CHAPTER 2

Rapid mycotoxin screening: An update on the evaluation of analytical performances and relevant criteria in relation to the method scope

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Abstract

Rapid screening methods play a key role in the control of the mycotoxin contamination in food and feed products. These methods are applied by official control laboratories or by food and feed business operators, albeit often for different purposes. Depending on the later application, different requirements for the method validation study are required. In order to harmonise the validation procedures for screening methods especially for official control, the EU has issued legal requirements for mycotoxins and another class of important analytes, namely pharmacologically active substances. The corresponding guidelines are compared, and similarities and differences are elaborated. Also business operators utilise rapid screening methods, but often require a quantitative estimate for their decision. In such a case, the use of adapted validation strategies may be necessary. Likewise, quantitative estimates from such methods are also required when using them for larger monitoring programmes that may serve as basis for subsequent risk assessment studies. When using rapid screening methods for classifying samples into compliant and suspect positive, prior knowledge about the expected contamination level should be considered to decide whether a specific method fits the purpose. The approach is based on Bayesian statistics and its application using real world examples is presented.

Keywords

mycotoxins – method validation – fitness for purpose – comparison of guidelines – Bayesian statistics

1 Introduction

When employing a rapid screening method for a specific measurement exercise, it is crucial for the user to ensure the suitability of the method for the given project before implementation. Typically, this assurance is obtained from the results of method validation studies. Within this chapter, we aim to present the essential elements of two screening guidelines established by the European Union for measuring mycotoxins and residues of pharmacologically active substances. We included the guideline of the latter group of analytes in this chapter, because components of the corresponding validation concept merit assessment for their suitability within the domain of mycotoxin analysis. The criteria for screening methods are outlined in EU legal documents, but mandatory only when applied for official control purposes. Nevertheless, these criteria may also prove beneficial for measurement campaigns conducted by food and feed business operators. Screening methods may be based on a qualitative evaluation such as the visual inspection of a dip stick or deliver a numerical value suitable for further statistical assessment. For the purpose of this chapter, our focus is exclusively on the latter category of methods. Additionally, screening methods, often referred to as rapid techniques allowing sample preparation and analysis in few minutes, may possess the capability to provide a quantitative estimate of the mass fraction. The most commonly used techniques for routine screening of mycotoxins are lateral flow devices ELISA, fluorescence polarization immunoassay and even LC-MS (Lattanzio et al 2019, Regulation 519/2014/EU), which is the applied detection technique in the CEN standard method EN 17279:2019 – *Multimethod for the screening of aflatoxin B1, deoxynivalenol, fumonisin B1 and B2, ochratoxin A, T-2 toxin, HT-2 toxin and zearalenone in foodstuffs, excluding foods for infants and young children, by LC-MS/MS*. In such methods LC-MS analysis is generally performed via a one-point/one shot calibration. The analytical results derived from these methods can then be employed for purposes beyond exclusive separation into two distinct classes. Lastly, we show how including additional information about the anticipated levels of the target analytes in the sample can help in deciding, whether a specific test will yield the desired performance.

2 Legal requirements for screening methods

2.1 *Screening methods for mycotoxins*

The use of semi-quantitative screening methods for the determination of mycotoxins in food and feed in the European Union is specified by Commission Regulation (EU) No 519/2014. By applying screening methods on a test item, samples are either compliant (negative samples) or potentially containing mycotoxins above the *screening target concentration*, e.g. a legal limit, requiring further analysis through a confirmatory method. The validation concept is based on the principle that validation experiments are conducted on samples containing the target analyte at the

STC. From the result, a cut-off value applying equation (1) is calculated, ensuring that the rate of false-negative results remains within the acceptable limit of 5 %.

$$cut\text{-}off = R_{STC} \pm (t\text{-}value)_{0.05} \times SD_{STC} \quad (1)$$

R_{STC} is the response measured at the STC and can be the reading of a measurement device or a concentration obtained after calibration. SD_{STC} is the standard deviation obtained from the validation experiments at the STC. In the case that the response is proportional to the concentration, SD_{STC} multiplied with the corresponding t-value is subtracted from R_{STC}, while this component needs to be added to R_{STC}, if the test response is inversely proportional to the concentration. In recent years however, screening test deliver almost exclusively an estimate of the concentration, thus the precision component is subtracted from R_{STC}. Classification of unknown samples as compliant or suspect positive is based on comparing the analytical result with the cut-off value.

