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The impact of the final rinse on the cytoxicity of critical products submitted for processing*

O impacto do último enxágue na citotoxicidade de produtos críticos passíveis de processamento

Impacto del último enjuague en la citotoxicidad de productos críticos pasibles de procesamiento

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ABSTRACT

Objective: To assess the cytotoxicity of products subsequent to a cleaning process based on a validated standard operating procedure (SOP), and a final rinse with different types of water: tap, deionized, distilled, treated by reverse osmosis and ultra-purified. **Method:** This was an experimental and laboratory study. The sample consisted of 130 hydrodissection cannulas, 26 per experimental group, characterized according to type of water used in the final rinse. The samples were submitted to internal and external contamination challenge with a solution containing 20% defibrinated sheep blood and 80% of sodium chloride 0.9%. Next, the lumens were filled with a ophthalmic viscosurgical device, remaining exposed for 50 minutes, and then were processed according to the validated SOP. Cytotoxicity was assessed using neutral red uptake assay. **Results:** No cytoxicity was detected in the sample extracts. **Conclusion:** The samples did not display signs of cytotoxicity, regardless of final rinse quality. The results obtained were reached by using only a validated cleaning operating procedure, based on the scientific literature, and on official recommendations and related regulation.

DESCRIPTORS

Surgical Instruments; Disinfection; Water Quality; Nursing.

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INTRODUCTION

Contemporary knowledge considers cleaning to be a fundamental step in assuring the effective processing of health products[1]. Due to its importance in the success of disinfection and sterilization, critical products must be cleaned according to Standard Operating Procedures (SOPs), based on updated scientific references and pertinent regulations[2].

Among the steps of cleaning SOPs, rinsing is one of the critical aspects, as it is a procedure that ensures the removal of remaining residue, whether organic or inorganic. Based on this theory, the water used both in cleaning and in rinsing critical products must not increase the bioburden of the products and must not cause recontamination due to the presence of residue. Thus, the Association for the Advancement of Medical Instrumentation[3] established that the final rinse of critical products must be conducted with high-purity water, which requires controlling bacterial contamination, endotoxins, total organic carbon, pH, hardness, resistivity, total dissolved solids, chloride, iron, copper, manganese, color and turbidity. This measure aims not only to control toxic syndromes and pyrogenic reactions among patients, but also to conserve surgical instruments.

In the Central Sterile Supply Department of hospitals in Brazil, products are usually rinsed with running tap water. In light of this reality, the Brazilian Health Surveillance Agency (ANVISA) released Collegiate Directory Resolution (RDC) number 15 establishing that the final rinsing of critical health products used in orthopedic and ophthalmological implant surgeries, and cardiovascular and neurological surgeries must be done with purified water. The resolution also determines water quality control through the measurement of water hardness, pH, chloride ions, copper, iron, manganese and microbial load[2].

Although this legislation is clear regarding the monitoring of water control, there are still questions regarding the actual impact of these contaminants on safety in instrument processing. Because RDC ANVISA no. 15[2] does not establish a minimum acceptable standard for purified water, interpreting the values obtained by health services is difficult. Furthermore, the relationship between the final rinse quality and the safety of critical products has not been shown experimentally, as it is a difficult variable to isolate.

In light of the problem represented by toxic syndromes supposedly caused by instruments rinsed without water quality control, the present study aimed to assess the cytotoxicity of products submitted to overcome contamination by the process of cleaning based on a validated SOP and final rinsing with different types of water: tap, deionized, distilled, reverse osmosis, and ultra-purified in order to demonstrate their potential to cause cell injury and death.

METHOD

Type of study: Experimental laboratory

Samples: Sample size was determined with the help of a statistician and was based on the effect of the treatments on the mean cell viability of experimental groups. To this end, a standard deviation of 40% was used within the group, with a 0.387 effect size, and 0.4 being the maximum value used for sample size calculation[4]. For the experiment, 130 hydrodissection cannulas were used, 26 per experimental group, characterized according to the type of water used in the final rinse: tap, deionized, distilled, reverse osmosis and ultra-purified water. The cannula were chosen due to their complex design: 4.0 cm in length, 0.6 mm in diameter with a 0.2 mm flattened opening at the distal end; they were also chosen because they come in contact with ophthalmic viscosurgical devices, which are difficult to remove from the instruments and can even render the cannula inoperable due to total obstruction should the solution dry up inside the lumen.

