Evaluation methods of disinfectants

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Scope of review

- Definition of disinfectant
- Factors involved in disinfectant activities and tests
- Characteristics of disinfectant evaluation tests
- Classification and type of tests
  - Type of tested microorganisms
  - Type of methodology
  - Type of test objective
- Example of tests

Definition

- **Disinfectant**
  - **No universally accepted definitions of disinfectants**
    - **Minimum requirement: 3-Log reduction of effective microorganisms excluding spores**
  - **Low-level disinfectant**
    - **Most of vegetative bacteria**
    - **Lipid-enveloped virus (HIV)**
  - **Intermediate-level**
    - **Nonlipid or small virus (Poliovirus)**
  - **High-level**
    - ***Mycobacteria* spp. including *M. tuberculosis***
- **Sterilant**
  - **6-Log reduction of the most resistant spores achieved at the half-time of the regular cycle**

Characteristics of disinfectants

- Materials used for elimination of microorganisms
  - Physical aspect (Cleaning effect)
  - Biologic aspect (Antimicrobial activity)
- Diverse applications and regulations
  - Medical, veterinary, food, industrial, etc
  - Various application methods
  - Regulations differ from country to country
    - Associations of Official Analytical Chemists, Assications Francaise de Normalization, British Standards Institution, etc

Factors involved in the activity of disinfectants

- Factors linked with the antimicrobial agents
- Factors linked with the microorganisms
  - Nature of the microorganisms
  - Physiologic state of the microorganisms
  - Microbial communities (Flora) and biofilms
  - Natural and acquired resistance
- Physical, physicochemical, and chemical environment of microorganisms
  - Concentration
  - Temperature
  - Presence of organic matter
  - Electrolytes
  - Additives and components of excipients

Factors interfering with evaluation tests and criteria for activity

- Test strains: Representativeness and reliability
- Criteria for activity
- Critical point in the test method
  - Preparation of inocula
  - Neutralization or washing by membrane filtration
  - Detection and count of survivors
    - Quantitative recovery of microorganisms
    - Size of the sampling
    - Subculture and revival of survivors
  - Physical parameters of exposure: time, temperature, humidity, pH

Characteristics of disinfectants tests

- **Different from antimicrobial agent evaluation**
  - **Minimum inhibitory concentration cannot be used**
    - **Not inhibition but killing**

    → Neutralizer or elimination of disinfectants required
  - **Different mechanisms and of activity**
    - **Non-linear killing curve of disinfectant**
    - **The rate of log killing decrease as the inoculum decrease**
  - **Inhibitory effect and postexposure effect**
- **Broad spectrum of efficiency according to in-use environment**
  - **Time, concentration, pH and temperature**
  - **Type and amount of microorganisms**

- **No acceptable method for the objective evaluation of disinfection procedure**
- **Various and numerous specific test methods**
- **Only extensive study of disinfectant capabilities**
  - **Biologic aspect was evaluated**
    - **Bactericidal, fungicidal, viricidal, sporicidal effect, etc**
  - **Standard strains of different species should cover**
    - **A sufficiently large spectrum**
    - **At least the targeted area**
  - **Technical considerations**
    - **Standardized methods to obtain reproducible results**
    - **The means of detection of surviving microbes**

Classification based one the type of microorganisms

- Bactericidal test
- Mycobactericidal test
  - Tuberculocidal test
- Sporicidal test
  - Bacterial spores are highly resistant to chemical agent
- Fungicidal test
- Virucidal test

Bactericidal test

Gram-positive bacteria		Gram-negative bacteria	
S. aureus	AOAC, AFNOR, BSI, DGHM, CEN	*P. aeruginosa*	AOAC, AFNOR, BSI, DGHM, CEN
E. faecium	AOAC	*S. typhi* and *S. cholerasuis*	AOAC and BSI
E. faecalis	Dutch suspension test	*S. typhimurium*	Dutch suspension test and CEN
E. hirae	AFNOR and CEN	*P. mirabililis*	DGHM and Dutch suspension test
		P. vulgaris	BSI
		E. coli	AFNOR, DGHM and CEN

