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THE EFFICACY OF A PNEUMONIA ACTION TEAM (PAT) ON THE TIMELINESS OF ANTIBIOTIC ADMINISTRATION IN PATIENTS WITH AN ADMITTING DIAGNOSIS OF PNEUMONIA.

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BACKGROUND: Health Care Financing Administration (HCFA) Region X, conducted a Pneumonia treatment study in 1995 and 1996 of antibiotic administration within four (4) hours of arrival. The findings were, in Region X, 45.7% of the time patients with an admitting diagnosis of pneumonia received the first dose of antibiotic within four (4) hours of arrival. The infection control department requested a Pneumonia Action Team (PAT) be sanctioned to determine: 1) timeliness of antibiotic administration in our facility; and 2) opportunities for improvement. A multidisciplinary team with members from Administration, Admitting, Education, Emergency, Infection Control, Medical Staff, Pathology, Pharmacy, Pulmonary, and Radiology was formed.

FINDINGS: The team looked at documentation, equipment, people, education, and procedures affecting the timeliness of antibiotic administration. The end result was, 77% of the time patients with an admitting diagnosis of pneumonia received the first dose of antibiotic within four (4) hours of arrival.

CURRENT ACTIONS/RECOMMENDATIONS: 1) Focus on evaluation of admission process to reduce delay in antibiotic administration. 2) Focus on physician orders that may create a delay, ie: obtain sputum culture first.

CONCLUSION: The focus of an interdisciplinary team in this issue has been effective in decreasing the time from admission to receipt of first antibiotic dose. Improved patient outcome and decrease in length of stay can be achieved through cooperation and collaboration from all areas.

ANTIMICROBIAL TEAM ROUNDS INFLUENCES PRESCRIBING BEHAVIOR.

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Facilitating appropriate, cost-effective antimicrobial therapy is commonly cited as a necessity to optimize infection management, minimize the emergence of resistant organisms and enhance outcomes. A reduction in the use of vancomycin and promotion of

A COMPREHENSIVE COMPARISON OF THE *IN VITRO* AND *IN VIVO* ANTIMICROBIAL EFFECTIVENESS OF TRICLOSAN, CHLORHEXIDINE, ALCOHOL/CHLORHEXIDINE, AND POLOXAMER IODINE TOPICAL FORMULATIONS

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Background:

The 1995 APIC Guideline for Handwashing and Hand Antisepsis in Health Care Settings recommended a thorough review of effectiveness of topical antimicrobial formulations in order to pair each desired use pattern with a suitable product. This task may prove difficult due to the use of a variety of different test methods, test organisms, or test conditions. Using consistent test methodology, an extensive comparison of *in vitro* and *in vivo* antimicrobial activity of triclosan, chlorhexidine, alcohol/chlorhexidine, and poloxamer iodine formulations provides a tool for selection of products appropriate for healthcare applications.

Methods:

The following formulations were examined in standard antimicrobial tests: 1% triclosan (PREVACARE* Health Care Personnel Handwash), 4% chlorhexidine gluconate (PREVACARE* S Surgical Scrub Antimicrobial Skin Cleanser), 0.5% chlorhexidine gluconate/70% w/w alcohol (PREVACARE* R Germicidal Hand Rinse), 0.75% available poloxamer iodine surgical scrub solution, and 1% available poloxamer iodine. The formulations were evaluated using standard minimum inhibitory concentration and time-kill tests to assess *in vitro* spectrums of activity. In vivo methods were utilized to assess immediate, residual and persistent antimicrobial activity.

Minimum Inhibitory Concentration Test (Figure 1):

1) NCCLS Document M7-A3 "Methods for Dilution Antimicrobial Susceptibility Test for Bacteria that Grow Aerobically.
2) Food and Drug Administration (FDA). 1974. OTC Topical Antimicrobial Products and Drug and Cosmetic Products Proposed Rules. 39 Federal Register 33102 - 33141. September 6, 1974.
3) Murray, P.R., E.D. Barron, M.A. Pfaller, F.C. Tenover, and R.H. Yolken (Eds.). 1995. Manual of Clinical Microbiology, 6th Edition, ASM Press, Washington, D.C., 1482 pages.
4) Espinel-Ingroff, A., K. Dawson, M. Pfaller, E. Anaisse, B. Breslin, d. Dixon, A. Fothergill, V. Paetznick, J. Peter, M. Rinaldi, and T. Walsh. 1995. Comparison and Collaborative Evaluation of Standardization of Antifungal Susceptibility Testing for Filamentous Fungi. Antimicrobial Agents Chemotherapy. 39(2):314-319.

Time Kill Test (Figure 2):

1) American Society of Testing and Materials. 1991. E1054-91 Standard Practices for Evaluating Inactivation of Antimicrobial Agents Used in Disinfectant, Sanitizer, Antiseptic, or Preserved Products. Annual Book of ASTM Standards. Vol.11.04.
2) Food and Drug Administration (FDA). 1974. OTC Topical Antimicrobial Products and Drug and Cosmetic Products Proposed Rules. 39 Federal Register 33102 - 33141. September 6, 1974.
3) Murray, P.R., E.D. Barron, M.A. Pfaller, F.C. Tenover, and R.H. Yolken (Eds.). 1995. Manual of Clinical Microbiology, 6th Edition, ASM Press, Washington, D.C., 1482 pages.
4) Pujol, I., J. Guarro, C. Llop, L. Soler, and J. Fernandez-Ballart. 1996. Comparison study of broth macrodilution and microdilution susceptibility tests for the filamentous fungi. Antimicrobial Agents chemotherapy. 40(9):2106-2110.

Health Care Personnel Handwash Test (Figure 3):

1) American Society of Testing and Materials. 1994. E1174-94 Standard Test Method for Evaluation of Health Care Personnel Handwash Formulation. Annual Book of ASTM Standards. Vol.11.04.

Surgical Scrub Test (Figure 4):

1) American Society of Testing and Materials. 1991. E1115-91 Standard Test Method for Evaluation of Surgical Hand Scrub Formulations. Annual Book of ASTM Standards. Vol.11.04.

Discussion:

- The results demonstrated the unique *in vitro* antimicrobial spectrum of the formulations on Gram positive, Gram negative, fungi, and yeast.
- The test results demonstrated excellent in vitro bactericidal activity against ATCC and fresh clinical isolates.
- Consistent antimicrobial activity against laboratory strains and clinical isolates.
- Formulations exhibited excellent activity against drug resistant bacteria such as VRE, MRSA, and PRSP.
- The *in vivo* results illustrated the similarities and differences among the immediate, residual and persistent antimicrobial activity of the formulations.
- A comparison of *in vitro* and *in vivo* data illustrated the inability to correlate any given formulations *in vitro* activity to *in vivo* activity.

Conclusion:

Using consistent test methodology, the antimicrobial attributes of topical antimicrobial formulations may be compared for use as a tool in selection of products for specific applications in the healthcare settings, such as, in areas with endemic multiply-resistant bacteria.

Figure 1: The Minimum Inhibitory Concentration Test (MIC) results for each of the following test formulations reported as the part per million (ppm) of the active ingredient: 1% triclosan (PREVACARE* Health Care Personnel Handwash), 4% chlorhexidine gluconate (PREVACARE* S Surgical Scrub Antimicrobial Skin Cleanser),and 0.5% chlorhexidine gluconate/70% alcohol (PREVACARE* R Germicidal Hand Rinse). (MIC data not available for 1% available poloxamer iodine).

Microorganism	Source	MIC (ppm)		
		1% Triclosan (10,000ppm)	4% CHG (40,000ppm)	0.5% CHG/ 70% Alcohol (5,000ppm)
Bacterial Strains:				
Acinteobacter lwoffii	ATCC 15309	5000	156.25	2500.00
Bacteroides fragilis	ATCC 25285	4.88	13.02	9.77
Burkholderia cepacia	ATCC 25416	5000	52.08	78.13
Corynebacterium jeikeium	ATCC 43734	9.77	2.44	2.44
Clostridium difficle	ATCC 9689	6.51	-	-
Enterobacter aerogenes	ATCC 13048	5000	3.91	3.26
Enterobacter cloacae	ATCC 13047	0.81	2.44	4.88
Enterococcus faecium	ATCC 19434	9.77	9.77	9.77
Enterococcus faecalis	ATCC 29212	4.88	4.88	4.88
Enterococcus faecalis	ATCC 19433	6.51	9.77	13.02
Enterococcus faecalis	Clinical	9.79	9.77	6.51
Escherichia coli	ATCC 11229	0.31	1.22	1.95
Escherichia coli (0157)	Clinical	1.22	3.26	2.44
Escherichia coli	ATCC 25922	0.61	3.26	4.88
Escherichia coli	Clinical	0.61	9.53	2.44
Escherichia coli (0157, H7)	Clinical	n/a	3.26	2 44
Haemophilus influenzae	ATCC 19418	0.31	4.88	4.88
Klebsiella oxytoca	ATCC 43165	2.44	9.77	7.81
Klebsiella pneumoniae	ATCC 11296	0.81	1.63	2.44
Micrococcus luteus	ATCC 7468	4 88	2.44	2.44
Micrococcus luteus	ATCC 4698	4.88	2.44	1 63
Micrococcus luteus	Clinical	5000	10,000.00	2500 00
Proteus mirabilis	ATCC 7002	2 44	39.06	26.04
Pseudomonas aeruginosa	ATCC 15442	5000	78.13	39 06
Pseudomonas aeruginosa	ATCC 27853	2500	26 04	19.53
Pseudomonas aeruginosa	ATCC 9027	2500	13.02	9.77
Pseudomonas aeruginosa	Clinical	5000	39.06	39.06
Salmonella typhimurium	ATCC 14028	0.2	-	-
Serratia marcescens	Clinical	5000	78.13	78.13
Shigella flexneri	Clinical	<0 15	-	-
Staphylococus aureus	ATCC 6538	0.49	52.08	2.44
Staphylococus aureus	ATCC 29213	0.15	1.22	2.44
Staphylococus aureus	Clinical	3.26	-	-
Staphylococcus epidermidis	ATCC 12228	4.88	4.88	19.53
Staphylococcus epidermidis	Clinical	5000	3.26	3 26
Staphylococcus haemolyticus	Clinical	625	3.26	4.88
Staphylococcus hominis	ATCC 27844	2500	3.26	2.44
Strenotrophomonas maltophilia	ATCC 13637	2500	9.77	9.77
Streptococcus pneumoniae	ATCC 6303	1.95	9.77	9.77
Streptococcus pyogenes	ATCC 19615	78.13	6.51	4.88
Streptococcus pyogenes	Clinical	78.13	52.08	1.22
Antibiotic-resistant Bacterial Strains:				
Acinetobacter calcoaceticus	Clinical	0.61	2.44	2.44
Bacteroides fragilis	Clinical	3.26	13.02	19.53
Enterobacter cloacae	Clinical	0.81	13.02	9.77
Enterococcus faecalis (MDR)	ATCC 51299	9.77	6.51	9.77
Enterococcus faecium	Clinical	5000	4.88	4.88
Enterococcus faecium (VREF)	ATCC 51599	13.02	13.02	13.02
Haemophilus influenzae	Clinical	19.53	9.77	9.77
Pseudomonas aeruginosa (R)	Clinical	5000	9.77	9.77
Staphylococus aureus	Clinical	3.26	3 26	9.77
Staphylococcus aureus (MRSA)	Clinical	0.61	6.51	4.88
Staphylococcus saprophyticus	Clinical	9.77	9.77	13.02
Streptococcus pneumoniae	Clinical	6.51	3.26	0.98
Fungal Strains:				
Candida tropicalis	ATCC 750	2500	500.00	2500.00
Blastomyces dermatitidis	ATCC 14112	5000	10,000.00	2500.00
Epidermophyton floccosum	ATCC 52062	1.63	9.77	9.77
Microsporum audouinii	ATCC 42558	2.44	19.53	19.53
Microsporum canis	ATCC 14055	4.88	13.02	9.77
Trichophyton mentagrophytes	ATCC 26323	4.88	19.53	19.53
Trichophyton rubrum	ATCC 52024	2.44	13.02	9.77
Trichophyton tonsurans	ATCC 28942	2.44	9.77	9.77

ATCC - American Type Culture Collection

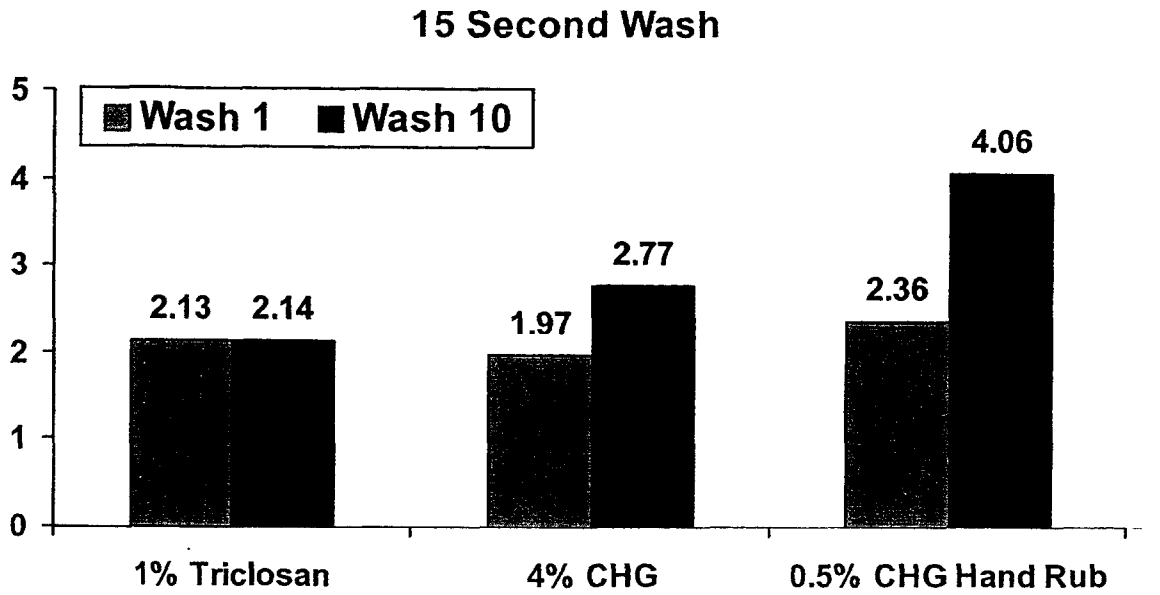
Figure 2: The Time Kill Test results for each of the following test formulations at full strength(*) and at a 50% dilution reported as the percent kill: 1% triclosan (PREVACARE* Health Care Personnel Handwash), 4% chlorhexidine gluconate (PREVACARE* S Surgical Scrub Antimicrobial Skin Cleanser), 0.5% chlorhexidine gluconate/70% alcohol (PREVACARE* R Germicidal Hand Rinse), and 1% available poloxamer iodine.

Microorganism	Source	Percent Kill (%)									
		1% Triclosan (10,000ppm)		4% CHG (40,000ppm)		0.5% CHG/ 70%Alcohol (5,000ppm)		1% avail. Poloxamer iodine (10,000ppm)			
		15s	30s	15s	30s	15s	30s	0s	60s		
Bacterial Strains:											
Acinteobacter lwoffii	ATCC 15309	>99 99	>99.99	27 56	>99 99	>99.99	>99 99	-	-		
Bacteroides fragilis	ATCC 25285	>99.99	>99 99	>99 99	>99.99	>99 99	>99.99	99 9999	99.9999		
Burkholderia cepacia	ATCC 25416	87 37	95.46	35 96	36 84	>99.99	>99 99	-	-		
Corynebacterium jeikeium	ATCC 43734	>99 99	>99.99	>99 99	>99.99	>99.99	>99.99	-	-		
Clostridium difficile*	ATCC 9689	99 9998	99.9998	-	-	>99.99	>99.99	-	-		
Enterobacter aerogenes	ATCC 13048	92 04	96 86	>99.99	>99 99	>99.99	>99 99	-	-		
Enterobacter cloacae	ATCC 13047	98 46	99 08	>99 99	>99.99	>99 99	>99.99	99.9999	99.9999		
Enterococcus faecium	ATCC 19434	>99.99	>99 99	91 64	99 96	>99.99	>99 99	99.9999	99.9999		
Enterococcus faecalis	ATCC 29212	>99 99	>99 99	96 81	99.90	>99.99	>99.99	64.0	99.9999		
Enterococcus faecalis	ATCC 19433	>99.99	>99.99	99 41	99.93	>99.99	>99.99	-	-		
Enterococcus faecalis	Clinical	>99 99	>99 99	97.09	99 95	>99.99	>99 99	31.3291	99.0411		
Escherichia coli	ATCC 11229	>99.99	>99 99	>99 99	>99 99	>99.99	>99.99	99 9999	99.9999		
Escherichia coli	Clinical	>99.99	>99.99	>99 99	>99 99	>99.99	>99 99	99 9999	99.9999		
Escherichia coli	ATCC 25922	>99 99	>99.99	>99.99	>99.99	>99.99	>99 99	99.9999	99.9999		
Escherichia coli	Clinical	>99 99	>99.99	-	-	-	-	-	-		
Escherichia coli (0157, H7)*	Clinical	99.9999	99 9999	>99.99	>99.99	99 22	>99.99	99.9999	99.9999		
Haemophilus influenzae	ATCC 19418	>99.99	>99 99	>99 99	>99 99	>99.99	>99.99	99.9992	99.9999		
Klebsiella oxytoca	ATCC 43165	99 01	99.88	>99 99	>99 99	>99.99	>99.99	99.9999	99.9999		
Klebsiella pneumoniae	ATCC 11296	>99.99	>99 99	>99.99	>99 99	>99.99	>99.99	99.9999	99.9999		
Micrococcus luteus	ATCC 7468	99.99	99.99	>99 99	>99 99	>99.99	>99.99	78.8043	99.9991		
Micrococcus luteus	ATCC 4698	>99.99	>99 99	>99.99	>99 99	>99.99	>99.99	99.9999	99.9999		
Micrococcus luteus	Clinical	99.97	>99.99	-	-	-	-	-	-		
Proteus mirabilis	ATCC 7002	62.98	98.54	>99.99	>99 99	>99.99	>99.99	99.9999	99 9999		
Pseudomonas aeruginosa	ATCC 15442	>99.99	>99.99	>99.99	>99 99	>99.99	>99.99	99.9999	99.9999		
Pseudomonas aeruginosa	ATCC 27853	>99.99	>99.99	>99.99	>99 99	>99.99	>99.99	99.9999	99.9999		
Pseudomonas aeruginosa	ATCC 9027	>99.99	>99 99	>99 99	>99 99	>99.99	>99.99	-	-		
Pseudomonas aeruginosa	Clinical	>99 99	>99.99	>99 99	>99.99	>99.99	>99.99	99.9999	99 9999		
Salmonella typhimurium*	ATCC 14028	99.9999	99.9999	-	-	-	-	-	-		
Serratia marcescens*	Clinical	90 7	99.9	>99.99	>99.99	>99.99	>99.99	99.9999	99.9999		
Shigella flexneri*	Clinical	99.9999	99 9999	-	-	-	-	-	-		
Staphylococus aureus	ATCC 6538	>99.99	>99.99	91.98	99.64	>99 99	>99.99	99.9999	99.9999		
Staphylococus aureus	ATCC 29213	>99.99	>99.99	97.14	99.29	>99 99	>99.99	99.9999	99.9999		
Staphylococus aureus	Clinical	>99.99	>99.99	36 73	72 65	>99.99	>99.99	99.9999	99.9999		
Staphylococcus epidermidis	ATCC 12228	>99.99	>99 99	>99.99	>99 99	>99 99	>99.99	99.9999	99.9999		
Staphylococcus epidermidis	Clinical	97.19	98.92	>99 99	>99 99	>99 99	>99.99	99.9999	99.9999		
Staphylococcus haemolyticus	Clinical	91.04	99 00	>99 99	>99 99	-	-	99.9999	99.9998		
Staphylococcus hominis	ATCC 27844	>99.99	>99 99	>99.99	>99.99	>99.99	>99.99	99 9999	99.9999		
Strenotrophomonas maltophilia	ATCC 13637	>99.99	>99 99	>99 99	>99.99	>99.99	>99.99	-	-		
Streptococcus pneumoniae	ATCC 6303	>99.99	>99 99	>99.99	>99.99	>99.99	>99.99	99.9988	99.9988		
Streptococcus pyogenes	ATCC 19615	>99.97	>99.97	89 16	99 97	>99.99	>99.99	99.9999	99.9999		
Streptococcus pyogenes	Clinical	>99.98	>99 98	99 98	99 98	>99.99	>99.99	99.9982	99.9982		

Figure 2: (Continued).

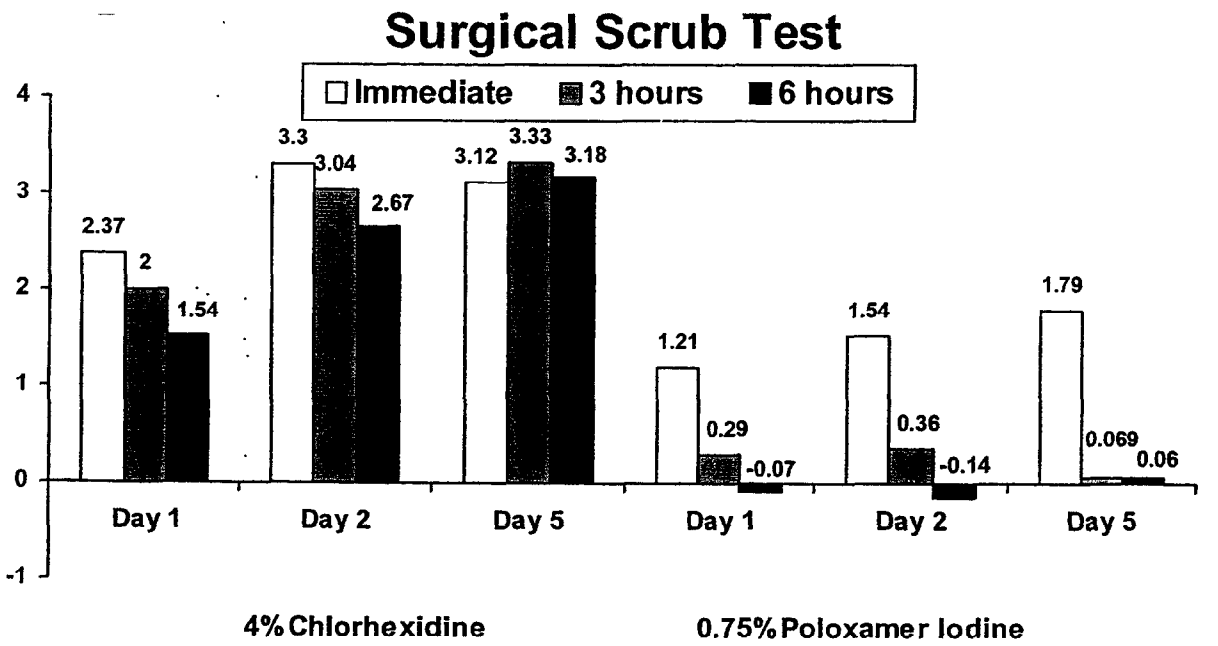
Microorganism	Source	Percent Kill (%)									
		1% Triclosan (10,000ppm)		4% CHG (40,000ppm)		0.5% CHG/ 70%Alcohol (5,000ppm)		1% avail. Poloxamer iodine (10,000ppm)			
		15s	30s	15s	30s	15s	30s	0s	60s		
Antibiotic-resistant Bacterial Strains:											
Acinetobacter calcoaceticus	Clinical	74 58	>99.99	>99 99	>99 99	>99.99	>99.99	-	-		

Figure 3: The Health Care Personnel Handwash results for each of the following test formulations reported as the percent kill: 1% triclosan (PREVACARE* Health Care Personnel Handwash), 4% chlorhexidine gluconate (PREVACARE* S Surgical Scrub Antimicrobial Skin Cleanser), and 0.5% chlorhexidine



gluconate/70% alcohol (PREVACARE* R Germicidal Hand Rinse).

