Review of Standard for Evaluating the Effectiveness of Contact Lens Disinfectants

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ABSTRACT: A new standard has been developed by the International Organization for Standardization (ISO) for evaluating contact lens disinfectants. This paper reviews the ISO 14729 standard and applies it to marketed products. The historical significance, justifications, scope, interpretations, application, and worthiness are discussed. The standard provides a more consistent procedure and a higher standard for contact lens disinfectants. As a result, more effective contact lens disinfectants have been marketed.

Keywords: contact lens, disinfectants, antimicrobial, ISO, method, procedure, standards

Introduction

The international regulatory hurdles for approval of a new contact lens care product were formidable in the 1980s and early 1990s. National bodies were requiring specific, national tests to be conducted. These requirements were in response to concerns over the contamination of lens care products spurred by reports documenting contamination of lenses during consumer use (20,30,31,47,54), lens cases (6,11,19,24,27), and solutions (3,5,9,12).

Several countries had specific sets of standards or guidelines which a contact lens disinfecting product (CL disinfectant) had to meet in order for the governing regulatory body to grant approval to market in their country. The Food and Drug Administration (FDA) guidelines were the most extensive regulations governing the approval of contact lens disinfectants at that time (49,50) and they had a significant impact on the development of the new international standard. The FDA required that a CL disinfectant be tested by a D-Value test and D-Values be calculated (42), although no specific criteria were set (43). A D-Value is the calculated time required to reduce a population of microorganisms by 90% or one-$\log_{10}$ (log). In addition, the FDA required no survivors after a lens was treated according to label instructions with the CL disinfectant and accessory products (e.g., cleaners, rinsing solutions) when tested by the Microbial Challenge Multi-Item Test (MCMIT) (44). On the other hand, the French guidelines (15,16,17) required that a CL disinfectant show $\geq$ 5 log reduction on contaminated lenses without the help of a cleaning (rubbing) and rinsing step (similar to hard surface disinfectants). The British (26) had different standards, requiring that a CL disinfectant meet their preservative effectiveness standards (i.e., a 2-log reduction at 6 hours and a 3-log reduction at 24 hours for bacteria, and a 2-log reduction of fungi by 7 days). The Dutch (13) had yet another standard, mandating a 3-log reduction of bacteria within the use period and a 2-log reduction of the fungi at 7 days (Tables 1 and 2). This diversity of national standards meant that products had to be tested by multiple tests that were frequently ill defined.

Why Was the ISO Standard Developed?

Microbiology methods for testing CL disinfectants needed to be harmonized for three reasons. First, accurate estimation of antimicrobial efficacy required precisely controlled and reproducible conditions. The different conditions mandated in different tests may have affected the apparent efficacy of the CL disinfectants. Secondly, the expense associated with the multiple testing requirements hampered the introduction of new products. Agreement was needed as to how to establish efficacy criteria for these products. The third reason is the consideration that contact lenses are inserted

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Table 1. Historical review of antimicrobial activity standards for contact lens disinfection solutions.

Key Elements	Regulatory Documents/Guidelines		
	US FDA PMA Guidelines (49)	British (MCA) Guidelines (26)	Dutch FDA Guidelines (13)
Challenge Organisms	*Staphylococcus epidermidis* (ATCC 17917) *Pseudomonas aeruginosa* (ATCC 15442) *Serratia marcescens* (ATCC 14041) *Candida albicans* (ATCC 10231) *Aspergillus fumigatus* (ATCC 10894) *Herpes simplex* (ATCC VR 260)	*Staphylococcus aureus* (ATCC 6538) *Pseudomonas aeruginosa* (ATCC 9027) *Candida albicans* (ATCC 10231) *Aspergillus niger* (ATCC 16404)	*Staphylococcus aureus* (ATCC 6538) *Pseudomonas aeruginosa* (ATCC 15442) *Escherichia coli* (ATCC11229) *Candida albicans* (ATCC 10231) *Aspergillus niger* (ATCC 16404)
Inoculum	0.5-2.0 X 10^6 CFU/ml	10^6 CFU/ml	10^5 CFU/ml
Diluent	Phosphate buffered saline with neutralizers for test	0.1% Peptone with Neutralizers	Inactivating Fluid for first dilution (5 minute contact time), saline thereafter
Sampling Regimen	Sample at various time intervals	Include intended use time and extend to 28 days	Include intended use time or 5 minutes for bacteria and 7 days for fungi, no re-growth out to 28 days.
Recovery Media	Not specified	Bacteria: Soya Tryptone Agar (TSA) with inactivators. Fungi: Sabouraud Dextrose Agar	Bacteria/Yeast: Soya Tryptone Agar (TSA) Mold: Malt Extract Agar
Incubation	Not specified	Bacteria: 72 hr at 30-35°C Fungi: 5 days at 20-25°C	Bacteria/Yeast: 72 hr at 37°C Mold: 5 days at 24°C
Assessment of Effectiveness	Disinfection cycle calculated from the number of "D-values" to bring the level of contamination to a log value of 0, plus three additional "D-values."	Bacteria: 2-$\log_1$ unit reduction by 6 hours, and 3-log reduction by 24 hours. Fungi: 2-log unit reduction by 7 days	Bacteria: 3-log unit reduction within use period Fungi: 2-log unit reduction in 7 days

Table 2. Historical review of lens challenge testing criteria.

