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Formulation and efficacy testing of disinfectant in different culture media for usage in pharmaceutical facilities

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Abstract

Objective : Maintaining and regulating the sterility of pharmaceutical manufacturing operations requires both cleaning and disinfecting surfaces. The antiseptics and disinfectants are employed to limit the development of microorganisms to lessen contamination in the finished product. Choosing disinfectants, making sure that the ones chosen have the appropriate qualities, and routinely evaluating the disinfectants' efficacy are some of the more laborious responsibilities encountered by pharmaceutical companies. The disinfectant was investigated and approved for use in sterile industrial facilities in this study. The W.H.O. and USP. regulation requirements served as the foundation for the validation. The effectiveness of the disinfectants was examined using three different techniques: swab analysis, membrane filtration and direct inoculation, and agar diffusion, commonly referred to as the ditch plate method. Findings: These techniques showed that the disinfectant was effective against common fungi and bacteria. The disinfectant 1%v/v solution began to function in both swab analysis and membrane filtration in less than ten minutes. The corresponding disinfectant's zone of inhibition was perfectly formed using the agar diffusion method. In conclusion, the protocols outlined in IP and W.H.O. criteria were followed to validate the disinfectant. The outcomes of various techniques were determined to be promising, the procedure was verified, and the disinfectant proved effective for the intended use.

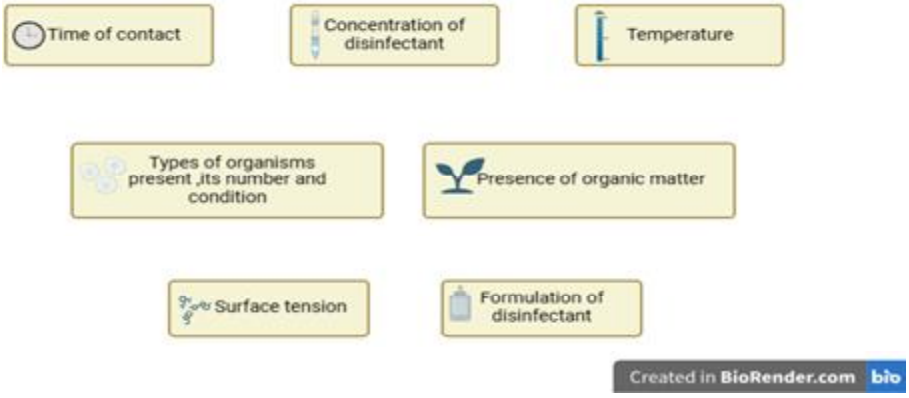
Key words : Validation, Disinfectant, Antimicrobial agent, Efficacy, Microbial Contamination.

Both antiseptics and disinfectants are extensively used in hospitals, other healthcare facilities, and the pharmaceutical industry to primarily control and prevent the growth of microorganisms on both inanimate objects and live tissues[21]. By employing it in different quantities, it can also be utilized to stop different organisms from contaminating pharmaceutical products. These are crucial components of infection control strategies that work to stop nosocomial infections. However, the main difficulty lies in selecting suitable disinfectants and antiseptics because different bacteria react differently to various disinfectants and antiseptics. There are numerous approaches available for researching how disinfectants and antiseptics work on microorganisms, particularly bacteria. The disinfectant works by doing the following lysis followed by the leakage of intracellular components, disruption of cell homeostasis, alteration of membrane properties, inhibition of enzymes, electron transport, oxidative phosphorylation, interaction with macromolecules, impacts on macromolecular biosynthesis processes, and interaction with genetic materials are some of the mechanisms that lead to the death of microbes. Many of these behaviors provide useful information for identifying and assessing the use of combination disinfectants and antiseptics[2]. The phrase "biocide" refers to a broad-spectrum chemical agent that renders microorganisms inactive. Other terms, such as "static," which describes agents that inhibit growth (such as bacteriostatic, fungistatic, and prostatic), and "cidal," which refers to agents that kill the target organism (such as sporicidal, virucidal, and bactericidal), can be more specific because biocides vary in their antimicrobial activity. Antiseptics are biocides, or agents, that kill or prevent the growth of microorganisms in or on living tissue (such as hand washes and surgical scrubs used by medical personnel). Disinfectants, on the other hand, are identical to antiseptics in that they are biocides or products intended to sterilize inanimate objects or surfaces. While they are typically not sporicidal, disinfectants can be sporostatic. Sterilization can be achieved using chemical or physical means. Proliferation of microbes in manufactured goods, such as food and medicine. Many biocides are employed in cleaning operations; in these instances, cleaning refers to the actual physical removal of foreign material from a surface[4].

