v. 2, n. 14, 2022

Journal of Agricultural Sciences Research

BACTERIOLOGICAL TESTING ON THE EFFICIENCY OF DIFFERENT BRANDS OF 70% ALCOHOL IN DISINFECTION OF MICROBIOLOGY LABORATORY SURFACES IN PANDEMIC TIMES

Gabriela Neves Vianna
Aluna de medicina veterinária da UNIFESO

Carolina Riscado Pombo
Professor of Veterinary Medicine at UFF

Alfredo Artur Pinheiro Junior
Professor of Veterinary Medicine at UNIFESO

Cecília Riscado Pombo
Professor of Veterinary Medicine at UNIFESO

All content in this magazine is licensed under a Creative Commons Attribution License. Attribution-Non-Commercial-Non-Derivatives 4.0 International (CC BY-NC-ND 4.0).

Abstract: With the Covid-19 pandemic and the hygiene measures established by health bodies, such as the use of 70% alcohol in hand and surface hygiene, the product was out of stock in the market. In the urgency of measures that promote the adaptation of companies to production to reestablish its availability, the Health Surveillance Agency (ANVISA) and the National Institute of Metrology, Quality and Technology (INMETRO) had to define new regulations for production, labeling and sale. The new measures allow production without registration with ANVISA and without the certificates, previously mandatory, on the labels. In addition to allowing the sale of the product at 70% in markets and pharmacies, prohibited since 2002. Therefore, the objective of the present study was to test the efficiency of different brands of 70% alcohol in liquid and gel presentation after the new ANVISA and INMETRO The products were tested against bacteria, *Staphylococcus aureus* and *Escherichia coli* in the cleaning of benches in the UNIFESO Microbiology laboratory. Ten brands of 70% alcohol in liquid presentation and ten brands in gel were analyzed. The labels were analyzed, taking into account the safety aspects that are still mandatory. Brand alcoholmetry was performed in liquid presentation. In both presentations, products were found to be inefficient for the bacteria tested and with irregularities in the labeling. A brand in the liquid presentation had lower alcohol content than described on the label. This indicates the need for inspection by the competent bodies in the production and sale of 70% alcohol.
Keywords: Alcohol. Bacteriological testing. Pandemic.

INTRODUCTION

The evolutionary history of planet Earth is marked by several pandemic events that affect the general population (1) Outbreaks such as the Bubonic Plague, caused by the bacterium: *Yersinia pestis,* that ravaged 14th century Europe and resulted in millions of deaths. In addition to the cholera outbreak, due to the action of the bacteria: *Vibrio cholerae*, which still mutates and occasionally affects the population, as well as the Spanish Flu, caused by a dyspnea mutation of the Influenza virus, which in 1918 resulted in the death of millions of people. Therefore, there are numerous examples of events suffered by the population caused by the action of microorganisms. In the most recent scenario, in 2019, a new fast-spreading virus emerged, called SARS-CoV-2, which is named after the Severe Acute Respiratory Syndrome it causes, the acronym being derived from English: *Severe Acute Respiratory Syndrome Coronavirus 2*, the Covid-19. This pathogen causes symptoms such as fatigue, fever, dry cough, which can lead to or, as already mentioned, in more severe cases, Severe Acute Respiratory Syndrome (SARS) (2) in addition to other varied symptoms. The main route of transmission is through droplets of saliva. With the rapid spread across the planet, the Covid 19 virus, which derives from the term: *Corona Virus Disease 2019,* which resulted in millions of deaths worldwide. Faced with the Public Health crisis, at a global level, it was necessary to take measures to prevent the contagion of such a virus, such as distancing between individuals, use of masks, frequent hand hygiene with soap and water and the use of 70% alcohol for washing hands and utensils (2). With the population's great demand for 70% alcohol, the competent national authorities, such as the National Health Surveillance Agency (ANVISA) and the National Institute of Metrology, Quality and Technology (INMETRO), issued new regulations that altered the authorizations for production of sanitizers, including 70% alcohol products, liquids and gels, in order to meet the

