Environmental Pollution Monitoring of Std Nucleic Acid Amplification Laboratory Based on Real-Time Quantitative Analysis

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Abstract: PCR has developed rapidly since its inception and has important applications in various fields. Through temperature cycling in three stages of denaturation, annealing and extension, a very small amount of DNA can be amplified millions of times, which has extremely high detection sensitivity. In this paper, real-time fluorescence quantitative PCR was used to monitor the smear samples of laboratory environment and instruments and equipment. After thoroughly cleaning and disinfecting the polluted laboratory environment, instruments and equipment, etc., the test results of pollutant removal were confirmed by experiments. Except that 4 samples were not rechecked and 2 samples continued to be false negative, there were no pollution results in 36 consecutive tests. In the same period, there was no correlation between the qualified rate of environmental monitoring and the positive rate of mixed detection and split detection, which reflected that the overall environment of the nucleic acid amplification testing laboratory in our province was better. The results show that environmental monitoring of STD nucleic acid amplification laboratory is beneficial to improve the accuracy of detection results, and also protects the laboratory personnel and environment.

1. Introduction

PCR nucleic acid detection laboratory is a reliable screening and confirmation method for virus detection, which has been widely used in medical and other fields, especially during this Covid-19 virus, as an important means for patients' diagnosis and detection. The country is also strengthening the infrastructure construction in this area, but the nucleic acid detection process is easy to be polluted, and the whole operation needs to be in a strict experimental environment to ensure the accuracy of data results, without missing or wrongly detecting suspected cases. The air conditioning design of this kind of laboratory directly affects the testing environment of the laboratory, but the biggest problem at present is how to accurately control the pressure and air distribution of the laboratory, which is the key to be solved urgently, so I put forward a complete air conditioning design scheme to ensure a good laboratory environment. In this project, I will study the types of ventilation and air conditioning systems, the layout of vents, the construction and debugging points by means of data inquiry, field investigation and CFD (Computational Fluid Software) simulation analysis, and put forward constructive ideas and suggestions, hoping to provide reference for similar projects.

Due to the high sensitivity of PCR technology, it has a high amplification level in vitro, so every link from sample preparation to result generation in the laboratory process, improper handling, will cause laboratory pollution. In order to effectively monitor whether the laboratory is polluted, the extremely small amount of pollution that may occur in any link in the experimental process is enough to lead to false positive results [1-3].

Real-time quantitative PCR technology is a new technology developed in recent years. Real-time quantitative PCR technology is developed from traditional PCR technology. Its basic principles are the same, but the main difference is its quantitative system. Combined with the actual situation of environmental pollution monitoring in our laboratory, some treatment strategies were put forward for

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monitoring, discovery and removal of environmental pollution in nucleic acid laboratory for reference.

2. The Application of Real-Time Pcr

Real-time PCR technology has been widely used not only in various fields of molecular biology, but also as a diagnostic method in clinical practice. Its application range involves the quantification of DNA, mRNA and virus load, the analysis of nucleic acid polymorphism, the analysis of gene mutation and many other fields. Literature [4] describes in detail the real-time PCR determination of cytokine gene expression. Cytokine is a kind of regulatory protein, and the change of its quantity is often closely related to inflammatory reaction, autoimmune diseases and transplant rejection. In addition, the sensitivity of commonly used protein detection methods (such as ELlSA) is difficult to detect very small amounts of protein products, so it is very meaningful to use PCR for quantitative gene diagnosis.

CDNA microarray and differential expression PCR are two key techniques for analyzing gene expression changes, but these two techniques can only analyze gene expression changes qualitatively, but not quantitatively. Literature [5-6] successfully quantified the changes of gene expression by using real-time quantitative PCR technology, which not only proved the effectiveness of the above two methods, but also provided a new technology with high throughput to detect the changes of gene expression.

