### DIRECT MICROSCOPIC SOMATIC CELL COUNT GUIDELINE

Rules and Examples for Counting Somatic Cells in Milk

The following images are scans of slides prepared by the FDA Laboratory Quality Assurance Team (LQAT) for use in training for the Direct Microscopic Somatic Cell Count (DMSCC) procedure as outlined in Form FDA-2400d. When viewing these images, keep in mind that the focus is fixed, whereas the actual counting procedure requires constant adjustment of the microscope's fine focus. This guideline can be used to help properly identify and count somatic cells in milk and should facilitate standardization of analysts performing the DMSCC procedure.

# RULES FOR IDENTIFYING AND COUNTING SOMATIC CELLS DMSCC – Single Strip Procedure (Form FDA-2400d)

### 1. Cells possess a nucleus stained dark blue

The <u>nuclear mass</u> of a cell, composed of one or more nuclear units, should stain dark blue with L-W stain. The intensity of "dark" blue may vary with staining technique and cells. The nuclear mass generally has no recognizable form, though generally appears as a solid blue unit. In some cells it may be granular in appearance. Some distortion may be expected. Count each dark blue mass, which bears resemblance to a typical nucleus. The nuclear mass of a leucocyte is polylobed and the lobes (units) are bridged by nuclear material. These multi-lobed nuclei with bridges are counted as one cell.

The <u>cytoplasm</u> which normally surrounds each nucleus may (a) be stained light blue, (b) not stained and appear as a clear zone or (c) have disintegrated and not be present.

# 2. Cells are generally $\geq$ 8 microns; do not count cells < 4 microns; count fragments only if > 50% of nuclear material is visible.

The nuclear mass of a countable somatic cell is generally eight microns or greater in size. Cells or fragments (cell that has been obviously damaged or degraded) may appear that are smaller. Do not count cells with nuclei of less than 4 microns. Fragmented cells are counted only if more than fifty percent of the nuclear mass is visible (at least four microns). Do not count cells without a nucleus.

## 3. Cluster of cells counted as one unless nuclear units are clearly separated.

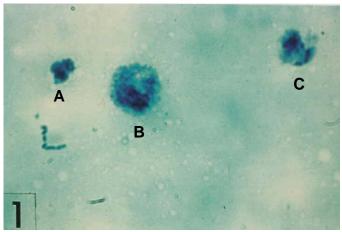
Use the fine focus to determine if nuclear bridges are present. If a clear area can be seen surrounding any of the nuclear masses in the cluster, count each clearly delineated nuclear mass as one cell. If the mass shows no clear differentiation, or there are bridges evident, count those attached as one.

# 4. Count cells touching only the top <u>or</u> the bottom edge of a horizontal strip (or left <u>or</u> right edge for vertical edge of the strip).

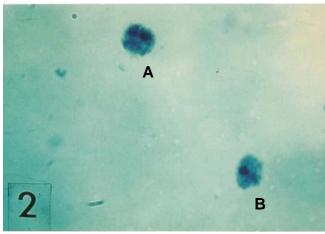
Nuclear masses that touch the edge or go outside of the strip boundaries should only be counted from one side of the strip to avoid counting an area larger than specified. Count cells that touch only one boundary of the strip; for horizontal strip counting, select either the upper <u>or</u> the lower edge; for vertical strips, select either the left or the right edge. Do not count cells that touch the other boundary.

#### 5. If in Doubt, Do Not Count Questionable Cells!

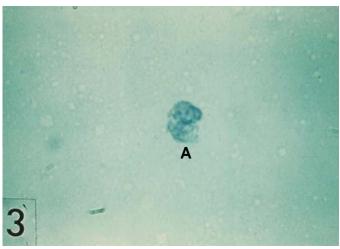
This Guideline was adapted from an FDA-LQAT's DMSCC training slide set and was originally prepared in printable format by James E. Fitts of NYS Department of Agriculture & Markets, Milk Control (04/98). This update was prepared by J. Fitts, Agriculture & Markets, and S. Murphy, Department of Food Science, Cornell University (07/04).



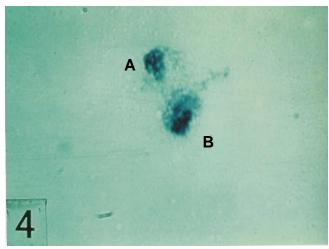
1. "A," "B" & "C" are all countable cells (3 cells).



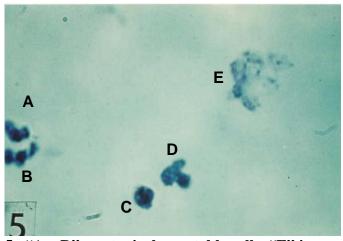
2. "A" & "B" are countable cells (2 cells).



