Original Article

LAL Test에서 위양성을 나타내는 원인들에 대한 고찰

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Consideration About the Bacterial Endotoxin Test Showing False Positive Test Result When Performing LAL Test

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Purpose: Since radiopharmaceuticals are intended for human administration, it is imperative that we should undergo quality control very strictly. Now almost all the PET laboratories have adopted Bacterial Endotoxin Test as the stand quality control method to monitor whether pyrogen is free or not in the product vial containing crude solution. The aim of this study is to find out the reason why false positive result is observed when using commercially available test vial. **Materials and Methods:** For this experiment, we used commercially available single test kit (Associates of Cape Code. Inc. USA) and we made pH samples by mixing each buffer whose pH ranges are 1.0 to 12.0. Otherwise we made Ethanol samples diluted with distilled water. After making test samples, it added 0.2 mL to the test vial. Assay mixture in the test vial was incubated in a water bath (Chang Shin Co. KOR) for 60 min at 35±2℃. **Results:** After incubation period (60±1℃), we inverted the test vial about 180° To know what pH and how many percentage of Ethanol (Fisher Scientific Korea. Ltd) will affect the reaction. With pH buffer, false positive result was observed at pH 1.0 to 5.0 and 7.7 to 12.6 but at pH 5.2 to.7.5, the test results show negative. It's very strange that we couldn't observe negative test result with Tris buffer at pH 8.4, 8.6, 8.8, 9.0. in other case Ethanol, the test result was seen with 5 to 10% Ethanol. But to my surprise we could see very thick gel formation with 100% Ethanol. **Conclusions:** In this study, we could notice that pH which is too much acidic or alkalic or high concentrated Ethanol would affect Bacterial Endotoxin Test result. As you know, LAL test is sensitive and very reliable method. Therefore, we are needed to elicit the accurate test result as possible as we can. **(Korean J Nucl Med Technol 2009;13(3):156-158)**

Key Words : Bacterial Endotoxin Test, LAL test, Pyrogen, Radiophamaceutical

Introduction

As with conventional drug, radiopharmaceuticals are human injectable drugs. All radiopharmaceuticals must pass several quality control tests, before dispensing for human administration. With that in mind, regular check should be made for the sterility, apyrogenicity and radiochemical purity of all radiopharmaceuticals. LAL test is sophisticated, rapid, sensitive and easy to use method recommended for quantification and qualification in the produced product containing crude solution. This method used the Limuls amebocyte lysate originated from blood of horseshoe crab, Limuls Polyphemus, the principal of this test is based on the fact that opaque gel formation will be occurred, if endotoxin-type pyrogen produced by gram negative bacilli is being. The thicker gel is formated, the greater concentration of pyrogens are being in the samples. But we should remind that the test result should be reported as negative, even if gel formation is occurred but collapsed after inversion. Fred Bang reported in 1956 that gram negative

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Fig. 1. pH buffer samples and pH meter.

bacilli such as Enterobacteriae cause blood to clot. That reaction is enzyme and the enzymes are located in granules in the amebocytes. It was later recognized that Animal's blood cell, mobile cell called amebocytes, contains granules with a clotting factor known as coagulogen. This is released outside the cell when lipopolysaccharide that is unique structural component of the bacterial cell is encountered. If endotoxin enters the circulatory system, it causes a toxic reaction with the sufferer having a high temperature and a low blood pressure. This may lead to endotoxic shock, which may be fatal. When undergoing the LAL test as a quality control test, sometimes we may experience the problem showing false positive result which should not be confused with normal result. Unfortunately, we hadn't had any chance to know the reason what was the affecting factor to show false positive result. The aim of this study is to find out the affecting factors of showing showing false positive result when doing the LAL test Which is officially termed as Bacterial Endotoxin Test.

Materials and Methods

For this experiment, we used single test vials of pyrotell (Associates of Cape Code. Inc. USA) containing 0.2 mL lysophilized pyrotell which is commercially available. There is only an aqueous extract of amebocytes of Limuls Polyphemus, 1.5% V/V of 25% human serum, albumin, 3%

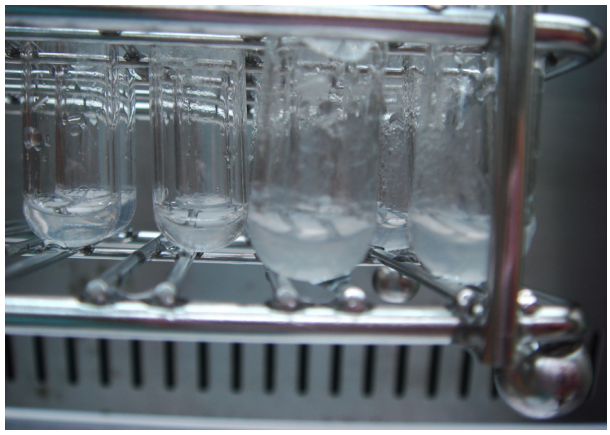

Fig. 2. false positive result.

