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Case study: Assessing Aber Countstar performance at low and high yeast concentrations in comparison with the Hemocytometer

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Introduction:

Determining the cell concentration of a yeast sample at low cell densities poses a significant challenge for various methods of analysis. Analysis of yeast cell concentrations as low as $5x10^4$ (50,000) cells/ml – $1x10^5$ (100,000) cells/ml is of increasing significance as a growing number of breweries opt for bottle conditioning their end product as opposed to forced carbonation. This method entails the use of yeast concentrations far lower than that of fermentation, though it is vital that these concentrations are measured to ensure a stable and consistent quality of brew.

Hemocytometry is capable of determining, with accuracy cell concentrations as low as $1x10^6$ cells/ml, as the small scale of the graticule increases variations and results in unreliable counts.

Other manufacturers have tried to overcome this issue, by increasing the size of the imaging field (though still relatively small). However, there is little evidence of these solutions performing better than the Hemocytometer.

The Aber Countstar is unique in its approach using slide based image analysis and the 'gold standard' method of using methylene blue or methylene violet to assess a sample's total, live and dead cell concentration (cells/ml), viability (%) average diameter (µm), aggregations (%) etc. It samples a volume over x8 larger than that of its closest competitor, while the image field is x2 as large. This allows the Aber Countstar to assess cell concentration and viability consistently at levels that challenge alternative methods.

Figure 1: Countstar instrument with five chamber slide.

The greater image field of the Countstar gives it a wide cell concentration range which extends from $3x10^7$ cells/ml to $5x10^4$ cells/ml.

Case study:

In a recent case study, the Aber Countstar was assessed for its consistency at various dilutions representative of the working range of the Countstar. A repeatability test was conducted to compare the Countstar to a Hemocytometer at concentrations of $5x10^4$, $1x10^5$, $1x10^6$ and $1x10^7$ cells/ml.

This demonstrated the reliable functioning of the Countstar within its recommended working range. However, it is also important to understand and assess the repeatability and accuracy of measurement at the lower end of the concentration range.

The Aber Countstar Yeast was used for assessing repeatability at the lower concentration region of its working range. Analysis was carried out using the Countstar software (version 2.7). Dried brewer's yeast (Young's) was used for the tests. Please contact Aber Instruments if interested in the detailed methodology.

Results:

