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White Paper

Inish Mini-Bar

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Abstract

There is an unmet need for a rapid, accurate and portable device to analyse contamination levels in draught beer. The Inish Mini-Bar provides a solution to this problem as it can analyse levels of contamination (bacteria, yeast, and debris) in beer, on-site and directly from the tap. A trial was carried out where the Inish Mini-Bar was used to test levels of contamination in 40 beer samples from bars around Dublin. Agar plating was carried out on the same samples to assess the reliability and accuracy of the results gathered from the Inish Mini-Bar. The results from Inish Mini-Bar and agar plating correlated 80% of the time, when comparing whether lines were clean or contaminated.

Introduction – Limitations in methods for Draught beer quality control

Bacterial contamination of draught beer can affect both the flavour and presentation of the product. Given this, contamination of beer can have negative implications for the brewer, bar owner and customer.

Breweries can have confidence that the bottled beer that the customer tastes is exactly as it was intended, meeting the breweries' strict quality control program. However, the same cannot be said of draught beer. While kegs are sterile leaving the breweries, the moment they are connected to a beer line (and in some cases disconnected and reconnected multiple times), there is a risk of contamination.

At present, agar plating is considered to the be gold standard method of detecting levels of contamination in beer served from the tap. However, this method has some limitations. For example, agar plating is time consuming and lab-based, meaning tests cannot be performed on-site. Additionally, agar plating requires experienced personnel to carry out the tests and it can take 3-5 days to generate results.

Another commonly used method of beer line contamination analysis is carried out using an ATP Luminometer Assay. ATP luminometers provide a more rapid test than agar plating, however, they do not measure levels of contamination directly from the beer. Instead ATP Luminometers measure residual bacteria from water rinsed through the line and not from the beer itself. Additionally, ATP luminometers require 15-30 minutes of sample preparation, leading to a higher man power cost and increasing the downtime of the line. Furthermore, the ATP luminometer test is subjective as the more the line is flushed out with water the lower the RLU reading is, leading to less reliable results across different bars.

Breweries want a rapid diagnostic test that will that will enable them to assess and monitor beer quality from every tap at every outlet. They will then have greater control over the quality of beer being delivered to their customers. Given the research linking beer quality to brewery profits, there is a clear case for investment in technologies that improve their quality control programs.

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To overcome the limitations of current testing methods a rapid, portable, robust and easy to use, low cost test is needed.

The Inish Mini-Bar – Overcoming limitations

The Inish Mini-Bar is a portable device that allows the on-site analysis of contamination levels in draught beer, directly from the tap. It can rapidly analyse the levels of yeast, bacteria and debris in samples. As it stands, there are no methods available to detect levels of bacteria in draught beer on-site in bars and restaurants. In contrast to existing methods, it is low-cost, rapid and effective.

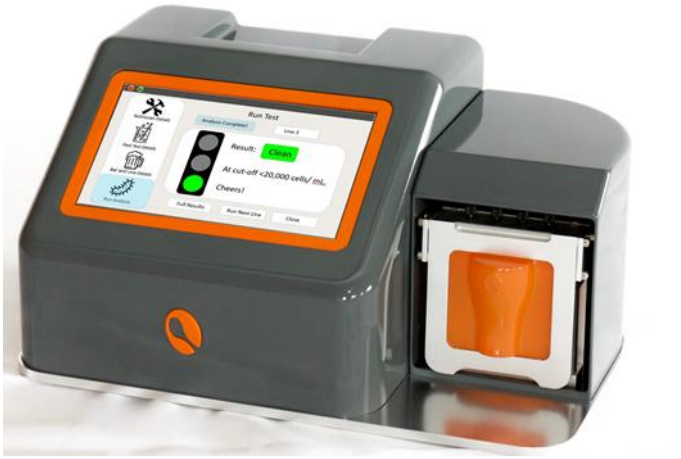

Figure 1. Image of the Inish Mini-Bar with a prototype graphical user interface.

The Inish Mini-Bar integrates microfluidic sample handling & impedance detection on chip with advanced electronics. It is a robust device, with an easy to use touch-screen display. Using impedance detection in combination with microfluidics allows for sample handling to be automated. A test takes approximately 60-90 seconds and results are generated immediately thereafter. Impedance detection allows bacteria, yeast and debris to be counted and analysed. Importantly the Inish Mini-Bar requires minimal sample preparation and the results are easy to interpret. A red light indicates that the sample contains above 20,000 cells/mL and as such lines need to be cleaned immediately. A green light indicates that the sample contains less than 20,000 cells/mL and that lines are clean.

