not apparent in control cysts that were not exposed to a disinfectant.

**Discussion.** Effective disinfection of *Acanthamoeba* is essential for safe lens wear. Clinical cases of *Acanthamoeba* keratitis in patients who wear rigid gas-permeable contact lenses, as well as the previously reported problems that originate from homemade saline solutions, underscore the importance of lens care formulations for effective cyst inactivation. The viable counting technique presented here is available to test lens care solutions in search of more effective chemical disinfection methods. The experimental data presented in this report show the applicability of this technique to produce quantitative data not accomplished by previously published methods.

**Key words:** *Acanthamoeba* enumeration, cyst inactivation, disinfection, rigid gas-permeable contact lenses, contact lens solutions

**Acknowledgments.** The authors thank Dr. Thomas J. Byers for advice and facilities, and Ms. Laurie Haldeman for typing the manuscript.

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**References**

than on crofilcon and perflcon; on etafilcon than on crofilcon, bufilcon, and perflcon; and on perflcon than on crofilcon and bufilcon. The results did not show a consistent relationship between hydration and surface charge and *P. aeruginosa* adherence. Among the SCLs tested, no one lens had a decisive advantage over another, because all, both new and worn, can bind amounts of *P. aeruginosa* that could potentially produce bacterial keratitis on predisposed eyes. Invest Ophthalmol Vis Sci 32:657–662, 1991

Injective ulcerative keratitis is one of the most severe hazards of hydrogel soft contact lens (SCL) wear. Extended-wear SCLs carry the highest risk. In the literature on the mechanism of adherence of *Pseudomonas aeruginosa* to new and used soft contact lenses (SCLs), one encounters some apparent contradictions. Thus, Butrus et al. found that *P. aeruginosa* adhered preferentially to the heterogeneous, grossly visible deposits. In addition, although Stern and Zam reported that the treatment of the SCLs with proteins, and particularly mucin, enhanced the adherence of *P. aeruginosa* to the SCLs. Butrus et al. found that the mucin-coated SCLs inhibited adherence of the bacteria to the lenses, but both groups agreed that adherence was increased when the SCLs were presoaked in human tears was enhanced in some SCLs and inhibited in other SCLs obtained from different individuals. Dart and Baden lob10 observed that the degree of deposits on human-worn, heavily deposited SCLs had no effect on the adherence of *P. aeruginosa*.

In addition, although Stern and Zam reported that the treatment of the SCLs with proteins, and particularly mucin, enhanced the adherence of *P. aeruginosa* to the SCLs, Butrus et al. found that the mucin-coated SCLs inhibited adherence of the bacteria to the lenses, but both groups agreed that adherence was increased when the SCLs were presoaked in protein solutions. Miller et al. agreed with Stern and Zam that in general, the adherence of *P. aeruginosa* to SCLs was enhanced when the lenses were presoaked in solutions of mucin and proteins. Although these studies were conducted with different SCLs, different history of wear and material of construction, probably different bacterial strains, and different methods of bacteria quantification, it appeared that *P. aeruginosa* attached readily not only to previously worn lenses but also to unused, never-worn SCLs.

SCLs in the eye are readily coated with tear proteins, mucus, and lipids. Used SCLs can have high levels of grossly invisible protein coatings and heterogeneous, grossly visible deposits. In addition, *P. aeruginosa* adheres preferentially to the heterogeneous, grossly visible deposits. Therefore, if one examines bacterial adherence to SCLs with heterogeneous deposits or to lenses with a diffuse protein coating, one might find different results in the amount of attached bacteria. The uncertainty remains in whether the coating that forms on all used lenses is a significant factor in SCL-induced *P. aeruginosa* keratitis. Aswad et al. found that in the rabbit cornea stressed by lid closure, a significantly greater incidence of bacterial keratitis developed in eyes fitted with used contaminated SCLs than in eyes fitted with new contaminated SCLs. These results conflicted with those of Koch et al. who, with a similar animal model, found no difference in the rate of infection of rabbit eyes fitted with *P. aeruginosa*-contaminated new or used SCLs.