Key Elements	Regulatory Documents/Guidelines	
	US FDA PMA Guidelines (49)	**French Guidelines (16,17)**
Objective of Test	Evaluate effectiveness of the contact lens care regimen	Evaluate effectiveness of the contact lens disinfectant or lens care regimen
Challenge Organisms	*Staphylococcus epidermidis* (ATCC 17917) *Pseudomonas aeruginosa* (ATCC 15442) *Serratia marcescens* (ATCC 14041) *Candida albicans* (ATCC 10231) *Aspergillus fumigatus* (ATCC 10894) *Herpes simplex* (ATCC VR 260)	*Staphylococcus aureus* *Pseudomonas aeruginosa* *Escherichia coli* *Candida albicans* *Aspergillus niger*
Inoculum Level	0.5 – 2 X 10^6 CFU/lens in organic soil	1.0 X 10^5 CFU/lens in organic soil
Recovery Method	Sterility Test, subculture lens and soak solution separately in liquid growth media specified.	Membrane filtration of solution and lens, quantify survivors.
Incubation	14 days at optimal temperature	Bacteria: 30-35°C Fungi: 20-25°C 24 hours, 48 hours, 5 days
Recovery Media	Suitable growth medium containing neutralizers	Membrane filters placed on Soybean-Casein Digest Agar (bacteria) or Sabouraud 4% Dextrose Agar (fungi)
Number of Lenses Tested per Organism	Total of 20 lenses from Groups 1, 2, 3, and 4 per organism	2
Assessment of Effectiveness	All lenses and solutions must be negative (sterile)	≥ 5 log unit reduction of the organisms on the lenses (*A. niger* reduction may not be required)

onto the ocular surface; therefore, a disinfecting solution can not be required to be so strong as to damage the patient's eye.

This harmonization effort fell under the ISO umbrella. The United States provided major support in the development these standards. The ISO 14729 procedure, "Microbiological Requirements and Test Methods for Products and Regimens for Hygienic Management of Contact Lenses," for testing of CL disinfectants emerged.

Developing the Standard

In the 1980s representatives from several CL disinfectant manufacturers began to draft potential standards and criteria. A group of microbiologists from several of the companies in the United States worked together to provide procedural details which would give consistent results. The recommendations were taken to the ISO committee (22) and regulatory bodies of the world. Comments from the groups were incorporated into the

standards. This was the basis for the current ISO 14729 standards.

Justifications

There were several areas of debate about the procedure based on differences in historical perspective and usage. The United States FDA wanted organic soil to be included in the testing of CL disinfecting procedures. The United Kingdom Medicines Control agency (26) thought that *Acanthamoeba* should be included. The inclusion of virucidal testing was included in the older FDA disinfection procedures but was less of a concern. These topics were annexed as informative technical reports into the resulting ISO 14729 procedure as described below.

A laboratory prepared organic soil composed of a mixture of killed *Saccharomyces cerevisiae* and inactivated fetal bovine serum was part of the earlier FDA guidelines (49,50). This procedure recommended testing lenses coated with microorganisms in this organic soil mixtures by the label directions for use of the CL disinfection regimen (7,28). Several representatives argued that testing efficacy of the CL disinfectant solution in the presence of organic soil provided useful information about whether the product may become neutralized by organic material that is likely to be present on contact lenses removed from the eye. The counter argument was that the use of organic soil was not useful because there was not a consistent method for preparing soil and none of the laboratory prepared soils accurately modeled tear components (2,18). Additionally, even tears themselves varied in composition from person to person (18,51,53). Finally, since the rubbing and rinsing steps removed soil, it was probably of little value in the Regimen Test. As a compromise, the use of organic soil in the Regimen Test is an option in an informative technical report in an Annex of the procedure. The practical outcome of this compromise is that the use of soil is required in the United States, but is not required in Europe.

Concern about *Acanthamoeba* infections in the U.K. prompted the debate over the inclusion of a requirement to *Acanthamoeba* activity. However, the incidence of *Acanthamoeba* is very low (29,41) and there are no standardized methods for testing *Acanthamoeba* (4). Therefore, this protozoan is not required as a challenge organism.

Testing CL disinfectants for virucidal activity was not included in the ISO 14729. The procedure is for testing CL disinfectants for individual use. Viruses are obligate intracellular parasites and do not replicate or proliferate on contact lenses (34). Testing products with viruses is important in relation to trial lens procedures. Trial lenses are reused between multiple patients, following proper disinfection. However, spread of HIV, hepatitis, and adenovirus between patients is a concern (8,10,52).

Scope of the ISO 14729 Standard

The ISO 14729 disinfection procedure evaluates the relative efficacy of the CL disinfection system. Two basic principles have been accepted. The first was that the new procedure would be based on establishing criteria that are realistic to achieve, with the understanding that the innate antimicrobial

Figure 1. Flow Chart for Stand Alone and Regimen Tests. *This chart diagrams the relationship between the Stand Alone Test and the Regimen Test in the evaluation of a new contact lens disinfectant. A summary of the criteria is provided in Table 4.*

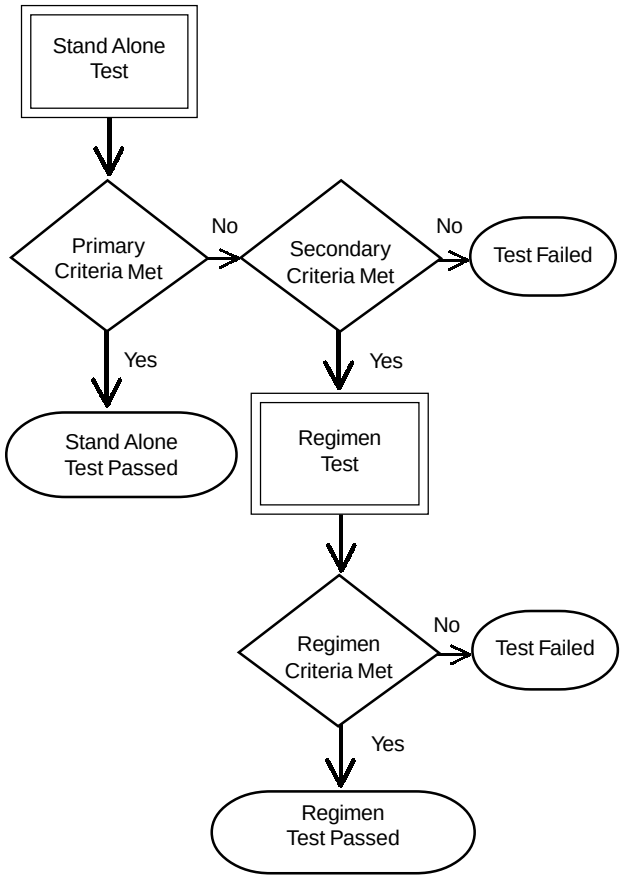

Table 3. Summary of performance requirements for ISO 14729 contact lens disinfection procedures. *Passage of the Stand Alone criteria allows the disinfecting solution to be marketed as a disinfectant. This requires reduction of the challenge organism by the stated log unit values at the end of the disinfection period. Less potent disinfectants may be tested as part of a regimen if they meet the "Regimen qualification" criteria outlined.*

