Key words：多施設共同研究，細菌性結膜炎，検出菌，薬剤感受性．multicenter study, bacterial conjunctivitis, bacterial isolates, drug sensitivity.

はじめに

細菌性結膜炎に対する抗菌薬の選択・投与方法は，起炎菌を検出したうえでその細菌に最も感受性のある薬剤を選択することである．しかし日常臨床では，患者の苦痛の早期軽減や社会生活への影響を考慮して，起炎菌の検出を待たずに治療を行う場合がほとんどであり，起炎菌の同定を行う前に汎用されている抗菌点眼薬を処方するのが現状である．一方，細菌の抗菌薬感受性には経年変化が認められること，近年メチシリン耐性黄色ブドウ球菌（MRSA）などの耐性菌による感染症の拡大に伴い，耐性菌対策が必須であることから，日常臨床における抗菌薬選択の重要性は高く，細菌性結膜炎の起炎菌の動向を把握しておくことは意義あることと思われる．

そこで，筆者らCore-Network of Ocular Infection（COI）のメンバーは，多施設における細菌性結膜炎の検出菌の動向と薬剤感受性の現状を把握し，今後の抗菌薬投与の指標となる有益な情報を得るために，新たな共同研究組織であるCOI細菌性結膜炎検出菌スタディグループを組織した．そして，2004年11月より2009年までの5年間，全国27施設を受診し，その臨床所見から細菌性結膜炎と診断された症例615例を対象に，結膜から検体を採取して同一施設で培養を実施し，症例背景（年齢，地域，受診施設），検出菌種，薬剤感受性について検討を行った．

初年度の結果についてはすでに報告した[1]が，今回，5年間の予定調査期間を終了したので，その結果を報告する．

I 対象および方法

対象は，全国の約27施設（大橋眼科［北海道］，くろさき眼科［新潟県］，栃尾郷病院［新潟県］，阿部眼科［秋田県］，東京医科大学［東京都］，東京医科大学八王子医療センター［東京都］，東邦大学［東京都］，とだ眼科［埼玉県］，鹿嶋眼科クリニック［茨城県］，いずみ記念病院［東京都］，上沼田クリニック［東京都］，ルミネはたの眼科［神奈川県］，稲田登戸病院［神奈川県］，いこま眼科医院［石川県］，バプテスト眼科クリニック［京都府］，大橋眼科［大阪府］，岡本眼科クリニック［愛媛県］，愛媛大学［愛媛県］，鷹の子病院［愛媛県］，町田病院［高知県］，魚谷眼科医院［鳥取県］，大分県立病院［大分県］，新別府病院［大分県］，NTT西日本九州病院［熊本県］，熊本赤十字病院［熊本県］，熊本大学［熊本県］，中頭病院［沖縄県］．ただし，研究参加年数が4年以下の施設も含む．）を，初年度（第1回：2004年11月，第2回：2005年2月，第3回：2005年5月，第4回：2005年8月），2年度（第5回：2006年2月，第6回：2006年11月），3年度（第7回：2007年11月），4年度（第8回：2008年11月，第9回：2009年2月），5年度（第10回：2009年11月～12月）の各調査期間に受診し，その臨床所見から細菌性結膜炎と診断された患者である．

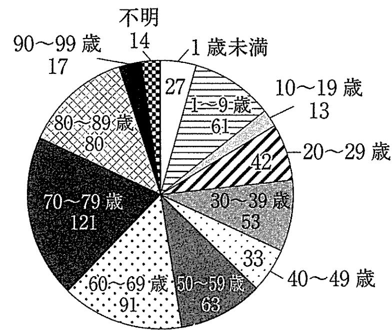


図1 症例の年齢分布（期間合計）

症例総数は615例（男性266例，女性344例，不明5例）で，年齢は生後0～99歳（平均年齢52.2歳）で，年齢不明を除き50.2%（309名）が60歳以上であった（図1）．また，7.2%（44例）がコンタクトレンズ（CL）を装用していた．

患者から同意を得た後，症状の重いほうの片眼の結膜を擦過して採取した検体を，輸送用培地「AMIES CARBON」を用いて阪大微生物病研究会（阪大微研）に送付し，好気・嫌気培養を行い，細菌の分離・同定を行った．そして，検出菌，地域別の検出菌，施設別の検出菌，年齢別の検出菌，季節別の検出菌，CL装用の有無による検出菌のそれぞれの内訳を検討した．また，検出菌に対して日本化学療法学会の標準法により，レボフロキサシン（LVFX），ミクロノマイシン（MCR），エリスロマイシン（EM），クロラムフェニコール（CP），スルベニシリンナトリウム（SBPC），塩酸セフメノキシム（CMX）の6剤の最小発育阻止濃度（MIC）を測定し，その結果を累積発育阻止率曲線で表した．なお，調査期間中，MCRの製造中止に伴い，4年度からはトブラマイシン（TOB）に変更した．さらに，今回の研究では，結膜炎以外の外眼部疾患を有する症例および参加施設の受診以前に抗菌薬が投与されていた症例は除外した．

II 結　　果

1. 細菌分離率

全症例615例のなかで細菌が分離されたのは587例（細菌陽性率95.4%）であり，男性263例，女性319例で，年齢は生後0～99歳（平均年齢52.2歳）であった．

2. 検出菌の種類と頻度

細菌が分離された587例から1,156株の細菌が検出された(1症例当たり1～8株). 初年度から5年度までのすべての検出菌のうち最も多かったのは, *Staphylococcus epidermidis* (*S. epidermidis*) 223株(19.3%), ついで*Propionibacterium acnes* (*P. acnes*) 166株(14.4%), *Streptococcus* spp. 150株(13.0%), *Staphylococcus aureus* (*S. aureus*) 125株(10.8%), *Corynebacterium* spp. 122株(10.6%), *Haemophilus influenzae* 53株(4.6%), *Moraxella* spp. 40株(3.5%)であった(図2). *S. aureus* 125株中, メチシリン感受性黄色ブドウ球菌(MSSA)が99株, メチシリン耐性黄色ブドウ球菌(MRSA)が26株であった. 嫌気性菌は178株で, そのうちの169株が*Propionibacterium* spp.であった. グラム陽性菌が全体の63.6%を占めていた. 経年変化では, 初年度は, 検体総数が429株で*S. epidermidis* が102株(23.7%)と最も高頻度に検出され, ついで*S. aureus* 66株(15.4%), *Streptococcus* spp. 59株(13.8%), *P. acnes* 40株(9.3%)の順であった. 2年度から5年度までは*P. acnes*

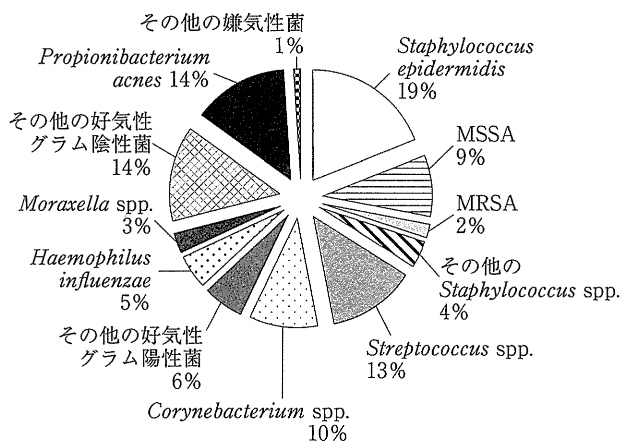


図2 検出菌の種類(期間合計)

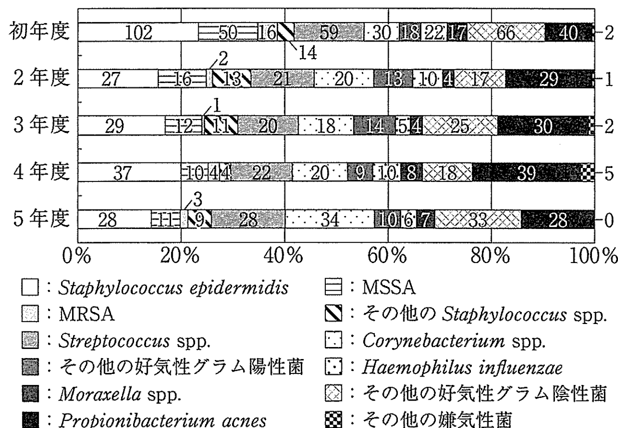


図3 検出菌の経年変化(主要菌種別)

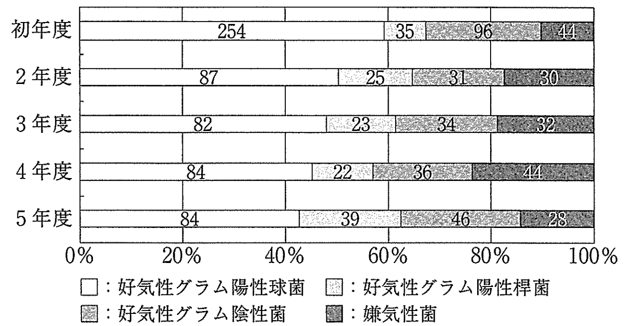


図4 検出菌の内訳・経年変化(グラム染色別)

が最も多く, 次いで*S. epidermidis* の順であったが, 5年間を通して大きな傾向の変化は認められなかった(図3).

グラム染色別の検出菌の内訳・経年変化については, 初年度, グラム陽性球菌が59.2%(254株)と最多であったが, 2年度50.2%(87株), 3年度47.9%(82株), 4年度45.1%(84株), 5年度42.6%(84株)と, 初年度から5年度まで検出菌の約50%はグラム陽性球菌で占められていた(図4). グラム陽性球菌は5年間を通して最も多く検出されていたものの, 経年的には検出比率が減少した.

3. 地域別の検出菌内訳・経年変化(グラム染色別)

地域別(北海道・東北, 関東, 中部, 関西, 中国・四国, 九州・沖縄)検出菌の内訳・経年変化は, グラム陽性球菌が地域・年度を問わず高頻度であった.

初年度は, 関西地域でグラム陰性菌が少なく, 関西・関東で嫌気性菌の比率がやや高かった. しかし, 2年度以降は地域間で参加施設の偏り(施設数, 施設のタイプ)が生じたために, 地域によってはばらつきがみられたものの, 全体的な検出菌の頻度については, 経年的, 地域的に大きな差は認められなかった(図5).

4. 施設別の検出菌内訳・経年変化(グラム染色別)

全症例615例の施設別内訳は, 大学病院57例, 総合病院127例, 眼科クリニック431例であった. 施設別の検出菌内訳・経年変化は, 5年間を通じ, 眼科クリニック, 総合病院ではグラム陽性球菌の割合が突出していた. 大学病院では, 検体数が少ないため, 各検出菌の頻度に大きなばらつきがみられ, 一定の傾向を得ることはできなかった(図6).

