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# EVALUATION OF MICROBIAL GROWTH ON SINGLE-USE VITRECTOMY PROBES REPROCESSED IN HEALTHCARE PRACTICE

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# Evaluation of microbial growth on single-use vitrectomy probes reprocessed in healthcare practice

AVALIAÇÃO DO CRESCIMENTO MICROBIANO EM SONDAS DE USO ÚNICO PARA VITRECTOMIA REPROCESSADAS NA PRÁTICA ASSISTENCIAL

EVALUACIÓN DE CRECIMIENTO MICROBIANO EN SONDAS DE USO ÚNICO PARA VITRECTOMÍA RECICLADAS EN LA PRÁCTICA ASISTENCIAL

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## ABSTRACT

The aim of this study was to evaluate the microbial growth on single-use vitrectomy probes reprocessed in healthcare practice. We investigated nine vitrectomy probes that had been reused and reprocessed using different methods. The samples were sectioned, individually, in portions of 3.5 cm, totaling 979 sampling units (extensions, connectors and vitrectomy cutters), which were inoculated in culture medium and incubated at 37°C for 14 days. The results showed microbial growth on 57 (5.8%) sample units, 25 of which had been sterilized using ethylene oxide, 16 by hydrogen peroxide plasma, and 16 by low-temperature steam and formaldehyde. Seventeen microbial species were identified. The most prevalent were: *Micrococcus* spp., coagulase-negative *Staphylococcus*, *Pseudomonas* spp., and *Bacillus subtilis*. The reuse of single-use vitrectomy probes was shown to be unsafe, therefore this practice is not recommended.

## DESCRIPTORS

Probe  
Vitrectomy  
Equipment reuse  
Sterilization  
Surgical wound  
infection  
Endophthalmitis

## RESUMO

O objetivo deste estudo foi avaliar o crescimento microbiano em sondas para vitrectomia de uso único, reprocessadas na prática assistencial. Foram investigadas nove sondas reusadas e reprocessadas por diferentes métodos. As sondas foram segmentadas, individualmente, em porções de 3,5 cm, totalizando em 979 unidades amostrais (extensões, conectores e ponteiros) inoculadas em meio de cultura e incubadas a 37°C, por 14 dias. Os resultados mostraram crescimento microbiano em 57 (5,8%) unidades amostrais, das quais, 25 foram esterilizadas por Óxido de Etileno, 16 por Plasma de Peróxido de Hidrogênio e 16 por Vapor à Baixa Temperatura e Formaldeído. Foram identificadas 17 espécies microbianas, sendo as mais prevalentes o *Micrococcus* spp., *Staphylococcus* coagulase negativa, *Pseudomonas* spp. e *Bacillus subtilis*. O reuso de sondas de uso único para vitrectomia não se mostrou seguro, portanto tal prática não é recomendada.

## DESCRIPTORES

Sonda  
Vitrectomia  
Reutilização de equipamento  
Esterilização  
Infecção da ferida operatória  
Endoftalmite

## RESUMEN

Este estudio objetivó evaluar el crecimiento microbiano en sondas para vitrectomía de uso único recicladas en la práctica asistencial. Se investigaron nueve sondas reutilizadas y recicladas mediante diferentes métodos. Las sondas fueron segmentadas individualmente en porciones de 3,5 cm, totalizándose 979 unidades de muestra (extensiones, conectores y punteras), inoculadas en medio de cultivo e incubadas a 37°C por 14 días. Los resultados demostraron crecimiento microbiano en 57 (5,8%) unidades de muestra, 25 de las cuales habían sido esterilizadas con óxido de etileno, 16 con plasma de peróxido de hidrógeno y 16 por vapor a baja temperatura y formaldehído. Se identificaron 17 especies microbianas, prevaleciendo el *Micrococcus* spp, *Staphylococcus* coagulasa negativo, *Pseudomonas* spp y *Bacillus subtilis*. La reutilización de sondas de uso único para vitrectomía no demostró seguridad, por lo que la práctica no es recomendable.

