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EURL MMP
European Union Reference Laboratory for *Milk and Milk Products*

Guidelines for identifying and counting somatic cells in cow's milk by EN ISO 13366-1

Pictures Library

27 September 2017

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Introduction

These guidelines have been drafted by EURL Milk & Milk Products (MMP), in collaboration with the Dutch NRL MMP (RIKILT Institute of Food Safety) and the German NRL MMP (Max Rübner-Institut) to give clarification and some guidance to NRLs MMP for the implementation of the current Standard EN ISO 13366-1, in the identification and counting of somatic cells in milk.

These guidelines include in particular photographs of slides prepared by EURL MMP and NL-NRL for Direct Microscopic Somatic Cell Count (DMSCC) according to the Standard EN ISO 13366-1. It is important to note that microscope focus is fixed on the images. An accurate counting requires constant adjustment of the microscope's fine focus.

The presence of cells clusters in milk has been discussed within this Working Group. This phenomenon may be due to a severe mastitis and/or an insufficient homogeneity of milk samples during preparation of slides. US/FDA (Fitts and Murphy, 2004) prescribes a method to count clusters of cells. It consists in adjusting the fine focus to determine whether nuclear bridges are present and if cells are surrounded by a visible cytoplasm. According to EN ISO 13366-1, if bridges are obvious and nuclear units are not clearly separated, the cluster must be counted as one cell. This procedure does not take into account the quantity of material that would represent one cell, and implies to be able to clearly identify bridges. These two aspects have been discussed by the Working Group.

The conclusion of the work mentioned in the former paragraph is that some cell pictures are actually controversial and there is a need for clear definitions. For this reason, these guidelines recommend to report only tentative counting results for samples with a high number of clustered (or disintegrated) cells.

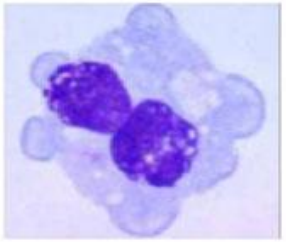
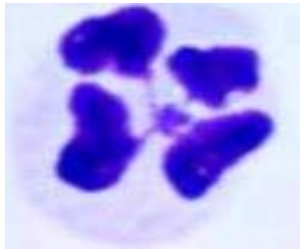
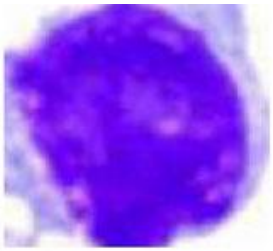
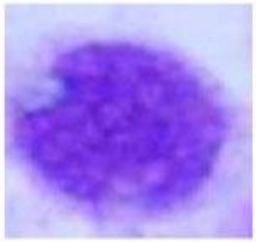
Reference papers

- Fitts J. & Murphy S. (2004). Direct microscopic somatic cell count guideline - Rules and examples for counting cells in milk. https://www.wifss.ucdavis.edu/wp-content/uploads/2016/09/RulesforIdentifyingCellCount.pdf

- Sarikaya H., Prgomet C., Pfaffl M.W., Bruckmaier R.M, (2004). Differentiation of leukocytes in bovine milk. Milchwissenschaft (59), 586-589.

- Zlotnik I. (1947). Types of cells present in cow's milk. J. Comp. Path. (57), 196-208.

Reference papers

	Macrophages	PMNs	Lymphocytes	Epithelial cells
Visual aspect of stained somatic cells from cows				
Morphological characteristics	**8-30µm** **Many different forms of nucleus** **Cytoplasm 0.5 to 10 x bigger than nucleus**	**10-14µm** **Intensively stained lobulated nucleus** **Small cytoplasm, dense granules**	**5-10µm** **Intensively stained round nucleus** **Very little cytoplasm**	**10-14µm** **Round nucleus** **Cytoplasm weakly stained**

References

- EN ISO 13366-1/IDF 148-1
- Dr. med. vet. Anke Schröder, Institute for Food Quality and Safety, University of Veterinary Medicine Hannover, Foundation

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Cell isolation and identification

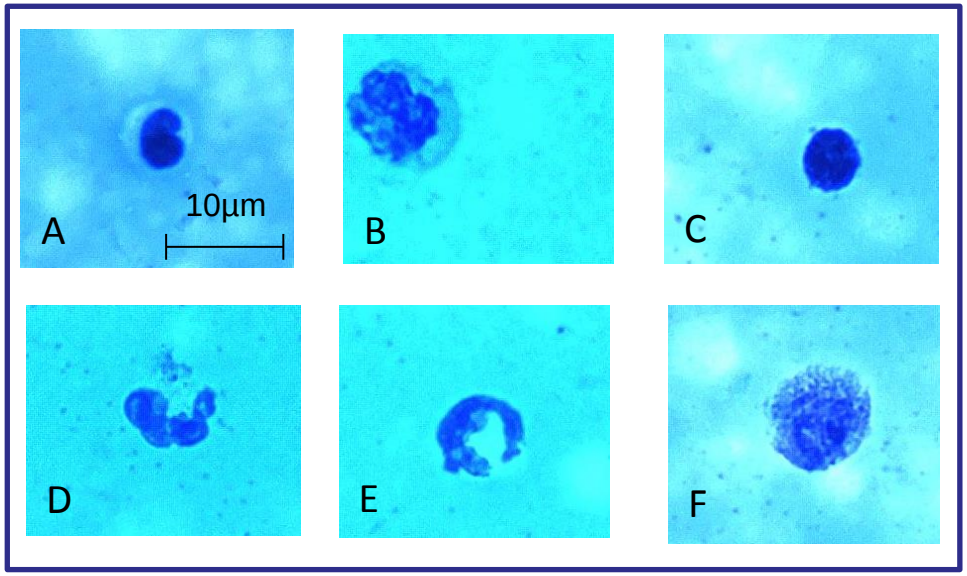

Fig 1: Pictures of somatic cells in raw cow's milk stained with Modified Newman-Lampert staining solution (Fluka, Nr 01375).
Microscope: Olympus BX51 (MICRO 1) **x 1000-fold magnification using immersion oil.** Capture software: ARCHIMED. Laboratory for Food Safety, Anses, France.

A-C: Lymphocytes (intensively stained round nucleus)
B: Macrophage (specific form of nucleus/big cytoplasma)
D-E: Neutrophils (lobulated nucleus)
F: Epithelial cell (large round nucleus)

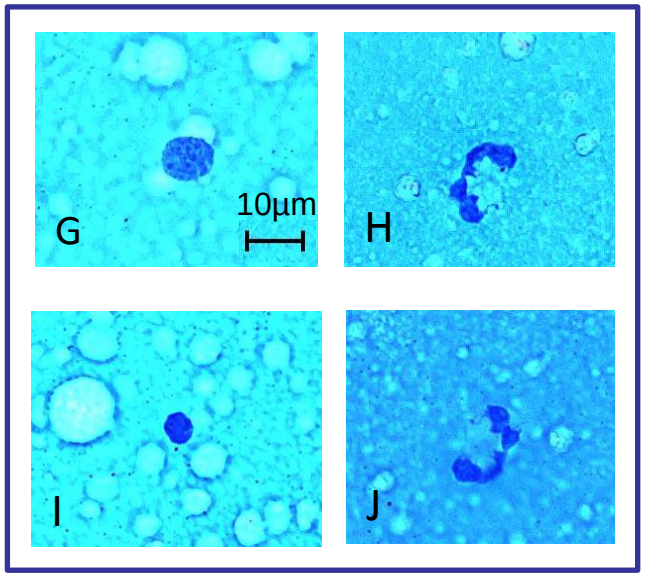

Fig 2: Pictures of somatic cells in raw cow's milk stained with Modified Newman-Lampert staining solution (Fluka, Nr 01375).
Microscope: Olympus BX51 (MICRO 1) **x 500-fold magnification.** Capture software: ARCHIMED
Laboratory for Food Safety, Anses, France.

G: Epithelial cell (round nucleus)
I : Lymphocyte (intensively stained round nucleus)
H-J : Neutrophils (lobulated nucleus)

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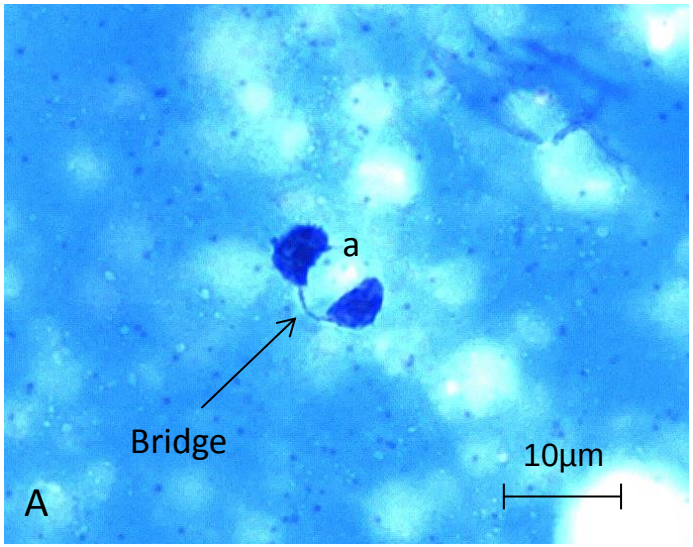
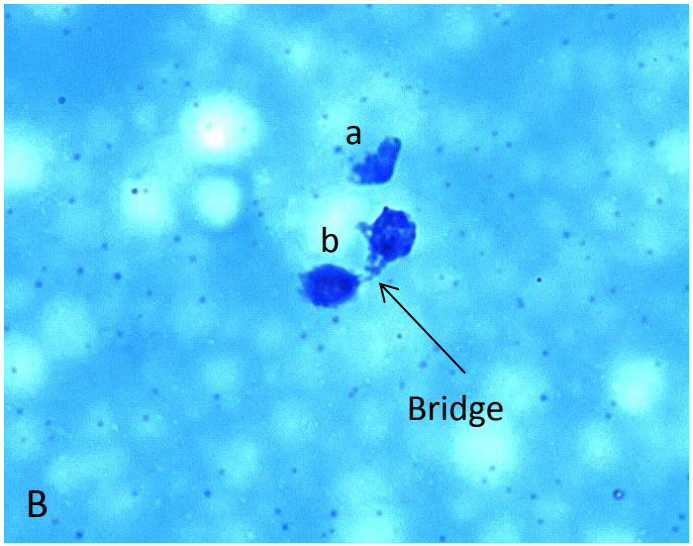
Specific feature of somatic cells : nuclear bridge



(1 cell)



(2 cells)

Raw cow's milk stained with Modified Newman-Lampert staining solution (Fluka, Nr 01375)
Microscope: Olympus BX51 (MICRO 1)
x 1000-fold magnification using immersion oil
Capture software: ARCHIMED
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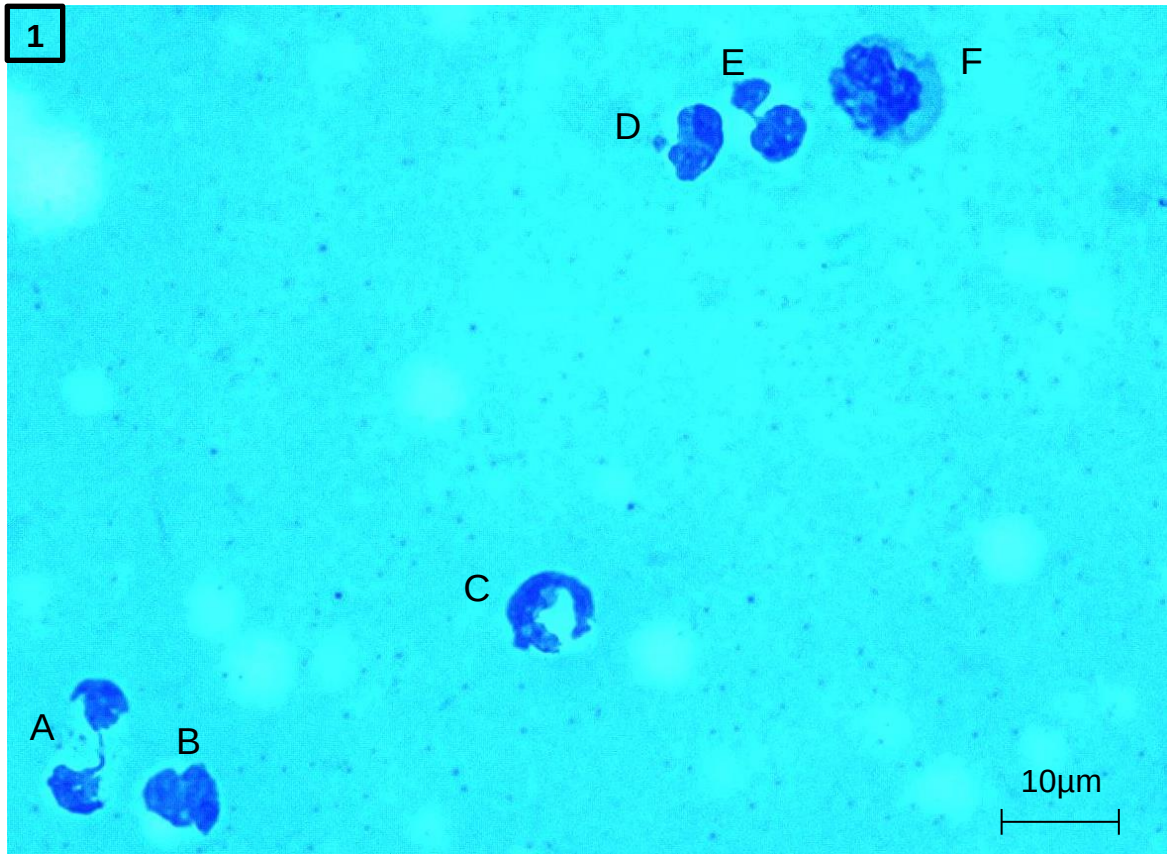
Somatic cells counting : x 1000-fold magnification with immersion oil



Count: 6 cells

- 'B-C-D-F' are typical countable cells
- 'A-E' are bi-lobed cells with nuclear brigdes, respectively counted as one cell

Raw cow's milk stained with Modified Newman-Lampert solution (Fluka, Nr 01375)
Microscope: Olympus BX51 (MICRO 1)
Capture software: ARCHIMED
Laboratory for Food Safety, Anses, France