5. 年齢別の検出菌内訳・経年変化(グラム染色別)

全症例615例中の年齢別内訳をみると, 65歳以上は282例(45.9%)であり, 細菌性結膜炎の半数を高齢者が占めた. 各年代(14歳以下, 15～64歳, 65歳以上)における検出菌の内訳・経年変化をみると, 各年代を通じてグラム陽性球菌が最も高頻度であり, 5年間を通してその傾向は変わらなかったものの, 15歳以上の年代ではグラム陽性球菌の割合が経年的に減少しており, 特に3年度以降ではその検出比率は半数を切っていた(図7).

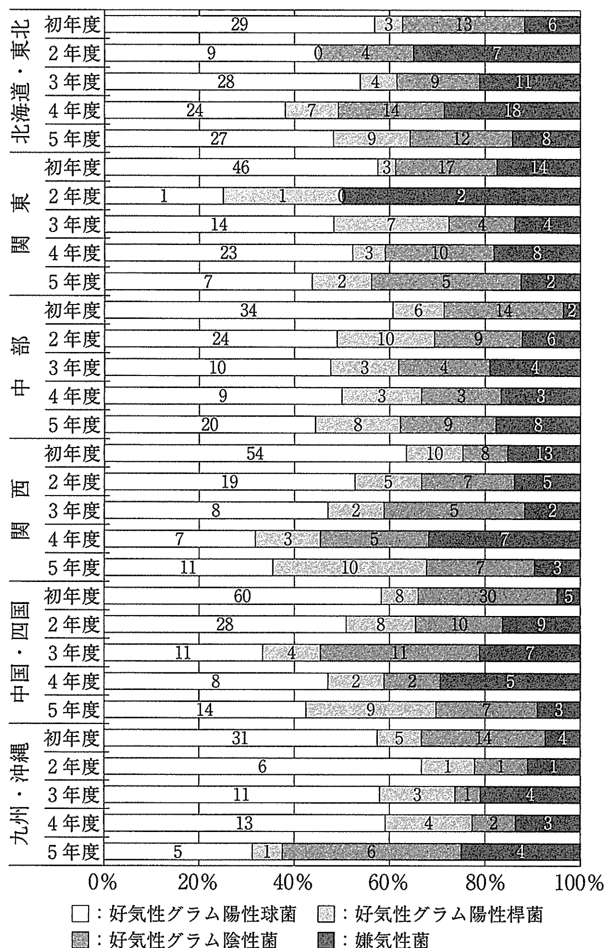


図5 地域別検出菌の内訳・経年変化（グラム染色別）

6. 季節別の検出菌内訳・経年変化

初年度に季節を4回に分けて行った調査では，2月にグラム陽性桿菌が少なく，嫌気性菌が多かった．冬期に多いとされる *Haemophilus influenzae* であるが，11月に6株，2月に6株，5月に6株，8月に4株検出されており，季節による大きな変化はみられなかった．なお，こうした初年度の結果[1]を受け，2年度以降では季節別の比較は行わなかった（図8）．

7. CL装用の有無との関連性

CLは88.5％が装用しておらず，装用者は7.2％にとどまった．CL装用の有無でグラム陽性菌と陰性菌の比率に大きな差はなかったが，CL装用者にグラム陽性桿菌が少なく，嫌気性菌が多い傾向を認めた（図9）．

8. 薬剤感受性

結膜炎由来臨床分類株である全検出菌1,156株（全菌種：初年度429株，2年度173株，3年度171株，4年度186株，5年度197株）に対するLVFX，MCR，TOB，EM，CP，SBPC，CMXの抗菌力を，累積発育阻止率曲線で示した（図10）．

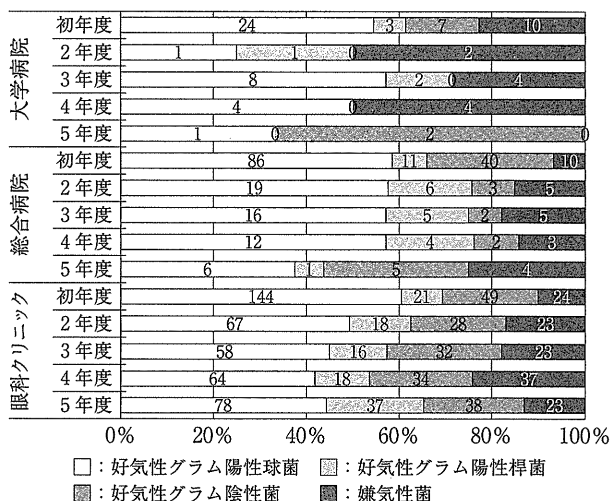


図6 施設別（眼科クリニック，総合病院，大学病院）検出菌の内訳・経年変化

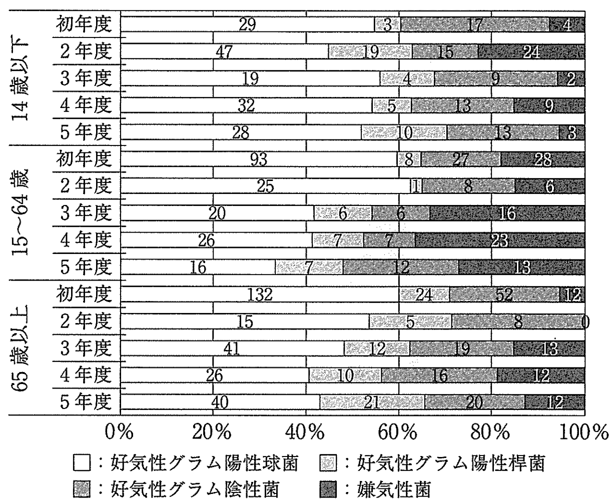


図7 年齢別の内訳・経年変化（グラム染色別）

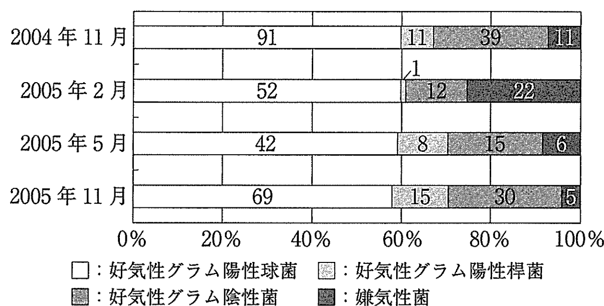


図8 季節別の検出菌内訳・経年変化（グラム染色別）

全体としての MIC_{80}，MIC_{90} はLVFX，CMXがその他の薬剤と比べて低い値となっており，結膜炎の主要な起炎菌に対する高い感受性が認められた．

全検出菌に対する各薬剤の抗菌力の経年変化を，累積発育

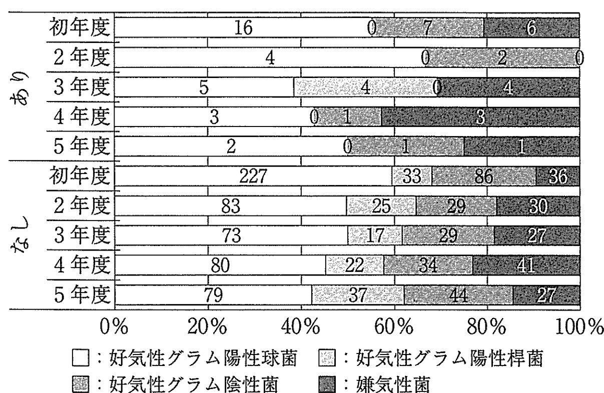
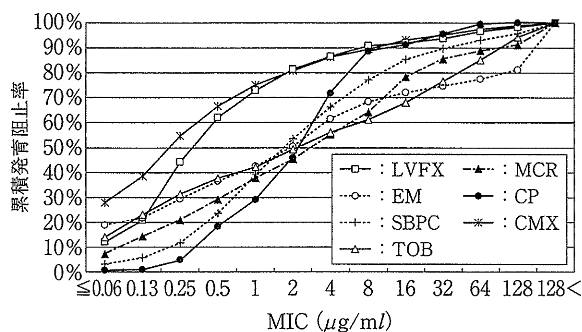


図9 CL装用の有無による検出菌内訳・経年変化（グラム染色別）



	Range	MIC_{80}	MIC_{90}
LVFX	≦0.06～128＜	2	8
MCR	≦0.06～128＜	32	128
TOB	≦0.06～128＜	64	128
EM	≦0.06～128＜	128	128＜
CP	≦0.06～128	8	16
SBPC	≦0.06～128＜	16	32
CMX	≦0.06～128＜	2	8

図10 全検出菌1,156株に対する全薬剤の累積発育阻止率曲線

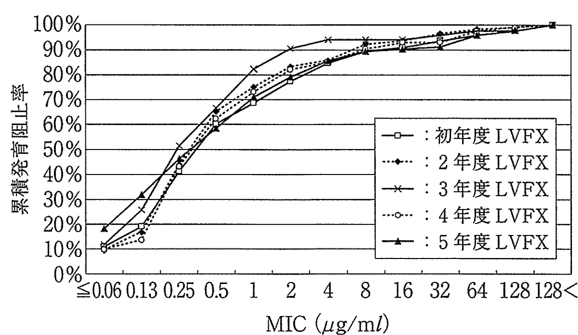
阻止率曲線で示した（図11～17）．

LVFXは5年間の調査期間で大きな変化はなく，累積発育阻止率曲線はほぼ同じパターンを描いた（図11）．MIC_{80}，MIC_{90}は低値を示しており，全検出菌に対する高い感受性が認められた．

MCR（初年度～4年度）およびTOB（4～5年度）は5年間の調査期間で大きな変化はなく，累積発育阻止率曲線はほぼ同じパターンを描いた（図12～13）．

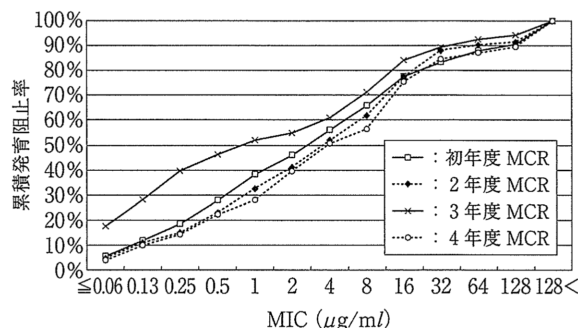
EM，CP，SBPCについても5年間の調査期間で大きな変化はなく，累積発育阻止率曲線はほぼ同じパターンであった（図14～16）．

CMXは5年間の調査期間で大きな変化はなく，累積発育阻止率曲線はほぼ同じパターンを描いた（図17）．MIC_{80}，MIC_{90}は低値を示しており，全検出菌に対する高い感受性が認められた．



	Range	MIC_{80}	MIC_{90}
初年度	≦0.06～128＜	4	8
2年度	≦0.06～128＜	2	8
3年度	≦0.06～128＜	1	2
4年度	≦0.06～128＜	2	8
5年度	≦0.06～128＜	4	16

