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Italian National Conversion table

Can farmer settlement be harmonized even further?

By
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Italy has recognized that varying bacteria conversion equations, which were used by the 29 Italian laboratories, could potentially lead to very unfair settlement of raw milk quality. Therefore a large scale project in 2 phases was carried out to establish a uniform conversion equation transforming the units from the routine analyzers: Individual Bacteria Counts(IBC´s) into reference units, Colony Forming Units(CFU´s)

Berte Asmussen, Raw Milk Connect, has talked to Dr Giuseppe Bolzoni about the work he carried out to bridge the gap between the Italian regions by developing a National Italian Conversion Equation. They also discussed how to harmonize handling of milk quality data within the EU.

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Q: First of all could you explain the concept of conversion, which transforms units from routine analyzers into reference units.

A: I try to explain in an easy way a big technical and scientific issue that, after about 30 years, has not yet been completely solved.

The analytical instruments for TBC, BactoScan and BactoCount, count the bacteria in the milk with high accuracy and rapidity, but give results with a measurement unit (Impulse) different from the official. The official measurement unit is the CFU that is the number of colonies that these bacteria form in a specific culture media, after 3 days of incubation at 30 ° C.

So, the instrumental results have to be transformed ("converted") in the official unit. The principal problems of this conversion are:

- different species of bacteria form colonies in different way : in some cases a single bacteria forms a colony and in other cases 100 or 1000 bacteria are necessary to form a colony. Moreover this behavior is not constant for each specie: metabolic conditions of the single microorganism may change it and is influenced by many factors like, storage conditions or milk composition;
- the instruments count also bacteria that don't form colonies with the reference method: a part of bacteria don't grow in the micro-environmental conditions of the reference method.

So, the conversion of the instrumental result in the official measurement unit can be obtained only with a statistical evaluation (estimate of the colonies that would be formed) that, typically, implies a high measurement uncertainty. The statistical result is the "conversion equation", a line (or a curve) that allows you to "imagine" how many colonies would be formed by the bacteria counted by the instruments in each sample of milk.

Analyzing many samples of milk with both methods it is possible to realize this comparison and calculate this equation. The choice of the samples to analyze is the most (but not the only) difficult thing to realize this comparison: the type of bacteria present in a milk sample and their way to form the colonies is, as mentioned, extremely variable (type of farm, bedding, milking, climate, etc.). You should select standardized samples between those that routinely arrive in your lab, but it is not possible to define scientifically the composition of the typical bacterial flora of these samples. For this reason, the concept of "geographical conversion" has developed, since the late 90s. Each lab created its own "conversion" on the basis of a number of samples considered "typical" of their own geographical area (but in many cases conversions realized by other laboratories or produced by manufacturer tools have been used).

Q: Dr Bolzoni – please explain why it was necessary to develop a national Italian conversion equation?

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A: It was necessary because of different final results (expressed in CFU) obtained, also in neighboring geographical areas, for the use of different conversion methods. The decision to start the realization of a single national conversion was born in 2008.



Our work started from a theoretical question: the type of bacteria in a milk sample is more influenced by the geographical area of the farm or by factors like the type of bedding, milking, disinfectants used, etc.? There is not a conclusive answer to this question, but I am firmly convinced that farms managed in a similar way (i.e. same bedding) will probably have similar milk bacteria (except, obviously, in extremely different weather conditions) and, on the contrary, farms located nearby, but for example with different bedding material or temperature of the tank, will probably have different bacterial flora. In this context, the definition of floras

"similar" or "different" is obviously referring to the way they would form colonies with the reference method.

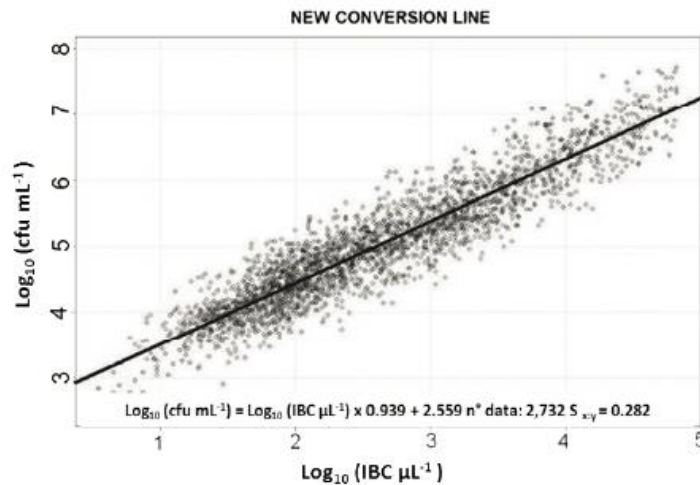
Q: Please summarize your experiences from this trial

A: The first goal was achieved with a significant help of the main Italian laboratories, both public and private, which analyzed more than 4,000 tank bulk milk samples with both methods and under controlled conditions; a detailed statistical analysis was conducted on these data (more than 28.000 analytical results!) and a conversion line representative of national milk was produced.

