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Monitoring of microbiology laboratory working environment

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ABSTRACT:

A total of 80 samples of in-use disinfectant solutions from floor mop bucket were collected over four working weeks and bacterial contamination measured by the *in-use* test technique to ascertain the level of sterility of three important rooms in the CSIR-Food Research Institute's accredited Microbiology Laboratory. Phenolic compound represented by the brand name Crusade® was used at a concentration of 4 % (v/v), with Tween 80 in diluent of Salt Peptone Solution as the neutralizer. High average bacteria survival levels were recorded early, during three and six hours of disinfection of the floors throughout the four weeks study period, with an average bacterial count of $\log 10^3$ cfu/ml. Later disinfection procedures at 9 and 24 hours resulted in the reduction in counts, with an average load of $\log 10^2$ cfu/ml. Comparative mean counts (cfu/ml) per day of disinfection showed that the microbial load during disinfection was high at the beginning of each working week, usually on Mondays and Tuesdays with noticeable reductions through Wednesdays and then lower counts on Thursdays and Fridays of each week. The high levels detected each Monday during the four weeks may be attributed to bacteria build up over the two non working days of Saturdays and Sundays when no cleaning and disinfecting activities were undertaken. None of the samples taken met the satisfactory limit of less than 250 cfu/ml after 24 hours of incubation at 30°C as described by earlier researchers for the *in-use* testing of working disinfectant.

Keywords:

laboratory monitoring, environment, *in-use test*, bacterial contamination, disinfectant.

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INTRODUCTION AND LITERATURE REVIEW

The monitoring of working environment of any testing or calibrating laboratory is a requirement of the International Organization for Standardization (ISO) and International Electro-technical Commission (IEC) Standard 17025:2005 (E). The ISO 17025 Standard is the current management system in place at the microbiology laboratory.

Determination of conduvcive environment for microbiology testing laboratories involves establishment of conditions within operational areas of activities that do not invalidate the results and thus meet internationally acceptable limits of criteria for the level of microbial load. The satisfactory fulfillment of this among other requirements, enhance the reliability of test results generated especially when the prescribed specific analysis for the corresponding standard test method is rigorously complied with.

Achieving the desired working environment depends to a large extent on the choice or selection of the appropriate cleaning and disinfecting agents that has to be complemented by equally more effective operational procedures with staff safety as paramount, especially during fumigation. These cleaning and disinfecting agents must essentially be user friendly in terms of safety as well as having maximum effectiveness against contact surfaces. The application of these agents is influenced by manufacture's attributes as well as their chemical composition of which their effectiveness on certain materials and situations have been evaluated using standardized tests (AOAC 1960, Kelsey and Sykes 1969).

As part of the laboratory commitment in meeting the required standards, monthly evaluation of the laboratory environment through initial stages of fumigation, de-fumigation, cleaning and disinfection are carried out. These activities are followed by microbiological examination of swabs and exposed plates on Plate Count Agar and Oxytetracycline Glucose Yeast Extract Agar (OGYEA) to monitor total counts as well as moulds and yeasts respectively.

When results obtained are found to be within the acceptable satisfactory limit, the testing officer as described in the management system operational manual approves and documents them. When the results fail to meet the required limits, corrective measures are taken by repeating fumigation, de-fumigation, cleaning and disinfecting exercises in order to meet the acceptable criteria limit. This study was therefore carried out to determine the sterility of the working environment in the internationally accredited microbiology laboratory of CSIR-Food Research Institute.

Control and management of the laboratory environment is necessary to reduce to minimum contaminating microorganisms. Environmental monitoring aims to determine if a site of concern is a source of pollution/contamination (Au *et al*., 2000), by using standard acceptable procedures, such as measurement of total bacterial load and pathogens, percentage total coliforms and pathogens such as *E. coli.*

The routine environmental monitoring program is a critical aspect of documenting the state of control of the microbiology laboratory facility. The qualification or requalification of an aseptic facility depends in large part on the demonstration of controlled microbial conditions; especially for selection of sample sites for environmental monitoring (Farquharson, 2002).

In determining the appropriate parameters of an environmental monitoring programme, the scope and purpose of the programme are important in the microbiology laboratory, the purpose of which is to document the state of control of the facility (FDA, 2004), so that any analyses carried out for clients is deemed to have occurred in a sterile environment. Environmental monitoring is directed to promptly identify potential routes of contamination, allowing for implementation of corrections before contamination occurs (FDA, 2004). Environmental monitoring data provides information on the quality of the analytical environment under which samples are examined and also the state of control of the laboratory. However, the trend of the data is the critical aspect (Hussong and Madsen, 2004); thus pristine environmental monitoring data for an aseptic analytic laboratory specifically addresses the state of control of that facility.

