

# A predictive model to evaluate the impact of the cooling profile on growth of psychrotrophic bacteria in raw milk from conventional and robotic milking

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This Research Communication explores the usefulness of predictive modelling to explain bacterial behaviour during cooling. A simple dynamic lag phase model was developed and validated. The model takes into account the effect of the cooling profile on the lag phase and growth in bulk tank milk. The time before the start of cooling was the most critical and should not exceed 1 h. The cooling rate between 30 and approximately 10 °C was the second most critical period. Cooling from 30 to 10 °C within 2 h ensured minimal growth of psychrotrophic bacteria in the milk. The cooling rate between 10 and 4 °C (the slowest phase of cooling) was of surprisingly little importance. Given a normal cooling profile to 10 °C, several hours of prolonged cooling time made practically no difference in psychrotrophic counts. This behaviour can be explained by the time/temperature dependence of the work needed by the bacteria to complete the lag phase at low temperature. For milk quality advisors, it is important to know that slow cooling below 10 °C does not result in high total counts of bacteria. In practice, slow cooling is occasionally found at farms with robotic milking. However, when comparing psychrotrophic growth in bulk milk tanks designed for robotic milking or conventional milking, the model predicted less growth for robotic milking for identical cooling profiles. It is proposed that due to the different rates of milk entering the tank, fewer bacteria will exit the lag phase during robotic milking and they will be more diluted than in conventional milking systems. At present, there is no international standard that specifies the cooling profile in robotic systems. The information on the insignificant effect of the cooling rate below 10 °C may be useful in the development of a standard.

**Keywords:** Predictive modelling, bacterial growth, milk cooling, conventional milking, robotic milking.

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Raw milk must be kept under refrigerated conditions to avoid spoilage. However, psychrotrophic gram negative bacteria, mainly *Pseudomonas* spp and some *Enterobacteriaceae* will grow (De Jonghe et al. 2013). Therefore, time/temperature control is essential to limit bacterial growth in the bulk milk tank at the farm. According to the European food hygiene legislation (Anon, 2004), milk must be cooled to 6 °C if the milk is collected every second day and to 8 °C for milk collected daily. European standard SS-EN 13732 (Anon, 2013) specifies the required cooling time from 35 to 4 °C. For a tank designed for four milkings and a performance temperature of 25 °C, the

specified cooling time is 3 h. Under practical milking conditions a common requirement in Sweden is that the milk must be cooled to 4 °C within two to 3 h from the end of milking. However, there is no standard for the cooling of milk in automatic milking systems. During the last two decades, an increase in herd size, and the use of large silo tanks for milk storage along with an increased proportion of farms with robotic milking has been seen. There is some concern among milk quality advisors that slower cooling profiles than desirable are observed. Important factors governing the growth of psychrotrophic bacteria are the length of the lag phase which depends on the cooling profile and the final milk temperature. In order to better understand the impact of these factors a dynamic growth model based on psychrotrophic bacteria during cooling and storage in bulk milk tanks was developed.

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## Materials and methods

### Predictive models

**Conventional milking.** Published information on growth rates of psychrotrophic bacteria or total counts at refrigeration temperatures in raw milk was sought (see online Supplementary Table 1). Most publications recorded growth rates at only one temperature and even fewer examined milk from different farms. None systematically reported on the length of the lag phase. However, (Tatini et al. 1991) developed a model based on the square root equation of Ratkowsky et al. (1982). Growth was recorded at 7, 13, 21 and 30 °C for 84 native raw milk samples. They expanded their model with data from other investigations of raw milk (see further references in Tatini et al. 1991):

$$\sqrt{\mu_{\max}} = 0.0303 \times (T - (-7.34)),$$

where  $\mu_{\max}$  is the maximum specific growth rate,  $T$  is the storage temperature in °C and  $T_0$  is  $-7.34$  °C.  $T_0$  is a temperature obtained by extrapolation of the equation to zero growth rate. This equation was in good agreement with growth rates found in a study of the recontamination flora of 46 different samples of pasteurised milk (Griffiths & Phillips, 1988), see below. In addition, these authors published a square root model for the length of the lag phase.

