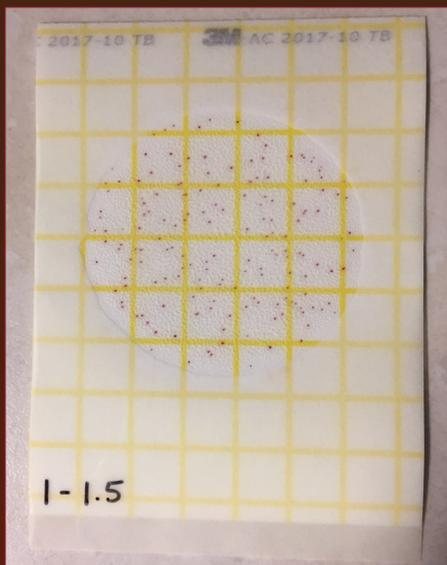


# Use of Petrifilm for Milk or Colostrum Total Plate and Coliform Counts

## Calf Care Audit Toolkit



### FAST FACTS

- *Monitoring bacterial counts in colostrum and milk are critical control points*
- *Petrifilm can be used for total plate and coliform counts*
- *Two simple dilutions are all that is needed*

Knowing the quality of the colostrum and milk fed to calves by evaluating bacterial contamination is a critical control point for calf rearing. High colostrum bacterial counts (>100,000 CFUs/ml for total aerobic bacteria and >10,000 CFUs/ml for coliform bacteria) are correlated with poor IgG absorption (Johnson 2007; Armengol 2016). The choices for culture include using standard agar plate methods for bulk tank milk within your practice, sending samples to another lab, or using Petrifilm (3M, St Paul MN) within your practice. There are several advantages to using Petrifilm: it is easily stored, creates less biohazardous waste, fits well in a small cooler and can be incubated in a portable incubator. The disadvantage is they are packaged in 50 and 25 films per package (total and coliform, respectively) and once open are stored at room temperature and can be destabilized by temperature and humidity.

The purpose of this factsheet is to provide dairy veterinarians with simplified procedures so that they can use Petrifilm for their Calf Care Audits to evaluate colostrum and calf milk quality.

### Sampling

Samples can be collected into 50 ml sterile milk tubes by the farm personnel and placed in the refrigerator. The best samples to evaluate are those “to be fed” to the calves to see what the calves are getting. Since these are samples for assessing bacterial contamination, it is important that farm personnel are shown how to carefully collect the samples to avoid extraneous contamination. Also it is important to limit the time between collection and culture since cold-tolerant bacteria will multiply during storage in a refrigerator.



### CONTACT

Vetextension@vetmed.wsu.edu

Authors: Dale A. Moore and William M. Sischo

This work is partly supported by USDA NIFA Grant No. 2015-68003-22998



United States  
Department of  
Agriculture

National Institute  
of Food and  
Agriculture



**PLATING PETRIFILM**

Dilution Methods

From our experience, total bacterial counts in colostrum can routinely range between 10,000 and over 100,000 CFUs/ml which cannot be quantified on a direct plating of colostrum to Petrifilm and the components of colostrum including fats interfere with plating. That means we need to dilute the sample. Although in the research lab they do many serial dilutions; two dilutions (1:125 for estimating coliform bacterial density and 1:3250 for estimating total aerobic bacteria) can provide you with sufficient information to assess colostrum bacterial quality. For milk, dilutions are likely to be different depending on source (non-saleable, saleable, pasteurized, milk replacer).

For the 1:125 dilution:

- Place 4 mL sterile saline in a sterile tube
- Add 32  $\mu$ L of well mixed (invert/shaken at least 10 times) colostrum/milk
- Vortex or invert the mixture several times (it needs to be well mixed)
- Plate 1 mL on coliform PetriFilm using the stamp that comes with the media and following directions for properly spreading the sample across the film.

For the 1:3250 dilution:

- Place 2.5 mL saline in a sterile tube
- Add 100  $\mu$ L from the 1:125 dilution
- Vortex or invert mixture several time (it needs to be well mixed)
- Plate 1 mL on aerobic PetriFilm using the stamp that comes with the media and following directions for properly spreading the sample across the film.

