Limulus Amebocyte Lysate

PYROCHROME®

Manufactured by:
ASSOCIATES OF CAPE COD INCORPORATED
124 Bernard E. Saint Jean Drive • E. Falmouth, MA 02536 USA

Telephone: (508) 540-3444
Toll-Free: (888) 395-2221
Fax: (508) 540-8680
Technical Support: (800) 848-3248
Customer Service: (800) 525-8378

PN000856 rev4 Oct 2019

PYROCHROME®

for the Detection and Quantification of Gram Negative Bacterial Endotoxins (Lipopolysaccharides)

The *Limulus* amebocyte lysate (LAL) test, when used according to U.S. Food and Drug Administration (FDA) guidelines (1), may be used for (1) the end-point testing of "human injectable drugs (including biological products), animal injectable drugs, and medical devices." The LAL test is recommended for the quantitation of endotoxin in raw materials used in production, including water, and for in-process monitoring of endotoxin levels. The USP Bacterial Endotoxins Test (2) is the official LAL test referenced in specific USP monographs and is harmonized with equivalent chapters in European Pharmacopoeia (EP) (3) and the Japanese Pharmacopoeia (JP) (4).

Summary of Test

Limulus amebocyte lysate is an aqueous extract of blood cells (amebocytes) from the horseshoe crab *Limulus* polyphemus. In the presence of endotoxin, factors in LAL are activated in a proteolytic cascade that results in the cleavage of a colorless artificial peptide substrate present in Pyrochrome® LAL. Proteolytic cleavage of the substrate liberates para-nitroaniline (pNA), which is yellow and absorbs at 405 nm. The test is performed by adding a volume of Pyrochrome® to a volume of specimen and incubating the reaction mixture at 37°C. The greater the endotoxin concentration in the specimen, the faster pNA will be produced. Pyrochrome® can be used to quantify endotoxin concentration in two ways (5). In the kinetic method, the time taken to reach a particular absorbance at 405 nm (the onset time) is determined. Higher endotoxin concentrations give shorter onset times. The assay requires specialized instrumentation to incubate multiple samples at a controlled temperature (usually 37°C) and to take optical density readings at regular intervals. Standard curves may be constructed by plotting the log onset time against the log concentration of standard endotoxin and are used to calculate endotoxin concentrations in specimens. Alternatively, in the endpoint chromogenic method, the amount of pNA released can be measured following a fixed incubation period. A standard curve, consisting of measured optical density plotted against known standard endotoxin concentration, is used to determine concentrations in specimens.

Pyrochrome® test methods are rapid, specific, easy to perform, and highly sensitive. The detection limit depends on the method and instrumentation used and may be as sensitive as 0.001 Endotoxin Units (EU) per mL. If the linear regression standard curve passes all acceptance criteria, standard curves may also be constructed using polynomial regression.

History and Biologic Principle

Howell described the clotting of *Limulus* blood in 1885 (6). In the 1950's, at the Marine Biological Laboratory, Woods Hole, MA, Bang discovered that gram negative bacteria cause *Limulus* blood to clot (7). Levin and Bang later determined that the reaction is enzymatic and that the enzymes are located in granules in the amebocytes (8, 9). They showed that clotting is initiated by a unique structural component of the bacterial cell wall called endotoxin or lipopolysaccharide (10). Current understanding is that the reaction consists of a cascade of enzyme activation steps terminating in the cleavage of the protein, coagulogen. The insoluble cleavage product of coagulogen (coagulin) coalesces by ionic interaction. If sufficient coagulin forms, turbidity appears followed by the formation of a gel-clot. This interaction forms the basis of an assay for endotoxin termed the *Limulus* amebocyte lysate (LAL) test. In 1977, Japanese investigators discovered that endotoxin-activated LAL would also cleave small chromogenic peptides which contained an amino acid cleavage site similar to coagulogen and the chromophore, paranitroanilide (11). Cleavage results in the release of pNA, which is yellow and absorbs light at 405 nm. In this chromogenic adaptation of the LAL assay, coagulogen concentration is reduced by dilution to minimize interference when chromogenic substrate is added to the LAL. Thus, when endotoxin is added to chromogenic LAL reagent, a color is formed in preference to turbidity or a gel-clot. In all versions of the LAL assay (gel-clot, turbidimetric, and chromogenic), the greater the amount of endotoxin present, the faster the endpoint (gel-clot, turbidity, or color) develops. More information about the LAL assay types, reaction, and applications is available in the literature (12, 13, 14).