図11 全検出菌1,156株に対するLVFXの累積発育阻止率曲線（全菌種：初年度429株，2年度173株，3年度171株，4年度186株，5年度197株）



	Range	MIC_{80}	MIC_{90}
初年度	≦0.06～128＜	32	128
2年度	≦0.06～128＜	32	64
3年度	≦0.06～128＜	16	64
4年度	≦0.06～128＜	32	128＜

図12 全検出菌959株に対するMCRの累積発育阻止率曲線（全菌種：初年度429株，2年度173株，3年度171株，4年度186株）

つぎに，細菌性結膜炎に対して最も広く使用されているLVFXの主要検出菌に対する抗菌力について，累積発育阻止率曲線で示した（図18～22）．

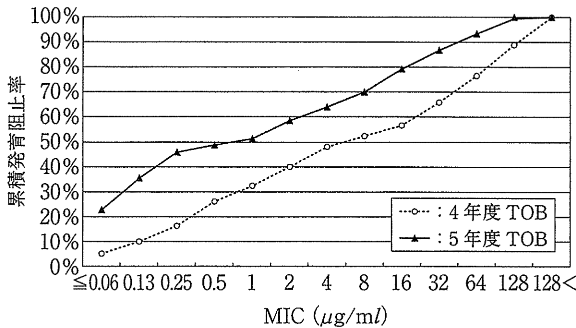
S. epidermidis 221株（初年度100株，2年度27株，3年度29株，4年度37株，5年度28株）では，年度間にて多少の変動は認められるものの，LVFXは*S. epidermidis*に対する高い感受性を5年間を通して維持していた（図18）．

P. acnes 166株（初年度40株，2年度29株，3年度30株，4年度39株，5年度28株）および*S. aureus*（MSSA）101株（初年度50株，2年度16株，3年度12株，4年度10株，

5年度11株）では，5年間を通して左に強くシフトした同様の曲線を描いており，*P. acnes* およびMSSAに対するLVFXのきわめて高い感受性が示された（図19，20）．

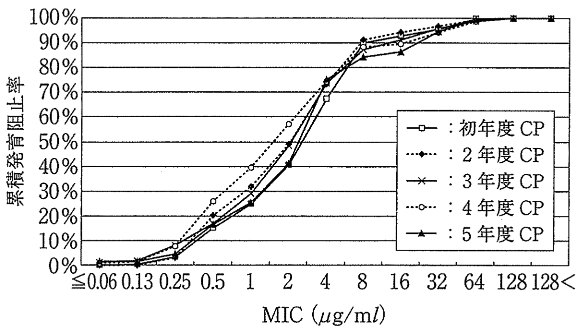
Streptococcus spp. 150株（初年度59株，2年度21株，3年度20株，4年度22株，5年度28株）は，曲線が左にシフトしており，*Streptococcus* spp. に対するLVFXの高い感受性が示された（図21）．

Corynebacterium spp. 118株（初年度30株，2年度20株，3年度18株，4年度20株，5年度30株）では，LVFXの感受性は低かったものの5年間の変化はほとんど認められず，LVFXに対する耐性化は進行していないと考えられた（図22）．



	Range	MIC_{80}	MIC_{90}
4年度	≦0.06～128<	128	128<
5年度	≦0.06～128<	32	64

図13 全検出菌383株に対するTOBの累積発育阻止率曲線
（全菌種：4年度186株，5年度197株）

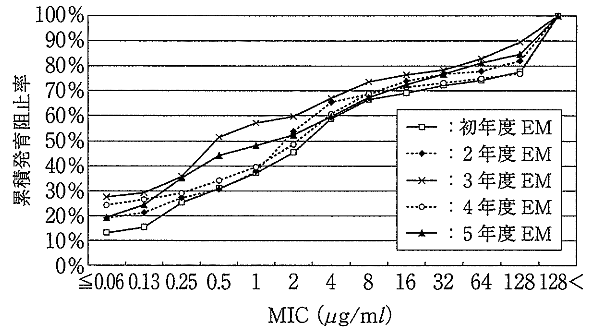


	Range	MIC_{80}	MIC_{90}
初年度	0.25～64	8	8
2年度	0.25～128	8	8
3年度	≦0.06～128	8	16
4年度	≦0.06～128	8	32
5年度	≦0.06～128	8	32

図15 全検出菌1,156株に対するCPの累積発育阻止率曲線
（全菌種：初年度429株，2年度173株，3年度171株，4年度186株，5年度197株）

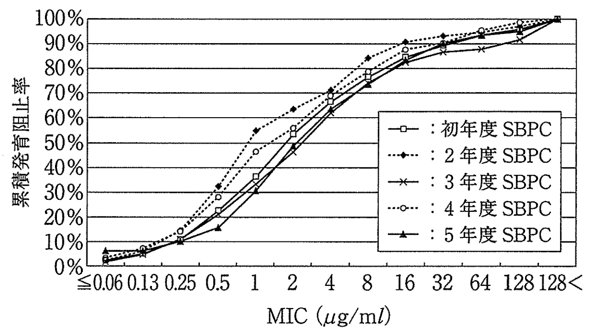
III 考　　按

細菌性結膜炎は，眼感染症のなかで最も高頻度に発症する疾患であるが，日常診療で結膜炎症例の起炎菌を確定することは困難である．今回のスタディは5年間にわたる全国多施設による細菌性結膜炎の細菌の検出状況と薬剤感受性の検討であり，2007年の本スタディグループの報告[1]に引き続き，細菌性結膜炎の現状把握と今後の適切な治療薬選択につながる臨床上有用な情報と考えられる．眼感染症における多施設



	Range	MIC_{80}	MIC_{90}
初年度	≦0.06～128<	128<	128<
2年度	≦0.06～128<	128	128
3年度	≦0.06～128<	64	128
4年度	≦0.06～128<	128<	128<
5年度	≦0.06～128<	64	128

図14 全検出菌1,156株に対するEMの累積発育阻止率曲線
（全菌種：初年度429株，2年度173株，3年度171株，4年度186株，5年度197株）

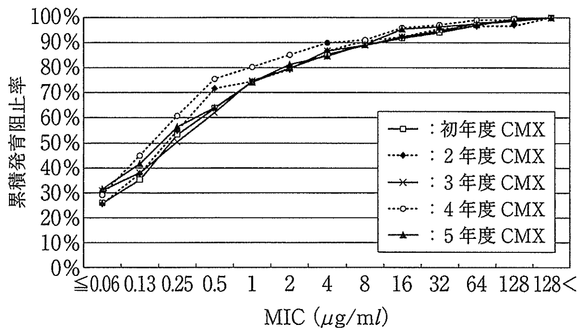


	Range	MIC_{80}	MIC_{90}
初年度	≦0.06～128<	16	64
2年度	≦0.06～128<	8	16
3年度	≦0.06～128<	16	128
4年度	≦0.06～128<	16	32
5年度	≦0.06～128<	16	32

図16 全検出菌1,156株に対するSBPCの累積発育阻止率曲線（全菌種：初年度429株，2年度173株，3年度171株，4年度186株，5年度197株）

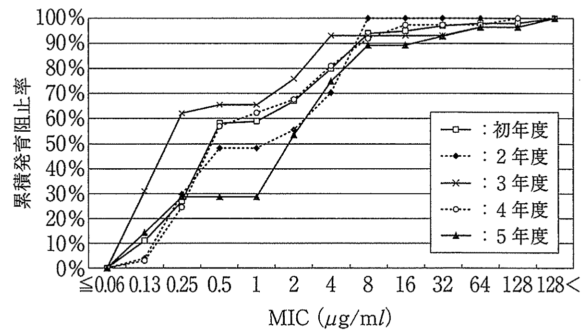
スタディとしては，眼感染症学会による感染性角膜炎サーベイランス[2,3]があり，感染性角膜炎診療ガイドライン[4]の礎となった．本スタディは同一の全国多施設において5年間細菌性結膜炎の動向を観察した結果であり，意義深いものと考えられる．

まず5年間にわたる細菌性結膜炎の細菌の検出状況についてであるが，起炎菌の累積頻度は，*S. epidermidis*（19.3%），*P. acnes*（14.5%），*Streptococcus* spp.（13.0%），*S. aureus*（10.8%），*Corynebacterium* spp.（10.5%），*Haemophilus influenzae*（4.6%），*Moraxella* spp.（2.7%）であり，*S. aureus* ではMSSAが79%，MRSAが21%であった．西澤らは検出菌データの多いものから順に，*S. epidermidis*, *S. aureus*, *Streptococcus* spp., *Propionibacterium* spp., *Corynebacterium* spp., *Haemophilus influenzae* とレビューしている[1,5〜10]が，本スタディとほぼ同様の結果を示しており，わが国における細菌性結膜炎の検出菌はこれら7菌種が4分の3を占めているものと推測される．また，細菌性結膜炎は世代により検出菌と臨床経過が異なり，小児では*Haemophilus*



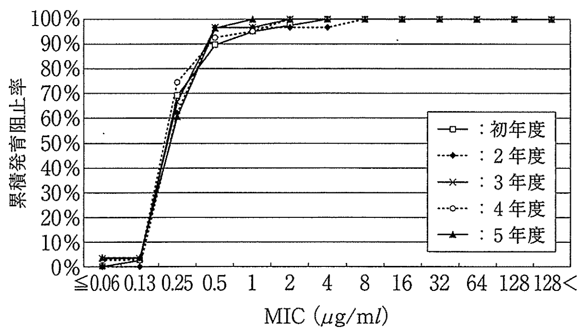
	Range	MIC80	MIC90
初年度	≦0.06〜128<	2	16
2年度	≦0.06〜128<	4	8
3年度	≦0.06〜128<	2	16
4年度	≦0.06〜128<	1	4
5年度	≦0.06〜128<	2	16

図17　全検出菌1,156株に対するCMXの累積発育阻止率曲線（全菌種：初年度429株，2年度173株，3年度171株，4年度186株，5年度197株）



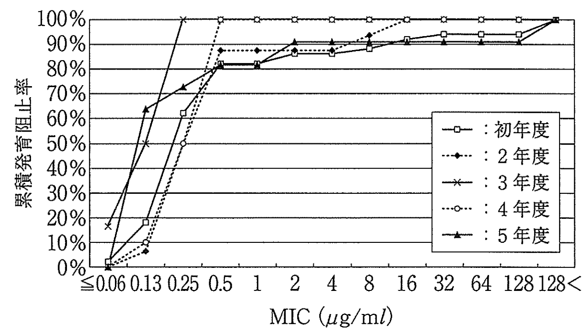
	Range	MIC80	MIC90
初年度	0.13〜128<	4	8
2年度	0.13〜8	8	8
3年度	0.13〜128<	4	4
4年度	0.13〜128	4	8
5年度	0.13〜128<	8	32

