FINAL REPORT

A Study to Determine the BactoScan Conversion Factor for the United Kingdom

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AGRI-FOOD AND BIOSCIENCES INSTITUTE

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ACKNOWLEDGEMENTS

The authors would like to thank the companies who undertook the analyses using routine samples during this study. They also wish to thank the laboratory staff who participated in discussions, and who undertook analysis for the study.

GLOSSARY

ACMSF	Advisory Committee on the Microbiological Safety of Food
BPW	Buffered peptone water
°C	Degree Celsius
cfu	colony forming units
DEFRA	Department for Environment, Food and Rural Affairs
DH	Department of Health
EC	European Commission
EURL	European Union Reference Laboratory
EQA	External Quality Assurance
FSA	Food Standards Agency
g	Gram
GLP	Good Laboratory Practice
h	Hour(s)
IBC	Individual bacterial count
IQC	Internal Quality Control
ISO	International Standards Organisation
LIMS	Laboratory Information Management System
ml	Millilitres
NML	National Milk Laboratories
PHLS	Public Health Laboratory Services
QC	Quality Control
TBC	Total bacterial count
TVC	Total viable count
UDF	United Dairy Farmers

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SUMMARY

In EU legislation referring to the microbiological standard of raw bovine milk the units ‘colony forming units (cfu)’ are cited when describing numbers of bacteria, as enumeration is based on conventional microbiological plate counting (Regulation (EC) No 853/2004 Annex III, Section IX). Such plate counts yield a total viable count (TVC) of bacteria which is normally expressed as cfu/cm^3. However, in the commercial world, virtually all work to determine the numbers of bacteria in raw milk is undertaken by automated equipment using flow cytometry. Such systems separate bacteria into individual cells prior to enumeration and hence the output is defined as individual bacterial counts (IBC). There is therefore a need for a conversion factor to convert IBC to TVC, to demonstrate that legislative standards are being met.

The UK lacks such a conversion factor, which is a requirement of EC legislation (Regulation (EC) No 2073/2005), and this was noted in the recommendations of the EU report ‘Final Report Of An Audit Carried Out In The United Kingdom From 08 To 19 April 2013’ ref. DG(SANCO)/2013-6872-MR (Anon. 2013). Accordingly the Food Standards Agency requested that the UK National Reference Laboratory for Milk and Milk Products, based at the Agri-Food and Biosciences Institute, Newforge Lane, Belfast undertake the task of determining a conversion factor for the UK. The study aimed to determine the equation relating conventional microbiological counts (TVC) of bacteria in raw milk to the results obtained from BactoScan equipment, which produce an individual bacterial count (IBC). The equation is referred to as the BactoScan conversion factor and takes the form:

$$\text{Log}_{10}\,(\text{TVC}) = m \times \text{Log}_{10}\,(\text{IBC}) + c$$

m = slope, and c = constant

In the UK high throughput automated bacterial counters for the determination of bacteria in raw milk are only found in three laboratories, and all are BactoScan machines. Hence this study is subsequently referred to as the determination of the BactoScan conversion factor. The three laboratories all agreed to participate in this study, and were based in Glasgow, Wolverhampton and Ballymena and thus analyse milk from Scotland, England, Wales and Northern Ireland. Therefore milk samples from across the UK were analysed.

Since the BactoScan machines are designed for a very high throughput of samples it was essential to use the equipment in commercial premises, which process high numbers of samples, in order to obtain valid results. Routine samples of raw milk were analysed in duplicate by the BactoScan, and using conventional plate counting as described in BS EN ISO 4833:2003 (Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of microorganisms -- Colony-count technique at 30 degrees C) (Anon. 2003). The mean value of the duplicates was then analysed, using regression analysis, to give the conversion factor.

Overall, 1,799 samples were analysed and regression analysis of the dataset gave the equation:

$$\mathbf{Log_{10}\,(TVC) = 0.9151x\ Log_{10}\,(IBC) - 0.5696}$$

(r^2 =0.6694)

Thus the equation above constitutes the conversion factor for the UK. This result was compared to the results of a pan-EU study undertaken by the European Union Reference Laboratory (Guillier et al, 2016), and was seen to fall within the confidence interval (95%) of the EU harmonised conversion equation, supporting its validity.

Enumeration of bacteria in raw milk

The microbiological quality of raw milk can be determined by classical microbiological methodology whereby a sample is diluted appropriately and applied to a solid medium and incubated, with bacterial colonies being subsequently counted. Currently, the relevant method is BS EN ISO 4833-1:2013 Microbiology of the food chain -- Horizontal method for the enumeration of microorganisms -- Part 1: Colony count at 30 degrees C by the pour plate technique (Anon. 2013b). However, this method is labour intensive and requires that samples are incubated for 72h, so that there is a considerable delay between commencing the analysis and obtaining the final results. It should also be noted that the results of this enumeration are usually referred to as the total viable count (TVC) which is reported in colony forming units (cfu) per unit volume.

The nature of the ‘unit’ forming a colony can range from a single bacterium, to an aggregate of bacterial cells adhering to some particulate matter. The sample material, raw milk in the case of this study, will be thoroughly mixed during preparation for enumeration but total dispersal of all bacteria in the sample is improbable. It should also be noted that some bacteria grow in chains, or aggregates such as tetrads, hence, once again, a single colony can arise from several bacteria. Therefore total viable counts are reported as cfu to more accurately reflect what is actually being enumerated.

Since conventional microbiological enumeration requires significant resources to obtain a result, and only after a significant period of incubation, alternatives have

been developed. One such method is based on the use of a flow cytometer, whereby a liquid sample is passed through a fine tube, where a laser illuminates the particles which can then be detected and enumerated. Using a fixed flow rate a finite volume of sample will pass through the system per unit of time, allowing the number of particles/volume to be calculated. To enumerate bacteria in milk the structure of the milk is destroyed using reagents specific to the company supplying the enumeration equipment, and all bacteria stained using a fluorescent dye which does not bind to any other particles in the milk. In addition some of the reagents function is to separate bacteria from any particles present, and each other. Thus in the flow cytometer the bacteria are counted as individual cells and the results are presented as the individual bacterial count, or IBC.

It can be seen that the IBC will normally be greater than the total viable count, and to relate the IBC to the cfu a regression equation is calculated of Log_{10} (TVC) against Log_{10} (IBC). The relationship is linear giving the equation:

$$Log_{10}\,(TVC) = m \times Log_{10}\,(IBC) + c$$

m = slope, and c = constant.

Thus to relate the results of flow cytometry counts to conventional microbiology results appropriate samples must be analysed by both methods. The methodology for milk is described in BS ISO 21187:2004 Milk -- Quantitative determination of bacteriological quality -- Guidance for establishing and verifying a conversion relationship between routine method results and anchor method results (Anon. 2004). The data can then be appropriately transformed and statistically analysed to give an equation as noted above.