	Average Log Reduction at Disinfection Time				
	Fungi		Bacteria		
Criteria	Fs[a]	Ca	Sm	Pa	Sa
Primary Criteria of Stand Alone Test	1.0	1.0	3.0	3.0	3.0
Secondary Criteria of Stand Alone Test (Regimen Qualification)	Stasis[b]	Stasis	*> 1*[c]	*≥ 1*	*≥ 1*
			Sm + Pa + Sa = 5.0[c]		
Regimen Criteria	≤10 CFU[d]	≤10 CFU	≤10 CFU	≤10 CFU	≤10 CFU

[a] Fs *Fusarium solani* ATCC 36301
Ca - *Candida albicans* ATCC 10231
Sm - *Serratia marcescens* ATCC 13880
Pa - *Pseudomonas aeruginosa* ATCC 9027
Sa - *Staphylococcus aureus* ATCC 6538

[b] Stasis at the soaking time

[c] The minimum acceptable log reduction provided by the sum of the averages is a minimum of 5.0 log reduction for all three bacteria within the recommended soak time with a minimum average of 1.0 log reduction for any single bacterial type.

[d] Equivalent to an average of not more than 10 CFU per lens type/storage solution combination

activity of the disinfectant would be assisted by the contact lens rubbing and rinsing regimen (23). Secondly, the new procedure would allow for approval of a CL disinfectant based on its activity within the lens care regimen.

The final procedure consists of two parts (Figure 1). The first part evaluates the innate antimicrobial activity of the disinfecting solution alone (hence, it was named the "Stand Alone Test"). If a product meets the primary criteria, it is exempted from further testing by the Regimen Test. It is assumed that when used with the rubbing and rinsing steps (which removes 10^3 to 10^4 microorganisms from the lens), the disinfectant will be capable of passing a more stringent regimen criteria (21,39,40). The second part (if required) is the Regimen Test. The Regimen Test evaluates the antimicrobial efficacy of the entire regimen described in the package insert (e.g., rubbing, rinsing, and disinfecting) (Table 3).

The secondary criteria required that the concentration of each bacterial species be reduced by a minimum 1.0 log unit, and that the sum of the log reductions of the three challenge bacteria had to exceed 5.0 log units for the disinfectant to be eligible for evaluation by the Regimen Test. This was believed to be the minimal level of activity acceptable for a CL disinfectant, even allowing for the mechanical cleansing of the lens.

The ISO disinfection test has several roots. The Stand Alone Test is based on pre-existing antimicrobial activity tests. These procedures include the FDA D-Value Test, the British Pharmacopoeia Test for Preservative Efficacy, and the Dutch disinfection test (Table 1). The primary criteria of the Stand Alone Test are intended for CL disinfection products with a higher level of antimicrobial activity. If the CL disinfectant meets the primary criteria, then testing by the Regimen Test is not required. The assumption is made that the rubbing and rinsing

Table 4. Summary of ISO 14729 procedure. *ISO/FDIA 14729. Microbiological Requirements and Test Methods for Products and Regimens for Hygienic Management of Contact Lenses.*

Key Elements	Stand Alone Test				Regimen Test			
Challenge Organisms	*Staphylococcus aureus* (ATCC 6538) *Pseudomonas aeruginosa* (ATCC 9027) *Serratia marcescens* (ATCC 13880) *Candida albicans* (ATCC 10231) *Fusarium solani* (ATCC 36031)				*Staphylococcus aureus* (ATCC 6538) *Pseudomonas aeruginosa* (ATCC 9027) *Serratia marcescens* (ATCC 13880) *Candida albicans* (ATCC 10231) *Fusarium solani* (ATCC 36031)			
Inoculum	1.0×10^5 to 1.0×10^6 CFU/ml Not more than 5 passes from depository stock Separate inoculum for each lot of product				1.0×10^5 to 1.0×10^6 CFU/ml Not more than 5 passes from depository stock Separate inoculum for each lot of product			
Growth Conditions	Bacteria	18-24 hr	30-35°C	TSA	Bacteria	18-24 hr	30-35°C	TSA
	Yeast	42-48 hr or 18-24 hr	20-25°C or 30-35°	SDA	Yeast	42-48 hr or 18-24 hr	20-25°C or 30-35°	SDA
	Mold	10-14 days	20-25°C	PDA	Mold	10-14 days	20-25°C	PDA
Diluent	Dulbecco's phosphate buffered saline or suitable diluent (with Tween for mold) for cultures Validated neutralizing medium for test				Dulbecco's phosphate buffered saline or suitable diluent (with Tween for mold) for cultures Validated neutralizing medium for test			
Neutralizers	Required and validated				Required and validated			
Number lots	3 lots of product in final packaging				3 lots of product in final packaging			
Lenses	Not required				Total of 8 lenses per lot per organism 8 lenses from a single soft lens type or 4 lenses each from Groups 1 and 4 soft lens types or 4 lenses each from two RGP polymer lens types			
Organic soil	Not required				Optional, when used: killed *Saccharomyces cerevesiae* in inactivated bovine or horse serum			
Regimen	Not done				Follow manufacturer's instructions			
Sampling	Sample at 25%, 50%, 75% and 100% of minimum disinfection time for all organisms, and additionally at 400% for fungi				At disinfection time, membrane filtration of each lens and treatment solution and filter to plate of solid medium; lens placed separately into agar medium overlay.			
Recovery Media	Suitable recovery medium				Suitable recovery medium			
Assessment of Effectiveness	Primary Criteria				Primary Criteria			
	Bacteria	Average 3.0-log reduction at disinfection time			Average of NMT 10 CFU per lens per lens group and per organism			
	Fungi	Average 1.0-log reduction at disinfection time						
	Secondary Criteria							
	Bacteria	Sum of averages is 5.0-log reduction for bacteria with minimum of 1.0-log reduction at disinfection time						
	Fungi	Stasis						

