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Rapid microbiological testing method

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ABSTRACT

Classical microbiological methods currently have unacceptably long cycle times. Rapid microbiological strategies are accessible within the marketplace for about 10 years and are mostly used in the clinical laboratory and in food industries. However, their reapplication in the pharma industry has wide range of advantage. A comparison with ancient strategies to be conjointly performed. During this review, data concerning the validation of RMM strategies described within the document was given in addition as proof of the issue of validation of those strategies. A comparison with ancient strategies is additionally mentioned. This data is beneficial to the industries and in the labs which will doubtless be adopted. These strategies for microorganism free products. This methodology for microorganism identification will be delicate, accurate and fast. How the laboratory should be maintained for carrying out different tests, there should be good hygienic condition maintained. This article also includes different methods for identifying bacteria which is present in drug products as well as the material which are used for doing test. Presence of bacteria may affect the activity of drug product and bio availability may decrease and potency the drug may loss. How the laboratory should be maintained for carrying out different tests, there should be good hygienic condition maintained.



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INTRODUCTION

Rapid microbiological methodology was introduced to the pharmaceutical industry for trade concerning twenty years ago. Pharmaceutical company firms were scared of these is ways and regulation accep-

tance of those methodologies. Ten years ago, initial submission of rapid microbiological methodology for regulatory approval comes concerning within the US. Ancient methodology of microorganism detection tend to be effortful and take over on a daily basis to get outcome. This methodology for microorganism identification will be delicate, accurate and fast. Microbiological laboratory could be a complicated operation and involves within the performance of different form of laboratory methodology. Several of these methodologies were employed in the microbiological laboratory, in a number of the cases this square measure known as typical or ancient methodology.

MICROBIOLOGICAL METHODS

Whether you wish to confirm sterility, confirm antimicrobial effectiveness, microorganism contam-

ination or bio burden, analyse endotoxins or establish flora some different types of standard tests are performed. The laboratory should be totally equipped to research all the variations of pharmaceutical product (sterile and non-sterile) and medical devices. The clean rooms are maintained for contamination free testing. The bio burden employed to estimate total variety of viable being and therefore the absence of specific pathogens. The sterility test is employed to sight the presence of viable being. This testing methodology fall under varied classes, one involves unleash tests for drug product like microorganism limit testing of non-sterile drug product and sterility testing of ophthalmic and parenteral. Several examples for rapid microbiological methodology are in use, which will be helpful within the pharmaceutical industries. (Henriques and Cardoso, 2019)

CONVENTIONAL METHODS

Three varieties of microbiological evolution conducted, one is for determination of presence of organism (presence-absence test) if any organism present then should determine how much is present (environmental test) and eventually identification of organism which is present (identification test). (Shintani, 2014)

1. Presence-absence test
2. Enumeration test
3. Identification test

Presence-absence test

This technology confirms whether microbes is present in a given sample for the assessment sterility testing is one among the standard test.

Test procedure

1. Collect 100ml of sample in a sterile container. Use aseptic technique to prevent contamination.
2. Add sample to the fill line of sterilizing sampling bottle.
3. Use the ampule beaker to aseptically open a broth ampule. Add the contents of the ampule to the bottle.
4. Incubate the sample at $35 \pm 0.5^\circ \text{C}$ ($95 \pm 0.9^\circ \text{F}$) for 24 hours.
5. After 24 hours, look for colour change. If there is no colour change, Incubate the sample for an additional 24 hours. If there is no colour change after 48 hours, test results is negative.

6. If there is a colour change, the result is presumptively to be positive and then absorbed under UV. (Microbiology Guide, 2019)

Enumeration Test

Performed to sight the number of microbes present in the sample. The results that square measure obtained are distracted by several characteristics that as well as tests condition matter, condition maintained throughout incubation. In typical methodology the variability are going to be high. This test is to confirm counts by watching and different tests.

General Procedure

Carry out determination beneath condition planned to get rid of extraneous microorganism that will lead to pollute substance that is used for testing. The precautionary measures are chosen to avoid polluting of product such that , they should not cause effects on other microorganisms.

Product which is examined, if it is having activity against some microbes, such type of microbes can be removed or neutralized. Inactivation's square measure is preferred for their efficaciousness. Any more active substance that are used in experiment, if they don't contain toxicity for microbes and for their affinity with in activator used should be displayed.