The principles of using flow cytometry to enumerate the bacterial flora of raw milk are outlined at :

http://www.fossna.com/~/media/files/documents/industrysolution/brochuresanddatasheet/bactoscanfc/bactoscanfcsolutionbrochuregb-pdf.ashx

Current study

In the UK flow cytometry is used to determine the bacterial quality of most commercial raw milks, and this takes place in three laboratories. All of these laboratories use BactoScan equipment produced by Foss. In 2001 the results of a study; ‘A Comparison Of Bactoscan Counts On Raw Bovine Milk Against Equivalent Total Viable Counts Obtained By The Agar Pour Plate Method’ were published by ADAS (Appendix 7). The study was based on analyses of raw milks in England and Wales and it concluded that there were many confounding factors which mitigated against the calculation of standard conversions factors relating BactoScan individual bacterial counts (IBC) and total viable counts (TVC) obtained by the ISO standard plate counting method. However, in the rest of the EU such conversion factors were derived in at least twelve countries, resulting in requests from DG Sanco (now called DG Santé) of the EU Commission that the UK follow suit. The aim of the Commission is to have a single conversion factor for EU member states.

Specifically, an audit was carried out by the EU Food and Veterinary Office (FVO) in the UK from 8 to 19 April 2013, to evaluate the official controls related to the production and storage of raw milk and dairy products. The audit noted the use of

BactoScan equipment in determining the quality of most of the UK milk production. and the FVO recommendations stemming from the audit were made in report DG(SANCO)/2009-8225-MR, (available at http://ec.europa.eu/food/fvo/act_getPDF.cfm?PDF_ID=10619) in which the lack of a conversion factor was raised.

The Food Standards Agency responded to the recommendations of report ref. DG(SANCO)/2013-6872-MR (available at file:///C:/Users/0552932/Downloads/ap%202013-6872%20UK%20-%20public%20health-milk-%20and%20dairy%20products%20(1).pdf) noting that the UK NRL had initiated a programme to obtain a conversion factor.

Currently in the UK BactoScan equipment is in use in three laboratories, which all have ISO 17025 accreditation for its use (Appendix 6), where it is used to determine the individual bacterial counts (IBC) of samples of raw milk. National Milk Laboratories (NML) employ the equipment in Hillington, Glasgow, and Four Ashes near Wolverhampton. These laboratories process samples from the majority of farms in Scotland, and England & Wales (Appendices 2 and 6). Thus data for both of these jurisdictions could be obtained by undertaking studies in the NML laboratories. The company was approached by the NRL and agreed to participate in the study. It should be noted that the Hillington laboratory analyses samples from Scotland, plus Cumbria and Lancaster, and samples analysed in this study were to be drawn at random from all routine samples. Thus when samples below are described as being from Scotland, this refers to the majority of the samples, and a small proportion will be from the two northern counties of England.

In Northern Ireland, United Dairy Farmers (UDF), have a BactoScan system installed in their Pennybridge laboratory and hence data from Northern Ireland milks could be determined, based on analyses conducted in this lab. Again the company responded positively when requested to participate in the study, albeit after a delay to ensure adequate laboratory resources. Background information on UDF is included in Appendices 3 and 6.

With the agreed participation of NML and UDF the study to determine a conversion equation for the BactoScan could proceed. Whilst only Foss equipment was available in the salient labs in this study the manufacturers of the Bentley BactoCount system requested that a comparison of their equipment was included, and with the agreement of the FSA and the collaboration of NML that study was undertaken, in Hillington, as part of this project.

Methodology

The methodology was based on obtaining numbers of bacteria in raw milk using conventional plate counts as described in BS EN ISO 4833:2003 (Anon. 2003), and comparing these counts to those obtained from the BactoScan. The procedures to compare the anchor method (BS EN ISO 4833:2003) with the BactoScan (or any similar device) are described in BS ISO 21187:2004 (Anon. 2004). This study was designed to be compliant with the requirements of the latter document. It should be noted that BS EN ISO 4833:2003 was updated, with minor changes, in 2013 (Anon. 2013b) but that the UDF laboratory was using BS EN ISO 4833:2003 as part of its

ISO 17025 calibration procedures for the BactoScan. Since it was agreed with the FSA that use of the 2003 standard method would not have any significant effect on the conversion equation obtained it was used in all three laboratories during this study.

As required by BS ISO 21187:2004 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 (ISO 4833:2003) at the premises of National Milk Laboratories (NML), Laches Close, Four Ashes, Wolverhampton, WV10 7DZ, and 32 Kelvin Avenue, Hillington Park, Glasgow, G52 4LT. Analyses also took place at the United Dairy Farmers laboratory in the Group Technical Centre, Pennybridge Industrial Estate, Larne Road, Ballymena, BT42 3HB. The ISO standard 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 were required to analyse 800 milk samples over a period of approximately one year, and the results were provided to AFBI on a monthly basis.

All samples were taken at random from the routine workload of the laboratories.

A study to compare IBC results from BactoScan and BactoCount equipment was also undertaken. Samples of raw milk (n = 1,000) were analysed in duplicate on both BactoScan and BactoCount machines in Hillington. The latter equipment was installed by Bentley specifically to undertake this study, which was undertaken from 11th November until 18th December 2014 (Appendix 1).

Results and discussion

The three participating laboratories initially analysed 800 samples. Sampling at Wolverhampton was initiated in September 2014 and concluded in July 2015. For Hillington sampling initiated in October 2014 and concluded in September 2015. Pennybridge sampling was undertaken from 27 April 2015 until 5th February 2016. Whilst the study was planned to be undertaken over one calendar year, operational conditions at the laboratories dictated the rate at which samples could be analysed. Lab managers increased the rate of sampling for the study when resources permitted, to ensure the work would not be unduly delayed later by factors such as staff absences, or increased levels of other contractual duties. This led to work being undertaken within 11 months at Wolverhampton and Pennybridge.

Problems with the Hillington dataset led to a second round of sampling, from February to May 2016 during which a further 200 samples were analysed, as discussed below.

Statistical analysis of all results was undertaken by Dr Alan Gordon, of Biometrics & Information Systems Branch, AFBI, using Genstat. The three datasets were analysed separately, and in combination. For some samples incomplete data was obtained and due to the need to have duplicate results for both analyses not being met fewer than 800 valid results were obtained. This was expected and the reason why the total of 800 samples was chosen to accommodate the loss of up to 7% of results.

Preliminary analyses of the data indicated that the TVC values for Hillington were unusually high, and on further investigation this dataset was discarded. NML undertook detailed investigations into the problem but no definitive cause was determined. However, following the analysis of the 800 samples, a robust review of procedures was undertaken and further quality assurance measures were put in place, with the results presented to the project team. In view of time constraints it was agreed that a further two hundred samples should be analysed, from February to May 2016, Table 1. Taking account of the quality assurance samples analysed during this period, these results were seen to be acceptable, and incorporated into the study.

Laboratory	**Mean Log_{10} counts**		**Valid samples**
	TVC	**IBC**	
Wolverhampton	3.74	4.77	799
Hillington	3.67	4.40	200
Pennybridge	3.77	4.75	800

Table 1. Log_{10} values for mean counts obtained by BactoScan (IBC) and conventional plate counting (TVC) by the three participating laboratories.

The locations of the farms from which samples were obtained are presented in Appendix 4.