Features of the Perfect Disinfectant :

1) Firstly, they ought to be microbicidal.
2) Usability is a must.
3) The activity should involve detergents.
4) These need to be non-toxic and need only the barest minimum in safety precautions.
5) They ought not to irritate.
6) The disinfectants that are utilized ought to be safe for surfaces. It implies they ought to be free of corrosion.
7) It should have the ability to act quickly.
8) Must involve activity while organic matter is present[3].
9) Should be active when hard water is present.
10) It should remain steady in a range of temperatures and circumstances.
11) Activity that remains.
12) It ought to be reasonably priced.

Factors Affecting the Disinfectant Efficacy



Instruments used :

S.no.	Equipment/Instrument
1	Hot air oven
2	Ultra sonicator
3	Laminar air flow unit
4	Colony counter
5	Dry bath
6	Incubator
7	Autoclave
8	Weighing balance
9	Heating mantle

10	Microscope
11	Pump
12	Sonicator
14	Petri-plates
15	Glass bottles
16	Filtration assembly
17	Volumetric plates
18	Test tube
19	Measuring cylinder
20	Bath sonicator

Preparation of disinfectant

Materials	concentration	Uses
Benzalkonium Chloride	2%	Surfactant
Lauryl Alcohol Ethoxylate	3%	Wetting Agent
Sodium bicarbonate	1%	Optimize pH levels
Coco amido propyl butane	1%	Detergent
Tetrasodium EDTA	0.1%	Antimicrobial
Denatonium benzoate	1%	Disinfectant
Ethyl alcohol	2%	Antiseptic
Silicon	0.1%	Emulsion
Citric acid	1%	Chelating agent

Procedure for preparation of disinfectant:

1. Add 50 ml of distilled water(TDS around 0.3 ppm or less and ph around 7) in the 250ml beaker.
2. Add 2ml of benzalkonium chloride in the beaker and leave for 1 hr till the exothermic reaction stops.
3. Add 3ml of lauryl alcohol ethoxylate.
4. Add 1mgof sodium bicarbonate.
5. Incorporate 1mlof coco amido propyl butane.
6. Add 0.1 ml of tetrasodium EDTA to the beaker.
7. Add 2 ml of ethyl alcohol into the solution as it will enhance the disinfectant properties.
8. Add I ml of denatonium benzoate to the solution.
9. Incorporate 0.1 ml of silicon emulsion into the solution.
10. Make up the volume up to 100ml with distilled water.
11. Adjust the pH (6-8) by using citric acid.
12. Thoroughly mix all the ingredients until uniform using a sonicator.

Different Culture media used :
Cetrimide Agar :

1. Weigh 4.53 g of powder in a clean conical flask having 1 ml Glycerol in it.
2. Add 100 ml ofdistilled water. Boil to dissolve.
3. Adjust the pH if necessary with IN NaOH/ IN HCl to about 7.0 to 7.4.
4. Sterilize by autoclaving at 121°C for 15 min. & 15 lbs pressure.
5. Pour the agar into sterile petri dishes aseptically.
6. Allow to solidify and incubate overnight. Store the plates in the refrigerator[7].

McConkey Agar :

1. Suspend 4.95 g of dehydrated medium in 1000 ml purified water.
2. Heat the medium and dissolve it completely.
3. Adjust the pH if necessary with IN NaOH/IN HCl to 6.9 to 7.3.
4. Sterilize by autoclaving at 121°C and 15 lbs pressure for 15 min [6].