new demands. Remembering that ANVISA is responsible for the regulation, control and inspection of products and services that involve risk to public health and INMETRO is responsible for carrying out national metrology and quality policies, in addition to verifying and inspecting compliance with technical and the legal standards, in which refers to unit of measurement, measurement methods, materialized measurements, measuring instruments and pre-measured products. As SARS-CoV2 is an enveloped virus with a phospholipid bilayer, the use of 70% alcohol promotes the denaturation of the outer cell membrane protein by dehydration by the hygroscopic and hydrophilic action of alcohol. The percentage of water in the substance helps the entry of alcohol into the cell, which acts by denaturing the nucleic acids and, consequently, inactivating the virus. The same process also occurs in bacteria. In view of this, the inspection process of substances to define whether the product correctly presents the proportion of water and alcohol is of paramount importance to guarantee the effective germicidal action of 70% alcohol. The objective of this work was to test the efficiency of different brands of 70% alcohol in liquid and gel presentations for cleaning surfaces in the Microbiology laboratory of the Faculty of Veterinary Medicine - UNIFESO, in order to verify the quality of the product that reaches the consumer after the new guidelines and releases by Organs competent bodies in times of a pandemic.

METHODOLOGY

The work was carried out at the Serra dos Órgãos University Center – UNIFESO, in the Microbiology laboratory at Campus Quinta do Paraíso. Initially, pre-experiments were carried out, during the months of October and November, in order to establish an adequate methodology in view of the conditions of the laboratory present on the Campus. Subsequently, with the methodology fully defined, the experiment itself was carried out during the month of December 2020. Ten different brands of 70% liquid alcohol and ten different brands of 70% gel alcohol acquired in commercial establishments in the municipalities of Teresópolis were used. and Guapimirim. The samples chosen for testing were the ones with the greatest relevance in the local market, being found in most of the markets and pharmacies of the two cities mentioned. Among the samples, the gel and liquid alcohols available for use in the 2020 elections (2 samples) were also analyzed. In addition, alcohol gel produced internally by students of the Pharmacy course at UNIFESO (1 sample). After the acquisition, the samples were sent to the laboratory, always maintaining the commercialization conditions. The products to be tested were separated into two groups, liquid presentation and gel presentation, identified by their initial: L for the liquid group and G for the gel group. Then each brand, within its given group, received a number. As in the examples: 1G For brand 1 in Gel presentation; 1L For brand 1 of the Liquid presentation group. The bench areas were identified in the same way as the products that would be tested, however, in their representation we also use the reference of which bacteria was used for their due contamination. Areas where Gram positive bacteria were used in the sanitizer efficiency tests received the "positive" sign (+) in their naming, whereas the areas where Gram negative bacteria were used received the "negative" sign (-). Example of identification of areas used for product testing: 1 G + For area where mark 1 of the gel presentation group will be tested after contamination by Gram positive bacteria; 1 L - For test area of brand 1 of the liquid group using Gram negative bacteria for contamination. And

