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1 **Effect of povidone iodine contact lens disinfecting solution on orthokeratology lens and**
2 **lens case contamination and organisms in the microbiome of the conjunctiva**

3

4 **Keywords.** Povidone iodine, contamination, colonisation, lens case, orthokeratology

5

6 **Abstract**

7 **Purpose.** To compare lens cleaning routines using a povidone iodine-based rigid lens

8 disinfecting solution and its effect on conjunctival colonisation, and lens and lens case

9 contamination.

10 **Methods.** Participants, aged 6-10 years, receiving orthokeratology treatment were

11 randomised to four lens cleaning routines: with and without the use of daily and/or weekly

12 cleaners, which were performed by their parents. Conjunctival colonisation was compared

13 before lens wear and at 1-, 3-, and 6-month after commencement of lens wear.

14 Contamination of lenses and lens cases was investigated at these times. Organisms were

15 identified using MALDI-TOF mass spectrometry.

16 **Results.** Of the 76 participants who completed the study, conjunctival colonization was

17 present in 24 (32%) at baseline. Of the remaining 52 participants, 34 consistently yielded no

18 growth. Participants positive at baseline were statistically more likely to be colonized after

19 commencement of lens wear ($p=0.020$). Overall, colonization rate was reduced to 15%

20 (11/72) after 6-month lens wear, which reached significance for initially colonized

21 participants ($p < 0.001$). Few cultures yielded potential ocular pathogens, with notably no

22 *Pseudomonas aeruginosa*. Contamination rates of both lenses and lens cases were also low,

23 with few isolations of ocular pathogens. No significant differences were observed between
24 cleaning regimes for conjunctival colonization or contamination of lenses or cases.

25 **Conclusions.** Disinfection for rigid and ortho-k lens wearers may be effectively achieved with
26 the use of povidone iodine-based solution, apparently regardless of cleaning routine
27 adopted in the current study. The absence of pathogens in the conjunctiva, lenses, and lens
28 cases in the great majority of samples indicates that it can improve the safety of overnight
29 lens wear.

30 Introduction

31 Multipurpose solutions (MPS) are the most commonly prescribed regimen to clean,
32 disinfect, and rinse rigid contact lenses. Traditionally, these solutions are based on
33 quaternary ammonium compounds (QAC) (e.g. polyquad) and biguanides (e.g.
34 polyhexamethylene biguanide (PHMB)). Hydrogen peroxide (H_2O_2) can also be used to
35 disinfect contact lenses. In recent years, solutions containing povidone iodine (PI) have been
36 introduced, initially for soft lenses,¹⁻⁵ and later expanded to include rigid lenses.⁶⁻⁷ PI has
37 been safely used as a disinfectant for ophthalmic operations and prophylactic eyedrops for
38 neonates for many years.⁸ PI-based soft and rigid lens solutions have been reported to be as
39 effective against *Pseudomonas aeruginosa* as other MPS and H_2O_2 -based systems.^{5,6,9} These
40 solutions were also reported to be effective against *Acanthamoeba*.^{2,6} The more recently
41 introduced formulation for RGP lenses consists of an anionic surfactant and PI solution, to
42 which neutralizing tablets containing sodium sulphite and proteolytic enzyme are added.

43 Major problems affecting reusable contact lens wear are compliance with disinfecting
44 regime and care of the lens case. Numerous studies have reported high levels of
45 contamination of lens cases, ranging from 30% to over 80%.^{3,10-12} This is partly due to the
46 build-up of organisms into a biofilm in the case, which are not as easily killed as planktonic
47 organisms.^{14,15} In contrast to QACs and biguanides, both PI and H_2O_2 are able to kill
48 organisms in biofilms formed in lens cases.⁷ In addition, oxidizing disinfecting solutions use
49 specialized cases and obvious changes in the colour (e.g. PI becoming colourless) or
50 characteristic (e.g. Bubbling with H_2O_2) of the solutions discourage topping up. Topping up
51 of solutions leads to dilution of active agents, reducing microbiocidal activity and increasing
52 biofilm formation.¹⁶ Failing to clean the lens case daily and replace it regularly (preferably