Real-time quantitative PCR technology not only enhances the study of gene quantity change, but also enhances the study of gene quality change. For example, literature [7] has established a real-time PCR method called Methylight, which uses specific primers and/or Taqman probes to detect whether cytosine in CpG island is methylated. Studies have shown that this method can detect cells containing hypermethylated genes from esophageal mucosa of esophageal cancer patients.

Intuitive quantitative analysis of samples is a main application direction of real-time quantitative PCR technology. The aforementioned DNA-binding dyes, molecular beacons, hybridization probes and TaqMan probes have little difference in sensitivity, but they are definite in specificity. Because probes are involved in hybridization process, they have higher specificity than pure DNA-binding dyes. Meanwhile, reports show that molecular beacons have higher specificity than linear probes [8]. Intuitive quantitative analysis of samples is a main application direction of real-time quantitative PCR technology. The aforementioned DNA-binding dyes, molecular beacons, hybridization probes and TaqMan probes have little difference in sensitivity, but they are definite in specificity. Because probes are involved in hybridization process, they have higher specificity than pure DNA-binding dyes. Meanwhile, reports show that molecular beacons have higher specificity than linear probes [8].

At present, the application of real-time quantitative PCR in clinical diagnosis is mainly reflected in the quantitative determination of virus carried by infected tissues or cells. This technology can detect both DNA and RNA viruses, and now there are standard operation manuals and various commercial detection kits. However, it must be made clear that there is no uniform standard of virus load in the world, and there are also various controversies about its standard curve and quantitative accuracy.

3. Materials and Methods

3.1 Experimental Method

Using PCR fluorescence method, STD virus nucleic acid was routinely detected in laboratory. The kit was produced by Shanghai Shuoshi Biotechnology Co., Ltd. and operated in strict accordance with the kit instructions.

3.2 Method of Sampling

Add 1mL sterile deionized water into 2mL Eppendoff tube, use sterile artificial fiber to sample swabs, dip them in deionized water, roll sampling the parts to be sampled, put the sampled swabs into

the swab tube, rinse repeatedly, squeeze the swabs as dry as possible along the tube wall, discard the swabs, and use the swab eluate for DAN detection of CT and NG.

3.3 Sampling Location

Six swabs were used to sample 22 monitoring points, such as instruments and equipment in four areas of PCR laboratory, among which swab1 took the experimental table top, clean bench table top, refrigerator door handle and sample feeder surface of the first area; Swab2 takes the sample feeder surfaces of different ranges in the second area (4 in total); Swab3 takes the experimental table top of the second area, the table top of the biosafety cabinet, the door handle of the refrigerator, the inner wall of the incubator and the inner hole of the centrifuge; Swab4 takes the three-zone experimental table and the inner hole of PCR amplifier; Swab5 takes the surface of the four-zone sampler, the table top of the fume hood, the door handle of the refrigerator and the surface of the washing machine; Swab6 takes the four-zone experimental table top, the inner wall of the incubator and the detection surface of the enzyme-linked instrument.

3.4 Real-Time Fluorescence Quantitative Rt-Pcr Detection

The STD nucleic acid detection kit was used to detect the STD nucleic acid in the RNA extracts of samples on ABI Stepone plus real-time fluorescence quantitative PCR instrument. The reaction system and conditions were in accordance with the reagent instructions, and the template amount was 5μL RNA. The experimental operation and reaction conditions were strictly carried out according to the kit instructions.

3.5 Fruit Judgment Standard

Reasonable distribution of sampling points according to the spatial layout, work flow and operation characteristics of each partition of STD nucleic acid amplification testing laboratory; To investigate the influence of environmental cleaning and disinfection of STD nucleic acid amplification laboratory on monitoring results.

3.6 Statistical Method

Statistical software SPSS 23.0 was used for analysis, and the data obtained were expressed as cases (n), percentage (%) and mean standard error ($\bar{x} \pm s$). T test or $\chi 2$ test was used to compare the differences among the data involved, Pearson was used to evaluate the correlation (R value) between the qualified rate of environmental monitoring and the positive rate of mixed test and split test, and $P < 0.05$ indicated that the difference was statistically significant.

