ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Advantages and effectiveness of bacterial culture in medical laboratories.

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Manuscript Info

Manuscript History:

Received: 18 June 2015
Final Accepted: 22 July 2015
Published Online: August 2015

Key words:

DCA agar, EMB agar, *E. coli, S. aureus,* TB

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Abstract

In current times, the microbial culture is an efficient technique utilized in the diagnosis of the infectious diseases. According to Pasirayi *et al*. (2011), the culturing of the microorganisms revealing their characteristic traits and significant information as population size, infection producing strength, metabolism, etc. is useful in the treatment and prevention of several infectious diseases. The bacterial culture is also useful in producing effective antibodies and other pharmaceutical products. This research is conducted to find out how far the methods of cell culture processes used in medical laboratories are effective in deriving the desired level of outcomes from the bacterial culture. This research aims to find out the advantages and effectiveness of bacterial culture in medical laboratories through an assessment of the various methods used in bacterial culture in a medical laboratory.

INTRODUCTION

The process of providing predetermined reproducing platforms for the microbiological organisms as bacteria to allow their growth under the controlled laboratory conditions is referred to as microbial culture. Talaro & Chess (2012) highlighted the use of bacterial culture as a primary diagnostic method and tool for determining the reasons of infectious diseases. This process is efficient in selecting or suppressing specific bacteria as per need.The study identifies the effective methods used in the laboratories alongwith an identification of their effectiveness. As pinpointed by Aydin & Aksoy (2013), the bacterial cultures are useful in obtaining antigens to develop serological assays and vaccines. The study of the bacterial growth mechanisms, characteristic traits of their adaptation and reproduction in a new media through cell culture is gaining importance (Jeng*et al*.2012).The major advantages of cell culture is that it enables isolation of specific bacteria for futher studies, determination of their strength of influence, identifying the population size from a sample and their relevant properties as metabolic characteristics are useful in treatment and prevention of several infectious diseases. A mutation selected in a bacterial population can sweep the entire population in 24-48 hours. Thus, the application of the suitable method of culture is significant.

Cell culture concept:

As per the view of McGarrity & Kotani (2013), the cell culture is a technique used in the molecular biology to enable the discovery of significant details in a rapid existing way, in the fields of cytology, toxicology, virology, cytochemistry, and immunology. The method of cell culture is important to facilitate the growth of cells out of their natural environment by controlling and monitoring the physiochemical environment of the cell. The physiochemical environment of the cell comprises of temperature and pH. Ohgaki & Kleihues (2013) identified the 2 major types of cell cultures as primary and secondary cell culture. The primary cell culture comprises of adherent cells and suspension cells. Sub-culturing of a primary cell culture is a secondary cell culture also known as cell line. The main purpose of the secondary cell culture is the provision of fresh nutrients and space for cell growth of the cell lines.

These cell lines are of two types, the finite cell lines with a limited life span and the continuous cell lines with the property of ploidy (Moodle.Kent.ac.uk 2014).

Methods and outcomes of the cell culture:
Sample collection, selection and processing comprise of the major cell culture method to drive the desired outcome. The samples can be selected depending upon indications and need to be representative of disease process and hence, need to be collected prior to the administration of antimicrobial agents (Health & Kiss 2007). A sample collected for the examination is rapidly transported to the laboratory influence test results. It is said that the swabs are popular for sample collections, but yields very small sample for correct microbial testing and need to be applied just to collect samples from mucous membranes and skin membranes, as they have a diverse and large indigenous flora, individual effort need to be put to reduce the contamination of the sample during sample collection (Sorokulova et al. 2014). The skin needs to be disinfected prior aspirating or noting a lesion. Few examples of such techniques are puncture of suprapubic puncture with the aspiration of urine and piercing of the transtracheal area with aspiration of lower respiratory secretions. Samples collected via an invasive method, typically these are collected intraoperatively, necessitate special attention. The inflammation type present can direct the form of microbial analysis performed. Suppose if a caseous granuloma is noticed histopathologically, microbial testing need consist fungal and mycobacterial cultures. The doctor needs to get various specimens for analysis from individual smaller lesions or from a single big lesion. If an eruption is found , the doctor should collect some milliliters of pus along with scraps of the abscess wall for microbial analysis. It is always recommended to collect sample prior the antibiotic administration.
Another bacterial culture method is liquid culture. In this method, selected bacteria culture is dissolved in liquid broth that means a nutrient medium. These are perfect for the preparation of antimicrobial assay. The researcher could inoculate broth with any type of bacterial culture and allow culture to grow overnight. Sometimes shaker is used for uniform bacterial growth (Etyang et al. 2010). A detail examination and through patient history is essential, yet lab tests support the doctors to come to a diagnostic conclusion. The gold standard laboratory test is said to be the culture of bacterial species with the antibiotic sensitivity testing.
Challenges in cell culture:
Infection manifestation
The clinical symptoms of an infection reflect the association between a causative microorganism and a host. The interaction can be affected by microbial virulence factors and the immune status of the host. Indication differs as per the infection severity and site of infection. Therefore, diagnosis is essential that is a composite of information, physical examination, including history, laboratory data and radiographic findings. Infection manifestations depend upon different factors, consisting the route of microbial entry; system tropisms of microorganisms; gender; age; microbial virulence; patient immunological status or disease and presence of implanted materials (Deters 2009).
Microbial basis of infection
Infections are caused by parasites, fungi, viruses and bacteria. The pathogens are endogenous (from normal flora) or exogenous (from animal, human or environmental sources).In case of endogenous infections, a bacterium is a part of patients indigenous flora. Exogenous infections can take place when bacteria are aspired from the upper towards the lower respiratory area or while it enters the mucosal or skin barriers as a result of surgery or trauma (Casadevall & Pirofski 2000).In case of exogenous infections the bacteria are obtained from water or soil. The differential analysis depends on physical examination, careful history, laboratory studies with a selection of roper subjects of microbial analysis. This allows doctors to recommend tests for microorganisms which are expected to be the reason for the infections.

