



## Hygiene in the home: relating bugs and behaviour

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### Abstract

Much infectious intestinal disease (IID) arises in the home environment. If programmes to prevent infection are to be effective it is essential to both identify the particular practices that risk disease transmission, and to understand the reasons for these practices. An in-depth, multidisciplinary study of carer and child hygiene in the domestic environment in the Wirral, UK, employed structured observation, surface swabbing for polio vaccine virus and enteric marker organisms, semi-structured interviews, projective interviews and focus group discussions. Observations revealed that child carers washed hands with soap after changing a dirty nappy on 43% of occasions, and that one in five toilet users did not wash hands with soap afterwards. Microbiological samples were taken from household surfaces at sites thought likely to be involved in the transfer of faecal material. 15% of bathroom samples showed contamination with polio vaccine virus. Nappy changing took place mainly in living rooms. Contact with living room surfaces and objects during nappy changing was frequent and evidence of faecal contamination was found in 12% of living room samples. Evidence of faecal contamination was also found in kitchens, again on surfaces thought likely to be involved in the transmission of faeces (taps and soap dispensers). Key factors motivating hygiene were the desire to give a good impression to others, protection of the child and aesthetics. In this setting, the particular risk practices to be addressed included washing hands with soap after stool and nappy contact and preventing the transfer of pathogenic organisms to the kitchen. The occasion of the birth of a child may be a privileged moment for the promotion of safer home hygiene practices. Using polio vaccine virus as an indicator of faecal contamination produces results that could be used in large-scale studies of household disease transmission. A better understanding of the household transmission of the agents of IID using multidisciplinary methods is needed if effective hygiene promotion programmes are to be designed.

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### Introduction

In the UK there are estimated to be over nine million cases of infectious intestinal disease (IID) every year (Wheeler et al., 1999). The transmission of pathogenic agents of IID via foods and in kitchens has been documented (Dawkins, Bolton, & Hutchinson, 1984;

Dewitt, Broekhuizen, & Kamplmacher, 1979; Humphrey, Martin, & Whitehead, 1994; Mendes & Lynch, 1978; Scott & Bloomfield, 1993). However, there remains a strong suspicion that much infection originates in the home, where human excreta are the primary source of infection (Curtis, Cairncross, & Yonli, 2000; Feachem, 1984). Sockett looked at 2766 *Salmonella* outbreaks occurring over a 2 year period and classed 86% of these as family outbreaks (Sockett et al., 1993). Data from the Netherlands, Germany and Spain indicate that more than 50% of reported outbreaks of

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1 gastro-intestinal infection arose in the home (Scott & Bloomfield, 1993). In the USA, Daniels et al. (2000) describe an outbreak of viral gastroenteritis that resulted from the household transmission of Norwalk like virus to a food handler from her child. Despite the obvious health implications, few studies have investigated the transmission of disease via the faecal-oral route within the home.

9 The principle habitat and place of reproduction of the common endemic IID pathogens is the human gut (Curtis et al., 2000). Fig. 1 shows how these pathogens enter the domestic environment in human faeces and transit to new hosts. In order to design effective interventions against IID it is necessary to pick out those particular practices which permit the transmission of IID agents. It is also necessary to understand why these practices occur and what might motivate a change in hygiene behaviour. Designing interventions thus requires skill not just in microbiology and epidemiology, but also in disciplines such as anthropology and consumer research.

11 Although several studies have reported the effects of various forms of hygiene promotion on behaviour (e.g. Alam, Wojtyniak, Henry, & Rahaman, 1989; Aziz et al., 1990; Ahmed, Zeitlin, Beiser, Super, & Gershoff, 1993; Shahid, Greenough, Samadi, Huq, & Rahman, 1996) fewer have focussed on trying to understand existing hygiene behaviours. Work in Burkina Faso suggests that water availability has an important influence on faeces disposal practices, but also points to the importance of the social environment and the desire for social approval

57 in motivating hygiene behaviour (Curtis et al., 1995). In developed country settings, work on domestic hygiene behaviour has largely been confined to experimental studies of the potential for food handling practices to spread pathogens in the kitchen environment (e.g. Cogan, Bloomfield, & Humphrey, 1999).

63 A multidisciplinary study of the hygiene practices of mothers and children was carried out in North-West England. This small-scale, intensive study aimed to pinpoint particular risk practices and to understand what motivated domestic hygiene behaviour. A secondary objective was to develop the methodology for research into home hygiene (Curtis et al., 1997). Beyond being one of the first multidisciplinary studies of home hygiene in the UK, the study was innovative in a number of respects. Firstly, polio vaccine virus was used as an indicator of viral contamination from faeces. Viruses are a prime cause of infant diarrhoea, especially in the winter in Europe (Ryan et al., 1996). However, studies of pathogenic viruses in the domestic environment have proved impractical because of the low rate of viral isolation (Bellamy et al., 1998). By recruiting families with a recently vaccinated infant it was possible to identify the spread of viruses of faecal origin in the domestic environment. Secondly, because questionnaires are notoriously poor at eliciting what people actually do, structured observation was employed to document hygiene practices in the home. Thirdly, the study sought to combine the skills and techniques of industrial and academic microbiological and behavioural scientists.

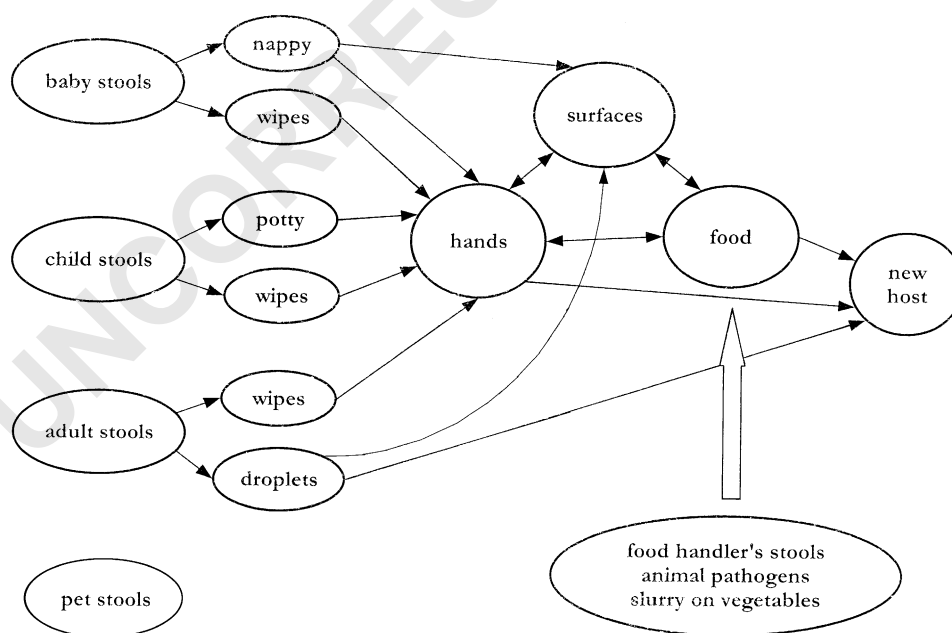


Fig. 1. Routes of transmission of IID agents.