steps of a regimen can be relied upon to lower the contact lens bioburden to levels sufficient to ensure efficacy when used according to label instructions. Therefore, further testing of that product would not be necessary when it is to be used in a standard lens care regimen that includes rubbing and rinsing of the lens. The primary criteria of the Stand Alone Test and Regimen Test criteria were readily agreed upon. However, some other products marketed at that time showed weaker antimicrobial activity. Therefore, the group agreed to add secondary criteria to the Stand Alone Test as a qualification for the Regimen Test. Addition of the secondary criteria allowed weaker CL disinfection systems to be marketed as part of a regimen. These products had proven safe and effective over time. In addition, the secondary criteria were designed to prevent products, such as unpreserved saline solutions, from being marketed as the disinfecting part of a regimen. Therefore, if the disinfectant alone exhibits a lower level of activity, then the entire regimen is tested to ensure efficacy. A two-part or two-tier approach is not unique. The criteria of European Pharmacopoeia preservation efficacy test uses two levels of preservative activity (14). The Regimen Test is based on the FDA MCMIT and the French AFNOR lens challenge test (Table 2). The Regimen Test evaluates the effect of the entire regimen, including the rubbing, rinsing, and disinfection steps as a unit. The ISO 14729 Regimen Test uses the best of two procedures: The FDA MCMIT which evaluates the effect of the disinfection process to eradicate microorganisms and the French AFNOR Test which uses membrane filtration for recovery and has criteria of a greater than a 5-log reduction.

The Stand Alone Test

The Stand Alone Test evaluates the innate antimicrobial activity of the CL disinfectant within the recommended disinfection time. With this procedure, three lots of product are evaluated. In the test, each lot of product is challenged with a large challenge (10^6 CFU/ml) of five different microorganisms (Table 4). The microorganisms include Gram-negative bacteria (*Serratia marcescens* and *Pseudomonas aeruginosa*), Gram-positive bacteria (*Staphylococcus aureus),* yeast (*Candida albicans*), and mold (*Fusarium solani*). The product is sampled for survivors at 25, 50, 75, and 100% of the disinfection time for bacteria, and then additionally at 400% of the disinfection time for yeast and mold. The additional sample time was included to provide assurance on the reliability of the study. If the disinfectant meets the requirements of the Stand Alone Test, the product can be labeled as a CL disinfectant.

The Stand Alone Test is designed to increase the stringency and the reproducibility of the test without sacrificing the disinfecting capability of the lens care regimen. Several changes were made in the base FDA document with these goals in mind:

1. *Choice of challenge microorganisms*

   The Stand Alone test is conducted with a specific set of microorganisms chosen as index organisms similar in scope to those of the previously used regulatory tests. The most significant change is the use of the fungus *Fusarium solani* to replace the spoilage organism *Aspergillus fumigatus* in the battery of challenge organisms. This change was justified on two counts. First, the fungus *Fusarium* is an ocular pathogen (25). Second, an index organism should display an intermediate spectrum of susceptibility to disinfecting agents, allowing differentiation among solutions. *Fusarium solani* is clearly preferable to *Aspergillus* in this regard. Finally, efficacy against *Aspergillus* is demonstrated in the test for preservation efficacy, also required for regulatory approval of a new disinfecting solution. Another important change was the control of organism growth conditions. The ISO 14729 procedure has specific requirements for the number of passes from the original stock culture and specific growth media, temperature and incubation times.

2. *Description of efficacy*

   A second major change is in the abandonment of the calculated D-Values to describe efficacy. A D-Value is the calculated time to reduce a population of microorganisms by 90% or by one-log unit. D-Values are usually applied to thermal disinfection where the kill rate is linear (32). Since the kill rates of CL disinfectants are not linear, D-Values are neither predictive of

antimicrobial activity nor are they reproducible for CL disinfectants. The method for determining acceptability in the ISO 14729 procedure is a requirement for a specific log reduction of each challenge species in order to demonstrate efficacy. This method is desirable because it is simple and reproducible. It has been used as a method to show preservative efficacy for many years.

3. *Required validation*

A detailed method is provided for validating the assay. The validation requires demonstration of the ability of the test system to recover survivors (33,46,45).

4. *Number of lots tested*

Data from three lots are required and each lot is tested with separate inoculum. This provides more information on the performance of the product.

5. *Low level disinfectants may qualify for the "Regimen" test*

It was recognized that CL disinfectants must have low toxicity because of use in the eye. The benefits to the patient from these systems containing less toxic chemicals outweigh any increased hazard due to lower bacterial efficacy. A provision was made to allow solutions to be marketed as part of a recognized regimen if the disinfecting solution alone showed a minimum standard of efficacy. If a product meets these minimal standards, then it qualifies to be tested by the Regimen Test. This minimal entry standard is in the ISO 14729 procedure to assure that any candidate CL disinfectant has at least a minimal level of antimicrobial activity. It was well known that the contact lens rubbing and rinsing steps are capable of reducing the microbial load from an initial inoculum of 10^6 CFU/lens to about 10^3 CFU/lens (21,39,44). Thus, the combination of preliminary regimen steps, such rubbing and rinsing, and the active disinfection step provide excellent removal and kill of microorganisms.

The Regimen Test

The Regimen Test was designed to evaluate the efficacy of a disinfecting solution in a regimen (Table 4). The contact lens care regimen as a whole is evaluated, including the rubbing, rinsing, disinfecting, and any other identified manipulations. After treating each lens by the steps outlined in the contact lens care regimen, the number of surviving organisms on the lens and in the solution is determined by membrane filtration.

