

# Methods for the Evaluation of Hand Disinfectants

Dr. Manfred Rotter, University of Vienna, Austria

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## Methods for the Evaluation of Hand Disinfectants



Manfred L. Rotter

Institute of Hygiene and Medical Microbiology  
Medical University of Vienna

Hosted by Paul Webber  
[paul@webbertraining.com](mailto:paul@webbertraining.com)  
[www.webbertraining.com](http://www.webbertraining.com)

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## Evaluation of Hand Disinfectants (1) Parameter: Reduction of bioburden

### ➤ *In vitro*

- MIC of disinfectant vs. selected strains
- MBC of disinfectant vs. selected strains
- Quantitative suspension tests for (bacteri-, fungi-, viru-)cidal properties
- Kill-time studies suspension tests

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## Evaluation of Hand Disinfectants (2)

### ➤ *In vivo*

- Controlled laboratory tests simulating practical conditions on hands of volunteers
- Field trials

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## Evaluation of Hand Disinfectants (3) Parameter: Reduction of infections

### ➤ Clinical trial

- Comparative trial with the aim  
⇒ Novum > Reference
- Equivalence study

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### SAMPLE SIZE NECESSARY FOR SIGNIFICANT DIFFERENCE OF PROPORTIONS (Example for comparative trial)

|  |                    |
|--|--------------------|
| PRESENT RATIO OF HAND-TRANSMITTED NOSOCOMIAL INFECTIONS $p_1$      | 2 %                |
| Desired reduction of infection ratio $p_2$                         | 50 %               |
| NEW RATIO OF HAND-TRANSMITTED NOSOCOMIAL INFECTIONS intended $p_2$ | 1 %                |
| Level of significance (one-sided)                                  | $\alpha = 5 %$     |
| Power of statistical test  | $1 - \beta = 90 %$ |
| SAMPLE SIZE<br>(= Number patients per experimental arm)            | 2500               |

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## Laboratory Test Methods

Hygienic Hand Disinfection:  
(Hygienic Hand *Wash* and Hygienic Hand *Rub*)

*In vitro*

*In vivo*

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## Bactericidal Properties of Hand Disinfectants Proposed Suspension test prEN 12054

*In vitro*

Test organisms

*Staphylococcus aureus*  
*Pseudomonas aeruginosa*  
*Escherichia coli* K 12  
*Enterococcus hirae*

Temperature

20°C

Contact time

- hygienic

1 min (optional 0,5 min)

- surgical

5 min (optional 1,2,3,4 min)

Requirement

reduction: rub 5.0 lg,  
wash 3.0 lg

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## HYGIENIC HANDRUB/HANDWASH

REFERENCE  
ONE HALF OF  
VOLUNTEERS

UNDER TEST  
OTHER HALF OF  
VOLUNTEERS

CONTAMINATION  
*E. COLI* MTC  
1000  
AIR DRY (2min)

PRE-DISINFECTION  
SAMPLING (1min)

DISINFECTION

POST-DISINFECTION  
SAMPLING (1min)

In 2nd test: same volunteers  
perform test with changed roles

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## Hygienic Handwash with antiseptic soap – EN 1499. Simulating practical Conditions

*In vivo*

Volunteers

12-15

Test organism

*Escherichia coli* K 12

Recovery

Fingertip rub before and after treatment

Application

- product

30 or 60 s, according to manufacturer

- reference

60 s handwash with unmedicated soap

Requirement

Product significantly ( $p = 0.01$  unidirectional)  
more efficacious than soap

Discrimination

Means  $\geq 0.5$  lg different (Power: 0.90)

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## Hygienic Handrub – EN 1500 Simulating practical Conditions

*In vivo*

Volunteers

12-15

Test organism

*Escherichia coli* K 12

Recovery

Fingertip rub before and after treatment

Application:

- product

30 or 60 s, according to manufacturer

- reference

2 x 30s (=60 s) handrub with 2x3 ml

60% (vol) 2-propanol

Requirement

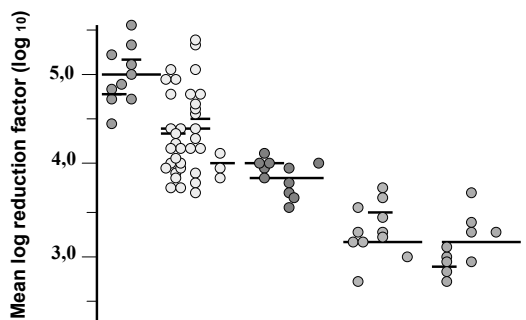
Product not significantly ( $p = 0.1$ , unidir.)  
less efficacious than 2-propanol 60%, 1min

Discrimination

Means  $\geq 0.6$  lg different (Power: 0.95)

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Place ...  
Preparation: n-Propanol 50%  
Iso-Propanol 60% Standard  
Ethanol 70% + chlorhex. 0,5 %  
Povidone-I-0,75% soap  
Phenolic soap

## Hygienic Hand Disinfection – ANOVA: non-standardized Results (lg RF<sub>Pi</sub>): 5 agents, 5 repetitions, 2 laboratories, with 15 volunteers in each

| Source of Variation | DF  | Mean Squares | F     | P       |
|---------------------|-----|--------------|-------|---------|
| Agents (5)          | 4   | 102,4        | 327,5 | <0,0001 |
| Volunteers (15)     | 14  | 2,6          | 8,1   | <0,0001 |
| Laboratories (2)    | 1   | 5,8          | 18,6  | <0,0001 |
| PxV                 | 42  | 0,7          | 2,2   | <0,0001 |
| VxL                 | 3   | 3,4          | 10,8  | <0,0001 |
| PxL                 | 14  | 3,1          | 10,0  | <0,0001 |
| PxVxL               | 42  | 0,7          | 2,4   | <0,0001 |
| Error               | 470 | 0,3          |       |         |

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**Hygienic Hand Disinfection – ANOVA:**  
**standardized results (lg RF<sub>Pre</sub>-lg RF<sub>Post</sub>):**  
4 [products–reference], 5 repetitions, 2 laboratories,  
with 15 volunteers in each

| Source of Variation    | DF         | Mean SQ    | F     | P        |
|------------------------|------------|------------|-------|----------|
| Products-Reference (4) | 3          | 90,2       | 117,5 | <0,00001 |
| Volunteers (15)        | 14         | 2,7        | 3,5   | <0,00001 |
| Laboratories (2)       | 1          | 3,5        | 4,6   | n.s.     |
| PxV                    | 42         | 0,9        | 1,1   | n.s.     |
| VxL                    | 3          | 1,6        | 2,1   | n.s.     |
| PxL                    | 14         | 1,7        | 2,1   | <0,01    |
| PxVxL                  | 42         | 0,9        | 1,2   | n.s.     |
| <b>Error</b>           | <b>466</b> | <b>0,8</b> |       |          |

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**Health-Care Antiseptic Drug Products-FDA (1)**

*In vitro*

- **Antibacterial spectrum** of
  - active ingredient
  - carrier
  - both
- **MICs** with 20 microbial species, 50 strains of each (50% fresh clinical strains)
  - 9 gramnegative spp.
  - 10 grampositive spp.
  - Candida (incl. C. albicans) (= approx. 1000 strains)
- **Development of resistance study**
- **Time-kill study** (suspension test) with selected strains for 0, 3, 6, 9, 12, 15, 20, 30 min

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**Health-Care Antiseptic Drug Products - FDA (2)**  
Antiseptic handwash and Health-Care Personnel handwash  
(originally ASTM E 1174)

*In vivo*

- **Volunteers (N):** approx. total of 108
  - Test preparation: approx. 54
  - Positive control: approx. 54
- **Contaminant bacterium:** *Serratia marcescens*, (*E. coli*)
- **Contamination and application:** 10 times on an experimental day (Test and control in parallel)
- **Samplings:**
  - after 1st contamination (baseline)
  - after 1st, 3rd, 7th, 10th wash (rub)
- **Required reduction within 5min:**
  - after 1st handwash: 2 lg
  - after 10th handwash: 3 lg