1	Table 1		57
	Types of data collected		
3	Method of data collection	Size of sample	59
5	Structured observation	10 Households (33 nappy changes, 21 toilet uses)	61
	Semi-structured interviews	10 Adults responsible for child care in study households	
7	Projective interviews	5 Adults responsible for child care in study households	63
	Focus group discussion	5 Adults responsible for child care in study households	
9	Virology	10 Households, 250 samples	65
	Bacteriology	10 Households, 75 samples	

11 **Methods** 67

13  
 15 A combination of study methods including structured  
 17 observation, surface virology and microbiology, semi-  
 19 structured interviews, projective interviews and a focus  
 group discussion were employed to study the hygiene  
 practices of carer-child couples in 10 households. The  
 methods used are summarised in Table 1.

21 *Recruitment of participants* 77

23 The study was carried out in the Wirral in North-West  
 25 England. Ten households were recruited by word of  
 27 mouth from amongst those attending two local GP  
 29 clinics or via personal contact. A condition of recruit-  
 31 ment was that households contained an infant aged  
 33 below 3 months who had received a polio vaccine within  
 35 the past 2 weeks, and a toddler under the age of 3 years.  
 37 Recruits were given a description of the study and signed  
 a consent form. Each household was visited three times  
 and the child carer then attended the research centre for  
 an in-depth interview or focus group discussion. Timings  
 of visits with respect to the polio vaccination dates are  
 shown in Table 2. At the end of the study volunteers  
 were paid a small sum in compensation for their time.

39 *Structured observation* 95

41 Structured observation was used to collect data on  
 43 hygiene practices in the home. The technique, drawn  
 45 from studies of animal behaviour and anthropology, is  
 used to record specific, predetermined aspects of  
 behaviour (Altman, 1973; Borgerhoff-Mulder & Caro,  
 1985; Martin & Bateson, 1986; Curtis et al., 1993).

47 Each household was visited by one of two observers  
 49 on three separate days. An interval of 1–15 days was  
 51 allowed between visits. The observer explained to the  
 53 child carer that the purpose of the study was to  
 55 investigate how the health of children could best be  
 promoted. On each visit the observer sat for 3 hs in the  
 lounge or kitchen whilst child carers were asked to carry  
 on their daily activities as normal. The start time for the  
 observation periods was varied across the three visits.  
 The earliest start time was 08:30 and the latest 17:00. In  
 order to reduce the extent to which the presence of the

observer would influence the behaviour of the subjects,  
 the same observer carried out all observations of a single  
 household. In one household, however, observations  
 were carried out by both observers. During the  
 observation periods nappy changing, potty use and  
 toilet use was recorded whenever it occurred.

At each occurrence of nappy changing the following  
 information was recorded:

- identity of the individuals, 77
- time and location of changing, 79
- the surface on which the child was placed, 79
- the condition of the nappy (dry, wet or containing  
faeces), 81
- where the dirty nappy was placed and how it was  
disposed of, 83
- when, how and how often hands were washed during  
and after nappy changing. 85

On occasions when the nappy contained faeces, a list  
 was made of items or surfaces touched by the person  
 changing the nappy during and after nappy changing.

At each occurrence of toilet or potty use the following  
 information was recorded:

- identity of the individual using the toilet/potty, 91
- whether hands were washed afterwards, 93

Table 2  
 Timing of visits with respect to polio vaccination dates (days  
 since most recent polio vaccine)<sup>a</sup> 95

Household ID number	Visit 1	Visit 2	Visit 3	
101	79	85	100	
102	26	33	6	
103	25	30	38	101
104	27	33	34	
105	13	15	21	103
106	2	12	16	
107	23	27	30	105
108	4	8	18	
109	2	6	18	107
110	3	5	6	
Median (range)	18 (2–79)	21 (5–85)	19.5 (6–100)	109

<sup>a</sup> In all cases but one the most recent polio vaccination was  
 also the first vaccination. The exception was ID 102, visit 3. In 111

1 ● if hand-washing occurred, where it took place,  
3 whether soap was used and whether and how the  
hands were dried.

5 When it was not possible to observe hand-washing  
7 directly, the sound of a running tap or the presence of  
wet taps or a damp towel was used to infer that hand-  
9 washing had taken place. Soap use was inferred by  
inspecting whether soap or dispensers were wet shortly  
11 after hand-washing.

#### 11 *Microbiology*

13 At the end of each of the three observation visits  
15 surface swab samples were taken. Samples were taken  
from sites that were thought likely to be involved in the  
17 transfer of faecal material. These included nappy  
changing areas, potty use locations and toilets. In this  
19 exploratory study, sampling was non-random, purposive  
and opportunistic.

21 Samples for virology were collected on every visit,  
some sites being sampled on more than one occasion.  
23 Samples for bacteriology were collected on the final visit  
only. When possible, a small sample of faecal material  
25 from the child's nappy was also collected to check for  
the presence of oral polio vaccine virus.

27 Sampling and viral and bacterial isolation followed  
the protocol for microbiology described in Appendix A.  
29 Briefly, at the end of each observation visit sterile  
moistened swabs were used to sample 10–12 sites of  
31 potential faecal contamination. Swab tips were broken  
off and returned to the lab in transport medium on ice.  
33 From here they were spun down and separated. After  
centrifugation, Poliovirus in the supernatant was identi-  
35 fied using standard cell culture techniques and the  
remaining pellet was re-suspended, frozen and trans-  
37 ferred for bacteriology. Quantitative cultures were  
carried out and bacteria isolated on the basis of Gram's  
39 stain and cultural characteristics.

#### 41 *Semi-structured interviews*

43 During the third visit to each household, a semi-  
structured interview was carried out with the adult  
45 responsible for the majority of nappy changing. These  
interviews followed a predetermined schedule and  
47 covered the topics of domestic hygiene, attitudes to  
faeces, nappy changing behaviour, hand-washing prac-  
49 tices, domestic cleaning practices and perceptions of  
pathogens. Interviews were tape-recorded and tran-  
51 scribed.

