Microbiology proficiency-testing scheme in wine

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INTRODUCTION

Wine is a complex product, both on the microbiological level and on the physical-chemical one. Many microorganisms may be present and constitute a complex microbial ecosystem difficult to apprehend. The main goal of the microbiological analysis of wine and must is to ensure higher quality of wines, allowing the detection of any defect during the different production phases and on the final product.

The methods developed for detection and quantification of microorganisms in wines can be grouped into 3 main categories: microscopic techniques (Malassez cell, epifluorescence), microbial enumeration by cultural medium (Petri dish) and PCR (Polymerase Chain Reaction), method based on identification of microorganisms by their DNA.

The number of laboratories performing microbiological analyses of wines has gradually increased in recent years, principally for the quantification of *Brettanomyces*. However, the lack of regular proficiency-testing schemes (PTS) in this field is an obstacle for the performance monitoring of the laboratories. The complexity of wine matrix in term of bacterial ecology is a factor to be taken into account in developing a proficiency-testing scheme, especially during the preparation of stable and homogeneous samples.

This work describes the design and the implementation of PTS for the analyses of wine samples spiked with yeasts. The goal of this PTS is to allow laboratories to demonstrate the reliability of their results and to compare each other analytical data and protocols used for the enumeration of *Brettanomyces* in wine.

METHODOLOGY

The setting up of a proficiency test can be schematized by 3 main steps: preparation of the samples, analyses by the laboratories and statistical treatment of the data.

SAMPLES PRODUCTION

One of the fundamental aspects for the implementation of a PT is the preparation of homogenous and stable samples.

For this PT, samples were prepared by spiking a batch of homogenized red wine with a suspension of *Dekkera bruxellensis* in well controlled proportions.

According to the requirements of the ISO 13528 [1], homogeneity of the samples was verified by experimental studies on 10 samples *in duplo* taken randomly across a batch of samples. Stability of the product was proved by analyzing 3 samples *in duplo* during 7 days. For both studies, the analyses were performed according to the Compendium of the OIV (International Organization of Vine and Wine [2]).

ANALYSES

Samples were shipped at (5±3) °C to the laboratories participating to the test together with a standard sample for monitoring the temperature.

A reply form was made available to allow the laboratories to return their analysis results.

Moreover, participants are invited to enter in the reply form some complementary information such as the date of the beginning of the analysis, growth medium used, incubation temperature and time and the type of plating.

Given the stability of the product, the participants were invited to analyze the samples as soon as possible after the reception.

STATISTICAL TREATMENT

The statistical treatments were conducted according to ISO 13528 standard [1]. The assigned values (X) were estimated using the robust means of the results. The proficiencies of each laboratory were evaluated thanks to tolerance values (TV) of twice the standard deviations. The results (x) could be evaluated and classified through z-scores:

$z \leq |2|$: satisfactory

$|2| < z \leq |3|$: questionable

$z > |3|$: unsatisfactory.

where : $z = \frac{x - xpt}{(TV/2)}$

RESULTS and DISCUSSION

The results of the homogeneity check are summarized graphically in Figure 1. These data show that the samples are homogenous enough to meet the requirements of the test, with a gap between the minimal and maximal values of 0,460 CFU/mL in log.

The analyses results of the stability checks showed a satisfactory recovery rate considering the expected concentration after storing the samples at (5±3) °C for 7 days (Table 1). The variability of the performed method can explain the difference between the results collected from D0 to D7.

Sixteen laboratories out of twenty gave their results together with useful information for the interpretation of the data. An assigned value (x_{pt}) of 2.462 log(CFU/mL) was calculated from the robust mean of the all returned results except for those given after the deadline and the result obtained using the PCR method (as this method differs in principle from the analytical method used by the others laboratories which are based on culture microbial growth). The main statistical parameters of this PT are summarized in Table 2.

The laboratories' results are shown as histograms in Figure 3. On this graph, assigned value and tolerance interval are indicated in the x-axis and the results of the laboratories are shown in different colors as a function of the performed method: OIV method (8 laboratories, green), internal method (7 laboratories, bleu) and PCR method (1 laboratory, orange). The means by method are also calculated and are shown in Table 3.

Participants use different growth media from many suppliers (see Figure 2) and the major part of the laboratories performs the enumeration in surface. Incubation temperature and time vary from 25 to 30 °C and from 4 to 12 days respectively. However no tendency was highlighted as a function of the growth media or the performed incubation conditions.

