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**STUDIES ON THE POTENTIAL OF HANDS AS VEHICLES FOR THE
SPREAD OF SELECTED HUMAN PATHOGENIC VIRUSES AND
BACTERIA**

A thesis submitted to the
School of Graduate Studies & Research
University of Ottawa

In partial fulfillment of the requirements for the degree of
Doctor of Philosophy
Department of Microbiology and Immunology
Faculty of Medicine

Shamim Alam Ansari



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UNIVERSITÉ D'OTTAWA
UNIVERSITY OF OTTAWA

Dedicated to my parents and my sister Tahira

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ABSTRACT

In this study, a simple protocol was developed to test the survival of a human rotavirus (HRV), rhinovirus type 14 (RV-14), and parainfluenzavirus type 3 (HPIV-3) as well as *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) on human hands. HRV and *E. coli* were suspended in 10% feces in normal saline, whereas RV-14, HPIV-3 and *S. aureus* were suspended in bovine mucin (5 mg/mL) in normal saline. Ten μ L of the test suspension was placed on each fingerpad. One mL of 20% tryptose phosphate broth (TPB) in Earle's balanced salt solution (EBSS) was used as an eluent for HRV, *E. coli* and *S. aureus*; EBSS alone was used for the elution of RV-14 and HPIV-3.

At the end of each experiment with HRV, HPIV-3, *E. coli* and *S. aureus*, hands of the volunteers were decontaminated by thoroughly rinsing them with 70% ethanol followed by washing with a germicidal liquid soap; for RV-14, hands were first rinsed in 5% acetic acid and then washed with the germicidal soap.

The HRV experiments were carried out on six adult volunteers. After 20, 60, and 260 minutes of virus inoculation, approximately 57, 43, and 7%, respectively, of the input infectious virus could be recovered from their fingerpads. The survival of RV-14 and HPIV-3 on hands was compared using the four adult volunteers for both agents. HPIV-3 lost nearly all of its infectivity within the first 60 minutes on the fingerpads. In contrast to this, nearly 16% of infectious RV-14 could be recovered even after 3 hours.

The poor survival of HPIV-3 on human hands prompted us to investigate its survival on stainless steel disks (1 cm in diam.) under

ambient conditions. After 20 minutes, nearly 38% of the input infectious virus could be recovered from the disks. On the other hand, 67% of RV-14 remained viable on the disks after 20 minutes under the same conditions of temperature and relative humidity (RH). The ability of the two respiratory viruses to survive well on non-porous inanimate surfaces indicates that, once contaminated, these surfaces may act as a potential virus source.

E. coli and *S. aureus* survival was tested on the hands of three volunteers. After 60 minutes on the fingerpads, only 3.5% of the input viable *E. coli* remained detectable. *S. aureus* was found to be more stable and after 60 minutes nearly 41% of it remained viable.

To study the patterns of virus transfer, the following three experimental models were developed and tested: finger-to-finger, finger-to-disk and disk-to-finger. For virus transfer, the inoculum was allowed to dry on the donor surface for the desired length of time and virus transfer was attempted by keeping the donor surface in contact with the target surface (recipient) for 5 seconds at a pressure of approximately 1 kg/cm².

HRV transfer was studied 20 and 60 minutes after contamination of the donor surface and 16% and 1.8%, respectively, of the residual infectious virus could be transferred from contaminated fingerpads to clean disks; conversely, when a clean fingerpad was pressed against a contaminated disk after 20 and 60 minutes, virus transfer was 17% and 1.6%, respectively. Contact between a contaminated finger and a second clean finger 20 and 60 minutes after virus inoculation resulted in the

transfer of 7% and 2.8%, respectively, of the residual infectious virus. These results suggest that human hands and environmental surfaces, singly or in combination, have the potential to spread rotaviral infections, particularly in institutional settings.

RV-14 and HPIV-3 transfer experiments were conducted after 20 minutes of contamination only. Irrespective of the type of donor or target surface, only 0.7 to 0.9% of RV-14 was transferred. Transfer of HPIV-3 from finger-to-finger or finger-to-disk could not be demonstrated, but 1.5% of the residual infectious HPIV-3 was transferred from disks to fingers. The comparatively rapid loss of HPIV-3 infectivity on hands may reduce their potential as vehicles for transmission. These results also suggest a role for environmental surfaces in the contamination of hands with respiratory viruses.

Ten hand-washing agents and tap water were compared for their ability to eliminate HRV from the fingerpads of an adult volunteer; three volunteers were used to test four of these hand-washing agents and tap water against *E. coli* and *S. aureus*. The contaminated site was exposed to the test product for 10 seconds, rinsed in tap water, and dried on a paper towel. The residual inoculum was then eluted for assay. Selected agents were also tested in parallel by a whole-hand method in which the palms of both hands were contaminated.

Alcohols (70%) alone or with Savlon reduced HRV titre by >99%, whereas the reductions by Provioline, Dettol, and Hibisol ranged from 95-97%. Aqueous solutions of chlorhexidine gluconate were significantly less effective for virus elimination than 70% alcohols. Savlon in tap water

(1:200) was found to be much less effective for eliminating HRV (80.6%) than for *E. coli* (98.9%) or *S. aureus* (96.2%). Tap water alone and liquid soap reduced HRV by 83.6% and 72.5%, respectively, whereas *E. coli* was reduced by 90% and 68.7%, and *S. aureus* by 99% and 99.4%, respectively. The results of the whole-hand method agreed well with those of the fingerpad protocol.

Since washing hands with ordinary liquid soap and water was found to be relatively ineffective for the elimination of HRV, we tested to determine if the use of such a hand-washing agent could result in the spread of localized HRV contamination to a wider area of the hands during washing. One hand was contaminated with fecally-suspended HRV and the inoculum allowed to dry. Both hands were then washed together using the soap and water and dried. Infectious HRV could be recovered from the second hand.

We compared the efficiency of paper-, cloth- and an electric blow dryer in further reducing the level of infectious rotavirus and *E. coli* remaining on fingerpads washed with either 70% isopropanol, Savlon in water (1:200), an unmedicated liquid soap, or tap water alone. The contaminated area on the fingerpads of a volunteer was exposed to the hand-washing agent for 10 seconds and then rinsed in 40°C tap water. The washed areas were dried for 10 seconds using one of the 3 methods. Irrespective of the hand-washing agent used, warm air drying produced the greatest and the cloth the smallest reduction in the numbers of both test organisms. These findings indicate the importance of the proper

drying of washed hands, particularly when less effective hand-washing agents are used.

The results of this study show that HRV, RV-14 and *S. aureus* can survive on human hands long enough to permit their spread through hands. In contrast to this, parainfluenzaviruses and *E. coli* appeared to be limited in their capacity to survive on hands. The differences found in the efficacy of commonly used hand-washing agents against HRV and the bacteria tested point to the importance of testing such products by proper *in vivo* protocols using representative viruses.

TABLE OF CONTENTS

| | <u>Page</u> |
|--|--------------------|
| ACKNOWLEDGEMENTS | i |
| ABSTRACT | ii |
| TABLE OF CONTENTS | viii |
| LIST OF TABLES | xiii |
| LIST OF FIGURES | xv |
| LIST OF ABBREVIATIONS | xvii |
| LITERATURE REVIEW | 1 |
| I. The Role of Human Hands in the Spread of Infectious Diseases | 2 |
| 1. Hospitals | 7 |
| 2. Daycare centres | 11 |
| 3. Nursing homes | 12 |
| 4. Other institutions | 13 |
| 5. Rotaviruses | 15 |
| 6. Rhinoviruses | 18 |
| 7. Parainfluenzaviruses | 20 |
| 8. <i>Escherichia coli</i> | 24 |
| 9. <i>Staphylococcus aureus</i> | 26 |
| II. Hygienic Hand-washing | 29 |
| III. Hand drying Methods | 34 |
| OBJECTIVES | 35 |

| | |
|---------------------------------|----|
| MATERIALS AND METHODS | 37 |
| I. Materials | 38 |
| 1. Biohazard containment | 38 |
| 2. Cells | 38 |
| a) MA-104 | 38 |
| b) HeLa | 39 |
| 3. Viruses | 39 |
| a) Rotaviruses | 39 |
| b) Rhinovirus | 40 |
| c) Parainfluenzavirus | 41 |
| 4. Bacteria | 41 |
| a) <i>Escherichia coli</i> | 41 |
| b) <i>Staphylococcus aureus</i> | 42 |
| 5. Organic loads | 42 |
| a) feces | 42 |
| b) mucin | 42 |
| 6. Eluents | 42 |
| a) Tryptose phosphate broth | 42 |
| b) EBSS | 43 |
| 7. Stainless steel disks | 43 |
| 8. Volunteers | 43 |
| 9. Hand-washing agents | 44 |
| 10. Hand-drying agents | 44 |
| II. Methods | 46 |
| 1. Plaque assays | 46 |
| a) Rotavirus | 46 |

| | |
|---|-----------|
| b) Rhinovirus | 47 |
| c) Parainfluenzavirus | 48 |
| 2. Protocol to study survival of viruses and bacteria on hands | 48 |
| a) Rotavirus | 48 |
| b) Rhinovirus | 54 |
| c) Parainfluenzavirus | 54 |
| d) <i>E. coli</i> | 59 |
| e) <i>S. aureus</i> | 59 |
| 3. Procedure for testing virus survival on inanimate surfaces | 59 |
| 4. Procedure for testing virus transfer | 60 |
| 5. Hygienic hand-washing | 60 |
| a) Fingerpad protocol | 60 |
| b) Whole-hand protocol | 67 |
| c) Removal of cytotoxicity | 68 |
| d) Removal of bacterial and fungal contaminants | 68 |
| 6. Hand-drying | 68 |
| 7. Statistical analysis | 69 |
| a) Survival experiments | 69 |
| b) Hygienic hand-washing | 69 |
| c) Hand-drying study | 70 |
| RESULTS | 73 |
| I. Development of methods | 74 |
| 1. Virus elution from fingerpads | 74 |
| 2. Efficiency of virus recovery | 76 |
| II. Survival of viruses and bacteria on fingerpads | 76 |
| 1. Rotavirus survival | 76 |

| | |
|---|-----|
| 2. Rhinovirus survival | 78 |
| a) Rhinovirus survival on fingerpads | 78 |
| b) Rhinovirus survival on disks | 81 |
| 3. Parainfluenzavirus survival | 81 |
| a) Parainfluenzavirus survival on fingerpads | 81 |
| b) Parainfluenzavirus survival on disks | 86 |
| 4. <i>E. coli</i> survival on fingerpads | 86 |
| 5. <i>S. aureus</i> survival on fingerpads | 86 |
| III. Study of Virus transfer between contaminated and clean surfaces | 92 |
| 1. Rotavirus transfer | 92 |
| 2. Rhinovirus transfer | 95 |
| 3. Parainfluenzavirus transfer | 98 |
| IV. Hygienic hand-washing | 98 |
| 1. <i>In vivo</i> testing of hand-washing agents against HRV | 100 |
| 2. <i>In vivo</i> testing of hand-washing agents against HRV and <i>E. coli</i> | 103 |
| 3. Comparison of the whole-hand method with the fingerpad protocol | 104 |
| 4. Spread of localized contamination from one hand to another | 106 |
| 5. <i>In vivo</i> testing of hand-washing agents against <i>s. aureus</i> | 108 |
| V. The efficacy of hand-drying methods | 108 |
| DISCUSSION | 117 |
| 1. The fingerpad protocol | 119 |
| 2. Survival of viruses and bacteria on hands | 121 |

| | |
|---|------------|
| a) Rotavirus | 122 |
| b) Rhinovirus and Parainfluenzavirus | 123 |
| c) <i>E. coli</i> and <i>S. aureus</i> | 125 |
| 3. Virus survival on inanimate surfaces | 129 |
| a) Rhinovirus and Parainfluenzavirus | 129 |
| 4. Virus transfer from contaminated surfaces to clean surfaces | 131 |
| a) Rotavirus | 131 |
| b) Rhinovirus and Parainfluenzavirus | 133 |
| 5. Antisepsis | 135 |
| 6. Drying of washed hands | 144 |
| CONCLUDING REMARKS | 148 |
| REFERENCES | 152 |
| APPENDIX I | 195 |
| APPENDIX II | 196 |
| APPENDIX III | 195 |

LIST OF TABLES

| | <u>Page</u> |
|--|--------------------|
| 1. Hand-washing agents tested | 45 |
| 2. Efficiency of various eluents in rotavirus recovery from experimentally-contaminated fingerpads | 75 |
| 3. Efficiency of virus recovery from washed and unwashed hands | 77 |
| 4. Rotavirus survival on experimentally-contaminated fingerpads | 79 |
| 5. Survival of RV-14 on fingerpads | 80 |
| 6. Survival of RV-14 on stainless steel disks | 82 |
| 7. Survival of HPIV-3 on fingerpads | 83 |
| 8. Survival of HPIV-3 on fingerpads within 10 min of deposition | 87 |
| 9. Survival of HPIV-3 on stainless steel disks | 88 |
| 10. Survival of <i>E. coli</i> on experimentally-contaminated fingerpads | 91 |
| 11. Survival of <i>S. aureus</i> on experimentally-contaminated fingerpads | 93 |
| 12. Transfer of infectious rotavirus from contaminated to clean surfaces in three separate models | 94 |
| 13. Transfer of RV-14 from contaminated to clean surfaces 20 min after virus deposition | 96 |
| 14. Total detectable virus in RV-14 transfer experiments | 97 |
| 15. Transfer of HPIV-3 from contaminated disks to clean fingers min after virus deposition | 99 |

| | |
|--|-----|
| 16. <i>In vivo</i> efficacy of hand-washing agents against human rotavirus using the fingerpad protocol | 101 |
| 17. <i>In vivo</i> efficacy of hand-washing agents against human rotavirus using the fingerpad protocol without the drying step | 102 |
| 18. Comparison of the <i>in vivo</i> efficacy of hand-washing agents against human rotavirus and <i>E. coli</i> using the fingerpad protocol | 105 |
| 19. Comparison of the whole-hand (WH) and fingerpad (FP) protocols | 107 |
| 20. Rotavirus spread from contaminated -left hand to clean right hand during hand washing | 109 |
| 21. <i>In vivo</i> efficacy of hand-washing agents against <i>S. aureus</i> in fingerpad protocol | 110 |
| 22. Comparison of <i>in vivo</i> efficacy of hand-washing agents against <i>S. aureus</i> and <i>E. coli</i> using the fingerpad protocol | 111 |
| 23. Relative efficacy of the hand-washing agents in eliminating rotavirus and <i>E. coli</i> from fingerpads without the drying step | 113 |

LIST OF FIGURES

| | <u>Page</u> |
|--|--------------------|
| 1. Vehicles involved in the spread of enteric infections | 3 |
| 2. Vehicles involved in the spread of respiratory infections | 5 |
| 3. Demarcated area with dried inocula (10 uL) on the fingerpads were made by pressing vials against finger | 50 |
| 4. Positioning of the vial (with 1 mL of eluent) on the fingerpad for virus recovery | 50 |
| 5. Flow diagram of the procedure for testing human rotavirus survival on fingerpads | 52 |
| 6. Flow diagram of the procedure for testing RV-14 survival on fingerpads | 55 |
| 7. Flow diagram of the procedure for testing HPIV-3 survival on fingerpads | 57 |
| 8. Flow diagram of the procedure to study transfer of viruses from experimentally contaminated to clean surfaces | 61 |
| 9. <i>In vivo</i> protocol for testing the hand-washing agents against viruses | 63 |
| 10. Flow diagram of the procedure for testing hand-washing agents by whole-hand method | 65 |
| 11. Illustration of the procedure to compare the efficacy of hand-washing and drying methods against viruses | 71 |
| 12. Comparison of the survival of HPIV-3 and RV-14 on the fingerpads of adult volunteers | 84 |
| 13. Comparison of the survival of HPIV-3 on fingerpads and | |

| | |
|--|-----|
| stainless steel disks | 89 |
| 14. Recovery of <i>E. coli</i> (a) and rotavirus (b) from fingerpads after washing and drying | 114 |
| 15. Survival of viruses and bacteria on fingerpads | 126 |

LIST OF ABBREVIATIONS

| | |
|-------------------|----------------------------------|
| BM | bovine mucin |
| BUDR | 5-bromodeoxyuridine |
| °C | degree centigrade |
| CFU | colony forming unit |
| cm | centimeter |
| CPE | cytopathic effect |
| CO ₂ | carbon dioxide |
| DEAE-Dextran | diethylaminoethyl-dextran |
| EBSS | Earle balanced salt solution |
| <i>E. coli</i> | <i>Escherichia coli</i> |
| EMEM | Eagle's minimal essential medium |
| EV-70 | Enterovirus 70 |
| FCS | fetal calf serum |
| g | gravitational force |
| h | hour |
| HBV | Hepatitis B virus |
| HPIV-3 | Human parainfluenzavirus type 3 |
| HRV | Human rotavirus |
| HSV | Herpes simplex virus |
| L | litre |
| m | metre |
| mg | milligram |
| MgCl ₂ | magnesium chloride |
| min | minutes |

| | |
|--------------------|-------------------------------------|
| mL | millilitre |
| NaOH | sodium hydroxide |
| nm | nanometre |
| p | probability |
| PBS | phosphate buffered saline |
| PFU | plaque forming unit |
| ppm | parts per million |
| RH | relative humidity |
| RV-14 | rhinovirus type 14 |
| RV-39 | rhinovirus type 39 |
| RSV | respiratory syncytial virus |
| SA-11 | Simian rotavirus |
| SD | standard deviation |
| <i>S. aureus</i> | <i>Staphylococcus aureus</i> |
| TCID ₅₀ | tissue culture infective dose (50%) |
| TPB | tryptose phosphate broth |
| ug | microgram |
| uL | microlitre |
| v/v | volume for volume |
| w/v | weight for volume |

LITERATURE REVIEW

I. THE ROLE OF HUMAN HANDS IN THE SPREAD OF INFECTIOUS DISEASES

The first step in the spread of an infection is the deposition of the causative agent at the portal of its entry in an amount sufficient to initiate the process of infection. The various vehicles involved in the spread of enteric and respiratory infections are depicted in Figures 1 and 2, respectively.

Hands are particularly important in domestic settings and in institutions where care-givers have frequent contact with young children (Adler, 1988; Ekanem et al., 1983; Hendley, 1973; Lemp et al., 1984). In those daycare centers where the same staff was involved in diaper changing and cooking, the frequency of outbreaks of infections was significantly higher (Hadler et al., 1980; 1982). Viral antigens (Samadi et al., 1983) as well as infectious viral particles (Faix, 1985; Hutto et al., 1986; Keswick et al., 1983a; Pickering et al., 1986) have been recovered from the hands of care-givers. It should, however, be noted here that properly controlled studies to generate direct evidence in this regard are particularly difficult to design and even harder to conduct mainly because of ethical considerations, numbers of variables involved and budgetary constraints.

In the following paragraphs a review of outbreaks of infectious diseases, especially enteric and respiratory infections, in institutional settings is presented to provide the basis for consideration of the potential role of hands in the spread of infectious diseases. It may also reflect the difficulties involved in demonstrating unequivocally that hands play a direct role in the spread of infectious diseases.

Figure 1. Vehicles involved in the spread of enteric infections.
(broken lines indicate that transmission of pathogens through these route is possible but evidence for it is lacking)

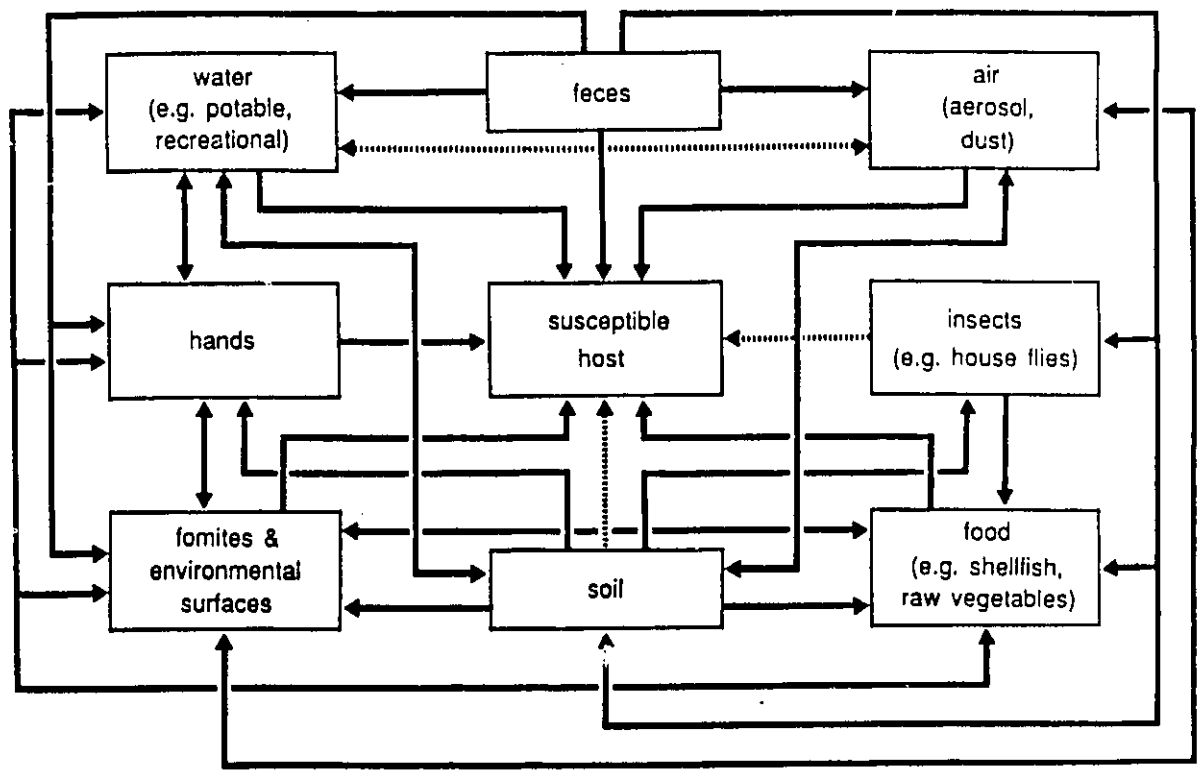
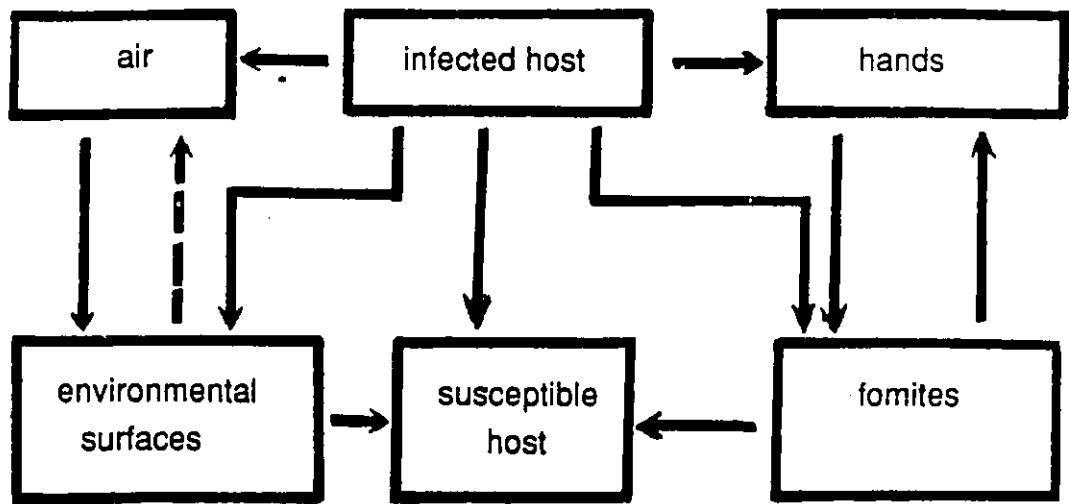


Figure 2. Vehicles involved in the spread of respiratory infections.
(broken line indicates that transmission of respiratory pathogens through this route is possible but evidence for it is lacking)



1. Hospitals:

Outbreaks of nosocomial infections (Allen and Ford-Jones, 1990; Coovadia et al., 1989; Davies et al., 1987; Ford-Jones, 1987; Jones and Martin, 1987; Singh-Naz et al., 1990) continue to be a serious problem. Such infections not only increase the morbidity rate but because of the extended hospital stay, add a significant economic burden (Matson and Estes, 1990). In the United States, approximately two million nosocomial infections occur annually in acute-care patients (Haley et al., 1985). In Canada, nosocomial infections are estimated to cost the health-care system 3 hundred million to 1 billion dollars annually (Losos and Trotman, 1984). The actual figure may be much higher because the impact of viral infections is often underestimated due to the difficulties and limitations associated with viral diagnosis (Brandt et al., 1986; Dagan et al., 1985; Jarvis et al., 1984).

Outbreaks of bacterial and viral infections in hospital pediatric wards and nurseries (Boyer et al., 1975; Davies et al., 1987; de Torres et al., 1978; Eisenbach et al., 1972; Gardner et al., 1973; Meissner et al., 1984; Mufson et al., 1973; Pacini et al., 1987; Rabkin et al., 1988; Valenti and Wehrle, 1986; Vial et al., 1988) and in neonatal intensive-care units (Rotbart et al., 1983; Klein et al., 1984; Tufvesson et al., 1986; Valenti et al., 1982) are common (Ford-Jones, 1987). There are reports of nosocomial viral infections in adult patients as well (Mathur et al., 1980; Marrie et al., 1982; Rahman, 1979). Children and adults with underlying immunosuppressive or debilitating conditions are at particular risk of contracting nosocomial infections (Donowitz, 1987; Hughes and Jarvis, 1987). In a prospective study, viruses were found to be responsible for 14.3% of all nosocomial infections in a pediatric

hospital (Welliver and McLaughlin, 1984). The most common infections were respiratory and gastrointestinal, amounting to 23.9% and 16.8%, respectively, of all infections. Viruses caused 1/3 of all nosocomial infections in the infant ward, prolonging hospital stay and causing death due to respiratory and congestive heart failure.

Rotaviruses have been described as a frequent cause of nosocomial diarrhea among children (Blaufuss, 1983; Chapin et al., 1983; Dennehy and Peter, 1985; Di Matteo et al., 1989; Hjelt et al., 1985; Matson and Estes, 1990; Middleton et al., 1977; Noone and Banatvala, 1983; Ryder et al., 1977). Attack rates of nosocomial rotavirus infection have been reported to be as high as 17% on infant wards, and the associated gastroenteritis usually prolonged hospitalization by several days (Dennehy and Peter, 1983; Flewett, 1982; Noone and Banatvala, 1983).

There are reports of possible involvement of contaminated hands in nosocomial outbreaks of rotavirus gastroenteritis. In an earlier study (Samadi et al., 1983) in a health clinic, hand-washings were sampled for the presence of rotaviruses; rotavirus antigen was detected in 78% of the hand-washings of those attending to infants with rotaviral diarrhea. Moreover, rotavirus antigen was also identified in hand-washings of 19% of the personnel not in direct contact with rotavirus-infected infants. This indicates that not only the hands of care-givers can be contaminated with rotaviruses but in fact, virus can be transferred to other personnel as well. Hospital staff may also be involved in spreading rotaviruses from one area of the hospital to another via contaminated hands and garments (Cubitt et al., 1980; Middleton, 1982).

Enteric adenoviruses also cause nosocomial infections (Chany et al., 1958; Vihma, 1969; Yolken et al., 1982); persons with congenital

immunodeficiency and immunosuppressed cancer or transplant patients may be at particular risk of severe, or even fatal, adenovirus infections (Stalder et al., 1977; Yolken, 1983). However, like CMV, some of the infections in such patients may be due to the reactivation of an indigenous virus (Shields et al., 1985). Nevertheless several outbreaks of adenovirus-associated epidemic keratoconjunctivitis in health-care settings have been reported to be caused by fomites and hands have been implicated as important vehicles in such outbreaks (Harrison et al., 1979; Hendley, 1973; Levandowski and Rubenis, 1981; Straube et al., 1983; Tullo and Higgins, 1980).

Members of the family Picornaviridae are also involved in nosocomial infections. So far, there are published reports of at least 18 outbreaks of echovirus infections in neonatal nurseries (Carolane et al., 1985; Hughes and Jarvis, 1987; Kinney et al., 1986; Modlin, 1986; Rabkin et al., 1988). An echovirus infected adult or a vertically infected neonate was thought to be the source for subsequent nosocomial spread of echovirus infection to other infants (Davies et al., 1979; Mertens et al., 1982; Nagington et al., 1978; 1983). It was suggested that the echovirus spread among the infants might have occurred via contaminated **hands** of health-care personnel (Modlin, 1988).

In recent years, there has been an increase in the reports on hepatitis A virus-related outbreaks of disease in hospitals (Goodman et al., 1982; Noble et al., 1984; Orenstein et al., 1981; Reed et al., 1984). One outbreak in a neonatal intensive care unit was believed to have been spread by contaminated **hands** of the nursing staff (Klein et al., 1984).

Nosocomial rhinovirus infections have regularly been reported (Kellener et al., 1988; Krilov et al., 1986; Valenti et al., 1982) and there is

considerable experimental evidence for **hands** as the vehicles for rhinovirus colds (Hendley and Gwaltney, 1988; Gwaltney et al., 1978).

Respiratory syncytial virus (RSV), parainfluenzaviruses and respiratory adenoviruses are also frequently involved in nosocomial infections (Meissner et al., 1984; Ford-Jones, 1987; Hall, 1983; Singh-Naz et al., 1990; Valenti and Wehrle, 1986). RSV appears to be the most common cause of hospital-acquired respiratory infection in nurseries and on pediatric wards during community outbreaks (Chanock et al., 1989). One study has shown that 45% of infants who stayed in a hospital for 1 week or more became infected with RSV. Furthermore, all of the infants who were hospitalized for 4 weeks or more, contracted RSV-associated respiratory infections (Hall et al., 1975).

Parainfluenzaviruses are second only to RSV as important causes of lower respiratory tract disease in young children, and commonly reinfect older children and adults to produce upper respiratory illnesses (Bisno et al., 1970; Glezen et al., 1989). They are commonly detected in cases of nosocomial infections in children on pediatric wards (Gardner et al., 1973; Mufson et al., 1973; Sims, 1981; Welliver and McLaughlin, 1984) and in neonatal units (Meissner et al., 1984).

The major mode of transmission of RSV was thought to be through droplet nuclei or small particle aerosols, but more recent evidence indicates that the spread is rather by large droplet or fomites, requiring either close contact with infected infants or contamination of hands and subsequent self-inoculation (Hall, 1980; Hall and Douglas, 1981a; 1981b).

S. aureus and *E. coli* are also important nosocomial bacterial pathogens. In recent years, methicillin-resistant strains of *S. aureus*

(MRSA) have become a major cause of nosocomial infections (Farrington et al., 1990). These organisms are frequently recovered from the **hands** of hospital personnel (Eisenbach et al., 1972; Rammelkamp et al., 1964; Sprunt et al., 1973). Boyer et al. (1975) recovered fecal coliforms from the hands of nursery personnel during an outbreak of gastroenteritis. Direct contact with contaminated articles was shown to be the cause of hand contamination; the use of disposable gloves by the nursery staff is believed to have resulted in the control of the outbreak.

2. Daycare centres:

Outbreaks of a variety of enteric infections frequently occur in daycare centres for infants and children and are caused by a variety of agents such as *Giardia* (Black et al., 1977; Pickering et al., 1981; Polis et al., 1986), *Shigella* sp. (Pickering et al., 1981; Weisman et al., 1975), *E. coli* (Bower et al., 1989; Ekanem et al., 1983; Paulozzi et al., 1986; Spika et al., 1986; Wenigen et al., 1983), *Campylobacter* sp. (Blaser and Reller, 1981), rotaviruses (Bartlett et al., 1988; O'Ryan et al., 1990; Pickering et al., 1981; Pickering et al., 1986), and hepatitis A virus (Hadler and McFarland, 1986).

Respiratory pathogens most commonly involved in daycare centres are *Hemophilus influenzae* type B (Ward et al., 1978), RSV (Hall et al., 1980), parainfluenzaviruses (Denny and Clyde, 1986) and rhinoviruses (Gwaltney, 1989). Daycare centres also appear to be conducive to the transmission of cytomegalovirus (CMV) infections (Adler, 1988; Hutto et al., 1986; Pass et al., 1984; 1986). CMV was recovered from the hands of three children and one of seven teachers and from toys during a random survey in a daycare center (Hutto et al., 1986). Survival of CMV was demonstrated on diapers, toys and hands of daycare personnel (Faix,

1985; Hutto et al., 1986; Schupfer et al., 1986).

Asymptomatic viral infections are also common in daycare centres (Barron-Romero et al., 1985; Friedman et al., 1988; Pickering et al., 1988). In some outbreaks of diarrhea in daycare centres, multiple pathogens have been isolated from children with diarrhea (Archer, 1989; Ekanem et al., 1983).

Although enteric viruses and bacteria have been isolated from environmental surfaces (Ekanem et al., 1983; Keswick et al., 1983a; Pancic et al., 1980; Wenigen et al., 1983), contaminated hands of children attending daycare centre as well as their care-givers are probably the main vehicle for the spread of infections. Some of the respiratory viruses may also spread via fomites-hand transmission routes (Gwaltney and Hendley, 1982; Hall et al., 1980). Children and staff infected in daycare centres may infect their close contacts in domestic settings (Barron-Romero et al., 1985; Hadler and McFarland, 1986; Pass and Hutto, 1986; Vernon et al., 1982).

3. Nursing homes:

Nursing homes provide another suitable environment for infectious diseases and there are frequent reports of outbreaks of enteric and respiratory diseases in such institutions (Arroyo et al., 1984; Carter et al., 1987; Fauvel et al., 1980; Halvorsrud and Orstavik, 1980; Jackson et al., 1990; Kaplan et al., 1982; Lewis et al., 1989; Mathur et al., 1980; Patriarca et al., 1986; Ryan et al., 1986; Tulloch et al., 1973). In some nursing institutions, enteric viruses were associated with 34% of the cases of diarrhea, whereas bacteria accounted for only 14% (Penland and Penington, 1980).

Carter et al. (1987) reported a severe outbreak of *E. coli* O157:H7-

associated hemorrhagic colitis in a nursing home. The source of the outbreak was contaminated sandwich meat but the secondary spread occurred through person-to-person contact and resulted in the deaths of an overall 19 (35%) of the affected residents.

4. Other institutions:

Outbreaks of infectious diseases also occur in schools (Hara et al., 1976; Koopman, 1978; Suttmoller et al., 1982), military barracks (Bancroft and Lemon, 1984), jails (McFarlane and Embil, 1982), and centres for the mentally retarded (Lehman et al., 1978; Matthew et al., 1973; Pavia et al., 1990). To what extent hands are involved in outbreaks of these diseases in such institutional settings is not fully understood.

Koopman (1978) reported that at least 44% of diarrhea cases in school children in Cali, Colombia, were related to unhygienic toilet conditions. Ekanem et al. (1983) found wide spread contamination of classroom objects (especially toys and classroom floors) with fecal bacteria. They isolated fecal coliforms from the hands of children and teachers with greater frequency during outbreak periods than on routine sampling; recovery rates of fecal coliforms were higher in teachers caring for non-toilet trained infants than those responsible for older age groups. A total of 11 outbreaks occurred in the five centres during the study period. Rotavirus was identified in four of the outbreaks in a total of 19 children, 13 of whom were ill at the time of specimen collection. They concluded that contaminated hands of children and staff probably played an important role in the dissemination of fecal bacteria and viruses in the school studied.

A study conducted during a non-outbreak period detected the presence of rotaviruses on environmental surfaces and on the hands of a

teacher (Keswick et al., 1983a). These observations suggest that several agents, including rotavirus, can remain infectious on environmental surfaces long enough to be transmitted to susceptible individuals. Though there are several laboratory studies carried out to investigate the survival of rotaviruses on various environmental surfaces and in air (Ijaz et al., 1985; Prince et al., 1986; Sattar et al., 1986), no information is available as to how long and to what extent rotaviruses can survive on human hands and how efficiently these viruses can be transferred from contaminated to clean surfaces. Black et al. (1981) demonstrated a significant reduction in diarrhea rates in daycare centres where a rigorous hand-washing program was instituted.

In view of the information presented above, it is clear that the outbreaks of certain enteric and respiratory infections are frequent especially in institutional settings. Isolation of enteric and respiratory pathogens from surrounding environmental surfaces and hands of personnel strongly suggests that these surfaces and human hands may play a vehicular role in the spread of infectious diseases not only within these centres but also to the household contacts and eventually into the community.

In order to establish the role of human hands or environmental surfaces in the spread of infectious diseases, it is very important to know how well an infectious agent survives on inanimate surfaces and hands, and how well it can be transferred from contaminated surfaces including human hands to clean surfaces or hands. Once the role of hands is established, the next logical approach would be to investigate the efficacy of various antiseptics against an infectious agent aimed at finding reliable chemical agents to interrupt the transmission of infectious

diseases. Finally with this information effective hand-washing guidelines can be formulated. This study was therefore undertaken to provide answers to these questions. In this study, two enteric pathogens (human rotaviruses, and *E. coli*), two respiratory pathogens (human rhinovirus type 14 and human parainfluenzavirus type 3) and *S. aureus*, a representative Gram-positive bacterium, were selected. The selection of the test organisms was based on their biological characteristics as well as their relative significance in institutional outbreaks. This information is briefly summarized in the following section.

5. Rotaviruses

On a global basis, rotaviruses are the most important viral pathogens in diarrheal disease requiring treatment or hospitalization of young children (Kapikian and Chanock, 1989). In developing countries, they account for nearly 6% of all diarrheal episodes and 20% of all diarrhea-associated deaths in children under 5 years of age (de Zoysa and Feachem, 1985). In industrialized countries as well, rotaviral gastroenteritis is a major cause of hospitalization of infants and young children (Brandt et al., 1983; Di Matteo et al., 1989; Donelli et al., 1988; Kapikian and Chanock, 1989; Madeley, 1983; Middleton et al., 1977). In the U.S. (Ho et al., 1988), rotaviral infections result in 50-90 deaths and about 66,000 hospitalizations each year.

Rotaviruses are members of the family Reoviridae. They are icosahedral viruses with two capsid layers and a diameter of 65-75 nm. The viral genome is composed of 11 segments of double-stranded RNA (Kapikian and Chanock, 1990). The majority of human and animal rotaviruses known share a common group antigen and are termed group A rotaviruses. This group contains at least 6 serotypes of human rotaviru-

ses (Kapikian and Chanock, 1990). Non-group A rotaviruses belong to groups B-F depending on their group specific antigen (Estes and Cohen, 1989). Present lack of information has made it difficult to assess the relative significance of non-group A rotaviruses in human disease. Rotaviral infection in the non-immune host generally results in profuse diarrhea with or without vomiting, fever or respiratory illness (Kapikian and Chanock, 1989). Virus shedding in the feces occurs and usually lasts for 5-7 days. In severe cases, rapid dehydration can lead to renal shutdown and death (Carlson et al., 1978; McCormack, 1982).