Farrington (2005) observed that it is undeniable that data generated and particularly the trending of these data show the state of control of a facility. He indicated that the regulatory concern over contamination from environment makes sense, but must be applied with judgment and scientific rigor. However, the major problem with environmental monitoring data is the fundamental imprecision and variability of these data. This imprecision renders the data all but useless as quantitative predictors of the system, but valuable as raw data for the determination of trends in the

facility as a whole. Consequently the importance of considering locations that poses the most microbiological risk to the sample is a key part of the monitoring program. It is therefore important to monitor the microbiological quality of the critical areas in the laboratory; such that air and surface samples should be taken at locations where significant activity or product exposure occurs during analysis of clients' samples. Critical surfaces that come in contact with the sample should remain sterile throughout analytical operations. When identifying critical sites to be sampled, consideration should be given to the points of contamination risk in the analytical process. Additionally, environmental monitoring locations should be described in Standard Operating Procedures (SOPs) with sufficient detail to allow for reproducible sampling of a given location surveyed. Written SOPs should also address elements such as frequency of sampling, when the samples were taken, duration of sampling, sample size (e.g., surface area), specific sampling equipment and techniques, alert and action levels, and appropriate response to deviations from alert or action levels (Bordner *et al*., 1978). This means that the sites used in the routine environmental monitoring programme must be justified and identified (FDA, 2008; CDC, 2010).

For specification of the number of sites for qualification studies in environmental monitoring, the International Organization for Standardization (ISO, 1999) described a method to determine the number of sampling sites for site qualification. The determination of the minimum number of sample sites by the following equation is recommended:

N_L=ÖA

Where,
NL is the minimum number of sampling locations (rounded up to a whole number)
A is the area of the clean room or zone in meters^2.

Passive air sampling by settle plates procedure is a frequently used to measure clean room or controlled zone monitoring. Settle plates have several advantages chief among them is the ability to remain in continuous exposure for up to four hours (EU, 2008) where extended exposure times must be demonstrated via demonstration of the growth promoting capabilities of the aged and exposed media. In addition, passive viable monitoring (settle plates) is not disruptive to the immediate environment and so may possibly sample sites to very near product exposure points. Besides, settle plates are not as prone to variation among different vendors but also are active samplers (Yao and Mainelis, 2006).

MATERIALS AND METHODS

Equipment

The equipment used were Incubators (Memmert, GMBH, Germany, model ICP 600 set at 30°C and 25°C), Autoclave (Prioclave, Ltd., model PS/ LAC/ EN 150), Hot air oven (Elektrohelios , model 28562) and Micropipette (Finnpipette, Labsystem, model 4,500).

Sampling Sites

Inoculation Rooms I and II and the Reading room of the Microbiology laboratory were investigated. Inoculation room I (coded IR1) has dimensions of 3.0 x 4.5 m, and partitioned by a wooden door from inoculation room II. Inoculation Room I is used for microbiological analysis of non-pathogenic microorganisms. Inoculation room 2 (coded IR2), adjacent to IR1 has the same dimensions and used for the analysis of pathogenic microorganisms. The reading room (coded RL) is a room with dimensions of 6.0 x 9.0 m where sensitive equipment like microscopes, incubators, refrigerators, colony counter and a freeze dryer are located. This room is also used for reading of plates, microscopy and biochemical examination of cultures.

Methods

Fumigation

Two to three grams of Potassium permanganate crystals were placed in each of 90 mm-diameter Petri dishes positioned at the four corners of each room. To each dish with the crystals 3.0 ml of 37.0 % Formalin was added quickly and the doors were shut immediately. The frequency of fumigation was once in every month as required per instructions in the Microbiology Technical Manual. This was preferably done over the weekend for 48 hours to allow for maximum diffusion of the gases into openings and surfaces.

Safety Precaution

In order to ensure protection of the respiratory tract, eyes and skin, nose mask, gloves and goggles were used since Formalin is a strong irritant and toxic reactant. All the doors leading into the rooms being fumigated were closed and boldly labeled *FUMIGATION IN PROGRESS. DO NOT OPEN.*

De-fumigation

De-fumigation was carried out by placing

dilute ammonium solution in 500 ml capacity beaker which by chemical affinity absorbs the fumes generated normally.

Cleaning and disinfecting of floors

Laboratory floors were first mopped with detergent solutions and then with disinfectant solution prepared as 200.0 ml of crusade (a phenolic-based) in 5.0 litres of clean tap water. The contact time allowed between the disinfectant and the floor surfaces was five minutes, before subsequently mopping with clean potable tap water.