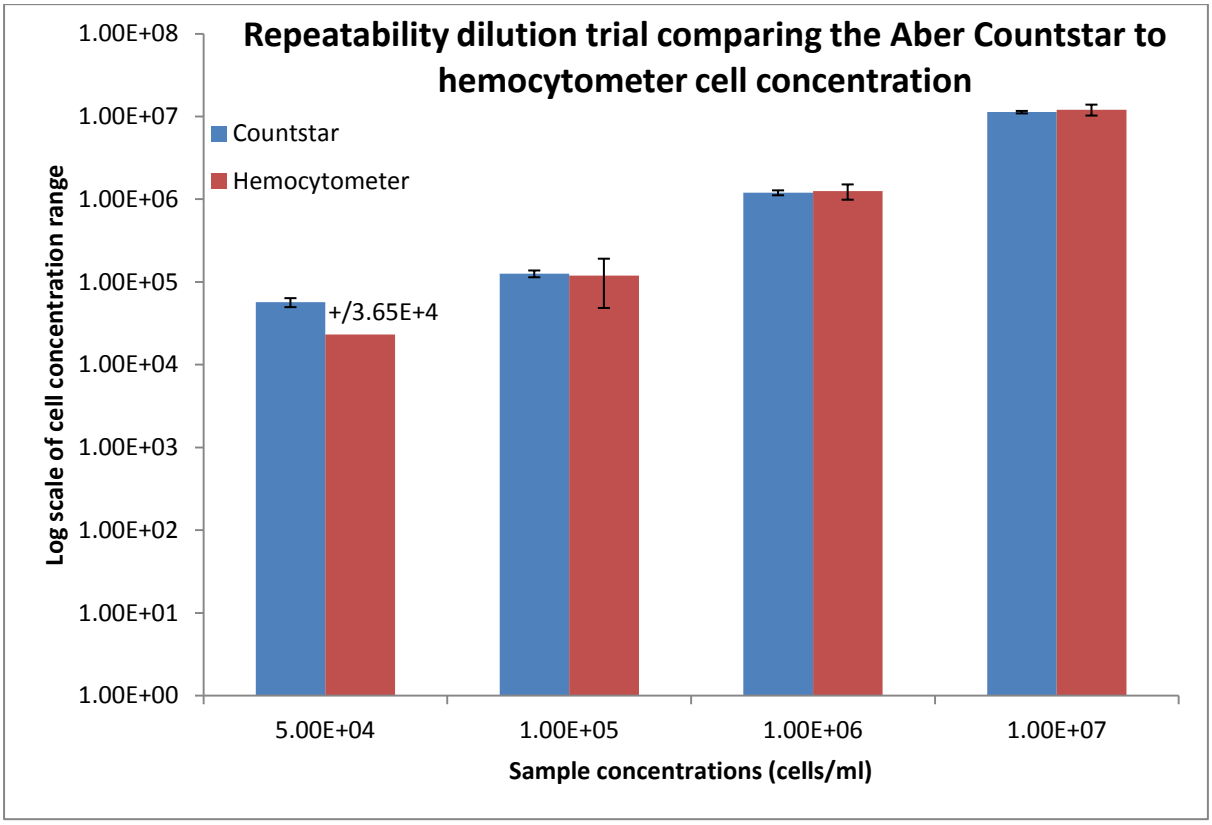

Figure 2: Mean average concentration of 30 repeat yeast samples diluted to approximately $5x10^4$, $1x10^5$, $1x10^6$ and $1x10^7$ cells/ml. Direct comparison of the Aber Countstar to Hemocytometer with standard deviation (SD). Figure values seen below (Fig. 2).

Results express a high SD for the Hemocytometer (poor repeatability) with a decrease in the SD as the concentration increases. Similarly the Countstar has a higher SD at low concentrations which gradually decreases as the concentration increases.
Though when comparing the SD for the two methods used, at each concentration the Countstar is far more repeatable with a lower SD in particular at the lowest concentrations.

Dilution	Countstar $\bar{x}$	Countstar SD	Hemocytometer $\bar{x}$	Hemocytometer SD
$5x10^4$	$5.68x10^4$	$7.07x10^3$	$2.33x10^4$	$3.65x10^4$
$1x10^5$	$1.26x10^5$	$1.19x10^4$	$1.20x10^5$	$7.14x10^4$
$1x10^6$	$1.20x10^6$	$8.18x10^4$	$1.25x10^6$	$2.62x10^5$
$1x10^7$	$1.13x10^7$	$3.89x10^5$	$1.21x10^7$	$1.83x10^6$

Figure 3: Values for Fig. 1 with the sample dilution, Countstar mean average, Countstar SD, Hemocytometer mean average and Hemocytometer SD from left to right respectively.