Reinfections with rotaviruses frequently occur (Bishop et al., 1983). A study in Argentina (Grinstein et al., 1989) showed at least 61% of rotavirus infections to be reinfections. Neonates and children are often asymptomatic rotavirus excretors (Barron-Romero et al., 1985; Champsaur et al., 1984; Vial et al., 1988). In adults, infections due to group A rotaviruses are generally milder than those by non-group A rotaviruses (Hrdy, 1987; Hung, 1988; Kapikian and Chanock, 1989; Su et al., 1986; Wang et al., 1985).

Rotaviruses are mainly transmitted from man to man by the fecal-oral route. In natural settings transmission from animals to man has not been demonstrated, although non-group A rotaviruses which caused epidemic diarrhea in adults in China (Hung et al., 1984) were also found in contaminated water and in pigs. Experimentally, rotaviruses can cross species barriers (Bishop, 1986; Mebus et al., 1976; Middleton et al., 1976; Torres and Lin, 1986; Wyatt et al., 1976). In volunteer studies, ingestion of viable rotavirus particles can infect humans (Kapikian et al., 1983; Ward et al., 1986) and challenge studies in both humans (Ward et al., 1986) and animals (Graham et al., 1987) show the minimal infective

dose to be as little as one cell culture infective unit.

As has been mentioned above, outbreaks of rotaviral infection are common in infants and young children in domestic (Champsaur et al., 1984; Grinstein et al., 1989), and institutional settings (Allen and Ford-Jones, 1990; Chan et al., 1989; Cone et al., 1988; Di Matteo et al., 1989; Ford-Jones, 1987; Guerrant et al., 1990; Hjelt et al., 1985; Maderova et al., 1987; Middleton et al., 1977; Noone and Banatvala, 1983; Raad et al., 1990; Roggeri et al., 1989; Bartlett et al., 1988; Barron-Romero et al., 1985; Guerrant et al., 1990; Keswick et al., 1983b; Pickering et al., 1981; Brown et al., 1989; Chiba et al., 1981; Hara et al., 1976; Linhares et al., 1983; Matsumoto et al., 1989).

One of the prerequisites for the vehicular spread of any virus is its ability to survive in or on a given vehicle and, in general terms, the chances of virus transmission by that vehicle increase in direct proportion to the length of virus survival. Apart from the nature of the vehicle itself, there are certain other factors which influence, directly or indirectly, the capacity of viruses to survive. Figure 1 shows the vehicles which may play a role in the spread of rotaviral gastroenteritis. It is almost certain that insects such as house flies mechanically spread rotaviruses under conditions of poor sanitation and hygiene.

Although it has been suggested that air may play a role in the spread of rotaviral gastroenteritis in institutional settings (Brandt et al., 1982; Brandt et al., 1984; Flewett, 1982; Fragosa et al., 1986; Goldwater et al., 1979; Nigro and Midulla, 1983), so far, there is no direct evidence to support this view. There is only one published report where airborne spread of an outbreak of rotaviral diarrhea in an island population was documented based on retrospective epidemiological evidence (Foster et

al., 1980).

Rotavirus spread is believed to have occurred through water in a number of outbreaks (Hopkins et al., 1984; Hung et al., 1984; Linhares et al., 1981; Lycke et al., 1978; Morens et al., 1979; Murphy et al., 1982; Suttmoller et al., 1982; Tulchinsky et al., 1984; Zamotin et al., 1981).

6. Rhinoviruses

Rhinoviruses, also known as "common cold viruses", account for about 30% to 50% of all respiratory illness (Couch, 1990). These are small (20-30 nm in diameter) non-enveloped, single stranded RNA viruses of the family Picornaviridae (Gwaltney, 1989). Since their first recognition, more than 100 serotypes have so far been described in the literature.

In the temperate regions, rhinoviruses are most prevalent from spring through early fall. Several rhinovirus serotypes often circulate simultaneously within a given population. In a Chicago nursery school, 14 different serotypes were isolated during the academic year, but 10 did not spread beyond the index case; only three types disseminated widely; infecting more than 40% of the children. Although the pattern of local prevalence of certain serotypes in a particular time interval seems well established (Fox et al., 1985), it is not clear whether some serotypes spread more easily than others.

Ordinarily, rhinoviruses cause mild upper respiratory illness. However, these organisms can cause serious respiratory disease, especially acute exacerbations of asthma. It has been suggested that rhinoviruses are the most common cause of asthma attacks in children aged 4 and older and that respiratory syncytial virus is most important in children of younger ages. Another recent report suggests a significant association

between rhinovirus infection and pulmonary disease in hospitalized infants and children (Krillov et al., 1986). Rhinoviruses also cause lower respiratory infections among adults. These studies confirm that rhinoviruses are capable of causing illnesses that are far more serious than generally realized.

It is well established that thousands of virus particles per mL are recovered from nasal washings of infected persons (D'Alessio et al., 1976; Douglas et al., 1966) and that less than one tissue culture infective dose can infect adults when given via aerosol (Cate et al., 1965), or inoculation of the nasal passages, or the mucosa of the anterior nares (Couch et al., 1966; D'Alessio et al., 1976; D'Alessio et al., 1984).

Epidemiological studies have shown that homes, schools, and other semi-closed institutions provide excellent sources of rhinovirus colds in the general population (D'Alessio et al., 1976, 1984; Meschievitz et al., 1984; Gwaltney, 1989), and that school-age children have higher rates of rhinovirus infection and are, therefore, considered as important reservoirs of these viruses (Beem, 1969; Gwaltney, 1989; Pereira et al., 1967; Fox et al., 1985).

The mechanism(s) of spread of these respiratory viruses are not clear. Based on the epidemiologic data, transmission of a rhinovirus cold is believed to occur through the airborne route or by direct person-to-person contact or indirectly via hands and fomites; but which route is more efficient for rhinovirus spread is still unresolved. In the last 15 years, data generated in this regard, have created two different schools of thought; one stresses the role of hand contact followed by self-inoculation as eminent in rhinovirus transmission (Gwaltney, 1989) and the other emphasizes air as the most important vehicle for rhinovirus

spread (Dick et al., 1987; Jennings et al., 1988).

Rhinoviruses have been recovered from environmental surfaces (Reed, 1975) and hands (D'Alessio et al., 1976; Hendley et al., 1973), and they are capable of surviving on surfaces for days (Hendley et al., 1973) and under conditions of non-use, rhinovirus type 13 (RV-13) dried on the skin surface of hands survived up to 3 hours (Hendley et al., 1973). These viruses have been shown to survive for at least 3 hours on experimentally-contaminated formica, stainless steel, nylon and plastic surfaces (Hendley et al., 1973; Reagan et al., 1981; Reed, 1975; Sattar et al., 1987).

Studies on the survival of rhinovirus type 14 on stainless steel disks in our laboratory have shown that RH affects greatly the survival capacity of these viruses. The half-life of the mucin suspended virus at high ($80\pm 5\%$), medium ($50\pm 5\%$) and low ($20\pm 5\%$) RH were 1.42, 0.55, and 0.24 hours, respectively (Sattar et al., 1987).

Spontaneous hand-nose movements are quite common (Hendley et al., 1973), thereby facilitating self-inoculation with virus on hands or contamination of hands with virus.

Successful transmission of rhinovirus infections was accomplished by direct transfer of nasal mucus via the fingers (Gwaltney et al., 1978) or through fomites (Gwaltney and Hendley, 1982). Transmission by the air-borne route was more difficult (Gwaltney et al., 1978) and only one of 12 persons was infected by 15 minutes of contact-free exposure to a rhinovirus infected volunteer seated at the same table.

7. Parainfluenzaviruses

Parainfluenzaviruses are 150-200 nm in diameter; they are enveloped viruses and belong to the genus *Paramyxovirus* of the family

Paramyxoviridae (Chanock and McIntosh, 1990). These viruses possess a negative single-stranded RNA genome. So far, there are four antigenic types of human parainfluenzaviruses recognized. Among the respiratory viruses, parainfluenzaviruses are important etiologic agents causing upper as well as lower respiratory tract infections of young children. Among older children and adults, they commonly reinfect to produce upper respiratory illnesses (Glezen et al., 1984). Types 1, 2, and 3 were shown to cause croup and type 3 is responsible for severe lower respiratory illnesses such as pneumonia and bronchiolitis (Chanock and Parrot, 1965; Chanock et al., 1963; Glezen and Denny, 1973; Glezen et al., 1984; Kim et al., 1961; Korppi et al., 1988); infections with type 4 parainfluenzavirus are detected infrequently, and associated illnesses are usually mild.

Although mortality due to parainfluenzavirus infections is quite low, the high morbidity rates associated with them result in significant economic loss every year (Merigan, 1982). Parainfluenzaviruses are second only to the respiratory syncytial virus in causing lower respiratory tract infections in young children (Chanock and McIntosh, 1990). In Europe and North America parainfluenzavirus type 3 is the most common parainfluenzavirus isolated from severely ill infants (Glezen et al., 1989). The morbidity of parainfluenzaviral infections may be more prominent in the elderly and chronically ill. Outbreaks of parainfluenza in nursing homes have been associated with an increased incidence of pneumonia and hospitalization (CDC, 1978).

Parainfluenzavirus types 1 and 2 cause community outbreaks in the fall, often in alternate years; type 3 used to be endemic throughout the year, but recently has shown clustering during spring and lasting

into the fall (Glezen et al., 1984). Several animal species appear to be natural hosts to parainfluenzaviruses, with the exception of parainfluenzavirus type 4, which has been found only in humans. Naturally-acquired antibodies to parainfluenzaviruses are found in cows, rodents, monkeys, and rabbits.

Apart from the infections observed in the general population (Denny and Clyde, 1986; Glezen and Denny, 1973; Hendley et al., 1969; Korppi et al., 1988; Monto, 1968), these viruses frequently cause outbreaks in hospitals (DeFabritus et al., 1979 Gardner et al., 1973; Glezen et al., 1971; Meissner et al., 1984; Mufson et al., 1973; Singh-Naz et al., 1990; Valenti et al., 1982), chronic care facilities (CDC, 1978), institutions for the mentally retarded (Jackson et al., 1990), schools (Beem, 1969; Pereira et al., 1967), daycare centres (Denny and Clyde, 1986; Loda et al., 1972), and other institutional settings (Hamre et al., 1966).

Infections caused by these viruses cause much sneezing, coughing, and production of profuse nasal discharge. Nasal discharges from children with primary parainfluenzavirus infections have been found to contain approximately 1000 TCID₅₀/mL of infectious virus (Hall, 1977). The secretions disseminated through coughs and sneezes are mostly in the form of particles greater than 15 μ m in diameter (Couch et al., 1966). In order for particles of this size to be inhaled, close person-to-person contact is required (Couch et al., 1966). The routes of inoculation that occur naturally have not been studied completely. Adults have been infected experimentally by intranasal and oropharyngeal inoculation, but the eye and anterior oral cavity as sites of inoculation have not been similarly examined (Kapikian et al., 1961; Tyrrell et al., 1959).

The actual mode of parainfluenza virus transmission, whether by direct person-to-person contact or via large droplet, or small-particle aerosol or indirectly via fomites or hands remains unknown. During parainfluenza outbreaks in hospitals and in nursing homes, clustering of cases and occurrence of illness in personnel having close contact with patients, suggest direct person-to-person transmission of virus (Chanock and McIntosh, 1990).

Airborne parainfluenzaviruses, at room temperature, have been found to survive better at 20% RH than when the RH is at 50 or 80% (Miller and Artenstein, 1967). Studies carried out in South Polar environment by Parkinson et al. (1983) showed that parainfluenzavirus type 1, with an initial titre of $3.78 \log_{10} \text{TCID}_{50}/\text{mL}$, was completely inactivated after 4 days indoors and after 7 days outdoors. Parainfluenzavirus types 2 and 3, with initial titres of 5.58 and 5.38 $\log_{10} \text{TCID}_{50}/\text{mL}$, respectively, were inactivated after 7 and 12 days, respectively, at room temperature, and after 17 days of storage outside. In that study, viruses were applied to the bottom inner surface of plastic Petri plates with a nebulizer. Hanks balanced salt solution containing 0.5% gelatin was added to the nebulizer chamber together with 1.0 mL of the stock virus solution. Although no statistical differences between virus inactivation at room temperature and outside storage were demonstrated, viruses in the warmer inside environment consistently showed earlier inactivation.

A recent paper (Brady et al., 1990) on the survival of parainfluenzaviruses on environmental surfaces reports that the virus can be recovered from facial tissue up to 2 hours and from formica and steel surfaces parainfluenzavirus type 2 was recoverable 2 hours after ino-

cultivation when the inoculum was allowed to dry. However, when the inocula on these non-porous surfaces were kept moist, parainfluenzavirus could be recovered as long as 10 hours after contamination. There are no reports on the survival of parainfluenzaviruses on human hands.

8. *Escherichia coli*

Escherichia coli (*E. coli*) is a Gram negative, rod-shaped bacterium which normally inhabits the gut of humans and warm-blooded animals. In this ecologic setting, *E. coli* is considered to be a part of the normal microbiological flora, except for a few divergent strains that can cause severe gastrointestinal tract and urinary tract infections. So far, five different types of diarrheagenic *E. coli* have been identified (Levine, 1987). They are designated as enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enterohemorrhagic *E. coli* (EHEC), and enteroadherent *E. coli* (EAEC). EAEC is still not well characterized. Serotyping of *E. coli* is based on the type of somatic (O) and flagellar (H) antigens they carry. There are currently 164 'O' and 56 'H' serogroups. These diarrheagenic strains differ in their pathogenesis, clinical features, epidemiology and O:H serotypes (Levine, 1987).

E. coli spreads through the fecal-oral route (Harris et al., 1985; Kessner et al., 1962; Paulozzi et al., 1986). Animal to human transmission has also been reported (Smith et al., 1965). *E. coli* has frequently been isolated from hands of hospital personnel and hands are suggested to be a major vehicle of their spread (Eisenbach et al., 1972; Salzman et al., 1967; Sprunt et al., 1973). In hospitals, EPEC has been shown to be transmitted by direct contact, by air or dust, and via fomites (DuPont, 1982). Asymptomatic adult excretors of EPEC strains have been blamed for the spread of the disease in infants and young children

(Kessner et al., 1962). Possible spread of *E. coli* through the respiratory route has been suggested by some workers (Boris et al., 1964; Kessner et al., 1962).

Diarrheagenic *E. coli* strains are important causes of gastrointestinal illness throughout the world. EPEC-associated outbreaks of diarrhea occur mostly in hospitals and daycare centres (Bower et al., 1989; Paulozzi et al., 1986), but community-based outbreaks (Boris et al., 1964; Echeverria et al., 1987; Kessner et al., 1962) and sporadic cases (Ulshen and Rollo, 1980) are also frequently observed. Studies of diarrhea cases based on hospital surveys have indicated that EPEC strains are responsible for 10 to 40% of illness (Boris et al., 1964; Gurwith and Williams, 1977). In developing countries, EPEC is a common cause of endemic infantile diarrhea (Charaemorakot et al., 1987; Echeverria et al., 1987; Moyenuddin and Rahman, 1985). These strains are also potential etiologic agents of nosocomial diarrhea of infants in developed countries (DuPont, 1982).

ETEC is most prevalent in North America, contributing up to 86% of the cases of diarrhea (Echeverria et al., 1975; Gurwith and Williams, 1977; Kapikian et al., 1976; Pickering et al., 1978). ETEC strains may cause epidemics of diarrhea in hospital nurseries (Guerrant et al., 1976; Ryder et al., 1976). ETEC is involved in 40-70% cases of diarrhea in United States travelers to Latin America (DuPont et al., 1976; Gorbach et al., 1975; Merson et al., 1976). ETEC infection with high morbidity and mortality is more common in Third World countries .

EHEC have been associated with outbreaks of enteric disease in schools, daycare centres (Spika et al., 1986), nursing homes (Carter et al., 1987; Ryan et al., 1986; Stewart et al., 1983), and in the community

(Lopez et al., 1989; Rogers et al., 1986; Tarr and Hickman, 1987). Many of these outbreaks were related to the consumption of beef (Riley et al., 1983; Ryan et al., 1986; Stewart et al., 1983). Though results from earlier investigations suggested that EHEC spread via food (Riley et al., 1983; Ryan et al., 1986), outbreaks in nursing homes in Canada, have shown both common-food and secondary person-to-person transmission (Waters, 1985; Carter et al., 1987; Hockin et al., 1987; Stewart et al., 1983). Similarly, person-to-person transmission appeared to be the predominant mode of spread of hemorrhagic colitis in daycare centre outbreaks in North Carolina (Spika et al., 1986). The patterns of food-borne transmission of EHEC in the community and institutions and secondary person-to-person spread are similar to the mode of spread observed with EPEC (Paulozzi et al., 1986) and EIEC (Marier et al., 1973).

EIEC has been incriminated in traveler's diarrhea (Wanger et al., 1988). This organism is also a common cause of illness in pediatric populations in certain developing countries (Taylor et al., 1986). More recently EIEC has been well documented as a cause of diarrheal disease in outbreaks with contaminated cheese, on a cruise ship, and in a mental institution (Harris et al., 1985; Snyder et al., 1984; Tulloch et al., 1973).

EAEC has also been documented in traveler's diarrhea (DuPont, 1982). There is increasing evidence of diarrhea in infants and young children due to EAEC (Nataro et al., 1987).

9. *Staphylococcus aureus*

Staphylococcus aureus (*S. aureus*) is a Gram-positive bacterium. *S. aureus* strains can be divided into four major categories: (1) penicillin-sensitive (2) penicillin-resistant that are susceptible to methicillin,

oxacillin, nafcillin and the cephalosporins; (3) strains with "borderline" resistance; and (4) MRSA that are resistant to methicillin, oxacillin and nafcillin and should be considered resistant to cephalosporins. This organism is an important human pathogen throughout the world. Epidemics by MRSA strains are frequently involved in outbreaks in hospitals (Farrington et al., 1990), especially in new-born nurseries (Coovadia et al., 1989), and in tertiary-care facilities (Ribner et al., 1989)). MRSA is also a problem in nursing homes (Storch et al., 1987). Food-poisoning by enterotoxin of coagulase-positive strains of *S. aureus* is another problem in many countries.

Rammelkamp et al. (1964) have described elegantly how the spread of *S. aureus* occurs in neonatal nurseries. They found that staphylococci do not frequently spread from a colonized baby to other infants in a nursery unless the babies are cared for by the same personnel. Only 3 of 91 infants acquired staphylococci from a colonized baby. However, when two nurses who had picked up staphylococci on their hands were allowed to handle non-colonized babies using routine techniques, 20% acquired their care-taker's strain. Hands of hospital personnel have been incriminated as the major vehicle in the transmission of MRSA from one patient to another within hospitals (Crossley et al., 1979; Peacock et al., 1980; Rammelkamp et al., 1964). In these studies, MRSA was found on the hands of employees who had contact with infected patients.

Airborne transmission of MRSA can occur; MRSA have been recovered from air samples taken near patients colonized or infected with MRSA (Crossley et al., 1979; Peacock et al., 1980). How frequently such transmission occurs is not clear. Possible airborne transmission of MRSA has been reported in burn units where care of burn-patients with MRSA

may lead to aerosolization of these organisms leading to contamination of environmental surfaces (Crossley et al., 1979; Rutala et al., 1983). Approximately 1 to 6% of hospital personnel involved in caring for patients with MRSA appeared to carry the organism in their anterior nares for varying periods of time (Locksley et al., 1982; Peacock et al., 1980). However, the mechanisms involved in the acquisition of these strains has not been fully documented. These individuals can be potential sources for MRSA transmission to other patients.

S. aureus has been isolated from various environmental surfaces within hospitals during several MRSA epidemics (Bartzokas et al., 1984; Crossley et al., 1979; Farrington et al., 1990). The major reservoir for MRSA within hospitals is MRSA-colonized and infected patients; especially MRSA outbreaks in intensive care units (ICU) and burn units often serve as important sources for nosocomial transmission of the organism (Boyce, 1989; Crossley et al., 1979; Locksley et al., 1982; Peacock et al., 1980).

How efficiently *S. aureus* survives on hands is not known. In a comprehensive study, Lowbury and Fox (1953) demonstrated the survival of *S. aureus* on glass slides. They suspended it in horse serum, normal saline, water or oleic acid and 100 minutes after contamination of the slides, the mean percentage survival of *S. aureus* were 81.9%, 47.9%, 35.7% and 17.4%, respectively. They also demonstrated that the Gram-positives survive better than Gram-negatives (*Pseudomonas pyocyanea*). However, they did not study the survival of this organism on hands. Several studies tested hand antiseptics against *S. aureus* (Ayliffe et al., 1988; Casewell et al., 1988; Ojajarvi, 1980).

II. HYGIENIC HAND-WASHING

The information summarized above clearly indicates that hands are potential vehicles for the spread of many infectious agents. They also focus on the importance of hygienic hand-washing in preventing outbreaks of infectious diseases, particularly in institutional settings. The importance of hand-washing in infection control has been recognized since the mid-nineteenth century. Semmelweis (1861) was the first to point to the role of contaminated hands in the transmission of purpural fever and he implemented strict hand-washing with chlorine water; this resulted in a rapid and remarkable reduction in the mortality rates in women.

The term 'hygienic hand-washing' is used to describe the washing of hands, particularly by health-care personnel, in order to eliminate transient microorganisms. Implementation of proper hygienic hand-washing has, in fact, been known to result in the control and prevention of outbreaks of institutionally-acquired infections (Black et al., 1981; Knittle et al., 1975; Koopman, 1978; Massanari and Hierholzer, 1984; Onesko and Wienke, 1987; Pacini et al., 1987; Raad et al., 1990; Salzman et al., 1967; Steere and Mallison, 1975). At the same time, a number of community-based prospective studies have also shown significant reductions in the numbers of cases of enteric diseases upon introduction of proper hygienic hand-washing (Feachem, 1984; Han and Hlaing, 1989; Khan, 1982; Taylor and Greenough, 1989). Hygienic hand-washing is therefore regarded as the single most important method of controlling and preventing disease spread in the community in general

and in hospitals, day-care centers, and nursing homes in particular.

The effectiveness of a hand-washing agent can be measured by determining the extent of reduction in disease outbreaks in a particular setting before and after the introduction of the test agent (Onesko and Wienke, 1987). Such field trials are expensive and complicated because many variables need to be taken into consideration and they do not permit the testing of more than one agent at a time.

The other approach is to first determine the germicidal efficacy of a product through proper laboratory-based *in vivo* tests using representative organisms. Such studies can readily assess the relative efficacy of several products in simultaneous tests. Products found to be effective can then be incorporated in subsequent field studies. Good correlation has been observed in results of laboratory-based and field studies (Ojajarvi, 1980).

Much has been published on the chemical disinfection of inanimate surfaces contaminated with viruses or bacteria. However, it is generally not possible to predict with any degree of confidence if an agent shown to be effective on such surfaces will also work well on human hands (Schurmann and Eggers, 1983). Therefore, it is important to test hand-washing agents for their germicidal efficacy in properly designed *in vivo* studies.

Hygienic hand-washing is primarily aimed at removing transient microflora, whereas a surgical scrub or a pre-operative skin antiseptic is expected to inactivate/remove resident microflora as well. Therefore, methods used to assess hygienic hand-wash agents are quite different

from those for testing surgical scrubs and pre-operative skin preps. Since this discussion relates specifically to agents used for hygienic hand-washing, protocols for testing other types of agents will not be considered here.

The efficacy of a hygienic hand-wash agent can be measured by the reduction in the numbers of naturally present or experimentally placed transient microorganisms on the skin. The post-treatment reduction in microbial load on hands is a direct measure of the efficacy of the treatment.

The first study for testing hand-wash agents was that of Price (1938). Since then, many others have addressed this issue, but using bacteria mainly alone as test organisms. In the methods reported, either one whole hand or a large area of the surface of one hand was contaminated with the test organism. This step in the protocol introduces considerable variability in the testing procedure because of the difficulties in the proper recovery of the test organism from the relatively large surface area contaminated and its uneven exposure to the test product. Recovery of the test organism before and after treatment of hands was either by rinsing of the entire contaminated area in an eluent (Price, 1938) or through the use of glass rings (Story, 1952). The ring was placed on one part of the contaminated surface and held in place by the subject and a known amount of eluent placed inside the ring. Another person would rub the confined area of the skin under the eluent with a glass spreader and 1.0 mL of the eluate would then be withdrawn for bacterial counts.

Studies published in the past 16 years (Ali and Maibach, 1980; Ayliffe et al., 1975; Lilly et al., 1979; Lilly and Lowbury, 1974; Michaud et al., 1976; Ojajarvi, 1976; Nicoletti et al., 1990; Peterson, 1973; Rotter et al., 1974; Rotter et al., 1986) are based mainly on the experimental contamination of fingers only; recovery of the test bacteria was carried out either by swabbing the skin (Peterson, 1973), rubbing the fingers together in an eluent (Ayliffe et al., 1978; Ojajarvi, 1976; Rotter et al., 1974) or by pressing the fingers directly on the surface of an agar plate (Ojajarvi, 1980).

Among the presently available protocols for testing hand-washing agents against bacteria, the 'Vienna Test Model' of Rotter (1986) and the 'Birmingham Test Model' of Ayliffe et al. (1978) are widely used in Europe and the UK, respectively. In the Vienna Model, fingers are immersed to the mid-carpal joint in a culture of *E. coli* and then the contaminated fingers are sampled by putting them in an eluent. For the test proper, the fingers are first contaminated as described above, the hand-washing agent is rubbed over the whole hand for 1 minute and the fingers then immersed in the eluent. In this test protocol, 60% isopropanol (v/v) is used as the standard to compare the germicidal efficacy of other agents.

In the Birmingham Model (Ayliffe et al., 1990), the procedure is similar to that in the Vienna Model, except that after drying the inoculum for 2 minutes, the fingertips are moistened in running tap water and the hand-washing agent is then applied for 30 seconds. Finally, residual bacteria are sampled after washing in running tap water for 30 seconds and drying with two paper towels for a total of 15 seconds. Ethanol and

ordinary soap and water are used as the standard here.

The protocols referred to above invariably ignore the use of an organic load in the medium used to suspend the test organism before contamination of hands. In the Vienna Model, both the hands are rubbed together with the test agent for 1 minute, but after the treatment residual bacteria are eluted from the fingertips only; this does not account for the bacteria which are spread over those areas of the hands that are not eluted.

In these protocols, the exposure time of the test organism to the hand-wash agent is also longer than in field situations. In one study, the mean contact time with the hand-wash agent recorded in normal hand-washing by health-care personnel was 8 seconds (Quraishi et al., 1984). Other studies (Daschner, 1988; Sprunt et al., 1973) have reported similarly short periods of contact with the hand-wash agent. The guidelines from the U.S. Center for Disease Control (Garner and Favero, 1985) recommend a 10 second exposure to the hand-wash agent.

The use of *E. coli* as the sole test organism in *in vivo* protocols to test hand-wash agents may not be justified. Whereas it may serve well as a representative of Gram negative bacterial species, at least *S. aureus* should also be included to assess the bacteria killing/removing potential of the formulations under test. Further *in vivo* testing with a suitable virus is considered necessary to establish a formulation's broad-spectrum efficacy and suitability for field application.

In vivo testing of hand-washing agents against viruses has been conducted to a limited degree (Cliver and Kostenbader 1984; Eggers,

1990; Schurmann and Eggers, 1983; 1985) and studies with rhinoviruses (Dick et al., 1986; Hayden et al., 1984; 1985; Hendley et al., 1978) have shown that the use of effective chemicals can prevent the spread of rhinovirus colds.

III. HAND-DRYING METHODS

The use of an effective hand-washing agent and proper compliance with the hand-washing guidelines are generally the only factors considered crucial in controlling and preventing the spread of infections by hands. The importance of the role of hand-drying in hygienic hand-washing has largely been overlooked and there are rare reports where the efficacy of various drying methods has been tested and discussed (Blackmore, 1987; Davis et al., 1969; Matthews and Newsom, 1987). Furthermore, these studies deal with bacteria only and virtually nothing is known on the efficacy of various hand-drying methods in eliminating viruses from hands.

OBJECTIVES

OBJECTIVES

Viruses continue to cause outbreaks of gastrointestinal and respiratory diseases in institutional settings such as hospitals and daycare centres. Indirect evidence suggests that hands play an important role as vehicles in such outbreaks. However, there has been a general lack of information with regards to the following basic questions:

1) How well do viruses, known to cause outbreaks of disease in institutional settings, survive on human hands?

2) How does their capacity to survive on hands compare with that of bacteria frequently incriminated as nosocomial pathogens?

3) Does the extent of virus survival on hands vary from person to person?

4) Does transfer of infectious virus occur between hands and inanimate surfaces?

5) How effective are commonly used hygienic hand-washing agents in the elimination of viral contamination from hands?

6) Is the *in vitro* testing of hygienic hand-washing agents using viruses predictive of their efficacy on the skin?

7) Is the *in vivo* testing of hygienic hand-washing agents using bacteria predictive of their efficacy against viruses on skin?

8) What role does the drying of washed hands play in the reduction of viral and bacterial contamination of the skin?

At the very out-set, it was noted that appropriate protocols for experiments to address these important issues were unavailable. Consequently, it was first necessary to develop the required methods. The main objectives of this investigation were, therefore, to develop the needed protocols and then apply them to answering the above questions.

MATERIALS AND METHODS

I. MATERIALS

1. BIOHAZARD CONTAINMENT

All experimental work with cell cultures, viruses and bacteria was carried out according to the Laboratory Safety Guidelines of the Medical Research Council of Canada (1990).

2. CELLS

a) **MA-104**: A seed culture of this cell line, derived from embryonic rhesus monkey (*Macaca mulatta*) kidneys, was originally provided to us by Dr. H. Malherbe of Gull Laboratories, Salt Lake City, UT, U.S.A.

The MA-104 line was routinely cultivated in 75 cm² plastic flasks (Costar, Cambridge, MA, U.S.A.) using Eagle's (AutoPow) minimal essential medium (EMEM; Flow Laboratories, Rockville, MD, U.S.A.). EMEM was prepared in deionized distilled water according to the manufacturer's instructions and autoclave sterilized in 460 mL quantities in 500 mL Pyrex bottles and stored at 4°C. For cell culture, complete EMEM was prepared by supplementing 460 mL of EMEM with 13.4 mL of a 7.5% (w/v) solution of sodium bicarbonate (Gibco Laboratories, Grand Island, NY, U.S.A.), 5 mL of a 200 mM solution of L-glutamine (Gibco) and 25 mL (5%) of fetal bovine serum (FBS; Gibco); gentamicin (Cidomycin, Roussel Canada Ltd., Montreal, Québec) was then added to a final concentration of 50 ug/mL. For cell passage, MA-104 monolayers were washed twice with sterile Ca⁺⁺- and Mg⁺⁺-free phosphate buffered saline (pH 7.2). Each monolayer then received 1.5 mL of a commercial (Gibco) solution of 0.25% trypsin and 1 mM EDTA (ethylenediaminetetraacetic acid) and reincubated for 5 min. After thorough pipetting, the cell

suspension was generally divided equally into three 75 cm² cell culture flasks (Costar). Twenty five mL of the constituted EMEM was then added to each flask. Complete monolayers were formed usually within 72 hours at 37°C.

b) **HeLa**: A seed culture of the A-5 strain of these cells, designated A-5 HeLa was kindly provided to us by Dr. B. Korant of E.I. du Pont de Nemours & Co., Wilmington, DE, U.S.A. They were also routinely grown in 75 cm² plastic flasks (Costar) in EMEM of the same composition as used for the MA-104 line. However, the FBS used was first heat treated at 56°C for 30 min in a water bath. Cell monolayers were trypsinized and passaged at a split ratio of 1:3 once every 72 hours as described above.

3. VIRUSES

a) **Rotavirus**: A cell culture-adapted (Wyatt et al., 1980) human rotavirus (Wa strain, subgroup II, serotype 1), designated HRV, was kindly provided to us by Dr. R. G. Wyatt (National Institutes of Health, Bethesda, MD., U.S.A.). The virus was plaque purified in MA-104 cells once in our laboratory. For preparing virus pools, semi-confluent monolayers of MA-104 cells in 75 cm² flasks were washed three times with Earle's balanced salt solution (EBSS) and then infected with the virus at a multiplicity of infection (m.o.i.) of about 0.01. The inoculated monolayers were then reincubated at 37°C on a rotating platform (Heidolph, Germany) for one hour to allow for virus adsorption. Each flask then received 15 mL of complete EMEM, without FBS but with 5.0 ug/mL of trypsin (1:250; ICN Nutritional Biochemicals Co., Cleveland, OH, U.S.A.) and was reincubated at 37°C. More than 75% of each mono-

layer showed cytopathic effects (CPE) within 72 hours of virus inoculation. The infected cultures were then frozen (-20°C) and thawed (22°C) three times before centrifugation of the cell culture harvest at 1000 xg for 15 min. The supernatant was aliquoted (0.5 mL) in 1 mL vials (Sarstedt Canada Inc., St. Laurent, Quebec H4S 1B2) and stored at -80°C.

b) Rhinovirus: Strain 1059 of human rhinovirus type 14 (RV-14) was obtained through the courtesy of Dr. J. Gwaltney, Jr. (University of Virginia, Charlottesville, VA, U.S.A.). The virus was plaque purified once in A-5 HeLa cells. The identity of the virus was further confirmed by us through virus neutralization using a typing serum (NIH Research Reference Reagents Catalog No. V1303-501-563) obtained from the National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD, U.S.A.

For the preparation of virus pools, each monolayer of A-5 HeLa cells in 75 cm² flasks was inoculated with 0.2 mL of the virus to give a m.o.i. of about 0.01. The inoculated flasks were held at 33°C for 1 hour to allow for virus adsorption. At the end of this period, 15 mL of the complete EMEM containing 2% heat-inactivated FBS was added to each flask. After 48 hours at 33°C, nearly all the cells in the monolayer showed virus-induced degeneration; the infected cultures were then frozen and thawed three times to help in virus release. The cell culture harvest was centrifuged at 1,000 xg for 15 min to remove cellular debris. The supernatant was aliquoted in 0.1 mL quantities and stored at -80°C.

c) Parainfluenzavirus: Strain 47885 of human parainfluenzavirus type 3 (HPIV-3) was obtained from the National Institute of Allergy and Infectious Diseases, NIH. The MA-104 cell line was used for its cultivation and assay. To prepare virus pools in cell monolayers grown in 75-cm² plastic flasks (Costar), the growth medium was discarded and the cells were infected with 0.2 mL of the virus to give a m.o.i. of about 0.01. The inoculated flasks were then incubated at 37°C for an hour for virus adsorption. Fifteen mL of the complete EMEM with only 2% FBS was added to each virus-infected culture and the bottles were reincubated at 37°C. After 48 hours, the virus-containing culture medium was removed, aliquoted and stored at -80°C. Due to the relatively fragile nature of HPIV-3, the infected cultures were not subjected to the usual freeze-thaw cycles.

4. BACTERIA

a) Escherichia coli: The strain of *Escherichia coli* (*E. coli*) selected for this study was isolated from the fecal sample used for suspending the rotavirus (see below). For initial isolation, 0.5 mL of the clarified fecal sample was streaked over the surface of an mFC (modified fecal coliform) agar (Difco Laboratories, Detroit, MI, U.S.A.) plate and incubated at 37°C for 18 hours. Colonies were then transferred to tryptose phosphate broth (Difco) tubes and incubated at 37°C for over night culture. The test suspension of the bacterium was prepared by mixing an 18-hour broth culture with the fecal sample at a 1:10 ratio. Stock cultures of the bacterium were prepared on tryptose soy agar slants (Difco) and kept at 4°C.

b) **Staphylococcus aureus**: A methicillin/penicillin-sensitive strain (ATCC # 6538) of *Staphylococcus aureus* (*S. aureus*), obtained from Dr. Tim Cusack of Lehn and Fink Co., Montvale, NJ, U.S.A., was used throughout this study. Its antibiotic sensitivity pattern was further confirmed by Mr. Frank Chan of the Bacteriology Lab., Children's Hospital of Eastern Ontario, Ottawa, Ontario. The test suspension of the bacterium was prepared by mixing a 20-hour culture, grown in tryptic soy broth (Difco) with the suspending medium at a 1:10 ratio. Stock cultures of *S. aureus* were prepared on tryptose soy agar slants (Difco) and kept at 4°C.

5. ORGANIC LOADS

a) **Feces**: The fecal sample used to suspend the rotavirus and *E. coli* was obtained from a healthy 1-year-old female child. A 10% (w/v) suspension of the feces was prepared in normal saline. The suspension was centrifuged at 1,000 x g for 15 min to remove coarse particulate matter and the supernatant was collected and stored at -20°C.

b) **Mucin**: Lyophilized bovine mucin (Sigma Chemicals; Product No. M-4503) was used as the organic load (Diem and Lentner, 1970) for *S. aureus*, HPIV-3 and RV-14. A solution of 5 mg/mL of mucin was prepared in normal saline and filtered through a 0.2 µm pore diameter membrane filter (Nalge Co., Rochester, NY, U.S.A.) and the filtrate stored frozen at - 20°C. For each experiment, the test virus was diluted 1:10 in the sterile mucin solution.

6. ELUENTS

a) **Tryptose phosphate broth**: In preliminary experiments to test

rotavirus survival on hands, 1X tryptose phosphate broth (TPB), EBSS, and 20 and 50% TPB in EBSS, all adjusted to a pH of 7.2, were tested as eluents of virus from fingerpads; 20% TPB in EBSS was found to give a consistently high recovery of rotavirus. It was therefore used as the eluent in all subsequent survival and antisepsis experiments with the rotavirus, *E. coli* and *S. aureus*.

b) **EBSS**: In the experiments with RV-14 and HPIV-3, EBSS alone was used as the eluent throughout.

7. STAINLESS STEEL DISKS

Clean and sterile stainless disks (1 cm diameter) were used in the virus transfer experiments as representatives of nonporous inanimate surfaces. These disks were punched out of polished stainless steel sheets purchased locally. Prior to experimental contamination with the virus suspension, the disks were cleaned by sonication for 10 min in a 7X cleaning solution (Linbro, Flow Laboratories) followed by thorough rinsing in running distilled water. They were then soaked in 95% ethanol for an hour and air dried and autoclave sterilized in a screw-capped glass bottle.