also about the areas, the control regions were separated. Being a positive control area (+) and another negative control (-). Such areas received the identifications of "Control +" for the positive control area and "Control –" for the negative control area. In the experiment carried out, the alcoholmetry tests were carried out and, later, the tests to verify the growth or non-bacterial growth in the contaminated and sanitized areas with the aforementioned marks. With this respective order of realization. Thus, before carrying out the bench tests, we carried out tests on the alcoholic percentages of liquid products, using a Gay Lussac alcoholometer, in order to check if the presentation expressed on the label faithfully corresponds to the product. Alcoholmetry was performed by placing the liquid sample in a beaker and placing it in a water bath until it reached 20°C, as measured by a thermometer. After reaching the necessary temperature, we place the alcoholic solution in a 500 ml beaker, insert the alcoholometer in the beaker and turn it clockwise. The percentage presented when the alcoholometer stabilizes was converted using the table that presents the data of different hydroalcoholic solutions x Gay Lussac (3). In the second stage of the experiment, we demarcated the granite countertops with a circular area of 30 cm in radius, around the Bunsen burner, so that the safety area was delimited (4). The circle was divided into six equal areas: 4 (four) for testing the samples; 1 (one) for the positive control; 1 (one) for the negative control. Two circular areas were demarcated, one on each bench, in order to carry out the experiments against Gram negative bacteria on one bench and Gram positive bacteria on the other. For each group of samples tested, the experimentation areas were cleaned and sanitized using sterile gauze and 70% alcohol, which had an efficient bactericidal potential. This process was carried out with the Bunsen burner on to reduce the chances of environmental contamination after the cleaning process. Before each contamination of the areas for carrying out the experimental tests, a period was given for the evaporation of the alcohol used in the cleaning and sanitizing process so that it did not interfere with the experimentation. For the contamination of the test areas, strains of *Escherichia coli* (Gram negative) and *Staphylococcus aureus* (Gram positive) isolated from samples processed in the laboratory. Confirmation of isolation of *Escherichia coli* was made by tests carried out in Rugai with Lysine from Laborclin and Costa Vernim, the latter produced in the college's own laboratory specifically for testing (5). It was also carried out, for the strains of *Staphylococcus aureus,* positive catalase test. The strains were maintained on selective media for each bacterial group: Eosin Methylene Blue Agar (EMB) (for Gram negative) and Mannitol Salt Agar (Mannitol) (for Gram positive). For the application of the bacteria in the test areas, the sample of each bacterial group was transferred to tubes containing Brain Heart Infusion Broth (BHI Broth), separately: one tube containing the Gram Positive sample and the other containing the Gram negative sample. The contamination of the positive control areas and the testing areas of each product was carried out using a sterile swab dipped in the BHI-broth solution, containing the diluted strain, covering the entire test area by zig-zag movements in four different directions, waiting for the drying time of the contaminated surface. After the surface was dry, with the aid of a barrier in the limits of the test area, 1 (one) ml of the tested product was sprayed. Between the tests of the different samples of alcohol and the different contaminating strains, the barrier was sanitized with the alcohol of efficient bactericidal function, mentioned above, for the initial cleaning of the benches. The

evaporation time of the product was respected, so that new experiments could be carried out and its use in the test area. For the alcohol gel samples, a sterile swab was used to spread the product evenly over the entire surface. After the drying time of the applied alcohol, a sterile dry swab was collected from each area, seeded in a plate with Müeller Hinton (MH) culture medium, properly identified and incubated in a bacteriological oven at 36°C +/- 1° C for 24 hours. At the end of the laboratory tests, the labeling analyzes of the tested products were carried out to verify the labels and containers and whether they are being produced and marketed in accordance with the INMETRO ordinances in force: Decree, number 269, of August 5, 2008 and Ordinance Number: 270, of August 5, 2008 (6)(7). Both establish methods for evaluating packages of 0.1 to 5.0 liters of ethyl alcohol containers for safety and user performance. Ordinance Number: 353, of November 12, 2020, was also used as a reference, which is temporary and will be in force as long as the Covid-19 pandemic lasts in the country (8). This ordinance releases the necessary certification established in Ordinance Number: 270 of 2008, which becomes voluntary, however, it does not release the other security requirements provided for in the previous ordinances. Basic statistical analyzes were performed in percentages to evaluate the data.

RESULT AND DISCUSSION

Among the 10 (ten) brands of 70% liquid alcohol analyzed, two of them have the RDC 350 of March 19, 2020 from ANVISA on their label, which formalizes the release of the sale of products without prior authorization from the National Health Surveillance Agency. This data shows that more companies started to produce and sell 70% alcohol at the time of a pandemic, as a way of supplying the market and an opportunity for economic growth. This corroborates the information given by (9), which discusses the restructuring and re-equipment of various industries, including other branches, for the production of sanitizers. Regarding packaging and certifications, 70% (7) of the tested products had INMETRO certificates provided for in Ordinance Number: 270 of 2008, and 30% (3) did not have them. However, the information presented is justified by Ordinance Number: 353 of the year 2020, which makes the certification described in Ordinance Number: 270/2008 voluntary during the pandemic. Also, according to Ordinance Number: 353/2020, the safety requirements provided for in Ordinances Number: 269 and Number: 270 of 2008 remain mandatory on product labels. In the analysis of the brands selected for the experiment, 60% (6) presented on their labels all the safety requirements defined in the aforementioned Ordinances. Meanwhile, 40% (4) had some type of pending, such as: lack of destination of the product in the main range and absence of safety phrases, such as, care for children and animals. The safety requirements defined by the Ordinances are of great importance when we consider the information presented by (10) who talks about the prohibition of the sale of 70% alcohol in markets and pharmacies, in 2002, by RDC 46 of ANVISA. The decision to ban the sale at the time aimed to minimize domestic accidents, especially with children, that occurred in the country. One of the samples that present RDC 350/2020 on its label, had a product registration with ANVISA until the year 2018, thus being able to claim not to have updated its registration by the voluntariness given in the new RDC, which only came into force in 2020. However, the same brand does not present the certificates, also voluntary during the pandemic, defined in Ordinance Number: 270/2002 and also presents pending issues in relation to the requirements, still mandatory,

