

Determination of the BactoScan Conversion Factor for the United Kingdom

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Abstract

The microbiological quality of almost all commercially produced raw milk in the United Kingdom of Great Britain and Northern Ireland (UK) is determined by flow cytometry using BactoScan equipment. However, European Commission standards for raw milk are laid down as standard plate counts in Regulation (EC) No 853/2004 Annex III, Section IX, hence there is a need to have a conversion factor between the IBC determined by commercial companies, and the TVC cited in legislation. Using methodology based on BS EN ISO 4833:2003, this study undertook to determine the conversion factor. Three commercial laboratories using BactoScan equipment, and accredited to ISO17025 for their use, undertook duplicate analysis of 1,800 routine samples of raw milk from farms across the UK to determine IBC values. In addition duplicate bacterial enumeration of all samples, using BS EN ISO 4833:2003, was undertaken to determine TVCs. Overall, 1,799 valid results were obtained and regression analysis of the IBC against the TVC results, as \log_{10} values, gave the equation:

$$\text{Log}_{10}(\text{TVC}) = 0.9151x \text{Log}_{10}(\text{IBC}) - 0.5696 \quad (r^2 = 0.6694).$$

Hence this relationship constitutes the UK BactoScan conversion factor which is to be used to convert IBC values for raw milk determined in commercial premises into valid TVC results with reference to European Commission legislation.

Keywords: *BactoScan, conversion factor, individual bacterial counts (IBC), raw milk, total viable count*

Introduction

The microbiological quality of bovine raw milk is defined, in the legislation of many countries, in terms of the total viable count (TVC) of bacteria per millilitre of milk [1]. In the European Union, the legal limit for the TVC of raw milk (in this report 'milk' will only refer to bovine milk) is 100 000 CFU ml⁻¹ [2]. Because the standard microbiological plating method requires considerable resources, and takes over three days to deliver a result, alternative methods have been developed for the determination of TVC in raw milk. Flow cytometry can have a high throughput of samples and rapid production of results and has

therefore been adopted to provide automated routine microbiological analyses [3, 4].

In the UK flow cytometry is used to determine the bacterial quality of almost all commercially produced raw milks, and this takes place in three laboratories: one in England, one in Scotland and one in Northern Ireland. All of these laboratories use BactoScan equipment (Foss UK Ltd, Birchwood, Warrington WA3 6AE, UK), and the laboratories all have enumeration procedures accredited to ISO17025. In the UK, where payments for raw milk use the microbiological quality of milk as a factor, these are based on individual bacterial counts (IBC) as determined by BactoScan equipment. Hence, commercially, there has been no need for a conversion factor to convert IBC values to total viable counts (TVC) at 30°C obtained by the ISO standard plate counting method. However, EC legislation concerning the microbiological quality of raw milk is based on the use of the former values (Regulation (EC) No 853/2004 Annex III, Section IX) [5].

Consequently the EU Food and Veterinary Office (FVO) requested that a UK conversion factor for the calculation of TVC results from IBC values was determined [6], and the Food Standards Agency of the UK commissioned the UK National Reference Laboratory for Milk and Milk Products (NRLMMP), based in Food Microbiology Branch, the Agri-Food and Biosciences Institute (AFBI), to obtain this conversion factor [7].

The NRLMMP approached the commercial companies using BactoScan equipment to determine IBC values for raw milk, and all agreed to participate in a study to determine a BactoScan conversion factor for the UK. These laboratories are estimated to analyse samples from over 99% of the UK bovine milk production. The procedures to compare the anchor method for the enumeration of bacteria in raw milk at 30°C (BS EN ISO 4833:2003, [8]) with an alternative method, such as the BactoScan, are described in BS ISO 21187:2004 [9]. This study was designed to be compliant with the latter standard.

Materials and methods

Total viable counts (TVC) of bacteria in raw milk at 30°C were determined using conventional plate counts as described in BS EN ISO 4833:2003 [8] and individual bacterial counts (IBC) using BactoScan equipment, as recommended by the manufacturers.

Samples of raw milk submitted for routine determination of individual

bacterial counts (IBC) were analysed in duplicate using a BactoScan, and standard plate count methodology [8] at the three participating laboratories. The ISO 21187:2004 standard [9] requires that 'Preferably, analysis by both methods should be carried out using the same test sample, within a short interval of time'. For the purposes of this study that interval of time was defined as one hour.

All three laboratories (one in England, one in Scotland and one in Northern Ireland) were required to analyse a total of 1800 milk samples over a period of approximately one year, and the results were provided to AFBI on a monthly basis. All samples were taken from the routine workload of the laboratories, based on a schedule designed to spread sampling events evenly over a period of one year, to meet the requirements of BS ISO 21187:2004 [9] that a wide range of samples is taken to ensure that they represent, inter alia, the variation in "the geographical regions and over the year".

All statistical analyses were undertaken by the Biometrics & Information Systems Branch, AFBI, using Genstat Release 18.1 for Windows (VSN International Ltd, Hemel Hempstead, HP2 4TP, UK). Note that enumeration results were transformed to Log_{10} to meet the requirements of BS ISO 21187:2004 [9] for data analysis. The latter documents states: 'Before any calculation is made, a scatter diagram (i.e. plotted distribution of two-dimensional arrays) of observed values should be checked visually to obtain a first impression of the character of the relationship. The scatter diagram will show whether the relationship between the results of both methods tends to be linear over the whole range. If not, an appropriate data transformation should be used to achieve a linear relationship.'