Figure 4: The figure depicts the results of two Surgical Scrub Tests for the following test formulations: 4% chlorhexidine gluconate (PREVACARE* S Surgical Scrub Antimicrobial Skin Cleanser), and 0.75% available poloxamer iodine.



Technical Report

Health Care Personnel Handwash with 1% Triclosan

PREVACARE*
Skin Care Products

Johnson & Johnson
MEDICAL
Division of Ethicon, Inc.

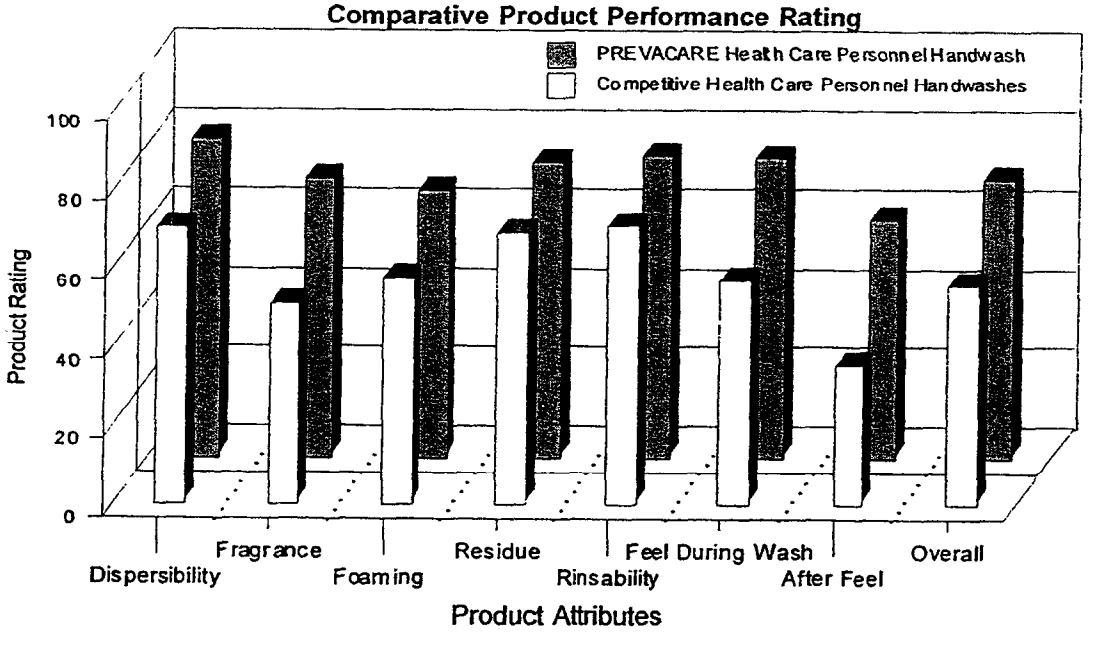
ARLINGTON, TEXAS 76004-3130

PREVACARE* Health Care Personnel Handwash is *Preferred* By Health Care Providers

OBJECTIVE: An independent analysis was conducted in eleven US hospitals to assess the response of health care workers to PREVACARE* Health Care Personnel Handwash as compared to their existing products.

STUDY DESIGN:
1) To initiate the study, 207 participants were asked to evaluate the attributes of the current skincare product.
2) Current products were replaced with unmarked PREVACARE Health Care Personnel Handwash.
3) After 7 days of consecutive use, participants were asked to evaluate the attributes of the new product.
4) Both current and test product assessments were collected and statistically evaluated.
5) The results are illustrated in the following figure.

SUMMARY: ***PREVACARE Health Care Personnel Handwash is preferred by healthcare providers for dispersibility fragrance, residual film, rinseability, foam generation, feel of product during and after use.***



Typical Physical Properties of PREVACARE Health Care Personnel Handwash

Appearance	Clear, teal liquid
Odor	Pleasant fragrance
Approximate pH	5.5
Viscosity	Viscous liquid (Approx. 2,500 cps @ 25°C)
Triclosan Content	1.0%w/w
Antimicrobial Activity	Broad Spectrum, Fast-acting, Persistent

*Trademark

In Vitro Antimicrobial Testing

Gram Positive • Gram Negative • Antibiotic-Resistant Bacteria
Yeast • Fungi • Viruses

OBJECTIVE: An independent laboratory measured the *in vitro* antimicrobial activity of PREVACARE Health Care Personnel Handwash against Gram positive, Gram negative, and Antibiotic-resistant bacteria, yeast, fungi, and viruses using a Minimum Inhibitory Concentration test and a Time Kill method.

TEST DESIGN: **Minimum Inhibitory Concentration Test**

Reference: National Committee on Clinical Laboratory Standard M7 Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically.

1) Prepare 15 serial dilutions of PREVACARE Health Care Personnel Handwash (10,000 ppm) for each organism.
2) Challenge each dilution with 1 x 10^6 cfu test organism/ml.
3) Incubate to achieve appropriate growth for each organism.
4) Observe tubes for turbidity (cloudiness) or growth. The lowest concentration having no turbidity is the minimum inhibitory concentration (MIC). The results are shown in the following Table. (This test was not conducted on viral cultures due to the limitations presented by their growth requirements.

TEST DESIGN: **Time Kill Test**

Reference: American Society of Testing and Materials draft Standard Test Method for The Assessment of the Rapid Germicidal Activity (Time-Kill) Activity for Antibacterial Wash Products.

1) Prepare a 50% (1:2) dilution of PREVACARE Health Care Personnel Handwash.
2) Challenge diluted handwash with 1 x 10^8 cfu test organism/ml.
3) Following contact times of 15, 30 and 60 seconds, neutralize (stop) the antimicrobial activity of the Handwash through dilution in the appropriate growth media.
4) Recover and quantitate the surviving test organisms for each contact time.
5) Calculate the percent kill for each contact time against each test organism. The results are shown in the following Table.

FDA REFERENCE: FDA Tentative Final Monograph Tentative Final Monograph for Health-Care Antiseptic Drug Products; Proposed Rule. June 17, 1994.

TESTS RESULTS:

Microorganism	Source	Percent Kill (%)			MIC (ppm)
		15s	30s	60s	Handwash (10,000ppm)
Bacterial Strains:					
Acinteobacter lwoffii	ATCC 15309	>99.99	>99.99	>99.99	5000
Bacteroides fragilis	ATCC 25285	>99.99	>99.99	>99.99	4.88
Burkholderia cepacia	ATCC 25416	87.37	95.46	99.89	5000
Corynebacterium jeikeium	ATCC 43734	>99.99	>99.99	>99.99	9.77
Enterobacter aerogenes	ATCC 13048	92.04	96.86	99.78	5000
Enterobacter cloacae	ATCC 13047	98.46	99.08	>99.99	0.81
Enterococcus faecium	ATCC 19434	>99.99	>99.99	>99.99	9.77
Enterococcus faecalis	ATCC 29212	>99.99	>99.99	>99.99	4.88
Enterococcus faecalis	ATCC 19433	>99.99	>99.99	>99.99	6.51
Enterococcus faecalis	Clinical	>99.99	>99.99	>99.99	9.77
Escherichia coli	ATCC 11229	>99.99	>99.99	>99.99	0.31
Escherichia coli	Clinical	>99.99	>99.99	>99.99	1.22
Escherichia coli	ATCC 25922	>99.99	>99.99	>99.99	0.61
Escherichia coli	Clinical	>99.99	>99.99	>99.99	0.61
Haemophilus influenzae	ATCC 19418	>99.99	>99.99	>99.99	0.31

Microorganism	Source	Percent Kill (%)			MIC (ppm)
		15s	30s	60s	Handwash (10,000ppm)
Klebsiella oxytoca	ATCC 43165	99.01	99.88	99.96	2.44
Klebsiella pneumoniae	ATCC 11296	>99.99	>99.99	>99.99	0.81
Micrococcus luteus	ATCC 7468	99.99	99.99	99.99	4.88
Micrococcus luteus	ATCC 4698	>99.99	>99.99	>99.99	4.88
Micrococcus luteus	Clinical	99.97	>99.99	>99.99	5000
Proteus mirabilis	ATCC 7002	62.98	98.54	>99.99	2.44
Pseudomonas aeruginosa	ATCC 15442	>99.99	>99.99	>99.99	5000
Pseudomonas aeruginosa	ATCC 27853	>99.99	>99.99	>99.99	2500
Pseudomonas aeruginosa	ATCC 9027	>99.99	>99.99	>99.99	2500
Pseudomonas aeruginosa	Clinical	>99.99	>99.99	>99.99	5000
Staphylococcus aureus	ATCC 6538	>99.99	>99.99	>99.99	0.49
Staphylococcus aureus	ATCC 29213	>99.99	>99.99	>99.99	0.15
Staphylococcus aureus	Clinical	>99.99	>99.99	>99.99	3.26
Staphylococcus epidermidis	ATCC 12228	>99.99	>99.99	>99.99	4.88
Staphylococcus epidermidis	Clinical	97.19	98.92	99.27	5000
Staphylococcus haemolyticus	Clinical	91.04	99.00	>99.99	625
Staphylococcus hominis	ATCC 27844	>99.99	>99.99	>99.99	2500
Strenotrophomonas maltophilia	ATCC 13637	>99.99	>99.99	>99.99	2500
Streptococcus pneumoniae	ATCC 6303	>99.99	>99.99	>99.99	1.95
Streptococcus pyogenes	ATCC 19615	>99.97	>99.97	>99.97	78.13
Streptococcus pyogenes	Clinical	>99.98	>99.98	>99.98	78.13
Antibiotic-resistant Bacterial Strains*:					
Acinetobacter calcoaceticus	Clinical	74.58	>99.99	>99.99	0.61
Bacteroides fragilis	Clinical	99.95	>99.99	>99.99	3.26
Enterobacter cloacae	Clinical	>99.99	99.99	>99.99	0 81
Enterococcus faecalis (MDR)	ATCC 51299	>99.99	>99.99	>99.99	9.77
Enterococcus faecium	Clinical	99.75	>99.99	>99.99	5000
Enterococcus faecium (VREF)	ATCC 51559	99.80	>99.99	>99.99	13.02
Haemophilus influenzae	Clinical	>99.99	>99.99	>99.99	19.53
Pseudomonas aeruginosa (R)	Clinical	>99.99	>99.99	>99.99	5000
Staphylococcus aureus	Clinical	>99.99	>99.99	>99.99	3.26
Staphylococcus aureus (MRSA)	Clinical	>99.49	>99.99	>99.99	0.61
Staphylococcus saprophyticus	Clinical	>99.99	99.99	>99.99	9.77
Streptococcus pneumoniae	Clinical	>99.99	>99.99	>99.99	6.51
Yeast and Fungal Strains:					
Candida tropicalis	ATCC 750	73.90	75.79	88.24	2500
Blastomyces dermatitidis	ATCC 14112	89.25	93.00	97.43	5000
Epidermophyton floccosum	ATCC 52062	87.39	>99.13	>99.13	1.63
Microsporum audouinii	ATCC 42558	80.31	92.50	98.44	2.44
Microsporum canis	ATCC 14055	99.85	99.90	>99.96	4.88
Trichophyton mentagrophytes	ATCC 26323	64.38	63.56	84.11	4.88
Trichophyton rubrum	ATCC 52024	97.40	99.25	99.29	2.44
Trichophyton tonsurans	ATCC 28942	52.00	84.00	85.26	2.44
Viral Strains:					
HIV-1	Vanderbilt Univ.	≥99.9	≥99.9	-	n/a
Herpes Simplex Virus, Type 1	ATCC VR-733	≥99.994	≥99.998	-	n/a
Herpes Simplex Virus, Type 2	ATCC VR-734	≥99.97	≥99.97	-	n/a
Influenza Type A	ATCC VR-544	≥99.999	≥99.999	-	n/a

* - Antibiotic-resistance profile data on file.
ATCC-American Type Culture Collection

SUMMARY: ***PREVACARE Health Care Personnel Handwash demonstrated excellent broad spectrum and fast antimicrobial activity against Gram positive, Gram negative, and antibiotic-resistant bacteria, yeast, fungal, and viral pathogens.***

In Vivo Antimicrobial Testing: Health Care Personnel Handwash Study

OBJECTIVE: The *in vivo* antimicrobial activity of PREVACARE Health Care Personnel Handwash was measured against an antibiotic-resistant marker organism in two studies by an independent laboratory. The ASTM Health Care Personnel Handwash test simulates the removal of transient microorganisms accomplished through a handwashing frequency typical of health care providers. PREVACARE Health Care Personnel Handwash was designed to assist health care providers reduce the risk of nosocomial infections through proper handwashing.

TEST DESIGN: **Health Care Personnel Handwash Test**

Reference: American Society of Testing and Materials E1174 Standard Test Method for Evaluation of Health Care Personnel Handwash Formulation.

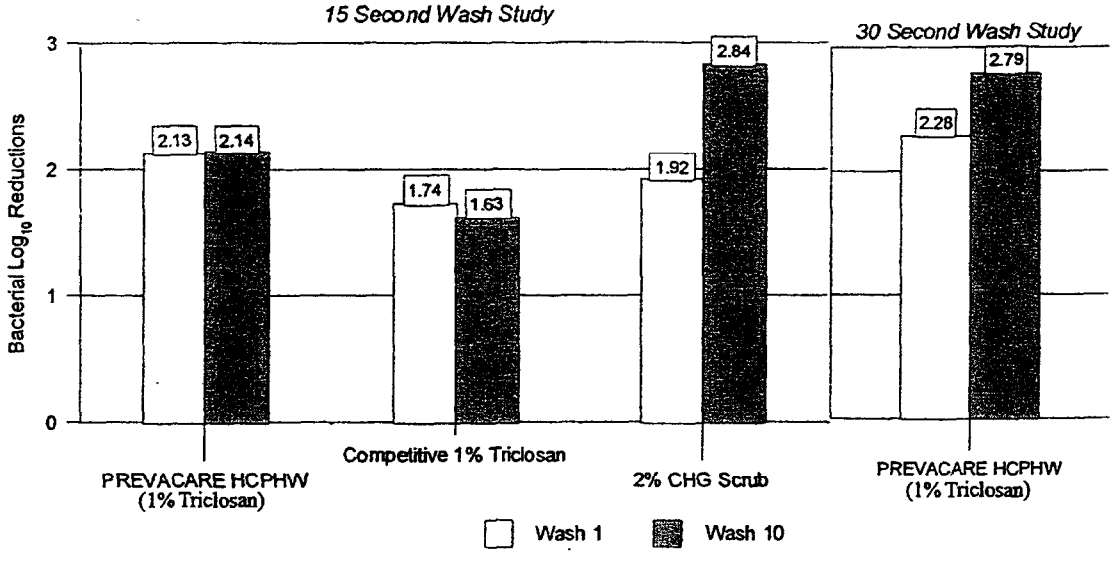
1) Human panelists were recruited and randomly assigned to each of the test formulations.
2) A 30 second cleansing wash is performed to remove dirt and oils.
3) Each subject spreads 5mls of *Serratia marcescens* (ATCC 14756) over the hands for 45 seconds. S. marcescens(ATCC 14756) is resistant to amoxicillin, ampicillin, cefuroxime, cephalothin, nitrofurantoin, and tetracycline.
4) The bacteria is allowed to air dry on the hands for 2 minutes.
5) The bacterial contamination (Steps 2-4) are repeated eleven times over the 8-10 hour study period.
6) Following the first contamination, a measurement of the total bacterial population (baseline) on each panelists hands is taken using the glove-massage technique described below (Steps 9-13).
7) Following the subsequent ten bacterial contaminations, each subject washed in a prescribed manner with 5 ml of the test material and a glove-massage technique was used to measure the surviving bacteria.
8) In Study 1, the wash time was 15 seconds and for Study 2, the time was 30 seconds.
9) Following a 30 second rinse, the hands were inserted into sterile, powder-free surgical gloves.
10) 75mls Stripping Solution is added to each glove.
11) The fluid-filled gloves are massaged for 1 minute to recover the surviving bacteria.
12) The glove fluid is further diluted, plated, and incubated in neutralizing media.
13) The surviving bacteria are enumerated and $\log_{10}$ reductions are calculated.
14) The results of the study are shown in the following table and figure.

FDA REFERENCE: FDA Tentative Final Monograph Tentative Final Monograph for Health-Care Antiseptic Drug Products; Proposed Rule. June 17, 1994.

TEST RESULTS:

	Formulation	Test Organism	Contact Time/ Cleanser Volume	No. of Subjects	Log$_{10}$ Reductions	
					Wash 1	Wash 10
Study 1	PREVACARE Health Care Personnel Handwash (1% Triclosan)	Serratia marcescens (ATCC 14756)	15 sec/5ml	8	2.13	2.14
	Competitive 1% Triclosan			6	1.74	1.63
	2% Chlorhexidine Surgical Scrub			9	1.92	2.84
Study 2	PREVACARE Health Care Personnel Handwash (1% Triclosan)	Serratia marcescens (ATCC 14756)	30sec/5ml	12	2.28	2.79

Health Care Personnel Handwash Study Results



SUMMARY:

PREVACARE Health Care Personnel Handwash demonstrates proven antimicrobial activity on the skin in 15 and 30 seconds.

PREVACARE Health Care Personnel Handwash effectively removes transient microbial pathogens from the hands following single and multiple handwashes.

PREVACARE Health Care Personnel Handwash demonstrated improved antimicrobial effectiveness over other 1% Triclosan Scrub formulations.

PREVACARE Health Care Personnel Handwash demonstrated similar effectiveness as a 2% Chlorhexidine gluconate formulation following a single wash at 15 seconds.

PREVACARE Health Care Personnel Handwash may be recommended for health care providers to assist in the reduction of the hand carriage of potential nosocomial pathogens.

PREVACARE Health Care Personnel Handwash Proven Safe and Mild for the Skin

OBJECTIVE: Four independent studies on human panelists were conducted to measure the gentleness of PREVACARE Health Care Personnel Handwash following repeated applications.

TEST DESIGN:

Primary Irritation Patch Test - This test was conducted on 104 human subjects to measure the irritation potential of the formulation following a 48 hour continuous, occlusive exposure.

Modified Draize Repeat Insult Patch Test - This test was conducted on 110 human subjects to assess the sensitization potential of the formulation following repeated applications.

Phototoxicity Patch Test - This test was conducted on 25 human subjects to determine the photoirritation potential of the formulation following 24 hour continuous occlusive exposure and 15 minutes of ultraviolet A treatment.

Photoallergy Patch Test - This test was conducted on 23 human subjects to assess the photosensitization potential of the formulation following repeated applications and Ultraviolet A and B doses.

SUMMARY:

PREVACARE Health Care Personnel Handwash demonstrated no dermal sensitization and minimal irritation typical of surfactant-based formulations.

PREVACARE Health Care Personnel Handwash is a gentle formula which is safe for repeated applications.

Technical Report

PREVACARE*
Skin Care Products

Antimicrobial Handwash with Triclosan

Johnson & Johnson
MEDICAL
Division of Ethicon, Inc.

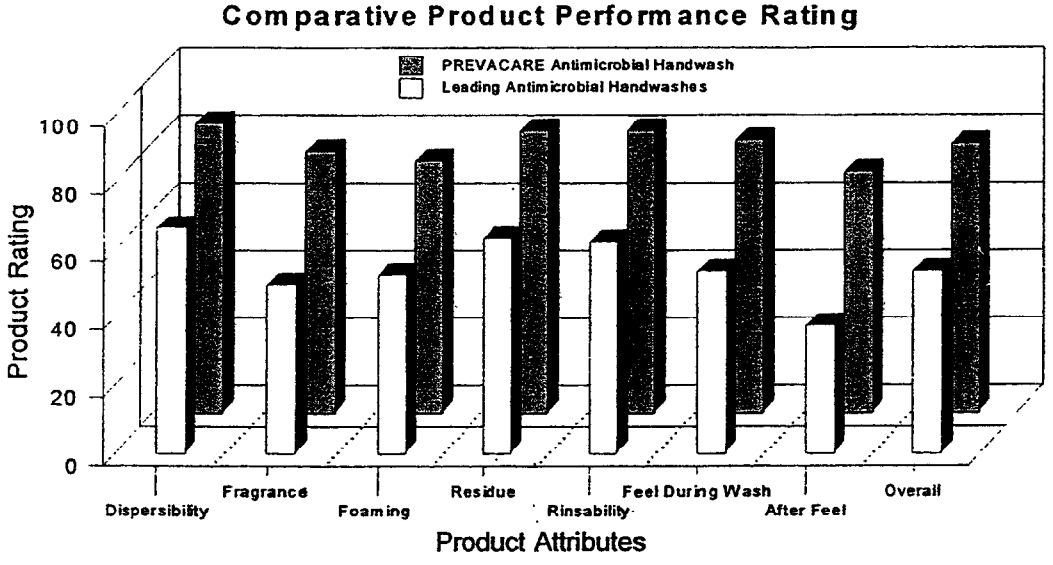
ARLINGTON, TEXAS 76004-3130

PREVACARE* Antimicrobial Handwash is *Preferred* By Health Care Providers

OBJECTIVE: An independent analysis was conducted in eleven U.S. hospitals to assess the response of health care workers to PREVACARE* Antimicrobial Handwash as compared to their existing products.

STUDY DESIGN:
1) To initiate the study, 183 participants were asked to evaluate the attributes of the current skincare product.
2) Current products were replaced with unmarked PREVACARE Antimicrobial Handwash.
3) After 7 days of consecutive use, participants were asked to evaluate the attributes of the new product.
4) Both current and test product assessments were collected and statistically evaluated.
5) The results are illustrated in the following figure.

SUMMARY: ***PREVACARE Antimicrobial Handwash is preferred by healthcare providers for dispersibility, fragrance, residual film, rinseability, foam generation, feel of product during and after use.***



Appearance	Clear, pale peach
Odor	Pleasant fragrance
Approximate pH	5.5
Viscosity	Viscous (Approx. 3,500 cps @ 25°C)
Triclosan Content	0.25%w/w

*Trademark

In Vitro Antimicrobial Testing

Gram Positive • Gram Negative • Antibiotic-Resistant Bacteria Yeast • Fungi

OBJECTIVE:

An independent laboratory measured the *in vitro* antimicrobial activity of PREVACARE Antimicrobial Handwash against Gram positive, Gram negative, and Antibiotic-resistant bacteria, yeast, and fungi using a Minimum Inhibitory Concentration test.

TEST DESIGN:

Minimum Inhibitory Concentration Test

Reference: National Committee on Clinical Laboratory Standard M7 Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically.

1) Prepare 15 serial dilutions of PREVACARE Antimicrobial Handwash (2500ppm) for each organism.
2) Challenge each dilution with 1 x 10^6 cfu test organism/ml.
3) Incubate to achieve appropriate growth for each organism.
4) Observe tubes for turbidity (cloudiness) or growth. The lowest concentration having no turbidity is the minimum inhibitory concentration (MIC). The results are shown in the following Table.

FDA REFERENCE:

FDA Tentative Final Monograph Tentative Final Monograph for Health-Care Antiseptic Drug Products; Proposed Rule. June 17, 1994.