図18　*S. epidermidis* 221株に対するLVFXの累積発育阻止率曲線（初年度100株，2年度27株，3年度29株，4年度37株，5年度28株）



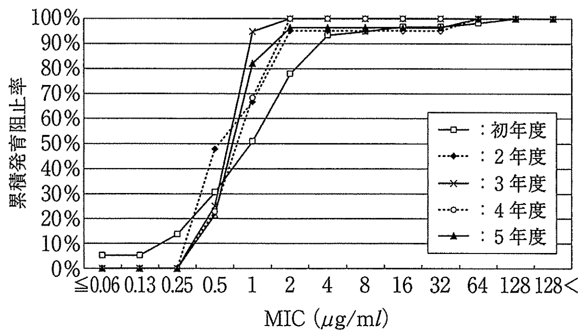
	Range	MIC80	MIC90
初年度	0.13〜4	0.5	0.5
2年度	0.25〜8	0.5	0.5
3年度	≦0.06〜2	0.5	0.5
4年度	≦0.06〜2	0.5	0.5
5年度	≦0.06〜1	0.5	0.5

図19　*P. acnes* 166株に対するLVFXの累積発育阻止率曲線（初年度40株，2年度29株，3年度30株，4年度39株，5年度28株）



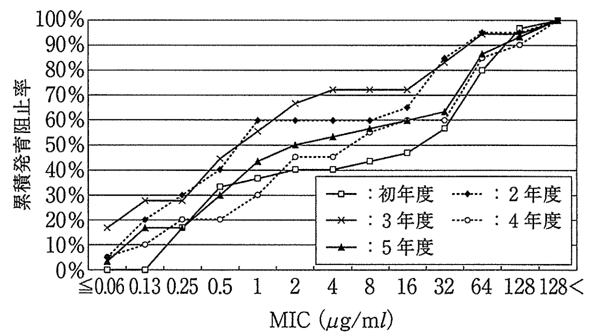
	Range	MIC80	MIC90
初年度	≦0.06〜128<	0.5	16
2年度	0.13〜16	0.5	8
3年度	≦0.06〜0.25	0.25	0.25
4年度	0.13〜0.5	0.5	0.5
5年度	0.13〜128<	0.5	2

図20　*S. aureus*（MSSA）99株に対するLVFXの累積発育阻止率曲線（初年度50株，2年度16株，3年度12株，4年度10株，5年度11株）



	Range	MIC_{80}	MIC_{90}
初年度	≦0.06〜128	4	4
2年度	0.5〜64	2	2
3年度	0.5〜2	1	1
4年度	0.5〜2	2	2
5年度	0.5〜64	1	2

図21 *Streptococcus* spp. 150株に対するLVFXの累積発育阻止率曲線（初年度59株，2年度21株，3年度20株，4年度22株，5年度28株）



	Range	MIC_{80}	MIC_{90}
初年度	0.13〜128<	64	128
2年度	≦0.06〜128<	32	64
3年度	≦0.06〜128<	32	64
4年度	≦0.06〜128<	64	128
5年度	≦0.06〜128<	64	128

図22 *Corynebacterium* spp. 118株に対するLVFXの累積発育阻止率曲線（初年度30株，2年度20株，3年度18株，4年度20株，5年度30株）

*influenzae*や，*S. pneumoniae*が多く，高齢者では*S. aureus*や*Corynebacterium* spp.が多いとされる[5]．本スタディでも，14歳以下では初年度にグラム陰性菌が32%を占め，その約半数が*Haemophilus influenzae*であったが，その後経年的にグラム陰性菌の割合は減少した．また，各年代を通じてグラム陽性球菌が最も高頻度であり，5年間を通してその傾向はかわらなかったものの，15歳以上の年代ではグラム陽性球菌の割合が経年的に減少していた．つぎに検出菌における地域差については，経年変化や地域別に一定の傾向はみられなかった．施設別では，眼科クリニック，総合病院ではグラム陽性球菌の割合が多く，大学病院では嫌気性菌が多いものの，各検出菌の頻度に大きなばらつきがみられ，一定の傾向はなかった．CL装用の有無については，88.5%が装用しておらず，装用者は7.2%にとどまり，CL装用の有無でグラム陽性菌と陰性菌の比率に大きな差はなかった．以上より，2007年の報告と同様，今日の細菌性結膜炎の主要検出菌は，*S. epidermidis*, *S. aureus*, *Streptococcus* spp., *Corynebacterium* spp., *Haemophilus* spp.と推察された．

全検出菌に対する薬剤感受性（MIC_{80}, MIC_{90}）は，LVFX, CMXがその他の薬剤と比べて低い値となっており，結膜炎の主要な起炎菌に対する高い感受性が認められた．また，この5年間の調査期間中に，細菌性結膜炎の主要検出菌に対する薬剤感受性に大きな変化がみられなかったことから，急速な菌の変化，耐性化の進行は生じていないと考えられた．本来，細菌性結膜炎に対する抗菌薬の選択，投与方法は，起炎菌を検出したうえで検出された細菌に対する最も抗菌力の強い薬剤を選択し使用することに尽きるが，日常臨床では，患者苦痛の軽減，quality of life (QOL) 低下の防止，感染拡大の阻止，病態の遷延化・難治化の阻止を治療の要点とし，起炎菌の検出を待たずに早期治療開始の必要性が迫られる．これらの事情を考慮すると，広域の抗菌スペクトルを示し，他の抗菌点眼薬と比較して高い感受性から，細菌性結膜炎の日常診療においてLVFX, CMXを第一選択としてよいと思われる．

以上のように，今回の5年間にわたる調査により，細菌性結膜炎の検出菌の急速な変化や耐性化は進行していないことが明らかとなったが，初年度の報告の考按で示したごとく，多剤耐性菌の出現や菌交代現象の要因としてあげられている抗菌薬の過剰投与や広域スペクトルを有する薬剤の濫用の弊害を常に念頭に置き，上記のような広域抗菌点眼薬の投与は必要最低限にとどめるべきであると考える．

COI細菌性結膜炎検出菌スタディグループ（50音順）———

注記：所属が眼科の場合は部門を省略，所属は調査参加当時のもの

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CLケア教室　第39回

コンタクトレンズケアの指導と定期検査の重要性
―最近の傾向から―

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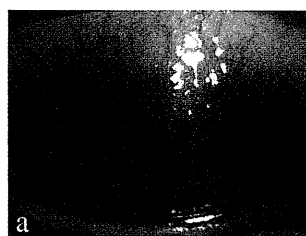
はじめに

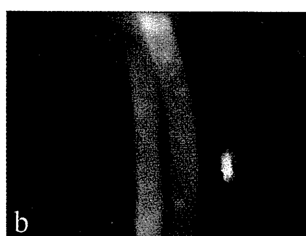
自分が使用しているコンタクトレンズ（以下　CL）名やケア用品の名前を知らないユーザーが非常に多い。処方時にCL名やケア用品の説明を受けているはずである。初めてCLを処方するときに処方者は一連のケア方法を説明するが，その後ユーザーがどこまで最初のケア方法を正しく守っているのか疑問の残るところである。はじめのころはユーザーも慎重で，指導されたCLケアを遵守しているが，慣れてくると気持ちの上で過信してしまいがちになり，正しいケア方法が守られていない場合が散見される。2009年12月に発表された国民生活センターの報告のなかで，頻回交換ソフトCL（以下　SCL）使用者の実態調査の結果をみると，CLケアの前に毎回石けんで手洗いをしていなかった人が65.5％もあるということが明らかになっている。更に，多目的用剤（以下　MPS）使用者のなかでこすり洗いを毎回しないという人が49.6％で，2/3の人はSCLのレンズケースを３カ月以内に交換していなかったことがわかった。CLケアの基本的注意点として，「レンズを取り扱う前は必ず石けんで手指を洗う」，「こすり洗いをする」，「レンズケースは1.5～３カ月に一度は新しいものと交換する」の三点が挙げられるが，この基本的注意点を遵守していない人は遵守した人に比較して，緑膿菌やアカントアメーバの検出率が高いという結果も出ている。正しいケアができていないことが明らかになっており，CLケアの基本的注意点が遵守されていないほど，緑膿菌やアカントアメーバの感染の機会が増えてしまうことが考えられる。

CLケアの指導と定期検査

CLの取り扱いに慣れてくるとケアに対する気配りが減り，手抜きケアとなってしまう。そういった意味でも定期検査は重要で，来院時に正しいケアができているかどうかを確認する必要がある。ところが，昨今はインターネットでの購入が非常に増えているように感じる。医師の診察を受けるようにという記載はあるが，実際はデータを自分で入力すれば，いとも簡単に購入できてしまう。インターネット上には様々なおしゃれ用カラーCLが氾濫している。洋服やアクセサリーと同じ雑品のごとく販売されている。先日も，友人が韓国のサイトで購入したおしゃれ用カラーCLをもらって装用し，合併症を来してから初めて眼科受診した例を経験した。ケアについて聞くとMPSは使っていたが，指導を受けていないためレンズを浸け置くだけであった。同じような例として，水道水で洗浄し，MPSに浸けて置いて使用していたという例もあった。処方せんがなくても前回と同じであれば販売するという販売店（もあると聞く）やインターネットでの購入を続けていると，正しいケアができているかどうかの確認ができず，眼疾患の早期発見が遅れてしまう。図１ａ～ｄは19歳，女性。６～７年前に初めてCLの処方を受け，その後，定期検査は全く受けず，ネットで購入を続けていた。１カ月交換のSCLを使用していたとのことであるが，レンズ名は

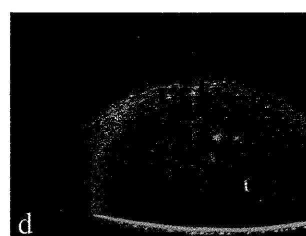


角膜上方からの新生血管の侵入と角膜浸潤



多数の角膜浸潤と角膜瘢痕

角膜全周から強い新生血管の侵入



多数のフルオレセイン染色

図１　定期検査を受けずに放置されていた症例

わからない（どうやって購入していたのか疑問である）とのことである。ケアについては，こすり洗いは行っていたが，軽く数回のみで，石けんでの手洗いは行っていなかった。自分でも調子の悪いときはあったが装用を続けていたとのことである。今回初めて，診察を希望して来院した。多数の角膜浸潤と角膜混濁および全周にわたる強い新生血管の侵入を認めていた。ケアについて指導を受けていても，次第にケアがおろそかになるというのに，雑品を購入するかのごとくネットなどで購入し，CL ケアの重要性について知識のないまま CL を使用し，定期検査を受けずにいると，本例のように眼疾患が存在していても気が付かないままで過ごしている場合がある。

更に，定期検査の受診率が悪いのはハード CL（以下 HCL）使用者である。レンズの破損，紛失など何かなければ受診しないユーザーが非常に多いように思われる。たとえどのような CL であっても，定期検査を受け，眼合併症の有無や CL の状態を観察するだけではなく，正しいケアを続けて行っているかをチェックすることが重要である。感染症のことを考えればできるだけ微生物に対する効果の強いケア用品を選択し，それを正しく使うよう指導しなければならない。更には，CL 関連角膜感染症の原因となる環境菌による影響を考えると，ケアを行っている場所，洗面所などの水回りを清潔に保つことにも注意を払うよう指導が必要になってくる。先日の第54回日本コンタクトレンズ学会では，レンズやケア用品を保存する温度についても議論されていた。近年は HCL に対しても除菌効果の期待できるケア用品が出ている。市販でケア用品を購入する際には，一番低価格のものを選択する場合が多いということを耳にするが，できるだけ微生物に対する効果の高いケア用品を購入し，正しい方法でケアを行うようにユーザーを指導するべきであると考える。

おわりに

処方する側も多忙ななか，CL についての説明とケア用品の特徴やケアの方法を説明し理解されたと思っていても，ユーザーはそのとき安く販売されているケア用品を購入する。その上にケアが手抜きになり，やがて気が緩んだころに角膜感染症が忍び寄る。処方医は正しいケアの指導と定期検査の重要性(ネットで購入を続けることの危険性)を常に心にとどめ，それをユーザーに啓発していかなければならない。

Utility of Real-Time Polymerase Chain Reaction in Diagnosing and Treating *Acanthamoeba* Keratitis

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Purpose: Using real-time polymerase chain reaction (PCR), we detected *Acanthamoeba* and monitored the changes in *Acanthamoeba* DNA copy number over the treatment course in patients suspected of *Acanthamoeba* keratitis (AK).

