

Directions for Use

Intended Use:

PyroTest™ helps pharmacists who extemporaneously compound reasonable quantities of drugs upon receipt of a valid prescription for an individually identified patient from a licensed practitioner.

Introduction:

The test reagent contains an extract prepared from the circulating amebocyte found in the blood of the American horseshoe crab, *Limulus polyphemus*. Gram-negative bacterial endotoxin catalyzes the activation of a series of enzymes contained within the *Limulus* amebocyte lysate (LAL). If the endotoxin is present in significant quantity this activation results in the formation of a gelatinous clot.

Materials Supplied:

- **1 ASSAY VIAL** (white cap) containing the *Limulus* amebocyte lysate. This vial is reconstituted with 0.25 ml of prediluted test sample. Sensitivity = 0.125 EU/mL
- **1 POSITIVE CONTROL VIAL** (blue cap) containing the *Limulus* amebocyte lysate plus a known amount of gram-negative bacteria endotoxin. This vial is reconstituted with 0.25 mL of prediluted test sample.
- **2 DILUTION VIALS** containing 30 ml of endotoxin-free water. These vials are used as needed to dilute the test sample prior to testing. Refer to the tables below for the appropriate dilution procedure.
- **2 DISPOSABLE SYRINGES** to be used for sample handling.

Test Inhibition:

Some ingredients interfere with the LAL test. The PyroTest may not provide reliable results when attempting to detect excessive endotoxins in these materials:

Suspensions, emulsions, phenols, lipids, chelators, antibiotics, surfactants, alcohol's with a final concentration (after dilution) of greater than 0.1%, concentrated acids and bases, some oils (other than castor, cottonseed, peanut, soybean, sesame)

Test Volume Required:

The correct volume of sample required to use the PyroTest is found by referring to Tables I, II, or III:

1. Table I is the preferred alternative. It is based on published USP Endotoxin Limits and the starting concentration of the sample in mg/mL.
2. Tables II or III are used in the absence of a USP Endotoxin Limit. Use the route of administration and infusion rate in mL/hour.

Dilution Procedures, all Tables:

The test sample usually has to be diluted once or twice before being injected into the ASSAY and POSITIVE CONTROL VIALS. Refer to the appropriate table and follow the dilution steps listed for that EU/mL or mL/hour value.

Sample With No Dilutions Required:

1. Using one of the syringes provided, inject 0.25 mL of sample into the ASSAY VIAL (White Cap). Gently agitate until entire contents are reconstituted.
2. Using the same syringe, inject 0.25 mL of sample into the POSITIVE CONTROL VIAL (Blue Cap). Gently agitate until entire contents are reconstituted.
3. Proceed to Incubation and Observation Procedure.

Sample That Requires One Dilution:

1. Locate either the Endotoxin Release Limit or Dose.
2. Inject the volume of sample indicated in "First Dilution" into one of the dilution vials. Vigorously shake the vial several times to mix the test sample and endotoxin-free diluent. Safely discard the empty syringe.

3. If only one dilution is required, proceed to step #4, directly below. If two (2) dilutions are required, proceed to Samples that Require Two Dilutions.
4. Using the syringe provided, inject 0.25 mL of diluted sample into the ASSAY VIAL (White Cap). Gently agitate until entire contents are reconstituted.
5. Using the same syringe, inject 0.25 mL of sample into the POSITIVE CONTROL VIAL (Blue Cap). Gently agitate until entire contents are reconstituted.
6. Proceed to Incubation and Observation Procedure.

Samples That Require Two Dilutions:

1. Using the syringe provided, inject the volume of sample indicated in "Second Dilution" into the second dilution vial. Vigorously shake the vial several times to mix the test sample and endotoxin-free diluent. Safely discard the empty syringe.
2. Using the second syringe provided, inject 0.25 mL of diluted sample into the ASSAY VIAL (White Cap). Gently agitate until entire contents are reconstituted.
3. Using the same syringe, inject 0.25 mL sample into the POSITIVE CONTROL VIAL (Blue Cap). Gently agitate until entire contents are reconstituted.
4. Proceed to Incubation and Observation Procedure.