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**Standard Test Method for Determining the Bacteria Eliminating Effectiveness of Hygienic Handwash and Handrub. ASTM E 2276**

*In vivo*  
(Similar to “Virus-eliminating“ Test acc. to ASTM E-1838)

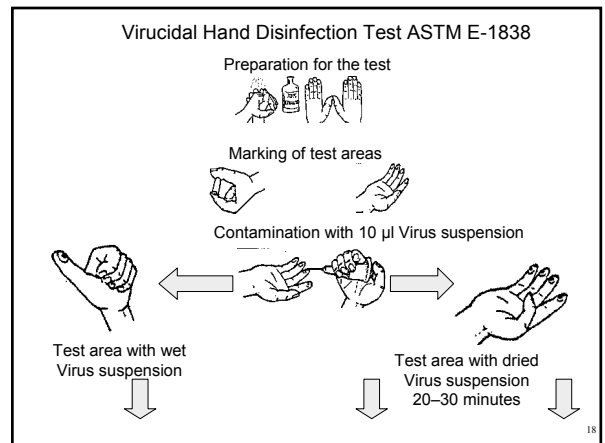
Finger Pads including thumbs of at least 2 volunteers  
Test bacteria: *S. marcescens*, *E. coli*, *S. aureus*, *S.epiderm.*  
Requirement: not defined, but in comparison to a negative and positive control

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**Virucidal Hand Disinfection Tests**

| Suspension Tests  |            | In vivo Tests                          |                                   |
|-------------------|------------|--|-----------------------------------|
|                   |            | Finger pad                             | Whole hand                        |
| <u>prEN 14476</u> | <u>DVV</u> | <u>ASTM E-1838</u><br>(acc. to Sattar) | <u>ASTM E-2011</u><br>(Steinmann) |
| Polio 1           | Polio 1    | Adeno (human 4)                        | Rota (human)                      |
| Adeno 5           | Adeno      | Rota (human) Wa                        | Rhino (human)                     |
|                   | Vaccinia   | Rhino (human) 37                       | Hepatitis A HM-175                |
|                   | SV 40      | Hepatitis A HM-175                     |                                   |
| 4.0 lg            | 4.0 lg     | not defined                            | not defined                       |

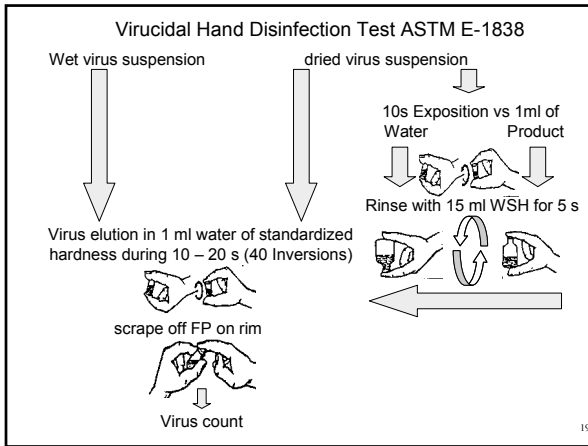
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## Laboratory Test Methods

