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CONFERENCE PAPER

MICROBIOLOGICAL EXAMINATION AND PROFICIENCY TESTING IN DAIRY LABORATORIES

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This paper considers the main factors in the assessment of microbiological examination of food and discusses a few points related to validation of quantitative and qualitative microbiological methods. Within the scope of accredited methods, the author defines the terms such as *conform reference*, *equivalence of reference method*, and *in-house method*. The paper describes evaluation of a routine method with respect to the official method based on results obtained by automatic epifluorescent microscopy using the BactoScan 8000 instrument for determination of bacteriological quality of milk and provides general guidance for the establishment of a conversion relationship between the two methods. The paper gives an overview of the quality assurance aspects involved in the application of the routine method and concludes with an example of interlaboratory proficiency study for the epifluorescent microscopic method which is regularly applied in dairy laboratories.

Key words:
conform reference, dairy laboratories, epifluorescent miscroscopy, microbiological methods, proficiency testing, quality assurance

The most important aspects in the assessment of microbiological examination are the validation of microbiological methods, implementation of quality controls, use of the terms conform, equivalent of, and in-house method and arrangements regarding the scope of accredited methods. The paper will present the practical use of quality assurance aspects in the application of routine method using the BactoScan 8000

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instrument for quantitative determination of the bacteriological quality of milk. Special emphasis will be placed on the importance of interlaboratory proficiency testing.

VALIDATION OF MICROBIOLOGICAL METHODS

The term validation is used for a process demonstrating that a particular method is suitable for the intended purpose. There are few sources in the field of microbiology that cover method evaluation. Nevertheless, methods submitted for accreditation must be validated. The validation of microbiological methods should not rely on the same principle as of chemical methods. This is why the Dutch Accreditation Council organises the preparation of the international Explanatory Document on Microbiology (RvA-T2) that would include a selection of performance characteristics dependent on the testing method.

In reality, only in a very limited number of cases will a laboratory fully develop and validate a new microbiological method. Often the laboratory itself applies limited validation. When a laboratory adopts a reference method or a method that has been developed and validated elsewhere, it has to demonstrate that it can apply the method properly in its own environment and obtain correct results. The verification of limited validation must be accomplished by the use of proper internal and external quality controls.

Microbiological methods are divided into either qualitative methods that demonstrate the presence or absence of the target microorganism, either directly or indirectly, in a defined quantity of test material or quantitative methods that determine the number of microorganisms present by direct enumeration (colony forming units) or indirectly (most probable number counts, colour absorbance, impedance) in a defined quantity of material. When a reference method is used, a laboratory should demonstrate its competence to meet the performance characteristics described in the national or international standard. For qualitative microbiological standard methods the performance characteristic is the limit of detection, while for quantitative microbiological standard methods these are trueness, repeatability, and reproducibility.

Microbiological investigations may be undertaken using alternative (rapid) methods such as immunological, molecular biological, or instrumental. The validation of these methods includes the assessment of their equivalence to the corresponding reference method (1).

Equivalence can be applied to the use of alternative (rapid) confirmation techniques; use of a different range of colony count than stated in the relevant standard; use of a different method for counting colonies or for calculation and expression of results than those stated in the relevant standard; and use of alternative (rapid) methods validated according to the relevant standard.

The *in-house method* is applicable to the use of a non-standard methods or the use of a standard method on a matrix that differs from the one specified by the standard method. When an in-house method is adopted it is recommended to select a non-standard method that has already been validated by a national or international organisation, or an accepted method that has already been in use in particular professional branches (e.g. the International Dairy Federation, IDF).

APLICATION OF QUALITY CONTROLS

One requirement for competence of laboratories in the International standard (2) is that a laboratory should ensure the quality of its results by using and assessing different quality controls. In the field of microbiology the use of correct quality controls is of particular importance, because the translation of the performance characteristic to microbiological examination is not always possible and it depends on the test matrix.

The terms »trueness« and »precision«, for example, are more difficult to define for microbiological examination than for analytical chemistry investigations. However, it is possible to demonstrate the technical control of the microbiological methods through control samples. Good microbiological reference samples are not yet available for all types of microorganisms.