TEST RESULTS:

Microorganism	Source	MIC (ppm) PREVACARE Antimicrobial Handwash (2500 ppm)
Bacterial Strains:		
Acinetobacter baumannii	ATCC 19606	52.08
Acientobacter lwoffi	ATCC 15309	78.13
Alcaligenes xylosoxidans	ATCC 27061	13.02
Bacteroides fragilis	ATCC 25285	2.44
Burkholderia cepacia	ATCC 25416	312.5
Corynebacterium jeikeium	ATCC 43734	208.33
Enterobacter aerogenes	ATCC 13048	1250
Enterobacter cloacae	ATCC 13047	0.2
Enterococcus faecium	ATCC 19434	312.5
Enterococcus faecalis	ATCC 29212	312.5
Enterococcus faecalis	Clinical	625
Enterococcus faecalis	ATCC 19433	208.33
Escherichia coli	ATCC 11229	0.15
Escherichia coli (0157)	Clinical	1.22
Escherichia coli	ATCC 25922	0.31
Escherichia coli	Clinical	0.31
Haemophilus infuenzae	ATCC 19418	0.2
Klebsiella oxytoca	ATCC 43165	0.81
Klebsiella pneumoniae	ATCC 11296	0.31
Micrococcus luteus	ATCC 7468	1250
Micrococcus luteus	Clinical	1250
Micrococcus luteus	ATCC 4698	3.26
Proteus mirabilis	ATCC 7002	1.63
Proteus mirabilis	Clinical	0.61
Pseudomonas aeruginosa	ATCC 15442	312.5
Pseudomonas aeruginosa	Clinical	156.25
Pseudomonas aeruginosa	ATCC 9027	312.5

Microorganism	Source	MIC (ppm) PREVACARE Antimicrobial Handwash (2500 ppm)
Serratia marcescens	ATCC 14756	312.5
Staphylococcus aureus	ATCC 6538	19.53
Staphylococcus aureus	ATCC 29213	1250
Staphylococcus epidermidis	ATCC 12228	833.33
Staphylococcus epidermidis	Clinical	1250
Staphylococcus haemolyticus	ATCC 29970	625
Staphylococcus haemolyticus	Clinical	3.26
Staphylococcus hominis	ATCC 27844	312.5
Staphylococcus hominis	Clinical	3.26
Staphylococcus saprophyticus	ATCC 15305	0.31
Stenotrophomonas maltophilia	ATCC 13637	312.5
Streptococcus pneumoniae	ATCC 6303	0.61
Streptococcus pyogenes	ATCC 19615	4.88
Streptococcus pyogenes	Clinical	0.81
Acinetobacter calcoaceticus	Clinical	0.31
Bacteroides fragilis	Clinical	0.2
Enterobacter cloacae	Clinical	0.61
Enterococcus faecalis (MDR)	ATCC 51299	416.67
Enterococcus faecium	Clinical	1250
Enterococcus faecium (VRE)	ATCC 51559	312.5
Haemophilus influenzae	Clinical	312.5
Klebsiella oxytoca	Clinical	1.95
Klebsiella pneumoniae	Clinical	0.81
Pseudomonas aeruginosa (R)	Clinical	312.5
Serratia marcescens	Clinical	625
Staphylococcus aureus (MRSA)	ATCC 33592	1250
Staphylococcus aureus (MRSA)	Clinical	3.27
Staphylococcus aureus	Clinical	312.5
Staphylococcus saprophyticus	Clinical	312.5
Strptococcus pneumoniae	Clinical	1.63

* - Antibiotic-resistance profile data on file.

Microorganism	Source	MIC (ppm) PREVACARE Antimicrobial Handwash (2500 ppm)
Yeast and Fungal Strains:		
Candida albicans	ATCC 10231	6.51
Candida albicans	Clinical	1250
Candida tropicalis	ATCC 750	1250
Candida tropicalis	Clinical	1250
Aspergillus fumigatus	ATCC 66638	625
Aspergillus niger	ATCC 16404	1.22
Aspergillus versicolor	ATCC 10072	6.25
Blastomyces dermatitidis	ATCC 14112	1250
Epidermophyton floccosum	ATCC 52062	0.61
Microsporum audouinii	ATCC 42558	0.81
Microsporum canis	ATCC 14055	1.63
Microsporum gypseum	ATCC 10215	1.22
Penicillium notatum	ATCC 9478	78.13
Penicillium roquefortii	ATCC 10110	78.13
Rhizopus stolonifer	ATCC 14038	4.88
Trichoderma viride	ATCC 26802	0.81
Trichophyton ajelloi	ATCC 28454	1.22
Trichophyton mentagrophytes	ATCC 26323	2.44
Trichophyton rubrum	ATCC 52024	1.63
Trichophyton tonsurans	ATCC 28942	1.22

ATCC - American Type Culture Collection

SUMMARY:

PREVACARE Antimicrobial Handwash demonstrated excellent broad spectrum antimicrobial activity against Gram positive, Gram negative, and antibiotic-resistant bacteria, yeast, and fungal pathogens.

In vivo Antimicrobial Testing: Health Care Personnel Handwash Study

OBJECTIVE: The *in vivo* antimicrobial activity of PREVACARE Antimicrobial Handwash was measured against an antibiotic-resistant marker organism in two identical studies by an independent laboratory. The ASTM Health Care Personnel Handwash test simulates the removal of transient microorganisms accomplished through a handwashing frequency typical of health care providers. PREVACARE Antimicrobial Handwash was designed to provide an exceptionally mild handwash with broad spectrum antimicrobial activity.

TEST DESIGN: **Health Care Personnel Handwash Test**

Reference: American Society of Testing and Materials E1174 Standard Test Method for Evaluation of Health Care Personnel Handwash Formulation.

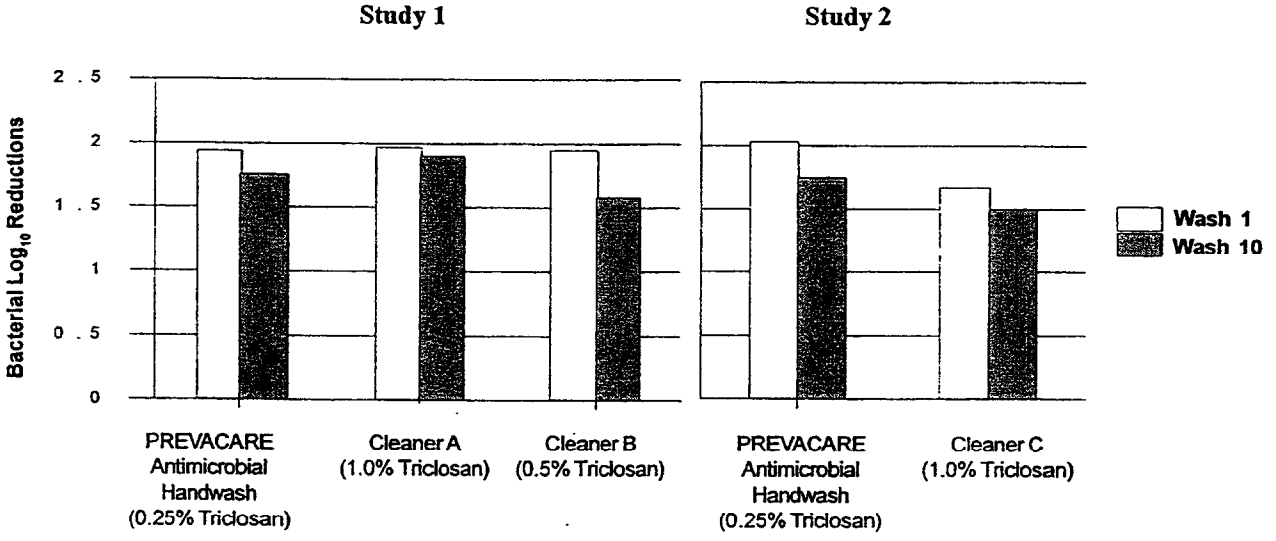
1) Human panelists were recruited and randomly assigned to each of the test formulations.
2) A 30 second cleansing wash was performed to remove dirt and oils.
3) Each subject spread 5mls of *Serratia marcescens* (ATCC 14756) over the hands for 45 seconds. *S. marcescens* (ATCC 14756) is resistant to amoxicillin, ampicillin, cefuroxime, cephalothin, nitrofurantoin, and tetracycline.
4) The bacteria was allowed to air dry on the hands for 2 minutes.
5) The bacterial contamination (Steps 2 -4) are repeated eleven times over the 8-10 hour study period.
6) Following the first contamination, a measurement of the total bacterial population (baseline) on each panelists hands is taken using the glove-massage technique described below (Steps 9-13).
7) Following the subsequent ten bacterial contaminations, each subject washed for 15 seconds in a prescribed manner with 5 ml of the test formulation and a glove-massage technique was used to measure the surviving bacteria.
9) Following a 30 second rinse, the hands were inserted into sterile, plastic bags.
10) 75mls Stripping Solution was added to each glove.
11) The fluid-filled plastic bags were massaged for 1 minute to recover the surviving bacteria.
12) The massage fluid was further diluted, plated, and incubated in neutralizing media.
13) The surviving bacteria were enumerated and $\log_{10}$ reductions are calculated.
14) The results of the two studies are shown in the following table and figure.

FDA REFERENCE: FDA Tentative Final Monograph Tentative Final Monograph for Health-Care Antiseptic Drug Products; Proposed Rule. June 17, 1994.

TEST RESULTS:

	Formulation	Test Organism	Contact Time/ Cleanser Volume	No. of Subjects	$\log_{10}$ Reductions	
					Wash 1	Wash 10
Study 1	PREVACARE Antimicrobial Handwash (0.25 % Triclosan)	Serratia marcescens (ATCC 14756)	15sec/5ml	12	1.94	1.75
	Cleanser A (1 % Triclosan)			12	1.97	1.89
	Cleanser B (0.5 % Triclosan)			12	1.95	1.57
Study 2	PREVACARE Antimicrobial Handwash (0.25 % Triclosan)	Serratia marcescens (ATCC 14756)	15 sec/5ml	12	2.03	1.75
	Cleanser C (1 % Triclosan)			12	1.67	1.50

Health Care Personnel Handwash Study Results with 15 Second Wash Time



SUMMARY:

PREVACARE Antimicrobial Handwash demonstrated proven antimicrobial activity on the skin in 15 seconds.

PREVACARE Antimicrobial Handwash effectively removed transient microbial pathogens from the hands following single and multiple handwashes.

PREVACARE Antimicrobial Handwash demonstrated improved antimicrobial effectiveness over other 0.5% Triclosan formulations following single and multiple washes at 15 seconds.

PREVACARE Antimicrobial Handwash demonstrated similar effectiveness as a 1% Triclosan formulation following single and multiple washes at 15 seconds.

PREVACARE Antimicrobial Handwash

Proven Safe and Mild for the Skin

OBJECTIVE:

Four independent studies on human panelists were conducted to measure the gentleness of PREVACARE Antimicrobial Handwash following single and repeated applications.

TEST DESIGN:

Primary Irritation Patch Test - This test was conducted on 104 human subjects to measure the irritation potential of the formulation following a 48 hour continuous, occlusive exposure.

Modified Draize Repeat Insult Patch Test - This test was conducted on 203 human subjects to assess the sensitization potential of the formulation following repeated applications.

Phototoxicity Patch Test - This test was conducted on 10 human subjects to determine the photoirritation potential of the formulation following 24 hour continuous occlusive exposure and 15 minutes of Ultraviolet A treatment.

Photoallergy Patch Test - This test was conducted on 30 human subjects to assess the photosensitization potential of the formulation following repeated applications and ultraviolet A and B doses.

SUMMARY:

PREVACARE Antimicrobial Handwash demonstrated minimal irritation typical of surfactant-based formulations.

PREVACARE Antimicrobial Handwash is a gentle formula which is safe for repeated applications.

Johnson & Johnson
MEDICAL
Division of Ethicon, Inc.

ARLINGTON, TEXAS 76004-3130

SKC-TRAMH

Technical Report

Antimicrobial Hand Gel

PREVACARE* Skin Care Products

Johnson & Johnson
MEDICAL
Division of Ethicon, Inc.
*Trademark

1998a

PREVACARE* Antimicrobial Hand Gel

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Typical Physical Properties of PREVACARE* Antimicrobial Hand Gel

Appearance	White gel
Odor	Pleasant fragrance
Approximate pH	6.5
Specific Gravity	Approx. 0.9 @ 25°C
Viscosity	Viscous Gel (Approx. 17,000cps @ 25°C)
Alcohol Content	60% (v/v) ethyl alcohol
Antimicrobial Activity	Broad Spectrum, Fast-acting, Persistent

*Trademark

In Vitro Antimicrobial Testing

Gram Positive ⌘ Gram Negative ⌘ Antibiotic-Resistant Bacteria Spore-forming Bacteria ⌘ Foodborne Pathogens ⌘ Mycobacteria Yeast ⌘ Fungi ⌘ Viruses

OBJECTIVE: An independent laboratory measured the *in vitro* antimicrobial activity of PREVACARE* Antimicrobial Hand Gel against Gram positive, Gram negative, antibiotic-resistant, and spore-forming bacteria, foodborne pathogens, mycobacteria, yeast, fungi, and viruses using Minimum Inhibitory Concentration and Time Kill test methods.

TEST DESIGN: **Minimum Inhibitory Concentration (MIC) Test**

Reference: National Committee on Clinical Laboratory Standard M7 Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically.

1) Prepare serial dilutions of PREVACARE* Antimicrobial Hand Gel for each organism.
2) Challenge each dilution with 1 x 10^6 cfu test organism/ml.
3) Incubate to achieve appropriate growth for each organism.
4) Observe tubes for turbidity (cloudiness) or growth. For bacterial species, the lowest dilution having no turbidity is the minimum inhibitory concentration (MIC). For fungal species, the lowest dilution showing inhibition of approximately 75% of the fungal growth as compared visually to a control. The results are shown in the following Table. (This test was not conducted on viral cultures due to the limitations presented by their growth requirements.)

T DESIGN: **Time Kill Test**

Reference: American Society of Testing and Materials draft Standard Test Method for The Assessment of the Rapid Germicidal Activity (Time-Kill) Activity for Antibacterial Wash Products.

1) Challenge PREVACARE* Antimicrobial Hand Gel with 1 x 10^8 cfu test organism/ml. This results in 99% (v/v) concentration of the hand gel. For mycobacteria strains, the inoculum level ranged from 1 x 10^4 to 1 x 10^8 cfu test organism/ml.
2) Following contact times of immediate testing, 15, 30 and 60 seconds, neutralize to quench antimicrobial activity of the hand gel through dilution in the appropriate growth media.
3) Recover and quantitate the surviving test organisms for each contact time.
4) Calculate the percent kill for each contact time against each test organism. The results are shown in the following Table.

FDA REFERENCE: FDA Tentative Final Monograph Tentative Final Monograph for Health-Care Antiseptic Drug Products; Proposed Rule. June 17, 1994.

TEST RESULTS:

Microorganism	Source	Percent Kill (%)				MIC (ppm)
		Immediate	15s	30s	60s	PREVACARE* Gel (600,000 ppm)
Bacterial Strains:						
Acinetobacter baumannii	ATCC 19606	99.9998	99.9998	99.9998	99.9998	9,375
Acinteobacter lwoffii	ATCC 15309	-	99.9998	99.9998	99.9998	391
Alcaligenes xylosoxidans	ATCC 27061	99.9999	99.9999	99.9999	99.9999	18,750
Bacillus cereus ♦ §	ATCC 14579	-	99.9999	99.9999	99.9999	2,344
Bacteroides fragilis	ATCC 25285	99.9999	99.9999	99.9999	99.9999	1,172
Bacteroides fragilis	Clinical	99.9999	99.9999	99.9999	99.9999	1,172
Burkholderia cepacia	ATCC 25416	-	99.9999	99.9999	99.9999	37,500
Campylobacter jejuni §	ATCC 29428	-	99.9989	99.9989	99.9989	1,172
Clostridium difficile ♦	ATCC 9689	-	99.9997	99.9997	99.9997	1,172

§ - As identified in the U.S. Public Health Department's Top Ten Foodborne Pathogens which include *Campylobacter jejuni, Escherichia coli* 0157:H7, *Listeria monocytogenes, Clostridium perfringens, Salmonella typhimurium, Staphylococcus aureus, Shigella flexneri, Vibrio vulnificus, Yersinia enterocolitica.*
♦ - Spore-forming bacteria include *Bacillus cereus, Clostridium difficile,* and *Clostridium perfringens.*

*Trademark

Microorganism	Source	Percent Kill (%)				MIC (ppm)
		Immediate	15s	30s	60s	PREVACARE* Gel (600,000 ppm)
Bacterial Strains (Continued):						
Clostridium perfringens ϕ §	ATCC 13124	-	99.9998	99.9998	99.9998	1,172
Corynebacterium jeikeium	ATCC 43734	-	99.9998	99.9998	99.9998	4,687
Enterobacter aerogenes	ATCC 13048	-	99.9999	99.9999	99.9999	18,750
Enterobacter cloacae	ATCC 13047	99.9999	99.9999	99.9999	99.9999	12,500
Enterococcus faecalis	ATCC 19433	-	99.9999	99.9999	99.9999	25,000
Enterococcus faecalis	Clinical	99.9999	99.9999	99.9999	99.9999	1,172
Enterococcus faecalis	ATCC 29212	99.9999	99.9999	99.9999	99.9999	586
Enterococcus faecium	ATCC 19434	99.9999	99.9999	99.9999	99.9999	586
Escherichia coli §	ATCC 11229	99.9999	99.9999	99.9999	99.9999	3,125
Escherichia coli §	Clinical	99.9997	99.9999	99.9999	99.9999	12,500
Escherichia coli §	ATCC 25922	99.9999	99.9999	99.9999	99.9999	9,375
Escherichia coli (0157, H7) §	Clinical	99.9999	99.9999	99.9999	99.9999	6,250
Haemophilus influenzae	ATCC 19418	99.9999	99.9999	99.9999	99.9999	18,750
Klebsiella oxytoca	ATCC 43165	99.9999	99.9999	99.9999	99.9999	12,500
Klebsiella pneumoniae	ATCC 11296	99.9999	99.9999	99.9999	99.9999	2,344
Listeria monocytogenes §	ATCC 7644	-	99.9999	99.9999	99.9999	293
Micrococcus luteus	ATCC 7468	99.9989	99.9996	99.9996	99.9996	73
Micrococcus luteus	ATCC 4698	99.9999	99.9999	99.9999	99.9999	49
Micrococcus luteus (Prsm)	Clinical	99.9999	99.9999	99.9999	99.9999	1,172
Mycobacterium avium	ATCC49601	-	99.9999	99.9999	99.9999	-
Mycobacterium bovis	ATCC 27291	-	99.9972	99.9972	99.9972	-
Mycobacterium chelonae	ATCC 14472	-	99.9944	99.9944	99.9944	-
Mycobacterium smegmatis	ATCC 14468	-	99.9887	99.9887	99.9887	-
Mycobacterium tuberculosis	ATCC 35818	-	99.9355	99.9355	99.9355	-
Plesiomonas shigelloides	ATCC 14029	-	99.9999	99.9999	99.9999	9,375
Proteus mirabilis	ATCC 7002	99.9999	99.9999	99.9999	99.9999	25,000
Proteus mirabilis	Clinical	99.9999	99.9999	99.9999	99.9999	18,750
Pseudomonas aeruginosa	ATCC 15442	99.9999	99.9999	99.9999	99.9999	37,500
Pseudomonas aeruginosa	Clinical	99.9999	99.9999	99.9999	99.9999	37,500
Pseudomonas aeruginosa	ATCC 27853	99.9999	99.9999	99.9999	99.9999	37,500
Pseudomonas aeruginosa	ATCC 9027	-	99.9999	99.9999	99.9999	37,500
Salmonella typhimurium §	ATCC 14028	-	99.9999	99.9999	99.9999	6,250
Serratia marcescens	ATCC 14756	99.9999	99.9999	99.9999	99.9999	18,750
Shigella flexneri §	ATCC 33948	-	99.9999	99.9999	99.9999	4,687
Staphylococcus aureus §	ATCC 6538	99.9999	99.9999	99.9999	99.9999	293
Staphylococcus aureus §	ATCC 29213	99.9999	99.9999	99.9999	99.9999	781
Staphylococcus epidermidis	ATCC 12228	99.9999	99.9999	99.9999	99.9999	4,687
Staphylococcus haemolyticus	ATCC 29970	99.9999	99.9999	99.9999	99.9999	391
Staphylococcus hominis	ATCC 27844	99.9999	99.9999	99.9999	99.9999	293
Staphylococcus hominis	Clinical	99.9999	99.9999	99.9999	99.9999	781
Staphylococcus saprophyticus	ATCC 15305	99.9999	99.9999	99.9999	99.9999	195
Stenotrophomonas maltophilia	ATCC 13637	-	99.9986	99.9997	99.9997	4,687
Streptococcus pneumoniae	ATCC 6303	99.9943	99.9943	99.9943	99.9943	391
Streptococcus pyogenes	ATCC 19615	99.9993	99.9993	99.9993	99.9993	391
Streptococcus pyogenes	Clinical	99.9999	99.9999	99.9999	99.9999	586
Vibrio vulnificus §	ATCC 27562	-	99.9999	99.9999	99.9999	4,687
Yersinia enterocolitica §	ATCC 9610	-	99.9999	99.9999	99.9999	12,500

Mycobacteria Strains (Ω Antibiotic resistance profile data on file.):						
Mycobacterium avium	ATCC49601	-	99.9999	99.9999	99.9999	-
Mycobacterium bovis	ATCC 27291	-	99.9972	99.9972	99.9972	-
Mycobacterium bovis Ω	ATCC 35746	-	99.9969	99.9969	99.9969	-
Mycobacterium bovis Ω	ATCC 35747	-	99.9710	99.9710	99.9710	-
Mycobacterium chelonae	ATCC 14472	-	99.9944	99.9944	99.9944	-
Mycobacterium smegmatis	ATCC 14468	-	99.9887	99.9887	99.9887	-
Mycobacterium tuberculosis	ATCC 35818	-	99.9355	99.9355	99.9355	-

Prsm - Presumptive Identification

§ - As identified in the U.S. Public Health Department's Top Ten Foodborne Pathogens which include *Campylobacter jejuni, Escherichia coli* 0157:H7, *Listeria monocytogenes. Clostridium perfringens, Salmonella typhimurium, Staphylococcus aureus, Shigella flexneri, Vibrio vulnificus, Yersinia enterocolitica.*

ϕ - Spore-forming bacteria include *Bacillus cereus, Clostridium difficile,* and *Clostridium perfringens.*

*Trademark

Microorganism	Source	Percent Kill (%)				MIC (ppm)
		Immediate	15s	30s	60s	PREVACARE* Gel (600,000 ppm)
Antibiotic-resistant Bacterial Strains (Antibiotic resistance profile data on file.):						
Acinetobacter baumannii	Clinical	99.9999	99.9999	99.9999	99.9999	4,687
Enterobacter cloacae	Clinical	99.9999	99.9999	99.9999	99.9999	18,750
Enterococcus faecalis (VRE)	ATCC 51299	-	99.9999	99.9999	99.9999	1,562
Enterococcus faecium (VREF)	ATCC 51559	-	99.9999	99.9999	99.9999	293
Enterococcus faecium (VREF)	Clinical	99.9999	99.9999	99.9999	99.9999	586
Haemophilus influenzae	Clinical	99.9999	99.9999	99.9999	99.9999	2,344
Klebsiella oxytoca	Clinical	99.9999	99.9999	99.9999	99.9999	18,750
Klebsiella pneumoniae	Clinical	99.9999	99.9999	99.9999	99.9999	9,375
Pseudomonas aeruginosa (MDR)	Clinical	99.9999	99.9999	99.9999	99.9999	37,500
Serratia marcescens	Clinical	99.9999	99.9999	99.9999	99.9999	25,000
Staphylococcus aureus	Clinical	99.9998	99.9998	99.9998	99.9998	391
Staphylococcus aureus (MRSA)	ATCC 33592	-	99.9999	99.9999	99.9999	3,125
Staphylococcus aureus (V/MRSA)	Clinical	99.9999	99.9999	99.9999	99.9999	586
Staphylococcus aureus (MRSA)	Clinical	99.9999	99.9999	99.9999	99.9999	1,172
Staphylococcus epidermidis	Clinical	99.9999	99.9999	99.9999	99.9999	1,172
Staphylococcus haemolyticus	Clinical	99.9999	99.9999	99.9999	99.9999	1,172
Staphylococcus saprophyticus	Clinical	99.9998	99.9998	99.9998	99.9998	2,344
Streptococcus pneumoniae (PRSP)	Clinical	99.9998	99.9998	99.9998	99.9998	293
Yeast and Fungal Strains:						
Candida albicans	ATCC 10231	99.9999	99.9999	99.9999	99.9999	12,500
Candida albicans	Clinical	99.9999	99.9998	99.9998	99.9999	4,687
Candida tropicalis	ATCC 750	99.9999	99.9999	99.9999	99.9999	2,344
Candida tropicalis	Clinical	99.9999	99.9999	99.9999	99.9999	1,172
Aspergillus fumigatus	ATCC 66638	-	99.9998	99.9998	99.9998	4,687
Aspergillus niger	ATCC 16404	-	92.5	98.7596	99.8192	9,375
Aspergillus versicolor	ATCC 10072	-	99.9999	99.9999	99.9999	9,375
Blastomyces dermatitidis	ATCC 14112	-	99.9667	99.9667	99.9667	2,344
Epidermophyton floccosum	ATCC 52062	-	99.9936	99.9936	99.9936	75,000
Microsporum audouinii	ATCC 42558	-	99.9706	99.9706	99.9706	3,125
Microsporum canis	ATCC 14055	-	99.9981	99.9981	99.9981	12,500
Microsporum gypseum	ATCC 10215	-	99.9967	99.9967	99.9967	4,687
Penicillium notatum	ATCC 9478	-	99.9999	99.9999	99.9999	18,750
Penicillium roqueforti	ATCC 10110	-	99.9998	99.9998	99.9998	4,687
Rhizopus stolonifer	ATCC 14038	-	99.9867	99.9867	99.9867	37,500
Trichoderma viride	ATCC 26802	-	99.9996	99.9996	99.9996	4,687
Trichophyton ajelloi	ATCC 28454	-	99.9974	99.9974	99.9974	9,375
Trichophyton mentagrophytes	ATCC 26323	-	95.1756	98.2366	99.6412	9,375
Trichophyton rubrum	ATCC 52024	-	99.9958	99.9958	99.9958	9,375
Trichophyton tonsurans	ATCC 28942	-	99.9971	99.9971	99.9971	4,687
Viral Strains:						
Herpes Simplex, Type 1	ATCC VR-733	-	99.97	99.98	99.98	-
Herpes Simplex, Type 2	ATCC VR-734	-	99.97	99.98	99.98	-
HIV-1	Vanderbilt Univ.	-	99.9	99.9	99.9	-
Influenza, Type A_2	ATCC VR-544	-	99.99	99.994	99.997	-
Rhinovirus, Type 37	ATCC VR-1147	-	99.994	99.99	99.997	-
Rotavirus	ATCC VR-2018	-	99.9	99.9	99.9	-
Feline Panleukopenia	ATCC VR-648	-	90.0	90.0	90.0	-

ATCC - American Type Culture Collection

SUMMARY: *PREVACARE* Antimicrobial Hand Gel demonstrated excellent broad spectrum and fast antimicrobial activity against Gram positive, Gram negative, antibiotic-resistant, and spore-forming bacteria, foodborne bacterial pathogens, mycobacteria, yeast, fungal, and viral pathogens.*

*Trademark

In Vivo Antimicrobial Testing: Health Care Personnel Handwash (Rub)

OBJECTIVE: The *in vivo* antimicrobial activity of PREVACARE* Antimicrobial Hand Gel was measured on 11 human subjects against an antibiotic-resistant marker organism (*Serratia marcescens*) by an independent laboratory. The ASTM Health Care Personnel Handwash test modified for a waterless hand rub formulation measures antimicrobial effectiveness of topical formulations against transient microorganisms. PREVACARE* Antimicrobial Hand Gel was designed to assist health care providers reduce the risk of nosocomial infections through reduction of transient flora when water is not readily available.

