

Vitroids™ and Lenticule® discs

How to use certified reference materials (CRMs) to perform a spiking protocol for method verification.

Case

Spiking protocol preparation to reach a low level of *Salmonella* inoculation:

3-5 CFU/test portion as requested in ISO 16140-3 (*Protocol for the verification of reference methods and validated alternative methods*) when applying Protocol #3.

Choose an appropriate [CRMs](#), for this case [VT000292 *Salmonella enterica* subsp. *enterica* serovar Abony WDCM 00029](#) is selected, as an example. This product has a mean value range between **15-80 CFU**.

To verify which is the precise value of a specific lot, its [certificate](#) must be checked.

From the certificate of lot BCCG2046, as an example

Sample: <i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Abony WDCM 00029 VT000292		
Certified value (geometric mean value)	Expanded uncertainty (log ₁₀ value)	Expected range
4.9E+01 cfu per disc	0.03	2.4E+01 - 9.8E+01 cfu per disc

In this case the certified value is 49 CFU of *Salmonella* per disc.

Protocol

1. Prepare the initial suspension of the sample to be inoculated (25 g sample + 225 mL diluent).
2. In a separate vial, rehydrate one vitroid in 10 mL Buffered Peptone Water (BPW). Do not spend more than 10-15 minutes for rehydration and spiking (point 3), because the bacteria can start growing in BPW. If the reference material contains 49 cfu per disc (with uncertainty: from 24 cfu to 98 cfu), this *Salmonella* suspension will contain **4,9 cfu/mL** (with uncertainty from 2,4 to 9,8 cfu/mL).
3. Spike the initial suspension (25 g sample + 225 diluent) with 500 microliters of the homogenized *Salmonella* suspension. Considering the reference value, the sample finally would be spiked with a reference value of **2,45 cfu/25 g sample** (with uncertainty between 1,2 and 4,9 cfu/25 g).
It is possible to use a higher amount of *Salmonella* suspension for spiking: for example, if using 800 microliters, the reference value will be still valid (**3,92 cfu/25 g sample**) with better chance to obtain positive results, but the uncertainty of the spiking level will be slightly above the 5 cfu/sample (between 1,92 and 7,84 cfu/25 g).

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