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Evaluation of Brazilian medical devices using agar diffusion cytotoxicity assay

Avaliação de dispositivos médicos brasileiros utilizando o ensaio de citotoxicidade pela difusão em ágar

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Over the last decades, governmental actions and mechanisms created to protect consumer rights have been linked to a growing effort to guarantee the quality and reliability of products. Samples of condoms (latex), medical devices and blood bags (PVC - polyvinyl chloride) have been tested using the agar diffusion assay. This assay evaluates the cytotoxicity induced by biomaterials by measuring the biological reactivity of mammalian cell cultures in contact with these materials. PVC is used in the production of medical devices because of its specific properties, such as flexibility, obtained after the addition of plasticizers (phthalates), which can cause toxicity even at low doses. Latex is a natural elastomer used for surgical gloves and condoms with a formulation that includes dispersion of liquid latex and chemicals, such as antioxidants and a vulcanizing accelerator, both of which are able to induce cytotoxicity. Samples were analyzed by the National Institute of Quality Control in Health - INCQS of the Oswaldo Cruz Foundation (Fiocruz) in accordance with the governmental sanitary surveillance actions on respect to the quality control. We observed an increase in the quality of the products in relation to the results of the agar diffusion assay during the period between 2000 and 2007. This situation, together with other actions, reflects in an improvement in the quality of products that can be translated in the health of the population. Rev. Bras. Hematol. Hemoter. 2009;31(2):84-87.

Key words: *Cytotoxicity; L929 cells; latex; medical devices; PVC.*

Introdution

The sector of biomaterials covers about 8000 types of products, ranging from simple bandages through life maintaining implantable devices, from equipment to screen and diagnose diseases and health conditions to the most sophisticated diagnostic imaging and minimal invasive surgery equipment.

Medicines and medical devices bring widespread benefits to patients and the community but their main characteristic must be the absence of both toxicity and adverse responses. A cautious approach is needed where there is scientific uncertainty about the existence or the extent of risks, but reasonable grounds of possible severe adverse effects.

Polyvinyl chloride (PVC) is one of the most widely used plastic materials. The physical properties of PVC can be readily modified by the introduction of additives and plasticizers. This means that the product is utilized in diverse applications such as in construction, tubing, coating,

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packaging and medical devices[1] however these chemicals also change the chemical consistency of PVC. Plasticizers that must be added to make PVC flexible are additives of special concern. The Food and Drug Administration (FDA) recognizes that much medical devices that utilizes PVC containing diethylhexyl phthalate (DEHP) should not be used in ways that result in significant human exposure to the chemical.[2] However, vinyl bags used in neonatal intensive care units have been shown to leach DEHP.[3] Xu *et al*.[4] confirmed that some PVC emulsion particles (e.g., E3, E8) cause cytotoxicity. In vitro tests have shown that these particles stimulate the release of inflammatory mediators in pulmonary cells.

Latex is a natural elastomer used for surgical gloves and condoms. Liquid latex is mixed with other chemicals to produce a latex compound that is suitable for manufacturing medical products. These chemicals include an antioxidant, a sulfur-based vulcanizing agent, and a vulcanizing accelerator. Accelerators are chemicals that increase both the rate and extent of cross-linking in the latex compound during vulcanization. Latex condoms are produced by dipping plastic, ceramic, stainless steel or glass mandrels mounted on a conveyor into a latex formulation. Most are lubricated with silicone or a water-based lubricant, and include a spermicidal. The most commonly used spermicidal is N-9, a water-soluble detergent (surfactant) that interacts with cell membranes, killing sperm, bacteria and some viruses. N-9, a nonoxynol, can be an ethoxylated alkyl phenol or a nonylphenyl ether.[5]

Regulatory agencies around the world, including the Brazilian National Sanitary Surveillance Agency (Anvisa) through the actions to guarantee the sanitary safety of products recommend the use of specific tests in order to evaluate the cytotoxicity induced by medical devices. In Brazil these tests are performed by the National Institute of Quality Control in Health - INCQS at Oswaldo Cruz Foundation - Fiocruz and here we present the cytotoxicity results for condoms, medical devices (intravenous catheters and infusion sets) and blood bags observed during 8 years.

