Why use Individual Bacteria Count instead of Colony Forming Units?

- Because it will assist the milk producer better in improving milk quality!

In most countries worldwide the bacteriological quality of raw milk is informed in Colony Forming Units (CFU) even though bacteria analyzers have recorded milk quality in Individual Bacteria Counts (IBC) for more than 20 years. The reason for this is that the Standard Plate Count (SPC) method, which is the reference/anchor method, provides results in CFU. And 20 years ago it was more correct and convenient to convert results from the new proprietary methods into the well known CFU units.

In this and the coming articles I will argue why I believe we can offer the dairy farmers better assistance to improve the bacteriological quality of his raw milk by offering the bacterial counts in Individual Bacteria Counts (IBC). A part from being much cheaper per sample at high sample volumes and more environmentally friendly these methods also offer the farmers a potential for being quicker in solving upcoming hygiene challenges. But changing between IBC’s and CFU’s (or vice versa if this was the case) will only offer a rough estimate. So it may blur the results and it for sure makes it impossible to compare results across borders!
Introduction

Years back when the first BactoScans were introduced, the first conversion equations were established. Not too many years later the EU hygiene directive was included in the Codex Alimentarius, stating that raw milk for human consumption must be below 100,000 CFU/ml. The purpose of this was to improve milk quality - and it has for sure worked! Total bacteria count has decreased dramatically since then! But it is now more than 20 years ago, and it is time to move on, - benefitting from all the experiences we have gained since then!

Read here why we lose information and waste resources by converting between IBC's and CFU's. Learn from the countries that chose to do differently and get suggestions for how a simpler and cheaper quality assurance system can be established!

If coming up with these suggestions 10-15 years back I would probably be accused of favoring the equipment supplier. Today, in a well established market with more suppliers of equipment present, - and probably more to come, this argument is no longer relevant. Instead it is important that IDF/ISO establish up to date procedures for testing new equipment and for establishing quality assurance systems for existing solutions which will work across borders and do not blur the information to the farmer!

CFU and IBC, definitions.

Total Bacterial Count (TBC) is determined in Colony Forming Units (CFU) when using the anchor method/reference method/standard plate count method for determination of total bacteria in milk. However, today most laboratories are using flow cytometers for determination of total bacteria in raw milk, and these instruments are counting the number of Individual Bacteria Count (IBC) in milk. The difference is illustrated in figure 2. When plating the milk, the bacteria colonies which can be counted after 72 hours each originates from a unit (a CFU) consisting of a variable number of individual bacteria in one cluster. When analyzing the same sample with a flow cytometer these individual clusters are dispersed and the (approximate) number of individual bacteria (IBC) will be counted.
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Therefore the number of IBC’s will usually be significantly higher than the corresponding number in CFU. How much will depend on a number of parameters.

Fundamental differences between the two methods

It is important to be aware that there are fundamental differences between above mentioned two methods!

The standard plate count (SPC) method count all colonies that grow and form a visible colony when plated on agar for 72 hours at 30 degrees. The flow cytometer will count all particles that, when hit by laser light, reflect a fluorescent pulse, after incubation with a DNA specific fluorescent dye for some minutes.

In general it is said that the SPC method will detect approximately 80% of all live bacteria, - the remaining bacteria did not grow (quick enough) in the used media, but might well do if incubated on a different agar or at a different temperature for a different period of time. For the flow cytometer there are weaknesses too. Some bacteria give too small pulses, for instance Strep uberis, are counted in too low numbers. So both methods have strong and weak points.

However, worldwide there is a need for quick, repeatable and reproducible methods, and as a consequence, at present, instruments based on flow cytometry have completely taken over from the standard plate count methods. This means that we must try to get the maximum information out of the analyses carried out by these instruments in the simplest possible way!

However, when analyzing a sample set to establish a conversion equation between the two methods the expected correlation (R^2) on log10 transformed data will be in the range of 70-80% (see below example). This means that the conversion made between the two methods will be a rough estimate only for many milk samples. Let’s look into one example which clearly illustrates this:
How big is the difference between the two methods?