8. VOLUNTEERS

The approval of our university's Human Research Ethics Committee was obtained before the start of this study (see Appendix I). All volunteers were briefed on the possible hazards of dealing with infectious agents prior to their signing a consent form (see Appendix II). After each experiment, the hands of the volunteers were decontaminated thoroughly using effective antiviral or antibacterial agents.

In rotavirus survival studies, six adult (three male and three female) members of the departmental staff participated. Three males and one female participated in RV-14 and HPIV-3 survival experiments; the same four volunteers were used in RV-14 transfer experiments. In viral and bacterial antiseptis studies, one male and two female volunteers participated.

9. HAND-WASHING AGENTS

Several antiseptic formulations, an unmedicated liquid soap, and tap water were tested for their efficacy in removing the rotavirus from experimentally contaminated fingerpads. Details of the hand-washing agents tested are given in Table 1. Isopropanol (70%), Savlon in 70% ethanol (1:30), Savlon in tap water (1:200), liquid soap and tap water alone were also tested against *E. coli* and *S. aureus*. These five agents were also used in whole-hand washing experiments.

10. HAND-DRYING AGENTS

The following three hand-drying agents were tested. Rolls of ordinary unbleached paper towels (Boudreault, Inc., Hull, Quebec) and regular looped cotton towels were purchased locally. A no-touch electric hand blow dryer (Model # NTC-126) was kindly loaned to us by its manufacturer World Dryer Limited, Mississauga, Ontario; its blower motor operated at 3450 revolutions per minute to deliver 5200 linear feet of warm air per minute over the hands being dried.

TABLE 1.
Hand-washing agents tested

| Active Ingredient (s) | Concentration | Dilution | Product name (Manufacturer) |
|---|--|----------------------------|--|
| Isopropanol | 70.0%.v/v | none | not applicable |
| Ethanol | 70.0% v/v | none | not applicable |
| Chlorhexidine gluconate and Cetrimide | 1.5% w/v 15.0% w/v | 1:30 in 70% isopropanol | Savlon (Ayerst, Montreal Quebec, Canada) |
| " " " | " " | 1:30 in 70% ethanol | " " |
| " " " | " " | 1:200 in tap water | " " |
| Chlorhexidine gluconate and Isopropanol | 0.5% w/v 70.0% w/w | none | Hibisol (Ayerst) |
| Chlorhexidine gluconate and isopropanol | 2.0% w/v 4.0% w/v | none | Cida-Stat (Huntington, Bramalea Ontario, Canada) |
| Povidone-iodine | 10.0% w/v (1% free I ₂) | none | Providine (Rougier, Montreal Quebec, Canada) |
| p-chloro-m- xylenol and isopropanol | 4.8% w/v 9.4% v/v | none | Dettol (Reckitt & Colman Lachine, Quebec, Canada) |
| Liquid soap | 100% | 1:10 in water | Ivory (Procter & Gamble, Toronto Ontario, Canada) |
| Tap water | - | - | - |

II. METHODS

1. PLAQUE ASSAYS

a) **Rotavirus:** The rotavirus was plaque assayed using the technique of Ramia and Sattar (1980). Monolayers of MA-104 cells were prepared in 12-well plastic plates (Costar) by seeding each well with approximately 5.0×10^4 cells in 2.0 mL of the complete EMEM; the plates were then sealed individually in plastic freezer bags (Philips Electronics Ltd., Toronto, Ontario, Canada) using an electric bag-sealer (Philips) and incubated at 37°C in a walk-in incubator. Confluent monolayers were generally ready for use within 48 hours.

Prior to virus inoculation, the monolayers were washed with three changes of EBSS (pH 7.2) to remove the serum present in the growth medium. The last EBSS wash was left on the cells and the plates held at 37°C for at least two hours. The EBSS was then removed and each well received 0.1 mL of the appropriate virus dilution; EBSS was used as the diluent. At least three wells were inoculated with each dilution to be tested and the cell control wells received 0.1 mL of EBSS. The inoculated plates were held for 1 hour at 37°C in an atmosphere of 5% CO₂ to facilitate virus adsorption. At the end of this period, each monolayer received 2 mL of an overlay containing EMEM (without serum), 0.6% agarose type II (Sigma) and trypsin (Nutritional Biochemical Co.) at a concentration of 5.0 ug/mL. The plates were left in the laminar flow hood for about 15 min to allow the overlay medium to solidify. Finally, the plates were resealed in plastic bags and held upright in a walk-in incubator (37 °C) for 72 hours.

About 3.0 mL of a 10% (v/v) solution of Formalin (BDH Chemicals, Toronto, Ontario) in normal saline was added to each well and the plates were kept for 2 hours at room temperature to fix the monolayers and inactivate the virus. The agar overlay was then removed, the fixed monolayers were stained with a 0.1% aqueous solution of crystal violet and washed in running tap water. Virus plaques were counted and the titre expressed as plaque forming unit (PFU)/mL.

b) Rhinovirus: The method described by Fiala (1968) was used for the plaque assay of RV-14. Monolayers of A-5 HeLa cells were prepared in 12-well plastic cell culture plates (Costar) by seeding each well with approximately 5×10^4 cells in 2.0 mL of complete EMEM. The seeded plates were incubated at 37°C in an atmosphere of 5% CO₂ and they were generally ready for use within 48 hours. Ten-fold dilutions of the samples were prepared in EBSS for virus titration. For plaque assay, the growth medium was removed from the wells and each one of them received 0.1 mL of an appropriate dilution of the test sample; at least 3 wells were used for each dilution. The inoculated plates were then incubated at 33°C for an hour for virus adsorption. At the end of this period, each well received 2.0 mL of an overlay containing M-199 (Flow Labs.) 0.22% NaHCO₃, 100 ug/mL of 5-bromodeoxyuridine (Calbiochem, San Diego, CA, U.S.A.), 50 ug/mL DEAE-dextran (Sigma), 30 mM MgCl₂ (Fisher Scientific) and 0.9% Oxoid agar No. 1 (Oxoid Limited, Basingstoke, Hampshire, England). After the solidification of the overlay, the plates were sealed in plastic bags (Philips Electronics) and reincubated in upright position for 72 hours at 33°C. The monolayers

were then fixed and stained as described above for the rotavirus plaque assay.

c) **Parainfluenzavirus:** Monolayers of MA-104 cells, prepared in 12-well plastic plates, were used for HPIV-3 plaque assay. The samples to be assayed were diluted in EBSS. The growth medium was removed and each well received 0.1 mL of the appropriate dilution, using at least three wells for each dilution tested. The inoculated plates were then incubated at 37°C in a 5% CO₂ atmosphere for one hour to allow for virus adsorption. At the end of this period, each well received 2.0 mL of an overlay containing EMEM, 2% FBS and 0.6% agarose. The plates were sealed in plastic bags (Philips) and incubated at 37°C for 4 days. The monolayers were fixed and stained as described above for rotavirus plaque assay.

2. PROTOCOL TO STUDY SURVIVAL OF VIRUSES AND BACTERIA ON HANDS

a) **Rotavirus:** The pads of the index, middle, ring, and little fingers on the left hand of the volunteers were designated as F1, F2, F3, and F4, respectively. Before the fingerpads were contaminated with the virus, volunteers were asked to wash their hands in running tap water, rinse them with 70% ethanol and allow them to air dry.

The fingerpads were pressed over the mouth (8 mm inside diameter) of an empty plastic vial (Sarstedt; No. 72.694.006) to demarcate the area where the virus inoculum was to be deposited. Virus suspended in feces (10 uL) was then placed in the center of the marked area on each fingerpad. The inocula were allowed to dry by keeping the

contaminated hand in a laminar flow hood. Figure 3 shows the fingerpads with the dried inocula. Once the inocula were dry, the volunteers were permitted to move about and perform any tasks as long as contact with the contaminated fingerpads could be avoided.

For virus elution, the contaminated area was placed over the mouth of a plastic vial containing 1.0 mL of the eluent, and the vial was inverted (Fig. 4) to soak the dried virus inoculum for 5 sec. This was followed by 20 repeated inversions of the vial while it was still in place, another 5 sec of soaking, and a further 20 inversions. Finally, the vial with contaminated finger still over it, was turned upright, and the fingerpad was scraped against the inside rim of the vial to recover as much as possible of the liquid. The eluates were then plaque assayed. The fingers were then decontaminated by thorough rinsing in 70% ethanol before they were washed with antiseptic soap and water. This step in the procedure was strictly followed for the safety of volunteers.

HRV survival on hands was tested by placing 10 μ L (1×10^3 to 1×10^4 PFU) of fecally-suspended virus on each fingerpad and eluting the inocula from at least two fingerpads at each sampling period. The flow chart for the rotavirus survival experiments is given in Figure 5.

In the rotavirus survival experiments, four fingers of one hand of each individual were inoculated with the virus suspension. Virus was eluted from two fingers at 20 min after deposition and from the other two fingers at 260 min after deposition of the virus suspension. Rotavirus survival at 60 min after deposition was determined in a subsequent trial with three fingers of each volunteer.

Figure 3. Demarcated area with dried inocula (10 μ L) on the fingerpads were made by pressing vials against finger.

Figure 4. Positioning of the vial (with 1 mL of eluent) on the fingerpad for virus recovery.

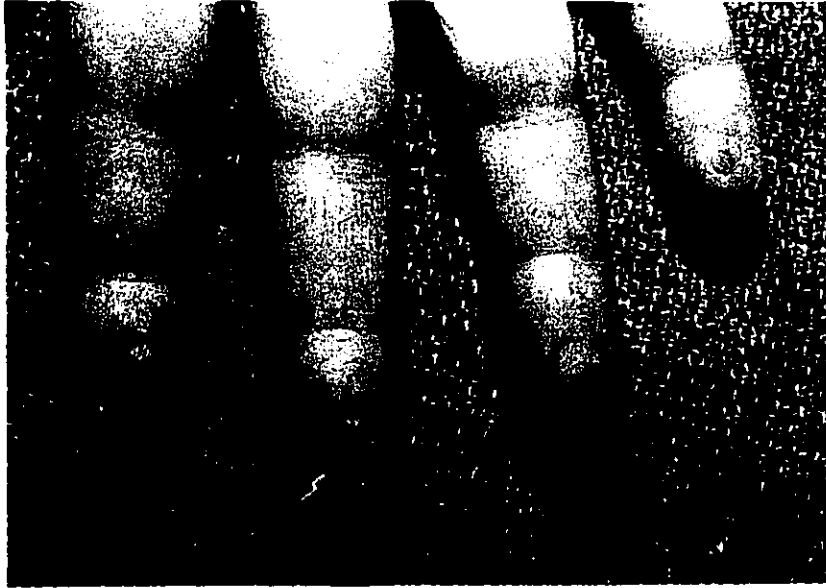
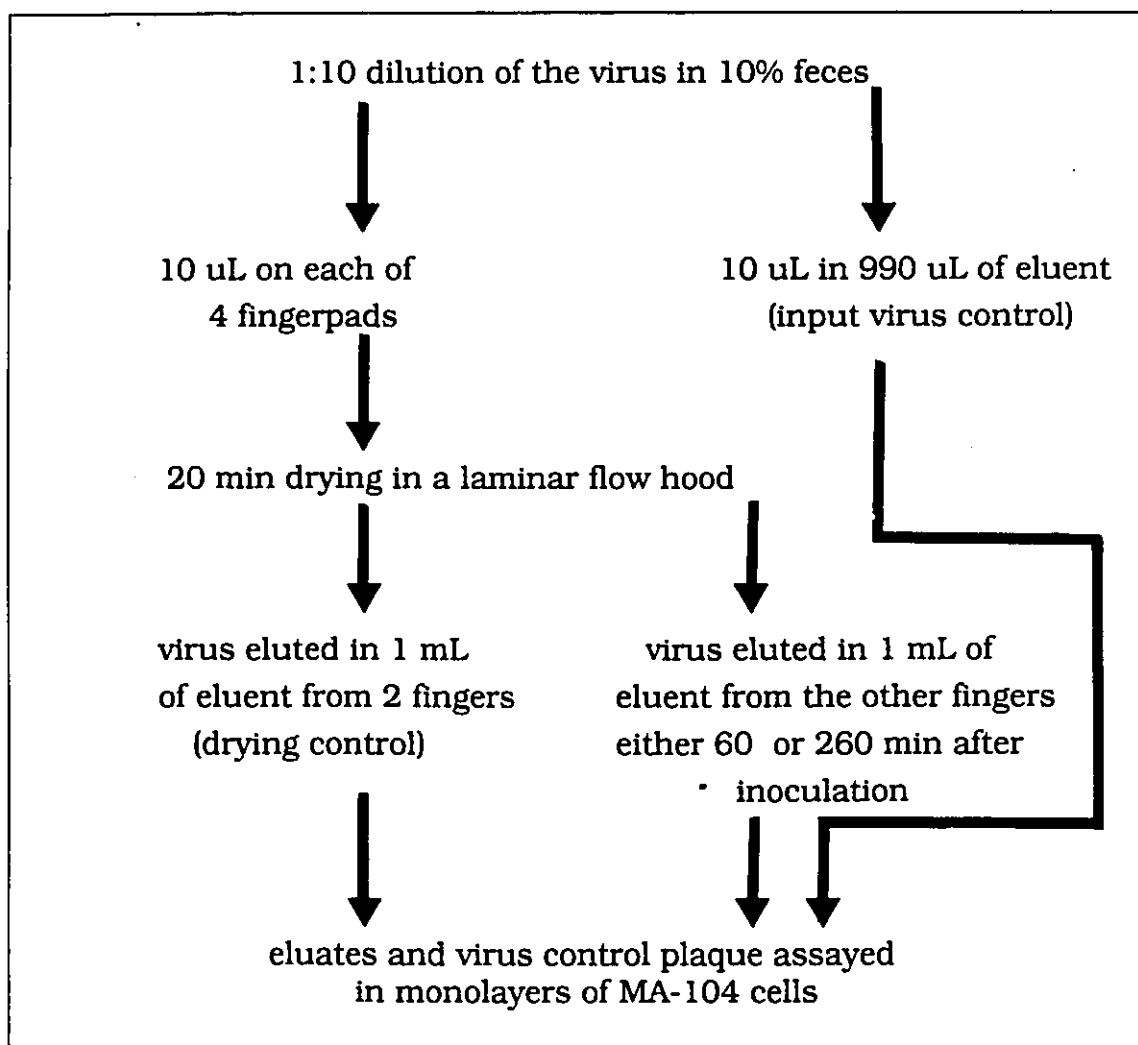


Figure 5. Flow diagram of procedure for testing human rotavirus survival on fingerpads.



b) **Rhinovirus:** Fingerpad contamination and virus elution in RV-14 survival studies was the same as described above for rotavirus. However, RV-14 survival was tested for 180 min instead of the 260 min used for rotavirus. Each of the five fingers of the left hand was contaminated with 10 uL of the RV-14-mucin suspension. At the end of each experiment, hands were decontaminated by thorough rinsing with 5% acetic acid followed by washing with an antiseptic soap and running tap water. The flow chart for RV-14 survival experiments is given in Figure 6. At least three experiments were conducted on each volunteer.

c) **Parainfluenzavirus:** Virus survival was tested at 5 different time points (0, 10, 20, 30, and 60 min). The flow chart is given in Figure 7. Ten microlitre of the virus-mucin suspension (6.0×10^4 to 5.4×10^5 PFU) was placed on the index finger of the right hand and immediately eluted in 1.0 mL of EBSS for the zero min baseline count. The middle finger of the right hand was then inoculated and virus was eluted after 10 min. The selected three fingers of the left hand were then contaminated with 10 uL of the virus suspension and virus eluted after 20, 30, and 60 min. Samples were kept on ice until plaque assayed. Experiments were carried out at least three times on each volunteer.

In another set of experiments, HPIV-3 survival testing was confined to a shorter time frame and virus was eluted 0, 1, 3, 7 and 10 min after the contamination of fingerpads. These experiments were carried out on one volunteer. After elution of the virus, hands were decontaminated with 70% ethanol and then washed with antiseptic soap and water. This experiment was repeated at least three times.

Figure 6. Flow diagram of procedure for testing RV-14 survival on fingerpads (drying step omitted).

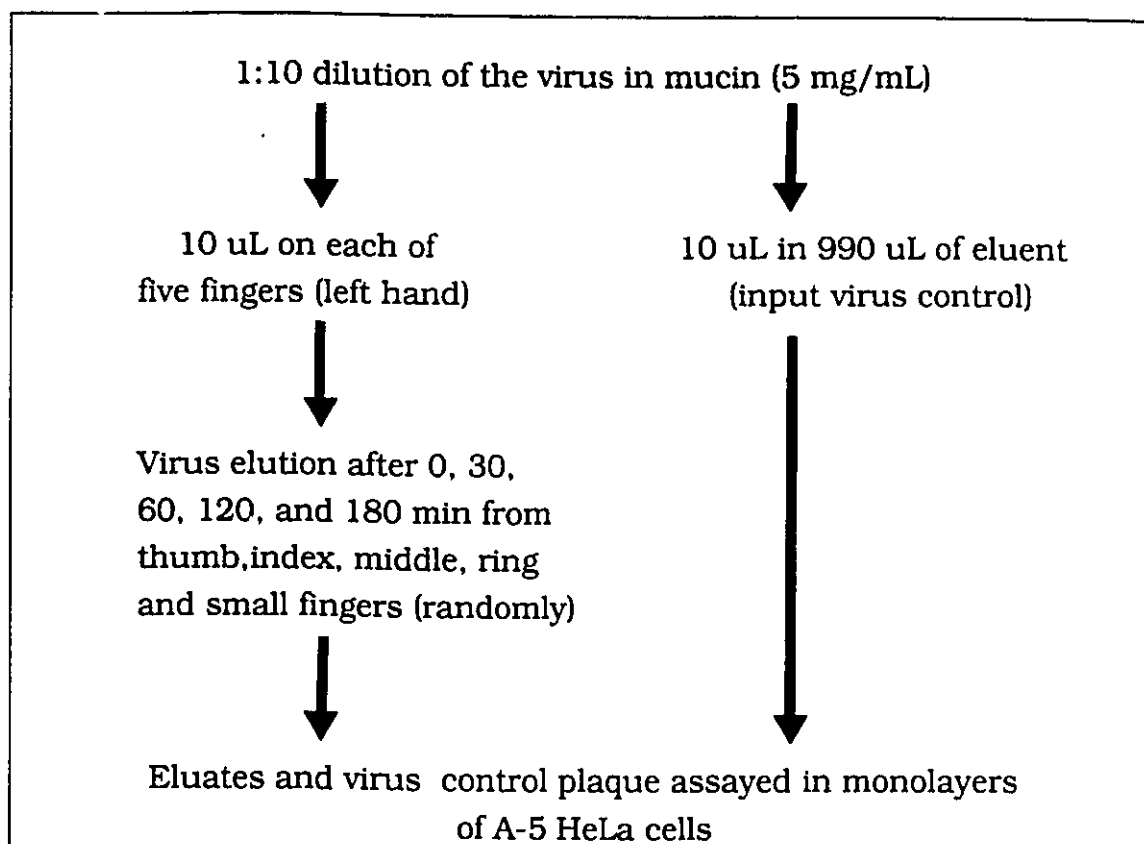
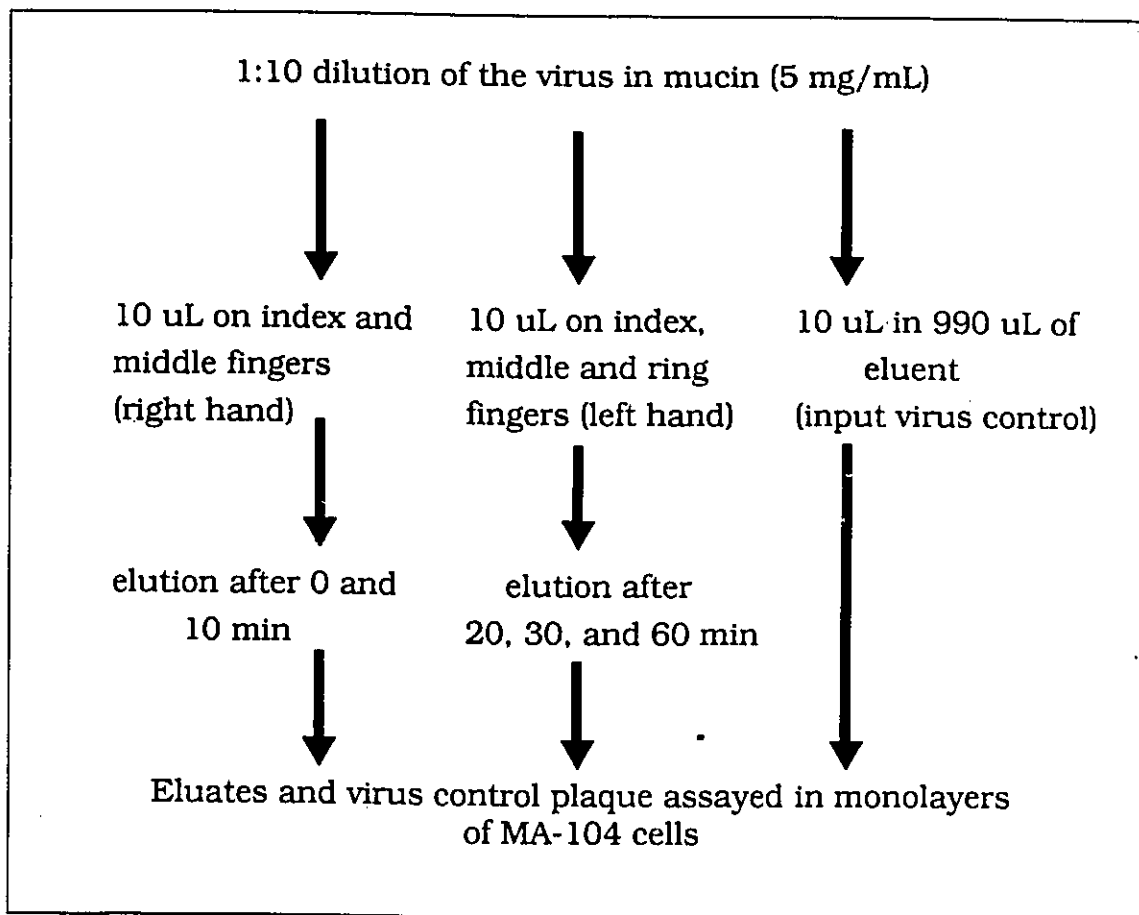


Figure 7. Flow diagram of procedure for testing HPIV-3 survival on fingerpads (illustrating time differences).



d) *E. coli*: *E. coli* survival on fingerpads was tested for up to 1 hour with 20 min and 60 min sampling times; the procedure was the same as for rotavirus survival testing (Fig. 5). Ten μ L of *E. coli* suspension in feces was placed on the thumb and each fingerpad of the left hand. The inoculum was eluted with 1.0 mL of 20% TPB from the thumb immediately (base titre). From F1 and F2 *E. coli* was eluted 20 min after deposition and from F3 and F4, 60 min after contamination. At the end of each experiment, eluates were titrated on mFC agar plates.

e) *S. aureus*: For survival study on hands, *S. aureus* was suspended in diluted bovine mucin. After contamination of fingerpads, the inoculum was eluted with 1.0 mL of EBSS and titrated on mannitol salt agar plates. The rest of the procedure was the same as stated above for *E. coli*.

3. PROCEDURE FOR TESTING VIRUS SURVIVAL ON INANIMATE SURFACES

The survival capacity of RV-14 and HPIV-3 was tested on stainless steel disks. RV-14 survival was tested only for a 20 min duration. HPIV-3 survival was tested for upto 60 min with sampling at 0, 1, 4, 7, 10, 20, 30, and 60 min after deposition of the virus on cleaned and sterilized stainless steel disks. The protocol involved the inoculation of the test virus suspension on several disks placed inside an operating laminar flow hood. At designated time points, virus was eluted by placing each disk in a glass vial containing 1.0 mL EBSS. The vial was then sonicated for 10 min in a sonication bath (Branson, 1200; 50\60 HZ; Branson Ultrasonic Corp., CT. U.S.A.).

4. PROCEDURE FOR TESTING VIRUS TRANSFER

Three models of virus transfer were tested: a) from a contaminated fingerpad to a clean disk, b) from a contaminated disk to a clean fingerpad and c) from a contaminated fingerpad to a clean fingerpad.

Ten μL of HRV (2×10^4 to 8×10^4 PFU), RV-14 (6×10^4 to 1.0×10^5 PFU) or HPIV-3 (2.85×10^4 to 7.0×10^4 PFU) was used to contaminate each clean fingerpad or stainless steel disk. After letting the inoculum dry for the required length of time under ambient conditions, contact was made between the donor and target surface for 5 seconds using a pressure of approximately 1 kg/cm^2 . The extent of virus transfer was then determined by virus recovery from the recipient surface. The virus remaining on the donor surface after transfer was also determined in a separate set of experiments with RV-14 only. To recover the virus from disk, each disk was placed separately in vials containing 1.0 mL of 20% TPB or EBSS then the vials were sonicated for 10 min in a sonicator bath (Branson). The flow chart for the three types of transfer experiments is given in Figure 8.

4. HYGIENIC HAND-WASHING

a) **Fingerpad protocol:** This is a modification of the method developed by us to study rotavirus survival on human hands. The details of this procedure are given in Figure 9. The same basic procedure was also used for working with *E. coli* and *S. aureus*. After the washing treatment, residual test organisms were eluted with 1.0 mL of 20% (v/v) of TPB in EBSS, and quantitation of the organisms was performed immediately after elution to minimize loss due to storage. At the end of

Figure 8. Flow diagram of procedure to study transfer of viruses from experimentally contaminated to clean surfaces.

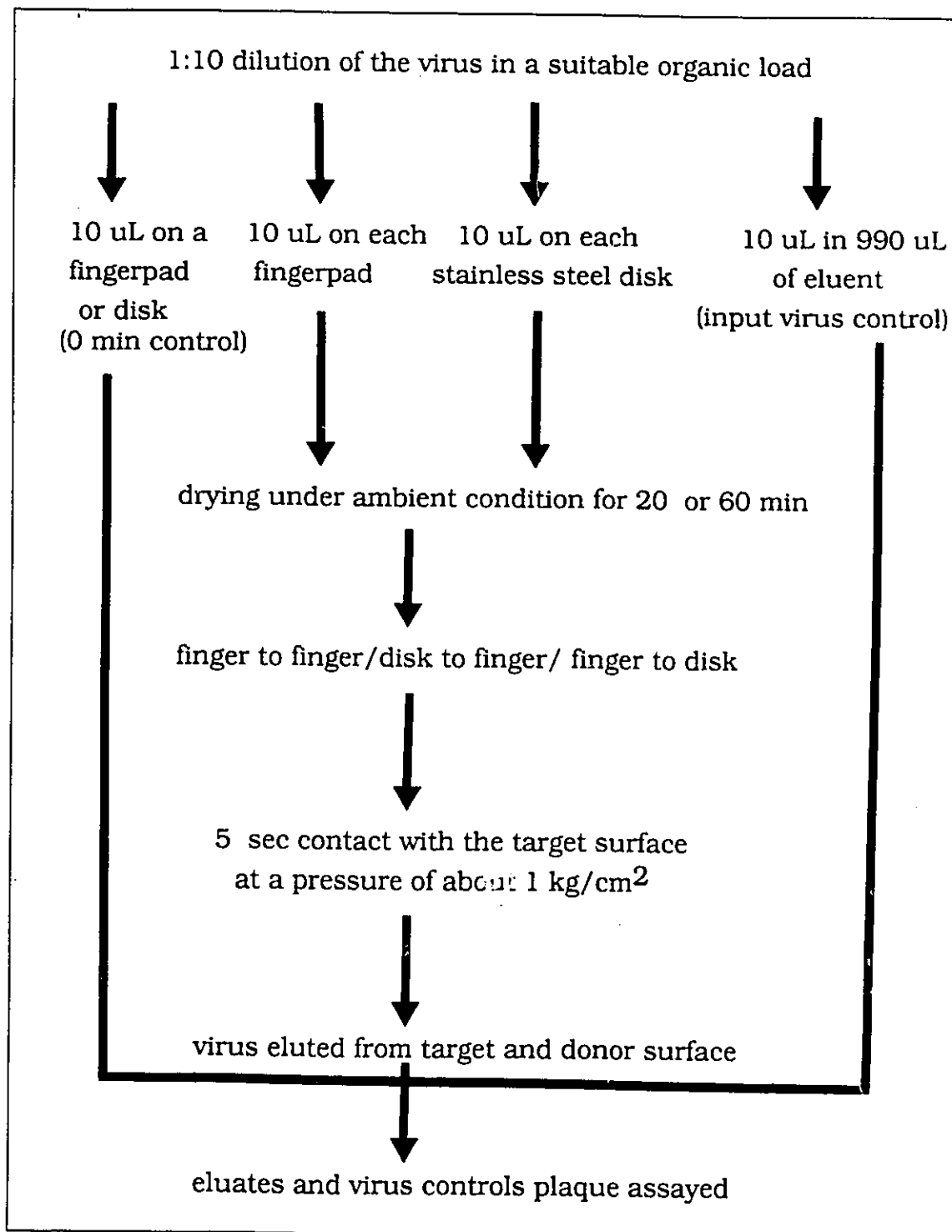


Figure 9. *In vivo* protocol for testing the hand-washing agents against viruses.

PROCEDURE FOR IN VIVO TESTING OF HANDWASHING AGENTS

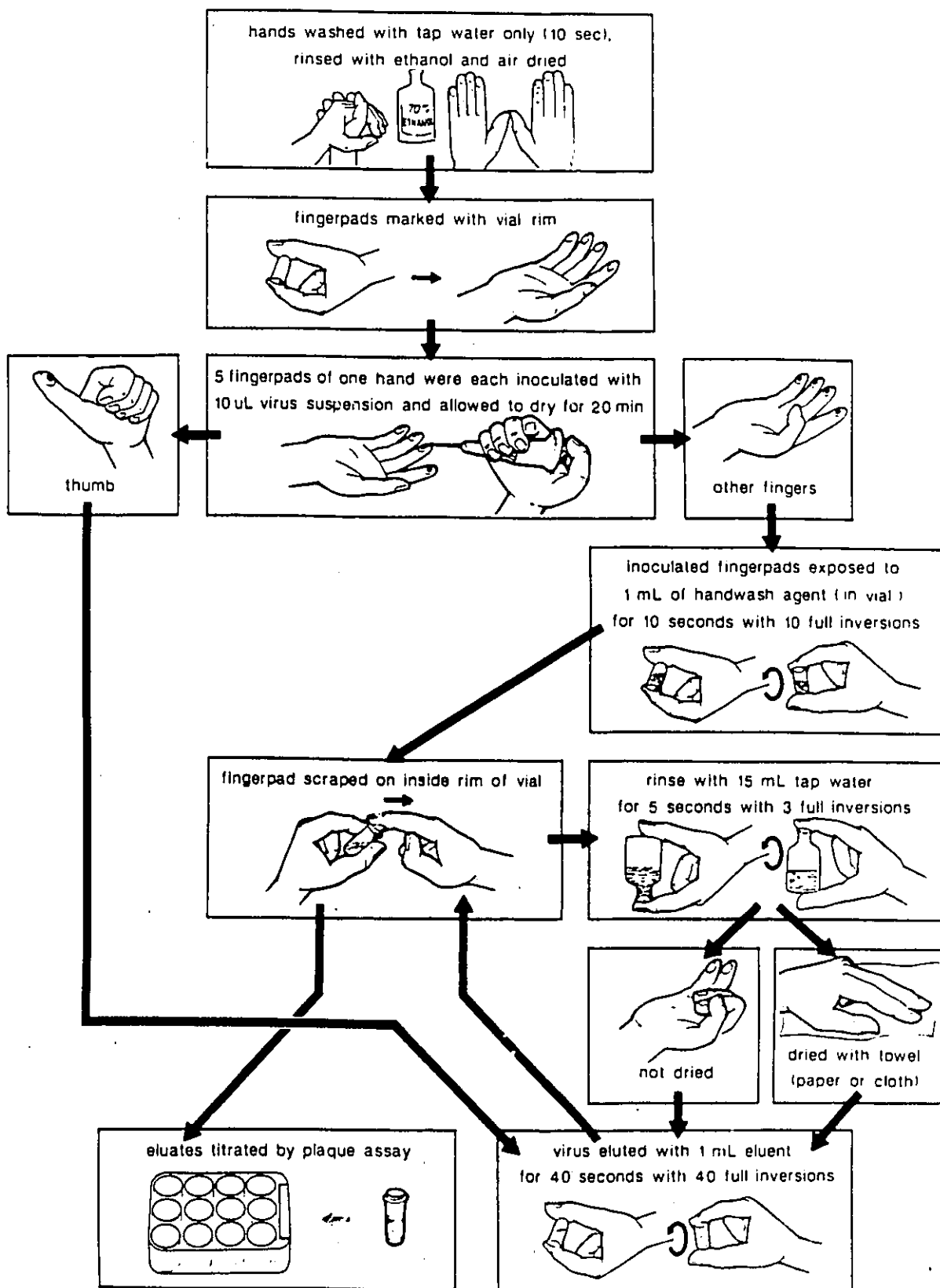
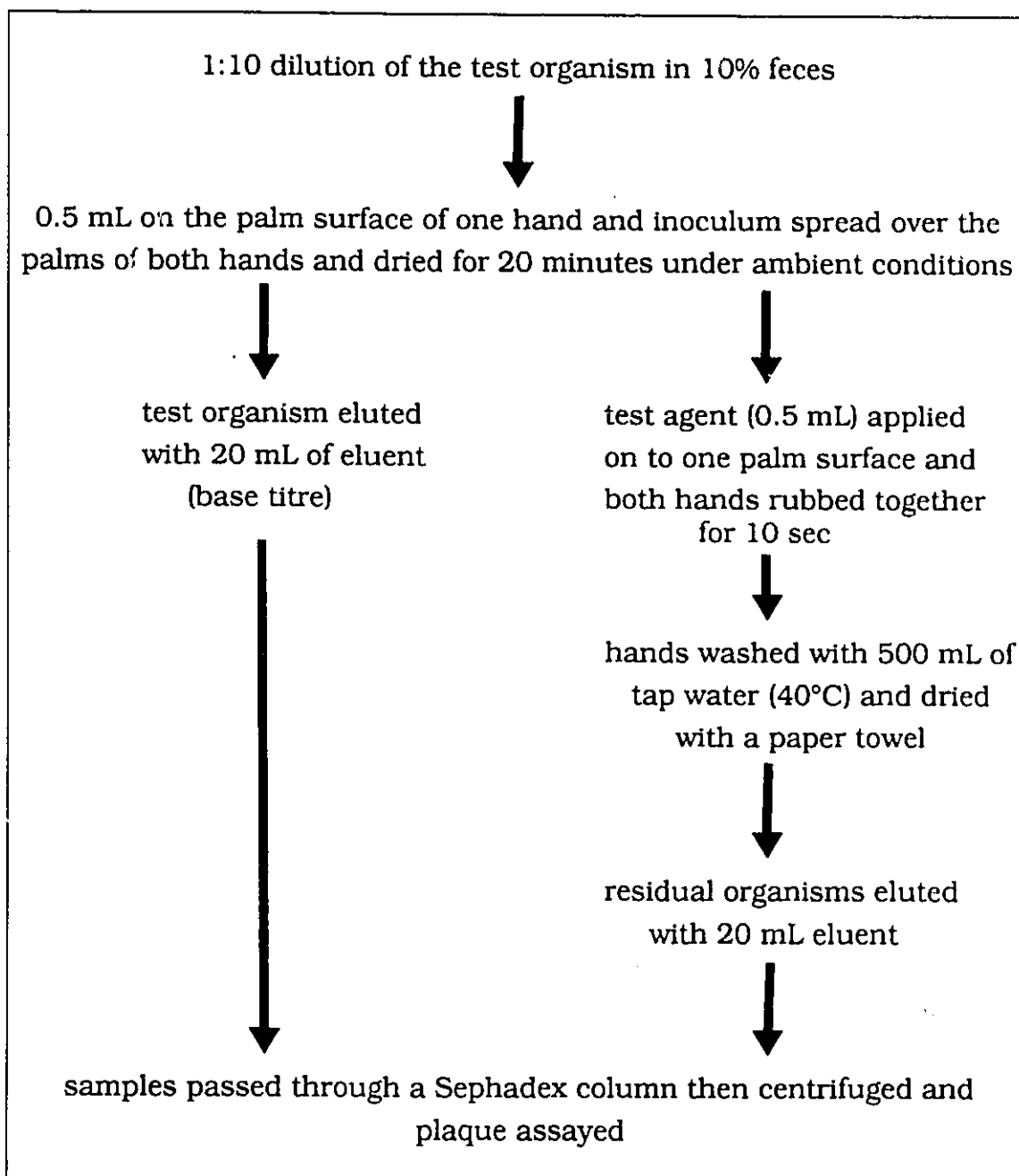


Figure 10. Flow diagram of procedure for testing hand-washing agents by whole-hand method.



each experiment, the contaminated fingerpads of the volunteers were thoroughly rinsed with 70% ethanol and then washed with an antiseptic soap and water.

b) **Whole-hand protocol:** The following whole-hand washing protocol (Fig. 10) was used to ascertain that the results obtained with our fingerpad protocol were representative of the normal hand-washing situation. The volunteers did not wash or decontaminate their hands before the start of the experiment and 0.5 mL of the fecal suspension of the rotavirus or *E. coli* was placed on the palm surface of one hand. The volunteer was then required to spread the inoculum over the entire palm surface of both hands by gently rubbing them together, and the inoculum was allowed to dry for 20 min under ambient conditions. To obtain the base titre of the test organism present at the end of this drying period, 20 mL of the eluent (20% TPB in EBSS) was poured on the hands while they were being rubbed together over the mouth (27 cm in dia.) of a plastic funnel in order to bring the eluent in contact with the entire contaminated surface. Generally, more than 18 mL of the eluate could be collected by this procedure. To test the efficacy of a hand-washing agent, the hands were contaminated with the test organism and dried as described above. Then, 0.5 mL of the test agent was placed on the palm surface of one hand and the volunteer required to rub his or her hands for 10 sec covering the entire contaminated surface. The hands were then washed, by pouring 500 mL of tap water at 40°C over them, and then dried with a paper towel. The residual test organism was eluted from the washed and dried hands as described above.

c) **Removal of cytotoxicity:** Some antiseptic formulations and hand-washing agents proved to be toxic for MA-104 cells. The gel filtration technique of Blackwell and Chen (1970) was used to remove toxic components from the eluates. The gel filter bed was prepared in the following manner: Twenty grams of Sephadex LH-20 (Pharmacia) was soaked in 50 mL of phosphate buffered saline overnight at 4°C. Five mL of the liquid swollen beads were transferred into a 7.0 mL Centriflo plastic cone (Amicon, Lexington, MA, U.S.A.). The cone was then held in a sterile 50.0 mL centrifuge tube (Product No. 25331; Corning Glass Works, Corning, NY, U.S.A.) and centrifuged at 1000 xg for 10 min at 4°C to pack the beads. For detoxification, the cone containing the prepared gel was transferred to a new sterile 50.0 mL tube, the sample layered on the surface of the gel and the cone re-centrifuged as described above. The filtrate collected in the centrifuge tube was then transferred to smaller tubes.

d) **Removal of bacterial and fungal contaminants:** In order to remove any fungal or bacterial contamination, the filtrates from the LH-20 columns were centrifuged at 14,000 xg for 10 min and the supernatant was used to inoculate the MA-104 cell monolayers.