53 monthly) is also a major factor in contamination.^{11,17} Investigation of contact lens
54 contamination has shown somewhat lower rates compared to lens cases, but few of these
55 studies have investigated rigid lenses.^{11,13} This may be attributable to the fall in popularity
56 of rigid lenses until the development of modern orthokeratology (ortho-k) for myopia
57 control and use of scleral lenses.^{18,19} Lower contamination has been suggested to be linked
58 with patients' awareness that the lens will be in contact with the cornea.¹¹ Prevention of
59 lens contamination is of particular importance for ortho-k users as this is an overnight
60 modality, in which lenses are reused for up to a year.^{20,21}

61 In recent years, there has been an upsurge in interest in the microbiome present in various
62 sites of the human body.²² Much of this attention has been focused on the gut and the
63 mouth, with fewer studies addressing the eye. A study investigating the ocular microbiome
64 of lens wearers showed an increased risk for ocular pathogens, in particular, gram-negative
65 bacteria.²³ It has been suggested that use of MPS and other disinfecting solutions for
66 contact lens care leads to transfer of the active agents into the eye, which may select for a
67 more limited microbiome in the conjunctiva.^{12,13} Some practitioners advise the use of saline
68 to rinse the lenses before insertion to avoid irritation and other effects of residual MPS in
69 the eye.^{24,25} However, partly to streamlining of the lens care process, manufacturers
70 generally do not suggest the use of saline for rinsing. MPS at low concentration may still kill
71 or retard the growth of organisms, changing the balance of the microbiome.²⁶ More
72 importantly, low levels of disinfectant lead to tolerance or development of resistance in
73 bacteria to the active agents, in particular, QACs and biguanides.^{27,28} This phenomenon has
74 been observed in both cross-sectional and longitudinal comparisons of spectacle wearers
75 and ortho-k patients.^{29,30} Survival of such organisms may explain the increased levels of
76 coagulase-negative staphylococci (CNS) in the conjunctiva of contact lens wearers.^{13,31}

77 However, the effects of the use of PI-based disinfecting solutions on the conjunctival
78 microbiome have not been reported. As PI is an oxidising agent, development of resistance
79 to PI-based disinfecting solutions is unlikely, but selection or removal of species may occur if
80 introduced into the eye.

81 The current study aimed to investigate the effect of various cleaning routines on the
82 contamination rate of lenses and cases of ortho-k participants using a PI disinfecting
83 solution over six months of lens wear. In addition, the effect on colonization of the lower
84 palpebral conjunctiva in children before and after ortho-k lens wear was determined.

85

86 **Methods**

87 In this 6-month prospective study, eligible participants were randomly assigned into one of
88 the four study groups, with variations in lens care procedures, as shown in Table 1. They
89 were required to carefully follow their instructions for lens care. Each participant was
90 required to attend on five occasions, which included two pre-lens visits to determine the
91 baseline microbiome of the conjunctiva and three visits after 1, 3, and 6 months of lens
92 wear. At the latter visits, samples were collected from the lenses and lens cases to
93 determine levels of contamination and organisms present. Colonization in the conjunctiva
94 was also determined at each of these visits.

95

96 *Participants*

97 Eighty participants with low myopia (-4.00D to -0.75D), aged 6-10 years, were randomly
98 assigned to one of the four study groups before commencing ortho-k lens wear. All

99 participants were concurrently participating in two myopia control studies (ClinicalTrials.gov
100 registration numbers: NCT02955927 and NCT03191942) using Katt BE free ortho-k lenses
101 (Precision Technology Services, Vancouver, B.C., Canada) made from Boston XO material (Dk
102 100 units) They were invited to participate in study when they had learnt lens handling and
103 pending for lens delivery. Informed consent for this study were obtained from the parents
104 prior to the commencement of the study. Tenets of the Declaration of Helsinki revised in
105 2013 were followed. The study was approved by the Departmental Research Committee of
106 the School of Optometry, The Hong Kong Polytechnic University (approval number:
107 HSEARS20170430002) and registered at ClinicalTrials.gov (registration number:
108 NCT03193255).