- Exposure time: 30 sec to 15 minutes
- Interfering substance: simulating practical condition
- Interpretation criteria
  - 5-log reduction in 5 minutes

Mycobactericidal test

Tuberculocidal test		Mycobactericidal test	
M. tuberculosis	DGHM	*M. Smegmatis*	AOAC
M. bovis BCG	AOAC, EPA	*M. avium-intracellulare*	CEN
M. terrae	DGHM, CEN, AOAC		

- **Devices treated with**
  - **2% horse serum (Proteinaceous load)**
  - **10^5 to 10^6 CFU of *M. terrae* or other *Mycobacteria* spp.**
- **Exposure time: 15 to 60 minutes**
  - **Vary according to the test strain**
- **Interpretation criteria**
  - **4-log reduction**

Sporicidal test

- **Test organisms**
  - ***B. cereus, B. subtilis* and *Clostridium sporogenes***
- **Spore inoculum is important**
  - **Spore only, not vegetative cells**
  - **Heating the suspension for 1 to 5 min at 80 to 85°C**
- **Interpretative criteria**
  - **Basic activity test**
    - **5-log reduction in 1 hour at 20 °C or in 5 min at 75 °C**
  - **Application test**
    - **3-log reduction in 1 hour at 20 °C**

Fungicidal activity

Yeast form		Mold form	
Candida albicans	AFNOR, DGHM	*Aspergillus niger*	AFNOR, CEN
Saccharomyces cerevisae	Dutch standard	*Trichophyton mentagrophytes*	DGHM, AOAC

- **Several test in many countries**
  - **Suspension test**
  - **Carrier test**
- **Interpretative criteria**
  - **4-log reduction in 15 minutes (or more)**

Virucidal test

- **Characteristics of virucidal test**
  - **Measurement of the infectious power of the virus**
  - **Difficulties in cell culture**
    - **Disinfectant itself is highly toxic**

    →Cell lysis and inhibition of viral penetration and replication

    →Dilution of virus-disinfectant mixture required
    - **Neutralizers can also have cytotoxic effects**
- **Tested organisms (AFNOR)**
  - **Enterovirus, adenovirus and poxvirus**
- **Interpretative criteria**
  - **4-log reduction in 15 to 60 minutes**

Classification based on methodologic principle

- **The experimental conditions precisely described**
  - **Contact time, temperature, interfering substances, reference strains and inoculum**
- **Insufficient reproducibility**
  - **Repeatability (Within laboratory): $0.25 \leq SD \leq 1.21$**
  - **Reproducibility (Between laboratory): $0.31 \leq SD \leq 1.54$**
- **Types of methodologic test**
  - **Suspension test**
  - **Carrier test**
  - **In-use and Field test**
  - **Test on Biofilms**

Suspension test

- **Objective**
  - **The potential activity of a disinfectant**
  - **The activity in conditions simulating practice**
  - **All types of microorganisms can be tested**
- **Method**
  - **Mixing fixed volumes of known dilutions of disinfectant with a fixed volume of inoculum**
  - **Defined contact time (Incubation)**
  - **Culture for counting**

Carrier test

- **Objective**
  - **Disinfectants potentials for surface and instrument under conditions that simulate the application**
  - **Bacteria, fungi and spore can be tested**
- **Method**
  - **Classified by the mode of application of the product**
    - **Disinfectant directly dropped on the carrier surface**
      - **Product used for surface disinfection (glass, stainless steel, plastic)**
      - **Recommended method and time at room temperature**
      - **After incubation, culture for survivor quantitation**
    - **Carrier immersed in the disinfectant dilution**
    - **Disinfectant dispersed by spraying or aerosol**
  - **Evaluated activity varied according to application method**
  - **During drying step, some bacteria died → Control required**

In-use and Field test

- Objective
  - Efficiency of a disinfectant applied to a defined procedure
  - Test of the disinfectant and procedure of application
  - Verify the application conditions in suspension or carrier test
  - Difficulty in compare several procedures
- Method
  - Test carried out in procedure in field such as hospitals, etc
  - After disinfection, residual contamination detected
- Interpretation
  - Endpoint interpretation (No survivors)
  - Not quantitative approach to the reduction of the flora