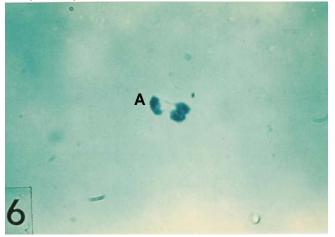
3. "A" is a countable cell (1 cell).



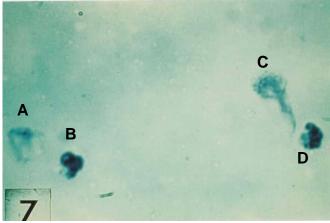
4. "A" & "B" are countable cells, both surrounded by disintegrating cytoplasm. (2 cells)



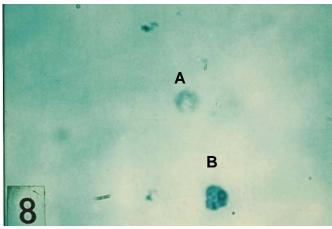
5. "A – D" are typical countable cells, "E" is cellular debris and is not counted. (4 cells)



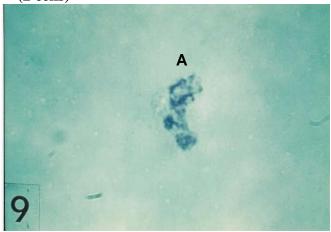
6. "A" is one cell with nuclear lobes connected by a nuclear bridge. Counted as 1 cell. (1 cell)



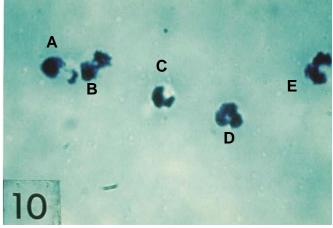
7. "A" & "C" are cytoplasmic debris and are not counted. "B" & "D" are typical cells. (2 cells)



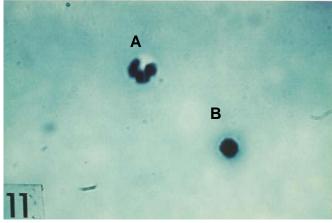
8. "A" is a "ghost" cell (no nuclear material) and not counted. "B" is a countable cell. (1 cell)



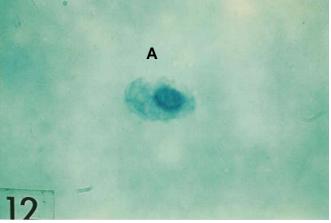
9. "A" is one cell with disintegrating nucleus, countable. (1 cell)



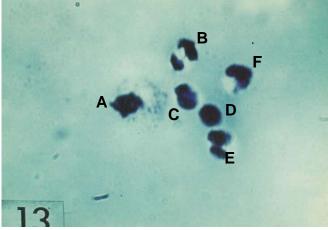
10. "A-E" are typical cells, all with multiple lobes. (5 cells)



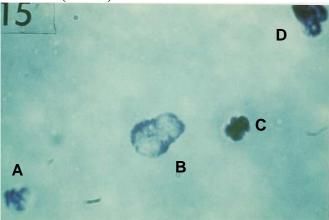
11. "A" is a typical cell. "B" is a typical monocyte. Both are counted. (2 cells)



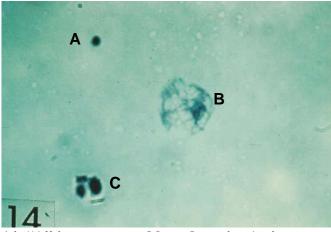
12. "A" is a single cell with large visible cytoplasm. (1 cell)



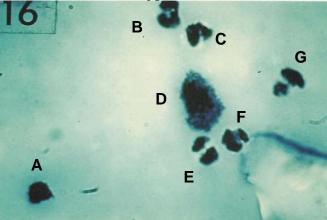
13. All are countable cells. "B" & "E" have bilobed nuclei with bridges, each counted as one. (6 cells)



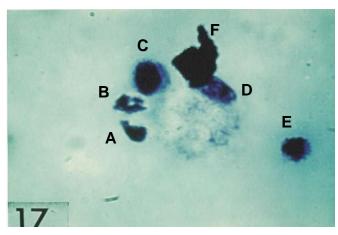
15. "A," "C" & "D" are countable cells. "B" is cytoplasmic debris not counted. (3 cells)



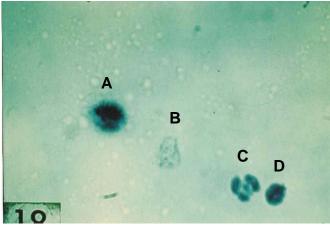
14. "A" is not counted based on size (< 4 microns). "B" though disintegrating, is counted. "C" is a typical cell. (2 cells)