Methods: Subjects were 6 patients (average age, 26.2 years) suspected of AK at the Kinki University Outpatient Clinic. For detection of *Acanthamoeba*, patients' corneal scrapings were collected for smear analysis, culture, and real-time PCR. After the diagnosis of AK was confirmed, treatment was initiated based on the quantitative result of the real-time PCR.

Results: Both the smear and culture were positive for *Acanthamoeba* in 4 cases and negative in 2 cases (agreement in 3 cases and disagreement in 2 cases). By real-time PCR, all 6 cases were positive for *Acanthamoeba* with an average DNA copy number of $4.8 \pm 9.1 \times 10^3$ copies per sample. We further monitored the variation in the *Acanthamoeba* DNA copy number over the treatment course and successfully treated all the patients. DNA copy number provided a parallel with other clinical features of AK.

Conclusions: Real-time PCR can be a useful method for a rapid and precise diagnosis of AK. Moreover, utility of the *Acanthamoeba* DNA copy number obtained by real-time PCR can help ophthalmologists in making the best treatment decision.

Key Words: *Acanthamoeba*, keratitis, real-time PCR, culture, detection and quantification of pathogens

(*Cornea* 2011;30:1233–1237)

Acanthamoeba keratitis (AK) is a potentially blinding disease that is often misdiagnosed and does not respond well to treatment. Because of the delayed diagnosis and improper treatment, patients with AK are often left with loss of vision caused by corneal opacity.[1] Furthermore, Centers for Disease Control and Prevention of the United States reported that cases of AK have overwhelmingly increased in recent years, especially among contact lens users.[2] Therefore, a method that enables a quick and accurate diagnosis of AK is indispensable.

Smear examination and culture using patients' corneal scraping are the conventional diagnostic methods to detect *Acanthamoeba*.[3–5] However, culture faces challenging problems such as a long turnaround time, and results of smear examination largely depend on the laboratory technician's skill. Besides smears and cultures, polymerase chain reaction (PCR)[6–9] and confocal microscopy[10] have been increasingly used for the detection of *Acanthamoeba*, but they cannot quantitate the pathogens.

Several studies have reported the quantification of *Acanthamoeba* using real-time PCR.[11–14] Thompson et al[13] validated 2 real-time PCR methods (the Rivière and Qvarnstrom assays) for the confirmation of AK by laboratory diagnosis. They also compared both assays with a battery of positive and negative samples and further validated real-time PCR for clinical application with a subset of true-positive and true-negative samples. Kandori et al[14] reported 2 cases of AK successfully diagnosed only by real-time PCR. In their 2 cases, culture and microscopic examination yielded a negative result and could not grow any pathogens. Although they used real-time PCR to qualitatively detect *Acanthamoeba* DNA, the quantitative results of the real-time PCR were not indicated or used for the diagnosis or treatment of AK in their study.

In this study, we performed real-time PCR to detect and quantitate *Acanthamoeba* in patients' corneal scrapings. To our knowledge, this is the first real-time PCR report that not only detects *Acanthamoeba* but also quantitates the *Acanthamoeba* DNA copy number. By monitoring the *Acanthamoeba* DNA copy number over the treatment course, we achieved excellent treatment outcomes in all 6 patients and reported 2 cases here.

PATIENTS AND METHODS

Subjects and Sample Collection

Subjects were 6 patients (4 men and 2 women; age range: 22 to 32 years; average: 26.2 years) suspected of AK based on the clinical signs presented at the Kinki University Outpatient Clinic between June 2008 and September 2009. All patients gave their informed consent to real-time PCR with their corneal scrapings.

For sample collection, the subject was under local anesthesia with 0.4% oxybuprocaine eyedrops, and an eye

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speculum was inserted before sampling. Corneal scrapings were collected for culture, smear examination, and real-time PCR. We used a stainless rounded blade (Feather disposable scalpel number 15; Feather Safety Razor, Co, Ltd, Gifu, Japan) to scrape the lesion (Fig. 1). A small amount of sample (about 5–10 μg) was collected and placed in 500 μL of saline solution immediately. The samples were kept at 2°C to 5°C until further processing. Real-time PCR was performed within 2 days after the sample was collected to determine the DNA copy number. All sterile precautions were taken to avoid contamination during the sample collection.

Microscopy and Culture

Corneal scrapings were cultured on blood agar medium (Nissui Pharmaceutical, Co, Ltd, Tokyo, Japan), chocolate agar medium (Biomerieux Japan, Tokyo, Japan), glucose agar medium (Biomerieux Japan), or nutrient agar medium (Nissui Pharmaceutical, Co, Ltd) supplied with *Escherichia coli* and were subjected to smear analysis with Fungiflora Y stain or direct microscopic examination.

Real-Time PCR

From the corneal scrapings collected for real-time PCR, DNA was extracted using EXTARGEN2 (Tosoh, Tokyo, Japan). The PCR assay used TaqMan probe, and the primers and probe were designed against *Acanthamoeba* 18S ribosomal RNA.[12] The primers were (forward) CCCAGATCG TTTACCGTGAA and (reverse) TAAATATTAATgCCCC-CAACTATCC, and the probe was CTGCCACCGAATACAT-TAGCATGG. The PCR assay was conducted on an ABI PRISM 2000 Sequence Detector, and the cycling settings were 40 cycles of 95°C for 10 seconds, 95°C for 5 seconds, and 60°C for 30 seconds. The assay was completed in about 2 hours. A positive control plasmid was used to construct a standard curve of the threshold cycle (C_t) values with a linear range from 1.0×10^1 to 1.0×10^6 copies. Relative copy number was also calculated by the standard curve (Fig. 2). We quantitated *Acanthamoeba* and monitored the *Acanthamoeba* DNA copy number for both detection and treatment of AK.

RESULTS

All 6 subjects were soft contact lens (SCL) users and were observed with radial keratoneuritis (Table 1), which is one of the pathognomonic clinical features of AK.

The smear and culture both showed positive results in 4 cases (case numbers 1, 3, 4, and 6 for smear and case numbers 2, 3, 4, and 6 for culture; Table 1). By real-time PCR, all 6 patients were positive for *Acanthamoeba* with an average DNA copy number of $4.8 \pm 9.1 \times 10^3$ copies per sample and a maximum of 2.5×10^4 copies per sample (Table 1).

CASE REPORTS

Case 1

The patient was a 27-year-old man, who used replacement 30-day SCLs and suffered from eye pain in the middle of May 2008. On June 2, superficial punctate keratitis and conjunctival injection were initially observed in both eyes. Corneal edema was also seen in the left eye, and the patient had difficulty opening his right eye because of severe pain (Fig. 3). Culture of the patient's discharge was positive for *Corynebacterium* sp. On June 16, the smear result of the corneal scraping from the left eye was positive for *Acanthamoeba* (Fig. 4). Real-time PCR also detected *Acanthamoeba* with 2.5×10^4 copies of DNA per sample. With the patient's consent, we also performed real-time PCR on the contact lens solution sample (about 1.0 mL) collected from the lens case. A positive result with 4.3×10^3 copies of DNA per sample was obtained.

Based on the real-time PCR results, treatment for *Acanthamoeba* was started with 0.05% chlorhexidine eyedrops 8 times per day, 0.03% miconazole eyedrops 8 times per day, and 0.5% levofloxacin eyedrops 4 times per day. Corneal scraping for real-time PCR was performed twice a week on the left eye during the treatment course.

The patient's symptoms were improved. The eye pain was gone, and the corneal inflammation and edema were alleviated.

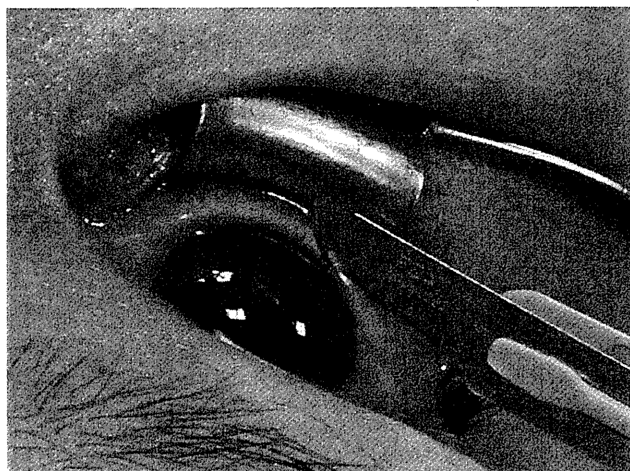

FIGURE 1. Photograph showing the setting for corneal scraping.

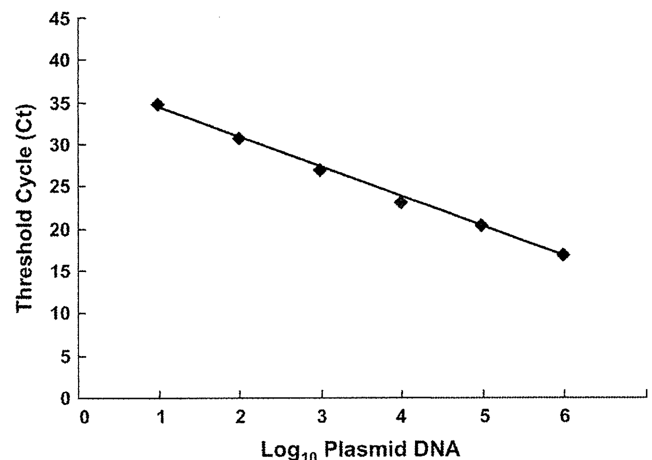

FIGURE 2. C_t values derived from the real-time PCR were compared with a positive control plasmid with a linear range from 1.0×10^1 to 1.0×10^6 copies.

