

Direct Microscopic Examination of Milk From Small Ruminants

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ABSTRACT

This guidelines explains the reasons, and methods, for performing direct microscopic somatic cell counting of milk from dairy animals with apocrine mammary systems. Sheep and goats are common examples of this type of dairy animal. The Guideline has a large number of photographs of actual milk smears to give accurate examples.

PREFACE

This guideline was prepared by: Lead Author, Daniel L. Scruton, Vermont Agency of Agriculture, Food and Markets, Frank Fillman, Jackson-Mitchell, California, Lynn Hinckley, University of Connecticut, Debora Miller Leach, consultant, Bebe Zabilansky, Bruns Brothers and with special thanks to the Connecticut Veterinary Medical Diagnostic Laboratory of the University of Connecticut.

GUIDELINE PREPARATION AND REVIEW PROCESS

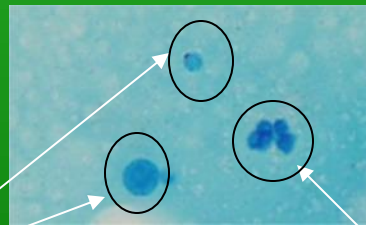
Guideline development within Dairy Practices Council (DPC) is unique and requires several levels of peer review. The first step in the process of guideline development starts with a Task Force subcommittee made up of individuals from industry, regulatory and education interested in and knowledgeable about the subject to be addressed. Drafts, called 'white copies', are circulated until all members are satisfied with the text. The final white copy may then be distributed to the entire task force, DPC Executive Board, state and federal regulators, education members, industry members and anyone else the DPC Executive Vice President and the Task Force Director feels would add to the strength of the review. Following final white copy review and correction the next step in the process requires a yellow cover draft that is circulated to the member Regulatory Agency representatives that are referred to as "Key Sanitarians". The Key Sanitarians may suggest changes and insert footnotes if their state standards and regulations differ from the text. After final review and editing the Guideline is distributed in the distinctive DPC green cover to people worldwide. These guidelines represent the state of the knowledge at the time they are written.

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Why is somatic cell count (SCC) testing not the same as used with cattle?

- Sheep and Goats produce milk differently than cattle. They have an apocrine mammary and the normal secretory process results in the shedding of cytoplasmic particles. The end result is more cellular material in the milk. Some of these particles are difficult to differentiate from white blood cells.



Non-nucleated Cell Fragments

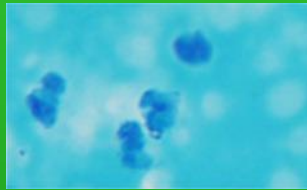
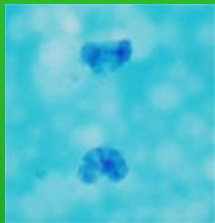
White Blood Cells

Different Types of Secretory Systems



Merocrine

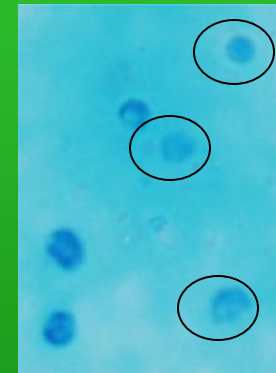
Cattle, Water Buffalo



Note the absence of crescent and non-nucleated cells.

Apocrine

Goats, Sheep



Note the non-nucleated and crescent cells. Circled cells are not counted for SCC.



Proper Staining Methods

To accurately measure the SCC of milk from animals with apocrine mammarys you need to use a staining method that differentiates nucleated cells from cytoplasmic particles (non-nucleated). The official stain recognized for use in regulatory testing in goats is Pyronin Y- Methyl Green (PY). Also, the preparation method of the stain formulation will affect both the color ranges and color contrast seen on the slide, and may cause variations; i.e., pink to light purple versus pink to blue. Commercially prepared stain is available from several scientific supply companies. The procedure is performed one of two ways:

1. Standard Methods

- *Follow the procedure in the current addition of Standard Methods for the Examination of Dairy Products by the American Public Health Association*



Proper Staining Methods

2. “New York Modification” to the Pyronin Y staining method is performed with the following steps at the specified duration:

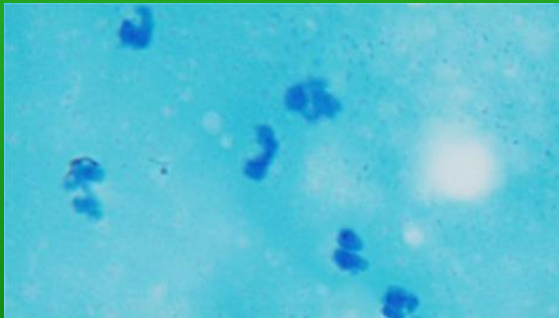
- | | | |
|----|---------------------------------|---------------|
| a) | Carnoy’s Fixative | 5 minutes |
| b) | 50% Ethanol | 1 minute |
| c) | 30% Ethanol | 1 minute |
| d) | H ₂ O | 1 minute |
| e) | Stain (Pyronin Y- Methyl Green) | 6 minutes |
| f) | Dry completely | |
| g) | Butanol | flush briefly |
| h) | Xylene | flush briefly |

Developed by James Fitts and Gary Davis, New York State Department of Agriculture and Markets.

