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Original article

Evaluation of the microbiological quality of the environment in a laboratory of microbiology candidate to the accreditation

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ABSTRACT

The microbiological quality of the environment of two laboratories of microbiological quality control of the agro alimentary products, the first candidate to the accreditation and the second not candidate to the accreditation was evaluated. The samples of air were taken by aspiring the air, through physiological water of a biocollector, rooms of laboratories to a flow of 2L/min. The test sample selection of surfaces was carried out by cleaning. In these samples, the totals aerobic Germs mesophilic, Yeasts and Moulds, Totals coliforms, Staphylococcus aureus were sought and counted. On the whole, 120 samples were analyzed including 45 samples of surfaces and 15 samples of air of each laboratory, for three months. The results show that in the Laboratory in Process of Accreditation the concentration of the Mesophilic Aerobic Bacteria total and the Yeasts Moulds respectively 42 UFC / 25cm^2 and 37 UFC / 25cm^2 and with 84% of samples in conformity. In the air of the same laboratory, the concentrations were of 10UFC/1000cm ^{3} of esophilic Aerobic Bacteria and 10 UFC/1000cm ^{3} of Yeasts Moulds with 100% of conformity. On the other hand, the Laboratory Not in Process of Accreditation, concentration of the Mesophilic Aerobic Bacteria and the Yeasts and Moulds respectively 128 UFC / 25cm^2 and 45 UFC / 25cm^2 and with 60% of samples in conformity. In the air of the same laboratory, the concentrations were of 1UFC/1000cm^3 of

Mesophilic Aerobic Bacteria and 42 UFC/1000cm ^{3} of Yeasts Moulds with 60% of conformity. The standards of microbiological quality were not still reached in the two laboratories; however those of the candidate to the accreditation were twice closer to the standards of the accreditation than the no laboratory in Process of accreditation.

1. Introduction

In Africa, many laboratories do microbiological quality control of foodstuffs. However, to be valid, results from these laboratories must the required international standards. The reliability of microbiological test results are related: (i) to skilled personnel, quality of materials, and on the other hand very tightly to the environment (air, all surfaces directly involved in the realization of the test). Atmospheric microbial contamination consisting mainly by bacteria, moulds and yeasts, and rarely pathogens (Hartemann, 2004). The only compliance with microbiological criteria on a sample cannot guarantee its quality at the end of the test, it goes along with the assurance of the application of good hygiene practices in the test environment (Pierre, 2005). According AFNOR; floors, walls, ceilings, and furniture testing laboratory must be regularly controlled to avoid the appearance of defects of continuity that create privileged fouling areas and are sources of contamination. The principles governing the export of food products from developing countries to the EU, involve four essential elements out of which the credibility of the technical content of the controls. Thus the "quality program of UEMOA", developed by UEMOA in partnership with EU and ONUDI has provided substantial support to strengthen: standardization activities, accreditation laboratories and certification of country member' firms (Mouillet 2010). This accreditation, which is none other than the set of features and activities that give one laboratory to provide satisfactory products and services to quality requirements. This study aimed to assess the risk of contamination to manage these risks in an appropriate and consistent manner. This was to assess the microbiological quality of the environment of two microbiology laboratories, the first in the process of accreditation and the second is not in the process of accreditation.

2. Materials and methods

2.1. Samples collection

2.1.1. Collection of surface samples

Sampling was carried out by the swab method. A swab dipped in 5 ml of sterile saline solution diluted to 1/1000 was used to clean a surface of 20cm2. The swab was then immediately put in its case, all enclosed in the ice to finally be transported. Faith in the laboratory, the samples were stored directly and immediately analyzed.

2.1.2. Collection of airs samples

A biocollector (Air Pollution Sampling & Measurement brand LaMotte) was used for the collection of air samples in the laboratories. 1000 cm3 air sample was aspired, from the laboratory's ambient air, through physiological water in the device at a rate of 2 L /min. The sample, consisting of microorganisms of the air volume sucked suspended in physiological saline, was subjected to two serial tenfold dilutions before analysis.

2.1.3. Sample preparation and analysis

Mesophilic Aerobic Bacteria, Yeast and Moulds, Total coliforms and Staphylococcus were searched and counted in each sample dilution.