109 All lens procedures, including insertion, removal, and cleaning, were performed by the
110 parents. All lenses were rinsed with saline (cleadew rinsing solution, Ophtecs Japan Inc.,
111 Tokyo, Japan), followed by putting a drop of unpreserved artificial tears (Teare, Ophtecs
112 Japan Inc., Tokyo, Japan) on the back surface of the lens before insertion. All participants
113 were instructed to use PI-based solution (cleadewGP, Ophtecs Inc., Japan) for disinfection
114 after lens removal in the morning, using the prescribed method for their group as shown in
115 Table 1.

116 All solutions and accessories were replaced monthly and upon presentation of their used
117 bottles at follow up visits, to ensure compliance. Parents were also required to disinfect the
118 lens cases weekly by soaking in freshly boiled water for 10 mins, before following the daily
119 routine of air-drying before sleep. Participants were required to use and care for their lenses
120 as instructed and attend the aftercare visits. Compliance with handling procedures were

121 reviewed by asking the parents/participants to describe the routine procedures for lens
122 insertion at night and for lens removal and cleaning in the morning.

123 Lens surface deposits and scratches, contamination of lenses and cases, and colonization of
124 the conjunctiva were determined for each participant at the post lens-wear visits. To ensure
125 safe ortho-k lens wear, any participant whose lenses displayed significant surface deposition
126 was excluded from the study and advised to follow a more stringent care routine.

127

128 *Samples*

129 Samples from the conjunctiva and the lens were collected from the left eye of each
130 participant, using the sterile Remel BactiSwabs (Thermo Fisher Scientific, Massachusetts,
131 US). Before sampling, swabs were moistened with sterile phosphate buffered saline (PBS)
132 and then immediately placed in sterile tubes containing Amies agar before refrigeration at
133 0-4°C. Conjunctival samples were taken by gently rolling the swabs on the lower palpebral
134 conjunctiva. Participants were asked to look up and their lower eyelid was gently reverted
135 to avoid touching the cornea or the eyelashes. The ortho-k lenses, after disinfection for at
136 least four hours, were removed from the lens cases with sterile tweezers. Lens samples
137 were taken by gently swabbing the concave surface of the lens. The lens cases containing
138 solution were sealed, labelled, and refrigerated together with the conjunctival and lens
139 swabs. They were transferred to the laboratory within 48 hours.

140 Swabs were moistened with the solution in the lens case and the remaining solution poured
141 away, before samples were collected from all inner parts of one compartment of the lens
142 case. Two separate swabs were used, one for the inner lens cap and holder and one for the

143 main body, then combined in a bijou bottle containing brain heart infusion medium for
144 incubation and subsequent culture.

145 The lens case was then gently rinsed twice with PBS to remove planktonic bacteria before
146 draining for 10 minutes. A 250- μ L aliquot of 0.4% crystal violet was added to the unsampled
147 compartment of the case and gently dispersed. The stain was gently poured away after two
148 minutes and the compartment gently rinsed twice with PBS to remove excess crystal violet.
149 After drying for 30 minutes at room temperature, the stained biofilm was dissolved in 200
150 μ L of absolute alcohol, transferred to a microlitre plate, and the optical density (OD)
151 determined spectrophotometrically (Genesys 20, Thermo Scientific) at 600 nm.

152

153 *Microbial assessment*

154 All swabs were placed in bijou bottles containing sterile brain heart infusion broth. They
155 were vortexed for 10 seconds and incubated overnight. Conjunctival samples were sub-
156 cultured onto blood agar (37°C aerobically and anaerobically) and chocolate agar (37°C in
157 5% CO₂). Lens samples were cultured on blood agar (37°C aerobically and anaerobically),
158 whereas samples from lens cases were cultured on blood agar (37°C aerobically and
159 anaerobically), chocolate agar (37°C in 5% CO₂), and MacConkey agar (37°C aerobically).
160 Individual colonies were analysed using a matrix-assisted laser desorption/ionization time-
161 of-flight (MALDI-TOF) mass spectrometer (Bruker Microflex LT/SH system; Bruker Corp,
162 Billerica, MA) for bacterial identification.