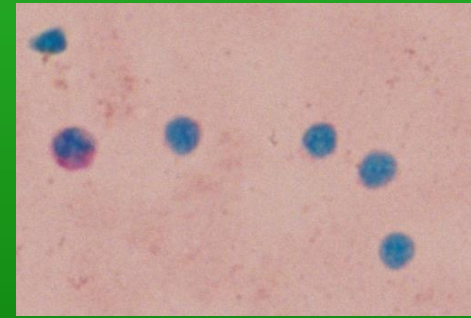


Stain Comparisons

- MB - Methylene Blue standard cow stain
- PY – Pyronin Y - Methyl Green stain for confirming high goat milk SCC



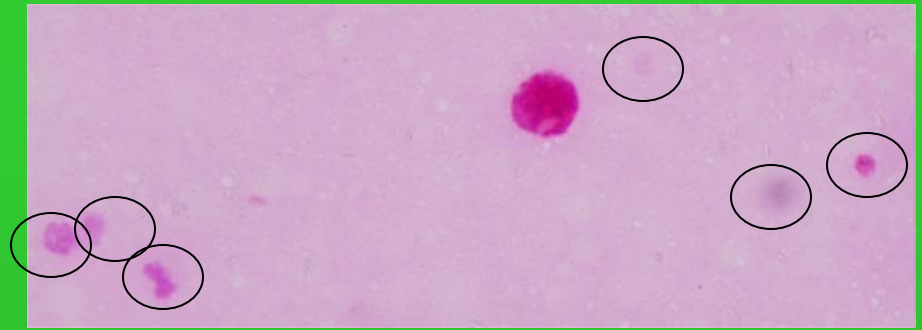
MB



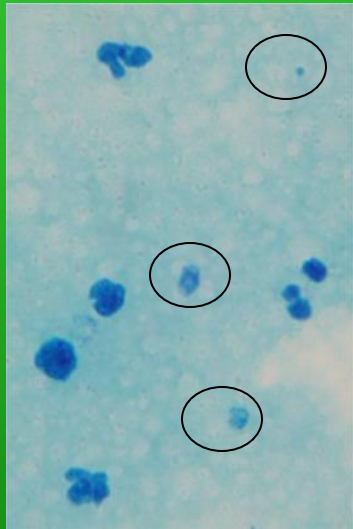
PY

Goat Milk

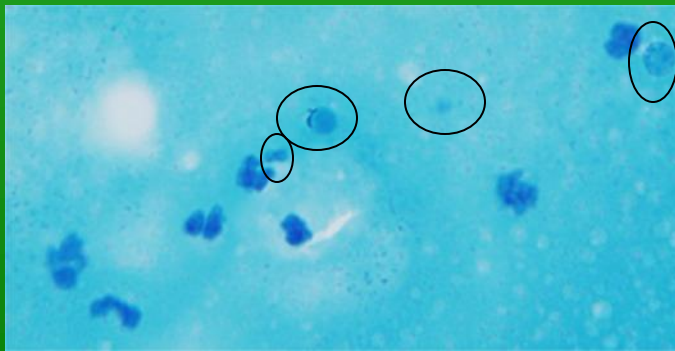
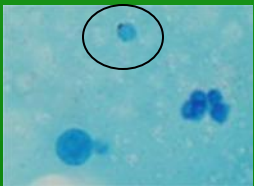
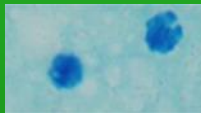
Objects inside of the circles are not counted.
Note the non-nucleated cells on PY are much fainter.



PY



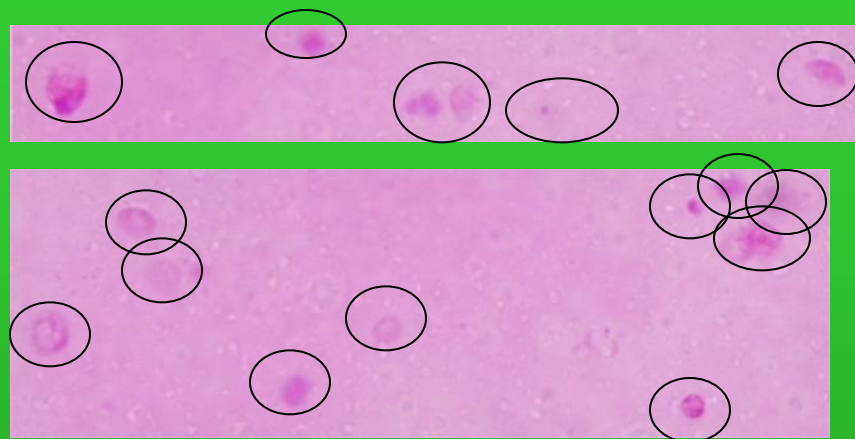
MB



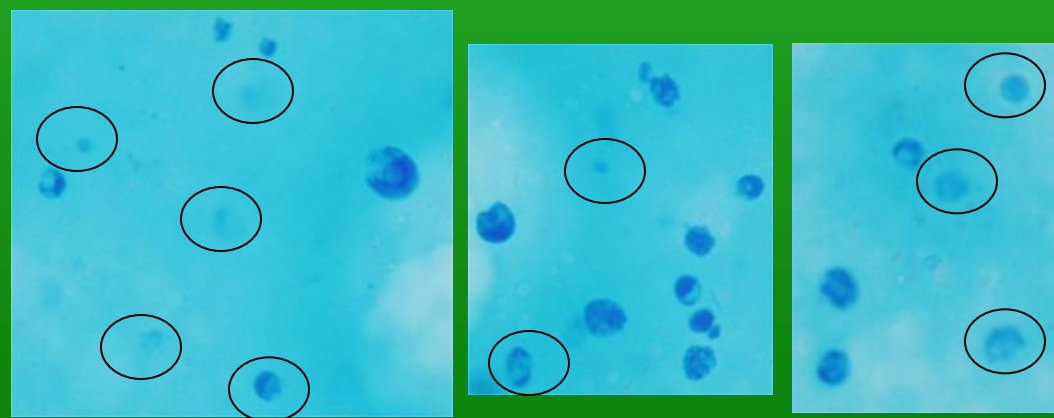
Sheep Milk

Objects inside of the circles are not counted.
Note the non-nucleated cells on PY are much fainter than on MB.