Three particular analyses results have been marked green, blue and red.

<table>
<thead>
<tr>
<th>Analyses result</th>
<th>IBC log</th>
<th>IBC/ml</th>
<th>CFU log</th>
<th>CFU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>green</td>
<td>4.2</td>
<td>16000</td>
<td>3.6</td>
<td>4000</td>
</tr>
<tr>
<td>blue</td>
<td>4.8</td>
<td>63000</td>
<td>3.6</td>
<td>4000</td>
</tr>
<tr>
<td>red</td>
<td>4.8</td>
<td>63000</td>
<td>4.7</td>
<td>50000</td>
</tr>
</tbody>
</table>

In the figure it is seen that the green and the blue result give the same result with standard plate count (log 3.6=4000 CFU/ml) but quite different IBC results, log4.2=16000 and log 4.8=63000 IBC/ml. The blue and the red result give the same IBC result, log 4.8=63000 IBC/ml but log 3.6=4000 and log 4.7=50000 CFU/ml. All results may well be correct, - and not caused by differences in sample handling procedures. From the known differences between the two methods the difference between the green and blue sample could be explained by the presence of many psychrotropic or microaerophilic (grow behind milk filters) bacteria in the blue sample (not well detected by the SPC method).

The explanation of the difference between the blue and the red sample could be the presence of many Strep uberis bacteria in the red sample. It is known that these bacteria are not well detected by the flow cytometers.
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<td>4.2</td>
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<tr>
<td>blue</td>
<td>4.8</td>
<td>63000</td>
<td>3.6</td>
<td>4000</td>
<td>4.8</td>
<td>63000</td>
<td>4.1</td>
<td>12500</td>
<td></td>
</tr>
<tr>
<td>red</td>
<td>4.8</td>
<td>63000</td>
<td>4.7</td>
<td>5000</td>
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<td>63000</td>
<td>4.1</td>
<td>12500</td>
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Table 2: Analyses results together with communication to the farmer

But what about the results communicated to the farmer once this conversion line/equation is used? It is important to note that if we do not use the routine method, - but the SPC method, then the green and the blue result would be equal to appr log 3.6 = 4000 CFU/ml. Now using the routine method the results are 16,000 and 63,000 IBC/ml, - or when converted, 2500 CFU/ml and 12500 CFU/ml.

For the blue and the red sample it is the other way round! The SPC method sees a difference whereas the routine method does not. The result communicated will be 12,500 CFU/ml (63,000 IBC/ml) but results with the SPC method would be 4000 and 50,000 CFU/ml! So for many samples there are big differences between the two methods and it varies from sample to sample which method detects what!

This is also illustrated well in the study made by Dr H.G Walte, the Max Rubner Institute in Germany. In below table SPC and BactoScan analyses have been made on a number of pure cultures. It is seen that all cultures except S. uberis has a conversion factor >1. The vertical red line corresponds to 100,000 CFU/ml in the German conversion. There is a tendency that many Gram + /coci have a conversion factor below average whereas G-/rods have a conversion factor above average between BSC FC and the anchor method. For Strep uberis only appr 60,000 IBC corresponds to 100,000 CFU, indicating that this bacteria is underestimated.

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It is fruit for thought that whereas the correlation is 0.7-0.8 between the routine methods and the anchor method the correlation ($R^2$) between the two routine methods used at present, BactoScan and BactoCount, in several tests is found to be appr 95%. This provokes the idea that it might be more relevant to establish a “fixed relationship” between the, at present, two methods instead of wasting time and information by estimating the number of CFU/ml. It will never be more that a rough estimate due to the limited correlation to the SPC method and due to the fact that the SPC method is not very repeatable and especially reproducible between labs.

Read in the next article about the differences in conversion equations between countries. Some convert, some don’t, - some have decided a conversion equation can cover almost a continent; some have one table per region in the country. But for all countries it is important that raw milk stay below the same level, - 100.000CFU/ml!