163

164 *Treatment of data*

165 Baseline age and sex differences among the four study groups were tested using one-way
166 ANOVA and chi-square test, respectively. Differences in levels of colonization in the lower
167 conjunctiva before and after lens wear were determined by McNemar's tests. Friedman
168 tests were performed to evaluate changes in colonization and contamination levels over
169 time, whereas repeated measures ANOVA (RM ANOVA) was used to evaluate the changes in
170 OD after ortho-k lens wear for each of the four study groups. Binary logistic regression
171 (enter method) was performed to determine the effect of factors on the risk of baseline
172 colonization, and the risks of contamination of lenses and lens cases after ortho-k lens wear.

173

174 **Results**

175 Of the 80 participants who completed the baseline visits, one participant from Group 3 was
176 excluded before lens delivery, because of recurrent corneal staining. The demographic data
177 of the 79 participants are shown in Table 2. There were no significant differences in age and
178 sex among the four groups of participants. The mean \pm SD age was 9.1 ± 1.1 years and 66%
179 were female. Three participants developed adverse events after the 3-month visit and were
180 terminated from the study and did not attend the 6-month visit. Colonization and
181 contamination rate of these three participants were not included in the 6-month analysis.

182

183 *Conjunctival colonization*

184 Baseline samples collected for three participants, one from Group 3 and two from Group 4,
185 were discarded due to delayed laboratory processing. Conjunctival colonization was
186 detected in 24 participants (32%), before lens wear (Figure 1). Result of the binary logistic

187 regression model investigating the effect of age, sex and study groups on the risk of baseline
188 colonization was statistically insignificant ($\chi^2(5) = 4.60$, $p = 0.466$).

189 After commencement of lens wear, samples from 34 of 52 participants negative at baseline,
190 consistently yielded no growth. Positive cultures were obtained from the remaining 18.

191 Figure 1 shows results of 42 participants with positive cultures. Twenty-four (32%)
192 participants yielded positive cultures at baseline and 15 (63%) had at least one positive
193 culture after commencement of ortho-k: 10 at one visit only; four at two visits; and only one
194 at all visits. Fifty-two participants were negative at baseline, of whom 18 subsequently
195 yielded positive cultures (15 on one visit only) (see Figure 1). Overall, colonization rate was
196 somewhat reduced from 32% at baseline to 15% after six months of lens wear, but this
197 change did not reach significance (Friedman test, $p = 0.079$). If only initially colonised
198 participants are considered, only 42% were colonized after one month and 25% on the two
199 later visits. Colonization was not affected by ortho-k lens wear (McNemar's test; $p = 0.122$),
200 i.e. participants with colonization before lens wear were more likely to continue to yield
201 positive cultures after lens wear (OR: 3.1; 95% CI: 1.1 to 8.6; $p = 0.027$) (Table 3).

202 At baseline, only four participants yielded a potential ocular pathogen, *Streptococcus*
203 *pneumoniae*, *Staphylococcus aureus*, and *Acinetobacter*. After commencement of lens wear,
204 cultures from five participants yielded an ocular pathogen on one occasion only, one at 1-
205 month visit, and two at each of the subsequent visits. Three of these isolates were *S. aureus*,
206 the remaining one being *S. pneumoniae*. A wide range of opportunistic or non-pathogenic
207 organisms were identified from the conjunctival swabs among the 39 participants with
208 positive colonization. *Micrococcus luteus* was the most commonly isolated organism (28%;
209 21/76), both before and after lens wear.

210

211 *Contamination*

212 Some participants failed to bring their lenses and lens cases with them at the three
213 scheduled visits, so only 74, 76, and 72 lenses and cases were analysed. Measurements of
214 OD to indicate presence of biofilm in the lens cases yielded very similar results at all three
215 post-wear visits and between groups (RM-ANOVA, time: $p = 0.116$; interaction: $p = 0.84$).