of Ordinance Number: 269 of 2002. Another sample does not have on its label RDC 350/2020 and does not even show registration in the net presentation, with the company responsible for registration with ANVISA only for the production of 70% alcohol in gel. In the alcoholmetry performed, it was only possible to analyze the samples that contained a volume equal to or greater than 500 (five hundred) ml (milliliters) by the size of the beaker necessary for the correct process using the alcoholometer, therefore, of the ten liquid samples of the experiment, nine were tested. Thus, of the nine samples tested, 11.11% (1) of the samples showed alcoholmetry below the standard of 70% as they were being presented. According to (11), the concentration of 70% of the alcoholic substance is ideal for the antimicrobial effect of the product because its percentage of hydration helps in the process of denaturing the protein. The study of (12) corroborates with (11), reporting that after a series of tests with different percentages, the most efficient concentration needs the volume of water contained in the presentation of 70%. Therefore, the studies demonstrate that the sample of the present experimental research may not present a satisfactory antimicrobial effect due to the low concentration. Regarding the efficiency tests in the disinfection of laboratory surfaces, 70% (7) of the samples showed a favorable result in the elimination of *Staphylococcus aureus*, and 30% (3) were not efficient against *S. aureus*. Two of the inefficient samples were not registered for the production of liquid alcohol. One of them being the company that had its registration expired in 2018. Which shows that at least 2/3 (two thirds) of the samples that presented problems in the elimination of *Staphylococcus aureus* on surfaces can only be present on the market due to new releases during the pandemic. In the elimination test of the *Escherichia coli*, one sample did not show satisfactory disinfection. Being the same brand with expired registration problems, which did not present the certificates described in Ordinance Number: 270 of 2008 and also did not present all the security requirements of Ordinance Number: 269 of 2008, both from INMETRO. In addition to not having been successful in eliminating*Staphylococcus aureus.*

This brand stood out in the market, during the times of a pandemic, for the voluminous donation of its products to hospitals, entities and the public transport sector. As indicated by (13), for hospitals, and by (14), for laboratories dealing with microbial activity, due to its disinfecting effect, 70% alcohol needs to present satisfactory efficiency in fighting bacteria. When the product does not pose a risk to its users. In addition, as described by (11), the indiscriminate use of alcohol can generate bacterial resistance, which often also happens with the use of contaminated disinfectant solutions. According to (15) to avoid the indiscriminate use of these products, periodic quality assessment programs are necessary. The difference in the number of samples that did not show efficiency against the bacteria: *Staphylococcus aureus* and the bacteria: *Escherichia coli* may be related to the much greater thickness of peptidoglycan present in the cell membrane of Gram positive bacteria when compared to Gram negative bacteria, as described by (16) and by (17). This can compromise the dehydration process of the outer cell membrane so that alcohol can enter the cytoplasm and promote bacterial protein denaturation, as described by (12). This way, it is possible to analyze that, with ANVISA's RDC 350/2020 and INMETRO's Ordinance Number: 353/2020, quality inspection of products that reach consumers becomes even more important. The same brand that showed all the problems, had a validity of 36 months. However, with only 14

months after its manufacture and with the correct storage as described by the label, it presented solid particles in its liquid medium. According to (18), the denaturing agent that must be added to liquid formulations comes in two forms: crystals and liquid. The use of the solid denaturing agent, in the form of crystals, does not offer solubility as good as the use of the liquid form, which may justify the appearance of solid particles in the product. the previous registration, did not present the INMETRO certificates. This same brand was pending in relation to safety requirements and was banned and withdrawn from the market in Rio de Janeiro in October 2020 by the Undersecretary of Surveillance, Sanitary Inspection and Zoonosis Control of the municipality. This event occurred after purchasing the sample for the experiment. Thus, among the four samples that had not previously been registered with ANVISA, two of which had RDC 350/2020 on their labels that justified their operation, two samples had problems. One of them being inefficient to the two bacteria and with problems in the mandatory aspects of labeling and the other one outside the safety standards of the labels and still suffered interdiction and recall of products in markets. In the experiment, ten different brands were chosen from those tested in liquid presentation for the alcohol gel tests. Of the ten brands tested, 50% (5) had valid registrations, 10% (1) of the samples had their registration canceled, 10% (1) were still in the process of obtaining registration and in 10% (1) none were found. registration process at all and 10% (1) had their production canceled by ANVISA after purchase for the experiment.