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TABLE 1. Patient Characteristics and Results for the Detection and Quantification of *Acanthamoeba*

Case Number	Age (yr)	Sex	Radial Keratoneuritis	Types of CLs Used	Smear Result	Culture Result	Maximum DNA Copy Number
1	27	M	+	30-day SCL	Positive	Negative	2.5×10^4
2	29	M	+	1-day SCL*	Negative	Positive	1.1×10^2
3	22	M	+	2-week SCL	Positive	Positive	2.8×10^2
4	24	M	+	30-day SCL	Positive	Positive	1.1×10^3
5	23	F	+	2-week SCL	Negative	Negative	1.8×10^3
6	32	F	+	2-week SCL	Positive	Positive	6.1×10^2

*The 1-day SCLs used by the patient in case 2 were actually stored in CL solution and used for 3–4 days.
+, Presence of radial keratoneuritis; CL, contact lens.

Overall, the *Acanthamoeba* DNA copy number declined over the treatment course (Fig. 5), and finally, no *Acanthamoeba* was detected 35 days after the treatment was initiated.

Case 2

The patient was a 29-year-old man, who often used 1-day SCLs for about 3 days. In January 2009, he experienced pain and conjunctival injection in the left eye when he wore the lenses (Fig. 6). He was treated with topical medications at another clinic but the symptoms did not improve. The patient visited our clinic on January 22 and presented with corneal opacity and conjunctival injection in the left eye. No causative pathogens were confirmed by culture. Based on the clinical findings, we suspected *Acanthamoeba* and started treatment with 0.2% fluconazole, 0.03% miconazole, and 0.05% chlorhexidine eyedrops. On February 13, culture and real-time PCR using the corneal scrapings were performed, and both the methods were positive for *Acanthamoeba*. *Acanthamoeba* DNA of 1.1×10^2 copies per sample was obtained by real-time PCR.

Based on the positive results, 0.05% chlorhexidine eyedrops 8 times per day, 0.03% miconazole eyedrops 8 times per day, and 0.5% levofloxacin eyedrops 4 times per day were started. Like case 1, corneal scraping for real-time PCR was performed on the left eye twice a week during the treatment course. In time, the patient's symptoms were improved. The pain was gone, and the corneal inflammation and edema were eased. The *Acanthamoeba* DNA copy number also declined over the treatment period in this case, and finally, absence of *Acanthamoeba* was confirmed 38 days after the initiation of the medical treatment (Fig. 7).

DISCUSSION

Our current results clearly demonstrate that real-time PCR could be a useful supplemental method to the conventional smear and culture techniques for a more rapid and accurate diagnosis of AK. Particularly, the DNA copy number obtained by real-time PCR could serve as a good index of treatment outcomes and a guide to a better antiamoebic regimen (Figs. 5, 7).

Unlike PCR that uses agarose gel electrophoresis for the preparation and analysis of DNA, real-time PCR can quantitate DNA at the site of the corneal scraping. By monitoring the DNA copy number, an accurate diagnosis and a tailored antiamoebic regimen can be achieved in the early stage of the disease, and if necessary, the treatment modality can be modified accordingly during the course. Even at the end of the treatment, *Acanthamoeba* DNA copy number can help identify the presence or absence of *Acanthamoeba*. All these clinical judgments can be facilitated by quantitative real-time PCR result.

The primers and probe used in this study have been clinically validated by Thompson et al[13] for the laboratory diagnosis of AK. Although Kandori et al[14] reported 2 cases of AK diagnosed only by real-time PCR, they only used this

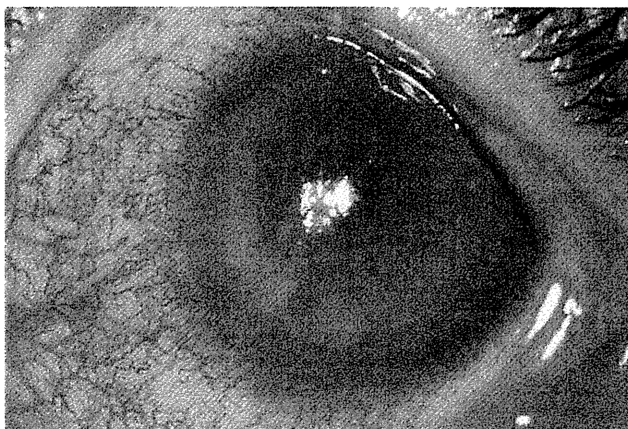

FIGURE 3. Photograph of the left eye of the patient in case 1 taken before treatment. Corneal opacity and edema were observed in the left eye.

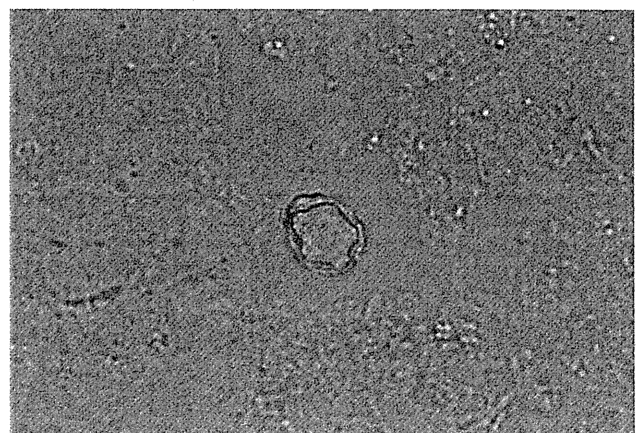

FIGURE 4. Photograph of the smear result taken on June 16, 2008. *Acanthamoeba* was detected by smear examination using the corneal scraping from the left eye.

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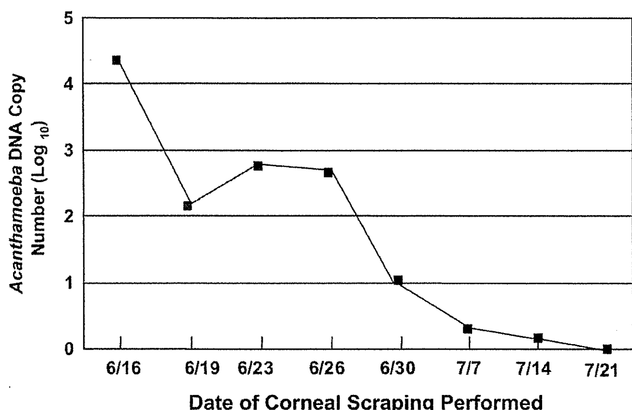

FIGURE 5. Variation in DNA copy number in case 1. Overall, the *Acanthamoeba* DNA copy number declined over the course, and finally, absence of *Acanthamoeba* was confirmed 35 days after the treatment started.

method to identify the presence of *Acanthamoeba* and did not measure the *Acanthamoeba* DNA copy number in the study. Unlike these previous studies, we used the *Acanthamoeba* DNA copy number for the purposes of diagnosis and treatment and reported an average DNA copy number of $4.8 \pm 9.1 \times 10^3$ copies per sample for a clinical diagnosis of AK and a maximum of 2.5×10^4 copies per sample through the course (Table 1). The quantitative result of real-time PCR is particularly useful and crucial in tailoring an antiamoebic treatment for patients because this disease tends to resist treatment. However, the use of a single set of primers and probe may be inadequate to detect all the potential *Acanthamoeba* species. In this study, we used the primers and probe designed by Qvarnstrom et al.[12] According to Thompson et al[13] who have compared the Rivière et al[11] and Qvarnstrom et al[12] real-time PCR methods, both real-time PCR assays are useful for diagnosing AK with overall agreement and clinical sensitivities and specificities of 100%. We therefore consider it possible to obtain a PCR result equivalent to our current findings with the Rivière et al[11] real-time PCR.

In case 1, culture did not detect *Acanthamoeba*. We also performed smear examination with Fungiflora Y stain, and no definite result was obtained because of the technician's inexperience. *Acanthamoeba* was subsequently detected by both real-time PCR and direct microscopic examination. In case 2, real-time PCR also showed a positive result despite the negative smear result. These results were concordant with our previous findings in which real-time PCR has proven useful in detecting the causative pathogens, although culture has failed to do so in patients with other corneal infections.[15] In case 1, we also successfully quantitated *Acanthamoeba* DNA using the multipurpose solution (MPS) sample taken from the patient's contact lens case. This not only validated the reported association between the MPS use and AK[16,17] but also demonstrated the consistent results between the PCR assays that used the MPS sample and corneal scraping. Because *Acanthamoeba* exists ubiquitously, samples from various sources can be used for clinical testing with real-time PCR in addition to the samples collected by corneal scraping, which is a painful procedure for patients.

Regardless of its usefulness, real-time PCR has some limitations such as sampling contamination and low DNA copy number. Regarding sampling contamination, we consider it reasonable to regard *Acanthamoeba* as the causative pathogen on the confirmation of its presence because *Acanthamoeba* is not part of the normal ocular surface flora. We previously performed real-time PCR for *Acanthamoeba* on tear samples collected from 10 eyes of 5 normal subjects, and none of the samples detected any *Acanthamoeba* DNA (data not shown). Because the measured DNA copy number depends on the amount of sample collected, the method used for sample collection should be carefully evaluated in addition to the condition of the cornea and the severity of the infection.[18]

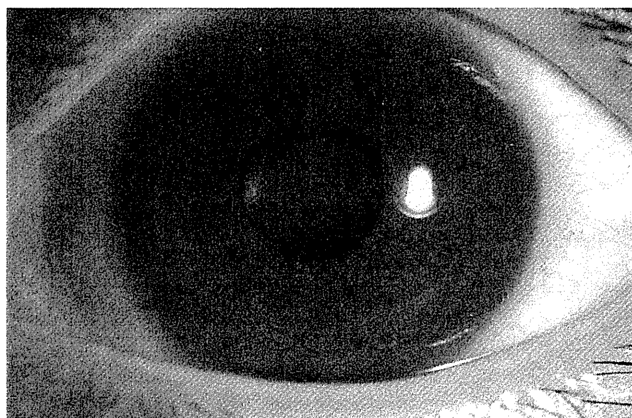

FIGURE 6. Photograph of the patient in case 2 taken on February 13, 2009 before treatment. The initial examination revealed corneal opacity and conjunctival injection in the left eye.

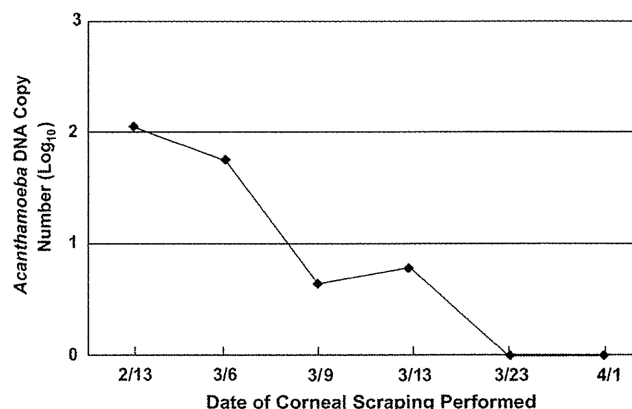

FIGURE 7. Variation in DNA copy number in case 2 similar to case 1; the *Acanthamoeba* DNA copy number also declined over the course, and finally, no DNA was detected 38 days after the treatment was initiated.