Classification based on the objective

- **Basic test**
  - To evaluate the intrinsic qualities of a disinfectants
  - Bactericidal, fungicidal, sporicidal or virucidal tests
- **Application test**
  - To determine the best conditions of application
  - Common and specified strains in a defined procedure
  - Concentration, exposure time and temperature
- **In-use and field test**
  - To evaluate the result of a disinfectant
  - To verify the efficacy in a defined procedure

Basic bactericidal activity

Method and reference	EN 1040 (1997) – Phase 1 suspension test	
	Dilution-neutralization (Preferentially)	Membrane filtration (If neutralization failed)
Objective	Basic bactericidal activity of chemical disinfectants and antiseptics (Classification as bactericidal)	
Strains	*P. aeruginosa* ATCC 15442 *S. aureus* ATCC 6538	
Inoculum	Adjusted suspension of agar culture obtained under specified conditions Number of viable cells in presence of antiseptic or disinfectant: 1.5 x 10^7 to 5 x 10^7 cfu/mL	
Diluent for the product and tested concentrations	Distilled water; the product test solution is 1.25 times the required test concentration (maximum tested 80%). At least three-dilutions including at least two-dilutions in the active range	
Contact time and temperature	1, 5, 15, 30, 45, or 60 min at 20°C	

Method and reference	Dilution-neutralization (Preferentially)	Membrane filtration (If neutralization failed)
Elimination of the tested product in the subcultures	Transfer of microorganism-disinfectant mixture (1 mL) into suitable neutralizer validated simultaneously – (Dilution: 1/10)	Transfer of microorganism-disinfectant mixture (0.1 mL) into filtration apparatus. Wash under conditions validated simultaneously with possible use of neutralizer – (Dilution: 1/10)
Count of survivors	Survivor count in the neutralized mixture by inclusion in agar medium	Membrane placed on agar medium
Incubation	Incubation at 36°C or 37°C for 48h	
Interpretation	Check that all controls fit with the fixed values and that the neutralizer (or the filtration procedure) is validated. Calculate the reduction in viability. The product passes the test if it demonstrates a 10^5 (or more) reduction in viability within 60 minutes (or less) with both microoragnisms. Further tests (phase 2, step 1 and step 2) are required to qualify the product for a specific use.	

Basic fungicidal activity

Method and reference	EN 1275 (1997) – Phase 1 suspension test	
	Dilution-neutralization (Preferentially)	Membrane filtration (If neutralization failed)
Objective	Basic fungicidal activity of chemical disinfectants and antiseptics (Classification as fungicidal)	
Strains	*C. albicans* ATCC 10231 *A. niger* ATCC 16404	
Inoculum	Adjusted suspension of agar culture obtained under specified conditions Number of viable cells in presence of antiseptic or disinfectant: 1.5 x 10^6 to 5 x 10^6 cfu/mL	
Diluent for the product and tested concentrations	Distilled water; the product test solution is 1.25 times the required test concentration (maximum tested 80%). At least three-dilutions including at least two-dilutions in the active range	
Contact time and temperature	5, 15, 30 or 60 min at 20°C	

Method and reference	Dilution-neutralization (Preferentially)	Membrane filtration (If neutralization failed)
Elimination of the tested product in the subcultures	Transfer of microorganism-disinfectant mixture (1 mL) into suitable neutralizer validated simultaneously – (Dilution: 1/10)	Transfer of microorganism-disinfectant mixture (0.1 mL) into filtration apparatus. Wash under conditions validated simultaneously with possible use of neutralizer – (Dilution: 1/10)
Count of survivors	Survivor count in the neutralized mixture by inclusion in agar medium	Membrane placed on agar medium
Incubation	Incubation at 30°C (Further 24h or more with *A. niger*)	
Interpretation	Check that all controls fit with the fixed values and that the neutralizer (or the filtration procedure) is validated. Calculate the reduction in viability. The product passes the test if it demonstrates a 10^4 (or more) reduction in viability within 60 minutes (or less) with both microoragnisms. Further tests (phase 2, step 1 and step 2) are required to qualify the product for a specific use.	