Cleaning and disinfecting of bench-tops and tables

Detergent solution was prepared in 2.0 litre capacity open plastic bowls. Clean (autoclaved) hand towels were dipped into the solution and used to clean the tables, followed by cleaning with potable tap water and then finally with disinfectant (TIMSEN PCS with active ingredient concentration of 40 % dry bead form of n-Alkyl[60 % C14, 30 % C16, 5 % C18] dimethyl benzyl ammonium chlorides). The contact time allowed between the bench surfaces and the disinfectant was five minutes.

Microbiological Examination

Preparation of Swab Templates

Templates of 50 mm^2 area were made from hard card. These were then wrapped in aluminum foil before autoclaving at 121°C for 30 minutes in an Autoclave (Prioclave Ltd, model PS/LAC/EN 150).

Swabbing

Each template was removed aseptically and placed on the bench surface. A sterile swab stick was dipped in diluent of Salt Peptone Solution [SPS, prepared from Peptone (Oxoid L 37) and Sodium Chloride (Merck K 26025300)] then squeezed on the inside of the test tube wall to remove excess liquid. With the template pressed firmly on the bench surface, the moist swab stick was used to run severally over the test area (50 mm^2) and then placed back into the test tube. The end of the swab stick protruding from the test tube was broken off before closing the test tube with cotton wool or cap.

Aerobic Plate Count, Mould and Yeast Count

Aerobic Plate Count was analyzed by the method of NMKL 86 (2006), and the Yeast and Mould Count by ISO 7954 (1987). One millilitre (1.0 ml) of the swab preparation in SPS was inoculated into a 90 mm diameter sterile petri dish by means of Micropipette (Finnpipette, Labsystem, Model 4,500). Another 1.0 ml of the swab preparation in SPS was inoculated into a second 90 mm-diameter sterile petri dish. One petri dish was mixed with molten Plate Count Agar (PCA, Oxoid CM 325) maintained at 45°C for enumeration of total bacteria count. The other petri dish was mixed with molten Oxytetracyline Glucose Yeast Extract Agar (OGYEA, Oxoid CM 545, with supplement Oxoid SR 0073) at 45°C for Mould and Yeast count. Both petri dishes were allowed to set at room temperature (25°C). The inoculation and subsequent plating procedures were repeated for swab preparations taken from the various swab sites and surfaces.

The PCA plates were incubated at 30°C for three days, while the OGYEA plates were incubated at 25°C for five days in Memmert incubator (GMBH, Germany and model ICP 600). All glassware (petri dishes) was sterilized using Hot air oven (Elektrohelios, model 28562). The media and diluents were sterilized in an autoclave (Prioclave Ltd, Model PS/LAC/EN 150).

Preparation of Exposed Plate for PCA and OGYEA

Three plates each were prepared from molten PCA and OGYEA. The plates were then dried at 55°C for 30 minutes. One set of PCA and OGYEA plates was placed at each of three locations within the room under monitoring. The plates were then exposed by opening the top lids for ten minutes and then closed immediately.

The PCA plates were incubated at 30°C for three days while the OGYEA plates were incubated at 25°C for five days in Memmert incubator (GMBH, Germany, Model ICP 600).

RESULTS AND DISCUSSIONS

Total aerobic plate count of swabs on Plate Count Agar (PCA)

The results obtained for examination of swabs on Plate Count Agar/50 square millimetre (50mm^2) of aerobic bacteria population of the Inoculation rooms 1 (R1) and 2 (R2), and also the Reading laboratory (RL) is as shown in Table 1. The average aerobic counts of Inoculation Room 1, Inoculation Room 2 and the Reading laboratory ranged between 15.7 – 32.7, 15.7 – 27.0 and 10.0 – 19.0 cfu/50mm^2 respectively for the 12 months of investigation. This indicates that the level of contamination in the Reading laboratory is less than in both Inoculation rooms 1 and 2. The trend will be described as R1>R2>RL (Fig.1). It was observed that the contamination level in R1 was the highest for most months, followed by R2, with RL

recording the least levels of bacteria load from the 50 mm^2 swab sites. Inoculation room R1 which recorded the heaviest microbial load has more human activity, since it is the room where most samples received from food industries are first deposited and analyzed. Inoculation room R1 therefore experiences more personnel activity than R2 and RL. Room R2 where analysis for pathogenic organisms usually has few industrial samples sent there, as compared to R1.

The least microbial load in the Reading Laboratory was expected as only reading of plates is carried out in the room. These plates would have been removed from the incubators and may not cross-contaminate the room as compared to the activities in both R1 and R2.