Table I: For Products with a USP Endotoxin Limit

1. Multiply the USP Endotoxin Limit (typically in EU / mg) by the concentration of the undiluted solution to be tested. Answer should be in EU / mL.
2. In the chart below, locate the EU / mL value which approaches but is **not greater than** the EU / mL limit calculated above.
3. Dilute solution as indicated using the 30 mL vials of endotoxin-free water.

Endotoxin Release Limit (EU / mL)	First Dilution (mL)	Second Dilution (mL)	Resultant Dilution	Maximum Valid Dilution
1.5	NONE	NONE	0	12
3.5	2.00	NONE	16	28
4.0	1.00	NONE	31	32
6.0	0.65	NONE	47	48
8.0	0.50	NONE	61	64
10.0	0.40	NONE	76	80
10.5	0.40	NONE	76	84
12.5	0.35	NONE	87	100
15.0	0.30	NONE	101	120
25.0	0.20	NONE	151	200
35.0	0.15	NONE	201	280
70.0	0.10	NONE	301	560
100.0	0.05	NONE	601	800
150.0	0.80	1.00	1194	1200
200.0	0.60	1.00	1581	1600
250.0	0.60	0.80	1964	2000
300.0	0.40	1.00	2356	2400
350.0	0.50	0.70	2675	2800
400.0	0.30	1.00	3131	3200
450.0	0.30	0.90	3468	3600
500.0	0.30	0.80	3889	4000
600.0	0.20	1.00	4681	4800
700.0	0.20	0.90	5184	5600
800.0	0.30	0.50	6161	6400
900.0	0.20	0.70	6622	7200
1000.0	0.20	0.60	7701	8000
1200.0	0.10	1.00	9331	9600
1400.0	0.10	0.90	10334	11200
1600.0	0.25	0.30	12221	12800
1800.0	0.10	0.70	13201	14400
2000.0	0.10	0.60	15351	16000
2500.0	0.10	0.50	18361	20000
3000.0	0.10	0.40	22876	24000
3500.0	0.10	0.40	22876	28000
5000.0	0.10	0.30	30401	40000
7000.0	0.10	0.20	45451	56000
8000.0	0.10	0.15	60501	64000
20000.0	0.10	0.10	90601	160000

Table II: Intrathecal Injection

1. Use Table I if the drug being tested has a USP Endotoxin Limit.
2. Dose = mLs used for single intrathecal injection or hourly mL infusion rate.
3. In the chart below, locate the dose which approaches but is **not less than** the mL /hour rate.
4. Dilute solution as indicated using the endotoxin-free vials provided.

Dose (mLs)	First Dilution (mL)	Second Dilution (mL)	Resultant Dilution	Endotoxin Release Limit (EU / mL)	Maximum Valid Dilution
0.0125	0.35	0.3	8758	1120	8960
0.025	0.7	0.3	4430	560	4480
0.05	0.60	0.70	2237	280.0	2240.0
0.10	0.90	1.00	1064	140.0	1120.0
0.15	0.05	NONE	601	93.3	746.7
0.20	0.10	NONE	301	70.0	560.0
0.40	0.15	NONE	201	35.0	280.0
1.0	0.30	NONE	101	14.0	112.0
1.5	0.45	NONE	68	9.3	74.7
2.0	0.55	NONE	56	7.0	56.0
2.5	0.70	NONE	44	5.6	44.8
3.0	0.85	NONE	36	4.7	37.3
3.5	1.00	NONE	31	4.0	32.0
4.0	2.00	NONE	16	3.5	28.0
8.0	NONE	NONE	0	1.8	14.0

Table III: Systemic Injection

1. Use Table I if the drug being tested has a USP Endotoxin Limit.
2. Dose = mLs used for single systemic injection or hourly mL infusion rate.
3. In the chart below, locate the dose which approaches but is **not less than** the mL /hour rate.
4. Dilute solution as indicated using the endotoxin-free vials provided.

