Performance Evaluation of the GeneDisc® Method for Detection and Identification of Wine Spoilage Yeast

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Introduction

Monitoring wine spoilage yeast is a concern for major industries who value quality product delivery and brand protection. Detection is commonly based on traditional culture method using selective enrichment, biochemical tests and microscopy. Conventional methods can be challenging because a high level of expertise is required and screening/identification may require up to 10 days.

Pall GeneDisc Technologies offers an innovative solution to accelerate the time to result from 2 h without enrichment or to 28 h / 72 h with enrichment. The GeneDisc system is based on real-time PCR (polymerase chain reaction), which facilitates the screening of spoilage yeast and the identification of major spoilers (Figure 1).

Yeast Screening		
Well	Dye 1	Dye 2
1	Inhibition control	
2	Yeast Screening	
3		

Yeast Identification		
Well	Dye 1	Dye 2
1	Inhibition control	*Saccharomyces cerevisiae diastaticus*
2	*Saccharomyces cerevisiae*	*Saccharomyces* spp.
3	*Brettanomyces bruxellensis*	*Saccharomycodes* spp.
4	*Brettanomyces* spp.	*Saccharomyces pastorianus*
5	*Schizosaccharomyces* spp.	*Zygosaccharomyces* spp.
6	*Candida* spp. *Pichia* spp.	*Zygosaccharomyces bailii*

Figure 1 : GeneDisc Plate Layout

Ready-to-use reagents and automatic interpretation of results make this platform easy to implement. Operator training can take less than a day.

The goal of this study was to evaluate performance of this new method in terms of specificity sensitivity and time to result compared with the plating method.

Materials and Methods

Sample Preparation and PCR Analysis

Wine samples were filtered through a Microcheck® II beverage monitor Metricel® black membrane 0.45 µm, 10 mL of wort broth was added to the Microcheck II Beverage monitor and the samples were incubated for 28 h – 72 h at 25 °C. The membrane was transferred into a GeneDisc Extraction Pack Food 01 for yeast lysis. The processed samples were loaded onto Yeast Screening and Yeast ID GeneDisc Plates and run on the GeneDisc Cycler as shown in figure 2.

Figure 2 : Protocol Workflow for Wine Samples

After a one hour PCR run, data was automatically processed by the GeneDisc Cycler. An example of result interpretation is displayed (Figure 3).

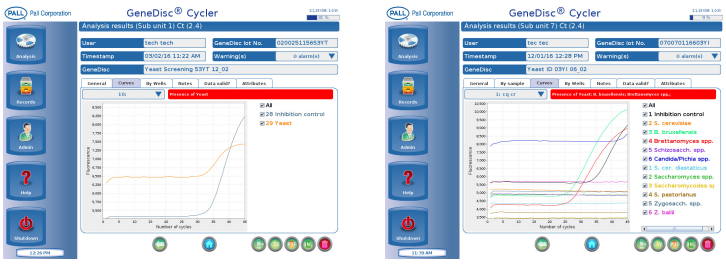

Figure 3 : Display of PCR results on (i) Yeast Screening and (ii) Yeast ID GD plates

The method was validated with a large panel of wines including red, rosé, white, sparkling white, peach white, sweet white, raspberry rosé, grapefruit rosé & sangria wines.

Specificity

Specificity of the PCR assays was determined by spiking samples with 21 bacterial and mold strains that may be present in wine and 85 spoilage yeast strains.

Sensitivity

Sensitivity was evaluated with calibrated range of cells. Spiking of wine samples with low levels of collection yeasts was also performed; enumeration was completed by plating and microscope.

Validation of the Time to Result

A time to result below 2 h was validated for samples without enrichment and colony identification.

When the detection of down to 1 cell / sample is required, an enrichment is needed. To evaluate the time to result after enrichment, spiked samples were tested and the time to result was compared to a culture method (WLN agar plates at 25 °C).

Results

Specificity

Inclusivity and exclusivity were tested using 106 strains reported in table 1.

Other bacteria and molds that may be present in wine were tested to demonstrate the lack of cross reactions.