Results and discussion

All three laboratories had ISO 17025 accreditation for the determination of IBC, hence they participated in external quality assurance (EQA) programmes, and the results of these were provided to AFBI to verify their competence. Overall, 1,799 valid results were obtained and statistical analysis of these was undertaken, Figure 1. Simple linear regression analysis of the data, as Log_{10} values, was undertaken and the conversion equation was determined as (standard error in brackets):

$$\text{Log}_{10}(\text{TVC}) = 0.9151 (0.0152) \text{Log}_{10}(\text{IBC}) - 0.5696 (0.0721) \\ (r^2 = 0.6694)$$

Further statistical analyses, using multiple regression analysis, was undertaken to determine if there was an effect of seasonality on the conversion equation, but this was found to be non-significant using an F-test.

Raw milk is legally required to have a TVC (30°C) value less than 100,000 cfu/mL, as defined in Regulation (EC) No 853/2004 of the European Parliament [5] and of the Council laying down specific rules on the hygiene of foodstuffs. To convert the IBC values determined for commercially produced raw milk in the UK the above equation can be used. Using the UK conversion equation the IBC corresponding to the legal limit of 100,000 cfu/mL is 1.22×10^6 per mL.

Conclusions

Raw milk samples (n = 1,799) were analysed in three laboratories located in Northern Ireland, England and Scotland and the bacterial count at 30°C determined using conventional microbiology and flow cytometry, the latter using BactoScan equipment. The resulting data was analysed to determine a BactoScan conversion factor. The BactoScan conversion

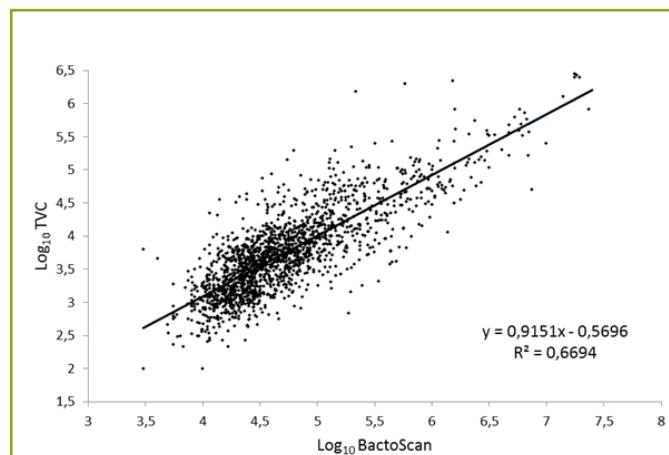


Figure 1: UK dataset of IBC and TVC for 1,799 samples of raw milk with the conversion line shown

factor for the United Kingdom of Great Britain and Northern Ireland was determined to be:

$$\text{Log}_{10}(\text{TVC}) = 0.9151 \text{Log}_{10}(\text{IBC}) - 0.5696.$$

This determination meets the requirement of EU Food and Veterinary Office (FVO) for the UK to determine this conversion factor, and therefore commercially determined IBC values can be converted to legally required TVC values using this factor.

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Conflict of Interest

The authors declare that they have no competing interests.

References

- Desmaures N, M Gueguen. Monitoring the microbiology of high quality milk by monthly sampling over 2 years. *J Dairy Res.* 1997; 64:271-280 doi:10.1017/s0022029996002130
- European Commission. Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2005:338:0001:0026:EN:PDF> Accessed April 2017.
- Gunasekera TS, PV Attfield, DA Veal. A flow cytometry method for rapid detection and enumeration of total bacteria in milk. *App Env Microbiol.* 2000; 66:1228-1232 doi:10.1128/aem.66.3.1228-1232.2000
- Suhren G, HG Walte. First experiences with automatic flow cytometric determination of total bacterial count in raw milk. *Int Dairy Fed Bull* 2000; 50:249-275.
- European Commission. Commission Regulation (EC) No 853/2004 Annex III, Section IX. [http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32004R0853R\(01\)#nr27-L_2004226EN.01004001-E0004](http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32004R0853R(01)#nr27-L_2004226EN.01004001-E0004). Accessed April 2017.
- European Commission. Food and Veterinary office, EU. Final report of an audit carried out in the United Kingdom from 08 to 19 April 2013 in order to evaluate the follow-up action taken by the

competent authorities with regard to official controls related to the safety of food of animal origin, in particular milk and dairy products. http://ec.europa.eu/food/fvo/act_getPDF.cfm?PDF_ID=10619 Accessed April 2017.

7. Food Standards Agency. Response of the Competent Authorities of the United Kingdom to report ref. DG(SANCO)/2013-6872-MR. 2013. http://ec.europa.eu/food/fvo/act_getPDFannx.cfm?ANX_ID=7365 Accessed April 2017.

8. BSI. Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of microorganisms -- Colony-count technique at 30 degrees C. BS EN ISO 4833:2003. British Standards Institute, London. 2003.
9. BSI. Milk -- Quantitative determination of bacteriological quality -- Guidance for establishing and verifying a conversion relationship between routine method results and anchor method results. BS ISO 21187:2004. British Standards Institute, London. 2004.