6. HAND-DRYING

A protocol developed earlier in our laboratory to test the *in vivo* efficacy of hand-washing agents against viruses and bacteria was used. The details of this procedure are given in Figure 11.

A uniform method was followed for each experiment. For cloth- and paper-towels, right after the washing and rinsing steps, each fingerpad

was pressed onto the towel for 10 sec. In the case of electric blow drying, the fingerpad was held for 10 sec against the blowing warm air-current. Residual rotavirus or *E. coli* was eluted with 1.0 mL of EBSS. At the end of each experiment, the contaminated fingerpads were thoroughly rinsed with 70% ethanol and then washed with soap and water.

7. STATISTICAL ANALYSES

a) **Survival experiments:** A computerized statistical program, Statistical Analysis System (SAS), was used to analyze the data. In survival experiments, nested analyses of variance were conducted on the data in order to ascertain the amount of variation attributable to sampling times, volunteers and fingers in these experiments.

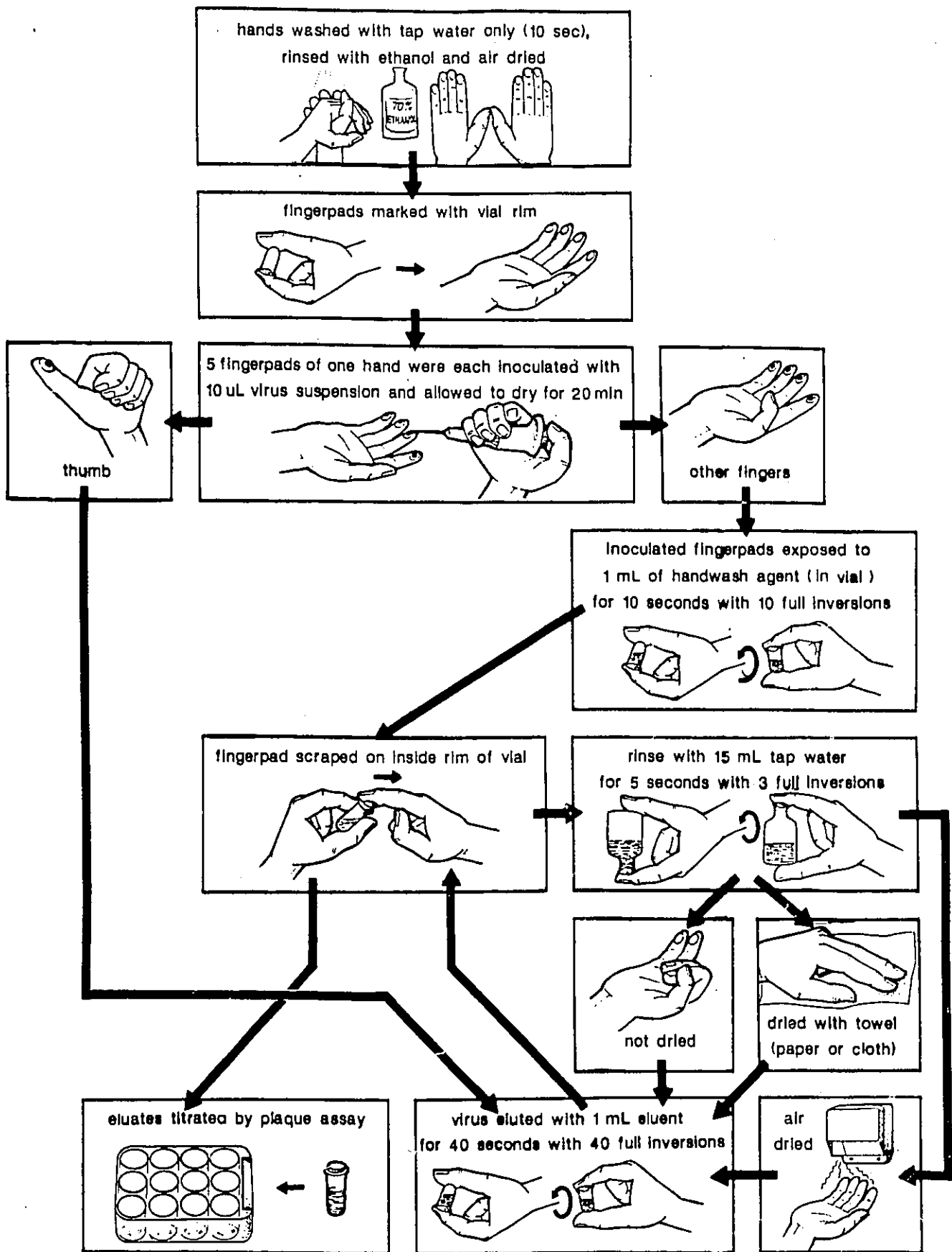
b) **Hygienic hand-washing:** For the fingerpad protocol, the testing of each hand-washing agent and each one of the two microorganisms was conducted at least three times using two fingerpads for treatment with the test agent in each trial. Therefore, the results presented in Table 16 are the mean of 6 observations for each hand-washing agent using one volunteer, and those given in Table 17 and Table 20 are based on the mean of 6 observations using 3 volunteers. For the whole-hand protocol (Table 18), each experiment was repeated 3 times and the volunteer used was the same as in experiments summarized in Table 16.

Results were statistically analyzed using the two-way analysis of variance based on the arcsin transformation (Cohen, 1977) of the raw data i.e. the percent of the organisms removed. The arcsin transformation was utilized in order to stabilize the variance. Comparisons of the means obtained with the different hand-washing

agents were made by Tukey's w-procedure (Steel and Torrie, 1960). This procedure allows for multiple comparisons among the means, while controlling for the error rate at the desired level permitting grouping of the data (Appendix III).

c) **Hand-drying study:** Experiments were repeated at least three times with each virus and bacterium and with each of the four hand-washing agents. These experiments were conducted on one adult male volunteer's hands. Results were statistically analyzed by using two-way analysis of variance based on the arcsin transformation of the raw data as stated above.

Figure 11. Illustration of the procedure used to compare the efficacy of hand-washing agents and drying methods against viruses.



RESULTS

I. DEVELOPMENT OF METHODS

Although virus survival on human hands has been tested (Gwaltney et al., 1978; Hall et al., 1980; Hendley et al, 1973; Reed, 1975), the findings of such investigations warrant caution in interpretation because the experimental design and certain materials used in these studies were unrealistic. Moreover, it was not known how long rotaviruses could survive on human hands and how readily transfer of infectious rotavirus could occur between hands and environmental surfaces. Therefore, the initial objective of this phase of the study was to develop a simple, reliable and reproducible system to assess quantitatively the survival of viruses on hands. Additional experiments were conducted to compare virus survival with that of two important bacterial pathogens.

Fingerpads were chosen as the site for experimental contamination because it permitted i) the use of microlitre quantities of the infectious agent being tested, ii) the inclusion of proper controls and sufficient numbers of replicates in the same test, iii) the elution of the test virus or bacterium with only 1.0 mL of eluent, iv) the exposure of the entire experimentally contaminated area on each fingerpad to the eluent for efficient and reproducible elution of the test agent, and v) ready decontamination of the skin after the test for the safety of the volunteers.

1. Virus elution from fingerpads: For these developmental studies, rotavirus was used as the test agent and the eluents tested for its recovery from fingerpads were i) 100% TPB, ii) 50% TPB in EBSS, iii) 20% TPB in EBSS and iv) EBSS alone. The results of these tests are presented in Table 2. Virus recovery with 20% TPB, at 0 min and 20 min after fingerpad contamination, was found to be significantly better

Table 2.
**EFFICIENCY OF VARIOUS ELUENTS IN ROTAVIRUS
 RECOVERY FROM EXPERIMENTALLY CONTAMINATED
 FINGERPADS**

| Eluent | Input PFU x10 ⁵ | Virus recovery (SD) | | | | Tukey* grouping |
|-------------|----------------------------------|-------------------------|-------|-------------------------|-------|--------------------|
| | | at 0 min | | at 20 min | | |
| | | PFU x10 ⁵ | % | PFU x10 ⁵ | % | |
| TPB 100% | 8.97 (1.49) | 6.97 (1.47) | 77.70 | 3.05 (0.23) | 34.00 | C |
| TPB 50% | 6.73 (0.83) | 5.64 (0.56) | 83.80 | 2.57 (0.07) | 38.19 | B |
| TPB 20% | 6.79 (0.62) | 5.81 (0.56) | 85.57 | 3.55 (0.07) | 52.28 | A |
| EBSS | 6.23 (1.41) | 5.14 (1.11) | 82.50 | 2.54 (0.09) | 40.77 | B |

* Eluents with the same Tukey groupings do not have significantly different percentages of recovery ($\alpha = 0.05$)

Ten μ L of fecally-suspended rotavirus was placed on the thumb and all the fingers of the left hand. From the thumb, virus was eluted immediately after its deposition (0 minute). The inoculum was allowed to remain for 20 minutes on the fingerpads and then eluted. For readings after 0 minutes, n= 3; after 20 minutes, n=12.

($\alpha=0.05$) than with the other three eluents. Therefore, in subsequent rotavirus experiments 20% TPB in EBSS was used as the eluent. TPB has been shown to be harmless to rotavirus infectivity (Ramia and Sattar, 1980).

2. Efficiency of virus recovery: For survival experiments, volunteers were asked to wash their hands in running tap water and then rinse them with 70% ethanol and allow them to air dry. The washing and ethanol rinsing of hands before the deposition of the virus on fingerpads were considered necessary to minimize the influence of accumulated materials, such as emollients, on virus survival and to reduce the possibility of any background levels of naturally present virus or bacteria on the hands and to provide a fairly uniform condition between volunteers. Because of the possible effect of this pretreatment of the skin on the virus survival study, it was felt necessary to compare virus survival under both conditions.

The results summarized in Table 3 show that 20 minutes after drying, $48.74 \pm 4.65\%$ of the inoculated rotavirus could be recovered from unwashed hands, whereas $50.95 \pm 5.05\%$ of the deposited virus could be recovered from hands which were washed before the start of the experiment. Statistical analysis using the t-test showed no significant difference in percent survival of rotaviruses on washed or unwashed hands ($p < 0.8579$). All subsequent survival experiments were carried out after washing hands with water and then rinsing them with 70% ethanol.

II. SURVIVAL OF VIRUSES AND BACTERIA ON FINGERPADS

1. Rotavirus: These experiments were conducted on the hands of three male and three female volunteers. The survival of HRV on fingerpads was determined 20, 60 and 260 min after deposition of the virus suspension on fingerpads. Each experiment was repeated at least

Table 3.

EFFICIENCY OF VIRUS RECOVERY FROM WASHED AND UNWASHED FINGERPADS

| Expt. No. | Virus PFU at 0 min $\times 10^4$ | % virus recovered 20 min after drying | | | |
|-----------------------|----------------------------------|---------------------------------------|-----------------|-----------------|-----------------|
| | | unwashed finger | | washed finger | |
| | | 1 | 2 | 3 | 4 |
| 1 | 3.58 | 43.12 | 49.72 | 56.15 | 48.88 |
| 2 | 2.01 | 52.74 | 47.76 | 57.21 | 46.27 |
| 3 | 2.15 | 44.19 | 54.88 | 45.12 | 52.09 |
| Overall mean: (SD) | | 46.68 (5.27) | 50.79 (3.68) | 52.83 (6.70) | 49.08 (2.92) |

Before inoculation, finger 3 and 4 and the thumb were washed with water and rinsed with 70% ethanol and finger 1 and 2 kept unwashed. Ten μL of fecally-suspended rotavirus was placed on the thumb and each of the four fingers of left hand. From the thumb virus eluted immediately after its deposition (0 minute). After 20 min, virus eluted from each fingerpads separately and plaque assayed.

three times with each individual on different days. The results of these experiments are summarised in Table 4. At 20, 60 and 260 minutes after virus inoculation, 57.0%, 42.6% and 7.1% respectively of the input infectious virus could be recovered from the contaminated fingers.

There are a number of variables involved in interpreting these results such as volunteers, sampling time, and fingers. Analysis of the variance components in each trial showed that 90%, 95.4% and 65.8% of the total variation at 20, 60 and 260 min, respectively, was attributable to random error and variation between volunteers was responsible for 10%, 4.6% and 33.9%, respectively, of the total. These analyses also showed that there was little or no finger-to-finger variation.

2. Rhinovirus survival

a) **RV-14 survival on fingerpads:** RV-14 survival was tested for a duration of 3 hours on the fingerpads of four volunteers. Ten uL of the virus suspension in mucin (2.1×10^4 to 1.1×10^5 PFU) was inoculated on the marked areas of the middle finger of the right hand and thumb and four fingers of the left hand and eluted at 0, 20, 30, 60, 120, and 180 minutes after contamination (see Fig. 5). Each experiment was repeated at least three times on each volunteer.

The data summarized in Table 5 indicate that RV-14 could survive for several hours on hands; after 3 hours, 10.5%-22.7% of the 0 minute virus titre remained infectious on the fingerpads. This represents 2,000-25,000 PFU, which is an amount considerably higher than the minimal infective dose of rhinoviruses (1 TCID₅₀) by the nasal or conjunctival route (Couch et al., 1990; Cate et al., 1965). Twenty minutes after virus deposition, when the inoculum became visibly dry, about 65.0% of the 0 min virus titre could be recovered. The overall loss in virus

Table 4.

**ROTAVIRUS SURVIVAL ON EXPERIMENTALLY-
CONTAMINATED FINGERPADS**

| Volunteer | 20 min | | 60 min | | 260 min | |
|----------------|--------|----------------------|--------|----------------------|---------|----------------------|
| | n* | Mean % survival (SD) | n* | Mean % survival (SD) | n* | Mean % survival (SD) |
| 1 | 6 | 85.3 (47.33) | 3 | 50.7 (18.98) | 6 | 8.5 (5.16) |
| 2 | 6 | 62.7 (14.89) | 3 | 37.2 (12.50) | 6 | 1.8 (2.02) |
| 3 | 6 | 58.8 (51.57) | 3 | 41.3 (5.04) | 6 | 4.4 (3.13) |
| 4 | 6 | 52.9 (34.33) | 2 | 30.3 (10.33) | 6 | 14.3 (7.35) |
| 5 | 12 | 51.1 (23.32) | 3 | 40.3 (5.42) | 12 | 10.2 (9.72) |
| 6 ^a | 6 | 37.3 (21.02) | 3 | 51.9 (14.96) | 6 | 0.02 (0.04) |
| Overall mean | | 57.0 (36.61) | | 42.6 (12.68) | | 7.1 (7.98) |

^a One volunteer was different for the 60-minute sampling time.

* number of observations

Ten μ L of fecally-suspended rotavirus was placed on four fingers of the left hand of each volunteer. Virus was eluted from two fingerpads at 20 minutes after deposition and from the two other fingerpads at 260 minutes after deposition of the virus suspension. Virus survival at 60 minutes was determined in a subsequent trial with three fingers of each volunteer.

Table 5.
SURVIVAL OF RV-14 ON FINGERPADS

| Volunteer | % virus recovered after | | | | |
|---------------|-------------------------|----------------|----------------|----------------|---------------|
| | 20 min | 30 min | 60 min | 120 min | 180min |
| 1 | 73.1 | 67.9 | 32.0 | 17.2 | 14.0 |
| 2 | 53.4 | 40.8 | 28.4 | 16.3 | 10.5 |
| 3 | 65.3 | 56.6 | 39.5 | 20.7 | 15.1 |
| 4 | 66.3 | 65.6 | 51.1 | 37.9 | 22.7 |
| Mean: (SD) | 64.5 (8.2) | 57.7 (12.3) | 37.8 (10.0) | 23.0 (10.1) | 15.6 (5.1) |

Ten uL of mucin-suspended RV-14 was inoculated on the thumb and each fingerpad of the left hand. Twenty, 30, 60, 120, and 180 minutes after deposition, the inoculum was eluted and titrated. Percent virus recovered at each time point is the average of at least three observations on each volunteer.

infectivity was gradual and after 30, 60, 120, and 180 minutes infectious virus recoveries were $57.7 \pm 12.3\%$, $37.8 \pm 10.0\%$, $22.0 \pm 10.6\%$ and $15.6 \pm 5.1\%$, respectively.

b) Rhinovirus survival on disks: The ability of RV-14 to survive on inanimate surfaces was tested using stainless steel disks. Experiments were conducted under ambient temperature and RH conditions on different days. The results summarized in Table 6 show that 20 minutes after deposition, $66.6 \pm 13.2\%$ of the initial input infectious virus was recoverable from the disks. These results compare favorably with rhinovirus survival on fingerpads.

3. Parainfluenzavirus survival

a) Parainfluenzavirus survival on fingerpads: The amount of input HPIV-3 on each fingerpad ranged from 1.3×10^5 to 5.5×10^5 PFU and initially, survival of this virus on fingers was tested over a period of 3 hours. Repeated trials showed that infectious virus was virtually undetectable 1 hour after its deposition on the fingerpads. In view of this, the duration of HPIV-3 survival on the hands of the four volunteers was examined during 60 minutes (Table 7). Virus infectivity dropped very rapidly within the first 10 minutes when only 5.3% of the input infectious virus was detectable. After 1 hour the virus infectivity was barely detectable. In Figure 12 the survival of HPIV-3 is compared with that of RV-14; the differences in the survival of these two viruses on fingerpads were found to be statistically highly significant ($p = 0.0001$).

Since the virus inoculum was visibly wet after 10 minutes on the fingerpad, the relatively rapid loss of HPIV-3 infectivity on the skin was unlikely to be due to the drying of the inoculum alone. To further explore this rapid inactivation of HPIV-3 on fingers, additional experiments on

Table 6.**SURVIVAL OF RV-14 ON STAINLESS STEEL DISKS**

| n | % PFU recovered (SD) at | | % survival |
|----|---------------------------|----------------------------|------------------|
| | 0 min x10 ⁴ | 20 min x10 ⁴ | |
| 15 | 2.52 (0.53) | 1.69 (0.51) | 66.61 (13.18) |

Ten μ L of mucin-suspended RV-14 was placed on four clean and sterilized stainless steel disks. Virus eluted with 1.0 mL of EBSS from one disk immediately (input PFU) and from the remaining three disks 20 minutes after virus deposition. Experiments were conducted on five separate days.

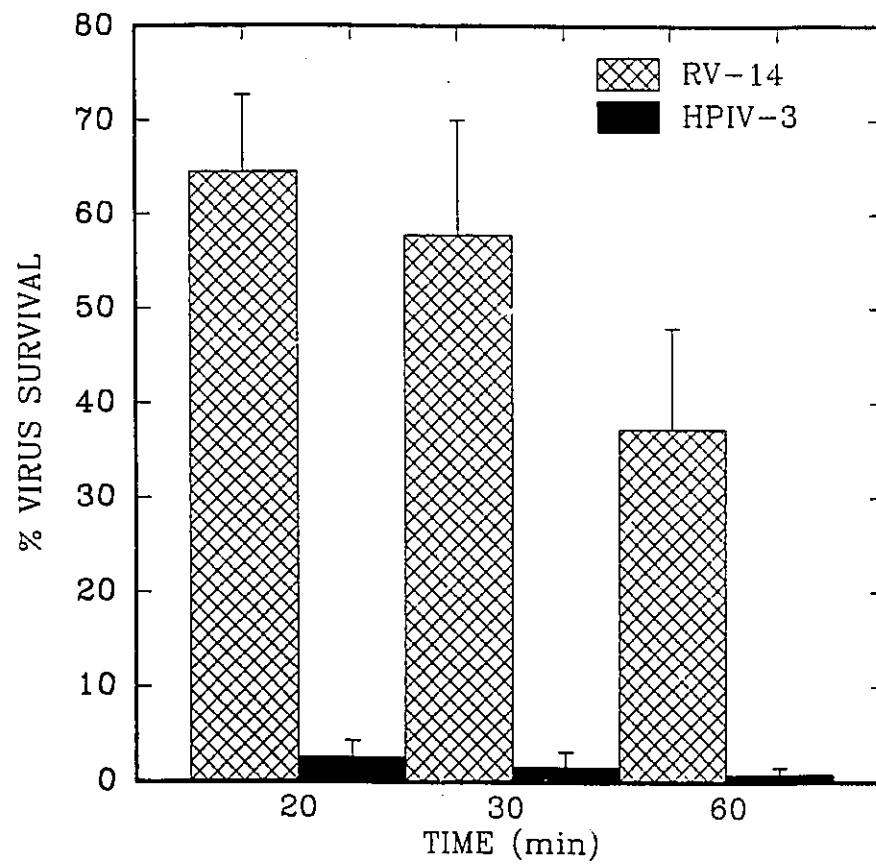
Table 7.
SURVIVAL OF HPIV-3 ON FINGERPADS

| Volunteer | % recovery after | | | |
|-----------|------------------|--------|--------|--------|
| | 10 min | 20 min | 30 min | 60 min |
| 1 | 9.6 | 4.9 | 3.9 | 1.9 |
| 2 | 2.4 | 0.9 | 0.7 | 0.3 |
| 3 | 5.6 | 1.7 | 0.9 | 0.6 |
| 4 | 3.4 | 2.6 | 0.4 | 0.3 |
| Mean: | 5.3 | 2.5 | 1.5 | 0.8 |
| (SD) | (3.1) | (1.7) | (1.6) | (0.8) |

Ten uL of mucin-suspended HPIV-3 was inoculated on the thumb and all fingerpads of the left hand. From the thumb, virus was eluted immediately after deposition (0 minute). From fingers 1, 2, 3, and 4, virus was eluted 10, 20, 30, and 60 minutes after inoculation, respectively, and titrated.

Figure 12. Comparison of the survival of HPIV-3 and RV-14 on the fingerpads of adult volunteers.

(Procedures for testing RV-14 and HPIV-3 survival on fingerpads are outlined in the footnotes for Tables 5 and 7, respectively)



one volunteer were performed to examine virus survival during the first 10 minutes after deposition. These results are shown in Table 8.

It is interesting to note that the loss in virus infectivity during first 10 min exposure to fingerpads was rapid and showed a typical linear decay in HPIV-3 infectivity.

b) HPIV-3 survival on disks: The rapid loss of HPIV-3 infectivity on hands prompted us to investigate its survival on disks under different ambient conditions ($55\pm 5\%$ RH and $22\pm 2^{\circ}\text{C}$). Results presented in Table 9 show that 1 hour after deposition, $12.2 \pm 2.73\%$ infectious virus was still detectable on steel disks. When these results were compared with the fingerpad survival, there was a marked difference (Figure 13). This difference was significant ($\alpha=0.05$) and demonstrates a clearly different behavior to that seen with rhinoviruses.

4. E. coli survival on fingerpads: The 3 volunteers in *E. coli* survival study had previously participated in the rotavirus survival experiments. The results summarized in Table 10 show that 20 minutes after deposition, only $8.5 \pm 3.2\%$ of the input CFU could be recovered and 60 minutes after drying on hands this was reduced to only $3.5 \pm 0.6\%$ of the inoculated viable bacteria. These findings clearly show that *E. coli* survives poorly on human hands when compared to HRV.

5. S. aureus survival on fingerpads: The survival of *S. aureus* was tested by the same protocol on the hands of the same three volunteers used in the experiments with *E. coli*. However, instead of feces, mucin was used to suspend the bacteria before experimental contamination of fingerpads since this was felt to more accurately reflect the *in vivo* situation.

Table 8.**SURVIVAL OF HPIV-3 ON FINGERPADS WITHIN
10 MINUTES OF DEPOSITION**

| Expt. no. | PFU at 0 min X10 ⁵ | % recovery after | | | |
|--------------|---|------------------|---------|--------|--------|
| | | 1 min | 4 min | 7 min | 10 min |
| 1 | 1.7 | 82.65 | 57.80 | 29.48 | 7.54 |
| 2 | 1.3 | 84.12 | 25.09 | 16.32 | 11.17 |
| 3 | 1.8 | 65.93 | 24.72 | 23.54 | 10.00 |
| 4 | 0.6 | 94.90 | 63.20 | 20.50 | 2.00 |
| 5 | 1.2 | 86.21 | 45.34 | 40.43 | 10.40 |
| Mean | | 82.76 | 43.23 | 26.05 | 8.22 |
| (SD) | | (10.54) | (17.94) | (9.36) | (3.73) |

Ten uL of mucin-suspended HPIV-3 was placed on the thumb and eluted immediately with 1.0 mL of EBSS (0 minute). On fingers 1, 2, 3 and 4, virus suspension was deposited for 1, 4, 7 and 10 min duration respectively and titrated. For each time point, virus deposition and elution was made separately on separate fingers.

Table 9.

SURVIVAL OF HPIV-3 ON STAINLESS STEEL DISKS

| Expt. no. | PFU at 0 minute X10 ⁵ | % PFU recovered at time (min) | | | | | | |
|---------------|--|-------------------------------|--------|--------|--------|--------|--------|--------|
| | | 1 | 4 | 7 | 10 | 20 | 30 | 60 |
| 1 | 0.20 | 88.33 | 71.00 | 61.00 | 43.66 | 37.11 | 32.05 | 16.21 |
| 2 | 0.63 | 81.56 | 60.17 | 54.31 | 45.00 | 29.05 | 14.31 | 9.13 |
| 3 | 0.47 | 80.83 | 64.67 | 63.97 | 53.11 | 39.95 | 17.67 | 10.32 |
| 4 | 0.53 | 84.33 | 66.00 | 59.66 | 51.63 | 42.67 | 20.17 | 12.33 |
| 5 | 3.00 | 86.16 | 68.00 | 57.20 | 49.13 | 40.33 | 18.56 | 13.00 |
| Overall mean: | | 84.24 | 65.97 | 59.23 | 48.51 | 37.82 | 20.55 | 12.20 |
| (SD) | | (3.13) | (4.02) | (3.68) | (4.10) | (5.29) | (6.77) | (2.73) |

Ten μ L of mucin-suspended HPIV-3 was placed on disks. After specified time intervals, virus was eluted by transferring the disk into a plastic test tube containing 1.0 mL of EBSS and then sonicated for 10 minutes.

Figure 13. Comparison of the survival of HPIV-3 on fingerpads and stainless steel disks.

(Procedures for testing HPIV-3 survival on fingerpads and stainless steel disks are outlined in the footnotes for Tables 7 and 9, respectively)

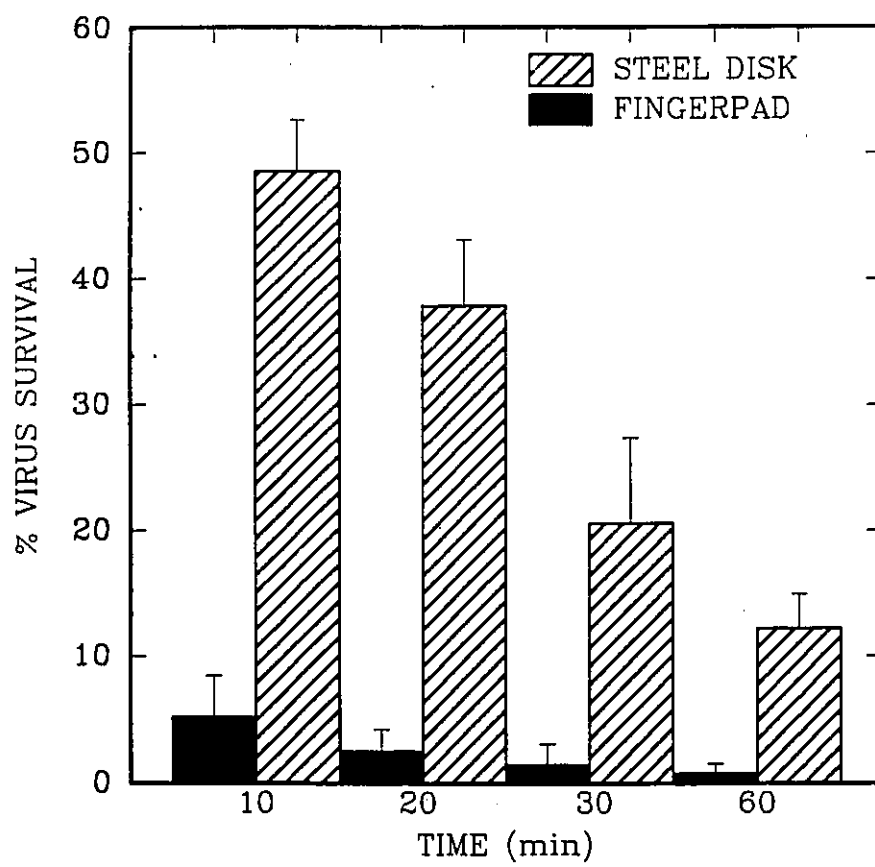


Table 10.**SURVIVAL OF *E. coli* ON EXPERIMENTALLY-CONTAMINATED FINGERPADS**

| Volunteer | % CFU recovered (SD) after | | | |
|---------------|----------------------------|----------------|---|----------------|
| | n | 20 min | n | 60 min |
| 1 | 10 | 8.44 (2.75) | 6 | 3.32 (0.51) |
| 2 | 10 | 8.35 (3.37) | 6 | 3.96 (0.76) |
| 3 | 10 | 8.59 (3.32) | 6 | 3.28 (0.52) |
| Overall mean: | | 8.46 | | 3.52 |
| (SD) | | (3.15) | | (0.60) |

Ten-uL of fecally-suspended *E. coli* was inoculated on the thumb and all fingerpads of the left hand. From the thumb, the inoculum was eluted immediately (base titre). After 20 min, the inoculum was eluted from fingers 1 and 2, after 60 min from fingers 3 and 4 separately and titrated on mFC agar plates. Colonies were counted 18 hours after incubation at 37°C.

The findings of these experiments are summarized in Table 11. *S. aureus* was found to survive on human hands much better than *E. coli*. Twenty and 60 minutes after deposition, $58.4 \pm 12.2\%$ and $41.3 \pm 5.9\%$, respectively, of the recoverable CFU at 0 minute were still detectable. Statistical analysis of the data show significant difference between *S. aureus* and *E. coli* survival on hands at $\alpha = 0.05$ level.

III. STUDY OF VIRUS TRANSFER BETWEEN CONTAMINATED AND CLEAN SURFACES

1. Rotavirus transfer: The findings of the foregoing experiments clearly established the fact that HRV could retain its infectivity on human hands for several hours. It has also been shown that HRV can survive on non-porous inanimate surfaces for several days (Sattar et al., 1986). In the following set of experiments we tested the extent to which transfer of infectious virus could occur through contact between a clean and a rotavirus-contaminated surface. The experiments were designed to demonstrate virus transfer from finger to finger, disk to finger and finger to disk.

The efficiency of rotavirus transfer in each model was tested 20 and 60 minutes after deposition on fingerpads or on stainless steel disks. Five volunteers participated in the 20-minute transfer experiments and six volunteers for the 60-minute transfer. Table 12 summarizes the results of these experiments.

The quantities of infectious virus transferred from contaminated fingerpads to clean disks and from contaminated disks to clean fingerpads were quite similar. Twenty minutes after virus inoculation 16.1% to 16.8% of the recoverable virus at 20 min could be transferred to and from finger and inanimate surface (disk) and 60 minutes post inoculation

Table 11.**SURVIVAL OF *S. aureus* ON EXPERIMENTALLY-CONTAMINATED FINGERPADS**

| Volunteer | % CFU recovered (SD) after | | | |
|-----------------------|----------------------------|------------------|----|------------------|
| | n | 20 min | n | 60 min |
| 1 | 15 | 55.97 (10.56) | 10 | 43.94 (7.25) |
| 2 | 15 | 64.71 (15.42) | 10 | 40.90 (6.66) |
| 3 | 15 | 54.47 (10.52) | 10 | 39.04 (3.86) |
| overall mean: (SD) | | 58.38 (12.17) | | 41.29 (5.92) |

Ten- μ L mucin-suspended *S. aureus* was placed on the thumb and all fingerpads of the left hand. The inoculum was eluted from the thumb immediately (0 minute). From fingers 1 and 2, the inoculum was eluted 20 min after deposition and from fingers 3 and 4 after 60 min. Mannitol salt agar plates were inoculated and bacterial colonies counted after incubation for 24 hours at 37°C.

Table 12.

**TRANSFER OF INFECTIOUS ROTAVIRUS FROM
CONTAMINATED TO CLEAN SURFACES IN THREE
SEPARATE MODELS**

| Time (min) between virus contamination and transfer | Number of volunteers | % infectious virus transferred (SD) | | |
|--|-------------------------|-------------------------------------|-------------------|---------------------|
| | | Finger to disk | Disk to finger | Finger to finger |
| 20 | 5 | 16.1 (5.43) | 16.8 (5.17) | 6.6 (2.61) |
| 60 | 6 | 1.8 (1.50) | 1.6 (1.18) | 2.8 (2.11) |

Three fingerpads of each volunteer were tested in each experiment. Ten uL of fecally-suspended rotavirus (2×10^4 to 8×10^4 PFU) was used to contaminate each donor surface. A 5-second contact with pressure (1 Kg/cm^2) was made between the donor and target surface either 20 min or 60 min after virus contamination. The extent of virus transfer was determined by recovery of infectious virus from the target surface.

such transfer amounted to 1.6% to 1.8%. At 20 and 60 minutes after inoculation, 6.6% and 2.8%, respectively, of the input infectious virus could be transferred from a contaminated to a clean finger. In all three models of virus transmission tested, HRV transfer 20 minutes after deposition was higher when compared with that at 60 minutes. At 20 minutes the least amount of virus transferred was observed in the finger-to-finger model. This amount of infectious virus, however, was at least 300 PFU, which is considerably higher than the estimated minimal infective dose (1 tissue culture infective dose) for rotaviruses (Graham et al., 1987; Ward et al., 1986). Virus transfer was not tested when the inoculum was still visibly wet because, it was expected, based on previous reports (Reed, 1975), that the transfer of virus from a wet surface will be better as compared to that from a dry surface.

2. Rhinovirus transfer: The transfer of RV-14 between surfaces was also determined according to the procedure described above. The results summarized in Table 13 show that, irrespective of the nature of the contaminated surfaces tested, the amount of virus transferred from finger to finger, disk to finger or finger to disk was surprisingly similar in contrast to the HRV results.

Since less than 1.0% of the recoverable virus at 20 minutes after drying could be transferred from the donor to target surface, it was considered important to account for the infectious virus still remaining on the donor surface. Therefore, in a series of experiments using one volunteer, virus was eluted from the target as well as the donor surface after virus transfer was carried out. Data presented in Table 14 show that, after the contact, as much as 72-92% of the virus which survived 20 minutes drying could be accounted for.

Table 13.

TRANSFER OF RV-14 FROM CONTAMINATED TO CLEAN SURFACES 20 MINUTES AFTER VIRUS DEPOSITION

| Volunteer | % virus transferred | | |
|-----------|---------------------|----------------|------------------|
| | Finger to disk | Disk to finger | Finger to finger |
| 1 | 1.4 | 0.6 | 0.9 |
| 2 | 0.7 | 0.5 | 0.6 |
| 3 | 0.8 | 0.7 | 0.7 |
| 4 | 0.7 | 0.8 | 0.7 |
| Mean | 0.9 | 0.7 | 0.7 |
| (SD) | (0.3) | (0.1) | (0.1) |

Ten uL mucin-suspended rhinovirus (6.0×10^4 to 1.0×10^5 PFU) was deposited on the thumb, all fingers of left hand and on cleaned steel disks. After 20 minutes, virus was eluted from the thumb and a disk (base titre). For virus transfer, contact was made between donor and recipient surfaces for 5 seconds with a pressure of approximately 1 kg/cm². Virus eluted from the target surface and plaque assayed.

Table 14.**TOTAL RV-14 DETECTABLE IN TRANSFER EXPERIMENTS**

| Mode of transfer | PFU on donor at 20 min X10 ⁴ | % virus transferred to target | % virus recovered from donor after transfer | Total virus recovered* |
|------------------|---|-------------------------------|---|------------------------|
| Finger-to-disk | 2.86 (0.33) | 0.89 (0.17) | 70.95 (15.21) | 71.85 (15.07) |
| Disk-to-finger | 2.33 (0.77) | 16.39 (4.84) | 74.12 (4.80) | 90.51 (0.64) |
| Finger-to-finger | 2.55 (0.66) | 0.67 (0.04) | 81.44 (4.89) | 82.10 (4.95) |

Procedure for virus transfer is the same as that outlined in the footnote for Table 13. After contact, virus was eluted from both the donor and target surfaces and plaque assayed.

* Target + donor % recovery.

It should be noted here that the disk-to-finger transfer results shown in these experiments were much higher than those obtained for the same transfer mode in the previous experiments (Table 13). This may have been due to the influence of the ambient RH; the earlier experiments with a low recovery took place in a low RH (35%) in contrast to the latter at high RH (65%).

3. Parainfluenzavirus transfer: In spite of its poor survival on hands, we attempted to determine if any infectious HPIV-3 could be transferred from contaminated hands to clean hands or disks 20 minutes after drying. Repeated experiments did not show any detectable virus transfer from finger to finger or finger to disk. However, nearly $1.5 \pm 0.98\%$ of infectious virus was transferred from contaminated disks to clean hands (Table 15). This represents a minimum of 204 PFU which is nearly 2.5 times the minimal infective dose for parainfluenzaviruses (Smith et al., 1966).

IV. HYGIENIC HAND-WASHING

So far, *in vivo* testing of hygienic hand-wash agents has been conducted mainly with bacteria (Ayliffe et al., 1988; Casewell et al., 1988; Ojajarvi, 1980). Limited testing of such products against viruses on experimentally-contaminated human hands has been carried out (Cliver and Kostenbader, 1984; Hayden et al., 1984; Hendley et al., 1978; Schurmann and Eggers, 1983) using wide variations in the test protocols in the absence of a standardized and universally acceptable method. Our study was, therefore, aimed at designing a simple *in vivo* protocol, using fingerpads, to assess the virus eliminating efficiency of hand-washing agents. The new protocol (Fig. 9; page 63) was then used to undertake a comparative study of the efficiency of the hand-washing

Table 15.