Further modelling was done based on data from Griffiths & Phillips (1988). In order to develop a dynamic growth model the duration of the lag phase ( $\lambda$ ) as a function of the temperature profile must be calculated. The growth rate equation was:

$$\sqrt{\mu_{\max}} = 0.031 \times (T - (-6.9))$$

The relation between the lag phase and storage temperature was expressed:

$$\sqrt{1/\lambda} = 0.015 \times (T - (5.7))$$

with the length of the lag phase in hours.

The equations were used to calculate  $h_0$ .  $H_0$  is related to the physiological state of the cell and the adaptation work needed to complete the lag phase (Baranyi & Roberts, 1994). The average  $h_0$  was estimated at 4.9 ( $\pm$ SD 0.6,  $n = 22$ ).

The lag phase under dynamic conditions of cooling of raw milk was estimated by time – temperature integration based on the procedure of Koutsoumanis et al. (2010).

$$h_0 = \lambda \times \mu$$

$$\lambda = 4.9 / (0.031 \times (T - (-6.9))^2)$$

$$\int_0^{t(\text{lag})} \frac{1}{T_{\text{lag}}} dt = 1$$

where  $t(\text{lag})$  is the total lag time and  $T_{\text{lag}}$  is the part of the lag time consumed at a certain time interval  $dt$  at the

corresponding temperature  $T_t$

$$\int_0^{t(\text{lag})} (4.9 / (0.031 \times (T_t - (-6.9))^2)) dt = 1$$

The calculations were made in Microsoft Excel 2010. The time and temperature data of the cooling profile was entered and divided into suitable time intervals (5–15 min depending on the registrations in the management software on the farm). The average temperature of each interval was calculated by linear interpolation. The lag phase work was estimated for each interval and the times were added until the lag phase was completed. The bacterial growth for the time remaining at 4 °C until milk collection (normally 48 h) was calculated.

$$^{10}\text{Log } N = ^{10}\text{log } N_0 + (48 - \lambda) \times \mu_{\max} / \ln 10$$

where  $N_0$  is the initial concentration of psychrotrophic bacteria/ml in milk. Certain assumptions have been made for the calculations: The growth equation was assumed to be log-linear up to 30 °C. For temperatures above 30 °C  $\mu_{\max}$  was kept constant at  $\mu_{\max 30^\circ\text{C}}$  which was assumed to be the optimal growth rate (Langeveld & Cuperus, 1980). This, however, is of little consequence for the predictions as the time above 30 °C is short and  $\mu_{\max}$  can be assumed to be lower above the optimum temperature. In conventional milking systems, milking is performed four times during 48 h. In the model calculations, the milk volume at each milking was assumed to be identical and the initial number of psychrotrophic bacteria was assumed to be identical each time (100 cfu/ml in order to compare different scenarios). Growth occurred only in milk from the first milking, due to long lag phases at low temperatures for the following milkings. Therefore, the final bacterial counts are the arithmetic average of four batches of milk, three of which contained 100 cfu/ml of bacteria.

**Robotic milking.** In robotic milking only milk from a single cow enters the bulk milk tank at each time interval. The bulk tank milk is continuously diluted with milk from the subsequent cows (to simplify calculations all milk was presumed to contain 100 psychrotrophic bacteria/ml initially, cf. De Jonghe et al. (2013) and O'Connell et al. (2016). For calculations, the time temperature profile was entered in Excel and the initial calculations were made according to the procedure above for conventional milking. Growth of bacteria in milk in each time interval and corresponding volume of milk were then treated as separate entities with individual length of the lag phase. Lag phase data was obtained by stepwise deletion of the previous step to obtain the length of the lag phase in the following step in Excel in the conventional model. Growth was calculated for the time remaining at 4 °C up to 48 h for each time interval. When no growth occurred (i.e. the lag phase was not completed), the concentration of bacteria remained at 100 cfu/ml. The number of bacteria/ml in each time interval was multiplied with the volume. A weighted average for the milk tank was calculated considering the total volume of milk.