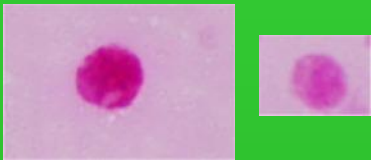
PY



MB



What Types of Cells are Counted?



Eosinophil



Lymphocytes



Polymorphonuclear (PMN)



Fragments are counted only if more than 50% of the nuclear material is visible. See FDA DMSCC 2400 form for guidance.

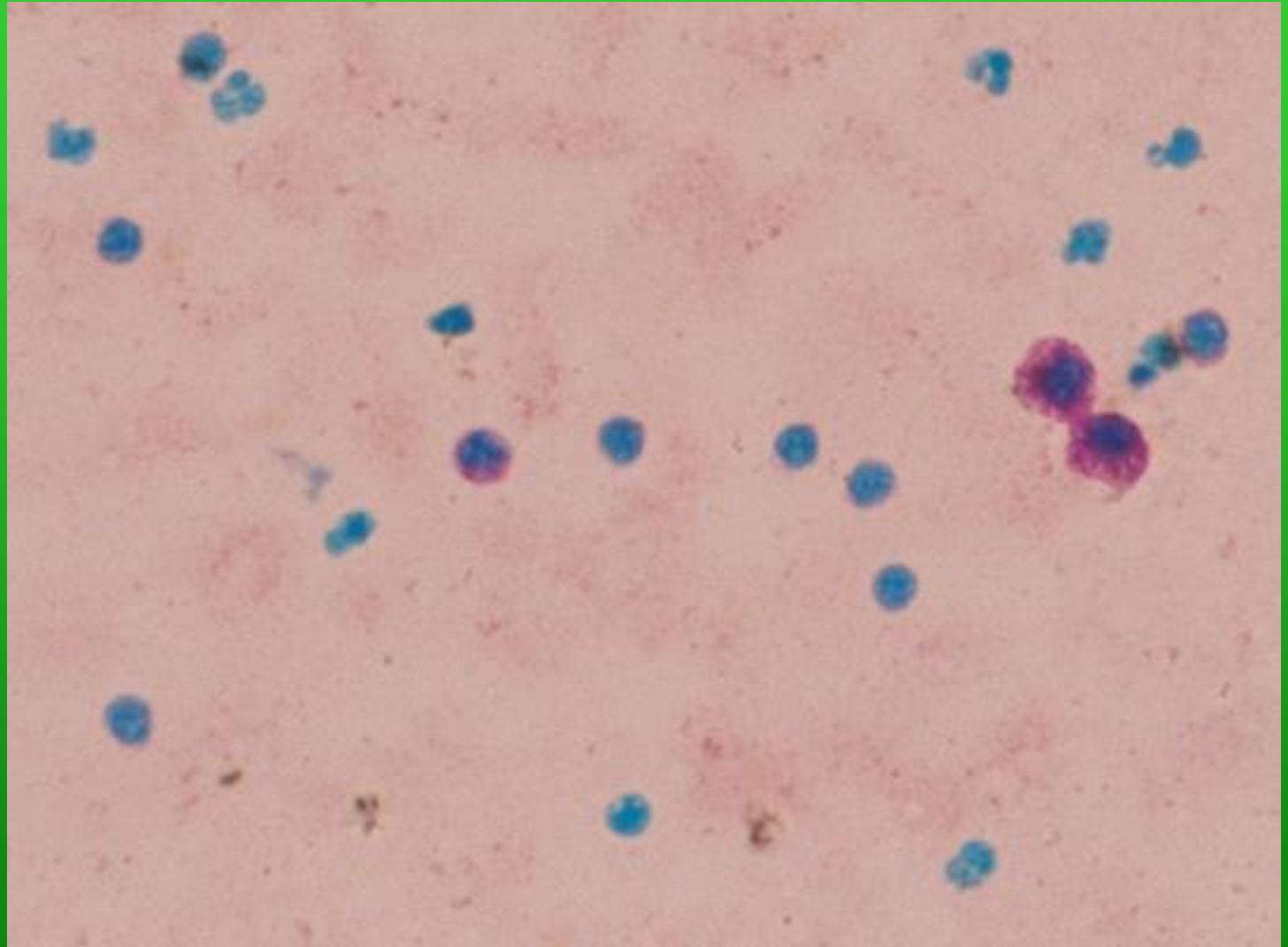
What Types of Cells are NOT Counted?



