the cartridge were entered as previously described. After all the information was entered, the word VERIFICATION TEST was entered under sample identification followed by lot number and dilution. The endotoxin verification was performed by loading 0.025 mL of a reference standard endotoxin (RSE) solution containing 0.1 EU/mL to a test cartridge with endotoxin ranges from 1 to 0.01 EU/mL. To confirm the verification test the average reported value (sample value) must fall within the range of 50–200% of the known nominal value and the recovery of the spiked values (spike recovery) must be within 50–200%. Furthermore, the coefficient of variation for the negative product control channels (sample CV) must be <25% and the coefficient of variation for the positive product control channels (spike CV) must be <25%.

Comparison of the PTS™ and the Bacterial Endotoxin Test (Gel-Clot)

Samples: To compare the traditional gel-clot method with the PTS™, samples of WFI from different sampling ports and finished biopharmaceutical products were analyzed as described below.

Traditional Bacterial Endotoxin Test: The gel-clot (4) reagents were composed of buffers and LAL of 0.03 EU/mL sensitivity (Associates of Cape Cod [ACC], Falmouth, MA, or Charles River Laboratories [CRL], Charleston, SC). CSE was diluted to 1.0, 0.06, 0.03, 0.015, and 0.0075 EU/mL, respectively. Dilutions were prepared daily in endotoxin-free sterile WFI from reconstituted CSE stock solutions (10,000 EU/mL). Product positive control (PPC) samples were prepared by spiking no more than 10% of the total sample volume with 10 EU/mL. WFI samples were analyzed at 1:1 dilutions while 1:10 dilutions of prod-

ucts in endotoxin-free sterile WFI were tested. All glassware used in the study was depyrogenated for a minimum of 1 h at 250 °C. Samples (0.10 mL) were added to depyrogenated tubes containing 0.10 mL of lysate. After addition, samples were incubated for 1 h at 37 °C. The end point value was the lowest concentration of endotoxin at which the lysate formed a solid gel-clot upon inversion (4, 5).

PTS™ Test Procedure

The procedure was previously described in the performance qualification test. Quadruplicate 0.025-mL aliquots of WFI and product dilutions described above were loaded into the four sample reservoirs of the cartridge. The reader draws and mixes the sample with the LAL reagent in two wells (sample wells) and positive product control in the other two wells (spike wells). LAL of a sensitivity of 0.01 EU/mL was used for all PTS™ analysis (Charles River Laboratories, Charleston, SC).

The sample is then incubated at 37 °C, inside the reader, and combined with the chromogenic substrate. After mixing, the optical density of the wells are measured and analyzed against an internally archived standard curve. The archived standard curve specific for each batch of cartridges is constructed using the log of the reaction time against the log of the concentration. The sample and spike values are calculated by interpolation of the standard curve using the reaction times. The system simultaneously performs testing in duplicate and averages the results. Acceptance criteria for a valid assay is an archived curve correlation coefficient of > -0.980, a coefficient of variation of $<25\%$, and PPC (spike) recovery of 50–200%.

Results and Discussions

The installation qualification of the PTS™ was initiated by the collection and verification of all related documents supplied by the vendor. These documents were the Endosafe® PTS™ user guide and certificate of calibration. All calibrations were verified to be in compliance with National Institute of Standards and Technology (NIST) standards and recalibration intervals for critical instrumentation were established. A standard operating procedure was developed for operation and maintenance of the system. All information regarding the manufacturer's name, serial number of the PTS™ kinetic reader, printer, and utility verification was documented and verified (Table I). The installation of the reader and printer was successfully completed and verified.

Once the installation qualification was completed, the operation qualification was initiated by the system self-test. The test was based upon the capability of the system to reach the testing temperature of 37 °C. Compendial endotoxin testing is currently performed at 37 °C. When the temperature did not reach 37 °C and a test cartridge was inserted, the system signaled the message REMOVE CARTRIDGE. The cartridge was then removed and the test was repeated two additional times. When the reader temperature reached 37 °C, the system allowed the insertion of a test cartridge by messaging INSERT CARTRIDGE. Then the system self-test was completed. This result demonstrated that the system will never start the testing without reaching 37 °C.

