SAÚDE: ASPECTOS GERAIS

Volume 1

Organizador
Daniel Luís Viana Cruz

EDITORA
OMNIS SCIENTIA

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PREFÁCIO

O bem-estar das pessoas depende de diferentes fatores, como os fatores genéticos, o ambiente, o estilo de vida e a assistência médica. Desta forma, a saúde deve ser mantida, por meio da aplicação da Ciência da Saúde e pelo modo em que cada indivíduo vive, assim como a sociedade em geral.

A visão integrativa em saúde é fundamental para a melhoria de vida da população, uma vez que aborda uma visão ampla sobre as áreas da saúde, de forma conjunta. Desta forma, o presente livro retrata informações sobre a promoção e educação em saúde, urgência e emergência, saúde do idoso, saúde do trabalhador, saúde bucal, acidentes no transito, acidentes ofídicos, queimaduras, viroses, síndromes, doenças autoimunes, entre outras.

Em nossos livros selecionamos um dos capítulos para premiação como forma de incentivo para os autores, e entre os excelentes trabalhos selecionados para compor este livro, o premiado foi o capítulo 17, intitulado “ADESÃO AO TRATAMENTO MEDICAMENTOSO DE PESSOAS VIVENDO COM DIABETES MELLITUS TIPO 2”.

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THEORETICAL-PRACTICAL VISUALIZATION OF THE MICROBIOLOGICAL GLASS LIDES CONSERVATION PROFILE OFTHE INSTITUTIONAL COLLECTION OF A PRIVATE UNIVERSITY

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ABSTRACT: The Microbiology laboratories of Higher Education Institutions develop activities with a variety of microorganisms and contaminated materials, and some laboratory techniques require more specific conservation measures, since they are of fundamental importance for the study of microbiology, such as slide staining. In this perspective, it is necessary to adopt a set of preventive measures within a program of good practices in handling and conserving the materials used. The present study objective heoretical and practical visualization of the conservation profile of microbiological glass slides of the institutional collection of a private university located in the metropolitan region of Belém, Pará, Brazil. This is a documentary study of a retrospective nature and primary qualitative and quantitative basis, the material worked on was acquired by an educational institution with the aim of meeting the visual and practical needs of microbiology. The slides containers were not found in the proper position, they were horizontally inside an MDF cabinet, with several other materials, which may have compromised its quality. In view of the parameters already discussed on the subject, it is possible to establish that good laboratory practices stand out as the necessary foundation for the realization of active methodological and teaching strategies, which aim to contextualize academic knowledge in a technical and exploratory perspective for the development of the student's theoretical questioning.

KEYWORDS: Glass Slides Conservation, Biodegradation, Microorganisms.

INTRODUCTION

The study of microbiology belongs to a field of biology responsible for contemplating the morphological and physiological aspects of microorganisms, including unicellular (organisms formed by a single cell) and prokaryotes (living beings whose cell nucleus is not bounded by a membrane), such as bacteria and fungi, The word Microbiology is derived from the Greek: mikros ("small"), bios ("life"), and logos ("science"). This Science studies microscopic organisms and their biological activities, that is, they verify the diverse forms, structures, reproduction, biochemical-physiological aspects, and their relationship with each other and with the host, which can be beneficial and harmful. Microbiology treats single-celled microscopic organisms, where all vital processes are performed in a single cell. Regardless of the complexity of any organism, the cell is, and must be considered, the basic unit of life. All living cells are basically composed of: protoplasm (from the Greek: first substance formed), colloidal organic complex consisting mainly of proteins, lipids and nucleic acids;

limiting membranes or cell wall; and a nucleus or an equivalent nuclear substance. (AKKERMANS, VAN ELSAS, DE BRUIJN, 1996).

In this regard, the discipline of microbiology taught in institutions of higher education, constitutes one of the fundamental branches of basic sciences, with the knowledge and detailed study of microorganisms and their essential functions to establish their use in varied applications, from the medical field, food, environmental, agricultural and industrial. Thus, microbiology is consolidated as one of the pillars of health sciences (ALBERTS et al, 2006; TORTORA, 2017).