Determination of mesophilic aerobic bacteria: to count the total bacteria in samples, 1 ml of each sample dilution was deposited in the bottom of two Petri dishes and then 15 ml of PCA medium was poured on the surface, well mixed by rotary movements and dried, then a second layer of PCA was cast and dried at room temperature before being incubated. All plates were incubated for 72 hours overturned in the oven of 30 ° C (NF EN ISO4833: 2003).

Determination of total coliforms: From all sample dilutions, 1 ml was deposited in the bottom of two Petri dishes and a volume 15 ml medium agar neutral red crystal violet and bile (VRBL) has been poured and mixed well by rotary movements and dried, and then a second layer of VRBL was cast and dried at room temperature before being incubated. The plates were incubated for 24 hours in an oven at 30 ° C (NF ISO 4832: 2006).

Determination of Staphylococcus: 0.1 ml was deposited on the surface of Bair Parker culture medium with egg yolk and potassium tellurite, well spread with a spreader and then left at room temperature before being incubated inverted for 48 hours in the oven to 37 ° C, according to Standard NF V08 057: 2009.

Determination of yeast and moulds: The way, 0.1ml samples were deposited on the surface of the glucose agar with chloramphenicol each Petri dish, although spread with a spreader and then dried at the temperature of laboratory before being incubated inverted at 25 ° C for 72 hours to 120 hours, according to the NF VO8 059: 2002.

3. Results and discussions

3.1. Quality of surfaces in the laboratory in accreditation process (lap) and the laboratory not in accreditation process (LNAP)

Table 1

Average concentration of the mesophilic aerobic bacteria, yeasts and moulds, totals coliforms and staphylococcus on surfaces at LAP and at LNAP.

Parameters	Laboratory in accreditation process (LAP)	Laboratory not in accreditation process (LNAP)	EU Norms
Mesophilic Aerobic Bacteria	42.67	128.2	25 UFC/25 cm^2
Yeasts/Moulds	37.56	45.78	25 UFC/25 cm^2
Totals Coliforms	10.00	15.56	25 UFC/25 cm^2
Staphylococcus	10.00	10.67	25 UFC/25 cm^2

In table 1, at LAP surfaces level, average concentrations obtained with respect to total Mesophilic Aerobic bacteria and Yeasts / Moulds are significantly higher than normal concentrations required. Those obtained in relation to Total Coliforms and Staphylococcus aureus are below the norm.

In LNAP, average concentrations obtained at surfaces compared to sprouts Mesophilic Aerobic Totals and Yeasts / Moulds are by far higher than normal concentrations recommended by cons, those obtained in relation to Total Coliforms and Staphylococcus aureus are below standard.

Table 2

Percentage of surface comformity in the LAP and LNAP.

	% of surface comformity in LAP	% of surface comformity in LNAP
Mesophilic Aerobic Bacteria	87	71
Yeasts/Moulds	87	71
Totals Coliforms	100	93
Staphylococcus	100	98

3.2. Quality of the air with the LAP and the LNAP

At LAP, the concentration of studied microorganisms varies between 10 and 10.63 with the low values are obtened for coliforms and Staphylococcus (10) and the highest number for the total aerobic germs (table 3). Compared to the EN norms for these microorganisms, the average air concentrations obtained are well below the standard required for all parameters.

At LAP, the quantities of studied microorganisms varies between 10 and 10.63 with the low number for coliforms and Staphylococcus (10) and the highest number for the total aerobic germs (table 3). Compared to the EN norms for these microorganisms, the average air concentrations obtained are well below the standard required for all parameters.

In LNAP, average concentrations obtained at surfaces compared to sprouts Mesophilic Aerobic Totals and Yeasts / Moulds are by far higher than normal concentrations recommended by cons, those obtained in relation to Total Coliforms and Staphylococcus aureus are below standard.

Table 3
Average concentration of the Aerobic germs mesophilic, yeasts and moulds, Totals Coliforms, Staphylococcus in the samples of air of the LAP and LNAP and acceptable maximum concentration.

	Laboratory in accreditation process (LAP)	Laboratory not in accreditation process (LNAP)	EU Norms
Mesophilic Aerobic Bacteria	10,73	50,67	15 UFC/1000 cm^3
Yeasts/Moulds	10,67	42	15 UFC/1000 cm^3
Totals Coliforms	10	10,67	15 UFC/1000 cm^3
Staphylococcus	10	10	15 UFC/1000 cm^3

Table 4
Percentage of conformity of the samples of air in the LAP and LNAP.