Following the completion of this test, the initiation acceptability test was performed to challenge the system by inserting a test cartridge into the reader and entering an invalid cartridge lot number and calibration code. The system did not allow the reaction to go forward when the wrong lot number and calibration code were entered, but only when the correct information was entered did the system proceed to complete the test. One of the major features of the PTS™ is its portability, which was tested by the battery operation test. When the battery was fully charged, the reader was turned off and disconnected from the power supply. After turning the reader back on, it reached the testing temperature of 37 °C. These results demonstrated that the battery was operational and that the system can be used to test WFI at distant sampling ports away from the laboratory. Point-of-use testing is a major advantage due to the fact that if a problem is detected onsite, faster corrective actions can be implemented and the lines can be quarantined as soon as possible.

The performance qualification of the system was initiated by determining the capability of the reader to pump the samples into the well of the inserted test cartridges. Samples of WFI were added to blank cartridges. All wells were found to be completely filled with the samples pulled into the optical wells of the test cartridges. The kinetic noise test was performed to confirm that the reaction baseline remains stable during a kinetic test. This is performed again on blank test cartridges that contained no LAL or endotoxin. The onset times for all four channels on the test report were

TABLE I
Installation Qualification Test Results

Procedure	Expected Results	Results as Expected (Yes/No)
System Documentation Verification	Complete documentation of specifications, manuals, reports (passivation report, welding check report, etc.), and certifications (ASME code, certificate of materials) associated with the equipment were collected and verified.	YES
Equipment/Component List	Complete documentation of equipment/component based on information from engineering drawings, serial numbers, nameplate, purchase orders and equipment specifications was verified.	YES
Instrument Calibration Verification	Complete documentation of all monitoring and control instrumentation associated with the system was collected. Designated instruments as "critical" or "non-critical." Verified that the critical instruments have been calibrated and the standards used are National Institute of Standards and Technology (NIST) traceable. Specified the location of the calibration records.	YES
Utility Verification	All utilities required for operation of the system as defined in the operating manuals are available and conform to specifications.	YES
Installation Verification	The components are correctly installed according to the procedures listed	YES

greater than (>) 1200 s. These results confirmed that the baseline remains stable. The results of the calibration verification/qualification test confirmed the linearity, accuracy, and range of the PTS™ instrument and test cartridges. The cartridges contained all reagents, LAL, and calibrated endotoxin concentrations. For this study cartridge batches were released based upon linearity and range using endotoxin standard solutions with nominal concentrations of 1, 0.1, and 0.01 EU/mL. All testing results were found to be in compliance with the acceptance criteria described in Table III. Once the calibration verification/qualification test was completed, the endotoxin verification test was performed with a RSE standard concentration of 0.1 EU/mL using a cartridge with an archived standard curve range of 1–0.01 EU/mL. Samples of 0.025 mL of the RSE standard 0.1 EU/mL solution were added to the cartridges wells. All testing results were found to be in compliance with the acceptance criteria described in Table III.

To determine the endotoxin concentration in WFI and biopharmaceutical products, the Endosafe® PTS™ was evaluated and compared to results obtained with the traditional gel-clot. Thirty-seven WFI samples were analyzed by both methods using 1:1 dilutions. Four different analysts participated in the water testing. The gel-clot test was performed using a lysate sensitivity of 0.03 EU/mL while the PTS™ was performed with a lysate sensitivity of 0.01 EU/mL. The United States Pharmacopoeia (USP) limit for endotoxin concentration in WFI is 0.25 EU/mL. All water samples were found to be negative by using the gel-clot test and PTS™. All the results obtained with the four different analysts were within the acceptance criteria described in Table IV. Endotoxin values were way below the required limits with ≤0.03 EU/mL for the gel-clot and ≤0.01 EU/mL for the PTS™ (Table IV). Although the PTS™ system was 3 times more sensitive than the BET, there were no problems using a more sensitive assay to monitor WFI because no interfering substances were detected. If needed, different test cartridges are available with different sensitivies ranging from 0.1 EU/mL to 0.005 EU/mL. For an assay to be valid by the PTS™ the percent of recovery of the spike endotoxin must be within 50–

TABLE II
Operation Qualification Test Results

Procedure	Expected Results	Results As Expected (Yes/No)
System Self Test	The reader initiates a "**SYSTEM SELF TEST**" and temperature starts increasing till it reaches 37 °C; the reader displays "**SELF TEST OK**", then display "**INSERT CARTRIDGE**"	YES
Initiation Acceptability Test A test cartridge was inserted into reader prior to the temperature reaching 37 °C.	The reader displays "**REMOVE CARTRIDGE**" and will not initiate the test.	YES
Information Input Acceptability Test	"**INVALID LOT #**" will be displayed on the screen and the reader will again display "**ENTER LOT #**"	YES
Invalid lot number and calibration codes of test cartridges were entered	"**INVALID CODE!**" will be displayed on the screen and the reader will again display "**ENTER CAL-CODE #**"	
Battery Operation Test After the reader is turned off and disconnected from power supply, it completes the system self-test and ascends to 37 °C utilizing battery power after is back on.	The reader displays "**SELF TEST OK**", then display "**INSERT CARTRIDGE**"	YES

200%. Recoveries for WFI samples ranged from 50 to 149%, which validated all results. None of the WFI samples showed any interference with the assay.