#### 53 *Projective interview*

55 Five participants were later interviewed individually  
using projective techniques developed for use in

consumer research. Participants were asked to collect 57  
pictures cut from magazines of images that represented 59  
hygiene. An experienced interviewer then discussed with 59  
the interviewee the reasons why those images had been 61  
chosen, the images that the participants would have 61  
liked to add, the story told by the images, feelings about 63  
the images and the issues raised. The discussions were 63  
recorded and transcribed. 65

#### 65 *Focus group discussion*

67 The other five respondents were invited to attend a 67  
focus group discussion during which they were asked to 69  
make collages of images that were felt to be dirty, 69  
unhygienic or unhealthy. An experienced facilitator 71  
discussed the collages with the group. The tape 71  
recording of the discussion was transcribed. 73

75 Transcripts of all of the interviews and the focus 75  
group were first reviewed manually for insight into 77  
hygiene motivation. They were then pooled and entered 77  
into QSR\*NUDIST. Statements were classified, coded 79  
and tabulated according to subject. This was carried out 79  
by a social scientist (AB) who had not been involved in 81  
the data collection. 81

#### 83 **Results**

85 **Table 3** shows the demographic characteristics of the 85  
10 households involved in the study. In a total of 30 87  
observation/sampling visits 33 nappy changes, 3 uses of 87  
a potty and 21 uses of the toilet were noted. 89

#### 89 *Potential risk practices*

#### 91 *Structured observation*

93 A total of 21 toilet visits were observed in 7 93  
households. Hand-washing following use of the toilet 95  
usually took place in the bathroom or toilet and could 95  
not be directly observed. However, it proved easy to 97  
infer hand-washing by listening for the sound of running 97  
water and by inspecting the taps, soap and towel 99  
immediately afterward. Hand-washing after toilet use 99  
was almost universal and the only individuals not to 101  
wash hands were children. However, hands were washed 101  
with soap after only 81% of toilet uses. These results are 103  
shown in **Table 4**. Hands were usually washed then dried 103  
on a towel. Potty use was observed on 3 occasions in 2 105  
households. This took place once in the living room and 105  
twice in the kitchen. On one occasion the mother wiped 107  
the child and then washed both hands with soap in the 107  
kitchen sink. On the other two occasions the child was 109  
not cleaned. 109

111 Thirty-three nappy changes were observed. Almost all 111  
changes took place on a plastic mat or towel in the living 111  
room (see **Table 5**). Nappy changes took place amongst

1 Table 3  
Demographic characteristics of study households

3

Demographic characteristic	Frequency in sample
<i>Housing type</i>	
Semi-detached	7
Detached	2
Apartment	1
<i>Number of household residents<sup>a</sup></i>	
3	2
4	6
5	2
<i>Household income</i>	
11–15K	2
16–20K	4
21–25K	2
> 31K	1
No data	1
<i>Mother's employment<sup>b</sup></i>	
Part-time	3
Full-time	3
Not employed	3
No data	1
<i>Father's employment<sup>c</sup></i>	
Part-time	2
Full-time	6
No data	2
<i>Mother's age<sup>b</sup></i>	
	Mean 30 (Range 18–38)
<i>Father's age<sup>c</sup></i>	
	Mean 31 (Range 24–36)

35 <sup>a</sup>In every household this includes both parents and one or  
two or three children.

37 <sup>b</sup>Mother' refers to the mother of the infant in the  
participating households.

39 <sup>c</sup>Father' refers to the father of the infant in the participating  
households.

43 a variety of everyday household objects and contact with  
these objects during changes was common (see Table 6).  
45 Hands were not washed on 13 occasions following

47 Table 4  
49 Hand-washing after toilet use

	Number of observations	Number of households	Not washed	Rinsed	Washed with soap (%)	
51						
53	Parent	11	6	0	1	10 (91)
	Child	8	4	2	1	5 (63)
	Other	2	2	0	0	2 (100)
55	Total	21	10	2	2	17 (81)

nappy changing. On only 43% of occasions did carers wash hands with soap after finishing the process of nappy changing, usually in the kitchen. The presence of faeces in the nappy did not increase the likelihood of hand-washing. These results are shown in Table 7.

#### Virology

Fifteen samples of faecal material were collected from nappies. All faecal samples were positive for poliovirus.

Two hundred and thirty-four domestic surface samples were collected and tested for poliovirus. Some of the sites for virology sampling were visited on more than one occasion and the results for virology are therefore presented as percentage of samples rather than percentage of sites sampled. A total of 13% of environmental samples were positive. 15% of bathroom samples were positive, 12% of living room samples and 10% of kitchen samples (see Table 8). Most frequently contaminated were bathroom taps, door handles, toilet flushes, liquid soap dispensers, nappy changing equipment and potties.

#### Bacteriology

The 73 samples from the third household visits were tested. Evidence of faecal contamination (*E. coli* or enterococci) was found at 19% of sites. 30% of living room sites, 20% of bathroom and 6% of kitchen sites were positive for faecal indicator bacteria. The number of sites sampled was small, but toilet flushes and potties were most frequently contaminated. Nappy changing equipment such as mats and wipes boxes also showed evidence of faecal contamination. These results are shown in Table 8.

#### Hygiene motivation

The aim of the qualitative research was to generate insights into the motivations underlying hygiene and of the potential risk practices that were observed. The questions that we wished to answer were 'What is understood by hygiene?', 'What influences home hygiene practices?', and 'What influences hand-washing and nappy changing behaviour?' Because it has a bearing on the methodology of future such studies, we also wished to determine how carers had felt about being

1 Table 5 57  
Where nappies were changed

Nappy change location (%)		Nappy change surface (%)		
5 Living room	28 (85)	Changing mat	25 (76)	61
Bathroom	2 (6)	Changer's knee	4 (12)	
7 Bedroom	1 (3)	Towel	2 (6)	63
Dining room	1 (3)	Carpet	1 (3)	
9 Other	1 (3)	Child standing	1 (3)	65
Total	33 (100)	Total	33 (100)	67

13 Table 6 69  
15 Items touched during 11 nappy changes in which the nappy contained faeces

Item touched	Number of nappy changes	
17 Changing mat	10	73
Wipes	7	
19 Nappy bag	4	75
Changer's clothes	4	
21 Cotton wool	3	77
Carpet	3	
23 Outside bin	3	79
Cotton wool bag/box	2	
25 Toys	2	81
27 Other (baby lotion, sponge, spray can, water bowl, food for toddler, phone, baby gym, spectacles, kitchen bin, mail, furniture, door handle)	1 each	83

29 Table 7 85  
31 Hand-washing during and after nappy changing

	All changes (%)	Changes with faeces in nappy (%)	
35 Hands washed after changing	17 (52)	8 (50)	91
37 Hands washed with soap after changing	14 (42)	6 (38)	93
Hands washed during changing	2 (6)	1 (3)	
39 Hands not washed during or after changing	13 (39)	7 (44)	95
Missing data	1 (3)	0 (0)	
41 Total	33 (100)	16 (100)	97

43  
45 observed and whether they thought that this had 101  
47 influenced their behaviour. Table 9 gives an illustrative 103  
selection of responses.

