

Table 1. Comparison of selected hand hygiene efficacy test methods by key step or variable

Key Step or Variable	ASTM E2783 (Time Kill)	EN 1276	Chlorine Equivalency former USDA E2/E3 rating)	ASTM E1174	ASTM E2755	ASTM E2946	ASTM E2011	EN 1499	EN 1500
Vitro/vivo	In Vitro	In Vitro	In Vitro	In Vivo	In Vivo	In Vivo	In Vivo	In Vivo	In Vivo
Purpose / Target Application in Design	"In vitro" hand hygiene product evaluation	"In vitro" antimicrobial activity of disinfectants and hand hygiene products	<ul style="list-style-type: none"> "In vitro" designed to test efficacy of halogen based disinfectants and sanitizers 	"In vivo" product evaluation ("healthcare personnel hand wash")	"In vivo" activity of hand hygiene personnel hand rubs	"In vivo" activity of food handler hand hygiene formulations	"In vivo" antiviral activity of hand hygiene formulations	"In vivo" hand washes – ensure a minimum performance standard	"In vivo" hand rubs – ensure a minimum performance standard
Test Organism(s)	Any BSL 1 or 2 organisms; we could recommend a specific list that are highly food relevant (e.g. e. Coli, listeria, salmonella, etc.)	<i>Ps. aeruginosa</i> ATCC 15442, <i>E. coli</i> ATCC 10536, <i>S. aureus</i> ATCC 6538, <i>Enterococcus hirae</i> ATCC 10541	<i>S. aureus</i> ATCC 6538 <i>S. typhi</i> ATCC 6539	<i>Serratia marcescens</i> and <i>E. coli</i>	<i>Serratia marcescens</i> ATCC 14756 <i>S. aureus</i> ATCC 6538, or 33591	<i>E. coli</i> ATCC 11229	Human Rotavirus, Human Rhinovirus Type 37, Feline calicivirus, Human Adenovirus Type 5	<i>E. coli</i> K12 NCTC 10538	<i>E. coli</i> K12 NCTC 10538

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Soil Type(s):	None	Flexible: Can be chosen based on the condition of use	Inoculated broth	4.5 mL of inoculums in nutrient broth	0.2 mL of inoculum in nutrient broth	Beef broth is “moderate” soil, Hamburger is “heavy” soil	Bovine serum	Inoculated broth	Inoculated broth
Soil Load (Quantity):	Volume of the inoculum in Nutrient broth used	0.3g/L clean conditions; 3 g/L dirty conditions	10 µl of inoculated broth for tube 1 and total 100 µl for tube 10	4.5 mL of inoculums in Nutrient broth	0.2 mL of inoculum in nutrient broth	4.5 mL of Beef broth for moderate soil Handling contaminated hamburger for 2 min	5% in the virus inoculum	Amount of inoculated broth which ends up on the hands during immersion of the hands	None specifically added. Just dried TSB from inoculating broth
Method of Contamination:	Inoculation of the product	Inoculation of the product	Inoculation of the product	3-1.5 mL of an overnight broth culture of the test organism	200µl of a concentrated broth suspension of the test organism	4.5 mL of Beef broth for moderate soil Handling contaminated hamburger for 2 min	1.5 mL of the suspension, 90 sec spread, 90 sec dry Or 20µL of virus suspension on each finger tip	Immersion into seeded broth	Immersion into seeded broth
Baseline Recovery (Pre-Test Value):	Not specified	1.5×10^8 - 5×10^8	N/A	5×10^8 - 1×10^9 Liquid suspension used for contamination. Recovery is not specified	$\geq 10^8$ cfu/hand (Usually 8.5-9.0 log ₁₀ cfu/hand)	Suspension 1×10^8	The virus “pull” shall contain $\geq 10^7$ infective unit/mL	Inoculum 2×10^8 - 2×10^9 Log pre-values at least 5	Inoculum 2×10^8 - 2×10^9 Log pre-values at least 5 per mL