The guideline also requires the additional analysis of negative control samples, enabling the estimation of the rate of false-positive results by calculating the corresponding t-value.

$$t\text{-}value = \frac{(cut\text{-}off) - mean_{neg}}{SD_{neg}} \quad (2)$$

where $mean_{neg}$ and SD_{neg} are the mean response and corresponding standard deviation of the negative control samples, which is blank test material. Depending on the requirements of a specific measurement campaign, samples containing a defined fractions of the STC (e.g. at 50 % of the STC) may be included in the validation study. Corresponding rates of false positive results of these samples would be estimated using equation (2). It is essential to highlight that while the rate of false-positive results may not impact the ability to identify suspect positive results, it can influence the economic implications of the test.

The guideline is applicable to quite different analytical formats and does not set criteria for method performance characteristics, with the only requirement of a limit for the rate of false negative result of 5 %. The regulation mentions as examples ELISA, lateral flow devices and physicochemical methods including mass spectrometry. The guideline specifically applies to screening tests that generate numerical results, but it could either be a response obtained for instance from a dip stick reader or an estimate of the mass fraction of the target analyte. In respect to method performance characteristics, the regulation explicitly specifies that in the rate of false negative and false positive results, method performance characteristics such as sensitivity, selectivity and precision are embedded.

The guideline can be easily implemented by conducting single laboratory validation (Lattanzio et al. 2018) and interlaboratory studies (Lattanzio et al. 2014). In the latter case, the outcome of the study would be a cross-laboratory estimate of the cut-off value.

2.2 *Screening methods for residues of pharmacologically active substances in food of animal origin as specified for official control in the EU*

Since the publication of Commission Decision in 2002, the criteria approach for the selection of appropriate analytical screening and confirmatory methods for various groups of substances is valid within the EU. Rather than establishing a set of standard analytical methods to be applied within the frame of official control, criteria are defined for various performance criteria, such as precision, trueness and identification of the target analytes. An important component of this concept is the introduction of the decision limit CCα, and the detection capability CCβ. The decision limit is above the legal limit and specifies the concentration at which a sample is considered non-compliant, with maximum probability of α that the sample is actually compliant. Typically, this value of a false positive is 5 %. This criterion corresponds to the requirement in mycotoxin analysis that the expanded measurement uncertainty needs to be subtracted from the measured concentration before concluding that the sample is non-compliant. Furthermore, this document specifies CCβ, which tackles the question at which concentration above CCα, the probability of a false negative results is 5 %. While the use of screening methods has been explicitly mentioned in this document, some questions such as the determination of CCβ for these methods have not been specified. In 2021, the Commission Decision has been significantly revised via Commission Implementing Regulation (EU) 2021/808, limiting the criteria for the selection of methods to residues of pharmacologically active substances. While the principal concept of the criteria concept was kept, more details have been included for screening methods, thus underlining the important role of these methods for control exercises: The general applicability of the detection capability has been substituted by CCβ exclusively for *screening* methods. In addition, the Regulation introduced the screening target concentration (STC), which is the concentration at which a sample is classified as screen positive requiring the application of confirmatory methods. Essentially, both parameters are below the legal limit in order to limit the probability of false negative results to 5 %. Furthermore, the Regulation specifies different validation parameters for qualitative, semi-quantitative and quantitative screening methods. In 2023, the EURLs in charge of these substances (EURLs for Residues of Veterinary Medical Products, 2023) issued a guidance document about the correct implementation of the provisions including further explanations of the terminology used. Classification into semi-quantitative and quantitative methods is based on the principle of the methods (e.g. physicochemical versus immunoassay) and calibration procedure applied (e.g. one point calibration versus calibration curve including the sample response). Detailed information is provided on how to calculate the STC and CCβ, depending on the classification of the screening method under investigation. When validating screening methods that use a calibration curve for quantification, the EU Regulation also requires that the assessed precision and trueness of the screening methods comply with the corresponding criteria set for confirmatory methods.