Throughout the twelve months of study, it was observed that increase in counts occurred during times of maximum human activity in the laboratory and coincided with the time students were allowed into the laboratory for attachment training and other research activities (January-February, May - June and September – November) (Table 1). Thus the increased levels may be due to contamination from the body and clothes of the students as well as personnel entering the laboratory for various activities. Such relationship in levels of contamination of indoor air in the laboratory is in accordance with earlier studies (Toivola *et al*., 2002; Karwowska, 2003; Fleischer *et al*., 2006) where the heaviest bacterial contamination was observed in heavily populated workplaces. Bhatia and Vishwakarma (2010) observed similar correlation and indicated that concentrated population in indoor environment is a factor for the content of airborne microbes, where the load is highly influenced by the number of occupants and their activity.

To ensure maintenance of laboratory sterility, it is critical that aseptic techniques should be used at all times. Such techniques include ensuring that only materials that have been sterilized will have contact with sterile items and surfaces; as in using sterile instruments like forceps to handle sterilized materials. In critical areas like the inoculation and reading rooms of the laboratory, unacceptable turbulence due to rapid movement that will disrupt control parameters to sterility is avoided or minimized. Thus the principle of slow, careful movement is encouraged in these clean rooms to avoid contamination. This accounts for the findings in this study that the recommended acceptable criteria limits for both swab examination (100 cfu/50 mm^2) and for exposed plate examinations (20 cfu/10 minutes) were met.

Training and retraining of staff have been ensured such that personnel are vested in aseptic techniques in the laboratory. Also routine evaluation by supervisory personnel of staff performance and conformance to written procedures and aseptic techniques being critical to trusting the results of the laboratory has been paramount. Thus for instance, only personnel donning the proper clean outfit (white gown) are permitted to enter the laboratory, especially such critical areas (Korczynski, 1992) in order to avoid contamination. Thus the comparatively low counts throughout the twelve months are acceptable. Insistence in the use of the laboratory coat when working in the clean rooms is due to the provision of a barrier between the body and exposed sterile materials to prevent contamination from particles or microorganisms shed from the body. Thus covering of the hair on the head with a hood; covering of even the beard or moustache, as well as wearing elastic gloves on the hands as a barrier during decontamination procedures all enhance reduction in cross-contamination from the individual (USP, 1999). In addition, personnel performing procedures in these rooms are prevented from speaking or coughing (Guzewich and Ross, 1999), picking the nose, mouth or ear during analytical procedures since they are in direct proximity of the critical areas and may transfer enteric and respiratory pathogens. While pathogens carried on the hands may be a major source of contamination in the laboratory (Taylor and Holah, 2000), air as a medium may be laden with dust, straw-type debris and even insects (Brown, 1996) which may also account for some of the contaminating microorganisms. However, data generated over the twelve months monitoring (Table 1) and the trend show the state of control of the microbiology laboratory in accordance with observation by Farrington (2005); and with the total aerobic plate count of the 50 mm^2 swabs sites on Plate Count Agar (PCA) indicating that the Reading room was less contaminated than both Inoculation rooms (**Table 1**).

This is expected as more human interaction and activity are carried out in both Inoculation rooms as compared to that in the Reading room; especially as food samples from industries are brought into the inoculation rooms for analysis.

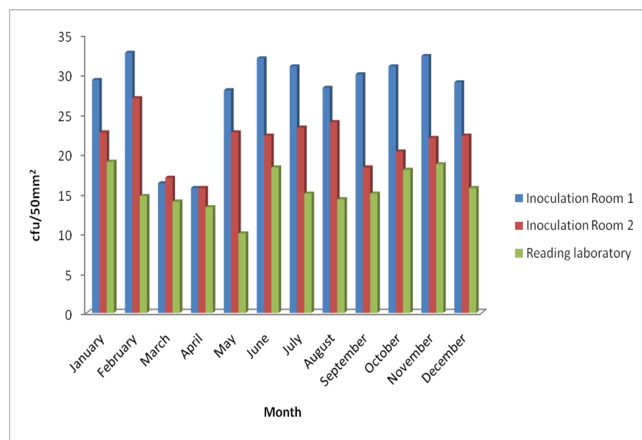
Table 1. Mean aerobic bacteria population in swabs of Inoculation Rooms 1, 2 and Reading room

Month	Average Plate count (Mean cfu/50 mm^2)		
	Inoculation Room 1	Inoculation Room 2	Reading laboratory
January	29.3	22.7	19.0
February	32.7	27.0	14.7
March	16.3	17.0	14.0
April	15.7	15.7	13.3
May	28.0	22.7	10.0
June	32.0	22.3	18.3
July	31.0	23.3	15.0
August	28.3	24.0	14.3
September	30.0	18.3	15.0
October	31.0	20.3	18.0
November	32.3	22.0	18.7
December	29.0	22.3	15.7

Figure 1 therefore shows that the Reading room being a place for mere observation and reading of incubated plates would have the least average bacteria load comparatively, indicating less contamination by microorganisms throughout the study period.

