

***Limulus* Amebocyte Lysate Testing: Adapting It for Determination of Bacterial Endotoxin in ^{99m}Tc-Labeled Radiopharmaceuticals at a Hospital Radiopharmacy**

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A bacterial endotoxin test (BET) is required to detect or quantify bacterial endotoxin that may be present in radiopharmaceutical preparations. The test uses *Limulus* amebocyte lysate, which, in the presence of bacterial endotoxin and divalent calcium ions, causes the formation of a coagulin gel. ^{99m}Tc-labeled radiopharmaceuticals have chelating ligands such as diethylene triamine pentaacetic acid (DTPA), ethylene dicysteine (EC), L,L-ethyl cysteinyl dimer (ECD), N-[2,4,6-trimethyl-3 bromoacetanilid] iminodiacetic acid (mebrofenin), dimercapto succinic acid-III (DMSA-III), dimercapto succinic acid-V (DMSA-V), and several others, which form a coordination complex with Na-^{99m}Tc-O₄ in the presence of reducing agents. During BET by the gel-clot method, the free sulfhydryl (–SH) and carboxyl (–COOH) in some of the chelating agents in the final ^{99m}Tc-labeled radiopharmaceuticals decrease the free divalent calcium ion concentration, which in turn inhibits coagulin gel formation. This study was designed using the premise that addition of calcium chloride solution to the reaction mixture would nullify this effect. **Methods:** We present here the data obtained from BET assay analysis of ^{99m}Tc-labeled radiopharmaceuticals and the cold kits from which they are made (EC, ECD, methoxyisobutylisonitrile, DTPA, mebrofenin, methylene diphosphonic acid [MDP], DMSA-III, and DMSA-V) using 2 different dilutions, maximum valid dilution (MVD) and half maximum valid dilution (MVD/2), with and without the addition of calcium chloride at a final concentration of 300 μM. **Results:** It was observed that at MVD and MVD/2 all of the ^{99m}Tc-labeled kits exhibited interference in coagulin gel formation with the exception of ^{99m}Tc-methoxyisobutylisonitrile, ^{99m}Tc-MDP, ^{99m}Tc-mebrofenin, and ^{99m}Tc-ECD. However, only the cold kits of methoxyisobutylisonitrile and MDP did not show inhibition. An addition of calcium chloride solution nullified this interference at both MVD and MVD/2 in all of the ^{99m}Tc-labeled radiopharmaceuticals in which interference was observed. **Conclusion:** In practice, *Limulus* amebocyte lysate testing is not a method of choice for ^{99m}Tc-labeled radiopharmaceuticals because these radiopharmaceuticals exhibit interference. However, our study proves the hypothesis that the addition of calcium chloride can circumvent this problem. The addition of calcium chloride

provides an enhanced biologic quality control testing option for the final formulation of ^{99m}Tc-labeled radiopharmaceuticals at the hospital radiopharmacy end.

Key Words: BET; transglutaminase; radiopharmaceuticals; ^{99m}Tc

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In the centralized radiopharmacy laboratories of hospitals, different generator-based radiopharmaceuticals such as ^{99m}Tc-, ⁶⁸Ga-, and ⁹⁰Y-labeled molecules are being used for various diagnostic and therapeutic purposes. ^{99m}Tc-labeled radiopharmaceuticals have wider applications in SPECT and planar imaging with γ cameras because of their short half-life (6.03 h) and pure γ emission (140 keV). It is mandatory for hospital radiopharmacies to monitor the bacterial endotoxin levels of a ^{99m}Tc-labeled radiopharmaceutical before its release for human use and ensure that it is within the permissible limits. For radiopharmaceutical products not administered intrathecally, the endotoxin limit (EL) is calculated as 175 EU/V, per mL, where EU is endotoxin unit and V is the maximum volume of injection (1,2). For this study, we set the EL as 25 EU/mL, with a V of 7 mL (the maximum volume of ^{99m}Tc-O₄ that can be added to a cold radiopharmaceutical kit). The kit includes a 10-mL vial, and it is presumed that all 7 mL will be injected into 1 patient. The standard method used for assessing the bacterial endotoxin levels in any injectable formulation is a bacterial endotoxin test (BET), which is based on gel-clot formation of *Limulus* amebocyte lysate (LAL) reagent in the presence of bacterial endotoxin (1,2). The LAL reagent, which is obtained from aqueous extracts of circulating amebocytes in the horseshoe crab (*Limulus polyphemus*), contains an intracellular coagulation system (clotting factor) that is triggered by lipopolysaccharides derived from gram-negative bacterial cell walls (3). The coagulation system includes 3 serine protease zymogens—factor C, factor B, and proclotting enzyme—along with protein that can form gel-clot. Bacterial endotoxins initiate the