The significance of the contact lens coating in relation to bacterial attachment and infectivity of SCLs is controversial, and the role of the SCL material and surface charges in SCL infectivity has not been well examined. Miller and Ahearn compared the adherence of *P. aeruginosa* to SCLs of various water content and polymer compositions with the use of presumably new SCLs that belong to the four groups within the Food and Drug Administration (FDA) classification. They found that degrees of bacterial adherence varied on the SCLs. There was no correlation between bacterial adherence and hydration of the SCLs, and bacterial adherence was lower on ionic than on nonionic SCLs. However, they did not evaluate their results statistically. Miller et al. examined the effect of human tear coatings on adherence of *P. aeruginosa* to various SCLs and sometimes found apparently higher or lower numbers of bacteria on the used than on the new lenses. These results have additional uncertainty because of the lack of statistical evaluation of the results. We sought to determine the rate of attachment of *P. aeruginosa* on new and rabbit-worn SCLs of different groups in the FDA hydrogel SCL classification. For the in vivo tear coating of the SCLs before bacterial contamination, we used rabbit-worn SCLs under a tarsorrhaphy with the intent to diminish the variables encountered when contact lenses were obtained from human subjects. In the latter case, the lenses had been rejected due to premature spoilage, or were old, heavily deposited, or poorly tolerated SCLs.

**Materials and Methods.** We used six different brands of SCLs that represent three FDA classification groups and one lens (bufilcon-Elite) that does not fit into the FDA classification (Table 1). The SCLs were divided into five sets, each of which contained all seven brands: three sets of new SCLs and two sets of rabbit-worn SCLs.

A single strain of *P. aeruginosa*, harvested from a human corneal ulcer, was used for all experiments. The harvested bacteria were stored at −70°C in tryptic soy broth (Difco, Detroit, MI) and 5% glycerol (Sigma, St. Louis, MO) in 5-ml portions. Bacteria taken in five loops from one of the original portions of the bacterial suspension were grown overnight at 37°C without agitation in 100 ml of sterilized tryptic soy broth (3 g/100 ml distilled water), spun down at full speed for 10 min (Centrifuge Model HN, Interna-
Table 1. SCLs used in the experiments

<table>
<thead>
<tr>
<th>FDA group</th>
<th>USAN*</th>
<th>Trade name</th>
<th>Chemical name†</th>
<th>% H2O</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Low-water, nonionic</td>
<td>Polymacon</td>
<td>Sequence</td>
<td>Poly(hydroxyethyl methacrylate)</td>
<td>38.6</td>
</tr>
<tr>
<td>I Low-water, nonionic</td>
<td>Crofilcon A</td>
<td>CSI</td>
<td>Poly(glyceryl methacrylate-co-methyl methacrylate)</td>
<td>39.0</td>
</tr>
<tr>
<td>II High-water, nonionic</td>
<td>Lidofilcon A</td>
<td>Sauflon 70</td>
<td>Poly(vinyl pyrrolidone-co-methyl methacrylate)</td>
<td>70.0</td>
</tr>
<tr>
<td>IV High-water, ionic</td>
<td>Bufilcon A</td>
<td>Hydrocurve II</td>
<td>Poly(hydroxyethyl methacrylate-co-diactone acrylamide-co-methacrylic acid)</td>
<td>55.0</td>
</tr>
<tr>
<td>IV High-water, ionic</td>
<td>Etafilcon A</td>
<td>Acuvue</td>
<td>Poly(hydroxyethyl methacrylate-co-sodium methacrylate)</td>
<td>58.0</td>
</tr>
<tr>
<td>IV High-water, ionic</td>
<td>Perfilcon A</td>
<td>Permalens</td>
<td>Poly(hydroxyethyl methacrylate-co-vinylpyrrolidone-co-methacrylic acid)</td>
<td>71.0</td>
</tr>
<tr>
<td>None High-water, ionic polymer, nonionic surface</td>
<td>Bufilcon A</td>
<td>Hydrocurve Elite</td>
<td>Same as Hydrocurve II</td>
<td>55.0</td>
</tr>
</tbody>
</table>