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. The results, which were regarded as commercial, in confidence, were presented to the project team for inspection and showed that the BactoScan equipment was being correctly used

since the results from the analysis of EQA samples were all within acceptable tolerances. Thus the IBC results from all three labs were supported by quality assurance schemes. Overall, 1,799 results were obtained and statistical analysis of these was undertaken, Figure 1.

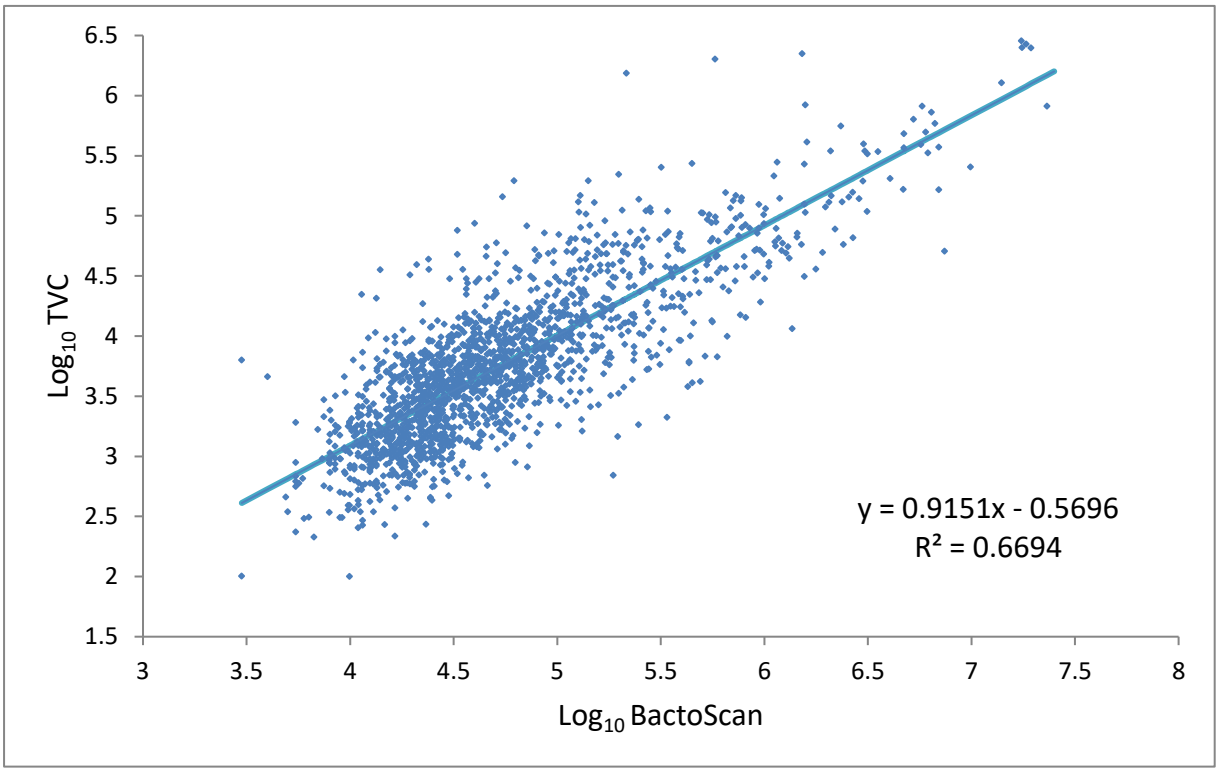


Fig 1. Dataset for the UK with conversion line shown.

The conversion equation for the UK is therefore:

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

The EURL produced a report on conversion factors based on results from twelve countries (European harmonisation of conversion equations between instrumental methods (flow cytometers) and reference method for the determination of total flora in raw cow's milk, available at: https://sites.anses.fr/en/sites/lait). The report (Guillier et al 2016) proposed a unified EU conversion equation:

EURL proposed Conversion: Log_{10} (TVC) = 0.850 Log_{10} (IBC) + 0.185

This is presented, along with the UK conversion equation, on Figure 2.

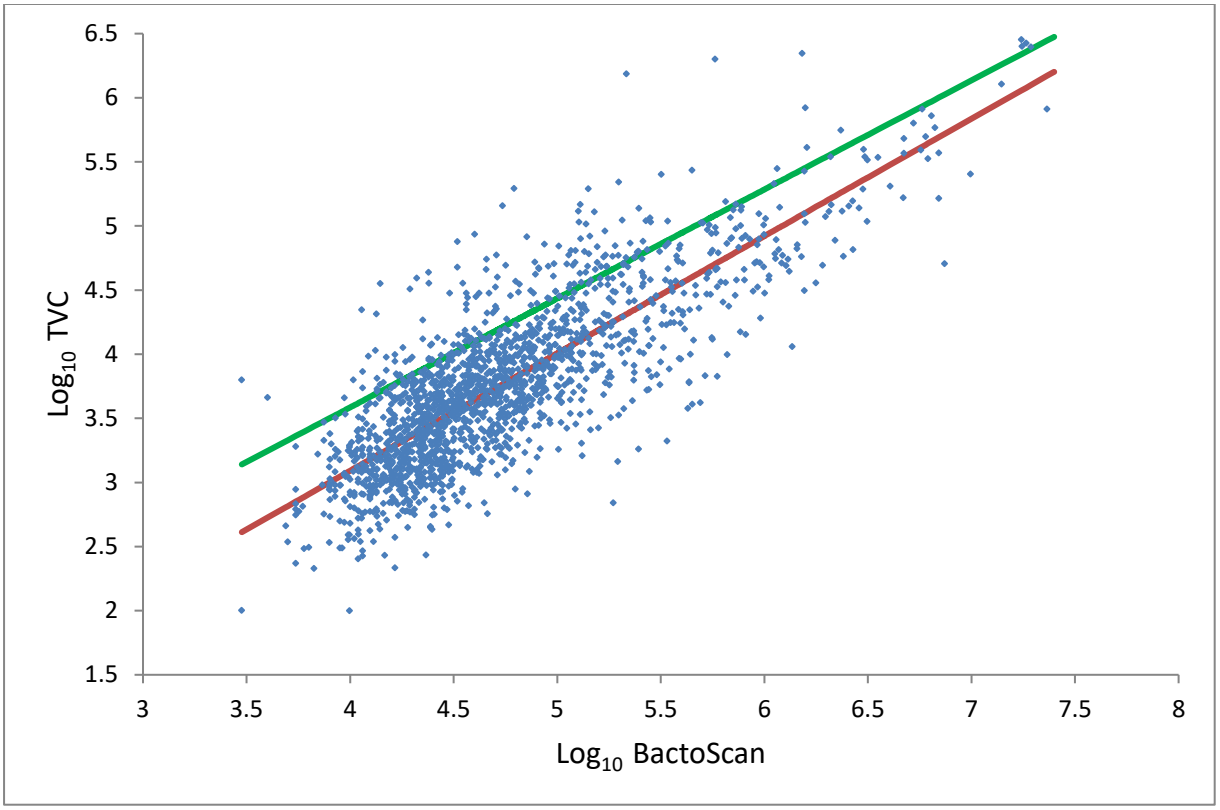


Fig 2. Dataset and conversion line for the UK (red) shown with the EURL derived conversion line (green).

