

**Supplementary Document S3.** In-use test / Kelsey and Maurer test procedure (Kelsey & Maurer, 1966; Khor & Jegathesan, 1977)

The Kelsey and Maurer “in-use” test is a test that determines if an antiseptic/disinfectant is bacteriologically contaminated in the hospital practice, for the duration of its use. The test consists of:

- To dilute the antiseptic/disinfectant (original article describes the test for an antiseptic for disinfectants from buckets at the end of cleaning session, liquid disinfectant squeezed from mops, from brushes, liquids in which urine bottles are rinsed and stored, laboratory discard jars, linen storage tanks ...) to be tested at 1/10 (1 ml of disinfectant in 9 ml of Ringer’s solution or culture broth with neutralizer for non-phenolic disinfectants): the diluent for alcohols, aldehydes, chlorine-based, and phenol compounds is usually nutrient broth, while for chlorhexidine, quaternary ammonium compounds, iodophors, and antiseptic soaps, nutrient broth is supplemented with neutralizer (e.g. Tween 80) is used as diluent. The test was further extended to disinfectants in stock containers. The appropriate neutralizer can be found in the manufacturer indications.
- The dilution is inoculated in duplicate on nutrient agar plates within an hour: ten drops (one drop = 20-25 µl) are inoculated on each agar plate
- One of the plates is incubated at 35-37°C for three days, and the second plate at room temperature for 7 days
- For either plates, when more than 5 colonies are detected after incubation, the tested antiseptic/disinfectant is considered “failed” to the “in-use” test; the antiseptic/disinfectant is ineffective for use in the hospital. This cut-off corresponds to more than  $2.5 \times 2 \log_{10}$  Colony Forming Units/ml of tested (undiluted) antiseptic/disinfectant.

For a growth of less than 5 colonies on either agar plates, the antiseptic/disinfectant is considered not contaminated.