**Surgical Hand Disinfection:**  
(Surgical Hand *Wash* and Surgical Hand *Rub*)

*In vitro*  
*In vivo*

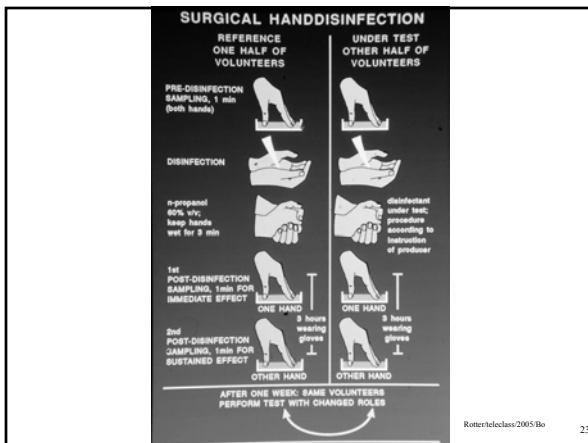
## Laboratory Test Methods

**Surgical Hand Disinfection:**  
*In vitro*

*Same Test Method as for Hygienic Hand Disinfection*

## Laboratory Test Methods

**Surgical Hand Disinfection:**  
*In vivo*



**Surgical Handrub/wash – prEN 12791**  
**Simulating practical conditions**

*In vivo*

Volunteers (N): 18-20

Test organism: Resident skin flora

Recovery: Fingertip rub before and after treatment

Application of

- Product: acc. to manufacturer, max.5 min handrub/wash
- Reference: 3 min handrub with 60%/vol 1-propanol (nx3 ml)

Immediate effect sample: immediately after end of treatment (one hand)

3-hours effect sample: 3 hrs after end of treatment (other [gloved] hand)

Requirement: Product not significantly (imm: p=0.1; 3-hrs: 2p =0.01) less efficacious than reference

Discrimination: imm. Effect: Means  $\geq 0.5$  lg different (Power: 0.95)

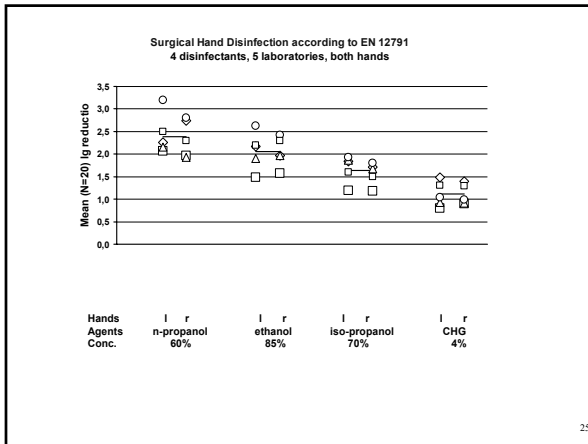
Sustained effect: Optional claim: At 3 hrs, product significantly (p=0.01, unidirectional) more efficacious than reference

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Surgical Hand Disinfection - ANOVA :  
non-standardized results (lgRF<sub>i</sub>);  
4 agents , 2 hands, 5 laboratories, 20 volunteers in each

| Source of Variation | DF | F     | p         |
|---------------------|----|-------|-----------|
| 1 Agents (4)        | 3  | 53,97 | < 0,001 * |
| 2 Laboratories (5)  | 4  | 5,22  | < 0,001 * |
| 3 Hands (2)         | 1  | 4,93  | 0,029     |
| 1 x 2               | 12 | 1,67  | 0,073     |
| 1 x 3               | 4  | 1,37  | 0,249     |
| 2 x 3               | 3  | 0,66  | 0,579     |
| 1 x 2 x 3           | 12 | 1,55  | 0,107     |