Materials and Method

In accordance with ISO10993-5, the Agar Diffusion Test is designed to determine the biological reactivity of mammalian cell cultures following contact with elastomeric plastics and other polymeric materials.[6]

Sample preparation

A total of seventy products, including twenty-five samples of blood bags, ten catheters, ten infusion sets and twenty-five condoms of all types from all the different manufacturers were assessed for cytotoxicity using the agar diffusion cytotoxicity assay. All products were analyzed by the INCQS in accordance with the governmental sanitary surveillance actions on respect to the quality control. Portions of the samples to be tested had flat surfaces of not more than 25 mm² in surface area. The sterile samples were added in duplicate to L929 cultures in contact with the solidified agar surface. The biomaterial samples were randomized, and the assay technician was blinded to the identity of the material.

The positive control used was a portion of latex from a Lemgruber 200 tourniquet (Brazil). The negative control was a USP reference plastic (USP - USA), lot G-8.

Cell culture preparation

Multiple cultures of L-929 mammalian fibroblast cells (ATCC cell line CCL1, NCTC clone L-929, USA) were maintained in Minimal Essential Medium (MEM, Sigma, USA) containing Earle's salts and 5% fetal calf serum (FCS, Gibco, USA) with a seeding density of about 10^5 cells/mL. The cultures were incubated at 37 ± 1ºC in a humidified incubator with a 5 ± 1% carbon dioxide atmosphere for a minimum of 24 hours until a monolayer of more than 80% confluence was obtained in 35-mm diameter dishes.[7]

After incubation, the agar layer (0.9% Bacto-Agar, Difco, Brazil) supplemented with MEM in 5% FCS and vital neutral-red stain (0.005% final concentration) was added replacing the medium. The agar layer acts as a cushion to protect cells from mechanical damage while allowing the diffusion of leachable chemicals from the polymeric specimens. Staining characterizes viable cells with a red color in its cytoplasm.

Interpretation of the results

The biological reactivity or cytotoxicity (cellular degeneration and malformation) was classified as grades 0 to 4. Grade 0 represents no reactivity zone (discoloured cells) around or under the sample; 1 (slight reactivity) with a reactivity zone limited to an area under the sample; 2 (mild reactivity) with a zone extending less than 0.5 cm beyond the sample; 3 (moderate reactivity): reactivity zone extending 0.5 to 1.0 cm beyond the specimen and grade 4 (severe reactivity): reactivity zone extending more than 1.0 cm beyond the sample.

The cell culture system should be suitable to detect the negative control (grade 0 - no reactivity) and the positive control (at least grade 3 - moderate). The halo was measured using a digital pachymeter (0.01 mm and 150 mm - Absolute Digimatic, Mitutoyo, Japan).

Results

Figures 1 to 3 present the results obtained during the period 2000-2007 of samples received to be evaluated for their capacity to cause cytotoxic effects.

Figure 1 shows the agar diffusion test results for condoms received in 2004, 2006 and 2007. We observed an improvement in the quality by the significant reduction of cell cytotoxic response during 2007 ($P < 0.05$). Since 2004,

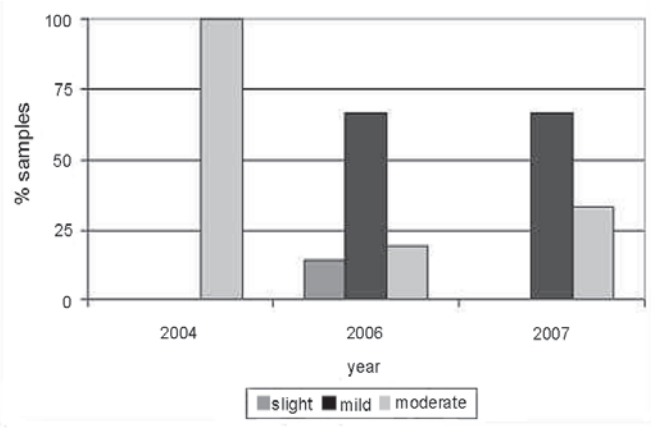

Figure 1. Percentage of samples of condoms with cytotoxic responses during 2004-2007