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In conclusion, real-time PCR seems to be a promising supplemental method to the conventional culture and smear examination for a more rapid and accurate diagnosis of AK. Moreover, its quantitative result can play a vital role in determining treatment outcomes. Clinical features, PCR result, and findings of the routine smear and culture should all be carefully considered in treating this vision-threatening disease.

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LABORATORY INVESTIGATION

Efficacy of commercial soft contact lens disinfectant solutions against *Acanthamoeba*

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Abstract

Purpose To investigate the relative efficacy of Japanese commercial soft contact lens disinfectant solutions against *Acanthamoeba* trophozoites and cysts.

Materials and methods Eight types of multipurpose solution (MPS), two types of hydrogen peroxide solution, and one povidone–iodine solution were evaluated to determine their effect against *Acanthamoeba* trophozoites and cysts (ATCC 50514). *Acanthamoeba* cysts were cultured in encystment medium for either 1 or 2 weeks (1 and 2-week-old cysts). The trophozoites and cysts were treated with each disinfectant solution for 0, 2, 4, 8, or 24 h. After performing four tenfold serial dilutions of each test solution, dilutions were cultured for 10 days. The number of surviving organisms was calculated using the trimmed Spearman–Karber method.

Results Among the MPS tested, only four were effective against trophozoites after treatment for 4 h, and none was effective against 2-week-old cysts. Hydrogen peroxide had a significant effect on trophozoites and 1-week-old cysts, but not on 2-week-old cysts. In contrast, povidone–iodine caused a 2.6 log reduction in 2-week-old cysts.

Conclusions MPS were found to have limited efficacy against trophozoites and no efficacy against 2-week-old cysts. Only povidone–iodine had any efficacy against 2-week-old cysts.

Keywords *Acanthamoeba* · Soft contact lenses (SCL) · Disinfectant solution · Multipurpose solution (MPS) · Mature cysts

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Introduction

Acanthamoeba, a genus of free-living protozoa, is known to cause a painful and potentially blinding form of keratitis on invading the cornea. *Acanthamoeba* keratitis (AK) occurs most commonly in contact lens wearers, with studies estimating that 90% of AK patients are soft contact lens (SCL) users [1]. Approximately 1 in 30,000 SCL users develops AK [2], and the incidence of the condition has increased dramatically in recent years [3]. A national survey performed jointly by the Japanese Contact Lens and Ocular Infection Societies revealed that, with *Pseudomonas aeruginosa*, *Acanthamoeba* is now the leading cause of serious cases of contact lens-related keratitis in Japan [4]. The recent increase in incidence of AK has been attributed to several factors, including the rising number of frequent replacement SCL wearers and widespread noncompliance with rubbing and rinsing regimens [5–8]. In addition to these factors, the use of ineffective SCL disinfectant solutions is also suspected to be closely linked with the recent increase in cases of AK. The United States Center for Disease Control and Prevention reports that in a 2006

outbreak of AK in Illinois, 21 out of the 39 reported cases of AK were associated with the use of one brand of disinfectant solution, resulting in a voluntary global recall of the product [9].

Several types of SCL disinfectant solution are currently commercially available, including povidone–iodine and hydrogen peroxide-based solutions, and multipurpose solutions (MPS). To facilitate lens cleaning, rinsing, disinfection, and storage, these disinfectant solutions are formulated with a variety of different surfactants, buffers, stabilizing agents, and isotonizing agents. Some of these inactive ingredients attenuate or potentiate the activity of the disinfectant, and, therefore, the specific formulation of each product has been found to significantly affect its efficacy as a disinfectant [10–12].

Although numerous studies have been performed to evaluate the effects of different SCL disinfectant solutions on *Acanthamoeba* [10–17], differences in methodology have made it difficult to compare the results of these studies and reach conclusions regarding the relative efficacy of the products. This situation is partially because there is no standardized method for evaluating the efficacy of lens care products against *Acanthamoeba*. Although the International Organization for Standardization (ISO) has adopted the Stand Alone Test (ISO 14729), a standard method for testing the disinfectant efficacy of lens-care products during development, the method does not include a specific protocol for *Acanthamoeba*. This is particularly important because of the unique characteristics of *Acanthamoeba*. The organism can assume two different forms during its life cycle: the motile, metabolically active trophozoite, and the dormant cyst, which forms as a protective response to environmental stress. Cysts are known to be more resistant to disinfectants than trophozoites [10, 14] and the maturity of the cyst may also have an effect on its sensitivity to disinfectant solutions. Because the effect of disinfectants on *Acanthamoeba* is strongly affected by the developmental stage of the organism, it seems likely that differences in the stage of organisms used in previous investigations may have led to discrepancies in their results [10, 11, 14].

In Japan, several varieties of SCL disinfectant solutions are commercially available. A comprehensive and objective investigation of the efficacy of these products against *Acanthamoeba* trophozoites or cysts has not yet been performed, however. In this study, we investigated the relative disinfectant efficacy of eight types of MPS, two types of hydrogen peroxide solutions, and one povidone–iodine solution currently on the market using *Acanthamoeba* trophozoites and cysts of different maturity (1 and 2-week-old cysts). The log reduction method used in this study is simple, yields quantitative results, and has been used for evaluation of the disinfectant efficacy of contact lens solutions in other previously reported research [11, 12]. In addition, the strain of *A. castellanii* (ATCC 50514) used in this study is an established pathogenic strain which has been used in several previous studies [11, 18, 19]. Using this common strain of *Acanthamoeba* and the log reduction method, we were able to perform a quantitative investigation of the efficacy of SCL disinfectant solutions.

Materials and methods

Commercial soft contact lens disinfectant solutions

The commercial SCL disinfectant solutions examined in our investigation are shown in Table 1. Of the eight MPS tested, six (MPS 1, 2, 3, 4, 7, and 8) use 1.0 ppm polyhexamethylene biguanide (PHMB) as the disinfectant. MPS 6 contains 1.1 ppm PHMB and MPS 5 contains 11 ppm polydronium chloride (Polyquad). Hydrogen Peroxide Solution 1 and Povidone–Iodine Solution 1 are accompanied by neutralizing tablets to be added to the disinfectant solution at the onset of disinfection, whereas Hydrogen Peroxide Solution 2 uses a special container and platinum disks to achieve neutralization.

With the exception of MPS 4, all MPS had a recommended disinfection time of at least 4 h. The recommended disinfection time for MPS 4 was 10 min. The hydrogen peroxide and povidone–iodine solutions had recommended disinfection times of 6 and 4 h, respectively, for adequate disinfection and neutralization. All solutions contained inactive ingredients, including buffers, stabilizing agents, isotonizing agents, and surfactants, in addition to the disinfectant. The main inactive ingredients in each solution are shown in Table 2.

Acanthamoeba trophozoites and cysts

Acanthamoeba castellanii (ATCC 50514) was used in this study. The trophozoites were cultured at 25°C in a peptone–yeast extract–glucose (PYG) medium (ATCC medium 712) in a tissue culture flask (Becton–Dickinson, Tokyo, Japan). Encystment was induced by transferring the trophozoites from PYG medium to Neff's constant-pH encystment medium [20] and incubating them at 25°C. Cysts which were incubated in the encystment medium for 1 week were designated 1-week-old cysts whereas cysts incubated in the medium for 2 weeks were designated 2-week-old cysts.

To determine whether different strains of *Acanthamoeba* respond differently to the disinfectant solutions, we also evaluated the efficacy of selected disinfectant solutions (MPS 1, 5, 6, and Povidone–Iodine Solution 1) against *Acanthamoeba castellanii* (ATCC 50370), another established pathogenic strain.

Table 1 Commercial soft contact lens disinfectant solutions used in the disinfectant efficacy test

	Disinfectant	Concentration (*w*/*v*)	Recommended disinfection time[d]	Product name	Manufacturer or distributor
MPS 1	PHMB[a]	1.0 ppm	≥4 h	Complete® Double Moist	AMO, Inc.
MPS 2	PHMB[a]	1.0 ppm	≥4 h	Bioclen® Zero	Ophtecs Corp.
MPS 3	PHMB[a]	1.0 ppm	≥4 h	Seedo Softcare	SEED Co., Ltd.
MPS 4	PHMB[a]	1.0 ppm	≥10 min	Fresh Look® Care 10 min	CIBA VISION
MPS 5	Polyquad[b]	11 ppm	≥4 h	Optifree® Plus	Alcon Japan, Ltd.
MPS 6	PHMB[c]	1.1 ppm	≥4 h	Renu® Multiplus	Bausch and Lomb Japan Company, Ltd.
MPS 7	PHMB[a]	1.0 ppm	≥4 h	Epica® Cold	Menicon Co., Ltd.
MPS 8	PHMB[a]	1.0 ppm	≥4 h	Rohto C Cube® Soft One® Moist i	Rohto Pharmaceutical Co., Ltd.
Hydrogen Peroxide Solution 1	Hydrogen peroxide	3.0%	≥6 h	Concept® One-Step	AMO, Inc.
Hydrogen Peroxide Solution 2	Hydrogen peroxide	3.42%	≥6 h	AO Sept®	CIBA VISION
Povidone–Iodine Solution 1	Povidone–iodine	0.5%	≥4 h	Bioclen®FR	Ophtecs Corp.

[a] Polyhexamethylene biguanide

[b] Polydronium chloride

[c] Dymed®

[d] Time indicated on the packaging of each product. For hydrogen peroxide or povidone–iodine solutions, the time necessary for neutralization is included

Table 2 Main inactive ingredients in soft contact lens disinfectant solutions

	Buffering agent	Stabilizing agent	Isotonizing agent	Surfactants (moisturizing/cleansing agents)
MPS 1	–	EDTA	+	+
MPS 2	Borate	Polylysine	+	Poloxamer[e]
MPS 3	+	–	+	+
MPS 4	Phosphate[c]	EDTA	NaCl, KCl[c]	Poloxamer[e]
MPS 5	Borate, citrate[c]	EDTA	NaCl[c]	Poloxamine[c]
MPS 6	Borate, Phosphate[c]	EDTA	NaCl[c]	Poloxamine, Hydranate®
MPS 7	–	EDTA	Propylene glycol, Alpha hydroxyl acid, AMPD, amino acids[c]	Propylene glycol, POE hydrogenated castor oil[c]
MPS 8	+	EDTA	+	Poloxamer[c]
Hydrogen Peroxide Solution 1[a]	+[d]	–	+[d]	–
Hydrogen Peroxide Solution 2	Phosphate[c]	+	NaCl[c]	Poloxamer[c,e]
Povidone–Iodine Solution 1[b]	Borate[c]	EDTA	NaCl[c]	Poloxamer[c,e]

+ Other inactive ingredients (exact formulation unknown); – inactive ingredients not present or unknown

[a] Neutralizing tablets also include catalase, lubricants, coloring agents, and coating agents

[b] Disinfectant granules or neutralizing tablets also include sodium sulfite, diluents, foaming agents, lubricants, and coating agents

[c] Silvany et al. [17]

[d] Included in the neutralizing tablets

[e] Poloxamer: polyoxyethylene polyoxypropylene glycol

All procedures involving the organisms were carried out in Biosafety Level 2 laboratories.

