1 Scope

This methodology provides a process for evaluating compatibility of solutions used for disinfection with contact lenses and lens cases using an antimicrobial efficacy endpoint. Specifically, the microbiological effect of the antimicrobial agent(s) while in the presence of the lens cases and/or lenses will be evaluated as described in the soak step of the label instructions. This applies only to lens cases with side-to-side horizontal wells.

This test methodology will be assessed as part of a ring test; based upon the data generated, determination of what will be included in the final test methodology will be made.

2 Normative References

ISO 14729
**3 Terms and definitions**

CFU = Colony Forming Unit  
Dulbecco’s Phosphate Buffered Saline (DPBS)  
Dulbecco’s Phosphate Buffered Saline w/0.05% polysorbate 80 (DPBST)  
International Organization for Standardization = ISO  
Sabouraud Dextrose Agar = SDA  
Tryptic Soy Agar = TSA

**4 Principle**

The antimicrobial efficacy of the test solution in combination with a lens and a lens case will be evaluated at various times following inoculation with organisms in the presence of organic soil. New lenses and new lens cases with no preconditioning shall be used unless otherwise justified. This test will simulate microbial contamination introduced by patient handling.

Place a lens in a well of a lens case and inoculate each lens with \(1.0 \times 10^5 - 1.0 \times 10^6\) cfu; leave inoculum in contact with the lens for 3 – 10 minutes and dispense the appropriate volume (minimum of 2 mL.) of the test solution into each well. The inoculated lenses in solutions will be allowed to soak for various storage times (the labeled regimen soaking period, at 24 hours, at 7 days and at the maximum labeled storage in the lens case) in order to evaluate the effects of the lens case and the lens on the antimicrobial activity of the test solution. A separate set of lens case wells shall be prepared for each time point; three wells shall be evaluated for each unique test condition. Prepare one well per unique sample for chemical analysis; analyze solution from each well in triplicate. Additional time points may be evaluated. One lot of each test component will be evaluated in this test.

Collect sufficient volumes of the test solution in triplicate at each time point for analysis of preservative content.
A variety of lenses shall be evaluated, e.g. group I, group IV, and representative silicone hydrogel materials. The lens case(s) recommended for use with the test solution shall be evaluated at a minimum.

All five challenge organisms specified in ISO 14729 shall be used.

Log reductions will be evaluated for all exposure times.

5 Rationale

These studies are designed to simulate the recommended soaking and storage periods wherein the contaminating microorganisms are introduced by patient handling.

6 Methodology

Refer to ISO 14729 for media, challenge organisms, culture maintenance, test equipment, and other details for conducting the Stand Alone Test with the exception of using lens cases for the microbial challenge.

6.1 Test Procedure

6.1.1

Conduct the test using lens types representative of those with which the solution is intended to be used, e.g. low water nonionic lens (Group I), high water ionic lens (Group IV) and representative silicone hydrogel lens materials. Use -3.00D lenses. New and unused lenses with no preconditioning shall be used unless otherwise justified.
The lens cases recommended for use with the test solution shall be evaluated at a minimum. The lens cases used in this test shall be new and unused with no preconditioning unless otherwise justified. Prepare three lens case wells per lens type per time point to be examined for the test samples; additionally, prepare control lens cases for each evaluation time point without lenses. Therefore, for evaluation of one lens case with one solution and one lens type, a total of 6 lens case wells (three wells for the test and three wells for the control) will be prepared for each of the five challenge organisms for each time point to be evaluated or 24 lens case wells for a minimum of four time points per challenge organism. Additional time points may be evaluated. If more than one lens type is evaluated in a test, only one set of control lens cases is required per inoculum preparation. The inoculum shall be prepared using organic soil as specified in Normative Annex A.

6.1.2 Prepare the lens cases (test and control) by removing the caps. Aseptically remove a new, unused lens from its sterile packaging, aseptically blot the lens on sterile gauze and place one lens inside each test well with the concave side up. Prepare three lens case wells without lenses for use as controls.

NOTE: Care should be taken to keep the shape of the lens concave.

6.1.3 Inoculate each test and control well with 0.10 mL of the inoculum suspension prepared with organic soil to result in a final count of between $1.0 \times 10^5$ and $1.0 \times 10^6$ cfu per well. Gently dispense the inoculum directly onto the concave surface of the lens for the test wells and into the well for each control well and cover the wells.
6.1.4 Leave the inoculum in contact with the lens for 3 – 10 minutes and then aseptically dispense a known volume of the test solution gently into each test and control lens case well so that each lens is completely immersed in the solution. Each well shall contain a minimum of 2 mL unless otherwise justified. Do not agitate the contents of the lens case at this time since contamination of the cap may occur.

