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Method Name: Determination of bovine lactoferrin in Infant and Adult/Pediatric Nutritional Formula

Approved by: Stakeholder Panel for Infant Formula and Adult Nutritionals
Final version date:
Effective date:

Intended Use: Reference method for dispute resolution.

1. **Applicability:**
Determination of intact bovine lactoferrin concentration in all forms of infant, adult and /or pediatric formulas (powders, ready-to-feed liquids and liquid concentrates).
The method must be able to distinguish intact lactoferrin from its hydrolyzed forms.

2. **Analytical Technique:**
Any analytical technique that meets the method performance requirements and specified conditions is acceptable. For the purpose of this SMPR, lactoferrin must be in the soluble fraction of: (a) reconstituted powder products (25 g into 200 g Type I water, 40 degrees Celsius), (b) "ready-to-feed" liquids "as is", or (c) liquid concentrates diluted 1:1 by weight using water

3. **Definitions:**

Accuracy[1]
The closeness of agreement between the average of an infinite number of replicates measured quantity values and a reference quantity value, if available.

Adult/Pediatric Formula
Nutritionally complete, specially formulated food, consumed in liquid form, which may constitute the sole source of nourishment, made from any combination of milk, soy, rice, whey, hydrolyzed protein, starch, and amino acids, with and without intact protein.

Infant formula
Breast-milk substitute specially manufactured to satisfy, by itself, the nutritional requirements of infants during the first months of life up to the introduction of appropriate complementary feeding[2], made from any combination of milk, soy, rice, whey, hydrolyzed protein, starch, and amino acids, with and without intact protein.

Limit of Detection (LOD)
The minimum concentration or mass of analyte that can be detected in a given matrix with no greater than 5% false positive risk and 5% false negative risk.

Limit of Quantitation (LOQ)
The minimum concentration or mass of analyte in a given matrix that can be reported as a quantitative result.

[1] Corresponds to the VIM definition for "trueness".
[2] Codex Standard 72 – 1981.

Repeatability

Variation arising when all efforts are made to keep conditions constant by using the same instrument and operator and repeating during a short time period. Expressed as the repeatability standard deviation (SD_r); or % repeatability relative standard deviation (%RSD_r).

Reproducibility

The standard deviation or relative standard deviation calculated from among-laboratory data. Expressed as the reproducibility relative standard deviation (SD_R); or % reproducibility relative standard deviation (% RSD_R).

Bovine Lactoferrin

Bovine lactoferrin is also referred to as Lactotransferrin (EC:3.4.21.-) and has CAS number: 146897-68-9. The amino acid sequence (without signal peptide) is displayed in Figure 1.

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     1          11         21         31         41         51
     |          |          |          |          |          |
   1                    A PRKNVRWCTI SQPEWFKCRR WQWRMKKLGA PSITCVRRAF    60
  61 ALECIRAIAE KKADAVTLDG GMVFEAGRDP YKLRPVAAEI YGTKESPQTH YYAVAVVKKG   120
 121 SNFQLDQLQG RKSCHTGLGR SAGWIIPMGI LRPYLSWTES LEPLQGAVAK FFSASCVPCI   180
 181 DRQAYPNLCQ LCKGEGENQC ACSSREPYFG YSGAFKCLQD GAGDVAFVKE TTVFENLPEK   240
 241 ADRDQYELLC LNNSRAPVDA FKECHLAQVP SHAVVARSVD GKEDLIWKLL SKAQEKFGKN   300
 301 KSRSFQLFGS PPGQRDLLFK DSALGFLRIP SKVDSALYLG SRYLTTLKNL RETAEEVKAR   360
 361 YTRVVWCAVG PEEQKKCQQW SQQSGQNVTC ATASTTDDCI VLVLKGEADA LNLDGGYIYT   420
 421 AGKCGLVPVL AENRKSSKHS SLDCVLRPTE GYLAVAVVKK ANEGLTWNSL KDKKSCHTAV   480
 481 DRTAGWNIPM GLIVNQTGSC AFDEFFSQSC APGADPKSRL CALCAGDDQG LDKCVPNSKE   540
 541 KYYGYTGAFR CLAEDVGDVA FVKNDTVWEN TNGESTADWA KNLNREDFRL LCLDGTRKPV   600
 601 TEAQSCHLAV APNHAVVSRS DRAAHVKQVL LHQQALFGKN GKNCPDKFCL FKSETKNLLF   660
 661 NDNTECLAKL GGRPTYEEYL GTEYVTAIAN LKKCSTSPLL EACAFLTR
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Figure 1: Amino acid sequence of bovine lactoferrin (UniProtKB - P24627 (TRFL_BOVIN))

4. Method Performance Requirements:

Analytical range	4-200 mg / 100g
Limit of Quantitation (LOQ)	4 mg / 100g
Recovery	90-110 %
Repeatability (RSD_r)	<6%
Reproducibility (RSD_R)	<9%
Concentrations apply to: a) 'ready-to-feed" liquids "as is"; b) re-constituted powders (reported as 25 g into 200 g of water); c) liquid concentrates diluted 1:1 by weight.	

5. System suitability tests and/or analytical quality control:

Suitable methods will include blank check samples, and check standards at the lowest point, midrange point and top of the analytical range.

6. Reference Material(s):

No infant formula certified reference material is available at this time.

7. **Validation Guidance**:
   Recommended level of validation: *Official Methods of Analysis*^{SM}.

8. **Maximum Time-To-Result**:
   No maximum time**.**