**TRANSFER OF HPIV-3 FROM CONTAMINATED DISKS TO
CLEAN FINGERS 20 MINUTES AFTER VIRUS DEPOSITION**

| Expt. no. | PFU at 0 min on disk X10 ⁴ | PFU remaining after 20 min X10 ⁴ | PFU transferred to finger X10 ² | % virus transferred |
|--------------|--|---|--|------------------------|
| 1 | 2.85 | 0.79 | 2.31 | 2.61 |
| 2 | 6.66 | 2.25 | 2.04 | 0.91 |
| 3 | 7.00 | 3.02 | 2.82 | 0.93 |
| Mean | 5.50 | 2.02 | 2.39 | 1.48 |
| (SD) | (2.30) | (1.13) | (0.40) | (0.98) |

Ten uL of mucin-suspended HPIV-3 was placed on five cleaned and sterilized stainless steel disks. Virus was eluted from one disk immediately (0 min) and from another 20 min after deposition (base titre). Clean fingerpads were pressed over the contaminated disks separately for 5 sec with a pressure of approximately 1 kg/cm², for virus transfer to occur. Virus eluted from the recipient fingerpads and plaque assayed.

agents to remove or inactivate the rotavirus, *E. coli* and *S. aureus*. Finally, the results obtained with the fingerpad protocol were compared with those obtained using the conventional 'whole-hand' procedure (Fig. 10; page 65).

1. In vivo testing of hand-washing agents against HRV:

Initially, HRV was used in the fingerpad protocol to test 11 different hand-wash agents; 9 antiseptic formulations, an unmedicated liquid soap and tap water alone. In these experiments, four contaminated fingers of one volunteer were used; after a 10-second contact with the test agent, fingerpads were rinsed with tap water and the residual virus eluted with or without paper towel-drying. Results of rotavirus reduction after hand-washing treatment and with drying or without drying are shown in Tables 16 and 17, respectively.

Seventy percent isopropanol and 70% ethanol alone, as well as Savlon containing 70% of either one of the alcohols, were able to reduce the infectivity titre of the virus by 99.0% or more. Hibisol, which contains chlorhexidine gluconate and 70% isopropanol, eliminated 95.3% of the virus. Dettol and Provioline produced a 96.2% and 96.5% reduction in the virus titre, respectively. Cida-Stat and Savlon in water (1:200) gave the lowest levels of virus removal of all the antiseptics tested, as they were able to reduce the virus titre by only 80.3% and 78.3%, respectively. Surprisingly, and in terms of mean percent virus removal, the liquid soap (86.9%) and tap water alone (83.6%) were as good as, if not better than, Cida-Stat and Savlon in water.

More virus was recovered when fingerpads were sampled without drying them with paper towel. As shown in Table 17, 70% alcohols were the most effective in eliminating rotaviruses from fingerpads whereas

Table 16.

**IN VIVO EFFICACY OF HANDWASHING AGENTS
AGAINST HUMAN ROTAVIRUS USING THE FINGERPAD
PROTOCOL**

| Handwashing agent | % reduction Mean (SD) | Tukey grouping ^a |
|-------------------------------------|--------------------------|-----------------------------|
| Isopropanol (70%) | 99.8 (0.18) | A |
| Ethanol (70%) | 99.8 (0.19) | A |
| Savlon (1:30) in 70% isopropanol | 99.4 (0.84) | A |
| Savlon (1:30) in 70% ethanol | 99.1 (0.50) | A |
| Providine | 96.5 (2.65) | A B |
| Hibisol | 95.3 (2.15) | A B C |
| Dettol | 96.2 (3.62) | A B C |
| Liquid soap | 86.9 (2.42) | B C D |
| Cida-Stat | 80.3 (3.51) | D |
| Savlon (1:200) in water | 78.3 (5.55) | D |
| Tap water (control) | 83.6 (3.49) | D |

^a Agents with the same Tukey groupings do not have significantly different percentages of reduction ($\alpha = 0.05$).

Ten uL of fecally-suspended virus was added on the thumb and all fingers of the left hand. After 20 minutes, virus was eluted from the thumb without washing (base titre); from two fingers after washing and drying.

Table 17.

**IN VIVO EFFICACY OF HANDWASHING AGENTS
AGAINST HUMAN ROTAVIRUS USING THE FINGERPAD
PROTOCOL WITHOUT THE DRYING STEP**

| Handwashing agent | % reduction Mean (SD). | Tukey grouping ^a |
|-------------------------------------|---------------------------|-----------------------------|
| Isopropanol (70%) | 99.0 (0.81) | A |
| Ethanol (70%) | 99.0 (0.70) | A |
| Savlon (1:30) in 70% isopropanol | 98.0 (1.24) | A |
| Savlon (1:30) in 70% ethanol | 92.5 (4.46) | A B |
| Proviiodine | 86.0 (8.55) | B C |
| Dettol | 83.7 (6.25) | B C |
| Liquid soap | 75.8 (4.07) | C D |
| Tap water (control) | 73.3 (9.67) | C D |
| Cida-Stat | 70.6 (1.86) | C D |
| Savlon (1:200) in water | 45.2 (13.79) | E |

^a Agents with the same Tukey groupings do not have significantly different percentages of reduction ($\alpha = 0.05$).

Ten uL of fecally-suspended virus was added on the thumb and all fingers of the left hand. After 20 minutes, virus was eluted from the thumb without washing (base titre) and from two fingers after washing but without drying.

formulations containing water were the least effective in reducing the HRV contamination from fingerpads. For example, washing with Savlon in water (1:200) removed only 45.2% of the challenge titre, whereas removal of HRV by liquid soap (75.8%) and tap water alone (73.3%) are comparable. These results suggest the importance of paper towel drying especially when virus contaminated hands are washed with an ineffective hand-washing agent.

The percentages of virus removal by various hand-washing agents were compared statistically using Tukey's procedure (Table 16 and 17). Those means designated by the same letter under the Tukey grouping were considered not significantly different from each other at the $\alpha = 0.05$ level. No differences were seen among the hand-washing agents represented by the letter A. Likewise, no differences could be demonstrated among those with the letter D. Group A, primarily the alcohol-containing formulations and those solutions with high concentrations of iodine or p-chloro-m-xyleneol (PCMX), were significantly different from group D, which included the aqueous chlorhexidine salts, soap and water and water alone. However, multiple comparisons also showed the existence of intermediate groups B and C, which showed some overlap with the means obtained with hand-washing agents in groups A and D. Despite these overlaps, the alcohols or alcohol-containing formulations were clearly superior to water and the aqueous solutions of chlorhexidine salts in reducing the levels of HRV. In Table 17, Savlon in water (1:200) appeared to be the least effective (group E).

2. In vivo testing of hand-washing agents against HRV and *E. coli*:

In a separate set of experiments, using three volunteers and the fingerpad protocol, five hand-wash agents (three antiseptics, the soap,

and the tap water alone) were compared for their ability to eliminate rotavirus and *E. coli* (Table 18). The purpose for including three volunteers in this part of the study was to test for possible person-to-person variation in the results.

Twenty minutes after inoculation of the test organism on the fingerpads, 57% and 7% of the original inoculum for HRV and *E. coli*, respectively, were detectable. Therefore, the challenge titres of the test organisms for the hand-washing agents were $1.5 \pm 0.4 \times 10^4$ PFU and $9.9 \pm 1.6 \times 10^4$ CFU for HRV and *E. coli*, respectively.

The results of these experiments are shown in Table 18. Surprisingly, tap water appeared to be more effective in removing *E. coli* from fingerpads than liquid soap. The differences in the mean percent removals between HRV and *E. coli* were not statistically significant for the alcohols, the alcoholic solution of Savlon, the soap or tap water alone. However, there was a statistically significant difference ($p = <0.0001$) between the mean percent removals of *E. coli* (98.9%) and the rotavirus (80.6%) by Savlon in water (1:200).

Statistical analyses of the data summarized in Table 18 also showed that the person-to-person variations in mean percent reductions by a hand-washing agent against a given organism were not significant ($p = 0.14$).

3. Comparison of the whole-hand method with the fingerpad protocol:

In order to determine if the fingerpad protocol was representative of the normal hand-washing practice, selected hand-washing agents were tested by the whole-hand protocol for their ability to eliminate HRV and *E. coli*. In these experiments, on an average, $1.8 \pm 0.9 \times 10^4$ PFU and

Table 18.

**COMPARISON OF *IN VIVO* EFFICACY OF HAND-WASHING
AGENTS AGAINST ROTAVIRUS (HRV) and *E. coli* (EC)
USING
THE FINGERPAD PROTOCOL**

| Mean % reduction in titer with: | | | | | | | | | | |
|---------------------------------|--------------------|-------|------------------------------------|-------|-----------------------------------|-------|----------------|--------|--------------|-------|
| Volun- teer | Isopropanol 70% | | Savlon in 70% ethanol (1:30) | | Savlon in tap water (1:200) | | Liquid soap | | Tap water | |
| | HRV | EC | HRV | EC | HRV | EC | HRV | EC | HRV | EC |
| 1 | 99.8 | 99.0 | 99.1 | 98.7 | 77.5 | 99.6 | 97.1 | 60.8 | 83.6 | 90.0 |
| 2 | 99.6 | 99.7 | 98.8 | 99.1 | 77.3 | 97.5 | 91.4 | 80.0 | 82.2 | 88.1 |
| 3 | 99.5 | 99.2 | 99.4 | 99.0 | 87.0 | 99.6 | 68.1 | 64.7 | 84.9 | 91.8 |
| Mean: | 99.6 | 99.3 | 99.1 | 98.9 | 80.6 | 98.9 | 72.5 | 68.7 | 83.6 | 90.0 |
| (SD) | (0.2) | (0.4) | (0.3) | (0.2) | (5.5) | (1.2) | (5.1) | (10.5) | (1.4) | (1.9) |

Ten μ L of fecally-suspended HRV or *E. coli* was placed on the thumb and all the fingers of the left hand. The test procedure was the same as outlined in the footnote of Table 16.

$5.7 \pm 3.2 \times 10^4$ CFU of HRV and *E. coli*, respectively, were used as the challenge titres. The results of these experiments are summarized in Table 19.

In both the protocols, 70% isopropanol and the alcoholic solution of Savlon reduced the titres of the two test organisms by at least 98.0%. Even though the overall removal of the virus and *E. coli* by the other hand-washing agents and tap water alone was not as high as that seen with the alcohols and alcoholic Savlon, there was no significant difference between the results obtained with the whole-hand and the fingerpad protocols.

The wide variations observed between the fingerpad and whole-hand experiments with liquid soap and *E. coli* were not found to be significant at the $\alpha = 0.05$ level. With Savlon in water (1:200), of interest because it is commonly used, the results of the whole-hand experiments were similar to those of the fingerpad protocol. Furthermore, Savlon in water (1:200) was found to be significantly less effective for the elimination of the virus (84.8%) when compared to *E. coli* (96.6%).

4. Spread of localized contamination from one hand to another:

In the previous experiments, as much as 7% of infectious HRV remained viable on the hands after washing with soap. This raised the possibility that use of soap might serve to spread localized virus contamination over a wider area of the hands being washed. To investigate this further, experiments were carried out not only with soap but also with isopropanol and tap water as controls.

One hand was contaminated with HRV and after 20 minutes drying, the hands were washed together using the agent under test. The initially uncontaminated hand was eluted to recover any infectious virus.

Table 19.

**COMPARISON OF THE WHOLE-HAND (WH) AND
FINGERPAD (FP) PROTOCOLS**

| Handwashing agent | Organism | % reduction mean (SD) | | Statistically significant difference |
|------------------------------|----------------|-----------------------|-------------|--------------------------------------|
| | | FP | WH | |
| 70% isopropanol | <i>E. coli</i> | 99.0 (1.5) | >99.9 (0.1) | no |
| | Rotavirus | 98.8 (0.2) | 99.9 (0.1) | no |
| Savlon (1:30) in 70% ethanol | <i>E. coli</i> | 98.7 (0.3) | >99.9 (0.1) | no |
| | Rotavirus | 99.1 (0.5) | 98.9 (0.6) | no |
| Savlon in tap water (1:200) | <i>E. coli</i> | 99.6 (0.1) | 96.6 (2.2) | no |
| | Rotavirus | 78.3 (5.6) | 84.8 (6.8) | no |
| Liquid Soap (1:10) | <i>E. coli</i> | 60.8 (36.8) | 94.5 (3.2) | no |
| | Rotavirus | 86.8 (2.4) | 93.8 (3.9) | no |

In the whole handwashing protocol, 0.5 mL of the fecally-suspended virus or bacterium was placed on the palm of one hand and then spread over the entire palm surface of both hands by gently rubbing them together. After 20 min, virus or bacterium was eluted with 20 mL of 20% TPB either without any treatment (control) or after washing with the test agent and paper-towel drying. Before plaque assay, samples were passed through a column of Sephadex LH-20 to remove cytotoxicity, and centrifuged at 14,000 xg for 10 min to remove bacteria and fungi.

For the details of the fingerpad protocol, please refer to the footnote for Table 16.

The results summarized in Table 20 show that washing with 70% isopropanol did not result in spread of the virus to the clean hand, whereas 24 and 43 PFU of HRV were transferred to the clean hand during washing with liquid soap or tap water alone, respectively.

5. In vivo testing of hand-washing agents against *S. aureus*:

Five hand-washing agents were tested for their capacity to eliminate *S. aureus* from experimentally-contaminated fingerpads. The data presented in Table 20 show that 70% isopropanol and Savlon in 70% ethanol were as efficient for *S. aureus* elimination as they were for *E. coli*. Tukey's procedure was used to group agents according to their observed differences; the percent reductions by isopropanol, Savlon in 70% ethanol, liquid soap and tap water alone were not significantly different at $\alpha = 0.05$ level (Table 21). It was clear that Savlon in water did not belong in this grouping. Results of these experiments comparing the efficacy of hand-washing agents against the two different bacteria, are presented in Table 22. It can be seen that removal of *E. coli* by liquid soap and tap water alone was less efficient compared to *S. aureus* reduction by these agents. Reduction of the *S. aureus* titre by liquid soap ($p = 0.0006$) and by tap water alone ($p = 0.0001$) was significantly higher than that for *E. coli*.

V. THE EFFICACY OF HAND-DRYING METHODS

We showed earlier that HRV could remain infectious on human hands for at least 4 hours and that a number of commonly used hand-washing agents were relatively ineffective in getting rid of this virus. We also noted that drying of hands with a paper towel produced a substantial reduction in the number of rotavirus PFU remaining on washed hands. In view of these early observations, the following experiments

Table 20.

**ROTAVIRUS SPREAD FROM CONTAMINATED LEFT HAND
TO CLEAN RIGHT HAND DURING HAND-WASHING**

| Hand-wash agents tested | Input virus (PFU) | Virus PFU recovered after 20 min | PFU recovered (%) | |
|-------------------------|-------------------|----------------------------------|--------------------------|-----------------------------|
| | | | from left hand (primary) | from right hand (secondary) |
| Isopropanol (70%) | 30,300 | 6,950 | 12 (0.2) | 0 |
| Liquid soap | 27,300 | 5,460 | 129 (2.4) | 24 (0.4) |
| Tap water | 21,100 | 4,640 | 240 (5.3) | 43 (0.9) |

0.5 mL fecally-suspended rotavirus was spread on the left hand. After 20 min, both hands were rubbed together with 0.5 mL of the test agent for 10 sec and then rinsed with 500 mL of 40°C tap water. After drying with paper towel for 10 sec, residual virus was eluted separately from each hand with 20 mL of 20% TPB. Eluates were passed through a Sephadex LH-20 column to remove cytotoxicity and then centrifuged at 14,000 x g for 10 min to remove bacteria and fungi before plaque assay.

Table 21.**IN VIVO EFFICACY OF HAND-WASHING AGENTS AGAINST
S. aureus IN THE FINGERPAD PROTOCOL**

| Hand-washing agent | n | % reduction (SD) | Tukey grouping* |
|------------------------------|---|------------------|-----------------|
| Isopropanol 70% | 9 | >99.99 (0.00) | A |
| Savlon in 70% ethanol (1:30) | 9 | 99.97 (0.01) | A |
| Liquid soap | 9 | 99.38 (0.54) | A |
| Tap water | 9 | 99.02 (0.47) | A |
| Savlon in tap water (1:200) | 9 | 96.16 (4.94) | B |

*Means with the same letter are not significantly different.

Ten uL of mucin-suspended *S. aureus* was placed on the thumb and all fingerpads of the left hand. After 20 min drying, the inoculum was eluted from the thumb without washing (base titre); from the fingers, residual bacteria were eluted after washing and drying with paper-towel.

Table 22.

**COMPARISON OF *IN VIVO* EFFICACY OF HAND-WASHING
AGENTS AGAINST *S. aureus* (SA) and *E. coli* (EC) USING
THE FINGERPAD PROTOCOL**

| Mean % reduction in titre with: | | | | | | | | | | |
|---------------------------------|--------------------|-------|------------------------------------|-------|-----------------------------------|-------|----------------|-------|--------------|-------|
| Volun- teer | Isopropanol 70% | | Savlon in 70% ethanol (1:30) | | Savlon in tap water (1:200) | | Liquid soap | | Tap water | |
| | SA | EC | SA | EC | SA | EC | SA | EC | SA | EC |
| 1 | 99.99 | 98.95 | 99.97 | 98.66 | 90.55 | 99.59 | 98.76 | 60.80 | 99.01 | 90.00 |
| 2 | 99.99 | 99.69 | 99.98 | 99.13 | 98.05 | 97.58 | 99.74 | 80.60 | 99.50 | 88.06 |
| 3 | 99.99 | 99.15 | 99.96 | 99.02 | 99.87 | 99.46 | 99.64 | 64.69 | 98.56 | 91.79 |
| Mean | 99.99 | 99.26 | 99.97 | 98.94 | 96.16 | 98.88 | 99.38 | 68.70 | 99.02 | 89.95 |
| (SD) | 0.01 | 0.38 | 0.01 | 0.25 | 4.94 | 1.12 | 0.54 | 10.49 | 0.47 | 1.87 |

Procedures for *E. coli* and *S. aureus* experiments are the same as outlined in the footnotes for Tables 17 and 21, respectively.

were undertaken to compare the rotavirus-eliminating efficiency of cloth towels and electric warm air with that of paper towels. Similar experiments were also conducted with *E. coli*.

The rotavirus survived much better than *E. coli* during the initial 20-minute drying of the inoculum. As can be seen from Table 4, 57% of rotavirus PFU as compared to 8.5% *E. coli* CFU (Table 10) remained detectable after 20 minutes. To compensate for the greater reduction in its viability during the drying of the inoculum, a larger number of *E. coli* CFU was placed on the fingerpad.

Table 23 shows the relative efficiency of the hand-washing agents in the elimination of the rotavirus and *E. coli* without any additional drying of the washed fingerpads. All hand-washing agents tested proved to be more effective against *E. coli* than against rotavirus. Except for 70% isopropanol, these differences were found to be statistically significant ($\alpha = 0.05$). The difference was particularly pronounced in the case of Savlon, where the reduction in *E. coli* CFU was $94.68 \pm 1.91\%$ as compared to $72.12 \pm 6.13\%$ for rotavirus PFU. Tap water alone was found to be nearly as effective as liquid soap for the removal of both test organisms, and comparable to Savlon for the elimination of the rotavirus. These findings reinforce the earlier results presented for rotavirus and *E. coli* in the previous section (Table 18).

Drying of washed fingerpads always resulted in a further reduction of both test organisms. Figure 14 summarizes the results of these experiments. Irrespective of the hand-washing agent used, electric warm air-drying produced the highest, and cloth-drying the lowest, reduction in the numbers of both test organisms. For example, after washing with the soap and water and no drying, the reduction in rotavirus was

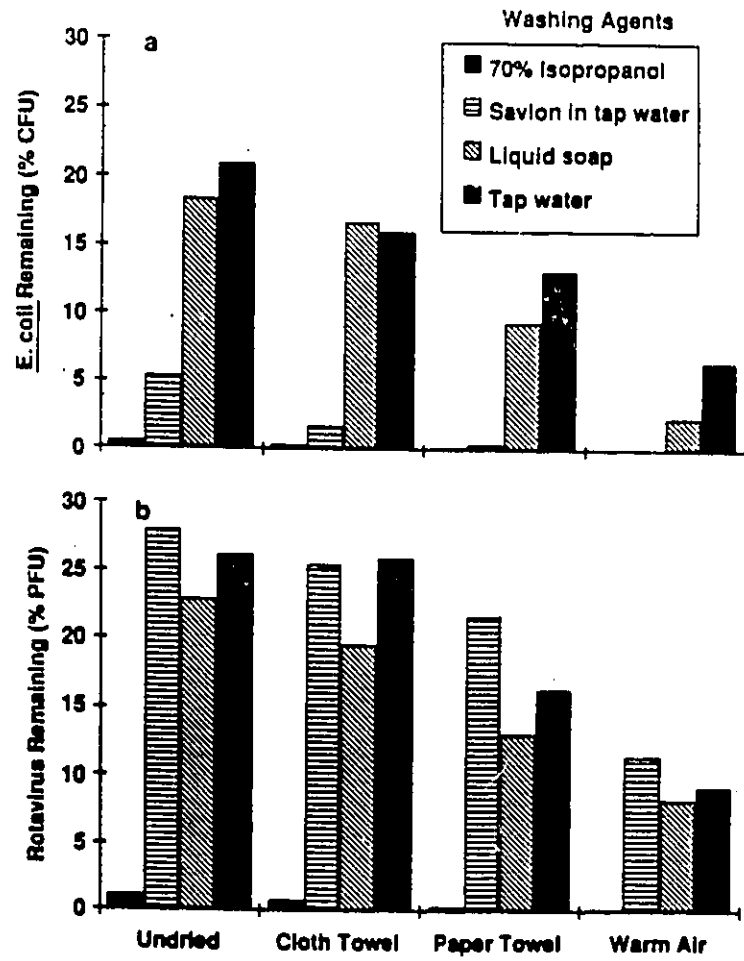
Table 23.

**RELATIVE EFFICACY OF THE HAND-WASHING AGENTS IN
ELIMINATING ROTAVIRUS AND *E. coli* FROM FINGERPADS
WITHOUT THE DRYING STEP**

| Hand-washing agent | % reduction (SD) | |
|-----------------------------|------------------|--------------------|
| | rotavirus PFU | <i>E. coli</i> CFU |
| 70% isopropanol | 98.90 (0.06) | 99.43 (0.25) |
| Savlon (1:200) in tap water | 72.12 (6.13) | 94.68 (1.91) |
| Unmedicated liquid soap | 77.11 (3.77) | 81.86 (3.55) |
| Tap water alone | 73.80 (5.52) | 78.99 (3.52) |

For procedure see footnote for Table 17. After washing with these agents, residual organisms were eluted without drying the washed area.

Figure 14. Recovery of *Escherichia coli* (a) and rotavirus (b) from fingerpads after washing and drying.



approximately 77%; when the washed hands were warm air-dried, the reduction ($91.74 \pm 0.81\%$) was greater when compared to the paper ($86.90 \pm 2.42\%$) or cloth ($80.40 \pm 0.14\%$). The reduction in the levels of residual contamination by warm air-drying was significantly different ($p=0.0001$) when compared to the two other methods with all the agents tested, except for 70% isopropanol.

DISCUSSION

Many infectious diseases can now be prevented through the proper use of safe and effective vaccines (Melnick, 1989). Improvements in the treatment and supply of potable water and in the collection, treatment and disposal of sewage have also resulted in a drastic reduction in the number of cases of enteric diseases such as hepatitis A. However, in spite of these and other developments in public health and general improvements in standards of hygiene, many viral enteric and respiratory infections continue to spread, particularly in institutional settings (Allen and Ford-Jones, 1990; Gwaltney and Hendley, 1982; Hall, 1983; Pentland and Penington, 1980). Whereas air may play a role in the spread of these infections (Hall, 1983; Dick et al., 1987; Gwaltney et al., 1978), it is generally believed that hands are the primary vehicles in such settings (Black et al., 1981; Ekanem et al., 1983; Samadi et al., 1983). There is also increasing evidence to suggest that environmental surfaces and fomites may also be involved as vehicles in institutional settings either directly or in conjunction with hands and possibly air (Dick et al., 1987; Jennings et al., 1988; Keswick et al., 1983a, 1983b; Pancic et al., 1980; Sattar et al., 1986).

Studies to determine the precise role and relative significance of hands, environmental surfaces and fomites in the transmission of pathogenic microorganisms are difficult to design and even harder to conduct mainly because of ethical considerations, number of variables involved and budgetary constraints. The unpredictability of infectious disease outbreaks in institutional settings also makes it difficult to plan and allocate necessary resources for such investigations. Attempts that

have been made so far have been confined mainly to certain types of bacterial diseases (Knittle et al., 1975; Onesko and Wienke, 1987; White et al., 1989). Therefore, with some notable exceptions, such as rhinoviruses (Hendley et al., 1973; Reed, 1975), virtually nothing has been reported, in precise quantitative terms, about how well human pathogenic viruses survive on hands, how they are transferred to and from hands and the efficacy of commonly used hygienic hand-washing agents in their elimination. Such information would be essential to elucidate the role of hands as vehicles for viral infections. One of the principal objectives of this study was therefore, to investigate how well rota-, parainfluenza- and rhinoviruses could survive on human hands. Their survival was then compared with that of *E. coli* and *S. aureus*.

1. The fingerpad protocol

Fingers may be the most important part of the hand in the spread of pathogenic microorganisms. They frequently come in contact with infectious fluids or surfaces and fomites contaminated directly or by large particle aerosols. Such acquisition and carriage of pathogens by the fingers may result in inoculation of self or others, particularly when caring for the sick, the young and the elderly.

The fingerpad protocol used here was developed in our laboratory as a simple, reproducible and quantitative method in order to study the role of hands in the transmission of pathogenic viruses and bacteria. The results of this study show that this protocol could be readily used for the study of enteric viruses and respiratory viruses as well as bacteria.

The nature of the medium used for suspending the test organism can influence its survival (England, 1982) on hands and environmental surfaces. The most appropriate suspending medium for laboratory-based studies is the body fluid in which the organism is normally discharged. However, such body fluids from naturally-infected individuals may not be available in sufficient quantities and with high enough infectious titres for experimental contamination. In the absence of such naturally-discharged pathogen-containing body fluids, organisms to be studied are experimentally added to a variety of suspending media. The choice of the suspending medium is governed by its ready availability, harmlessness to the test organism's infectivity and the assay system as well as its similarity to the body fluid(s) in which the test organism is normally discharged. In this study, we used either feces or bovine mucin in normal saline instead of suspending the test organisms in cell culture media or buffered salt solutions (Brady et al., 1990; Reed, 1975).

EBSS, with or without TPB, was used as an eluent because it was not only harmless to microbial infectivity but also highly efficient in the recovery of the viruses and bacteria tested here. These eluents are readily available, safe for contact with the skin and harmless to the cell cultures used. Elution of the virus inoculum immediately from the experimentally-contaminated finger or disk generally resulted in a recovery of 80-95% of the input infectious virus. Although it is recognized that the efficiency of elution may vary inversely with the length of time the virus or the bacterium is on the contaminated surface, this would be confounded with the inherent loss of infectivity and would not be possible to

determine without the help of a suitable microorganism-incorporated radio-active tracer. Whereas this would be feasible in experiments on inanimate surfaces, the use of such labelled microorganisms on human hands would be restricted. In an earlier study in this laboratory (Sattar et al., 1986), 90-100% of radioactively-labelled rotavirus could be eluted from metal disks using TPB.

The washing and ethanol rinsing of hands before the deposition of the virus on fingerpads were considered necessary to minimize the influence of accumulated materials, such as natural oils or emollients, on virus and bacterial survival. It also served to eliminate the possibility of any background levels of virus or bacteria on the hands of the volunteers.

2. Survival of viruses and bacteria on hands

In general terms, the potential of a vehicle to spread a given infectious agent is directly related to the capacity of the agent to survive in or on that vehicle. Hands have long been implicated in the spread of infectious diseases, and are often suggested as the most important vehicle (Hendley and Gwaltney, 1988; Adler, 1986; Hutto et al., 1986; Black et al., 1981; Modlin, 1988). In spite of this, their role in the spread of enteric and respiratory viral diseases remains unclear, and there is relatively little information on the capacity of enteric and respiratory viruses to survive on hands. Most of the evidence in this regard comes from work with rhinoviruses (Hendley et al., 1973; Gwaltney et al., 1978; Reed, 1975) and respiratory syncytial virus (Hall et al., 1980; 1981; Hall and Douglas; 1981b).

a) **Rotavirus:** Before the present study, no information was available on rotavirus survival on human hands. The results of this study show that these viruses can retain their infectivity on human skin for several hours. For the survival experiments, the first sampling time of 20 minutes was selected because this was the minimum period required for the inocula to become visibly dry. The choice of the last sampling time, 4 hours post-drying of inocula, was based on the expected maximum period between hand-washing for institutional staff and those in their care. During this period care-givers would most likely make direct contact with several susceptible individuals in institutional settings. There were also scheduling difficulties for the volunteers if the experiments were to be conducted for periods longer than 4.5 hours.

Although the experiments to study rotavirus survival on hands were conducted under ambient conditions (approximately $22\pm 2^{\circ}\text{C}$ and $45\pm 5\%$ RH), the exact temperature and moisture level of the skin could not be determined. In view of this, it is difficult to make a direct comparison between human rotavirus inactivation on the skin surface and that previously observed under defined conditions of temperature and RH for the same virus dried onto inanimate surfaces (Sattar et al., 1986).

A 10% suspension of feces was used to suspend the virus in these experiments because the large amounts of particulate matter and the thicker consistency made more concentrated stool samples harder to work with. The 10-uL inoculum used here was large enough to be measured with reasonable accuracy, and yet it was considered small enough

to represent a realistic amount of contamination that could be acquired during contact from fecally-contaminated material.

Cases of rotaviral diarrhea have been found to excrete up to 10^{11} virus particles per gram of feces (Flewett and Woode, 1978), and it has been demonstrated that at least 1 of every 4×10^4 of these particles is infectious for cell culture (Ward et al., 1984). In this study, the level of infectious rotavirus in the 10-uL inoculum was, therefore, within the range one might encounter in field situations. It has also been shown that the minimum infective dose for animal (Graham et al., 1987) as well as human (Ward et al., 1986) rotaviruses may be as low as 1 cell culture infective unit. Even though less than 10% of the input infectious virus remained detectable on hands after 260 min, this remaining fraction still represented between 130 and 1700 PFU.

The survival of HRV was consistently poorer on the hands of certain volunteers. This may be due to differences in the biochemistry, physiology, and topography of the skin from one individual to another. Whereas this requires further investigation, we noted that, one volunteer who tended to perspire excessively gave more variable results for virus survival.

b) Rhinovirus and Parainfluenzavirus: Rhinoviruses are ubiquitous agents and are among the most common viral respiratory pathogens of man. They are capable of causing upper respiratory tract infections at all ages. The exact mechanisms of transmission of these viruses still remain controversial. Although survival of rhinoviruses on human hands has been studied previously, we regard certain aspects of

the experimental design to be flawed. For example, in one study (Hendley et al., 1973), rhinovirus type 39 was suspended in either Hanks' balanced salt solution or normal saline and placed on the palm or the back surface of the hand; the deposited virus was recovered by simple washing of the contaminated area with a broth and no attempt was made to quantitate the infectious virus recovered 1 and 3 hours after deposition. In testing the survival capacity of respiratory viruses, use of balanced salt solution or normal saline as a virus suspending medium may be inappropriate due to the absence of an organic load.

The minimal infective dose for rhinoviruses through the nasal route has been found to be less than 1 TCID₅₀ (Couch, 1990); humans can also be readily infected by rhinoviruses through the conjunctival route (Bynoe et al., 1961; Winther et al., 1986). The findings of this study show that on average 13,500 PFU of RV-14 could be recovered from human hands after 3 hours. This suggests that RV-14 contaminated hands may have the potential to spread infectious virus particles for several hours.

The capacity of parainfluenzaviruses to survive on human hands was unknown before this study. The results of our quantitative tests using the fingerpad protocol clearly show that HPIV-3 could not persist on human skin as well as RV-14 did (Fig. 15) under ambient conditions on the hands of the same set of volunteers. The reduction in the infectivity titre of HPIV-3 was apparent well before the inoculum became dry on the contaminated fingerpads; within 10 minutes of deposition on fingerpads, >90% of HPIV-3 infectivity was lost. This suggests that if

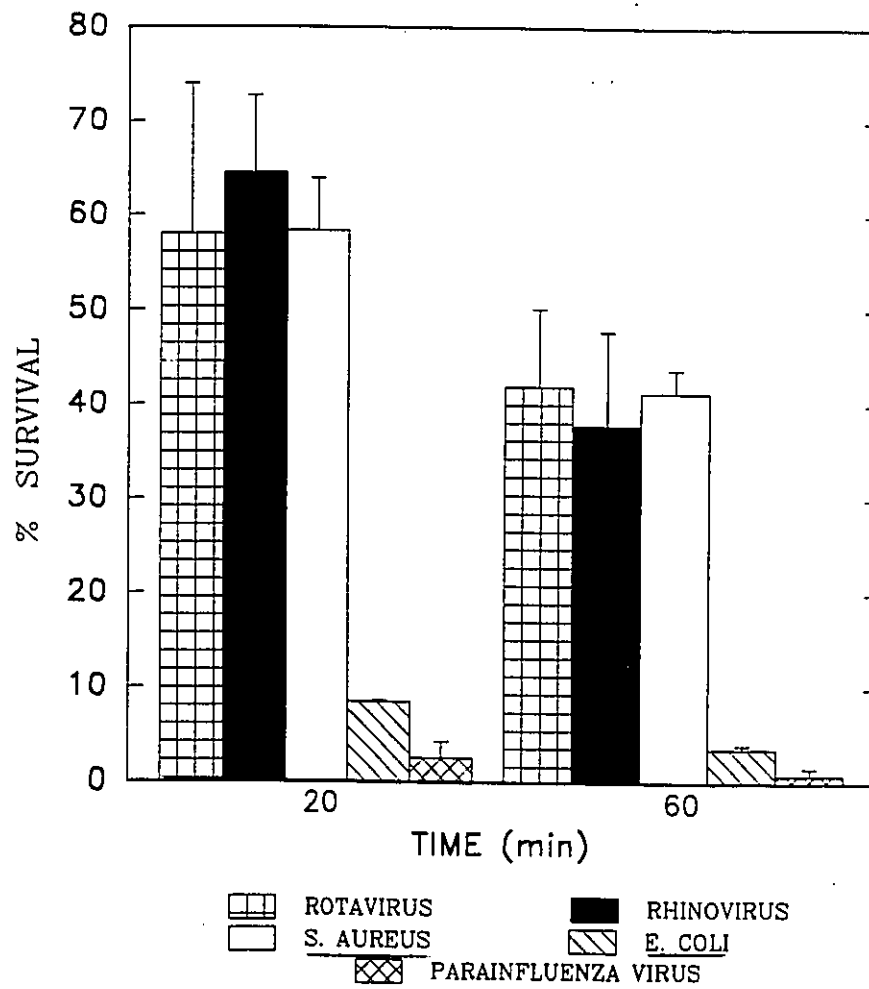
spread of parainfluenzaviruses by hands is to occur, it may only be within the first few minutes after their contamination with the infectious material. Limited testing with parainfluenzaviruses shows their minimal infective dose for humans by the intranasal route to be about 80 TCID₅₀ (Smith et al., 1966). In view of this, hands would appear to play a limited role as vehicles for parainfluenzaviruses. The reasons for the rapid loss of HPIV-3 on fingerpads are not known. As an enveloped virus, it may be subject to rapid inactivation by fatty acids and lipases on the skin.

In contrast to survival on hands, HPIV-3 was found to survive better on non-porous environmental surfaces (Table 9). One hour after deposition on the disks >12% infectious HPIV-3 was detectable as compared to 0.8% on human hands. It is, therefore, possible that environmental surfaces, after their contamination with respiratory secretions may serve as potential sources for HPIV-3 spread.

c) ***E. coli* and *S. aureus***: *E. coli* was used as a representative of Gram-negative fecal bacteria. It is also an important nosocomial pathogen (Paulozzi et al., 1986; Salzman et al., 1967). *S. aureus* was chosen because it is not only an important nosocomial pathogen, but it is a Gram-positive bacterium normally found on human skin.

S. aureus survived better than *E. coli* on the hands of the volunteers. Only $8.46 \pm 3.15\%$ and $3.52 \pm 0.60\%$ of the input *E. coli* CFU remained viable after 20 and 60 min, respectively. Whereas, for the same time periods, the recovery of *S. aureus* CFU was $58.4 \pm 2.2\%$ and $41.3 \pm 5.9\%$, respectively. *S. aureus* survival on hands was found to be remarkably similar to that of the rotavirus (Fig. 15).

Figure 15. Survival of viruses and bacteria on fingerpads.



There are only a few published reports where survival of various Gram-positive and Gram-negative bacteria was studied on hands (Ayliffe et al., 1978; 1988; Casewell and Desai, 1983; Gontijo Filho et al., 1985; Paulo et al., 1985; Pether and Gilbert, 1971). Whereas these studies also indicate poor survival of *E. coli* and better survival of *S. aureus*, we consider the suspending media (distilled water or broth) and the methods of skin sampling used in these earlier studies to be inappropriate. To our knowledge, our study is the first one where fecally-suspended *E. coli* was used to determine its survival on hands. Poor survival of *E. coli* on hands and its high minimal infective dose (DuPont et al., 1971) suggest that the spread of these organisms through hands may be infrequent.

In this study, *S. aureus* was suspended in bovine mucin because this organism is frequently found in the anterior nares (Williams, 1963). It is important to note that in most of the previous studies *S. aureus* was suspended in saline or in distilled water (Lilly and Lowbury, 1978) or in culture broth (Ayliffe et al., 1978; 1990).

The average numbers of bacteria used to contaminate each fingerpad were 6.5×10^6 and 4.6×10^6 CFU for *E. coli* and *S. aureus*, respectively. These figures are considered quite realistic because up to 10^7 viable cells of *S. aureus* and Gram-negative bacilli have been recovered from the hands of nurses (Ayliffe et al., 1978). Findings of this study provide strong evidence that *S. aureus* is more stable on hands than *E. coli* and reinforce the potential role of hands at least for the transmission of *S. aureus*.

