



HEALTH PRODUCTS AND FOOD BRANCH

OTTAWA

SUPPLEMENT TO THE PROCEDURE FOR THE DEVELOPMENT AND MANAGEMENT
OF FOOD MICROBIOLOGICAL METHODS

Annex 4.3

EXPERIMENTAL LAYOUT FOR THE VALIDATION OF INDIRECT QUALITATIVE MICROBIOLOGICAL
METHODS

1. **Application**

The following information is offered as *an informative supplement* to the *Procedure for the Development and Management of Food Microbiological Methods : Part 4 Guidelines For The Evaluation of Qualitative Food Microbiological Methods*. The following supplement describes the experimental layout required by the *Microbiological Methods Committee* during the validation of a new indirect alternative method in comparison with a reference cultural method. An indirect method means that the result of the method is not an isolated and characterized bacterial colony. Indirect methods typically detect the nucleic acids or proteins or an organism.

2. **Procedure**

2.1 General requirements

Samples are considered paired when both the alternative and reference methods start from the same analytical portion in one common primary enrichment. When each method starts with a different enrichment, the samples are said to be unpaired. A different experimental layout must be applied to each situation.

For each food type in the study, a minimum of 45 samples must be tested by each method. Twenty samples must be inoculated at a level sufficiently low such that fractional recovery is obtained (25% - 75% positives by either or both methods). Twenty more samples should be inoculated at a level up to 1 log higher than the low level. Finally, 5 samples should be left uninoculated to serve as negative controls. The actual inoculum concentration required to reach fractionality will vary according to the food type and will need to be adjusted.

Within a food type, include as many different food items as possible so as to cover the variability within that food type. In any case, a minimum of 3 different food types per food category is required.

All results of the alternative method must be confirmed by a suitable reference method. The way confirmation is done will vary depending on method pairing and is described in more details below.

2.2 Experimental layout for paired samples

2.2.1 Sample preparation and spiking

When the methods are paired, only one set of 45 samples is required for each food type since both methods use the same analytical portions. The overall experimental layout is summarized in Figure 1.

The individual portions may be prepared in advance into separate bags or suitable containers. Inoculate each portion with a suitable inoculum (use sterile cell suspension buffer for negative controls) and equilibrate as per Part 4. Foods that are processed should be inoculated with a cell culture that has been stressed in a way that closely mimics said processing (see Annex 4.2).

Perform the reference and alternative methods on each analytical portion. This is usually done by first immersing the portions in a suitable culture broth followed by incubation at a set temperature. To be considered paired, this initial step must be identical for both methods (*i.e.*, one analytical portion in one primary enrichment that is subsequently split). However the incubation period may differ. After the primary enrichment, the methods may (and will usually) diverge. For example, the broth could be plated onto selective media for the reference method, and subjected to DNA extraction and PCR for the alternative method.

2.2.2 Confirmation of alternative method results for paired samples

All the results of the alternative method must be confirmed by a suitable reference method. For paired samples this is usually accomplished automatically by virtue of the reference method being applied on the same analytical portions as the alternative method.

It may happen that for a given analytical portion, the alternative method yields a presumptive positive result while the reference method is negative, thus resulting in an apparent false positive. Under these circumstances, it is possible to go above and beyond the reference method to try and confirm the presumptive result. This is called sheer persistence and it involves the use of any means that will ultimately allow the isolation and characterization of a target analyte colony. These may include the plating of a large number of replicates, re-incubation in various broths, the use of immunomagnetic separation, etc.

If positive confirmation is ultimately obtained by sheer persistence, an absolute positive result is scored. However the initial result of the reference method must remain unchanged.

2.3 Experimental layout for unpaired samples

2.3.1 Sample preparation and spiking

When the methods are unpaired, two sets of 45 samples must be prepared and analyzed in parallel. Otherwise, the portions are prepared, inoculated and equilibrated as described in section 2.2.1 for paired samples. The samples for both methods must be prepared simultaneously and randomly assigned to one method or the other. One set of samples is used for the reference method, the other set is used for the alternative method. The overall experimental layout is summarized in Figure 2.

2.3.2 Confirmation of alternative method results for unpaired samples

All the results of the alternative method must be confirmed by a suitable reference method. However, unlike paired samples, the reference method results can not be used for this purpose because the analyses are performed on different analytical portions.

For confirmation, a portion of the alternative method enrichment must be diverted towards the reference method at the earliest possible stage. This is usually done at the end of the primary enrichment of the alternative method where an aliquot will be taken and the reference method is followed. Hence two data sets will be obtained from the alternative method samples: the presumptive results and the results of the confirmation. Both are different from the reference method results which are obtained from a different sample set.