## Results and discussion

### Validation of the model

Publications on growth rates of psychrotrophic bacteria in raw milk are not common. In online Supplementary Table 1, data on growth rates at 4 and 8 °C (calculated from tables and figures) are presented. Most are valid for a mixed flora in raw milk and range from  $\mu_{\max} = 0.02$  to  $0.05 \text{ h}^{-1}$  ( $10^0$  log scale) at 4 °C. The model predicted  $\mu_{\max} = 0.05 \text{ h}^{-1}$  at 4 and  $0.09 \text{ h}^{-1}$  at 8 °C. Single strains of *Pseudomonas fluorescens* grow in raw goat's milk with  $\mu_{\max}$  ranging from  $0.017$ – $0.036 \text{ h}^{-1}$  and two strains of *Ps. fragi* with  $\mu_{\max} = 0.042$  and  $0.060$ , respectively. It can be assumed that the fastest growing or most competitive strain will dominate in bulk tank milk (Tatini et al. 1991). De Jonghe et al. (2013) studied growth of pseudomonads over time in raw milk in a simulated dairy chain from farm bulk tank to dairy silo at optimal (3.5 °C) or suboptimal storage temperature (6 °C). The predictions of the model were similar to their results with respect to lag times and growth rates. O'Connell et al. (2016) studied the effect of storage temperature and duration on the microbiological quality of bulk tank milk. They emphasised that dilution of bulk tank milk from subsequent milkings has to be taken into account to obtain correct results. Their results are in line with the predictions of the model.

### Three phases during cooling

Cooling curves were collected from tanks for conventional milking for evaluation (see example in online Supplementary Fig. 1a). Cooling cannot be started immediately when the first milk enters the tank, due to the risk of freezing. A certain volume of milk e.g. 5% of the tank volume must be collected before cooling can commence. During this first phase, the milk is warm at approximately 30 °C. In a second phase, the cooling progresses rapidly due to effective cooling of the initial volume of milk. In a third phase cooling continues at a slower rate to 4 °C, since warm milk continues to enter the tank during milking. Based on the shape of actual cooling curves simplified linear three phase cooling curves were created (Phase 1: 30 °C; phase 2: 30–10 °C; phase 3: 10–4 °C, see online Supplementary Fig. 1b), to explore the impact of the three phases on growth under standardised cooling conditions. Actual temperature profiles may differ, but still have a similar shape. In tanks for robotic milking, various technical solutions enable restricted cooling of milk until full cooling capacity can be applied.