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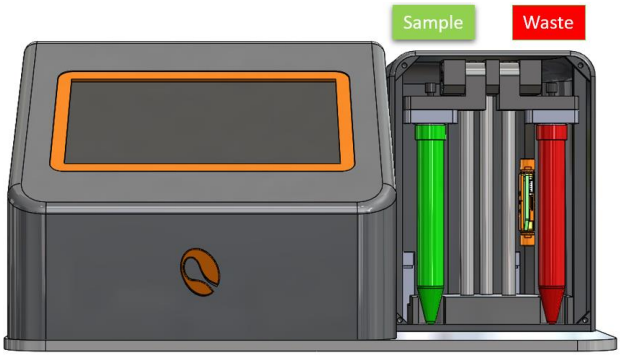
Device specifications

Performance Specifications	
General Specifications	• Easy sample preparation: consumable tubes provided for sample analysis and instrument rinsing • Portable • Provides analysis in minutes • User friendly software • Option to save analysis results to external PC / USB drive
Sample	Beer- no line rinsing required
Operating modes	Bacteria counting and yeast counting (can be executed at the same time
Measurement time	10 min (includes sample degassing)
Bacteria Counting	5,000 cells/mL- 100,000 cells/mL
Bacteria Counting precision	CV 5%
Bacteria Counting accuracy:	Different for ranges: 5,000-20,000 cells/mL; 20,000- 100,000 cells/mL

Operating Specification	
Sample Volume	Up to 11mL
Sample Layout	 **Sample Tube:** 15mL Centrifuge Tube **Waste Tube:** 15mL Centrifuge Tube
Sample analysis	2 minutes
Rinsing cycle time	1 minute

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Technical Specifications	
Power options	12V, Battery powered with charging option. Charging power supply provided.
LCD touch screen	7-inch, resolution 800x600 pixels with capacitive touch
External interfaces	Ethernet and USB 3.0
Weight	5 kg
Size	220mm x 345mm x 179mm

Inish Mini-Bar Vs Agar plating benchmarking trial

A benchmarking trial was carried out using 40 samples from bars around Dublin. The Inish Mini-Bar was used to test levels of contamination in specific beer lines in each bar. The same samples were also tested using agar plating so that the results generated using the Inish Mini-Bar could be benchmarked against the agar plate results, allowing the accuracy of the Inish Mini-Bar results to be determined. For the benchmarking trial 40 samples were analysed from bars around Dublin.

The number of cells per mL (indicating contamination levels) was determined using the Inish Mini-Bar and then compared to results from Agar plating of the same sample. The objectives of this trial were to determine whether the Inish Mini-Bar could replicate results obtained from agar plating and indicate if lines were contaminated (above 20,000 cells/mL) or clean (below 20,000 cells/mL) (Figure 4).

Methods

Samples were collected from specific lines in selected bars across a range of dates (Figure 3). Samples were collected and analysed on the same day using the Inish Mini-Bar and were plated that same day for agar plating. The samples were split in two; One half was used for agar plating and the other half for Inish Mini-Bar analysis.

Agar plating – Bacteria counting

Liquefication of NVV-A Agar

- NVV-A Agar bottles were liquefied in a 95°C water bath for 40-45 minutes and then allowed to cool to approximately 45°C. 300µL of 2-Phenylethanol and 250µL of Cycloheximide were added and swirled gently to mix.

Sample Preparation

- All preparations were done in the Biosafety hood
- Sterile lager beer that had been filtered using a 2µm filter was spiked with a known concentration of *lactobacillus* and poured into a 15mL tube. This was mixed by votexing for 5 seconds. This was subsequently used as the D0 dilution for the positive control.
- Three 15 mL tubes were labelled from D1 to D3. 9mL of filtered beer was added to D1, D2 and D3. The sample D0 was serially diluted to give D1 (1:10), D2 (1:100), D3 (1:1000).

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- The same process was carried out for all trade samples collected on the day of the experiment.

Pour plate method

- Petri dishes were labelled appropriately (D0-D3). 1mL of sample (D0, D1, D2, D3) was added to each petri dish and NBB-A Agar (20 mL) was poured into each petri dish. The dish was rotated gently to mix the contents of the plate.