Cytoplasmic particles, very small cells (bacteria), non-nucleated fragments or debris

Practice Slide Goat A

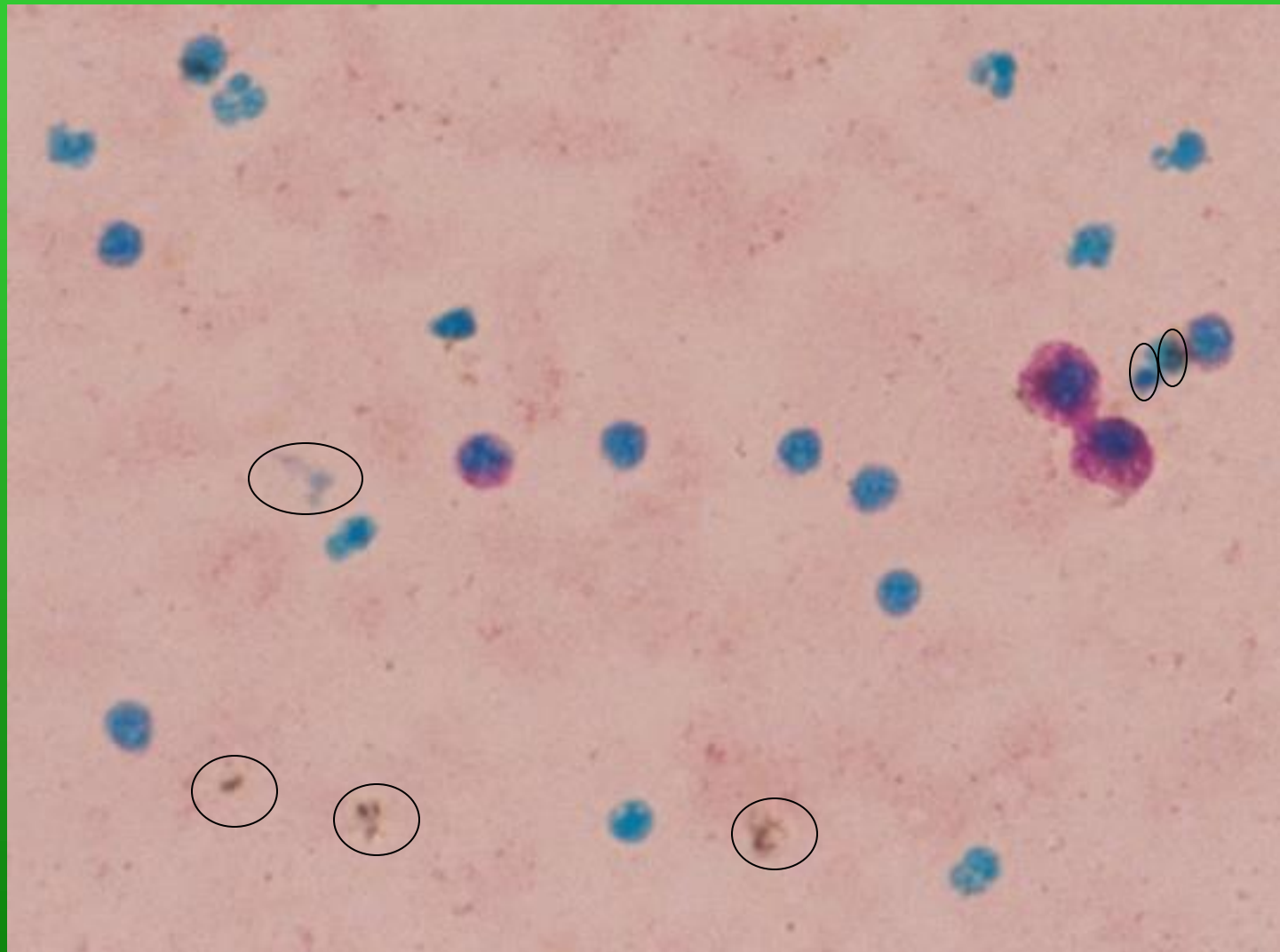
How many countable cells do you see?



