FEDERAL SERVICE FOR SURVEILLANCE ON CONSUMER RIGHTS PROTECTION
AND HUMAN WELLBEING
FEDERAL STATE-FUNDED INSTITUTION OF SCIENCE
CENTRAL SCIENTIFIC RESEARCH INSTITUTE OF EPIDEMIOLOGY
OKPO 01897593 PSRN 1027700046615 TIN/RRC 7720024671/772001001
Novogireevskaya street 3a, Moscow 111123 Russia Tel. (495) 672-10-69, fax (495) 304-22-09, email: crie@pcr.ru, www.crie.ru

10.12.2013 Moscow No. 03/724

Att. of Chief external expert
in clinical laboratory medicine
of the Russian Ministry of Health
Professor **A.G. Kochetov** M.D.

Director
of Scientific Research Institute of Disinfectology
of Rospotrebnadzor
Professor N.**V. Shestopalov** M.D.

On the problem of decontaminating PCR laboratory rooms

Informational letter

One of the most acute problems of microbiology departments at healthcare facilities worldwide is the laboratory rooms' contamination by microorganisms, their metabolic byproducts, toxins, and nucleic acids, whose outspread is of extreme importance at sites of polymerase chain reactions analysis. A polymerase chain reaction (PCR) is a highly sensitive method that allows for determining even one copy of the DNA fragment of the researched microorganism. This calls for especially thorough arrangement of a PCR laboratory and brings to the forefront the problem of protecting the reaction mixture from the DNA molecules from the environment, capable of becoming the reaction target and yielding false positive results.

If trace quantities of positive DNA (DNA amplification products – amplicons, DNA template used for positive control, positive DNA of the clinical sample) get into the reaction tube, it leads to amplification of a specific DNA segment during the PCR and thus to false positive results.

In course of work, two types of contamination are possible:

1) Cross-contamination from sample to sample (in course of specimens handling or during reaction mixture dropping) that leads to sporadic false positive results;
2) Contamination with amplification products (amplicons) which is of greatest importance as during PCR, amplicons are accumulated in vast numbers and are perfect targets for re-amplification.

Usually it is very hard to determine the contamination source; this requires great money and time expenditures. There are several methods for combating this phenomenon. One of them is exposure of DNA molecules to photochemical influence. For this, psoralen or isopsoralen is used, that is activated by short-time exposure to ultraviolet light. DNA molecules modified by these compounds cannot participate in the amplification reaction.

However, as is well known, no biological or chemical reaction proceeds with 100 % efficiency, thus after inactivation of the amplification products, at least several of billions of amplified segment copies remain intact, which significantly reduces the method's value. Besides, there is always a risk of cross-contamination from one sample to another during their processing.

Thus, this method can only partially eliminate the contamination source and does not warrant the absence of false positive results.

Current cumulative experience of laboratory work using PCR for diagnostics allows for determining main requirements to arranging such laboratories, that actually run the tests, and disinfection practices.

The Methodical guidelines "Work organization for PCR analysis of the material contaminated with pathogenic biological agents of risk groups III and IV" dated March 4, 2004, recommends the following measures if the laboratory is contaminated (Appendix 3):

- Disposal of all reagents in the contaminated area;
- Disposal of all test materials on any processing stages (except initial);
- Terminal cleaning, chemical and ultraviolet disinfection of all laboratory surfaces;
- Chemical and ultraviolet disinfection of furniture, working surfaces, equipment's and devices' frames.

In accordance with the said Methodical guidelines, as well as Methodical guidelines on use of germicidal lamps for air and indoor surfaces disinfection No. 11-16/03-06 dated February 28, 1995, all work spaces of PCR laboratories must be equipped with ultraviolet lamps with radiation peak in the 260 nm region (DB-60 type lamps) at a rate of 2.5 W per 1 m^3. These lamps should be used for treatment for 1 hour before work start and 1 hour after work completion. No PCR analysis is allowed before the decontamination is complete.