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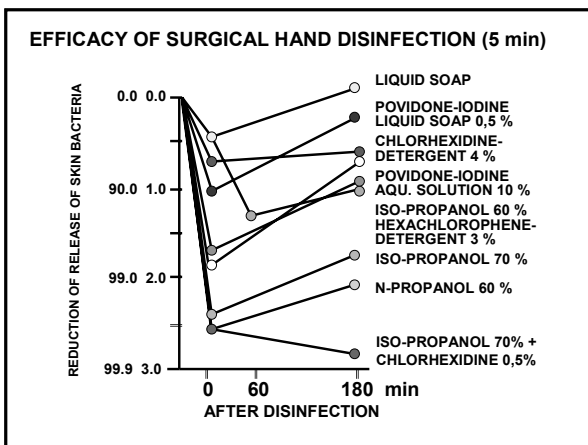
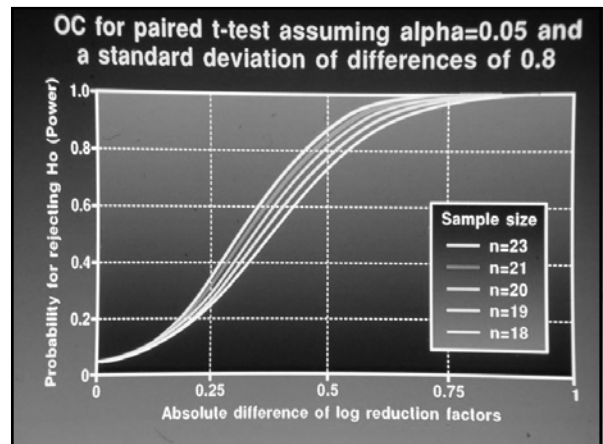
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Surgical Hand Disinfection - ANOVA :  
standardized results (lgRF<sub>Pi</sub> - lgRF<sub>Ri</sub>):  
3 [products -reference], 5 laboratories, 2 hands,  
20 volunteers in each

| Source of Variation        | DF | F     | p         |
|----------------------------|----|-------|-----------|
| 1 [Products-Reference] (3) | 2  | 42,16 | < 0,001 * |
| 2 Laboratories (5)         | 4  | 1,67  | 0,162     |
| 3 Hands (2)                | 1  | 0,70  | 0,404     |
| 1 x 2                      | 8  | 1,66  | 0,109     |
| 1 x 3                      | 4  | 3,41  | 0,012     |
| 2 x 3                      | 2  | 0,63  | 0,533     |
| 1 x 2 x 3                  | 8  | 0,40  | 0,918     |

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Surgical Hand Disinfection: FDA (orig. ASTM E 1115)

Volunteers (N): approx: 100 (150)  
per arm:

- Test: approx. 50
- Positive control: approx. 50
- ( Placebo ) ( 50 )

Testbacteria: normal resident handflora

Application of Product:  
acc. to manufacturer's instruction or  
without any: apply product 2 x 5 min, then  
rinse hands for 1 min

„Baseline“:  
rinse hands for 30 s, wash hands for 30s,  
rinse hands for 30s

Positive control: FDA-approved antiseptic; all parameters  
as product; concurrent testing

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## Surgical Hand Disinfection: FDA *in-vivo* model Schedule for disinfecting and sampling and required lg bacterial reduction

| Sampling<br>Times (hrs) | Day of test periode |    |   |   |     |
|-------------------------|---------------------|----|---|---|-----|
|                         | 1                   | 2  | 3 | 4 | 5   |
| 1/60                    | lg                  | ⊗  | x | x | ⊗   |
| 3                       | O                   | ⊗  | x | x | O   |
| 6                       | <bl                 | lg | x | x | Olg |

X: Desinfection: Day 1 (1/60), day 2, 3, 4 (1/60, 3, 6), day 5 (1/60)

O: Sampling: Day 1, 2 and 5: after 1/60, 3 and 6 hrs with gloves

bl: Baseline

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Thank You for Your Attention

Further Information is available!

## Other 2005 Teleclasses

For more information, refer to  
[www.webbertraining.com/schedule.cfm](http://www.webbertraining.com/schedule.cfm)

- April 21 – Creutzfeldt-Jakob Disease: Recommendations for Disinfection and Sterilization with Dr. William Rutala
- April 28 – Overcoming the Resistance of Biofilms with Dr. Peter Gilbert  
Sponsored by Virox Technologies Inc. [www.virox.com](http://www.virox.com)
- May 19 – Antiseptic Practice & Procedure with Susan Crow  
Sponsored by 3M Canada [www.3m.ca](http://www.3m.ca)
- May 26 – Canadian Response to West Nile Virus with Dr. Paul Sockett
- June 7 – Measuring the Cost of Hospital Infection with Dr. Barry Cookson

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