Reagent

Pyrochrome® is packaged at 3.2 mL/vial in lyophilized form. It contains an aqueous extract of amebocytes of *Limulus* polyphemus, stabilizer, salts, buffer and chromogenic substrate.

Pyrochrome® is not labeled with a specific sensitivity. Sensitivity in a given test (designated λ) is the lowest endotoxin concentration used to construct the standard curve. The greatest sensitivity, λ, of Pyrochrome® is 0.001 EU/mL in a kinetic assay and 0.005 EU/mL in an endpoint assay. Pyrochrome® is for in-vitro diagnostic purposes only. It is not intended for the diagnosis of endotoxemia in humans. The toxicity of Pyrochrome® has not been determined. However, prolonged or repeated contact of LAL with the skin has resulted in a Type I allergic reaction in some individuals (15). Thus, caution should be exercised when handling Pyrochrome®.

Reconstitution Procedure:

1. Gently tap the vial of Pyrochrome® to cause loose material to fall to the bottom. Break the vacuum by lifting the gray stopper. Do not contaminate the mouth of the vial. Remove and discard the stopper; do not inject through or reuse the stopper. A small amount of LAL powder remaining on the stopper will not affect the test.

2. Reconstitute Pyrochrome® with 3.2 mL Pyrochrome® Buffer or Glucashield® buffer (available separately from Associates of Cape Cod, Inc.). To obtain a sensitivity of 0.001 EU/mL in a microplate reader, it is necessary to reconstitute Pyrochrome® with Glucashield®. This sensitivity can be attained with either Pyrochrome® reconstitution buffer or Glucashield® buffer in a tube reader. The pellet will take a few minutes to go into solution, therefore, rehydrate at least 5 minutes prior to use. Swirl vial to insure homogeneity but avoid vigorous mixing that may cause excessive foaming and a loss of sensitivity. Cover the vial with Parafilm M® and store cold (2-8°C) when not in use. Pyrochrome® must be used within 8 hours of reconstitution.

Storage Conditions

Lyophilized Pyrochrome® is relatively stable and, if stored properly, will retain full activity through the expiration date on the label. Store the product at 2-8°C. Temperatures in excess of 37°C can cause rapid deterioration of lyophilized Pyrochrome® as evidenced by loss of sensitivity and a distinct yellowing of the product. Pyrochrome® is shipped in insulated containers to protect against high temperatures. Pyrochrome®, prior to reconstitution, is light sensitive and should be stored in the dark

Reconstituted Pyrochrome® is usually clear and slightly opalescent. An occasional lot will exhibit a slight, uniform turbidity. The presence of small fibers or strands does not indicate contamination nor affect activity; however, flocculent precipitation or a distinct yellow color indicates deterioration and the reagent should not be used. Reconstituted Pyrochrome® is less stable than the lyophilized product; vials may be held for up to 8 hours at 2-8°C. Note that it may be necessary to use freshly reconstituted reagent to obtain maximum sensitivity. Reconstituted Pyrochrome® cannot be frozen.

Specimen Collection and Preparation

Specimens should be collected aseptically in containers that are free of detectable endotoxin. Reused, depyrogenated glassware or sterile, disposable, polystyrene or polyethylene terephthalate (PET) plastics are recommended to minimize adsorption of endotoxin to container surfaces. Not all plastic containers are free of detectable endotoxin. In addition, extractable substances from some plastics may interfere with the test. Labware should be tested for acceptability by randomly selecting containers from a batch, rinsing them with a small volume of LAL Reagent Water (LRW) at room temperature for one hour, and testing the rinse as a specimen. The rinse should contain significantly less endotoxin than the lowest standard concentration to be used. Also, the rinse should neither inhibit nor enhance the test as determined by recovery of a known amount of added endotoxin. The pH of the reaction mixture (a volume of specimen or specimen dilution mixed with an equal volume of Pyrochrome®) should be 6 to 8. Adjust the pH of the specimen with HCl, NaOH, or buffer (free of detectable endotoxin). Dilute concentrated HCl or NaOH with LRW to an appropriate concentration and use a volume that will not lead to significant dilution of the test specimen. If a precipitate forms in the sample upon pH adjustment, dilute the sample (not to exceed the MVD – see "Limitations of Procedure") before adjusting the pH. Do not adjust the pH of unbuffered saline or water. Note that dilution alone may overcome pH problems.