Contamination: Aseptically, the samples were submitted to internal and external contamination challenge with 20% defibrinated sheep blood and 80% 0.9% sodium chloride solution. Next, the lumen was filled with an ophthalmic viscosurgical device that remained in contact for 50 minutes (enough time for it to dry up, promoting extreme conditions of soiling and difficult cleaning).

Cleaning: Cleaning was conducted according to a specific protocol for complex-shaped devices and validated through cytotoxicity tests[5]: Presoaking in tap water for approximately five minutes; cleaning the lumen with high-pressure water jet until cleared, followed by a five-second continuous flow; suction of content inside cannula with a 10 mL syringe, as per the manufacturer's recommendation; backwashing with enzymatic detergent in ultrasonic cleaner for 15 minutes at 50 °C; when necessary, clearing the lumen with a metal rod, according to the manufacturer's recommendations; rinsing with the type of water for the group in consideration, with a 10 mL syringe; drying in a compressed air dryer; individually wrapped in surgical grade paper and film; and sterilization in an autoclave at 135 °C for 5 minutes.

Control groups: Positive control – Samples submitted to challenge contamination, immersed in tap water and enzymatic detergent solution with five enzymes, wrapped and sterilized; Negative Control – Samples without any processing, wrapping or sterilization; and Comparative control – same as experimental groups, minus the drying phase. Each group consisted of three samples.

Treatment of water used in final rinse: The tap water was obtained directly from the water supply, with no additional treatment. For the other types of water, the following equipment were used: a Cristófoli® Distiller, Purify® mixed-bed deionizer, MilliQ® Direct8® with a Biopak® polisher (this equipment was used to obtain reverse osmosis and ultra-purified water). The present research did not aim to determine which equipment is best for purifying water. All of these technologies, when used separately, present advantages and disadvantages regarding the analyzed contaminants, pointing to the need of adapting water treatment systems according to each health service. Thus, the variation observed in the values was expected and served to indicate the possible element involved in the supposed cytotoxicity related to the final rinse. The microbiological and physical-chemical characterization of each type of water used is represented in the data of Table 1.

Table 1 - Physical, chemical and microbiological characteristics of the type of water used in this study and threshold limit values established by the AAMI (2007) - São Paulo, São Paulo, Brazil, 2014.

Characteristics	Threshold limit values according to the AAMI (2007)				Values obtained for type of water used in the present study				
	Potable water	Soft water	Deionized water	High-purity water	Tap water	Distilled water	Deionized water	RO** Water	Ultra-purified water
Bacteria (CFU/mL)	<200	<200	<200	≤10	220	100	530	150	3
Endotoxins (EU/mL)	NA*	NA*	NA*	<10	4.70	0.101	1.000	0.912	<0.05
Total organic carbon (mg/L)	<1.0	<1.0	<1.0	<0.05	<1.0	<1.00	3.34	<1.00	0.002
pH	6.5 a 8.5	6.5 a 8.5	NA*	NA*	6.65	7.16	NA*	NA*	NA*
Hardness (CaCO3 in ppm)	<150	<10	<1.0	<1.0	22.7	<1.50	<1.50	<1.50	<1.50
Resistivity (MΩ-cm)	NA*	NA*	>1.0	>1.0	NA*	0.073	1.25	0.555	18.2
Total dissolved solids (CaCO3 in mg/L)	<500	<500	<0.4	<0.4	<100	<100	<100	<100	<100
Chloride (mg/L)	<250	<250	<1.0	<0.2	6.18	<1.00	<1.00	<1.00	<1.0
Iron (mg/L)	<0.3	<0.3	<0.2	<0.2	<0.1	<0.1	<0.10	<0.10	<0.10
Copper (mg/L)	<0.1	<0.1	<0.1	<0.1	<0.04	<0.01	<0.01	<0.01	<0.01
Manganese (mg/L)	<0.1	<0.1	<0.1	<0.1	<0.01	<0.01	<0.01	<0.01	<0.01

*NA: Does not apply.
**Reverse osmosis.

Cytotoxicity test: Due to the composition of the sample, the methodological framework used was neutral red vital dye uptake, which uses cellular viability as a parameter [6]. To estimate the possible difference in percentage of cell viability between experimental and control groups, we extracted samples at the highest possible concentration.

