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The Italian experience for alternative method (flow cytometers) for TBC: study of the reproducibility in the years 2003-2016 and focus on the improvement after the implementation of a unique conversion system at national level

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INTRODUCTION

Results of BactoScan and BactoCount are given in impulses, whereas the TBC is officially expressed in CFU/ml, making a conversion system necessary to transfer results onto the official scale. In Italy, these instruments were introduced at the beginning of the 1980s. Today they are used in more than 50 labs and Bactoscan FC accounts for almost the totality of the instruments. The initial huge number of different conversions applied by laboratories has been responsible of a remarkable impact on the final reproducibility of the results reported in CFU/ml notwithstanding the excellent precision of these instruments.

In the years the number of conversions was gradually reduced. In 2008, the NRC-BMQ (IZSLER) launched the first part of the project for the evaluation of a single conversion line with the collaboration of 15 Italian laboratories. In the following years, the work was corroborated by the participation of the Italian NRL-MMP (ISS) and a new recruitment of 33 public and private laboratories throughout the country. In 2012, a single national conversion equation for BactoScan FC was elaborated and validated for bovine milk and it was gradually implemented throughout the country (G.Bolzoni et al. 2015. New national conversion line for Bactoscan FC in Italy: a step forward. Italian Journal of Food Science 2, 27).

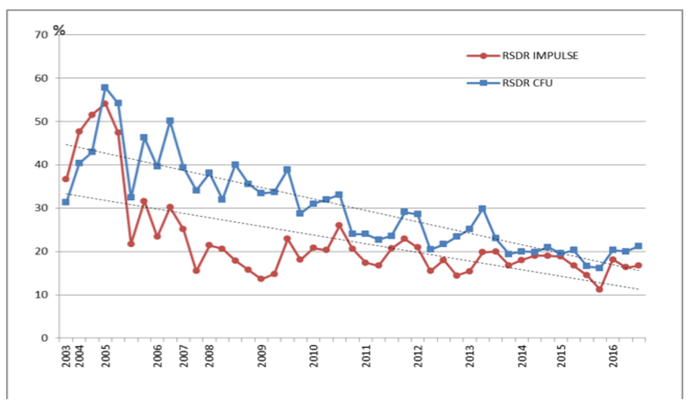
OBJECTIVES

This work aimed at assessing at national level, the effect on reproducibility of TBC (in CFU/ml) due to the gradual reduction of the number of different conversion equations in use in Italy, till the adoption of the single conversion line.

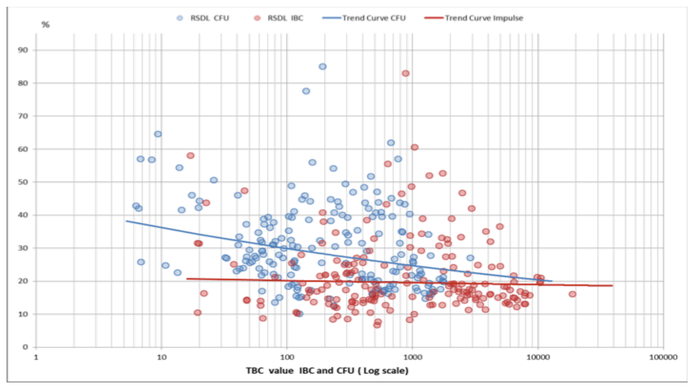
METHODOLOGY

A statistical analysis was applied on the results of about 50 PTs organized by AIA in Italy in the years 2003-2016 and for which laboratories had to provide results in impulses and in CFU/ml, according to their conversion equation in use.