Application test: bactericidal acitivity

Method and reference	EN 1276 (1997) – Phase 2 Step 1 suspension test	
	Dilution-neutralization (Preferentially)	Membrane filtration (If neutralization failed)
Objective	Bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic, and institutional areas	
Strains	*P. aeruginosa* ATCC 15442 *E. coli* ATCC 10536 *S. aureus* ATCC 6538 *E. hirae* ATCC 10541 Additional strains: *S. typhimurium, Lactobacillus brevis, E. cloacae* or any suitable strain according to the prescriptions of the standard	
Inoculum	Adjusted suspension of agar culture obtained under specified conditions Number of viable cells in presence of antiseptic or disinfectant: 1.5 x 10^7 to 5 x 10^7 cfu/mL	

Method and reference	Dilution-neutralization (Preferentially)	Membrane filtration (If neutralization failed)
Diluent for the product and tested concentrations	Standardized hard water (Distilled water for products ready to use). The product test solution is 1.25 times the required test concentration (maximum tested 80%). At least three-dilutions including at least one in the active range and one in the inactive range.	
Interfering substances	Bovine albumin: clean (0.3 g/L) and dirty (3 g/L) conditions; skimmed milk (1% v/v); yeast extract; sucrose; buffers, sodium laury sulfate according to the use of the product	
Contact time and temperature	5 min and additional time: 1, 15, 30, and 60 min 20°C and additional temperatures: 4°C, 10°C, or 40°C	
Elimination of the tested product in the subcultures	Transfer of microorganism-disinfectant mixture (1 mL) into suitable neutralizer validated simultaneously – (Dilution: 1/10)	Transfer of microorganism-disinfectant mixture (0.1 mL) into filtration apparatus. Wash under conditions validated simultaneously with possible use of neutralizer – (Dilution: 1/10)

Method and reference	Dilution-neutralization (Preferentially)	Membrane filtration (If neutralization failed)
Count of survivors	Survivor count in the neutralized mixture by inclusion in agar medium	Membrane placed on agar medium
Incubation	Incubation at 36°C or 37°C for 48h (or adapted conditions for additional strains)	
Interpretation	Check that all controls fit with the fixed values and that the neutralizer (or the filtration procedure) is validated. Calculate the reduction in viability for each strain and each experimental condition. • For general application: the product is bactericidal at the concentration that demonstrates a 10^5 (or more) reduction in viability within 5 min at 20°C, in clean or dirty condition, with each of the four reference strains. • For specific application: the product is bactericidal at the concentration that demonstrates a 10^5 (or more) reduction in viability within 5 min at 20°C, with each of the four reference strains and, if needed, with additional strains, in additional experimental conditions (exposure time, temperature, interfering substances). Further tests (phase 2, step 2) are required to qualify the product for a specific use.	

Application test: quantitative surface test bactericidal acitivity

Method and reference	WI 216028 (CEN Enquiry 1999) – Phase 2 Step 2 surface test
	Surface test without mechanical action
Objective	Bactericidal activity on surfaces of chemical disinfectants and antiseptics used in food, industrial, domestic, and institutional areas
Strains	*P. aeruginosa* ATCC 15442 *E. coli* ATCC 10536 *S. aureus* ATCC 6538 *E. hirae* ATCC 10541 Additional strains: *S. typhimurium, Lactobacillus brevis, E. cloacae* or any suitable strain according to the prescriptions of the standard
Inoculum	Adjusted suspension of agar culture obtained under specified conditions 1.5×10^8 to 5×10^8 cfu/mL Adjusted suspension mixed with an equal volume of the chosen interfering substance 0.05 mL of the mixture dropped on the carrier, dried at 37°C

