Direct Microscopic Examination of Milk From Small Ruminants

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THE DAIRY PRACTICES COUNCIL®
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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

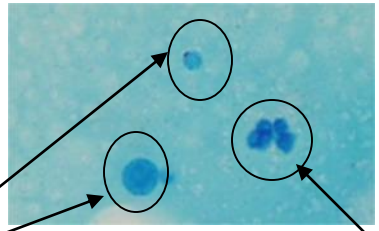
The Dairy Practices Council (DPC) Guideline development and update process is unique and requires several levels of peer review. The first step starts with a Task Force subcommittee made up of individuals from industry, regulatory and educational institutions interested in and knowledgeable about the subject to be addressed. Drafts, called “white copies,” are circulated until all members of the subcommittee are satisfied with the content. The final “white copy” may be further distributed to the entire Task Force; DPC Executive Board; state and federal regulators; educational and industry members; and anyone else the Task Force Director and/or the DPC Executive Vice President feel would add strength to the review. Following final “white copy” review and corrections, the next step requires a “yellow cover” draft to be circulated to representatives of participating Regulatory Agencies referred to as “Key Sanitarians.” 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 DPC members and made available for purchase to others. These guidelines represent our state of the knowledge at the time they are written. Currently, DPC Guidelines are primarily distributed electronically in pdf format without colored covers, but the process and designation of the steps remains the same. Contributors listed affiliations are at the time of their contribution.

DISCLAIMER

The DPC is not responsible for the use or application of the information provided in this Guideline. It is the responsibility of the user to ensure that the information addresses their needs and that any action taken complies with appropriate regulations and standards.

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.

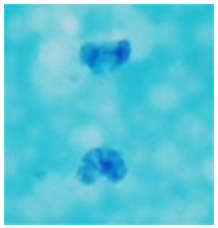


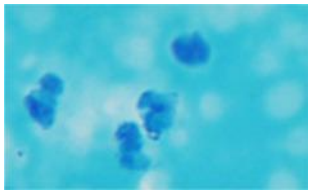
Non-nucleated Cell Fragments

White Blood Cells

Different Types of Secretory Systems

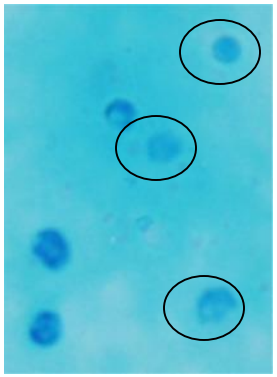
Merocrine
Cattle, Water Buffalo

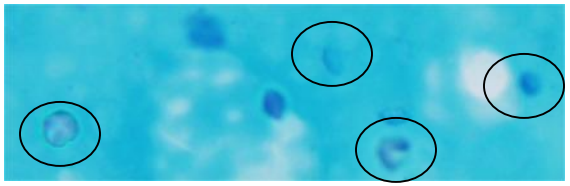




Note the absence of crescent and non-nucleated cells.

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 mammaries 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

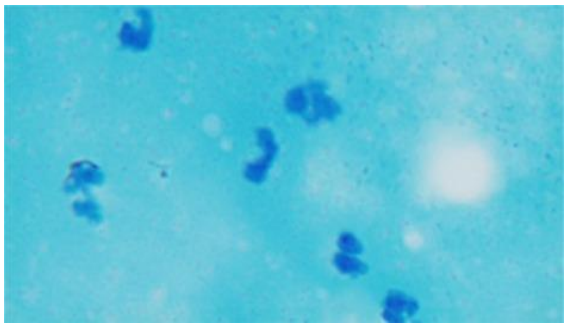
2. "New York Modification" to the Pyronin Y staining method is performed with the following steps at the specified duration:

a)	Carnoy's Fixative	5 minutes
b)	50% Ethanol	1 minute
c)	30% Ethanol	1 minute
d)	H_20	1 minute
e)	Stain (Pyronin Y- Methyl Green)	6 minutes
f)	Dry completely	
g)	Butanol	flush briefly
h)	Xylene	flush briefly