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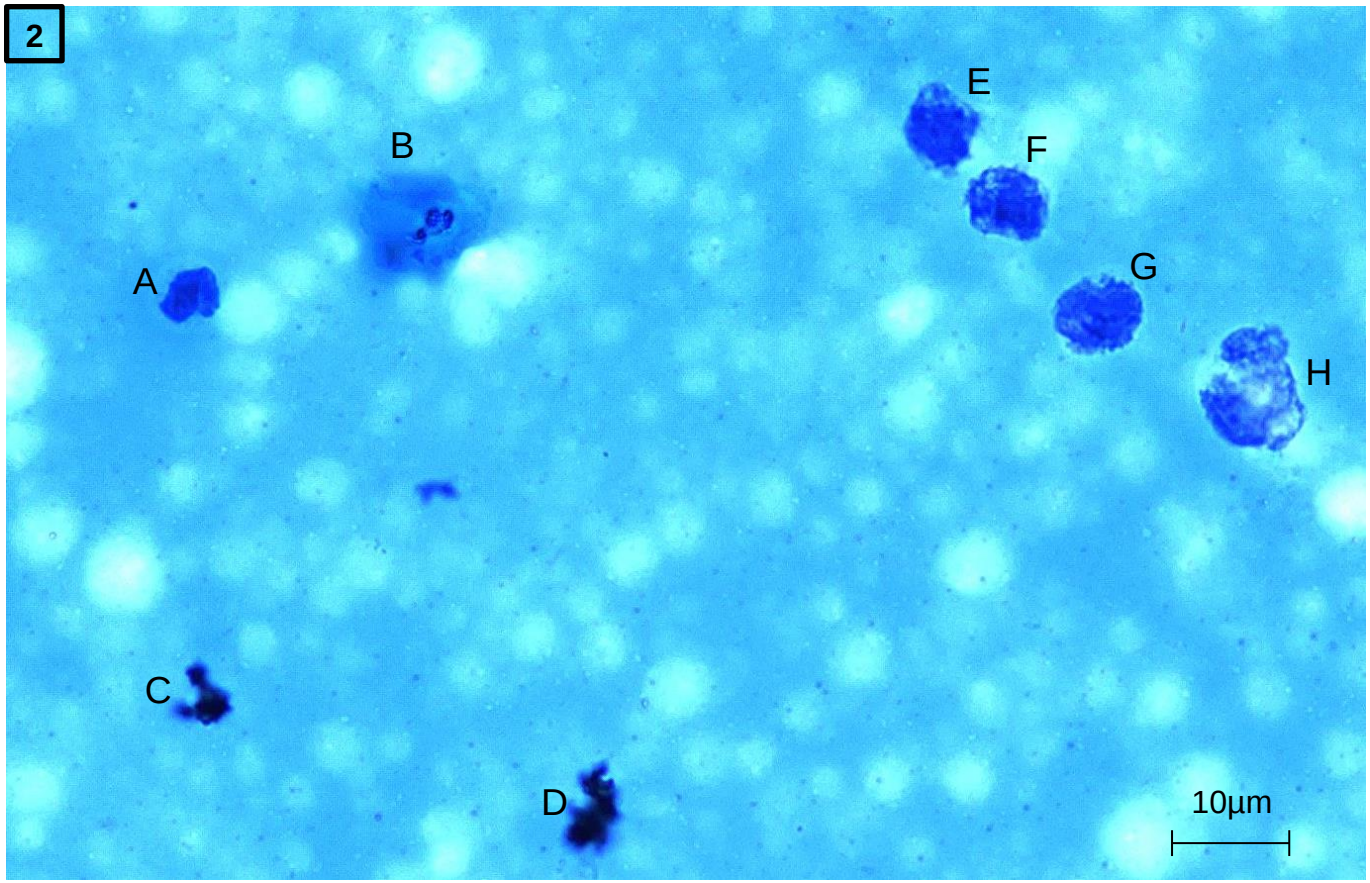

Count: 6 cells

- **'A-E-F-G-H' are typical countable cells**
- **'B' is a disintegrating cell, though countable**
- **'C-D' are residues of staining solution**

Raw cow's milk stained with modified Newman-Lampert solution (Fluka, Nr 01375)
Microscope: Olympus BX51 (MICRO 1)
x 1000-fold magnification using immersion oil
Capture software: ARCHIMED
Laboratory for Food Safety, Anses, France

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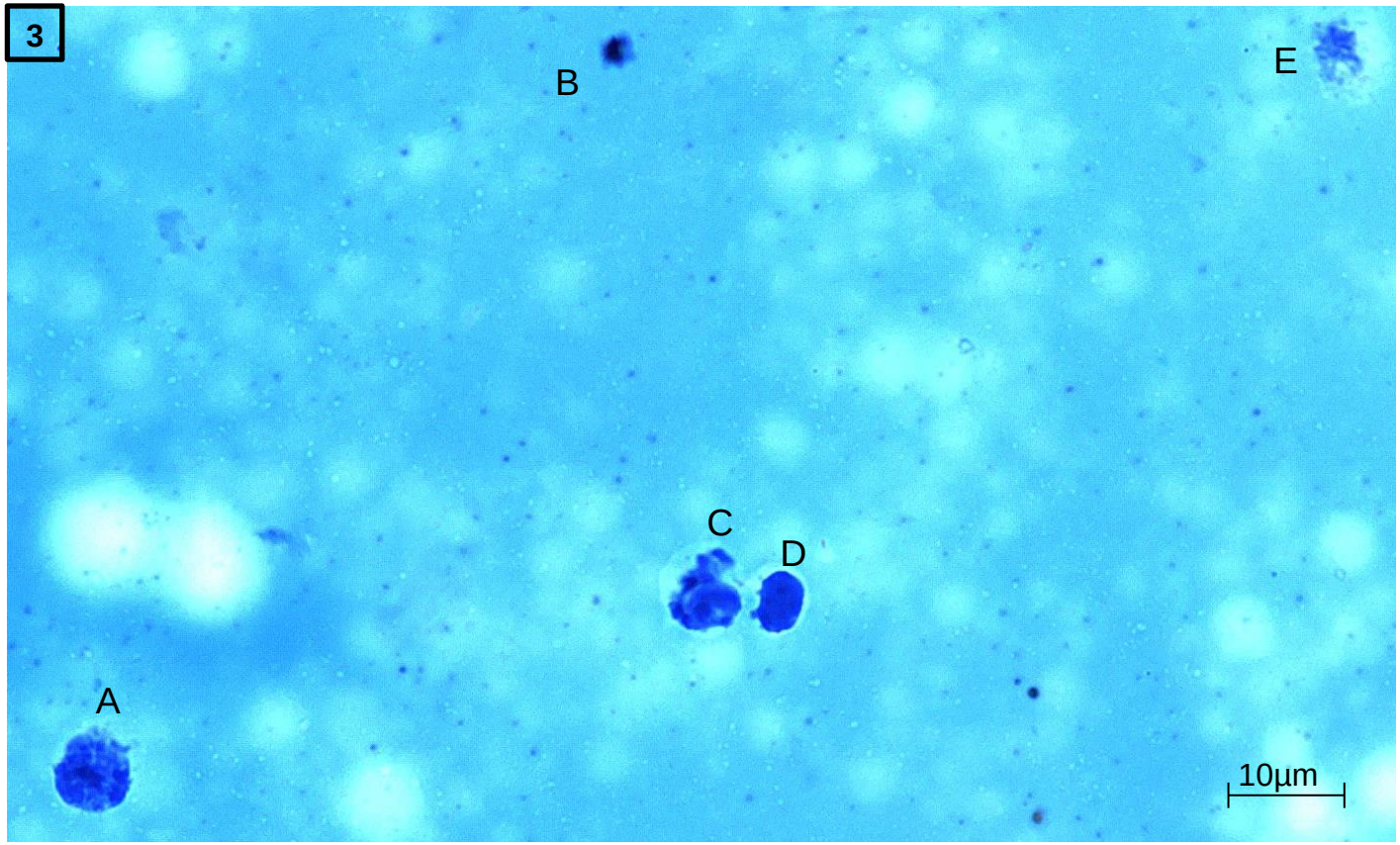

Count: 4 cells

- **'A' is a typical countable cell**
- **'C-D' are two countable cells with a visible cytoplasm**
- **'B' is a residue of staining solution, not a countable cell**
- **'E' is a disintegrating cell with a visible cytoplasm**

Raw cow's milk stained with Modified Newman-Lampert solution (Fluka, Nr 01375)
Microscope: Olympus BX51 (MICRO 1)
x 1000-fold magnification using immersion oil
Capture software: ARCHIMED
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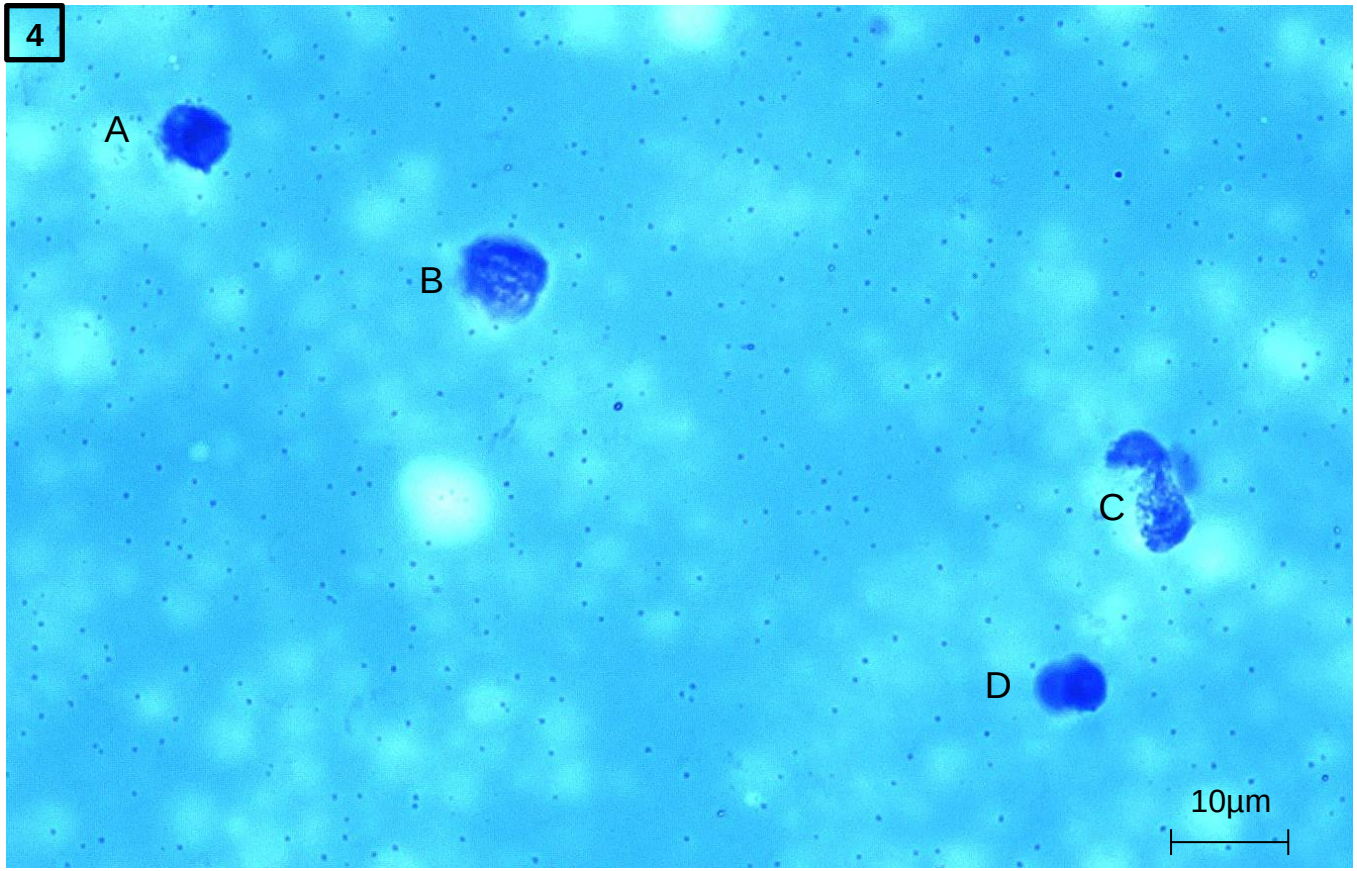

Count: 4 cells

- 'A-B-C-D' are typical countable cells

Raw cow's milk stained with Modified Newman-Lampert solution (Fluka, Nr 01375)
Microscope: Olympus BX51 (MICRO 1)
x 1000-fold magnification using immersion oil
Capture software: ARCHIMED
Laboratory for Food Safety, Anses, France

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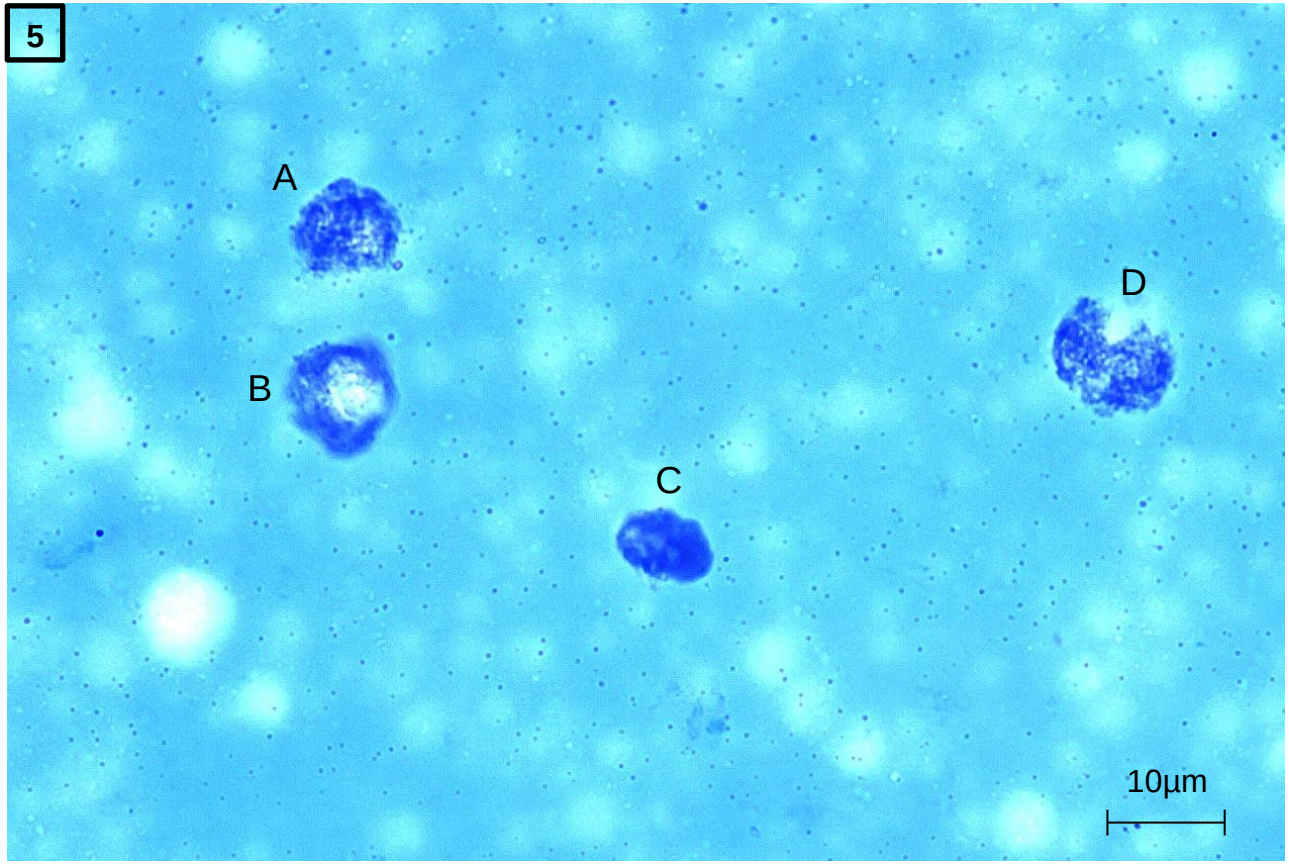