The theoretical-practical pedagogical approaches responsible for making the transmission of knowledge according to the needs of the students are susceptible to a recurrent reassessment, alteration and redesign, in order to attract the attention and engagement of the students, as observed in the technical rearrangement for conducting practical classes in the laboratories (PARANÁ, 2008). In microbiology, conditions are unfavorable as regards the development of practical classes due to the lack of laboratory structure, material quality and specific storage and maintenance techniques. In addition, when the infrastructure is favorable for the development of practical classes, the quality of the material used, such as microscopy slides, in addition to being insufficient to meet the demand, have ineffective conservation quality for prolonging the survival of stored microorganisms (DA SILVA, MORAIS, & CUNHA, 2011).

The Microbiology laboratories of Higher Education Institutions (HEI) develop activities with a variety of microorganisms and contaminated materials, in this perspective, it is necessary to adopt a set of preventive measures within a program of good practices in handling and conservation of the materials used, in order to guarantee protection in the development of activities, in the handling of the equipment and in the conservation of the material, as well as in the manipulation by the students, teachers and technicians and researchers, in order to avoid the contamination of their experiments and the laboratory environment (AUGUST, 1990; MINISTRY OF HEALTH, 1999).

Laboratory safety in the academic environment has developed over the years for good technique, need for adequate preparation and training of those who manipulate (BORGES, LIMA, 2007). From the moment of material collection, storage, transportation, to continuous use in practical classes. Such security is based on the adoption of procedures that aim at the integrity of the material that is used during the techniques of promotion to the critical development of the student regarding the practical observation of preventive measures and care of the slides used during the learning process (BORGES, LIMA, 2007).

Some laboratory techniques demand more specific conservation measures, since they are of fundamental importance for the study of microbiology, such as the realization of staining methods on slides that end up being daily used in the ways more varied and for the most diverse purposes, through these stains and fixations on slides it is possible, for a certain time, to visualize bacteria and fungi, both for health purposes and for teaching (TRUJILLO et al, 2012, MINISTRY OF HEALTH, 1999). However, little is found in the literature on its storage and durability. Thus, the present work aims to carry out a theoretical and practical survey of the profile of conservation of microbiological sheets of

the institutional collection of a private university located in the metropolitan region of Belém, Pará, Brazil

METHODS

The material analyzed was acquired by a HEI located in the city of Belém-PA, with the objective of meeting the visual and practical needs of microbiology, the samples were being stored at the time of collection in plastic boxes with dividers, kept in a MDF cabinet, without an apparent order, with several other materials, being handled freely and continuously by students and teachers since march 2008. About 900 prepared glass slides were analyzed, divided into 30 boxes with 30 samples each. The boxes contained the same microorganisms properly ordered and cataloged **(Table 1)**.

Table 1: Ordered and cataloged microorganisms present in the collected boxes

1 - Anthrax spore	*16 - Mouse Salmonella typih*
2 - Bacillus anthracis	*17 - Mycobacterium tuberculosis*
3 - Bacillus subtilis	*18 - Neisseria gonorrhoeae (gonococcus)*
4 - Bacteria Three type smear	*19 – Pneumococcus*
5 - Bordetella pertrussis	*20 – Proteus*
6 - Botulinum spore	*21 - Pseudomonas aeruginosa*
7 - Candida albicans	*22 - Pylorus spira bacillus*
8 - Corynebacterium diphtherine	*23 - Rhizobium meliloti*
9 - Clostridium botulinum	*24 - Salmonella paratyphi*
10 - Clostridium tetanus (C. tetati)	*25 - Salmonella typhi*
11 - Cryptococus neoformans	*26 – Staphylococcus*
12 - Dysentery bacteria	*27 - Streptococcus aureus*
13 - Escherichia coli (E. coli)	*28 – Streptococcus*
14 – Leptospira	*29 - Tetanus spore*
15 - Lymphocyte transfomation	*30 - Vibrio cholera*

The method used was to view the glass slides one by one, from September to November, on the a Leica DM 500 optical microscope, starting with the 10x objective and later with the 40x objective, ending with the 100x objective, when it was necessary.