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Test Article Application Details:	N/A	N/A	N/A	5 mL of the test product during handwashing using 40°C water for 1 min handwashing	1.5 ml of a test material (calculations for foaming materials provided)	5 mL of the test material Wash for 30±5 sec, rinse for 30±5 sec	Volume specified by manufacturer	3 ml applied and washed for 30 or 60 sec +15 sec rinse or following manufacturer instructions	3 ml applied and rubbed for 30 seconds, then sampled
Number of Subjects / Replicates (Minimum, Recommended)	N/A	N/A	N/A	Not specified FDA CDER asks for at least 12 subjects	At least 8 subjects Total depends on number of test materials, study purpose, and regulatory requirements governing the study.	At least 8 subjects	At least 6 subjects	At least 12 subjects	18-22 subjects
Internal Reference:	None	None	Referenced Chlorine solution	None	None	None	None	Soft soap (British Pharmacopoeia 1993) 200g/L	2x3ml of 60% isopropanol rubbed for 60 seconds total
Acceptance Criteria:	None	5 log reduction	Test article is at least equivalent to 50 ppm chlorine	None in the test method. Per 2015 FDA HC TFM: 2 Logs after the 1 st application, 3 Logs after 10 th application	None in the test method.	None in the test method.	None in the test method	Statistically non-inferior to the reference product	Statistically non-inferior to the reference product

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Can bland Handwash be a benchmark?	Yes, not in the test method	N/A	N/A	Yes, not in the test method	N/A	Yes, not in the test method	Yes, not in the test method	N/A	N/A
Product dilution	Undiluted	Undiluted	Undiluted	Undiluted	Undiluted	Undiluted	Undiluted	Undiluted	Undiluted
Contact time	Flexible; most typical is 15 sec, 30 sec and 60 sec.	5 min	1, 2.5 and 5 min	30 sec lather + 30 sec rinse	1.5 mL application volume, Rub until hands are dry. Or manufacturer's recommendations	30±5 sec	10-20 sec for handwash, 20-30 sec for hand rub, or other times representative use condition time	30 or 60 sec +15 sec rinse or following manufacturer instructions	30 sec

Table 2. Comparison of selected hand hygiene test methods by strengths and limitations and suitability for inclusion in Model Food Code

Method	Strengths	Limitations	Expected variability and reproducibility	Relevance and Fit for Food Code (H/M/L)	Recommended for CFP & Food Code
ASTM E2783 (Time Kill)	<p>“In vitro” test, relatively inexpensive, can be run with many organisms and by many labs with good reproducibility.</p> <p>Large amount of data and experience using this method</p>	<p>“In vitro” test (i.e. results will not necessarily predict real world hand hygiene results or the <i>in-vivo</i> methods)</p>	<p>Results more variable when the product has high foam; results are highly dependent of the mixing technique</p>	<p>High: Good screening test, should be required as a means to ensure broad spectrum antimicrobial effectiveness before “in vivo” testing.</p>	Yes
Chlorine Equivalency	<p>“In vitro” test. Long history of use</p>	<p>Risks posed by working with <i>S. typhi</i> (typhoid fever)</p> <p>Data is not relevant for hand antiseptics in general, especially those that do not contain halogen based active ingredients</p>	<p>Products with border line efficacy have high variability in results</p>	<p>Low</p>	No
EN 1276	<p>“In vitro” test</p> <p>Includes options of soils to be added, based on the industry. Could be tested for clean and dirty conditions</p>	<p>Some of microorganisms are not relevant for food retail use</p> <p>The test method is not designed for chemistries affected by soil</p>	<p>No</p>	<p>Low</p>	No
ASTM 1174	<p>“In vivo” test</p>	<p>Designed for healthcare</p>	<p>Fair reproducibility</p>	<p>Medium</p>	No

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Method	Strengths	Limitations	Expected variability and reproducibility	Relevance and Fit for Food Code (H/M/L)	Recommended for CFP & Food Code
	A lot of data available for this test	applications No soil used besides the inoculum broth <i>E. coli</i> (not <i>Serratia</i>) should be required for food retail application	Cannot compare across tests		
ASTM E2755	"In vivo"	Price of the test (relatively expensive) Some of microorganisms are not relevant for food retail use	Fair reproducibility Cannot compare across tests	Medium	No
ASTM E2946	"In vivo" test Designed for food handler applications (bacteria) Two different food relevant soils (moderate and heavy)	Recently released, so limited experience with the method	Fair reproducibility Cannot compare across tests	High	Yes
ASTM E2011	"In vivo" test	No soil used besides the inoculum broth Viruses only Viruses are not included in FDA CDER Monograph for hand antiseptics.	Fair reproducibility Cannot compare across tests	Medium (viruses only)	No

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Method	Strengths	Limitations	Expected variability and reproducibility	Relevance and Fit for Food Code (H/M/L)	Recommended for CFP & Food Code
EN 1499	"In vivo" test	Designed for healthcare applications Limited history of use in US	No	Low	No
EN 1500	"In vivo" test	Designed for healthcare applications Limited history of use in US	No	Low	No