The Regimen Test is a carrier test, loosely based upon the MCMIT. A carrier test is a test where objects, such as contact lenses, are inoculated with microorganisms and tested according label in structions for use. There are several important points of differentiation from the MCMIT, however, as detailed below:

1. *Choice of challenge microorganisms*

The Regimen Test uses the same challenge organisms as the Stand Alone Test. Again, the major change is the use of *Fusarium solani* as the challenge organism in place of the spoilage organism *Aspergillus fumigatus*.

2. *Organic load*

The organic load is optional in this test, in contrast to the requirements of the FDA and the French lens challenge tests. The organic load consists of inactivated bovine serum and killed *Saccharomyces cerevisiae*. It is a laboratory model for deposits accumulated during wear, such as proteins, lipids, mucins, and other organic components (53). However, a laboratory prepared organic load is not the same as that found in tear deposits. (2,18).

3. *Three lots*

The efficacy of the regimen against each organism must be demonstrated with three different manufactured batches of product. Previously, the FDA allowed testing of only a single lot of product.

4. *Quantitative recovery*

The microorganisms from all solutions and lenses are recovered by membrane filtration and plated onto an agar surface. Passing the test is determined by recovery of a minimal number of microorganisms. The ISO 14729 Regimen Test requires that for each microbial species, the average count for all lots tested be no more than (NMT) 10 survivors for each lens-solution combination per species. A total of 24 lenses per microbial species are tested. This is an improvement over the MCMIT because the ISO 14729 procedure quantitates the survivors, whereas the MCMIT was based on turbidity (indicating microbial growth), which could have resulted from only a few microorganisms, or several million.

5. *Validation requirements*

The quantitative recovery allows a far more precise validation protocol than with liquid recovery. The Regimen Test is validated to detect low numbers of viable microorganisms from the test solution.

The design of CL disinfecting solutions should take into consideration the event of non-compliance. For example, the disinfection time should be appropriate for contact lens wear. Likewise, if the rubbing step is eliminated, the product should be tested under stress conditions (23). Although outside the scope of the ISO standard, additional testing of the CL disinfectant, such as other microorganisms, high levels of organisms, and organic soil, may be considered depending on the particular patient instructions for use of the product.

Interpretation of the ISO Standard

The ISO 14729 standard has two tiers of criteria (Figure 1). The first tier is the Stand Alone Test and the second tier is the Regimen Test. The Stand Alone test gives insight into the performance of the CL disinfecting solution without the aid of the proven regimen steps, such as rubbing, rinsing, and disinfection.

The Stand Alone Test has two sets of criteria, the primary and secondary. If the CL disinfecting solution reduces the level of bacteria by an average greater than or equal to 3.0 log units and the level of fungi by an average greater than or equal to 1.0

Table 5. Comparison of ISO 14729 performance criteria for bacteria at disinfection time.

Product Contribution	Primary Criteria of Stand Alone Test per each of 3 bacteria		Regimen Test Criteria for per each of 5 organisms	
CL disinfectant alone	***Example: bacteria***			
	Average 3.0 log reduction			
	Inoculum	1,000,000 CFU/ml		
	Survivors	1,000 CFU/ml		
	Example: fungi			
	Average 1.0 log reduction			
	Inoculum	1,000,000 CFU/ml		
	Survivors	100,000 CFU/ml		
CL disinfectant regimen (including all label instructions)			Average NMT 10 CFU/lens	
			Example: bacteria and fungi	
			Inoculum	1,000,000 CFU/lens
			Survivors	10 CFU/lens

log units within the recommended soaking period, then the CL disinfectant meets the primary criteria of the Stand Alone Test (Table 3). Meeting the primary criteria simply means that the CL disinfectant is expected to pass the Regimen criteria, if it should be tested. Therefore, if the CL disinfecting solution meets the primary criteria, it is exempted from further testing and can be labeled as a disinfectant.

If the CL disinfectant fails to meet the primary criteria, but passes the secondary criteria, then it qualifies to be tested by the second-tier Regimen Test. The secondary criteria are met if the sum of the average log reductions for the three challenge bacteria is at least 5.0 log units within the recommended soaking period with a minimum of a 1.0 log reduction for any single bacteria (Table 3). The product cannot be classified as a CL disinfecting solution or as part of a CL disinfecting regimen according to the ISO 14729 standard unless it meets the both the secondary criteria of the Stand Alone test and the Regimen Test (Figure 1).

If the CL disinfectant meets this minimal level of activity, it qualifies to be tested by the Regimen Test. The Regimen Test evaluates the label instructions in the package insert for the entire disinfecting system or regimen. The Regimen Test requires that the population of microorganisms be brought to an average level of NMT 10 CFU/lens (Table 3). If the CL disinfecting regimen meets the Regimen criteria, it can be labeled as part of a CL disinfecting system.

The Regimen Test actually has more stringent criteria than the Stand Alone Test, because these criteria include the contribution of mechanical cleaning of the contact lens. A level of NMT 10 CFU/lens (Regimen Test) is approximately a 5 to 6-log reduction per microbial species, depending on the inoculum size. In comparison, the log reduction required to pass the primary criteria of the Stand Alone test is a 3.0-log reduction for bacteria and 1.0-log reduction for fungi. Whereas, the Regimen allows no more than an average of 10 survivors (CFU/lens), the primary criteria of the Stand Alone test allows up to 1 X 10^5 (100,000) survivors of some species (CFU/lens) (Table 5). However, the CL disinfecting solutions that passes the primary criteria are expected to also pass the Regimen criteria because of the contribution of the rubbing and rinsing steps to the removal of microorganisms.

The Difference a Regimen Makes

A regimen is any process by which a lens is disinfected. It is the use directions on the label of each CL disinfecting product. Examples of label instructions from several different marketed products are summarized in Table 6.