Under present-day conditions and with regard to rapid growth of both PCR laboratories number and tests number in each laboratory (over 40 million tests per year in Russia, 2013), such prolonged downtime of a laboratory is a factor that reduces the quantity and often the quality of PCR tests. In this connection, perfecting the disinfection measures for inactivation of DNA molecules in the air and on the surfaces is a task of utmost importance.

Until now, the main equipment type for disinfecting PCR laboratory rooms have been open-type ultraviolet irradiators that use low-pressure mercury vapor lamps as monochromatic radiation source. This ultraviolet technology, widely used for indoor air decontamination, excludes open surfaces, which has been reflected in the requirements of the Guideline R 3.5.1904-04 "Use of ultraviolet germicidal irradiation for indoor air decontamination". Besides, according to numerous users such lamps are insufficiently effective against not only PCR products, but also highly resistant microorganisms, in particular resistant nosocomial strains and mold fungi, which reduces the bactericidal efficiency of indoor air decontamination.

In this connection, the appearing of new state-of-art technology should be noted – the pulsed plasma-optical technology based on exposure of objects to high-intensity ultraviolet light of continuous spectrum. On the basis of this technology pulsed ultraviolet units with xenon lamps for rapid decontamination of rooms have been developed and implemented into practical healthcare. The units are marked by high efficiency against broad spectrum of microorganisms of any resistance degree regardless of their growth and spread stage. They are intended for work in the absence of people, are environmentally safe and have automated control of the disinfection process and the remote control, which is specifically important for convenience and safety of the personnel. Ultrashort decontamination time allows for using the units in cases when urgent disinfection is required.

The research conducted by the Scientific Research Institute of Epidemiology of Rospotrebnadzor together with the Federal Clinical Research Center of Children's Hematology, Oncology and Immunology named after Dmitry Rogachov of the Russian Ministry of Health has demonstrated that treating the PCR laboratory rooms with a UV unit that uses a xenon lamp to generate pulsed high-intensity continuous spectrum UV light ensures more than threefold reduction of false positive results number. The efficiency of the continuous spectrum UV light with regard to destructing the PCR products has been tested at molecular biology laboratory of the Federal Clinical Research Center of Children's Hematology, Oncology and Immunology. For a period of 1 month, a pulsed UV unit was run for 10 minutes before starting the work at the PCR laboratory, that weekly tests over 200 samples of patients' biomaterial for over 50 various pathogens of viral and bacterial nature.

Experimental studying the mechanism of amplicons inactivation under the influence of continuous spectrum UV light has proved that short-time treatment of DNA amplicons in different physical states (solution, dry substance) results in complete inactivation of the amplicon. After exposure to continuous spectrum UV light, the DNA amplicons have completely lost the ability to act as a template for the polymerase chain reaction specific for this amplicon. Studying the inactivation mechanism with help of mass-spectrometry (MALDI-TOF) has shown that the leading inactivation mechanism was the molecule fragmentation of DNA amplicons.

Thus, use of pulsed UV units allows for:

- Ensuring the safety for the personnel of bacteriological laboratory working with biological agents of risk groups III and IV;
- Decontaminating the biological laboratory rooms from all types of microorganisms, including their highly resistant strains and multi-resistant forms, with the efficiency of over 99.9 %;
- Ensuring the quality and reliability of sample preparation and PCR analysis due to highly efficient inactivation of microbiological contaminants;
- Significantly reducing the false positive results number of PCR analysis;

- Increasing the PCR analysis performance rate due to disinfection time reduction.

It is evident that the new pulsed plasma-optical technology und the units on its basis are a significantly more efficient, reliable and convenient method of laboratories treatment in comparison to other existing methods.

Director of the Institute
Russian Academy of Medical Sciences member, Professor V.I. Pokrovskiy

Head of the Healthcare-Associated Infections laboratory, M.D. A.V. Tutelyan