216 Contamination rates of the lenses were fairly low and constant during the first 6 months (1-
217 month: 16%; 3-month: 5%; 6-month: 10%) (Friedman test, $p = 0.113$). The most frequently
218 isolated organism was *M. luteus*. Ocular pathogens were only isolated from lenses of two
219 participants at the 1-month visit: one with *Acinetobacter* and the other with *S. pneumoniae*.
220 There were no ocular pathogens observed on lenses at 3-month and 6-month visits.

221 The contamination rates of the lens cases were slightly higher than that of lenses (1-month:
222 24%; 3-month: 13%; 6-month: 19%), but again the change in the contamination rate over
223 time was insignificant (Friedman test, $p = 0.674$). Only one case yielded a potential pathogen
224 (*Acinetobacter*) at the 6-month visit. All other isolates were opportunists. Binary logistic
225 regressions were performed to evaluate the effect of age, sex, study group, baseline
226 colonization and OD on contaminations of lenses and lens cases. It was revealed that
227 contamination of lenses was not associated with these factors ($\chi^2(9) = 10.33$, $p = 0.324$).

228 However, the regression model was significant for contamination of lens cases ($\chi^2(9) =$
229 23.64 , $p = 0.005$, Nagelkerke $R^2 = 0.412$). Contamination of lens cases was associated with
230 increasing age ($p = 0.021$) and assignment to Group 4 ($p = 0.011$), but not with the other
231 variables ($p > 0.056$). Participants with contaminated lens cases were statistically older but
232 the difference was clinically insignificant (9.3 ± 1.0 years vs. 8.8 ± 1.2 years). The odds of

233 contamination of the lens cases was higher in Group 4 than in Group 1 (OR = 12.45; 95% CI:
234 1.79-87.01), but differences between other groups did not reach significance ($p > 0.254$).
235 The contamination rates varied substantially between visits for all groups (see Table 4).

236

237 **Discussion**

238 This study demonstrated that the rate of conjunctival colonization decreased after
239 commencing lens wear and contamination rates of lenses and lens cases were low,
240 indicating that the PI-based disinfecting solution offers a good alternative for rigid lens care.
241 Pre-lens wear colonization was observed in 31.6% of participants, which is in agreement
242 with the reports by Sankaridurg et al.³¹ (36%) and Iskeleli et al.³² (30%). Colonization was not
243 affected by sex, age, or cleaning regime. At baseline, opportunistic organisms were carried
244 by 27.6% of participants, while pathogens were only present in 5.3%. Following lens wear,
245 rates of colonization with opportunists showed a downward trend, falling to 20.3%, 15.8%,
246 and 12.5% at 1-, 3-, and 6-month visits, respectively, although this trend did not reach
247 significance (Friedman test, $p = 0.131$). This decrease in positive cultures after commencing
248 lens wear has been reported by previous studies.^{33,34} However, it was observed, whilst
249 colonization was not associated with lens wear, those who were colonized at baseline were
250 significantly more likely to be colonized after commencing ortho-k treatment (see Figure 1).
251 A study on extended wear RGP lenses has shown that the number of participants with
252 positive conjunctival cultures decreased after 2-month of lens wear compared to baseline,
253 although there was an increase in isolates of potential pathogens.³⁵ However, other studies
254 have reported conflicting results. Zhang et al.³⁶ reported no differences between contact
255 lens wearers and non-wearers, although the abundance of some organisms was reduced in

256 ortho-k wearers. In contrast, Stapleton et al.³⁷ found that a decrease in colonization only
257 occurred after a considerable period extended soft contact lens wear.