Seventeen product samples were tested both with the gel-clot and PTS™ at 1:10 dilutions. Three different analysts participated in the product testing. No pyrogenic substances were found in any of the products tested by any of the analysts (Table V). All values obtained with the gel-clot and PTS™ were within allowable limits for all products. Spike recoveries for all products using the PTS™ ranged from 72 to 194%. (Table V). Again the higher sensitivity of the PTS™ did not affect the results of the assay.

There was a 100% correlation between the gel-clot and PTS™. However, the PTS™ assay delivered results within 15 min while the gel-clot took 1 h to complete. This represented a 75% reduction in the time needed to evaluate the presence or absence of pyrogens in WFI and finished products. However, if a more complex sample is analyzed and several dilutions are tested, then more than one cartridge must be used to complete the test and the time savings might be compromised. With the gel-clot multiple sample dilutions can be analyzed while the PTS™ can analyze one sample at a time. However, a new system for example, a multi-cartridge system (MCS), has been developed, which analyzes up to 5 samples at a time. A regular gel-clot analysis requires accessories such as LRW, tubes, pipettes, tips, microplates, sample containers, etc. To perform the assay, a control CSE is always tested to determine the lysate sensitivity but the test cartridges contained the preloaded CSE endotoxin concentrations, LAL reagent, buffers, and synthetic chromogenic substrate based upon the sensitivity of the cartridges used. Furthermore, in the gel-clot method, the CSE spikes are added to samples for the determination of possible inhibition/enhancement effects. In this study the lysate sensitivity was 0.01 EU/mL so the reference standard concentrations were 1.00, 0.10, and 0.01 EU/mL.

TABLE III
Performance Qualification Test Results

Procedures	Expected Results	Action as Expected (Yes/No)
Internal Pump Performance Test Samples of 25 microliters of water for injection were added to 4 wells in test cartridge.	Pump pulled samples into optical well of test cartridges.	YES
Kinetic Noise Test Samples of 25 microliters of water for injection were added to 4 wells in test cartridge.	The onset times for all four channels on the test report should be greater than 1200 seconds.	YES
Calibration Verification/Qualification Test	a) The onset times for the negative product control channels (channels 1 and 3) must be greater than (>) the mean onset time for the lowest point on the archived standard curve.	YES
Samples of 25 microliters of water for injection were added to 4 wells in test cartridge.	b) Recovery of the spiked values (spike recovery) must be within 50%–200%.	
	c) The coefficient of variation for the negative product control channels (sample CV) must be <25%.	
	d) The coefficient of variation for the positive product control channels (spike CV) must be <25%.	
Endotoxin Verification Test Results	a) The average reported value (sample value) must fall within the range of 50%–200% of the know nominal value.	YES
Samples of 25 microliters of 0.1 EU/ml endotoxin solution were added to 4 wells in test cartridge.	b) Recovery of the spiked values (spike recovery) must be within 50%–200%.	
	c) The coefficient of variation for the negative product control channels (sample CV) must be <25%.	
	d) The coefficient of variation for the positive product control channels (spike CV) must be <25%.	

The PTS™ cartridges were already preloaded with all required reagents, which reduce the possibility of exogenous endotoxin contamination during the handling and manipulation of samples. The cartridges are FDA-licensed chromogenic LAL. The system did not need to run the endotoxin standards and PPC preparations. Rapid monitoring of in-product testing and water can alert production personnel of potential problems before they become critical. Corrective action can be taken as soon as possible to reduce pyrogen load and levels of endotoxin. The portability and simplicity of the PTS™ allows point of use testing of WFI samples near the ports and quick training of personnel. Previous studies with the PTS™ testing of cell therapy samples indicated a problem with the spike recovery of some of the samples tested. However, it was not determined if there was inhibition or enhancement of the reaction that caused the problem (18). Based upon the spike recovery values obtained in this study, we did not see any interference with spike recoveries in the samples analyzed. However, the samples analyzed in this study were WFI and biopharmaceutical products, which contained less inhibitory substances than cell therapy products.