2.3 *Comparison of method performance characteristics required in the different guidelines*

While the main purpose of screening methods does not depend on the actual field of application, the validation requirements for the measurement of mycotoxins on the one hand and pharmacologically active substances on the other hand differ significantly. The minimum set of validation experiments for mycotoxins includes measurement at the STC and at concentrations below the STC. Based on these results, the screening test can be applied under real world conditions for the specific purpose of classifying samples into compliant and suspect positive. The differences of the precision and trueness of the various methods have exclusively an impact on the rate of false positive results and the user needs then to decide, whether the expected rate is acceptable for the specific measurement exercise. Further specifications for the measured precision at the various levels are therefore not considered relevant. Moreover, this Regulation utilises the technical term *semi*-quantitative, without providing a clear definition. Considering the fact that setting the cut-off value is based on a *one-point* calculation from the measurements at the STC, the definition of semi-quantitative methods as given in the guidance document on screening method validation for pharmaceutical active substances (EURLs for Residues of Veterinary Medical Products. 2023) may also apply in this context. It is important to emphasize that the validation guideline for mycotoxins is specifically designed for the application of screening methods in classifying samples against legal limits. The assessment of the screening method's capability to provide an estimate of mycotoxin levels across a wide range is beyond the scope of this guideline and requires additional validation experiments.

In contrast to mycotoxins, a significant higher number of performance characteristics are required for pharmacologically active substances as shown in Table 2.1, including even the need for compliance with the criteria. An important strength of this approach is its flexibility, allowing a screening method that successfully meets validation criteria to be applied beyond its original screening purpose. These could be scenarios like monitoring campaigns, where the obtained information on the concentration can significantly refine the interpretation of the results. Such validation procedures could therefore be considered as model for other areas of food and feed safety, such as the screening for mycotoxins. However, merging these guidelines is not an easy task, as some expressions such as the STC have different meanings.

3 Field of application of rapid methods and method performances characteristics

The guidance documents for the use of screening methods established by European legislation focus on the use within the frame of official control. That means

TABLE 2.1 Required method performance characteristics for semi-quantitative and quantitative screening methods of pharmacologically active substances according to Commission Implementation Regulation (EU) 2021/808 (European Commission, 2021). The precision and trueness of quantitative methods need to comply with the criteria set in this Regulation, while for semi-quantitative methods the precision has to be assessed, but compliance with these criteria is not necessary and indicated by the brackets (x).

Method Performance Characteristics	Semi-quantitative	Quantitative
CCβ	x	x
Trueness		x
Precision	(x)	x
Relative matrix effect/absolute recovery		x
Selectivity/Specificity	x	x
Stability	x	x
Ruggedness	x	x

that the primary objective is to classify the samples into just *two* classes: Samples that are considered as negative without further investigation and suspect positive that require the application of confirmatory analysis. For such a scenario, the performance of the screening methods in terms of delivering a quantitative estimate of the mass fraction is less relevant. However, rapid methods are also applied by food and feed businesses operators with objectives that differ from the previously described scenario. In an overview (Davis end Tanyi, 2021) various types of users from the industry perspective and their corresponding requirements for the rapid methods are presented. There are for instance applications where samples are analysed from material still on the truck, thus requiring a quick decision on accepting or rejecting the whole batch. Like in official control, the emphasis is on the separation into two classes. On the other hand, there are also situations where the user requires a more precise quantitative estimate of the mycotoxin content to decide on the later use of the test material. For instance, when conducting routine monitoring campaigns or when classifying the material into more than 2 categories. Such risk management programs may be adjusted to adverse effects of the mycotoxin content to specific animals. These kinds of measurement exercises are performed under less time pressure and are often done in a laboratory with trained personnel. Under such circumstances, rapid methods compete with other analytical methods such as liquid chromatography but are often the preferred choice given their simplicity and high sample throughput. When using rapid methods for such purpose, the validation study needs to include the full set of characteristics such as limit of detection/quantification, specificity, precision and trueness.