258 In the current study, pathogens remained rare after commencement of lens wear, with only
259 three incidences of colonization with *S. aureus* and two with *S. pneumoniae*. This low rate of
260 colonization with ocular pathogens is encouraging, as it indicates no increase in risk of
261 infection associated with ortho-k lens wear. Interestingly, colonization with pathogens was
262 transient, with no participant being colonized on more than one occasion. The presence of
263 pathogens was much lower than in an earlier study investigating effects of ortho-k lens wear
264 on periorbital colonization, in which 39% of participants (9/23) were colonized with
265 potentially pathogenic organisms.¹³ This study was also conducted in Hong Kong, but lens
266 disinfection was performed using a PHMB-based solution. It is possible that colonization of
267 the conjunctiva in the current study was reduced as a result of use of a PI-based disinfecting
268 solution. The pathogens isolated from the conjunctiva are those frequently present in the
269 nasal cavity or naso-pharynx^{13,38} and may be transferred to the eye directly by fingers or
270 contaminated lens. It is therefore vital for practitioners to stress the importance of hand
271 washing before lens handling and to remind patients to avoid touching their eyes.

272 The rate of lens contamination was low, in comparison to previous studies,^{35,39} although
273 there do not appear to have been reports on rigid lenses in recent years. Notably there was
274 no difference between the groups, indicating that the cleaning regime had little impact on
275 the contamination level within six months of lens wear. Although this reflects adequate
276 disinfection by the solutions, it is also possible that participants and/or their parents may
277 pay particular attention to lens hygiene, as they are aware that the lens will be inserted
278 directly into the eye. Some parents of Group 1 (no-rub) participants questioned this cleaning

279 strategy as they thought failure to rub would result in lenses not being properly cleaned.
280 Although they were instructed to continue with their allocated strategy as this was the
281 manufacturer's recommended cleaning procedure, this may have resulted in some
282 participants in Group 1 not complying with their designated procedures. The lack of
283 differences between cleaning groups may also be attributable to the presence of an anionic
284 surfactant and proteolytic enzyme in the system, which may eliminate the need for rubbing
285 and use of additional cleaning products. However, as ortho-k lenses are used for a
286 prolonged period of up to one year, rubbing and protein treatment may be required to
287 remove more stubborn deposits, which can build up over time.⁴⁰

288 It was encouraging to note that only two participants' lenses yielded potential pathogens at
289 the 1-month visit and no further isolates occurred at the 3- or 6-month visits. This reflects
290 both adequate cleaning and disinfection of the lenses. Although most studies have reported
291 limited transient contamination of contact lenses with pathogens, there are some reports of
292 high levels of contamination with such organisms in soft lenses.^{41,42} This was attributed to
293 the presence of biofilm, which may not be easily eliminated by MPS. Presence of pathogens
294 was associated with corneal infiltrative events, but whether this was causative or a
295 consequence remains unresolved.

296 As with lenses, overall contamination of lens cases, at 18.9%, was much lower than
297 previously reported in rigid lens cases.^{12,43} In a review, Szczotka-Flynn et al.¹² reported an
298 overall contamination rate of greater than 50% in lens cases. The review includes studies by
299 Donzis et al.⁴⁴ and Devonshire et al.,⁴⁵ who reported contamination rates of 41% and 78%,
300 respectively.

301 However, a previous study, comparing lens case designs used conventional flat cases and a
302 cylindrical case, noted that the inner surface contamination was much lower in cylindrical
303 than in flat cases.⁴³ The cases provided by the manufacturer of the PI-based solution is a
304 cylindrical case with a slightly smaller diameter, which would prevent insertion of fingers
305 into the chambers. The lens holder, which is attached to the lid, is inserted into the inter-
306 connected chambers after these are filled with the solution. The results indicated that the
307 lens case was disinfected simultaneously with the lenses. However, a recent study³ of use of
308 a PI-based solution for soft lenses reported a similar overall contamination rate (70%) to
309 findings with MPS disinfecting solutions.^{46,47} Notably, the level of contamination in the PI
310 study (soft lens) was very low and considered insignificant for 73% of these cases and gram-
311 positive and fungal contamination was lower than for other disinfecting solutions. It is
312 possible that the high rate of contamination, albeit low level, may be partly attributable to
313 the rather complex lens case design that does require considerable finger contact to insert
314 or withdraw the lenses. It has been recently reported that the use of the PI-solution in the
315 cylindrical lens case, provided for the RGP version of the solution, resulted in complete loss
316 of viability of both *S. aureus* and *Pseudomonas aeruginosa* biofilms.⁷ With respect to
317 biofilm, only the PI-based solution and hydrogen peroxide system were able to achieve such
318 results. It is important to note that biofilm must be assessed by culture or viability as
319 measurements of OD, following crystal violet staining, can lead to false positive results due
320 to presence of dead organisms or residual stain in complex lens case designs.⁷ It is notable
321 that very few incidences of isolation of pathogens were noted in lens cases, with only one
322 participant having a case with *Acinetobacter* at the 6-month visit. Most pathogens are
323 strong biofilm producers⁴² and the ability of this PI-based solution to kill organisms in the