During analysis of food samples brought into the inoculation rooms from industry, there is the possibility of minute particles falling on the swab areas. Such particles may likely encourage the growth of microbial cells that could aggregate into masses and entrap debris, nutrients and other microorganisms to form a microbial biofilm (IFT, 1994). This may have accounted for the comparatively larger numbers of counts in the two inoculation rooms (**Fig. 1**); especially as these biofilms have been reported to increase the resistance of embedded microorganism (IFT, 1994), with adverse conditions further stimulating microorganisms to grow in the biofilms (Van der Wende *et al*., 1989; Van der Wende and Characklis, 1990) and also Langeveld *et al*. (1995) who observed the growth of thermophilic *Streptococcus thermophilus*.

Fig. 1. Average bacteria load on Plate Count Agar from swab sites for twelve calendar months



Yeast and mould counts of swabs on Oxytetracycline Glucose Yeast Extract Agar

The results obtained for the mean population of yeasts and moulds from 50 square millimeter swab areas on Oxytetracycline Glucose Yeast Extract Agar (OGYEA) plates are as shown in **Table 2.** It was observed that yeasts and moulds for R1, R2 and RL ranged between 5.0 – 12.0, 4.7 – 11.7 and 4.0 – 10.3 cfu/50mm^2 respectively for 12 months (Table 2). The trend followed the same pattern as for the aerobic mesophilic bacteria count where the contamination level in R1 was the highest for most months, followed by R2, with RL recording the least levels of yeasts and mould load from the 50 mm^2 swab sites. The trend can be described as R1>R2>RL (Fig.2). Significantly, it was observed that both the yeast and mould counts and also the total aerobic plate counts were lowest for the months of March and April (Fig. 1 and Fig. 2). This may be due to reduced activity in the laboratory and effective sanitation and disinfection during that period. However, the acceptable criteria limits of 100 cfu/50 mm^2 were met for all the swab examination for both aerobic bacteria (Fig. 1) and also the yeasts and moulds (Fig. 2); since none of the results either reached or exceeded this limit.

The low results for the yeasts and moulds recorded in this study (Fig. 2) indicate that the surfaces and air in the laboratory did not have large population of mould spores to cause multiplication

Table 2. Mean Population of Yeasts and Moulds in swabs of Inoculation Rooms 1, 2 and Reading Laboratory

Month	Average Yeasts and Mould Counts (Mean cfu/50 mm^2)		
	Inoculation Room 1	Inoculation Room 2	Reading laboratory
January	8.7	7.0	7.0
February	10.3	10.7	9.3
March	5.0	4.7	4.0
April	5.7	5.0	4.0
May	8.6	8.0	7.0
June	9.3	8.0	8.0
July	7.7	7.7	8.3
August	9.0	7.3	7.3
September	8.0	8.7	6.0
October	10.3	10.7	7.3
November	12.0	11.7	10.0
December	11.0	7.7	10.3

of numbers. it could therefore be reported that the validity of these test results emanating from such an accredited laboratory would be trusted and reproducible since the processes, systems and procedures are maintained. Thus an instituted vigilant and responsive monitoring program for both personnel and surfaces is performed regularly with documentation of findings. This is augmented with follow-up and investigative procedures that include more sampling, increased observation and retraining regimes; that include also maintenance of personal hygiene of personnel like regular hand washing. These activities are therefore reflected in the low bacteria, yeasts and mould numbers in this study. Acceptable monitoring methods used for assessing the environmental quality in the microbiology laboratory include surface, active air and passive air (settling plates) monitoring. Environmental monitoring of surfaces involves sampling contact surfaces, floors, walls and equipment on a regular schedule by means of touch plates, swabs and contact plates. With active monitoring, assessment and documentation of microbial air quality is carried out with membrane (gelatin) samples, although other methods like impaction and centrifugal are used in other laboratories. These different devices have their individual advantages and disadvantages even though they are all used to measure the number of organisms per volume of air sampled. In this laboratory, these devices are used strictly according to the manufacturer's specifications to ensure compliance and reliability of results. In the months of February, October and November, higher counts in yeasts and moulds were recorded (Fig. 2) in line with the increased activity in the laboratory. This indicated that as human activity increased, the air carried greater numbers of yeasts and moulds in the laboratory.