In the field of risk assessment concerning mycotoxin-contaminated food, there exists a high demand for analytical results derived from a larger number of samples. Typically, these assessments rely on monitoring programs conducted in well-

equipped laboratories, employing techniques such as high-performance liquid chromatography coupled to mass spectrometry. However, risk assessment programmes in certain regions, notably some African countries, could benefit from the use of lateral flow devices (LFDs). It is essential to emphasize that utilizing LFDs for risk assessment purposes requires different fitness-for-purpose criteria compared to situations involving their application for classifying samples into two categories near legal limits. Specifically, the validation exercise should address the performance of LFDs in more complex food matrices (processed food rather than raw materials), at significantly lower mycotoxin levels than legal thresholds and the occurrence of false positive results at negligible low levels of mycotoxins. Furthermore, prior to their application, the potential impact of analytical results associated with higher uncertainty on risk assessment requires thorough evaluation.

Regardless of the specific guideline utilised in the validation phase of the analytical method, the reliable application of this method under routine conditions requires the periodic analysis of samples of known content, to verify laboratory performances as well as possible reproducibility variability between test kits production lots (Lattanzio et al, 2018). This will ensure that the performance profile as assessed during the validation exercise will remain consistent.

4 Fitness for purpose by the application of Bayesian statistics

The primary objective of conducting method validation studies is to evaluate the performance characteristics of the methods in question. These performance characteristics serve as essential benchmarks for users to judge on the method's suitability in achieving the intended measurement objectives. As discussed in preceding sections, typical performance indicators encompass precision, trueness, and the rates of false negative and false positive outcomes. In this chapter we intend to elaborate on the question, whether these particulars deliver adequate information for users to make correct decisions about the method's alignment with their requirements, focusing on the use of screening methods for classification of samples into compliant and suspect positive. To address this question, we turn our attention to exemplars of outcomes from previous validation studies performed on lateral flow immunoassays. These immunoassays underwent evaluation in accordance with Commission Regulation (EU) No 519/2014. Specifically, cut-off values were determined through the analysis of samples containing the target analytes at the STC, while values for false suspect rate were derived from the assessment of samples containing the analytes at various mass fractions lower than the STC. These samples are wrongly classified as false suspect, because they are actually compliant.

In Table 2.2, we present the percentage of false suspect results exhibited by a specific immunoassay designed to deliver a quantitative response (Lattanzio et al. 2013), specifically to DON and T-2+HT2 in wheat. Notably, there is no calibration

TABLE 2.2 Performance profile of a screening method expressed in terms of rate of false positive results measured on blanks, 25 % and 50 % of the mass fraction level of interest (Lattanzio et al. 2013). The level of interest corresponds to the STC.

Content of target analyte	Rate of false positive results (%): DON in wheat	Rate of false positive results (%): T-2+HT2 in wheat
Blank	0.27	0.3
25 %	12	6.2
50 %	88	23

curve created and the response is directly compared against a cut-off value to establish, whether the sample is suspect or not. Depending on the mass fractions of the test samples employed in the validation study, the corresponding false suspect rate ranged from 0.27 % to a very high value of 88 %. An additional study (Lattanzio et al. 2018) examined an immunoassay specifically developed for the quantitative estimation of aflatoxin mass fractions in maize. When using this type of immunoassays, samples with an estimated mass fraction above the cut-off value are considered suspect. In conjunction with trueness and precision assessments, the false suspect rate was determined to be 14 % for samples containing 50 % of the STC of 4 mg kg^{-1}. This increased false suspect rate may lead to the conclusion that these tests lack the required specificity, thereby questioning their suitability for the intended purpose. However, our aim herein is to demonstrate that drawing definitive conclusions about the fitness of the screening method based solely on results obtained from the method validation study would be premature and may even lead to potential misinterpretations. Rather, a comprehensive evaluation requires the incorporation of supplementary information to arrive at a conclusive assessment.

When addressing the challenge of evaluating the suitability of these screening methods for the intended purpose, we need to introduce the concept of *conditional* probability. The application of this concept to screening test is shown in Figure 2.1.