Of the samples analyzed, 10% (1) were produced by students of the pharmacy course at UNIFESO, being an experimental sample. This shows information given by (19) that the new releases of production and sale of 70% alcohol were aimed at increasing the number of producers in the country in order to reduce market shortages. When talking about the certification seals of Ordinance nº 270/2008 INMETRO, 70% (7) of the brands did not have the certificates, 20% (2) of the samples had them and 10% (1) were in the experimental phase. Remembering that the certification imposed in said Ordinance became voluntary during the pandemic by Ordinance Number: 353 of 2020 INMETRO. Regarding safety requirements, which remain mandatory, 70% (7) of the brands had some pending, 20% (2) were in accordance with all requirements and 10% (1) was an experimental sample. While, in the liquid samples, the most recurrent issue between the brands was the lack of warning about the necessary care to avoid accidents with children and animals, in the gel presentations the biggest problem was the lack of the CEATOX (Toxicological Assistance Center) number. Within the set that had pending issues, 60% (6) did not have the contact number on their labels and 50% did not have all the security phrases. However, despite the fact that alcohol is one of the safest antiseptics precisely because of its low toxicity, according to (20), and its commercialization in gel was started precisely because it predisposes to a lower risk of accidents according to (21), the requirements, including the CEATOX number and safety phrases must appear on their labels, following INMETRO's Ordinances Number: 270 and number 269, both from 2008. Regarding the visual differences of the product: 60% (6) of the samples had opaque packaging and 40% (4) were in transparent packaging and 70% (7) of the brands had the color of the transparent substance, while 30% (3) blue. It is important to analyze that 10% (1) of the brands had an indicative label for use by children and compared the product to the SLIME children's toy, even promoting a product viscosity that resembled the toy. Which can

be related to what was said by (22) and (23) about the changes that brands promoted in their products in order to please the target market at the time of exponential sales growth. Regarding the efficiency tests of the products to eliminate bacteria on previously contaminated surfaces, 90% (9) of the samples showed efficiency against Staphylococcus aureus and Escherichia coli bacteria. And, 10% (1) did not show satisfactory results in inhibiting the growth of any of the bacteria, which, contrary to expectations, showed significant growth. As (11) portrayed, alcohol is of great importance because it is effective in everyday actions such as cleaning hands and environments. (22) states that the use of alcohols produced with poor quality inputs or in disagreement with what is established by official quality standards can lead to the development of bacteria and not their elimination, as occurred in this experiment. Therefore, studies show that there are risks of using products without the proper quality, both in their daily use and in potentially more contaminated environments such as hospitals and laboratories. The brand that did not show efficiency against any of the bacteria, was the same for the use of children and had its registration canceled by the audit still in 2020. The brand, for which no registration process was found, had its production prohibited , in 2020, by the National Health Surveillance Agency. One of the brands was recalled in 2020, after the Rio de Janeiro State Department of Consumer Protection and Defense (PROCON-RJ) found irregularities in the product, such as the lack of a Technical Responsible on the label. The tested batch was within the defined for recall. Two other brands tested were banned by the State Department of Consumer Protection and Defense of Santa Catarina (PROCON-SC) and by the state's Civil Police, who claimed to find non-standard products. The samples were taken to Universidade Blumenau for further tests requested by PROCON. One of the companies contests the allegations and presented reports on the production and quality of the product. The other company did not respond. Two of the ten brands tested were those made available during the 2020 electoral process in the municipality of Guapimirim, for use by voters and poll workers. The aforementioned brands did not present any problem between the efficiency tests and labeling analysis. That is, among the ten brands tested, nine were on the national market, and among them 55.55% (5) had problems in relation to the official standards established by ANVISA and INMETRO.