Count: 4 cells

- 'A-B-C-D' are typical countable cells

Raw cow's milk stained with Modified Newman-Lampert solution (Fluka, Nr 01375)
Microscope: Olympus BX51 (MICRO 1)
x 1000-fold magnification using immersion oil
Capture software: ARCHIMED
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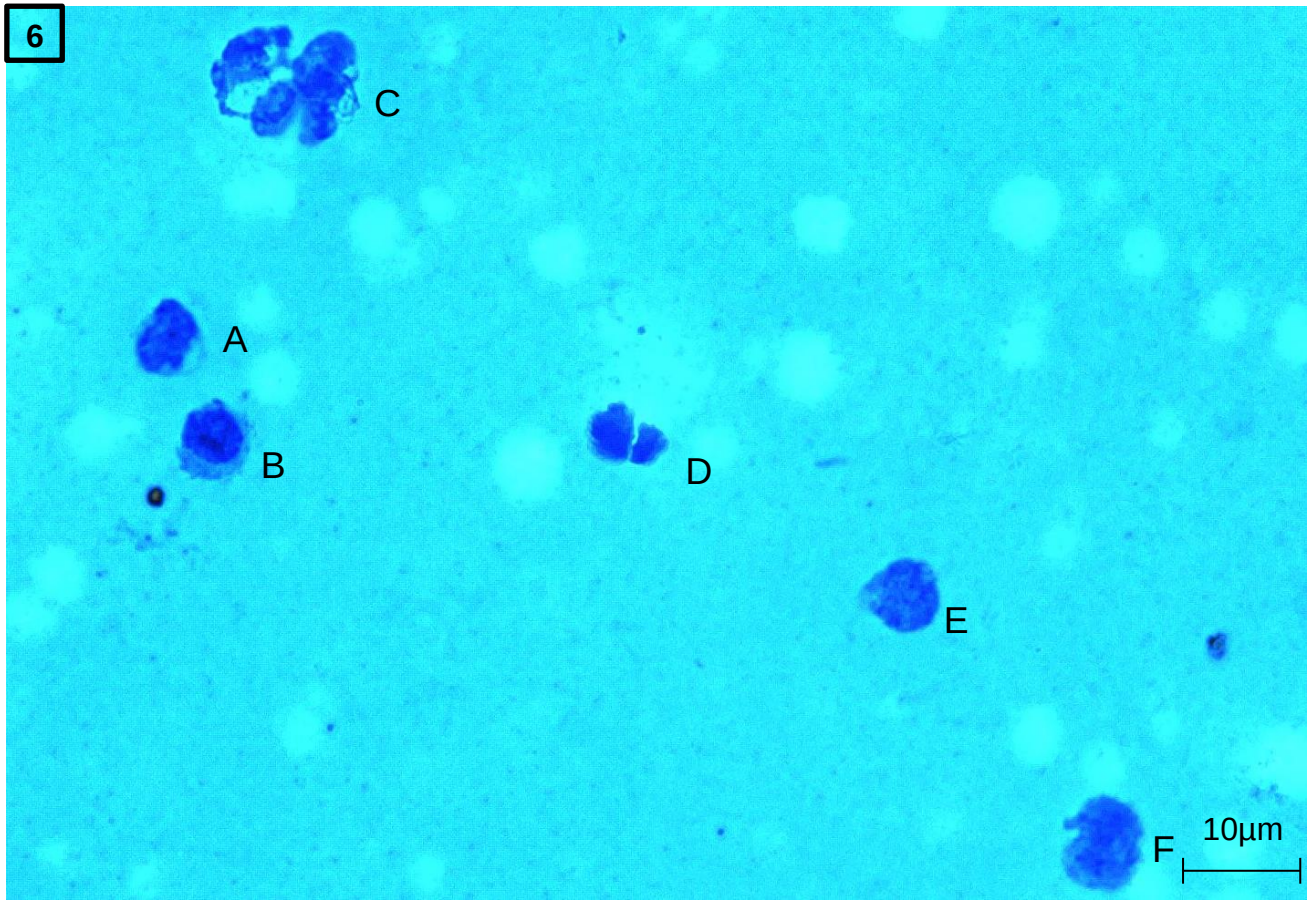

Count: 6 cells

- 'A-B-E-F' are mononuclear countable cells
- 'C' is a multi-lobed cell, with visible nuclear bridges
(due to the high quantity of material, this is a tentative cell count)
- 'D' is a bi-lobed cell

Raw cow's milk stained with Modified Newman-Lampert solution (Fluka, Nr 01375)
Microscope: Olympus BX51 (MICRO 1)
x 1000-fold magnification using immersion oil
Capture software: ARCHIMED
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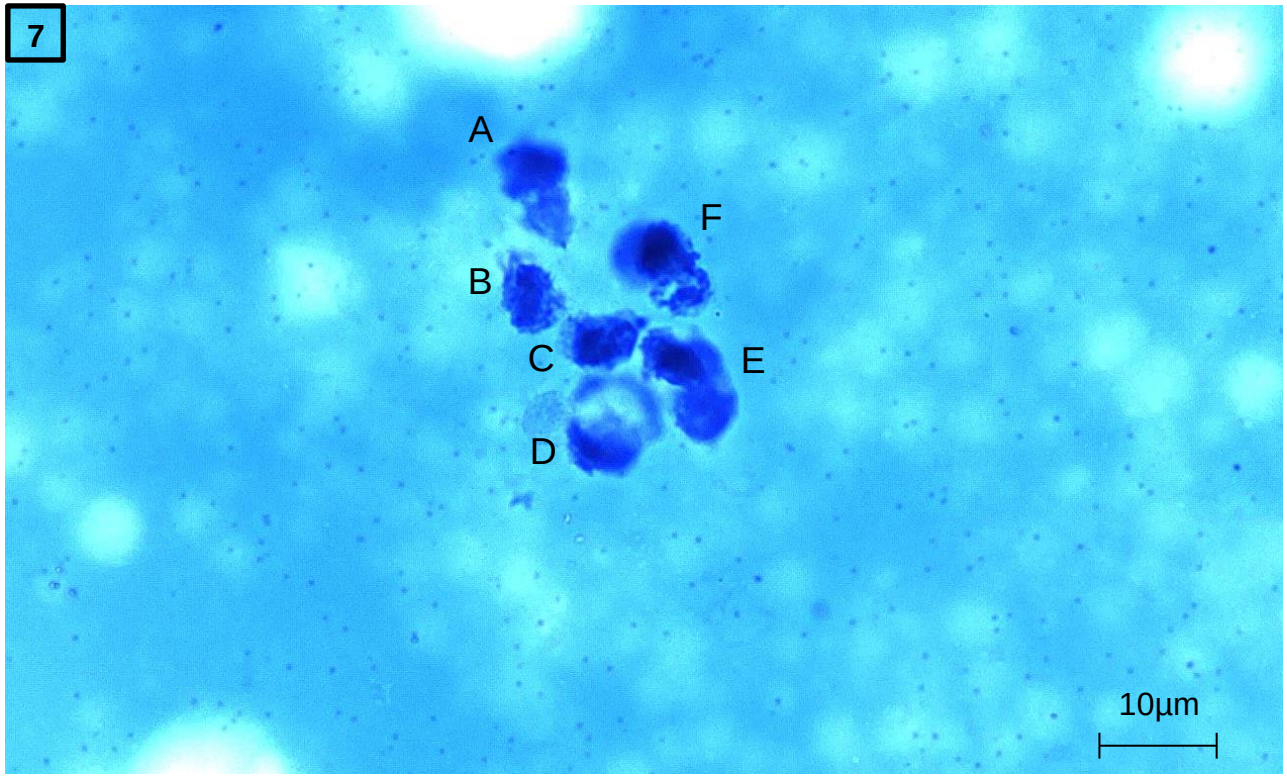

Count: 6 cells

- 'A' is a countable cells
- 'B-C-D-E-F' is a clump of cells counted individually (clear nuclear differentiation)

Raw cow's milk stained with Modified Newman-Lampert solution (Fluka, Nr 01375)
Microscope: Olympus BX51 (MICRO 1)
x 1000-fold magnification using immersion oil
Capture software: ARCHIMED
Laboratory for Food Safety, Anses, France

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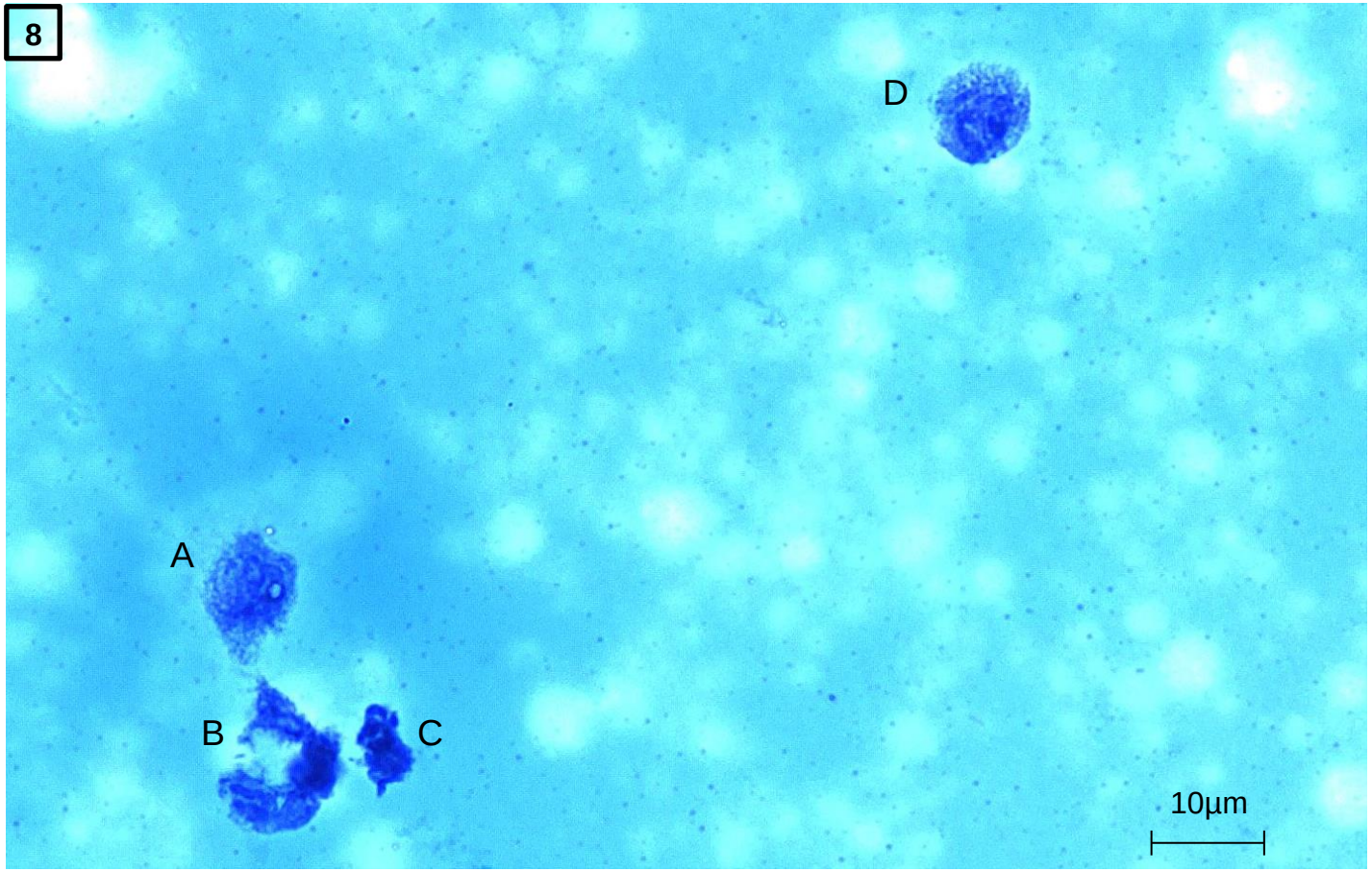

Count: 4 cells

- 'A-B-C' form 3 cells counted individually
- 'D' is a typical countable cell

Raw cow's milk stained with Modified Newman-Lampert solution (Fluka, Nr 01375)
Microscope: Olympus BX51 (MICRO 1)
x 1000-fold magnification using immersion oil
Capture software: ARCHIMED
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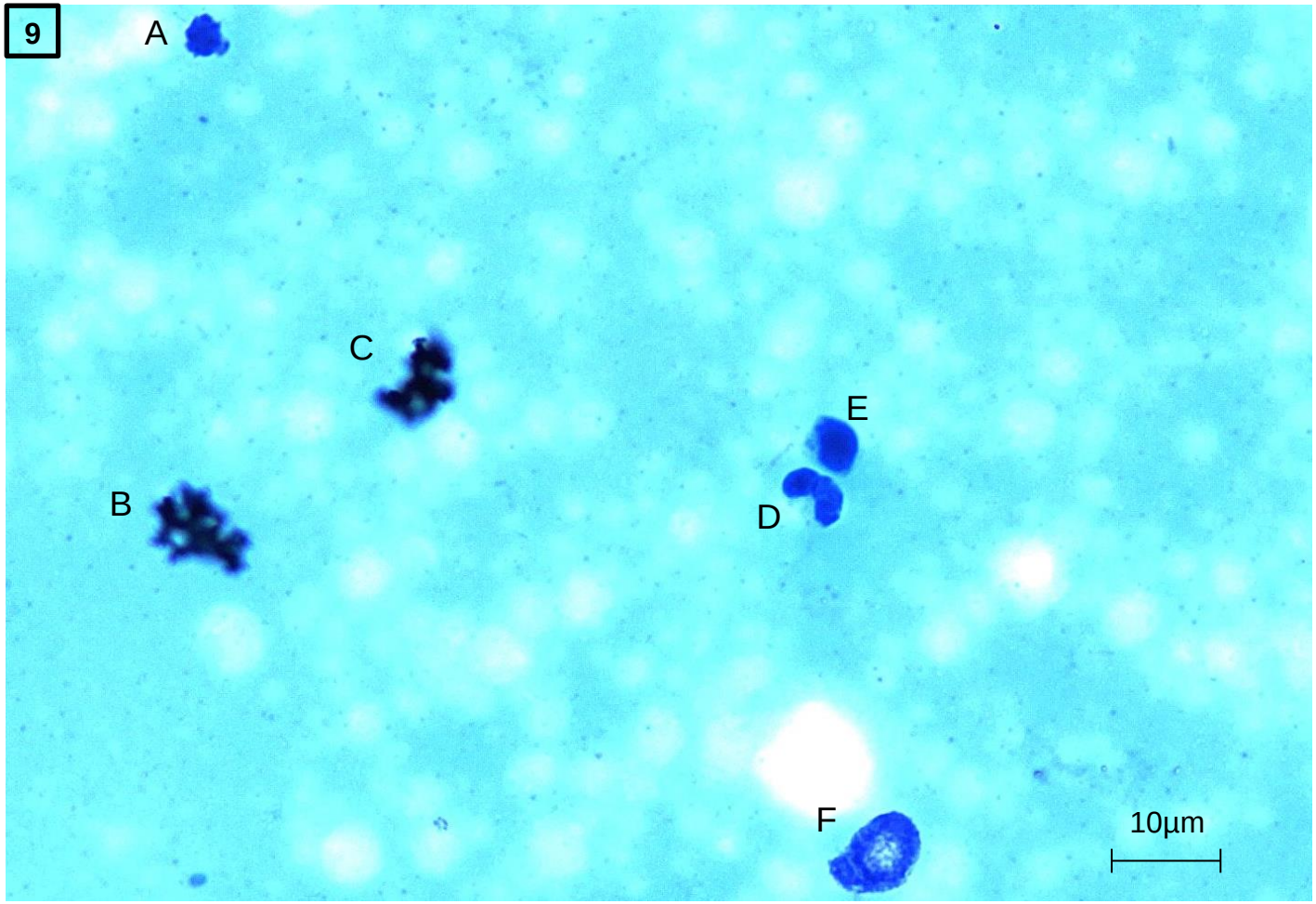