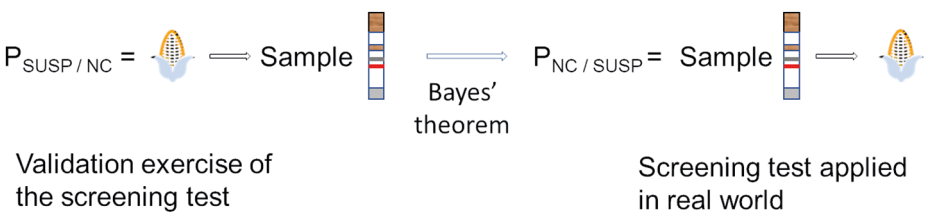

FIGURE 2.1 Application of Bayes' theorem and conditional probability for a screening test on the analysis of mycotoxins in maize samples. $P_{SUSP/NC}$: Probability of suspect result from the validation study. This represents the probability that a screening test yields a suspect result when analysing a known non-compliant sample. $P_{SUSP/NC}$: Probability of non-compliance with a suspect result under real-world conditions. This indicates the probability that an unknown sample is non-compliant when the screening test produces a suspect result.

All these validation studies have in common that probabilities for correct and false suspect results are determined on samples containing the analyte at specific and known mass fractions. The results from the validation studies of the immunoassay, as an illustration, tackle inquiries like "What is the probability ($P_{SUSP/NC}$) of encountering *correct* suspect results when the sample is non-compliant containing the analyte with a mass fraction above the STC?", or "What is the probability of encountering *false* suspect results when the sample contains the target analyte with a mass fraction at 50 % of the STC?" To put it another way, these particular values for the rate of suspect results are solely valid under the condition that the samples contain the analyte at the specified mass fraction. However, when the end users of the method analyse a substantial number of samples – such as 1000 – their focus shifts to the *converse* inquiry: "What is the probability ($P_{NC/SUSP}$) of non-compliance of the sample under the *condition* that the immunoassay produces a suspect result?" For a more in-depth discussion, it is crucial to emphasize that these two probabilities ($P_{SUSP/NC}$ versus $P_{NC/SUSP}$) are different and should not be confused. While the initial query is tackled through method validation employing precisely defined samples, resolving the second inquiry requires considering both the method performance and the expected contamination level of material to be analysed. In general, detailed information on the contamination level of this material remains unknown prior to the analysis. However, opting for the utilisation of screening methods requires the presumption that the majority of samples comply with legal limits. This arises from the logical implication that if one assumes that a majority of samples contain mycotoxins surpassing the legal limits, the most reasonable decision would be to circumvent the use of screening methods and instead consistently employ confirmatory techniques. While such assumptions about the presumed contamination level are frequently accessible, these pieces of information are generally omitted during the evaluation of correct and false suspect rates obtained in method performance studies. However, tackling the aforementioned second question in a comprehensive manner requires the inclusion of the contamination assumption, also referred to as prior knowledge.

The linkage between the method performance characteristics in terms of probability of false negative and suspect positive rates and prior knowledge concerning the contamination level is established through Bayesian statistics. Bayesian statistics is frequently applied in quite different areas and the use in chemistry and analytical science is elaborated in various publications (Wilkes 2022, Armstrong and Hibbert 2009), which also introduce the Bayes theorem.

$$P_{NC/SUSP} = \frac{P_{SUSP/NC} \times P_{Prior\ probability}}{P_{Marginal}} \tag{3}$$

Let us explain the four components comprising the equation in question. (1) The expression on the left of the equation is the probability that the analysed sample is non-compliant based on a suspect result of the screening test. This particular prob-

ability is the central piece of information sought by end-users when applying the test on unknown samples. This term is also called *posterior* probability. (2) $P_{SUSP/NC}$ is the probability of a suspect response when analysing samples with the analyte at STC and obtained in the validation study of the test. (3) $P_{Prior\ probability}$ is the assumed *prior* probability of the non-compliant samples in the entire population of all samples, which may be derived, for instance, from previously conducted monitoring programs involving analogous sample types. (4) $P_{Marginal}$ is the probability of a suspect result obtained from the analysis of a randomly selected sample and is the sum of the joint probability of two events: the first refers to the estimated mass fraction distribution, and the second relates to the probability of the screening test yielding a suspect result for samples with specific mass fractions of the analyte. In more specific terms, the calculation for $P_{Marginal}$ involves (a) multiplying the assumed fraction of non-compliant samples by the corresponding probability of a correct suspect result and (b) multiplying the portion of samples with a mass fraction below the specified threshold concentration (STC) by the rate of false suspect results. Finally, the mathematical products are summed up to derive the value of $P_{Marginal}$. It is important to note that the effective application of this concept requires the estimation of the rate of suspect results of both non-compliant samples and samples with various mass fraction during the validation process.