Soybean Casein Digest Medium :

1. Weigh 30 g of dehydrated powder of Soyabean Casein Digest Medium and dissolve in l000 ml, I purified water.
2. Boil to dissolve the medium completely. Adjust the pH if necessary with IN NaOH/ IN HCI% solution so that the final pH achieved is 7.1 to 7.5.
3. Dipense the medium in 100 ml quantities in sterile Scott bottles. Autoclave at 121'C 3 for 15 min. at 15 lbs pressure.
4. Cool and Label each bottle indicating batch no. of the medium, and date of preparation.
5. Date of completion of pre-incubation and use before date. Transfer the media for pre-incubation.
6. Store at a temperature of 20-25°C [1]

Evaluation Test for disinfectant :

Minimum Inhibitory Concentration (MIC) Testing : This technique establishes the disinfectant's lowest concentration at which a certain microorganism's growth is inhibited.

1. Prepare several distilled water dilutions of the disinfectant.

2. Prepare dilutions of the disinfectant solution at 0.5%, 1%, 1.5%, 2%, and 4% to cover a range of concentrations.
3. Microbial inoculum preparation :

Macrodilution: Standard test tubes are filled with 1 ml of broth medium.

4. Making the antimicrobial agent stock solution: The antimicrobial agents are prepared in concentrations of 0.5%, 1%, 1.5%, 2%, and 4%, and their effectiveness against the same pathogenic microorganisms is assessed[13].

5. In these experiments, there are two controls.

Growth control : There are no antimicrobial agents present, and there is bacterial suspension. With antimicrobial agents removed, this is used to observe the growth of bacteria in real-time.

Sterile control : It is devoid of test bacteria and antibacterial agents, containing only a growth medium. It is anticipated that after incubation, there will be no bacterial growth.

For 18 hours, test tubes were incubated at 37°C. Utilizing a spectrophotometer, determine the sample's turbidity following incubation. Turbidity was present in the sample with low or no antimicrobial activity, but not in the test sample with strong antimicrobial activity, suggesting that the growth of microorganisms is inhibited. When examined under a spectrophotometer, no turbidity was observed, indicating that the minimum inhibitory concentration was determined to be 1.5% [12].

Testing for Zone of Inhibition :

1. Get the Petri plate agar media ready. Optimal conditions for bacterial growth were achieved by incubating these Petri plates at 36°C for a duration of 18-24 hours.

3. Apply various disinfectant concentrations to media plates (0.5%, 1%, 1.5%, 2%, 4%, 6%, 8%, and 10%etc.) after the incubation period.

4. A larger zone of inhibition forms on treated media plates with higher disinfectant concentrations, or vice versa[10].

5. Metric rulers were used to measure the zone of inhibition.

6. On a culture plate that has been contaminated with bacteria, a disc or strip containing a certain amount of disinfectant is placed. The zone of inhibition—the region around the disc where bacterial growth is inhibited—is measured to assess the disinfectant's effectiveness[18].

Chick-Martin test-

1. It is a widely used technique to assess how well disinfectants work against microbes.
2. In a petri dish, prepare agar media for the growth of P.aeruginosa in different growth media.
3. Next, adddisinfectant of variousconcentrations of disinfectant (1,0.95,0.82,0.75,0.64).
4. Before incubating at 37°C for a predetermined amount of time, let the sample stand on the bench for 30 minutes at room temperature[12].
5. Use phenol at various concentrations (2,1.86, upto 1.21).
6. Then find out the phenol coefficient of the test disinfectant by calculating it using the phenol coefficient formula (Phenol Coefficient = Concentration of phenol/ Concentration of disinfectant[3].