324 biofilm can prevent their survival in the lens case. Notably none of the lens cases in this
325 study yielded *P. aeruginosa*.

326 Both hydrogen peroxide system and PI-based solutions are strongly microbiocidal and
327 require neutralization following disinfection, which must be done correctly to ensure safety.
328 There is a clear colour change when neutralization has been achieved with PI and some
329 hydrogen peroxide systems. However, other hydrogen peroxide systems, relying on a
330 neutralizing disc within the case, may fail if used beyond the recommended period of use,
331 leading to potential ocular damage.⁴⁸ In addition to its strong disinfecting ability, use of PI-
332 based solution has other advantages, including no report of bacterial resistance due to
333 genetic changes, i.e., *qac* genes⁴⁹ and improved comfort for day wear lenses.³

334 The main limitation of this study is the inability to determine complete compliance with
335 cleaning instructions provided to each group, correct rinsing procedures, and intermittent
336 cleaning of lens cases. As solutions were provided free of charge to participants, it is
337 unlikely that they would have substituted an alternative disinfecting solution or continued
338 use of a lens case beyond a month. Caution must also be applied for comparison of studies
339 of contamination of lens cases with earlier reports: active agents and their concentrations
340 have changed considerably in the last decades for soft contact lens solutions; manufacturers
341 have paid most attention to effectiveness against *Pseudomonas*, as it was the leading of
342 microbial keratitis; and in contrast with current practice, a new lens case was not provided
343 with the purchase of each bottle of disinfecting solution. Therefore, the age of cases tested
344 in previous reports of lens case contamination may be variable and include some that may
345 have been used for several months (or even longer!). In addition to changing of lens cases,
346 some participants had to replace their lenses during the study period due to fitting issues,

347 breakages, or loss. Both of these problems could potentially have resulted in lower rates of
348 contamination.

349 In conclusion, PI-based solution offers a viable alternative system of disinfection for rigid
350 and ortho-k lens wearers. Unexpectedly, there was no difference in contamination rates
351 among the cleaning routines used. The absence of pathogens in the great majority of
352 samples cultured and the dramatically reduced contamination rate compared with those
353 previously reported indicate that use of PI-based solution can improve the safety of
354 overnight lens wear.

355

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362

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487

488 **Figure Legend**

489 **Figure 1.** Conjunctival colonization of participants in the four study groups at different visits.

490 Only one participant (ID #19) had both pathogenic and opportunistic pathogens in baseline

491 visit

492

493 **Table 1. Lens case cleaning regimes**

Group 1	Group 2	Group 3	Group 4
No rub	Rub with cleadewGP	Rub with daily cleaner	Rub with daily cleaner and weekly protein removal
Disinfect lenses without cleaning after removal every morning	Rub lenses with cleadew GP solution and rinse with saline after removal every morning before disinfection	Rub the lenses with daily cleaner* and rinse with saline after removal every morning before disinfection	Rub lenses with daily cleaner* and rinse with saline after removal every morning before disinfection and use P-AB weekly