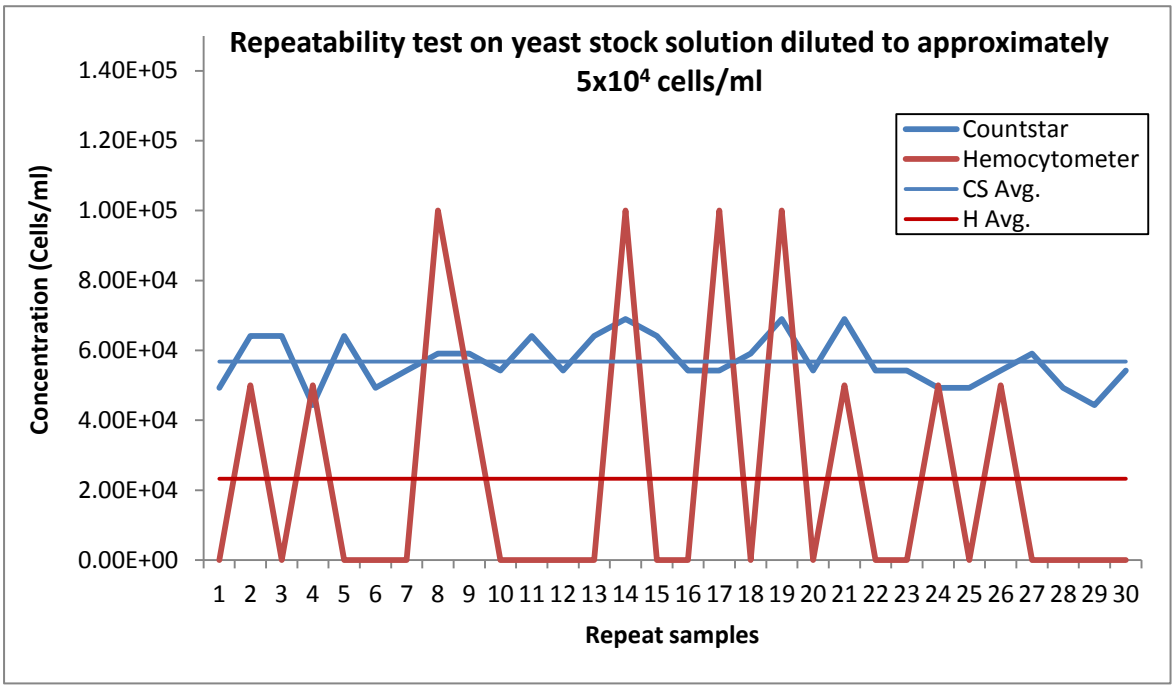

Figure 4: A total of 30 repeat samples for the Aber Countstar and the Hemocytometer at a concentration of 5x10⁴ cells/ml with a mean average line. SD for Countstar (+/12.45%) and Hemocytometer (+/156.65%).