Incubation of NBB-A Agar plates

- The reaction zone of an Anaerotest strip was moistened with one drop of distilled water and put into the Anaerobic jar.
- The agar plates are stacked (upside down) in the plate holder of the Aneorojar. The plate holder was then placed in the Anaerojar.
- Water (35mL) was slowly poured with a measuring cylinder evenly over AnaerocultA special paper over a period of 15-20 seconds, holding the AnaerocultA as horizontal as possible.
- The moist AnaerocultA was then placed in the Anaerojar immediately. The Anaerojar was tightly sealed and incubated at 30°C for 5 days in the incubator.

Plate Count

- After 5 days of incubation, the lid of Anaerojar was opened and the bacterial colonies were counted. The number of colonies counted was multiplied by the appropriate dilution factor to get the number of cells/mL.

Agar plating – Aerobic yeast and bacteria counting

- Pre-prepared WLN sterile agar plates were used
- The samples prepared as described in the sample preparation section above were used here.

Spread plate method

- 100 µL of each dilution (D0, D1, D2 and D3) was transferred to the surface of culture plates. This was repeated in duplicate (one set for aerobic bacteria and the other for the yeast).
- Each sample was spread on the corresponding Agar plate using a sterile L-shaped loop.
- One set of plates was incubated at 30°C for 48-72h under aerobic conditions to count yeast colonies, the other set was incubated at 30°C for 48-72h under aerobic conditions to count aerobic bacteria colonies.

Plate count

- After 3 days of incubation, the plates were removed from incubators and the yeast/aerobic bacteria colonies were counted.
- In order to calculate yeast/aerobic bacteria concentration, the following formula was used: Concentration = Number of colonies x Dilution Factor/ Volume plated

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Inish Mini-Bar analysis

Sample preparation

- The trade sample was taken directly from the tap.
- Canned beer spiked with known concentrations (500,000-2,000,000 cells/ mL) of bacteria was used as the positive control.
- Filtered beer containing no contaminants was used as the negative control.

Samples degassed

- 5 mL of sample was poured into a pre-prepared 15 mL tube containing 400 uL of PBS (25X).
- The lid was placed on tube and shaken for 10 seconds. The lid was then removed to allow gas escape.
- This was repeated 3 times to ensure beer was degassed.

Analysis

- The sample was inserted into Inish Mini-Bar and analysed for ~90 seconds at a flow rate of 50 µL/min.
- Line cleanliness is indicated straight away.
- Results were further analysed using CytoSpec software. The FCS files from the Inish Mini-Bar were opened and the bacteria and yeast populations were gated appropriately.
- The number of events contained in the gate was then multiplied by a constant of 10 to account for the dilution factor and the events lost in the debris.

Figure 2. Simplified overview of the Inish Mini-Bar protocol.
Samples are taken directly from the tap. They are inserted into the Inish Mini-Bar and analysed in 60-90 seconds. The Inish Mini-Bar will then indicate if lines are clean or contaminated.