Count: 4 cells

- 'A-F' are typical countable cells
- 'B-C' are residues of staining solution
- 'D-E' forms 2 cells counted individually

Raw cow's milk stained with Modified Newman-Lampert solution (Fluka, Nr 01375)
Microscope: Olympus BX51 (MICRO 1)
x 1000-fold magnification using immersion oil
Capture software: ARCHIMED
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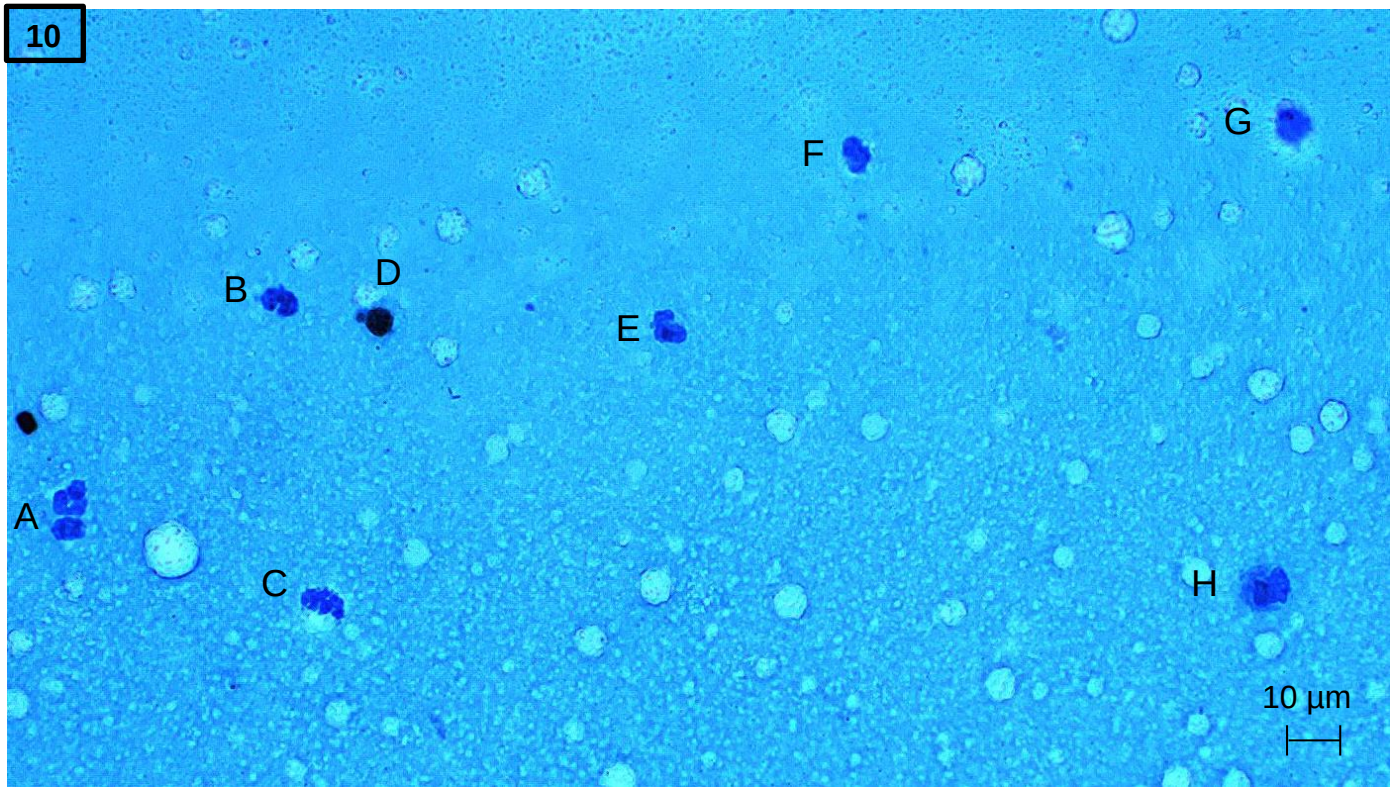
Somatic cells counting : x 500-fold magnification



Count: 7 cells

- **'A' is a bi-lobed cell**
- **'B-C-E-F-G-H' are typical countable cells**
- **'D' is a residue of staining solution**

Raw cow's milk stained with Modified Newman-Lampert solution (Fluka, Nr 01375)
Microscope: Olympus BX51 (MICRO 1)
Capture software: ARCHIMED
Laboratory for Food Safety, Anses, France

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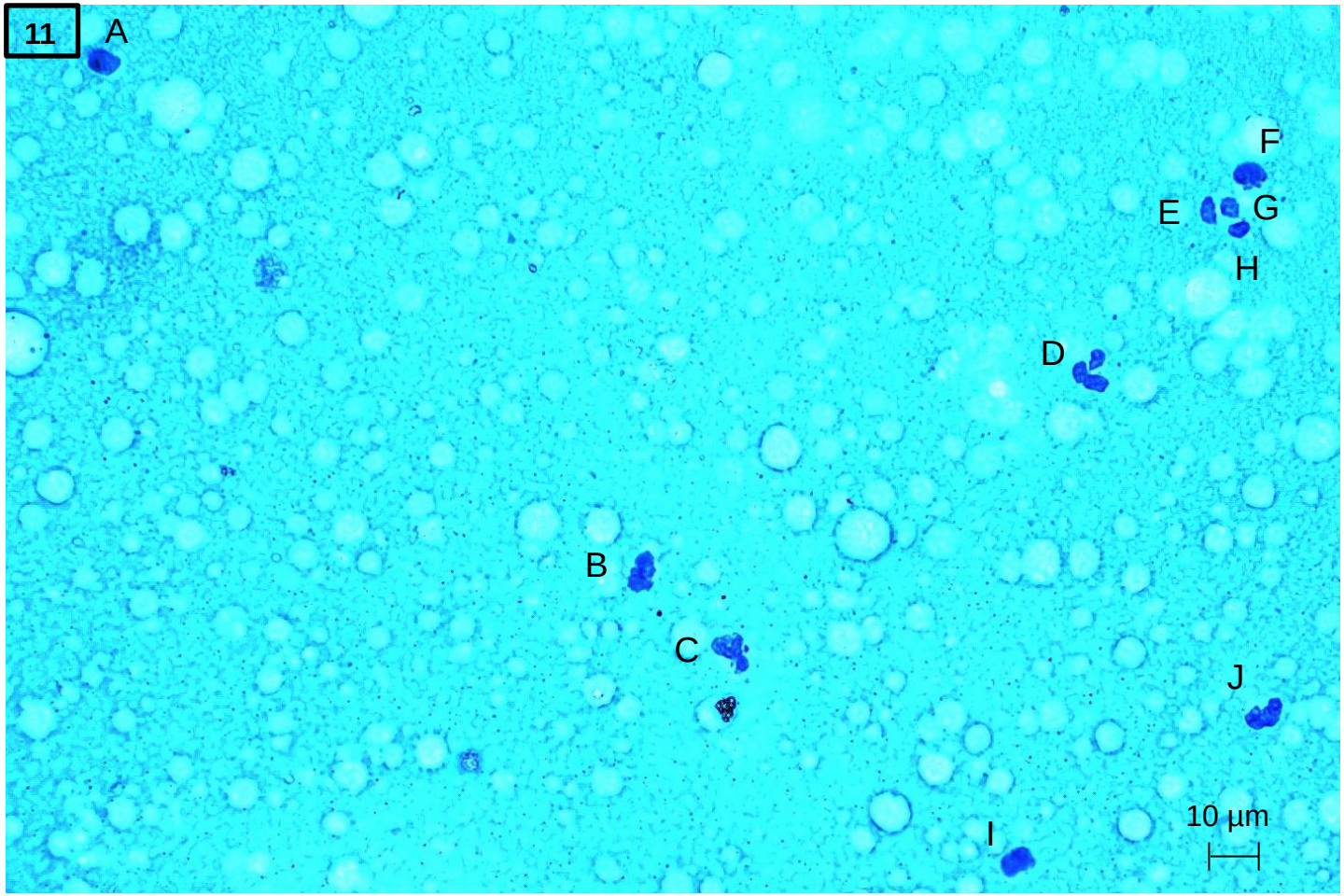
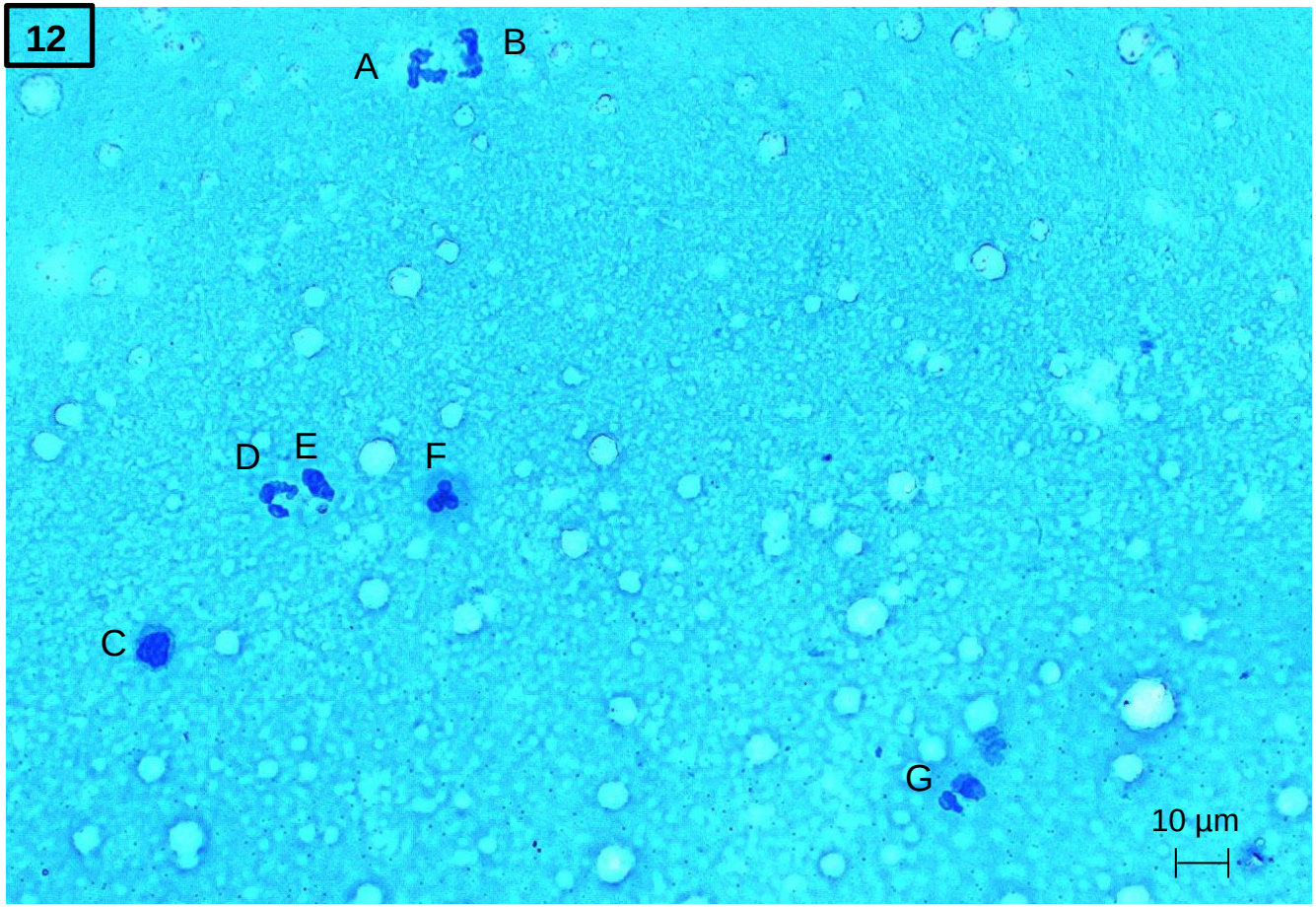


Count: 10 cells

- 'A-B-C-E-F-G-H-I-J' are typical countable cells
- 'D' is a multi-lobed cell

Raw cow's milk stained with Modified Newman-Lampert solution (Fluka, Nr 01375)
Microscope: Olympus BX51 (MICRO 1)
x 500-fold magnification
Capture software: ARCHIMED
Laboratory for Food Safety, Anses, France

Pictures Library

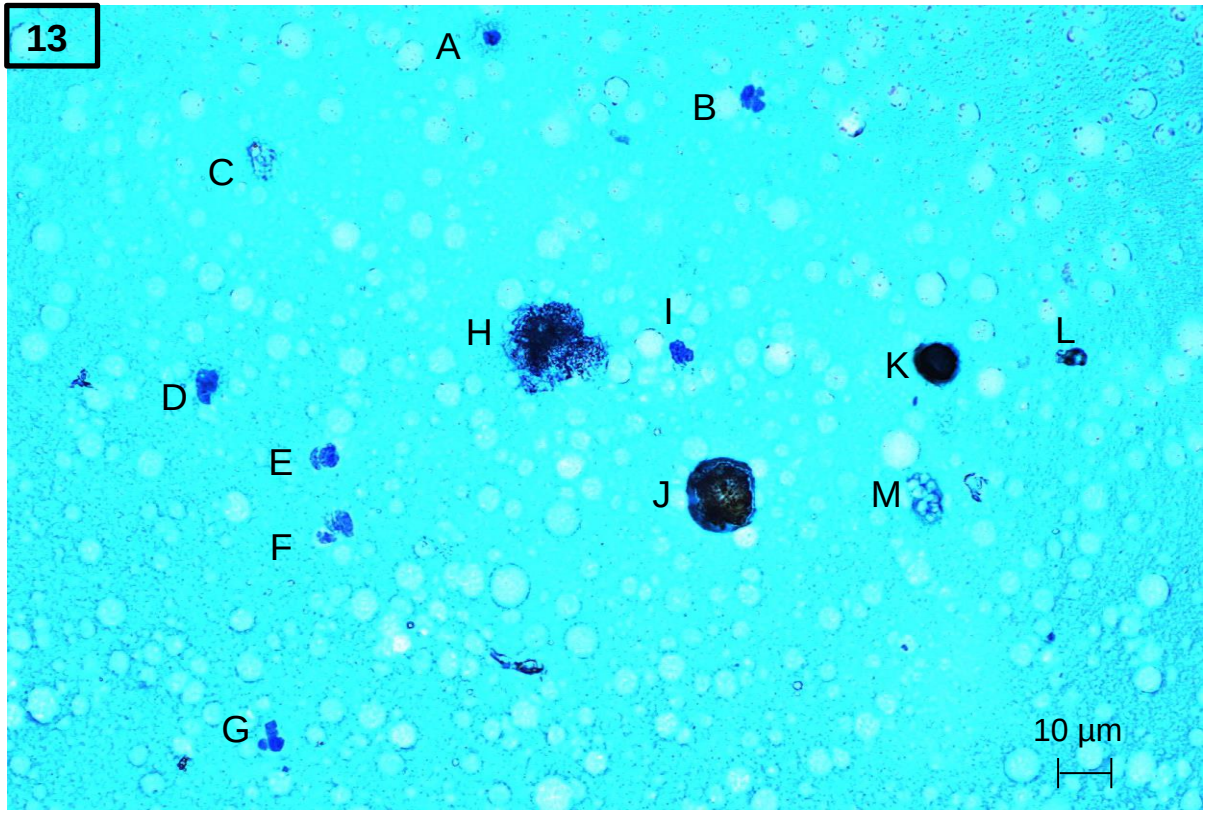


Count: 7 cells

- 'A-B-C-D-E-F 'are countable cells
- 'G' is a bi-lobed countable cell with a nuclear bridge