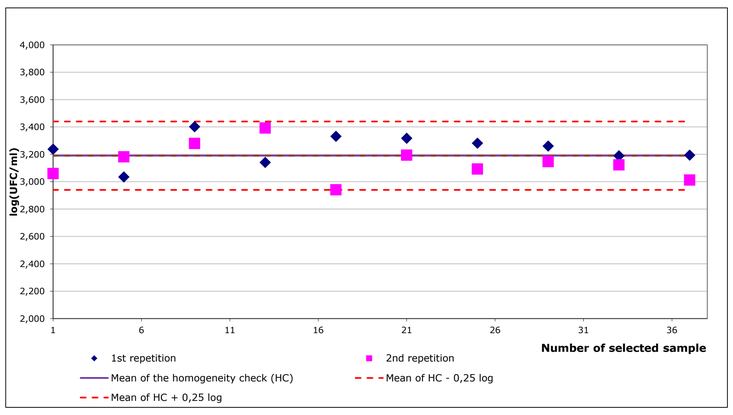

Figure 1. Homogeneity check

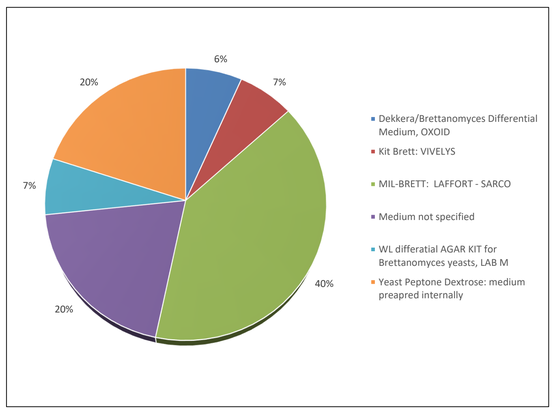

Figure 2. Different media used by the laboratories

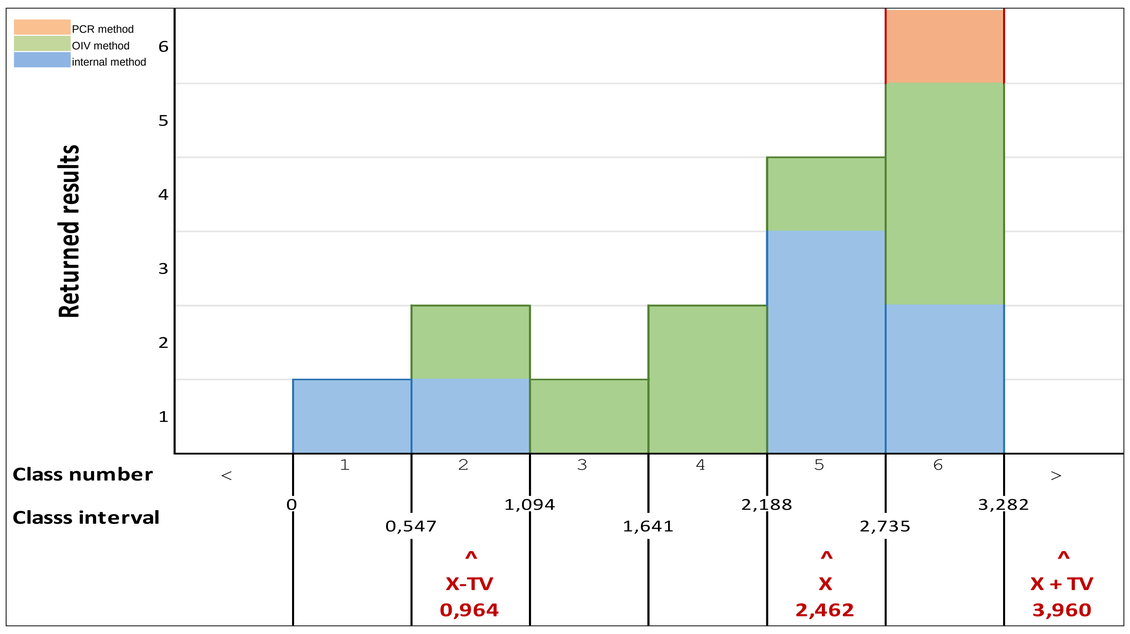

Figure 3. PT results represented as a histogram

Day of analysis	D0	D1	D3	D4	D7
Mean - log(UFC/mL)	2.781	2.405	2.723	2.825	2.463

Table 1. Average results of the enumeration of *Brettanomyces* in wine after 7 days at (5±3) °C

Statistical parameter	Value
Assigned value for proficiency testing (x_{pt})	2,462, log(CFU/mL)
Standard uncertainty of the assigned value (u_{xpt})	0,270, log(CFU/mL)
Robust standard deviation of the results (s^*_x), from all the results which participated to the estimation of the assigned value	0,749, log(CFU/mL)
Number of results (p_x)	12
Coefficient of variation (CV_x)	30%
Standard deviation for proficiency assessment (SDPA), characteristic of dispersion related to the evaluation of the results	0,749, log(CFU/mL)
Tolerance value, two times the standard deviation for proficiency assessment. It is a maximum tolerated deviation from the assigned value (VT = 2 x SDPA)	1,498, log(CFU/mL)
Upper limit of the tolerance interval (Assigned value + tolerance value). Value of the parameter over which the result x is considered as untrue.	3,960, log(CFU/mL)
Lower limit of the tolerance interval (Assigned value - tolerance value). Value of the criterion below which the result x is considered as untrue.	0,964, log(CFU/mL)
Number of untrue results (p_D)	2

Table 2. Summary of the statistical treatment of the data

	OIV method	Internal method	PCR method
Means, x^*_m	2.182	2.060	2.916
Standard uncertainty of the means, $u_{x^*_m}$	0.397	0.680	-
Standard deviation of the results, s^*_m	0.898	1.438	-
Number of the results, p_m	8	7	1

Table 3. Means by used method in PT, log(CFU/mL)

CONCLUSION

A PT for *Brettanomyces* analysis in wine, gathering twenty laboratories around the world, was successfully implemented and the results were published in an interlaboratory comparison report distributed to the participants. This first PT has been transformed into a regular PTS, including 3 tests per year. Moreover, the analyses of lactic and acetic bacteria are also added in this regular PT. Laboratories can now monitor punctually and/or continuously through time the reliability of their results and obtain recognition of their analytical procedures by the accreditation bodies for microbiological analyses of wines.

REFERENCES

(1) **ISO 13528:2015** - Statistical methods for use in proficiency testing by interlaboratory comparisons

(2) [OIV] International Organization for Vine and Wine 2017. Compendium of International Methods of Wine and Must Analysis, Vol. 2, section 4.

(3) **ISO 17043:2010** - Conformity assessment - General requirements for proficiency testing.