

# On-farm evaluation of the neonatal hygiene management protocol using ATP bioluminescence

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## INTRODUCTION

- ▶ A proper hygiene management protocol can assist in the protection of newborn calves by reducing the load of pathogenic bacteria to which the calves are exposed.
- ▶ The Royal GD in the Netherlands proposes the "Hygiene Check Kalf" to obtain an idea about the bacterial load in the calf's environment. This "Hygiene Check" is based on routine bacteriologic culturing techniques.
- ▶ Adenosine triphosphate (ATP) bioluminescence has been advocated as a simple and useful method for the evaluation of the degree of microbiological contamination of surfaces and liquids.

## OBJECTIVE

The objective of the present study was to examine whether an ATP bioluminescence meter can be used as an on-farm tool to assess the degree of microbiological contamination of the newborn calf's environment.

## MATERIALS AND METHODS

- ▶ On 10 dairy farms in the Netherlands a Royal GD "Hygiene Check Kalf" was performed to evaluate the cleaning and disinfection protocol used in the calf raising units.
- ▶ On the same occasion ATP bioluminescence measurements, expressed as the number of relative light units (RLU) were performed on samples of the calf pens, the colostrum administration material (bottles, nipples, oesophageal tube) and the milk buckets using UltraSnap ATP swabs and a SystemSURE Plus meter (Hygiena™, California, USA).
- ▶ Optimal cut-off points for the ATP bioluminescence method to classify the calf hutches, the colostrum administration material (Tube/Bottle) and the colostrum collection material (Bucket) as "clean" or "contaminated" were determined using the "Hygiene Check Kalf" method as reference. Statistical analysis was performed in R (R Core Team 2022).

Clean calf pen does not correlate with clean colostrum/feeding equipment.

ATP bioluminescence measurements can be used as an on-farm screening tool to evaluate the cleaning and disinfection protocol used in the calf raising units on Dutch dairy farms.



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## RESULTS

The ATP value of the calf pen showed a negative correlation with the ATP value of the colostrum collection (Bucket) and administration material (Tube/Bottle). The bacterial contamination of colostrum collection and administration materials was positively correlated (Fig 1).

Optimal cut-off values and corresponding sensitivity, specificity and ROC curve AUC for the ATP bioluminescence method are summarized in Table 1.

Cut-off points for the ATP bioluminescence value determined using 100% sensitivity (no false negative results) for the calf pen, colostrum administration and collection material are presented in Table 2.

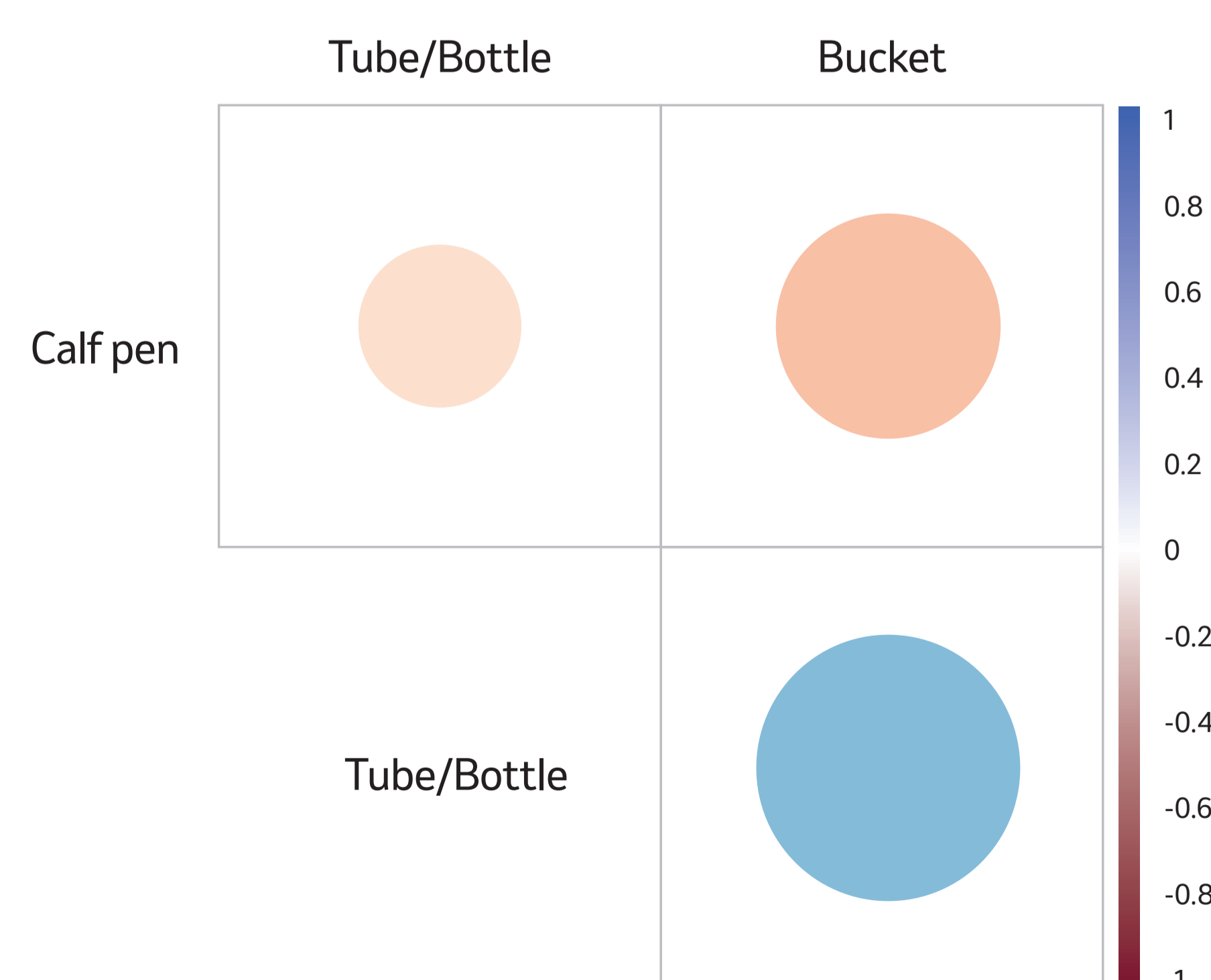
TABLE 1. Cutoff values for ATP value of calf pens, colostrum administration (Tube/Bottle) and colostrum collection (Bucket) material based on classification "contaminated" or "not contaminated" as determined by total bacterial count (Hygiene Check Kalf)

| Equipment   | Cut-off value (RLU) | Sensitivity (%) | Specificity (%) | ROC-curve AUC (%) |
|-------------|---------------------|-----------------|-----------------|-------------------|
| Calf pen    | 88                  | 80.0            | 76.9            | 82.3              |
| Tube/Bottle | 416                 | 75.0            | 66.7            | 54.0              |
| Bucket      | 655                 | 71.4            | 66.7            | 76.2              |

TABLE 2. Cutoff values for ATP value of calf pens, colostrum administration (Tube/Bottle) and colostrum collection (Bucket) material based on classification "contaminated" or "not contaminated" as determined by total bacterial count (Hygiene Check Kalf) using Maximum Sensitivity

| Equipment   | Optimal cut-off value (RLU) | Sensitivity (%) |
|-------------|-----------------------------|-----------------|
| Calf pen    | 50                          | 100.0           |
| Tube/Bottle | 10                          | 100.0           |
| Bucket      | 150                         | 100.0           |

FIGURE 1. Correlation matrix of ATP value (RLU) of calf pen, colostrum administration (Tube/Bottle) and colostrum collection material (Bucket)



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