The Preliminary Incubation Count for Raw Milk
- An Old Test Revisited -

The Preliminary Incubation (PI) Count for raw milk has been used by a number of laboratories, cooperatives and dairy companies as a tool to potentially detect inadequate hygiene practices on the farm that might be missed by the Standard Plate Count (SPC) procedure. The test requires incubating or “stressing” the milk sample at 55°F (12.8°C) for 18 hours, after which an SPC or Plate Loop Count (PLC) is performed (Standard Methods for the Examination of Dairy Products, 15th ed. 1985). The goal is to detect bacteria that grow during the PI, thus the PI count should always be compared to a fresh SPC. The PI count is based on the theory that the normal microbial flora of the cow (e.g., skin and teat bacteria) will not grow substantially when held at this temperature/time combination, whereas certain microorganisms associated with inadequate hygiene practices could grow to significant levels under these conditions when present in the milk. Factors that can influence the results of this test include the precision of the incubation temperature/time, the age and storage history of the milk sample, as well as the type(s), initial numbers, stage(s) of growth and optimum growth temperatures of the microflora present in the sample. Because of these factors, and the fact that this test relies on the detection of microorganisms capable of growth under the conditions of the test, results may vary. The counts of the test should be interpreted and used with caution. It is important that the results of this test be compared to the bacteria count (SPC) of the fresh, unincubated sample, as high SPC/PLC counts will also result in high PI Counts, without significant microbial growth during the preliminary incubation step. Despite these concerns, many have used the test and have found the information to be useful in farm quality programs.

What are acceptable levels for PI Counts?

The 15th edition of Standard Methods (1985), the last edition that included the PI test (most recent is the 17th ed., 2004), suggests a maximum allowable PI Count of 200,000 cfu/ml. Some milk handlers use counts of 50,000-100,000/ml as their limits; some have used even lower. In a study of over 850 individual NY samples, 45% had PI counts of less than 50,000. Another approach sometimes used is to consider a milk suspect if the PI count is higher than the SPC by a designated amount (e.g., PI count ≥ 3 - 4 times the SPC). For example, if a producer sample had an SPC of 8,000/ml and a PI count of 9,000/ml, no substantial increase occurred and the PI count would not imply poor hygiene practices; if the PI count were 32,000/ml or greater (i.e., 4 times the SPC), this might suggest that procedures on the farm could be improved. On the other hand, if a producer sample had an SPC of 100,000/ml and a PI count of 110,000/ml, the PI count, while greater than 100,000/ml, provides no additional information, as no substantial growth occurred. The SPC alone would indicate a bacterial problem caused by organisms that grow poorly at 55°F within 18 hrs.

The selection of appropriate PI “cut-off” values for those that use the test is sometimes difficult; there is little scientific data on the subject. Values in current use are based mostly on experience and an effort to use the test to improve milk quality. As stated above, because the test is based on microbial growth at a specific temperature over a specified time, results can be dependent on a number of variables. Selecting a meaningful cut-off value is challenging. Proper interpretation of PI results may require more of an “intuitive” approach than just using established set values. For some, this test is considered to be a useful tool as a potential indicator of poor production methods and to mark trends in producer quality (e.g., spikes in counts). Others have had difficulty interpreting the test and/or tracking causes of what might be labeled as a “high” PI count. It is most useful when accompanied by other tests, observations and inspections; experience is essential. When used as such, it may be instrumental in helping improve overall microbial counts of farm raw milk.
**Trouble-Shooting PI Counts:**

A number of guidelines have been developed over the years for trouble-shooting high PI counts. In general they have focused on most of the traditional sources of bacteria in milk that are not considered the normal flora of the cow. For the most part, mastitis organisms are not considered to be likely causes of increases in PI counts over the SPC values, although there may be exceptions. General areas include:

a. Cow cleanliness:
   - teat/teat-end/udder condition & cleanliness, pre-milking hygiene procedures, condition of milk filters

b. Equipment cleanliness/adequate procedures/design:
   - washed after each milking/pick-up; appropriate water temperatures & detergents; emphasis put on bulk tanks, outlet valves, receiver jars & components, vacuum to receiver and worn rubber parts

c. Sanitization procedures:
   - should be just prior to milking with correct sanitizer levels; no untreated water prior to milking

d. Milk Cooling & Bulk Tank Cleanliness
   - cooled to <40°F, blends not to exceed 50°F (preferably <45°F); tank washed after each pick-up

e. Water quality/use of untreated water
   - check for coliforms & psychrotrophic bacteria counts; all rinses before milking contains sanitizer

The goal of the PI count has been to detect hygiene inadequacies during farm production that may not be apparent with the SPC alone (e.g., covered up by cooling). To this end it has been used successfully by some field personnel in discovering deficiencies on the farm. It is not unusual however, that causes of high PI counts are not found when farms are inspected, even by those with experience, as high PI counts may come and go and specific causes may not be evident. The bacteria most commonly associated with high PI counts are Gram-negative psychrotrophs (i.e., grow under refrigeration) such as certain *Pseudomonas* spp., although other types of bacteria may be involved. These types of organisms occur in raw milk due to poor udder preparation, inadequate equipment cleaning and sanitizing procedures and possibly from contaminated water sources. Failure to sanitize milking systems just prior to milking has been shown to be associated with elevated PI counts. Since the test selects for bacteria that grow at cool temperatures, the bulk tank and associated components are often suspect. In addition, prolonged or marginal cooling (~45°F) may select for psychrotrophs, which can easily become the dominant flora in refrigerated raw milk. Based on these concepts, the PI procedure has been used as an indicator of inadequate practices on the farm. To ensure that results reflect the farm, utmost care should be used when collecting and handling samples, as poor procedures (e.g., dirty sampler, poor sample cooling, prolonged storage before testing) may influence the results of the PI test.

**How Does the PI Count Relate to the Quality of Raw and Pasteurized Milk?**

Gram-negative psychrotrophic bacteria are often the cause of reduced shelf-life of pasteurized milks. These organisms however, generally do not survive pasteurization; their occurrence in pasteurized milk is almost always the result of post-pasteurization contamination. The presence of these organisms in raw milk should not influence pasteurized milk quality held for normal sell-by dates (~14-18 days) unless total counts (not PI counts) are well above the regulatory limit of 300,000 CFU/ml. The bacteria responsible for high PI counts are often present only in relatively low numbers in the unincubated milk, thus the PI count alone cannot be directly correlated with the flavor quality of the raw milk or the quality or shelf-life of the processed product. A more useful gauge of the potential influence of the raw milk microflora on the quality of dairy products is the total bacteria count of the raw milk at the time of processing (e.g., taken from the plant silo or surge tank). There is limited information on the relationship of PI counts to other test procedures (e.g., psychrotrophic spore counts) for raw milk or to bacterial spoilage of the raw or pasteurized milk; however, available studies show little correlation. Using the PI count of a sample to predict the quality of products made from this milk is not advised, as there is no research to support the use of this test in that manner.


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