CONCLUSIONS AND FINAL CONSIDERATIONS

Considering the results presented, it is possible to conclude that the samples tested showed, for the most part, efficiency in eliminating bacteria: *Staphylococcus aureus* e *Escherichia coli*. Regarding alcoholmetry in liquid presentations, these presented correct values, as described on the labels, in most of the analyzed samples. When dealing with the basic requirements that are still mandatory, in times of a pandemic, listed in Ordinances Number: 269 and Number: 270, both of 2008-INMETRO, and maintained by Ordinance Number: 353 of 2020-INMETRO, most of the analyzed samples did not meet all the requirements. Samples ineffective against bacteria were also considered to have labeling issues. Of the 10 (ten) samples tested in the liquid presentation, one had a problem with withdrawing from the market after the purchase for the experiment. Among the 10 (ten) gel samples tested, 5 (five) had problems between collection, recall, canceled registration and production ban. This demonstrates that the new releases were of paramount importance to quickly avoid

market shortages, however, they create a greater need for inspections and quality tests in order to ensure the safety of product users. It is recommended that new studies be carried out to analyze the efficiency of products with a greater number of samples. And, especially after the Covid-19 pandemic, if companies that started producing 70% alcohol using the new ANIVSA and INMETRO releases that allowed production without registration and without certification will adapt to mandatory standards in non-pandemic times. In addition, to analyze whether the ban on the sale of 70% alcohol to the general population issued by RDC 46 of 2002 and temporarily revoked in times of pandemic by RDC 350 of 2020 will continue in force.

THANKS

I thank the institution Centro Universitário Serra dos Órgãos – UNIFESO, for the quality teaching and for the opportunity to publish this work and I thank all those directly and indirectly involved in the construction of this work.

REFERENCES

1. Senhoras EM. Coronavírus e o papel das pandemias na história humana. Boletim de Conjuntura. 2020; 1 (1): 31-34.

2. BRASIL, Ministério da Saúde. Diretriz de 08 de abril de 2020. Diretrizes para diagnóstico e tratamento da Covid-19: guia de diretrizes. 2.ed. Brasília, DF: Ministério da Saúde, 08 de abril de 2020.

3. Faria, LG.; Ferrarini, M.; Oliveira, AC.; Patricio, DCR.; Souza, APM.; Souza, PM. Comparativo de determinação do grau alcoólico entre densímetro de gay-lussac e título alcoométrico. In: Anais do II Simpósio de assistência farmacêutica, 2014. São Camilo, SP: Centro Universitário São Camilo, 2014.

4. Scaranto, MK. Plasma rico em plaquetas. 2002. 26f. Curso de especialização Periodontia, Universidade Federal de Santa Catarina, Santa Catarina, 2002.

5. Costa, GA.; Hofer, E. Isolamento e Identificação de Enterobactérias. Rio de Janeiro: Instituto Oswaldo Cruz, 1972. 120 p.

6. BRASIL, INMETRO. Portaria n. 269 de 05 de agosto de 2008. Diário Oficial da União, Brasília, 06 de agosto de 2008. Seção 1, p.52

7. BRASIL, INMETRO. Portaria n. 270 de 05 de agosto de 2008. Diário Oficial da União, Brasília, 06 de agosto de 2008. Seção 1, p.52.

8. BRASIL, INMETRO. Portaria n. 353 de 12 de novembro de 2020. Diário Oficial da União, Brasília, 24 de novembro de 2020. Seção 1, p.19.

9. Folha de Pernanbuco. Cervejarias artesanais produzem álcool para combate ao coronavírus. 2020. [acesso 12 jul 2021] Disponível em: <https://www.folhape.com.br/economia/cervejariasartesanais-produzem-alcool-para-combate-ao-coronavirus/137079/>.

10. Jusbrasil. Decisão: Mantida resolução da Anvisa que proíbe a comercialização de álcool líquido superior a 54° GL. 2016 [acesso 09 ago 2021] Disponível em: <https://trf-1.jusbrasil.com.br/noticias/413698063/decisao-mantida-resolucao-da-anvisa-que-proibe-a-comercializacao-de-alcool-liquido-superior-a-54deg-gl>.

