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Original Article

# Checking of potency of disinfectant at district level small microbiology laboratory with an example: make own laboratory standard

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ABSTRACT

Background: Disinfections must be able to destroy all type of pathogens. Disinfectants used in hospital set up play a major key role in the breaking the chain of infection transmission. It is very difficult to trust a new disinfectant for regular uses in hospital directly even though manufacturely claimed. Prior testing is not possible at every place of the world. Aim: To develop a simple method of microbiological challenge to disinfectants. This method can be easily done in small level microbiological laboratory with help of routine bacterial culture testing. Methods and materials: A new disinfectant's potency is checked against the effective older one's potency. The potency is checked with the help of a known bacterial organism at different concentrations of disinfectant solutions and also that of the micro organism. Here we have used sodium hypochlorite as the older disinfectant and staphylococcus aureus as the microorganism. Results: Effectively of the disinfectant is recorded as the ability to destruct the microorganism used in challenge test. Discussion: One can have gross idea of at least visible anti bacterial activity of a newly introduced disinfectant prior to use. Conclusion: The spectrum of disinfectant solution against the micro organism including virus, bacteria, and parasite may be very large. The effectively checking against all spectrums is very difficult for the advance laboratories too. Such type of testing at local level may help to checking of disinfectant solution -a key factor for infection prevention.



## 1. Introduction

The concept of Testing or checking the disinfect potency is very old. Robert Koch described a disinfectant test in the article Uber Desinfektion, in 1881. A silk thread was contaminated by submersion in a liquid culture of Bacillus Anthracis. After drying, the contaminated thread was immersed in several disinfectant solutions for a given exposure time. The thread was then cultured in a nutrient broth and no growth after incubation indicated activity of the disinfectant. He concluded from the comparisons of disinfectant solutions that mercuric chloride was the most active disinfectant. [1]

The testing of disinfectants has also helped for finding the cause of spread of infection. [2] Disinfectants should consistently remove or inactivate known or possible pathogens from inanimate objects. [1, 3] Hospitals should have their in built test method that can be easily applicable. Though disinfectant testing is important, available disinfectant tests [1, 4, 5] are very costly, complicated and some time even not applicable in small microbiological laboratories. The literatures related to hospital disinfectant testing are few in India. The test developed is here just simple modification of the older tests and becomes applicable for even in small set up of microbiology laboratories.

## 2.Method and materials

Here, we have tested a new disinfectant against the standard disinfectant the sodium hypochlorite to which the newer disinfectant is going to replace. The ideal disinfectant should be able to kill all type of micro organism. So the laboratory can use the any isolate from the clinical samples but same organism should be used for the standard and test disinfectants. Micro organism used here is *staphylococcus aureus.*

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The microbiological challenge is done by following the steps

· Prepare the staphylococcus aureus solution in sterile normal saline having opacity of. Mc Farlands 0.5 $BaSO_4$ standard and labeled as 1.

· Dilute 0.5 Mc Farlands solution in to 1:2, 1:4, 1:8, 1:16, and 1:32 in sterile normal saline by using double dilution technique as shown in figure 1. These concentrations are labeled as 2, 3, 4 5, 6 respectively.

· Same way, Take the both the disinfectant solutions in concentration of 1:1,1:2, 1:4,1:8,1:16, each 1ml in sterile test tube. These disinfectant concentrations are labeled as A, B, C, D E, and F respectively. The diluent for the disinfectants should be peptone water. Peptone water provides activity of disinfectant in presence of the organic matter up to some extent. Mouth of the test tube should be covered. Prepare 5 sets of each concentration of the both the solution.

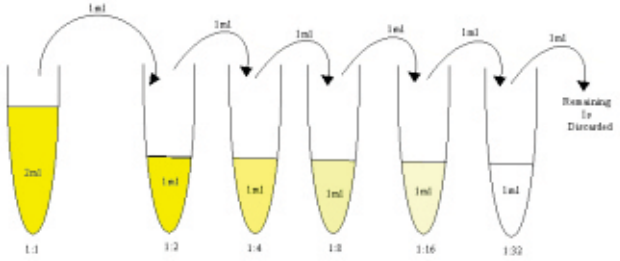
· Each set is inoculated with 100µl of organism of each concentration. A to F into 1 to 6. e.g.1:1 concentration of micro organism is inoculated in to the disinfectant solution having concentration of 1:1 to 1:32.like wise for other concentration of microorganism too.

· After inoculation the test tubes are kept at room temperature for the defined contact time required for the disinfection e.g. 30 minute for sodium hypochlorite. The contact time for the new disinfectant was 5 minute as per manufacture guide line.