The EU conversion line clearly differs from the UK result, although the UK line lies within the 95% confidence limits of the EU equation. The practical significance of adopting the EU equation would be that IBC values in the UK would convert to higher TVC values than at present. 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 and of the Council laying down specific rules on the hygiene of foodstuffs (853/2004). Using the EU conversion the IBC corresponding to 100,000 cfu/ml is 4.62 x 10^5 per ml, but with the UK conversion the IBC is 1.22 x 10^6

per ml, a factor of 2.64 times higher. Therefore adoption of the EU conversion would markedly increase the TVC reported for a given IBC result.

Conversion lines for the three UK datasets were also calculated (Appendix 5) and compared with the UK conversion equation in terms of the predicted Log_{10} (TVC) values from BactoScan results, Figure 3

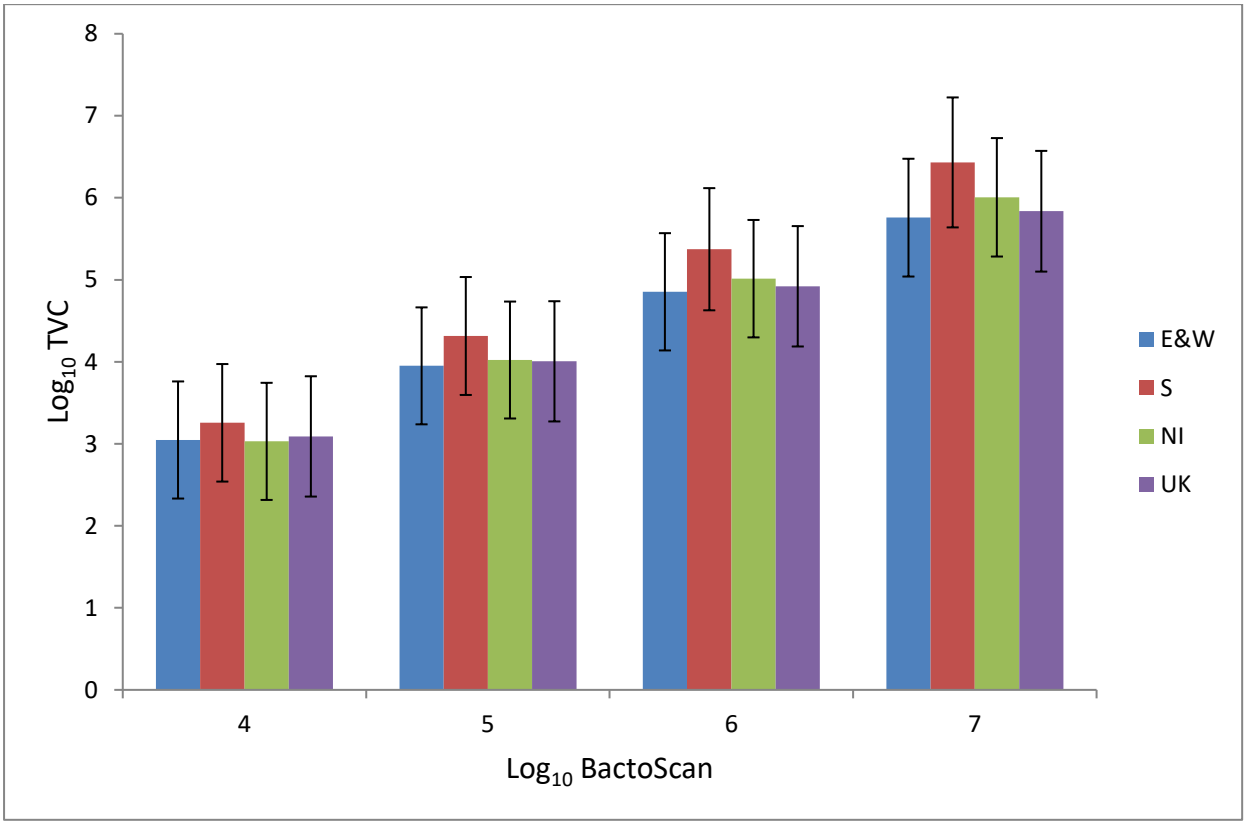


Figure 3. Predicted Log_{10} (TVC) values calculated from the regression equations of the UK datasets: England and Wales (E&W), Scotland (S), Northern Ireland (NI) and the complete United Kingdom dataset (UK). Error bars indicate confidence intervals, 95%.

It can be seen that, taking the error bars into account, similar predicted results are obtained from the regional datasets. Further statistical analyses found no significant effect of seasons on the conversion equation.

Conclusions.

Raw milk samples (n = 1,799) were analysed in three laboratories located in Northern Ireland, England and Wales and Scotland and the data analysed to determine a BactoScan conversion factor. The BactoScan conversion equation for the United Kingdom of Great Britain and Northern Ireland was determined to be:

$$Log_{10}(TVC) = 0.9151\ Log_{10}(IBC) - 0.5696$$

References.

Anon. 2003. 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.

Anon. 2004. 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.

Anon. 2004. Commission Regulation (EC) No 853/2004 Annex III, Section IX, available at http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32004R0853R(01)#ntr27-L_2004226EN.01004001-E0004.

Anon. 2005. Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. Available at: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2005:338:0001:0026:EN:PDF

Anon. 2013. 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. Available at: http://ec.europa.eu/food/fvo/act_getPDF.cfm?PDF_ID=10619

Anon. 2013b Microbiology of the food chain -- Horizontal method for the enumeration of microorganisms -- Part 1: Colony count at 30 degrees C by the pour plate technique. BS EN ISO 4833-1:2013. British Standards Institute, London.Guillier,L., Gnanou-Besse, N., Cauquil, A., Hennekine, R., Deperrois, V., Bouchez, P., Hennikinne, J-A., and Lombard, B. 2016**.** European harmonisation of conversion equations between instrumental methods (flow cytometers) and reference method for the determination of total flora in raw cow's milk EURL. Available at: https://sites.anses.fr/en/system/files/MMP-Cr-201602R.pdf

Appendix 1.

Comparison of Foss BactoScan and Bentley BactoCount.

Currently no major laboratory in the UK is using a BactoCount system to determine TVC of raw milk. Accordingly a study was undertaken to compare the results of BactoScan and BactoCount equipment for a set of duplicate samples. Given that both types of equipment require a steady, and high, throughput of samples to obtain optimal results the following methodology was used:

Bentley installed an appropriate machine at NML premises in Hillington and trained staff in its use. Samples of raw milk (n=1,000) were analysed in duplicate on both BactoScan and BactoCount machines and the data collected. The study was undertaken from 11th November until 18th December 2014. The data was analysed by a statistician of Biometrics & Information Systems Branch, AFBI, and the results are presented on Figure A1.