Quality criteria were established for a better organization, with two different categories, staining and presence of Fungi, since the coloring has no direct relationship with cases of funga l slides. In relation to the staining, it was classified as: Remnants - when there is staining residue in scattered spots; Little - when there are moderate stain clusters; Reasonable - when there are large points of stain along the slide, Great - when there are excellent concentrated points of stain and Absence - when there is no stain. Regarding the fungal slides, it was classified as: Presence - when there are no fungi.

RESULTS AND DISCUSSION

The results were inferred according to what was observed, with regard to the state of the glass slides, during the development of the work. Of the 900 slides, 344 are distributed in the subcategories with coloring, as can be seen in **Table 2**.

Table 2 – Staining

N	Slides	Remnant	Little	Reasonable	Great	Absence
1	*Botulinum spore*	0	0	0	0	30
2	*Clostridium botulinum*	0	0	0	0	30
3	*Pneumococcus*	0	0	0	0	30
4	*Streptococcus aureus*	0	0	0	0	30
5	*Streptococcus*	0	0	0	0	30
6	*Tetanus spore*	0	0	0	0	30
7	*Pylorus spira bacillus*	2	0	1	0	27
8	*Candida albicans*	3	1	0	0	26
9	*Cryptococus neoformans*	3	1	0	0	26
10	*Mycobacterium tuberculosis*	2	2	0	0	26
11	*Bacillus subtilis*	3	1	1	0	25
12	*Bacteria Three type smear*	5	1	0	0	24
13	*Corynebacterium diphtheria*	5	1	0	0	24
14	*Leptospira*	6	0	0	0	24
15	*Anthrax spore*	7	0	0	0	23
16	*Clostridium tetani (C. tetani)*	5	1	1	0	23
27	*Staphylococcus*	7	0	0	0	23
18	*Bacillus anthracis*	8	2	0	0	20
19	*Pseudomonas aeruginosa*	11	3	2	0	14
20	*Vibrio cholerae*	5	5	6	1	13
21	*Shigella bactéria*	6	11	3	0	10
22	*Mouse Salmonella typhi*	6	10	4	0	10
23	*Neisseria gonorrhoeae*	9	7	4	1	9
24	*Proteus*	9	4	8	1	8
25	*Rhizobium meliloti*	0	8	13	1	8
26	*Bordetella pertrussis*	8	8	6	1	7
27	*Salmonella paratyphi*	6	9	9	0	6
28	*Escherichia coli*	2	7	20	1	0
29	*Lymphocyte transfomation*	0	7	23	0	0
30	*Salmonella typhi*	7	4	15	4	0
	TOTAL	**126**	**92**	**116**	**10**	**556**

Regarding the microorganisms existing in the staining, it was observed that 90% did not have a defined shape, that is, only 90 samples, distributed in 13 types, contained defined bacteria, as can be seen in **Table 3**.

Table 3 – Defined microorganisms.

Microrganisms	Total
Salmonella typhi	19
Escherichia coli	18
Proteus	9
Rhizobium meliloti	9
Salmonella paratyphi	8
Vibrio cholera	7
Neisseria gonorrhoeae	5
Bordetella pertrussis	4
Mouse Salmonella typhi	4
Shigella bactéria	3
Pseudomonas aeruginosa	2
Bacillus subtilis	1
Helicobacter pylori	1

Regarding the results of the fungal slides, 49 were present and 851 were absent. For a better understanding below, it will be possible to glimpse examples of the states of the slides in the Staining category (Figure 1), Defined microorganism (Figure 2) and Fungal slides (Figure 3).