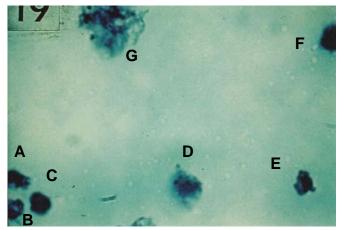
16. "A-G" are all countable cells. (7 cells)



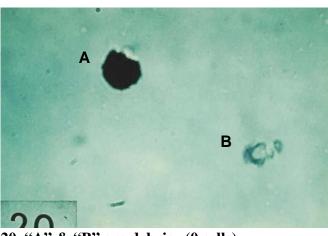
17. "A," "B," "C" & "E" are typical cells (4). "D" is a countable cell with large area of disintegrating cytoplasm (1 cell). "F" is debris, not a countable cell. (5 cells)



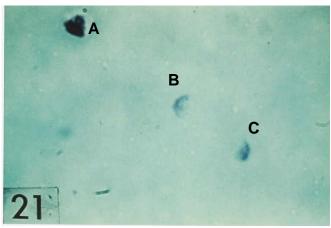
18. "A," "C" & "D" are typical cells. "B" is a ghost cell or debris and not counted.
(3 cells)



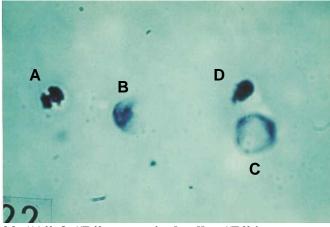
19. "A-G" are typical cells. (7 cells)



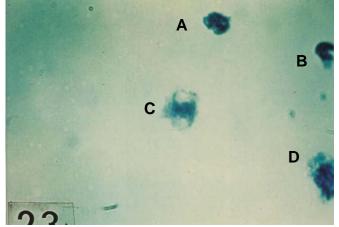
20. "A" & "B" are debris. (0 cells)



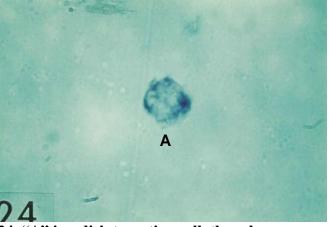
21. "A" is a typical countable cell. "B" & "C" are cytoplasmic debris not counted. (1 cell)



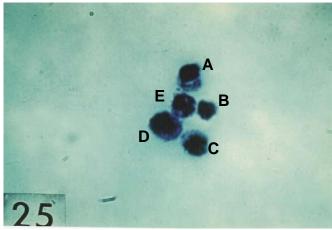
22. "A" & "D" are typical cells. "B" is a fragment greater than 50% (countable) and though "C" has disintegrating nuclear material, is countable. (4 cells)



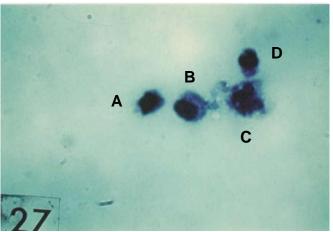
23. "A" & "B" are typical cells. "C" & "D" are countable cells showing early degeneration. (4 cells)



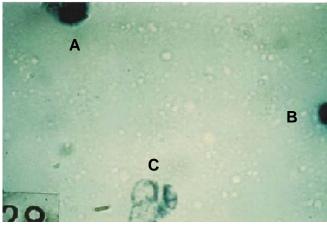
24. "A" is a disintegrating cell, though countable. (1 cell)



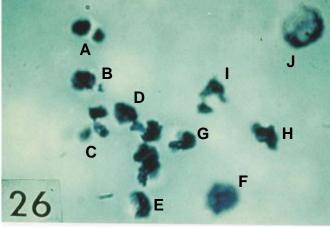
25. "A-E" is a typical clump of cells counted individually. (5 cells)



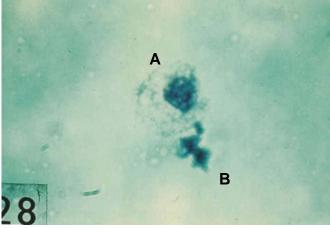
27. "A-D" are all typical countable cells. (4 cells)



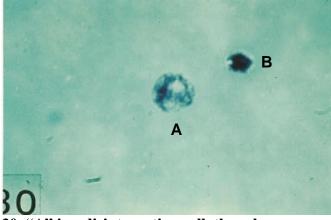
29. "A" & "B" are typical cells. "C" is a ghost cell. (2 cells)



26. "E," "F," "G" & "H" are mononuclear cells. "A," "B" & "I" are bi-lobed cells. "C" is a tri-lobed cell. "D" has 4 nuclear lobes. "J" is a countable disintegrating cell. (10 cells)



28. "A" is a typical cell showing cytoplasmic degeneration. "B" is cellular debris. (1 cell)



30. "A" is a disintegrating cell, though countable. "B" is a typical cell. (2 cells)