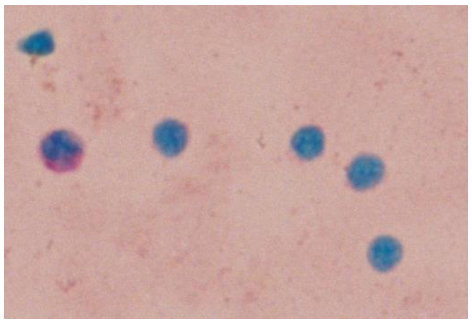
Developed by James Fitts and Gary Davis, New York State Department of Agriculture and Markets.

Stain Comparisons

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



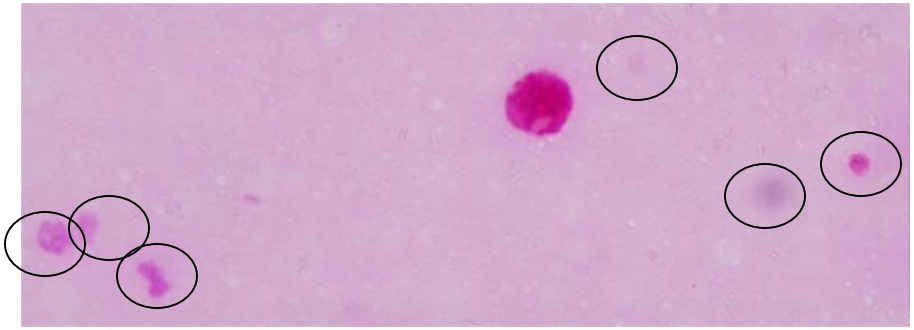
MB



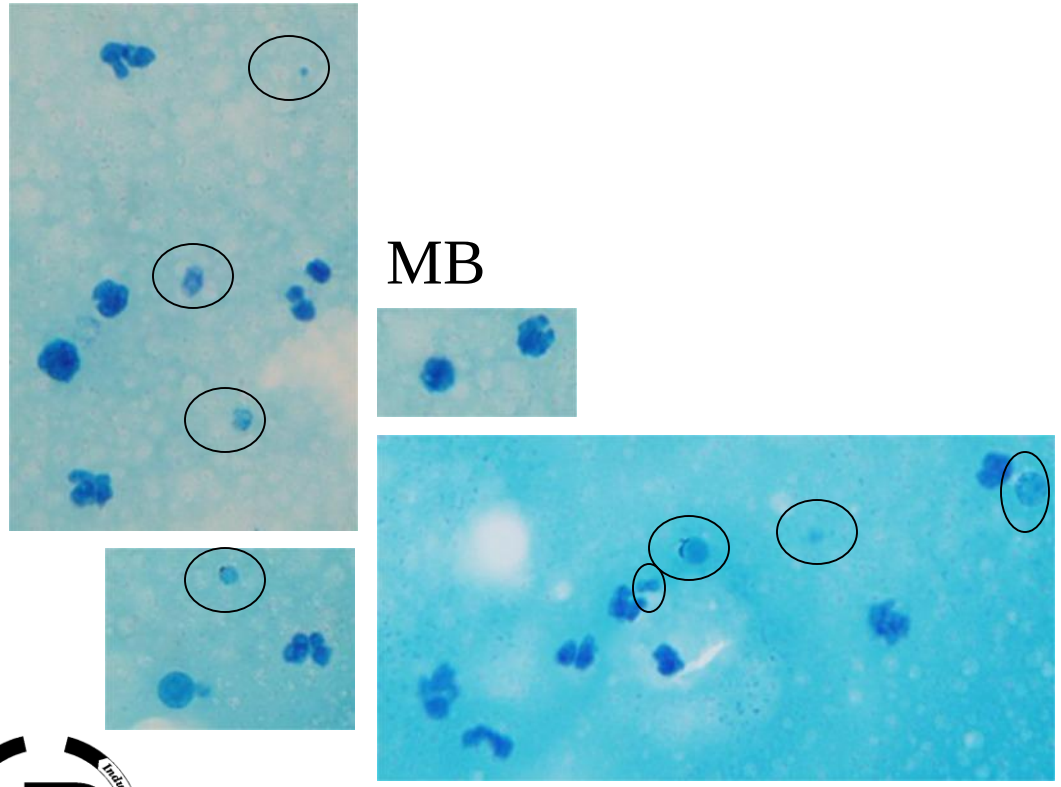
PY

Goat Milk

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



