Disinfectant Qualification Versus Claims

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ABSTRACT

Regulations, both within Europe and the United States, require disinfectant manufacturers to test disinfectants prior to sale. Regulations also require end-users to qualify disinfectants using a sound scientific approach. As regulations and methods continue to grow and evolve, it can be difficult to understand the requirements for the disinfectant supplier relative to the end user. This presentation will aid in understanding US, EU and rest of world regulations and testing requirements including why new methods are slow to evolve. It will also address the testing role, if any, of new and novel microbiological test methods versus older "Pasteur" microbiology tests while discussing what and how to test and other best practices to aid in the successful implementation of a rotational disinfectant program.

Introduction

Disinfectant testing can be complicated and technique-sensitive. The methods that exist for registration of disinfectants may not be applicable to end-user disinfectant qualification studies. Understanding the differences between the methods and each's purpose can be helpful in qualifying disinfectants and designing a DET evaluation.

USA Regulatory Test Guidelines

Disinfectants are registered with the United States Environmental Protection Agency as pesticides prior to sale. Suppliers are responsible for testing any targeted claims that will appear on a product label with mandates for each specific microorganism to be tested. This can create some confusion given the number of microbial species in existence and conferring activity against similar species. As regulatory standards, these methods represent worst-case conditions and pass/fail criteria that exceed what would be encountered in a controlled environment.

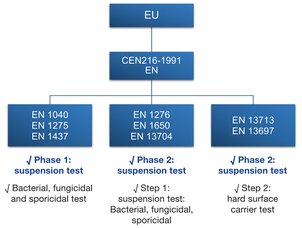
Many of these methods are qualitative but have been in place for decades and are difficult to change or improve because of the historical record of data generated to register existing products.

CLAIM	TEST REPLICATES	TEST METHOD(S)	TEST ORGANISMS	PASS/FAIL CRITERIA
Bactericidal	60 carriers per lot per microorganism 3 lots of product	AOAC Use-Dilution Method, (955.15), (964.02)	*Staphylococcus aureus* ATCC 6538 *Pseudomonas aeruginosa* ATCC 15442 Additional supplemental bacteria as required by claim	*S. aureus*, ≤3/60 positive *P. aeruginosa*, ≤6/60 positive All others, 0/10 positive
Fungicidal	Suspension test or 10 carriers per lot 2 lots of product	AOAC Fungicidal Activity Test (955.17) Modified AOAC Use-Dilution	*Trichophyton interdigitale* ATCC 9533	Complete kill / No Growth
Tuberculocidal	10 carriers per lot 2 lots of product	AOAC Tuberculocidal Activity Test (965.12)	*M. tuberculosis* var *bovis* (BCG)	No positive carriers
Virucidal	1-2 carriers per lot	ASTM E-1053 Test Method for Efficacy of Virucidal Agents Intended for Inanimate Environmental Surfaces	Specific virus claimed	≥3.0 log10 reduction
Sporicidal	60 carriers per lot per surface (x2) per microorganism 3 lots of product	AOAC Sporicidal Activity of Disinfectants (966.04)	*Bacillus subtilis* ATCC 19659 *Clostridium sporogenes* ATCC 3584	Complete kill on all carriers
Sanitizer for Non-Food Contact Surfaces	3 lots of product	ASTM E-1153 Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate, Hard, Nonporous Non-Food Contact Surfaces	*Staphylococcus aureus* ATCC 6538 *Pseudomonas aeruginosa* ATCC 4352 -or- *Enterobacter aerogenes* ATCC 13048	≥3.0 log10 reduction

Figure 1: US EPA claim requirements[1]

EU Regulatory Test Guidelines

European registration testing utilizes multiple phases and tiers for disinfectant testing. Phase 1 employs basic suspension tests while Phase 2 studies include both qualitative methods (step 1) and quantitative methods (step 2).



These newer standards allow for the use of quantitative methods that provide a greater level of scientific data than the qualitative pass/fail methods frequently used historically in the US.

They also allow for both the use of coupons and the testing of disinfectants in suspension with a log10 reduction value set as the pass/fail criteria which can provide greater information to both the supplier and end-user.

These methods continue to evolve for different application types and product versions.

	CLAIM	TEST METHOD(S)	TEST ORGANISMS	PASS/FAIL CRITERIA
Basic Suspension Tests	Bacteria	EN 1040	*Pseudomonas aeruginosa* ATCC 15442 *Staphylococcus aureus* ATCC 6538	≥5.0 log10 reduction
	Fungi	EN 1275	*Candida albicans* ATCC 10231 *Aspergillus brasiliensis* ATCC 16404	≥4.0 log10 reduction
	Spores	EN 14347	*Bacillus subtilis subsp. spizizenii* ATCC 6633 *Bacillus cereus* ATCC 12826	≥4.0 log10 reduction
Quantitative Suspension Tests	Bacteria	EN 1276	*Pseudomonas aeruginosa* ATCC 15442 *Staphylococcus aureus* ATCC 6538 *Escherichia coli* ATCC 10536 *Enterococcus hirae* ATCC 10541	≥5.0 log10 reduction
	Fungi	EN 1650	*Candida albicans* ATCC 10231 *Aspergillus brasiliensis* ATCC 16404	≥4.0 log10 reduction
	Spores	EN 13704	*Bacillus subtilis* ATCC 6633	≥3.0 log10 reduction
Hard Surface Test	Bacteria	EN 13697	*Pseudomonas aeruginosa* ATCC 15442 *Staphylococcus aureus* ATCC 6538 *Escherichia coli* ATCC 10536 *Enterococcus hirae* ATCC 10541	≥4.0 log10 reduction
	Fungi		*Candida albicans* ATCC 10231 *Aspergillus brasiliensis* ATCC 16404	≥3.0 log10 reduction