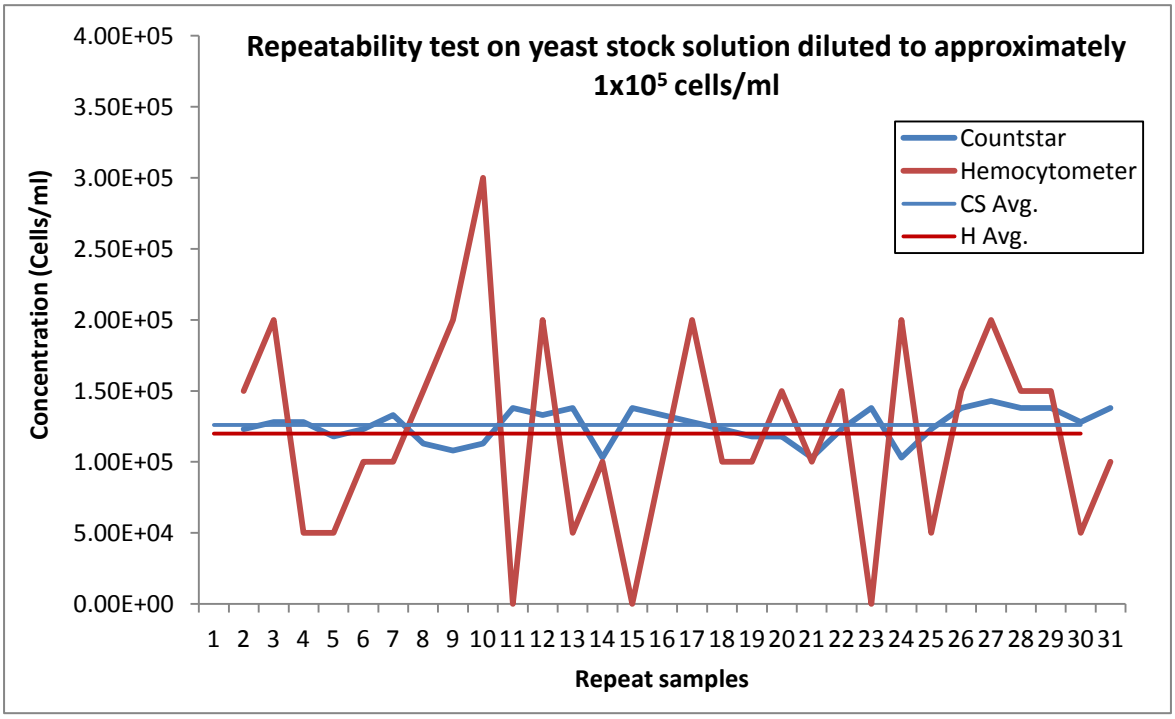

Figure 5: 30 repeat samples for the Aber Countstar and the Hemocytometer at a concentration of 1x10⁵ cells/ml with a mean average line. SD for Countstar (+/9.44%) and Hemocytometer (+/59. 5%).

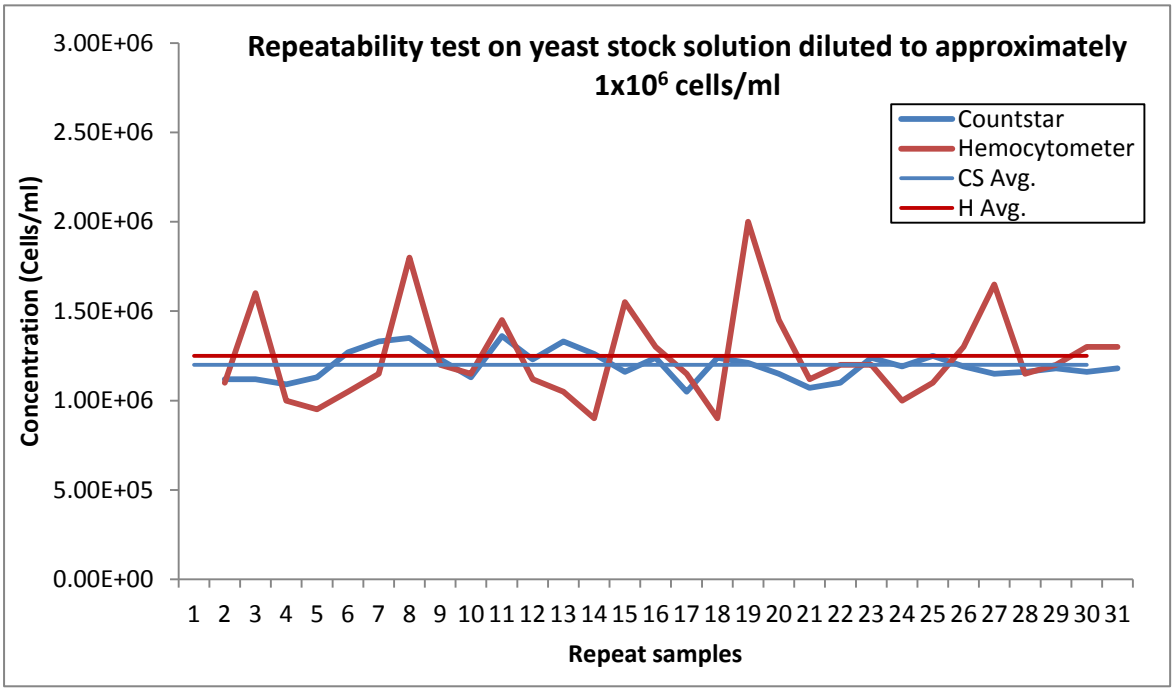

Figure 6: 30 repeat samples for the Aber Countstar and the Hemocytometer at a concentration of 1x10⁶ cells/ml with a mean average line. SD for Countstar (+/6.82%) and Hemocytometer (+/20.96%).