The release of WFI and products in biopharmaceutical operations requires endotoxin testing to provide infor-

TABLE IV
Endotoxin Analysis of Water for Injection by BET and PTS™

Sample	Dilution	PTS™ Endotoxin Value (EU/ml)	PPC Recovery Percent	BET Endotoxin Value (EU/ml)
WFI-1	1:1	<0.01	60	<0.03
WFI-2	1:1	<0.01	76	<0.03
WFI-3	1:1	<0.01	92	<0.03
WFI-4	1:1	<0.01	122	<0.03
WFI-5	1:1	<0.01	93	<0.03
WFI-6	1:1	<0.01	92	<0.03
WFI-7	1:1	<0.01	92	<0.03
WFI-8	1.1	<0.01	100	<0.03
WFI-9	1:1	<0.01	117	<0.03
WFI-10	1:1	<0.01	88	<0.03
WFI-11	1:1	<0.01	76	<0.03
WFI-12	1:1	<0.01	76	<0.03
WFI-13	1:1	<0.01	83	<0.03
WFI-14	1:1	<0.01	95	<0.03
WFI-15	1:1	<0.01	82	<0.03
WFI-16	1:1	<0.01	90	<0.03
WFI-17	1:1	<0.01	81	<0.03
WFI-18	1:1	<0.01	100	<0.03
WFI-19	1:1	<0.01	100	<0.03
WFI-20	1:1	<0.01	65	<0.03
WFI-21	1:1	<0.01	86	<0.03
WFI-22	1:1	<0.01	97	<0.03
WFI-23	1:1	<0.01	106	<0.03
WFI-24	1:1	<0.01	110	<0.03
WFI-25	1:1	<0.01	84	<0.03
WFI-26	1:1	<0.01	92	<0.03
WFI-27	1:1	<0.01	106	<0.03
WFI-28	1:1	<0.01	72	<0.03
WFI-29	1:1	<0.01	106	<0.03
WFI-30	1:1	<0.01	118	<0.03
WFI-31	1:1	<0.01	112	<0.03
WFI-32	1:1	<0.01	101	<0.03
WFI-33	1:1	<0.01	105	<0.03
WFI-34	1:1	<0.01	123	<0.03
WFI-35	1:1	<0.01	78	<0.03
WFI-36	1:1	<0.01	75	<0.03
WFI-37	1:1	<0.01	149	<0.03

PTS™ = portable testing system.
PPC = product positive control.
BET = bacterial endotoxin test.

TABLE V
Endotoxin Analysis of Biopharmaceutical Products by BET and PTS™

Product	Dilution	PTS™ Endotoxin Value (EU/ml)	PPC Recovery Percent	BET Endotoxin Value (EU/ml)
1	1:10	<0.10	93	<0.30
2	1:10	<0.10	72	<0.30
3	1:10	<0.15	115	<0.30
4	1:10	<0.10	92	<0.30
5	1:10	<0.10	117	<0.25
6	1:10	<0.10	87	<0.30
7	1:10	<0.10	148	<0.30
8	1:10	<0.10	76	<0.30
9	1:10	<0.10	71	<0.30
10	1:10	<0.10	102	<0.30
11	1:10	<0.10	73	<0.30
12	1:10	<0.10	149	<0.30
13	1:10	<0.10	130	<0.30
14	1:10	<0.10	136	<0.30
15	1:10	<0.10	109	<0.30
16	1:10	<0.10	130	<0.30
17	1:10	<0.10	194	<0.30

PTS™ = portable testing system.
PPC = product positive control.
BET = bacterial endotoxin test.

mation to support the safety and efficacy of the processes in the removal of pyrogenic substances. Results obtained with the PTS™ demonstrated close to real-time and faster analysis of endotoxin substances in biopharmaceutical samples, providing a rapid and accurate assessment of the manufacturing environment and processes. There is a limited throughput capacity when compared to the traditional 96-well capacity kinetic and chromogenic test. However, a new system (e.g., MCS) has been developed, which analyzes up to 5 samples at a time. Furthermore, the traditional kinetic and chromogenic systems require intensive training, standard curves, accessories, and reagents, which are not needed in the PTS™. We only compared the PTS™ system to the traditional gel-clot method and found that it was faster and easy to use. Staff training was fast and simple when compared to the gel-clot method. However, it will be interesting to compare the PTS™ to the traditional kinetic and chromogenic tests to study their variability regarding the % CV and % PPC.

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