TEST DESIGN:

Health Care Personnel Handwash Test

Reference: American Society of Testing and Materials E1174 Standard Test Method for Evaluation of Health Care Personnel Handwash Formulation.

1) Human subjects were recruited and randomly assigned to each formulation in the study.
2) A 30 second cleansing wash was performed to remove dirt and oils.
3) Each subject spread 5 ml of *Serratia marcescens* (ATCC 14756) at 1 x 10^8 cfu/ml over the hands for 45 seconds *S. marcescens* (ATCC 14756) is resistant to amoxicillin, ampicillin, cefuroxime, cephalothin, nitrofurantoin, and tetracycline.
4) The bacteria was allowed to air dry on the hands for 2 minutes.
5) The bacterial contamination (Steps 2-4) were repeated eleven times over the 8-10 hour study period.
6) Following the first contamination, a measurement of the total bacterial population (baseline) on each hand was taken using the glove/bag-massage technique described below (Steps 9-13).
7) Following the subsequent ten bacterial contaminations, each subject applied 5 ml of the test formulation. The subjects rubbed the hands together vigorously until dry.
8) Following the application, the hands were inserted into sterile, low bioburden plastic bags.
9) 75mls Stripping Solution was added to each bag. For Wash 10, a neutralizer was included in the Stripping Solution to quench antimicrobial activity.
10) The fluid-filled bags were massaged for 1 minute to recover the surviving bacteria.
11) The massage fluid was further diluted, plated, and incubated in neutralizing media.
12) The surviving bacteria were enumerated and $\log_{10}$ reductions were calculated.
13) The results of the study are shown in the following table and figure.

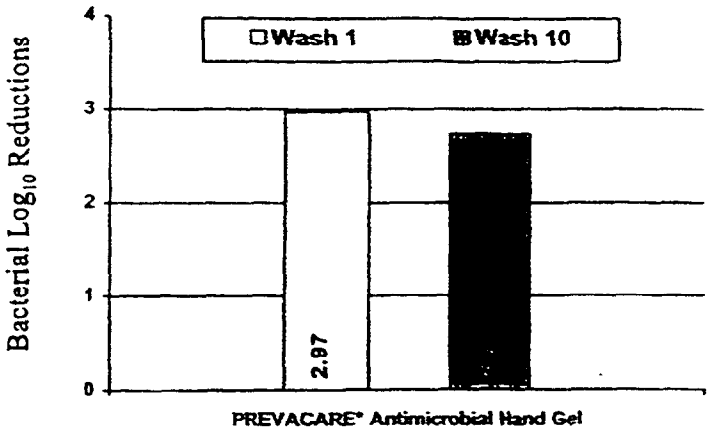
FDA REFERENCE: FDA Tentative Final Monograph Tentative Final Monograph for Health-Care Antiseptic Drug Products; Proposed Rule. June 17, 1994.

TEST RESULTS:

Formulation	Test Organism	Contact Time/ Gel Volume	No. of Subjects	$\log_{10}$ Reductions	
				Wash 1	Wash 10
PREVACARE* Antimicrobial Hand Gel (60% ethyl alcohol)	Serratia marcescens (ATCC 14756)	Until Dry / 5.0 ml	11	2.97	2.74

*Trademark

Health Care Personnel Handwash (Rub) Test Results



SUMMARY:

PREVACARE* Antimicrobial Hand Gel demonstrated excellent antimicrobial activity on the skin.

PREVACARE* Antimicrobial Hand Gel effectively reduced transient microbial pathogens from the hands following single and multiple uses.

PREVACARE* Antimicrobial Hand Gel may be recommended for health care providers to assist in the reduction of the hand carriage of potential nosocomial pathogens.

PREVACARE* Antimicrobial Hand Gel Demonstrated Safe and Mild for the Skin

OBJECTIVE:

Five independent studies were conducted to measure the safety and mildness of PREVACARE* Antimicrobial Hand Gel following single and repeated applications.

TEST DESIGN:

Primary Irritation Patch Test - This test was conducted on 103 human subjects to measure the irritation potential of the formulation following a 48 hour continuous, semi-occlusive exposure.

Repeated Insult Patch Test - This test was conducted on 214 human subjects to assess the sensitization potential of the formulation following repeated applications.

21-Day Cumulative Irritation Test - This test was to determine cumulative (21-day) dermal irritation potential on 25 human subjects following repeated daily applications.

Allergic Contact Dermatitis, Phototoxicity and Photoallergic Contact Dermatitis Test - This test was conducted to determine the potential to induce allergic contact dermatitis, phototoxicity, and phoallergic contact dermatitis in 26 human subjects.

Acute Oral Toxicity LD_{50} Test - To determine the acute oral safety of the formulation according to the Cosmetic, Toiletry, and Fragrance Association Safety Testing Guidelines.

SUMMARY:

PREVACARE* Antimicrobial Hand Gel was found non-irritating, non-sensitizing, non-photosensitizing, and non-phototoxic.

PREVACARE* Antimicrobial Hand Gel was not a primary skin irritant, did not induce allergic contact dermatitis, and was non-toxic by oral ingestion.

*Trademark

In Vivo Antimicrobial Testing: Surgical Scrub Test

OBJECTIVE: The immediate and persistent (residual) *in vivo* antimicrobial activity of PREVACARE* Antimicrobial Hand Gel was measured against resident skin flora by an independent laboratory on 36 human subjects. The ASTM Surgical Scrub Test assesses the utility and effectiveness of formulations for surgical scrubbing.

TEST DESIGN:

Surgical Scrub Test

Reference: American Society of Testing and Materials E1115 Standard Test Method for Evaluation of Surgical Hand Scrub Formulations.

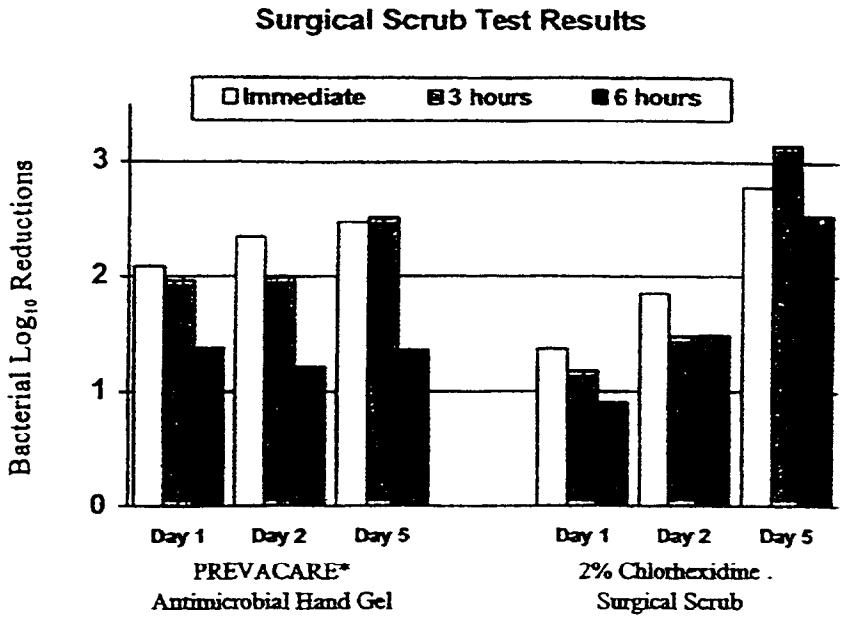
1) Human subjects were recruited, prescreened for baseline bacterial counts 1.5 x 10^5 cfu/hand, and randomly assigned to each formulation in the study.
2) To initiate the study (Day 1), the subjects performed a surgical scrub procedure using 5.0 ml of the test material.
3) For PREVACARE* Antimicrobial Hand Gel, the subjects distributed the test material over the hands and lower third of the forearms until dry. No scrub brushes were utilized. The procedure was repeated using an additional 5.0 ml of gel. For the 2% Chlorhexidine Scrub, two 3 minute surgical scrubs were performed using a scrub brush and 5.0 ml of test material for each scrub.
4) Following the scrub, the hands were inserted into sterile, powder-free surgical gloves.
5) 75mls Stripping Solution was added to the gloves immediately, after 3 hours, or after 6 hours.
6) The fluid-filled gloves were massaged for 1 minute to recover the surviving bacteria.
7) The glove fluid was further diluted, plated, and incubated in neutralizing media.
8) The surviving bacteria were enumerated and $\log_{10}$ reductions were calculated.
9) The surgical scrub (Steps 2-4) was repeated eleven times over the 5 day study. The hands were sampled three times following the initial scrub (Day 1), the second scrub (Day 2), and the final scrub (Day 5).
10) The results of the study are shown in the following table and figure.

FDA REFERENCE: FDA Tentative Final Monograph Tentative Final Monograph for Health-Care Antiseptic Drug Products; Proposed Rule. June 17, 1994.

TEST RESULTS:

Formulation	Contact Time/ Volume	No. of Subjects	Study Day /Scrub No.	$\log_{10}$ Reductions		
				Immediate	3 hour	6 hour
PREVACARE* Antimicrobial Hand Gel (60% ethyl alcohol)	Until Dry / 2 aliquots of 5.0 ml	18	Day 1 / Scrub 1	2.09	1.97	1.38
			Day 2 / Scrub 2	2.35	1.99	1.22
			Day 5 / Scrub 11	2.48	2.52	1.36
2% Chlorhexidine Surgical Scrub	6 min / 2 aliquots of 5.0 ml	18	Day 1 / Scrub 1	1.37	1.18	0.91
			Day 2 / Scrub 2	1.85	1.48	1.49
			Day 5 / Scrub 11	2.78	3.15	2.54

*Trademark



MMARY: ***PREVACARE* Antimicrobial Hand Gel demonstrated fast-acting, persistent (residual) antimicrobial activity on the skin against resident bacterial flora.***

PREVACARE* Antimicrobial Hand Gel effectively reduces and prevents regrowth of resident skin flora under surgical gloves for up to 6 hours following single and multiple uses.

PREVACARE* Antimicrobial Hand Gel may be used between surgical procedures in accordance with hospital protocol.

PREVACARE* Antimicrobial Hand Gel Demonstrated Effective Preservative Activity

Gram Positive ⌘ Gram Negative ⌘ Yeast ⌘ Fungi

OBJECTIVE: The *in vitro* antimicrobial preservative activity of the formulation was measured against Gram positive, Gram negative, and antibiotic-resistant bacteria, yeast, and fungi.

TEST DESIGN: **United States Pharmacopeia Preservative Efficacy Test**
Reference: United States Pharmacopeia. 1997. Microbiological Tests: Antimicrobial Preservatives-Effectiveness. *In* United States Pharmacopeia 23, Chapter <51>. pp. 1681.

SUMMARY: ***PREVACARE* Antimicrobial Hand Gel is effectively preserved against Gram positive, Gram negative, and antibiotic-resistant bacteria, yeast, and fungal strains.***

*Trademark

In Vivo Antimicrobial Testing: Cup Scrub Test

OBJECTIVE: An independent laboratory measured the persistent, *in vivo* bactericidal activity of PREVACARE* Antimicrobial Hand Gel against *Staphylococcus aureus* inoculated onto the skin of 48 human subjects in two clinical studies. The ASTM Cup Scrub Test measures immediate and persistent bactericidal activity of topical formulations against normal skin flora or marker test organisms.

TEST DESIGN: **Cup Scrub Test**

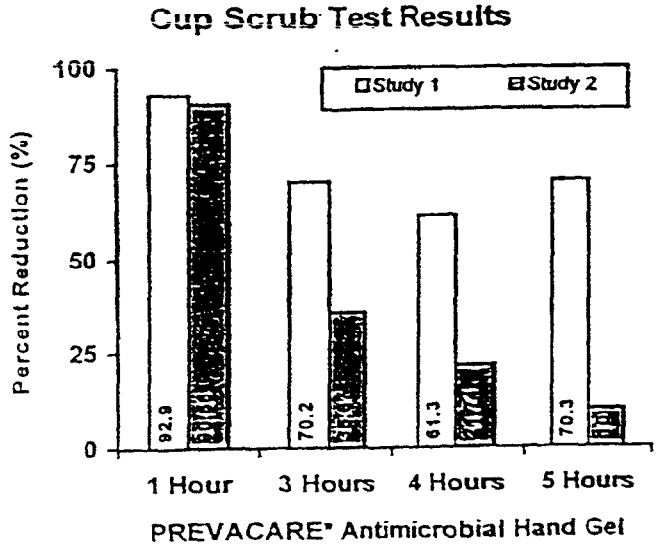
Reference: American Society of Testing and Materials E1874 Standard Test Method for The Evaluation of Antibacterial Washes By Cup Scrub Technique.

1) Human subjects were recruited and randomly assigned within each of the studies.
2) Following preparation of the forearms, the subjects applied 2.5 ml PREVACARE* Antimicrobial Hand Gel over the surface of one forearm from wrist to elbow using even pressure. The other forearm served as the untreated control.
3) Following air drying, *Staphylococcus aureus* (ATCC 6538) was inoculated at 1, 3, 4, or 5 hours after application of the gel onto the forearm at three delineated test sites. For Study 1, approximately 1 x 10^4 cfu was evenly applied to each test site. For Study 2, approximately 1 x 10^6 cfu was applied to each test site.
4) The inoculated sites were immediately occluded for 30 minutes.
5) Following occlusion, the surviving bacteria were recovered from the test and control sites using glass cups or cylinders pressed firmly against each site.
6) The surviving bacteria were enumerated and $\log_{10}$ reductions were calculated.
7) The results of the study are shown in the following table and figure.

TEST RESULTS:

Formulation	Test Organism	Gel Volume	Subjects	Elapsed Time After Use	Percent Reduction	
					Study 1	Study 2
Baseline	*S. aureus* (ATCC 6538)	Untreated	48	n/a	(3.0 $\log_{10}$)	(5.0 $\log_{10}$)
PREVACARE* Antimicrobial Hand Gel (60% ethyl alcohol)		2.5 ml	12	1 Hour	92.9% †	90.56% †
			12	3 Hours	70.2% †	35.67% †
			12	4 Hours	61.3% †	21.74%
			12	5 Hours	70.3% †	10.00%

† - Statistically significant bactericidal activity.



SUMMARY:

PREVACARE* Antimicrobial Hand Gel demonstrated persistent bactericidal activity of greater than 1 log (90%) reduction against Staphylococcus aureus on the skin for up to 1 hour (Study 1 and 2).

PREVACARE* Antimicrobial Hand Gel may be recommended for health care providers to assist in the reduction of hand carriage of potential nosocomial pathogens.

*Trademark

In Vivo Antimicrobial Testing: Agar Patch Test

OBJECTIVE: An independent laboratory measured the skin substantivity and residual bacteriostatic activity of PREVACARE* Antimicrobial Hand Gel against *Staphylococcus aureus* inoculated onto the skin of 48 human subjects. The ASTM Agar Patch Test measures immediate and persistent bacteriostatic activity of topical formulations against marker test organisms applied to the skin on agar plates.

TEST DESIGN: **Agar Patch Test**

Reference: American Society of Testing and Materials E1882 Standard Test Method for The Evaluation of Antibacterial Washes By Agar Patch Technique.

1) Human subjects were recruited and randomly assigned within each study.

2) Following preparation of the forearms, the subjects applied 2.5 ml PREVACARE* Antimicrobial Hand Gel over the surface of one forearm from wrist to elbow using even pressure. The other forearm served as the untreated control.

3) Agar plates inoculated with 200-300 cfu *Staphylococcus aureus* (ATCC 6538) were attached to the subjects' forearms in triplicate at 1, 3, 4, or 5 hours after gel application. The plates remained on the arm approximately 30 minutes.

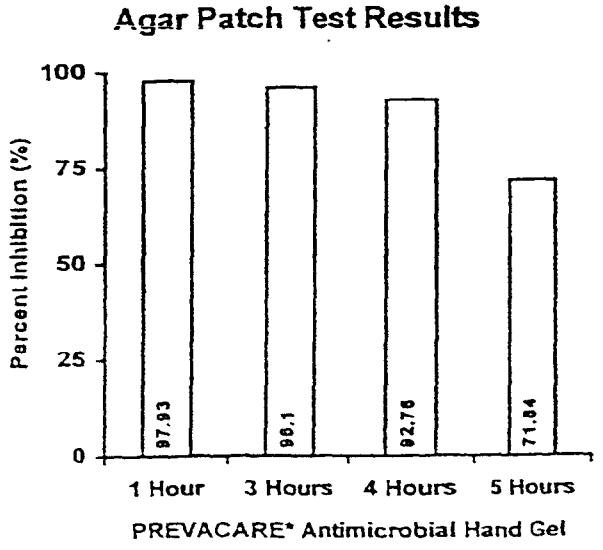
4) The surviving bacteria were enumerated and percent inhibition from the untreated control was calculated.

5) The results of the study are shown in the following table and figure.

RESULTS:

Formulation	Test Organism	Gel Volume	Subjects	Elapsed Time After Use	Percent Inhibition
PREVACARE* Antimicrobial Hand Gel (60% ethyl alcohol)	*S. aureus* (ATCC 6538)	2.5 ml	12	1 Hour	97.93 †
			12	3 Hours	96.10 †
			12	4 Hours	92.76 †
			12	5 Hours	71.84 †
Competitive Hand Rinse (62% alcohol)			12	1 Hour	00.00

† - Statistically significant bacteriostatic activity.



SUMMARY:

PREVACARE* Antimicrobial Hand Gel demonstrated persistent bacteriostatic activity against Staphylococcus aureus on the skin for up to 4 hours.

*Trademark

In Vivo Antimicrobial Testing: Agar Patch Wash Off Test

OBJECTIVE: An independent laboratory measured the skin substantivity and residual bacteriostatic activity of PREVACARE* Antimicrobial Hand Gel against *Staphylococcus aureus* inoculated onto the skin of 48 human subjects following washing with soap and water or rinsing with water alone. The ASTM Agar Patch Test measures immediate and persistent bacteriostatic activity of topical formulations against marker test organisms applied to the skin on agar plates.

TEST DESIGN:

Antimicrobial Agar Patch Test

Reference: American Society of Testing and Materials E1882 Standard Test Method for The Evaluation of Antibacterial Washes By Agar Patch Technique.

1) Human subjects were recruited and randomly assigned within each study.

2) Following preparation of the forearms, the subjects applied 2.5 ml PREVACARE* Antimicrobial Hand Gel over the surface of each forearm from wrist to elbow using even pressure. The other forearm served as the non-rinsed control.

3) One of the subjects' forearms was randomly assigned to wash with soap and water or rinse with water alone. The wash/rinse procedure occurred immediately or 1 hour following application of the gel. The water rinse was performed by placing the forearm under flowing water for 30 seconds and allowing the arm to air dry. The soap and water wash was performed by briefly wetting the arms, rubbing a non-antimicrobial bar soap over the arm for 5 seconds, lathering for 10 seconds, and rinsing under flowing water for 30 seconds.

4) Five minutes following washing/rinsing, agar plates inoculated with 200-300 cfu *Staphylococcus aureus* (ATCC 6538) were attached to the subjects' forearms in triplicate. The plates remained on the arm approximately 30 minutes.

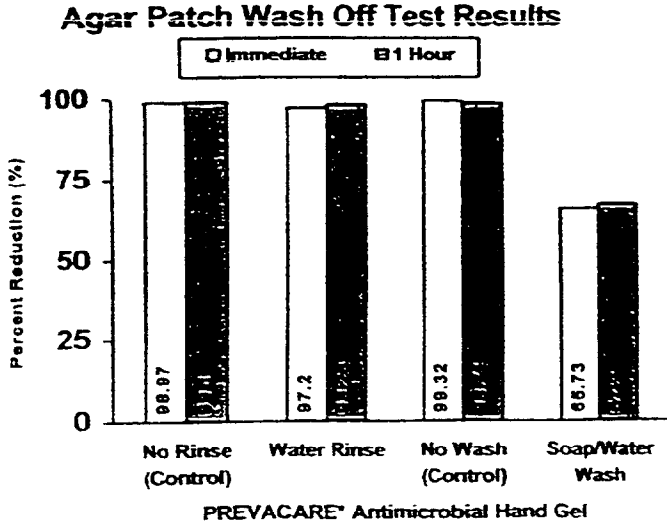
5) The surviving bacteria were enumerated and $\log_{10}$ reductions were calculated.

6) The results of the study are shown in the following table and figure.

TEST RESULTS:

Formulation	Test Organism	Gel Volume	Subjects	Procedure	Percent Reduction	
					Immediate	1 Hour
PREVACARE* Antimicrobial Hand Gel (60% ethyl alcohol)	*S. aureus* (ATCC 6538)	2.5 ml	24	No Rinse (Control)	98.97 †	99.10 †
				Water Rinse	97.20 †	98.26 †
			24	No Wash (Control)	99.32 †	98.71 †
				Soap/Water Wash	65.73 †	67.20 †

† - Statistically significant bacteriostatic activity.