PY

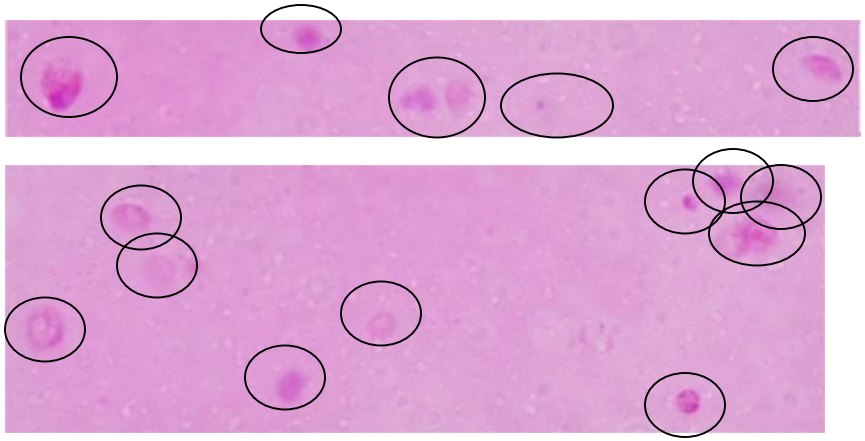


MB

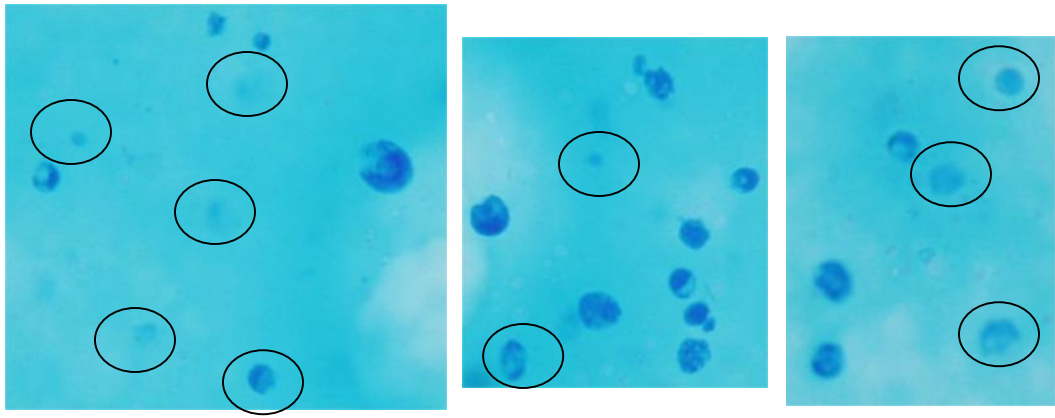
Sheep Milk

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

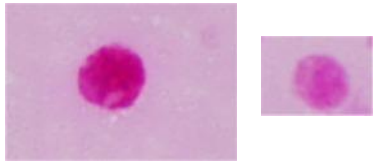
PY



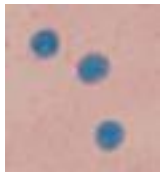
MB



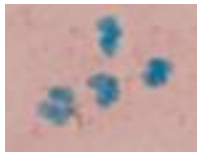
What Types of Cells are Counted?