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Results

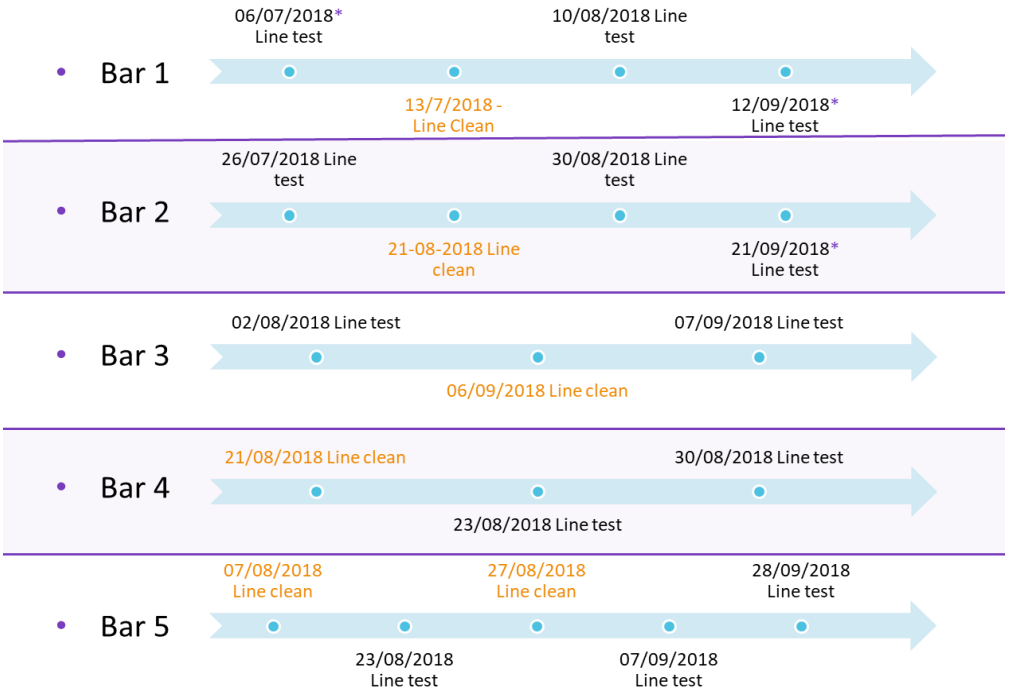

***Figure 3. Dates of line cleans and tests**. Note that dates marked with * indicates that Inish Mini-Bar results could not be used from these dates due to technical and sample difficulties.*

Figure 3 shows the dates of line testing for each bar and the dates that lines were cleaned (when known) for each Bar. In total 5 bars were tested. The bars ranged from ones with good cleaning regimes to ones with poor cleaning regimes so that a spread of contamination levels could be analysed.

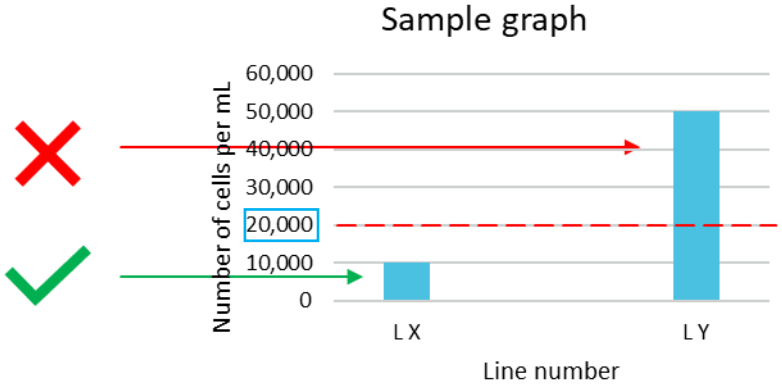

Figure 4. Sample graph of results.

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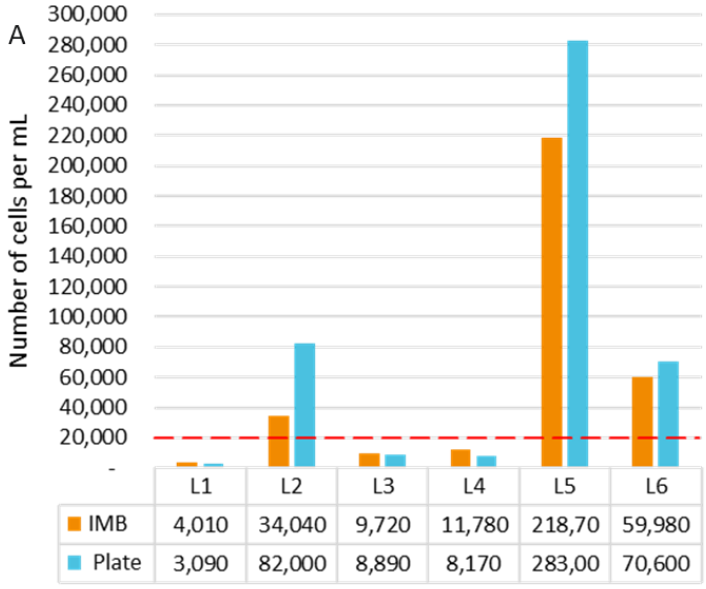
When levels of contamination are below 20,000 cells/mL lines are indicated to be contaminated by the Inish Mini-Bar and need to be cleaned immediately. When levels of cells are below 20,000 cells/mL lines are indicated to be clean by the Inish Mini-Bar and lines need not be cleaned.