Dose (mLs)	First Dilution (mL)	Second Dilution (mL)	Resultant Dilution	Endotoxin Release Limit (EU / mL)	Maximum Valid Dilution
0.05	0.10	0.20	45451.0	7000.0	56000.0
0.10	0.10	0.40	22876.0	3500.0	28000.0
0.15	0.10	0.50	18361.0	2333.3	18666.7
0.20	0.10	0.70	13201.0	1750.0	14000.0
0.25	0.10	0.90	10334.3	1400.0	11200.0
0.30	0.10	1.00	9331.0	1166.7	9333.3
0.35	0.20	0.60	7701.0	1000.0	8000.0
0.40	0.20	0.70	6622.4	875.0	7000.0
0.45	0.30	0.50	6161.0	777.8	6222.2
0.50	0.20	0.90	5184.3	700.0	5600.0
1.0	0.50	0.70	2675.3	350.0	2800.0
1.5	0.60	0.90	1751.0	233.3	1866.7
2.0	0.70	1.00	1359.6	175.0	1400.0
2.5	0.90	1.00	1064.3	140.0	1120.0
3.0	0.05	NONE	601.0	116.7	933.3
6.0	0.10	NONE	301.0	58.3	466.7
10.0	0.15	NONE	201.0	35.0	280.0
15.0	0.20	NONE	151.0	23.3	186.7
20.0	0.25	NONE	121.0	17.5	140.0
25.0	0.30	NONE	101.0	14.0	112.0
30.0	0.35	NONE	86.7	11.7	93.3
35.0	0.40	NONE	76.0	10.0	80.0
40.0	0.45	NONE	67.7	8.8	70.0
45.0	0.50	NONE	61.0	7.8	62.2
50.0	0.55	NONE	55.5	7.0	56.0
60.0	0.70	NONE	43.9	5.8	46.7
70.0	0.80	NONE	38.5	5.0	40.0
80.0	0.90	NONE	34.3	4.4	35.0
90.0	1.00	NONE	31.0	3.9	31.1
100.0	2.00	NONE	16.0	3.5	28.0
200.0	NONE	NONE	0.0	1.8	14.0

Incubation and Observation Procedure: ALL SAMPLES

1. Use PyroTest aluminum vial block, #INC004, to ensure proper heat transfer to samples. Preheat block for at least 20 minutes or leave in incubator between tests.
2. Incubate both the ASSAY VIAL and the POSITIVE CONTROL VIAL, **undisturbed** for 60 minutes at 37° C.
3. At the end of the incubation period, gently remove vials from the aluminum block and invert each vial 180 degrees. Observe the reaction mixture for gel formation. A positive reaction is indicated by a firm gel which remains intact when the vial is inverted. A partial gel is a negative.

Interpretation of results: ALL SAMPLES

POSITIVE CONTROL VIAL

A positive result observed in the **POSITIVE CONTROL VIAL** indicates that the assay was performed properly and that the dilution of the test sample does not interfere with the accurate detection of gram-negative endotoxin.

A positive result should always be observed in the **POSITIVE CONTROL VIAL**.

ASSAY VIAL

A positive result in the **ASSAY VIAL** indicates the test sample contains more than the **maximal allowable endotoxin concentration**.

A negative result in the **ASSAY VIAL** indicates that the endotoxin content in the test sample is within acceptable limits.

Documentation of Results: ALL SAMPLES

Enter results on PyroTest™ Log Sheet.

Q.I.medical, inc

Nevada City, CA 95959
530-265-4820 Fax 530-265-9416
Technical Services 800-837-8361
E-mail: info@qimedical.com
www.qimedical.com

Cat. #PT5000
5 tests/percase