Evaluation of the efficacy of soft contact lens disinfectant solutions using the log reduction method

Pre-cultured trophozoites or cysts were collected from a flask, and after centrifugation (150*g*, 10 min) the organisms were suspended in 1/4 Ringer's solution (Nihon Pharmaceutical, Tokyo, Japan) at a concentration of 5×10^6 organisms/ml. Each MPS, hydrogen peroxide solution, or povidone–iodine solution (10 ml) was inoculated with 100 μl amoeba suspension so the final concentration was 5×10^4 organisms/ml. Control samples were also prepared with 1/4 Ringer's solution. Subsequently, each sample was incubated at 25°C for 2, 4, 8, or 24 h in a 15-ml conical tube (Becton–Dickinson) or a special container, if provided by the manufacturer (Hydrogen Peroxide Solution 2).

Immediately after the incubation period, samples containing PHMB or Polyquad were neutralized with Dey-Engley Neutralizing Broth (Sigma, St Louis, MO, USA) at a ratio of 9 parts Dey-Engley Neutralizing Broth to 1 part test solution. Dey-Engley Neutralizing Broth was also added to the hydrogen peroxide, povidone–iodine, and control samples (1/4 Ringer's solution) in the same manner to maintain consistent conditions in all samples. For hydrogen peroxide and povidone–iodine samples, neutralization was carried out in accordance with the manufacturer's directions with either neutralizing tablets or platinum disks immediately after inoculation of the amoeba suspension. The special container provided by the manufacturer, which contained a platinum disk neutralization system, was used for Hydrogen Peroxide Solution 2.

A 0-h sample for each MPS was prepared by neutralizing the test solution with Dey-Engley Neutralizing Broth immediately after inoculation of *Acanthamoeba*. 0 h samples for hydrogen peroxide and povidone–iodine samples were prepared using solution which had already been neutralized with either neutralizing tablets or platinum disks.

After neutralization with the Dey-Engley Neutralizing Broth, the log reduction method was used to evaluate the efficacy of each solution [11, 12]. Briefly, tenfold serial dilutions of each test solution were performed with PYG medium, resulting in four dilutions with theoretical maximum final concentrations of 5×10^3, 5×10^2, 5×10^1, and 5×10^0 amoeba/ml. Four 200-μl aliquots of each dilution were transferred to separate wells in a 96 well plate (Corning International, Tokyo, Japan), so that each dilution could be tested in quadruplicate. In order to provide organisms in the first dilution (9 parts Dey Engley Neutralizing Broth: 1 part test solution) with the nutrients necessary to proliferate, 80 μl PYG medium was added to each well. The 96-well plates were incubated at 25°C. Samples containing trophozoites were incubated for 1 week whereas those containing cysts were incubated for 3 weeks. At the end of the incubation period, amoebal growth in the wells was confirmed by use of a phase-contrast microscope. The wells containing amoebal growth were counted, and the number of surviving organisms in each test solution was calculated using the Spearman–Karber equation as described elsewhere [21].

The decrease in the number of organisms in each test solution was determined relative to the baseline number of organisms detected in each solution immediately after inoculation (0 h). This value was calculated for each solution after 2, 4, 8, and 24 h of incubation, and expressed as a log reduction value. Results are presented as mean ± SEM. One-way ANOVA and Dunnett's test were used to compare the difference between the log reduction values for the control (1/4 Ringer's solution) and each of the test solutions at each time point. $P < 0.05$ was used to indicate statistical significance.

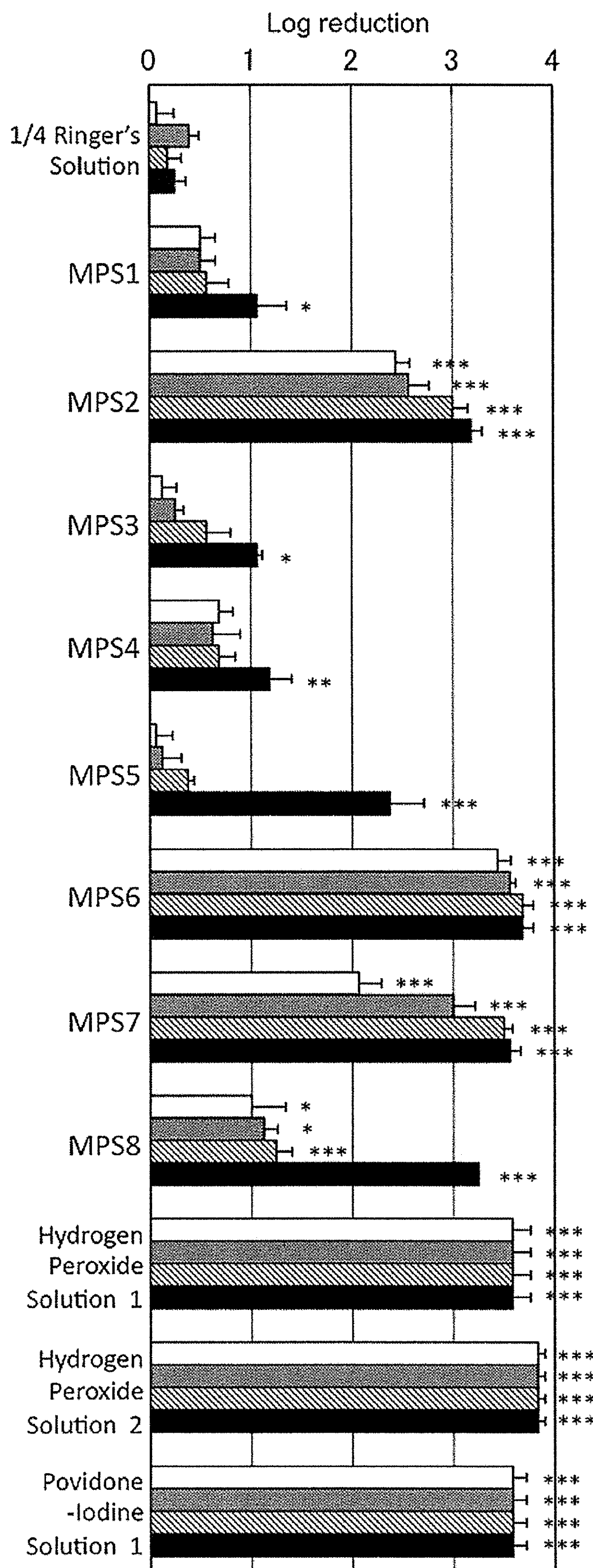
Verification of the log reduction method

To verify the reliability of the log reduction method used in this study, we evaluated the efficacy of selected disinfectant solutions (MPS 1, 5, 6, and Povidone–Iodine Solution 1) against *A. castellanii* (ATCC 50514) by another method. Briefly, *Acanthamoeba* trophozoites and 2-week-old cysts were suspended in 1/4 Ringer's solution or disinfectant solution for 4 h, then each sample was used to inoculate agar plates coated with *E. coli*, and incubated at 25°C for 2 weeks. The efficacy of the solutions were recorded as positive or negative as described elsewhere [22].

Results

Efficacy of soft contact lens disinfectant solutions against *Acanthamoeba* trophozoites

The eight types of MPS examined in this study had different effectiveness against *Acanthamoeba* trophozoites (Fig. 1). MPS 1, 3, and 4 (PHMB, 1.0 ppm) and MPS 5 (Polyquad, 11 ppm) were relatively ineffective against trophozoites. When trophozoites were treated with these solutions for the manufacturer's recommended disinfection time (10 min–4 h), no statistically significant differences were observed between the log reduction values for these solutions and the control (1/4 Ringer's solution). When trophozoites were incubated in these solutions for 24 h, a 1.1–2.4 log reduction was achieved. In contrast, MPS 2 and 7 (PHMB, 1.0 ppm) and MPS 6 (PHMB, 1.1 ppm) had



◀ **Fig. 1** Efficacy of commercial soft contact lens disinfectant solutions against *Acanthamoeba* trophozoites (ATCC 50514). Eight types of multipurpose solution (MPS; $n = 4$), two types of hydrogen peroxide solution ($n = 3$), and one povidone–iodine solution ($n = 3$) were examined to determine their efficacy against *Acanthamoeba* trophozoites. 1/4 Ringer's solution was used as the control ($n = 7$). *White bar* 2-h treatment; *gray bar* 4-h treatment; *hatched bar* 8-h treatment; *black bar* 24-h treatment. The decrease in the number of surviving organisms in each solution was expressed logarithmically (log reduction value). The *error bars* indicate SEM. The log reduction value for each solution was compared with that for the control (*P value 0.01–0.05; **P value 0.001–0.01; ***P value <0.001)

relatively high biocidal activity against trophozoites; these solutions produced a 2.6–3.6 log reduction within the manufacturers' recommended disinfection time (4 h), a significantly greater decrease in surviving organisms than that found for the control ($P < 0.001$). MPS 8 (PHMB, 1.0 ppm) was moderately effective against *Acanthamoeba* trophozoites, giving a 1.1 log reduction within the 4 h recommended disinfection time, also significantly greater than that produced by the control ($P > 0.03$).

Both hydrogen peroxide solutions and the povidone–iodine solution had a greater disinfectant effect on trophozoites than the MPS (Fig. 1). Treatment with either hydrogen peroxide or povidone–iodine solution for 2 h yielded more than a 3 log reduction in trophozoites, significantly greater than that produced by the control ($P < 0.001$) (Fig. 1).

Efficacy of soft contact lens disinfectant solutions against 1-week-old cysts

Most of the MPS examined in this study had greater disinfectant efficacy against 1-week-old cysts than against trophozoites (Figs. 1, 2), although the efficacy of each type of MPS varied greatly. MPS 1 and 3 (PHMB, 1.0 ppm) and MPS 5 (Polyquad, 11 ppm) were relatively ineffective against 1-week-old cysts, and no statistically significant differences were observed between the log reduction values for these solutions and the control (1/4 Ringer's solution). In contrast, MPS 2, 4, and 7 (PHMB, 1.0 ppm) and MPS 6 (PHMB, 1.1 ppm) had greater biocidal activity against 1-week-old cysts. These solutions produced 2.8–3.3 log reductions in 1-week-old cysts within the manufacturer's recommended disinfection time (4 h), significantly greater than that produced by the control ($P < 0.001$). MPS 8 (PHMB, 1.0 ppm) was moderately effective against 1-week-old cysts, giving a 1.9 log reduction after 4 h of treatment, also significantly greater than that produced by the control ($P = 0.006$).

Both the hydrogen peroxide solutions and the povidone–iodine solution had greater disinfectant effects than the MPS on 1-week-old cysts (Fig. 2). Treatment for 2 h with either the hydrogen peroxide solutions or the povidone–