Figure 1 - (in sequence): Stain 1 – Bacillus anthracis (Remnant); 2 – Neisseria gonorrhoeae (Little); 3 – Escherichia coli (Reasonable); 4 Salmonella typhi (Great)

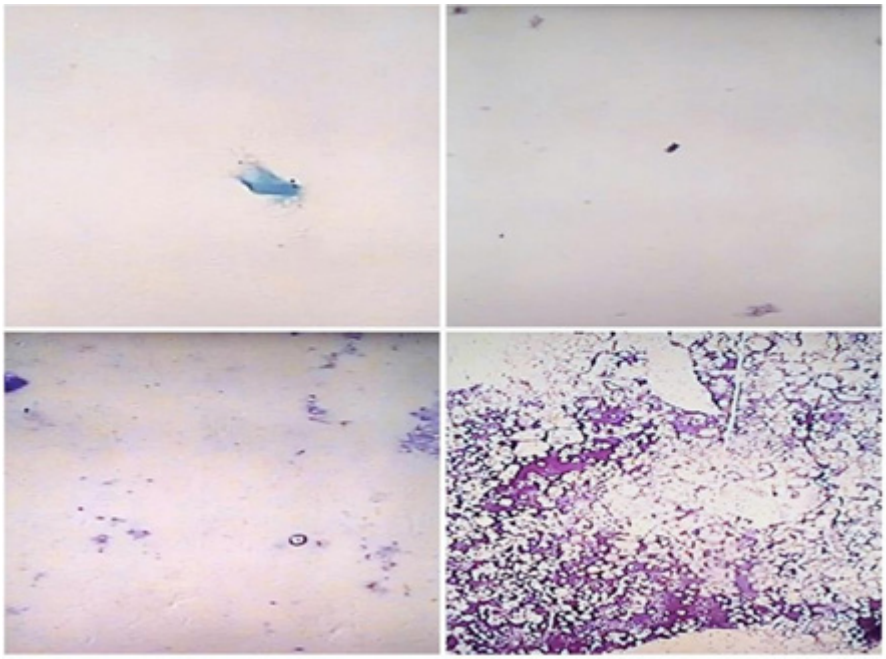


Figure 2. *Salmonella typhi*

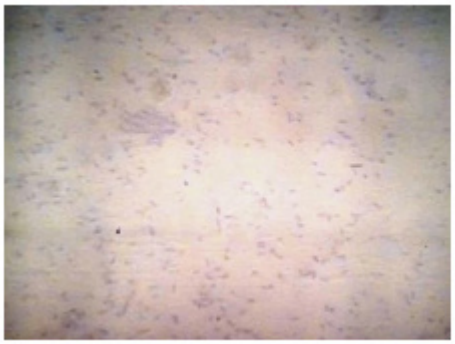
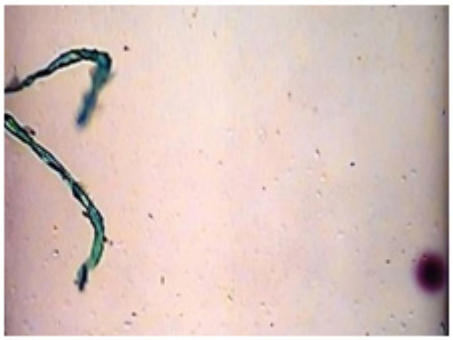