For the coliform Petrifilm, incubate for 24 hr at 35°C and for the Total Plate Aerobic Petrifilm, incubate for 48 hr at 32°C. If you want to estimate the portion of coliforms that are *E. coli* then incubate the coliform Petrifilm another 24 hours.

Reading the Petrifilm

The next step is to count the colonies. Have a fine point sharpie pen handy to mark the ones you counted. Both Petrifilms are gridded into a total of 20 squares. For the **aerobic total count (TPC)**, if there are fewer than 100 colonies (roughly 5 CFU per square), count the colonies in all the squares. If the plate has more than 100 colonies, pick 3 representative squares on the film, average the number of colonies in those 3 squares and multiply by 20. The actual count or the estimated count is multiplied by 3250 to get the total number of CFUs per ml of the original sample. As an example:

Aerobic Counts if > 100 colonies	
Count 3 squares & take average	# of Colonies
Square 1	11
Square 2	7
Square 3	13
Square Average	10.3

Estimated CFU on Petrifilm	207
<b>Estimated CFU/ml in Sample</b>	<b>671,667</b>

**PORTABLE INCUBATOR**



If you multiplied 207 by 3250, the TPC is 671,667. If the endpoint for your recommendation is to know if the sample has more than 100,000 CFU/ml then any sample with more than 30 colonies on the film will have a count greater than 100,000. The range of CFU you can comfortably estimate is between 16,250-650,000 (actual counts on the film = 5-200).

For the coliform count, if less than 100 colonies, count all the colonies in all the squares. If the count is greater than 100, then count all the colonies in 3 representative squares, average, multiply the average by 20 and then multiply the estimated plate count by 125. For example:

Coliform Counts if >100 colonies	
Count 3 squares & take average	# of Colonies
Square 1	7
Square 2	3
Square 3	6
Square Average	5.3

Estimated CFU on Petrifilm	107
<b>Estimated CFU/ml in Sample</b>	<b>13,333</b>

Multiply 107 by 125 for a total coliform count of 13,333 CFU/ml. If your goal is to identify samples with counts greater than 10,000 CFU/ml then anything greater than 80 CFU on the film will have a count greater than 10,000. The range of CFU you can comfortably estimate is between 625-25,000 CFU (actual counts on the film 5-200).

For detailed instructions on plating and reading, see the Interpretation Guide provided by the company. Coliform guide: <http://multimedia.3m.com/mws/media/2362460/petrifilm-ecoli-coliform-interpretation-guide.pdf>  
 For the TPC Interpretation Guide: <http://multimedia.3m.com/mws/media/2361940/petrifilm-aerobic-interpretation-guide.pdf>

#### Interpretation

Recommendations for colostrum bacterial counts are <100,000 for TPC and <10,000 for coliforms (McGuirk 2004). For milk fed to calves, pre-pasteurized samples should be <1,000,000 CFUs/ml and post-pasteurization should be <20,000 CFU/ml (from the pasteurizer). We have the evidence that too much bacteria in colostrum and milk is unhealthy for calves. Monitoring the bacterial quality of colostrum and milk fed to calves is the first step. If the counts are higher than the goals, the next step is to review the processes of colostrum harvest and storage and the processes for handling and feeding of calf milk.

#### References

- Armengol, R., and L. Fraile. 2016. Colostrum and milk pasteurization improve health status and decrease mortality in neonatal calves receiving appropriate colostrum ingestion. *J Dairy Sci* 99:4718-4725.
- Johnson, J. L., S.M. Godden, T. Molitor, T. Ames, and D. Harman. 2007. Effects of feeding heat-treated colostrum on passive transfer of immune and nutritional parameters in neonatal calves. *J.Dairy Sci* 90: 5189-5198.
- McGuirk, S. and M. Collins. 2004. *Vet Clin North Am Food Anim Pract* 20 (3):593-603.



WSU Extension programs and employment are available to all without discrimination. Evidence of noncompliance may be reported through your local WSU Extension office.