Substances that denature proteins, chelate ions, bind endotoxin or alter endotoxin's hydrophobic state may interfere with the test. Interference may be detected as recovery of significantly more or less endotoxin than expected when a known amount of standard endotoxin is added to the specimen (see "Limitations of Procedure"). In most cases, dilution of the specimen will reduce the concentration and activity of interfering substances and still yield valid test results. Appropriate controls and dilution schemes are discussed under "Test Procedure." Specimens should be tested as soon as possible after collection. It may be advisable to freeze non-sterile specimens that will be stored or shipped before testing. Specimens expected to contain low concentrations of endotoxin should be tested for loss of endotoxin during storage.

Test Procedure

Test Reagents Supplied with Pyrochrome®

1. Pyrochrome® (see description under Reagent and Reconstitution Procedure above).

2. Pyrochrome® Reconstitution Buffer (catalog number C1500 only). Use the buffer to reconstitute Pyrochrome® as described above.

3. Glucashield® Reconstitution Buffer (catalog number CG1500 only). Use the buffer to reconstitute Pyrochrome® as described above.

Test Reagents Not Supplied with Pyrochrome®

1. Control Standard Endotoxin (CSE). (Associates of Cape Cod, Inc., catalog number EC010). Reconstitute CSE with the volume specified on the Certificate of Analysis (C of A, which gives the potency of the CSE) and as directed in the package insert. Follow the directions in the insert for use and storage of standard endotoxin. The potency of the CSE has been determined with respect to the U.S. Reference Standard Endotoxin (RSE) and is stated on the C of A. USP Endotoxin Reference Standard (identical to RSE) may be obtained from the U.S. Pharmacopeial Convention, Inc. Note: The C of A and the potency stated on it are specific to a combination of Pyrochrome® and CSE lot. A given lot of CSE may show different potencies (EU/ng) when tested with different lots of Pyrochrome®, Pyrotell, or other brands of LAL reagent (1). Similarly, different lots of CSE will likely have different potencies when tested with the same lot of Pyrochrome®. Be sure to use the correct C of A and potency.

   Use CSE to prepare dilutions of standard endotoxin from which to construct standard curves, for positive controls and for positive product (interference) controls.

2. LAL Reagent Water (LRW). Recommended sources include Associates of Cape Cod, Inc. (various sizes and packaging configurations available). Commercially available USP Sterile Water for Injection (sterile WFI) without bacteriostat or USP Water for Irrigation may be used provided that they have been shown to be acceptable for use as an LRW. The endotoxin limit for USP sterile WFI is only 0.25 EU/mL; therefore, sterile WFI may contain detectable endotoxin and be unsuitable for use.

   To certify that water is acceptable as an LRW, test it as a specimen with a positive product control (see item 1.c. in the section on "Controls"). Use certified LRW to make dilutions of standards, and to prepare positive controls (see items 1.a. and 1.b. under "Controls"). Construct a standard curve from the onset times or endpoint assays for the standards. The correlation coefficient should be at least 0.980 (absolute value). The endotoxin concentration of the water being tested can be estimated by extrapolation of the standard curve below the lowest endotoxin concentration and should be significantly less than that of the lowest standard. In addition, the endotoxin concentration of the positive product control should be within ± 25% of that of a positive control.

3. 50% acetic acid (for endpoint method). Prepare by adding a volume of glacial acetic acid to an equal volume of distilled or reverse osmosis (RO) water (LRW, see 2. above, may be used but is not required).

Materials and Equipment

1. Microplates. 96-well, covered microplates (available from Associates of Cape Cod, Inc., catalog number CA961). Plates should be certified or tested for endotoxin and/or glucan contamination and should not cause inhibition or enhancement. Check microplates before use and discard if scratches or other optical interference is observed on or in the bottoms of the wells.

2. Reaction tubes. Kinetic assays can be run in an incubating tube reader such as the Pyros Kinetix® Flex using 8 x 75 mm, depyrogenated, borosilicate glass culture tubes (Associates of Cape Cod, Inc. catalog number TK100). The end-point assays can also be run in 8 x 75 mm or 10 x 75 mm, depyrogenated, borosilicate glass culture tubes (Associates of Cape Cod, Inc. catalog numbers TK100 and TB050 respectively).

3. Optical reader capable of reading at 405 nm, or at 540-550 nm for the diazo method. For the kinetic method, use an incubating microplate reader, such as the BioTek® ELx808™ or the Molecular Devices VersaMax™, or a tube reader such as the Pyros Kinetix® Flex tube reader (Associates of Cape Cod, Inc. catalog numbers PKF32, PKF64 and PKF96). For the endpoint method, use a microplate reader to read microplates or, if running the test in tubes, a spectrophotometer with appropriate cuvettes.