Test procedure

1. Test solution filtration.
2. Rinse with a neutralizer agent named neutralizer buffer solution to test simplification.
3. In the last rinse add microorganism to the solution.
4. Place membranes in culture medium. (Moldenhauer, 2013)

Identification Test

Many system area units exist for identification of microbes. Some microbes can origin within the clinical setting, some of the techniques are classical test method for identification during other were automated.

1. Staining of the isolated microorganism
2. Motility testing
3. Biochemical testing
4. Serological
5. Phage writing
6. Identification disc testing
7. Semi-automated and automatic identification system

8. Molecular techniques

Staining of the isolated bacteria

Colouring the microorganism forms the leading and therefore the more vital stage within the recognition of microorganism. The extracted microorganism area unit is coloured by numerous strategies relying on the microorganism focussed.

Gram staining

There are 2 varieties Gram positive and Gram negative microorganism, Gram positive microorganism may be either coccus bacteria or bacillus or vibrio cholera. Gram positive unhealthful microorganism, Gram negative microorganism may be coccus or bacillus Gram negative unhealthful microorganism usual area unit *Escherichia.coli*, *Klebsiella pneumoniae* enterobacteria, *shigella flexneria* etc.

Motility testing

This test is done by doing a moisture scale and then it taken to absorb by using microscope. Motility of microorganism even is cross verified by inoculation the microorganism within the solid medium.

Organic chemistry test

The staining is followed by use of assorted organic chemical agent and to induce nearer to the recognition of microorganism. Various chemical trial so btainable for bacteria recognition. Little of their area unit needed to meet out relying on the microorganism.

Serology

This produces a vital stage in microorganism recognition. It always requires recognition of substance by means of catalyst or by doing light bioassays. Medical science is additionally costumed to ensure identification obtained by different strategies. As an example, enterobacteria species known by organic chemistry by using slide agglutination test is performed for serotype. One more sample being vibrio infectious disease.

Bacteriophage typing

Phage writing is a technique used to detection of one strain of the microorganism. It is known to find the supply eruption of contamination. The virus that infect microorganism area unit known as bacteriophage and a few of those will slowly cause one strain of microorganism.

The agar is added to culture and then it is dried. A line is drawn at the bottom of the Petri plate to conform totally various places. Vaccination of every sq. of criss cross is completed by a special bacteriophage. The bacteriophage unit permitted to dry, and then unit incubated. The bacteriophage place

can show a disc shaped clear appreance wherever the microorganisms are present, and often utilized in difference.

Identification disc testing

Disc dispersal technique is firstly focused to spot the against bacteria susceptibleness of the microorganism. It is conjointly useful in recognition of different microorganism for example, *Streptococci*, *Moraxella*, etc.

Semi-automated and automatic identification system

The colonies are obtained by isolating from this system. The recognises the microorganism and conjointly Carrie off the against bacteria susceptibleness trial for a similar. Sensitive Gram negative automotive recognition system, number of the Semi-automated and automatic recognition system accessible to microorganism recognition.

Molecular techniques

Molecular strategies which includes G+C online page, deoxyribonucleic acid-Deoxy ribonucleic interbreeding and deoxyribonucleic base order. These strategies don't seem to be used habitually in clinical lab. Amplification method is like enzyme chain reaction, and super molecule series, primarily build amplification area unit getting for detecting directly in the clinical lab that is used. Example: Cupid's disease, swamp fever, etc. ([Manual, 1998](#))

TYPES OF NEW TECHNOLOGY

Some of new technologies accessible that replace microbiological technique and their foundation in different sciences like chemistry, biological science, optics. This technology is categorised within the canal Drug Association. The PDA document publication and system was free and numerous spectrographic analysis varieties present and used. As additional technique had been developed, there is some strategies that engulf over one kind of automation in the method, and is also ready for finding, enumerate.