Extraction was conducted in sufficient volume to cover the sample, which was established at 2.8 mL of Eagle's minimum essential medium in Earle's balanced salt solution, with L-glutamine and 5% fetal bovine serum. In order to carry out extraction, initially, each sample was internally flushed with 2.8 mL of culture medium using a sterilized 5 mL syringe and was kept in a sterile test tube containing the same culture medium used in the flush. To ensure detachment of the residue, the tubes were submitted to three five-second cycles of ultrasonication, at a frequency of 40 kHz and 30 W potency, in addition to five minutes of orbital agitation[7]. Following these procedures, the tubes were kept in an incubator at 37 °C±1 °C for 24 hours.

To conduct the neutral red uptake assay, the following procedures were carried out: NCTC clone 929 cell suspensions [L Cell, derivative of strain L] (ATCC® CCL1™), in a concentration of approximately 2.5 x 10^5/mL in Eagle's minimum essential medium, in Earle's balance salt solution, 0.1 mM of nonessential amino acids, 1 mM of sodium pyruvate and 10% of bovine fetal serum were seeded in 0.2 mL volume in flat-bottomed 96-well microplates and then incubated for 24 h at 37 °C in a 5% CO_2 humid atmosphere for monolayer formation[6].

After the incubation period, the culture medium was disposed of and substituted by 0.2 mL of culture medium containing the sample extracts. The microplates were incubated once again in the incubator for 24 h at 37 °C, with 5% of CO_2. All of the extracts were tested three times. To ensure cell response to cytotoxic and noncytotoxic agents, a positive and negative control was conducted for each microplate. Latex extract was used as the toxic agent and filter paper extract as the atoxic agent. After incubation, the medium with the extracts was discarded and 0.2 mL of serum-free medium was added to each well, with 50 µg of neutral red dye. The microplates were incubated again for 3 h at 37 °C in order for the live cells to take up the neutral red dye. This medium was prepared 24 h before use and kept in the incubator at 37 °C. Before use, the medium was centrifuged at 1,500 RPM for 15 minutes to eliminate any formed crystals. At the end of the uptake period, the medium was removed and the cells washed twice with 0.2 mL preheated phosphate saline buffer solution at 37 °C and once with a 0.2 mL aqueous solution with 40% formaldehyde and 1% $CaCl_2$ to remove dye that was not taken up. This solution was then discarded and 0.2 mL of aqueous solution and 1% of acetic acid and 50% of ethanol were added to extract the dye. After 10 minutes of agitation, the microplates proceeded to optic density reading in a microplate reader, with wavelength at 540 nm and reference filter at 620 nm[6].

Data analysis and processing: The aim of this study was to assess the mean percentages of cell viability of the experimental groups after exposure to the sample extracts. Optical density (OD) was obtained for each group, by means of a microplate reader. Next, the mean OD measurement of the three readings was determined and, sequentially, the sample's cell viability. This was obtained by dividing the sample extract OD by the OD of the control cells, (cells in medium with no extract) and multiplying the result by 100. Once the cell viability of each sample was determined, the mean cell viability was calculated for each experimental group, which was used in the presentation and discussion of the results. This method allowed for the possibility of calculating the Cytotoxicity Index (CI_{50}), i.e., the concentration capable of causing 50% cell death[6].

RESULTS

The results indicated an absence of cytotoxicity in the sample extracts, with the lowest mean cell viability at 91%,

obtained from the samples rinsed with distilled water. However, there was no statistically significant difference when compared to the other types of rinse water. The CI_{50} could not be calculated as the extracts of the experimental groups presented cell viability higher than 50%. The other results are presented in Table 2.

Table 2 - Mean, standard deviation, maximum value, minimum value, and median of cell viability percentages obtained in the cytotoxicity tests for each experimental group, with sample extracts at 100% - São Paulo, São Paulo, Brazil, 2014.

Final rinse water	Mean (%)	Standard deviation (%)	Maximum (%)	Minimum (%)	Median (%)
Tap water	99	6	108	80	100
Distilled water	91	15	121	64	90
Deionized water	95	8	106	80	96
RO* water	98	8	116	83	98
Ultrapure water	100	6	106	75	101
Positive control	2	1	3	2	2
Negative control	97	2	99	94	97
Comparative control with no drying	91	12	105	82	87

*Reverse osmosis.

