APPLICATION OF EC JRC CERTIFIED REFERENCE MATERIAL WITH SOMATIC CELL COUNTING IN MILK

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Application of the EC JRC Certified Reference Material (CRM)
Transition from current anchoring systems

DRAFT 23-11-2020

Guidance on application of EC JRC Certified Reference Material for somatic cell counting in milk

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Background
Milk somatic cell count (SCC) is a widely used indicator for monitoring the udder health of several mammalian species and is relevant in food quality regulations, milk payment testing, farm management and breeding programmes (IDF, 2008). In February 2020 the EC Joint Research Centre (EC JRC) launched a new certified reference material (CRM ERM®-BD001) for somatic cell counting in milk. The launch is one of the tangible outcomes of a close cooperation of the International Dairy Federation (IDF), the International Committee for Animal Recording (ICAR) and EC JRC in developing solutions and tools to promote a better global equivalence in somatic cell counting in milk.
APPLICATION OF EC JRC CRM

A “gold standard” to:

Check on method performance
Check on calibration settings of routine methods
Assign reference values to Secondary Reference Materials (SRMs)
Use in proficiency testing
1. CHECK ON METHOD PERFORMANCE

For reference method users and routine method users

To verify whether their method operates correctly

After checking the basic functioning of the method*

Using appropriate reference value with EC JRC CRM and related uncertainty information

Check whether difference between mean measured value and stated reference value is smaller than expanded uncertainty: $\Delta \leq U$

*Checks on reference method functioning:

- Dimensions of micros
- Repeatability

(see ISO 13366-1|IDF 14)

<table>
<thead>
<tr>
<th>Certified value [cells/mL]</th>
<th>Uncertainty [cells/mL]</th>
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<tr>
<td>64000</td>
<td>800</td>
</tr>
<tr>
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<tr>
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<td>790</td>
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1. CHECKING/ADJUSTING CALIBRATION SETTINGS OF ROUTINE METHODS


Results with a routine method have to be traceable to a reference

Calibration and calibration maintenance of routine methods
LOWCHART FOR CHECKING/ADJUSTING CALIBRATION SETTINGS OF ROUTINE METHODS

Check on method functioning

- Preparation of calibration sample set

Verification of calibration settings

- OK
  - No adjustment of calibration settings necessary
  - Adjust calibration settings

- Not OK
  - Method or instrument maintenance/adjustment

*Checks on routine method functioning:
- Blank checks
- Carry over
- Method-specific critical aspects
- Repeatability

(see ISO 13366-2|IDF 148)
PREPARATION OF CALIBRATION SAMPLE SET

Use both EC JRC CRMs to prepare calibration sample set:

Reconstitute in water according to EC JRC instructions

- Sample low with 62 000 cells/mL
- Sample high with 1 166 000 cells/mL

Preparation of calibration sample set by mixing sample low and sample high in different ratios: 4:0 – 3:1 – 2:2 – 1:3 – 0:4

Corresponding reference values to be calculated

Sample set with five equidistant reference values
ROM CALIBRATION CHECK
TO SLOPE AND INTERCEPT ADJUSTMENT

Calibration check

\[ y = a + b \cdot x \]

Reference

Slope and intercept setting

\[ \text{est SCC} = \text{intercept} + \text{slope} \cdot \text{routine} \]

\( \text{slope} = 1, \text{intercept} = 0 \)
What is a SRM?

Hierarchy for metrological quality control (ISO Guide 80)

- **SI measurement**: Fundamental measurement of the parameter
- **Certified Reference Material**: RM designated or acknowledged as having the highest metrological qualities
- **Secondary Reference Material**: RM produced by the establishment of traceability to a primary standard or a SRM
- **In-House Reference Materials**: In-house prepared or commercial RM with appropriate stability and homogeneity
ASSIGNING REFERENCE VALUES TO SECONDARY REFERENCE MATERIALS (SRM)

Advised procedure in brief:

Prepare material suitable to serve as SRM (see ISO 13366-2|IDF 148-2)

Analyse a CRM and the SRM with about same cell count levels pairwise (n>15 pairs) with a precise method under repeatability conditions

From the average of the pairwise differences and the CRM reference value

SRM reference value

From the uncertainties of the CRM and pairwise differences

uncertainty with SRM reference value

Proficiency testing = comparing measuring results between laboratories.

Including EC JRC CRM (or a SRM) in PT schemes allows to compare:
- between laboratories
- against “gold standard”
- in time

with next speaker…..
No Certified Reference Material (‘gold standard’) available

Application of various SRMs, globally coming from more than 20 suppliers

Relying on instrument manufacturer settings and checks through proficiency testing

Adjusting calibrations based on results in proficiency testing

Somatic cell counting is a big ship, sailing with many anchors.
POSSIBLE ISSUES WITH RE-ANCHORING

Being:
robust and stable,
well characterised,
representative in behavior,
affordable,

The EC JRC CRM is now there to serve dairy stakeholders around the globe as the “common anchor” for somatic cell counting in milk.....

....but re-anchoring may in places cause a significant shift in counting levels.
TAKEHOLDERS IN SOMATIC CELL COUNTING

Competent authorities
Laboratories
Farmers
Veterinarians
Milk collectors and processors
Breeding organisations
Instrument manufacturers
Providers of secondary reference materials (SRM)
Providers of proficiency testing services
ASPECTS TO CONSIDER WITH RE-ANCHORING

Extent of the expected shift in counts
Need for reassessment of limits/targets (milk payment, udder health programs)
Secure interlinkage between past data and future data
Consequences for laboratory protocols
Consequences in laboratory proficiency testing
Timing of the transition (when, in one step or in multiple steps?)
Communication to stakeholders
The launch of the EC JRC CRM is a major step towards better global equivalence in somatic cell counting in milk. It can be applied in method performance verification, in calibration, in assigning values to SRM’s and in proficiency testing.

Re-anchoring to the EC JRC CRM may in some geographies come with a significant shift in counting levels. This should be prudently dealt with between involved local stakeholders to secure a proper landing. IDF and ICAR can assist with guidance in this.
THANK YOU TO ALL WHO CONTRIBUTED!

THANK YOU FOR YOUR ATTENTION!