4. Incubator capable of maintaining 37±1°C (required for the endpoint methods only). A dry block incubator for microplates (or tubes, as appropriate) is recommended. (A water bath can be used for the endpoint test tube method.) Incubators should be shown to have a uniform heat distribution.

5. Test tube racks to hold and/or incubate dilution and reaction tubes.

6. Pipets, micropipets with pipet tips (multichannel pipettors are useful when using microplates), or repeating pipettors with plastic syringe barrels. Disposable pipets and tips free of interfering endotoxins and glucans are recommended. Associates of Cape Cod, Inc., offers the Pyroclear® line of products, which are certified to be free of interfering endotoxins and glucans.

7. Vortex-type mixer.

8. Parafilm M® (American National Can™). The side in contact with the paper backing is typically free of detectable endotoxin.

9. Test tubes that are free of interfering endotoxin with adequate capacity for making dilutions of endotoxin standard or test specimen. See "Specimen Collection and Preparation" for containers suitable for dilutions.

10. Hot-air oven with capacity to heat to at least 250°C for depyrogenation of glassware. Commonly used depyrogenation cycles assure that all articles in the oven are exposed to minimum time and temperature of 30 minutes and 250°C (2, 16, 17).

Controls

Controls are necessary to insure a valid test. Recommended procedures are detailed by the FDA (1) and USP (2), EP (3) and JP (4).

1. Endotoxin controls.

a. Endotoxin standard series. Prepare a fresh set of dilutions from the stock endotoxin solution for each test. Do not use previously prepared and stored dilutions unless you have demonstrated the stability of that range of concentrations. Make dilutions in a geometric series to give the range of endotoxin concentrations required. Twofold dilutions are recommended for endpoint methods; greater dilutions can be used for the kinetic method. The lowest endotoxin concentration in any standard series is the detection limit of that particular test and is designated λ (Note: detection limits of 0.005 and 0.001 EU/mL are possible with Pyrochrome® in endpoint and kinetic tests respectively). To get to the range of standards required, use as few dilutions as possible with appropriate pipet volumes to maximize accuracy. Dilutions can be made in glass or suitable plastic test tubes or directly in a microplate. Maximum concentrations detected with Pyrochrome® are method dependent. For guidance, see the recommended incubation times available for Pyrochrome®.

b. Positive control (a single standard endotoxin concentration) should be included if the standard series (see a. above) is not prepared in the same way as the positive product controls (see c. below). The endotoxin concentration of the positive control should equal that of a standard from the middle of the standard curve. A value of 0.5 EU/mL would be appropriate for positive controls included with a standard series consisting of 0.005, 0.05, 0.5, 5 and 50 EU/mL. If a standard series is not included in a test, a positive control must be included to verify that it is appropriate to use the parameters of a previous (archived) standard curve to calculate endotoxin concentrations. Refer to the FDA guideline (1) under Routine Testing of Drugs by the LAL Test for details. The positive control should be prepared in water in the same manner as the PPC in product.

c. Positive product controls are inhibition/enhancement controls and consist of the specimen or dilution of specimen to which standard endotoxin is added. The concentration of added endotoxin in the test specimen should be the same as that of the positive control. See section b. above for selection of the appropriate endotoxin concentration for the positive product control. The added endotoxin is frequently referred to as a "spike."

2. Negative controls.
LRW negative controls should be included with each test.

Specimen Preparation - Determination of Test Dilution

If a test protocol has been developed previously for the type of specimen under test, make the necessary dilution for the assay and proceed as directed under "Performing the Test." If a protocol has not been developed for the specimen type, make a series of tenfold dilutions of the specimen. Do not exceed the Maximum Valid Dilution of the product by more than a factor of 10. (Refer to "Limitations of Procedure" below or the FDA Guideline (1) for explanation and calculation of Maximum Valid Dilution (MVD) and Minimum Valid Concentration (MVC). Prepare appropriate dilutions of all specimens with a positive product control for each one.

Performing the Test

Consistent technique is necessary to obtain satisfactory results. All controls and specimens should be tested in at least duplicate. Care should be taken to avoid contamination when setting up the test. Equipment should be appropriately calibrated and qualified to assure uniform incubation temperatures.

1. Prepare test instrumentation as necessary. In an automated system this usually involves entering sample descriptors, setting test parameters, and turning on the incubator set at 37°C.