Quality control can be implemented in a number of ways: internal serial (also called first line control) i.e. internal control of serial analyses assessed by technicians (using blanks, positive controls, negative controls, multiple observations); internal process (also called second line control) i.e. internal process control conducted by technicians, but assessed by laboratory management staff, e.g. the quality officer (using reference materials and spiked samples); and external (also called third line control) i.e. participation in an externally organised proficiency testing scheme. The quality controls will be described in the international Explanatory Document on Microbiology (RvA-T2 organised by Dutch Accreditation Council).

ROUTINE METHODS FOR QUANTITATIVE DETERMINATION OF THE BACTERIOLOGICAL QUALITY OF MILK

Particular routine methods are used for quantitative determination of the bacteriological quality of milk for a number different reasons such as: speed of analysis and/or response, ease of execution and/or automation, analytical attributes (specificity, limit of detection, limit of determination, repeatability, reproducibility, accuracy), extent to which the parameter of interest is expressed, and reduction of costs. Adequate operation of a routine method involves the application of quality assurance principles.

The below example of the use of a rapid microbiological method for routine work (3) that has been in use in Slovenia since 1995 (4) illustrates the evaluation of a routine method with a reference method for the determination of the bacteriological quality of milk based on automatic epifluorescent microscopy using the BactoScan 8000 instrument.

When introducing a routine method in order to comply with national and international regulations or standards, a number of issues is to be addressed:

- which criteria are essential for a routine method;
- what influencing factors should be considered when evaluating a routine method;
- how should a routine method replace a reference method;
- which constructive measures should be taken to improve the relationship between a reference and a routine method.

In most regulations for determination of the bacteriological quality of milk the reference method is the colony count at 30 C (5). However, many routine methods are applied, often with different methodological principles. The difference between the reference and routine values is expected to be less pronounced when the two methods are based on the same test principle.

The routine method must be evaluated as an estimate of the reference method according to general principles (6), either in-house or elsewhere.

In case of an expected deviation from the method of evaluation (e.g. milk composition, milk of other species, sample preservation, sample pre-treatment), the routine method must be validated additionally. The results of additional validation must be archived.

The laboratory must follow the following sets of control procedures: daily operating protocols, middle term controls, and long term controls. The daily protocol includes:

- start-up procedure for the instrument/equipment;
- start-up controls including limits;
- sample identification;
- sample storage and pre-treatment;
- sample checks (temperature, visible condition);
- sample analysis;
- routine checks during measurement (instrument settings, visual checks, temperature control, blanks, pilot samples);
- cleaning in between samples;
- shut-down procedure;
- truncation of results at the lower and upper limit of detection;
- evaluation and authorisation of results

Middle-term controls include:

- instrument checks;
- volume controls;
- carry-over controls;
- repeatability checks;
- within-lab reproducibility.

Long-term controls include:

- reproducibility;
- interlaboratory comparisons.

The purpose of interlaboratory comparisons is to establish that the precision characteristics of the laboratory's procedure are still in line with those recognised as feasible and achieved elsewhere and that the measured level is in agreement with that of the other participants. Conversion characteristics must express results in units of another method.

The results of interlaboratory comparisons are statistically evaluated. Graphical interpretations are frequently used. Figure 1 shows an evaluation of performance of laboratories that participated in international proficiency testing using the BactoScan instruments. It is presented as the Euclidian distance between accuracy and repeatability, which gives information about the performance characteristics of the instruments (7). Figure 2 illustrates a deviation of laboratory results from the total average. The figure has been taken from the report on cell count collaborative study organised by Milk Standard Service, Wangen, Germany.