Raw cow's milk stained with Modified Newman-Lampert solution (Fluka, Nr 01375)
Microscope: Olympus BX51 (MICRO 1)
X 500-fold magnification
Capture software: ARCHIMED
Laboratory for Food Safety, Anses, France

Pictures Library



Count: 7 cells

- 'A-B-D-E-F-G-I' are countable cells
- 'C-H-J-K-L-M' are debris or residues of staining solution

Raw cow's milk stained with Modified Newman-Lampert solution (Fluka, Nr 01375)
Microscope: Olympus BX51 (MICRO 1)
x 500-fold magnification
Capture software: ARCHIMED
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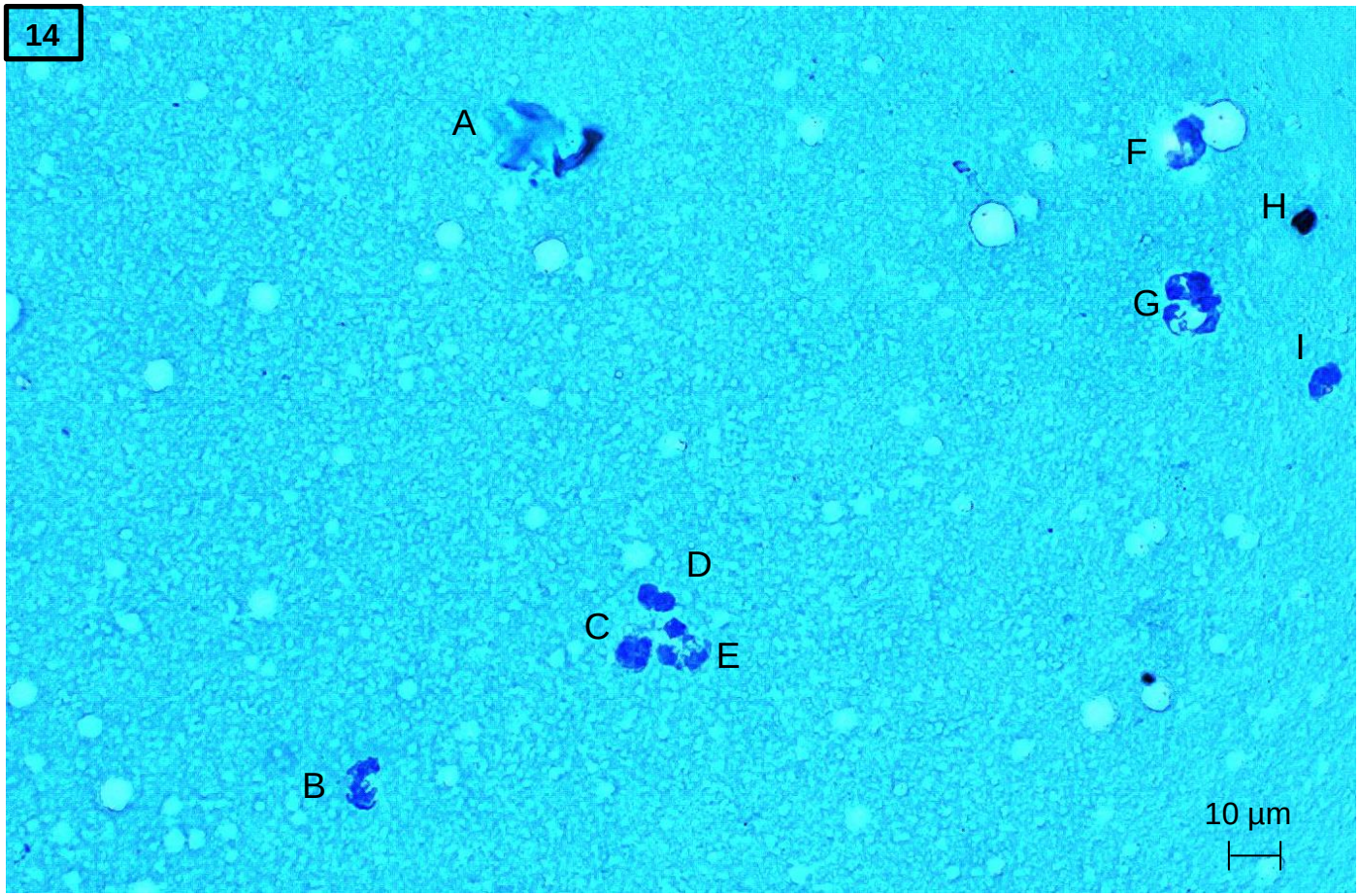

Count: 7 cells

- **'A-H' are residues of staining solution**
- **'B-C-D-F-I' are countable cells**
- **'E-G' are multi-lobed countable cells**

Raw cow's milk stained with Modified Newman-Lampert solution (Fluka, Nr 01375)
Microscope: Olympus BX51 (MICRO 1)
x 500-fold magnification
Capture software: ARCHIMED
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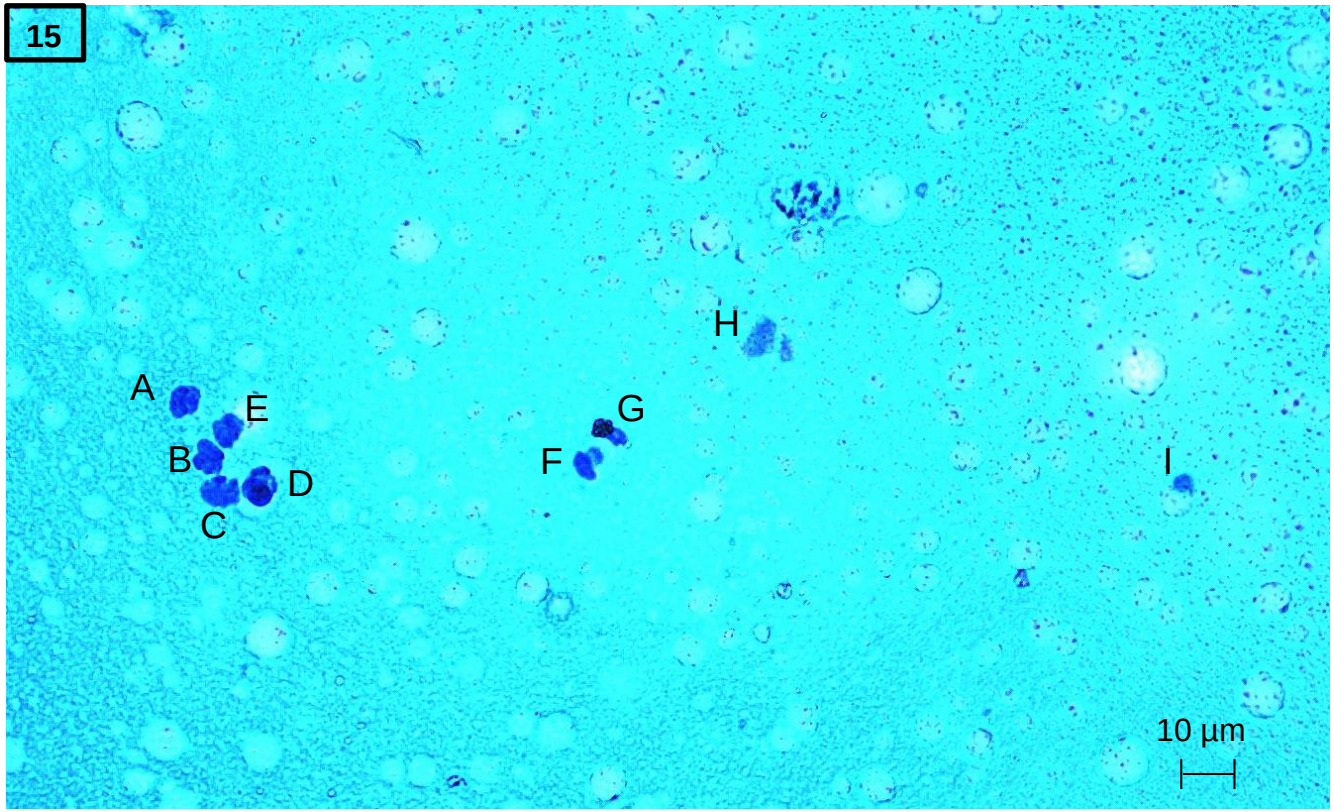

Count: 8 cells

- 'A-B-C-D-E' is a clump of cells counted individually
- 'F-G' are 2 typical cells
(G is not clearly visible as hidden behind a residue)
- 'H' is a countable cell showing degeneration
- 'I' is not counted based on size (< 4µm)

Raw cow's milk stained with Modified Newman-Lampert solution (Fluka, Nr 01375)
Microscope: Olympus BX51 (MICRO 1)
x500-fold magnification
Capture software: ARCHIMED
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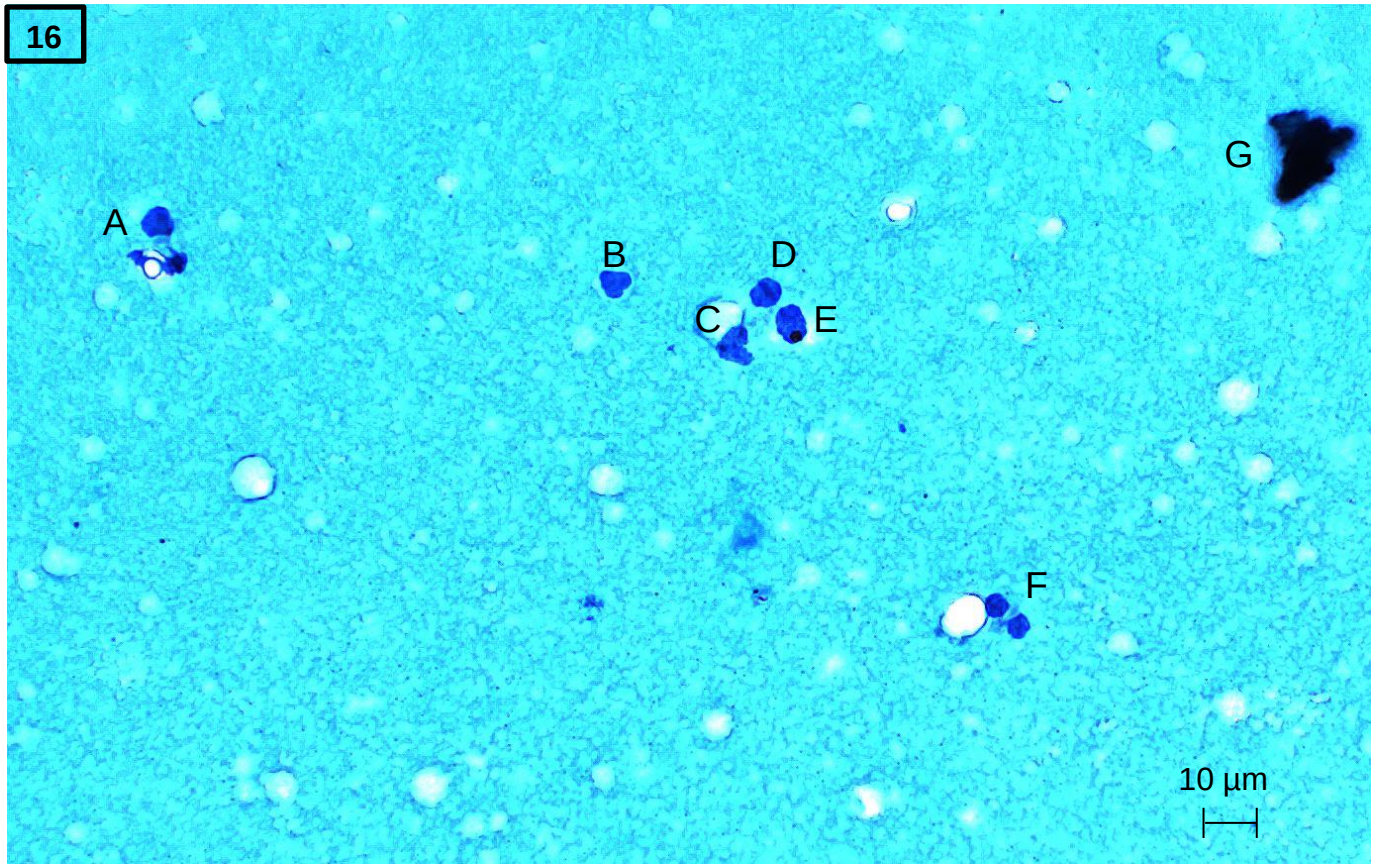
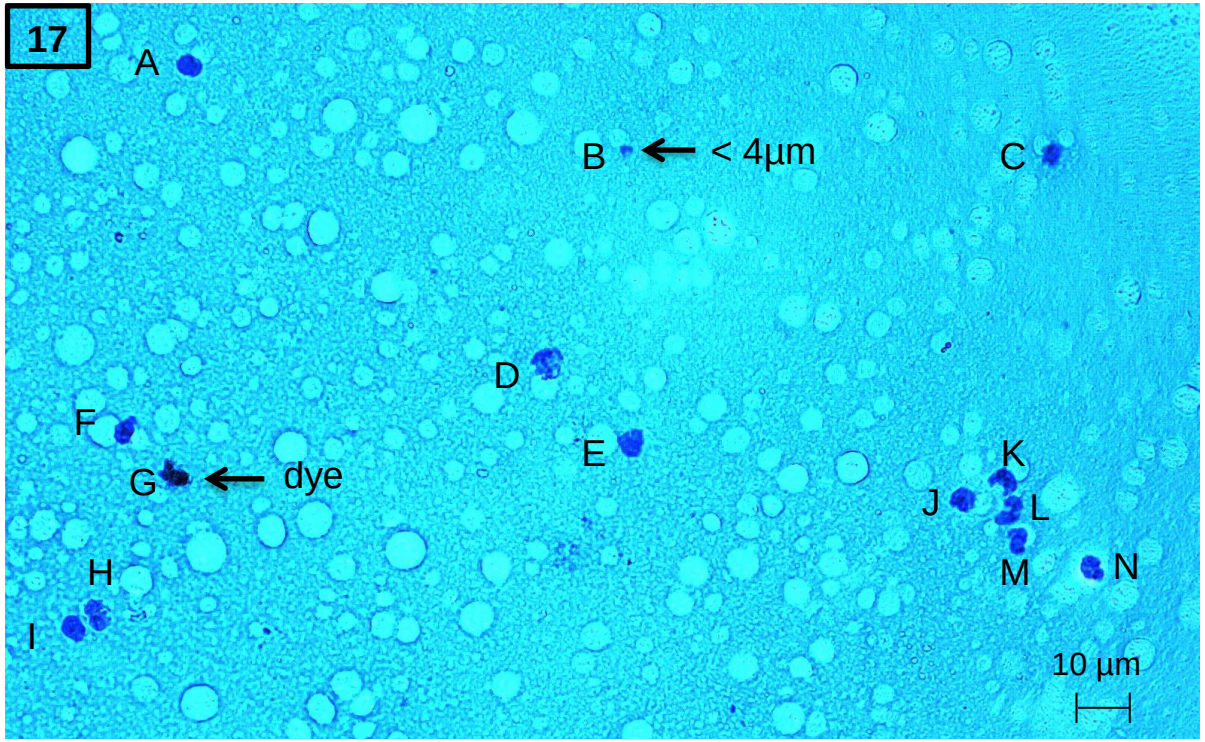