Now we apply the Bayes' theorem to the real-world example of DON and T-2+HT2 in wheat as given in Table 2.2. The performance profile of the test kits is taken from previous publications (Lattanzio et al. 2013). The screening test for this analyte/matrix combination is characterized by a high rate of false suspect of 88 % for sample containing DON at 50 % of the STC obtained in the validation exercise, thus flagging the screening test as potentially not fit for purpose. For the sake of this example, we use a prior probability of non-compliant samples of 5 %, while the mass fraction distribution below the STC were taken from EU monitoring results.

The outcomes of applying Bayes' theorem are presented in Table 2.3. The first column shows four mass ranges, aligned with the levels assessed during the validation study (Lattanzio et al., 2013). For example, the "50 % of STC" range encompasses all samples falling within 38 % to 62 % of the STC, with a corresponding probability of 0.5 % as denoted in the second column of the table. The forth column in Table 2.3 shows the probability of a suspect result (%) as specified in Table 2.2. For instance, this probability is 88 % for the range corresponding to 50 % of STC. The fifth column contains the product of these probabilities, used in calculating $P_{Marginal}$. For samples exceeding the STC, we adopt a value of 99 % for $P_{SUSP/NC}$ based on another validation study (Lattanzio et al., 2018). The calculated $P_{Marginal}$ value is 6.3 %, indicating a notably low probability that the analysed samples require further confirmatory testing. In fact, a substantial majority, approximately 93.7 %, are deemed compliant without additional analysis. When looking at the samples with a suspect positive result, the probability of a confirmation of these samples as non-compliant ($P_{NC/SUSP}$) is 78 %, with a 22 % chance of yielding a

TABLE 2.3 Application of the Bayes' theorem to the determination of DON in wheat (Lattanzio et al. 2013) as shown in Table 2.2. The values for the *Probability of suspect result* (%) are obtained from the validation study and the corresponding values for the *Probability of sample falling in the mass fraction range* (%) represent the prior knowledge on the expected contamination level of the material. STC = Screening target concentration. The posterior probability $P_{NC/SUSP}$ is calculated applying equation (3).

Content of mycotoxin	Mass fraction range as % of STC	Probability of samples falling in the mass fraction range (%)	Probability of suspect result (%)	Product of both probabilities (%)
Blank	0-12	89	0.27	0.24
25 % of STC	13-37	4.6	12	0.55
50 % of STC	38-62	0.5	88	0.40
100 % of STC	63-100	0.6	95	0.19
Above STC		5.0	$P_{SUSP/NC}$ = 99	4.95
			$P_{Marginal}$ (%), sum of the products	6.3
			$P_{Prior\ probability}$ (%)	5
			$P_{NC/SUSP}$ (%)	78

false suspect result. Although the latter may appear relatively high, it solely concerns approximately 6.3 % of all samples. This outcome stems from the fact that the elevated likelihood of false suspects of 88 % applies to just about 0.5 % of all samples. This analysis was repeated for the T-2+HT2 method in wheat (Table 2.2), characterized by notably lower probabilities of false suspect results in comparison to the prior example. Interestingly, the differences in $P_{NC/SUSP}$ and $P_{Marginal}$ are less pronounced, with corresponding values of 5.5 % and 86 %, respectively. A comparative illustration of these values between both methods is shown in Figure 2.2, clearly demonstrating that the screening tests meet the target criteria for both analytes. It effectively identifies the small fraction of non-compliant samples, correctly categorizing the majority as compliant.

It is essential to emphasize that these target statistics can be readily recalculated should new information concerning the actual contamination levels become available.