· When contact period is over, inoculums from each test tube taken and cultured into nutrient agar plate to see the viability of the organism. The plates are incubated for overnight incubation at $37^0$ C. The test tube can also be put in to the incubator to monitor the growth, how ever the final results are calculated from the growth on the plates. After 18 to24 hour the results are noted.

· 1:32 concentration of microorganism and 1:1 concentration of disinfectant are taken as positive and negative control respectively.

**Figure 1: method of dilution.**




## 3. Results

The growth of bacteria on the nutrient agar plate is noted according to concentrations e.g. A to F in 1 to 6 shown tin Tables 1&2.

**Table No. 1. Growth with sodium hypochlorite**

| Dis infectant | | Micro organism concentration | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1:1 | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 |
| | | 1 | 2 | 3 | 4 | 5 | 6 |
| 1:1 | A | G | NG | NG | NG | NG | NG |
| 1:2 | B | G | G | NG | NG | NG | NG |
| 1:4 | C | G | G | G | NG | NG | NG |
| 1:8 | D | G | G | G | G | G | NG |
| 1:16 | E | G | G | G | G | G | NG |
| 1:32 | F | G | G | G | G | G | G |

**G= Growth: NG= No Growth**

**Table 2: Growth with new disinfectant**

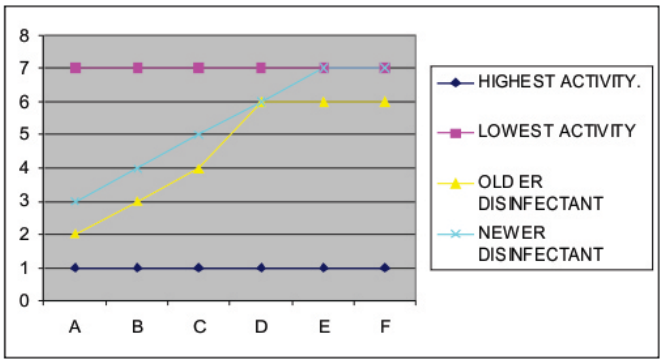
| Dis infectant | | Micro organism concentration | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1:1 | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 |
| | | 1 | 2 | 3 | 4 | 5 | 6 |
| 1:1 | A | G | NG | NG | NG | NG | NG |
| 1:2 | B | G | G | G | NG | NG | NG |
| 1:4 | C | G | G | G | G | NG | NG |
| 1:8 | D | G | G | G | G | G | NG |
| 1:16 | E | G | G | G | G | G | G |
| 1:32 | F | G | G | G | G | G | G |

From the above tables, by taking first no growth point in each row we make laboratory standard for each disinfectant e.g. A2 B3 C4 D6 E6 F7 for sodium hypochlorite and A3 B4 C5 D6 E7 F7. When there is growth even at 6 level dilutions, we take 7 for reading for disinfectant activity.

## 4.Discussion

Earlier attempts [6] of sterilization and disinfectant control have been simply mathematize in this test method and have been tried to make disinfectant activity in the form graphical manner. In this test, any disinfectant activity of A1 B1 C1 D1 E1 F1 suggests highest anti bacterial activity while A7 B7 C7 D7 E7 F7 is the lowest (or no) activity. Disinfectant activity can also see by graphical method too as shown in figure 2. Disinfectant having good antibacterial activity tends to remain near to base line highest activity line. As any disinfectant must be able to inactivate all type of bacteria, so one can use any own laboratory isolate e.g. Staphylococcus, Pseudomonas, E.coli, Klebsiella sp. etc. and no requirement of any ATTCC type standard strain. We can test any disinfectant (e.g. sodium hypochlorite, glutaraldehyde etc.), and also make own laboratory standard for that disinfectant

**Figure 2: Graphical antibacterial activity at different concentrations**




## 5. Conclusion

1. One can the also test the same disinfectant periodically to check the potency against its own standard.
2. One can compare different disinfectants by performing the test at same time with same organism.
3. The effectivity of the disinfectant is considered from the multiple factors, some of them are- cost, dilution factor recommended for the use, contact time of the disinfectant, toxicity, compliance etc.

**Limitation of this test**

1. Antibacterial standard made here is organism, disinfectant and laboratory specific. The organism can be preserved, however yearly new standard should be made even for the same organism.
2. The testing is done at very small scale level. Multiple data from the different part of the world is required its acceptibity of the test.
3. Claimed antivirus and other disinfectant activity can not be measured from this test.

[1] Testing of disinfectants: Sridhar rao available at. (www.microrao.com).

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[5] Hume, Emma B. et al. Soft Contact Lens Disinfection Solution Efficacy: Clinical Fusarium Isolates vs. ATCC 36031. Optometry & Vision Science.2009 86 (5) :415-419

[6] Joan F.Gardner, Margaret M Peel. Introduction to sterilization and disinfection control, 2ndedition, Churchill Livingstone, 1991.