Actual strata for each of the four regions are detailed in the table below:

Centre	West	Quebec	Ontario	Atlantic
<150,000	5	10	?	3
150-300,000	9	10	?	11
>300,000	3	9	?	4

Milking schedule: Herds following a 3x milking schedule were to be excluded, however some herds not originally on 3x milking did initiate this practice during the sampling period. The table below shows the farms that did milk cows 3x during the duration of the study and the dates during which 3x milking was undertaken.

Region	Herd	Dates began 3x milking	Dates ended 3x milking
Atlantic	6	1.1.07	30.4.07
Atlantic	6	1.9.07	present
Quebec	8	29.4.2007	25.7.2007
West	7	20.12.2007	continues
West	13	20.5.2007	4.8.2007

Initial Recruitment Guidelines

Farmers were given the option of exclude any animals from the sampling (valuable animals, show animals, etc.). Out of all the herds, only five cows from the Atlantic provinces cows were excluded, none from Ontario, one from the West, and none from Quebec.

Farm Retention

Because retention of farms for Year 1 was so successful (91 of 93 farms remained at the beginning of Year 2), the decision was taken to give farms that had been least compliant with sampling the option of discontinuing milk sampling. As of January 1st, 2008, therefore, thirteen farms were removed from the milk sampling for the Cohort, leaving 78 farms to be sampled in 2008. These farms were: Farms 13 and 17 in the Atlantics, Farms 1, 3, 9, 11 and 15 from Quebec, Farms 4, 15, 22 and 24 from Ontario and Farms 5 and 13 from the West.

Two other farms were lost in Year 2. In late January, Farm 16 in the West burned down and therefore exited the Cohort. On April 30th, Farm 10 in the West sold their herd, and in August, Farm 18 in the West also sold their herd. These two herds exited the Cohort at those respective times.

Although individual cow milk samples, bulk tank milk samples and health data were no longer collected on these farms, they did continue to participate in surveys and other data collections as required.

Sample size had previously been determined for a final sample size of 80 farms, with any extra farms being beneficial but not necessary to retain. The reduction in farm numbers, therefore, preserved the estimated statistical power while decreasing spending on the data collection.

Bulk Tank Bacteriology forms were issued and updated throughout 2007 as the protocol for bulk tank bacteriology changed. During this time, bulk tank samples were shipped from the various Centres to UPEI and validation of the bulk tank bacteriology protocols were undertaken. For this reason, not all samples will have identical Bulk Tank Bacteriology forms in hard copy.

Changes and Realisations to use of Milk Sample Submission, Bacteriology and Bulk Tank Bacteriology forms (also refer to NCDF SOP for Movement of Samples and Forms)

When cows chosen for sampling (dry cow/fresh cow or intensive lactating cow samples) had only three milking quarters, technicians usually submitted Bacteriology forms with a blank column (left without barcode) and inserted '98' into the 'Species' (code 0-99) box for 'Sample Not Received'. At times, an empty vial with barcode was issued in order to alert Bacteriology labs to the fact that the sample had not been lost but in fact that there had been no sample from the indicated quarter of that cow. If barcodes were issued but no sample was taken, no further data will exist for this sample.

Changes and Realisations to NCDF SOP for Movement of Samples and Forms and Movement of Bulk Tank Samples

Most movements detailed in these documents proceeded as planned. Ontario and Quebec Centres chose not to pre-bar-code sample vials for ease of entry when the samples were collected instead. These Centres instead applied the barcodes to sample vials when they were processed back at the Centre – all samples did have barcodes for use in data entry as well as for SCC analysis.

Ontario and Quebec also utilised computerised data entry and therefore did not transfer barcodes over to paperwork but printed these directly on the Sample Submission forms instead.

Movement of Samples and Forms

3. Centres were to label individual forms left on farms (e.g. Mastitis Sampling, Dry Cow and Fresh Cow and General Health forms) with farm-specific numbers, some of which would be repeated for each of the farms (for instance, Farm #3 in the Atlantics would have form C-2 and Farm #4 in the Atlantics would also have form C-2, but these forms would be individually recognisable because they would be A-3-C-2 and A-4-C-2 respectively).

4. Farmers often forgot to record a 'Mastitis Clinical Score' for the cows they sampled for Dry Cow/Fresh Cow and sometimes for Clinical Mastitis samples. When no clinical score was recorded, no score was entered into the database (i.e. a score of 0 was not assumed if there was no recorded score).

9, Unlike the forms that related to individual farms, Centres were to label Milk Sample Submission forms in a continuous series, starting with #1 and continuing. At times this series was disrupted in certain Centres, and these Centres were then instructed to begin again with a number higher in the series that had not been previously used and proceed from that number (e.g. If a Centre was double-labelling Milk Sample Submission forms from #345-398, they were instructed to just skip on to #400 and continue in a series from that higher number. Therefore, some numbers - #399 in the example above – might have been left out, but filing at the Administrative Centre was simplified.)

10. Again, in Ontario and Quebec, computerised entry was done, so no transfer of barcodes was accomplished – the barcode numbers were simply entered and printed out onto the Milk Sample Submission forms.

12. In 2008, L1, L2, L3, DC1 and DC2 samples were those from which aliquots were taken instead of M1 Clinical Samples, so two spare identical barcodes were included with these samples (see below - #15) for a total of four identical barcodes.

13. Again (see below - #15), since the type of samples sub-samples were taken from changed in 2008, this directive changed to: 'technicians should also include two spare barcodes (four for L1, L2, L3, DC1 and DC2 samples) of each sample number.