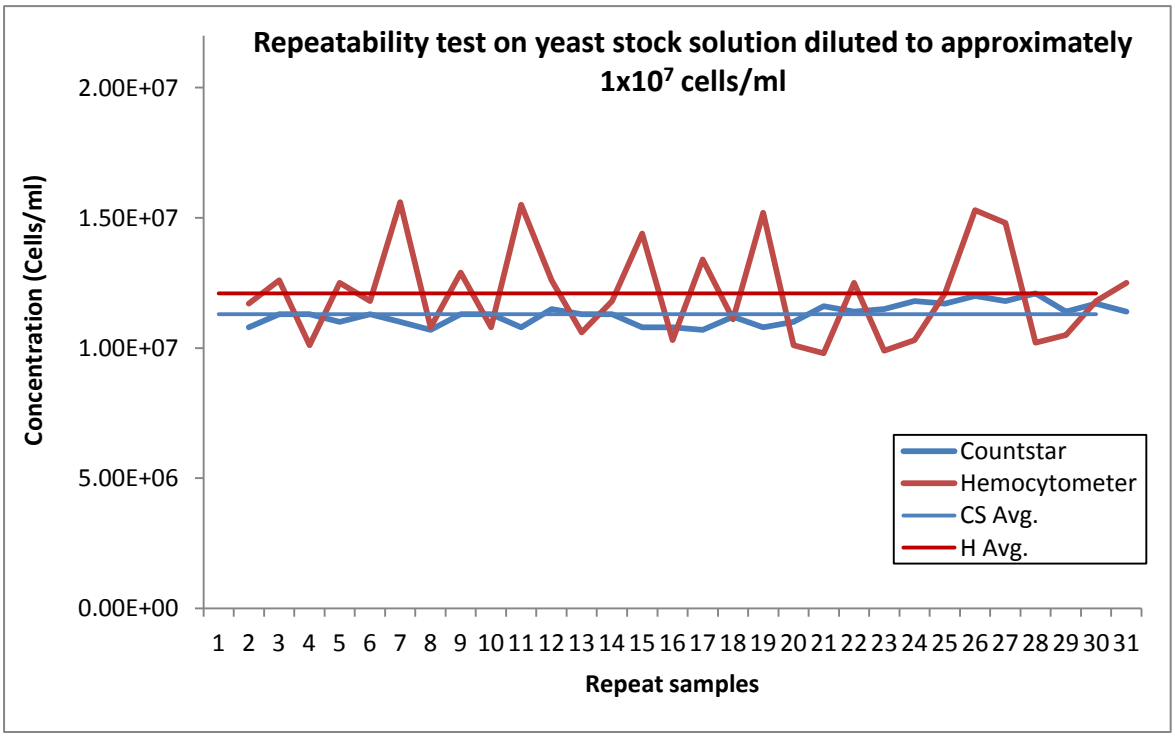

Figure 7: 30 repeat samples for the Aber Countstar and the Hemocytometer at a concentration of 1x10⁷ cells/ml with a mean average line. SD for Countstar (+/3.44%) and Hemocytometer (+/15.12%).

Approximate Cell Count	Percentage error For Countstar	Percentage error For Hemocytometer
$5x10^4$ cells/ml	(+/12.45%)	(+/156.65%)
$1x10^5$ cells/ml	(+/9.44%)	(+/59.5%)
$1x10^6$ cells/ml	(+/6.82%)	(+/20.96%)
$1x10^7$ cells/ml	(+/3.44%)	(+/15.12%)

Figure 8: Percentage error for the Countstar and Hemocytometer at $5x10^4$, $1x10^5$, $1x10^6$ and $1x10^7$ dilutions over 30 repeat samples for each method and dilution.

Conclusion:

It is evident from the results demonstrated above that the Aber Countstar measurements are more accurate, repeatable and reliable at low concentrations than that of the Hemocytometer, as well as at higher concentrations. The superior performance can be attributed to the increased imaging field of the Countstar which reduces the impact of infrequent clusters of cells or alternatively areas devoid of cells to be averaged out. Along with a greater imaging field, the Aber Countstar also eliminates individual operator variability through differing interpretations, allowing for more consistent and repeatable results.

Therefore, to summarise the case study, it was shown that the Aber Countstar is not only more reliable, accurate and repeatable through its entire working range ($5x10^4$ – $3x10^7$ cells/ml) than the Hemocytometer, but also at the lower end of its range ($5x10^4$ cells/ml). This will enable users to successfully analyse the yeast concentrations in applications such as bottle conditioned products.