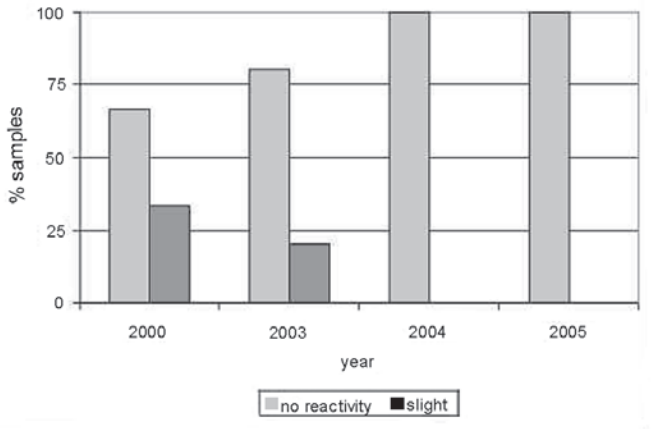

Figure 2. Evaluation of cell cytotoxicity induced by samples from medical equipment during 2000-2005

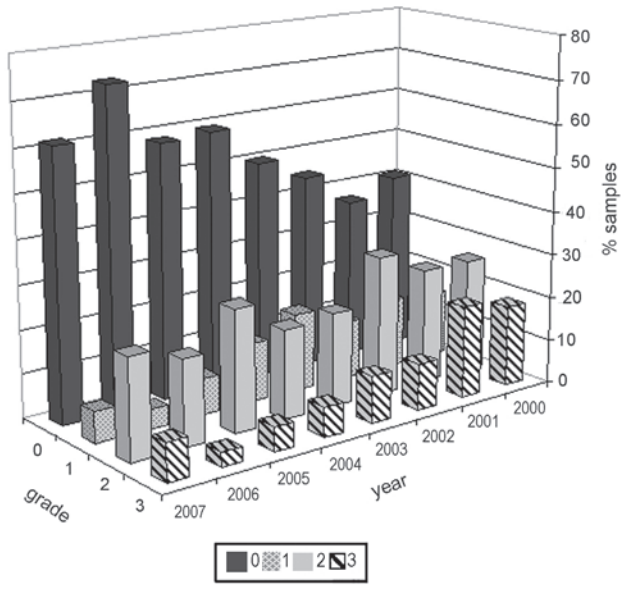

Figure 3. Percentage of blood bags presenting with cytotoxic responses during 2000-2007

12% of condoms presented a slight cytotoxic effect, 64% a mild cytotoxic effect and 24% a moderate cytotoxicity effect.

Medical devices (intravenous catheters and infusion sets) received during the years 2000 to 2005 presented an improvement in the quality, with 100% of samples without cytotoxicity effects during 2004-2005 (Figure 2). If we consider all the samples, 20% presented a slight reactivity to induce cytotoxicity.

Figure 3 represents a decrease in the cytotoxicity effects observed for the blood bags received during 2000-2007, and an increase in the percentage of samples classified as grade 0.

The positive control presented halo diameters of 0.5-1.0 cm and negative control zero (0) cm.

Discussion and Conclusion

The original FDA test method was used to determine the cytotoxic effect of medical materials and devices as part of quality control.[8] The modified direct cell culture testing method, which uses the mouse L-929 fibroblast cell line, has shown to be effective in determining the effects of short-term exposure to physiological concentrations of latex condom and glove surface materials. Anvisa was created in 1999 and structured following the FDA design to substitute the National Secretary of Sanitary Vigilance (SNVS) instituted by Decree nº 79,056 of 1976. In its capacity, the Anvisa, as the former SNVS, has enforcement powers similar to the FDA, including the concession and the cancellation of authorization of operation for drug, food and medical product manufacturers and distributors. The Brazilian agency was created as a public institution linked to the Ministry of Health under a management contract in accordance with the political governmental guidance to foster protection of the health of the population.