3. Virus survival on inanimate surfaces

a) Rhinovirus and Parainfluenzavirus: There are conflicting reports on the survival of parainfluenzaviruses on inanimate surfaces (Brady et al., 1990; Parkinson et al., 1983). Our finding that HPIV-3 survives poorly on hands stimulated the performance of a series of experiments to test the survival of this virus on stainless steel disks. The results (Table 9) indicate that HPIV-3 survives relatively longer on these disks than on hands.

A recent study (Brady et al., 1990) has shown that infectious parainfluenzaviruses can be recovered for up to 2 hours from stainless steel and plastic surfaces. A significantly longer survival of parainfluenzaviruses was reported by Parkinson et al. (1983). They sprayed suspensions of parainfluenzaviruses on plastic Petri plates, and these plates were then kept indoors (21.4°C and 3% RH) or outdoors (-22.4 to -33.2°C at 100% RH). Parainfluenzaviruses type 1, 2, and 3 survived for 4, 7, and 12 days indoors and 7, 12, and 17 days in the outside environment. A direct comparison of these results with our findings cannot be made because of the disparity in experimental design. But Parkinson's study indicates better survival of parainfluenzaviruses at lower temperature and high RH. During our study, the indoor RH was in the range of 50-60% and the room temperature was fairly constant at 22°C. The study conducted by Brady et al. (1990) was under ambient temperature, but the RH level was not reported.

Brady et al. (1990) consider drying as the cause for the reduction in parainfluenzavirus infectivity. Findings of our 10-minute survival

experiments suggest that drying may not be a critical factor since, within 4 minutes of deposition on fingerpads, when the deposited drop of virus suspension was still wet, >55% of the inoculated virus infectivity became undetectable. We may speculate that the virus is more sensitive to the atmospheric temperature, which might explain why in the South Polar environment at -22.4 to -33.2°C, HPIV-3 survived for 17 days (Parkinson et al., 1983). A relatively faster reduction on hands can be explained by the fact that the temperature of human hands is higher than the temperature of objects in indoor settings and secondly, the skin of hands secretes factors which may accelerate the inactivation process of these viruses.

A large proportion of the particles generated during sneezing and coughing is greater than 15 μm in diameter. Such particles tend to settle out of air rapidly and thus for direct virus transmission to occur, impaction of these particles on the nasopharynx of persons in the immediate vicinity of an acutely infected case may be necessary (Couch et al., 1966). Droplet nuclei containing viruses may remain suspended in air for extended periods. However, the capacity of human parainfluenzaviruses to survive in air is largely unknown. Miller and Artenstein, (1967) have shown that the biological decay rate of HPIV-3 in artificially-generated aerosols at 50% and 80% RH was 3.8% and 3.1% per minute, respectively, whereas at 20% RH, the decay rate was reduced to 0.53% per minute.

In the light of our present findings and previous published reports it can be speculated that the transmission of human parainfluen-

zaviruses occurs mainly by direct exposure to large airborne particles. Limited spread of these viruses through hands may be possible within few minutes of contamination; inanimate environmental surfaces appear to have better potential to spread parainfluenzaviruses.

4. Virus transfer from contaminated surfaces to clean surfaces

a) Rotavirus: The capacity of rotaviruses to survive well on hands prompted us to investigate their transfer from experimentally-contaminated to clean surfaces through contact. Results of these experiments clearly indicate that transfer of infectious rotavirus can occur readily between hands as well as between hands and nonporous inanimate surfaces.

Many types of porous and nonporous inanimate surfaces can act as rotavirus donors or target surfaces in institutional settings. However, earlier studies have shown that rotaviruses are more readily recovered from experimentally-contaminated nonporous inanimate surfaces and that the extent of virus survival is virtually the same on plastic, glass, and stainless steel (Sattar et al., 1986). In view of this, stainless steel disks were chosen here to represent nonporous inanimate surfaces.

In the transfer experiments, only one level of pressure was tested, and the contact between the surfaces was made without any friction or rubbing motion. We estimate that the pressure used here is generally what is applied in many routine functions such as turning doorknobs and shaking hands. Further testing would be required to determine if and how different levels of pressure and friction might influence virus transfer.

In all three models, rotavirus transfer after 20 minutes was higher than that at 60 minutes. This suggests that, although the inocula were visibly dry after 20 min, drying was incomplete. This is in agreement with previous observations with rhinoviruses (Reed, 1975) where virus transfer was greatest when the inoculum was still damp. At 20 min, the lowest percentage of transfer was observed in finger-to-finger contact. If chemicals on the skin are detrimental to rotavirus survival, then the virus transferred from finger-to-finger may be exposed to a second chemical challenge which would reduce the infectious virus recovered. The amount of infectious virus that could be transferred from a contaminated to clean surface even after 60 min drying was at least 3.0×10^2 PFU, which is considerably higher than the estimated minimal infective dose for human rotaviruses (Ward et al., 1986).

The results of this study strongly suggest that hands may play a vehicular role in rotavirus transmission. Since rotaviruses may remain infectious on inanimate surfaces for prolonged periods (Sattar et al., 1986), the roles of fomites and hands in their transmission may be complementary or synergistic. In institutions housing infants and young children the numbers of hand-to-hand, hand-to-object and object-to-hand contacts are inevitably high. Children frequently suck their fingers and put toys and other objects into their mouths; in one study, these activities were noted as often as every 2 to 3 minutes (Black et al., 1981). Such frequency of contacts may help to explain the high incidence of rotavirus infection and disease in susceptible populations of infants and young children.

b) **Rhinovirus and Parainfluenzavirus:** Repeated experiments failed to detect any HPIV-3 transfer from finger-to-disk or finger-to-finger 20 minutes after contamination. Transfer of HPIV-3 from disk-to-finger was demonstrated, indicating that the virus survived better on inanimate surfaces and such contaminated surfaces may play a role in dissemination of parainfluenzaviruses in institutional settings.

The transfer of HPIV-3 and RV-14 from disks to clean fingers supports a role for fomites in the contamination of hands with both viruses. The numbers of PFU transferred for HPIV-3 and RV-14, respectively, were about 200 and 170. This represents just over 3 minimal infective doses for HPIV-3 (Smith et al., 1966) and several doses for RV-14 (Couch, 1990). With a rapid loss of HPIV-3 infectivity on the skin, the chances of directly infecting susceptible hosts through hands are limited. Since no finger-to-finger or finger-to-disk transfer of the virus could be demonstrated when the inoculum was allowed to dry for 20 minutes, indirect passage of the virus from hands is even less likely to result in disease spread. In contrast to this, the small minimal infective dose for rhinoviruses, and their ability to remain viable on human skin for longer periods suggest that hands may play an important role in their spread.

In the experiments by Reed (1975), either phosphate buffered saline or tissue culture fluid was used for suspending the test rhinovirus and the contaminated surfaces were sampled for infectious virus 2-10 min after their inoculation. We believe that the differences in sampling time may have been responsible for the wide variations in the resulting rhinovirus survival and recovery from environmental surfaces and

fomites. We tested the transfer of rhinovirus from experimentally-contaminated to clean surfaces only after 20 min of drying, and in each experiment a uniform pressure was applied for 5 sec.

The issue of the principal route of rhinovirus transmission is still unresolved. Are rhinoviruses transmitted mainly through direct contact or through air? One view is that these viruses spread mainly through direct hand-to-hand or fomes-to-hand contact followed by self-inoculation (Hendley and Gwaltney, 1988); the other view is that rhinovirus transmission occurs chiefly by the aerosol route (Dick et al., 1987; Jennings et al., 1988). Earlier, Pancic et al. (1980) have demonstrated the recovery of 3 to 1,800 rhinoviruses PFU from recipient fingertips following contact with contaminated doorknobs or faucets touched earlier by a donor.

The results of the present study further confirm the fact that rhinoviruses not only survive for several hours on non-porous inanimate surfaces and on human hands but that transfer of virus can occur on contact with a contaminated surface. This suggests that hands and environmental surfaces have the potential to act as vehicles for rhinoviruses. To establish the role of air in rhinovirus transmission, isolation of the virus from the surrounding air is imperative, and this can be attempted with available technology (Park, 1980). Karim et al. (1985) have shown that in the air-borne state, RV-14 can survive for several hours only at RH levels higher than 80%; when the RH was low, virus became undetectable within 15 minutes of aerosolization. This indicates that, under conditions of high RH, rhinoviruses may remain viable in air

long enough to be inhaled in sufficient quantities by susceptible individuals.

Proper and regular hand-washing as well as disinfection of hard environmental surfaces may be crucial in minimizing the spread of rhinoviruses, particularly in institutional settings. Our earlier studies (Sattar et al., 1989) have shown that HPIV-3-contaminated hard non-porous surfaces are relatively easy to disinfect with a variety of commercial and non-commercial formulations. However, no information is available on the *in vivo* efficacy of antiseptics in dealing with HPIV-3-contaminated hands. Rhinoviruses have also been found to be fairly susceptible to chemical disinfectants in studies on inanimate surfaces and on hands (Carter et al., 1980; Dick et al., 1986; Hayden et al., 1984; 1985). We have also shown (Sattar et al., unpublished data) that a medicated liquid soap could reduce the infectivity of RV-14 by more than 99.0% on experimentally-contaminated fingerpads after a contact time of 10 sec.

5. Antisepsis

Hand-washing is regarded as the single most important procedure for preventing the transmission of infections (Black et al., 1981; Reybrouck, 1986; Steer and Malison, 1975). However, the desired impact of hand-washing depends not only on the regularity and thoroughness of the procedures used but also on the type of hand-washing agent selected. The continued occurrence of disease outbreaks, such as rotaviral gastroenteritis (Allen and Ford-Jones, 1990; Di Matteo et al., 1989; Guerrant et al., 1990; Hjelt et al., 1985; Maderova et al., 1987;

Pacini et al., 1987; Raad et al., 1990;), is believed to be due the poor compliance with hand-washing guidelines (Albert and Condie, 1981; Donowitz, 1987; Quraishi et al., 1984). But, a high degree of compliance alone may not be sufficient for proper infection control if the hand-washing agent is not effective in the removal or inactivation of important nosocomial pathogens. The findings of this study indicate that some commonly used hand-washing agents may be inadequate to deal effectively with viruses which are known to cause disease outbreaks in institutions.

In a field situation, antiseptics should be suitable for the inactivation of both viral and bacterial pathogens on contaminated skin. This may be particularly true when the minimal infective dose for some viral agents is extremely low. Furthermore, some viruses may also survive on human skin more readily than many types of bacteria.

Use of the whole hand for regular testing of antiseptics against viruses is not considered feasible. Apart from the relatively large volumes of a high-titered virus pool required for each experiment, determination of the recoverable titre of the organism at the end of 20 min after contamination and treatment of hands with the test product must be conducted at different times. For viruses, a detoxification (disinfectant removal) step is also required to protect the cell monolayer from cytotoxic effects of the residual disinfectant. Moreover, recovery of inoculated organisms from the whole hand is generally lower and more variable than from fingerpads. These factors make the whole-hand protocol inherently difficult to control.

On the other hand, the fingerpad method used in our studies represents a simpler and better way of testing antiseptics against viruses as well as bacteria. It not only permits the use of microlitre quantities of the infectious agent, but also allows the inclusion of appropriate controls and sufficient numbers of replicates in the same test. The results obtained with this protocol were reproducible and did not show any significant person-to-person variation; they were also in agreement with the findings based on the whole-hand technique. The suitability of using fingers as compared to whole hands in antiseptics experiments has also been reported by others (Schurmann and Eggers, 1983).

The results of this study are based on a relatively small number of volunteers. Similar experiments with a minimum of 10 volunteers may be necessary to test for person-to-person variation and also to convincingly demonstrate the potential of this technique as a standard *in vivo* protocol for testing the germicidal efficacy of hygienic hand-washing agents.

So far, much of the *in vivo* evaluation of handwashing agents has been conducted with bacteria; there are few published reports where they have been tested against viruses on either naturally- or experimentally-contaminated hands (Cliver and Kostenbader, 1984; Eggers, 1990; Hayden et al., 1984; Hendley et al., 1978; Schurmann and Eggers, 1983; Schurmann and Eggers, 1985). Furthermore, there is no standard *in vivo* protocol to test the virus- or bacteria-eliminating properties of hand-washing agents.

In commonly used suspension and carrier testing of germicides, a disinfectant is required to reduce the infectious titer of the test organism

by at least 99.9% to be considered effective. There is no such generally recognized criterion of efficacy for the *in vivo* testing of antiseptics for viruses. In order to establish a universally acceptable criterion of efficacy for antiseptics tested by *in vivo* protocols, it will be necessary to test a wide variety of formulations and several representative viruses. Carter et al., (1980) and Schurmann and Eggers (1983) reported that *in vitro* testing of antiseptics showed them to be more effective than they were in *in vivo* tests. These findings further reinforce the need for *in vivo* testing of antiseptics.

In reviewing critically the available published information on testing hand-washing agents, we found either no mention of the suspending medium or inappropriate media were used for suspending the test organism. In testing the efficacy of handwashing agents, the nature of the suspending medium is as crucial as noted earlier for survival studies. For example, rotavirus particles in feces are often found either as large aggregates or as clumps embedded in tissue fragments (Holmes, 1979). This may provide the virus considerable protection against the action of chemical disinfectants. It has also been suggested that the accumulation of organic matter on the skin surface during the course of a work-day may decrease the effectiveness of hand-washing agents (Lilly and Lowbury, 1978; Lowbury and Lilly, 1974).

Although surveys on health-care personnel have shown that the mean duration for total handwashing was about 8 seconds (Quraishi et al., 1984), we used the 10 second exposure of the contaminated area to the agent under test as recommended in the handwashing guidelines

from the U.S. Centers for Disease Control (Garner and Favero, 1985). This relatively short exposure time is also considered to be more suitable for testing antiseptics used for hygienic hand disinfection (Ayliffe et al., 1988; Quraishi et al., 1984; Reybrouck, 1986).

Rinsing of hands may be an important step in hand antisepsis; tap water is normally used in the field and we followed this practice in the testing protocol. Although it is acknowledged that the composition of tap water may vary geographically and temporally, neither distilled nor a standard hard water is believed to be more predictive than tap water of the results which would be obtained under natural conditions. Moreover, the organic or inorganic content of tap water could affect the elution of the test organism from the fingers.

It can be argued that the chlorine residual in tap water could play a role in the inactivation of pathogens on contaminated hands. We do not believe this to have been an important factor in this study as the chlorine residue in our tap water was low (0.05-0.2 ppm). Previous work in our laboratory has shown that rotaviruses are relatively resistant to chlorine in tap water (Raphael et al., 1987). Furthermore, the feces used for suspending the test organisms may have interfered with the action of such small amounts of chlorine by neutralization of the chlorine or protection of the test organisms, or both. However, inactivation by tap water could occur for some other viruses (Cliver and Kostenbader, 1984; Raphael et al., 1987) and bacteria. Therefore, we suggest that dechlorinated tap water could be a suitable rinse water for routine *in vivo* testing of antiseptics, but this needs further investigation.

The selection of a human rotavirus for this part of the study was based on several reasons. These viruses are not only among the major causes of acute gastroenteritis, but they are frequently implicated in outbreaks of the disease, even where chemical disinfection and antiseptics are regularly used for infection control (Pacini et al., 1987). They are also among the very few human pathogenic viruses whose minimal infective dose, based on human volunteer studies (Ward et al., 1986), has been shown to be one cell culture infective unit. They are capable of surviving on human hands for several hours and on non-porous inanimate surfaces for several days (Sattar et al., 1986). Infectious rotaviruses can be transferred readily between hands and inanimate objects, and both viable viruses (Keswick et al., 1983a) and their antigens (Samadi et al., 1983) have been demonstrated on the hands of care-givers. Considerable evidence is already available on the relative resistance of rotaviruses to chemical disinfectants (Lloyd-Evans et al., 1986; Springthorpe et al., 1986).

E. coli was chosen as representative of Gram-negative bacteria and some previous studies on antiseptics have used strains of this organism as a model (Ayliffe et al., 1988; Rotter, 1988).

The selection of *S. aureus* was based on the fact that it is the single most common agent involved in nosocomial infection and also regarded as the representative Gram-positive organism for testing of antiseptics and hard surface disinfectants (Ayliffe et al., 1988; Onesko and Weinke, 1987).

In this study, we used more than 100,000 CFU of *S. aureus* to contaminate the hand which is a realistic amount. Ayliffe et al. (1978) reported the recovery of 10^6 to 10^7 *S. aureus* and Gram-negative bacilli from the hands of nurses and Casewell et al. (1988) report contamination levels of 10^3 per finger from nurses in an intensive care unit.

The *S. aureus* used here was a methicillin- and trimethoprim-sensitive strain because of the possible hazards associated in handling methicillin-resistant *S. aureus* (MRSA). But, can the strain used be considered representative of *S. aureus* commonly involved in nosocomial outbreaks? Studies published in the past five years indicate that MRSA may be more resistant to certain types of antiseptics (Russell et al., 1986). Townsend et al. (1984) and Tennent et al. (1985) have also reported that the most common gentamicin-resistant plasmid of *S. aureus* codes for resistance to propamidine, quaternary ammonium compounds and ethidium bromide as well and suggested that the emergence of antibiotic-resistant strains was perhaps due to the extensive use of cationic disinfectants in the hospital environment.

Brumfitt et al. (1985) reported that MRSA strains were approximately four times as resistant to chlorhexidine, nine times as resistant to propamidine and three times as resistant to cetrimide as methicillin-sensitive strains. Topical treatment with chlorhexidine (body wash plus nasal cream) was unsuccessful in eliminating MRSA from persistent carriers. This aspect needs further investigation.

The sensitivity of the two test bacteria to most of the agents tested was more or less the same, except for tap water. Initially, when tap water

washing efficiency was tested on three volunteers hands, the overall percent reduction of *S. aureus* was significantly higher ($p = 0.0001$) compared to that for *E. coli*. Further work is needed to elucidate the reasons for these observed differences.

Alcohols, and formulations containing 70% alcohol, were the most effective in reducing rotaviral and bacterial contamination of hands. This is in agreement with findings of other investigators (Ayliffe et al., 1988; Rotter, 1988), and in Germany 60% isopropanol is used as an index for comparing the *in vivo* bactericidal efficacy of antiseptics (Rotter, 1988). Much of the loss in the infectivity of the virus and the bacterium on the skin surface, due to these antiseptics, may be the result of *in situ* inactivation. In the case of the other agents tested, it may have been predominantly due to wash-off, as discussed below. Furthermore, addition of emollients to alcohols or alcoholic solutions of chlorhexidine salts makes them less astringent and more widely acceptable (Larson, 1988). Although both Hibisol and Savlon contain 70% alcohol, at the recommended use dilution, Hibisol contains a higher concentration of chlorhexidine gluconate. In spite of this, Savlon performed better under the test conditions used. This may have been due to the presence of cetrimide as an additional active ingredient.

Dettol was chosen as a product containing p-chloro-m-xyleneol (PCMX). The concentration of PCMX in the undiluted product tested was higher than is usually found in PCMX-based handwashing agents (Larson, 1988). Like Dettol, Proviiodine is an antiseptic not routinely used for hygienic hand disinfection. However, it was selected because previous

studies of rotavirus disinfection (Springthorpe et al., 1986) showed it to be the most effective of the iodine-based formulations tested. Even though these products were found to be more effective than soap and aqueous chlorhexidine salts, the level of rotavirus removal by the more dilute solutions of PCMX or iodine-based products, formulated for hygienic handwashing, may not be as high.

Aqueous solutions of chlorhexidine gluconate (Savlon and Cida-Stat) are commonly used for handwashing; the results of this study clearly demonstrate that they may not be reliable antiseptics for pathogens such as rotaviruses. In fact, the virus removal by tap water alone was comparable to that achieved by Savlon in water. This suggests that the reduction in titre by this product may be due primarily to wash-off. Similar results for the action of aqueous chlorhexidine salts have been obtained in *in vitro* studies of rotavirus disinfection (Lloyd-Evans et al., 1986; Springthorpe et al., 1986).

Besides alcohols, the chlorhexidine formulations were significantly better than soap in removing *S. aureus* and *E. coli*. This result confirms other studies on the efficacy of this agent against *S. aureus* (Ayliffe et al., 1978; Ojajarvi, 1976; 1980; Lilly and Lowbury, 1978). However, removal of *E. coli* by soap in our protocol was not as effective as reported by others (Ayliffe et al., 1978; Ojajarvi, 1980; Nicoletti et al., 1990). This discrepancy in results may be due to differences in the test protocols. Chlorhexidine preparations were found to be superior to soap and water in reducing *Klebsiella aerogenes* from contaminated hands (Casewell et al., 1988).

The routine use of plain soap and water is considered by many to be quite adequate for the decontamination of hands in infection control. This is not borne out by the results reported here. Washing contaminated hands with almost any agent is clearly desirable, but it should be noted that use of a less effective product could actually lead to the spread of localized contamination over the entire surface of both hands (Schurmann and Eggers, 1985). Our finding that washing the rotavirus-contaminated left hand with liquid soap spread the virus onto the right hand during washing (Table 20), suggests that washing of rotavirus-contaminated hands with soap may be useless and gives a false sense of protection.

Regular and proper washing of hands by health-care personnel may be inadequate if ineffective products are relied upon. Therefore, selection of an antiseptic with demonstrated broad-spectrum efficacy is considered essential in conjunction with efforts to increase compliance with good handwashing practice. Proper testing of handwashing agents, against both viruses and bacteria, by an *in vivo* protocol is clearly desirable, and the fingerpad protocol presented here may be a suitable model for such standard tests.

6. Drying of washed hands

As has been mentioned earlier, there is a serious lack of published information on the *in vivo* efficacy of commonly used hand-washing agents. Even less is known about the role and relative efficiency of various methods of hand drying in the elimination of contamination. The

few reports that have been published relate exclusively to bacteria (Davis et al., 1969, Matthews and Newsom, 1987; Walker, 1953).

Blackmore (1987) reviewed the merits and weaknesses of drying washed hands with paper towels, cloth towels and electric air dryers and concluded that electric hand dryers could not be recommended for use in clinical areas because such dryers were relatively slow and noisy and their hygienic efficiency questionable. However, the results of this study clearly show that electric air blow dryers are superior to the other two drying methods for the elimination of bacteria as well as viruses after hygienic hand-washing. Walker (1953) also found electric air drying of hands to be better, and more cost-effective, than use of sterile cloth towels following preoperative scrubbing.

The main focus of this part of the study was to compare the efficacy of the three methods of hand drying in eliminating bacterial and viral contamination after hygienic hand-washing.

The use of 70% isopropanol could reduce the transient microflora to undetectable levels. In such cases, any one of the three methods of hand drying could be used. In contrast to this, the extent of reduction of residual contamination on washed hands during drying is more critical when less effective hand-washing agents are used. This is exemplified by the data on the unmedicated liquid soap (Fig. 14).

In these experiments, washed finger-pads were dried for 10 seconds. In the field, however, proper drying of both hands requires 20 to 30 seconds because of the much greater surface area. In the field, hands are normally rubbed against each other during warm air drying and

friction is often applied when they are dried with paper or cloth towel. We did not incorporate any friction in the hand drying step of our protocol because of the difficulties in standardizing and accurately representing field conditions. Whether friction contributes to a further reduction in the level of contamination during drying remains to be determined.

The exact reason(s) for the higher efficiency of the warm air dryer in reducing the bacterial and viral contamination are not understood at this stage. The blowing of warm air may lead to an accelerated dehydration of the skin surface, thereby irreversibly affecting the viability of the organisms tested. Furthermore, warm air may penetrate all the crevices inherent to the skin whereas absorbent towels may not reach such areas even though the skin appears dry.

'No-touch' dryers are desirable over the ones that require the pushing of a button or bar to initiate the drying cycle. This is particularly important in health-care institutions and food establishments where contact with such surfaces can recontaminate properly washed hands. Different brands of paper or cloth towels may have very different absorption characteristics, which could influence their capacity to remove contaminating microorganisms from washed hands. Such inherent differences in the paper and cloth towels used by us and other investigators (Davis et al., 1969; Blackmore, 1987) may account for the variations in the results obtained.

Surrounding air is recirculated by electric air dryers and it has been suggested that this may result in the dispersion of infective aerosols already present in the atmosphere, as well as those generated by hands

being rubbed together for drying (Walker, 1953). Matthews and Newsom (1987) compared bacterial aerosol generation during hand drying by paper towels and electric air dryers and concluded that electric air-drying was safe from a bacteriological point of view. Since viruses and bacteria behave very differently in the airborne state (Sattar and Ijaz, 1987), further studies may be necessary to determine if the use of electric air dryers increases the generation and dispersal of infectious viral aerosols.

The increasing popularity of alcohol-based waterless hand washing agents (Larson et al., 1986) or alcohols containing emollients (Nystrom, 1987) may simplify the handwashing process, and avoid the need to dry "washed" hands. However, handwashing agents requiring a water rinse and drying will continue to be used widely. The results reported here suggest that the method used for drying of washed hands may also play an important role in the overall reduction of transient microflora and should be considered in conjunction with both compliance with handwashing and the choice of the washing agent.

CONCLUDING REMARKS

This study was aimed at elucidating the role of hands in the spread of infectious diseases and was able to achieve its initial objectives. The protocols developed during this investigations were successfully applied to determine how well selected human pathogenic viruses and bacteria could survive on the hands of human volunteers and to compare the efficacy of hand-washing and hand-drying agents in the elimination of representative viruses and bacteria from experimentally-contaminated hands. The major conclusions from this study are as follows:

1) The ability of HRV and RV-14 to survive on human hands was similar and both of these viruses remained viable for at least three hours. In contrast to this, the infectivity of HPIV-3 became virtually undetectable within a few minutes of its deposition on the hands. This suggests that rotaviruses and rhinoviruses have a greater potential than parainfluenzaviruses for spread by means of contaminated hands.

2) The capacity of *S. aureus* to survive on human hands was similar to that of HRV and RV-14, whereas *E. coli* behaved much like HPIV-3 in this regard.

3) The extent of survival of a given virus or bacterium on hands did not differ noticeably from volunteer to volunteer. Even though the number of volunteers included in this study was small, the results indicate that individual differences in the physiology, biochemistry and topography of the skin do not have any apparent influence on the infectivity of the microorganisms tested.

4) RV-14 survived on stainless steel disks as well as it did on human hands. HPIV-3 survived somewhat better on the disks when compared to its survival on hands.

5) Contact between contaminated and clean fingers could result in the transfer of infectious HRV or RV-14; transfer of these viruses could also occur between fingers and metal disks. This suggests that hands and environmental surfaces may play a complementary or synergistic role in the spread of rota- and rhinoviruses.

6) The results of tests on hygienic hand-wash agents against HRV and *E. coli* using the fingerpad protocol developed in this study were predictive of those obtained using the conventional whole-hand method.

7) Several commonly used hygienic hand-wash agents were found to be relatively ineffective in the elimination of HRV from experimentally-contaminated fingerpads. Some of these agents proved to be much more effective against *E. coli* and *S. aureus*. This clearly points to the need for *in vivo* testing of hygienic hand-wash agents using representative viruses.

8) Washing hands with ordinary liquid soap and water was found to spread infectious HRV, placed on one hand, over a wider area of both hands. Therefore, the use of ineffective hand-wash agents, especially in health-care settings and daycare centres, may increase the risk of spreading pathogens, such as rotaviruses, through hands.

9) Drying of washed fingerpads by paper towel always resulted in a further reduction in the residual viral and bacterial contamination. This attested to the importance of the drying step in hygienic hand-washing. Further work in this regard showed electric warm air-drying to be superior to paper and cloth towels in the reduction of the residual contamination.

The findings summarized above raise many new questions and suggest several possible directions for future research. At the same time, the protocols developed and applied in this study show considerable

promise for use in many areas of basic and applied research as well as in the routine testing of hygienic hand-wash agents against viruses and bacteria.

Is the poor survival of HPIV-3 on human hands typical of enveloped viruses? Similarly, can the behavior of *E. coli* be considered representative of Gram-negative bacteria? Further testing with representative bacteria and viruses, which are safe for inoculation of the human skin, will be necessary to answer these questions.

The strain of *S. aureus* selected for this study was not antibiotic-resistant in view of its safety for the volunteers. Will the sensitivity of known antibiotic-resistant strains of this bacterium be the same to hygienic hand-wash agents as that seen with the strain used here? Do antibiotic-resistant strains of *S. aureus* survive better on human skin?

The survival of viruses on nonporous inanimate surfaces is influenced by RH and air temperature. Do these factors affect virus survival on human skin as well? Investigations of this nature would be very helpful in clarifying the reasons for the seasonality of outbreaks of viral infections.

What is the basis for the loss of viral and bacterial infectivity on human skin? Is the loss reversible? The dangers associated with the placement of radio-labelled microorganisms on human skin make such investigations difficult to conduct.

Hands are recognized as an important vehicle in the spread of hepatitis A virus. It would, therefore, be very useful to apply the protocols developed here to study the survival of this virus on human hands and assess the efficacy of hand-washing agents against it.

REFERENCES

REFERENCES

- Adler S. P. 1988. Cytomegalovirus transmission among children in day care, their mothers and caretakers. *Pediatr. Infect. Dis. J.* 7: 279-285.
- Adler S. P. 1986. Molecular epidemiology of cytomegalovirus: Evidence for viral transmission to parents from children infected at a day care center. *Pediatr. Infect. Dis.* 5: 315-318.
- Albert R. K. and F. Condie. 1981. Handwashing patterns in medical intensive care units. *N. Engl. J. Med.* 304: 1465-1466.
- Allen U. and E. L. Ford-Jones. 1990. Nosocomial infections in the pediatric patient: An update. *Am. J. Infect. Contr.* 18:176-193.
- Ali R. and H. I. Maibach. 1980. A comparison of the antimicrobial effect of 0.5% chlorhexidine (Hibistat) and 70% isopropyl alcohol on hands contaminated with *Serratia marcescens*. *Clin. Exp. Dermatol.* 5:197-201.
- Ansari S. A., S. A. Sattar, V. S. Springthorpe, G. Wells and W. Tostowaryk. 1989. In vivo protocol for testing efficacy of handwashing agents against viruses and bacteria: Experiments with rotavirus and *Escherichia coli*. *Appl. Environ. Microbiol.* 55: 3113-3118.
- Ansari S. A., S. A. Sattar, V. S. Springthorpe, G. A. Wells and W. Tostowaryk. 1988. Rotavirus survival on human hands and transfer of infectious virus to animate and nonporous inanimate surfaces. *J. Clin. Microbiol.* 26: 1513-1518.
- Archer, D. L. 1989. Disease in Day care: a public health problem for the entire community. *J. Environ. Health.* 51: 143-147.

- Arroyo J. C., B. Postic, A. Brown, et al. 1984. Influenza A/Philippines/2/82 outbreak in a nursing home: Limitations of influenza vaccination in the elderly. *Am. J. Infect. Contr.* 12: 329-334.
- Ayliffe G. A. J., J. R. Babb, K. Bridges, H. A. Lilly, E. J. L. Lowbury, J. Varney and M. D. Wilkins. 1975. Comparison of two methods for assessing the removal of total organisms and pathogens from the skin. *J. Hyg.* 75: 259-274.
- Ayliffe G. A. J., J. R. Babb, J. G. Davis and H. A. Lilly. 1988. Hand disinfection: A comparison of various agents in laboratory and ward studies. *J. Hosp. infect.* 11: 226-243.
- Ayliffe, G. A. J., J. R. Babb and A. H. Quoraishi. 1978. A test for hygienic hand disinfection. *J. Clin. Pathol.* 31: 923-928.
- Ayliffe G. A. J., J. R. Babb , and H. A. Lilly. 1981. Tests for hand disinfection. In: Disinfectants: their use and evaluation of effectiveness (SAB Technical Series No. 16) pp. 37-44.
- Ayliffe G. A. J., J. R. Babb, J. G. Davies, S. W. B. Newsom, C. Rowland, J. H. Platt and B. Mason. 1990. Hygienic hand disinfection tests in three laboratories. *J. Hosp. Infect.* 16: 141-149.
- Bancroft W. H. and S. M. Lemon. 1984. Hepatitis A from the military perspective. In: Hepatitis A. Edited by R. J. Gerety. Academic Press. Orlando, Florida. pp. 81-100.
- Barron-Romero B. L., J. Barreda-Gonzalez, R. Doval-Ugalde, J. Zermeno-Equia Liz and M. Huerta-Pena. 1985. Asymptomatic rotavirus infections in day-care centers. *J. Clin. Microbiol.* 22: 116-118.
- Bartlett A. V., R. R. Reves and L. K. Pickering. 1988. Rotavirus in infant-toddler day care centers: epidemiology relevant to disease control strategies. *J. Pediat.* 113: 435-441.

- Bartzokas C. A., J. H. Paton, M. F. Gibson et al. 1984. Control and eradication of methicillin-resistant *Staphylococcus aureus* on a surgical unit. *N. Engl. J. Med.* 311: 1422-1425.
- Beem M. O. 1969. Acute respiratory illness in nursery school children: A longitudinal study of the occurrence of illness and respiratory viruses. *Am. J. Epidemiol.* 90: 30-44.
- Bishop R. F.. 1986. Epidemiology of diarrheal disease caused by rotavirus. In: Development of vaccines and drugs against diarrhea. Edited by J. Holmgren, A. Lindberg, R. Mollby. Chartwell- Bratt Ltd, London, U.K. pp. 158-170.
- Bishop R. F., G. L. Barends, E. Cipriani and J. S. Lund. 1983. Clinical immunity after neonatal rotavirus infection. *N. Engl. J. Med.* 309: 72-76.
- Bisno A. L., N. P. Barratt, W. H. Swanston and L. P. Spence. 1970. An outbreak of acute respiratory disease in Trinidad associated with parainfluenza viruses. *Am. J. Epidemiol.* 91: 68-77.
- Black R. E., A. C. Dykes, and S. P. Sinclair. 1977. Giardiasis in day-care centers: evidence of person-to-person transmission. *Pediatr.* 60: 486-491.
- Black R. E., A. C. Dykes, K. E. Anderson, J. G. Wells, S. P. Sinclair, G. W. Gary Jr., M. H. Hatch and E. J. Gangarosa. 1981. Handwashing to prevent diarrhea in day-care centers. *Am. J. Epidemiol.* 113: 445-451.
- Blackmore M. A.. 1987. Hand-drying methods. *Nursing Times* 83: 71-74.
- Blake, R. E., A. C. Dykes, S. P. Sinclair, and J. G. Wells. 1977. Giardiasis in day care centers: evidence of person to person transmission. *Pediatr.* 60: 486-491.

- Blaser M. J. and L. B. Reller. 1981. *Campylobacter* enteritis. *N. Engl. J. Med.* 305: 1444-1452.
- Blaufuss H. 1983. Rotavirus infections in a pediatric clinic. *Eur. J. Clin. Microbiol.* 1: 51-53.
- Boris M., B. M. Thomason, V. D. Hines, T. S. Montague and T. F. Sellers. 1964. A community epidemic of enteropathogenic *Escherichia coli* O126:B16:NM gastroenteritis associated with asymptomatic respiratory infection. *Pediatr.* 33: 18-29.
- Bower J. R., B. L. Congeni, T. G. Cleary, R. T. Stone, B. E. Murray, J. J. Mathewson and L. K. Pickering. 1989. *Escherichia coli* O114: nonmotile as a pathogen in an outbreak of severe diarrhea associated with a day care center. *J. Infect. Dis.* 160: 243- 247.
- Boyce J. M. 1989. Methicillin-resistant *Staphylococcus aureus*: detection, epidemiology and control measures. *Infect. Dis. Clin. N. Am.* 3: 901-913.
- Boyer, K.M., N. J. Petersen, I. Farzaneh, C. P. Pattison, M. C. Hurt and J. E. Maynard. 1975. An outbreak of gastroenteritis due to *Escherichia coli* O142 in a neonatal nursery. *J. Pediatr.* 86: 919-927.
- Brade L., W. A. K. Schmidt and I. Gattert. 1981. Zur relativen Wirksamkeit von Desinfektionsmitteln gegenüber Rotaviren. *Zentralbl. Bakteriol. Hyg. I. Abt. Orig. B* 174: 151-159
- Brady M. T., J. Evans and J. Cuartas. 1990. Survival and disinfection of parainfluenza viruses on environmental surfaces. 18: 18-23.
- Brandt C. D., H. W. Kim, W. J. Rodriguez, J. O. Arrobio, B. C. Jeffries and R. H. Parrott. 1982. Rotavirus gastroenteritis and weather. *J. Clin. Microbiol.* 16: 478-482.

- Brandt C. D., H. W. Kim, W. J. Rodriguez, J. O. Arobio, B. C. Jeffries and R. H. Parrott. 1986. Simultaneous infections with different enteric and respiratory tract viruses. *J. Clin. Microbiol.* 23: 177-179.
- Brandt C. D., H. W. Kim, W. J. Rodriguez, J. O. Arrobio, B. C. Jeffries, Stallings EP, C. Lewis, A. J. Miles, R. M. Chanock, A. Z. Kapikian and R. H. Parrott. 1983. Pediatric viral gastroenteritis during eight years of study. *J. Clin. Microbiol.* 18: 71-78.
- Brandt C. D., R. H. Parrot, H. W. Kim and W. J. Rodriguez. 1984. Diarrhea viruses: Detection, specific identification and epidemiology. In: Medical Virology III. Edited by L. M. De la Maza, E. M. Peterson. Elsevier, New York. pp. 35-68.
- Brown D. W. G., L. Campbell, D. S. Tomkins and M. H. Hambling. 1989. School outbreaks of gastroenteritis due to atypical rotavirus (letter). *Lancet* ii: 737-738.
- Brumfitt W., S. Dixon and J. M. T. Hamilton-Miller. 1985. Resistance to antiseptics in methicillin and gentamicin resistant *Staphylococcus aureus*. *Lancet* i: 1442-1443.
- Bynoe M. L., D. Hobson, J. Horner, A. Kipps, G. C. Schild and D. A. J. Tyrrell. 1961. Inoculation of human volunteers with a strain of virus isolated from a common cold. *Lancet* i: 1194-1196.
- Carlson J. A. K., P. J. Middleton, M. T. Szymanski, J. Huber and M. Pidderin. 1978. Fatal gastroenteritis: An analysis of 21 cases. *Am. J. Dis. Child.* 132: 477-479.
- Carolane D. J., A. M. Long, P. A. McKeever, S. J. Hobbs and A. P. Roome. 1985. Prevention of spread of echovirus 6 in a special care baby unit. *Arch. Dis. Child.* 60: 674-676.
- Carter C. H., J. O. Hendley, L. A. Mika and J. M. Gwaltney. 1980. Rhinovirus inactivation by aqueous iodine *in vitro* and on skin. *Proc. Soc. Exp. Biol. Med.* 165: 380-383.

