

Supplementary file S1. Questionnaire to laboratories potentially interested to participate in the interlaboratory comparison.

Interlaboratory validation of thirteen qPCR methods to quantify adulterants in culinary spices and herbs

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EU SURVEY: qPCR methods to evaluate adulteration in major spices and herbs

Survey Invitation: Invitation to participate in an Interlaboratory Comparison Exercise for the validation of qPCR Detection Methods.

We cordially extend this formal invitation to your laboratory to participate in an Interlaboratory Comparison Exercise (ILC) focused on the validation of 13 qPCR detection methods for identifying adulterants in culinary herbs and spices. Your laboratory's expertise is highly regarded in this field, and your participation is desired to ensure the success and reliability of this exercise.

Background

Culinary herbs and spices serve as integral components of global cuisine, valued not only for their flavour-enhancing properties but also for their potential health benefits. However, the intricate and extensive supply chains associated with these commodities render them susceptible to adulteration and fraudulent practices. In light of this, the European Commission initiated a coordinated control plan to address the prevalence of adulterated herbs and spices within the European Union, underscoring the urgent need for robust detection methods which main outcomes were published [here](#).

Objective

The primary objective of this ILC is to validate rigorously 13 qPCR detection methods tailored to identify adulterants within six commonly utilized spices and herbs: **paprika, turmeric, saffron, cumin, oregano, and black pepper**. These methods are designed meticulously to ensure precise detection capabilities, distinguishing between inadvertent contamination and

deliberate adulteration. The outcome of the ILC will assess if the methods perform well and are fit for their intended purposes.

Your Participation

We extend this invitation to your laboratory knowing your expertise in this field. Your participation in this ILC is highly desired, as it not only contributes to the validation of crucial detection methods but also reinforces the integrity of the broader scientific community's efforts in combating food fraud and safeguarding consumer interests.

Survey Details

To facilitate your laboratory's seamless integration into this initiative, we kindly request your participation in a brief survey. Your responses will enable us to tailor the ILC to your laboratory's specificities and requirements. The survey is designed to be succinct, requiring only 2-5 minutes of your valuable time.

Next Steps

Upon receiving your survey responses, we will diligently proceed with the organization and coordination of the ILC. Detailed instructions and materials pertinent to participation will be promptly provided to your laboratory, ensuring clarity and coherence throughout the process. All the reagents for performing the qPCR validation analysis, including MasterMix, DNA samples, qPCR primers, will be provided by us, with no additional costs charged to you. We will also provide the protocol and a template to report the results.

The exercise consists of testing **13** methods for **6** different matrices. Each method requires qPCR analysis of 10 DNA templates in triplicates with two different primer pairs, totalling 66 samples, conducted on two different days, equivalent to two 96-well plates per method.

Your participation in this endeavour is deeply appreciated, and we eagerly anticipate your laboratory's contributions. Should you have any inquiries or necessitate further explanations, please do not hesitate to contact us directly.

Please use the 'Comment' box below to provide any additional information or remarks where appropriate. All reported data will be treated confidentially and will only be disclosed in anonymous form. Please respond to the questionnaire before **21st June 2024**. For any questions, please contact us via email at JRC-ILC-HERBS-SPICES@ec.europa.eu.

With sincere regards,

Laboratory information

Name of the laboratory:	Click or tap here to enter text.
Place of the laboratory:	Click or tap here to enter text.
Country:	Click or tap here to enter text.
Contact person:	Click or tap here to enter text.
Email:	Click or tap here to enter text.
Telephone number:	Click or tap here to enter text.
Address for dispatching materials:	Click or tap here to enter text.

Questionnaire

1. Would you participate to the Interlaboratory Comparison Exercise for testing the qPCR methods for detection of adulteration in herbs and spices?

☐ Yes

☐ No

2. How many methods (each consisting of qPCR analysis of 10 DNA templates in triplicates with two different primer pairs, totalling 66 samples, conducted on two different days, equivalent to two 96-well plates per method) would your laboratory be able to process in a single round?

☐ 1-5 (from 2 to 10 times 96-wells plates)

☐ 6-10 (from 12 to 20 times 96-wells plates)

☐ 11-13 (from 22 to 26 times 96-wells plates)

3. During which trimester(s) would your laboratory be available to participate in the Interlaboratory Exercise?

☐ October – December 2024?

☐ January - March 2025?

☐ April – June 2025?

☐ Other (please specify – N.B. end of ILC is June 2025: Click or tap here to enter text.)

4. Do you typically prepare qPCR plates manually (without a pipetting robot)? For the scope of the exercise, we would prefer a manual preparation, if possible.

☐ Yes

☐ No

5. Please select the type(s) of real-time PCR platform routinely used in your laboratory, preferably for the purpose of this ILC. We would prefer 96-well plate qPCR, if possible. The method has been developed for a 96-well plate and for this comparison exercise would be preferable this format.

☐ 96-wells

☐ 384-wells

☐ RotorGene Qiagen

☐ Other (please specify [Click or tap here to enter text.](#))

6. Is your staff routinely involved in preparing 96-well plates for qPCR analysis?

☐ Yes

☐ No

7. Are your laboratory equipment (pipettes, real-time thermocyclers, ect.) calibrated?

☐ Yes, calibrated annually

☐ Yes, but not annually

☐ Sometimes

☐ Never calibrated

Please add any additional comments or specifications regarding the calibration status of specific equipment (pipettes, real-time thermocyclers, etc.): [Click or tap here to enter text.](#)

8. Does your laboratory have a general interest or activity related to food fraud detection using molecular methods?

☐ Yes

☐ No

- 9. Please provide any additional comments or feedback regarding your laboratory's potential participation to the ILC:**

Click or tap here to enter text.