12.2 Calculation of the number of *E. coli* in the test sample

12.2.1 General case

If the difference between the results for the two measurement cells is less than or equal to 1 $\log_{10}$ unit, calculate the geometric mean of the two results. Round the final result (geometric mean) to two significant figures in accordance with ISO 7218.

12.2.2 Special cases

- If the result for one of the measurement cells is expressed as $<1.3 \times 10^2$ *E. coli* /100 g FIL, consider it to be equal to 1.3×10^2 *E. coli* /100 g FIL and calculate the geometric mean of the two results. Round the final result (geometric mean) to two significant figures.
- If the result for one of the measurement cells is expressed as $>4.0 \times 10^5$ *E. coli* /100 g FIL, consider it to be equal to 4.0×10^5 *E. coli* /100 g FIL and calculate the geometric mean of the two results. Round the final result (geometric mean) to two significant figures.
- If the difference between the results for the two measurement cells is more than 1 $\log_{10}$ unit, both results are considered invalid, and no final result is obtained.
- If the two measurement cells yield a result that is less than 1.3×10^2 *E. coli* /100 g FIL or if there is no DT, express the final result as $<6.7 \times 10^1$ *E. coli* /100 g FIL.
- If the two measurement cells yield a result that is more than 4.0×10^5 *E. coli* /100 g FIL, express the final result as $>4.0 \times 10^5$ *E. coli* /100 g FIL.

NOTE : At the time of the validation study of the impedance method (BacTrac 4300 analyser), the quantification limit was estimated experimentally at about 130 *E. coli* / 100 g. This limit fits with the theoretical quantification limit, corresponding to the presence of one bacterium in each of the two measurement cells, in other words 2 bacteria in a test portion of 1.5 g FIL (2×7.5 mL = 15 mL FIL diluted 1:10), that is 2/1.5 = 1.33 *E. coli* /g or, rounded to two significant figures, 130 *E. coli* /100 g FIL. For *E. coli* concentrations lower than this value, the BacTrac analyser cannot correctly quantify *E. coli* because of a deviation to linearity of the enumeration results compared with those obtained for concentrations higher than 130 *E. coli* / 100 g FIL.

At the time of this same study, the detection limit was also estimated experimentally at 44 *E. coli* /100 g FIL for oysters and 66 *E. coli* /100 g FIL for mussels, values close to the theoretical detection limit. The detection limit of the method has therefore been considered to be equal to the theoretical detection limit, namely one bacterium in a test portion of 1.5 g FIL (2×7.5 mL = 15 mL FIL diluted 1:10), that is 1/1.5 = 0.666 *E. coli* /g or, rounded to two significant figures, 67 *E. coli* /100 g FIL.