Count: 6 cells

- **'A-B-C-D-E' are typical countable cells**
- **'F' is a bi-lobed cell with a nuclear bridge**
- **'G' is a residue of staining solution, so not counted**

Raw cow's milk stained with Modified Newman-Lampert solution (Fluka, Nr 01375)
Microscope: Olympus BX51 (MICRO 1)
x500-fold magnification
Capture software: ARCHIMED
Laboratory for Food Safety, Anses, France

Pictures Library

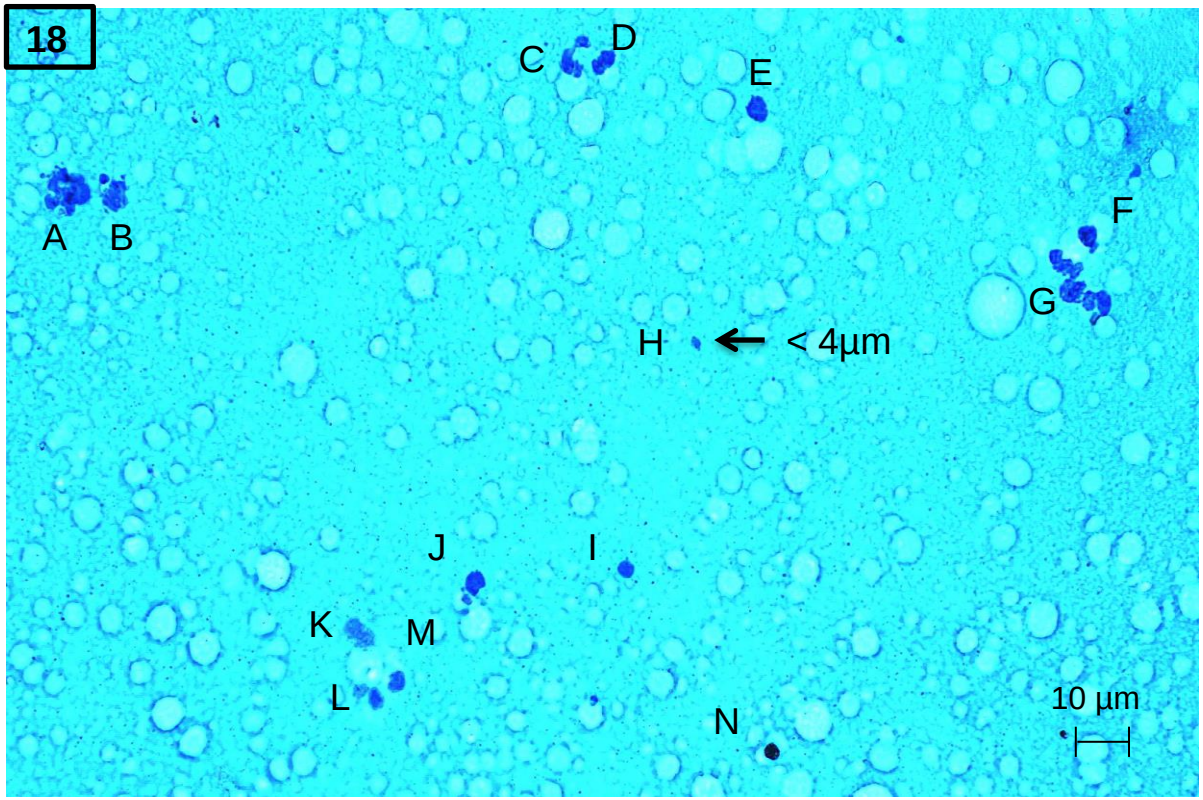


Count: 12 cells

- **'A-C-D-E-F-H-I-J-M-N are typical countable cells**
- **'B' is not counted based on size (< 4µm)**
- **'G' is a residue of staining solution so no counted**
- **'K-L' are two cells (no clear bridging)**

Raw cow's milk stained with Modified Newman-Lampert solution (Fluka, Nr 01375)
Microscope: Olympus BX51 (MICRO 1)
x500-fold magnification
Capture software: ARCHIMED
Laboratory for Food Safety, Anses, France

Pictures Library



Count: 12 cells

- 'A-B-C-D-E-I-J-K-L-M are countable cells
- 'G' is a multi-lobed cell counted as one (no clear nuclear differentiation)
- 'H' is not counted based on size (<4µm)
- 'N' is a residue of staining solution so not counted

Raw cow's milk stained with Modified Newman-Lampert solution (Fluka, Nr 01375)
Microscope: Olympus BX51 (MICRO 1)
X 500-fold magnification
Capture software: ARCHIMED
Laboratory for Food Safety, Anses, France

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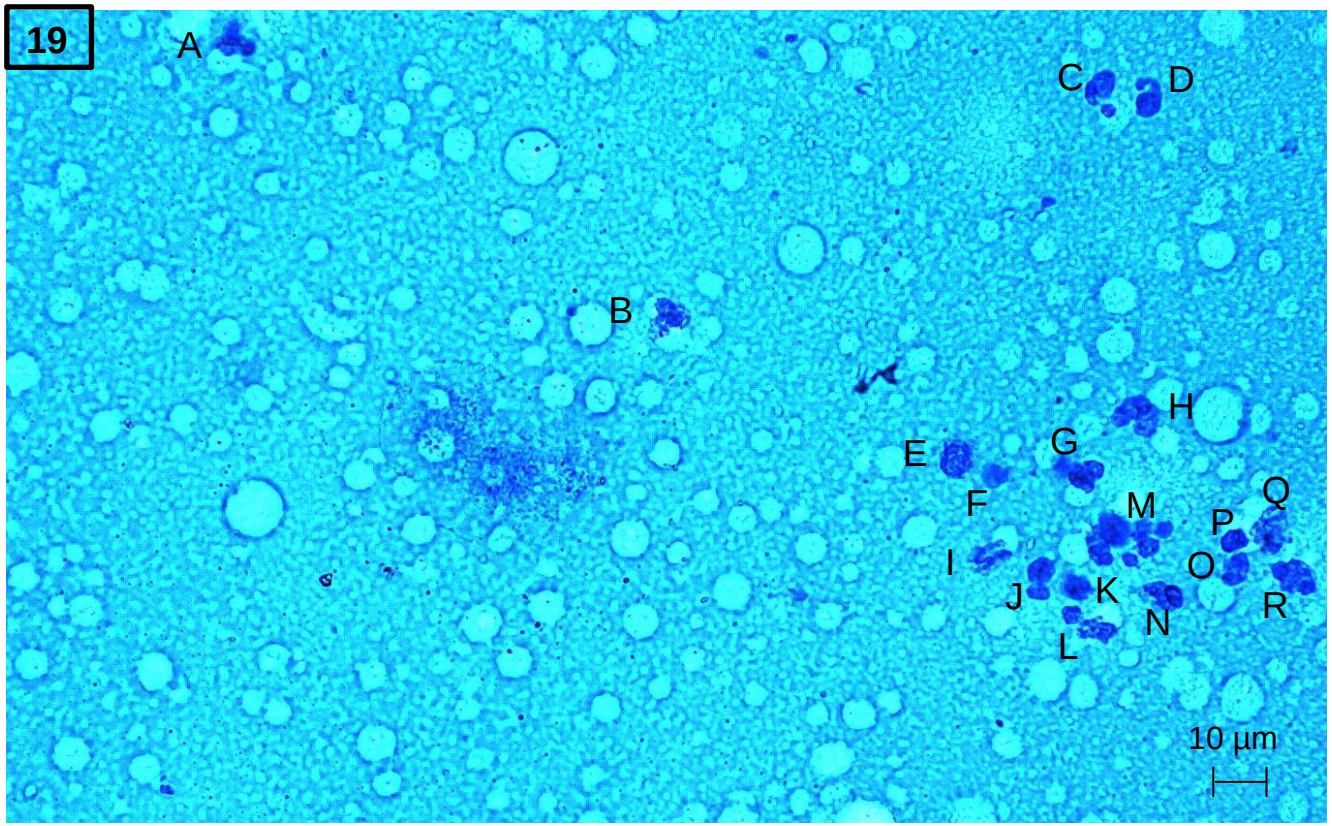

Count: 18 cells

- **'A-B-C-D-E-F-G-H-I-K-N-O-P-Q-R' are countable cells**
- **'J-L' are bi-lobed cells**
- **'M' is a multi-lobed cell**

Raw cow's mlik stained with Modified Newman-Lampert solution (Fluka, Nr 01375)
Microscope: Olympus BX51 (MICRO 1)
x500-fold magnification
Capture software: ARCHIMED
Laboratory for Food Safety, Anses, France

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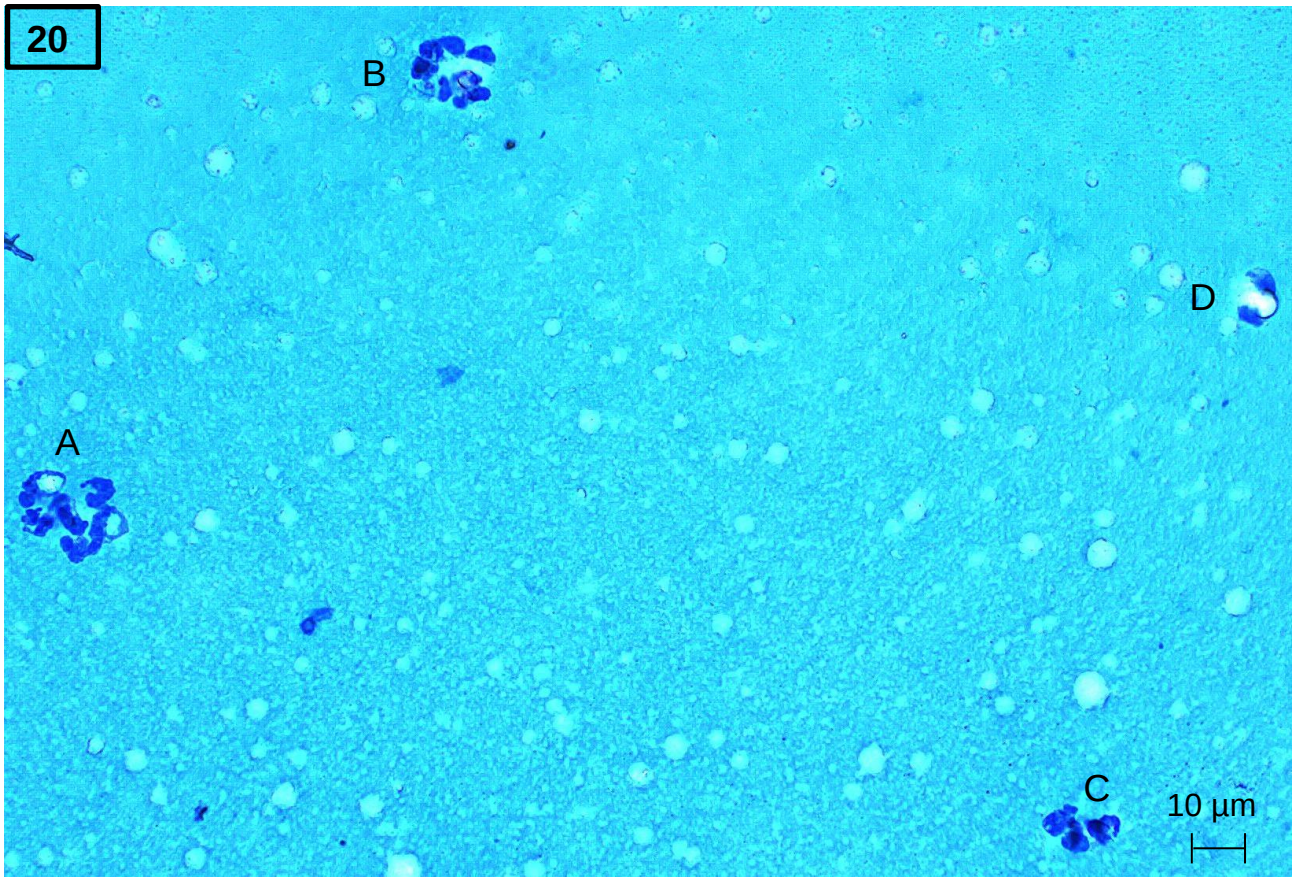

Raw cow's milk stained with Modified Newman-Lampert solution
(Fluka, Nr 01375)
Microscope: Olympus BX51 (MICRO 1)
X 500-fold magnification
Capture software: ARCHIMED
Laboratory for Food Safety, Anses, France

Count: 4 cells

A consensus was not found for this picture (cf. introduction). A clear nuclear differentiation is not visible for A, B and C. Considering the quantity of material, these clusters may contain more than one cell. Consequently, cell count is reported tentatively.