	%age of air conformity in LAP	% age of air conformity in LNAP
Mesophilic aerobic bacteria	100	60
Yeast/Moulds	100	60
Totals coliforms	100	93
Staphylococcus	100	100

4. Discussion

In the Laboratory in process to be accreditation, we determined the microbiological quality of the environment (air and surfaces). Surface samples tested gave average concentrations of: (i) 42,67 UFC/25 cm2 for total bacteria and 37.56 CFU/25 cm2 for yeasts and moulds which are above the EN norm, (ii) 10 CFU / 25 cm2 for coliforms; 10 CFU / 25 cm2 for Staphylococcus which respect the EN norm and a microbiological average rate of 0.13, 0.13, 0, 0, respectively for the parameters mesophilic aerobic bacteria, yeast and moulds, total coliforms and Staphylococcus.

These concentrations for mesophilic aerobic bacteria, yeast and mold are above the norm, however, the rates obtained from such analyzes correspond to those of Duffaye and al. (1992) obtained in their second quality control of the surfaces of hoods used in the preparation of injectables.

But with a different method, the resulting microbiological rates are lower than those obtained by Maia et al. (2008), with an average contamination rate of 0.4 at the local preparations of surfaces in the radio Pharmacy University Hospital TOWER,

The air samples analyzed gave average concentrations 10.73 UFC / 1000 cm3; 10.67 UFC / 1000 cm3; 10 CFU / 1000 cm3; 10 CFU / 1000 cm3 all below standard and microbiological average rates of 0.13; 0.07; 0; 0 for respectively total aerobic mesophilic bacteria, yeast and mould, total coliforms and staphylococcus.

These air results are different from those of average rates Duffaye et al., (1992), with the same method used. But are different from those of Chaari-chebel et al., (2007) who scored for air samples, more than 90% non-compliant rates in the operating room of the University Hospital of Monastir (Tunisia).

In the Laboratory not to be accredited, we determined the microbiological quality of the test environment and the surface samples analyzed gave microbiological averages of 128.22 CFU / 25 cm2; 45.78 CFU / 25 cm2; 15.56 CFU / 25 cm2; 10.67 CFU / 25 cm2; and a microbiological average rate of 0.29; 0.29 0.07; 0.02 respectively for mesophilic aerobic bacteria settings, yeast and moulds, total coliforms and staphylococcus.

The averages obtained for the determined microorganisms on these surface, are well above the required standard and average rates, but confirm those of Duffaye et al., (1991) obtained for their first quality control of surfaces of the laminar air flow hoods used for preparation of injectable drugs,

These air results are different from those found by MAIA et al., (2008) who obtained average concentrations of: 50.67 CFU / 1000 cm3, 42 UFC / 1000 cm3 10.67 UFC / 1000 cm3, 10 CFU / 1000 cm3 and an average rate of

0.4; 0.4 for aerobic mesophilic respectively, yeast and moulds, Microbiological; 0.07; 0 for total coliforms and staphylococcus.

With the same methods used, results of air samples analyzed differ from those of CHAARI-CHEBEL et al. (2007), who obtained the results of air samples over 90% in the non-compliant operating room of the University Hospital of Monastir (Tunisia), and the average concentrations which still exceed OMS standards. These differences can be explained partly by the lack of air treatment in the operating room of the hospital Monastir as mentioned by the author and also the number of samples processed.

With a different sampling method, the air average rates in the laboratory to be accredited and the one not to be accredited, are in accordance with those of Bussieres et al., (2006) who obtained 0.06 as microbiological average in the sterile preparation hood in the area of hemato-oncology in a tertiary care hospital in Quebec.

5. Conclusion

In view of the results, this study allowed us to conclude that in the laboratory in accreditation process, the microbiological concentration recommended is not yet achieved: 42.67 CFU / 25 cm2 et 37.56 CFU / 25 cm2 for respectively mesophilic aerobic germs, and yeasts and molds. However, we can say that the laboratory is fully impregnated in the quality approach towards accreditation of its food microbiology unit (80% and 100%) of conformity and the perfect application and cleaning protocols are enable continuous improvement of quickly reach the fixed standard values.

In the laboratory not in the process of accreditation, the recommended concentration is reached 128.22 CFU / 25 cm2 ; 45.78 CFU / 25 cm2, but the average compliance rates achieved are still much higher, so a good and perfect application of cleaning protocols and a continuous improvement will allow the laboratory to be in the standard.

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