13. Uncertainty of test results

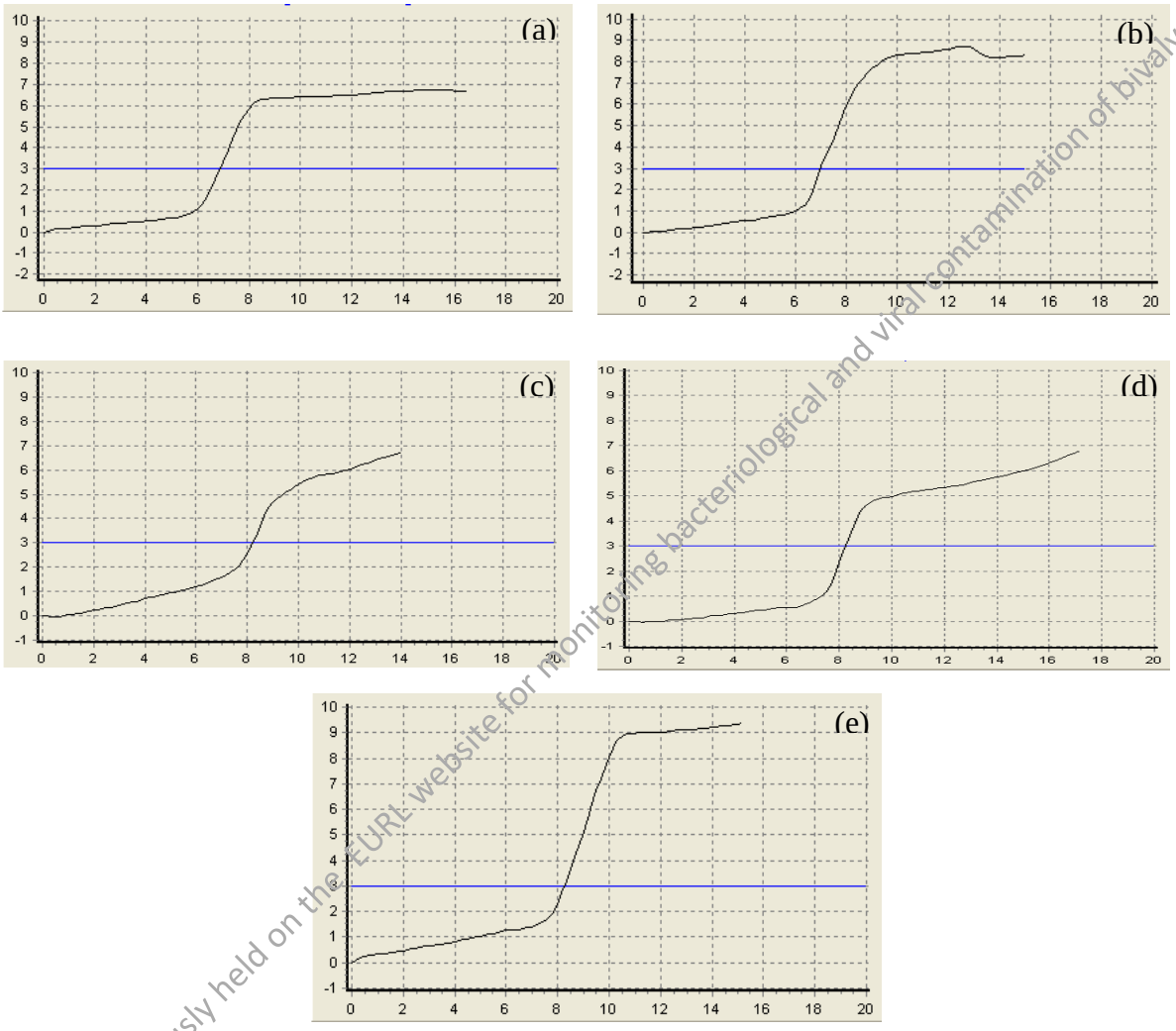
Uncertainty inherent in any test method, i.e. instruments, media, analyst performance etc can be assessed by the repeatability and reproducibility of test results. These should be monitored for instance through control tests analysed alongside sample tests, through in-house comparability testing between analysts and through external intercomparison exercises, which would highlight any uncertainties within the test methods.

Repeatability and reproducibility data are given in the validation report of the impedance technique and in standard NF V08-106.

14. Appendix: Examples of impedance variation curves (M value)

The next sections show different examples of impedance variation curves and the correct interpretation.

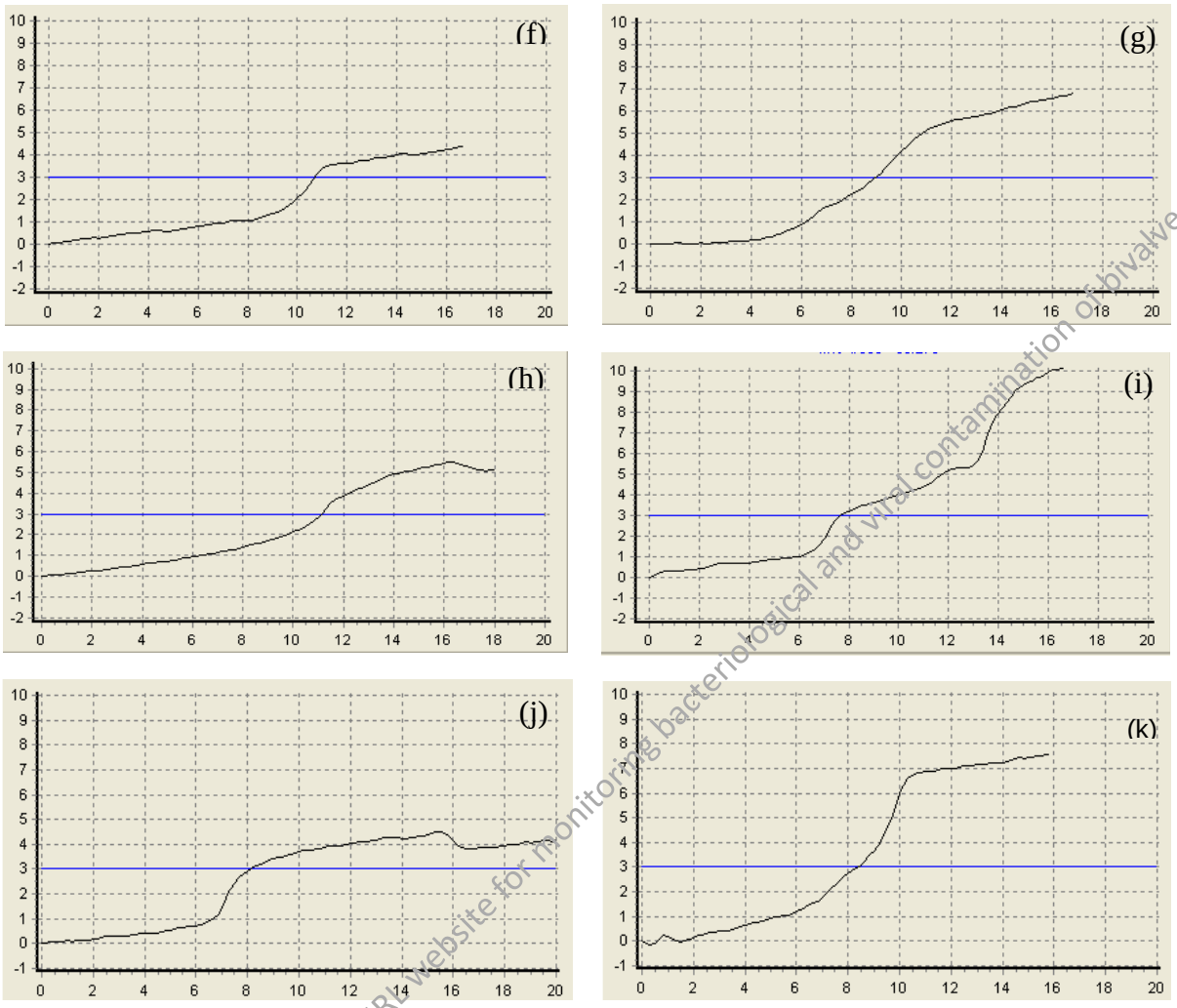
14.1 Impedance variation indicative of the presence of *E. coli*