Determining the efficacy of the disinfectant by membrane filtration method :

- Prepare test tubes having 9 ml of sterile distilled water.
- Add I ml of the use dilution for on disinfectant.
- Vortex the tube for 5 min
- Add 0.1 ml of any one culture into the test sample. samples. Efficacy of the Disinfectant by membrane filtration[5]
- Make the sample solutions in such a way that each contact time has two sets of samples.
- Give a contact time of 30 min [14].
- After the specified contact time, filter the samples through a 0.45u membrane.
- SCDA agar[5].
- Give three washes of 100 ml each with 1% sterile peptone water.
- After filtration with the help of the sterile forceps take the membrane and place it on SCDA agar.
- Incubate the plates
- After incubation count the total number of colonies present on the membrane.
- Note down the number of colonies.
- This will be the final count of the microbial culture[14].
- Select the plates that have nil count.
- Repeat the same procedures taking all the cultures to be tested.
- Contact time for the usage of the disinfectant will be sent based on the results which will have the least count.

Agar well diffusion method :

Agar dilution involves the incorporation of varying concentrations of antimicrobial agents into an agar medium, usually using serial twofold dilutions, followed by the application of a defined bacterial inoculum to the agar surface of the plate. These observations are often considered the most assureable for the detection of an MIC for the test bacterium/ antimicrobial/disinfectant combination[21].

Procedure :

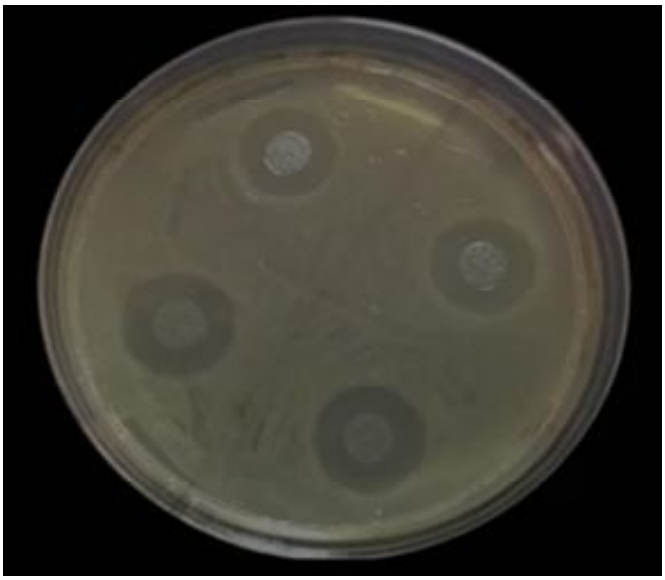
1. Prepare SCDA plates by preparing SCDA medium and aseptically pouring
2. Approximately 10-15 ml into sterile petri plates followed by solidifying at room
3. temperature[21].
4. After petrification, label all the plates with the name of the media, preparation batch number, and date of preparation[15].
5. Incubate the prepared plates at 30-35°C for 24-48 hr. And check if there is any contamination. If contamination occurs then discard the plates.
6. After incubation, the specified concentration of microorganisms to be challenged is spread over the surface.
7. The wells are prepared by using the sterile cork borer or a tip.
8. A fixed volume of the antimicrobial agent at the required concentration is introduced into the well.
9. Incubate the plates for 24 hr. The antimicrobial agent diffuses through the agar medium and a zone of inhibition is formed.
10. Measure the zone of inhibition[8]

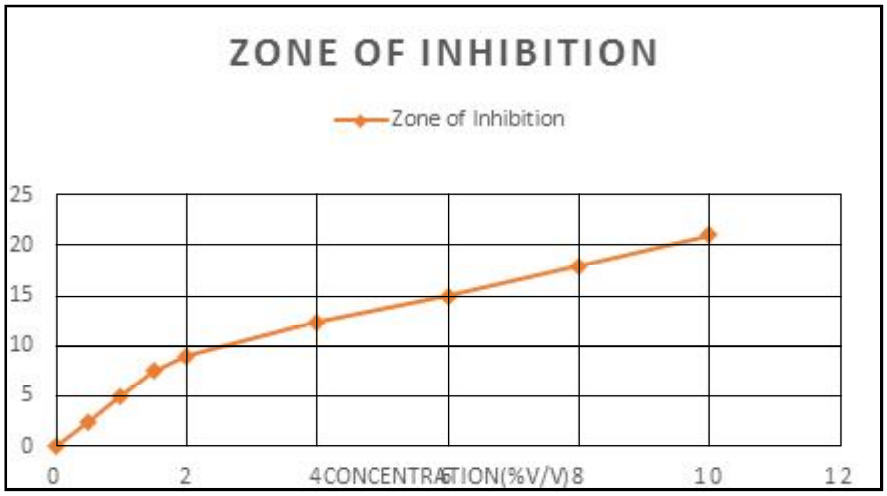
Growth promotion test of media(GPT) - A growth promotion test is a quality control method used to determine if a specific batch of culture media promotes the expected bacterium's development. This involves inoculating the media with specific microorganisms and incubating it under appropriate conditions to validate growth.