494 * O2 Daily Care Solution, Ophtecs Inc., Japan

495 P-AB: Progent A+B, Menicon Co, Japan

496

497

498 **Table 2. The mean (\pm SD) of baseline age and the number (percentage) of female**
 499 **participants at baseline**

	All	Group 1	Group 2	Group 3	Group 4	p-value
N	79	19	20	20	20	--
Age (years)	9.1 \pm 1.1	9.1 \pm 1.5	8.9 \pm 1.1	9.3 \pm 0.8	8.9 \pm 1.2	0.623 [^]
Female	52 (66%)	11(58%)	14 (70%)	13 (65%)	14 (70%)	0.839 [#]

500 [^] One-way ANOVA; [#] Chi-squared tests

501

502

503 **Table 3. Association of conjunctiva colonization before and after orthokeratology wear**

	Negative after ortho-k (n=43)	Positive after ortho-k (n=33)
Negative before ortho-k (n=52)	34 (65.4%)	18 (34.6%)
Positive before ortho-k (n=24)	9 (37.5%)	15 (62.5%)

504 McNemar's test; p = 0.122

505 **Table 4. Contamination rates in lens and lens cases**

	Overall	Post lens wear visit		
	contamination rate	1-month	3-month	6-month
Number of valid samples	222	74	76	72
Lens contamination	23/222 (10.4%)	12 (16%)	4 (5%)	7 (10%)
<i>Group 1</i>	4/53 (8%)	3/17 (18%)	0/19 (0%)	1/17 (6%)
<i>Group 2</i>	7/57 (12%)	4/19 (21%)	1/20 (5%)	2/18 (11%)
<i>Group 3</i>	4/55 (7%)	2/19 (11%)	0/18 (0%)	2/18 (11%)
<i>Group 4</i>	8/57 (14%)	3/19 (16%)	3/19 (16%)	2/19 (11%)
Case contamination	42/222 (18.9%)	18 (24%)	10 (13%)	14 (19%)
<i>Group 1</i>	7/53 (13%)	2/17 (12%)	1/19 (5%)	4/17 (24%)
<i>Group 2</i>	10/57 (18%)	6/19 (32%)	0/20 (0%)	4/18 (22%)
<i>Group 3</i>	9/55 (16%)	4/19 (21%)	3/18 (17%)	2/18 (11%)
<i>Group 4</i>	16/57 (28%)	6/19 (32%)	6/19 (32%)	4/19 (21%)

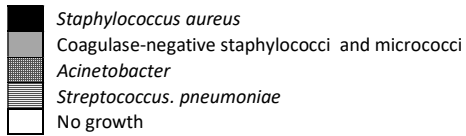
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No	Group	Visit			
		Baseline	1-month	3-month	6-month
1	1	■	■	■	■
2	1	■	■	■	■
3	1	■	■	■	■
4	2	■	■	■	■
5	2	■	■	■	■
6	2	■	■	■	■
7	2	■	■	■	■
8	2	■	■	■	■
9	2	■	■	■	■
10	2	■	■	■	■
11	2	■	■	■	■
12	3	■	■	■	■
13	3	■	■	■	■
14	3	■	■	■	■
15	3	■	■	■	■
16	3	■	■	■	■
17	3	■	■	■	■
18	4	■	■	■	■
19	4	■	■	■	■
20	4	■	■	■	■
21	4	■	■	■	■
22	4	■	■	■	■
23	4	■	■	■	■
24	4	■	■	■	■
25	1	■	■	■	■
26	1	■	■	■	■
27	1	■	■	■	■
28	1	■	■	■	■
29	1	■	■	■	■
30	1	■	■	■	■
31	1	■	■	■	■
32	2	■	■	■	■
33	2	■	■	■	■
34	2	■	■	■	■
35	3	■	■	■	■
36	3	■	■	■	■
37	3	■	■	■	■
38	3	■	■	■	■
39	4	■	■	■	■
40	4	■	■	■	■
41	4	■	■	■	■
42	4	■	■	■	■

43-76 were **negative** for all cultures

508



509

510

511 **Figure 1.**