The bacteria used in a medical laboratory:
Clostridium difficileculture: Costridium difficult is gram positive, anaerobic and pathogenic bacteria which is associated with the disease Antibiotic Associated Diarrhea (Rupnik et al. 2009). Culturing of the bacteria requires continuous maintenance of anaerobic environment.
Method of culture:
Clostridium difficile is enteric bacteria, so it is isolated from the stools of human. Perry et al. (2010) used the fecal filtrate for as the source of bacteria, whereas Wilcox (2013) used fresh watery stools as a sample.
After the sample collection, the culture media and the other equipments are sterilized. After the sterilization inoculation of the culture media is done using the sample and then culture plates are incubated in an anaerobic condition for 48 hrs (Dubberke & Burnham 2011).
Depending upon the culture media used colonies of different features appear on the plate after 2-3 days. Bayardelle (2009) used TCCFA agar media for isolation. It is selective media in which colonies of Clostridium difficile appear

yellow in color with filamentous edge. On the other hand Delmee (2005) used blood agar media using 7% sheep blood for the isolation of the bacteria in which the colonies of the bacteria appear as gray white in color having an appearance like ground glass. If the culture is growing well, then a characteristic horse-stable-like odor may be detected. Shin & Lee (2014), in their studies compared the application of the selective agar and Chrome, ID agar and proved that the Chrome, ID agar is more sensitive to the bacteria and can be used for effective isolation.

***Mycobacterium tuberculosis* culture:**

Mycobacterium tuberculosis is the causal organism of tuberculosis. This bacteria is Gram positive and aerobic in nature. **Methods of culture**

To culture the bacteria in the laboratory, the bacteria are isolated from the sputum of the affected persons. The required equipment's are sterilized and then the sputum id processed using NaCl and then inoculated in the culture media. Generally bottles are used for the culture instead of petri dishes. The culture media are then incubated at 37oC temperatures for 7-8 weeks.

There are various culture media that are used for the purpose. Löwenstein–Jensen medium is the most used medium for culture (Stephen & Kandhakumari 2014). It is a selective media in which the colonies appear as brown and granular. Saudi et al. (2012), used blood agar for culture. The colonies appear light gray and shiny. They showed that blood agar is better than the LJ medium for the culture of Mycobacterium tuberculosis. Again Kidenya et al. (2013) reported that the use of micro broth culture can significantly reduce the incubation time compared to LJ solid media.

***Streptococcus pyoge*nes culture:**

Streptococcus pyogenes is infectious Gram positive bacteria that are responsible for a number of diseases in human. It is beta hemolytic and facultative anaerobic bacteria.

Culture method:

The bacteria are collected from the saliva of infected persons. The necessary equipment's and the culture media are sterilized. Then the media are inoculated with the sample and incubated at 350C temperatures for 24- 48 hrs. In an atmosphere containing 5-10% CO_2. Various media are used for the culture. Lamagni et al. (2008) used human blood agar for the detection of the bacteria in which the colonies appear as clear zones due to beta hemolytic process. Again Neal et al. (2007) used Group A Selective Strep Agar using 5% sheep blood for the culture. Studies have shown that the Group A Selective Strep Agar is more selective compared to the human blood agar (gieseker et al. 2002).

Staphylococcus aureus

These are Gram positive bacteria that are associated with infection of the respiratory tract, skin and food poisoning.