A



	L1	L2	L3	L4	L5	L6
IMB	4,010	34,040	9,720	11,780	218,70	59,980
Plate	3,090	82,000	8,890	8,170	283,00	70,600

B

Line Number	Clean (●) (<20,000 cells/mL) or dirty (●) (>20,000 cells/mL)
L1	IMB ● Plate ●
L2	IMB ● Plate ●
L3	IMB ● Plate ●
L4	IMB ● Plate ●
L5	IMB ● Plate ●
L6	IMB ● Plate ●

Figure 5 Inish Mini-Bar Vs Agar plating. *Sample of results. (A) The red dotted line indicates 20,000 cells/mL. When levels of contamination rise above this the line is deemed to be contaminated. (B) indicates whether the lines were clean or dirty. Lines were tested on the 30/08/2018. Lines had been cleaned on the 30/08/2018, 9 days before testing.*

A sample of the results generated can be seen in Figure 5. As shown, the Inish Mini-Bar indicated that lines L2, L5 and L6 were contaminated. In this case the Inish Mini-Bar correlated with the agar plate method 100% of the time, indicating whether the lines needed to be cleaned or not. Despite lines being cleaned 9 days previously the level of contamination in some of the lines was above the level at which line cleaning is required. Line L5 contained substantial levels of contamination, over 10 times the amount needed for lines to be considered dirty, at which point they need to be cleaned immediately.

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Line	Bar 1 (T1)	Bar 2 (T1)	Bar2 (T2)	Bar 3 (T1)	Bar 3 (T2)	Bar 4 (T1)	Bar 4 (T2)	Bar 5 (T1)	Bar 5 (T2)	Bar 5 (T3)
L1	IMB ● Plate ●	IMB ● Plate ●	IMB ● Plate ●	IMB ● Plate ●	IMB ● Plate ●	IMB ● Plate ●		IMB ● Plate ●	IMB ● Plate ●	IMB ● Plate ●
L2	IMB ● Plate ●	IMB ● Plate ●	IMB ● Plate ●	IMB ● Plate ●	IMB ● Plate ●	IMB ● Plate ●	IMB ● Plate ●	IMB ● Plate ●	IMB ● Plate ●	IMB ● Plate ●
L3	IMB ● Plate ●	IMB ● Plate ●	IMB ● Plate ●	IMB ● Plate ●	IMB ● Plate ●	IMB ● Plate ●	IMB ● Plate ●			
L4	IMB ● Plate ●	IMB ● Plate ●	IMB ● Plate ●	IMB ● Plate ●	IMB ● Plate ●					
L5	IMB ● Plate ●	IMB ● Plate ●	IMB ● Plate ●	IMB ● Plate ●	IMB ● Plate ●					
L6	IMB ● Plate ●		IMB ● Plate ●		IMB ● Plate ●					
L7	IMB ● Plate ●									

***Figure 6. Lines tested across all bars**. Test (T) number is indicated in the top row along with bar number. A green dot indicates lines were shown to be clean (below 20,000 cells/mL) and a red dot indicates lines were shown to be contaminated (above 20,000 cells/mL).*

Figure 6 shows a summary of all tests carried out during the benchmarking trial. Of the 40 bars tested, 21 out of 40 lines (52%) contained more than 20,000 cells/mL and were therefore were contaminated. Furthermore, the Inish Mini-Bar correlated with the Agar plating 80% of the time. Figure 7 (below) shows each line tested, grouped by date and by bar showing the number of cells per mL as shown by the Inish Mini-Bar and the Agar plate results respectively.

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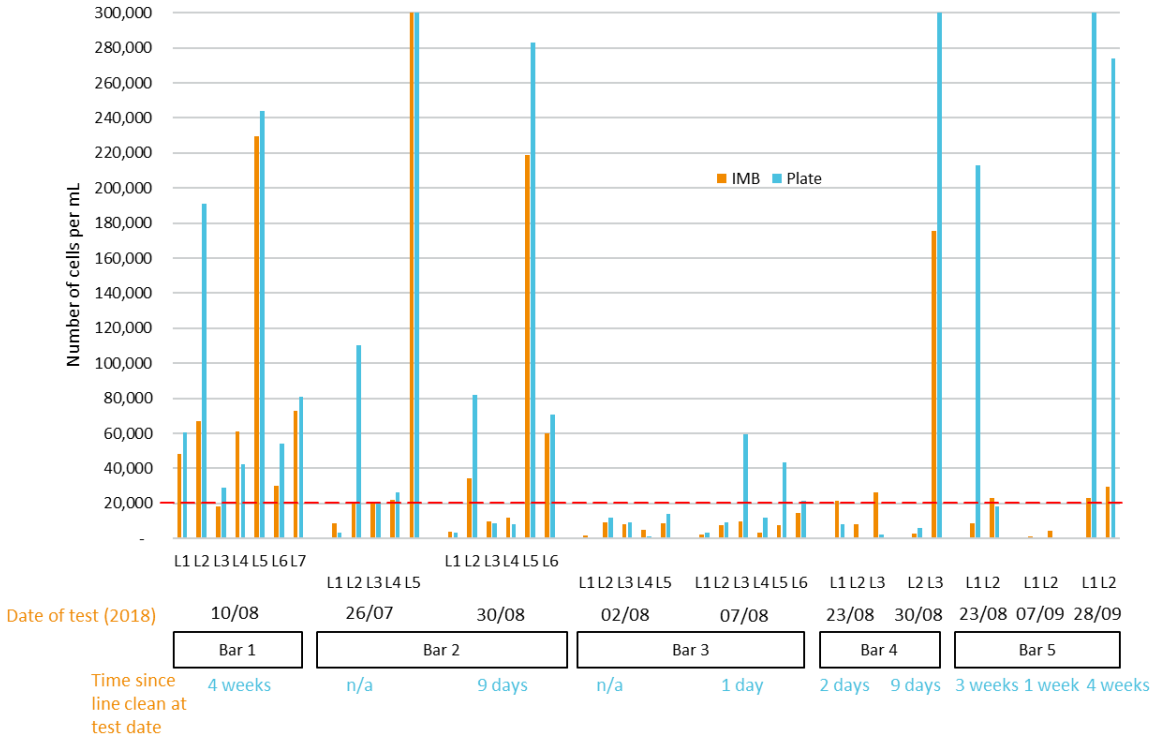