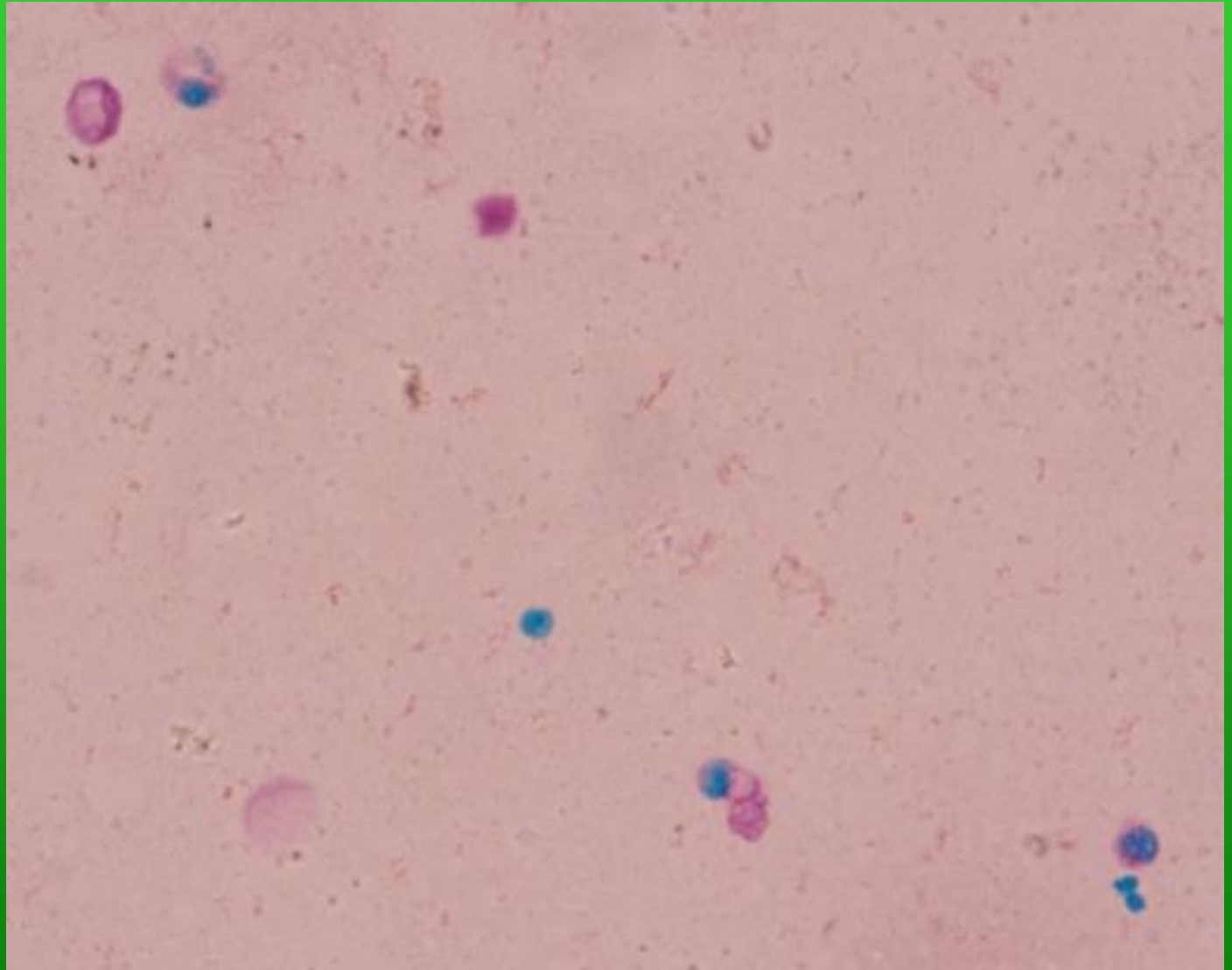
Answer Slide Goat A

19 countable
cells

Do not count
the circled
objects.

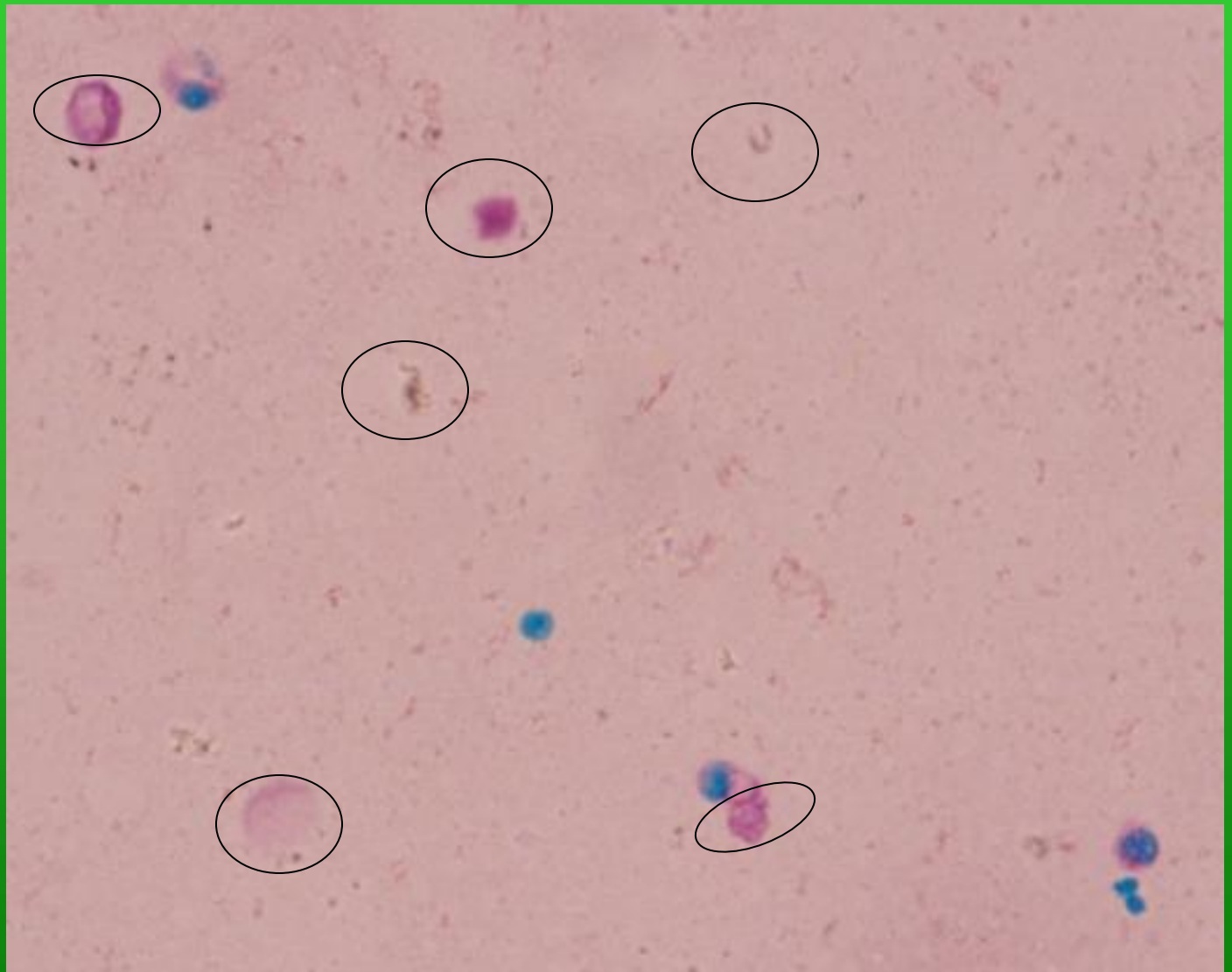


Practice Slide Goat B



How many
countable
cells do you
see?