Cultural method:

The bacterial sample is collected from food or from mucus of the infected person. The sample is then processed. After the sterilization of the culture media and other equipment, the processed sample is used to inoculate the culture media. The plated containing the culture is then inoculated at 35^0C temperature for 48 hrs.

Various media are used for this purpose. In some studies, Mannitol salt agar is used for S. aureus. It is a differential medium in which the colonies of the bacteria appear as small and surrounded by yellow zones. Again, Kim & Oh (2010) used blood agar for the isolation of the bacteria from food in which the bacteria produce pigments, golden yellow in color. Again Schoeller (2001) used Baird–Parker agar, which is also a selective agar medium for the bacteria and the colonies appear as black with clear surrounding zones.

Lactobacillus acidophilus

These bacteria are beneficial for the human and are used in the production of various yogurt, cheese and many more. These are Gram positive acid producing bacteria that are naturally present in our body.

Cultural method:

The sample can be collected from any product of the bacteria, i.e. yogurt or cheese. The culture media and the other equipment's are then sterilized. After that inoculation is doing in the culture media using the diluted sample. The media are then incubated at 37^oC for 24- 48hrs.

The main culture media that are used for the culture is the Lactobacillus MRS Agar media. It is an enriched and selective media. The pH of the media is maintained at 6.3- 6.7 for optimum growth of the bacteria. According to Sule et al. (2014) MRS agar is the only selective media that can be used to isolate Lactobacillus acidophilus. This media selectively permits the growth of the Lactobacillus acidophilus but for proper identification of the bacteria, other methods should be used. On the other hand, Haakensen et al. (2009) used the Lactobacillus Selection Agar Base for the isolation of Lactobacillus from food. It is also a highly selective medium. In this agar media, the colonies appear as large and white.

Method outline:

The current research follows the positivism philosophy for its conduction. Through the application of the deductive approach and descriptive design, the study efficiently executes the data collection and result derivation method. The primary and secondary data collection methods are efficient to collect relevant data. Through the online survey of laboratory professionals and personal interview of the eminent scientists, the research progress to identify the potential benefits and issues in context of cell culture. The utilization of the SPSS software and graphical representations enable clear and better understanding of the research results. Conducting the research through Gantt chart utilization enables successful completion of the study within specified time limit.

Research philosophy:

Research philosophy enables the initiation of the research through the correct path. Positivism is the philosophy supporting scientific ways of research conduction (Hopkins 2001). The positivism philosophy assists in cross-sectional survey as needed in this research. Observation is independent. Research advancement can be done by the hypothesis as well as a deduction. Thus, this philosophy is used in the conduction for this research.

Research approach:

There are two kinds of research approach, namely, deductive and inductive approach. This research follows the deductive approach. The aim is to test the theory only not to generate a new approach. Inductive approach is basically apprehensive with the generation of a new set of theory which emerge from the given data. The focus of inductive approach is to explore the new observable fact, which is not appropriate for this research. Deductive approach aims the testing theory (McCann et al. 2007). It mainly starts with the hypothesis. Thus, this research progress with the deductive approach considering the cell culture specific data to identify the effectiveness of the cell culture methods in the medical laboratories.

Sampling:

For the quantitative data collection, the random sampling technique is used to select 100 laboratory professionals for survey conduction. Electronically generated close-ended questionnaires are sent to the laboratory professionals through e-mails. For the qualitative data, selective sampling technique is used to conduct interviews with open-ended questions on 4 scientists researching on the bacterial culture subject.

Primary data collection:

Qualitative data collection

This research helps to obtain the deepest knowledge of the issue or the conversation of the bacterial cell culture. In qualitative data collection, data can be collected by taking the interviews of four scientists. Qualitative measures are generally used to accumulate data by a group discussion in the meeting or by interview. Four scientists have been taken for the data collection. Two peoples are from a microbiologist, one is from tissue culture specialist and the last one of the research scientist (Cullimore 2000). As per the questionnaire scientists has given their view about the bacterial cell culture or can say that science has given subjective knowledge about the bacterial cell research.

Quantitative data collection-:

This is basically requisite for the quantification of the amount. This data collection structure is rigid in nature and structured means it includes telephonic interview, online questionnaire, etc. Here 100 laboratory professional has been taken to the survey but out of 100 laboratories professional 60 professional are replying.

Secondary Data collection:

Secondary data collection researcher has taken the help from website, journals, and books to find out the outcomes of the sample.