Method and reference	Surface test without mechanical action
Diluent for the product and tested concentrations	Standardized hard water (Distilled water for products ready to use). At least three-dilutions including at least one in the active range and one in the inactive range. 0.1 mL of product dilution applied on the contaminated carrier
Contact time	5 min and additional time: 1, 15, 30, and 60 min
Temperature	18 °C to 25°C and additional temperatures: 4°C, 10°C, or 40°C
Interfering substances	Bovine albumin: clean (0.3 g/L) and dirty (3 g/L) conditions; skimmed milk (1% v/v); yeast extract; sucrose; buffers, sodium laury sulfate according to the use of the product
Carrier	Stainless steel discs (2cm diameter), washed, rinsed, and treated by isopropanol under specified conditions
Elimination of the tested product in the subcultures	Transfer of the carrier into suitable neutralizer (validated simultaneously) in presence of glass beads and shaking under specified conditions.

Method and reference	Surface test without mechanical action
Count of survivors	Survivor count in the neutralized mixture (undiluted and diluted) by inclusion in agar medium. Inclusion of the carrier in agar medium under specified conditions
Incubation	Incubation at 36°C or 37°C for 48 h
Interpretation	Check that the spontaneous death of inoculum cells on the carrier is less than 2 log. Check that the neutralizer validated. Check that for the active concentrations the number of cells. remaining on the carrier is less than 100. Calculate the reduction in viability for each strain and each experimental condition. • For general application: The product is bactericidal at the concentration that demonstrates a 10^4 (or more) reduction in viability within 5 min at 20 °C, in clean or dirty conditions, with the four reference strains. • For specific application: The product is bactericidal at the concentration that demonstrates a 10^4 (or more) reduction in viability within 5 min at 20 °C, in clean or dirty conditions, with the four reference strains, and, if needed, with additional strains, in additional experimental conditions (exposure time, temperature, interfering substances).

Hygienic handrub and handwash

- **To reduce the transient flora without necessarily affecting the resident skin flora**
- **Hygienic handrub**
  - **Treatment of hands with an antiseptic handrub**
  - **Broad spectrum and fast-acting, and persistent activity is not necessary.**
- **Hygienic handwash**
  - **Treatment of hands with an antiseptic handwash and water**
  - **Broad spectrum, but is usually less efficacious and acts more slowly than the hygienic handrub.**

Evaluation method disinfectant for hand hygiene

- **Classification of method to evaluate disinfectant**
  - **To evaluate elimination capability to transient pathogens from hands**
    - **Hands are experimentally contaminated with the test organism**
  - **To evaluate ability to reduce the naturally present resident flora**
- **Variations in protocols → Impossible of direct comparisons**
  - **Contamination of hand with a test organism before test**
  - **To contaminate fingers or hands**
  - **The volume of hand hygiene product applied**
  - **Contact time**
  - **The method used to recover the organism from the skin**

Standards for hand disinfectant evaluation

Region	Europe	USA/Canada
Name	European Standards (EN)	Standards of ASTM International (American Society for Testing and Materials)
Organization	European Committee for Standardization (CEN)	FDA, Health Canada
Protocols	EN 1499 (Hygienic handwash) EN 1500 (Hygienic handrub) EN 12791 (Surgical hand prep.)	ASTM E-1174 (Handwash) ASTM E-1838 (Fingerpad for virus) ASTM E-2276 (Fingerpad for bacteria) ASTM E-2613 (Fingerpad for fungi) ASTM E-2011 (Whole hand for virus) ASTM E-1115 (Surgical handrub)

Test method for handwash

Protocols	EN 1499	ASTM E-1174
Region	Europe	USA/Canada
Subject No.	12-15	*X*
Study design	Random cross-over	Baseline comparison
Test organisms	*E. coli* (K12)	*S. marcescens, E. coli*
Control	Soft soap	Baseline
Interpretation	Significant Log_{10} reduction	1^{st} use (< 5 min): 2-log_{10} reduction 10^{th} use (< 5 min): 3-log_{10} reduction
Hand preparation	• Hands washing (Soft soap) • Dry and immerse in broth • Remove excess fluid • Air dry for 3 min	• Hand contamination with 5 mL of suspension of test organisms