SUMMARY:

PREVACARE* Antimicrobial Hand Gel demonstrated immediate and residual antimicrobial activity against Staphylococcus aureus on the skin following rinsing with water.

PREVACARE* Antimicrobial Hand Gel demonstrated that residual bacteriostatic activity is not removed by water rinsing but may be readily removed with soap and water wash when desired.

*Trademark

PREVACARE* Antimicrobial Hand Gel *Moisturized* Skin After Single Application

OBJECTIVE: An independent clinical study on 40 human subjects was conducted to measure the moisturization efficacy on normal and moderately dry skin of single applications of PREVACARE* Antimicrobial Hand Gel. The study utilized dual instrument analysis to quantitatively measure the skin surface impedance to quantify the relative hydration of the stratum corneum.

TEST DESIGN: **Skin Moisturization Study**

1) 40 human subjects completed the study. The study included two groups based on the clinical characterization of exhibiting mild to moderate dry skin or normal, non-dry skin.

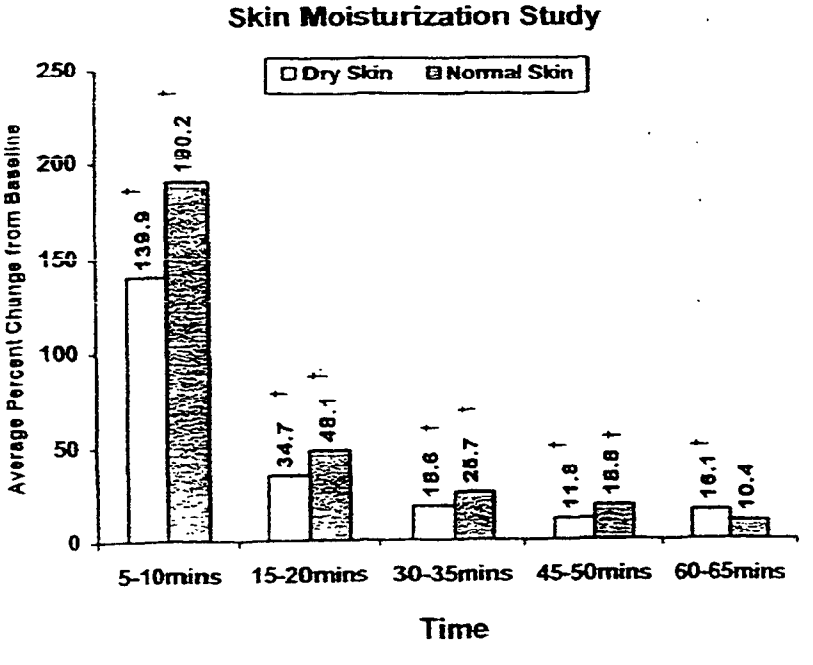
2) Using randomized sites on the lower leg and following equilibration to room conditions, instrumental analysis was performed on untreated sites to quantify the relative hydration of the stratum corneum (measure of skin moisturization content) prior to treatment with the test materials. Randomly, one leg was used for treatment and the other leg served as the untreated control.

3) A single application of 400 µl of test material was applied by clinical staff to the treatment site.

4) Instrumental measurements were performed on treated and untreated test sites at approximately 5-10, 15-20, 30-35, 45-50, and 60-65 minutes after application.

5) The results of the analysis are shown in the figure below.

TEST RESULTS: **For dry skin, PREVACARE* Antimicrobial Hand Gel demonstrated statistically significant immediate skin moisturization and for up to 1 hour following a single application. For normal skin, PREVACARE* Antimicrobial Hand Gel demonstrated statistically significant immediate skin moisturization and for up to 45 minutes following a single application.**



† - Statistically Significant Moisturization

SUMMARY:

A single application of PREVACARE* Antimicrobial Hand Gel was effective in significantly moisturizing normal and dry skin immediately and up to 1 hour after application.

PREVACARE* Antimicrobial Hand Gel moisturized skin after a single application.

*Trademark

PREVACARE* Antimicrobial Hand Gel Demonstrated Non-drying After Repeated Application

OBJECTIVE: An independent clinical study on 43 human subjects was conducted to measure the moisturization efficacy on normal and moderately dry skin of single and repeated applications of PREVACARE* Antimicrobial Hand Gel. The study utilized dual instrument analysis to quantitatively measure the skin surface impedance to quantify the relative hydration of the stratum corneum.

TEST DESIGN: **Skin Moisturization Study**

1) 43 human subjects completed the study. The study included two groups based on the clinical characterization of exhibiting mild to moderate dry skin or normal, non-dry skin.

2) Using randomized sites on the lower leg and following equilibration to room conditions, instrumental analysis was performed on untreated sites to quantify the relative hydration of the stratum corneum (measure of skin moisturization content) prior to treatment with the test materials.

3) The initial kinetic phase of the study involved a single application of 10 mg/cm^2 of test material to all subjects.

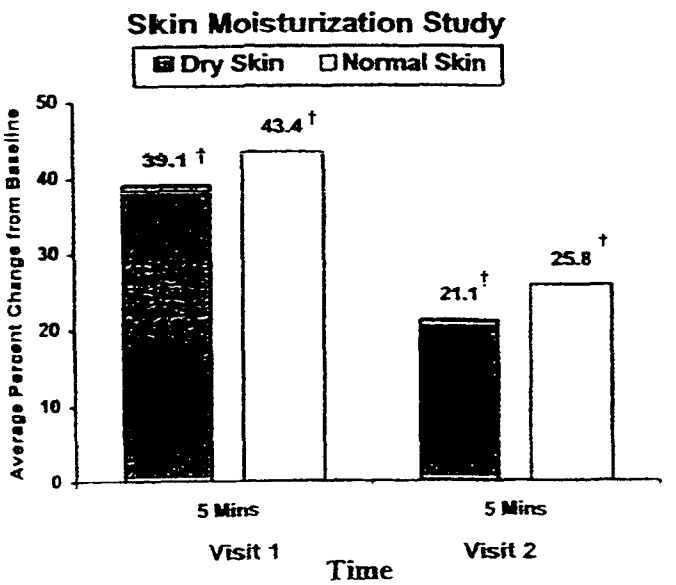
4) Instrumental measurements were performed on treated and untreated test sites at 5 mins, 1 and 3 hours after application.

5) Subjects were then provided with test material for home use on the test site four times daily for 7 days.

6) Following one week of usage, Steps 3 and 4 were repeated. Instrumental measurements were performed on treated and untreated test sites at 5 mins, 1 and 3 hours.

7) The results of the analysis are shown in the figure below.

TEST RESULTS: **The study revealed that a single and repeated applications of PREVACARE* Antimicrobial Hand Gel were effective in statistically significantly moisturizing the skin immediately after application for both the dry skin and normal non-dry skin subjects.**



† - Statistically Significant Moisturization

SUMMARY:

For normal and dry skin, PREVACARE* Antimicrobial Hand Gel demonstrated non-drying with immediate skin moisturization following single and repeated applications.

The percent increase in moisturization was statistically significant at 5 minutes among normal as well as dry skin population.

*Trademark

In Vivo Antimicrobial Testing: Antiviral Fingerpad Test

OBJECTIVE:

The *in vivo* antiviral activity of PREVACARE* Antimicrobial Hand Gel against Rhinovirus was measured using the ASTM Fingerpad Test by an independent laboratory. The method involves the inoculation of a concentrated viral challenge to the fingertips of 12 human subjects which is then allowed to dry and exposed to the topical preparation without scrubbing or agitation for 20 seconds.

This study and the *in vitro* antiviral data provide an initial profile of the formulations' antiviral spectrum, speed of inactivation, and activity on the skin. Additional research is required in this area to compile a complete profile of the antiviral activity of the formulation and the relationship of this data to the reduction of transmission of viral particles.

TEST DESIGN:

Antiviral Fingerpad Test

Reference: American Society of Testing and Materials E1838 Standard Test Method for Determining the Virus-Eliminating Effectiveness of Liquid Hygienic Handwash Agents Using the Fingerpads of Adult Volunteers.

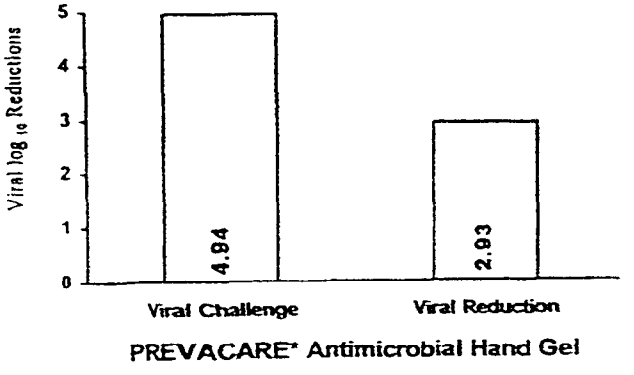
Test Method:

1) 12 Human subjects completed the study.
2) Following preparation of the hands, a mixture of Rhinovirus (approximately 1×10^5 pfu) and bovine mucin to simulate nasal secretions was inoculated onto each of the five fingerpads of both hands of each subject.
3) The viral inoculum was allowed to dry for 20-25 minutes.
4) The test material was applied to the test site via inversion of a small vial containing 1.0 ml of the test material onto the fingerpad for 20 seconds.
5) Following the 20 seconds contact, the inside rim of the vial was used to scrape the remaining liquid from the skin. The technique was repeated for each fingerpad for each subject.
6) A rinse was performed by inverting a vial containing 15 ml of 200 ppm hard water against the fingerpad for 5 full inversions.
7) Various controls are performed to measure the inoculum of virus, the amount of virus applied to the fingerpad, and the virus surviving after drying.
8) The surviving viral particles were eluted using the vial inversion method above.
9) The surviving viral particles were enumerated and $\log_{10}$ reductions were calculated.
10) The results of the study are shown in the following table and figure.

TEST RESULTS:

Formulation	Test Strain	No. of Subjects	Gel Volume	Contact Time	Log_{10} Dried Viral Challenge	Log_{10} Reduction
PREVACARE* Antimicrobial Hand Gel (60% ethyl alcohol)	Rhinovirus	12	1.0 ml	20 sec	4.94	2.93 † (Percent Reduction=99.88%)

† - Statistically significant activity



SUMMARY:

PREVACARE* Antimicrobial Hand Gel demonstrated a statistically significant reduction of Rhinovirus, the common cold virus, on the skin of human subjects.

PREVACARE* Antimicrobial Hand Gel has been demonstrated to assist in the reduction of the hand carriage of Rhinovirus, the common cold virus.

*Trademark

PREVACARE* Antimicrobial Hand Gel Demonstrated Compatible with Latex Gloves

OBJECTIVE: An analysis of the physical integrity of latex gloves was conducted to measure glove tear resistance and tensile strength following 6 hours of direct contact with PREVACARE* Antimicrobial Hand Gel.

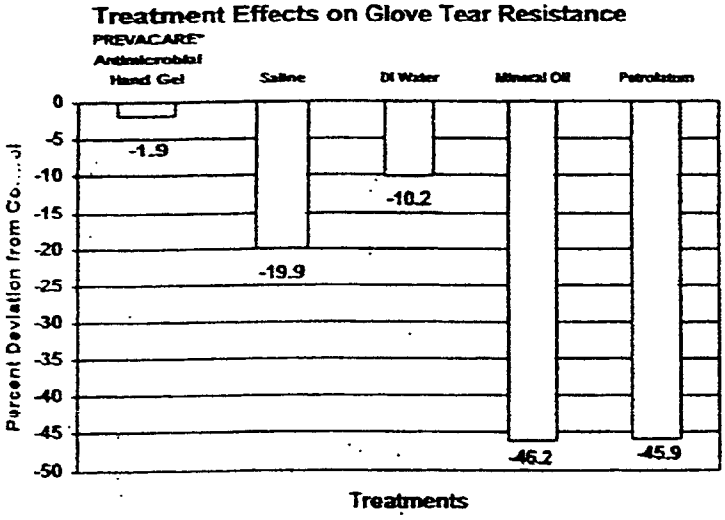
TEST DESIGN:

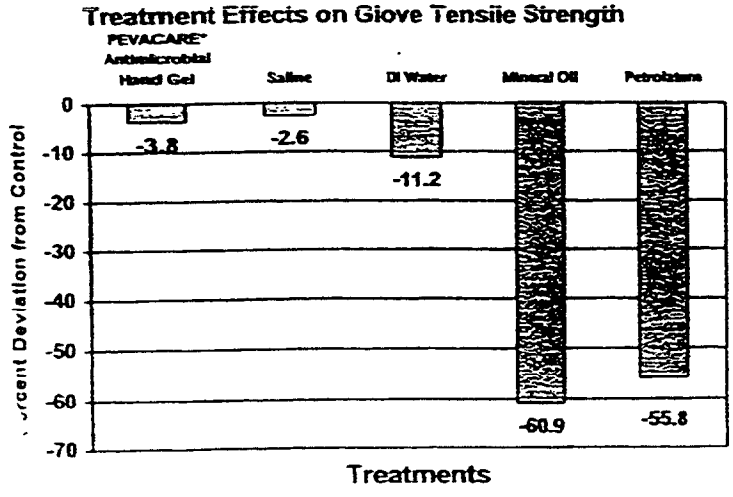
Latex Glove Tear Resistance Test

Reference: American Society of Testing and Materials. 1991. Standard Test Method D624-91 Tear Strength of conventional Vulcanized Rubber and Thermoplastic Elastomers.

Latex Glove Tensile Strength Test

Reference: American Society of Testing and Materials. 1992. Standard Test Method D412-92 Vulcanized Rubber and Thermoplastic Rubbers and Thermoplastic Elastomers - Tension.





1) Sections of 15 Johnson & Johnson Medical MICROTOUCH* Powdered Latex Surgical Gloves were treated with the test formulations and conditioned for 6 hours at 30°C.

2) Using an automated instrument, the tear resistance and tensile strength of the gloves were measured at 20°C. The tear resistance is the force per unit glove thickness necessary to tear the latex glove. The tensile strength is the maximum tensile stress needed to stretch the glove until it ruptures.

3) The results were statistically evaluated and are shown in the following figures.

TEST SUMMARY:

No statistical differences from the untreated controls were observed with PREVACARE* Antimicrobial Hand Gel.

SUMMARY:

PREVACARE* Antimicrobial Hand Gel does not alter, the physical integrity of latex gloves for 6 hours.

PREVACARE* Antimicrobial Hand Gel has been demonstrated to be compatible for use with latex gloves.

*Trademark

The Relationship Between Upper Respiratory Infections and Hospital Admissions for Asthma: A Time-trend Analysis

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We have shown that viruses are associated with 80 to 85% of asthma exacerbations in school-age children in the community. We hypothesize that viral infections are also associated with severe attacks of asthma precipitating hospital admissions. To investigate this, we conducted a time-trend analysis, comparing the seasonal patterns of respiratory infections and hospital admissions for asthma in adults and children. During a 1-yr study in the Southampton area of the United Kingdom, 108 school-age children monitored upper and lower respiratory symptoms and took peak expiratory flow rate (PEFR) recordings. From children reporting a symptomatic episode or a decrease in PEFR, samples were taken for detection of viruses and atypical bacteria. A total of 232 respiratory viruses and four atypical bacteria were detected. The half-monthly rates of upper respiratory infection were compared with the half-monthly rates for hospital admissions for asthma (International Classification of Diseases [ICD] code 493) for the same time period for the hospitals serving the areas from which the cohort of schoolchildren was drawn. The relationships of upper respiratory infections and hospital admissions for asthma with school attendance were studied. Strong correlations were found between the seasonal patterns of upper respiratory infections and hospital admissions for asthma ($r = 0.72$; $p < 0.0001$). This relationship was stronger for pediatric ($r = 0.68$; $p < 0.0001$) than for adult admissions ($r = 0.53$; $p < 0.01$). Upper respiratory infections and admissions for asthma were more frequent during periods of school attendance (87% of pediatric and 84% of total admissions), than during school holiday periods ($p < 0.001$). These relationships remained significant when allowance was made for linear trend and seasonal variation using multiple regression analysis ($p < 0.01$). Not surprisingly, school attendance, because it is a major factor in respiratory virus transmission, was found to be a major confounding variable in children. This study demonstrates that upper respiratory viral infections are strongly associated in time with hospital admissions for asthma in children and adults. Rhinoviruses were the major pathogen implicated, and the majority of viral infections and asthma admissions occurred during school attendance. **Johnston SL, Pattemore PK, Sanderson G, Smith S, Campbell MJ, Josephs LK, Cunningham A, Robinson BS, Myint SH, Ward ME, Tyrrell DAJ, Holgate ST. The relationship between upper respiratory infections and hospital admissions for asthma: a time-trend analysis.**

AM J RESPIR CRIT CARE MED 1996;154:654–60.

Asthma is a major cause of hospital admissions at all ages (1, 2), and accounts for around 2,000 deaths in the United Kingdom (UK) each year (3). There has been a substantial increase in asthma admission rates in both the UK (4) and the United States (5) over the past three decades. Previous hospital admissions for asthma are known to be the major risk factor for asthma mortality (6). Identification of the major cause and seasonal pattern of such admissions would have important implications in targeting future treatment strategies for asthma. Despite numerous previous studies on the subject, the epidemiologic evidence for an association between viral infections and asthma exacerbations is controversial: the weighted average viral detection rates in previous studies of exacerbations of asthma in children are 24% for incidental studies and 31.9% for prospective studies, whereas that in the three adult studies of asthma exacerbation is only 13.3% (7). Storr and Lenney cite indirect evidence of a role for virus infections, arguing that the peaks of pediatric hospital admissions for asthma that they observed at the start of each new school term suggested triggering of asthma by viruses that had been acquired during vacations and rapidly spread in school populations (8).

In many previous epidemiologic studies of viral infections and exacerbations of asthma, virus detection rates have been low, owing to methodologic problems in detecting and identifying respiratory viruses (9, 10), particularly rhinoviruses and coro-

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naviruses, which are thought to account for approximately two-thirds of common colds (11). We have recently developed polymerase chain reaction (PCR) assays for rhinoviruses (12), human coronaviruses (13), and *Chlamydia pneumoniae* (14). Using these assays in combination with comprehensive standard virologic techniques, we have recently reported that viral upper respiratory infections are associated with 80 to 85% of asthma exacerbations in children in the community (15). However, many of the children in this recent study were not diagnosed as asthmatic, and none of the episodes studied was of sufficient severity to lead to a hospital admission. Another study, in adults, has found similar results in adult asthmatic subjects in the community, although the viral detection rate was considerably lower (44% of exacerbations), possibly as a result of less intensive surveillance of the subjects, less comprehensive viral detection methods, and the use of nasal and throat swabs instead of nasal aspirate samples (16). Thus, although there is now good evidence of an important role for viral infections in asthma exacerbations in the community, the role of upper respiratory infections in severe asthma leading to hospital admissions remains unknown.

The children taking part in our previous study (15) form a sentinel cohort in which the prevalence of detected upper respiratory infection is likely to accurately reflect the prevalence in the community at that time. In order to examine the relationship between upper respiratory infections and severe attacks of asthma leading to hospital admission, we performed a time-trend analysis, comparing the seasonal trends for upper respiratory infections in the cohort of children studied and hospital admissions for asthma over the same time period in the hospitals serving the area from which the cohort of children was drawn. We also examined the relationship between school attendance and upper respiratory infection and hospital admission rates.

METHODS

Subjects

In a longitudinal study of the role of viral infections in children with respiratory symptoms, 114 children aged 9 to 11 yr (62 male and 52 female), who lived in the Southampton area of the UK and who had reported wheezing and/or persistent cough in response to a postal questionnaire, recorded daily upper and lower respiratory symptoms and twice daily peak expiratory flow rates (PEFR) for 1 yr (15). This study lasted from April 3, 1989 to May 7, 1990. Children recorded daily upper and lower respiratory tract symptoms and morning and evening PEFR. In the event of an episode of respiratory symptoms or a decrease in PEFR, the child was visited at home so that nasal aspirate and serum specimens could be obtained for virologic testing. Details of the selection of the cohort and of the surveillance methods have been published (15).

Viral Detection

Viruses were detected through cell culture with Ohio HeLa, Clone 16, MDCK, LLC-MK_2, and HEp2 cell lines; immunofluorescence assays for respiratory syncytial (RS) virus, influenza type A, parainfluenza types 1 through 3 and adenovirus. Enzyme linked immunosorbent assay (ELISA) was used for the same viruses and for coronaviruses 229E and OC43, and polymerase chain reaction (PCR) was used for picornaviruses (rhinovirus and enterovirus) and coronavirus. Full details of the methods used have been published (12, 13, 15).

Detection of Atypical Bacteria

Testing for *Mycoplasma pneumoniae* (MP) was done with a solid-phase reverse ELISA for the detection of IgM antibodies to MP in serum (Incstar, Wokingham, UK). The assay was done according to the manufacturer's instructions, and positive specimens were confirmed by repeat testing. *Chlamydia pneumoniae* (CP) was detected with a single-stage PCR using species-specific primers selected from the CP major outer membrane protein gene and flanking sequences (14). Appropriate controls were included in each run, and positive samples were confirmed by repeat testing with a sample taken from a different stored aliquot from the original positive sample.

Asthma Admission Data

Details concerning the dates of admission for all asthma admissions (International Classification of Diseases [ICD] code 493) to the hospitals in the Wessex Regional Health Authority (WRHA, one of 14 Health Regions in England) and the Southampton District Health Authority (SDHA, one of nine Health Districts in the WRHA) between April 1, 1989 and March 30, 1990 were obtained from the WRHA Information Directorate. Total numbers of asthma admissions for all ages, and asthma admissions for pediatric (< 20 yr of age) and adult (≥ 20 yr of age) age groups were obtained, and the numbers of admissions calculated for each half month (1st to 15th days, and 16th day to end of month) for the SDHA and WRHA for each age group. Population data for the SDHA and WRHA were obtained as the midyear estimates for 1989 from the UK Office of Population Censuses and Surveys. The interval of half-months was chosen as the optimum time frame to allow time trends to be discerned; however, for the analyses on school holidays, weekly intervals were used, since some holiday periods were only 1 wk in length.

Virus and Atypical Bacteria Detection Data

The number of symptomatic episodes reported by the cohort of schoolchildren from the Southampton area in which a proven upper respiratory viral or atypical bacterial infection occurred was calculated for each half month for the same time period as for asthma admissions. These data represent the seasonal pattern in the number of episodes of infection occurring in the cohort of schoolchildren. Because 17 of the episodes involved two or more infecting organisms (15), the total number of viral or atypical bacterial pathogens detected in the cohort per half-month was also calculated, so as to represent the total load of respiratory infections occurring in the community at that time.

School Holidays

The dates of the school holiday periods for the period from April 1, 1989 to March 30, 1990 were obtained from the UK Department of Education. Dates were adjusted to include the adjacent (following) weekend, in order to derive weekly rates of episodes of infection in the cohort of schoolchildren and similar weekly rates for asthma admissions (total, pediatric, and adult) for both the SDHA and the WRHA.

Statistical Analysis

Statistical analyses were done with the Minitab statistical package (Minitab Inc., State College, PA) and also, where indicated, by SPSS (SPSS Trends 6.1; SPSS Inc., Chicago, IL). Correlation analyses were done for the total number of asthma admissions for the region, and were then subdivided into pediatric and adult age groups. The dependent variable was the number of asthma admissions (total, pediatric, or adult) in each half-month or weekly time period, and the independent variable was the number of proven upper respiratory infections occurring in the cohort of children in the same time period, or the week/half-month periods immediately before or after it. In these analyses, episodes in which multiple infections were detected were treated as a single episode in order to reflect the number of episodes of infection detected in the cohort of schoolchildren. Similar analyses were done with the total number of pathogens per half-month as the independent variable, to reflect the total community load of upper respiratory infections. An important assumption governing the validity of the tests of significance is the independence of the residuals. This was tested using a Box-Pierce test, and in no case were the residuals found to be significantly correlated.