49 *What is understood by 'hygiene'?* 105

51 Hygiene was primarily described as cleanliness; 107  
53 keeping house, clothes and people clean. A typical 109  
55 response was: "I'd say it was cleanliness—everything 111  
was as clean as you could possibly make it." A hygienic 111  
house was described as looking tidy, bright and ordered. 111  
Unhygienic things looked untidy and dirty, contained

101 faeces, bacteria, or food remains, smelled bad or could 101  
103 spread disease or inflame allergies. The function of 103  
105 hygiene was described as protecting babies and to fight 105  
107 bacteria in kitchen and toilet. Hygiene was also thought 107  
109 to involve some personal effort. Viruses, lice and 109  
111 cigarette-ends were described as *not* unhygienic. 111

*What influences home hygiene practices?* 107

109 Respondents explained that most household cleaning 109  
111 was prompted by the sight of dirt. Cleaning sometimes 111  
111 followed a routine, but often had to be done opportu- 111  
111 nistically, when the baby was asleep or when children

Table 8 Microbiological results							57
Site	Virology			Bacteriology			59
	No. samples	No. positive	% Positive	No. samples	No. positive	% Positive	61
<i>Toilet/bathroom</i>							63
Door handle	4	2	50	7	1	14	65
Nappy bucket	3	1	33	1	1	100	67
Liquid soap dispenser	5	1	20	2	0	0	69
Toilet/bathroom taps	32	6	19	11	1	9	71
Toilet flush	29	5	17	11	4	36	73
Toilet rim	6	1	17	1	0	0	75
Toilet roll holder	5	0	0	2	1	50	77
Radiator (by toilet)	1	0	0	1	1	100	79
Other sites (bath, trainer seat, wall by toilet, door lock, baby bath, bin, radiator, toilet seat, toilet brush, etc.)	24	0	0	8	0	0	81
Total	109	16	15	44	9	20	83
<i>Living room</i>							85
Potty	10	3	33	6	2	30	87
Water bowl	3	1	33	—	—	—	89
Wipes box	14	2	14	5	1	20	91
Door handle	12	1	8	1	1	100	93
Changing mat	16	1	6	5	1	20	95
Other sites (Basin, baby bag, toy, plate, cotton wool bag)	14	0	0	3	0	0	97
Total	69	8	12	15	5	30	99
<i>Kitchen</i>							101
Liquid soap (kitchen)	2	1	50	2	0	0	103
Kitchen taps	17	2	12	9	1	11	105
Other sites (sink, worktop, washing up liquid, bin, cupboard door, etc.)	10	0	0	5	0	0	107
Total	29	3	10	16	1	6	109
<i>Other sites</i>							111
Newel post	2	1	50	1	0	0	113
Carers hands	23	2	9	7	1	14	115
Baby/child	2	0	0	3	0	0	117
Total	27	3	11	11	1	9	119
Overall total	234	30	13	86	16	19	121

were out. Toilets, bathrooms and kitchens required the most cleaning. Bleach was often used for toilets and antibacterial sprays for surfaces. An increased use of antibacterial products was reported as a result of having a new baby. Some mothers also reported cleaning more often because of the new baby; for example, one mother reported cleaning the kitchen several times a day because that was where things were prepared for her baby.

Another reported cleaning floors with a vacuum cleaner on most days now that her child spent much time on the carpet. The most frequently given reasons for cleaning were to improve the appearance of the house, especially to save embarrassment if a visitor was expected, and to remove smells and bacteria, especially from kitchens and toilets.

3	Question	Key findings	Examples from interviews and discussions	59
1	Table 9			57
	Hygiene motivation, illustrative responses			
5	What is understood by hygiene?	1. Cleaning, tidying, ordering	“Hygiene’s just being clean.” B09  “If you walked into my house and the first thing you saw that there was no dust on the side everything was neat and tidy, there was nothing lying around the floor—you look in the kitchen, all the surfaces are clean, all the dishes are washed in the cupboard, there’s no things round—you look in the lounge and it’s been hoovered and everything’s in its place, ... I think that would say hygiene.” BoB “Keeping myself clean and my clothes clean and... my personal space clean.” Projecti	61 63 65 67
13		2. Protecting babies from bacteria and allergens	“It’s like protecting against something that could cause harm—so being hygienic would be to protect in as many ways as you can if it’s something that would damage a baby, because I’m not worried about me.” B0B.  [Hygiene is] getting rid of... asthma, eczema, hay fever, rhinitis, it’s trying to combat those kind of things... B0B	69 71 73
19	What influences home hygiene?	1. Removing visible dirt	If there was sort of soap scum on the sink I’d clean it ... the toilet, I put toilet cleaner down it a couple of times a week and then if it looks dirty I clean it as well— B04	75 77
25		2. Removing smells and bacteria	[Reason for cleaning the toilet] “Well if I’m being honest with myself it has to be the look of it...” B06 “... eliminating some of the bacteria that are going to be around,... including E. Coli, Salmonella...the big ones that everyone knows about that are so hyped up that you can’t help but try and counteract those risks can you—I can’t,” B0B “I know sometimes you can smell it when you walk in—you can smell the clean, you know...” B09	79 81 83 85
31		3. What other people think	“... well it’s a small house isn’t it, you can smell everything as soon as you walk in—I don’t like it and I think if I can smell it, then other people will be able to.” B02 “I clean up every room because... my mum’ll ... might go in and have a look you know—see how tidy they are!... she’d say oh that room’s not very tidy...she’d check yes, she’d say oh that wasn’t very clean...” B08 “I think you just want people you know to think that you’re tidy and your house is tidy... I get very embarrassed if I notice at my house there’s something grotty...” B06 “The bathroom I clean about once a week or so, just have a general clean.” B04	87 89 91 93
37		4. Routine and/or opportunity	“I usually Hoover round and everything again on a Saturday [because] my husband takes the two children to his mums” B08 “If [my toddler] isn’t here I do tend to do a clean up then... rather than when he’s here, it’s easier because he wants to help all the time.” B02 “My kitchen I would do daily because to me that’s a food preparation—that’s where I do the key things for the baby.” B0B	95 97 99
43		5. Having a new baby	“I’m antibacterial mad at the moment, since I’ve had him, everything... like the dishcloths I’d buy anything, but I buy microban antibacterial do you know—everything is antibacterial—just to try and eliminate anything that I can do really. I never used antibacterial anything until I had the baby.” B09 “You wouldn’t forgive yourself if it had asthma because you haven’t kept hoovering up” B09 “Feel like I’m doing the best for the baby if I buy those kind of [anti-bacterial] things.” B09	101 103 105
51	What influences nappy changing behaviour?	1. Convenience	“He only ever gets done on the floor because he’s too wriggly” Projecti	107
55			“More convenience really, when we had just Sam we always changed nappies upstairs, just because it didn’t seem very nice really, the living room having a poeey smell and things like	109 111