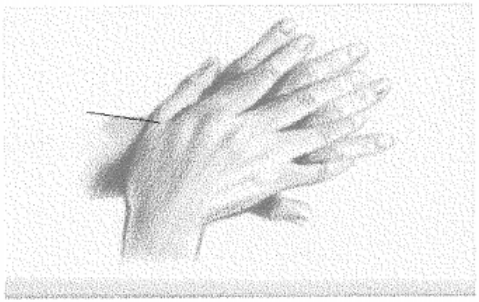
Protocols	EN 1499	ASTM E-1174
Baseline determination	• Kneading the fingertips of each hand separately for 60 seconds in 10 ml of broth without neutralizers.	X
Handwash	• Treated with the product (Within 1 min) • Teated with 20% solution of soft soap	• Treated with the product to hands and lower third of forearms
Final culture	• Recovery of bacteria for final values (see EN 1500).	• Hands and forearms rinsed with water • Nnumber of washes using 75 ml of eluent for each hand in glove • The eluates are tested for viable bacteria.

Chemical disinfectants and antiseptics- Hygienic handrub: EN 1500;1997)

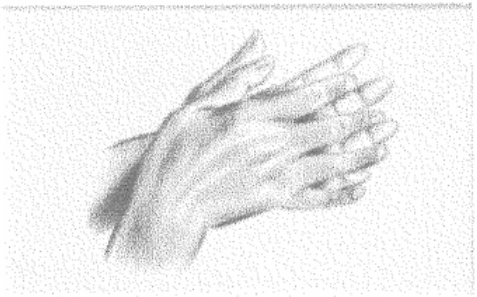
- **Objective**
  - Simulating test for hygienic handrub in practical conditions
  - Reduction of transient flora of artificially contaminated hands of volunteers (18-22 persons)
- **Strains**
  - *E. coli* K12 10083 (NCIMB)
  - Inoculum: 2 L of concentration of 2 x 10^8 and 2 x 10^9 cfu/mL
- **Application of the test organism**
  - Hands prepared by washing for 1 minute with soft soap
  - Dried thoroughly on paper towels
  - Hands were immersed to the mid-metacarpals for 5 sec, finger apart, to inoculum
  - Air dried for 3 min

- **Inoculation for Pre-values**
  - **Fingertips rubbed for 1 min in a Petri dish (10mL TSB)**
- **Application of disinfectant**
  - **European standards reference handrub procedure**
    - **3mL of disinfectant for 30 sec x 2 times**
    - **5 sec water rinsing of the finger from distal to proximal with fingertips upright under running tap water**
  - **Reference product: 60% propan-2-ol**
- **Sampling of survivors**
  - **After rinsing, immediately rubbing the fingertips and thumb for 1 min on the base of two Petri dishes containing media**
  - **Media: 10mL of TSB containing neutralizer**
- **Incubation**

1. Palm to palm



2. Left palm over right dorsum and right palm over left dorsum



3. Palm to palm, fingers interlaced

4. Backs of fingers to opposing palms with fingers interlocked

5. Rotational rubbing of right thumb clasped in left palm and vive versa

6. Rotational rubbing, backwards and forwards with clasped fingers of right hand in left palm and vice versa

- **Count the number of colony released from the fingertips of left and right hands of 15 volunteers before and after application**
- **Log10 conversion and mean of left and right hand**
- **Compare the effect of reference and tested disinfectant with statistical method**

Table 1 Reference handrub procedure - experimental results

Preparation: 60% propan-2-ol v/v
Application: Rub-in 3 ml/30s repeat once (6ml total over 60s)
Date of experiment: July 2004
Test organism: E. coli K 12 NCTC 10538
Suspension: 9.0×10^8