2. Add to the reaction vessel the appropriate volume of sample (negative control, endotoxin standard, specimen or positive product control) to give the appropriate ratio when Pyrochrome® is added (see 3. below).

   Tests run in a microplate reader: a 1:1 lysate to sample ratio is used. For optimal performance, use a volume of 0.05 mL of sample for any such method (kinetic, endpoint or diazo-coupling). Alternatively, 0.1 mL may be used for the kinetic and endpoint methods, though use of 0.05 mL is recommended to attain maximum sensitivity or for a wide range standard curve (greater than three log range).

   Tests run in the Pyros Kinetix® Flex tube reader: a lysate to sample ratio of either 1:1 or 1:4 may be used. A sample volume of 0.1 mL is required for tests run using a 1:1 ratio of Pyrochrome® LAL reagent to sample. For tests in the Pyros Kinetix® Flex tube reader in which a 1:4 ratio of Pyrochrome® LAL reagent to sample is used, the sample volume should be 0.2 mL.

3. Add Pyrochrome® as appropriate for the method and mix. Failure to mix adequately is a common cause of unsatisfactory tests. Most platereaders have a shake function.

   a. For a microplate, a 1:1 lysate to sample ratio is used and the volume of Pyrochrome® added is equal to the sample volume (see 2. above; typically 0.05 mL). Add Pyrochrome® as rapidly as possible to all samples and mix from 5 to 30 seconds. Either put the plate on an incubator block (for an endpoint test) or into an incubating microplate reader (for a kinetic test). Read the plate with the cover off.

   b. For methods using individual reaction tubes or cuvettes, the volume of Pyrochrome® added can either be equal to the sample volume (0.1 mL in a Pyros Kinetix® Flex tube reader) or one quarter for the sample volume (0.05 mL in a Pyros Kinetix® Flex tube reader). Important: the reaction time should be the same for all tubes. In the case of the Pyros Kinetix® Flex tube reader, the instrument detects the reaction tube upon insertion and initiates timing. To each reaction vessel in turn, add Pyrochrome®, mix for approximately 2 seconds and place the vessel into the incubating device (for endpoint tests) or tube reader (for kinetic tests).

      Regardless of whether microplates or reaction tubes are used, a repeating pipettor is convenient for adding Pyrochrome®.

      Care should be taken to avoid contaminating the vial of Pyrochrome® when handling the reagent.

4. Read the test.

   a. For the kinetic method, allow the test to run until all samples have incubated for significantly longer than the time required for the lowest standard endotoxin concentration to reach the onset OD405. Automated test systems will usually terminate the test after a preset period. Request recommended incubation times for guidance.

   b. For the endpoint method, time the incubation precisely, remove the plate or reaction tubes from the incubator, stop the reaction by adding 50% acetic acid (volume sufficient to give a final concentration 10% acetic acid; use 0.025 mL for sample/Pyrochrome® volumes of 0.05 mL) and read the optical density (OD) in a spectrophotometer or microplate reader (as appropriate) at 405 nm. The test should be set up and read so that the incubation time for each sample is the same (within ±30 seconds). The incubation period depends on the endotoxin concentration range desired and is likely to vary with different lots of Pyrochrome®. Preliminary tests may be necessary to determine the correct incubation period. See the recommended incubation times for guidance.

Preliminary tests may be necessary to determine the correct incubation period.

Results

1. **Preliminary calculations.**
   For the kinetic method, determine the time taken for specimens to reach a particular optical density threshold (usually 0.03 OD units) after any data corrections have been made. Optical density readings must be relative to an initial reading taken to be zero OD units. Many microplate readers have software packages that perform these calculations. The time taken to reach the OD value is called the onset time.

2. **Construct a standard curve.**
   a. For the kinetic method, construct a standard curve by regression of the log onset time on the log endotoxin concentration for the standards. The equation for the regression line describes the standard curve.

      Provided that the absolute value of the correlation coefficient for the standard curve is at least 0.980 (see point 2. under Interpretation below), a second degree/order polynomial (i.e. quadratic) regression line equation may be used to calculate endotoxin concentrations.

   b. For the endpoint method, construct a standard curve by plotting optical density readings against standard endotoxin concentrations.