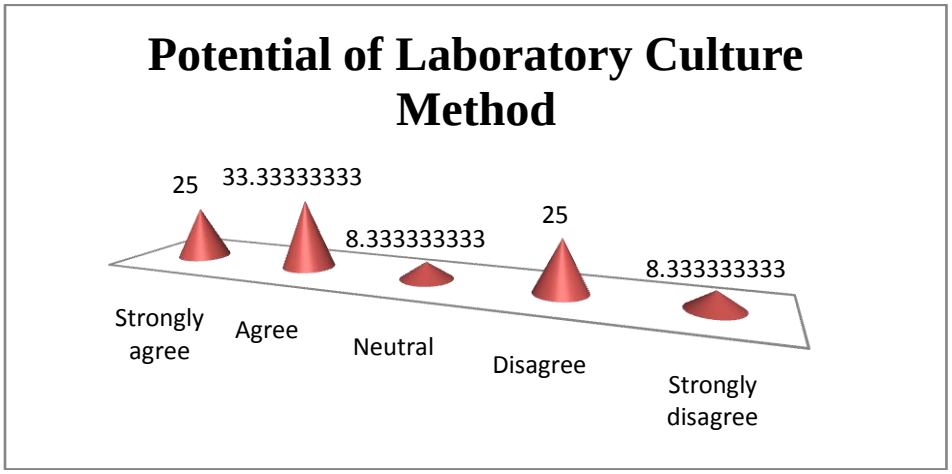
Results and findings:

The questions that are used for collecting quantitative data include-

1. *Do you think that the available laboratory culture methods have the potential to detect all the microorganisms in the chosen sample?*

Table 1: Potential of Laboratory Culture Method

Options	Total participants	No. of respondent participants	Frequency (%)
Strongly agree	60	15	25
Agree	60	20	33.333333
Neutral	60	5	8.3333333
Disagree	60	15	25
Strongly disagree	60	5	8.3333333



Key points and analysis:

According to the views of the laboratory professionals, most of them think that the available culture methods that are used in culturing bacteria has the potential to detect all the microorganisms that are present in a chosen sample. Whereas some of them disagree as they think that it is not possible to detect all the microorganisms.

2. According to your opinion, what is the effectiveness of the selective culture method to obtain pure cultures of bacteria?

Table 2: Effectiveness of Selective Culture Method

Options	Total participants	No. of respondent participants	Frequency
It is very effective	60	20	33.33%
It is effective	60	30	50.00%
Do not know	60	4	6.67%
It is not very effective	60	6	10.00%
It is not at all effective	60	0	0.00%

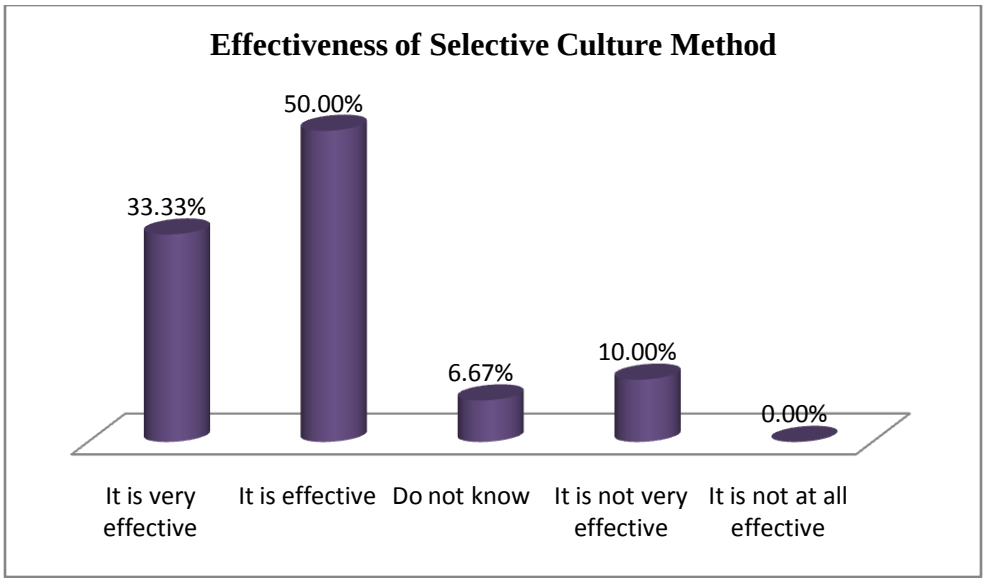


Figure No.: Effectiveness of Selective Culture Method

Key points and analysis:

The data show that most of the laboratory researchers think that the selective culture method can be used for obtaining the pure culture of a microorganism, as they are designed to inhibit all the other microbes except the selected one.