For unpaired samples, an alternative presumptive result may not match the confirmation result by the reference method. Sheer persistence may also be applied here. If sheer persistence is successful, a positive confirmation result will be scored. However, contrary to paired samples, this will have no effect on the results of the reference method.

2.4 Data interpretation

Once obtained, all raw data must be organized into tables and analyzed according to the document "*Supplement to the procedure for the development and management of food microbiological methods – Annex 4.4 : Procedure for the statistical evaluation and calculation of performance parameters of a new alternative qualitative method compared to a reference cultural method*" and "*Annex 4.1: Performance parameters of microbiological methods – note on sensitivity and specificity (absolute or relative)*" available on Health Canada's website.

Figure 1. Experimental layout for method validation using paired samples showing confirmation of alternative method results. The solid lines represent the reference method pathway, the dotted lines represent the alternative method pathway.

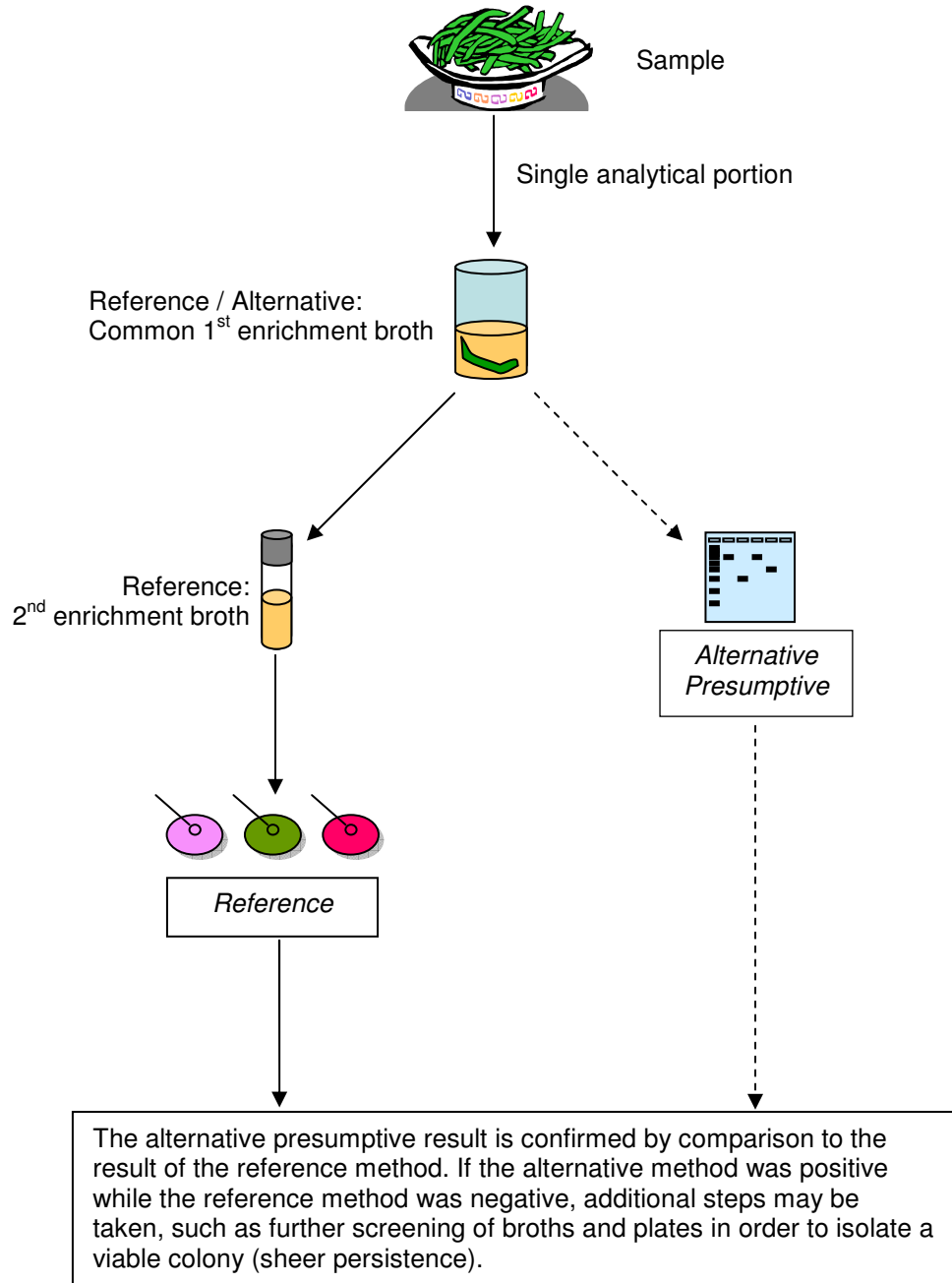
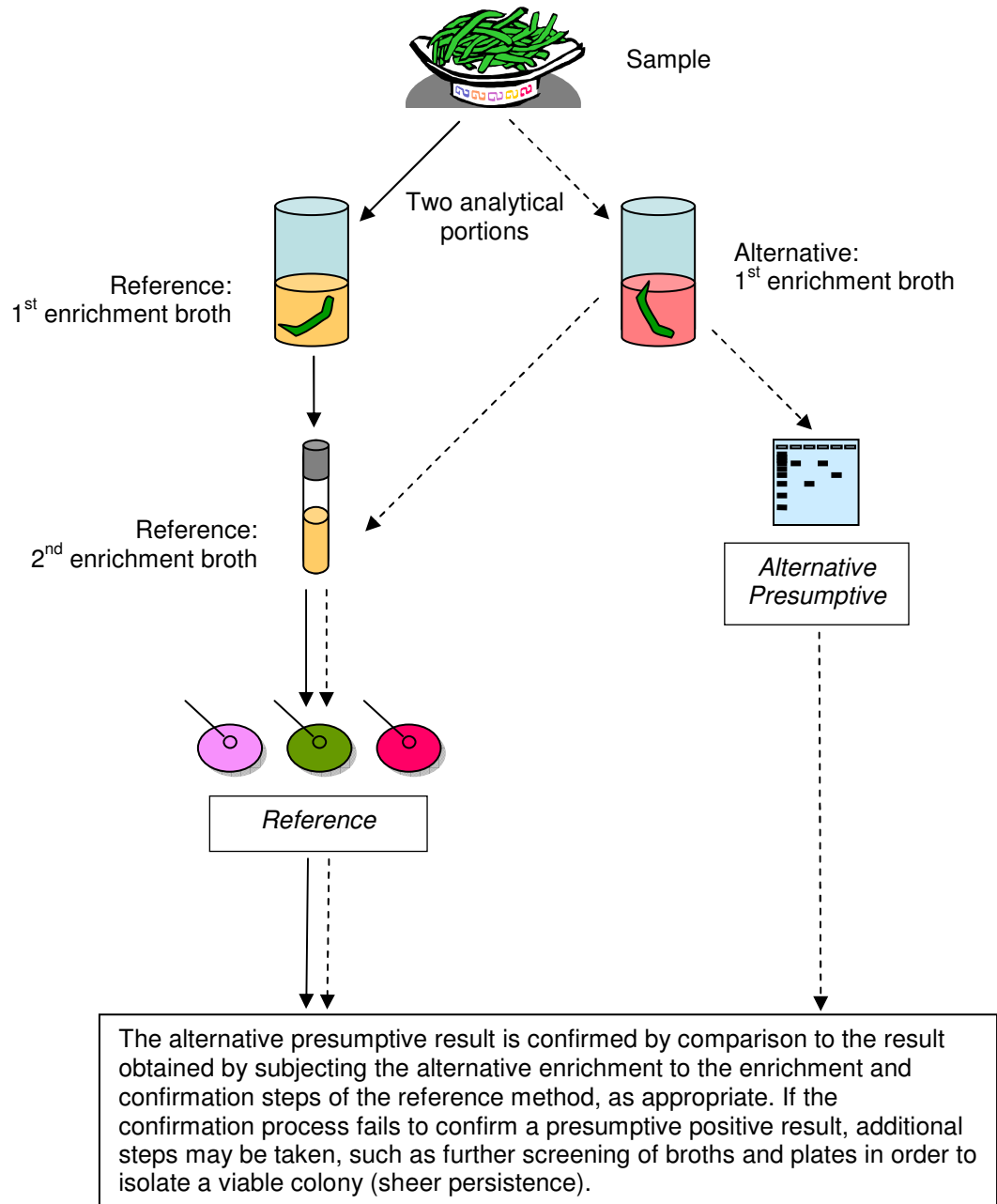


Figure 2. Experimental layout for method validation using unpaired samples showing confirmation of alternative method results. Solid lines represent the reference method pathway, dotted lines represent the alternative method pathway.



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