Exclusivity	*Acetobacter* sp. , *Aspergillus* spp. *Bacillus* spp., *Burkholderia cepacia, Byssochlamys* spp., *Citrobacter* spp., *Cladosporium cladosporioides, Cryptococcus neoformans, Escherichia coli, Fusarium oxysporum, Gluconobacter sp., Lactobacillus* spp., *Megasphaera* spp., *Mucor racemosus, Paecilomyces variotii, Pectinatus* spp., *Pediococcus* spp., *Penicillium* spp., *Pseudomonas aeruginosa, Salmonella enterica, Talaromyces flavus*
Inclusivity *(Yeast Screening and Yeast ID)*	*Aureobasidium pullulans, Blastobotrys adeninivorans, Brettanomyces anomalus , B. bruxellensis, B. custersianus, B. naardenensis, Candida albicans, C. davenportii, C. parapsilosis, C. sake, C. stellata, C. tropicalis, C. zemplinina, C. zeylanoïdes, Debaryomyces hansenii, Pichia fermentans , P. kudriavzevii, P. membranifaciens, Saccharomyces bayanus, S. eubayanus, S. cerevisiae, S. cer. diastaticus, S. uvarum*
Additional Inclusivity *(Yeast screening)*	*Candida glabrata, Citeromyces matritensis, Clavispora lusitaniae Farysizyma setubalensis, Hanseniaspora/Kloeckera* sp., *H. uvarum, Kluyveromyces marxianus, Metschnikowia pulcherrima, Meyerozyma guilliermondii, Rhodotorula* sp., *R. glutinis*
	100 % of conformity

Table 1: Assay Specificity – Inclusivity & Exclusivity – List of Strains Species

Sensitivity

Table 2 summarizes the results of the sensitivity studies. Limits of detection are reported in cells per PCR well. The lowest spiking levels tested for wine samples are also reported in cells / 750 ml samples.

Yeast PCR Assay	Limit of detection	Lowest spiking level
	Cells / PCR well	Cells / 750 ml sample[1] With enrichment
Yeast Screening	down to 1	down to 1
Saccharomyces cere. diastaticus	20 [2]	1[2]
Saccharomyces cerevisiae	1	1
Saccharomyces spp.	1	1
Brettanomyces bruxellensis	1	8
Saccharomycodes spp.	1	3
Brettanomyces spp.	1	8
Saccharomyces pastorianus	1	8
Schizosaccharomyces spp.	down to 5	4
Zygosaccharomyces spp.	down to 1	6
Candida-Pichia spp.	down to 10 (*Candida* spp.) down to 1 (*Pichia* spp.)	down to 1
Zygosaccharomyces bailii	1,200	6

[1] Based on sample preparation protocol with 750 mL of wine filtered [2] validated on beer sample

Table 2: Assay Sensitivity

Time to Result

For a sensitivity down to 1 cell per sample, an enrichment is required. Table 3 reports the comparison between the GeneDisc Method and culture method

		GeneDisc Method	
		28 h	72 h
Culture Method	2 days	*S. ludwigii* *S. cerevisiae*	
	3 days	*C. stellata* (10-100 cells) *P. fermentans* (10-100 cells) *S. eubayanus* *S. japonicus* (10-100 cells) *S. pastorianus*	
	Not detected	*P. fermentans* *S. japonicus*	*C. stellata* *B. bruxellensis* *Z. bisporus*

Table 3: GeneDisc Method Results for Spiked Wine Samples

The GeneDisc method had a shorter time to result for most of the yeast tested, moreover the enrichment time can be a powerful tool to set the sensitivity level.

Conclusion

The GeneDisc real-time PCR method for yeast is a fast, highly sensitive and specific method. Results from different wines types demonstrated the method was able to accurately detect and identify yeast even at low contamination levels.

Performance, easy-to-use design and ready-to-use reagents make the GeneDisc system a good monitoring tool for the wine industry. Shortened time to results compared to traditional methods can accelerate decision making for batch release and in process monitoring.

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