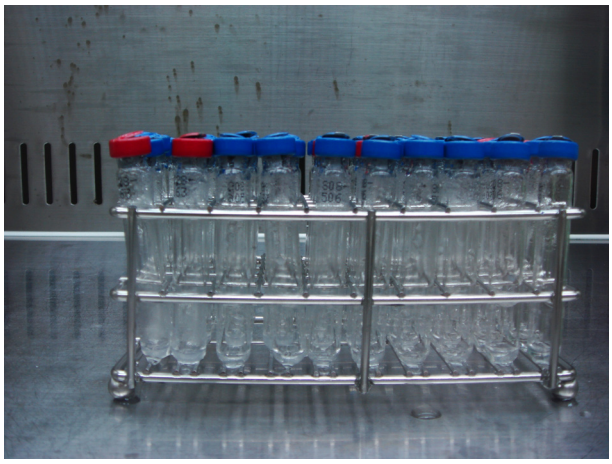

Fig. 3. negative result of LAL.

Nacl and other appropriate ions in the test kit. Prior to use, It should be always stored at -20 to +8℃ to minimize the deterioration of lysophilized pyrotell. We made pH samples by mixing each buffer, which includes HCl-KCl buffer ranging from 1.0 to 2.2, Glycine-HCl buffer ranging from 2.2 to 3.6, Acetate buffer ranging from 3.5 to 5.6, Phosphate buffer ranging from 5.7 to 8.0, Tris buffer ranging from 7.2 to 9.0 and Glycine-Sod.hydroxide-Sod.chloride buffer ranging from 8.4 to 12.8 to find out whether the variable range of pH affect false positive result or not.

After making pH samples, we checked the pH of each buffer with pH meter (Orion Research Inc. USA) to establish the accurate experimental condition (Fig. 1). Otherwise, we made 5%, 10%, 20%, 50%, 100% Ethanol respectively by diluting an absolute Ethanol (Fisher Scientific Korea. Ltd) to know whether organic solvent also affect false positive result or not. To do this test, we added 0.2

mL of the test specimen such as Ethanol and Buffer to a single test vial (STV) of pyrotell. After the pyrogen dissolved, an Assay mixture in the test vial was mixed vigorously and the STV was placed immediately in a sample rack and incubated in the water bath (Chang Shin. Co. KOR).

Result

After incubation period, we inverted and moved the test vial smoothly to know what pH and how many percentage of Ethanol will affect the reaction. With pH buffer, we could observe false positive results (Fig. 2) at pH 1.0 to 5.0 , 7.7 to 9.2 and 11.2 to 12.6. but at pH 5.2 to 7.5 that is preferable point which does not affect test result, the test result showed negative (Fig. 3). To my surprise, we could also see the negative result at pH 9.4 to 11.0. With Ethanol, the test result was negative with 5 to 10% Ethanol. And false positive result was seen with 20 to 50%. But we could see very thick gel formation with 100% Ethanol. In this test result, we could know that alcoholic solvents which contained so much concentration of organic solvent and pH which was far acidic and alkalic caused precipitation of the lysate.

Conclusion

All radiopharmaceuticals for human administration are required to be pyrogen free. Pyrogen are either protein or polysaccharide produced the metabolism of microorganisms. Bacterial products, the so-called endotoxin, are the main factors of pyrogen, but various chemicals also can add pyrogens to a radiophamaceutical solution. Following administration, pyrogen will produce symptoms of fever, chills, malaise, leukopenia, pain in joint, headache, and dilation of pupils. This reaction may be fatal, if sufferers who have cancer or immuno dificiency disease like AIDS are administrated radiophamceuticals containing pyrogen-type gram negative bacilli. Due to the state of their physical strength and immune system is much weaker than common person. Since pyrogens arise mainly from the metabolism of bacteria, the best thing to prevent pyrogenic contamination is to use sterile glassware, solutions, and equipment under aseptic conditions in any preparation procedure. So we are needed to follow quality control measures that are required of conventional drugs. In this experiment, we could acknowledge factors to affect false positive result which should not be confused with normal test result. LAL test has been very imperative method to find out whether pyrogen is being or not in radiophamaceuticals injected to the human. Therefore we should elicit the test data as reliable as possible by excluding these factors which influence in objective and reliable test result.

요 약

환자 몸에 투여되는 주사용제재으로서 약품 내 무균성이 요구되어진다. 현재 대부분의 방사성약품합성 실에서는 사용하기가 비교적 간편하고 민감도가 우수한 Bacterial Endotoxin Test로 방사성약품 내 발열성물질이 존재하는지의 유무를 실험한다. 그렇지만 테스트결과를 해석할 때 가끔씩 위 양성의 결과를 나타내서 결과를 해석 시 혼란을 줄 때도 있다. 이 위 양성의 원인을 알아보기 위해 pH영역이 다른 buffer와 Ethanol sample을 사용해서 실험을 해보았다. 그 결과 pH영역이 너무 산성이나 염기성인 1.0~5.0 그리고 11.2~12.6에서 위 양성의 결과를 나타냈고 Ethanol sample에 있어서는 20~50%에서는 위 양성의 결과를 100%에서는 완전한 양성의 결과를 나타내었다. Pyrogen은 면역력이 약한 환자들에게 있어서 두통이나 발열, 백혈구 감소증 등의 증상을 일으키고 심지어 합병증으로 인해 환자들에게 치명적인 반응을 일으키기도 한다. 그러므로 우리는 위 양성의 결과에 영향 미치는 이런 요인들을 최대한 배제함으로써 객관적이고 신뢰할만한 Pyrogen test결과를 도출하는게 필요하다.

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