3. Do you think the differential culture technique can be employed to completely differentiate the two organisms?

Table 3: Efficiency of Differential Culture Technique

Options	Total participants	No. of respondent participants	Frequency
Strongly agree	60	15	25.00%
Agree	60	35	58.33%
Neutral	60	6	10.00%
Disagree	60	3	5.00%
Strongly disagree	60	1	1.67%

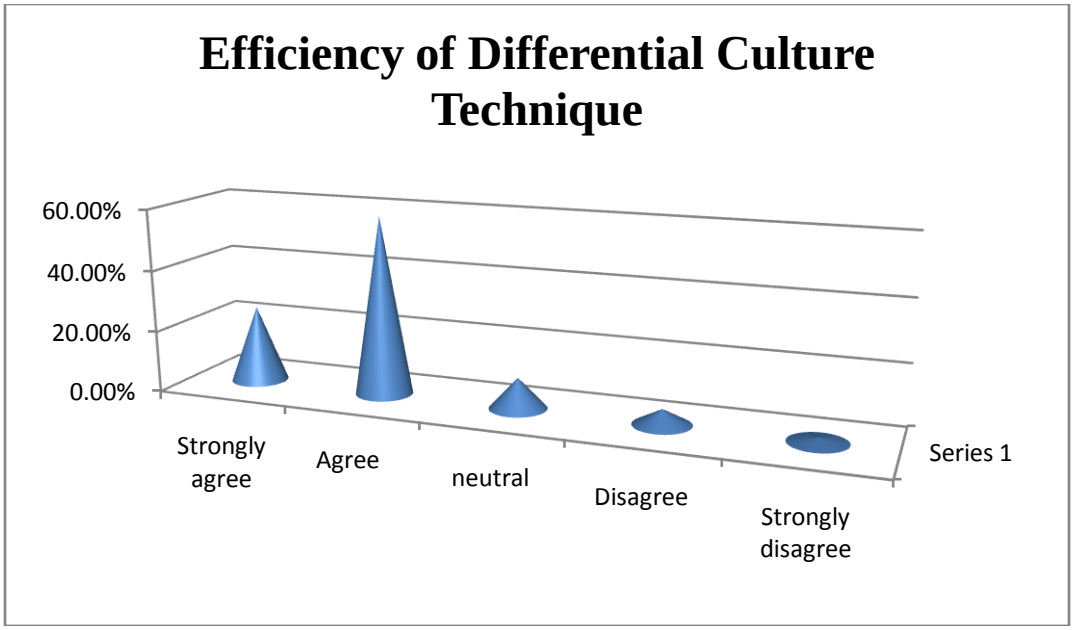


Figure No.: Efficiency of Differential Culture Technique

Key points and analysis
The collected data represent that most of the laboratory professionals think that the different media can be successfully used for the differentiation between the two or more cultures of bacteria, as they are designed such that different cultures show different colony morphology in that media.

4. Both liquid and the solid media are used for the isolation of bacteria. As per your opinion, which one is the best choice while culturing bacteria?

Table 4: Efficiency of Solid Media in Bacterial Culture

Options	Total participants	No. of respondent participants	Frequency
Solid media	60	40	66.67%
In liquid media	60	20	33.33%