Number of cfu's per plate from dilution 10^x counts obtained from 0.1 ml

Number of cfu's per plate from dilution 10^x counts obtained from 0.5 ml

Subject No.	Hand L/R	Pre-values 10^{-3}		10^{-4}		10^{-5}		10^{-6}		Post-values 10^{0}*		10^{1}*	
1	L	+	+	+	+	7	10	1	0	12	15	0	0
	R	+	+	+	+	13	5	0	2	17	23	1	4
2	L	+	+	61	42	9	6	1	1	10	8	1	0
	R	+	+	27	29	2	6	0	1	5	2	1	1
3	L	+	+	+	+	13	10	2	1	49	61	9	4
	R	+	+	+	+	5	11	1	0	76	76	15	8
4	L	+	+	+	+	8	7	0	0	0	0	0	0
	R	+	+	+	+	6	9	1	2	4	1	0	0
5	L	+	+	+	82	14	10	-	-	3	2	1	0
	R	+	+	60	87	9	8	-	-	17	12	1	0
6	L	+	+	93	77	0	12	0	2	69	76	10	2
	R	+	+	71	90	17	3	3	0	14	13	1	0
7	L	+	+	91	84	13	12	-	-	3	0	0	0
	R	+	+	93	93	17	11	-	-	1	0	0	0
8	L	+	+	88	91	8	9	-	-	55	10	0	0
	R	+	+	77	80	11	8	-	-	50	5	0	0
9	L	+	+	104	105	8	18	-	-	2	5	0	0
	R	+	+	444	104	7	13	-	-	6	5	0	2
10	L	+	+	46	31	5	4	-	-	1	0	0	0
	R	+	+	38	32	4	7	-	-	1	1	1	0
11	L	+	+	83	61	8	5	-	-	1	2	1	0
	R	+	+	81	68	19	8	-	-	1	1	0	0
12	L	+	+	31	36	2	2	-	-	47	38	4	1
	R	+	+	25	25	2	0	-	-	95	96	8	13
13	L	+	+	13	17	1	1	-	-	2	0	0	0
	R	+	+	29	24	5	2	-	-	1	0	0	0
14	L	+	+	+	+	21	17	-	-	+	+	69	64
	R	+	+	+	+	36	28	-	-	115	101	9	8
15	L	+	+	38	38	2	5	-	-	5	7	0	0
	R	+	+	35	32	5	1	-	-	9	8	0	0

Underlined: count used for further computation
+ = Too many to count
Spaces indicate no values available
• Counts obtained from 0.5ml

NB – in precount (10^{-6}) = no count available

Table 2 Test handrub procedure - experimental results

Preparation: Esence handrub disinfectant
Application: Rub-in 3 ml/30s repeat once (6ml total over 60s)
Date of experiment: July 2004
Test organism: E. coli K 12 NCTC 10538
Suspension: 9.0×10^8

Number of cfu's per plate from dilution 10^x counts obtained from 0.1 ml

Number of cfu's per plate from dilution 10^x counts obtained from 0.5 ml

Subject No.	Hand L/R	Pre-values 10^{-3}		10^{-4}		10^{-5}		10^{-6}		Post-values 10^{0}*		10^{1}*	
1	L	+	+	+	+	9	17	1	3	9	5	0	0
	R	+	+	+	+	10	23	0	1	23	23	3	2
2	L	+	+	18	19	1	3	0	1	1	2	0	0
	R	+	+	15	22	2	2	0	0	51	58	4	4
3	L	+	+	+	+	25	16	0	2	30	35	0	5
	R	+	+	+	+	8	14	0	2	33	56	6	6
4	L	+	+	+	+	7	11	0	0	1	0	0	0
	R	+	+	+	+	8	5	2	0	2	0	1	0
5	L	+	+	81	76	11	1	-	-	6	2	0	0
	R	+	+	83	97	17	16	-	-	2	1	0	0
6	L	+	+	88	78	10	3	0	0	13	17	0	1
	R	+	+	+	+	8	6	2	3	9	13	3	1
7	L	+	+	67	77	12	9	-	-	40	38	10	7
	R	+	+	96	82	12	13	-	-	10	16	0	0
8	L	+	+	90	87	9	6	-	-	51	70	7	10
	R	+	+	78	86	7	12	-	-	101	95	1	13
9	L	+	+	+	+	19	15	-	-	3	6	0	0
	R	+	+	99	112	16	12	-	-	8	12	1	0
10	L	+	+	68	64	7	8	-	-	8	2	0	0
	R	+	+	94	70	9	7	-	-	2	3	0	0
11	L	+	+	89	105	6	15	-	-	2	0	0	0
	R	+	+	69	68	8	10	-	-	7	9	0	0
12	L	+	+	13	9	1	2	-	-	2	14	2	0
	R	+	+	24	45	4	0	-	-	75	98	8	13
13	L	+	+	25	27	4	2	-	-	6	12	0	1
	R	+	+	18	18	1	0	-	-	11	14	0	0
14	L	+	+	100	83	15	8	-	-	122	111	11	18
	R	+	+	+	+	10	11	-	-	48	45	6	2
15	L	+	+	25	28	4	4	-	-	4	8	2	1
	R	+	+	17	18	3	1	-	-	5	4	1	0