DISCUSSION

The results of this study showed that using validated cleaning SOPs is essential to ensure safety regarding the cytotoxicity of critical products, even in extreme conditions of soil. This was observed through the mean cell viability presented in the experiment's positive control group, 2%.

The SOP used resulted in atoxic products, rendering indifferent the type of water used in the final rinse. However, we emphasize that the final rinse should be controlled. For example: by using predetermined volumes, and establishing how long technological apparatuses are used, such as high-pressure water jet pistol. Vague and subjective recommendations, such as *rinse abundantly* with no further details can limit the transposition of the results to the assistance as well as not observing any additional cleaning instructions provided by the manufacturer[5].

In the comparative control group with no drying, none of the samples presented cytotoxicity, suggesting that drying may not influence cytotoxicity, at least not for the period of an hour and a half between the end of the sample cleaning and the beginning of sample drying. Applying this data to other products of greater length or made with different materials requires validation.

Regarding the use of tap water for the final rinse, we must consider that its microbiological, physical and chemical characteristics are very diversified and depend on the analysis at the point of use. Although the present results did not display cytotoxicity, this type of water is still contraindicated due to lack of control of biological contaminants, such as endotoxins and Gram-negative bacteria, and the possibility of corroding the instrumentation. In other words, if contaminant control is not necessary for reasons related to adverse events, it is mandatory in order to better conserve surgical instruments.

Furthermore, there is the issue of seasonal and geographical variation of organic and inorganic contaminants in the water [8], in addition to biofilm that forms within the plumbing system. These facts demand that there be a constant quality monitoring routine in place, as required by RDC ANVISA no. 15[2].

The use of deionized water in the final rinse is controversial. The AAMI [3] recommends the use of deionized water in all the processing steps, except the final rinse. This recommendation is based on the limited treatment of water, which, in this case, aims to remove only inorganic contaminants. Thus, microbiological contamination may remain, as was observed in the deionized water used in this study, with 530 CFU/mL of heterotrophic bacteria. In this case, the use of a water filtration system at the end of the deionization process is indicated [3].

Although the mean cell viability found in this study was satisfactory, lack of control of microorganisms, total organic carbon and endotoxins still contraindicate the use of deionized water with no microbiological control, as well as tap water.

The use of distilled water is also a controversial theme especially regarding the need for its sterility. There is even one publication that states: "The only safe way to remove detergent residue is by cleaning with sterile water jets" without reference to primary studies [9]. For the American Society of Cataract and Refractive Surgery and the American Society of Ophthalmic Registered Nurses[10], when not specified by the manufacturer, ophthalmological instrumentation must be rinsed with deionized or sterile distilled water. This observation is supported by a study that demonstrated the absence of cytotoxicity in cannula processed and rinsed with sterile distilled water[5].

In the present study, the sterility of distilled water did not prove to be an essential condition for the absence of cytotoxicity, as the mean cell viability was 91%.

Regarding water treated by reverse osmosis, the mean cell viability was 98%, also demonstrating safety for use in the final rinse.

An important point to consider is related to the difficulties of properly storing water submitted to reverse osmosis or any other treatment. In general, water tanks are considered critical points of the water treatment system and must be constructed with inert material in order to avoid content contamination. Moreover, they must have appropriate characteristics and surface roughness to prevent residue adherence, the formation of biofilm and corrosion by disinfectants[11]. However, RDC ANVISA no.

15[2] did not mention this precaution or the importance of implementing water tank cleaning and sanitization protocols, essential for water quality control.

The ultra-purified water used in the present study met AAMI[3] microbiological standards. This water was not stored, being immediately collected and used after its purification. As expected, the samples rinsed in this water demonstrated 100% cell viability, attesting to one of its main applications, which is in the preparation of cell culture mediums.

Therefore, the results show that the final rinse of critical health products had little influence on cytotoxicity when the cleaning process was in accordance with a validated SOP. However, this result does not dismiss the need for controlling steam contaminants. Resolution *ANSI/AAMI/ISO* 17665-1, part 2[12] establishes threshold limits for contaminants measured in steam condensate. This document considers both the contaminants related to instrumentation corrosion as well as contamination. These aspects must also be taken into account by health services.

CONCLUSION

The hydrodissection cannula did not show signs of cytotoxicity, regardless of the final rinse water quality.