11. Bernardi, GA.; Costa, TCM. Avaliação da atividade antimicrobiana do álcool 70% em superfícies contaminadas. 2017 [acesso 22 jun. 2021] Disponível em: <https://www.crmv-pr.org.br/uploads/noticia/arquivos/Avaliacao-da-atividade-antimicrobiana-do-alcool-70-em-superficies-contaminadas.pdf>.

12. Cunha, HVF. Afinal, por que o álcool 70% é mais eficaz como bactericida que o álcool absoluto? 2016 [acesso 08 jul. 2021]. Disponível em: <https://foodsafetybrazil.org/afinal-por-que-o-alcool-70-e-mais-eficaz-como-bactericida-que-o-alcool-absoluto/>.

13. ANVISA (Agência Nacional de Vigilância Sanitária). **Segurança do paciente em serviços de saúde: limpeza e desinfecção de superfícies**. 1 ed. Brasília, DF, 2012 [acesso 11 jul. 2021] Disponível em: <https://www.pncq.org.br/uploads/2018/Manual_Limpeza_e_Desinfeccao_2012_(1).pdf>.

14. OMS (Organização Mundial da Saúde). **Manual de segurança biológica em laboratório**, 2004. [acesso 11 jul. 2021] Disponível em: <https://www.who.int/csr/resources/publications/biosafety/16007%20Add%201.pdf>.

15. Andrade, D.; Beraldo, CC.; Oliveira, BA.; Santos, LS. Álcoois: A produção do conhecimento com ênfase na sua atividade antimicrobiana. Medicina, Ribeirão Preto, SP, 35, p. 7-13, jan./mar. 2002.

16. Silva, NP.; Trabulsi, LR. Morfologia e Estrutura da Célula Bacteriana. In: TRABULSI. 2ed. MICROBIOLOGIA. São Paulo: Livraria Atheneu Editora, 1996. 3-11p.

17. Fox, A. The Bacterial Cell, 2016. In: HUNT, M. Microbiology and Immunology On-line. University of South Carolina School of Medicine. [acesso 11 ago. 2021] Disponível em: <https://www.microbiologybook.org/default.htm>.

18. Polyorganic. Porque Utilizar o Desnaturante Benzoato de Denatônio?. 2015 [acesso 22 ago. 2021] Disponível em: <https://polyorganic.com.br/porque-utilizar-o-desnaturante-benzoato-de-denatonio/>.

19. Agência Brasil. Anvisa libera fabricação e venda de álcool e desinfetantes. 2020. [acesso 12 jul. 2021]. Disponível em: <https://agenciabrasil.ebc.com.br/saude/noticia/2020-03/anvisa-libera-fabricacao-e-venda-de-alcool-e-desinfetantes>.

20. Flores, AF.; Menegaro, A.; Pinto, EP.; Sbardelotto, PRR.; Silva, FI.; Simer, P. Sanitizantes: Concentrações e aplicabilidade na indústria de alimentos. Scientia Agraria Paranaensis, p. 171-174, 2016.

21. Mesiano, ERAB.; Sanmartin, JA.; Santos, AAM; Verotti, M.P. Importância do álcool no controle de infecções em serviços de saúde. 2002. [acesso 22 jun. 2021]. Disponível em: <https://www.anvisa.gov.br/servicosaude/controle/controle_alcool.pdf>.

22. Rogenski, R. Conheça os vencedores do Caboré 2020. 2020. [acesso 30 ago. 2021]. Disponível em: <https://www.cabore.com.br/noticias2020/2020/12/02/conheca-os-vencedores-do-premio-cabore-2020/>.

23. Rocha, R. Covid fez players de limpeza anteciparem lançamentos. 2020. [acesso 30 ago. 2021]. Disponível em: <https://www.meioemensagem.com.br/home/marketing/2020/07/01/covid-fez-players-de-limpeza-anteciparem-lancamentos.html>.

24. Beux, MR. Álcool gel fora do padrão pode atuar como proliferador de bactérias, alerta microbiologista da UFPR. 2020. [acesso 11 jul. 2021]. Disponível em: <https://www.ufpr.br/portalufpr/noticias/alcool-gel-fora-do-padrao-pode-atuar-como-proliferador-de-bacterias-alerta-microbiologista-da-ufpr/>.