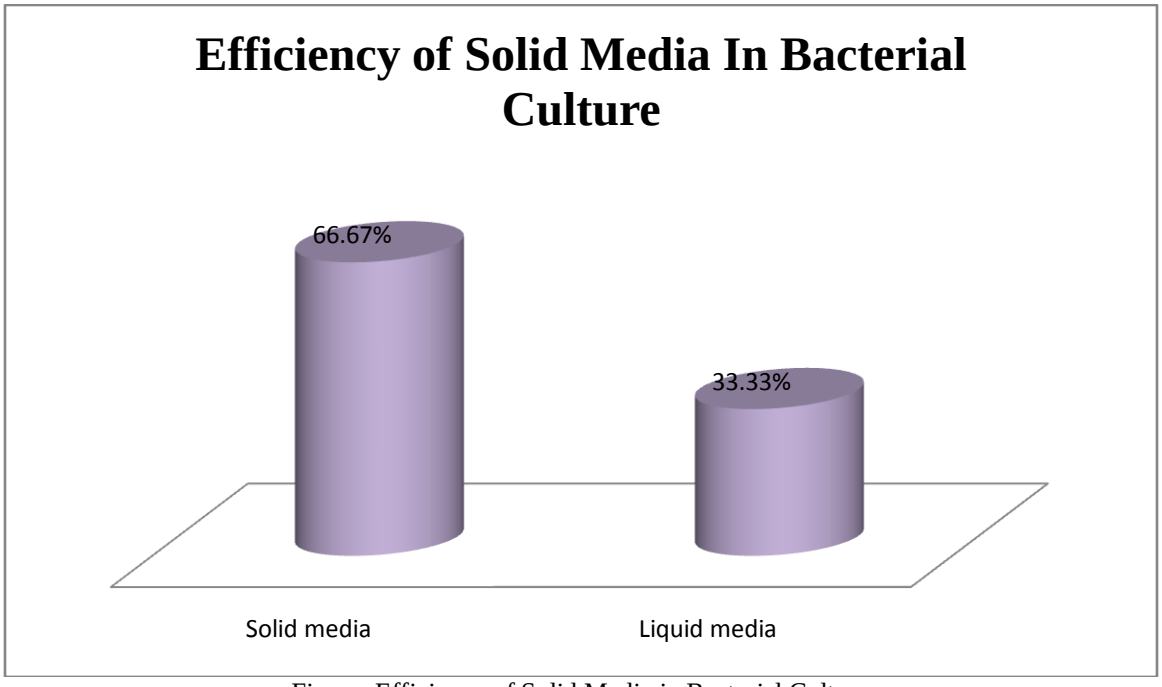


Figure: Efficiency of Solid Media in Bacterial Culture

Key points and analysis:
As pre the data collected, it can be seen that most of the laboratory professionals prefer using solid media while performing the bacterial culture methods, as they think that isolated colonies can be obtained in the solid culture method that can be further used for obtaining pure culture.

5. According to you, which is the best method of sterilization for the lab equipment's, physical method or the chemical method?

Table 5: Preference for Sterilization Method

Options	Total participants	No. of respondent participants	Frequency (%)
Physical methods	60	35	58.33%
Chemical methods	60	25	41.67%