The ISO 14729 infers that, if a CL disinfectant meets the primary criteria for disinfection of contact lenses, then the CL disinfectant is exempt from conducting the Regimen Test, because it is assumed that if the CL disinfectant were tested

Table 6. Comparison of regimens of marketed products.[a]

Regimen	Rubbing step	Rinsing step	Disinfecting step	Final Rinse	Storage Time
Product A	Clean with daily cleaner	Rinse thoroughly	Minimum of 6 hours or overnight	Rinse before insertion into eye	None
Product B	Rub each side of lens for 10 seconds with solution	Rinse thoroughly	Minimum of 4 hours	None	30 days
Product C	Rub for about 20 seconds	Rinse thoroughly	At least 6 hours or overnight	Rinse before wearing	30 days
Product D	Rub for 20 seconds	Rinse thoroughly	At least 4 hours	Rinse prior to insertion	Allowed but time not specified
Product E	Rub with daily cleaner	Rinse thoroughly	Minimum of 2 hours	Rinse before wearing	Up to 7 days
Product F	Rub each side of lens for 10 seconds	Rinse thoroughly	At least 4 hours of overnight	Rinse before wearing	None
Product G	Rub for 20 seconds	Rinse thoroughly	At least 4 hours	Rinse prior to insertion	Allowed but time not specified
Product H	None	Rinse 5 seconds	At least 6 hours or overnight	Rinse briefly	30 days

[a]Descriptions are summarized from marketed product labels.

Table 7. Comparison of Stand Alone Test results to Regimen Test results with and without Rubbing and Rinsing steps for a 3% hydrogen peroxide system utilizing simultaneous neutralization of the active.[a]

Organism	Average Log Reduction at Disinfection Time		
	Stand Alone (No lenses)[b]	Regimen With Rubbing and Rinsing Lenses	Regimen Without Rubbing and Rinsing Lenses
S. aureus	4.2	> 5.0[c]	3.7
P. aeruginosa	4.9	>5.0	5.0
S. marcescens	4.6	>5.0	2.9
C. albicans	3.3	>5.0	1.6
F. solani	2.3	>5.0	1.1

[a]Data originally presented in poster (36).

[b]The primary criteria of the Stand Alone Test is an average of a 3.0-log reduction of the three bacteria and an average of a 1.0-log reduction of the two fungi.

[c]The criteria are an average of NMT 10 CFU/lens, which is equivalent to approximately a 5-log reduction.

Table 8. Effect of soil on Regimen Test.

REGIMEN 1

LENS GROUP	WITH SOIL					WITHOUT SOIL				
	S. aureus	*P.aeruginosa*	*S. marcescens*	*C. albicans*	*F. solani*	*S. aureus*	*P. aeruginosa*	*S. marcescens*	*C. albicans*	*F. solani*
I	0	0	0	0	0	0	0	0	0	0
II	0	0	0	0	0	0	0	0	0	0
III	0	0	0	0	0	0	0	0	0	0
IV	0	0	0	0	0	0	0	0	0	0
Total number of survivors	0					0				

REGIMEN 2

LENS GROUP	WITH SOIL					WITHOUT SOIL				
	S. aureus	*P. aeruginosa*	*S. marcescens*	*C. albicans*	*F. solani*	*S. aureus*	*P. aeruginosa*	*S. marcescens*	*C. albicans*	*F. solani*
I	2	6	0	1	0	0	3	1	0	0
II	0	0	1	6	0	1	0	1	16	0
III	0	1	0	0	0	0	2	0	1	0
IV	0	0	0	0	0	1	2	0	0	0
Total number of survivors	17					28				

REGIMEN 3

LENS GROUP	WITH SOIL					WITHOUT SOIL				
	S. aureus	*P. aeruginosa*	*S. marcescens*	*C. albicans*	*F. solani*	*S. aureus*	*P. aeruginosa*	*S. marcescens*	*C. albicans*	*F. solani*
I	0	1	0	0	2	1	0	0	2	0
II	0	0	0	6	3	4	0	4	6	0
III	0	1	1	2	0	2	0	0	2	0
IV	2	0	0	0	2	2	0	2	3	0
Total number of survivors	20					28				

according to the label instructions, then it would pass the Regimen Test criteria. This does not mean that a CL disinfectant can be marketed without the appropriate rubbing and rinsing steps. For example, a 3% hydrogen peroxide system that meets the primary criteria of the ISO 14729 Stand Alone Test will also meet the Regimen Test criteria when tested with the appropriate rubbing and rinsing steps (Table 7). However, when the 3% hydrogen peroxide system is used without the aid of the rubbing and rinsing lenses, it will fail the Regimen Test (36).

Does Soil Make a Difference in the Regimen Test?

It has been documented in the literature that laboratory prepared soil can neutralize the effectiveness of some hospital disinfectants, such as iodophors, quaternary ammonium compounds (28), and chlorine (38). Similar findings were found for a PHMB preserved contact lens disinfectant when high loads of microorganisms were introduced into the product (48). However, the effect of soil is not seen when a rubbing or rinsing step is used prior to

disinfection, since most of the soil is removed prior to the disinfection step. Bell et.al., showed that there was no difference between tests conducted with lenses coated with soil and clean lenses (with no soil) (1). The results are summarized in Table 8.

Easy Regimen to Meet Patient Needs

What the contact lens user frequently requests is an easy to use regimen; a regimen where the lens does not have to be rubbed or cleaned prior to disinfection. As shown previously in the case of hydrogen peroxide systems, a lens cannot simply be dropped into a CL disinfectant and be expected to be disinfected. The ISO 14729 standard was with the assumption that CL disinfectants would be used with the standard rub, rinse, and disinfect steps. The lens must be treated according to label instructions, whatever that is. Products not using the standard regimen steps should prove their claim under stress conditions. One CL disinfectant regimen with no rubbing step reported its efficacy under stress conditions, such as efficacy against numerous clinical and environmental isolates other than the standard challenge organisms, using high loads of organisms, in the presence of soil, after storage at elevated temperatures, and during extended storage. This product was shown to meet both the primary criteria of the Stand Alone Test and the stringent Regimen Test requirements using a "no rub" regimen (35,36,37).