4. Result

4.1 Test Results of Ct and ng Dna in Each Area of Pcr Laboratory

In 36 tests, CT and NG results in zone 1 and zone 2 were all negative. The NG test results of swab4, swab4, and swab6 in three areas were uncertain four times, while others were all negative, and the re-examination results of uncertain samples were all negative. The results of swab5CT and NG in four districts were false negative 7 times, the false negative rate was 19.4%, and the others were negative; Of the 8 false negatives, 5 were rechecked, 4 were negative, 1 was false negative, and the other 3 were not rechecked. The results of swab6 CT and NG were false negative for 5 times, and the false negative rate was 13.9%; Two of them were rechecked, and one was negative, one was false positive, and the other was not rechecked. The test results showed that, except that 4 samples were not re-examined and 2 samples continued to be false negative, there were no contamination results in 36 consecutive tests (see Table 1).

Table 1 Test Results Of Ct and ng Dna in Each Area of Pcr Laboratory

Lab	Swab number	Detection site	Detection times	CT results			NG result		
				Positive times	Negative times	False negative number	Positive times	Negative times	False negative number
Zone 1	Swab 1	Experiment table	36	0	36		0	36	
		Superclean bench	36	0	36		0	36	
		Fridge	36	0	36		0	36	
		Sample feeder	36	0	36		0	36	
Zone 2	Swab 2	Sample feeder 1	36	0	36		0	36	
		Sample feeder 2	36	0	36		0	36	
		Sample feeder 3	36	0	35		0	36	
		Sample feeder 4	36	0	34		0	35	
	Swab 3	Experiment table	36	0	35		0	33	
		Biosafety cabinet	36	0	33	7	0	36	7
		Fridge	36	0	32		0	36	
		Incubator	36	0	36		0	36	
		Centrifugal machine	36	0	36		0	33	
Zone 3	Swab 4	Experiment table	36	0	36		0	35	
		PCR instrument	36	0	35		0	36	
Zone 4	Swab 5	Sampler	36	0	36		0	36	
		Fume hood	36	0	36		0	36	
		Refrigerator	36	0	34	5	0	35	5
	Swab 6	The experimental station	36	0	36		0	35	
		Incubator	36	0	36		0	36	
		Enzyme linked immunosorbent assay	36	0	36		0	6	

4.2 Correlation Comparison between Cleaning and Disinfection of Nucleic Acid Testing Laboratory and Monitoring Results

The results of this study (Table 2) also found that there was no correlation between the qualified rate of environmental monitoring and the positive rate of mixed detection and split detection in the same period, which reflected that the overall environment of the laboratory for nucleic acid amplification detection in our province was better. The analysis of the reasons may be related to timely finding the reasons for the low qualified rate of detection and reasonable treatment of indoor object surface and equipment pollution, so it did not cause adverse effects on the overall environment.

Table 2 Correlation Comparison Between Cleaning and Disinfection of Nucleic Acid Testing Laboratory and Monitoring Results

Different periods	Qualified rate of environmental monitoring(A)(%)	Positive rate of mixed test(B)(‰)	Correlation between (A) and (B) (R value)	Positive rate of mixed test(C)(‰)	(A) correlation with (B) (r value)
June 2019	100.00	4.21	r=0.217,P=0.473	41.00	r=0.351,P=0.263
August 2019	100.00	8.36		74.00	
January	100.00	4.21		55.00	

2020					
February 2020	100.00	9.01		76.00	
March 2021	100.00	4.47		51.00	
April 2021	98.00	11.65		88.00	

5. Discussion

In recent years, viral nucleic acid detection in STD nucleic acid amplification testing laboratories in China has been basically fully realized, which can effectively improve the detection rate of virus variants or occult viruses. Moreover, the combination of polymerase chain reaction (PCR) and routine enzyme-linked immunosorbent assay can further reduce the potential risk of virus transmission during blood transfusion [11-12]. However, in nucleic acid detection, the minimal environmental pollution will cause false positive results, which will easily lead to the waste of blood and reagents. Therefore, it is particularly important to strengthen and improve the environmental quality control of STD nucleic acid amplification laboratory.