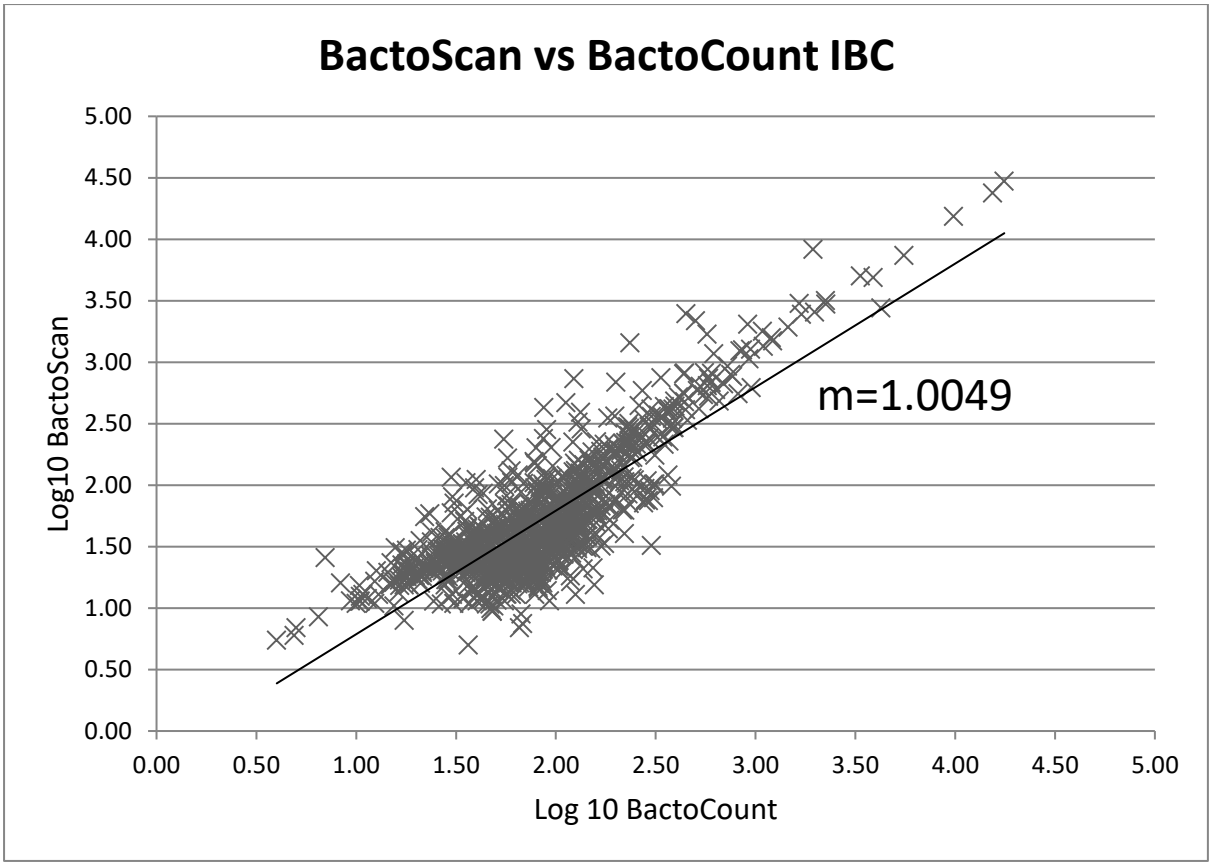


Figure A1. Plot of Log $_{10}$(IBC) data obtained using BactoScan and BactoCount systems.

A previous study undertaken in the Republic of Ireland showed a very high linear correlation between IBC results obtained on a BactoScan and those of a BactoCount system. In this study the correlation coefficient was close to 1, indicating an overall close agreement between the systems, but the intercept was -0.2175 and the R^2 value of 0.6963 showing variance between the systems, Figure A1. A statistical analysis, two sample T-test, showed the two systems cannot be regarded as equivalent, $p < 0.001$.

Appendix 2.

Background for National Milk Laboratories.

National Milk Laboratories was established in 2004 as a subsidiary company within the NMR Group plc. The business operates through two laboratories – one near Wolverhampton (Laches Close, Four Ashes, Wolverhampton, WV10 7DZ) and one in Glasgow (32 Kelvin Avenue, Hillington Park, Glasgow, G52 4LT). The core activity of the business is the provision of milk testing services to dairy processors to verify the compositional and hygienic quality of milk sourced off each supplying farm. This information is then used by milk purchasers to calculate milk payments made to individual supplying farms.

NML's service includes the collection of milk samples from depots across GB 7 days a week. All samples are transported in refrigerated vehicles and are registered on arrival at the lab. NML then ensures that milk samples are tested in accordance with dairy processor requirements (generally one test per week). Any samples not scheduled for routine testing are held in refrigerated storage for five days in case any follow up testing is deemed necessary following the routine test.

Both laboratories are accredited to standard ISO17025:2005 for Bactoscan FC testing by UKAS (UKAS numbers are 2051 for the Glasgow laboratory and 2700 for the Wolverhampton laboratory). Both laboratories operate microbiology labs offering a range of tests on bulk milk samples (including TBC, coliforms, thermodurics and psychrotrophs) all based on ISO methods.

Over the last 10 years the business has grown such that it now undertakes testing for 99% of GB dairy farmers. As a result the business currently receives milk samples from over 10,800 GB dairy farms.

Appendix 3. Background for United Dairy Farmers

United Dairy Farmers is the largest milk processor in Northern Ireland and has a UKAS accredited laboratory (ISO17025) analysing over one million milk samples a year for Quality Assurance, Payment on Quality, Dairy Herd Management, and Advisory purposes. Accreditation is held for IBC determination using the BactoScan and Bacterial count determination to ISO 4833:2003. Hence it is appropriately qualified to participate in this study.

The Laboratory tests milk samples for United Dairy Farmers, and also for other milk buyers. All independent testing is carried out on a confidential basis.

Although this is primarily a laboratory to analyse milk for payment and milk recording purposes, it also provides an extensive advisory function for dairy farmers

Appendix 4.

Location of farms sampled during the study.