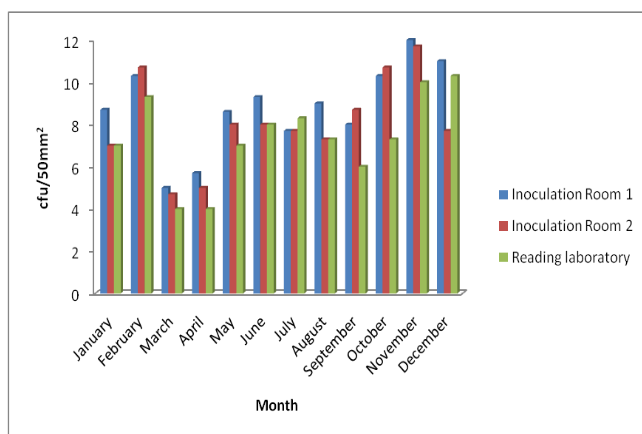
Increased microflora concentration in the outdoor air and the immediate vicinity of the laboratory due to increase in dust particles may affect the yeast and mould counts observed. When the outer doors leading to the laboratory are frequently opened during high human traffic, there is the possibility of introducing microorganisms indoors through dust particles from outdoors. The prevailing high temperatures and relative humidity were conducive to microorganisms during the time of the study, making it easy for bacteria and mould proliferation. During the onset of the rainy season (March/April), less dust particles laden with fungal spores and bacteria are blown around as in the hot season, thus accounting for decreased yeasts and mould during that period (Fig. 2). The higher counts occurred when the weather conditions were dryer and dustier (October – December) (**Fig. 2**).

As this study which was concerned with quanlitative and not quatitative aspects of contaminating microflora, further studies would be needed to specify what kind of organisms abound in the laboratory. This will aid in the kind of disinfection, sanitization and monitoring procedures to embark on in order to reduce the microbial load. It will also serve to provide information to personnel on possible health concerns due to fungal spores. In earlier studies (Hunter *et al*., 1988; Miller *et al*., 1988; Gniadek and Macura, 2003) observed *Scopulariopsis* spp. as a major cause of onychomycosis. Later Stryjakowska-Sekulska *et al*. (2007) isolated the same organism from an enclosed reading room. Having also observed high concentration of outdoor microflora like *Cladosporium* spp., and *Alternaria* spp. in the atmosphere, Stryjakowska-Sekulska *et al*. (2007) contended that this could influence indoor air contamination. Thus in this study, some outdoor microflora may be trapped in the enclosed laboratory environment, thus increasing the numbers of counts on the exposed petri dishes. Additionally, some "indoor moulds" like *Aspergillus* spp., *Penicillium* spp., Rhizopus spp. and *Mucor* spp. which are isolates from some food analysis carried out in the laboratory may also cause increased counts if their spores accidentally escape into the indoor air during reading of plates. Earlier studies have indicated that the microbiological quality of indoor air is formed by two main factors, namely the microbiological composition of outdoor air and also indoor air microbial sources (Abdel Hameed and Farag, 1999; Dharmage *et al*., 1999; Gutarowska and Jakubowska, 2002; Wojcik-Stopczynska *et al*., 2003). Although indoor air is not influenced by environment, season and the weather as much as outdoor air, such influence may provide significant high microbial contamination if access to the laboratory is continuously disturbed by continuous opening of the doors due to increased human traffic.

As there is no official specification for the fungal quality of indoor air as a reference for limits, it makes interpretation of the results of this study difficult. However, during a WHO Expert Meeting, Gorny and Dutkiewicz (2002) proposed maximum

fungal count of 5000 cfu/m^3 for indoors. The proposed limits may not be adaptable to the laboratory environment in this case since more stringent measures are taken to keep the place sterile as an internationally accredited facility. Therefore a reduced figure would be more acceptable as a reference value to suit our situation. The maximum bacteria count at any time in this study did not reach this limit. This augments the sterility measures adopted in the laboratory. In 2001, the American Industrial Hygiene Association (www.wondermakers.com) proposed a limit of 250 cfu/m^3 fungal organisms.

Fig. 2 Average Yeasts and Moulds count on OGYEA from swab sites for twelve calendar months



Aerobic mesophilic bacteria population on exposed PCA plates for 10 minutes

Figures-3 shows the trend of the aerobic mesophilic microorganisms on PCA plates exposed for 10 minutes for each of the 12 months investigated for Inoculation Rooms 1, 2 and Reading Laboratory. The range recorded for R1, R2 and RL were 7.3 – 11.7, 7.7 – 10.0 and 5.7 – 10.7 cfu/10 minutes exposure time. The highest bacteria count of 11.7 cfu/10 minutes was observed for R1 while the lowest count of 5.7 cfu/10 minutes was recorded for RL.