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activation of the cascading series of serine protease in LAL. The last activated enzyme in the series, the clotting enzyme, cleaves a peptide from an endogenous substrate coagulogen and forms a modified substrate coagulin in the presence of an enzyme transglutaminase. The activity of transglutaminase is Ca^{++} -dependent (4), and thus the coagulin gel formation by endotoxin requires the presence of free divalent calcium ions (Ca^{++}). $^{99\text{m}}\text{Tc}$ -labeled radiopharmaceuticals, which invariably use chelating agents, deplete the free divalent calcium ions normally present in the LAL reagent, thereby hindering transglutaminase activity and inhibiting coagulin gel formation.

None of the available pharmacopoeias describe any specified methods for performing a BET assay for $^{99\text{m}}\text{Tc}$ -labeled radiopharmaceuticals; however, according to the United States Pharmacopoeia (USP): “In the event of doubt or dispute, the final decision is made based on the gel-clot limit test unless otherwise indicated in the monograph for the product being tested” (1). In view of the USP information, the present study was performed with the gel-clot method for evaluation of inhibition and standardization of a BET assay, which can overcome the inhibition due to depletion of free divalent calcium ions in routinely used $^{99\text{m}}\text{Tc}$ -labeled radiopharmaceuticals and their corresponding cold kits (2,5,6). Validation of the gel-clot method defines the optimum test concentration or test dilution, preparation of samples and controls, and maintenance of test conditions and acceptance criteria. The first step in a BET assay by the gel-clot method is the identification of a suitable dilution or concentration of sample and controls for routine testing. Maximum valid dilution (MVD) is the maximum allowed dilution of a sample under study at which the EL can be determined (1). The MVD factor so obtained is the limit dilution factor for the test to be valid. To determine the extent of MVD that may be applied to overcome an interference problem without exceeding the limit of endotoxin concentration, 175 EU per injection of a 7-mL volume of $^{99\text{m}}\text{Tc}$ -labeled radiopharmaceuticals was used (1,5,6). Further, it was shown in this study that the inhibition of coagulin gel formation could be reversed

by the addition of a pyrogen-free solution of calcium chloride at a final concentration of 250–500 μM during the BET assay of $^{99\text{m}}\text{Tc}$ -labeled radiopharmaceuticals and their cold kits by gel-clot endpoint method.

MATERIALS AND METHODS

LAL reagent (sensitivity $[\lambda]$, 0.125 EU/mL), control standard endotoxin (stock solution concentration, 20 EU/mL), and LAL reagent water (endotoxin level < 0.001 EU/mL) were obtained from Charles River Laboratories. Endotoxin stock solution was prepared in accordance with the information stated in the certificate of analysis. The sterile and endotoxin-free 60 mM solution of calcium chloride (7) was prepared by dissolving calcium chloride crystals (E. Merck) in sterile endotoxin-free water (Charles River Laboratories) and filtering through a 0.20- μm Minisart sterile filter (Satarious Stedim Biotech GmbH). The cold kits for the $^{99\text{m}}\text{Tc}$ -labeled radiopharmaceuticals—diethylene triamine pentaacetic acid (DTPA), ethylene dicysteine (EC), L,L-ethyl cysteinyl dimer (ECD), *N*-[2,4,6-trimethyl-3 bromoacetanilid] iminodiacetic acid (mebrofenin), [tetrakis(2-methoxy-2 methyl propyl-1-isocyanide) copper(I)] tetrafluoroborate (methoxyisobutylisonitrile), methylene diphosphonic acid (MDP), dimercaptosuccinic acid-III (DMSA-III), and dimercaptosuccinic acid-V (DMSA-V)—were from the Board of Radiation and Isotope Technology. $^{99\text{m}}\text{Tc-O}_4^-$ for preparing the above $^{99\text{m}}\text{Tc}$ -labeled radiopharmaceuticals was obtained from the ^{99}Mo - $^{99\text{m}}\text{Tc}$ Geltech generator from the Board of Radiation and Isotope Technology. Cold kits for preparing $^{99\text{m}}\text{Tc}$ -labeled DTPA, EC, ECD, DMSA-III, DMSA-V, mebrofenin, methoxyisobutylisonitrile, and MDP injection were reconstituted with saline (screened in advance for endotoxin) and labeled with $^{99\text{m}}\text{Tc}$ as per the standard protocol provided with the respective cold kit.