6.1.5 Place the caps on the inoculated lens case wells tightly and store the inoculated lens cases at 20-25ºC. The temperature shall be monitored using a calibrated device and the temperature documented.

NOTE: Care should be taken in moving the inoculated lens cases since contamination of the caps may occur.

6.1.6 Prepare an inoculum baseline check for each challenge organism suspension. NOTE: It is suggested that 0.10 mL of the inoculum suspension be dispensed into a sterile tube containing a volume of DPBST equivalent to the volume of test solution contained in the lens case well. Vortex and serially dilute a 1.0 mL aliquot of the inoculated DPBST and plate out the dilutions in triplicate.

6.1.7 Solutions will be sampled at least at the minimum regimen soaking time(s) (±10 minutes), 24±1 hours, at 7±0.25 days and at the proposed maximum lens storage period ±0.25 days. Additional time points may be evaluated. If a regimen time point is less than 30 minutes, the sample must be taken...
within ±30 seconds. Solution from both test and control wells will be
sampled at each time point. Each lens case well will be sampled at one time
point only. The following procedure will be used at each sampling time:

6.1.7.1 Ensure each well is tightly capped.

6.1.7.2 Orient the case well perpendicular to the vortexing surface (hold the case
vertically with the inoculated well in contact with the surface of the vortex
instrument) and vortex each well separately immediately before sampling on
the high speed setting for a minimum of 30 seconds.

6.1.7.3 Using aseptic technique, immediately remove the lens taking care to shake
the excess liquid from the lens into the lens case well. Place the lens into 2
mL of neutralizing broth media, vortex for approximately 15 seconds,
remove the lens, place the lens on an agar plate (e.g. TSA for bacteria and
SDA for mould and yeast) and cover with an agar overlay. Incubate the
plates as described in 6.1.9.

6.1.7.4 Vortex and dispense 0.1 mL and 1.0 mL volumes of the neutralizing broth
(solution carryover) from 6.1.7.3 into separate petri plates followed by
addition of appropriate agar medium. Incubate the plates as described in
6.1.9.

6.1.7.5 Using aseptic technique, immediately remove a 1 mL aliquot from the
vortexed lens case well using a sterile pipet and dilute in 9 mL of a validated
neutralizing media.

6.1.7.6 Perform serial dilutions in validated neutralizing media. Mix each dilution
well by vortexing vigorously prior to preparing the subsequent dilutions.
Let stand to allow neutralization to be completed. Neutralization conditions
shall be based on recovery medium control testing (see 6.3.2.2, ISO 14729).
6.1.7.7 If an antimicrobial agent in the formulation cannot be adequately inactivated or neutralized, eliminate it using a validated membrane filtration procedure (see Annex B, ISO 14729)

6.1.8 Determine the viable count of organisms in appropriate dilutions by preparation of triplicate plates (unless otherwise justified) of a suitable recovery medium (e.g. TSA for bacteria and SDA for mould and yeast).

If membrane filtration has been employed to remove or neutralize antimicrobial agents, culture the membranes on these media as appropriate.

If the pour plate method is utilized, keep the agar for pour plates below 50°C prior to pouring.

The agar media used for determination of viable counts may also contain antimicrobial inactivators or neutralizers, if required.

6.1.9 Incubate bacterial recovery plates at 30–35°C. Incubate yeast recovery plates at 20-25°C or 30-35°C. Incubate mould recovery plates at 20-25°C. Incubation times for optimal recovery of bacteria, yeast and moulds shall be determined. Minimum incubation times shall be based on recovery medium control testing (see 6.3.2, ISO 14729). Record the number of all cfu observed on countable plates. For each plated lens sample, count all cfu on the agar plate regardless of whether they are on the lens or not on the lens.
NOTE: Plates should be observed periodically during incubation to prevent the occurrence of uncountable plates due to overgrowth.

NOTE: 2 Countable plates refer to 30 cfu/plate to 300 cfu/plate for bacteria and yeast, and 8 cfu/plate to 80 cfu/plate for moulds, except when colonies are observed only for the 10^0 or 10^-1 dilution plates. The absence of microorganisms shall be documented, e.g. by recording a “0” or "NR" (no recovery), when plates for all dilutions of a sample at a single time point have zero colonies.