Answer Slide Goat B

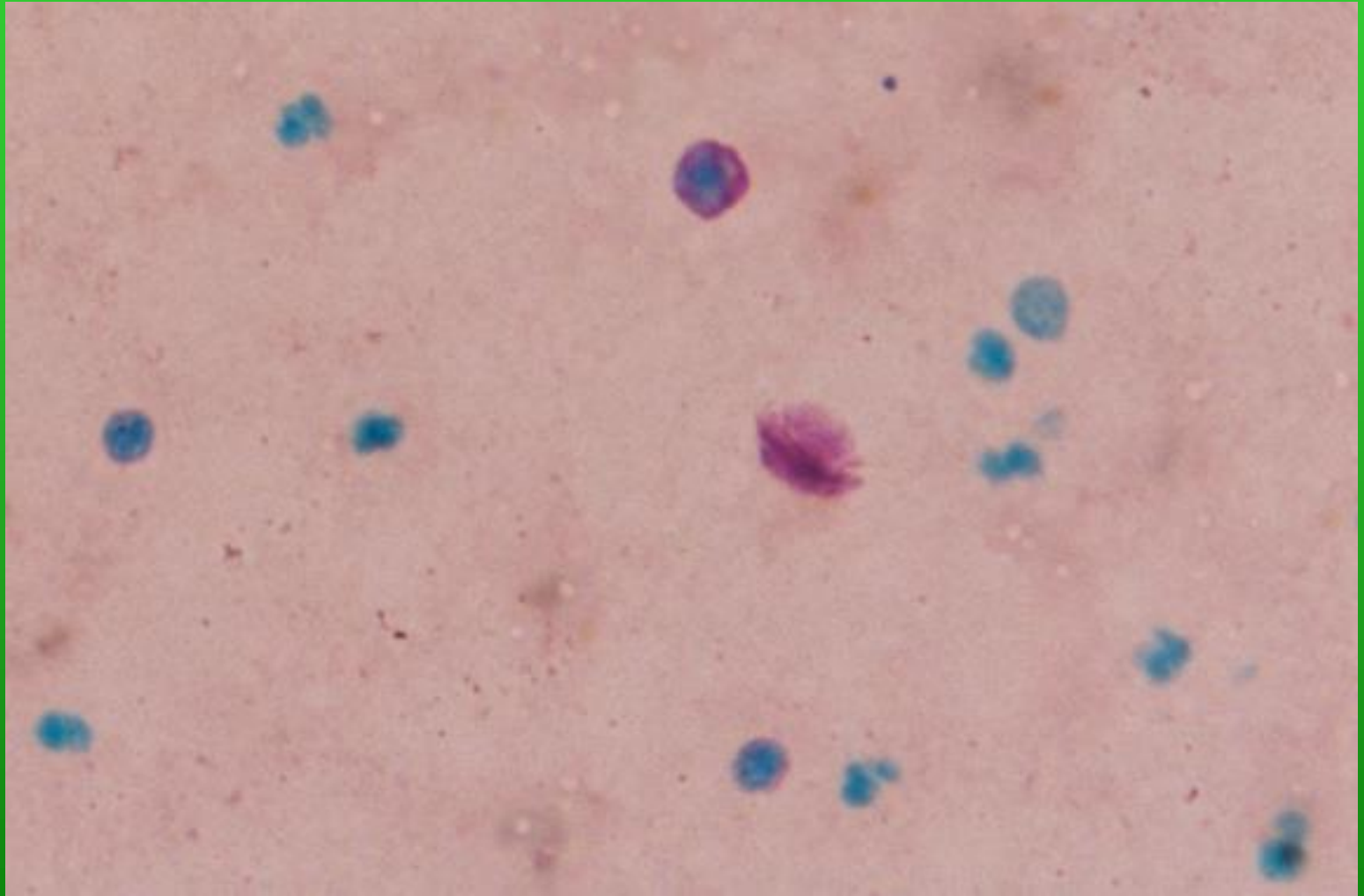


5 countable
cells

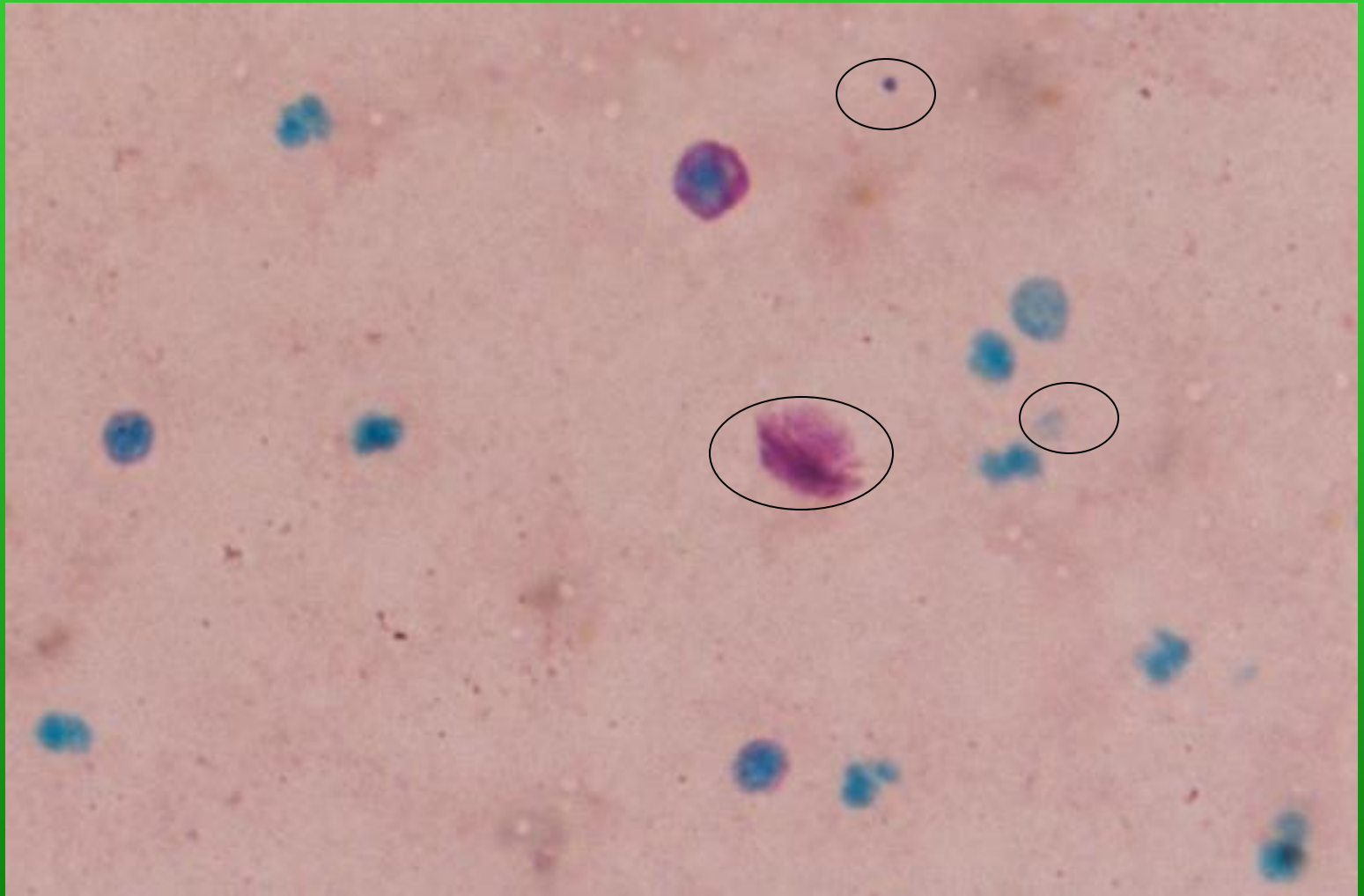
Do not
count the
circled
objects.

Practice Slide Goat C

How many countable cells do you see?