5 Conclusions

When using rapid screening methods for the detection of mycotoxins in food and feed, various aspects need to be considered prior to their application under real world conditions. While a detailed validation procedure has been established for

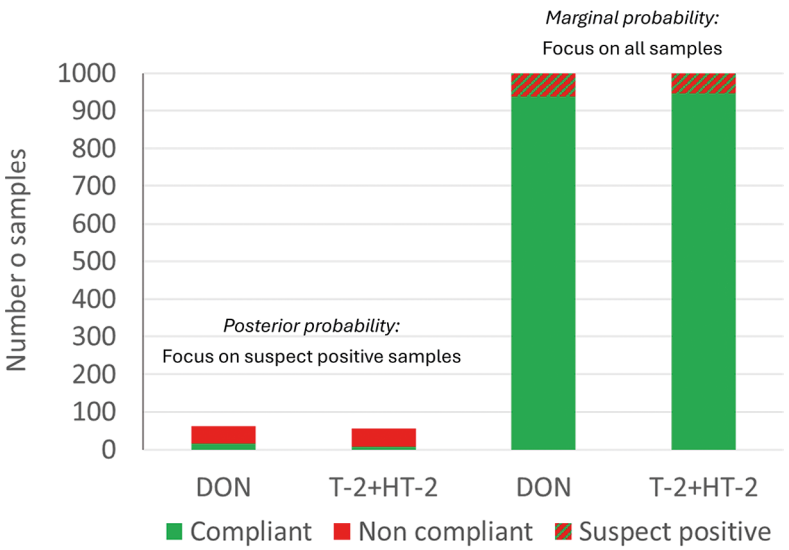

FIGURE 2.2 Comparison of the results from the application of Bayesian statistics to the hypothetical analysis of 1000 samples. The results demonstrate that for both methods the vast majority (green area) are correctly identified as compliant. Moreover, from the low number of suspect samples, the majority of samples (red area) is correctly non-compliant.

official control applications, these methods also find utility in other contexts. Business operator, for instance, may use the quantitative estimate of the mass fraction for further decisions on the use of the test material. In such cases, additional validation experiments may be necessary. The EU validation procedure for mycotoxins ensures that the rate of false negative results is limited to 5 % regardless of the precision profile of the screening test. However, methods with higher precision values exhibit a higher rate of false positive results of compliant samples. Consequently, it is advisable to assess the false positive rate at various concentration levels below legal limits. To predict the impact of the screening method's performance profile on economic aspects, supplementary information could be considered. Indeed, the assessment of the usefulness of a specific test also depends on factors such as the expected contamination level of the entire batch of material. For example, in situations where the contamination is anticipated to be low, with only a small fraction exhibiting high contamination, a test with a higher false positive rate may still be suitable. Conversely, if the majority of the batch is expected to have mycotoxin levels slightly below the legal limit, the screening test requires a lower false positive rate. Bayesian statistics is employed for the required calculations. As a main result of this assessment, the posterior probability delivers an estimate of the probability that an unknown sample is non-compliant based on a suspect positive result from the screening method. In addition, the marginal distribution gives an estimate of the expected portion of suspect positive samples from the screening analysis of all

samples. Both components aid users in evaluating the added value of employing the screening method. In the final conclusion about the added value of the screening method, the user may also consider additional aspects that are not yet addressed in this chapter. These aspects refer to general characteristics of the screening method such as cost or the need for trained personnel to carry out the analysis. For instance, the user may prefer either screening methods that can be applied by less trained personnel on-site or methods that require a laboratory environment, depending on the specific purpose of the measurement exercise. In the latter case, the required time for obtaining the result is longer but this drawback may be compensated by a better precision of the measured value. Also, the cost of the screening method play an important role, to decide how many false positive results are still acceptable that require the application of confirmatory methods. In summary, the suitability evaluation of a rapid screening method for a specific task depends on various aspects, encompassing the method's performance profile, available knowledge on expected contamination levels, and practical features like costs and complexity of executing the method protocol.

Acknowledgements

This work has been carried out in the FoodSafety4EU project. This project has received funding from the European Union's Horizon 2020 Research and Innovation programme under Grant Agreement No 101000613.

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