The relationships between school holiday periods and both upper respiratory infections and asthma admissions were examined by comparing the weekly rates of episodes of infection or asthma admissions in school term time and in holiday periods, using the chi-squared test.

Analysis for confounding variables was done through regression analyses with correlated errors (SPSS Trends 6.1; SPSS Inc., Chicago, IL). The confounders fitted were: (*1*) a linear trend, to allow for increases in both series; (*2*) a dummy variable, which took a value of 1 for a school holiday and of 0 otherwise; and (*3*) curves of Cos (wt) and Sin (wt), where w is $2\pi/52$ and t is time in weeks, to allow for seasonal variation.

RESULTS

For the period for which the corresponding asthma admission

TABLE 1

POPULATION AND ASTHMA ADMISSION DATA FOR THE YEAR APRIL 1989 TO MARCH 1990 FOR THE WESSEX REGIONAL HEALTH AUTHORITY (WRHA) AND SOUTHAMPTON DISTRICT HEALTH AUTHORITY (SDHA)

	WRHA			SDHA		
	Total	Pediatric	Adult	Total	Pediatric	Adult
Population	2,934,461	742,764	2,191,697	421,944	106,453	315,491
Asthma admissions	4,038	2,232	1,805	590	315	275
Admissions/1,000	1.38	3.0	0.82	1.4	2.96	0.87

data were collected (April 1, 1989 to March 30, 1990), there were 279 reported symptomatic episodes. From these, a total of 232 respiratory virus, three CP, and one MP detections were made during 221 (78.5%) proven upper respiratory infections. The median weekly number of episodes of infection was 4 (interquartile range [IQR] = 2 to 5). Full details of the types of viruses and descriptions of the asthma exacerbations associated have been reported elsewhere (15). The populations in the WRHA and the SDHA, and the numbers of admissions in each age group during the same period, are shown in Table 1. The median (IQR) number of asthma admissions per week among subjects under 20 yr of age for the SDHA and WRHA, respectively, were 5 (3 to 8.75) and 35 (29.25 to 50.5), and for subjects 20 yr of age and over were 5 (3 to 6.75) and 32 (25.5 to 40).

Strong correlations were found between the half-monthly rates of episodes of upper respiratory infection in the Southampton cohort of schoolchildren and the half-monthly rates for hospital admissions for asthma for both the WRHA and the SDHA (Figure 1). Similarly strong correlations were found when the asthma admissions were analyzed for the pediatric age group alone, and the correlations were still significant, although less strong, when asthma admissions were analyzed for the adult age group alone (Figure 1).

Similar but stronger correlations were found when the data were analyzed for the total number of infecting pathogens, to reflect the total community load of infections at that time. The correlations for the WRHA and the SDHA, respectively, were $r = 0.72$, $p < 0.0001$ and $r = 0.64$, $p < 0.001$ for total asthma admissions; $r = 0.68$, $p < 0.0001$ and $r = 0.58$, $p < 0.005$ for pediatric asthma admissions; and $r = 0.53$, $p < 0.01$ and $r = 0.44$, $p < 0.05$ for adult asthma admissions.

The seasonal patterns in the half-monthly rates of both total numbers of upper respiratory infecting organisms and of asthma admissions showed large fluctuations, with four identifiable peaks occurring in April, September/October, November/December, and January/February (Figure 2). There were clear relationships between the peaks of both respiratory infections and asthma admissions in all age groups for both the WRHA and the SDHA and the start of a new school term/half-term, although the relationship was clearly stronger for pediatric than for adult asthma admissions (Table 2).

The peaks occurred from 2 to 4 wk after the start of each new school term/half-term, with troughs occurring during school holiday periods (Figure 2). The numbers of asthma admissions occurring in the WRHA during school term-time were 3,384 (84% of the total for the year) for the total population, 1,943 (87%) for the pediatric population, and 1,441 (80%) for the adult population ($p < 0.001$).

A regression analysis allowing for the confounding variables of linear trend, seasonal variation, and school holiday periods and a correlated error confirmed these results. The unadjusted analysis showed a significant association between asthma admissions and respiratory infections. This relationship remained when allowing for the confounding variables, except for children in Southampton (probably a result of the low numbers in this group). However, in the case of pediatric admissions, the infection parameter alone was not significant when the confounding effect of school attendance was omitted (Table 3).

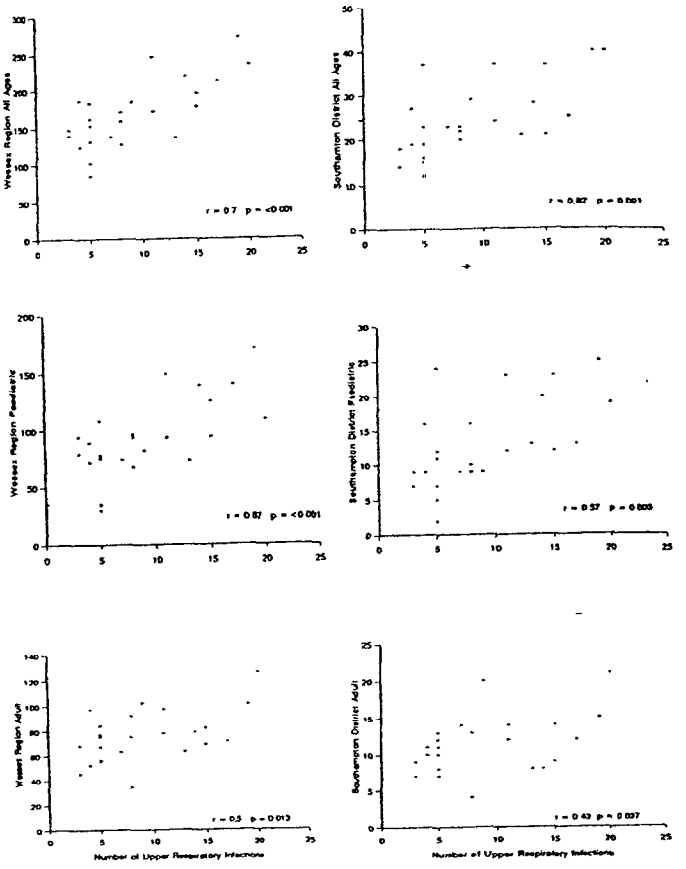

Figure 1. Correlations between numbers per half-month over the year April 1989 to March 1990 of episodes of upper respiratory tract infection in the cohort of Southampton schoolchildren and total, pediatric, and adult hospital admissions for asthma in the Southampton District Health Authority and Wessex Regional Health Authority.

The highest rates of asthma admissions were recorded during the 4-wk period spanning the last 2 wk of November and the first 2 wk of December. For the pediatric age group, there were 279 asthma admissions in those 4 wk (4.9/1,000/yr), and for the adult age group, there were 227 asthma admissions (1.3/1,000/yr). The total number of asthma admissions during that month was 506 (2.24/1,000/yr). The November/December peak occurred during a nationwide influenza epidemic (17), and the principal virus types detected during this peak were influenza virus, coronaviruses, and rhinoviruses (Figure 3). The peaks of April, September/October, and January/February were accounted for principally by rhinoviral infections, with RS virus, coronavirus, and parainfluenza viruses making minor contributions (Figure 3).

DISCUSSION

In this study, we have demonstrated that proven upper respiratory infections in a sentinel cohort of schoolchildren follow a seasonal pattern highly correlated with the seasonal pattern of hospital admissions for asthma in the district and region from which the cohort was drawn. These correlations were most strong for pediatric asthma admissions, but remained significant when

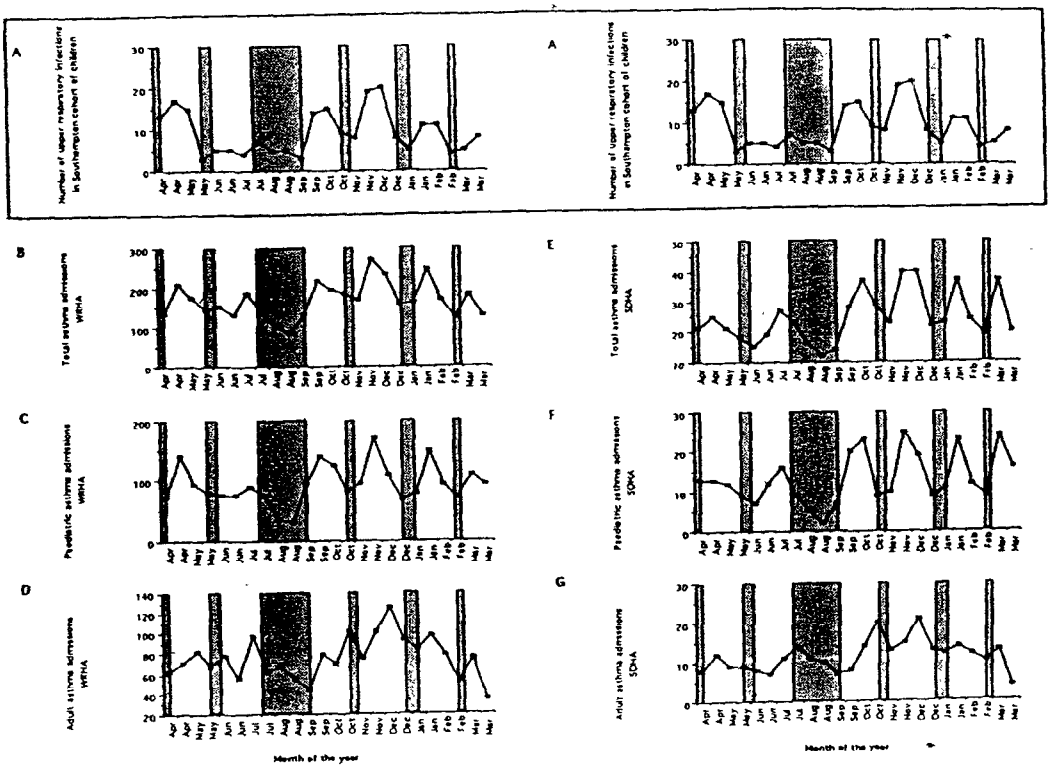

Figure 2. Relationship to school holidays (*shaded boxes*) and seasonal variations in numbers per half-month of episodes of upper respiratory tract infection (*A; bold box*) and total, pediatric, and adult hospital admissions for asthma for the Wessex Regional Health Authority (WRHA) (*B–D*), and Southampton District Health Authority (SDHA) (*E–G*) for the year April 1989 to March 1990.

adult asthma admissions alone were considered. In each case the correlations were strengthened by analyzing the asthma admissions in conjunction with the total numbers of infecting organisms detected (to reflect the total community load of upper respiratory infection at that time).

We also observed a very close relationship between the peaks of both respiratory infections and asthma admissions and the start of a new school term or half-term, with the vast majority of respiratory infections and asthma admissions occurring during school term time. Regression analysis showed that this was not due to seasonal confounding, and established school attendance as the major factor determining pediatric admissions but not adult admissions. Rhinoviruses were the major type of viru identified (15), and were associated with all of the four peak identified for viral infection or asthma admissions. Influenza vi rus was important in one of the peaks, and coronavirus, RS vi rus, and parainfluenza viruses were present in smaller number in the remaining three peaks.

The respiratory infections detected in the cohort during th year in which the study was done (April 1989 to March 1990 and which were used for analysis in the study, were 98.3% vira and 1.7% atypical bacterial, suggesting that the respiratory viruse were by far the most important factor in any correlations iden tified.

We were unable to allow for other possible confounding var ables, such as temperature, humidity, and allergen exposure, sin concurrent data for these parameters was not available to us. Ter perature and humidity are also known to be important facto (although much less important than school attendance) in t transmission of some respiratory viral infections, and also in t levels of airborne allergens such as pollens, fungal spores, a house dust mite levels in the home. These factors may therefc be associated with asthma admissions, both through their effe on viral transmission and indirectly. In England, the peak se sons for pollen exposures are February through May for t pollens and early May to the end of July for grasses, where for fungal spores the peak season is June through Septemb (20). House dust mite exposure in the home peaks in July a August, although high levels have also been reported throu November (21). Increases in asthma admissions in early July w seen for both the SDHA and WRHA and in both age whereas virus infections did not increase appreciably (Fig and there were major increases in both admissions and viral

TABLE 2

RELATIONSHIPS BETWEEN SCHOOL TERMS AND HOLIDAY PERIODS AND THE NUMBERS PER WEEK OF UPPER RESPIRATORY INFECTIONS AND ASTHMA ADMISSIONS IN THE YEAR APRIL 1989 TO MARCH 1990 FOR THE WESSEX REGIONAL HEALTH AUTHORITY (WRHA) AND SOUTHAMPTON DISTRICT HEALTH AUTHORITY (SDHA)

	School Holiday Periods	School Term Time	Chi squared p Value
Upper respiratory organisms, n/wk	2.27	4.78	0.008
Asthma admissions, n/wk			
WRHA			
Pediatric	25 5	47.4	< 0.001
Adult	30.6	35.1	0.2
SDHA			
Pediatric	2.7	6.9	< 0.001
Adult	4.6	5.2	0.6

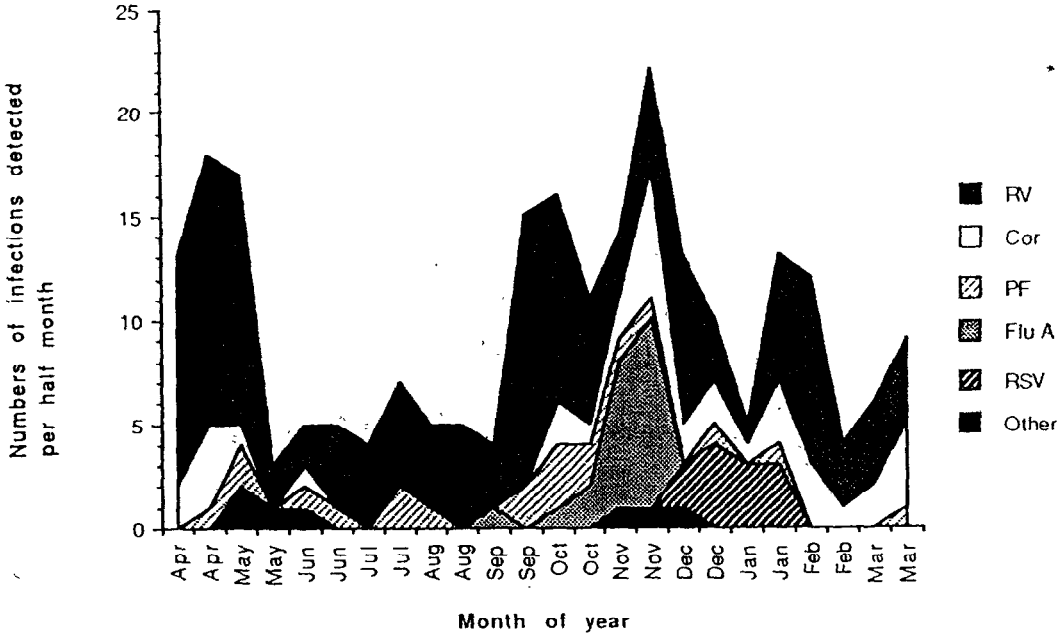

Figure 3. Seasonal variation in numbers of infecting upper respiratory tract organisms per half-month detected in the Southampton cohort of children in the year April 1989 to March 1990. Individual organisms are identified as shown: RV is rhinovirus, Cor is coronaviruses 229E and OC43, PF is parainfluenza viruses types 1 through 3, Flu A is Influenza type A, and RSV is respiratory syncytial virus.

fections in September. It is quite possible that the July peak in admissions is related to house dust mite and/or pollen exposure, and that both fungal spores and house dust mites contribute to the September peak in addition to the effect of viruses. There have been very few studies that have accurately and systematically examined the influence of weather or allergen exposures on asthma symptoms; two recent reports on asthma admissions, and on asthma symptoms and peak flow recordings, failed to find any relationship with airborne allergens, although in each study temperature was found to have a weak effect (22, 23). In neither study were the effects of viral infections investigated.

The seasonal patterns of asthma admissions that we observed for all ages during a single year in the WRHA were virtually identical to those previously reported by Storr and Lenney for the 11-yearly average of pediatric asthma admissions in the Brighton area (8), and to previously reported UK childhood data (24, 25).

TABLE 3

ESTIMATES BY REGRESSION ANALYSIS OF THE EFFECT OF CHANGE IN THE NUMBER OF UPPER RESPIRATORY INFECTIONS FROM 25TH TO 75TH CENTILE ON THE NUMBERS PER WEEK OF ASTHMA ADMISSIONS IN THE YEAR APRIL 1989 TO MARCH 1990 FOR THE WESSEX REGIONAL HEALTH AUTHORITY (WRHA) AND SOUTHAMPTON DISTRICT HEALTH AUTHORITY (SDHA), IN PEDIATRIC (< 20 YR) AND ADULT (≥ 20 YR) AGE GROUPS

	Coefficient	Effect*	t	p Value
Unadjusted				
WRHA				
Pediatric	2.650		3.84	0.001
Adult	1.639		3.47	0.001
SDHA				
Pediatric	0.336		2.18	0.034
Adult	0.305		2.72	0.009
Adjusted for linear trend, seasonality, and school holidays				
WRHA				
Pediatric	3.210	9.63	3.24	0.002
Adult	1.340	4.02	2.87	0.006
SDHA				
Pediatric	0.250	0.75	1.60	0.120
Adult	0.234	0.70	2.13	0.039
†WRHA				
Pediatric	1.244	3.73	1.70	0.096
†SDHA				
Pediatric	0.049	0.15	0.30	0.770

* Coefficient × interquartile range of episodes of infection.
† School attendance parameter omitted.

As reported by Storr and Lenney, we also observed clear patterns in the rates of asthma admissions and school holiday periods, with peaks occurring early in each school term or half-term and troughs in each school holiday (8). The observation of an identical pattern for respiratory infection rates in the present study lends support to the hypothesis formulated by Storr and Lenney, that viral respiratory infections were likely to be associated with asthma admissions. The correlations observed in the present study between asthma admission rates and viral infection rates confirm this to be true, not only for pediatric asthma admissions, but also for adult asthma admissions. A possible criticism of this study is the potential for false-positive virologic detection results with PCR. However, extensive precautions were taken to avoid contamination (12–14); the majority of the PCR-positive samples were confirmed as positive by another method, either ELISA or tissue culture (12–15); and the rates of asymptomatic infection and numbers of episodes involving detection of two or more organisms (15) make false-positive viral detections unlikely.

These data, along with our previously reported findings that viral infections are associated with 80 to 85% of exacerbations of asthma in children in the community (15), indicate that upper respiratory viral infections play a pivotal role in triggering asthma exacerbations of all grades of severity and in all age groups, including attacks leading to hospital admissions. Furthermore, because hospital admissions for asthma are the most important risk factor for asthma mortality (6), it is possible that upper respiratory viral infections contribute significantly to asthma mortality.

The most frequently identified virus type were rhinoviruses, which were important in each of the four peaks of viral infection and asthma admissions identified in the study. The recent identification of the major group rhinovirus receptor as intercellular adhesion molecule-1 (ICAM-1) (26) has improved the prospects for developing effective antirhinoviral therapy, including monoclonal antibodies to ICAM-1 (27–29) and solubilized ICAM-1 to block the receptor binding sites on the virus (30).

In the current absence of effective antiviral medication (31), the influence of antiasthmatic medication in virus-induced exacerbations of asthma requires further study. Glucocorticoids are of proven benefit in treating acute attacks of asthma in adults (32–35) and children (35–37). The effect of prophylactic glucocorticoid therapy in preventing severe asthma exacerbations precipitated by proven upper respiratory infection should now be studied. The data presented in this study suggest that particularly in children, any intervention would achieve most of its effect if employed in the first few weeks following a return to school.

In conclusion, this study has provided evidence that upper respiratory viral infections are strongly associated in time with exacerbations of asthma leading to hospital admission, in both adults and children. Rhinovirus is the most important virus type implicated, and the majority of upper respiratory viral infections and asthma admissions occur during periods of school attendance.

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Short communication

A novel approach using an attenuated recombinant vaccinia virus to test the antipoxviral effects of handsoaps

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Abstract

Evidence indicates an increase in nosocomial and household infections due to viruses (Jeffries, D.J., 1995. Viral hazards to and from health care workers. J. Hosp. Infect. 30, 140–155). An antiviral assay was developed for evaluating efficiency of handsoaps at inactivating cell-free and cell-associated virus. A recombinant vaccinia virus, lacking a virulence factor (Isaacs, S.N., Kotwal, G.J., Moss, B., 1992. Vaccinia virus complement-control protein prevents antibody-dependent complement-enhanced neutralization of infectivity and contributes to virulence. Proc. Natl. Acad. Sci. USA 89, 628–632), whose construction was described earlier (Kotwal, G.J., Isaacs, S.N., McKenzie, R., Frank, M.M., Moss, B., 1990. Inhibition of the complement cascade by the major secretory protein of vaccinia virus. Science 250, 827–830), was used as the representative poxvirus. Two antibacterial handsoaps, one surgical handsoap, one moisturizing handsoap, and a sanitizing agent were tested. An aliquot of the virus was mixed and incubated with soap, then titrated onto BSC-1 cells for incubation at 37°C for 48 h. The soaps' effect on cell-associated virus was tested similarly. The antibacterial soaps inactivated all cell-free virus in 1 min. The surgical soap was effective with a 5-min incubation. None of the soaps eliminated all of the cell-associated virus in 1 min. This safe and reproducible assay seems efficient to establish the comparative efficacy of household and surgical soaps.

Keywords: Handwashing; Surgical hand disinfection; Antiviral testing; Vaccinia virus

Viruses are a leading cause of infectious diseases originating from nosocomial and household infections. Handwashing is the most effective means of stopping the spread of nosocomial infection (Marcil, 1993). In general, antibacterial handsoaps are often employed for this task. Consequently, it was decided to compare the relative antipoxviral effectiveness of these household products.

Although experimentation to test antiviral effects with household products such as Lysol has

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been conducted, Lysol is a disinfectant spray, not a handsoap (Ward et al., 1991). In a study using bovine rotavirus, it seems the most efficient method for removing the virus from finger tips was alcoholic solutions versus the much less effective soap and water and disinfectant detergents; however, a comparison of antibacterial handsoaps was not established (Bellamy et al., 1993). In an unrelated study, alcohol products tested in the presence of blood resulted in significantly greater reductions in numbers of colony-forming units than both a detergent containing 4% chlorhexidine gluconate (the active ingredient in the surgical soap tested, Hibiclens®) and a non-antimicrobial soap; yet this study did not contain information concerning viruses (Larson and Bobo, 1992). Alcoholic soap-free hand cleansers have recently been made available to the general public, but handsoaps are more widely used in practice. Thus, those individuals that consciously make the effort to use an antibacterial handsoap to combat 'germs' neglect the possibility that these handsoaps may offer no protection in daily encounters with viruses.

Poxviruses are once again gaining clinical importance due to their inclusion in the list of bioterrorism (e.g. small pox), as emerging viruses (e.g. monkey pox) and as opportunistic agents in AIDS (molluscum contagiosum). A recombinant vaccinia virus, vSIGK3, whose origin was described previously (Kotwal et al., 1990), was used as the infectious agent because this virus is safe to work with in the laboratory and is stable at room temperature, and thetefore is a potential representative poxvirus for handsoap testing. Vaccinia is also ideal since it is a plaque-forming virus, which enables a rapid enumeration of viral titers.