* U.S. Adopted Name.
† All polymers are cross-linked with minor amounts of one of various cross-linking agents.

tional Equipment Co, Needham Heights, MA), and
washed in double-filtered phosphate-buffered saline (PBS) (pH 7.4), while being mixed in a vortex (Vortex
Genie, Scientific Industries, Springfield, MA) at full
speed. This cycle was completed three times. The
bacterial suspension was resuspended to an optical
density of 0.150 at 590 nm (Beckman DU® Spectro-
photometer), which is equivalent to a bacterial con-
centration of $1 \times 10^8$ colony-forming units (CFU)/
ml. The bacterial suspension was freshly made be-
fore each experiment.

Bacterial adherence to new SCLs: Three sets of new
SCLs were used in these experiments. Each lens was
cut into four segments under sterile conditions. Each
segment of each lens in every set was used to assay
bacterial adherence. The pieces of sterile lenses were
exposed to 5 ml of a $1 \times 10^8$ CFU/ml *P. aeruginosa*
suspension for 1 hr at room temperature, rinsed again
in a large volume of double-filtered PBS, and fixed in half-strength Karn-
ovsky’s fixative. The lenses were cut in half after the
fixation process and before critical point drying.

After alcohol dehydration and critical point drying,
the lenses were viewed under a scanning electron mi-
croscope (SEM) (AMR, Model 1000 A, Bedford,
MA) at 20 Kv and a fixed working distance of 12
mm. Twelve random areas of each lens piece were
photographed at a magnification of $\times1200$. The bac-
teria attached to the lens in each of the 12 areas were
counted and expressed as the number per unit area of
hydrated lens. The lens shrinkage from the hydrated
state (bacterial attachment) to the dehydrated state
(bacteria counting) was corrected according to Re-
fojo’s general relationship of dimension changes
with hydrogel hydration as follows: 18.0% for poly-
macon and crofilcon, 27.0% for bufilcon, 29.0% for
etafilcon, 38.0% ifor lidofilcon, and 38.5% for per-
filcon.

Results. The mean bacterial adherence for the var-
ious lenses are shown in Figure 1. Bacterial attach-
ment was uneven over the lens surface and caused
high standard deviations. The bacterial attachment
was not a normal (Gaussian) distribution. The Wil-
coxon rank-sum test was used for the statistical evalu-
ation of the results. For all lenses, there was a statisti-
cally significant difference ($P < 0.05$) in bacterial at-
achment between the unworn lenses and the lenses
worn for 24 hr. All lenses, regardless of type, showed
significantly more bacterial attachment when they
were unworn (new) (Fig. 2).

Examination at higher magnification showed a few
unidentified amorphous granules on some lenses, but
imidiately in PBS, and exposed to 5 ml of a $1 \times 10^8$
CFU/ml *P. aeruginosa* suspension for 1 hr at room
temperature, rinsed again in a large volume of dou-
ble-filtered PBS, and fixed in half-strength Karn-
ovsky’s fixative. The lenses were cut in half after the
fixation process and before critical point drying.
Fig. 1. Mean bacterial adherence (±SD) to new and worn soft hydrogel contact lenses. Solid bar, worn lens; cross-hatched bar, new lens. *Significant compared with worn lens of the same type (P < 0.05).

no large deposits, and the coating was never so thick that the surface pattern could not be visualized. The comparison of bacterial attachment on new and worn SCLs and the statistical differences, if any, at the significant level of P < 0.05, are shown in Table 2.

