Annex to:

EFSA (European Food Safety Authority), Clawin-Rädecker I, De Block J, Egger L, Willis C, Da Silva Felicio MT and Messens W, 2021. The use of alkaline phosphatase and possible alternative testing to verify pasteurisation of raw milk, colostrum, dairy and colostrum-based products. EFSA Journal 2021;19(4):6576, 73 pp. https://doi.org/10.2903/j.efsa.2021.6576

Annex A – Protocol for the assessment of the use of alkaline phosphatase and possible alternative testing to verify pasteurisation of raw milk, colostrum, dairy and colostrum-based products

Clarification of the Terms of Reference (ToRs)

Issues for clarification with the requestor included:

- The products to be assessed are milk, colostrum, dairy and colostrum-based products and can be derived from sheep, goats and, if possible, from other animal species such as solipeds and camelids, producing such products for human consumption.
- Definitions of raw milk, dairy products, colostrum and colostrum-based products from Regulation No (EC) 853/2004[1] apply.
- The relevant products to be considered are those immediately after thermal pasteurisation of milk or colostrum, in the processing plant or at farm level if the adequate equipment is in place, as well as the end products placed on the market (milk or colostrum for direct consumption and any products based on those such as yoghurt, cheese, ice cream, milk powder, cream, or fermented milk). End products placed on the market should be understood to be supplied 'at retail' or by 'direct supply to the final consumer'.
- Thermal pasteurisation will consider the legally defined treatment conditions defined in Regulation No (EC) 853/2004[1].
- This mandate concerns the evaluation of the potential use, and limitations, of ALP activity for the verification of pasteurisation and other possible uses of this test, e.g. to assess colostrum quality or immunoglobulin G (IgG) concentration, are excluded from this mandate.
- Alternative testing to verify thermal pasteurisation of the relevant products should include alternative methods to the ISO 11816-1:2013[2] standard for the determination of ALP activity, as well as possible alternatives to the determination of ALP activity.

[1] Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin. OJ L 139, 30.4.2004, p. 55–205.
[2] ISO 11816-1 [IDF 155-1:2013]. Milk and milk products – Determination of alkaline phosphatase activity – Part 1: Fluorimetric method for milk and milk-based drinks. International Organization for Standardization, Geneva, Switzerland.

Further description of the clarifications made with the requestor is provided in Section 1.2 of the scientific report. ToRs were considered clarified with the requestor on 4 February 2021.

Steps 1 and 2.1: Formulation of the problem and planning of the methods

ToRs	Step 1.1 Assessment questions (reflecting clarification of ToRs)	Step 1.2 Sub-questions	Step 1.3 Approach to be followed	Step 2.1 Evidence needs	Step 2.1 Description of method to be used
EFSA is requested to evaluate the use of ALP and possible alternative testing to verify thermal pasteurisation of milk, colostrum, dairy and colostrum-based products ('products') from sheep and goats. More specifically EFSA is requested: **ToR1**: to provide an overview of the scientific information available on the use and limitations of ALP testing for verifying pasteurisation in the above products derived from sheep and goats, compared to cattle. If information is available, the overview could be extended to products derived from other species such as solipeds and camelids, producing such products for human consumption	**AQ1**: What is the use and what are the limitations of ALP testing to verify thermal pasteurisation of milk or colostrum from sheep and goats (and other species such as solipeds and camelids, producing such products for human consumption), compared to cattle, both immediately after such treatment, as well as on the end products placed on the market (milk or colostrum for direct human consumption and milk or colostrum-based products such as yoghurt, cheese, ice cream, milk powder, cream, or fermented milk)?	None	Qualitative up to quantitative	(a) Literature review (b) Data from databases (c) Expert knowledge (d) Primary data collection (questionnaire)	(a) A literature search will be carried out in the Web of Science™ Core Collection to retrieve information on the use and limitations to ALP testing for verifying pasteurisation in the relevant non-bovine products. The search string to be used is TS=(milk* OR colostrum* OR cheese* OR dairy OR yoghurt* OR yogurt* OR (ice cream)) AND TS=(ALP or (alkaline phosphatase)) AND TS=(sheep* OR goat* OR soliped* OR camelid* OR horse* OR equine OR donkey* OR dromedar* OR camel* OR alpaca*). (b) ALP testing data from samples of milk from bovines and non-bovine species will be extracted from the database of Public Health England (PHE). These data will be used to derive the ALP levels after pasteurisation for sheep and goat milk (together with the data obtained from the questionnaire). (c) Apart from the literature search, relevant documents will also be identified and reviewed, based on the knowledge and expertise of the WG members. (d) A questionnaire will be used to gather information about the current usage of ALP and possible alternatives to verify pasteurisation of relevant products in the EU. The European Dairy Association (EDA) will be contacted to establish if there are any industries in the EU producing colostrum and/or colostrum-based products from non-bovine species. The initial ALP concentration in raw milk from various species and the thermal stability of ALP will be mainly derived through literature screening. To consider limits of ALP in pasteurised

ToRs	Step 1.1 Assessment questions (reflecting clarification of ToRs)	Step 1.2 Sub-questions	Step 1.3 Approach to be followed	Step 2.1 Evidence needs	Step 2.1 Description of method to be used
					milk of different animal species, the data will be summarized semi-quantitatively or, when feasible, quantitatively.
ToR 2: to list the possible alternative methods to the determination of ALP activity, and their possible limitations for the verification of pasteurisation of the products immediately after such treatment in the processing plant, as well as on the end product placed on the market	**AQ2:** What are the possible alternative methods to the determination of ALP activity, and their possible limitations, for the verification of thermal pasteurisation of milk or colostrum from sheep and goats, both immediately after such treatment, as well as on the end product placed on the market (as above)?	none	Qualitative	(a) Literature review (b) Data from databases (c) Expert knowledge (d) Primary data collection (questionnaire)	(a) The search described for AQ1 will be also used to retrieve information on the alternatives to ALP testing for verifying pasteurisation in the relevant non-bovine products. (b) ALP testing data from samples of milk from non-bovine species as extracted from the PHE database (see AQ1) will also be used to evaluate possible correlations between the Enterobacteriaceae counts and ALP levels; both measured after pasteurisation. (c) Apart from the literature search, relevant documents will also be identified and reviewed, based on the knowledge and expertise of the WG members. (d) The questionnaire described in AQ1 will be also used to gather information about the possible alternatives to verify pasteurisation of relevant products in the EU.

Step 2.2: Integration of evidence across sub-questions and remaining overall uncertainty

ToR/AQ	Step 2.2. Integration of evidence between sub-questions	Step 2.2. Addressing overall uncertainty
ToR1-AQ1	SQs have not been defined so there is no need for evidence integration across SQs.	There is no need to plan beforehand.
ToR1-AQ1	SQs have not been defined so there is no need for evidence integration across SQs.	There is no need to plan beforehand.