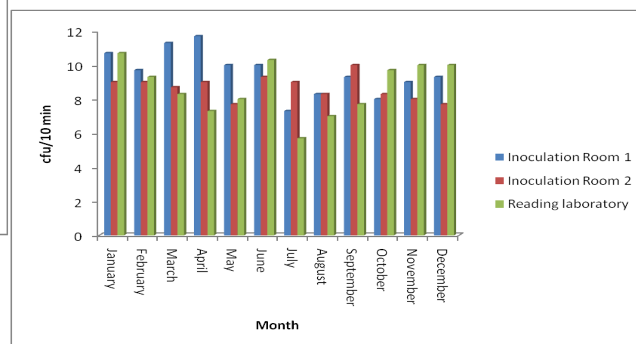
Yeasts and moulds population on exposed OGYEA plates for ten minutes

The population of yeasts and moulds on ten minutes exposed plates from R1, R2 and RL ranged between 5.3 – 7.7, 5.3 – 9.3 and 4.0 – 9.0 cfu/10minutes respectively (**Table 4**). The inoculation room R2 recorded the highest count of 9.3 cfu/10 minutes while the Reading laboratory RL recorded the lowest count of 4.0 cfu/10minutes (Fig. 4). Nevertheless the acceptable criteria limit for the exposed plate is less than twenty colony forming units per ten minutes (<20 cfu/10 min) of

Table 3. Aerobic Bacteria Population on exposed plates of PCA for ten minutes for Inoculation Rooms 1, 2 and Reading Laboratory for twelve calendar months

Month	Average Aerobic Bacteria Counts (Mean cfu/10 min)		
	Inoculation Room 1	Inoculation Room 2	Reading laboratory
January	10.7	9.0	10.7
February	9.7	9.0	9.3
March	11.3	8.7	8.3
April	11.7	9.0	7.3
May	10.0	7.7	8.0
June	10.0	9.3	10.3
July	7.3	9.0	5.7
August	8.3	8.3	7.0
September	9.3	10.0	7.7
October	8.0	8.3	9.7
November	9.0	8.0	10.0
December	9.3	7.7	10.0

Fig. 3 Average Aerobic Bacteria Population on exposed PCA Plates for 10 minutes for twelve calendar months



exposure. None of the plates exposed for both PCA and OGYEA reached or exceeded this value. Consequently the environmental monitoring regime would be considered effective and reliable. However, exposure of the petri dishes for longer periods as 15 minutes and enumeration of types of yeasts and moulds would enhance monitoring activities in the laboratory to achieve effective sterility levels.

In a study of the variability of airborne fungal flora and their monthly distribution in the atmosphere in five different locations for twelve months by means of the petri plate gravitational 15 minutes exposure method using Malt Extract Agar (MEA) and Rose Bengal Chloramphenicol Agar (RBCA), Suerdem and Yildirim (2009) identified 19 fungi genera (*Cladosporium*, *Alternaria*, *Penicillium*, *Phoma*, *Aspergillus*, *Botrytis*, *Chaetomium*, *Chrysosporium*, *Didymocladium*, *Doratomyces*, *Drechslera*, *Fusarium*, *Humicola*,

Mucor, *Rhizoctonia*, *Rhizopus*, *Sporotricum*, *Trichoderma*, *Ulocladium*), the most predominant being *Cladosporium* (27.5%), *Alternaria* (18.5%), *Mycellia sterilia* (13.5%), *Phoma* (7.9%), *Penicillium* (6.7%) and *Aspergillus* (5.9%), with some as important phythopathogens and aeroallergens (Larsen and Gravesen, 1991; Pasanen, 1992; En and Asan, 2001). However Lanjewar (2011) in exposing Petri dish with Potato Dextrose Agar medium for 15 min for 12 months observed *Alternaria alternata, Cladosporium and Aspergillus* species to be the most abundant species (13.66, 5.80 and 5.50% of the total, respectively).

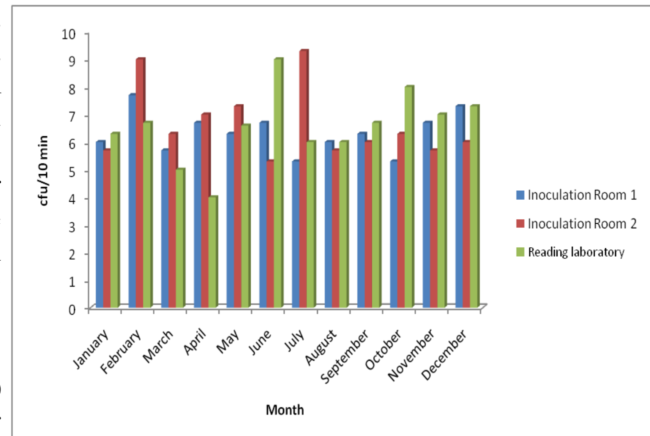
Stryjakowska-Sekulska *et al*. (2007) observed the microbiological quality of indoor air sampled to consist of bacteria and moulds such as *Staphylococcus* spp., *Micrococcus* spp., *Serratia* spp., *Aspergillus* spp., *Penicillium* spp., *Rhizopus* spp., *Cladosporium* spp. and *Alternaria* spp. among which pathogenic and strongly allergenic microorganisms were detected.