Table 9 (continued)			57
Question	Key findings	Examples from interviews and discussions	
		that, but now, I mean, three of them, it isn't convenient to go upstairs every time..." B04 [change upstairs] If it's in the night and I don't want to come downstairs. B05	59
	2. State of the nappy	"It's just not nice when it's pooey all over their backs and it takes ages to clean off and then you've got to give them a bath, but it's not particularly—it's not disgusting it's just you know—it's just a fact of life." B05	61
		"Sometimes when it's all up his back and over his clothes and... he needs a whole change, but his doesn't smell too bad with him being breast fed as well so it's not the smell—just sheer quantity." B10	63
	3. Nappy changing is bonding time	"I wouldn't say it was favourite thing—but actually with him yes, because it's a real bonding time and laughing and talking and that." B05	67
		"Because she feels freer without a nappy on so she smiles a lot more and it's time just like to talk to her—as if you're getting to know her—so it's nice." B07	69
		"When the baby's got her nappy off she likes to lie there and kick her legs about and she's quite happy, have a little smile and little giggle." B09	71
	4. Nappy contents not always seen as risky	"I wouldn't have thought [that what's in the nappy] is dangerous or harmful." B05	73
		"Well [what's in the nappy] would be harmful if like they ate it... it's full of bacteria... yes it's full of waste and...yes it's not the sort of thing you want hanging around." B06	75
		"They feel alright [after nappy changing] but I feel as if I need to go and wash them" B07	77
What factors influence hand-washing behaviour?	1. Feel a need to wash after certain activities	"You just have to wash your hands after you've been to the loo." B04	79
		whenever I've had a cigarette outside, I'll come in and ... I wash my hands, B0B	81
		if you've been into the garden touching anything out there, always wash your hands. B09	83
	2. Discomfort	"During I'm preparing food—just because I don't particularly like the feel on my hands you know if you're sticky or whatever." B066	85
	3. Smell	"When you've done...like the baby's nappies or whatever if it gets on your hands and you're walking down the road later you can still smell it—even though you've washed your hands it's just...seems to have got this incredible ability to keep the smell there." B03	87
	4. Having a baby	"...to get rid of the smell and the odours and anything that might be kind of lingering—because it's not good for him." B05	89
		"You seem to wash your hands more with having the baby." B08	91
		"Since having him I wash my hands all the time." B055	93
		"Just a bit frightened of more germs going about than anything because they've got no immune system really, have they, when they're under two." B08	95
		"I found I wash my hands a lot more than I would have....before I had the babies." Group	97
		"Because, like germs and bacteria left on your hands and then you put like your fingers in your mouth you could transmit all different germs." B07	99
	5. To prevent spread of bacteria	"If you go to the loo if you have diarrhoea for example and you go to the loo and don't wash your hands and then start eating an apple or something and you would have thought you'd be more likely to get worse or something." B0N	101
		No I don't.(always wash hands after nappy change) don't have time do you. B01	103
	6. Convenience	With food yes. Not always after toilet.... if something's distracted me. B10	105
		I've probably gone straight from seeing to one of the children—maybe wiped A's nose or whatever on to doing something else without thinking. B02	107
		" I try very sure to make sure I wash his hands before his meals, but you know being honest with you I don't always, quite often I'll sit him down and...you know.".... B06	109
How acceptable was the study?	1. Acceptable	"Erm... yes it was fine yes, once I got used to it, it was a bit strange to begin with, but once I got used to someone sitting in the house, then it was OK yes." B01	111

Table 9 (continued)

Question	Key findings	Examples from interviews and discussions
		“It doesn’t bother me—it would have bothered if you were going to come back and say, well really you should clean your house more—then it would bother me, but no it doesn’t bother me to think that you’ve taken swabs.” BO1
		“My thought was when I knew she was coming, I thought to myself well, what I’ve got to think is she’s not...she’s not judging me—she’s just merely doing her job. And that was it.” BO9
		“At first I was a bit, like—nervous because I didn’t know you, but you’re fine to get on with, so it was alright really.” BO7
		“Quite normal... quite fun—good conversation point over coffee or whatever with friends—quite amusing. It’s a bit of a... when you’re stuck in the kitchen with three kids, it’s quite fun having to entertain them—and a bit of a change different people to talk to and the money’s good (laughter)...the money’s handy.” BO3
	2. One participant found it stressful	“I felt very... quite nervous, which surprised me...I felt very nervous and when you were around although you weren’t, you know, intrusive at all. I felt the whole time gosh, I’m being watched, something’s being written and I did, I found it quite stressful.” BO6
		“I think cleaned the bathroom and the kitchen and hoovered the carpets—I didn’t know what she was going to be doing or why she was doing it at the time...” BO1
	3. Some people cleaned more	“No I didn’t [clean up], I spoke to G. who was also doing this study and she said I don’t think they’d appreciate it if you cleaned everywhere before they came.” BO2
	4. Some did not	“Tried my best—I did most things the same I think.” BO3
		“Well before she came I thought, I’ve just got to think I mustn’t run round and start cleaning madly because this lady’s coming because she just I thought wants to see normal everyday muck.” BO9
		“I couldn’t help but, you know, clean a bit probably more than I would normally or, you know, I was thinking if she was going to... I was more aware of say hygiene or what I was doing, I was very aware of myself and my routine...” BO6

#### *What influences nappy changing practices?*

The choice of room for nappy changing was determined largely by convenience. Thus, changes during the day were usually done in the sitting room, while at night or early in the morning nappies were changed upstairs in the bedroom. The kitchen was often seen as an unsuitable place for changing nappies, although the reasons given were as often connected with convenience and the comfort of the baby as with the possibility of contaminating a food preparation area. “It doesn’t smell nice and it’s not nice” said one Mother. “I don’t think it’s necessarily a cleanliness thing, I think it is more of a comfort”, said another. Although dirty nappies, dirty babies and the smell of faeces were considered unpleasant, nappy changing was often seen as a positive, bonding experience, which could be enjoyed by the mother and baby. “I don’t particularly like, you know, his poos, they’re horrible, but it’s nice when they’re all clean.” “...it’s a real bonding time and laughing and talking and that.” The observations confirmed that carers spent much of an, often prolonged, nappy changing time in communication and play with the baby.

#### *What influences hand-washing behaviour?*

The times when it was recognised as important to wash hands were; after changing nappies, or using the

toilet, and before preparing food, making up bottles or feeding a baby. Other occasions mentioned were after handling raw meat, smoking, using cleaning agents, gardening, cleaning a pet cage and putting something in the bin. Only one interviewee reported washing hands before eating. Although washing hands after toilet use or nappy changing and before food preparation was regarded as ideal, it was recognised that hand-washing does not always occur. Two mothers said they always washed their hands after changing a nappy after having been observed not to do so. The main reasons given for not washing hands were distraction with other tasks, especially children. The reasons given for washing hands were to remove smells, to remove faeces, to prevent the spread or ingestion of germs or bacteria and also to protect the baby from tobacco smells and dangerous chemicals. “...Again just to get rid of the smell and the odours and anything that might be kind of lingering—because it’s not good for him.” Interviewees generally reported an increase in hand-washing associated with having a new baby. “...since having him I wash my hands all the time.” “We should have shares in soap now (laughter).” Reasons given for the changes were the perceived weakness of the baby’s immune system and the desire to do what is best for the child. “Feel like I’m doing the best for the baby if I buy those kind of (antibacterial) things.”

1 *How did carers feel about being observed and did they*  
 2 *think their behaviour had changed as a result?*

3 The majority of participants were happy with the  
 4 methods used. Although the presence of an observer was  
 5 often felt to be strange initially, once this wore off  
 6 several participants reported enjoying the company and  
 7 attention. "Fine, it's been informal, pleasant—someone  
 8 to talk to, unobtrusive—it's been fine yes. I've enjoyed  
 9 it." Two participants reported feeling nervous initially  
 10 and one described the experience as stressful. "I felt very  
 11 nervous and when you were around although you  
 12 weren't, you know, intrusive at all, I felt the whole  
 13 time, gosh, I'm being watched, something's being  
 14 written and I did, I found it quite stressful." Most  
 15 participants felt that they should clean or tidy before the  
 16 first visit. This urge was resisted to varying degrees and  
 17 no extra effort was reported for subsequent visits. "I  
 18 tried not to, sort of like, do anything that I wouldn't  
 19 normally. I couldn't help but, you know, clean a bit,  
 20 probably more than I would normally." Participants  
 21 were interested in the microbiological sampling and  
 22 some reported that this process highlighted surfaces to  
 23 which they would pay more attention when cleaning in  
 24 future.