Pictures Library

Somatic cell counting : x 400-fold magnification

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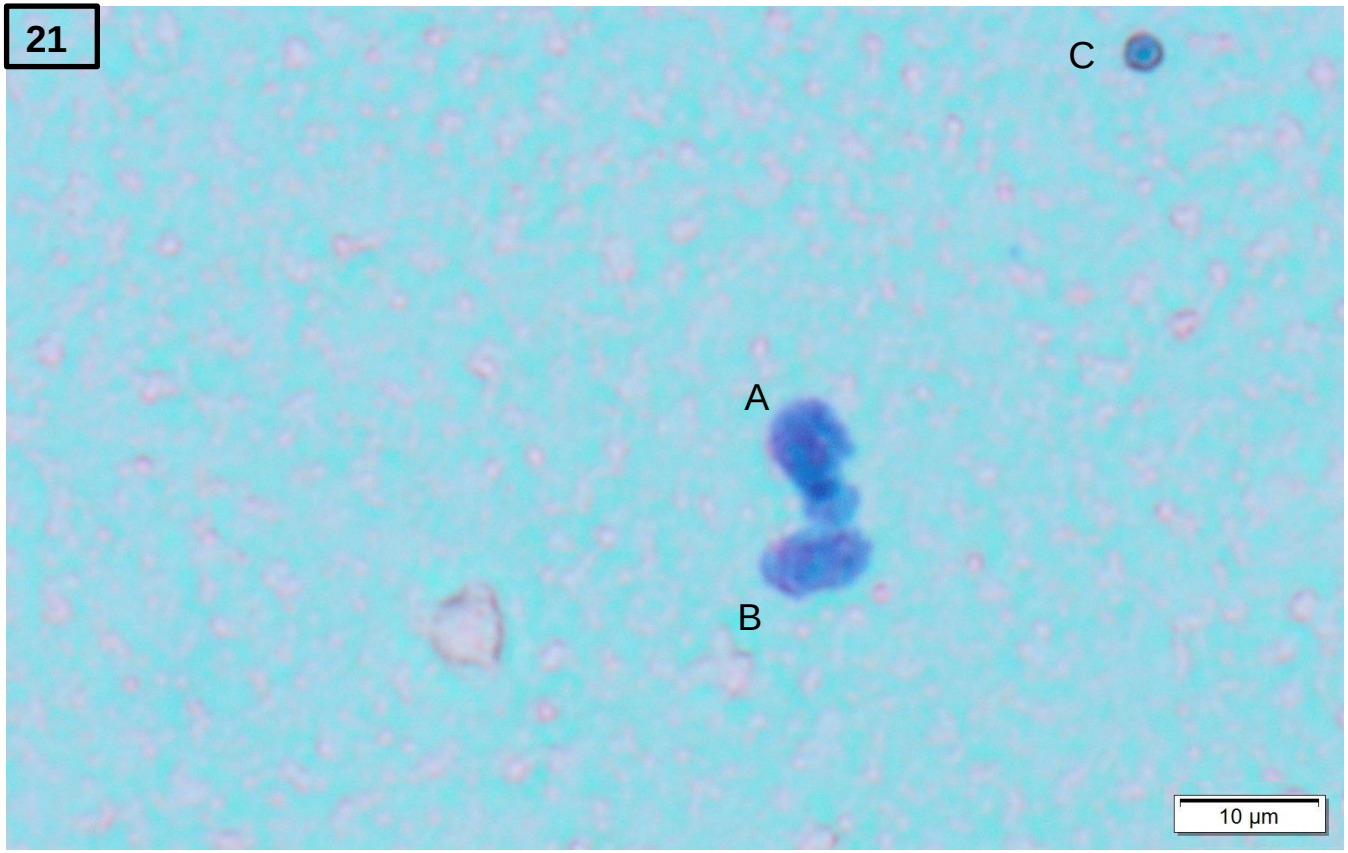

Count: 2 cells

- 'A-B' are two countable cells (needs focus for more certainty)
- 'C' is due to staining, it is not a cell

Semi-skimmed UHT milk, with added cell suspension in polypropylene glycol, bronopol stabilised milk
Stained with Modified Newman-Lampert solution (Fluka, Nr 01375)
Microscope: Olympus BX40
x 400-fold magnification
RIKILT Wageningen UR (Institute of Food Safety)

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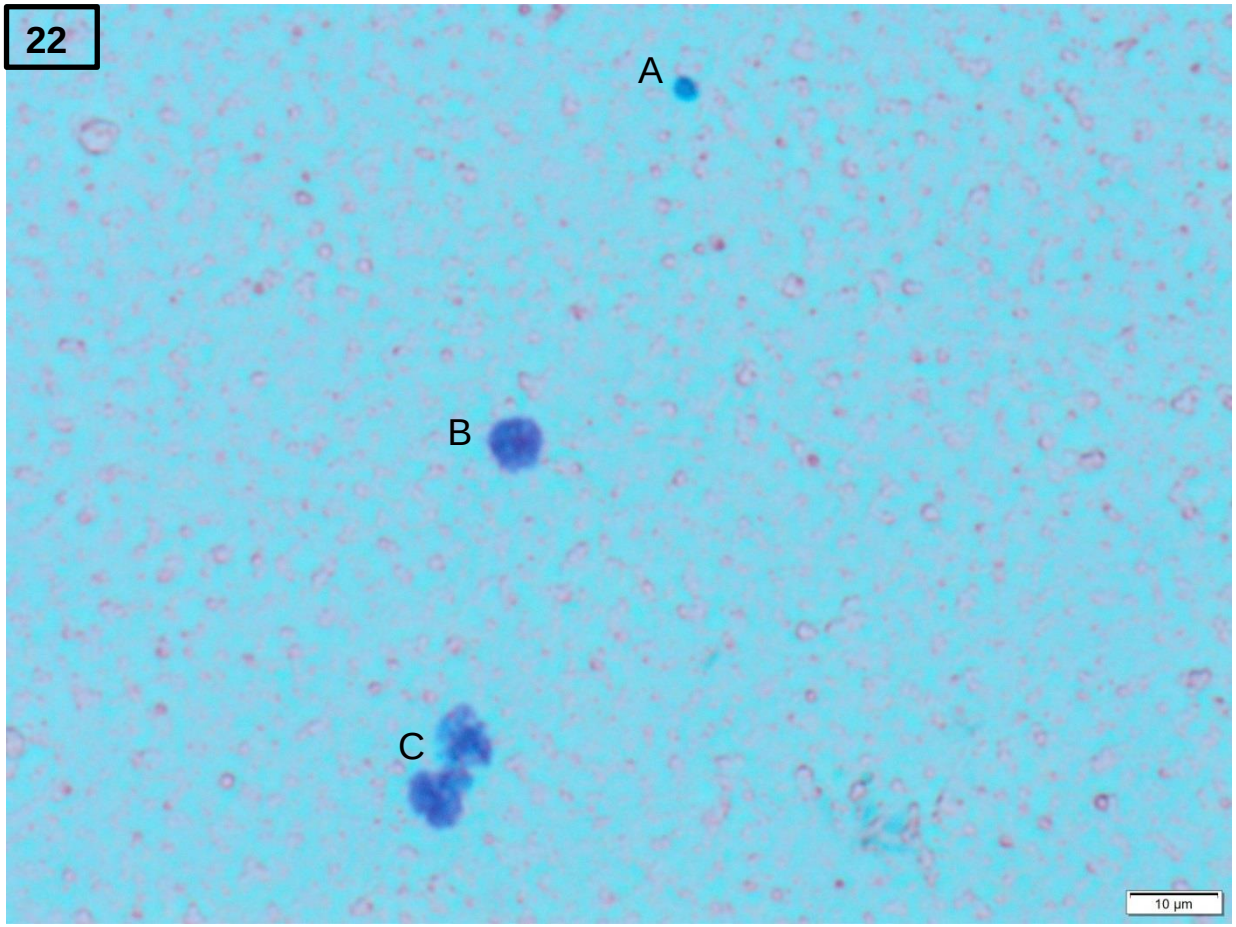

Count: 2 cells

- 'A' is not counted based on size (<4µm)
- 'B' is a typical mononuclear cell
- 'C' is a bi-lobed cell

Semi-skimmed UHT milk, with added cell suspension in polypropylene glycol, bronopol stabilised milk
Stained with Modified Newman-Lampert solution (Fluka, Nr 01375)
Microscope: Olympus BX40
x 400-fold magnification
RIKILT Wageningen UR (Institute of Food Safety)

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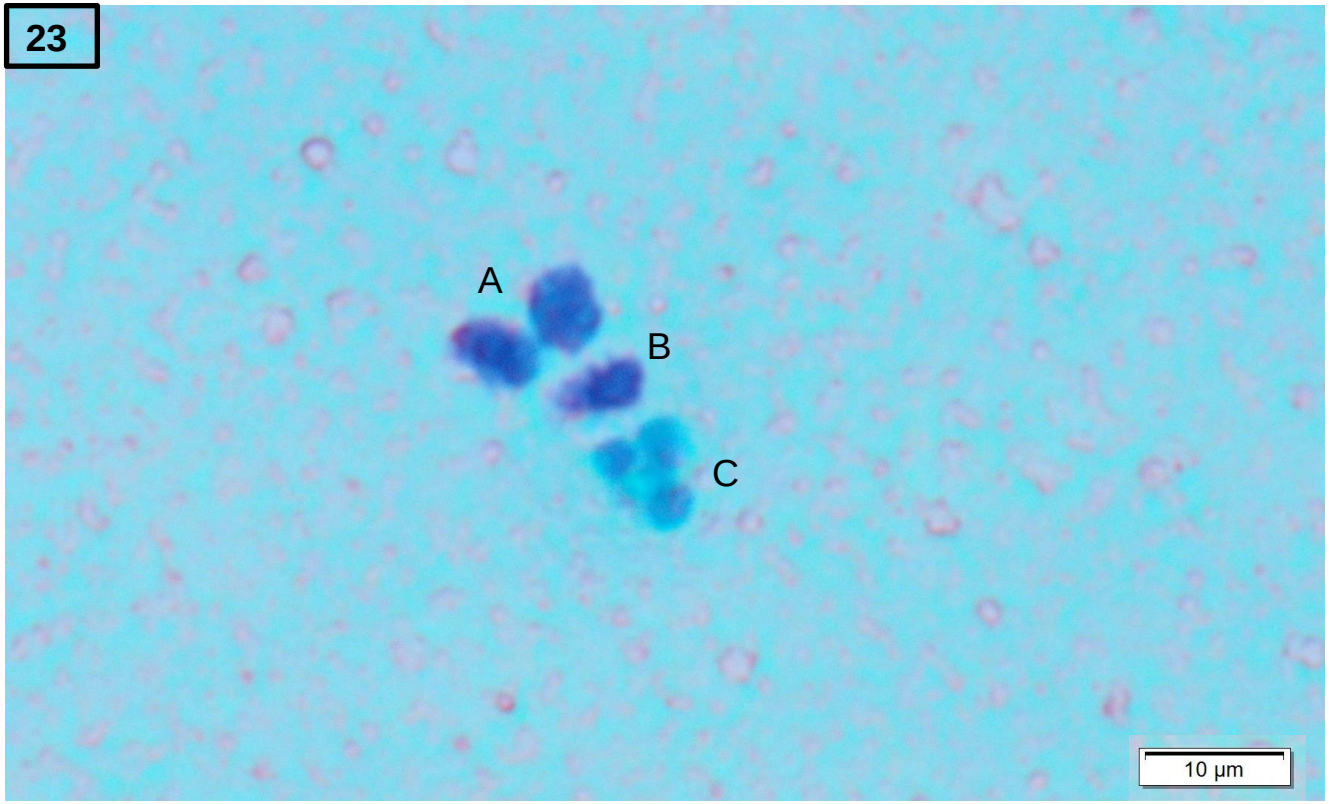

Count: 3 cells

- 'A' is a bi-lobed cell with a nuclear bridge
- 'B' is a mononuclear cell
- 'C' is perhaps a tri-lobed cell
(it can be not counted due to the lower colour)

Semi-skimmed UHT milk, with added cell suspension in polypropylene glycol, bronopol stabilised milk
Stained with Modified Newman-Lampert solution (Fluka, Nr 01375)
Microscope: Olympus BX40
x 400-fold magnification
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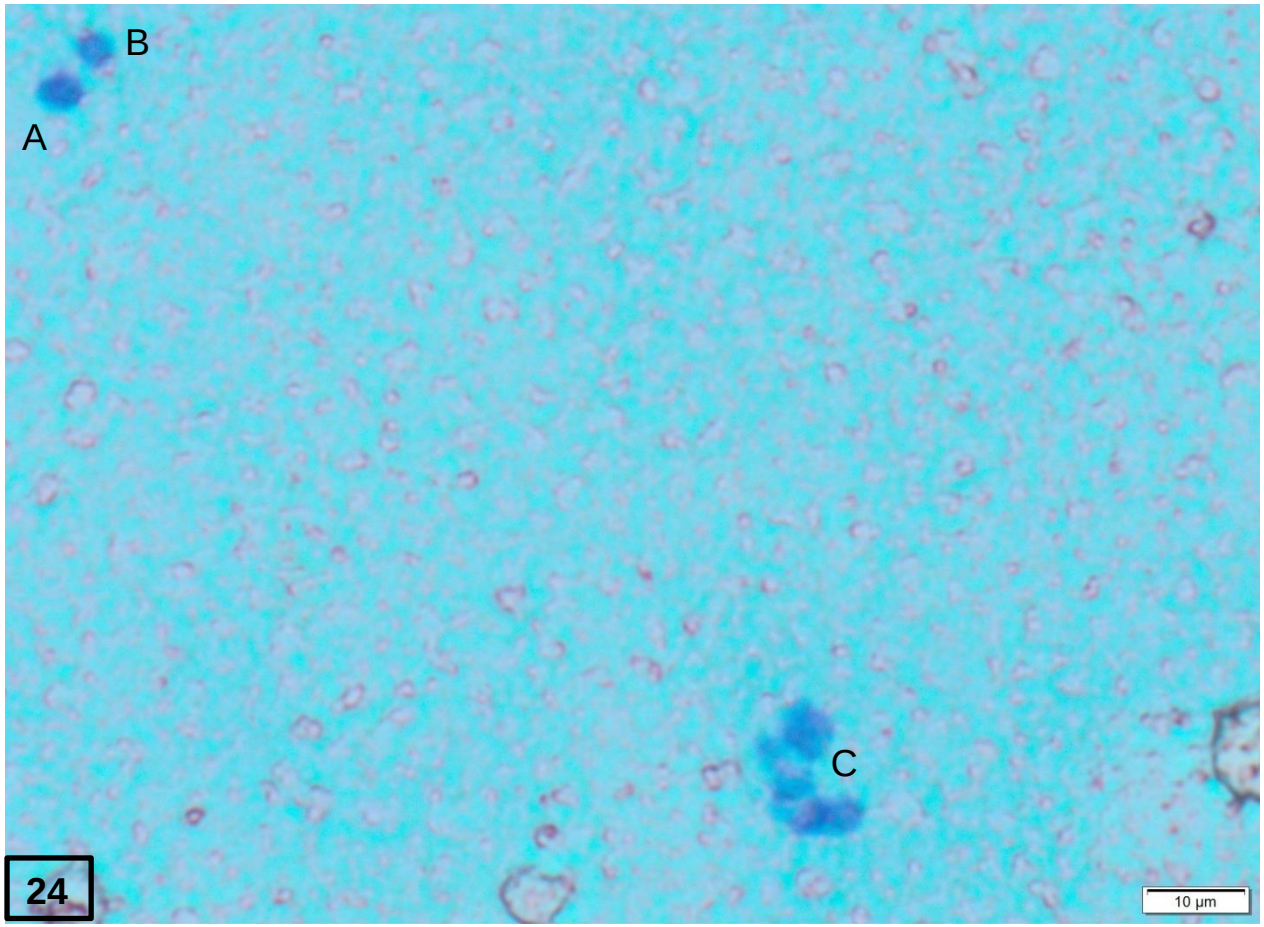

