

**5.5.2.2 Incubator**, capable of being controlled at  $(36 \pm 1) ^\circ\text{C}$ .

**5.3.2.3 pH-meter**, having an accuracy of calibration of  $\pm 0,1$  pH units at  $25 ^\circ\text{C}$ .

**5.5.2.4 Stopwatch**

**5.5.2.5 Graduated pipettes** of nominal capacities 10 ml and 1,0 ml and 0,1 ml. Calibrated automatic pipettes may be used.

**5.5.2.6 Petri dishes**, of diameter 90 mm

**5.5.2.7 Container** of sufficient capacity to take both hands immersed vertically up to the mid-metacarpals

**5.5.2.8 Bottles** of at least 1 l capacity

## **5.6 Procedure**

### **5.6.1 Preparation of the contamination fluid**

Grow the *E. coli* (see 5.4.1) in two tubes each containing 5 ml of TSB (see 5.4.2.4) for 18 h to 24 h at  $(36 \pm 1) ^\circ\text{C}$ . Inoculate these cultures into two bottles (see 5.5.2.8) with 1 l TSB (see 5.4.2.4) each and incubate for 18 h to 24 h at  $(36 \pm 1) ^\circ\text{C}$ . Pool the resulting bacterial suspension.

This contamination fluid shall contain between  $2 \times 10^8$  cfu/ml and  $2 \times 10^9$  cfu/ml which shall be confirmed by a standard quantitative culture technique in accordance with prEN 12054.

### **5.6.2 Application of the contamination fluid**

Prepare the hands by washing for 1 min with soft soap (see 5.4.2.6) to remove natural transients. Dry them thoroughly on paper towels. Pour the contamination fluid into the container (see 5.5.2.7) and immerse the hands up to the mid-metacarpals for 5 s with fingers spread apart. Carefully allow surplus liquid to drain back into the container.

**NOTE** : During this procedure care should be taken to avoid contamination of the immediate work area.

Allow the hands to dry in the air for 3 min, holding them in a horizontal position with the fingers spread out and rotating them to and fro to avoid the formation of droplets.

One batch of contamination fluid shall be used not longer than 3 h after the first subject's hands have been contaminated. Additionally, it shall be ensured that, in a test, all subjects' hands shall be treated with the same batch of contamination fluid, even if various products are tested against the reference product.