## DESCRIPTORES

Sonda  
Vitrectomía  
Equipo reutilizado  
Esterilización  
Infección de herida operatória  
Endoftalmitis

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## INTRODUCTION

At Brazilian health institutions, the reprocessing of single-use materials is frequent, although the risks of this practice has not been explored so as to guarantee safety in the use of these materials. The main justification for this practice is the high cost of materials<sup>(1)</sup>, like vitrectomy probes for example. In the Brazilian context, the reprocessing of single-use materials has been discussed and studied in view of different aspects, which are: ethical, legal, technical and safety. Until date, however, evidence does not permit a consensus<sup>(2)</sup>. Besides going against manufacturer indications for single use, the material traded as such is manufactured using raw materials that do not support aggressions inherent in cleaning and further sterilization, which can compromise functionality during subsequent use. In addition, these materials cannot be disassembled to permit the necessary cleaning, which is considered the main phase for the safety of the sterilized product.

In Brazil, the Ministry of Health, through the National Health Surveillance Agency (ANVISA), has been leading discussions and regulating reprocessing since 1985<sup>(3)</sup>. Despite the lack of official data or specific studies on the actual dimensions of this practice<sup>(4)</sup>, it is known that ophthalmology is one of the medical specialties that reuse single-use materials, including vitrectomy probes. Despite the gravity of infections when sight is affected, the reuse of these materials is frequent and often performed without any validation as to the safety of their reprocessing.

Vitrectomy probes are used in vitrectomies, surgical procedures performed to remove the vitreous humor, a jelly substance that fills up most of the eye<sup>(5)</sup>. This surgical procedure is performed to treat advanced cases of retinal detachment, diabetic retinopathy, inflammations and traumas leading to vitreous turbidity. Post-vitrectomy endophthalmitis rates in the reported situations are low, ranging between 0.02% and 0.05%<sup>(6-7)</sup>, when compared with endophthalmitis after cataract surgeries including vitrectomy, which range between 0.04% and 0.13%<sup>(8-10)</sup>. Attention is due to the risk when these vitrectomy probes

are used in cataract extraction surgeries that include vitrectomy, in which there is a possibility that the posterior capsule will break, entailing vitreous loss. These events are related with risk factors for endophthalmitis<sup>(5,11)</sup>.

Based on these risks, the reprocessing of single-use materials should be based on strong evidences for the absence of related risks, not only infection, but also the presence of endotoxins, toxic residues of cleaning products, material functionality and integrity. Despite these multiple potential risk factors, this research was limited to the safety assessment involving the sterility of single-use vitrectomy probes reused in care practice, reinforcing that these devices cannot be disassembled for cleaning and include narrow, long lumens, which represent a challenge for safe processing.

## OBJECTIVE

To assess microbial growth in single-use vitrectomy probes reprocessed in care practice.

## METHOD

Considering, *a priori*, that the reuse of single-use materials implies legal issues for the healthcare establishments that practice it, access to analysis material was obtained through the intentional rational method, requesting the help of the Chairwoman of the Brazilian Ophthalmology Nursing Society (SOBRENO). She intermediated the donation of vitrectomy probes, together with a short description of the cleaning and sterilization routine each device was submitted to (Figure 1).

Nine reprocessed vitrectomy probes were donated, properly wrapped and sterilized, coming from four institutions that constituted the experimental group. Single-use vitrectomy probes consist of two extensions measuring 215cm each, with a 1.5 mm diameters, linked by connectors and attached to a tip.

**Chart 1** – Description of cleaning and sterilization procedures for vitrectomy probes by the institutions that donated samples - São Paulo, 2009

I*	ROUTINES	
	CLEANING	STERILIZATION
A	Manual without disassembly, no internal friction, immersion in enzymatic detergent, rinsing, drying.	Hydrogen Peroxide Plasma (HPP) and Low-temperature Steam Formaldehyde (LTSF)
B	Idem A	Ethylene Oxide (ETO)
C	Manual without disassembly, no internal friction, immersion in enzymatic detergent, rinsing.	ETO
D	Cleaning not informed.	LTSF

\* I = Donating institution

The extensions of the new devices were individually segmented into 3.5-cm portions, in a laminar flow chapel, using an aseptic technique, with the help of scissors and disposable blades, both sterilized, totaling 979 sample

units, including 935 segments, 35 connectors and 9 tips. Sample size was analyzed with the help of a mathematician, adopting the two-tailed epidemiological research model, with a 90% sampling power,  $\alpha=0.5\%$  and  $\beta=10\%$ .

A new vitrectomy probe, brand ACCURUS®, originally sterilized by the manufacturer using Ethylene Oxide (ETO), was used as a negative control. Extensions were sectioned in the same way as in the experimental group, totaling 121 extensions, 4 connectors and 1 tip.