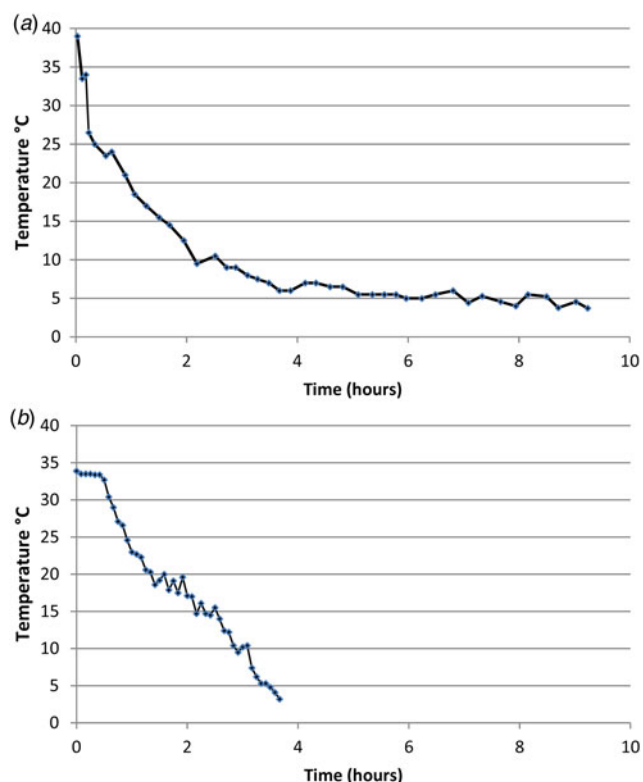
### Conventional milking

Simplified growth calculations for a conventional tank were made to explore the impact of the three phases. The initial bacterial counts in milk were set to 100 cfu/ml in all calculations. As expected, the time before cooling was the most critical. Under favourable conditions, i.e. cooling from

**Table 1.** Predicted growth of psychrotrophic bacteria in milk with different cooling rates between 10 and 4 °C

Condition*	Time		Cfu/ml† (after 48 h)
	10–4 °C (h)	Lag phase (h)	
A	1	28.4	310
A	4	27.1	350
B	1	24.2	460
B	4	22.8	520

\*A, 1 h at 30 °C, 1 h from 30–10 °C; B, 1 h at 30 °C, 2 h from 30–10 °C.  
†Initial level of bacteria was set 100 cfu (colony forming units)/ml.



**Fig. 1.** Cooling profiles in milk bulk tanks from two different robotic milking systems. Bacterial growth for 48 h was predicted from the temperature curves under conditions of conventional as well as robotic milking. (a) Long cooling time from 10 to 4 °C. Conventional milking: 400 cfu/ml. Robotic milking: 130 cfu/ml. (b) Somewhat slower initial cooling phase, very rapid cooling from 10 to 4 °C. Conventional milking: 530 cfu/ml; robotic milking: 160 cfu/ml.

30–10 °C and from 10–4 °C within 1 h each, there was almost no growth. However, there was notable growth which increased with the time at 30 °C, e.g. 4700/ml after 3 h with a cooling rate of 10 °C/h between 30 and 10 °C.

Online Supplementary Table 2 shows the effect of cooling rate during the second phase from 30 °C. Slow cooling leads to markedly reduced lag times and thus to increased bacterial counts. However, cooling at a rate of 10–20 °C/h from 30 to 10 °C seems acceptable (cf. Table 1 above).

The rate of cooling between 10 and 4 °C had surprisingly little effect on bacterial growth (Table 1) if the milk is cooled rapidly to 10 °C. Even 4 h of cooling time to 4° is of no consequence. This is important knowledge to milk advisors who encounter similar observations in practice in the absence of increased bacterial counts.

### Robotic milking

Cooling profiles from farms were collected and evaluated. First, calculations were made according to the procedure for a tank with conventional milking. Second, calculations were repeated, taking into account the restricted flow of milk during robotic milking (see materials and methods). Interestingly, bacterial growth was always less for robotic than for conventional milking (Fig. 1). In particular, the difference was largest when cooling profiles with extremely slow cooling were evaluated (data not shown). During robotic milking small volumes of milk from one cow enters the tank per time unit. In one technique, cooling is started immediately at a low intensity corresponding to the volume of milk. Only a few hundred litres of milk will enter the tank per hour during the initial cooling phase and will be cooled continuously. Therefore, a smaller fraction of the milk will experience high temperature as compared to conventional milking. Thus, only bacteria from this fraction will grow (and be further diluted), the remaining will not complete the lag phase. Other robotic milking systems have other solutions that achieve the same result. In conventional milking, approximately 1/4 of the volume will enter in a few hours. There will be more bacterial growth and the bacteria will be less diluted.

In conclusion, it is predicted that slow cooling rate of milk below 10 °C is of little importance for bacterial growth. It is proposed that bacterial growth in milk from robotic milking will result in lower bacterial counts than milk from conventional milking with the same cooling profile. The information on the insignificant effect of the cooling rate below 10 °C may be useful in the development of a standard.

### Supplementary material

The supplementary material for this article can be found at <https://doi.org/10.1017/S0022029917000334>.

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