15. Sub-samples of M1 samples were taken according to this protocol for most of 2007 (researchers are encouraged to consult the Database for actual dates). Sub-samples were also from June to December, 2008, according to the protocol below:

After all bacterial identification has been completed but BEFORE adding bronopol tablets, samples should be ready to be preserved for later use to validate a multiplex PCR assay to diagnose intramammary infection (Project #1, D. Scholl, Project leader).

Wearing gloves and using your fingers, pull out a small group of hairs at a time (8-10 hairs generally can easily be removed with each pull). The hair should come out effortlessly and you should be able to see the follicle as a grayish bulb at the end of the hair. Pluck groups of hairs until at least 40-60 hairs (with visible follicles) have been collected. Place and seal these hair samples in well-labeled Ziploc storage bags or small paper envelopes for storage and shipping (not provided - some people have found the paper envelopes less sticky and easier to handle and label in the warm weather). A barcode should be applied to the outside of the storage bag or envelope and the date and cow ID should be written on the bag or envelope. The sample can also be entered onto the Sample Submission form with the sample type for 'HF' for 'Hair Follicle'. The date of sampling and the ID of the cow should also be recorded. Copies of these forms along with the samples should be mailed to Bonnie Mallard. A copy should also be mailed to Isabelle Jodoin in the Administrative Centre and completed forms filed at the Regional Centre.

Expected Outcome

Immunized cows are expected to produce an antibody and cell-mediated immune response as per any standard vaccination procedure. On day 23 post-immunization, cows should have a small skin nodule indicative of cell-mediated immune response. This reaction is similar to the tuberculin skin test given to export cattle and the size of the nodules should be greatest between 24-72 hours following the ID injection of antigen. This nodule is expected to resolve thereafter. Otherwise, there should be no lingering visible signs associated with this set of immunizations.

This standard immunization protocol has been tested on over 1000 Holstein cows and there have been neither fatal responses nor detrimental consequences other than a small skin nodule following the DTH test. However, as with any vaccination procedure or cow treatment, there is always a risk that some cows may have an allergic or otherwise deleterious effect following immunization or vaccination. These are impossible to predict but are not considered a major concern. Should this rare event arise the producer would be compensated for the loss or damage to that cow.

Changes and Realisations to NCDF Immunogenetic protocol and sample collection

PEI sampled all farms during summer, 2007, at the same time as the summer intensive sampling period. Quebec sampled six farms from November 26th – December 21st, 2007 and six farms from May 5th to 30th, 2008. Ontario sampled five farms from October 3 – November 30th, 2007 and ? farms in August, 2008. The West sampled six farms from October 9th – November 3rd, 2007 and ten farms from November 5th – December 1st, 2007. Nova Scotia sampled four farms and New Brunswick sampled five farms at the same time as the 2008 summer intensive sampling period. All besides the PEI samplings in 2007 used only cows from the 2007 summer intensive sampling that were outside the window of +/-28 days around calving. Samplings occurring in 2008 sampled cows that were from the 2008 summer intensive sampling period. ??

Legend for Cohort Data Bank

Bacteriologie = Bacteriology Results – Column Headings
ValidationNum = Validation Number = record number of entries in this database
BarCode = unique, 8-digit barcode given to each sample individually
CultureDate = date the sample was cultured in the bacteriology lab
NbofCol_1 = number of colonies grown for Isolate 1 from this sample
NbofCol_2 = number of colonies grown for Isolate 2 from this sample
HemolysisType_1 = digit indicating type of hemolysis in Isolate 1 from this sample (refer to Bacteriology form for further details)

- **0** = **A** on bacteriology form = α hemolysis
- **1** = **B** = β hemolysis
- **2** = **C** = weak β hemolysis
- **3** = **D** = non-hemolytic
- **4** = **E** = double-zone hemolysis

HemolysisType_2 = digit indicating type of hemolysis in Isolate 2 from this sample (refer to Bacteriology form for further details and see above)
Specie_1 = digit indicating species of bacterium determined for Isolate 1 from this sample (refer to Bacteriology form for details)
Specie_2 = digit indicating species of bacterium determined for Isolate 2 from this sample (refer to Bacteriology form for details)
Status_1 = digit indicating bacteriologic status of the sample (refer to Bacteriology form for details)
SampleType = type of sample collected (for details see 'SOP for Movement of Samples and Forms' in NCDF Reference Manual

L1: First intensive sample from lactating cow, in either of four intensive sampling periods

L2-L7: Second through seventh intensive samples from lactating cows, in either of four intensive sampling periods

T1, T2: Dry cow samples 1, 2

V1, V2 : Fresh cow samples 1, 2

M1, M2, M3: Clinical samples 1, 2, 3

Animal_ID = corresponding number (identified as chain number in DHI) of enrolled animal (name in parentheses)
Quarter = quarter (or composite) from which sample has been taken

LF: left front, RF: right front, LR: left rear, RR: right rear, C: composite

DateSampleTaken = date sample was taken from the cow
DateSubmit = date sample was submitted to the bacteriology lab
DateSampleThawed_1: date sample thawed by the bacteriology lab
SecondDateFreezed = date sample was refrozen after bacteriology
DateShipSCC = date sample was shipped to the somatic cell count lab
DateSampleThawed_2 = date sample was thawed by somatic cell count lab
BT_MilkingNumber = number of milkings in the tank when the bulk tank sample was collected
BT_AM_PM = time when bulk tank sample was collected, after morning or afternoon milking

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