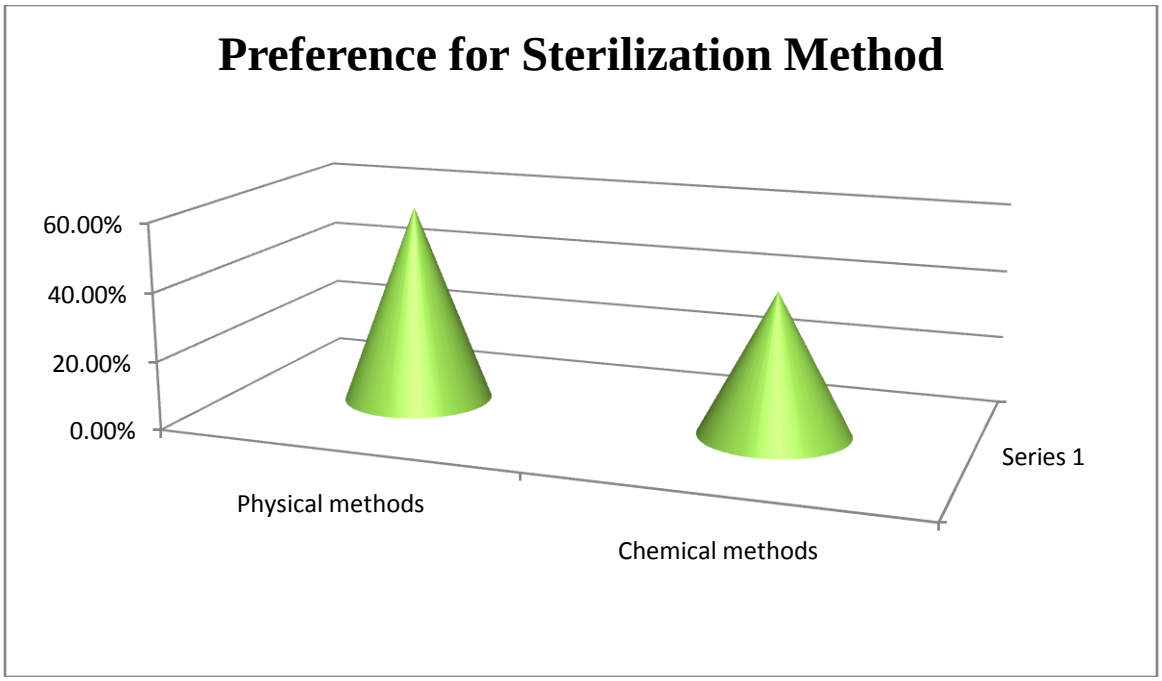


Figure no.: Preference for Sterilization Method

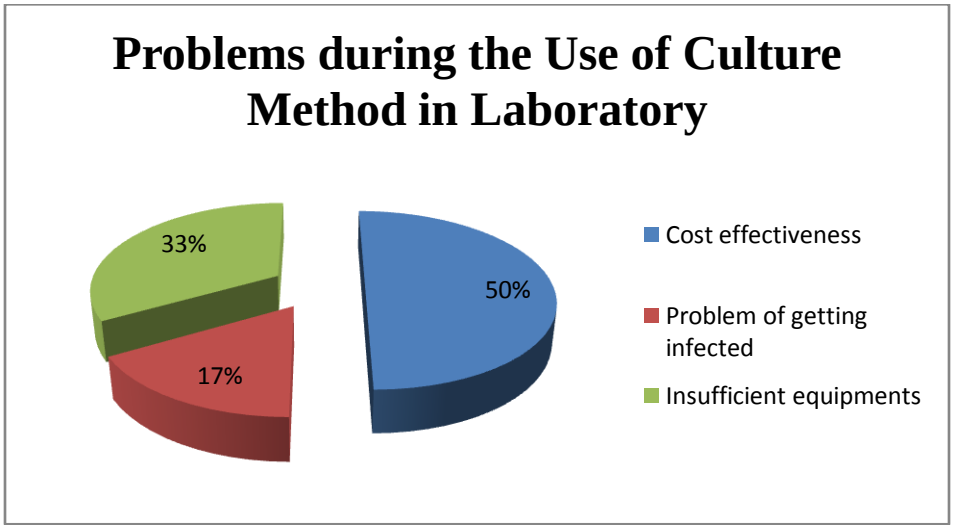
Key points and analysis:

The data obtained from the laboratory professionals represents that most of them prefer using the physical methods of sterilization for the lab equipment's, as the physical methods ensure complete sterilization; whereas some of the microbes may be resistant to the chemicals used for sterilization.

6. What *is the major problem that you have faced while using the culture methods for isolation and identification of bacteria in the laboratory?*

Table 6: Problems during the Use of Culture Method in Laboratory

Options	Total participants	No. of respondent participants	Frequency
Cost effectiveness	60	30	50.00%
The problem of getting infected	60	10	16.67%
Insufficient equipment's	60	20	33.33%



Key points and analysis:
While working in the laboratory, the professionals face many problems that they have to deal with. The major three problems that we have focused on are the cost effectiveness, problem of getting infected and the insufficiency of the availability of the equipment's. The data collected shows that, about 50% of the laboratory professionals are worried about the cost effectiveness, as most of the microbial equipment's, chemicals and instruments are very expensive. About 17% of the laboratory professionals face problems regarding the infection while handling pathogenic microbes and about 33% of the professionals are concerned with the availability of the equipment's in the laboratory.

Questions for qualitative data collection:

Question 1:
Why do you think that the cell culture methods are necessary and effective?
According to the view of the microbiologist, the cell culture methods are the basics of detecting or identifying any microorganisms associated with any type of diseases. Effective treatment of any microbial disease is possible only when the microbe is successfully isolated and identified. Proper identification is only possible using the culture methods. There is no other method available to isolate the microorganisms except for the culture method. Using the culture methods, the characteristics of the pathogens can be identified which is very helpful in designing the drugs against those pathogens.
The tissue culture specialists highlighted the fact that, the microscopic size of the cells of microbes makes it difficult to separate the different microbes physically. So, in order to isolate the microbes, they should be cultured and isolated using proper culture media. Again there are many types of microbes, some are helpful and some are harmful for humans. So, proper differentiation of the microorganisms is also very important, which can be done using the culture methods.
The scientist pointed out that, not only in the field of medical microbiology, the microorganisms are now a day an important part of the industry. Various microbial products are industrially manufactured using the microorganisms. This also requires culturing the microbes at a mass level. But, before using any microbe for industrial production, identification of its characteristics is very important, which is carried out in the laboratory using the cell culture method.
Question 2:
What are the major cultural procedures that you generally use in the laboratory?
The answers by the four eminent researchers highlighted some of the commonly used cultural methods in the laboratory.
For the bacterial culture, the two main methods include the solid culture method and the liquid culture method. In the solid culture method, the bacteria are grown on solid media containing agar. The growth of the bacteria in the solid media can be seen as the bacterial colonies. Each bacterial colony represents one bacterium. On the other hand, the liquid culture method is carried out using liquid media, in which no agar is present. The growth of bacteria can be measured by measuring the turbidity of the media. The researchers also emphasized that the solid media are often preferable for isolating, identifying and differentiating microorganisms. On the other hand liquid media are often preferred while preserving and maintaining bacterial culture.
The other methods that are used in the bacterial culture method, depends on the type of solid media that is used in the culturing process. Depending on the media used the bacterial culture method can be divided as a selective culture method, differential culture method, enriched culture method and minimal culture method.
According to the researchers, the selective media are mainly used for obtaining pure cultures of a particular microorganism. The differential culture method is used for separating two or more microorganisms from a mixed culture. In the enriched culture method, the medium id provided with nutrients that promote the growth and differentiation of the microorganisms. On the other hand, the minimal culture method is applied for limiting the growth of the microorganisms.
The researchers emphasized that, each of these methods is different and the results produced by these methods are also varied. These methods are applied according to the necessity of the research.
Question 3:
What kind of challenges do you face while working in the laboratory with the microorganisms?
When the researchers are asked about the challenges in the field of bacterial culture, various views are reflected from their answers.