Preliminary research with a 5-min incubation of 1×10^8 virus particles/ml of vSIGK3 and undiluted soap solution demonstrated that Dial® Antibacterial (Dial Corporation, Phoenix, AZ, USA), SoftSoap® Antibacterial (SSA; Colgate–Palmolive Company, New York, NY, USA), and a surgical soap, Hibiclens® (Zeneca Pharmaceuticals, Wilmington, DE, USA), effectively eliminated the virus. The active ingredient in Dial® and SSA is triclosan. Roccal II® 10% (Roccal) (National Laboratories Lehn & Fink Industrial Products Division of Sterile Drug Inc., Toledo, OH, USA), a sanitizing agent/germicide-algicide and deodorizer, was used as a comparison. Roccal's active ingredients are 12% Octylphenoxypolyethoxyethanol, 20% Alkyldimethylbenzylammonium chloride. These results suggested that all soaps were efficient at eliminating the infectivity of input virus in 5 min (data not shown). The rationale for choosing 1×10^8 particles/ml was that viruses such as hepatitis B may be present in titers of around 10^8 per ml of body fluid. Most other viruses do not exceed 10^6 particles per ml in body fluids (Lanphear, 1994).

In practice, very few people routinely wash their hands for 5 min. We therefore decided to test shorter reaction periods of 15, 30, and 60 s to estimate not only the effectiveness of the average person's handwashing (which could be shorter than the actual reaction periods), but also to determine the handsoaps' range of effectiveness. In addition, the handsoaps were diluted to a simulated working dilution of 1:20 with sterile water. Hibiclens® was not diluted since it is not diluted in normal use as a surgical soap. Another handsoap, SoftSoap® Moisturizing (SSM; Colgate–Palmolive Company, New York, NY, USA), was added to the study in order to observe the difference between it and SSA, and also because it functions as a negative control since it has no antimicrobial active ingredient.

The handsoaps were also tested using virus infected cells to determine their effects on cell-associated virus, simulating infectious blood or pus, since numerous viruses undoubtedly maintain their infectiousness on moist surfaces (Hilding, 1994). Soaps working efficiently in this manner would be an invaluable safety precaution in hospitals, daycares, schools, and homes, where body fluid-borne viruses are common. Studies have shown that blood remains in some areas of ungloved dental workers' hands, particularly subungual spaces, for at least 5 days — even with handwashing (Allen and Organ, 1982). Whether or not the remaining blood retains infectious properties after handwashing would be of importance to determine.

Pairs of six-well tissue culture plates (Falcon, Franklin Lakes, NJ, USA) containing confluent

monolayers of BSC-1 cells grown at 37°C were divided up into three, four-well sections. Section 1 received only virus, Section 2 received virus and handsoap, and Section 3 received only handsoap. Virus dilutions were added to a complete minimal essential medium (CMEM; Gibco BRL, Gaithesburg, MD, USA) containing 2.5% fetal bovine serum (FBS; Harlan, Indianapolis, IN, USA) with 1% penicillin, streptomycin, and fungizone.

All wells in Sections 1–3 had a total of 1 ml CMEM added, with virus and/or soap additions. For the initial dilution of 10^{-3} Section 1 1 μl vSIGK3 vaccinia virus (virus) was added to the 1 ml medium. This aliquot was then diluted to the 10^{-6}, 10^{-7}, and 10^{-8}. For use in Sections 2 and 3, the soaps first had to be brought to their average handwashing dilutions. The working dilution for the handsoaps was one part handsoap to 20 parts sterile water; Hibiclens® was not diluted since it is not diluted in normal use; Roccal was diluted for general disinfecting (one part cleaner to 1250 parts sterile water) as per manufacturer's instructions. Ten microliters of virus was added to 10 μl of any given handsoap solution, then incubated at room temperature for either 15, 30, or 60 s. The reaction period, or the amount of time that the virus alone is in contact with the working dilution of the given soap, ended when the mixture was added to the CMEM. For Section 2, the initial dilution of 10^{-2} was made by adding 20 μl virus + handsoap solution to 1 ml CMEM. This aliquot was then diluted down to 10^{-3}, 10^{-4}, and 10^{-5}. For the initial dilution of 10^{-2} of Section 3, 10 μl of the working dilution of a soap was added to 1 ml CMEM. This was then diluted down to 10^{-3}, 10^{-4}, and 10^{-5}.

In order to determine the titer of cell-associated virus, an additional plate of BSC-1 cells was grown at 37°C, but then infected at a multiplicity of 10 and harvested after 48 h, as described before (Joklik, 1962). The infected, scraped cells were centrifuged at $12\,500 \times g$. This pellet was suspended in 100 μl working dilution of the soap for a 60-s reaction period. Of this pellet + handsoap solution, 200 μl was added to 2 ml CMEM on the confluent monolayer to make the initial 10^{-2} dilution. This was then diluted down to 10^{-3}, 10^{-4}, and 10^{-5}. All plates, infected with either cell-free virus and cell-associated virus, were incubated at 37°C for 48 h, stained with crystal violet, and rinsed with water. Both sections of the experiment were conducted using Dial®, SSA, SSM, Hibiclens®, and Roccal.

To determine the number of surviving viral particles in the cell-free and intracellular viral solutions, the plaques from one well, visible after staining, were counted and then multiplied by the dilution factor. Plaque counts for Section 1 in the 60-s cell-free testing indicated that a consistent amount of virus was present in all trials; the average number of plaques was 1.5×10^{-8} per ml. None of the handsoap dilutions used adversely affected the cellular monolayer in the absence of virus. The raw data for cell-free and cell-associated virus was illustrated graphically (Fig. 1). Only the antibacterial soaps (Dial® and SSA) inactivated the cell-free virus in a 60-s reaction period. No significant inactivation was noted with the cell-associated virus in 60 s.

There was no greater than 2% difference between the titers obtained at different times with any given soap samples, suggesting a highly reproducible outcome when the protocol was strictly followed. It also suggested that there was no need for statistical analysis as long as the entire set of experiments was repeated at least twice. If the control virus used with a known titer did not come within ±2% of the expected titer, the experiment was repeated until the control virus was within ±2%.

There have been no systematic studies reported on the antiviral effects of handsoaps using a human virus. This study, however, presents an effective assay, which may be used for further investigations. It should be emphasized that vaccinia cannot directly be compared to other viruses because it is extremely stable to both physical and chemical agents. Few other viruses can survive 0.5% phenol for 2–3 days. Therefore while vaccinia is a representative poxvirus, it is not a representative virus.

It is possible to further study the soaps and their reaction with vaccinia. More significantly, it is crucial to determine what needs to be done to make the soaps more effective, such as manufacturers altering the active ingredients or individuals

modifying their hygiene habits to allow for an adequate reaction period. In addition, it must be questioned as to why Hibiclens®, the surgical scrub, was less effective than the antibacterial handsoaps, Dial® and SSA. It may result from a difference in active ingredients: Dial® and SSA share the active ingredient triclosan, whereas Hibiclens®'s active ingredient is chlorhexidine gluconate. Perhaps the reduced efficacy of the surgical scrub's active ingredient is the reason why alcohol-based antiseptics, which appear to be a successful alternative in surgery preparation to detergent-based antiseptics, are being researched for use with shorter duration handwashing (Pereira et al., 1997). Further research using this assay could also include alcohol-based hand cleansers, which are currently on the market.

Many infections occur due to unknown and unexplained causes-prime examples are HCV infection, which currently infects 3.5 million people in the United States alone (Neiblum and Boynton, 1996), and the common cold. The common cold's modes of transmission and level of communicability are difficult to pinpoint (Jaakkola and Heinonen, 1995) for all strains and progress towards effective cold treatment in the past century has been scarce (Hilding, 1994).

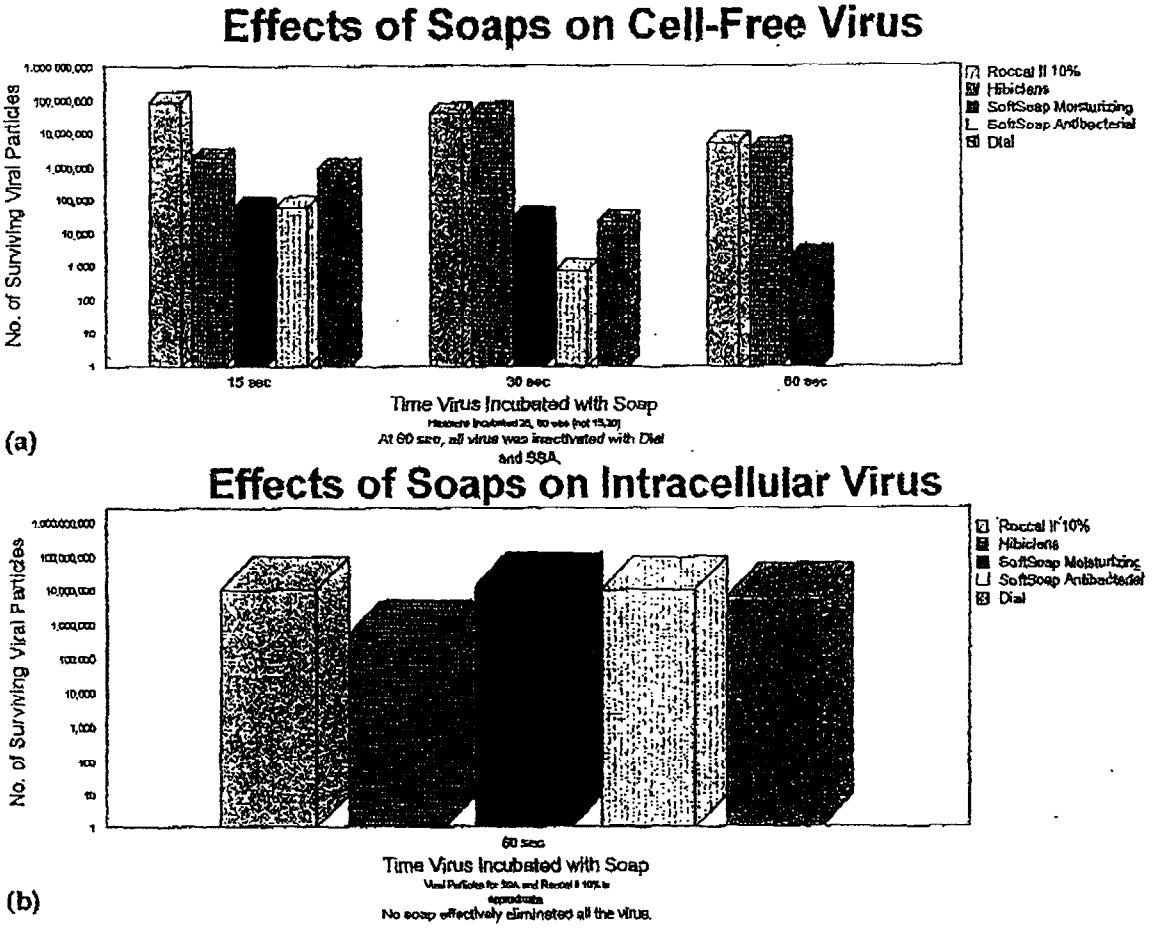

Fig. 1. a: Effects of soaps on cell-free virus. The graph indicates that as the reaction period increases, the number of surviving viral particles is reduced exponentially. At 60 s, all viral particles were inactivated by Dial® and SSA. b: Effects of soaps on intracellular virus. The graph indicates that the reaction period was not sufficient for any of the soaps to either eliminate or reduce the number of surviving viral particles. Note: the viral particle count for SSA and Roccal® was approximated.

Some researchers believe studies on a practical and efficient means to influence behavior in order to increase compliance with hand hygiene guidelines are needed more than elaborate and sophisticated studies on the effects of handwashing (Nystrom, 1994).

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The use of bacteriophage MS2 as a model system to evaluate virucidal hand disinfectants

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Summary: A model system which would closely reflect the resistance of poliovirus but could be easily performed in any microbiology laboratory would offer considerable advantages for rapidly screening hand decontamination products. The use of the bacteriophage MS2 as a simple model for virucidal testing has been evaluated. In suspension tests the sensitivity of MS2 to alcohols, organic acids and alkalis generally reflected that observed in studies using poliovirus. MS2 could be applied and recovered from the hands of volunteers with high efficiency. Furthermore MS2 proved to be a suitable replacement for *Escherichia coli* in a standard hand-decontamination test.

Keywords: Bacteriophage MS2; disinfectant test; virucide.

Introduction

The growing awareness of viruses in the environment and the identification of potential infection risks has resulted in the demand that disinfectants used for hard surfaces, instruments and hand hygiene should have adequate virucidal activity. Agreement has yet to be reached on a standard European suspension test for the virucidal activity of disinfectants but both the German (DVV[1]) and French (AFNOR[2]) methods include the non-enveloped poliovirus among their test strains because it is resistant to chemical inactivation. No standard virucidal hand disinfection test exists. A wide range of commonly used bactericidal hand disinfectants including 60% isopropanol have been shown to have very meagre activity against non-enveloped viruses.[3–6] Hence, there is an urgent need to develop formulations, particularly for hand disinfection, which have both virucidal and bactericidal activity.

Hand disinfection tests are currently performed using a live bacterial culture (usually *Escherichia coli*) which is applied to the skin of volunteers. The bacteria surviving a standard washing or disinfection procedure are recovered and because of the variability between individuals' skin

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properties and their washing techniques (even when a strictly defined method is used), panels of ten or more persons have to be used to obtain meaningful results.[7,8] Some handwashing trials with viruses applied to the skin have been reported but these have been confined to very small (1–3) numbers of volunteers.[3,6,9]

Virus tests are technically more difficult and more time consuming than those using bacteria or fungi and can only be carried out in laboratories with the necessary facilities. To assess the efficacy of virus-inactivating disinfectants, a tissue culture method is used to determine residual infectivity. The cytotoxicity of some products is such that it is not possible to demonstrate the required reduction in virus titre. The AFNOR protocol[2] advises physical separation of the virus, as an alternative to dilution, to try to minimize this problem. This complicates the procedure and adds to the time required to complete a test. Unfortunately, suspension tests appear to be a poor guide to the activity of disinfectants on the skin.[3]

Products will always have to be tested against standard viruses such as poliovirus and rotavirus. Nevertheless, it would be highly advantageous to have a rapid test procedure using a non-mammalian, non-infective agent when comparing hand disinfectants under realistic in-use conditions. In this report, the use of the bacterial virus (bacteriophage) MS2 is considered. This non-pathogenic virus can be easily propagated in any microbiology laboratory and the virus suspension can be stored refrigerated for many months without loss of infectivity. Surviving infectious particles are eaily counted on lawns of a sensitive strain of *E. coli*.

Methods

Bacteriophage propagation

MS2 (obtained from M. A. Linggood) was grown to high titres on *E. coli* H73FT$^+$. An exponentially growing culture of *E. coli* in nutrient broth (Oxoid) was divided into 20 ml aliquots and inoculated with 1 ml phage stock. After 16 hours incubation at 37°C, remaining cells and cell debris were removed by centrifugation at 10 000 ***g*** for 15 min. The supernatant containing the phage was filtered and the filtrate stored at <4°C. Prior to use, phage suspensions were allowed to equilibrate to room temperature.

Bacteriophage titre

Infectious particles were counted by using a soft agar overlay technique. Molten, soft (0·7%) nutrient agar was dispensed in 2·0 ml aliquots in glass bottles and held at 44°C. Phage-containing solutions were suitably diluted in 0·2% peptone water at 20°C and 0·1 ml added, together with 0·2 ml overnight culture of *E. coli* H73FT$^+$ to the molten agar. The contents were gently mixed and poured over the surface of a nutrient agar plate. Plaques were countable after 24 hours plaque forming units ml^{-1} (pfi

Cell culture and medium

Vero cell line (Africar. monkey were used in the production propagated as monolayers at (Northumbria Biologicals Lt containing 10% foetal calf se penicillin and 100 µg ml^{-1} stre same medium except that it co

Preparation of poliovirus suspe

The medium above confluent poliovirus type 1 (Mahoney st inoculated into the flasks. Vir after which the inoculum was Flasks were incubated at 3 observed. Virus was harveste followed by centrifugation for supernatant containing the vir

Poliovirus titre

Confluent monolayers of Ver plates in a 5% carbon dioxide virus were made in maintenar added to five replicate wells. which was incubated at 37°C i to 7 days for cytopathic effec Karber formula.[10]

Disinfection suspension tests wit

Suspension tests with poliovir DVV recommendations.[1] Typ soil agent was added to 8 ml di time at 20°C, 0·1 ml suspensior quenching agent (containing thiosulphate and 0·7 g lecithin active phage counted using th suspension tests were carried o times, an initial 100-fold dil medium. Subsequent 10-fold inoculated as described above.

were countable after 24 hours incubation at 37°C and results expressed as plaque forming units ml^{-1} (pfu ml^{-1}).

Cell culture and medium

Vero cell line (African monkey kidney) purchased from Flow Laboratories were used in the production of poliovirus suspensions. The cells were propagated as monolayers at 37°C in 750 ml plastic tissue culture flasks (Northumbria Biologicals Ltd) using Medium 199 with Earle's salts containing 10% foetal calf serum (FCS), 2 mM glutamine, 100 IU ml^{-1} penicillin and 100 µg ml^{-1} streptomycin. Cultures were maintained on the same medium except that it contained 2% FCS.

Preparation of poliovirus suspensions

The medium above confluent monolayers of Vero cells was removed and poliovirus type 1 (Mahoney strain) obtained from NATEC, Hamburg was inoculated into the flasks. Virus was allowed to absorb for 1 hour at 37°C, after which the inoculum was removed and maintenance medium added. Flasks were incubated at 37°C until 90–100% cytopathic effect was observed. Virus was harvested by two cycles of freezing and thawing followed by centrifugation for 15 min at 4°C to pellet the cell debris. The supernatant containing the virus was stored in aliquots at −70°C.

Poliovirus titre

Confluent monolayers of Vero cells were cultured in 96-well microtitre plates in a 5% carbon dioxide–95% air atmosphere. Ten-fold dilutions of virus were made in maintenance medium and 100 µl of each dilution were added to five replicate wells. Cell controls were included on each plate which was incubated at 37°C in a CO_2 incubator and observed daily for up to 7 days for cytopathic effect. The virus titre was calculated using the Karber formula.[10]

Disinfection suspension tests with MS2 and poliovirus type 1

Suspension tests with poliovirus and MS2 were performed according to the DVV recommendations.[1] Typically, 1 ml phage and 1 ml water or protein soil agent was added to 8 ml disinfectant solution. After the desired contact time at 20°C, 0·1 ml suspension was diluted in 9·9 ml 0·1% peptone water or quenching agent (containing 1 g peptone, 5 g Tween 80, 1 g sodium thiosulphate and 0·7 g lecithin per litre deionized water) and the remaining active phage counted using the soft agar overlay method. The poliovirus suspension tests were carried out in a similar way. After appropriate contact times, an initial 100-fold dilution was made into ice-cold maintenance medium. Subsequent 10-fold dilutions were made to 10^{-9} and cell cultures inoculated as described above.

Handwashing trial

A standard method for the assessment of hand disinfection was followed[7] except that each finger pad was inoculated with 20 µl MS2 (*c.* 6×10^9 pfu ml^{-1}). The handwashing products tested in this study (alcohol and non-medicated soap) were neutralized by dilution and it was not necessary to use a separate inactivator.

The ability to recover phage from the fingers was assessed by applying MS2 in 20-µl drops to each finger and thumb on both hands of a volunteer. Opposing fingers were rubbed together for 40 s and air dried for 80 s. All 10 digits were then sampled by rubbing them gently for 1 min in a large petri dish containing *c.* 50 glass beads and 100 ml nutrient broth. The broth was then serially diluted and samples assayed using the soft agar overlay method.

Results

Sensitivity of MS2 to alcohols and alkaline alcohols

In suspension tests, MS2 was relatively insensitive to a variety of alcohols during short contact times (2 min). Ethanol (70% v/v) was marginally more effective than isopropanol, whilst n-propanol was inactive (Table I). Ethanol at 50% produced only a 24% (mean of four determinations) reduction in pfus. The addition of low levels of sodium hydroxide greatly increased the phage inactivation. Sodium hydroxide alone was also an effective virucide at higher concentrations. These results reflect very closely data obtained using poliovirus (Table I).

Both poliovirus and MS2 were inactivated by aldehydes. Formaldehyde

Table I. *Inactivation of MS2 and poliovirus virus (type 1 Mahoney strain) by alcohols and alcohol + sodium hydroxide*

Test composition	Log reduction after 2 minutes	
	MS2	Poliovirus 1
70% Ethanol	0·70	0·60
70% Iso-propanol	0·41	ND
70% n-Propanol	0·00	ND
50% Ethanol	0·13	0·10
5 mM NaOH	0·34	0·20
20 mM NaOH	>5	>4·6
50 mM NaOH	>5	>4·6
50% Ethanol + 5 mM NaOH	>5	>4·6
50% Ethanol + 20 mM NaOH	>5	>4·6
4% Formaldehyde	>5	>3·1

ND, Not done.

(4% w/v) reduced the titre formaldehyde required 2 min

Evaluation of MS2 for hand d

In a preliminary experiment applied and recovered from th stock (titre $5{\cdot}6 \times 10^9$ pfu ml^{-1} described in methods), the rec disinfection procedure was t MS2, 3 ml 80% ethanol or 3 rubbed into the hands for 30 further 80 s. This procedure t *in situ*, i.e. there is no removal counts from three volunteers 7·0, and 50% alkaline ethanol

In practice, hands will disinfection or a combined w determine the contribution of with a larger test panel was Ayliffe and colleagues.[7] The n hands in each of the three trea soap and water, and disinfect indicated an acceptable low le and that significant differenc treatments. The log reductio ethanol and soap and water w noted that neither pro inactivating/removing the ph

Table II. *Log pfu of MS*

Subject	Control	Soap/wate
1	8·78	7·23
2	7·71	6·51
3	8·34	6·35
4	7·65	6·01
5	7·36	6·43
6	8·71	6·28
7	8·30	6·54
8	8·11	6·20
9	8·90	7·04
10	8·68	7·54
Mean LR		
SD		

* LR = Log reduction (control vs treatm

(4% w/v) reduced the titre of MS2 by >4 logs in 30 s whereas 1% formaldehyde required 2 min to achieve the same inactivation.

Evaluation of MS2 for hand disinfection tests

In a preliminary experiment it was found that MS2 phage could be easily applied and recovered from the fingers. When 20 µl drops of bacteriophage stock (titre $5{\cdot}6 \times 10^9$ pfu ml^{-1}) were applied to each finger and thumb (as described in methods), the recovery was $1{\cdot}4 \times 10^9$ per pair of hands. A hand disinfection procedure was then followed in which, after application of MS2, 3 ml 80% ethanol or 3 ml 50% ethanol (adjusted to pH 11·5) was rubbed into the hands for 30 s. Hands were sampled after air drying for a further 80 s. This procedure tests the ability of alcohols to inactivate phage *in situ*, i.e. there is no removal of phage by washing or rinsing. The mean log counts from three volunteers were control, 8·4 pfu $hands^{-1}$; 80% ethanol, 7·0, and 50% alkaline ethanol, 6·6.

In practice, hands will more often be washed with soap before disinfection or a combined washing and disinfection agent employed. To determine the contribution of rinsing on viral removal from the skin, a trial with a larger test panel was undertaken using the method described by Ayliffe and colleagues.[7] The numbers of phage particles recovered from the hands in each of the three treatments (control, washing with non-medicated soap and water, and disinfection with alkaline alcohol followed by rinsing) indicated an acceptable low level of variability within each treatment group, and that significant differences could be demonstrated between the three treatments. The log reductions in active phage observed with the alkaline ethanol and soap and water were 2·1 and 1·64 respectively. It should also be noted that neither procedure was particularly effective at inactivating/removing the phage.

Table II. *Log pfu of MS2 recovered from hands in a handwashing test*

Subject	Control	Soap/water	LR*	50% Alkaline ethanol	LR*
1	8·78	7·23	1·55	6·53	2·25
2	7·71	6·51	1·20	5·90	1·81
3	8·34	6·35	1·99	5·99	2·35
4	7·65	6·01	1·64	5·32	2·33
5	7·36	6·43	0 93	5·62	1·74
6	8·71	6·28	2·43	6·27	2·44
7	8·30	6·54	1·76	6·23	2·07
8	8·11	6·20	1·91	5·77	2·34
9	8·90	7·04	1·86	6·07	2·83
10	8·68	7·54	1·14	6·89	1·79
Mean LR			1·64		2·10
SD			±0·43		±0·38

* LR = Log reduction (control vs treatment).