Growth-Based Technologies

The presence of microbes is going to be depending upon the measure of attributes that needs for growth. A number of systems that is enclosed underneath this class is luminescence, quantitative analysis detection of carbon derived product, measure of changes in indefinite quantity pressure, and organic chemistry assay. Rapid method that use the utilization of growth-based policy unit, to the foremost half, drop the time in minutes at that are able to sight where the microbes can grow very actively. Presently used growth-based technique

still use standard fluid media. Which gives out result, similar sorts of appeal that ancient ways, area units may be used for growth-based rapid microbiological method. Examples embody bioburden sampling, microorganism Limit, surrounding observance, sterility testing. Samples of the core technique principle that area unit know using area unit supported electrical resistance biology, the analyse of greenhouse gas carbon dioxide, the usage of organic chemistry and supermolecule substance. (Moldenhauer, 2013)

Viability-based technologies

This type of technology permits personal to find viable variety of being used while not requiring growth of microorganism. It is able to induce totally different results by employing this technique for enumeration against growth based technology. This technique is probably going to incorporate microorganism which don't seems ready to be underneath the state utilized by growth based technology. Some system that area unit enclosed underneath this class area unit solid-phase optical device cytometer. We have return an extended means in terms for microbe qualification and for the detecting microorganism. Several within the trade are currently mistreatment viability-based techniques which might differs in between living cells from dead cells, and may even choose particular varieties of microbes mistreatment super molecule, catalyst. In several cases, directly labelling for various individual cell by using viability stains markers has been incontestable with that of no demand for growing of the cell. And since microbe developing isn't needed, like damaged cells, currently be simply and fast analysing. Labelling of microorganisms by viability will happen within hour or in few minutes, close to period of time detection and quantitative results is also earned.

In the cytometry, separate substance square measure calculated how they reacts with a light rays in the more slender cell flow. That to be employed in the microbial uses, microorganisms square measure should be labelled by using the viability marker, and therefore this labelled cell square measures will be injected in the cell flowing. Separate cell moving line, through a cantered light rays, and square measure quantified because the cell glow and low weight dispersed signals square measure identified. (Moldenhauer, 2013)

Artifact-based or component-based technologies

These techniques are primarily based on artifacts or elements using the existence of particular cellular elements. A number of the method victimisa-

tion this technique area unit carboxylic acid profiles, and detection of the fluorescent identification. The widest used strategies for toxin detection use genus *Limulus* Amebocyte Lysate several, for testing purpose instrument called bench-top is most used in laboratories, which need trial to be shifted from the producing base. Latest highly active in toxin instrument currently yield sampling is done by doing the point of use, with leads to as early as quarter-hour. LAL chemical agent which contain every cartridge and management customary toxin. The reactivity of the rapid microbiological method is 0.02-1.1 and 0.04-5.1 EU per cubic centimetre, and is food and drug administration approved is another to ancient LAL sampling strategies for end and finished substance unharness. Several firms are currently victimisation this technique is most used in pharmaceutical water system, inprocess substance, end products. (Moldenhauer, 2013)

VALIDATION AND QUALIFICATION

The objective for doing the validation for Associate in Nursing analysing technique that is for evolve that it's appropriate for use. Rapid microbiological method is compared with ancient strategies to prove that they're comparable.

Validation parameters

1. Limit of detection (LOD): LOD is one amongst the essential to outline what's thought-about to be mixed with some other substance. The limit for detecting is known by protection sample with series dilutions mixed with various other substance of viable type of contamination. Sampling is done with every challenge being victimisation Associate in Nursing quantity between ten and one hundred CFU.
2. At the certain determinate stages microorganism is needed to detect in time vary.
3. Quantification is done to determine how much does measured wort is close to true worth. (Working ICH Expert Group, 2006)
4. Dimensionality is that the capacity to get an identification signal that is which directly proportion to the colony forming units of microorganism during a testing, with appropriate end point. The analysis of dimensionality is assessed by the identification constant (r^2) that ought to be larger than 0.94 to think about that dimensionality is adequate.
5. The less colony forming units of organisms quantitative in cell growing media with good accuracy and preciseness.
6. Relation b/w the colony forming unit's count

when incubation and ancient technique count. The range should be more than 69%. ([Working ICH Expert Group, 2005](#))

Selection of organism for validation

The rapid microbiological technique is to test using microbes associated with the assembly and producing technique. organism belongs to the many categories: gram negative, gram positive bacteria, aerobic and anaerobic bacteria, identified within the beginning material, a number of the isolate square measure detected by in-process sampling isolates is identified by surrounding observation of producing space, conjointly extracts from the assembly space and also the microbes from the business sources that high nutrient media should be exposed regularly.

