Comparison of rapid detection of *Brettanomyces* in Australian wines using flow cytometry and PCR methods

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Background

- *Brettanomyces bruxellensis* is the major wine spoilage yeast.
- Plating onto selective media is considered the gold standard for *Brettanomyces* detection; however, results can take up to 10 days.
- Alternative, rapid methods are available and include PCR and flow cytometry-based methods.

Comparative results

- Of the 23 *Brettanomyces* negative wines, only one was positive by plating (6 cfu/mL) with both rapid methods returning a negative result.
- Comparative results for the 21 *Brettanomyces* positive wines determined that:
    - Flow cytometry results correlated with plating 67% of the time
    - PCR results correlated with plating 38% of the time
    - All three methods agreed 24% of the time

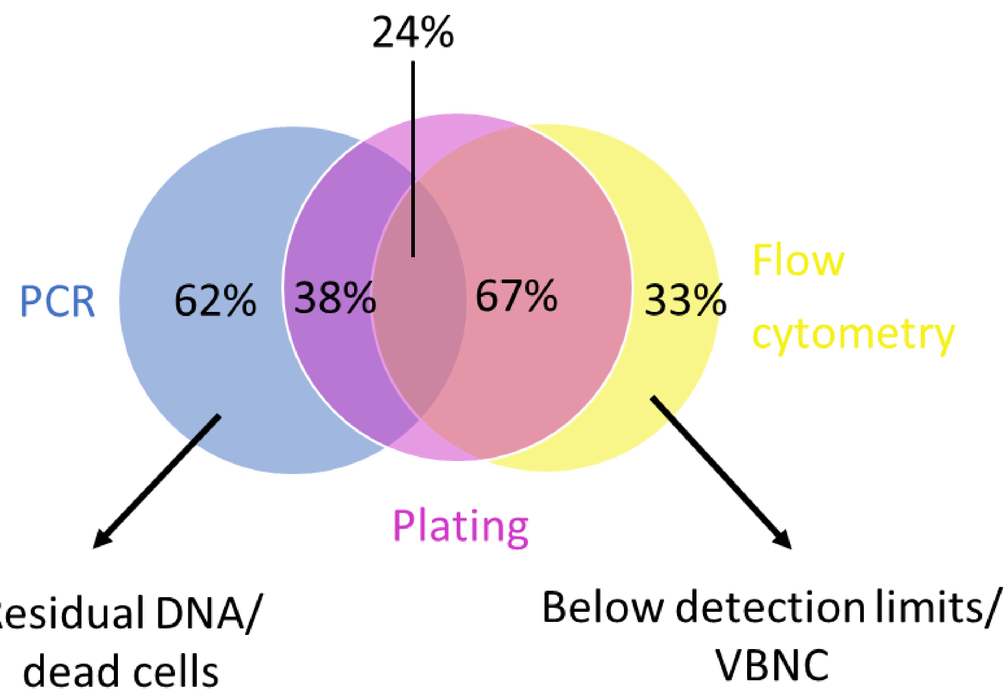

Figure 1. A summary of the consistency of results between the three methods. 21 wines were determined to be positive for *Brettanomyces* by PCR based methods. Wines were then analysed by flow cytometry and selective plating. PCR results agreed with plating 38% of the time. Flow cytometry results agreed with plating 67% of the time. All three methods agreed 24% of the time. Differences in results between PCR and plating were attributed to residual DNA and/or dead cells. Differences in results between flow cytometry and plating were attributed to cells being below detection limits and/or non culturable cells.

Conclusions

- Plating remains the gold standard for detection of *Brettanomyces*
- For rapid detection the flow cytometry method correlates more highly with plating
- The flow cytometry method is semi-quantitative

Method

- 44 Australian wines (23 *Brettanomyces* negative, 21 *Brettanomyces* positive) were analysed and compared by three detection methods:
    - Selective plating
    - PCR-based rapid detection method (VinoBRETT by Veriflow™)
    - Flow cytometry-based rapid detection method (Sysmex CyFlow™ BrettCount)
- Wines were determined to be positive or negative based on initial PCR results
- The quantitative nature of the two rapid methods was determined using serially diluted *Brettanomyces* positive wine.