Discussion

The bacteriophage MS2 can be used in any disinfectant test which currently employs bacteria, with very little additional effect. Results are obtained within 24 hours. The validity of using bacteriophages as a model for mammalian viruses depends on their sensitivity to disinfectants relative to more resistant mammalian viruses such as poliovirus and coxsackie viruses. We have shown that MS2, in common with other viruses,[5,11] is more resistant to alcohols than are bacteria and, in contrast to bacteria, the bacteriophage is more sensitive to inactivation by ethanol than by iso/n-propanol. Addition of alkali greatly enhances the effectiveness of 50% or greater ethanol against MS2. Similar results have been previously obtained with other viruses.[5] Further, the sensitivity of MS2 to formaldehyde is again similar to poliovirus[7] although coxsackie B4 virus is significantly more resistant.[5]

Poliovirus has been frequently chosen for disinfectant testing, both for its relatively high resistance to chemical inactivation and for its ability to produce high virus titres in culture. It shares its disinfectant resistance with other enteroviruses which are known to be transmissable by hand contact. Poliovirus is thus a useful 'benchmark' to which other test viruses, including bacteriophage MS2, can be compared.

MS2 was successfully used in hand decontamination tests and could be substituted for *E. coli* in the method described by Ayliffe *et al.*,[7] with good recovery of the virus and acceptable reproducibility within the test panel results. Poor recovery of some mammalian viruses from the hands has been reported by Schurmann & Eggers.[3] It is proposed that MS2 could be used as a 'model' virus for the initial screening of hand disinfectant products.

There is clearly a market demand for effective antiviral products and awareness that some existing antibacterial formulations may be inadequate.[3,6,11,12] The low log reductions in MS2 observed in our handwashing tests with soap and water and with alcohol are similar to those reported for enteroviruses[6] and clearly indicate that current decontamination methods are inadequate. The use of MS2 will allow us to screen antiviral compositions more rapidly than testing with animal viruses and additionally may allow the development and realistic testing of hand decontamination products. The general use of MS2 to assess antiviral disinfectants will require initial monitoring to establish that the comparability with poliovirus, reported here, holds in other circumstances.

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Etiology of community-acquired pneumonia in 254 hospitalized children

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Background. **Childhood community-acquired pneumonia is a common illness, but there have been relatively few comprehensive studies of the viral and bacterial etiology in developed countries. The aim of the present investigation was to determine the etiology of community-acquired pneumonia in hospitalized children by several laboratory methods.**

Methods. **In a 3-year prospective study a nasopharyngeal aspirate for viral studies and acute and convalescent serum samples for viral and bacterial serology were taken from 254 children with symptoms of acute infection and infiltrates compatible with pneumonia in the chest radiograph. The role of 17 microbes was investigated.**

Results. **A potential causative agent was detected in 215 (85%) of the 254 patients. Sixty-two percent of the patients had viral infection, 53% had bacterial infection and 30% had evidence of concomitant viral-bacterial infection. *Streptococcus pneumoniae* (37%), respiratory syncytial virus (29%) and rhinovirus (24%) were the most common agents associated with community-acquired pneumonia. Only one patient had a positive blood culture (*S. pneumoniae*) of 125 cultured. A dual viral infection was detected in 35 patients, and a dual bacterial infection was detected in 19 patients.**

Conclusions. **The possible causative agent of childhood community-acquired pneumonia can be detected in most cases. Further studies are warranted to determine what etiologic investigations would aid in the management of pneumonia. With effective immunization for *S. pneumoniae* and respiratory syncytial virus infections, more than one-half of the pneumonia cases in this study could have been prevented.**

INTRODUCTION

Childhood community-acquired pneumonia is a common illness, with an incidence of 36 to 40 episodes/1000 children/year in those <5 years of age and 11 to 16 episodes in children 5 to 14 years of age.[1, 2] It has been estimated that 2.5 million cases occur annually in Europe.[3] There have been relatively few comprehensive studies of the viral and bacterial etiology of childhood community-acquired pneumonia. This is explained by the fact that many bacteria and viruses can cause the illness and many of them can be detected by methods available only in research laboratories. Detailed information about the etiology is needed for treatment recommendations which vary a lot and are not based on solid data.[3] In addition control of pneumonia depends on an accurate understanding of the relative importance of etiologic agents.

We conducted a 3-year prospective study investigating the role of 17 microbes in the etiology of childhood community-acquired pneumonia. Several laboratory methods were used to obtain the maximal yield of microbiologic diagnoses.

METHODS

Patient population and clinical samples. From January 1, 1993, to December 31, 1995, 254 consecutive hospitalized children with community-acquired pneumonia were enrolled in the study at the Department of Pediatrics, Turku University Hospital. Acute and convalescent serum samples and a chest radiograph were available from all these patients. The diagnosis was based on simultaneous finding of fever (>37.5°C) and/or respiratory symptoms and infiltrates compatible with pneumonia in the chest radiograph. All patients were treated with antibiotics. Initially 296 patients were enrolled, but 42 children were excluded, 33 because a convalescent serum sample was not obtained and 9 because their chest radiographs did not have infiltrates compatible with pneumonia at the reevaluation done by 3 pediatric radiologists, or the radiographs were not available for the reevaluation.

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Each of 4 patients had 2 episodes of pneumonia that were analyzed separately in the study. Fifty-six percent of patients were male and 44% were female. The mean age of the patients was 3.8 years (range, 0.1 to 16.7 years). Thirty-seven children were <1 year of age, 71 between 1 and 2 years, 84 between 2 and 5 years and 62 were >5 years of age.

On admission a blood sample was collected for serologic analyses (n = 254). A nasopharyngeal sample was aspirated through the nostrils with a disposable mucus extractor. Two sterile cotton swabs were dipped into the mucus, and these were then placed in separate viral transport medium tubes for rhinovirus PCR assay (n = 190) and virus culture (n = 230). The rest of the mucus was used for virus antigen detection (n = 231). The patients returned 3 - 4 weeks after discharge when the second blood sample (n = 254) and the follow-up chest radiograph (n = 254) were taken.

Virologic studies. Virus culture was done by using the Ohio strain of HeLa cells and human foreskin fibroblasts according to routine procedure. Viral antigens [influenza A and B viruses, respiratory syncytial virus (RSV), parainfluenza virus types 1, 2 and 3 and adenovirus] in nasopharyngeal aspirates were detected by time-resolved fluoroimmunoassay with monoclonal antibodies.[4] Virus-specific serum antibody titers were determined by enzyme immunoassay (EIA) using antigen-coated solid phase and horseradish peroxidase-conjugated rabbit antihuman IgG (Dako, Glostrup, Denmark).[4] Human herpesvirus 6-IgG responses were detected by microimmunofluorescence.[5] The diagnosis of Epstein-Barr virus and varicella-zoster virus infections was based on routine determination of IgM antibodies.

Two reverse transcription-PCR assays were used for detection of rhinoviruses. The details of the methods have been reported earlier.[6,7] Nasopharyngeal aspirate samples collected during 1993 and 1994 were frozen at −70°C and tested by rhinovirus-PCR later. From January, 1995, rhinovirus PCR was done routinely from unfrozen samples.

Bacteriologic studies. On admission a blood sample was taken for blood culture from the patients who appeared to be more ill as judged by the care physician (n = 125). Bacterial antibody assays were carried out from acute and convalescent serum samples. IgG antibodies to pneumococcal pneumolysin and C-polysaccharide (CPS) were measured by EIA, and a 2-fold or higher rise in antibody titers between paired sera was considered diagnostic.[4] Circulating immune complexes containing pneumococcal CPS and pneumolysin were also measured from paired sera as described earlier.[8] In 120 healthy children immune complexes were present in <1% by the limits used.[8] Antibodies to nontypable *Haemophilus influenzae* and *Moraxella catarrhalis* were detected by EIA by using whole bacterial cell antigen, and a 3-fold or higher antibody rise between paired sera was considered diagnostic for acute infection.[4] In 188 healthy children positive bacterial antibody responses to the 3 bacteria developed in 0 to 2.7% of the cases in paired serum samples taken 1 month apart.[9] In addition in 200 young adults with common cold only 1 patient developed a positive response to pneumolysin and 1 patient to *H. influenzae* studied in serum samples taken 21 days apart.[4] IgM antibodies to *Mycoplasma pneumoniae* were measured by a commercial EIA kit with minor modifications (Platelia; Sanofi Diagnostics Pasteur S.A., Marnes la Coquette, France).[10] The IgM serology test was chosen because when compared with culture, serology and PCR it proved to be the most valuable tool for diagnosing *M. pneumoniae* infections.[10] IgG and IgM antibodies to chlamydial species were studied by a microimmunofluorescence method with elementary bodies of *Chlamydia pneumoniae* Kajaani 7 and *Chlamydia trachomatis* 1.2 as antigens.[4] The presence of IgM antibodies and/or a 4-fold or greater change in IgG levels between paired sera was considered diagnostic for acute chlamydial infection. Antistreptococcal antibodies (anti-streptolysin O and anti-deoxyribonuclease B) were measured by routine commercial laboratory tests.

Statistical analysis. The standard chi square test was used to compare the proportions among the groups.

RESULTS

A potential causative agent was detected in 215 (85%) of the 254 patients (Table 1). Evidence of viral infection was demonstrated in 62% and of bacterial infection in 53% of the patients. There were significantly more viral infections in children <2 years of age (80%) than in those >2 years of age (49%) ($P < 0.001$). No significant differences were found in the occurrence of bacterial etiology according to the age of the pa-

TABLE 1. Etiology of childhood community-acquired pneumonia (N = 254)

Age of Patients (yr)	Total No.	Viral	Bacterial	Mixed	Total No. with Etiology Detected
<2	108	86 (80)*	51 (47)	37 (34)	100 (93)
2–5	84	49 (58)	47 (56)	28 (33)	68 (81)
>5	62	23 (37)	36 (58)	12 (19)	47 (76)
Total	254	158 (62)	134 (53)	77 (30)	215 (85)

* Numbers in parentheses, percent.

TABLE 2. Viruses and bacteria in the etiology of childhood community-acquired pneumonia (N = 254)

Virus	No. of Patients	Bacterium	No. of Patients
Respiratory syncytial virus	73 (29)*	*Streptococcus pneumoniae*	93 (37)
Rhinovirus	58 (24)†	*Haemophilus influenzae*	22 (9)
Parainfluenza 1, 2, 3 viruses	25 (10)	*Mycoplasma pneumoniae*	17 (7)
Adenovirus	19 (7)	*Moraxella catarrhalis*	10 (4)
Influenza A and B viruses	10 (4)	*Chlamydia pneumoniae*	7 (3)
Coronavirus	7 (3)	*Streptococcus pyogenes*	3 (1)
Human herpesvirus 6	7 (3)	*Chlamydia trachomatis*	2 (1)
Epstein-Barr virus	1		
Varicella-zoster virus	1		

* Numbers in parentheses, percent.
† Number of patients studied = 238.

tients. Mixed viral-bacterial infection was detected in 30% of the patients. Sole viral infection was found in 32% and sole bacterial infection in 22% of the patients. In 153 (60%) patients the etiology could have been established within 2 working days by using viral antigen detection (n = 65 positive cases), rhinovirus PCR (n = 51) or a *M. pneumoniae* IgM test (n = 17) and by measuring pneumococcal pneumolysin and CPS immune complexes (n = 43) from the first serum sample.

Twelve viruses were associated with community-quired pneumonia. RSV (29%), rhinovirus (24%) and parainfluenza viruses (10%) were the most common agents (Table 2). The recovery rate of RSV by virus antigen detection was 73%, by virus culture it was 58% and by IgG serology it was 80% in 66 patients in whom all 3 tests were performed. Virus culture and sensitive virus antigen detection assays found 53 (80%) of 66 cases. There were no significant differences in the detection rates of RSV by various diagnostic tests in different patient age groups. RSV was found in 46% of the patients < 2 years of age compared with 11% of patients >5 years of age.

Seven bacterial genera were potential causative agents of community-acquired pneumonia (Table 2). Evidence of *Streptococcus pneumoniae,* the most common etiologic agent, was detected in 93 (37%) patients. Only 1 patient had blood culture positive (125 cultured) for *S. pneumoniae;* in the other 92 patients the diagnosis was based on serologic evidence of pneumococcal infection. By measuring only IgG responses to pneumolysin and CPS, 60 cases (65%) were found. When using only pneumolysin serology (IgG response and immune complexes), 77 (83%) cases were detected. There were no significant differences in the occurrence of pneumococcal pneumonia in different age groups of the patients. Several cases of *H. influenzae* (n = 22), *M. pneumoniae* (n = 17), *Moraxella catarrhalis* (n = 10) and *C. pneumoniae* (n = 7) infections were diagnosed, but their role was clearly less than that of *S. pneumoniae.* In 1 patient *Streptococcus pyogenes* was cultured from pleural fluid, whereas 2 developed a significant increase in antistreptococcal antibodies.

Evidence of two or more microbes associated with community-acquired pneumonia was found in 105 (41%) children. A mixed viral-bacterial infection was demonstrated in 30% of the patients. Nearly one-half of the patients with RSV-, rhinovirus-, parainfluenza virus- or adenovirus-associated pneumonia had evidence of concomitant bacterial infection (Table 3). *S. pneumoniae* was the most common bacterium associated with RSV (25%), rhinovirus (33%) and parainfluenza virus (28%) infections. Evidence of *H. influenzae* infection was detected in 18, 10 and 4% of these viral infections, respectively. *M. pneumoniae* or *C. pneumoniae* infections were rarely identified in virus-induced pneumonia. Of the patients with *S. pneumoniae* infection 46 of 93 (49%) had evidence of concomitant viral infection. Two bacteria as causative agents were found in 19 patients. A dual viral infection was detected in 35 patients.

The spring and fall RSV epidemics occurred in 1993 and 1995. RSV infection was associated with pneumonia in 45% of the cases in 1993, in 5% in 1994 and in 30% in 1995. Rhinovirus peaks were identified every year during late fall. The number of rhinovirus infections, as well as parainfluenza virus infections, adenovirus infections and influenza virus infections, did not differ significantly between the study years (data not shown). Interestingly pneumococcal infections were detected significantly more often during 1995 (45%) than during 1994 (33%) and 1993 (25%) (P = 0.02). A *M. pneumoniae* outbreak occurred in 1995 when 14 of 17 cases were detected.

TABLE 3. Mixed viral and bacterial infections in the etiology of community-acquired childhood pneumonia

Virus	No. of Patients	*Streptococcus pneumoniae*	*Haemophilus influenzae*	*Moraxella catarrhalis*	*Mycoplasma pneumoniae*	Total No.
Respiratory syncytial virus	73	18 (25)*	13 (18)	0 (0)	2 (3)	32 (44)
Rhinovirus	58	19 (33)	6 (10)	5 (9)	2 (3)	30 (52)
Parainfluenza viruses	25	7 (28)	1 (4)	3 (12)	0 (0)	12 (44)
Adenovirus	19	2 (11)	2 (11)	2 (11)	2 (11)	9 (47)
Influenza A and B viruses	10	5 (50)	1 (10)	1 (10)	1 (10)	6 (60)

* Numbers in parentheses, percent.

DISCUSSION

We found evidence of the etiology of community-acquired pneumonia in 85% of the hospitalized patients. Sixty-two percent of the patients had viral infection and 53% had bacterial infection. In previous studies the specific cause of pneumonia has been identified in 40 to 80% of cases, but no single study has included adequate studies for detection of all of the most common causative agents. In addition in many earlier studies, patient inclusion criteria were restrictive (Table 4).

The observations clearly show that for optimal yield several techniques should be used even for the detection of one microbe. RSV culture alone would have detected only 58% of the RSV cases found. In a recent study by Wubbel et al.[19] viral culture and direct fluorescent antibody tests from nasopharyngeal and pharyngeal swabs detected viruses in only 20% of 157 ambulatory children with community-acquired pneumonia compared with 62% in this study. Measurement of pneumolysin IgG antibodies found only 50% of 93 serologically verified pneumococcal infections. It has been shown earlier that identification of nonbacteremic pneumococcal infections in children is difficult and confusing because different assays give different results,[8, 16, 20] as was also seen in this study. Blood culture is positive only in 1 to 3% of the patients, and in clinical practice its use can be individualized to patients who appear ill. It is probable that we could have increased our yield with *S. pneumoniae* and with other microbes if PCR techniques could have been used more widely.[21, 22]

As in several previous studies RSV and *S. pneumoniae* were the most common etiologic agents of childhood pneumonia (Table 4). By preventing these two infections more than one-half of our pneumonia cases could have been prevented. A new finding is that in 58 patients (24%) rhinovirus was detected by culture or PCR from the nasopharynx, suggesting its common role in the etiopathogenesis of childhood pneumonia. Five studies have earlier identified rhinoviruses by culture in childhood pneumonia with 2 to 12% recovery rates.[11, 12, 16, 23, 24] Rhinoviruses have also been detected from bronchial samples, supporting their role in the etiology of pneumonia.[25] A part of the PCR-positive cases, however, may have resulted from carriage.[26]

There is increasing evidence that many childhood respiratory infections may be caused by more than one pathogen. The classic view is that viruses pave the way for bacterial infection. We found evidence of viral-bacterial infection in 30% of the children with pneumonia, a result which is in agreement with previous studies.[14, 15, 16, 18] Whether upper respiratory viral infection only predisposed to bacterial infection in the lung or whether the viruses were also involved in the

TABLE 4. Etiology (%) of childhood community-acquired pneumonia: summary of studies in which both viruses and bacteria were studied

Study	*N*	Country	Duration (mo)	Age	No. of Microbes Detected/No. Studied	Blood Culture Positive	*Streptococcus pneumoniae*	*Mycoplasma pneumoniae*	*Haemophilus influenzae*	*Moraxella catarrhalis*	Total Bacteria*	Respiratory Syncytial Virus	Adenovirus	Rhinovirus	Influenza A or B	Parainfluenza 1, 2, 3	Total Viruses	Mixed Viral-Bacterial	Etiology Detected (Total)
Ramsey et al.,[11] 1986	162†	US	20	Median 21 mo	17/17	1	18	8	7	NS	29	9	4	4	7	7	31	8	49
Turner et al.,[12] 1987	98†	US	16	59 <2 yr; 39 ≥2 yr	12/13	3	17	0	2	NS	19	28	1	2	2	5	39	10	48
Ausina et al.,[13] 1988	198†	Spain	12	Mean 5 yr	11/11	NS	15	40	2	NS	58	15	3	NS	4	5	27	NS	79
Claesson et al.,[14] 1989	336‡	Sweden	14	Medians 1 and 5 yr‡	11/12	1	13	10	1	NS	23	20	5	NS	3	3	29	4	48
Nohynek et al.,[15] 1991	121§	Finland	12	Median 1.8 yr	12/14	1	16	9	17	7	45	28	13	NS	3	7	45	20	69
Ruuskanen et al.[16] 1992	50§	Finland	8	Mean 4.4 yr	10/16	0	38	20	12	10	62	30	10	10	2	8	60	34	88
Gendrel et al.,[17] 1997	104¶	France	30	Mean 5.6 yr	10/11	8	13	41	NS	NS	55	10	4	NS	4	6	29	8	84
Heiskanen-Kosma et al.,[18] 1998	201‖	Finland	12	105 <5 yr; 96 ≥5 yr	11/13	NS	28	22	6	3	51	21	2	NS	0	3	25	10	66
Wubbel et al.,[19] 1999	168**	US	23	106 <5 yr	12/—	0	21	7	NS	NS	28	8	2	NS	3	4	20	3	43

* Single etiologic agents, not mentioned here, are added.
† From 19 to 21% of the patients were hospitalized.
‡ One-half of the patients were hospitalized; only serologic methods were used; medians of age given for hospitalized patients and outpatients, respectively.
§ All children were hospitalized.
¶ One-half of the patients were hospitalized; most patients with *M. pneumoniae* pneumonia were ambulatory.
‖ 32% of the patients were hospitalized.
** All patients were ambulatory.
NS, not studied.

lower respiratory tract infection remains to be clarified. Even dual viral[16, 20] or dual bacterial[27] infections in childhood community-acquired pneumonia have been demonstrated, as was also seen in this study.

Our study lasted 3 years which included two double-humped RSV epidemics (which is typical for RSV in Finland[28]) and three rhinovirus outbreaks. If the study had lasted for only 1 year, like most earlier studies, markedly different observations would have been obtained concerning the number of *S. pneumoniae*, RSV, *M. pneumoniae* and influenza virus infections. Interestingly the numbers of *S. pneumoniae* infections increased during the study years. This observation is in agreement with the results of Baer et al.,[29] who found an increase in invasive bacteremic pneumococcal infections during early 1990s in Tampere, Finland.

It is important to emphasize the limitations of this study. All our observations are indirect evidence of the etiology of childhood community-acquired pneumonia, and causality should be considered with some care. No attempt was made to collect samples from the lung by transthoracic needle aspiration. This procedure is potentially harmful, and we do not consider it ethically acceptable in a developed country where the outcome of the illness is good. In addition some of our virologic and bacteriologic methods, although reasonably well-validated,[4, 7, 8, 9, 22] have been mostly used in our research laboratories, and little comparative data exist from other laboratories. Furthermore we studied only hospitalized children and a study on outpatients might have given different results. In two large recent studies on ambulatory patients, *C. pneumoniae* or *M. pneumoniae* were detected in nearly one-half of the patients.[30, 31]

By using extensive diagnostic methods the possible causative agent of childhood community-acquired pneumonia can be detected in most cases. A wide array of viruses and bacteria can cause childhood community-acquired pneumonia, and many infections are mixed infections. In one-half of the cases, the etiology can be identified in 1 to 2 days. The observations raise the question of which of the numerous etiologic investigations available would aid in the management of pneumonia. One option is to treat all children with community-acquired pneumonia without any etiologic tests. In the few cases with an unsatisfactory response,[16] depending on the initial treatment, tests for *M. pneumoniae*, *C. pneumoniae* and viruses as well as a search for penicillin-resistant pneumococci may prove to be helpful.

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Predictive value of pneumococcal nasopharyngeal cultures for the assessment of nonresponsive acute otitis media in children

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Background. **Nonresponsive acute otitis media (NR-AOM) is reported in >10% of children with OM treated with antibiotics. Drug-resistant *treptococcus pneumoniae* is currently consid-ed the leading cause of antibiotic failures in OM. Nasopharyngeal colonization with *S. pneu-oniae* was found to increase significantly dur-g episodes of AOM.**

Objectives. **To investigate the nasopharyngeal olonization with *S. pneumoniae* during NR-OM and compare it with that found in AOM not cently treated with antibiotics (NT-AOM); to sess the predictive value of nasopharyngeal neumococcal cultures results for the bacteriologic assessment of NR-AOM.**

Materials and methods. **Patients age 3 to 48 months with NT-AOM and NR-AOM were prospectively studied. Simultaneous nasopharyngeal cultures for *S. pneumoniae* and middle ear fluid cultures were obtained at enrollment. Antibiotic susceptibility testing was performed in all *S. pneumoniae* isolates. Penicillin and ceftriaxone MICs for *S. pneumoniae* were determined by E-test. The sensitivity, specificity and positive and negative predictive values of positive or negative nasopharyngeal cultures for the presence of *S. pneumoniae* in middle ear fluid were calculated.**

Results. **We studied 362 and 217 children with NT-AOM and NR-AOM, respectively. Of the children with NT-AOM and NR-AOM, 95 and 97%, respectively, were younger than 2 years of age. *S. pneumoniae* was isolated in the nasopharynx of 66 and 58% of children with NT-AOM and NR-AOM, respectively. Penicillin-nonsusceptible *S. pneumoniae* was isolated more frequently from the nasopharynx of patients with NR-AOM than from those with NT-AOM (84% *vs.* 47%; $P < 0.01$). Antibiotic susceptibility patterns were similar for *S. pneumoniae* isolates recovered from the nasopharynx and those from the middle ear fluid in both NT-AOM and NR-AOM. A positive nasopharyngeal culture had only little predictive**

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