In a BET assay, it is required to calculate the MVD to establish the extent of dilution to avoid interfering test conditions: $\text{MVD} = \text{EL}/\lambda$ (where EL is the endotoxin limit of the radiopharmaceuticals and λ is the sensitivity of LAL reagent used in EU/mL). Thus, considering that the EL is 25 EU/mL (based on 7 mL being the maximum volume that is injected in a patient), the λ of the lysate used in the present study was 0.125 EU/mL and the permitted MVD 1:200. The detailed protocol for the assay is shown in Table 1.

The experiments were performed at a half MVD (MVD/2) of 1:100 and MVD of 1:200. However, the MVD calculation is based on the EL of 25 EU/mL, and in the study the preparations were

TABLE 1
Detailed Protocol for BET

Test serial no.	Assay tube (in duplicate)	LAL reagent water (μL)	Control standard endotoxin 4 λ (μL)	Test sample* (μL)	LAL reagent (μL)
1	Negative water control	100	—	—	100
2	Positive water control 2 λ	50	50	—	100
3	NPC [†]	50	—	50	100
4	PPC 2 λ [‡]	—	50	50	100
5	PPC 2 λ with calcium chloride [‡]	—	50	50 [‡]	100

*Repeated at MVD and MVD/2—that is, 1:66 and 1:33 dilutions.

[†]These tubes were set up for all $^{99\text{m}}\text{Tc}$ radiopharmaceuticals ($^{99\text{m}}\text{Tc}$ -DTPA, $^{99\text{m}}\text{Tc}$ -DMSA-III, $^{99\text{m}}\text{Tc}$ -DMSA-V, $^{99\text{m}}\text{Tc}$ -ECD, $^{99\text{m}}\text{Tc}$ -mebrofenin, $^{99\text{m}}\text{Tc}$ -methoxyisobutylisonitrile, and $^{99\text{m}}\text{Tc}$ -MDP) and their cold kits.

[‡]Calcium chloride at final concentration of 300 μM was incorporated in assay tube of test sample at final dilution.

Tests 3 and 4 were also performed initially for 60 mM stock solution of calcium chloride and saline (0.9% sodium chloride) to ensure absence of endotoxin in stock solutions.

diluted 3-fold (1 mL made to 3 mL). In view of this dilution, the MVD and half MVD for the present study were 1:66 and 1:33, respectively.

Reactions for positive product control (PPC) in the assay were set up by adding 50 μ L of 4 λ control standard endotoxin to all freshly prepared ^{99m}Tc -labeled radiopharmaceuticals and their corresponding cold kits. During standardization, instead of the separate addition of calcium chloride (screened in advance for endotoxin), its aliquots of 100 μ L at different concentrations of 5, 15, 30, and 60 mM were added to the first dilution tube of PPC for all the ^{99m}Tc -labeled radiopharmaceuticals and their cold kits. On the basis of our findings (data not shown), although any stock solution of calcium chloride can be used, in this study we chose a 60 mM stock solution of calcium chloride, which gives a final concentration of 250–500 μ M of calcium chloride in the assay tube.

PPC and negative product control (NPC) samples were incubated at 37°C ($\pm 1^\circ\text{C}$) for 60 min in a dry heating block. To demonstrate reproducibility of the results, 3 batches of each of the ^{99m}Tc -labeled radiopharmaceuticals and their cold kits were tested.

To validate that the addition of calcium chloride did not interfere in the gel-clot formation and also did not reduce the λ of the test, we performed assays with positive water control at different dilutions with and without calcium. The assay was also done with negative water control with and without calcium. The assay was repeated 14 times to study reproducibility.

