Spread 1,0 ml and 0,1 ml samples of the undiluted sampling fluid and 0,1 ml of a 10^{-1} dilution in TSB (see 5.4.2.4) containing neutralizer over the surface of TSA plates (see 5.4.2.3) using glass spreaders. If necessary, repeat with 0,1 ml of a 10^{-2} dilution. The interval between sampling and plating shall not exceed 30 min.

5.6.6 Incubation

Incubate all plates aerobically at (36 ± 1) °C for 18 h to 24 h. Count the colony forming units and reincubate for a further 24 h to detect any slow growing colonies.

5.7 Calculation

Record the number of colony forming units (cfu) per plate for each dilution step. Calculate the dilution factor by multiplying the sample dilution and the sample volume (in millilitres). Calculate the number of cfu per millilitre of sampling fluid by multiplying the plate count (cfu) by the dilution factor.

NOTE 1 : Whenever possible, the counts should be obtained from plates showing 15 to 300 colonies. With very efficient handrubs some counting plates for postvalues can show fewer than 15 colonies or no growth at all even if inoculated with 1 ml of undiluted sampling fluid (TSB, see 5.4.2.4). These values can then be accepted.

If suitable counts are obtained from two adjacent dilution steps, e.g. 299 colonies from 10^{-1} dilution and 31 from 10^{-2} dilution, the weighted arithmetic mean of both is calculated as follows :

$$\frac{(299 + 31)\ \text{cfu}}{1{,}1 \times 10^{-1}} = 3000\ \text{cfu / ml undiluted sampling fluid}$$

NOTE 2 : If colony counts of different dilution steps are grossly disproportional, insufficient neutralization of the antimicrobial agent should be suspected.

All viable counts per millilitre sampling fluid are transformed to decimal logarithms. For computational reasons values of "0" ($\log 0 = -\infty$) have to be set "1" ($\log 1 = 0$).

NOTE 3 : Since 0-values should be found only among postvalues and should occur only with the most active products, this adjustment can, at the worst, introduce a conservative bias of underestimating the antimicrobial efficiency of a product.

For both reference and test procedure the log counts from right and left hands of each subject shall be averaged separately for prevalues and postvalues.

NOTE 4 : This double weighting increases the precision of the measurement.

From the difference between this individual combined log prevalue and the log postvalue a log reduction factor is established for each subject.

Then, the two arithmetic means of all individual log reduction factors are calculated for both the reference and the test procedure.

If the data conforms to 5.8, compare the results for both procedures, P and R.