Figure 7. Summary of results. Inish Mini-Bar Vs Agar plate*. The name of each line is indicated directly beneath the graph. The date of testing is also displayed beneath that along with which bar was tested. The time since the line was last cleaned is also indicated.*

Discussion

As aforementioned, from the 40 samples tested from the 5 bars around Dublin, the Inish Mini-Bar and Agar plating results correlated 80% of the time. The Inish Mini-Bar was able to determine levels of bacteria, yeast and debris repeatably and reliably with high levels of accuracy. Additionally, the Inish Mini-Bar demonstrated clear advantages over Agar plating method with regards to speed and simplicity. The Inish Mini-bar is also able to discern levels of debris and dead cells which the agar plate cannot. It is yet to be seen if dead cells and debris have a substantial effect on the quality of beer served from the tap, but these contaminants could be important to measure.

Differences in results in Bar 3 between the Inish Mini-Bar and agar plating were due to high levels aerobic bacteria and high levels of debris in the second round of testing. The high levels of aerobic bacteria can be difficult to distinguish from debris and noise and this caused the differences seen in lines L3, L5 and L6 between the Inish Mini-Bar and agar plate results on the second round of testing. The debris seemed to be small and random in shape and size leading to the signal generated from the bacteria to be masked and thus the results from the Inish Mini-Bar to be skewed. This has since been improved.

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Interestingly, despite the lines having been cleaned the day before the second test was carried out, the lines remained contaminated. This may indicate that the lines weren't cleaned properly. If improvements are made in the ability of the Inish Mini-Bar to distinguish bacteria from debris, the Inish Mini-Bar could prove to be extremely useful in situations like this, where a test after the lines were cleaned could rapidly indicate if they were cleaned properly or if they needed to be cleaned again or possibly replaced, rather than waiting days for results from Agar plating.

In Bar 2, lines were cleaned 9 days before the second testing took place and 3 of the 6 lines tested were clean. The Inish Mini-bar correlated with agar plating 91% of the time over the two dates of testing. The one time the Inish Mini-Bar showed a different result to the agar plating it was only just below the cut-off point (20,000 cells/mL), with the Inish Mini-Bar showing 19,990 cells/mL. If this data had been analysed further, as is possible, then the technician could see that the lines were very close to containing more than 20,000 cell/mL and could judge that the lines needed to be cleaned. As the lines had been left uncleaned for a substantial amount of time all lines contained over 20,000 cells/mL as expected. Levels of contamination in L5 dropped substantially between the first and second test.

The Inish Mini-Bar and the agar plate results correlated 86% of the time in bar 1. There are only results shown for one testing date for bar 1. This is due to the fact that during the other two tests of bar 1 the sample was overly diluted in addition to the Inish Mini-Bar not functioning properly.

Concluding remarks

The Inish Mini-Bar has the potential to help beer companies increase profits, increase yield, reduce the number of lost customers and reduce reputation damage due to poor quality product. As such, the Inish Mini-Bar represents a robust, easy to use and accurate testing method perfect for on-site testing and monitoring of beer quality.