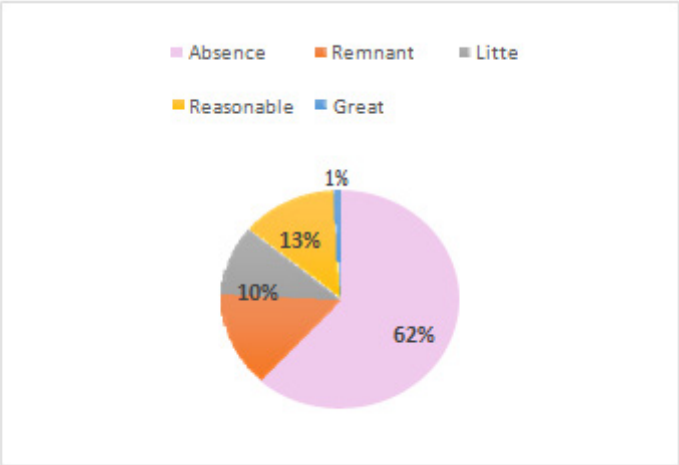
Figure 3. *Pneumococcus*



DISCUSSION

Although there are no data on the handling of the material, it is known that for more than 10 years these have been used by students and teachers of the HEI. Little is know in the literature about the storage and proper handling of ready-made glass slides, however, JOHANNES LIEDER GMBH & CO. KG - LIEDER, a German company that has been selling this material since 1955, guarantees that the slides must be stored in cool places keeping the boxes in an upright position so that the blades are horizontal, away from dust and sunlight in order to ensure quality. However, the slides containers were not found in the proper position, they were horizontally inside an MDF cabinet, with several other materials, which may have compromised its quality. The compromise of this quality can be found in the results according to the Staining category, in which 556 slides were not found, the others (344) are divided into Remnants (126), Little (92) Reasonable (116) and Great (10), as in graph A (Figure 4).

Figure 4. Graph A, representing the amount of coloring in percentage



The division of these categories was necessary due to the variable slide quality, as it is possible to quantify, according to graph A. It was observed that 62% correspond to the Absence subcategory, when there is no amount of stain in the slides, compared to the Great 1% subcategory that are slides suitable for viewing, liable for didactic use, so it can be seen that most of the slides are out of date.

Although there is more than one staining method for the slides already mentioned, the occurrence of defined microorganisms, about 100%, occurs in slides stained by the Gram method, with some specificities such as the example of *Rhizobium meliloti*, this is due to the fact there is more than one type of stain present, also called selectively. According to Capille (2017), Lugol or iodine-iodized solution, one of the elements used, acts as a mordant, that is, as a substance whose function is to maintain the durability of the color, which may justify the results found.

The specificities found in the slides, in relation to staining that differ from the traditional Gram method or not, are described in table 3.

Table 3 – Staining Specificities

Slides	Method for Staining
Rhizobium meliloti	Congo Red dye
Lymphocyte transfomation	May-Grunwald Giemsa
Cryptococus neoformans	China Nanquim
Corynebacterium diphtheriae	Albert-Laybourn
Mycobacterium tuberculosis	Ziehl-Neelsen
Leptospira	Fontana-Tribondeau

Fontes: (HUNGRIA et al, 1994; PEREIRA, 2002); (DOLES, 2018); (ANVISA, 2004) e (BRASIL, 2018).

It is noteworthy that the slides of Lymphocyte transformation despite having the specificity in their coloring, mentioned above, present expressive results regarding their quality of staining.

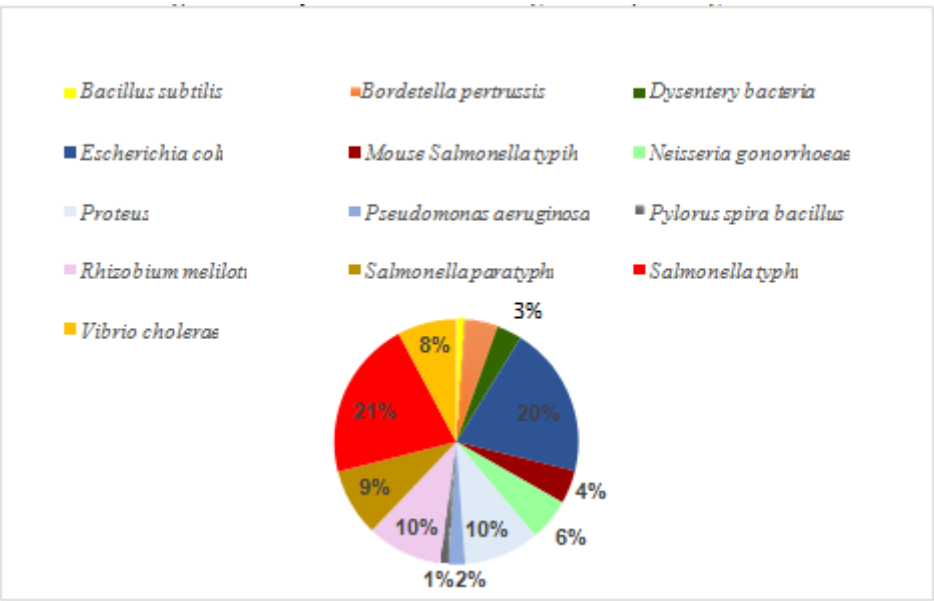
Several companies produce and sell the material, such as Histotech, Roster, Didática sp., Johannes Lieder and Histoplus, however only two of these give guarantees, Histotech and Johannes Lieder, although they are different guarantees, it is believed that fact that there are no Brazil norms that determine a minimum period of duration, howeve