Each sampling unit was individually and directly inoculated in Tryptic Soy Broth (TSB) culture medium and incubated at 37°C for 14 days, with daily turbidity reading. The microbiological identification of positive cultures was performed at the Microbiology Laboratory of the Hospital Infection Service at *Irmandade Santa Casa de Misericórdia de São Paulo*, Brazil. Samples that displayed turbidity were plated in blood Agar medium and incubated at 35°C ± 2°C for 7 days. Plates showing positive growth were identified according to the morphology and tinctorial property visualized in Gram coloring. To colonies of Gram-positive cocci, catalase, coagulase (Staphy Test®, Probac® do Brasil) or esculin hydrolysis, growth in

the presence of bile (Bile esculin) and growth in the presence of 6.5% NaCl 6,5% (Kit for Enterococci, Probac® do Brasil) tests were applied. Gram-negative bacilli were identified through the biochemical series, containing Triple Sugar Iron (TSI), motility, citrate, phenylalanine and indole. In this series, bacilli that revealed to be non-glucose fermenters were identified through the NF II Kit (Probac® do Brasil), applying the following tests: oxidase, culture in MacConkey, fermentation of O/F glucose, maltose and lactose, arginine and lysine decarboxylation and gelatin liquefaction.

## RESULTS

The nine reprocessed vitrectomy probes revealed microbial growth on some of their extensions, connectors and tips, according to the data displayed in Table 1.

**Table 1** – Distribution of microbial growth in vitrectomy probes reused according to the donating institution, respective sterilization methods and number of reuses - São Paulo, 2009

Institution	Number of Vitrectomes	Manufacturer	Sterilization	Number of Reuses	Microbial growth		
					Extensions	Connectors	Tips
A	3	Alcon®	HPP	6	6/110	0/4	1/1
		Accurus®	HPP	2	9/112	0/4	0/1
		Accurus®	LTSF	2	3/111	0/4	0/1
B	2	Alcon®	ETO	8	4/120	1/4	0/1
					4/116	0/4	0/1
C	2	Accurus®	ETO	10	12/89	0/4	0/1
					3/52	0/3	1/1
D	2	Alcon®	LTSF	ni*	4/114	0/4	0/1
		Accurus®			8/111	1/4	0/1
<b>Total</b>	<b>9</b>				<b>53/935</b>	<b>2/35</b>	<b>2/9</b>

\* Not informed

Fifty-seven positive samples were recovered from 979 sampling units. Among the 57 (5.8%) positive cultures, 53 came from extension segments and two from connectors. Two out of nine tips were contaminated.

In the comparison of the sterilization methods used for reprocessing the vitrectomy probes, the chi-square test ( $\chi^2$ ), whose result was 0.9951, proves that there was no statistically significant difference among the three sterilization methods used to reprocess the probes, which were: ETO, HPP, LTSF.

As for the recovered microorganisms, data are displayed in Figure 2 and Table 2.

Seventeen microbial species were isolated. In some sample, more than one microorganism was found. Micro-

scopic analysis revealed 16 bacterial and one fungal species, with 7 Gram-positive, 9 Gram-negative bacteria and one fungus, according to the data displayed in Table 2.

No microbial cultures were found in negative control sampling units (0/126).

## DISCUSSION

Infectious complications after posterior vitrectomy are rare. On average, it occurs in one in every 3,000 surgical procedures. The etiological agents most commonly mentioned in literature after posterior vitrectomy are coagulase negative *Staphylococcus*, *Proteus mirabilis* and *Enterococcus faecalis*<sup>(6-7)</sup>.

**Chart 2** – Distribution of microorganisms identified on the vitrectomy probes reused according to the donating institution and analyzed parts - São Paulo, 2009