Worthiness of the ISO Disinfection Standard

The ISO 14729 CL disinfection standard will be a very important standard. For the first time in the contact lens industry, there is consensus on the standard by which CL disinfectants must perform to be marketed within the U.S., Europe, and several other countries. There has been intense review, debate, and revisions from all parties. The standard for CL disinfectants is much higher than the past. The implementation of the standard will result in a more consistent quality between products. Today, whatever the regimen may be, CL disinfectants are tested by a standardized test procedure prior to approval or certification for marketing. The standard gives the consumer a better assurance of the performance of the products that they purchase.

Conclusions

The ISO 14729 procedure provides a more consistent procedure and a higher standard for CL disinfectants. The standard has influenced manufacturers to provide CL disinfectants that are now safer and more effective than ever. In addition, simpler to use disinfectants with good performance are now marketed. All are benefits to the consumer.

References

1. Bell, W. M., R. A. Rosenthal, and R. L. Abshire, "Effect of organic soil in regimen tests of marketed contact lens disinfection systems" ASM 99th General Meeting, No. Q-138, p. 560, American Society for Microbiology, Chicago, IL (1999).

2. Bohnert, J. L., T. Horbett, B. Ratner, and F. Royce, "Adsorption of proteins from artificial tear solutions to contact lens materials," *Invest. Ophthalmol. Vis.* Sci., **29,** 3620373 (1988).

3. Bowden, F. W., E. J. Cohen, J. J. Arentsen, and P. R. Laibson, "Patterns of lens care practices and lens product contamination in contact lens associated microbial keratitis," *CLAO J,*. **15,** 49-54 (1989).

4. Buck, S. L., R. A. Rosenthal, and B. A. Schlech, "Methods used to evaluate the effectiveness of contact lens care solutions and other compounds against *Acanthamoeba*: A review of the literature," *CLAO J.*, **26,** 72-84 (2000).

5. Campbell, R. C., "Inefficacy of soft contact lens disinfection techniques in the home environment," *Contact Lens Forum,* pp17-26 (Sept. 1990).

6. Caroline, P. J. and R. C. Campbell, "Strategies of microbial cell survival in contact lens cases," *Contact Lens Forum,* pp 28-36 (Sept. 1990).

7. Chick, H. and C. J. Martin, "The principles involved in the standardization of disinfectants and the influence of organic matter upon germicidal value," *J. Hyg.* (Camb), **8,** 654-697 (1908).

8. Cohen, E. J., "Is your office safe? Yes," *Cornea,* **9** (Suppl. 1), S41-S43 (1990).

9. Collins, M., J. Coulson, V. Shuley, and A. Bruce, "Contamination of disinfection solution bottles used by contact lens wearers," *CLAO J.*, **20**, 32-36 (1994).

10. Darrell, R. W. and G. B. Jacob, "Hepatitis surface antigen in human tears," *Arch. Ophthalmol.*, **96**, 674-676 (1978).

11. Dart, J., "Contamination of contact lens storage cases," *Br. J. Ophthalmol.*, **74**, 129-132 (1990).

12. Donzis, P. B., B. J. Mondino, B. A. Weissman,and D. A. Bruckner, "Microbial contaminationof contact lens care systems," *Am. J. Ophthalmol.*, **104**, 325-333 (1987).

13. Dutch Food and Drug Administration (Warenwet) "Appendix to the regulation of contact lens fluids and eye-washings," (1979).

14. European Pharmacopoeia, 2002, "Efficacy of Antimicrobial Preservation, 5.1.3," in *European Pharmacopoeia* 4th Ed., European Department for the Quality of Medicines within the Council of Europe, Strasbourg (2000) pp407-409.

15. French Guidelines (L'Association Francaise de Normalisation), "Constitution of product license submission dossier for product used for application and care of contact lenses," (Jan. 1985).

16. French AFNOR NF, "Water miscible and neutralizable antiseptics and disinfectants in liquid form. Determination of bactericidal activity," T 72-150 (1987).

17. French AFNOR NF, "Water miscible disinfectant used in liquid state. Germ-carrier method," T 72-190 (1989).

18. Gachon, A. M., J. Richard, and B. Dastugul, "Human tears; Normal protein pattern and individual protein determinants on adults," *Curr. Eye Res.*, **2**, 301-303 (1983).

19. Gray, T. B., R. T. M. Cursons, and J. F. Sherwan, "Acanthamoeba, bacterial, and fungal contamination of contact lens storage cases," *Br. J. Optom.*, **79,** 601-605 (1995).

20. Hart, D. E., W. Reindel, H. M. Proskin, and M. F. Mowrey-McKee, "Microbial contamination of hydrophilic contact lenses: quantitation and identification of microorganisms associated with contact lenses while on the eye," *Optom. Vis. Sci.*, **70,** 185-191 (1993).

21. Houlsby, R. D., M. Ghajar, and G. Chavez, "Microbiological evaluation of soft contact lens disinfecting solutions," *J. Am. Optom. Assoc.*, **55,** 205-211 (1984).

22. International Committee on Contact Lenses, "Contact lens maintenance systems," ICLC, **19,** 153-156 (1992).

23. ISO 14729, "Ophthalmic optics – Contact lens care products – Microbiological requirements and test methods for products and regimens for hygienic management of contact lenses," (2001).

24. Larkin, D. F. P., S. Kilvington, and D. L. Esty, "Contamination of contact lens storage cases by *Acanthamoeba* and bacteria," *Br. J. Ophthalmol.*, **74,** 133-135 (1990).

25. Liesegang, T. J. and R. K. Forster, "Spectrum of microbial keratitis in South Florida," *Am. J. Ophthalmol.*, **90,** 38-4 (1980).

26. Medicines Control Agency, "Chemical and pharmaceutical documentation. Microbiological studies," Annex VI, Chap. 4, page 67 (1989).

27. Midelfart, J., A. Midelfart, and L. Bevanger, "Microbial contamination of contact lens cases among medical students," *CLAO J.*, **22,** 21-24 (1996).