## 27 Discussion

29 Despite its importance for public health, IID trans-  
 30 mission in the home has been relatively little studied.  
 31 One reason for this neglect is that hygiene is a difficult  
 32 subject to study because it is private and morally bound.  
 33 Questionnaires are notoriously poor at eliciting what  
 34 people actually do. Observation can produce more valid  
 35 results, but is intrusive and expensive (Curtis et al.,  
 36 1993). Multiple agents of IID and their rarity and  
 37 transience make studies of pathogen transmission in situ  
 38 impractical. The alternative, to employ viral and  
 39 bacterial indicators of faecal contamination, is less than  
 40 ideal since pathogens and indicators do not behave  
 41 identically in the environment. This study suggests that  
 42 using multiple methods in concert to study home  
 43 hygiene can provide new insights and also information  
 44 for the design of hygiene promotion interventions.

## 47 Limitations of the study

49 This study has four main limitations. First, due to its  
 50 small size, and non-random, sampling methods, statis-  
 51 tically meaningful conclusions cannot be drawn about  
 52 patterns of contamination, and generalisations from the  
 53 qualitative data must also be treated with caution.  
 54 Second, we assumed that all virus detected was excreted  
 55 by the vaccinated infants in faeces, though nasophar-  
 angeal excretion is technically possible, as is infection

and excretion by other household members. Third, the  
 57 pattern of spread of poliovirus may not accurately  
 58 model the behaviour of pathogenic intestinal viruses  
 59 such as rotavirus in the domestic environment. Never-  
 60 theless, the study does highlight the potential for faecal  
 61 pathogens from any source to contaminate and survive  
 62 on household surfaces. Fourth, there is no perfect  
 63 method for recording what people actually do about  
 64 hygiene in their own homes, or of determining their  
 65 motivation. Behaviour is subject to reactive biases due  
 66 to the presence of an observer (Curtis et al., 1993) and  
 67 motivation can only be recorded as reported. This  
 68 favours rational explanation because respondents feel  
 69 they need to offer coherent explanation. Subconscious  
 70 and irrational motivation may determine behaviour, but  
 71 not be reported.

72 Multidisciplinary, cross-sectoral collaboration of the  
 73 sort exemplified by this study is complex to organise, co-  
 74 ordinate and analyse, and expensive in human resources.  
 75 Complexity and cost should not however, be expected to  
 76 grow in proportion to the sample size.

## 79 Potential risk practices

81 The study was based around the proposition that  
 82 there would be frequent opportunities for contamination  
 83 of the domestic environment with faecal pathogens.  
 84 Audit studies of bacterial and viral pathogens in  
 85 households have generally proven negative (Bellamy  
 86 et al., 1998; Finch, Prince, & Hawksworth, 1978; Scott,  
 87 Bloomfield, & Barlow, 1982), probably because the  
 88 excretion of infective materials is a rare event. However,  
 89 experimental studies with *Salmonella* and *E. coli* show  
 90 the potential for spread from toilets to bathrooms  
 91 surfaces and hands (Barker & Bloomfield, 2000; Gerba,  
 92 Wallis, & Melnick, 1975) and from hands to other  
 93 surfaces (Rheinbaben et al., 2000). We hypothesised that  
 94 we would find evidence of contamination by 'in vivo'  
 95 tracing of everyday contact with faeces, during defeca-  
 96 tion or childcare, and subsequent hand-washing, or lack  
 97 of it. Microbiological samples showed that faecal  
 98 contamination of the domestic environment does occur.  
 99 Faecal coliforms were found at a number of sites, not  
 100 only in toilets and bathrooms but also in kitchens and  
 101 on a variety of objects connected with nappy changing.  
 102 All households in the study contained an infant who had  
 103 recently received oral polio vaccine. These infants were  
 104 shedding poliovirus in faeces. This allowed poliovirus to  
 105 be used as an indicator of viral contamination originat-  
 106 ing from faeces. Such viral contamination was found in  
 107 toilets, bathrooms, kitchens and other household sites.  
 108 This is the first study, to our knowledge, to successfully  
 109 investigate the spread of viruses of faecal origin and to  
 110 specifically examine the microbial contamination asso-  
 111 ciated with nappy changing.