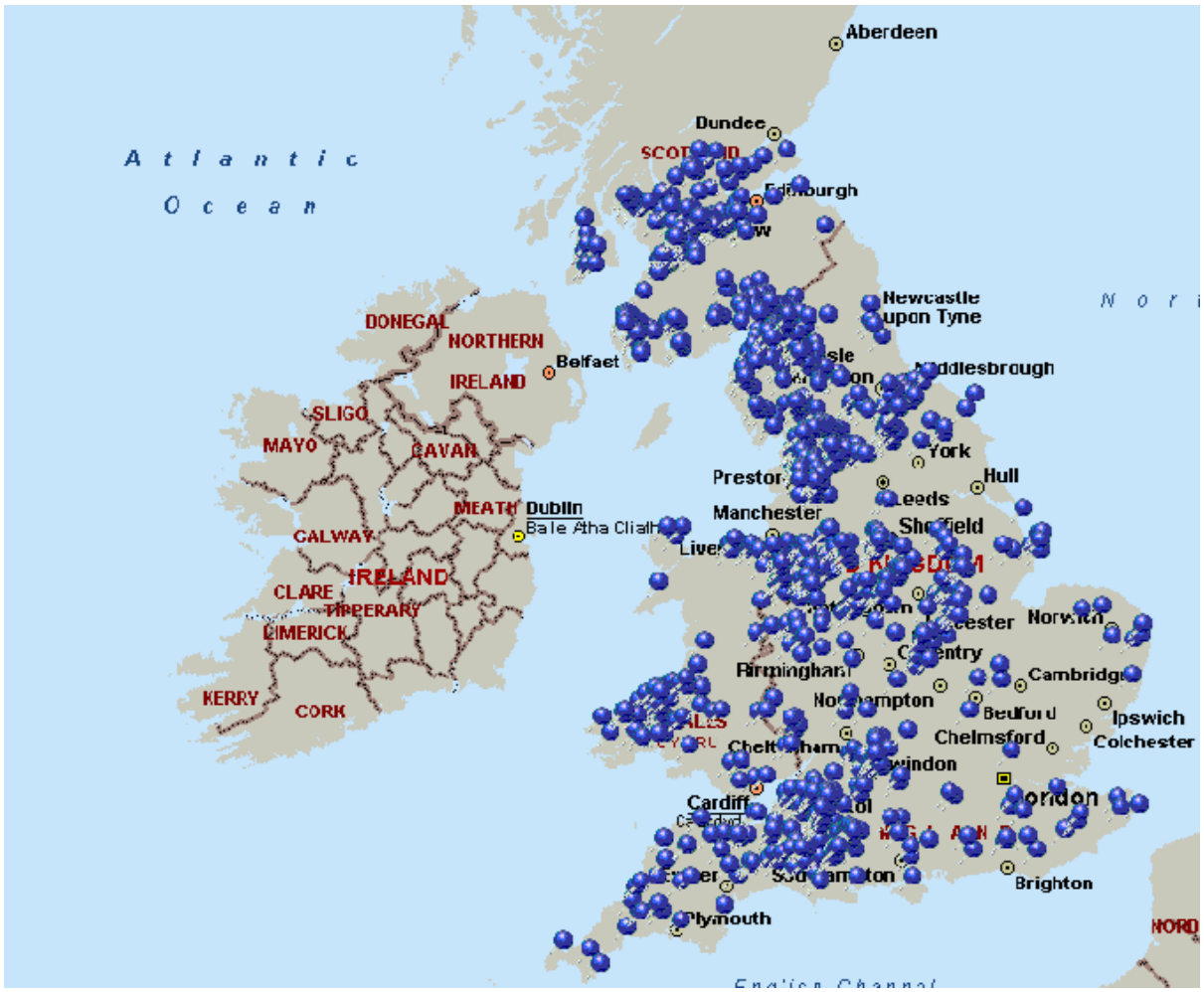


Fig A 2. Location of all dairy farms in England, Wales and Scotland farms sampled during this study.

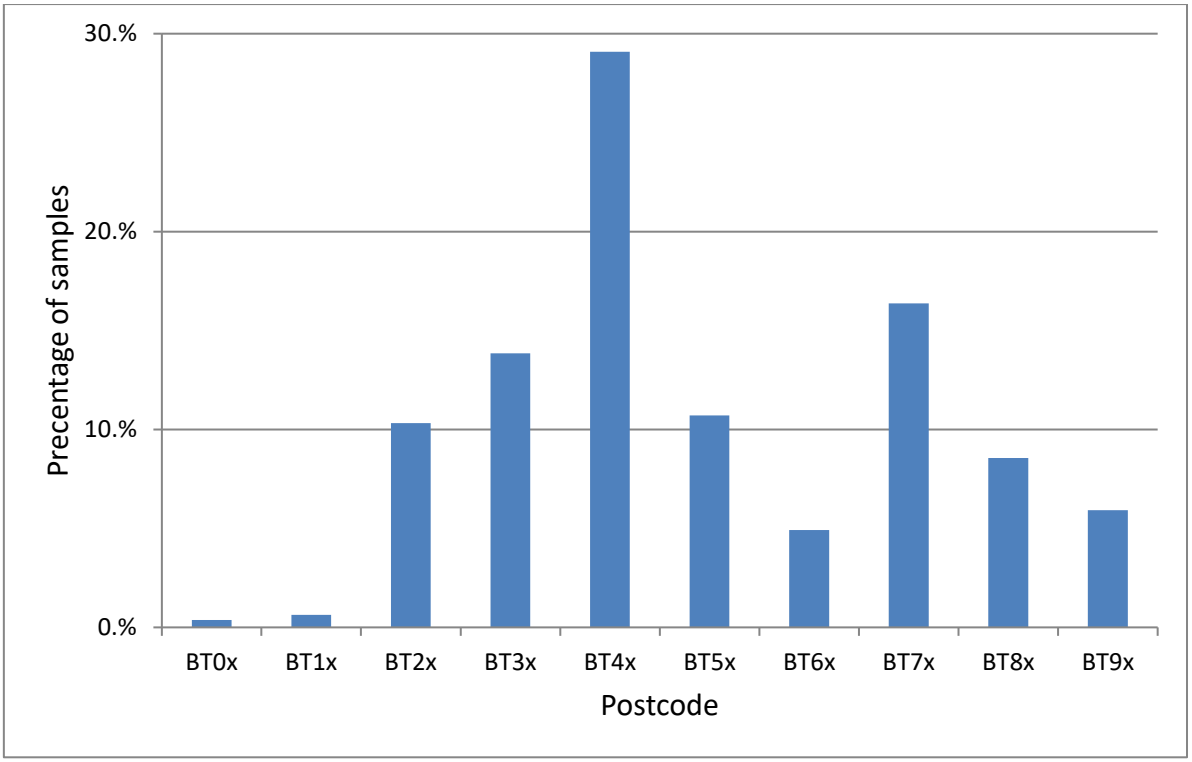


Fig A 3. Distribution of Northern Ireland farms sampled by postcode of farm.