The similar microbes will show a lot of completely different in growth, depends on the sources. Thus, the validation is done for all kinds materials used for doing rapid microbial tests and to know of growing beneath in 2 condition one in aerobic and one in anaerobic, particularly for product factory-made in enclosed system. ([Drug Food Administration, 2008](#))

Validation Procedure

The main purpose of doing of validation for associate in nursing analysing method that is to show that it is appropriate for its supposed reason. Thus, rapid microbiological method that should be contrast with ancient ways to show that they are perhaps that they supply less variability.

Polluted product can be analysed throughout the assembly is based on the cell product and microbes, many fault, like unable to control method streams and failing in antiseptic process technology, will leads to addition microbes. Thus, a significant estimation fort he assembly should be presented for spot and to avoid microorganism contamination. Contamination may takes place through following: supply material, data manipulating, process path, vessel that is used for cell culture. ([Agalloco, 1993](#))

Revalidation methodology

If there is any kind of changes in product which is being produced, together with change within the rapid microbiological method which will probably decrease or increase detection of viable form of microbes got to validate again the rapid microbiological method. Any difference in cell sorts, gather in the process, microbe growths media, vital process stage will probably have an effect on the identification of viable form of microbes. In the first validation may be planned to reduce the result of some change which is by planning assay with the suitable conditions that embrace celebrated modification for

doing validation again of the rapid microbiological method to be carryout when there are unit modified within the method that might probably decrease or increases detection of microbes. ([Drug Food Administration, 2008](#))

Regulatory Submissions

The appropriate pathway for fast biological science submissions to Food and Drug Administration is best determined through direct with the agency. As mentioned on top of, the PAT initiative recommends discussion with Food and Drug Administration concerning all aspects of implementation for brand spanning new method analytical ways. Till fast biological science ways become a lot of widespread within the pharmaceutical trade, it's going to be simplest to implement new fast ways on a post-approval basis. It's going to even be helpful to use a comparison protocol to implement fast biological science ways for multiple merchandise and/or facilities. A comparison protocol for associate degree analytical technique may be an elaborated arranges that specifies the validation experiments and acceptance criteria. The approval of a close validation protocol ought to greatly cut back requests for extra data to support the next changes. Associate degree approved comparison protocol may designate a reduced coverage class for changes coated by the comparison protocol. ([Easter, 2005](#))

Validation of other strategies

Validation studies of alternate microbiological strategies to take an outsized degree of variability into consideration. Once conducting microbiological testing by standard plate count, as an example, one oftentimes encounters a spread of results that's broader (%RSD 14 to 34) than ranges in normally used chemical assays (%RSD 1.5 to 3.5). Several standard microbiological strategies are subject to sampling error, dilution error, plating error, incubation error, and operator error. The USP goes on to state that the characteristics like accuracy, precision, specificity and strength are applicable to analytical strategies and fewer acceptable for alternate microbiological technique validation. Yet, the final gift restrictive expectation is to use these analytical performance characteristics to different fast microbiological technique validation. ([Miller, 2010](#))

Advantages

High Reliability

Whether square measure delivering product the on time, each time on to customers, makers want. A huge amount inventories will definitely guarantee steady flow steady, automatic microbe detection

defeats the necessity for such massive inventories by eliminating human errors inherent within the manual technique.

Product safety

Test for bio burden and environmental observation can offer result among hours, and also the final product may be analysed for sterility among 0.5 the time as ancient technique. This fast result offers internal control personnel for directly to contamination events.

Quicker Times to market

Pharmaceutical firms measure investment countless greenback on analysis and development, Market share and values drop dramatically when concluded, therefore innovative firms want quick, reliable strategies for quality assurance.

Lower price

Even when patents expire, this technique facilitates firms value. Product delivery and modest can reduce the prices.

New innovation

This detection permits nice innovation and growth among a company. With less time spent on repetitive and long tasks; some trained personnel will usually shift their attention toward new products. (Sandle, 2015)

CONCLUSIONS

Rapid microbiological method is one of the important method for assuring safety, drug purity, effectiveness of the drug. It is more important in building a good quality product. Some of the strategies are required for accepting microbiological drug quality, they are sampling, doing validation, continuous monitoring. It all gives information about the science, design and qualification of the rapid microbiological method. It is a key for improving environmental monitoring process.

Conflict of Interest

None.

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