Count: 3 cells

- 'A-B' are typical mononuclear cells
- 'C' is a multi-lobed cell

Semi-skimmed UHT milk, with added cell suspension in polypropylene glycol, bronopol stabilised
Stained with Modified Newman-Lampert stain solution (Fluka, Nr 01375)
Microscope: Olympus BX40
x 400-fold magnification
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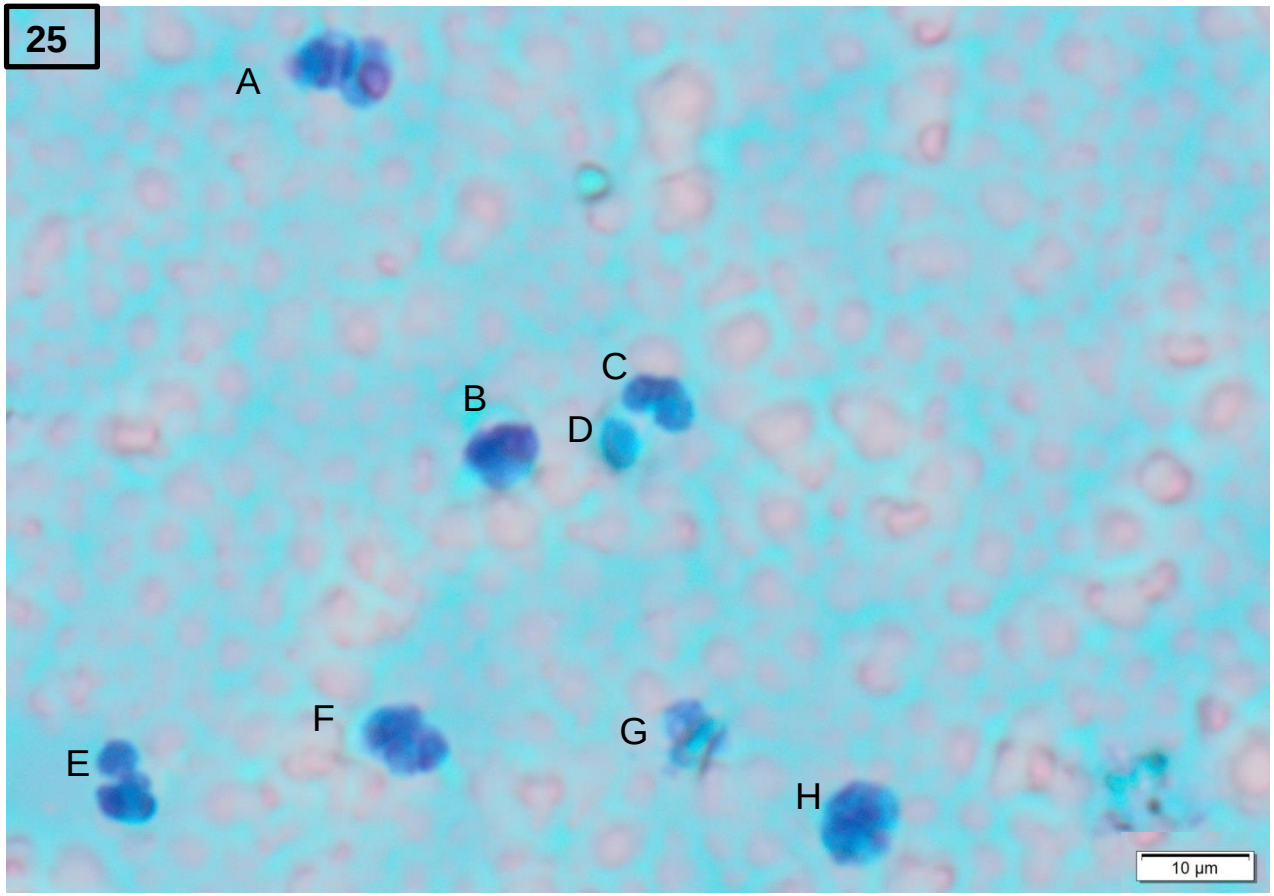

Count: 8 cells

- 'A-B-C-D-E-F-H' are countable cells
- 'G' is a disintegrated cell, though countable

Raw milk sample with 0.02% bronopol
Stained with Modified Newman-Lampert solution (Fluka, Nr 01375)
Microscope: Olympus BX40
x 400-fold magnification
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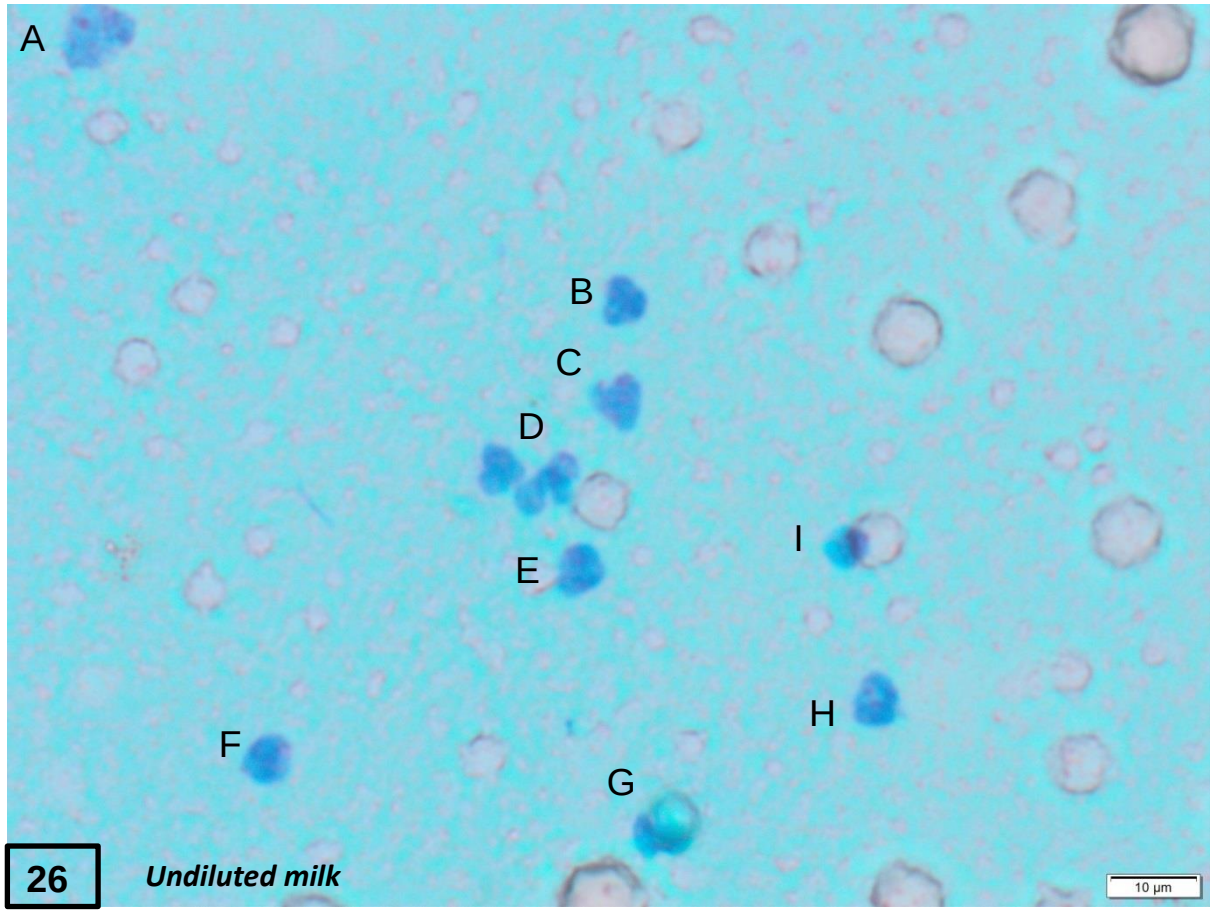
Somatic cell counting : Dilution effect using PBS



26 *Undiluted milk*

Pictures 27 – 28 – 29 are a series from a sample that needs dilution (> 2 millions cells /ml)

Count: 9 cells

- **'A-B-C-E-F-H-I' are typical mononuclear cells**
- **'G' is a disintegrating cell, though countable**
- **'D' is a multi-lobed cell**

Semi-skimmed UHT milk, with added cell suspension in polypropylene glycol, bronopol stabilised
Stained with Modified Newman-Lampert solution (Fluka, Nr 01375)
Microscope: Olympus BX40
x 400-fold magnification
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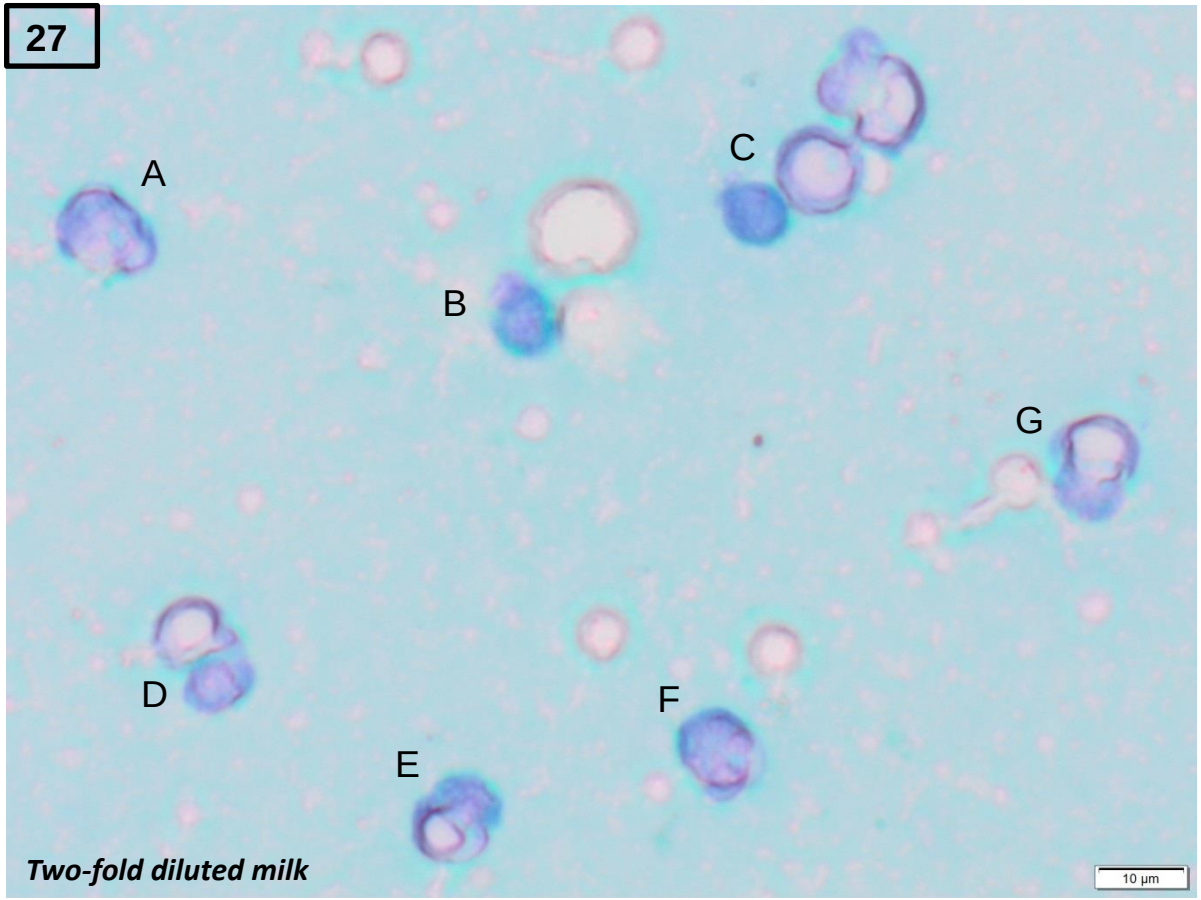

Count: 7 cells

- 'A-B-C-D-E-F-G are mononuclear countable cells

Semi-skimmed UHT milk, with added cell suspension in polypropylene glycol, bronopol stabilised – 2 fold diluted
Stained with Modified Newman-lampert solution (Fluka, Nr 01375)
Microscope: Olympus BX40
x 400-fold magnification
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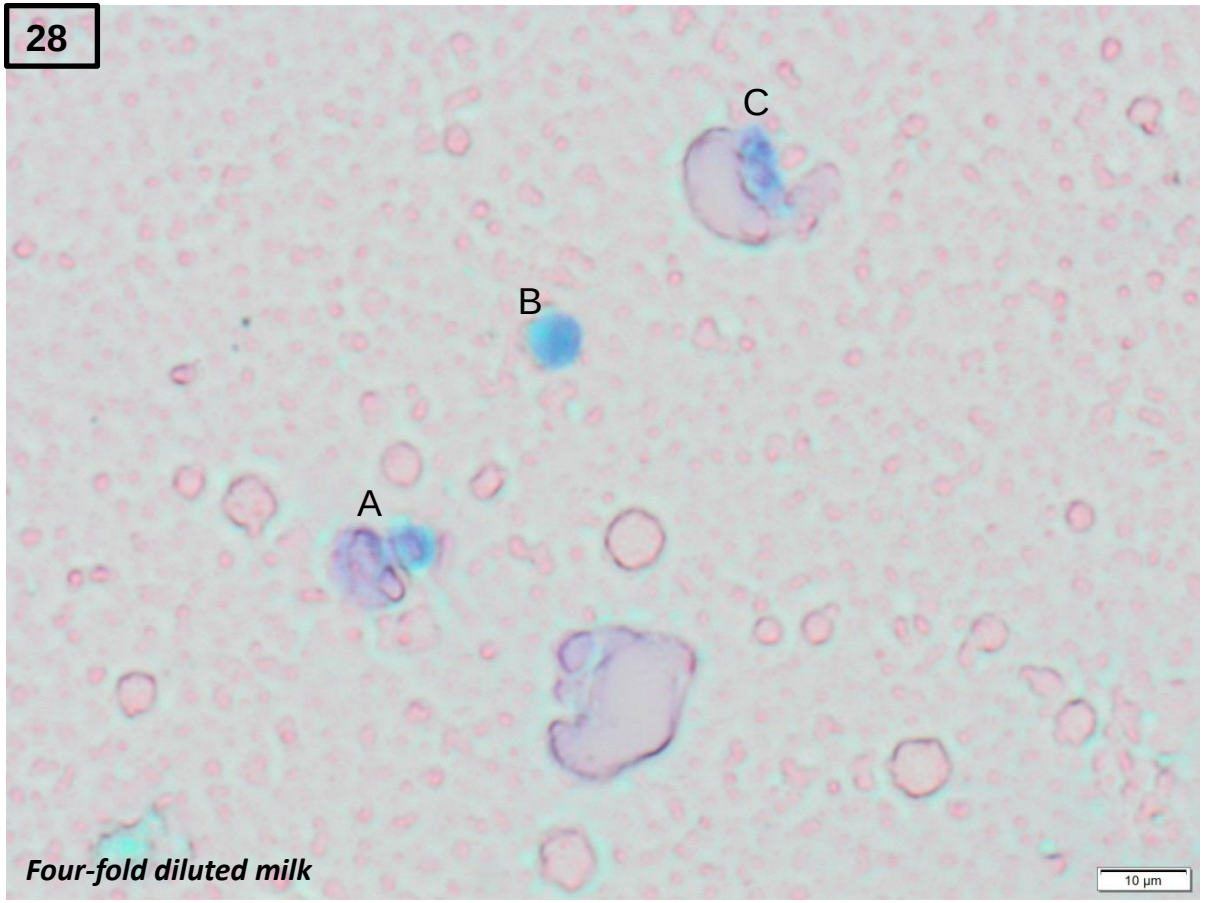

Four-fold diluted milk

Count: 3 cells

- 'A-B-C' are countable bleached cells

Semi-skimmed UHT milk, with added cell suspension in polypropylene glycol, bronopol stabilised – 4 fold diluted
Stained with Modified Newman-Lampert solution (Fluka, Nr 01375)
Microscope: Olympus BX40
x 400-fold magnification
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