RESULTS

Table 2 shows results obtained for different ^{99m}Tc -labeled radiopharmaceuticals and their cold kits at MVD and MVD/2 in the BET assay. Assays were performed for 3 consecutive batches, and results were consistent. No NPC tubes showed any gel-clot formation, indicating that the endotoxin levels in all the tested samples were less than 25 EU/mL. In PPC tubes at MVD, without calcium chloride, it was observed that the cold kit for mebrofenin showed a semisolid gel-clot, the

cold kits for methoxyisobutylisonitrile and MDP showed firm gel-clots, and the rest of the cold kits had no gel-clot formation, indicating inhibition in the assay. After labeling with ^{99m}Tc , in the presence of endotoxin (PPC), the ^{99m}Tc -mebrofenin showed a firm gel-clot. As expected, ^{99m}Tc -methoxyisobutylisonitrile and ^{99m}Tc -MDP gave firm gel-clots. Also ^{99m}Tc -ECD showed a firm gel-clot, and ^{99m}Tc -EC and ^{99m}Tc -DTPA showed semisolid gel-clots, whereas their cold kits showed inhibition. None of the other cold kits showed any reversal of gel-clot inhibition after labeling with ^{99m}Tc . At MVD/2 in the PPC without calcium chloride, all cold kits except methoxyisobutylisonitrile and MDP showed an absence of gel-clot. Additionally at MVD/2, in PPC without calcium chloride, all ^{99m}Tc -labeled radiopharmaceuticals showed inhibition except ^{99m}Tc -MDP, ^{99m}Tc -mebrofenin, ^{99m}Tc -methoxyisobutylisonitrile, and ^{99m}Tc -ECD, which gave firm gel-clots.

After the addition of calcium chloride, PPC at MVD and MVD/2 for all ^{99m}Tc -labeled radiopharmaceuticals and their cold kits showed clot formation, indicating that the inhibition of the assay observed earlier was successfully reversed. Table 3 shows the results obtained for the gel-clot test performed at different concentrations of standard endotoxin concentrations from 4 λ to $\lambda/4$, demonstrating that the presence of calcium chloride does not interfere in the assay. Further, the experiments gave consistent results over 14 replications, indicating the validity and reproducibility of the procedure to reverse the inhibition of gel-clot formation by adding calcium chloride.

DISCUSSION

It has been previously observed that during the validation of a BET assay performed by the gel-clot method, some of

TABLE 2
Results for Different ^{99m}Tc -Labeled Radiopharmaceuticals and Their Cold Kits at MVD and MVD/2 in BET Assay

Product analyzed	MVD				MVD/2			
	NPC		PPC		NPC		PPC	
	Without CaCl_2	With CaCl_2	Without CaCl_2	With CaCl_2	Without CaCl_2	With CaCl_2	Without CaCl_2	With CaCl_2
^{99m}Tc -EC	–	–	+	++	–	–	–	++
EC cold kit	–	–	–	++	–	–	–	++
^{99m}Tc -DTPA	–	–	+	++	–	–	–	++
DTPA cold kit	–	–	–	++	–	–	–	++
^{99m}Tc -ECD	–	–	++	++	–	–	++	++
ECD cold kit	–	–	–	++	–	–	–	++
^{99m}Tc -DMSA-III	–	–	–	++	–	–	–	++
DMSA-III cold kit	–	–	–	++	–	–	–	++
^{99m}Tc -DMSA-V	–	–	–	++	–	–	–	++
DMSA-V cold kit	–	–	–	++	–	–	–	++
^{99m}Tc -mebrofenin	–	–	++	ND	–	–	++	ND
Mebrofenin cold kit	–	–	+	++	–	–	–	++
^{99m}Tc -methoxyisobutylisonitrile	–	–	++	ND	–	–	++	ND
methoxyisobutylisonitrile cold kit	–	–	++	ND	–	–	++	ND
^{99m}Tc -MDP	–	–	++	ND	–	–	++	ND
MDP cold kit	–	–	++	ND	–	–	++	ND

– = no gel-clot; + = semisolid gel-clot; ++ = firm gel-clot; ND = not done.