According to the microbiologist, the cost is the major factor that comes on the way of the effective research. All the equipment's and instruments used in the culture methods are very costly. The media, staining reagents and other chemicals that are employed while culturing bacteria are also costly, which makes the overall research process a costly matter to handle. He also emphasized on the fact that with the increasing discovery of new microbes, the demand of new technologies in the field of cell culture is increasing which will further increase the overall cost of the research.

While the microbiologist emphasized on the cost effectiveness of the method, the scientist reflected on the fact of getting infected while handling the pathogenic microbes. He said that many of the research fellows, working under him face this problem, even after following the proper techniques of sterilization.

The tissue culture specialists identified the problem of contamination. He stated that though all the procedures are followed, it is often seen that the culture plates are contaminated by other microorganisms or mites, which in turn affects the whole culture method. He also pointed out another problem with the storing of culture, as most of the laboratories fail to provide proper storage facilities which hamper the overall research process.

Question 4:

How do you overcome those challenges in the laboratory?

When the microbiologist is asked regarding the methods and measures that they use in order to overcome the problem, he said that, it is very difficult to reduce the cost of the equipment's and the materials that are used in the cell culture method. Again the newer technologies will also be costly. So, he emphasized on the efficient use of the resources that are available in the laboratory for the culture method. This will be able to reduce the overall cost of research to some extent.

While talking about overcoming the challenges faced in the laboratory, the scientists discussed that, in order reduce the incidence of infection during handling the pathogenic organisms, the sterilization methods should be employed properly and efficiently. Sterilization is one of the most important parts of the bacterial culture method. It is applied in each and every step of the method. So, proper care and efficiency is needed in order to reduce the chances of infection.

The tissue culture specialist stated that in order to reduce the chances of contamination, the laboratories should be cleaned and decontaminated in regular intervals. The sterilization techniques should be properly and efficiently followed. The laboratories should develop their storage techniques and methods for long time storage of the bacterial cultures.

Conclusion:

The qualitative and the quantitative data clearly showed that there are varied opinions regarding the usefulness of a particular culture method and the preferences regarding the choice of methods and the sterilization techniques. The data also showed that the problems that the laboratory professionals and the eminent microbiologists or scientists face during the research are more or less similar. They all are concerned regarding the cost of the microbial equipment's and the materials. They also showed their concern regarding the risk of getting infected while handling pathogenic microorganisms in the laboratory. Again the risk of contamination of the cultures by other bacteria or mites is another issue of concern. All these issues are needed to be addressed properly in order to make the research environment safe as well as cost effective. The measures that the scientists use to solve these problems are also very important. It is possible to control the risk of contamination or infection, but reducing the cost is difficult. So, efficient use of the resources is necessary to reduce the cost to some extent, as advised by the microbiologist.

Limitations of research:

The research faces issues as time limitations and budget constraint. Since the research conduction and completion was to be within the specified time limit, a number of minute details collected had to be overlooked. Consideration of those details could have further enriched the process of result derivation and formation of the final opinion on the research topic. Another major issue is that of financial limitation. The method of cell culture and research conducted on the topic is highly expensive. The software used SPSS is also expensive. The limitation of budget restricts the use of more efficient software and observation of advanced cell culture methods. This again declines the quality of the research result.

Recommendations:

Based on the issues identified in the research in context of cell culture, the following recommendations are expected to prove effective.

Adoption of more efficient anti-contamination measures:

The establishment of a specific program for avoiding contamination in a cell culture laboratory can prove highly effective. Identifying the needs of the working conditions and adopting relevant measures to meet those needs through the program assist largely in the aim of contamination prevention. Based on the previous issues faced by the professionals in the laboratory the relevant measures need to be applied. Identification of problem through record keeping, reviewing the records, discussing and finalizing the resolving measures through group discussion is highly recommendable. The steps as utilizing good aseptic techniques, highly focusing on the laboratory cleanliness and strategic use of frozen cell repository, using antibiotics sparingly and routine monitoring of contamination are highly effective in resolving the issue of contamination for cell cultures.

Future scope of research:

The current research is conducted on the bacteria culture. It enables the identification of the effectiveness of the culture methods with their significant details. However, the research on overall tissue culture can highlight significant data regarding the culture methods.

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