6.1.10 Determine the average number of colony forming units on countable plates from 6.1.9.

6.1.11 Calculate the average cfu/mL of test solution and multiply by the number of milliliters of test solution per lens case well to obtain the average total cfu/per lens case well for each well of the test solution.

6.1.12 Calculate the average cfu of inoculum per lens case well.

6.1.13 Calculate the average cfu per lens (6.1.7.3).

6.1.14 Calculate the average cfu per solution carryover samples per well (6.1.7.4).

6.1.15 Calculate the total number of survivors per lens case well (solution survivors (6.1.11) + lens survivors (6.1.13) + solution carryover survivors (6.1.14)). Then calculate the log survivors for each well. Calculate the average log survivors for the triplicate wells for each unique test sample as follows:

\[(\text{log survivors well 1} + \text{log survivors well 2} + \text{log survivors well 3}) \div 3\]
6.1.16 Subtract the log total survivors (6.1.15) from the log inoculum per lens case well (6.1.12) to determine the log reduction at each time point for each unique lens/lens case combination per solution (log inoculum/well – log survivors/well). Calculate the log reduction for each well. Then calculate the average log reduction for the triplicate wells for each unique test sample as follows:

\[(\text{log reduction well 1} + \text{log reduction well 2} + \text{log reduction well 3}) \div 3\]
Preparation of challenge organisms in organic soil

A.1. Materials and reagents

A.1.1 Organism for organic soil
A.1.1.1 *Saccharomyces cerevisiae* (S.c.) of unspecified strain

A.1.2 Culture media and reagents
A.1.2.1 Dulbecco's Phosphate Buffered Saline without calcium chloride and magnesium chloride (DPBS): 200 mg/l KCl, 200 mg/l KH$_2$PO$_4$, 8000 mg/l NaCl, and 2160 mg/l Na$_2$HPO$_4$ • 7H$_2$O
A.1.2.2 Sabouraud Dextrose Agar (SDA) slants
A.1.2.3 Heat-inactivated bovine serum

A.1.3 Test equipment
A.1.3.1 The following common laboratory equipment is required: sterile pipettes, swabs, tubes, petri dishes (90 mm to 100 mm x 20 mm), etc., incubator, and suitable instruments for spectrophotometric determination of cell density, for colony counting, and for centrifugation.

A.2 Preparation of heat-killed yeast
A.2.1 Culture the S.c. on SDA slants for 42 - 48 hours at 20-25°C. Harvest S.c. from the SDA slants in DPBS, centrifuge, decant supernatant, add fresh DPBS, centrifuge again and resuspend in DPBS. Adjust the concentration to $1 \times 10^7$ – $1 \times 10^8$ cfu/mL and heat kill at 100±2°C for 10 minutes.
No further manipulations of the heat-killed yeast shall take place. Store under refrigeration (2° C to 8° C) until day of use.

A.3 Preparation of organic soil

A.3.1 On the day of use, centrifuge an aliquot of the heat-killed yeast preparation (A.2) at no more than 5000 x g for a maximum of 30 minutes and decant the supernatant; add an equal aliquot of the heat- inactivated bovine serum and resuspend the heat-killed yeast. (Example: If centrifuging 1 mL of heat-killed yeast above, use 1 mL of heat-inactivated bovine serum to resuspend the heat-killed yeast pellet.)

A.3.2 Dilute 1 mL of this organic soil preparation into 9 mL DPBS. This constitutes what is called organic soil preparation.

A.4 Preparation of challenge organisms in organic soil

A.4.1 Prepare individual challenge organisms per ISO 14729 section 6.2. After harvesting, the cultured organisms may be washed using centrifugation. The bacterial suspensions may be filtered (e.g. 3 µm to 5 µm pore size) to produce a single-cell dispersion. Adjust all challenge cell suspensions with DPBS to a concentration of between 1 x 10⁷ cfu/ml and 1 x 10⁸ cfu/ml using a spectrophotometer, centrifuge to pellet the organisms, decant the supernatant and resuspend the organisms in the organic soil preparation (A.3.2). The actual concentration of colony forming units per ml must be determined for each suspension, e.g. by the plate count method, at the time of the test. The maximum storage time for the prepared inoculum is two hours at room temperature only. Do not refrigerate.