March 25, 2021 To Senate Agriculture Committee From Dr. Andrea Etter, Assistant Professor, UVM, Nutrition and Food Sciences Dept.

The common pathogens found in raw milk include *Listeria monocytogenes*, pathogenic *Escherichia coli* (that is, with *stx* genes capable of causing kidney failure), *Salmonella enterica*, and *Campylobacter*. While all of these pathogens cause human illness, the quantity of bacteria needed to cause illness varies widely among them. Below are there estimates of the number of bacteria needed to cause illness in an "average" human (i.e., healthy adult)

Pathogenic *E. coli* (with *stx* genes): 1-100 bacteria *Listeria monocytogenes:* 1-100 bacteria *Salmonella:* best estimates are 1,000-100,000 bacteria *Campylobacter:* 500-10,000 bacteria

Traditionally, selective microbiological growth media (broths, solid agar) has been used to detect these pathogens in foods. However, this process is quite slow, with results taking 3-7 days. DNA or RNA-based methods, typically based on polymerase chain reaction (PCR) are considerably faster, with a turnaround time of 1-3, depending on capabilities of the laboratory and the type of sample. They are also much more sensitive at detecting pathogens, able to detect as few as 1 bacteria in a standard 25 gram sample of food.

However, not all PCR methods are the same. Regular PCR can detect pathogens when they are present at about 10,000 bacteria/sample, as the DNA present must be visible on a gel. In contrast, quantitative PCR can detect down to a single bacterium in the original sample, because the detection is based on a probe that fluoresces when it binds to and copies a section of the DNA/RNA of the pathogen of interest. This fluorescence is detected by the machine (this is the same technology currently used in Covid-19 PCR tests) and recorded. This fluorescence also provides information on the quantity of the target pathogen present. Coded within the machine's programming is a threshold for florescence that essentially is the line between background "noise" and actual, real data. The sooner the fluorescence level in a sample passes that threshold, the more pathogen was present in it.

I'm afraid I don't know exactly how qPCR would cost. There are labs that do it: <u>https://www.elslab.com/services/pathogen-testing/</u> and <u>https://certified-</u> <u>laboratories.com/microbiology/</u>, but none of them post costs. My back of the envelope calculations are rough, but as follows:

- I would think material costs for qPCR would be about \$7-10 a sample (cost of DNA/RNA extraction kit, fluorescent probe, plates to run the PCR in, etc)
- Equipment is expensive, but a one-time purchase (\$19-30,000 for a basic qPCR machine)

• Labor would be around 1-2 hours per sample (this depends on many factors, such as the speed of the technician, buts these tests are usually done in 96-well plates, so multiple samples are processed simultaneously).

Consequently, it's probably reasonable to expect **a minimum cost of \$25-50/sample** for testing for four pathogens/sample.

Whether or not the qPCR testing could replace the current tests depend on whether the state wants to focus solely on safety or not. The information gained from total bacterial counts/APC would be essentially covered with regular qPCR pathogen testing, however, I'm not aware of a way to do somatic cell counts by qPCR, so if they wanted to move to all-PCR testing, they'd have to drop that measure of milk quality.