the variation is great, from 5 to 20 years for the permanence of the coloration, as long as the adequate storage measures are followed according to the Johannes Lieder company.

Regarding microorganisms, although the table includes bacteria and fungi, only bacteria were found, due to the time they were in use, more than 10 years. Fungi are simply stained, that is, only one colorant is used, without the use of a mordant, they are more susceptible to degradation.

Most bacteria are stained by the Gram method (with more than one dye), which makes them less susceptible to degradation by the time variable. It was noticed, from the result of the slides with defined microorganism (Graph B) (Figure 5), that 99%, with regard to the total of slides, are gram negative, possibly due to the fact that they have two membranes, as stated by Madigan et al (2016), the function of the membrane is to prevent proteins from diffusing away from the cell, if we associate with the fact that these proteins are also stained, it is possible to say that these two membranes prevent the staining from dissipating quickly.

Figure 5 - Graph B - Defined Microorganism in percentage.



Escherichia coli and *Salmonella typhi*, have expressive results in terms of quantity, as shown in graph B, together accounting for 41% of the total slides with defined bacteria. Fungal slides, it can be seen that 95% are not affected by fungi, which is a positive point, when compared to the presence of color 38%, and the poor storage and handling of the slides, since fungi are considered as microorganisms that, regardless of their constitution, can attack all types of material. (SINGH et al, 1995; BORTOLETO et al, 2002; PALETTA et al, 2005; ROSA et al, 2008. Therefore, when comparing stain

defined Microorganism and Fungal slides, it is possible to state that time, as well as stored and utilization, limit the good quality and durability of the prepared slides.

CONCLUSION

There is a shortage both in the literature and in laws on storage and duration of ready glass slides, little is found about the commercialization and importance of such material with regard to its use. However, there is an ongoing search for methods that enable the handling of materials in a laboratory, with regard to their use, transport and storage, given that it was possible to observe in relation to the results obtained, that the level of conservation of the slides was below what was necessary for the demand for practical classes.

Throughout the work it was found that all slides that contained a defined microorganism were stained by the gram method, although with specificities. Among these, only the bacterium Bacillus subtilis is categorized as gram positive, and all others as gram negative. In the literature, nothing was found that could answer this question clearly, only the particularities and differences between the two types were found, which implies a hypothesis that it is due to the double membrane present in the negatives. The time of the slides, since they were acquired by the institution, accounted for more than 10 years, linked to inadequate storage, unknown handling and the amount of didactic practices made it realize that this variable (time), is directly linked to the quality and durability of the stain and the defined microorganisms analyzed. This study was the first to be conducted on the evaluation of ready-made microbiological slides.

In view of the parameters already discussed on the subject, it is possible to establish that good laboratory practices stand out as the necessary foundation for the realization of active methodological and teaching strategies, which aim to contextualize academic knowledge in a technical and exploratory perspective for the development of the student's theoretical questioning, as well as the interdisciplinarity and decompartmentalization of the content of classroom, thus encouraging reasoning and the will to learn

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