**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 | * “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.)* | *Ps. aeruginosa ATCC 15442, E. coli ATCC 10536,* *S. aureus ATCC 6538, Enterococcus hirae ATCC 10541* | *S. aureus ATCC 6538**S. typhi ATCC 6539* | *Serratia marcescens and E. coli* | *Serratia marcescens ATCC 14756**S. aureus ATCC 6538, or 33591* | *E. coli ATCC 11229* | *Human Rotavirus, Human Rhinovirus Type 37, Feline calicivirus, Human Adenovirus Type 5* | *E. coli K12 NCTC 10538* | *E. coli K12 NCTC 10538* |
| **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 soilHandling 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 soilHandling contaminated hamburger for 2 min | 1.5 mL of the suspension, 90 sec spread, 90 sec dryOr 20µL of virus suspension on each finger tip | Immersion into seeded broth | Immersion into seeded broth |
| **Baseline Recovery (Pre-Test Value):** | Not specified | 1.5x108-5x108 | N/A | 5x108-1x109Liquid suspension used for contamination. Recovery is not specified | ≥108 cfu/hand (Usually 8.5-9.0 log10 cfu/hand) | Suspension 1x108  | The virus “pull” shall contain ≥107 infective unit/mL | Inoculum 2x108-2x109Log pre-values at least 5 | Inoculum 2x108-2x 109Log pre-values at least 5 per mL |
| **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 materialWash 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 specifiedFDA 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 1st application, 3 Logs after 10th 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 |
| **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)** | “In vitro” test, relatively inexpensive, can be run with many organisms and by many labs with good reproducibility. Large amount of data and experience using this method | “In vitro” test (i.e. results will not necessarily predict real world hand hygiene results or the *in-vivo* methods) | Results more variable when the product has high foam; results are highly dependent of the mixing technique | High: Good screening test, should be required as a means to ensure broad spectrum antimicrobial effectiveness before “in vivo” testing. | **Yes** |
| **Chlorine Equivalency**  | “In vitro” test. Long history of use | Risks posed by working with *S. typhi* (typhoid fever)Data is not relevant for hand antiseptics in general, especially those that do not contain halogen based active ingredients | Products with border line efficacy have high variability in results  | Low | No |
| **EN 1276** | “In vitro” testIncludes options of soils to be added, based on the industry. Could be tested for clean and dirty conditions | Some of microorganisms are not relevant for food retail useThe test method is not designed for chemistries affected by soil | No | Low | No |
| **ASTM 1174** | “In vivo” testA lot of data available for this test | Designed for healthcare applicationsNo soil used besides the inoculum broth*E. coli* (not *Serratia*) should be required for food retail application | Fair reproducibilityCannot compare across tests | Medium | No |
| **ASTM E2755** | “In vivo” | Price of the test (relatively expensive)Some of microorganisms are not relevant for food retail use | Fair reproducibilityCannot 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 reproducibilityCannot compare across tests | High | **Yes** |
| **ASTM E2011** | “In vivo” test | No soil used besides the inoculum brothViruses onlyViruses are not included in FDA CDER Monograph for hand antiseptics. | Fair reproducibilityCannot compare across tests | Medium (viruses only) | No |
| **EN 1499** | “In vivo” test | Designed for healthcare applicationsLimited history of use in US | No | Low | No |
| **EN 1500** | “In vivo” test | Designed for healthcare applicationsLimited history of use in US | No | Low | No |