Institution	Sample	Microorganisms
A	Extensions	<i>Bacillus subtilis</i> , <i>Chryseobacterium</i> spp, <i>Micrococcus</i> spp, coagulase (-) <i>Staphylococcus</i> , <i>Acinetobacter lwoffii</i> , <i>Streptococcus Grupo Viridans</i> , <i>Micrococcus</i> spp, <i>Moraxella</i> spp, Non-sporulated Gram (+) bacillus, <i>Pseudomonas</i> spp, <i>Enterococcus faecalis</i> , <i>Trychophyton</i> spp
	Connectors	No growth
	Tips	<i>Bacillus subtilis</i>
B	Extensions	<i>Trychophyton</i> spp, coagulase (-) <i>Staphylococcus</i> , <i>Pseudomonas fluorescens</i> , <i>Acinetobacter lwoffii</i> , <i>Micrococcus</i> spp, <i>Achromobacter xylosoxidans</i>
	Connectors	<i>Moraxella</i> spp
	Tips	No growth
C	Extensions	Non-sporulated Gram (+) bacillus, <i>Pseudomonas fluorescens</i> , <i>Methylobacterium</i> spp, coagulase (-) <i>Staphylococcus</i> , <i>Burkholderia cepacia</i> , <i>Achromobacter xylosoxidans</i> , <i>Bacillus subtilis</i> , <i>Pseudomonas putida</i> , <i>Micrococcus</i> spp
	Connectors	<i>Flavobacterium</i> spp
	Tips	No growth
D	Extensions	<i>Micrococcus</i> spp, Non-sporulated Gram (+) bacillus, <i>Pseudomonas fluorescens</i> , <i>Micrococcus</i> spp, <i>Acinetobacter lwoffii</i> , <i>Bacillus subtilis</i> , coagulase (-) <i>Staphylococcus</i> , <i>Methylobacterium</i> spp, <i>Chryseobacterium</i> spp
	Connectors	No growth
	Tips	<i>Micrococcus</i> spp

**Table 2** – Distribution of microorganisms identified in vitrectomy probes reused in decreasing order of growth frequency per microbial group - São Paulo, 2009

Microorganism	Number of samples	%
<b>Gram Positive</b>		
<i>Micrococcus</i> spp	14	20.9
Coagulase negative <i>Staphylococcus</i>	8	11.9
<i>Bacillus subtilis</i>	6	9.0
Non-sporulated Gram positive bacilli	6	9.0
<i>Streptococcus</i> Grupo Viridans	4	6.0
Other Gram positive cocci	2	3.0
<i>Enterococcus faecalis</i>	1	1.5
<b>Gram Negative</b>		
<i>Pseudomonas</i> spp	8	12.0
<i>Acinetobacter lwoffii</i>	3	4.5
<i>Chryseobacterium</i> spp	3	4.5
<i>Achromobacter xylosoxidans</i>	2	3.0
<i>Methylobacterium</i> spp	2	3.0
<i>Moraxella</i> spp	2	3.0
<i>Burkholderia cepacia</i>	1	1.4
<i>Flavobacterium</i> spp	1	1.4
Gram negative cocci	1	1.4
<b>Fungi</b>		
<i>Trychophyton</i> spp	3	4.5
<b>Total</b>	<b>67</b>	<b>100.0</b>

Most microorganisms found in this study belong to the skin and mucosa microbiota, such as *Micrococcus* and coagulase negative *Staphylococcus*, the latter coinciding with the ophthalmologic infection agents appointed in literature. Possible water, soil and operating surface contaminants were also identified though, such as Gram-positive bacilli, *Pseudomonas* and *Acinetobacter* species, based on which it can be inferred that a range of healthcare materials serve

as contamination sources and that adequate cleaning and sterilization methods should guarantee their elimination. Effective internal channel cleaning of vitrectomy probes is very difficult, due to their long extension (215 cm) and narrow diameter (1.5 mm). Therefore, manual methods, without friction of their surfaces, as found in practice, are insufficient. Cleaning any health product with these characteristics represents the main challenge for professionals at processing units. The cleaning technique for this material shape should involve disassembly of the product, exposure to detergent, manual mechanic and complementary automated action (ultrasonic washer with retro-flux), rinsing, drying and visual inspection<sup>(13)</sup>. In this study, the devices were not submitted to this cleaning standard. In all cases, only manual cleaning occurred, without disassembly of the device, as the material does permit this; without internal friction, using brushes, immersion in enzymatic detergent, rinsing and drying, which one of the institutions that donated the samples did not perform. In the processing of these devices, drying is a step that cannot be neglected, mainly in materials submitted to sterilization in ETO. Both ETO and its sub-products ethylene chlorohydrin and ethylene glycol are extremely irritating to tissue. Ethylene glycol is a sub-product that slowly results from the reaction between ETO and water<sup>(14)</sup>. Thus, in materials that are not dried adequately, the final quantity of this substance could represent an additional risk when reprocessing the devices.