- Carter, A. O., A. A. Borozyk, J. A. Carlson, B. Harvey, J. C. Hockin, M. A. Karamali, C. Krishnan, D. A. Korn and N. H. Lior. 1987. A severe outbreak of *Escherichia coli* O157:H7- associated hemorrhagic colitis in a nursing home. *N. Engl. J. Med.* 317: 1496-1500.
- Casewell M. W., M. M. Law and N. Desai. 1988. A laboratory model for testing agents for hygienic hand disinfection: handwashing and chlorhexidine for the removal of *Klebsiella*. *J. Hosp. Infect.* 12: 163-175.
- Cate T. R., R. B. Couch, W. F. Fleet, W. R. Griffith, P. J. Gerone and V. Knight. 1965. Production of tracheobronchitis in volunteers with rhinovirus in small-particle aerosol. *Am. J. Epidemiol.* 81: 95-105.
- CDC. Center for Disease Control. 1978. Parainfluenza outbreaks in extended-care facilities-United States. *Morbidity and Mortality Weekly Report*. 27: 475-476.
- Champsaur H., E. Questiaux, J. Prevot, M. Henry-amar, D. Goldzmidh, M. Bourjouane and C. Bach. 1984. Rotavirus carriage, asymptomatic infection and disease in the first two years of life. *J. Infect. Dis.* 149: 667-674.
- Chan R. C., J. C. Tam, T. F. Fok and G. L. French. 1989. RNA-electrophoresis as a typing method for nosocomial infection in a special-care baby unit. *J. Hosp. Infect.* 13: 367-375.
- Chanock R. M. 1956. Association of a new type of cytopathogenic myxovirus with infantile croup. *J. Exp. Med.* 104: 555-576.
- Chanock R. M. and R. H. Parrot. 1965. Acute respiratory disease in infancy and childhood: present understanding and prospect for prevention. *Pediatrics*. 36: 21-39.
- Chanock R. M., R. H. Parrot, K. M. Johnson, A. Z. Kapikian and J. A. Bell. 1963. Myxoviruses: parainfluenza. *Am. Rev. Respir. Dis.* 88: 152-166.

- Chanock R. M. and K. McIntosh. 1990. Parainfluenza viruses. In: Fields Virology. Edited by B. N. Fields, D. M. Knipe, R. M. Chanock, M. S. Hirsch J. L. Melnick, T. P. Monath, B. Roizman. Raven Press, New York. pp. 963-988.
- Chanock R. M., K. McIntosh, B. R. Murphy and R. H. Parrott. 1989. Respiratory syncytial virus. In: Viral Infections of Humans: Epidemiology and Control. Edited by A. S. Evans. Plenum Publishing Corp., New York. pp. 525-544.
- Chany C., P. Lepine, M. Lelong, L. T. Vinh, P. Satge, and J. Virat. 1958. Severe and fatal pneumonia in infants and young children associated with adenovirus infections. *Am. J. Hyg.* 67: 367-378.
- Chapin M., J. Yatabe, and J. D. Cherry. 1983. An outbreak of rotavirus gastroenteritis on a pediatric unit. *Am. J. Infect. Contr.* 11: 88-90.
- Charkaeomarakot A., P. Echeverria, D. N. Taylor, K. A. Bettelheim, N. R. Blacklow, O. Sethabutr, J. Seriwatana and J. Kaper. 1987. HeLa cell-adherent *Escherichia coli* in children with diarrhea in Thailand. *J. Infect. Dis.* 156: 669-672.
- Chiba S., R. Kogasaka, Y. Sakuma and T. Nakao. 1981. Long-term observation of viral gastroenteritis in an orphanage: multiple pathogens and different clinical features [abstract no. P16/04]. In: Fifth International Congress of Virology. Strasbourg, France, Aug 2-7.
- Cliver D. O. and K. D. Kostenbader. 1984. Disinfection virus on hands for prevention of food-borne disease. *Intrn. J. Food Microbiol.* 1: 75-87.
- Cohen J. 1977. *Statistical Power Analysis for the Behavioral Sciences*. New York, Academic Press.
- Coovadia Y. M., R. H. Bhana, A. P. Johnson, I. Haffejee and R. R. Marples. 1989. A laboratory-confirmed outbreak of rifampicin-

methicillin resistant *Staphylococcus aureus* (RMRSA) in a newborn nursery. *J. Hosp. Infect.* 14:303-312.

Cone R., K. Mohan, M. Thouless and L. Corey. 1988. Nosocomial transmission of rotavirus infection. *Pediatr. Infect. Dis. J.* 7: 103-109.

Couch R. B. 1990. Rhinoviruses. In: Fields Virology. Edited by B. N. Fields, D. M. Knipe, R. M. Chanock, M. S. Hirsch, J. L. Melnick, T. P. Monath and B. Roizman. Raven Press Ltd., New York. pp. 607-629.

Couch R. B., T. R. Cate, R. G. Douglas Jr., P. J. Gerone and V. Knight. 1966. Effect of route of inoculation on experimental respiratory viral disease in volunteers and evidence for airborne transmission. *Bacteriol. Rev.* 30: 517-529.

Crossley K., B. Landesman and D. Zaske. 1979. An outbreak of infections caused by strains of *Staphylococcus aureus* resistant to methicillin and aminoglycosides. II. Epidemiologic studies. *J. Infect. Dis.* 139: 280-287.

Cubitt W. D., D. A. McSwiggan and H. Holzel. 1980. An outbreak of rotavirus infection in a long-stay ward of a geriatric hospital. *J. Clin. Pathol.* 33: 306-308.

D'Alessio D. J., C. K. Meschievitz, J. A. Peterson, C. R. Dick and E. C. Dick. 1984. Short-duration exposure and the transmission of rhinovirus colds. *J. Infect. Dis.* 150: 189-194.

D'Alessio D. J., J. A. Peterson, C. R. Dick and E. C. Dick. 1976. Transmission of experimental rhinovirus colds in volunteer married couples. *J. Infect. Dis.* 133: 28-36.

D'Angelo L. J., J. C. Hierholzer, R. C. Holman, J. D. Smith. 1981. Epidemic keratoconjunctivitis caused by adenovirus type 8: epidemiologic and laboratory aspects of a large outbreak. *Am. J. Epidemiol.* 114: 44-49.

- Dagan R. C. B. Hall and M. A. Menegus. 1985. Atypical bacterial infections explained by a concomitant virus infection. *Pediatr.* 76: 411-414.
- Daschner F. D. 1988. How cost-effective is the present use of antiseptics? *J. Hosp. Infect.* 11: Suppl A, 227-235.
- Davies D. P., C. A. Hughes, J. MacVicar, P. Hawkes and H. J. Mair. 1979. Echovirus-11 infection in special-care baby unit (letter). *Lancet* I: 96.
- Davies, E. A., A. N. Emmerson, G. M. Hogg, M. F. Patterson and M. D. Shields. 1987. An outbreak of infection with methicillin-resistant *Staphylococcus aureus* in a special care baby unit: value of topical mupirocin and of traditional methods of infection control. *J. Hosp. Infect.* 10: 120-128.
- Davis J. G., J. R. Blake, D. J. White et al. The types and numbers of bacteria left on hands after normal washing and drying by various common methods. *The Med Officer* 1969;October: 235-238.
- DeFabritus A. M., R. R. Riggio, D. S. David, L. B. Senterfit, J. S. Cheigh and K. H. Stenzel. 1979. Parainfluenza type 3 in a transplant unit. *J. Am. Med. Asso.* 241: 384-386.
- Dennehy P. H. and G. Peter. 1985. Risk factors associated with nosocomial rotavirus infection. *Am. J. Dis. Child.* 139: 935-939.
- Denny F. W. and W. A. Clyde. 1986. Acute lower respiratory tract infections in nonhospitalized children. *J. Pediatr.* 108: 635-646.
- de Torres B. V., R. M. de Ilja and J. Esparza. 1978. Epidemiological aspects of rotavirus infection in hospitalized Venezuelan children with gastroenteritis. *Am. J. Trop. Med. Hyg.* 27: 567-572.

- de Zoysa I. and R. G. Feachem. 1985. Interventions for the control of diarrheal diseases among young children: rotavirus and cholera immunization. Bull. WHO 63: 569-583.
- Dick E. C., S. U. Hossain, K. A. Mink, C. K. Meschievitz, S. B. Schultz, W. J. Raynor and S. L. Inhorn. 1986. Interruption of transmission of rhinovirus colds among human volunteers using virucidal paper hand-kerchiefs. J. Infect. Dis. 153: 352-356.
- Dick E. C., L. C. Jennings, K. A. Mink, C. D. Wartgow and S. L. Inhorn. 1987. Aerosol transmission of rhinovirus colds. J. Infect. Dis. 156:442-448.
- Diem K. and C. Lentner. 1970. Documenta Geigy Scientific Tables. Seventh edition. Basle, Switzerland: J. R. Geigy S. A.
- Di Matteo A., A. Sarasini, M. S. Scotta, M. Parea, G. Licardi and G. Gerna. 1989. Nosocomial outbreak of infant rotavirus diarrhea due to the appearance of a new serotype 4 strain. J. Med. Virol. 27: 100-104.
- Donelli G., F. M. Ruggeri, A. Tinari, M. L. Marziano, D. Menichella, D. Caione, C. Concato, G. Rocchi and S. Vella. 1988. A three-year diagnostic and epidemiological study on viral infantile diarrhoea in Rome. Epidemiol. Infect. 100: 311-320.
- Donowitz L. G. 1987. Handwashing technique in a pediatric intensive care unit. Am. J. Dis. Child. 141: 683-685.
- Douglas R. G., T. R. Cate, P. J. Gerone and R. B. Couch. 1966. Quantitative rhinovirus shedding patterns in volunteers. Am. Rev. Res. Dis. 94: 159-167.
- DuPont H. L. 1982. *Escherichia coli* diarrhea. In: Bacterial Infections of Humans. Edited by A. S. Evans and A. Feldman. Plenum Medical Book Co., New York. pp. 219-234.

- DuPont H. L., J. Olarte, D. G. Farns, L. K. Pickering, E. Galindo and G. Evans, Jr. 1976. Comparative susceptibility of Latin American and United States students to enteric pathogens. *N. Engl. J. Med.* 295: 1520-1521.
- DuPont H. L., S. B. Formal, R. B. Hornick, M. J. Snyder, J. P. Libonati, D. J. Sheahan, E. H. LaBrec and J. P. Kalas. 1971. Pathogenesis of *Escherichia coli* diarrhea. *N. Engl. J. Med.* 285: 1-9.
- Echeverria P., N. R. Blacklow and D. H. Smith. 1975. Role of heat-labile toxigenic *Escherichia coli* and reovirus-like agents in diarrhea in Boston children. *Lancet II*: 1113-1116.
- Echeverria P., D. N. Taylor, K. A. Bettelheim, A. Charaemorakot, S. Changchawalit, A. Thongcharoen and U. Leksomboon. 1987. HeLa cell-adherent enteropathogenic *Escherichia coli* in children under 1 year of age in Thailand. *J. Clin. Microbiol.* 25: 1472-1475.
- Eggers H. J. 1990. Experiments on antiviral activity of hand disinfectants. Some theoretical and practical considerations. *Zbl. Bakt.* 273: 36-51.
- Eisenbach K. D., R. M. Reber, D. V. Eitzmen and H. Baer. 1972. Nosocomial infections due to kanamycin-resistant R factor carrying enteric organisms in an intensive care nursery. *Pediatrics* 50: 395-402.
- Elazhary M. A. S. Y. and J. B. Derbyshire. 1979. Aerosol stability of bovine parainfluenza type 3 virus. *Can. J. Comp. Med.* 43: 295-304.
- Ekanem, E. E., H. L. DuPont, L. K. Pickering and B. J. Selwyn. 1983. Transmission dynamics of enteric bacteria in day care centers. *Am. J. Epidemiol.* 118: 562-572.
- England B. L. 1982. Detection of viruses on fomites. In: Methods in environmental Virology. Edited by C. P. Gerba and S. M. Goyal. Marcel Dekker Inc., New York. pp. 179-220.

- Espejo R. T., E. Calderon, N. Gonzalez, A. Salomon, A. Martuscelli and P. Romero. 1979. Presence of two distinct types of rotavirus in infants and young children hospitalized with acute gastroenteritis in Mexico City, 1977. *J. Infect. Dis.* 139: 474-477.
- Estes M. K. and J. Cohen. 1989. Rotavirus gene structure and function. *Microbiol. Rev.* 53: 410-449.
- Faix R. G. 1985. Survival of cytomegalovirus on environmental surfaces. *J. Pediatr.* 106: 649-652.
- Farrington M., J. Ling, T. Ling and G. L. French. 1990. Outbreaks of infection with methicillin-resistant *Staphylococcus aureus* on neonatal and burns units of a new hospital. *Epidemiol. Infect.* 105: 215-228.
- Fauvel, M, A. Chagnon and R. Svoire-Ranco. 1980. Rotavirus gastroenteritis outbreak in a senior citizen's home- Quebec. *Can. Dis. Wkly. Rep.* 6: 205.
- Feachem R. G. 1984. Interventions for the control of diarrhoeal diseases among children: Promotion of personal domestic hygiene. *Bull. W. H. O.* 62: 467-476.
- Fiala M. 1968. Plaque formation by 55 rhinovirus serotypes. *Appl. Microbiol.* 16: 1445-1450.
- Flewett T. H. 1982. Clinical features of rotavirus infections. In: Virus Infections of the Gastrointestinal Tract. Edited by D. A. J. Tyrrell and A. Z. Kapikian. Marcel Dekker Inc., New York. pp. 125-146.
- Flewett T. H., A. S. Bryden and H. Davies. 1974. Diagnostic electron microscopy of faeces. II Acute gastroenteritis associated with reovirus-like particles. *J. Clin. Pathol.* 27: 608-614.

- Flewett T. H. and G. N. Woode. 1978. The rotaviruses (brief review). *Arch. Virol.* 57: 1-23.
- Ford-Jones E. L. 1987. The special problems of nosocomial infection in the pediatric patient. In: Prevention and Control of Nosocomial Infections. Edited by R. P. Wenzel. Baltimore: The Williams & Wilkins Co., New York. pp. 494- 540.
- Ford-Jones E. L., C. M. Mindorff, J. M. Lingley, U. Allen, L. Navas, M. L. Patrick, R. Milner and R. Gold. 1989. Epidemiologic study of 4684 hospital-acquired infections in pediatric patients. *Pediatr. Infect. Dis. J.* 8: 668-675.
- Foster S. O., E. L. Palmer, G. W. Gary, Jr., M. L. Martin, K. L. Herrman, P. Beasley and J. Sampson. 1980. Gastroenteritis due to rotavirus in an isolated Pacific island group: an epidemic of 3,439 cases. *J. Infect. Dis.* 141: 32-39.
- Fox, J. P., M. K. Cooney, C. E. Hall and H. M. Foy. 1985. Rhinoviruses in Seattle families, 1975-79. *Am. J. Epidemiol.* 122: 830-847.
- Fragosa M., A. Kumar and D. L. Murray. 1986. Rotavirus in nasopharyngeal secretions of children with upper respiratory tract infections. *Diagn. Microbiol. Infect. Dis.* 4: 87-88.
- Friedman M. G., A. Galil, B. Sarov, M. Margalith, G. Katzir, K. Midthun, K. Taniguchi, S. Urasawa, A. Z. Kapikian, R. Edelman and I. Sarov. 1988. Two sequential outbreaks of rotavirus gastroenteritis: evidence for symptomatic and asymptomatic reinfection. *J. Infect. Dis.* 158: 814-821.
- Gardner P. S., S. D. M. Court, J. T. Brocklebank, M. A. P. S. Downham and D. Weightman. 1973. Virus cross-infection in paediatric wards. *Br. Med. J.* 2: 571-575.

- Garner J. S. and M. S. Favero. 1985. Guideline for handwashing and hospital environmental control, 1985. Centers for Disease Control, Atlanta.
- Glezen W. P. and F. W. Denny. 1973. Epidemiology of lower respiratory disease in children. *N. Engl. J. Med.* 288: 498-505.
- Glezen W. P., A. L. Frank, L. H. Taber and J. A. Kasel. 1984. Parainfluenza virus type 3: seasonality and risk of infection and reinfection in young children. *J. Infect. Dis.* 150: 851-857.
- Glezen W. P., F. A. Loda W. A. Clyde Jr, R. J. Senior, C. I. Sheaffer, W. G. Conley and F. W. Denny. 1971. Epidemiologic patterns of acute lower respiratory disease of children in a pediatric group practice. *J. Pediatr.* 78: 397-406.
- Glezen W. P., F. A. Loda and F. W. Denny. 1989. Parainfluenza viruses, In: Viral Infections of Humans. Edited by A. S. Evans. Plenum Publishing Corp., New York. pp. 493-507.
- Goldwater P. N., I. L. Chrystie and J. E. Banatvala. 1979. Rotaviruses and the respiratory tract. *Br. Med. J.* 2: 1551.
- Gontigo Filho P. P., M. Stumpf and C. L. Cardoso. 1985. Survival of gram-negative and gram-positive bacteria artificially applied on the hands. *J. Clin. Microbiol.* 21: 652-653.
- Goodman R. A., C. C. Carder, J. R. Allen, W. A. Orenstein and R. J. Finton. 1982. Nosocomial hepatitis A transmission by an adult patient with diarrhea. *Am. J. Med.* 73: 220-226.
- Gorbach S. L., B. H. Kean, D. G. Evans, D. J. Evans Jr. and D. Bessudo. 1975. Travellers' diarrhea and toxigenic *Escherichia coli*. *N. Engl. J. Med.* 292: 933-936.

- Gordon A. G. 1982. Rotavirus infections and the Sompe Syndrome (letter). *J. Infect. Dis.* 146: 117-118.
- Graham D. Y., G. R. Dufour and M. K. Estes. 1987. Minimal infective dose of rotavirus. *Arch. Virol.* 92: 261-271.
- Grinstein S., J. A. Gomez, J. A. Bercovich and E. L. Biscotti. 1989. Epidemiology of rotavirus infection and gastroenteritis in prospectively monitored Argentine families with young children. *Am. J. Epidemiol.* 130: 300-308.
- Guerrant R. L., M. D. Dickson, R. P. Wenzel and A. Z. Kapikian. 1976. Toxigenic bacterial diarrhea: Outbreak involving multiple bacterial strains. *J. Pediatr.* 89: 885-891.
- Guerrant R. L., J. M. Hughes, N. L. Lima and J. Crane. 1990. Diarrhea in developed and developing countries: magnitude, special settings, and etiologies. *Rev. Infect. Dis.* 12: S41-50.
- Gurwith M. J. and T. W. Williams. 1977. Gastroenteritis and Latin American. *J. Infect. Dis.* 135: 1003-1005.
- Gwaltney J. M. Jr. 1989. Rhinoviruses. In: Viral Infections of Humans. Edited by A. S. Evans. Plenum Publishing Corp., New York. pp. 593-615.
- Gwaltney, J. M. Jr. and J. O. Hendley. 1982. Transmission of experimental rhinovirus infection by contaminated surfaces. *Am. J. Epidemiol.* 116: 828-833.
- Gwaltney, J. M. Jr., P. B. Moskalski and J. O. Hendley. 1978. Hand-to-hand transmission of rhinovirus colds. *Ann. Intern. Med.* 88: 463-467.

- Gwaltney J. M. Jr., P. B. Moskalski, and J. O. Hendley. 1980. Interruption of experimental rhinovirus transmission. *J. infect. Dis.* 142: 811-815.
- Hadler S. C. and L. McFarland. 1986. Hepatitis in day-care centers: Epidemiology and prevention. *Rev. Infect. Dis.* 8: 548-557.
- Hadler S. C., H. M. Webster, J. J. Erben, J. E. Swanson and J. E. Maynard. 1980. Hepatitis A in day care centers: A community-wide assessment. *N. Engl. J. Med.* 302: 1222-1227.
- Hadler S. C., J. J. Erben, D. P. Francis, H. M. Webster, and J. E. Maynard. 1982. Risk factors for Hepatitis A in day-care centers. *J. Infect. Dis.* 145: 255-261.
- Haley R. W., D. H. Culver, J. W. White, W. M. Morgan and T. G. Emori. 1985. The nationwide nosocomial infection rate: a new need for vital statistics. *Am. J. Epidemiol.* 121: 159-167.
- Hall C. B. 1977. The shedding and spreading of respiratory syncytial virus. *Pediatr. Res.* 11: 236-239.
- Hall C. B. 1980. Prevention of infections with respiratory syncytial virus: the hopes and hurdles ahead. *Rev. Infect. Dis.* 2: 384-392.
- Hall C. B. 1982. Respiratory syncytial virus. *Pediatr. Infect. Dis.* 1(3)(Suppl) S33-S34.
- Hall, C. B. 1983. The nosocomial spread of respiratory syncytial viral infections. *Ann. Rev. Med.* 34: 311-319.
- Hall C. B. and R. G. Douglas. 1981a. Nosocomial respiratory syncytial viral infections: Should gowns and masks be used? *Am. J. Dis. Child.* 135: 512-515.

- Hall C. B. and R. G. Douglas. 1981b. Modes of transmission of respiratory syncytial virus. *J. Pediatr.* 99: 100-103.
- Hall C. B., R. G. Douglas and J. M. Geiman. 1975. Quantitative shedding patterns of respiratory syncytial virus in infants. *J. Infect. Dis.* 132: 151-156.
- Hall, C. B., R. G. Douglas Jr. and J. M. Geiman. 1980. Possible transmission by fomites of respiratory syncytial virus. *J. Infect. Dis.* 141: 98-102.
- Hall C. B., R. G. Douglas, K. C. Schnabl and J. M. Geiman. 1981. Infectivity of respiratory syncytial virus by various routes of inoculation. *Infect. Immun.* 33: 779-783.
- Halvorsud J. and I. Orstavik. 1980. An epidemic of rotavirus- associated gastroenteritis is in a nursing home for the elderly. *Scand. J. Infect. Dis.* 12: 161-164.
- Hamre D., A. P. Connelly and J. J. Procknow. 1966. Virologic studies of acute respiratory disease in young adults. IV. Virus isolations during four years of surveillance. *Am. J. Epidemiol.* 83: 238-249.
- Han A. M. and T. Hlaing. 1989. Prevention of diarrhea and dysentery by hand washing. *Trans. Royl. Soc. Trop. Med. Hyg.* 83: 128-131.
- Har A. M., T. Hlaing and M. L. Kyin. 1988. Hand washing intervention to reduce ascariasis in children. *Trans Royal Soc Trop Med Hyg.* 82: 153.
- Hara M., J. Mukoyama, T. Tsuruhara, Y. Saito and I. Tagaya. 1976. Duovirus in school children with gastroenteritis. *Lancet* I: 311.
- Harris J. R., J. Mariano, J. G. Wells, B. J. Payne, H. D. Donnell and M. L. Cohen. 1985. Person-to-person transmission in an outbreak of Enteroinvasive *Escherichia coli*. *Am. J. Epidemiol.* 122: 245- 252.

- Harrison H. R., P. Howe, L. Minnich and C. G. Ray. 1979. A cluster of adenovirus 19 infection with multiple clinical manifestations. *J. Pediatr.* 94: 917-919.
- Hayden G. F., D. Deforest, J. O. Hendley and J. M. Gwaltney. 1984. Inactivation of rhinovirus on human fingers by virucidal activity of glutaric acid. *Antimicrob. Chemother.* 26: 928-929.
- Hayden G. F., J. M. Gwaltney, D. F. Thacker and J. O. Hendley. 1985. Rhinovirus inactivation by nasal tissues treated with virucide. *Anti viral Res.* 5:103-109.
- Hendley J. O. 1973. Epidemic keratoconjunctivitis and hand washing. *N. Engl. J. Med.* 289: 1368-1369.
- Hendley J. O. and J. M. Gwaltney, Jr. 1988. Mechanisms of transmission of rhinovirus infections. *Epidemiol. Rev.* 10: 242-258.
- Hendley J. O., J. M. Gwaltney Jr. and W. S. Jordan Jr. 1969. Rhinovirus infection in an industrial population. IV. Infections within families of employees during two fall peaks of respiratory illness. *Am. J. Epidemiol.* 89: 184-196.
- Hendley J. O., L. A. Mika and J. M. Gwaltney. 1978. Evaluation of virucidal compounds for inactivation of rhinovirus on hands. *Antimicrob. Agents Chemother.* 14: 690-694.
- Hendley J. O., R. P. Wenzel and J. M. Gwaltney Jr. 1973. Transmission of rhinovirus colds by self-inoculation. *N. Engl. J. Med.* 288:1361-1364.
- Hjelt K., P. A. Krasilnikoff, P. C. Grauballe and S. W. Rasmussen. 1985. Nosocomial acute gastroenteritis in a pediatric department with special reference to rotavirus infection. *Acta Paed. Scand.* 74: 89-95.

- Ho M. S., R. I. Glass, P. F. Pinsky, N. Young-Okoh, W. M. Sappenfield, J. W. Buehler, N. Gunter and L. J. Anderson. 1988. Diarrheal deaths in American children, Are they preventable? *J. Am. Med. Asso.* 260: 3281-3285.
- Hockin J., H. Lior, L. Mueller, C. Davidson, P. Ashton and F. Wu. 1987. An outbreak of *Escherichia coli* O157:H7 diarrhea in a nursing home-Alberta. *Can. Dis. Wkly. Rep.* 13-45: 206.
- Holmes, I. H. 1979. Viral gastroenteritis. *Prog. Med. Virol.* 25: 1-36.
- Hopkins R. S., B. Gaspard, F. P. Williams, R. J. Karlin, G. Cukor and N. R. Blacklow. 1984. A community water-borne gastroenteritis outbreak: evidence for rotavirus as the agent. *Am. J. Pub. Hlth.* 74: 263-265.
- Hrdy D. B. 1987. Epidemiology of rotavirus infection in adults. *Rev. Infect. Dis.* 9: 461-469.
- Hughes J. M. and Jarvis W. R. 1987. Nosocomial gastrointestinal infections. In: Prevention and Control of Nosocomial Infections. Edited by R. P. Wenzel. Williams and Wilkins, Baltimore, MD., pp. 405- 439.
- Hung T. 1988. Rotavirus and adult diarrhea. *Adv. Virus Res.* 35: 193-218.
- Hung T., G. Chen, C. Wang, H. Yao, Z. Fang, T. Chao, Z. Chou, W. Ye, X. Chang, S. Den, X. Liang and W. Chang. 1984. Water-borne outbreak of rotavirus diarrhea in adults in China caused by a novel rotavirus. *Lancet* I: 1139-1142.
- Hutto C., E. A. Little, R. Ricks, J. D. Lee and R. F. Pass. 1986. Isolation of cytomegalovirus from toys and hands in day care center. *J. Infect. Dis.* 154: 527-530.

- Ijaz M. K., S. A. Sattar, C. M. Johnson-Lussenburg, V. S. Springthorpe and R. C. Nair. 1985. Effect of relative humidity, atmospheric temperature and suspending medium on the air borne survival of human rotavirus. *Can. J. Microbiol.* 31: 681-685.
- Jackson B. M., J. D. Smith and R. K. Sikes. 1990. Parainfluenza outbreak in retardation facility. *Am. J. Infect. Contr.* 18: 128-129.
- Jarvis W. R. 1987. Epidemiology of nosocomial infections in pediatric patients. *Pediatr. Infect. Dis. J.* 6: 344-351.
- Jarvis W. R., P. J. Middleton, and E. W. Gelfand. 1984. Significance of viral infections in severe combined immunodeficiency disease. *Pediatr. Infect. Dis.* 2: 187-192.
- Jennings L. C., E. C. Dick, K. A. Mink, C. D. Wartgow and S. L. Inhorn. 1988. Near disappearance of rhinovirus along a fomite transmission chain. *J. Infect. Dis.* 158:888-892.
- Jones M. R. and D. R. Martin. 1987. Outbreak of methicillin-resistant *Staphylococcus aureus* infection in a New Zealand hospital. *N. Z. Med. J.* 100: 369-373.
- Kapikian A. Z. and R. M. Chanock. 1989. Viral gastroenteritis. In: Viral Infections of Humans. Edited by A. S. Evans. Plenum Medical Book Co. pp. 293-340.
- Kapikian A. Z. and R. M. Chanock. 1990. Rotaviruses. In: Fields Virology. Edited by B. N. Fields, D. M. Knipe, R. M. Chanock, M. S. Hirsch, J. L. Melnick, T. P. Monath, B. Roizman. Raven Press, New York. pp. 1353-1404.
- Kapikian A. Z., R. M. Chanock, T. E. Reichelderfer, T. J. Ward, R. J. Huebner and J. A. Bell. 1961. Inoculation of human volunteers with parainfluenza type 3. *J. Am. Med. Assoc.* 18: 537-541.

- Kapikian A. Z., H. W. Kim, R. G. Wyatt, W. L. Cline, J. O. Arrobio, C. D. Brandt, W. J. Rodriguez, D. A. Sack, R. M. Chanock and R. H. Parritt. 1976. Human reovirus-like agent as the major pathogen associated with "winter" gastroenteritis in hospitalized infants and young children. *N. Engl. J. Med.* 294: 965-972 .
- Kapikian A. Z., R. G. Wyatt, M. M. Levine, R. W. Yolken, D. VanKirk, R. Dolin, H. B. Greenberg and R. M. Chanock. 1983. Oral administration of human rotavirus to volunteers: induction of illness and correlates of resistance. *J. Infect. Dis.* 147: 95-106.
- Kaplan J. E., L. B. Schonberger, G. Varano, N. Jackman, J. Bied, G. W. Gary. 1982. An outbreak of non-bacterial gastroenteritis in a nursing home: demonstration of person-to-person transmission by temporal clustering of cases. *Am. J. Epidemiol.* 116: 940-948.
- Karim Y. G., M. K. Ijaz, S. A. Sattar and C. M. Johnson-Lussenburg. 1985. Effect of relative humidity on the airborne survival of rhinovirus-14. *Can. J. Microbiol.* 31: 1058-1061.
- Kellner G., T. Popow-Kraupp, M. Kundi, C. Binder, H. Wallner and C. Kunz. 1988. Contribution of rhinoviruses and respiratory viral infections in childhood: A prospective study on a mentally hospitalized infant population. *J. Med. Virol.* 25: 455-469.
- Kessner D. M., H. J. Shaughnessy, J. Googins, C. M. Resmussen, M. J. Rose, A. L. Marshall, S. L. Andlman, J. B. Hall and P. J. Rosenbloom. 1962. An extensive community outbreak of diarrhea due to enteropathogenic *Escherichia coli* O11: B4. I. Epidemiologic studies. *Am. J. Hyg.* 76: 27-43.
- Keswick B. H., L. K. Pickering, H. L. DuPont and W. E. Woodward. 1983a. Survival and detection of rotaviruses on environmental surfaces in day-care centers. *Appl. Environ. Microbiol.* 46: 813-816.
- Keswick B. H., L. K. Pickering, H. L. DuPont and W. E. Woodward. 1983b. Prevalence of rotavirus in children in day-care centers. *J. Pediatr.* 103: 85-86.

- Keystone J. S., S. Krajaden and M. R. Warren. 1978. Person-to-person transmission of *Giardia lamblia* in day-care nurseries. *Can. Med. Assoc. J.* 118: 241-248.
- Khan M. U. 1982. Interruption of Shigellosis by hand washing. *Trans. Roy. Soc. Trop. Med. Hyg.* 76: 164-168.
- Kim H. W., A. J. Vargosko, R. M. Chanock, and R. H. Parrot. 1961. Parainfluenza 2(ca) virus etiology association with croup. *Pediatr.* 28: 614-621.
- Kinney J. S., E. McCray and J. E. Kaplan. 1986. Risk factors associated with echovirus 11 infection in a hospital nursery. *Pediatr. Infect. Dis.* 5: 192-197.
- Klein B. S., F. R. Dollete and R. H. Yolken. 1982. The role of respiratory syncytial virus and other viral pathogens in acute otitis media. *J. Pediat.* 101: 16-20.
- Klein B. S., J. A. Michaels and M. W. Rytel. 1984. Nosocomial hepatitis A. A multinursery outbreak in Wisconsin. *J. Am. Med. Asso.* 252: 2716-2721.
- Knittle M. A., D. V. Ertzman and H. Baer. 1975. Role of hand contamination of personnel in the epidemiology of gram-negative nosocomial infections. *Pediatr.* 86: 433-437.
- Koopman J. S. 1978. Diarrhea in school toilet hygiene in Cali, Colombia. *Am. J. Epidemiol.* 107: 412-420.
- Korppi M., P. Halonen, M. Kleemola and K. Launiala. 1988. The role of parainfluenza viruses in inspiratory difficulties in children. *Acta. Pediatr. Scand.* 77: 105-111.
- Krilov L., L. Pierik, E. Keller, K. Mahan, D. Watson, M. Hirsch, V. Hamparian and K. McIntosh. 1986. The association of rhinoviruses

- with lower respiratory tract diseases in hospitalized patients. *J. Med. Virol.* 19: 345-352.
- Kurtz J. B. and J. Boxall. 1976. A partial substitute for handwashing. *Nurs Times* 72: 332-333.
- Laboratory Biosafety Guidelines. 1990. Health and Welfare and Medical Research Council of Canada. Ottawa, Ontario, Canada.
- Larson E. 1988. A casual link between handwashing and risk of infection? Examination of the evidence. *Infect. Contr.* 9: 28-36.
- Larson E. L., P. I. Eke and B. E. Laughon. 1986. Efficacy of alcohol-based hand rinses under frequent use conditions. *Antimicrob. Agents Chemother.* 30: 542-544.
- Lehman N. I., D. L. B. Sharma and I. D. Gust. 1978. Prevalence of antibody to the hepatitis A virus in a large institution for the mentally retarded. *J. Med. Virol.* 2: 335-339.
- Lemp, G. F., W. E. Woodward, L. K. Pickering, P. S. Sullivan and H. L. DuPont. 1984. The relationship of staff to the incidence of diarrhea in day-care centers. *Am. J. Epidemiol.* 120: 750-758.
- Lewis D. C., N. F. Lighthfoot, W. D. Cubitt and S. A. Wilson. 1989. Outbreaks of astrovirus type 1 and rotavirus gastroenteritis in a geriatric in-patient population. *J. Hosp. Infect. Contr.* 14: 9-14.
- Levandowski R. A. and M. Rubenis. 1981. Nosocomial conjunctivitis caused by adenovirus type 4. *J. Infect. Dis.* 143: 28-31.
- Levine M. M. 1987. *Escherichia coli* that cause diarrhea: enterotoxigenic, enteropathogenic, enteroinvasive, enterohemorrhagic, and enteroadherent. *J. Infect. Dis.* 155: 377-389.

- Lilly H. A. and E. J. L. Lowbury. 1974. Disinfection of the skin with detergent preparations Igrasan DP 300 and other antiseptics. Br. Med. J. 4: 372-374.
- Lilly H. A. and E. J. L. Lowbury. 1978. Transient skin flora: their removal by cleansing or disinfection in relation to their mode of deposition. J. Clin. Pathol. 31: 919-922.
- Lilly H. A., E. J. L. Lowbury and M. D. Wilkins. 1979. Detergents compared with each other and with antiseptics as skin degerming agents. J. Hyg. 82: 89-93.
- Linhares A. C., F. E. Pinherio, R. B. Freitas, Y. B. Gabbay, J. A. Shirley and G. M. Beards. 1981. An outbreak of rotavirus diarrhoea among a non-immune, isolated South American Indian community. Am. J. Epidemiol. 113: 703-710.
- Linhares A. C., H. C. Monco, Y. B. Gaggay, V. L. C. de Araujo, A. C. Serruya and C. B. Loureiro. 1983. Acute diarrhea associated with rotavirus among the children living in Belem, Brazil. Trans. Roy. Soc. Trop. Med. Hyg. 77: 384-390.
- Lloyd-Evans N., V. S. Springthorpe and S. A. Sattar. 1986. Chemical disinfection of human rotavirus-contaminated inanimate surfaces. J. Hyg. 97: 163-173.
- Locksley R. M., M. L. Cohen, T. C. Quinn, L. S. Tompkins, M. B. Coyle, J. M. Kiriara and G. W. Counts. 1982. Multiply antibiotic-resistant *Staphylococcus aureus*: introduction, transmission, and evolution of nosocomial infection. Ann. Intern. Med. 97: 317-324.
- Loda F. A., W. P. Glezen and W. A. Clyde Jr. 1972. Respiratory disease in group day care. Pediatr. 48: 428-437.
- Lopez E. L., M. Diaz, S. Grinstein, S. Devoto, F. Mendilaharsu, B. E. Murray, S. Ashkenazi, E. Ruboglio, M. Woloj, M. Vasquez, M. Turco, L. K. Pikerling and T. G. Cleary. 1989. Hemolytic uremic syndrome

- and diarrhea in Argentine children: the role of shiga-like toxins. *J. Infect. Dis.* 160: 469-475.
- Losos J. and M. Trotman. 1984. Estimated economic burden of nosocomial infection. *Can. J. Publ. Hlth.* 75: 248-250.
- Lowbury E. J. L. and J. Fox. 1953. The influence of atmospheric drying on the survival of wound flora. *J. Hyg., Camb.* 51: 203-214.
- Lowbury E. J. L. and H. A. Lilly. 1974. The effect of blood on disinfection of surgeon's hands. *Br. J. Surg.* 61: 19-21.
- Lycke E., J. Blomberg, G. Berg, A. Eriksson and L. Madsen. 1978. Epidemic acute diarrhea in adults associated with infantile gastroenteritis. *Lancet II*: 1056-1057.
- Madeley C. R. 1983. Viruses and diarrhoea: Problems of proving causation. In: Medical Virology II. Edited by L. M. de la Maza, E. M. Peterson. Elsevier Biomedical. New York. pp. 81-109.
- Maderova E., V. Kesnerova, J. Cervenka and C. Pucekova. 1987. Prevalence of nosocomial infections in selected hospitals. *J. Hyg. Epidemiol. Microbiol. Immunol.* 31: 365-374.
- Marier R., J. G. Wells, R. C. Swanson, W. Callahan and I. J. Mehlmen. 1973. An outbreak of enteropathogenic *Escherichia coli* foodborne disease traced to imported French cheese. *Lancet II*: 1376-1378.
- Marples R. R. and A. M. Kligman. 1974. Methods for evaluating topical antimicrobial agents on human skin. *Antimicrob. Agent Chemother.* 5: 323-329.
- Marrie T. J., S. H. S. Lee, R. S. Faulkner, J. Etrier and C. H. Young. 1982. Rotavirus infection in a geriatric population. *Arch. Intern. Med.* 142: 313-316.