Comments: the impedance variation is indicative of the presence of *E. coli.* The line corresponding to the selected variation threshold (3 %) cuts the curve in its exponential part (the part that falls between the end of the baseline and the start of the plateau).

Conclusion: Retain the result given by BacEval program if it falls between $1.3x10^2$ and $4.0x10^5$ *E. coli* /100 g FIL.

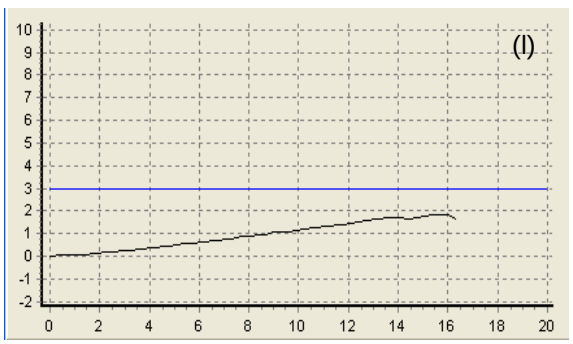
14.2 Impedance variation non-indicative of the presence of *E. coli*

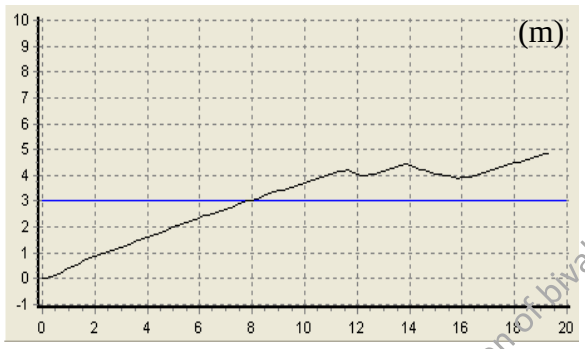


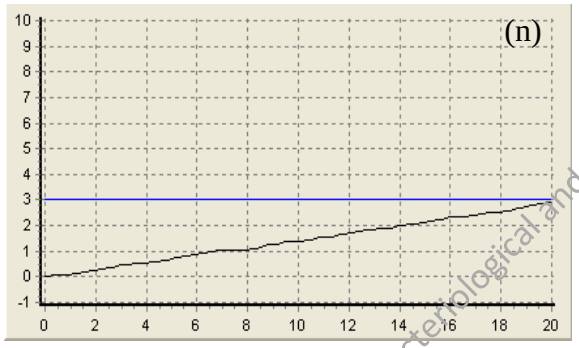
Comments: the impedance variation does not indicate the presence of *E. coli* because it is not steep enough or too small in its exponential part for curves (f), (g), (h), (i) and (j) or shows a slow change at the start of the exponential phase for curve (k). In addition, the result given by BacEval program for curves (f) and (h) is lower than $1.3x10^2$ *E. coli* /100 g FIL.

Conclusion: the result is expressed as $<1.3x10^2$ *E. coli* /100 g FIL.