- 1 The sample size in the present study is too small for  
 2 patterns of contamination to be linked to behavioural  
 3 patterns at the level of the individual household,  
 4 although the results suggest that this would be feasible  
 5 in a larger study. Nevertheless, the observational data  
 6 highlight some possible risk and protective practices.
- 7 The hands of adults were washed with soap after  
 8 using the toilet on the majority of occasions observed.  
 9 However, one in five uses of the toilet were not followed  
 10 by hand-washing with soap. We suspect that hand-  
 11 washing with soap may be overestimated for several  
 12 reasons. The current study took place during the  
 13 daytime when many family members were not present.  
 14 Those present were the infant's main caretaker (usually  
 15 the mother), and sometimes older siblings, not yet of  
 16 school age. These groups may be more concerned about  
 17 hand-washing or more compliant than older siblings or  
 18 spouses who were not present. Those who were present  
 19 in the home were aware that they were being observed  
 20 by a researcher with an interest in hygiene and might  
 21 have been more likely to live up to the ideal of washing  
 22 hands after using the toilet. The fact that a number of  
 23 bathroom and toilet sites including door handles, were  
 24 found to show signs of faecal contamination suggests  
 25 that hand-washing after using the toilet is not always  
 26 regularly practised.
- 27 Hand-washing after nappy changing stands in marked  
 28 contrast to that after toilet use. Hands were washed  
 29 following only 52%, and soap was used after only 43%  
 30 of nappy changings. Hand-washing behaviour was not  
 31 influenced by the presence of faeces in the dirty nappy.  
 32 Nappy changing generally took place in the living room.  
 33 It may be that the absence of convenient washing  
 34 facilities discouraged hand-washing and also that cues to  
 35 trigger hand-washing that may exist in a bathroom are  
 36 absent from the sitting room. This situation may have  
 37 been compounded in some cases by the presence of an  
 38 older child and the additional demands this placed on  
 39 the time and attention of the caretaker. Kitchen taps  
 40 were found to harbour enteric microbes on occasion.  
 41 Hand-washing in the kitchen after nappy changing may  
 42 represent a prime route for the transfer of pathogenic  
 43 organisms to new hosts.
- 44 Nappy changing took place in the midst of a variety  
 45 of everyday objects such as changing equipment,  
 46 furniture, toys and telephones, and contact was made  
 47 with these objects during nappy changing. Contact was  
 48 increased because of the prolongation of the nappy  
 49 changing process because it was enjoyed by mother and  
 50 child. These facts may combine with irregular hand-  
 51 washing to increase the risk of contamination of the  
 52 household environment with faeces from nappies.
- 53 Indicator organisms clustered at points where direct  
 54 contact with faecal material on hands was most likely;  
 55 toilet flushes, nappy mats and wipes, taps and potties.  
 Direct observation of behaviour combined with surface  
 sampling offers a powerful means of tracking the  
 transmission routes of infectious agents in the home.
- The frequency with which hands were not washed  
 after changing nappies was a striking finding of this  
 study, especially in view of the fact that participants  
 knew they were being observed by a researcher with an  
 interest in hygiene. The scale of the difference between  
 hand-washing after toilet use and after nappy changing  
 suggests that there is a difference in attitude towards  
 these activities. It has been noted in developing countries  
 that mothers often fail to regard child excreta as  
 dangerous (Kanki et al., 1994b). This seems also to be  
 the case in the UK. However, if children are the main  
 victims of infectious intestinal disease, their stools are  
 also the main source of diarrhoeal pathogens.
- Motivation for hygiene behaviour*
- From the perspective of the public health scientist,  
 pathogens cause disease, faeces are a potent source of  
 pathogens and hygiene behaviour is a rational response  
 to this disease threat. Hygiene thus serves to reduce the  
 likelihood of harmful contact with pathogens. The idea  
 that health behaviour is a rational response to a  
 perceived disease threat underlies models of individual  
 health behaviour such as the Health Belief Model  
 (Becker, 1974). However, from the perspective of the  
 person carrying out the practices, hygiene serves very  
 different purposes. The desire to nurture children, to  
 create a tidy and aesthetically pleasing environment, to  
 be rid of substances that occasion disgust and to be  
 respected by others, all influence hygiene behaviour.  
 Carers who described their hygiene behaviour in terms  
 of germ avoidance may have been offering rational  
 medical explanations for behaviours that are, in fact,  
 indulged in for other reasons (to remove a bad smell, to  
 demonstrate affection for a child, to avoid opprobrium  
 from visitors). The present study suggests that, where a  
 health motivation for hygiene behaviour exists, it is  
 likely to be concern about the health of a third party (the  
 baby) rather than the health of the caretaker.
- Efforts to promote safer hygiene might be more  
 effective if they built on the universal positive desire for  
 cleanliness, rather than relying on the traditional,  
 rational, but not very attractive, appeal to fear of gut  
 infection.
- The increase in hand-washing and use of antibacterial  
 products which was reported to follow the birth of a  
 baby suggests that this may be a time when parents are  
 particularly amenable to behaviour change. The time  
 following the birth of a baby may be especially  
 favourable for interventions aimed at changing hygiene  
 practices.
- It has been suggested by an increasing number of  
 authors that too much hygiene may have negative  
 impacts on health through failure to stimulate the

1 correct balance between the Th1 and Th2 components of  
 3 the immune response (Rook & Stanford, 1998). There is,  
 5 however, no evidence to suggest that the ingestion of the  
 7 pathogenic agents of IID has a net benefit to health. If  
 9 the hygiene hypothesis proves well-founded, it will  
 11 underline the need to target hygiene promotion much  
 13 more specifically at risk practices for intestinal infection,  
 15 such as failure to wash hands after stool contact, and  
 17 not at 'hygiene' in general. More studies such as this one  
 19 will therefore be needed.

#### Methodology: feasibility of a larger study

15 There is no perfect way to document private and  
 17 morally loaded behaviours such as those related to  
 19 hygiene. Interviews are notoriously poor at eliciting  
 21 behaviour and methods other than direct consensual  
 23 personal observation are difficult and unethical. Struc-  
 25 tured observation can give a good overall view of the  
 27 prevalence of particular behaviours without necessarily  
 29 painting a true picture of what usually happens in  
 31 individual households (Curtis et al., 1993, Cousens et al.,  
 1996). This is because people react to being observed,  
 often by trying to project an image as 'cleaner or more  
 hygienic' (Kanki et al., 1994a). Others are flustered by  
 the presence of an observer and forget to do what they  
 might otherwise routinely practice. The behaviour of  
 individuals also varies by time of day, week or year and  
 by mood and circumstance. These difficulties notwith-  
 standing, structured observation remains the best  
 available tool for documenting hygiene behaviour.

33 Observation and surface swabbing was accepted by 9  
 35 out of 10 child carers in the study. Four people who were  
 37 contacted did not wish to participate in the study, giving  
 39 a number of reasons, including lack of time. A high  
 41 refusal rate from a particular group could affect the  
 43 external validity of a larger study, especially if these were  
 45 the most or the least hygienic members of society.  
 47 External validity was aided by the choice of mothers of  
 49 infants in their first few months of life as the target  
 51 group. Mothers who normally work were at home for  
 53 maternity leave and could thus be represented in the  
 55 sample. Such mothers often welcomed the distraction of  
 having visitors. However, recent mothers are a particu-  
 lar sample of the population who have particular  
 concerns and habits. They may not be representative of  
 the population as a whole. Hence, the finding of  
 unexpectedly high levels of hand-washing after using  
 the toilet may be a reflection of our hypothesised  
 heightened sense of hygiene when a new baby is present  
 and not a true picture. The study took up a considerable  
 amount of participant's time and the remuneration was  
 important in motivating participants to continue to the  
 end.

Documenting the motivation for hygiene practice is  
 more complex. Using interviews, projective techniques

57 and focus groups gave some insight into why people do  
 59 what they do. However, such techniques rely on people  
 61 rationalising their own behaviour. Much behaviour is  
 63 automatic, habitual or determined by stimuli that are  
 65 not open to conscious scrutiny. Self-reports of motiva-  
 67 tion are therefore inherently limited in their scope. So,  
 69 for example, a mother might report that she washed her  
 71 hands to avoid germs for the sake of offering a rational  
 73 explanation, whilst her real motivation might be long  
 75 habit, the discomfort of sticky hands or their smell. Of  
 the three techniques, projective approaches, which  
 tackle the subject from an indirect angle were best at  
 eliciting underlying motivation. However none are ideal.  
 New developments in the understanding of the relation-  
 ship between emotion and behaviour may help to shed  
 light on these issues (Curtis & Biran, 2001).

#### Conclusions

77 The study was small and cannot be used for statistical  
 79 inference. However, a number of tentative conclusions  
 81 can be drawn.