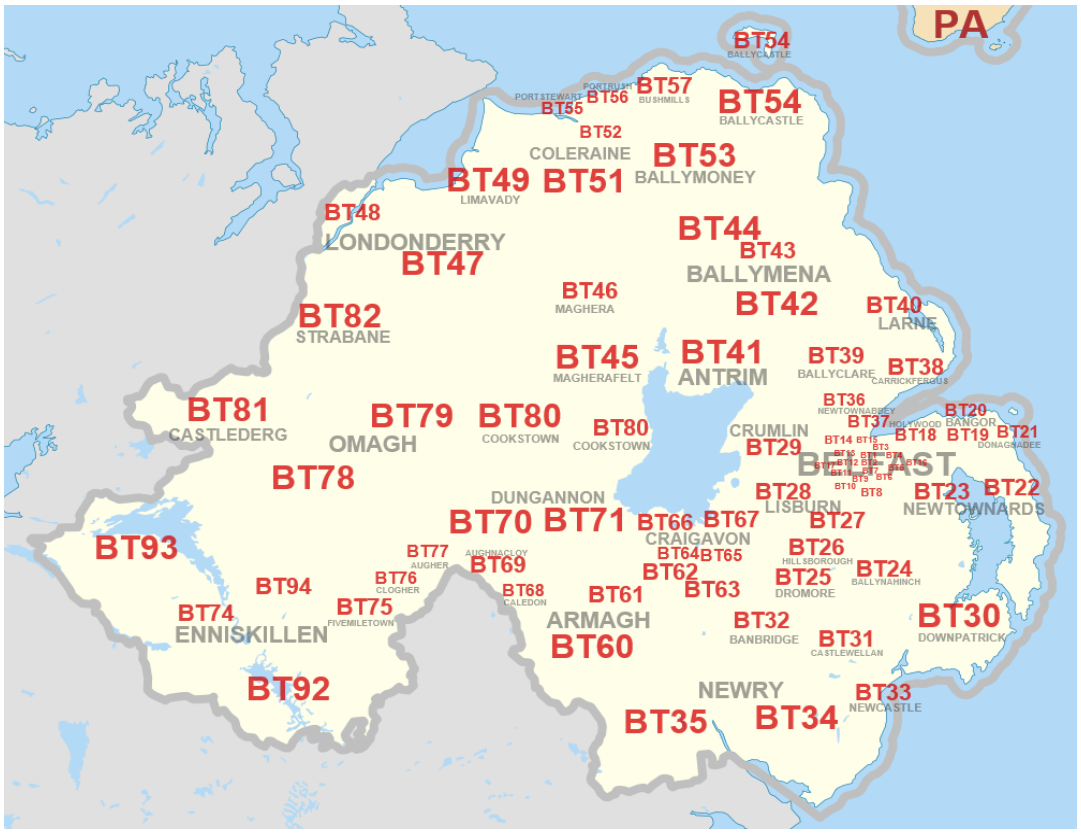


Fig A 4. Geographical distribution of postcodes of Northern Ireland.

Appendix 5.

Regression lines calculated from regional data.

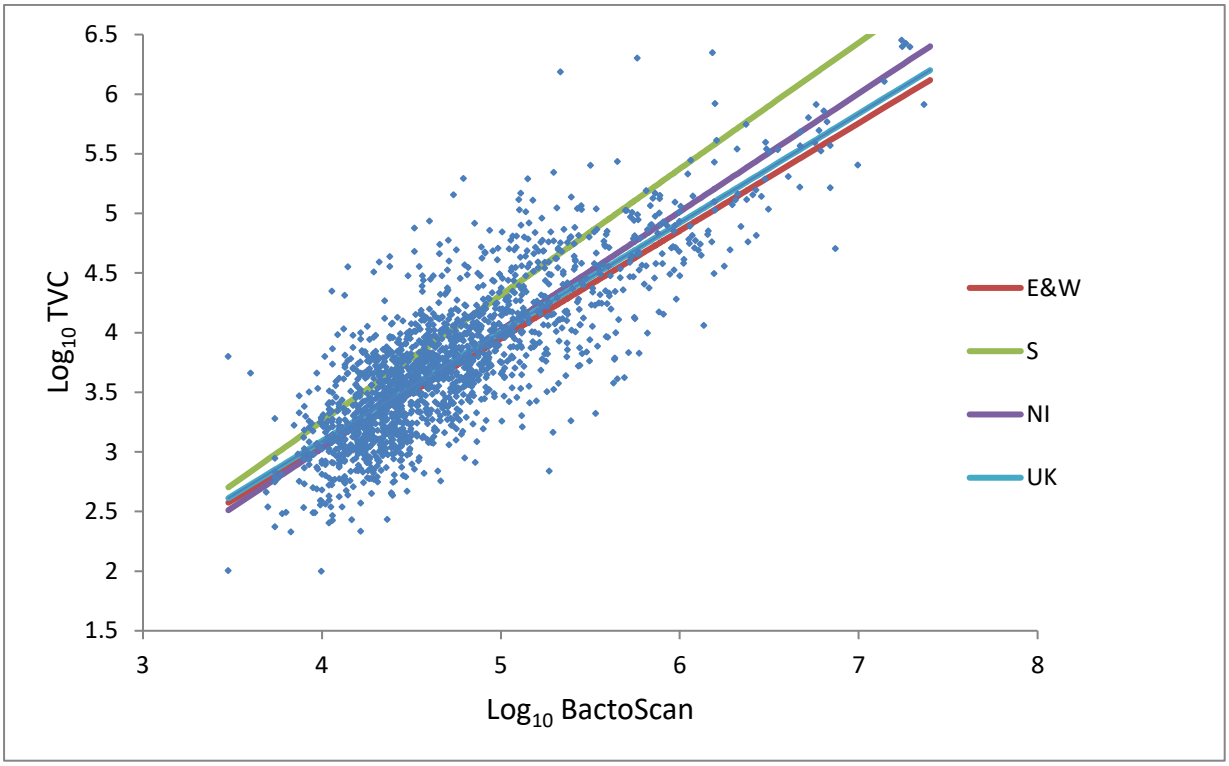
The linear regression equations for the three regions, or conversion factors, in the format Y = mX + c, where m is the slope and c the constant are shown on Table A1, as is the regression equation for all of the data.

Location	m	c
Northern Ireland	0.9919	-0.9380
England and Wales	0.9035	-0.5669
Scotland	1.0586	-0.9780
All results: UK	**0.9151**	**-0.5696**

Table A1. Conversion factors for Northern Ireland, England and Wales, Scotland, and the combined data.

The regressions lines from the regional data are shown below on Fig A5.

Fig A 5. Conversion factors for the regions studied. England and Wales (E&W), red:, Scotland (S), green; Northern Ireland (NI), purple; United Kingdom of Great Britain and Northern Ireland (UK), blue.



Appendix 6.

UKAS accreditation documents of the three participating laboratories

issued by

United Kingdom Accreditation Service

21 - 47 High Street, Feltham, Middlesex, TW13 4UN, UK

UKAS TESTING

2700

Accredited to ISO/IEC 17025:2005

National Milk Laboratories Limited

Issue No: 010 **Issue date:** 19 July 2012

Unit 26 - 28 Calibre Industrial Park
Laches Close
Four Ashes
Staffordshire
WV10 7DZ

Contact: Mr Andrew Dungey
Tel: +44 (0)190 274 9920
Fax: +44 (0)190 274 9938
E-Mail: andrewd@nationalmilklabs.co.uk
Website: http://www.nationalmilklaboratories.co.uk

Testing performed at the above address only

DETAIL OF ACCREDITATION

Materials/Products tested	Type of test/Properties measured/Range of measurement	Standard specifications/ Equipment/Techniques used
DAIRY PRODUCTS	Chemical Tests	
Milk, Raw and Semi-Skimmed	Butterfat	MLAB/539 using Combi-Foss 6000 Fourier Transform Infra Red Analyser
Liquid milk, all types	Freezing point depression (FPD)	MLAB/505 using freezing point cryoscope
Milk, raw	Protein	MLAB/539 using Combi-Foss 6000 Fourier Transfer Infra Red Analyser
	Casein	
	Somatic cell count	
	Urea	
DAIRY PRODUCTS	Microbiological Tests	
Milk, raw	Antibiotics and anti-microbial residues, presence/absence test	MLAB/501 by Bacillus stearothermophilus var calidoctis sensitivity by Delvotest
	Total Bacterial Count	MLAB/537 using Bactoscan FC by Flow cytometry
Bovine blood, serum, plasma Raw bovine milk	*Mycobacterium avium* subspecies *paratuberculosis*	M/LAB555 using Indirect ELISA (IDEXX)