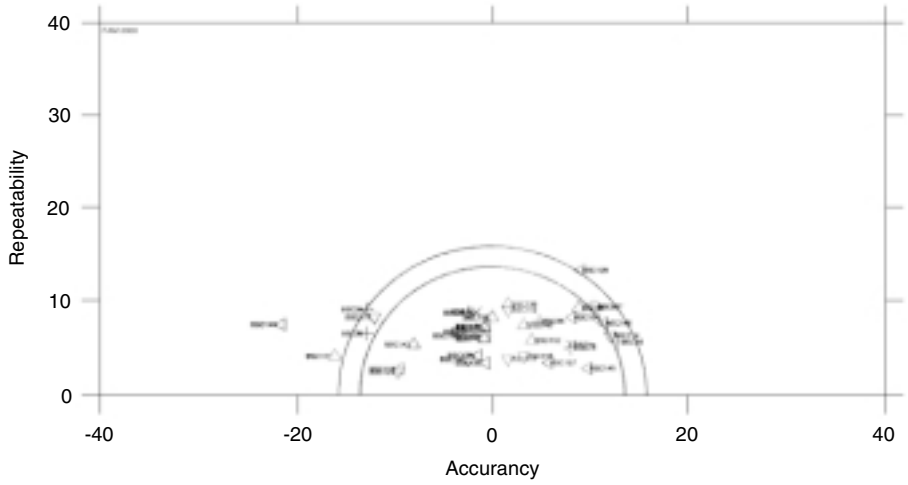

Figure 1 *Evaluation of performance of individual laboratories using the BactoScan instruments*

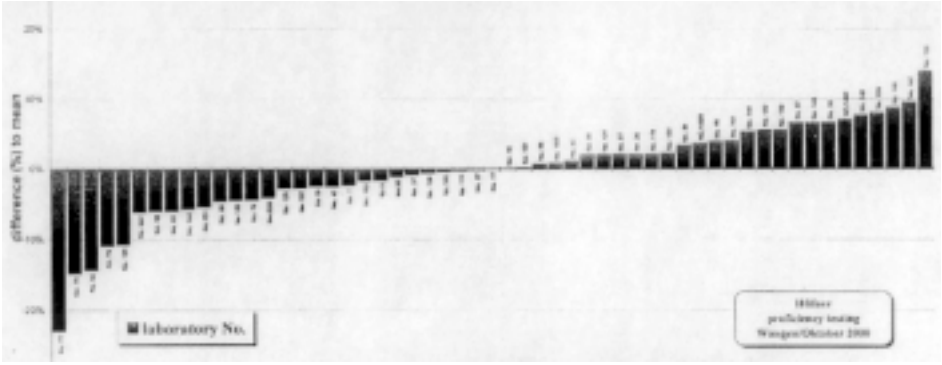

Figure 2 *Cell count proficiency testing – difference (%) from the mean of control milk samples*

In order to make comparable the use of routine methods the International Standards Organization and AWI are preparing a new standard proposal »Milk-Quantitative determination of bacteriological quality – Protocol for establishing a conversion relationship between routine method result and anchor method results and its verification« (8).

CONCLUSIONS

In the assessment of microbiological examination the choice and the evaluation of a method, and the use of a quality assurance system are very important. This is why every laboratory should rely on good laboratory practice, protocols, and standards. Our experiences suggests that it is equally important to perform quality control of the reference plate count method as a prerequisite for a reliable evaluation of an alternative microbiological method. The performance of test methods is evaluated through participation in interlaboratory proficiency studies.

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Sažetak

MIKROBIOLOŠKO ISPITIVANJE I PROSUDBA VRSNOSTI LABORATORIJA ZA MLIJEČNE PROIZVODE

U ovome su članku prikazani glavni čimbenici u procjeni mikrobiološkog ispitivanja hrane. Prikazano je nekoliko točaka validacije kvantitativnih i kvalitativnih mikrobioloških metoda. Osim toga, definiraju se izrazi »sukladnost« i »istovjetnost« referentne metode s »internom« metodom u smislu akreditacija metoda. Članak opisuje kako evaluirati rutinsku metodu u odnosu na referentne/službene metode s obzirom na rezultate dobivene brojenjem automatskim epifluorescentnim mikroskopom s pomoću uređaja BactoScan 8000 namijenjenog za utvrđivanje bakteriološke kakvoće mlijeka te daje opće upute za usporedbu navedenih metoda. Tu je i pregled aspekata osiguranja kakvoće koji se uzimaju u obzir u primjeni rutinske metode te primjer međulaboratorijske prosudbe vrsnosti uz pomoć epifluorescentne mikroskopske metode koja je uobičajena u laboratorijima za mliječne proizvode.

Ključne riječi:
BactoScan 8000, kontrola mlijeka, osiguranje kakvoće

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