The main pollution sources that cause the pollution of STD nucleic acid amplification laboratory include cross-contamination among samples, pollution of PCR reagents, pollution of PCR amplification products, pollution of cloning plasmids in experiments, pollution of staff, pollution caused by incomplete disinfection, etc. Due to the high sensitivity and specificity of PCR method, a large number of PNA products can be amplified in vitro, and the amplification products pollute the reagents, instruments and ventilation systems in the laboratory, resulting in laboratory pollution and false positive results.

Great attention should also be paid to the false negative in PCR amplification. The detection results show that the frequency of false negative in SWAB 5 and 6 in the four areas is 19.4% and 13.9% respectively. After searching for the reason, it is found that the residue of sodium hypochlorite on the surface of the article is caused. After repeatedly wiping the surface of the article with clear water, no false negative results appear, indicating that sodium hypochlorite can affect the PCR amplification reaction. Although the causes of PCR false negative are complex, such as the loss of target nucleic acid, the residue of extraction reagent, the incomplete removal of inhibitors in samples, the temperature difference between the wells of the amplifiers, etc. However, false negative results can be found in time by setting an internal control system (internal standard) in the reaction system.

Because of the high cost of PCR reagents, it is also possible to mix multiple samples in an experimental area as one sample for testing, and then test the positive comprehensive samples separately, thus saving the cost and time while monitoring the environmental pollution in the laboratory.

To prevent false positive results, the laboratory should formulate corresponding quality assurance measures, such as wiping the workbench with 1: 10 84 disinfectant, spraying 1: 50 84 disinfectant inside the laboratory, and mopping the floor with 1: 50 84 disinfectant after settling in the air. It is recommended not to wipe or mop the floor with clear water that day, but to wipe the floor with clear water the next morning. Laboratory consumables (except those sealed with self-sealing bags), which can be sterilized, are sterilized once with standby sterilization pot. Add the prepared 84 disinfectant (1: 50) instead of the cracking solution into the cracking solution tank to complete the process of adding the cracking solution. After the machine sucks a certain amount of 84 disinfectant every time, pause for ten minutes, let the 84 disinfectant volatilize into the channel, and repeat it four times. The gun tip used in this process also has no filter element. After that, wash the 84 disinfectant solution (1:50) in the lysis solution tank with water and replace it with distilled water to repeat the above procedure. (This kind of method is not recommended under normal circumstances, and should be used carefully only when pollution cannot be removed. 84 disinfectant is corrosive, and long-term action will rust the inside of the channel and cause damage to the machine; After treating the machine with this method, no nucleic acid experiment will be done on the same day).

6. Summary

PCR technology has become an important technical means in the field of disease prevention and control. Prevention is the key to prevent the pollution of PCR laboratory: formulate scientific and reasonable PCR laboratory related systems, procedures, standard operating procedures and record forms, and continuously improve and perfect these documents; Pre-job training and assessment of inspectors in PCR laboratory and regular and irregular personnel supervision; PCR laboratory pollution can be avoided to a great extent, but the possibility of pollution still exists. In a word, the occasional equipment pollution has not caused adverse effects on the environment of STD nucleic acid amplification testing laboratory, but the staff of STD nucleic acid amplification testing laboratory still need to be vigilant, pay attention to the implementation of environmental disinfection measures, evaluate and improve various systems in stages, and effectively guarantee environmental safety and the accuracy of testing results for the benefit of the people.

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