Latex causes allergic reactions in susceptible individuals but little is known regarding the cytotoxic effects of other additives. The use of condoms to prevent sexually transmitted diseases, especially HIV, is widely encouraged. Condoms contain latex, spermicidal lubricants (such as dimethyl-siliconium) and other unspecified compounds, such as dyes and flavoring. In 1995 the Center for Disease Control (CDC) of America reported 600 cases of type I latex sensitivity with at least 16 of the patients dying as a result of sensitization.[9] Between 1988 and 1992 the FDA received more than 1000 reports of adverse health effects caused by exposure to latex, including eight deaths.[10-11]

Many factors can affect the suitability of samples, for example the polymeric composition, processing and cleaning procedures, contact mediums, dyes, adhesive labels, absorption, adsorption, permeability of condoms and storage conditions and also additional chemicals that change the characteristics of consistency.

The manufacturers provide very little information regarding the type and amount of each compound added. It

is known that condoms that contained flavorings are more toxic than the others suggesting that these types of additives are not the only factors that may contribute to cytotoxicity.

The Medical Device Agency (MDA) identified the presence of dithiocarbamate vulcanization accelerators in latex gloves. MDA reported that the genotoxicity of these agents was due to the type used as well as the residue concentration. As condoms used in this study were from different manufacturers, little information is provided regarding the use of dithiocarbamate and the amount of residue present in each product. Thus, genotoxicity testing might also be justified in the evaluation of condoms. Besides testing a single chemical (e.g. dithiocarbamate) that may contribute to genotoxicity, synergistic effects with other chemicals should also be considered.[12]

Since 1980, the cytotoxicity of latex urinary catheters has been reported in clinical and experimental situations using various cell lines, such as urothelial cells, V79 cells and L-929 cells. PVC is widely used in the production of a vast array of medical devices because of its specific properties, including flexibility obtained after the addition of plasticizers such as phthalates. Of the 25 different phthalate esters, DEHP is one of the most commonly used in the production of medical devices. Since DEHP leaches into solutions stored in PVC containers in fractions varying from 10 to 15%, certain populations including dialysis patients, hemophiliacs, neonates and the developing fetus may have critical exposure to DEHP.[13]

In the case of plasticized PVC as a biomaterial in contact with blood, this challenge requires better understanding of the nature of plasticized PVC, the consequences of plasticizer selection and, in particular, surface characteristics.[14]

We observed an increase in the quality of the products in relation to the agar diffusion assay results during the period between 2000 and 2007. This situation, together with other actions, reflects an improvement in the quality of products that can be translated into health of the population.

Resumo

Durante as últimas décadas, a disseminação de ações governamentais e os mecanismos criados para proteger os direitos dos consumidores se associaram em crescentes esforços para garantir a qualidade e confiabilidade dos produtos. As amostras de preservativos (látex), dispositivos médicos e bolsas de sangue (PVC - cloreto polivinílico) foram testadas utilizando o ensaio de difusão em ágar. O teste avalia a citotoxicidade induzida por biomateriais, medindo a reatividade biológica de culturas de células de mamífero em contato com tais materiais. PVC é utilizado na produção de dispositivos médicos, devido às suas propriedades específicas, incluindo flexibilidade, obtida após a adição de plastificantes (ftalatos) que podem apresentar toxicidade mesmo em doses baixas. Látex é um elastômero natural utilizado em luvas cirúrgicas e preservativos que na sua formulação inclui a dispersão do látex líquido e de substâncias químicas, como antioxidantes e um acelerador de vulcanização, ambos capazes de induzir citotoxicidade. As amostras foram analisadas pelo Instituto Nacional de Controle de Qualidade em Saúde – INCQS da Fundação Oswaldo Cruz (Fiocruz) em atendimento às ações governamentais de vigilância sanitária no tocante ao controle de qualidade. Um aumento na qualidade dos produtos em relação aos resultados no ensaio de difusão em ágar foi observado no período compreendido entre 2000-2007. Esta situação, dentre outras ações, reflete uma melhoria na qualidade dos produtos que pode ser traduzida em saúde para a população. Rev. Bras. Hematol. Hemoter. 2009;31(2):84-87.

Palavras-chave*:Citotoxicidade; células L929; látex;dispositivos médicos; PVC.*

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