Medium	Organisms	Incubation Temprature	Result
Cetrimide agar	*Ps. aureginosa* ATCC 9027	30-35ºC	Growth
Soyabean casein digest agar	*B. subtilis* ATCC 6633	30-35ºC	Growth
MacConkeys broth	*E. coli* ATCC 8739	30-35ºC	Growth

Result for GPT

Zone of Inhibition





S. No.	Concentration of disinfectant(%v/v)	Zone of Inhibition
1	0	0
2	0.5	2.5
3	1	5
4	1.5	7.5
5	2	9
6	4	12.4
7	6	15
8	8	18
10	10	21

Method used log reduction Positive control

Results without disinfectant after incubation at 37°C for 48hrs

Microorganisms	Dilutions						Viable Count (CFU/ml)
	10^0	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	
E. coli	M	M	UC	UC	UC	145	$7.65 * 10^{-5}$
P. aeruginosa	M	M	UC	UC	UC	131	$8.21* 10^{-5}$
B. subtilis	M	M	UC	UC	UC	126	$8.26* 10^{-5}$

M = MATT GROWTH UC = UNCOUNTABLE CFU = COLONY FORMING UNITS

Result with Disinfectant for a contact time of 30 min.

Results with disinfectant after incubation at 37°C for 48hrs

Microor-ganisms	Conc (%v/v)	Dilutions						Log reduction contact time
		10^0	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	
E. coli	0.5	0	0	0	0	0	0	7 log reduction /30 min
	1	0	0	0	0	0	0	
	1.5	0	0	0	0	0	0	
P. aeruginosa	0.5	0	0	0	0	0	0	7 log reduction /30 min
	1	0	0	0	0	0	0	
	1.5	0	0	0	0	0	0	
B. subtilis	0	0	0	0	0	0	0	7 log reduction /30 min
	1	0	0	0	0	0	0	
	1.5	0	o	0	0	0	0	

The log reduction technique results show no microorganism growth at the lowest concentration of 0.5% VN, and the disinfectant is effective against all common bacteria strains for a 30-minute contact duration, determining the required contact duration for bacterial suspension.

Disinfectant efficacy testing of disinfectant by membrane filtration method

Chick Martin test of disinfectant at the increased concentration

Test disinfectant	Concentration (%)	Culture medias		
		1	2	3
Disinfectant	1	-	-	-
	0.95	-	-	-
	0.82	-	-	-
	0.75	+	+	+
	0.64	+	+	+
Phenol	2	-	-	-
	1.86	-	-	-
	1.62	-	-	-
	1.5	+	+	+
	1.21	+	+	+

Determination of chick martin coefficient for test disinfectant and phenol using P. aeuroginosa as test organism + growth in the recovery medium.Chickmartin coefficient for test disinfectant 1.75/0.87=2.01 (Phenol Coefficient = Concentration of phenol/ Concentration of disinfectant). A coefficient of 2.01 suggeststhe test disinfectant is effective at killing microorganisms.

To sum up, this study examined the creation and effectiveness evaluation of diverse culture media intended for use in pharmaceutical facilities. It is clear from thorough investigation and analysis that choosing the right culture media is essential to guaranteeing accurate and trustworthy microbiological testing in pharmaceutical production processes. The results highlight how crucial customized formulations and exacting testing procedures are to preserving regulatory compliance and quality control. It is essential to carry out more research and development in this field in the future to improve pharmaceutical quality assurance procedures and protect public health.

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