RESULTS

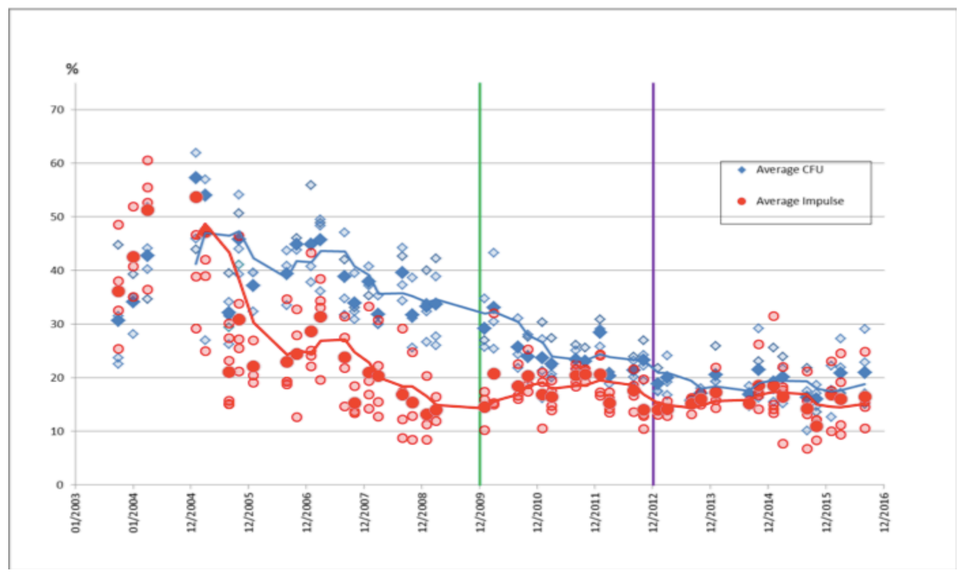


1. Average RSDR values of each PT (years 2003-2016) calculated on the valid results given both for the instrumental measurement unit (impulses) and after the conversion into CFU, as converted by each participant based on the conversion in use at the moment of PT. Impulse results are instrument-dependent. CFU/ml results depend also on the conversion mode



2. RSDL (relative SD among laboratories) reported for both the units of measure and as function of the level of contamination

RSDL is a component (likely conditioned, among the other causes, by the number of the conversions used) of the overall reproducibility of the PT (RSDR), for any given level, whereas RSDr is independent from the different conversions applied



3. RSDL values for both the units of measure, calculated for each individual sample (shaded indicators) and for the averaged values for each sample group (solid indicators). Mobile media lines (solid lines) are calculated as the rolling averages of 4 consecutive values. RSDL values calculated for each series of samples of each PT in the years 2003-2016.The two vertical lines represent the temporal phases of the entire project.

Impulses – indicator of instrumental performance. A reduction of the average RSDL (for impulses) from the initial PTs ≈ 50% to < 20% for the last ones is the main result of continuous monitoring, maintenance or modification of instruments carried out after the first rounds or implemented by outlier laboratories: the most significant improvement is concentrated in the beginning, goes on for the gradual replacing of BactoScan 8000 with FC model (completed in 2008) and reaches an evident stabilization in 2009.

CFU/ml - indicator of the conversion lines used by the participant laboratories. The delayed reduction of RSDL values (for CFU/ml) is the evident consequence of the decreasing RSDL values (for impulses) simultaneous to the instrument replacing and of the gradual reduction of the different conversions in use in the years. Although the milestones of this gradual process date back to 2010 and 2013, no clear change is evident in the trends: first for a progressive implementation of the new conversions after the dissemination of the results of the two phases of the project; second, many laboratories intentionally moved to the new conversion in 1-2 years to try to soften the possible impact on the historical levels of TBC results they had been producing in their geographic area.

RDSL Residual difference. The very low residual difference (average RSDL in the years 2013 -2016 = 20.6 for CFU and 16.5 for impulses) should disappear in the time. It is probably due to the persistence of different conversion systems (e.g.: 2 BactoCounts currently in use and probably few laboratories which, although participating in national rounds of PTs, till maintain their own conversion lines). Probably, since PT provider evaluates results in CFU and in impulses independently, the selection of lab outliers can have been different in the two series of results.

CONCLUSIONS

Information from the Italian periodic rounds on TBC with flow cytometry instruments since 2003 has highlighted a continuous improvement of the reproducibility of the results in CFU/ml obtained by the alternative method. This trend has become evident starting from 2008 with the first project for the evaluation of a single conversion and really satisfactory from 2012, after the project between CRQLB and NRL-MMP to evaluate, define and transfer a single conversion line at the national level. The performances of the Italian laboratories can now be considered established and demonstrate the uniformity of evaluation of this parameter throughout the country.

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