Table 4. Average Yeasts and Moulds Population on exposed plates of OGYEA for ten minutes for Inoculation Rooms 1, 2 and Reading Laboratory for twelve calendar months

Month	Average Yeasts and Mould Counts (Mean cfu/10 min)		
	Inoculation Room 1	Inoculation Room 2	Reading laboratory
January	6.0	5.7	6.3
February	7.7	9.0	6.7
March	5.7	6.3	5.0
April	6.7	7.0	4.0
May	6.3	7.3	6.6
June	6.7	5.3	9.0
July	5.3	9.3	6.0
August	6.0	5.7	6.0
September	6.3	6.0	6.7
October	5.3	6.3	8.0
November	6.7	5.7	7.0
December	7.3	6.0	7.3

Passive air monitoring using settling plates involve exposing the nutrient growth medium in the petri dishes to the environment so that microorganisms that settle onto the agar surface are detected qualitatively and/or quantitatively. Therefore such plates are positioned in areas which are viewed as having the greatest risk of causing contamination; and evaluation of the best media and exposure conditions that will produce optimum recovery of low levels of microorganisms from the environment are employed. To avoid migrating microorganisms from uncontrolled or lesser control

Fig. 4 Average Yeasts and Moulds Population on exposed OGYEA plates for 10 minutes for twelve calendar months



areas to the aseptic areas, the doors are kept shut between demarcated zones in the laboratory. The frequency of carrying out such procedures in the laboratory provides a valid database of contaminants and thus makes it easier to monitor, compare data, interpret and plan effective cleaning and sanitization programs to decontaminate the environment. Nevertheless, all media used in the laboratory are validated as capable of detecting the microorganisms (bacteria, yeasts and moulds) at documented conditions of incubation temperature and time. Also any media received in the laboratory are monitored for being capable of recovering such microorganisms by performance of growth promotion tests on prepared media. That provides added validation to the database obtained during environmental monitoring in the laboratory so that appropriate corrective actions can be instituted for suspect areas.

Farquharson (2002) reported that a cleanroom should be one with control on particle contamination, constructed and used as a way to minimize introduction; generation and retention of particles in the room and in which temperature, humidity, airflow patterns, air motion and pressure are controlled.

WHO (2011) indicated that environmental microbiological monitoring should reflect the facility used (room or isolator) and include a combination of air and surface sampling methods appropriate to the facility such as active air sampling; settle (exposure) plates; surface contact plates, swabs or flexible films; operator's gloved hand plates. In accordance with Arora (2004), the quality assurance laboratory under investigation is well lit with dust-free air-conditioned environment

where environmental conditions are monitored daily. Thus in line with Jimenez (2004), the environmental monitoring regime in this laboratory provides the evidence and documentation necessary to determine the efficiency of different systems to prevent microbial contamination in order to control the presence, distribution, and survival of microorganisms in such clean rooms and other controlled environments in the three rooms of the testing microbiology laboratory.

CONCLUSION AND RECOMMENDATIONS

Generally, the results obtained over the twelve months period of monitoring were satisfactory since none of the tests failed the acceptable criteria limit for the different microbiological examinations of swab and exposed plate. This may be an indication that adequate and effective fumigation was accomplished during each monitoring exercise.

The recommended acceptable criteria limits for swab examination is 100 cfu/50 mm^2, while for exposed plate examinations 20 cfu/10 minutes is accepted. These limits were met in all cases for swab and exposed plate examinations. However, there may be the need to extend the time of exposure of plates to one hour and the frequency of carrying out these activities to twice a month so as to maintain higher standards of acceptability. Other studies have exposed the plates for one hour and increased the frequency of monitoring (CGMP, 2008) to every other week. Also, sampling devices such as slit sampler and membrane filters necessary to enhance the reliability of the test results generated should be employed; as the most efficient approach in determining concentration of microorganisms in the air is by the use of these devices for the exposed settle plate test procedure.

It is recommended that:

1. The danger posed by accidental inhalation of Formalin during fumigation and also its irritation and toxic characteristics necessitates the use of protective clothing (gloves, goggles and mask). Subsequently, much more user friendly sterilizing agents should be explored and applied in order not to expose staff to danger.
2. A full complements of equipment necessary for delivering dependable results should be explored in the exposed plate test procedure; in particular with the exposure time, plates should be extended for 30 minutes at bi-monthly frequency, while still maintaining the acceptable criteria limit of 20 cfu/min of exposure.

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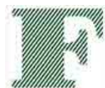

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