- Massanari R. M. and W. J. Hierholzer. 1984. A cross-over comparison of antiseptic soaps on nosocomial infection rates in intensive care units. *Am. J. Infect. Contr.* 12: 247-248.
- Mathur U. N., D. W. Bentley and C. B. Hall. 1980. Concurrent respiratory syncytial virus and Influenza A infections in the institutionalized elderly and chronically ill. *Ann. Intern. Med.* 93: 49-52.
- Matson D. O. and M. K. Estes. 1990. Impact of rotavirus infection at a large pediatric hospital. *J. Infect. Dis.* 162: 598-604.
- Matsumoto K., M. Hatano, K. Kobayashi, A. Hasegawa, S. Yamazaki, S. Nakata, S. Chiba and Y. Kimura. 1989. An outbreak of gastroenteritis associated with acute rotaviral infection in children. *J. Infect. Dis.* 160: 611-615.
- Matthew E. B., D. E. Dietzman, D. L. Madden, S. J. Newman, J. L. Sever, B. N. Nagler, S. M. Bouton and M. Rostafinski. 1973. A major epidemic of infectious hepatitis in an institution for the mentally retarded. *Am. J. Epidemiol.* 98: 199-215.
- Matthews J. A. and S. W. B. Newsom. 1987. Hot air electric driers compared with paper towels for potential spread of airborne bacteria. *J. Hosp. Infect.* 9: 85-88.
- McCormack J. G. 1982. Clinical features of rotavirus gastroenteritis. *J. Infect. Dis.* 4: 167-174.
- McFarlane E. S. and J. A. Embile. 1982. Antibody to hepatitis A virus in young inmates. *J. Infect. Dis.* 146: 442.
- Mebus C. A., R. G. Wyatt, R. L. Sharpee, M. M. Sereno, A. R. Kalica, A. Z. Kapikian and M. J. Twiehaus. 1976. Diarrhea in gnotobiotic calves caused by the reovirus-like agent of human infantile gastroenteritis. *Infect. Immun.* 14: 471-474.

- Melnick J. L. 1989. Virus vaccines: principles and prospects. Bull. WHO 67: 105-112.
- Meissner H. C., S. A. Murray, M. A. Kiernan, D. R. Snyderman and K. McIntosh. 1984. A simultaneous outbreak of respiratory syncytial virus and parainfluenza virus type 3 in a newborn nursery. J. Pediatr. 104: 680-684.
- Merigan T. C. 1982. Respiratory viral infections of adults. In: Scientific American Medicine. Edited by E. Rubenstein, and D. D. Federman. Scientific American, New York. Chapter 7, section xxv: 1-10.
- Merson M. H., G. K. Morris, D. A. Sack, J. G. Wells, J. C. Feely, R. B. Sack, W. B. Creech, A. Z. Kapikian and E. J. Gangarosa. 1976. Travellers' diarrhea in Mexico. A prospective study of physicians and family members attending a congress. N. Engl. J. Med. 294: 1299-1305.
- Mertens T., H. Hager and H. J. Eggers. 1982. Epidemiology of an outbreak in a maternity unit of infections with an antigenic variant of echovirus 11. J. Med. Virol. 9: 81-91.
- Meschievitz C. K., S. B. Schultz and E. C. Dick. 1984. A model for obtaining predictable natural transmission of rhinoviruses in human volunteers. J. Infect. Dis. 150: 195-201.
- Michaud R. N., M. B. McGrath, and W. A. Goss. 1976. Application of a gloved-hand model for multiparameter measurements of skin-degerming activity. J. clin. Microbiol. 3: 406-413.
- Middleton P. J. 1982. Role of viruses in pediatric gastrointestinal disease and epidemiologic factors. In: Virus Infections of Gastrointestinal Tract. Edited by D. A. J. Tyrrell, and A. Z. Kapikian. Marcel Dekker Inc. New York. pp. 211-226.
- Middleton P. J., M. Petric, C. M. Hewitt, M.T. Szymanski, J. S. Tam 1976. Counter immunoelectro-osmophoresis for the detection of infantile

- gastroenteritis virus (orbi-group) and antibody. *J. Clin. Pathol.* 29: 191-197.
- Middleton P. J., M. T. Szymansky and M. Petric. 1977. Viruses associated with acute gastroenteritis in young children. *Am. J. Dis. Child.* 131: 733-737.
- Miller W. S. and M. S. Artenstein. 1967. Aerosol stability of three acute respiratory disease viruses. *Proc. Soc. Exp. Med.* 125: 222-227.
- Modlin J. F. 1986. Perinatal echovirus infection: Insights from a literature review of 61 cases of serious infection and 16 outbreaks in nurseries. *Rev. Infect. Dis.* 8: 918-926.
- Modlin J. F. 1988. Echovirus infections of newborn infants. *Pediatr. Infect. Dis. J.* 7: 311-312.
- Monto A. S. 1968. A community study of respiratory infections in the tropics. III. Introduction and transmission of infections within families. *Am. J. Epidemiol.* 88: 69-79.
- Morens D. M., R. M. Zweighaft, T. M. Vernon, G. W. Gary, J. J. Eslien, B. T. Wood, R. C. Holman and R. Dolin. 1979. A waterborne outbreak of gastroenteritis with secondary person-to-person spread association with a viral agent. *Lancet* I: 964-966.
- Moyenuddi M. and K. M. Rahman. 1985. Enteropathogenic *Escherichia coli* diarrhea in hospitalized children in Bangladesh. *J. Clin. Microbiol.* 22: 838-840.
- Mufson M. A., H. E. Mocega and H. E. Krause. 1973. Acquisition of parainfluenza 3 virus infection by hospitalized children. I. Frequencies, rates and temporal data. *J. Infect. Dis.* 138: 141-147.
- Murphy A., G. Grohman and M. Sexton. 1982. Viral gastroenteritis on Norfolk Island. In: Viral Diseases in South-East Asia and the Western

Pacific. Edited by J. S. Mackenzie. Academic Press, New York. pp. 437-440.

Nagington J., G. Gandy, J. Walker and J. J. Gray. 1983. Use of normal immunoglobulin in an echovirus 11 outbreak in a special-care baby unit. *Lancet* II: 443-446.

Nagington J., T. G. Wreghitt, G. Gandy, N. R. C. Robertson and P. J. Berry. 1978. Fatal echovirus 11 infections in outbreak in special-care baby unit. *Lancet* II: 725-728.

Nataro J. P., J. B. Kaper, and R. Robins-Browne. 1987. Patterns of adherence of diarrheagenic *E. coli* to HEp-2 cells. *Pediatr. Infect. Dis. J.* 6: 829-831.

Nicoletti G., V. Boghossian and R. Borland. 1990. Hygienic hand disinfection: a comparative study with chlorhexidine detergents and soap. *J. Hosp. Infect.* 15: 323-337.

Nigro G. and M. Midulla. 1983. Acute laryngitis associated with rotavirus gastroenteritis (letter). *J. Infect.* 7: 81-83.

Noble R. C., M. A. Kane, S. A. Reeves, and I. Roeckel. 1984. Posttransfusion hepatitis A in a neonatal intensive care unit. *J. Am. Med. Assoc.* 252: 2711-2715.

Noone C. and J. E. Banatvala. 1983. Hospital acquired rotaviral gastroenteritis in a general paediatric unit. *J. Hosp. Infect.* 4: 297-299.

Nystrom B. 1987. Hospital Infection control in Sweden. *Infect. Contr.* 8: 337-338.

Ojajarvi J. 1976. An evaluation of antiseptics used for hand disinfection in wards. *J. Hyg.* 76: 75-82.

- Ojajarvi J. 1980. Effectiveness of handwashing and disinfection methods in removing transient bacteria after patient nursing. *J. Hyg.* 85: 193-203.
- Onesko K. M. and E. C. Wienke. 1987. The analysis of the impact of mild low-iodine, lotion soap on the reduction of nosocomial methicillin-resistant *Staphylococcus aureus*: a new opportunity for surveillance by objectives. *Infect. Contr.* 8: 284-288.
- Orenstein W. A., E. Wu, J. Wilkins, K. Robinson, D. P. Francis, N. Timko, and R. Wayne. 1981. Hospital-acquired hepatitis A: report of and outbreak. *Pediatr.* 67: 494-497.
- O'Ryan M. L., D. O. Matson, M. K. Estes, A. V. Bartlett and L. K. Pickering. 1990. Molecular epidemiology of rotavirus in children attending day care centers in Houston. *J. Infect. Dis.* 162: 810-816.
- Pacini D. L., M. T. Brady, C. T. Budde, M. J. Connell, V. V. Hamparian and J. H. Hughes. 1987. Nosocomial rotaviral diarrhea: Pattern of spread on wards in children's hospital. *J. Med. Virol.* 23: 359-366.
- Pancic, F., D. C. Carpenter and P. E. Came. 1980. Role of infectious secretions in the transmission of rhinovirus. *J. Clin. Microbiol.* 12: 567-571.
- Park G. W. 1980. The use of a large volume air sampler for the detection of airborne respiratory viruses: a feasibility study. M. Sc. Thesis, University of Ottawa, Ottawa, Canada.
- Parkinson A. J., H. G. Muchmore, E. N. Scott and L. V. Scott. 1983. Survival of human parainfluenza viruses in the south polar environment. *Appl. Environ. Microbiol.* 46: 901-905.
- Pass R. F. and S. C. Hutto. 1986. Group daycare and cytomegalovirus infections of mothers and children. *Rev. Infect. Dis.* 8: 599-605.

- Pass R. F., S. C. Hutto, D. W. Reynolds and R. B. Polhill. 1984. Increased frequency of cytomegalovirus infection in children in group day care. *Pediatrics*. 74: 121-126.
- Patriarca P. A., J. A. Weber, R. A. Parker, W. A. Orenstein, W. N. Hall, A. P. Kendall and L. B. Sconberger. 1986. Risk factors for outbreaks of influenza in nursing homes: A case-control study. *Am. J. Epidemiol.* 124: 114-119.
- Paulo P. G. F., M. Stumpf and C. L. Cardoso. 1985. Survival of gram-negative and gram-positive bacteria artificially applied on the hands. *J. Clin. Microbiol.* 21: 652-653.
- Paulozzi, L. J., K. E. Johnson, L. M. Kamahale, C. R. Clausen, L. W. Riley and S. D. Helgerson. 1986. Diarrhea associated with adherent enteropathogenic *Escherichia coli* in an infant and toddler center, Seattle, Washington. *Pediatr.* 77: 296-300.
- Pavia A. T., C. R. Nichols, D. P. Green, R. V. Tauxe, S. Mottice, K. D. Greene, J. G. Wells, R. L. Siegler, E. D. Brewer, D. Hannon and P. A. Blake. 1990. Hemolytic-uremic syndrome during an outbreak of *Escherichia coli* O157:H7 infections in institutions for mentally retarded persons: clinical and epidemiologic observations. *J. Pediatr.* 116: 544-551.
- Peacock J. E. Jr., F. J. Marsik and R. P. Wenzel. 1980. Methicillin-resistant *Staphylococcus aureus*: Introduction and spread within a hospital. *Ann. Intern. Med.* 93: 526-532.
- Pether J. V. S. and R. J. Gilbert. 1971. The survival of salmonellas on finger-tips and transfer of the organisms to foods. *J. Hyg.* 69: 673-681.
- Pentland R., and C. R. Pennington. 1980. Acute diarrhea in the elderly. *Age Ageing*, 9: 90-92.

- Pereira M. A., B. E. Andrews and S. D. Gardner. 1967. A study on the virus aetiology of mild respiratory infections in the primary school child. *J. Hyg.* 65: 475-483.
- Peterson A. F. 1973. The microbiology of the hands, In: Developments in Industrial Microbiology. Published by American Institute of Biological Sciences. Washington D. C. pp. 125.
- Pickering L. K., A. V. Bartlett and W. E. Woodward. 1986. Acute infectious diarrhea among children in day care: epidemiology and control. *Rev. Infect. Dis.* 8: 539-547.
- Pickering L. K., A. V. Bartlett, R. R. Reves and A. Morrow. 1988. Asymptomatic excretion of rotavirus before and after rotavirus diarrhea in children in day care centers. *J. Pediatr.* 112: 361-365.
- Pickering L. K., D. G. Evans, H. L. DuPont, J. J. Vollet and D. J. Evans. 1981. Diarrhea caused by *Shigella*, rotavirus and *Giardia* in day-care centers: prospective study. *J. Pediatr.* 99: 51-56.
- Pickering L. K., D. J. Evans, O. Munoz, H. L. DuPont, P. Coella-Ramirez, J. G. Vollet, R. H. Conklin, J. Olarte and S. Kohl. 1978. Prospective study of enteropathogens in children with diarrhea in Houston and Mexico. *J. Pediatr.* 93: 383-388.
- Polis, M. A., C. U. Tuazon, D. W. Alling and E. Talmanis. 1986. Transmission of *Giardia lamblia* from a day care center to the community. *Am. J. Publ. Health.* 76: 1142-1144.
- Price P. B. 1938. The bacteriology of skin; a new quantitative test applied to a study of the bacterial flora and the disinfectant action of mechanical cleansing. *J. Infect. Dis.* 63: 301-318.
- Prince D. S., C. Astry, S. Vonderfecht, G. Jakab, S. Fu-Min and R. H. Yolken. 1986. Aerosol transmission of experimental rotavirus infection. *Pediatr. Infect. Dis.* 5: 218-222.

- Quraishi Z. A., M. McGuckin and F. X. Blais. 1984. Duration of handwashing in intensive care units: A descriptive study. *Am. J. Infect. Contr.* 11: 83-87.
- Rabkin C. S., E. E. Telzak, M. S. Ho, et al. 1988. Outbreak of Echovirus 11 infection in hospitalized neonates. *Pediatr. Infect. Dis. J.* 7: 186-190.
- Raad I. I., R. J. Sherertz, B. A. Russell and P. D. Reuman. 1990. Uncontrolled nosocomial rotavirus transmission during a community outbreak. *Am. J. Infect. Contr.* 18: 24-28.
- Rahman M. 1979. Outbreak of chicken pox and herpes zoster in a geriatric hospital. *Br. J. Clin. Pract.* 33: 291-293.
- Ramia S. and S. A. Sattar. 1980. Concentration of seeded simian rotavirus SA-11 from potable waters by using talc-Celite layers and hydroextraction. *Appl. Environ. Microbiol.* 39: 493-499.
- Rammalkamp C. H. Jr., E. A. Mortimer Jr. and E. Wolinsky. 1964. Transmission of streptococcal and staphylococcal infections. *Ann. Intern. Med.* 60: 753-758.
- Raphael R. A., S. A. Sattar and V. S. Springthorpe. 1987. Lack of human rotavirus inactivation by residual chlorine in municipal drinking water systems. *Rev. Internatl. des Sci. de l'eau* 3: 67-69.
- Reagan K. J., M. L. McGeady and R. L. Crowell. 1981. Persistence of human rhinovirus infectivity under diverse environmental conditions. *Appl. Environ. Microbiol.* 41: 618-620.
- Reed S. E. 1975. An investigation on the possible transmission of rhinovirus colds through indirect contact. *J. Hyg.* 75: 249-258.

- Reed C. M., T. L. Gustafson, J. Siegel, and P. Duer. 1984. Nosocomial transmission of hepatitis A from a hospital-acquired case. *Pediatr. Infect. Dis.* 3: 300-303.
- Reybrouck G. 1986. Handwashing and hand disinfection. *J. Hosp. Infect.* 8: 5-23.
- Ribner B. S., M. N. Landry, K. Kidd, M. Peninger and J. Riddick. 1989. Outbreak of multiply resistant *Staphylococcus aureus* in a pediatric intensive care unit after consolidation with a surgical intensive care unit. *Am. J. Infect. Contr.* 17: 244-249.
- Riley L. W., R. S. Remis, S. D. Helgerson, H. B. McGee, J. G. Wells, B. R. Davis, R. J. Herbert, E. S. Olcott, L. M. Johnson, N. T. Hargrett, P. A. Blake and M. L. Cohen. 1983. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. *N. Engl. J. Med.* 308: 681-685.
- Rogers M. F., G. W. Rutherford, S. R. Alexander, J. H. Diliberti, L. Foster, L. B. Schonberger and E. S. Hurwitz. 1986. A population-based study of hemolytic uremic syndrome in Oregon, 1979-1982. *Am. J. Epidemiol.* 123: 137-142.
- Rotbart H. A., M. J. Levin, R. H. Yolken, D. K. Manchester and J. Jantzen. 1983. An outbreak of rotavirus-associated neonatal necrotizing enterocolitis. *J. Pediatr.* 103: 454-459.
- Rotter M. 1988. Are models useful for testing hand disinfection. *J. Hosp. Infect.* 11:(suppl A) 236-243.
- Rotter M., W. Koller and G. Wewalka. 1980. Povidone-iodine and chlorhexidine gluconate containing detergents for disinfection of hands. *J. Hosp. Infect.* 1: 149-158.
- Rotter, M. H. Mittermayer and M. Kundi. 1974. Untersuchungen zum modell der kunstlich kontaminierten Hand-Vorschlag fur eine Prufmethode. *Zentralblatt Fur Bakteriologie und Hygiene (I. Abteilung Originale Reihe B)*. 159: 580-581.

- Rotter M., W. Koller, G. Wewalka, H. P. Werner, G. A. Ayliffe and J. R. Babb. 1986. Evaluation of procedures for hygienic hand disinfection, controlled parallel experiments on the Vienna test model. *J. Hyg.* 96: 27-37.
- Roggeri F. M., M. L. Marziano, A. Tinari, E. Salvatori and G. Donelli. 1989. Four-year study of rotavirus electropherotypes from cases of infantile diarrhea in Rome. *J. Clin. Microbiol.* 27: 1522-1526.
- Russell A. D., S. A. Hammond and J. R. Morgan. 1986. Bacterial resistance to antiseptics and disinfectants. *J. Hosp. Infect.* 7: 213-225.
- Ryan, C. A., R. V. Tauxe, G. W. Hosek, J. G. Wells, P. A. Stesz, H. W. McFadden Jr., P. W. Smith, G. F. Wright and P. A. Blake. 1986. *Escherichia coli* O157:H7 diarrhea in a nursing home: clinical, epidemiological and pathological findings. *J. Infect. Dis.* 154: 631-638.
- Ryder R. W., A. E. Buxton, I. K. Wachsmuth, E. Mason, and F. F. Barrett. 1977. Heat-stable enterotoxigenic *Escherichia coli* and necrotizing enterocolitis: lack of an association. *J. Pediatr.* 91: 302-303.
- Ryder R. W., I. K. Wachsmuth, A. E. Buxton, D. J. Evans, H. L. DuPont, E. Mason and F. F. Barrett. 1976. Infantile diarrhea produced by heat-stable enterotoxigenic *Escherichia coli*. *N. Engl. J. Med.* 295: 849-853.
- Salzman T. C., J. J. Clark and L. Klemm. 1967. Hand contamination of personnel as a mechanism of cross infection in nosocomial infection with antibiotic resistant *Escherichia coli* and *Klebsiella aerobacter*. *Antimicrob. Agents Chemother.* 7: 97-100.
- Samadi A. R., M. I. Haq and Q. S. Ahmed. 1983. Detection of rotavirus in handwashings of attendants of children with diarrhoea. *Br. Med. J.* 286: 188.

- Sattar S. A. and M. K. Ijaz. 1987. Spread of viral infections by aerosols. *CRC Crit. Rev. Environ. Contr.* 17: 89-131.
- Sattar S. A., Y. G. Karim, V. S. Springthorpe and C. M. Johnson-Lussenburg. 1987. Survival of human rhinovirus type 14 dried on to non-porous inanimate surfaces: effect of relative humidity and suspending medium. *Can. J. Microbiol.* 33: 802-806.
- Sattar S. A., N. Lloyd-Evans, V. S. Springthorpe and R. C. Nair. 1986. Institutional outbreaks of rotavirus diarrhoea: potential role of fomites and environmental surfaces as vehicles for virus transmission. *J. Hyg.* 96: 277-289.
- Sattar S. A., V. S. Springthorpe, Y. Karim and P. Loro. 1989. Chemical disinfection of non-porous inanimate surfaces experimentally contaminated with four human pathogenic viruses. *Epidemiol. Infect.* 102: 493-505.
- Schupfer P. C., J. R. Murphy and J. F. Bale Jr. 1986. Survival of Cytomegalovirus in paper diapers and saliva. *Pediatr. Infect. Dis.* 5: 677-679.
- Schurmann W. and H. J. Eggers. 1983. Antiviral activity of an alcoholic hand disinfectant: comparison of the *in vitro* suspension test with *in vivo* experiments on hands, and on individual fingertips. *Antiviral Res.* 3: 25-41.
- Schurmann W. and H. J. Eggers. 1985. An experimental study on the epidemiology of enteroviruses: water and soap washing of poliovirus 1-contaminated hands, its effectiveness and kinetics. *Med. Microbiol. Immunol.* 174: 221-236.
- Semmelweis I. The etiology, the concept and the prophylaxis of childbed fever. Pest, Calif.: Hartleben's Verlag-Expedition, 1861 (translation by FP Murphy, republished, Classics of Medicine Library, Birmingham, 1981).

- Shields A. F., R. C. Hackman, K. H. Fife, L. Corey and J. D. Meyers. 1985. Adenovirus infections in patients undergoing bone-marrow transplantation. *N. Engl. J. Med.* 312: 529-533.
- Sims D. G. 1981. A two-year prospective study of hospital-acquired respiratory infections on pediatric wards. *J. Hyg.* 86: 335-341.
- Singh-Naz N., M. Willy and N. Riggs. 1990. Outbreak of parainfluenza virus type 3 in a neonatal nursery. *Pediatr. Infect. Dis. J.* 9: 31-33.
- Smith M. H. D., K. W. Newell and J. Sulianti. 1965. Epidemiology of enteropathogenic *Escherichia coli* infection in non-hospitalized children. *Antimicrob. Agents Chemother.* 5: 77-83.
- Smith C. B., R. H. Purcell, J. A. Bellanti and R. M. Chanock. 1966. Protective effect of antibody to parainfluenza type 1 virus. *N. Engl. J. Med.* 275: 1145-1149.
- Snyder J. D., J. G. Wells, J. Yashuk, N. Puhr and P. A. Blake. 1984. Outbreak of invasive *Escherichia coli* gastroenteritis on a cruise ship. *Am. J. Trop. Med. Hyg.* 33: 281-284.
- Spika J. S., J. E. Parsons, D. Nordenberg, J. G. Wells, R. A. Gunn and P. A. Blake. 1986. Hemolytic uremic syndrome and diarrhea associated with *Escherichia coli* O157:H7 in a day care center. *J. Pediatr.* 109: 287-291.
- Springthorpe V. S., J. L. Grenier, N. Lloyd-Evans and S. A. Sattar. 1986. Chemical disinfection of human rotaviruses: efficacy of commercially available products in suspension tests. *J. Hyg.* 97: 139-161.
- Sprunt K., W. Redman and G. Leidy. 1973. Antibacterial effectiveness of routine handwashing. *Pediatr.* 52: 264-271.
- Stalder H., J. C. Hierholzer and M. N. Oxman. 1977. New human adenovirus (candidate adenovirus type 35) causing fatal

- disseminated infections in a renal transplant recipient. *J. Clin. Microbiol.* 6: 257- 265.
- Steel R. G. D. and J. H. Torrie. 1962. Principles and procedures of statistics. McGraw-Hill Book Co., Toronto.
- Steere A.C. and G. F. Mallison. 1975. Handwashing practices for the prevention of nosocomial infections. *Ann. Intern. Med.* 83: 683-690.
- Stewart P. J., W. Desormeaux and J. Chéné. 1983. Hemorrhagic colitis in a home for the aged-Ontario. *Can. Dis. Wkly. Rep.* 9-8: 29-32.
- Storch G. A., J. L. Radcliff, P. L. Meyer and J. H. Hinrichs. 1987. Methicillin resistant *Staphylococcus aureus* in a nursing home. *Infect. Contr.* 8: 24-29.
- Story P. 1952. Testing of skin disinfectants. *Br. Med. J.* 2: 1128- 1130.
- Straube R. C., M. A. Thompson, R. B. Van Dyke, G. Wadell, J. D. Connor, D. Wingard and S. A. Spector. 1983. Adenovirus type 7b in a children's hospital. *J. Infect. Dis.* 147: 814-819.
- Su C., Y. Wu, H. Shen, D. Wang, Y. Chen, D. Wu, L. He and Z. Yang. 1986. An outbreak of epidemic diarrhoea in adults caused by a new rotavirus in Anhui Province of China in the summer of 1983. *J. Med. Virol.* 18: 167-173.
- Sutmoller F., R. S. Azerado, M. D. Lacerda, O. M. Barth, H. G. Pereira, E. Hoffer and H. G. Schatzmayer. 1982. An outbreak of gastroenteritis caused by both rotavirus and *Shigella sonnei* in a private school in Rio de Janeiro. *J. Hyg.* 88: 285-293.
- Tarr P. I. and R. O. Hickman. 1987. Hemolytic uremic syndrome, epidemiology. A population-based study in King County, Washington. *Pediatr.* 80: 41-45.

- Taylor C. E. and W. B. Greenough. 1989. Control of diarrheal diseases. *Annu. Rev. Pub. Hlth.* 10: 221-244.
- Taylor D. N., P. Echeverria, T. Pal, O. Sethabutr, S. Saiborisuth, S. Sricharmorn, B. Rowe, J. Cross. 1986. The role of *Shigella* spp., enteroinvasive *Escherichia coli*, and other enteropathogens as causes of childhood dysentery in Thailand. *J. Infect. Dis.* 153: 1132- 1138.
- Tennent J. M., B. R. Lyon, M. T. Gillespie, J. W. May and R. A. Skurray. 1985. Cloning and expression of *Staphylococcus aureus* plasmid-mediated quaternary ammonium resistance in *Escherichia coli*. *Antimicrob. Agents Chemother.* 27: 79-83.
- Torres A. and J. H. Lin. 1986. Diarrheal response of gnotobiotic pigs after fetal infection and neonatal challenge with homologous and heterologous human rotavirus strains. *J. Virol.* 60: 1107-1112.
- Townsend D. E., N. Ashdown, J. M. Bradley, J. W. Pearman and W. B. Grubb. 1984. Australian methicillin-resistant *Staphylococcus aureus* in a London hospital. *Med. J. Austral.* ii: 339-340.
- Tufvesson B., S. Polberger, L. Svanberg and T. Sveger. 1986. A prospective study of rotavirus infections in neonatal and maternity wards. *Acta Pediat. Scand.* 75: 211-215.
- Tulchinsky T. H., I. Levine, T. A. Swartz. 1984. Waterborne enteric disease outbreaks in Israel. *Monogr. Virol.* 15: 61-69.
- Tullo A. B. and P. G. Higgins. 1980. An outbreak of adenovirus type 4 keratoconjunctivitis. *Br. J. Ophthalmol.* 64: 489-493.
- Tulloch E. F. Jr., K. J. Ryan, S. B. Formal and F. A. Franklin. 1973. Invasive enteropathogenic *Escherichia coli* dysentery: An outbreak in 28 adults. *Ann. Intern. Med.* 79: 13-17.

- Tyrrell D. A. J., M. L. Bynoe, K. Birkum, S. Petersen and M. S. Pereira. 1959. Inoculation of human volunteers with parainfluenza viruses 1 and 3 (HA₂ and HA₁). *Br. Med. J.* 2: 909-911.
- Ulshen M. H. and J. L. Rollo. 1980. Pathogenesis of *Escherichia coli* gastroenteritis in man: Another mechanism. *N. Engl. J. Med.* 302: 99- 101.
- Valenti W. M., T. A. Clarke, C. B. Hall, M. A. Menegus and D. L. Shapiro. 1982. Concurrent outbreaks of rhinovirus and respiratory syncytial virus in an intensive care nursery: epidemiology and associated risk factors. *J. Pediatr.* 100: 722- 726.
- Valenti W. M. and P. F. Wehrle. 1986. Selected viruses of nosocomial importance. In: Hospital Infections. Edited by J. V. Bennett and P. S. Brachman. Little Brown, Boston. pp. 531-559.
- Vernon A. A., C. Schable and D. Francis. 1982. A large outbreak of hepatitis A in a day-care center: association with non-toilet- trained children and persistence of IgM antibody to hepatitis A virus. *Am. J. Epidemiol.* 115: 325-331.
- Vial P. A., K. A. Kotloff and G. A. Losonsky. 1988. Molecular epidemiology of rotavirus infection in a room for convalescing newborns. *J. Infect. Dis.* 157: 668-673.
- Vihma L. 1969. Surveillance of acute viral respiratory diseases in children. *Acta Pediatr. Scand. Suppl.* 192: 1-52.
- Walker P. E. 1953. Mechanical air drying of hands following preoperative scrubbing. *Publ. Hlth. Rep.* 68: 317-319.
- Wang S., R. Cai, J. Chen, R. Li and R. Jiang. 1985. Etiologic studies of the 1983 and 1984 outbreaks of epidemic diarrhea in Guangxi. *Interviol.* 24: 140-146.

- Wanger, A. R., B. E. Murray, P. Echeverria, J. J. Mathewson and H. L. DuPont. 1988. Enteroinvasive *Escherichia coli* in Travelers with diarrhea. *J. Infect. Dis.* 158: 640-642.
- Ward R. L., D. I. Bernstein, E. C. Young, J. R. Sherwood, D. R. Knowlton and G. M. Schiff. 1986. Human rotavirus studies in volunteers: determination of infectious dose and serological response to infection. *J. Infect. Dis.* 154: 871-880.
- Ward J, G. Gorman, C. Phillips and D. Fraser. 1978. Hemophilus influenzae type b disease in a day-care center. *J. Pediatr.* 92: 713-717.
- Ward, R. L., D. R. Knowlton and M. J. Pierce. 1984. Efficiency of human rotavirus propagation in cell culture. *J. Clin. Microbiol.* 19: 748-753.
- Waters J. R. 1985. *Escherichia coli* O157:H7 and hemolytic uremic syndrome-Alberta, 1984. *Can. Dis. Wkly. Rep.* 11-29: 123-124.
- Weissman J. B., E. J. Gangarosa, A. Schmerler, R. L. Marier and J. N. Lewis. 1975. Shigellosis in day care centers. *Lancet.* I: 88-90.
- Welliver R. C. and S. McLaughlin. 1984. Unique epidemiology of nosocomial infections in a children's hospital. *Am. J. Dis. Child.* 138: 131-135.
- Wenigen, B. G., A. J. Ruttenber, R. A. Goodman, D. D. Juranek, S. P. Wahlquist and J. D. Smith. 1983. Fecal coliforms on environmental surfaces in two day care centers. *Appl. Environ. Microbiol.* 45: 733-735.
- White K. E., C. W. Hedberg, L. M. Edmonson, D. B. W. Jones, M. T. Osterholm and K. L. MacDonald. 1989. An outbreak of Giardiasis in a nursing home with evidence for multiple modes of transmission. *J. Infect. Dis.* 160: 298-304.

- Williams R. E. O. 1963. Healthy carriage of *Staphylococcus aureus*: its prevalence and importance. *Bacteriol. Rev.* 27: 56-71.
- Winther B., J. M. Gwaltney, N. Mygind, R. B. Turner and J. O. Hendley. 1986. Sites of Rhinovirus recovery after point inoculation of the upper airway. *J. Am. Med. Asso.* 256: 1763-1767.
- Wyatt R. G., D. L. Sly and W. T. London. 1976. Induction of diarrhea in clostrum-deprived newborn rhesus monkeys with the human reovirus- like agent of infantile gastroenteritis. *Arch. Virol.* 50: 17-27.
- Yolken R. H. 1983. Avidin-biotin radioimmunoassay for human rotavirus. *J. Infect. Dis.* 148: 942.
- Yolken R. H. and C. C. Franklin. 1985. Gastrointestinal adenovirus: An important cause of morbidity in patients with necrotizing enterocolitis and gastrointestinal surgery. *Pediatr. Infect. Dis.* 4: 42-47.
- Yolken R. H., M. Murphy. 1982. Sudden infant death syndrome associated with rotavirus infection. *J. Med. Virol.* 10: 291-296.
- Zamotin B. A., L. T. Libiyainen, F. L. Bortnik, E. P. Chernitskaya, Z. I. Emina, N. F. Rossikhin, V. I. Veselov, S. A. Kharyutkin, V. A. Egerev, V. A. Nikulin, Z. D. Dorozhkine, K. P. Kostyukova, G. V. Dorozhkin, V. G. Shironina, V. S. Chernyi, E. I. Agapov and V. I. Vlasor. 1981. Waterborne group infection of rotavirus etiology. *Microbiol. Epidemiol. Immunol.* 11: 99-101.
- Zissis G., J. P. Lambert, J. Fonteyne and D. deKegel. 1976. Child-mother transmission of rotavirus? (letter) *Lancet* I: 96.

APPENDIX I

UNIVERSITÉ D'OTTAWA
UNIVERSITY OF OTTAWA

ÉCOLE DES ÉTUDES SUPÉRIEURES ET DE LA RECHERCHE
SCHOOL OF GRADUATE STUDIES AND RESEARCH

CERTIFICATION OF INSTITUTIONAL ETHICS REVIEW COMMITTEE

This is to certify that the Institutional Ethics Review Committee of
 University of Ottawa has examined the research proposal by
 (name of institution)
 Dr. S.A. Sattar entitled Establishment of an In-vivo
 (name of applicant)
 Protocol for Evaluating the Virucidal and Bacterial Efficacy of Antiseptics
 (title of project)

and concludes that, in all respects, the proposed project meets appropriate standards of ethical acceptability

MEMBERS OF THE COMMITTEE

| Name (optional) | Position held | Department or discipline |
|------------------|-----------------|--------------------------|
| Ann B. Denis | Professor | Sociology |
| Jane Ledingham | Professor | Psychology |
| Viola Duff | Professor | Nursing |
| Edmond Rossier | Professor | Microbiology |
| Sanda Rodgers | Professor | Common Law |
| Beatrice Wickett | External Member | Elisabeth Bruyère Centre |
| Jean Farrall | Director | Research Services |

SIGNATURES

Date

Committee Chairperson

Jean Farrall

Department head or representative of
Institution

SERVICE DE LA RECHERCHE
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APPENDIX II

CONSENT FORM

TESTING OF ANTISEPTICS FOR THEIR VIRUCIDAL AND BACTERICIDAL EFFICACY

I _____ hereby consent to participate in this research on antiseptics.

I have been given the opportunity to read the basic objectives of the study and _____ has explained to me the procedure for the contamination and antiseptics of my hands. I have also had the opportunity to ask questions regarding the safety of these procedures and the nature of the microorganisms to be used in this study. If I have any cuts or abrasions on my hands, I will not be allowed to participate in this study till the skin has healed completely.

I will first be required to wash my hands with tap water and rinse them in 70% ethanol. A known amount of the virus or bacterium under test will be placed on the thumb- and fingerpads of my hands and the inoculum allowed to dry under ambient conditions. The contaminated area will be exposed for 10 seconds to the antiseptic under test, rinsed in water and dried with paper towel. Any remaining virus or the bacterium used for contaminating the hands will then be eluted from the skin with Earle balanced salt solution (EBSS).

To minimize the risk of any infectious virus or bacterium remaining on the hands at the end of the experiment, the contaminated areas of the hands will first be wiped with 70% isopropanol before thorough washing with soap and water.

I understand that there will be no direct benefit to me from participating in this study. I also understand that I may withdraw from this study at any time, even after signing this form, and this will in no way affect my status, working conditions or academic standing at the university.

It has also been made clear to me that I will not be identified in any way in any of the reports or publications that may arise out of this study.

Dr. E. Ferry, M.D., Professor, Department of Microbiology & Immunology (TEL: 707-6584), has agreed to act as the Medical Consultant for this investigation. He will be involved in the initial selection of the volunteers, and will also be available should any health problems arise which are related to these experiments.

Signature of volunteer

Date

Name of witness

Signature

Date

Principal investigator's name and address

Medical Consultant's signature

Date

Principal investigator's signature

Date

A COPY OF THE COMPLETED FORM IS TO BE PROVIDED TO THE VOLUNTEER. A COPY OF IT MAY ALSO BE MADE AVAILABLE TO THE AGENCY SPONSORING THE STUDY.

APPENDIX III

Multiple comparison method:

When testing the equality of several means, the usual ANOVA test tells if the means are significantly different from each other but does not say which means differ from which other means. Multiple comparison methods provide more detailed information about the differences among the means. A variety of multiple comparison methods are available with Statistical Analysis System (SAS), General Linear Model (GLM) or Analysis of Variance (ANOVA) procedures.

Tukey (1952; 1953) proposed a test, designed specifically for pairwise comparisons based on studentized range when sample sizes are equal. Tukey (1953) and Kramer (1956) independently proposed modifications for unequal sample sizes. The Tukey option in procedure GLM or ANOVA provides the required test statistics. Two means are considered significantly different by the Tukey-Kramer criterion if

$$|Y_i - Y_j| > q(\alpha; k, v) s \left\{ \left(\frac{1}{n_i} + \frac{1}{n_j} \right) / 2 \right\}^{1/2} \dots \dots \dots (I)$$

where $q(\alpha; K, v)$ is the α level critical value of a studentized range distribution of k independently distributed normal random variables with v degrees of freedom. For equal sample sizes, Tukey's method rejects the null hypothesis of equal population means if

$$|Y_i - Y_j| > q(\alpha; k, v) s / n \dots \dots \dots (II)$$

s in both the expressions (I & II) is the mean square error and obtained from the full Analysis of Variance table. As an example Table 2 of the result section is given below as generated by the GLM Procedure:

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: recovery

Alpha= 0.05 df= 44 MSE= 20.63492

Critical value of Studentized Range= 3.776

Minimum significant difference=4.4287

| Tukey Grouping | Mean | N | Eluent |
|----------------|--------|----|---------|
| A | 58.935 | 15 | 20% TPB |
| B | 49.135 | 15 | EBSS |
| B | 47.505 | 15 | 50% TPB |
| C | 42.911 | 15 | 1xTPB |

Means with the same letter are not significantly different.

For alpha= .05, k= 4 and v= 44, the Minimum Significant Difference (MSD) is obtained as follows:

$q(a;K,v) = 3.776$ (from Table in Tukey)

$MSE = s^2 = 20.63492$ (from Analysis of Variance table)

n= 15

$MSD = (3.776 \text{ sq. root of } 20.63492) / \text{sq. root of } 15 = 4.4287$

If the absolute difference between any pairs of means is greater than or equal to 4.4287 then those two means are significantly different from each other at alpha= .05 level of significance.

In the above Table we can see that 20% TPB is significantly different from the other three means (as marked by different letters); no difference between EBSS and 50% TPB (same letter), but these two are different from 1x TPB (marked by C). The Tukey option clearly provides all the above information and no additional computations are necessary.