Underlined: count used for further computation
+ = Too many to count
Spaces indicate no values available
• Counts obtained from 0.5ml

NB – in pre-count (10^{-6}) = no count available

Volunteer	REFERENCE propan 2 ol (60%) 3ml x 2 (Total =60s)			Ebiox EsenseHand Disinfectant 3ml x 1 (30s) then 3ml x 1 (30s) (Total = 60s)		
	X	Y	Z	X	Y	Z
1	6.91	1.52	5.39	7.13	1.42	5.71
2	6.67	1.14	5.53	6.28	1.23	5.05
3	6.97	2.18	4.79	7.10	1.89	5.21
4	6.97	0.35	6.62	6.94	0.15	6.79
5	6.97	1.08	5.89	6.98	0.69	6.29
6	6.91	1.70	5.21	7.04	1.40	5.64
7	7.05	0.24	6.81	6.99	1.75	5.24
8	6.94	1.78	5.16	6.93	2.20	4.73
9	7.05	0.95	6.10	7.16	1.13	6.03
10	6.64	0.15	6.49	6.88	0.85	6.03
11	6.93	0.39	6.54	6.95	0.75	6.20
12	6.34	2.07	4.27	6.28	1.77	4.51
13	6.30	0.15	6.15	6.26	1.33	4.93
14	7.40	2.71	4.69	7.02	2.18	4.84
15	6.53	1.16	5.37	6.40	1.02	5.38
mean	**6.84**	**1.17**	**5.67**	**6.82**	**1.32**	**5.50**
S	**0.29**	**0.81**	**0.78**	**0.33**	**0.58**	**0.66**
N	**15**	**15**	**15**	**15**	**15**	**15**

X = $\log_{10}$ pre values — mean value from left and right hands
Y = $\log_{10}$ post values — mean value from left and right hands
Z = X-Y $\log_{10}$ reduction
S = standard deviation
N = sample size

Fingerpad method (ASTM-E)

- Applied to handwash and handrub easily
- Lower risk to subjects
- 10 µL of test virus suspension in soil
- Apply to center of each thumb- and fingerpad
- Dried and exposed to 1 mL of test formulation for 10-30 s
- Eluation and culture or specific assay

ASTM	E-1838	E-2276	E-2613
Target	VIrus	Bacteria	Fungus
Organisms	Adeno, Rota, Rhino, Hepatitis A	*E. coli, S. marcescens, S. aureus, S. epidermidis*	*C. albicans, A. niger*

Test method for surgical hand prep

Protocols	EN 12791	ASTM E-1115
Region	Europe	USA/Canada
Subject No.	18-20	X
Study design	Cross-over design (1 week interval between reference and test runs)	Baseline comparison
Test organisms	Resident skin flora	Resident skin flora
Control	N-propanol 60% (3min)	Baseline
Interpretation	Not be inferior to Log_{10} reduction in immediate and 3 hour	Day 1 (< 1 min): 1-log_{10} reduction Day 2 (< 1 min): 2-log_{10} reduction Day 5 (< 1 min): 3-log_{10} reduction
Purspose	Test bactericidal effect	Reduction in bacterial flora Immediate and persistent Single or repetitive treatment