In this study, risk severity was expressed by the recovery of vegetative and not just sporulating microorganisms (*Bacillus subtilis*), indicating evident cleaning and sterilization flaws. As opposed to the heat sterilization method, in which the sterilizing agent is conducted, cold methods only act through contact between the sterilizing agent and the material surface. Inorganic and organic substance residues, including biofilms, can constitute physical barriers that lead to sterilization failure.

A study<sup>(15)</sup> that explored the presence of organic residues on surgical instruments submitted to immersion in enzymatic solution for 60 minutes, mechanical cleaning during the immersion, ultrasonic washing for 10 minutes, rinsing three times in tap water, lubrication, inspection of functioning, drying with compressed air and vertical positioning for final drying, detected the presence of residues in 84.3% (27/32), through visual microscope inspection. Based on these study results, the authors conclude that, even when using cleaning protocols to reprocess the materials, it is very hard to completely remove the residues. Considering the reprocessing of vitrectomy probes, which were submitted to a cleaning process that does not comply with best-practice recommendations, it is highlighted that health establishments should take great caution when making decisions on reprocessing and re-using disposable materials.

This research was limited to the assessment of potential infection risks, but other potential risks are associated with the reuse of disposable materials, described in literature, which go beyond infections, also evidencing pyrogenic reactions, adverse events deriving from toxic residues of processing products, functional performance errors and damage to the material's physical integrity<sup>(16)</sup>.

One very relevant aspect in the processing of health-care materials is the quality of rinsing water, which can be a source of endotoxins (when contaminated with Gram-negative microorganisms) and other organic and inorganic residues that can jeopardize processing safety<sup>(17)</sup>. Official recommendations indicate water treated through reverse osmosis, bacteria filter, and distilled water for the final rinsing. None of the institutions that donated the devices for this research mentioned this care. Concerning material used in ophthalmologic surgeries, specific recommendations exist for rinsing, which should be performed abundantly to remove detergent residues, while sterilized distilled water should be used for the final rinsing<sup>(18)</sup>. This recommendation is based on reports of post-surgical toxic eye syndromes, which the American Society of Cataract and Refractive Surgery – ASCRS - e a American Society of Ophthalmic Registered Nurses – ASORN<sup>(18)</sup> defined as TASS – Toxic Anterior Segment Syndrome.

The sterilization technologies that use low temperatures are indicated to sterilize thermosensitive materials. Since the 1950's, ETO has been used, while HPP and LTSF are relatively new methods. Research on the latter dates back to the 1990's and methods differ in terms of diffusibility power. In a way, the availability of these technologies favored the choice to reuse disposable materials with

thermosensitive raw material, in view of the possibility to reduce hospital costs<sup>(19-20)</sup>. The materials used in this study are thermosensitive, but include narrow and long lumens, which represent a challenge to diffuse the sterilizing agent. One of the sterilization methods, used at one of the donating institutions, HPP, is incompatible with the long extension of the material under analysis (215 cm).

These study results suggest the possibility of biofilm development on the internal surface of the devices. It is known that biofilm developed on health products can include Gram-positive (*Enterococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptococcus viridans*), Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Pseudomonas aeruginosa*) or fungi<sup>(21)</sup>. These microorganisms can come from patients and health professionals' skin, from tap water or other environmental sources<sup>(21)</sup>. Many of the microorganisms described in biofilm compositions are in accordance with those identified in this study (*Enterococcus faecalis*, coagulase-negative *Staphylococcus*, *Streptococcus viridans* and *Pseudomonas aeruginosa*). As the vitrectomy probes analyzed in this experiment were reused multiple times, between two and ten times, and without a validated cleaning protocol, the recovered microorganisms may have originated in biofilms developed in the narrow and long lumens of the device extensions, and also in connectors and tips.

The present study results leave no doubt as to the risk of reprocessing disposable vitrectomy probes, as performed by the institutions that donated the analyzed devices. The processing steps they adopted demonstrated deficiencies to guarantee material sterility, and no tests were performed to validate practice, in compliance with current legislation, Resolution No. 2606<sup>(22)</sup>. No studies were found in literature that assessed the technical feasibility of re-using vitrectomy probes and it is to be doubted whether favorable results would be achieved in view of the complexity of the device.

In the universe of disposable materials, some could be reused as their shape is simple, without internal spaces, functioning like new after reprocessing. According to the authors, the characteristics that would represent safe reuse do not apply to vitrectomy probes.

## CONCLUSION

The reprocessing of single-use vitrectomy probes was not safe under the conditions of this study. Therefore, this practice is not recommended. =



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