TABLE 3

Results of Gel-Clot Assay at Different Standard Endotoxin Concentrations in Presence and Absence of Calcium Chloride

Experiment no.	0.5 EU/mL (4λ)			0.25 EU/mL (2λ)			0.125 EU/mL (λ)			0.0625 EU/mL (λ/2)			0.03125 EU/mL (λ/4)		
	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3
1															
A	++	++	++	++	++	++	++	++	++	+	+	+	-	-	-
B	++	++	++	++	++	++	++	++	++	+	+	+	-	-	-
2															
A	++	++	++	++	++	++	++	++	++	+	+	+	-	-	-
B	++	++	++	++	++	++	++	++	++	+	+	+	-	-	-
3															
A	++	++	++	++	++	++	++	++	++	+	+	+	-	-	-
B	++	++	++	++	++	++	++	++	++	+	+	+	-	-	-
4															
A	++	++	++	++	++	++	++	++	++	+	+	+	-	-	-
B	++	++	++	++	++	++	++	++	++	+	+	+	-	-	-
5															
A	++	++	++	++	++	++	++	++	++	+	+	+	-	-	-
B	++	++	++	++	++	++	++	++	++	+	+	+	-	-	-
6															
A	++	++	++	++	++	++	++	++	++	+	+	+	-	-	-
B	++	++	++	++	++	++	++	++	++	+	+	+	-	-	-
7															
A	++	++	++	++	++	++	++	++	++	+	+	+	-	-	-
B	++	++	++	++	++	++	++	++	++	+	+	+	-	-	-
8															
A	++	++	++	++	++	++	++	++	++	+	+	+	-	-	-
B	++	++	++	++	++	++	++	++	++	+	+	+	-	-	-
9															
A	++	++	++	++	++	++	++	++	++	+	+	+	-	-	-
B	++	++	++	++	++	++	++	++	++	+	+	+	-	-	-
10															
A	++	++	++	++	++	++	++	++	++	+	+	+	-	-	-
B	++	++	++	++	++	++	++	++	++	+	+	+	-	-	-
11															
A	++	++	++	++	++	++	++	++	++	+	+	+	-	-	-
B	++	++	++	++	++	++	++	++	++	+	+	+	-	-	-
12															
A	++	++	++	++	++	++	++	++	++	+	+	+	-	-	-
B	++	++	++	++	++	++	++	++	++	+	+	+	-	-	-
13															
A	++	++	++	++	++	++	++	++	++	+	+	+	-	-	-
B	++	++	++	++	++	++	++	++	++	+	+	+	-	-	-
14															
A	++	++	++	++	++	++	++	++	++	+	+	+	-	-	-
B	++	++	++	++	++	++	++	++	++	+	+	+	-	-	-

T1 = assay tube 1; T2 = assay tube 2; T3 = assay tube 3; A = with CaCl₂; B = without CaCl₂; - = no gel-clot; + = semisolid gel-clot; ++ = firm gel-clot.

the ^{99m}Tc-labeled radiopharmaceuticals or their cold kits inhibit the extent of gel-clot formation significantly (7). To overcome this problem, some cold kit manufacturers use the rabbit pyrogen test as described in USP Chapter <151>, Pyrogenicity Test, which is not cost-effective for a nuclear medicine department because several rabbits have to be maintained in a special animal house. Additionally, the occurrence of false-positive, variable results; potential radioactive dose to the analyst; and the resulting radioactivity after administration to animals are common limitations with this test. The present study was planned to find a solution to this problem.

The importance of finding a solution to the limitations presented by this study is accentuated by the fact that the gel-clot limit test is recommended as a confirmatory test for bacterial endotoxin in ^{99m}Tc-labeled radiopharmaceuticals by the USP. Further, though BET by kinetic chromogenic techniques offers the advantage of greater dilution to resolve inhibition, this technique may introduce new problems because the chelating agents may interfere with the chemistry involved in color development. In addition, in the kinetic chromogenic method, the coagulase enzyme is required for releasing the chromogenic tag paranitroaniline from

the synthetic chromogenic peptide BOC-Leu-Gly-Arg-pNAA (paranitroanilide-pNAA) analog of coagulogen Z, and there is a competition between the chromogenic substrate and coagulogen for activated enzyme (8). Thus, the reaction is quite different from the mechanism involved in a gel-clot reaction, for which coagulin gel formation takes place in the presence of endotoxin due to the activity of Ca^{++} -dependent transglutaminase.