A breakdown by grape variety of wines analysed

Grape Variety	Number of wines	Grape Variety	Number of wines
Shiraz	8	Cabernet Blend	2
Pinot Noir	7	Cabernet Franc	1
Cabernet Sauvignon	5	Malbec	1
Merlot	1	Barbera	1
Mourvèdre	1	Grenache	1
Sauvignon Blanc	1	unknown red	13
Pinot Grigio	1	unknown white	1

Quantitative results

- Flow cytometry method is semi-quantitative
- PCR method is not quantitative

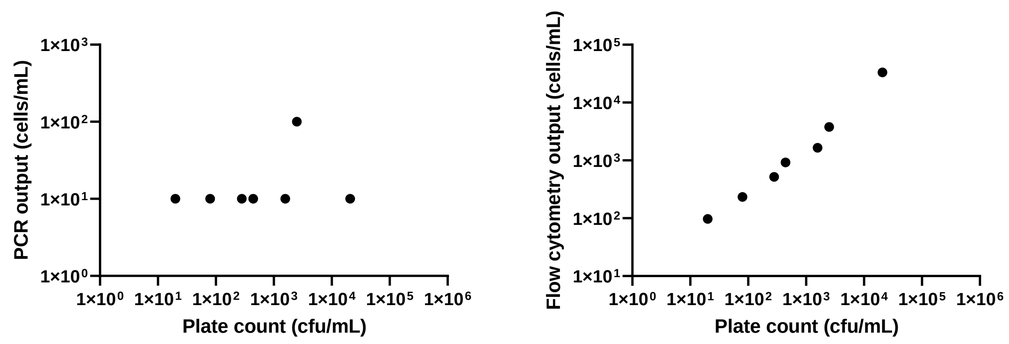

Figure 2. A comparison of the quantitative nature of two rapid *Brettanomyces* detection methods in serially diluted wine. A *Brettanomyces* positive wine was serially diluted in filtered wine and analysed by a PCR-based method (left) and a flow cytometry method (right). Each method was analysed against plating of each dilution onto selective media. PCR method output was set at 10 cells/mL (low) and 100 cells/mL (medium).

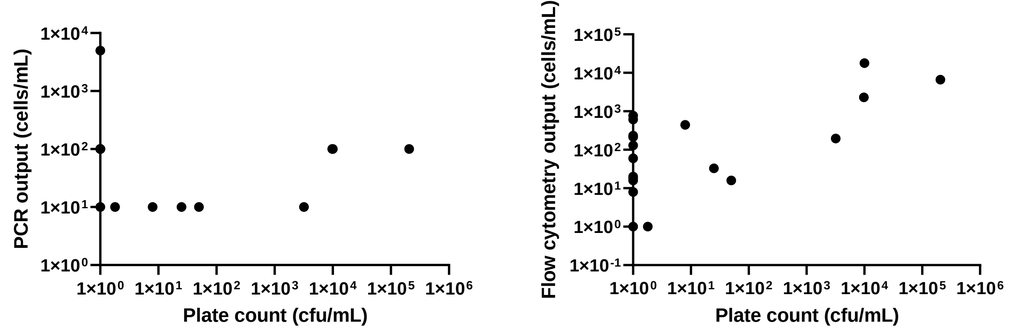

Figure 3. A comparison of the quantitative nature of two rapid *Brettanomyces* detection methods in *Brettanomyces* positive Australian wines. 21 Australian wines were determined to be *Brettanomyces* positive by a PCR-based method (left). Wines were analysed using a flow cytometry method (right) and the result from the two methods were compared with selective plating results. PCR method output was set at 10 cells/mL (low), 100 cells/mL (medium) and 5000 cells/mL (high).

This project was funded by Sysmex Australia.
For more information about rapid *Brettanomyces* analysis please visit the AWRI stand at WineTech.

Wine Australia

The AWRI is a member of the Wine Innovation Cluster.