28. Miner, N. A., E. Whitmoore, and M. McBee, "A quantitative organic "soil" neutralization test for disinfectants," *Dev. Ind. Microbiol.*, **16,** 23-30 (1975).

29. Moore, M.B. 1990. *Acanthamoeba* keratitis and contact lens wear: The patient is at fault. *Cornea*. **9** (Suppl. 1): S33-S35.

30. Mowrey-McKee, M. F., K. Monnat, H. J. Sampson, C. M. Smith, G. A. Davies, L. Mandt, and H. M. Proskin, "Microbial contamination of contact lenses, Part I: Quantitation of microbes on patient worn-and-handled lenses," *CLAO J.*, **18,** 87-91 (1992).

31. Mowrey-McKee, M. F., H. J. Sampson, and H. M. Proskin, "Microbial contamination of contact lenses, Part II: Quantitation of microbes after patient handling and after aseptic removal from the eye," *CLAO J.*, **18,** 240-244 (1992).

32. Pfluf, I. V. and C. F. Schmidt, "Thermal destruction of microorganisms," In *Disinfection, Sterilization and Preservation*, C. A. Lawrence and S. S. Block (Eds), Lea & Febiger, Philadelphia, PA (1968), pp63-105.

33. Proud, D. W. and S. V. W. Sutton, "Development of a universal diluting fluid for membrane filtration sterility testing," *Appl. Environ. Microbiol.,* **58,** 1035-1038 (1992).

34. Richmond, S. (Ed), "Virus disease of the eye," in *Principles of Virology,* Easty Year Book Medical Publishers, Inc., Chicago (1985), pp1-20.

35. Rosenthal, R. A., C. L. McAnally, L. S. McNamee, S. L. Buck, R. L. Schlitzer, and R. P. Stone, "Broad spectrum antimicrobial activity of a new multi-purpose disinfecting solution," *CLAO J.*, **26,** 120-126 (2000).

36. Rosenthal, R. A., C. L. McAnally, S. L. Buck, and B. A. Schlech, "Effectiveness of a new simple to use contact lens disinfection regimen," *Optom. Vis. Sci.*, **77** (Supplement 12a), 178, Poster Presentation at the American Academy of Optometry, Orlando, FL (2000).

37. Rosenthal, R. A., C. L. McAnally, S. L. Buck, and B. A. Schlech, "The capacity of a new contact lens care product to kill microorganisms," CLAO meeting, Las Vegas Nevada (2001).

38. Schunk, T. and R. Schweisfurth, "Disinfectant performance of oxidizing contact lens solutions: Quantitative suspension tests with organic soil contaminants," *Contactologia*, **11,** 84-89 (1989).

39. Shih, K. L., J. Hu, and M. J. Sibley, "The microbiological benefit of cleaning and rinsing contact lenses," *Int. Contact Lens Clinic*, **12,** 235-248 (1985).

40. Shovlin, J. P., "Resistant pathogens following disinfection: The significance of the 'contribution of elements'," *Intl. Contact Lens Clin.*, **16,** 126-128 (1989).

41. Stehr-Green, J. K., T. M. Bailey, and G. S. Visvesera, "The epidimiology of *Acantha-moeba* keratitis in the United States," *Am. J. Ophthalmol.*, **107,** 331-336 (1989).

42. Stumbo, C. R., "Nature of thermal death curves for P.A. 3679 and *Clostridium botulism*," *Food Technol.*, **4,** 321-326 (1950).

43. Sutton, S. V. W., R. J. Franco, M. F. Mowrey-McKee, S. C. Busschaert, J. Hamberger, and D. W. Proud, "D-value determinations are an inappropriate measure of disinfecting activity of common contact lens disinfecting solutions," *Appl. Environ. Microbiol.*, **57,** 2021-2026 (1991a).

44. Sutton, S. V. W., H. Proskin, D. A. Keister, and D. W. Proud, "A critical evaluation of the multi-item microbial challenge test in ophthalmic disinfectant testing," *CLAO J.*, **18,** 155-160 (1991b).

45. Sutton, S. V. W., T. Wrzosek, and D. W. Proud, "Neutralization efficacy of Dey-Engley medium in testing of contact lens disinfecting solutions," *J. Appl. Bact.*, **70,** 351-354 (1991c).

46. Sutton, S. V. W., "The evaluation of biocide neutralization in the determination of microbicidal efficacy of contact lens solutions," *Int. Contact Lens Clin.*, **19,** 167-173 (1992).

47. Tragakis, M. P., S. I. Brown, and D. B. Pearce, "Bacteriological studies of contamination associated with soft contact lenses," *Am. J. Ophthalmol.*, **75,** 496-499 (1973).

48. Tsai, T. and H. K. Dannelly, "How dangerous is noncompliance with multipurpose solutions?" *Contact Lens Spectrum*, pp48-50 (January 2000).

49. U. S. Food and Drug Administration, "DRAFT testing guidelines for class III soft (hydrophilic) contact lens solutions," FDA, Washington, D.C. (July 15, 1985).

50. U. S. Food and Drug Administration, *Guidance Document for Lens Care Products* (May 1997).

51. Van Haeringen, N. J., "Clinical biochemistry of tears," *Surv. Ophthalmol.*, **26,** 84-96 (1981).

52. Vogt, M. W., D. D. Ho, S. R. Bakar, J. P. Gilbard, R. T. Schooley, M. S. Hirsch, "Safe disinfection of contact lenses after contamination with HTLV-III," *Ophthalmol.*, **93,** 771-774 (1986).

53. Wedler, F. C., B. Illman, D. Horenskey, and M. Mowrey-McKee, "Analysis of protein and mucin components deposited on hydrophilic contact lenses," *Clin. Exp. Optom.*, **70,** 59-68 (1987).

54. Willcox, M. D. P., K. N. Power, F. Stapleton, C. Leitch, N. Harmis, and D. F. Sweeney, "Potential sources of bacteria that are isolated from contact lenses during wear," *Optom. Vis. Sci.*, **12,** 1030-1038 (1997).