END

Assessment Manager: BC/RO

Page 1 of 1

Schedule of Accreditation

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United Kingdom Accreditation Service

2 Pine Trees, Chertsey Lane, Staines-Upon-Thames, TW18 3HR

2051

Accredited to ISO/IEC 17025:2005

National Milk Laboratories Limited

Issue No: 018 **Issue date:** 18 April 2016

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Website:

Testing performed at the above address only

DETAIL OF ACCREDITATION

Materials/Products tested	Type of test/Properties measured/Range of measurement	Standard specifications/ Equipment/Techniques used
LIQUID MILK	<u>Compositional Tests</u>	In-House Documented Methods
	Butterfat content Protein content Lactose content Urea content Casein	Automated Infra-red analysis based on ISO 9622 / IDF 141C:2013 using the Combifoss 6000 Analyser
	Freezing Point Depression (FPD)	Lab-Op 34 based on BS EN ISO 5764:2009
	<u>Biological Tests</u>	
	Somatic cell count (SCC)	Automated fluorescent staining and electronic cell counting using the Fossomatic analyser based on BS EN ISO 13366-2:2006
	Total bacterial count	Documented in-house procedures using Bactoscan FC based on manufacturers' recommended method
	Detection of antibiotics and antimicrobial residues	MLAB/501, using Bacillus stearothermophilus by Delvotest using TECAN sampler

Schedule of Accreditation

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United Kingdom Accreditation Service

21 - 47 High Street, Feltham, Middlesex, TW13 4UN, UK

2700

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National Milk Laboratories Limited

Issue No: 010 **Issue date:** 19 July 2012

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Testing performed at the above address only

DETAIL OF ACCREDITATION

Materials/Products tested	Type of test/Properties measured/Range of measurement	Standard specifications/ Equipment/Techniques used
DAIRY PRODUCTS	Chemical Tests	
Milk, Raw and Semi-Skimmed	Butterfat	MLAB/539 using Combi-Foss 6000 Fourier Transform Infra Red Analyser
Liquid milk, all types	Freezing point depression (FPD)	MLAB/505 using freezing point cryoscope
Milk, raw	Protein	MLAB/539 using Combi-Foss 6000 Fourier Transfer Infra Red Analyser
	Casein	
	Somatic cell count	
	Urea	
DAIRY PRODUCTS	Microbiological Tests	
Milk, raw	Antibiotics and anti-microbial residues, presence/absence test	MLAB/501 by Bacillus stearothermophilus var calidoctis sensitivity by Delvotest
	Total Bacterial Count	MLAB/537 using Bactoscan FC by Flow cytometry
Bovine blood, serum, plasma Raw bovine milk	*Mycobacterium avium* subspecies *paratuberculosis*	M/LAB555 using Indirect ELISA (IDEXX)

END

Schedule of Accreditation

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United Dairy Farmers Ltd

Issue No:015 Issue date: 17 June 2014

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DETAIL OF ACCREDITATION

Materials/Products tested	Type of test/Properties measured/Range of measurement	Standard specifications/ Equipment/Techniques used
LIQUID MILK	<u>Chemical Tests</u>	
	Butterfat (2-10%) Lactose (2-10%) Protein (2-10%)	Documented In-House Method Section 4 based on BS ISO 9622:2013 using Mid Infra-red Analysis and FTIR Analysis based on the guidelines of IDF Standard 141:2013
	Freezing Point Depression	Documented In-house Method Section 6 based on ISO 5764:2009
	<u>Microbiological Tests</u>	
	Antibiotics 0.006-1 i.u. penicillin equivalent/ml	Documented In-house Method Section 5 based on IDF Bulletin No 258:1991 and Official Journal of the European Communities L93 Vol. 34:1991
	<u>Microbiological Tests</u>	
	Antibiotic residue (detection) covering Beta-lactam antibiotic types	Method Section 5 using CHARM MRL Beta-Lactam test
	Somatic Cell Count (up to 10^6 cells/ml)	Documented In-house Method Section 4 using Fluoro-Opto Electronic Method (Somascope) based on ISO 13366-2:2006
	Total Bacterial Count	Method Section 3 using BactoScan FC
END		

Appendix 7. ADAS BactoScan report of 2001.

A COMPARISON OF BACTOSCAN COUNTS ON RAW BOVINE MILK AGAINST EQUIVALENT TOTAL VIABLE COUNTS OBTAINED BY THE AGAR POUR PLATE METHOD

1. SUMMARY

Several years data on bacterial levels in raw bovine milk supplies throughout England and Wales held by ADAS have undergone detailed statistical analyses to determine differences between the agar plate method and BactoScan.

Total viable counts as determined by agar plating were compared against BactoScan counts for approximately one million milk samples. Statistical techniques were used to see whether season, geographical area or year significantly affected the relationship between the two methods. In addition, studies on individual farm supplies were used for comparison against geographical areas to determine the relative variabilities.

The enormous quantity of data caused logistical problems in terms of data analysis. Once statistical analysis was completed it became evident that considerable variations in the relationship between the two methods occurred between individual farms which made determining effects due to geographical area, season or year extremely difficult. What was apparent was that the BactoScan consistently gave much higher bacterial counts than agar plating. In addition it was concluded that for individual supplies BactoScan counts were more consistent than agar plate counts strongly indicating that BactoScan was a more accurate, reproducible and reliable means for determining the hygienic quality of raw bovine milk.

2. INTRODUCTION TO THE BACTOSCAN

The routine analysis of ex-farm bulk tank raw milk samples for payment and quality control purposes has been undertaken throughout the UK, EU and other countries for many years.

One of the tests undertaken is for total bacterial count (TBC) which indicates the hygiene of milk production and gives a rough estimate of milk quality in terms of product safety and suitability for processing.

Traditionally the number of bacteria in milk was determined by an automated agar pour plate culture method based on BS4285: Section 2.1:1984, the results being expressed as total viable count (TVC). This TVC test took 3 days to complete which often caused difficulties for both producers and processors due to the consequent lateness in identifying high bacterial count problems. The TVC test was also considered insufficiently accurate because it did not detect certain types of bacteria routinely present in raw milk. To overcome these problems over the last few years a new technique known as epifluorescent staining followed by direct microscopic counting for determining the numbers of bacteria in milk has been introduced. This test is undertaken using an automater analysis known as BactoScan manufactured by Foss Electric in Denmark. BactoScan enables the numbers of milk bacteria to be determined within 10 minutes of sample receipt giving obvious advantages in terms of turn-around of results. It also counts all the bacteria in milk thus giving a more accurate estimate of the hygienic quality.