In order for surgical instrumentation to be processed safely, it is fundamental that each step of the SOP be based on updated scientific references, on additional orientations provided by the manufacturer and on related regulation. To ensure the constancy of results, commercially available cleaning monitors must be used in order to identify possible mechanical failures of the ultrasonic cleaner, as the diameter of the samples used did not allow for the lumen to be cleaned manually and visually inspected, even with magnifying lenses, thus making it impossible for us to affirm that the instrumentation was free of organic residue. Thus, the use of cleaning monitors, as recommended by RDC ANVISA no. 15 of 2012 is one of the many essential items needed to guarantee the constancy of the results obtained with the SOPs.

Even though the results showed equivalence regarding the quality of final rinse water, this study did not investigate aspects related to the instrumentation's conservation. Therefore, the unrestricted use of tap water in the last rinse is still contraindicated. Furthermore, we emphasize that the variability of contaminants in tap water is difficult to foresee, as is the number of colony forming units in water submitted only to deionization.

In order to transpose these results to the assistance, we must take into consideration that the quality of final rinse water is contingent on a cleaning procedure based on validated SOP and that the results of this study are limited to new surgical instruments, which can be understood as *in a good state of conservation*. This fact is related to water contaminant control, as written in the conservation manuals that accompanied the instrumentation.

Considering that RDC ANVISA no. 15 of 2012 does not specify threshold values for contaminants in final rinse water of critical products, the values obtained in the quantification of contaminants for samples rinsed with distilled, reverse osmosis or ultra-purified water may be used as reference for meeting with the standards presented in article 74, together with endotoxin control.

RESUMO

Objetivo: Avaliar a citotoxicidade de produtos submetidos à contaminação desafio, limpeza baseada em procedimento operacional padrão (POP) validado e enxágue final em diferentes tipos de água: de torneira, deionizada, destilada, tratada por osmose reversa e ultrapurificada. **Método:** Estudo experimental e laboratorial. Foram utilizadas como amostras 130 cânulas de hidrodissecção, 26 por grupo experimental, caracterizados, de acordo com a água utilizada no último enxágue. As amostras foram submetidas à contaminação desafio interna e externamente por uma solução contendo 20% sangue de carneiro desfibrinado e 80% de Cloreto de Sódio a 0,9%. Em seguida, tiveram o lúmen preenchido por solução viscoelástica, permanecendo em contato com o contaminante por 50 minutos, sendo então, processadas, de acordo com um POP validado. A citotoxicidade foi avaliada pela captura do corante vital vermelho neutro. **Resultados:** Ausência de citotoxicidade nos extratos das amostras. **Conclusão:** As amostras não demonstraram citotoxicidade, independentemente da qualidade de água utilizada no último enxágue. Os resultados apresentados puderam ser alcançados unicamente por meio do uso de um procedimento operacional padrão de limpeza validado, baseado em literatura científica, em recomendações oficiais e na legislação relacionada.

DESCRITORES

Instrumentos Cirúrgicos; Desinfecção; Qualidade da Água; Enfermagem.

RESUMEN

Objetivo: Evaluar la citotoxicidad de productos sometidos a contaminación desafío, limpieza siguiendo procedimiento validado y enjuague en diferentes tipos de agua: de caño, desionizada, destilada, tratada por ósmosis inversa y ultrapurificada. **Método:** fueron utilizados 130 canulas de hidrosección, 26 por grupo experimental, caracterizadas por el tipo agua utilizada en el último enjuague. La muestras fueron contaminadas interna y externamente por una solución con 20% sangre de carnero desfibrinado y 80% de Cloruro de Sodio a 0,9%. Luego el lumen fue cubierto por la solución viscoelastica, permaneciendo en contacto con el contaminante 50 minutos y posteriormente procesados, siguiendo orientaciones del procedimiento padrón validado. El ensayo de citotoxicidad se realizó mediante la incorporación del colorante vital rojo neutro. **Resultados:** ausencia de toxicidad en extractos de las muestras. **Conclusión:** no hubo toxicidad en las muestras, independiente del agua utilizada en el último enjuague. Los resultados fueron alcanzados gracias al uso del procedimiento operacional padrón de limpieza validado, embasado en literatura científica, recomendaciones oficiales y en legislación relacionada.

DESCRIPTORES

Instrumentos Quirúrgicos; Desinfección; Calidad del Agua; Enfermería.

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