We found that some of the $^{99\text{m}}\text{Tc}$ -labeled radiopharmaceuticals ($^{99\text{m}}\text{Tc}$ -EC, $^{99\text{m}}\text{Tc}$ -DMSA-V, $^{99\text{m}}\text{Tc}$ -DMSA-III, and $^{99\text{m}}\text{Tc}$ -DTPA) and cold kits (EC, ECD, DTPA, DMSA-III, DMSA-V, and mebrofenin) inhibited the extent of gel-clot formation. However, inhibition was not observed in $^{99\text{m}}\text{Tc}$ -mebrofenin, $^{99\text{m}}\text{Tc}$ -methoxyisobutylisonitrile, $^{99\text{m}}\text{Tc}$ -ECD, and $^{99\text{m}}\text{Tc}$ -MDP or in cold kits of MDP and methoxyisobutylisonitrile. A LAL enzyme inhibition led to a false-negative BET result of the assay sample, and this inhibition was detected by spiking the test samples with purified endotoxin so that a 2λ concentration was achieved. It is known that the inhibition of BET assays for some of the $^{99\text{m}}\text{Tc}$ -labeled radiopharmaceuticals is due to depletion of Ca^{++} ions, and activity of transglutaminase is Ca^{++} -dependent and inhibited by the free thiol group in the reagents (4). $^{99\text{m}}\text{Tc}$ -DMSA-III and cold kits of EC, DMSA-III, and DMSA-V contain free carboxylic acid ($-\text{COOH}$) groups in addition to a free thiol group, and these showed inhibition of clot formation at MVD as well as at MVD/2. $^{99\text{m}}\text{Tc}$ -EC and $^{99\text{m}}\text{Tc}$ -DTPA, however, for which the free SH group is absent and only free COOH groups are present, showed partial inhibition (semisolid clot) at MVD and complete inhibition at MVD/2. This observation proved that free SH and COOH groups inhibit the LAL test probably by depleting Ca^{++} ion concentration, thereby inhibiting transglutaminase activity. Previously, we showed that the depletion of Ca^{++} ions is due to formation of mercaptides of calcium during the BET of $^{99\text{m}}\text{Tc}$ -labeled radiopharmaceuticals containing a free SH group (9), whereas the free COOH group in $^{99\text{m}}\text{Tc}$ -labeled radiopharmaceuticals forms its corresponding calcium salts (9).

The depletion of free Ca^{++} ions and formation of mercaptide prove the fact that the inhibition of a BET assay is dependent on free SH groups available.

The addition of $300\ \mu\text{M}$ calcium chloride in the final BET assay tube of all the above-mentioned $^{99\text{m}}\text{Tc}$ -labeled radiopharmaceuticals with free SH and COOH groups reversed the inhibition phenomenon and resulted in formation of gel-clot in PPC tubes. Finally, our results have proved that a $300\ \mu\text{M}$ concentration of calcium chloride, when incorporated during the BET assay for $^{99\text{m}}\text{Tc}$ -labeled radiopharmaceuticals having free SH, free COOH, and both of these groups, overcomes the inhibition of the assay. This concentration of calcium chloride exhibited consistent,

reliable, and reproducible reversal of inhibition by $-\text{SH}$ and $-\text{COOH}$ for all the $^{99\text{m}}\text{Tc}$ -labeled radiopharmaceuticals and their cold kits. This study has shown the feasibility of adding pyrogen-free calcium chloride solution to overcome inhibition observed in BET assays used for validation of $^{99\text{m}}\text{Tc}$ -labeled radiopharmaceuticals and their cold kits having free SH, free COOH, and both of these groups.

The identical and reproducible results of experiments, shown in Table 3, on gel-clot formation at different standard endotoxin concentrations, ranging from 4λ to $\lambda/4$, in the presence and absence of calcium chloride validates the fact that the calcium chloride does not interfere in the gel-clot assay. The results shown in Table 3 fulfil the compendial procedure for using calcium chloride to overcome inherent inhibition observed with several $^{99\text{m}}\text{Tc}$ radiopharmaceuticals and their cold kits during the LAL gel-clot assay for BET.

CONCLUSION

This study has shown the feasibility of adding calcium chloride solution to overcome inhibition observed in a LAL BET assay used for $^{99\text{m}}\text{Tc}$ -labeled radiopharmaceuticals and their cold kits having free SH, free COOH, and both of these groups. The adaptation of this modified LAL test method provides a reliable BET quality control testing option for the final formulation of $^{99\text{m}}\text{Tc}$ -labeled radiopharmaceuticals and their cold kits at hospital radiopharmacies and central radiopharmacies.

DISCLOSURE

No potential conflict of interest relevant to this article was reported.

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