1. Faecal-oral spread of pathogenic viruses and bacter-  
 83 ia in the home can and probably does occur.
2. Infants may be an important source of intestinal  
 85 infection. Poor hygiene in association with nappy  
 87 changing and potty use may be major risk practices  
 for IID.
3. Rigorous hand-washing after contact with faecal  
 89 material in toilets, nappies and potties, possibly  
 91 coupled with the decontamination of high-risk  
 93 surfaces may offer an effective means to break  
 95 domestic transmission routes of IID.
4. Hygienic behaviour is motivated by a number of  
 97 factors which include the desire to nurture a baby,  
 99 to create an attractive environment, to tidy and order,  
 to avoid things that are disgusting and to create a  
 good impression. Interventions to prevent the do-  
 mestic transmission of pathogens should focus on  
 existing motivation and habit and on facilitating  
 behaviour change.
5. Studying home hygiene is difficult, but not impos-  
 101 sible. This study was unique in using polio vaccine  
 103 virus as an indicator of viral contamination originat-  
 105 ing from an identifiable source within the home  
 107 (infant faeces). It overcomes the major problem of  
 109 previous studies which have been unable to pinpoint  
 the source of microbial contamination of household  
 surfaces. A larger study using the techniques piloted  
 here is feasible.
6. Combining the skills of industrial and academic  
 111 microbiological and behavioural scientists using  
 quantitative and qualitative techniques gives good

1	insight into what people do and why. The results can		
3	be used to design hygiene promotion interventions.		
5	<b>Acknowledgements</b>		
7	Thanks to collaborators Sue Fisher for field work,		
9	Debbie Stevens for lab work, Kwesi Tsiquaye for the		
11	idea of using polio vaccine virus. The contributions of		
13	Martin Jones, Sally Bloomfield and Sandy Cairncross		
15	are acknowledged. Funding was provided by Unilever		
17	Research.		
19	<b>Appendix A. microbiology protocols</b>		
21	<i>Preparation of transport medium</i>		
23	Virus Transport Medium (VTM) was prepared from		
25	Hanks Basal Salt Solution (HBSS) containing 0.7%		
27	Bovine Serum Albumin, the pH of the solution was		
29	adjusted by the addition of 1% sterile sodium bicarbonate		
31	solution (7.5%) 1.5 ml was added to sterile plastic		
33	bijou.		
35	<i>Sampling procedure</i>		
37	An area of 10 cm <sup>2</sup> was sampled using swabs		
39	moistened with Hanks BSS (HBSS). Swab tips were		
41	returned to the remaining HBSS and the tubes stored in		
43	ice until returned to the laboratory. Swabs were vortex		
45	mixed in the HBSS for 10 s and allowed to stand for		
47	1 min. The suspension was then transferred to a sterile		
49	Eppendorf tube and centrifuged at 13,000 rpm for		
51	20 min. The supernatant was transferred to a second		
53	sterile Eppendorf tube containing 10 µl of antibiotic		
55	solution for virus isolation. The pellet was re-suspended		
	in 1 ml sterile 10% glycerol and 1% peptone solution.		
	Samples stored at -80°C until sent to LSHTM for		
	bacteriology.		
	<i>Cell culture and media preparation for virus isolation</i>		
	Hep-2C cells (Human caucasian larynx carcinoma)		
	were obtained from the European Cell Culture Collec-		
	tion. Growth medium for the cells consisted of Eagles		
	minimal essential medium (with Earle's salts) supple-		
	mented with 10% Foetal Calf Serum (FCS), 1% (v/v)		
	100 × non-essential amino acids, 2.5 µml Amphotericin		
	B, 100 µg/ml Streptomycin, 50 I.U./ml penicillin, 20 mM		
	L-Glutamine. Cells were maintained on the above		
	medium but with the FCS reduced to 2%. Stocks of		
	cells were in 75 cm <sup>2</sup> cell culture bottles and passaged as		
	required. Plates (48-well) were prepared by sub-culturing		
	at a dilution to give confluent monolayers in 24-48 h.		
	<i>Monoclonal antibodies against poliovirus</i>		57
	These were obtained from Dr. Philip Minor (NIBSC)		59
	by Dr. Kwesi Tsiquaye. They were aliquoted and stored		61
	at 20°C until required, (Type 1: 425, Type 2 : 267 and		63
	Type 3: 495). The serum was titrated against the three		65
	serotypes of poliovirus to determine the level required to		67
	neutralise virus.		69
	<i>Preparation of faecal samples for virus isolation</i>		71
	An appropriate volume of Hanks' balanced salt		73
	solution (HBSS) (Sigma H8264) was added to give a		75
	10% (w/v) suspension and the sample vortex-mixed for		77
	30 s. The suspension was allowed to settle out for 15 min		79
	at 4°C then clarified by centrifugation for 30 min at		81
	1450g/4°C. The supernatant was decanted and anti-		83
	biotics added to give a final concentration of 1000-I.U./		85
	ml penicillin, 1000 µg/ml streptomycin (Sigma P0781)		87
	and 2.5 µg/ml amphotericin B (Sigma A2942). The		89
	sample was held at room temperature for 1 h then		91
	centrifuged for 2 h at 1450g/4°C. The supernatant was		93
	decanted and dilutions of 10-1 and 10-2 prepared in		95
	HBSS plus antibiotics and mixed with appropriate		97
	dilutions of monoclonal antibody, incubated for 1 h at		99
	37°C. The cells were inoculated with 100 µl undiluted		101
	and diluted suspension (treated with antiserum) or cell		103
	maintenance medium as a control. After 1 h incubation		105
	at 37°C, the inoculum was decanted and replaced with		
	0.5 ml overlay medium. The cells were observed daily,		
	and after 5 days the plates were fixed and stained.		
	<i>Overlay medium</i>		
	Carboxymethyl cellulose (CMC) (BDH Low viscos-		
	ity) was prepared by adding 1.5 g-85 ml deionised water		
	and sterilised by autoclaving at 121°C for 15 min. To the		
	solution of CMC the following were added:		
	10 ml Eagles MEM 10×,		
	3 ml sodium bicarbonate (7.5%),		
	1 ml glutamine 2000 mM),		
	1 ml penicillin/streptomycin		
	(10,000 units/10 mg/ml),		
	1 ml fungizone(250 µg/ml),		
	2 ml foetal calf serum.		
	<i>Fixation and staining</i>		
	Plates were fixed using 20% (v/v) formaldehyde		
	(500 µl/well), the formaldehyde was left in contact with		
	the cells for 1 h. After which it was removed by pipetting		
	and each well was washed five times with deionised		
	water (1 m/wash). Crystal violet stain was added		
	(0.25 ml/well) and left for 30 min. The stain was		

1 prepared from 0.2% Crystal Violet, in 20% ethanol  
made up to 100% with deionised water).

3 After staining the crystal violet was removed by  
pipetting and the wells washed with water ( $4 \times 0.25$  ml)  
5 to remove excess stain. The plates were air dried and the  
number of plaques per counted and recorded.

#### 9 Control virus

11 As a control for the neutralisation and plaque assay, a  
virus control was included in every batch of samples  
13 tested. This consisted of Polio type 1 Mahoney strain  
that had been cultured in Vero cells.

15 The samples were received frozen and stored at minus  
 $70^\circ$  until needed.

#### 17 Bacteriology: quantitative culture

19 The samples were thawed and tenfold serial dilutions  
21 were prepared in quarter strength Ringers solution. A  
0.1 ml sample of the original sample and of the original  
23 sample and of each of the dilutions were spread  
inoculated, using sterile glass spreaders, on Blood agar  
25 and MacConkey's agar plates. The plates were incu-  
bated overnight at  $37^\circ$ .

27 The colonies of bacteria that had developed were  
counted and identified, on the basis of Gram's stain and  
29 cultural characteristics.

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