Additionally, we have been able to obtain scientific information about the influence of some factors on the conversion: for example, the limited influence of seasonal conditions, the systematic differences between laboratories in the execution of the reference method or the high influence of preservatives on conversion. Moreover, we have verified the excellent uniformity in the use of analytical instruments in the different laboratories, thanks to proficiency tests organization.

The most important information however, concerns the measurement uncertainty of the conversion: it is high if realized by a single laboratory and, in consequence, it is higher if produced by many laboratories. For this reason the study of individual factors influence on the conversion (such as the time between milking and collection, or storage temperature of the samples) is almost insignificant and, moreover, would be impossible to manage continuous variations of the conversion equation in the lab's daily activity.

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National Italian conversion line – $\text{Log}_{10} \text{CFU/ml} = 0,939 \cdot (\text{LogIBC}/\mu\text{l}) + 2,559$

Q: What have been your conclusions from the project?

A: The national conversion doesn't eliminate all the possible analytical errors but, at least, homogenizes them at national level.

Actually over 90% of the Italian milk samples are analyzed with the national conversion line. The reference method was the "reference" for about a century, it is applied worldwide and is affordable even for small size laboratories and, finally, is the method on which international law is based. But we cannot neglect its limitations: it counts only those bacteria that can form a colony in predefined conditions and, above all, as all manual methods, it is subject to occasional mistakes by operators and/or systematic errors by single lab. For large laboratories it is also impractical (if not with exorbitant costs) analyzes hundreds or thousands of samples per day without automatic instruments.

Q: How was the new equation introduced?

A: Thanks to the first phase (concluded in 2010) the subsequent passage to the national conversion (2013) was markedly easier. Its adoption has been made compulsory by the National Reference Lab and, for most of the laboratories; the effect on the final results was almost imperceptible.

The process of adoption of the national conversion in Italy was gradual and differentiated. For some labs, the change was significant compared to their previous situation. For this reason in the first step of the project (2010) the use of the new conversion was optional and, some labs, opted

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for a gradual transition with periodic steps during a year. In this case meetings are made for farmers' associations and dairy industries to explain this innovation. We have provided for each participant lab, the information necessary to compare the previous situation to the new one in order to highlight the points of any significant change.

Q: What was the reaction of the labs and farmers-did some farmers get a different grading and did they receive any special assistance?

A: In 2013 the change concerned mainly the very high bacterial counts that normally affect a small percentage of the samples (<1-2%). The transition from maximum values of about 3,500,000 CFU/ml to 6-7000000 CFU/ml values surprised in the early stages but their frequency and their practical significance represented little more than curiosities. The situation was monitored by local laboratories and by our Reference Centre; periodically, collaborative trials (in collaboration with Italian Breeders Association) and statistical elaboration of comparison between the period before and after the conversion were made. I can briefly conclude that between 2013 and today there have been no noteworthy changes; while, for the previous period, the situation was variable depending on single lab that managed it independently.

Q: Do you validate the national conversion – if yes how?

A: Yes, the results of the project were shown in a report that was sent to all participating laboratories, submitted to the National Accreditation Authority for laboratories, and communicated to the Ministry of Health. The fact that all the geographic areas have been represented in our work and the type of the statistical elaboration that we made, allow also to some little laboratories , not participating to the project, to use the national conversion conducting a kind of secondary validation in order to verify the correspondence with the delivered milk samples.

Q: Do you have a suggestion as how to handle the current complex situation within EU, where more dairies have milk suppliers in several countries, raw milk is shipped across borders plus the requirement that all milk should have less than 100.000 CFU?

A: I work on the problem of conversion from more than 20 years, but I want to surprise you by saying that **“we could work without it”**. I will explain better:

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A national conversion is useful for many practical and scientific reasons, but it does not solve the problem at Community level. For this, I think that it would be feasible a program that includes:

- 1) the comparative study of the two instruments currently on the market, to define their equivalence or correlation factor
- 2) the study of an European conversion with a similar project to the one made by us at the national level.

At this point, it would also be possible to take a "revolutionary" decision: according this new European conversion line (accepting a broader estimation error than those available at national level), it could be defined a fixed level of Impulses that corresponds to the legal limit of 100,000 CFU / ml.

The daily analysis could be directly expressed in Impulses (having a higher degree of precision and accuracy for each sample) for all practical purposes (milk quality payment system, self-control, auditing, research). For the health assessment, the fixed and harmonized level of Impulse will be used for evaluation of compliance with the geometric mean limit of the Regulation EC 853/2004.

Proposed organization of TBC–testing of raw milk in EU:

1. Daily monitoring of Total Bacteria Count for farmer settlement, lab QC/QAI and auditing to be logged in IBC's
2. For monitoring of EU-regulatory level of 100.000 CFU/ml the corresponding level expressed in IBC's should be fixed by the competent authority

The full report, may be read on

<http://www.chiriottieditori.it/ojs/index.php/ijs/article/view/186/31>