Figure 3: European Test Methods[1]

Key Registration Methodology

AOAC Penicylinder Test[2]:

The AOAC Use Dilution Test (UDT) is the required method utilized to establish a disinfectant with the US EPA as a bactericide. The method utilizes stainless steel penicylinders, soaked in the bacterial inoculum which are added to the disinfectant for the established wet contact time. After exposure, the carriers are placed into neutralizing growth media and incubated for growth/no growth.

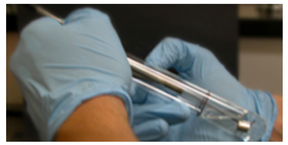


Image 1: Bactericidal UDT

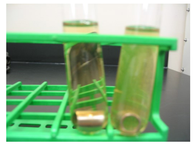


Image 2: Qualitative UDT results

AOAC Sporicidal Test[3]:

The method utilized for registering a sporicide in the United States requires complete kill on 720 tested carriers (suture loops and porcelain penicylinders) against spores of B. subtilis and C. sporogenes. This incredibly high challenge can often result in extended contact times for sporicidal label claims.

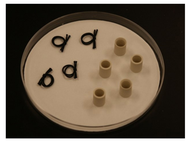


Image 3: Carriers for the AOAC Sporicidal Test

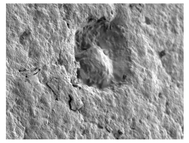


Image 4: SEM image of a porcelain penicylinder

EN 13697 Test[5]:

In Europe, the most widely used hard surface method is the BS EN 13697, Quantitative Non-porous Surface Test. This method, unlike many of the methods utilized by the US EPA, allows for calculation of a true log reduction value. Bacteria or fungi are dried onto a stainless steel disk, disinfectant is applied and survivors quantitated after the established contact time.

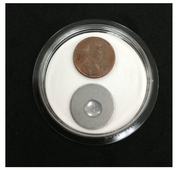


Image 5: Inoculated EN 13697 Carrier

Image 6: EN 13697 carrier treated with disinfectant

Spray and Wipe Methods

Specific methods exist both in the United States and Europe for testing spray and wipe products for registration and claims. These methods add additional variability because of factors including inconsistent spray patterns on a small surface and physical removal from a wipe beyond a disinfectant's capabilities.

End-User Qualification

FDA requires "The suitability, efficacy, and limitations of disinfecting agents and procedures should be assessed. The effectiveness of these disinfectants and procedures should be measured by their ability to ensure that potential contaminants are adequately removed from surfaces."[5]

Figure 4: Stages of Disinfectant Validation

In Vitro Testing / Disinfectant Efficacy Test (DET):

When conducting a DET evaluation, US AOAC methods are inappropriate for use given their challenge levels and qualitative endpoints. Although, some elements such as inoculum prep may be useful, a quantitated hard surface method must be employed for DET. EN-13697 and ASTM E2197 are two methods that can be easily adapted for Disinfectant Efficacy Test using both ATCC and in-house isolates and common surfaces found in the cleanroom. USP 43 <1072> is useful in determining log reductions.[6]

Carrier acceptance criteria USP 43 <1072>

- 2 log reduction bacterial and fungal spores
- 3 log reduction vegetative bacteria and yeast

Image 7: DET Test Coupon

In situ (Triple Clean)

After a shutdown is a great opportunity to generate data that shows your disinfectant rotation program is able to work well under worst-case conditions.

Utilizing real-world application techniques and equipment, an in-situ evaluation demonstrates disinfectant performance under the actual conditions found in the controlled environment versus the laboratory evaluation.

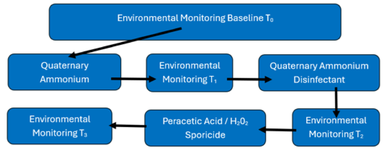


Figure 5: Potential in situ test plan

Conclusion

The methods employed by the US EPA were developed in the 1950's and have a long history of successful use for the registration of disinfectants.[7] Unfortunately, these older methods tend to be qualitative in nature and not applicable as an end-user qualification method. Efforts continue to update and modify the methods, but this process is time-consuming as any method bias that affects existing registrations must be avoided. The methods used in Europe are much newer and quantitative which make them a better choice for modification to meet qualification requirements. Understanding the different applications of the selected methods will help the end-user to both better interpret disinfectant label claims as well as select the appropriate experimental design for in vitro testing.

References

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3. Official Methods of Analysis (Revised 2013) 21st ED., AOAC INTERNATIONAL, Method 966.04, Gaithersburg, MD, Chapter 6
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6. USP, USP General Chapter <1072>, "Disinfectants and Antiseptics," USP 43-NF 38 (Rockville, MD, 2022)
7. Should the AOAC Use-Dilution Method Be Continued for Regulatory Purposes? Omidbakhsh: Journal of AOAC International Vol. 95, No. 2, 2012.