Discussion. Bacterial attachment to surfaces is believed to be dependent on the organism, the composition of the surface, and the substances that mediate binding. Klotz et al.19 stressed that different P. aeruginosa isolates can vary in hydrophobicity and that more hydrophobic organisms show a significantly increased tendency toward surface adherence. Van der Waals forces and brownian motion, as well as net surface charge, have been reported to determine bacterial attachment.6,20–23 However, we found that P. aeruginosa attach indifferently to two hydrogel SCLs (bufilcon A and bufilcon-Elite) of the same chemical composition and hydration, regardless of surface charge and whether worn or unworn. We found higher bacterial attachment on new high-water non-ionic lenses than on low-water nonionic lenses (eg, lidofilcon vs polymacon or crofilcon). However, after the lenses were worn, this relationship is reversed on lidofilcon vs crofilcon. On the other hand, when we compared the three ionic lenses, we found fewer bac-

Fig. 2. SEM polymacon Sequence (A, C) and bufilcon Hydrocurve Elite (B, D) soft contact lenses, new (A, B) and after 24 hr in rabbit eyes (C, D). Lenses were incubated in Pseudomonas aeruginosa suspensions of the same concentration and for the same time period.
iontophoretic surface was often lost after the lens was worn. In general, our results did not show a consistent difference on *Pseudomonas aeruginosa*.

### Table 2. Difference on *Pseudomonas aeruginosa* counts on unworn (U) and on worn (W) soft contact lenses

<table>
<thead>
<tr>
<th></th>
<th>Polymacon</th>
<th>Croflicon</th>
<th>Lidofilcon</th>
<th>Bufilcon</th>
<th>Bufilcon Elite</th>
<th>Etafilcon</th>
<th>Perfilcon</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>U</td>
<td>W</td>
<td>U</td>
<td>W</td>
<td>U</td>
<td>W</td>
<td>U</td>
</tr>
<tr>
<td>Polymacon</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Croflicon</td>
<td>=</td>
<td>=</td>
<td>=</td>
<td>=</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Lidofilcon</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>=</td>
<td>=</td>
<td>=</td>
</tr>
<tr>
<td>Bufilcon</td>
<td>(0.002)</td>
<td>(0.002)</td>
<td>(0.001)</td>
<td>—</td>
<td>=</td>
<td>=</td>
<td>(0.007)</td>
</tr>
<tr>
<td>Bufilcon Elite</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>+</td>
<td>=</td>
<td>=</td>
</tr>
<tr>
<td>Etafilcon</td>
<td>(0.007)</td>
<td>(0.007)</td>
<td>(0.006)</td>
<td>—</td>
<td>=</td>
<td>=</td>
<td>+</td>
</tr>
<tr>
<td>Perfilcon</td>
<td>(0.04)</td>
<td>(0.04)</td>
<td>(0.01)</td>
<td>—</td>
<td>=</td>
<td>=</td>
<td>+</td>
</tr>
</tbody>
</table>

* Indicates significant differences in pairwise comparison at the 0.05 significant level using the Wilcoxon rank-sum test, where the numbers of bacteria on the lenses in the row at the head of the table are significantly lower than on the lenses in the columns of the table.

The authors thank D.A. Kara for statistical evaluation and Dr. D. Korb and CooperVision for donating the lenses.

From the Eye Research Institute, the Department of Ophthalmology, Harvard Medical School, and Massachusetts Eye and Ear Infirmary, Boston, Massachusetts. Supported in part by grant La 616/1-1 of the Deutsche Forschungsgemeinschaft (CAL-B), and grants EY00327 and EY05799, from the National Eye Institute, National Institutes of Health, Bethesda, Maryland. Submitted for publication: December 19, 1989; accepted October 9, 1990. Correspondence to: Miguel F. Refojo, DSc, Eye Research Institute, 20 Staniford Street, Boston, MA 02114.

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