3. **Calculate the endotoxin concentrations.**
   Calculate endotoxin concentrations of all specimens (including standards and controls) using the equation for a straight line:

$$Y = aX + b \text{ rearranged as } X = (Y - b) / a$$

where:
Y = log onset time (kinetic method), or optical density (endpoint method)
X: Kinetic method: X = log endotoxin concentration (it is necessary to determine the antilog of X to obtain the endotoxin concentration)
Endpoint method: X = endotoxin concentration
a = slope of the line
b = the Y intercept

Multiply the endotoxin concentration calculated from the standard line by the dilution of the sample to express the concentration in terms of the original, undiluted sample.

These calculations are commonly performed by endotoxin testing software.

Interpretation

1. In order for a test to be valid, the endotoxin concentration of negative controls (estimated by extrapolation of the standard curve) should be significantly less than that of the lowest standard concentration.

2. The correlation coefficient for the standard curve included with the test should have an absolute value of at least 0.980.

3. The mean measured endotoxin concentration of positive controls (for verification of an archived curve) must be within 25% of the nominal concentration. Thus, if the positive control is 0.125 EU/mL, the measured concentration must be between 0.09375 and 0.15625 EU/mL.

4. Endotoxin concentrations can only be reported for the range from the lowest to the highest standard endotoxin concentration. Results for specimens that lie outside of this range should be reported as either less than the lowest standard concentration (not detectable) or greater than the highest standard concentration.

   For the endpoint method, valid endotoxin concentrations can only be calculated from OD values that lie on the linear portion of the standard curve.

5. In order to demonstrate that the specimen does not significantly interfere with the LAL/endotoxin reaction, the measured endotoxin concentration of the positive product control must be within 50 to 200% of the nominal concentration of the added endotoxin 'spike.' Before determining whether the spike is recovered within these limits, subtract the endotoxin concentration measured in the (unspiked) specimen. For example, in order to be considered free of significant interference, the measured endotoxin concentration in a 0.125 EU/mL positive product control (after subtraction of any endotoxin in the unspiked specimen) must be within the range 0.0625–0.25 EU/mL (50 to 200% of 0.125 EU/mL). If the measured endotoxin concentration in the unspiked specimen is 0.028 EU/mL and that in the positive product control is 0.163 EU/mL, the endotoxin attributable to the spike is 0.163 – 0.028 = 0.135 EU/mL. This is within the range and subject to other requirements being met; the test of the sample is valid.

Limitations of Procedure

The procedure is limited by the extent of the inhibition or enhancement capacity of the specimen under test. If the procedure cannot be validated (1, 2) at a specimen concentration that is greater than the minimum valid concentration (MVC), then the bacterial endotoxins test using LAL reagent cannot be substituted for the USP Pyrogen Test. The MVC is calculated as follows:

$$MVC = \frac{(\lambda)\ (\text{specimen dose})}{(\text{endotoxin tolerance limit})}$$

where λ is in EU/mL, specimen dose is in units per kg body weight, and the endotoxin tolerance limit is in EU/kg.

The maximum valid dilution (MVD) is the specimen dilution containing the MVC (1). It is calculated by dividing the initial specimen concentration by the MVC.

The endotoxin tolerance limit is 0.2 EU/kg for drugs with an intrathecal route of administration and 5 EU/kg for all other parenterally administered products. The limit for medical devices is expressed per mL of an extraction or rinse volume obtained as described in the USP (18) based on the limits for devices. For devices that do not contact cerebrospinal fluid, the limit is 20 EU/device; for those that do, it is 2.15 EU/ device. The limit for liquid devices is the same as that for drugs per the FDA guideline (1).

Some serine proteases (e.g. trypsin, activated blood factors) will cause a false positive result unless denatured (for example, by heat treatment) before testing. Yellow-colored materials such as animal serum, albumin, and plasma may interfere with pNA based chromogenic assay. For these products, use the diazo-coupling assay (Associates of Cape Cod, Inc. catalog number CD060). Excess turbidity in a sample may also interfere with the test.

Expected Values

Endotoxin in specimens can be quantified between the range of standard endotoxin concentrations used to construct the standard curve. If it is necessary to dilute the specimen to overcome any inhibition or enhancement, the least amount of endotoxin that can be detected will be increased accordingly. Materials derived from biological sources, even after biochemical purification, may still contain measurable levels of endotoxin. Water obtained by distillation, reverse osmosis, or ultrafiltration may contain less endotoxin than detectable as long as the purification process is operating correctly and the water is not contaminated after production.

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Our experienced staff will be pleased to discuss the practical and theoretical aspects of the LAL test.

Please call if you have questions about using Pyrochrome®. We will replace any of our products that do not perform to product specifications; contact Customer Service before returning product.