Answer Slide Goat C



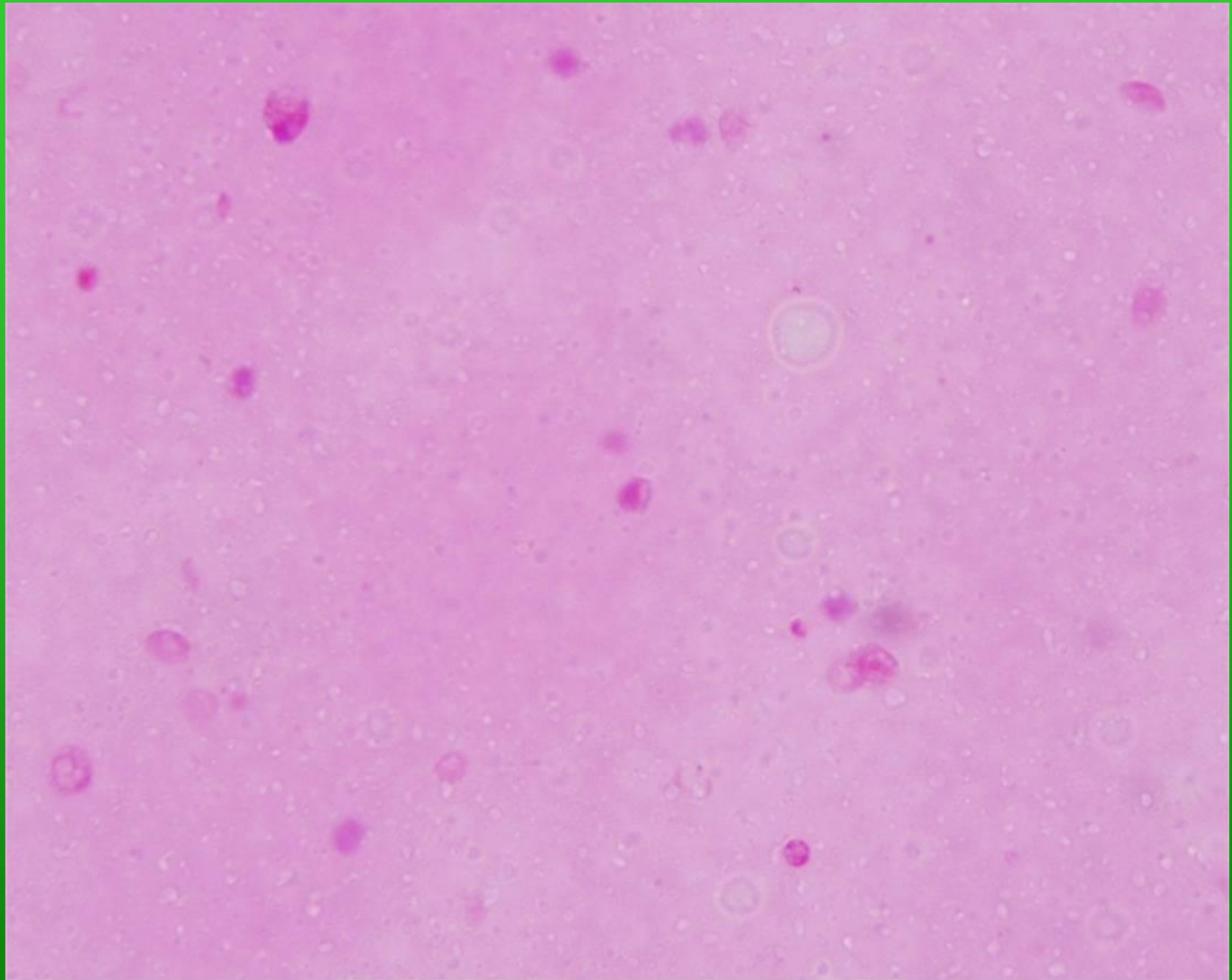
12
countable
cells

Do not
count the
circled
objects.

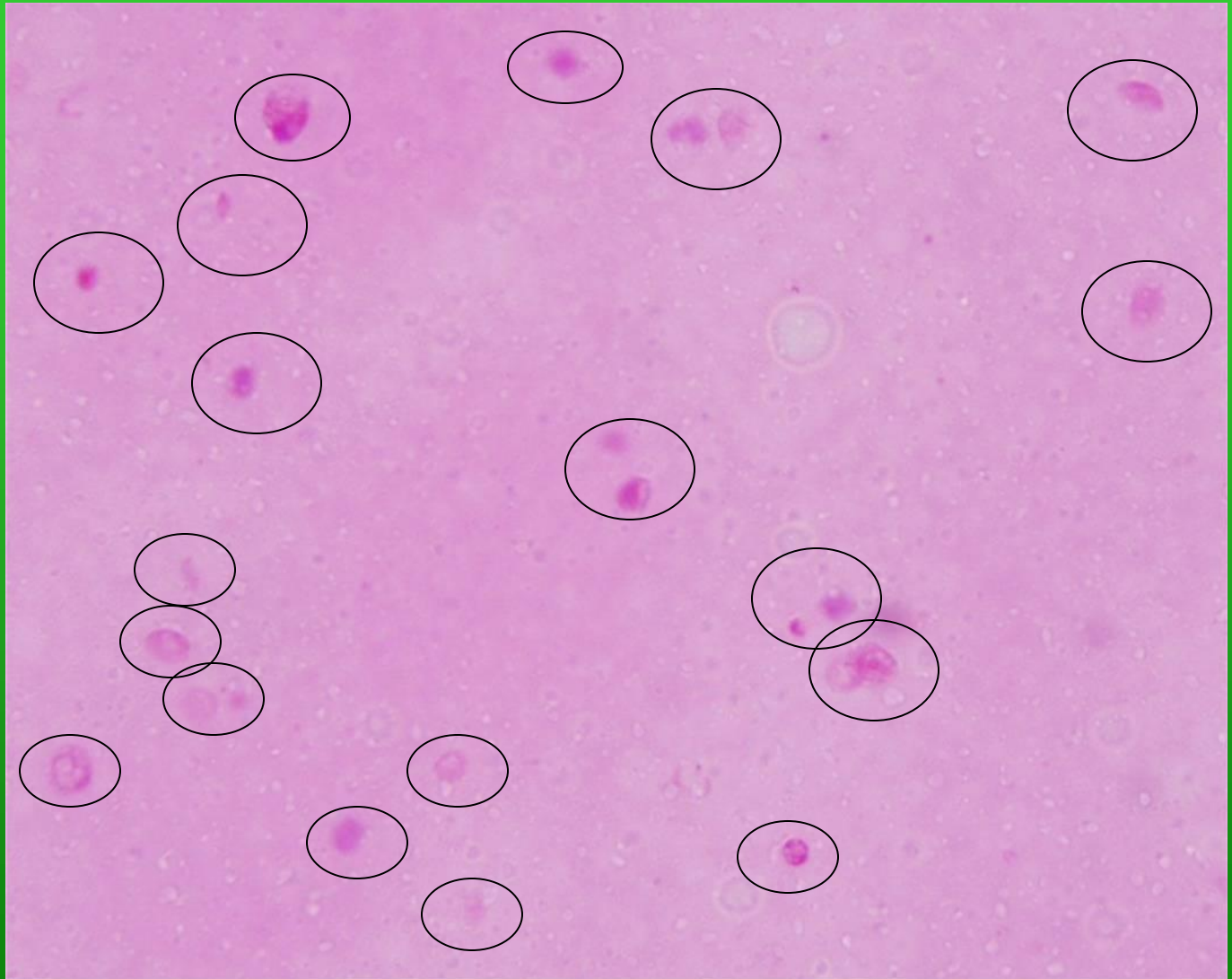


Practice Slide Sheep A

How many countable cells do you see?



Answer Slide Sheep A



0 countable
cells

Do not
count the
circled
objects.