14.3 Absence of impedance variation



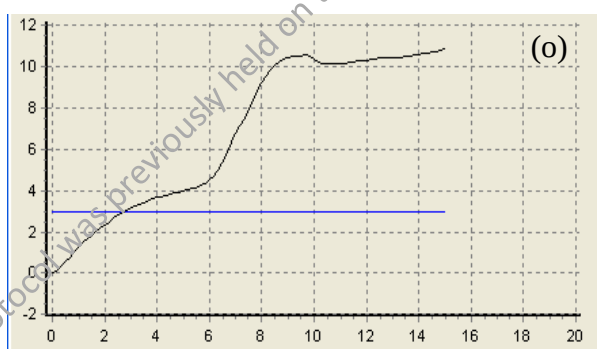


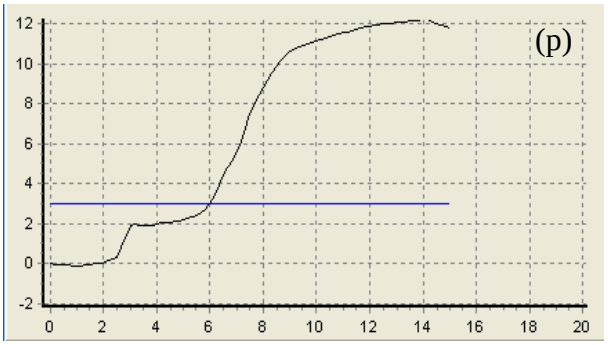


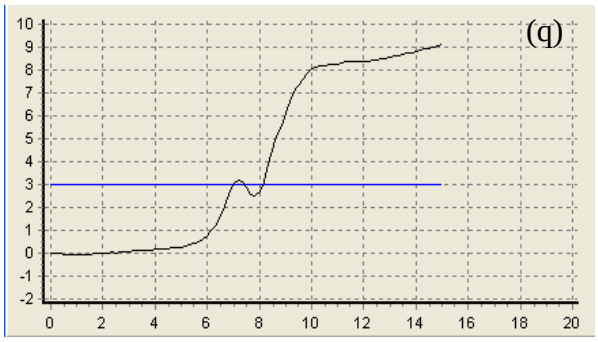
Comments: the increase of impedance is due to a drift of the baseline. The DT for the curve (m) is not valid.

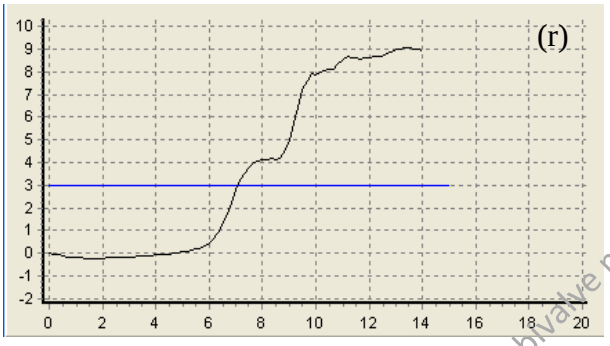
Conclusion: the result is expressed as <1.3x10^{2} *E. coli* /100 g FIL.

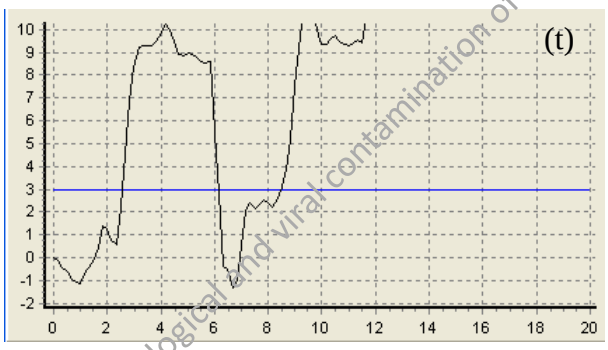
14.4 Non-interpretable impedance variation

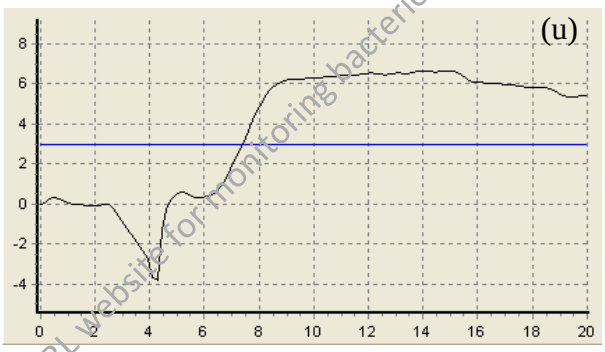












Comments: the impedance variation is atypical with a very strong drift of the baseline for curve (o), an impedance jump prior the exponential phase for curve (p) or two exponential phases for curves (q) and (r). Technical problems may cause atypical variation for curves (s), (t) and (u).

Conclusion: the result is considered invalid, and no result is obtained.