Eosinophil



Lymphocytes



Polymorphonuclear (PMN)

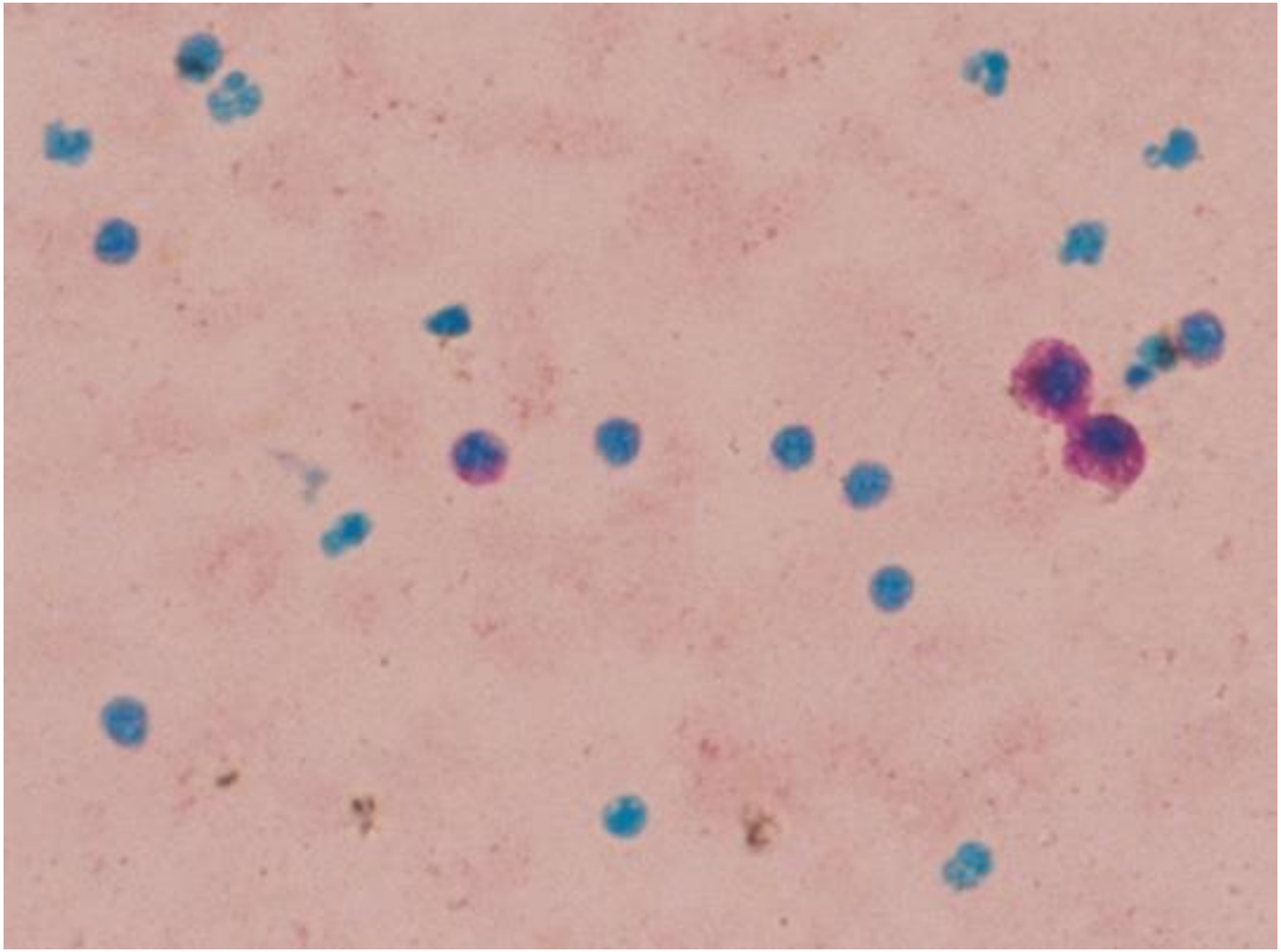
Fragments are counted only if more than 50% of the nuclear material is visible. See FDA DMSCC 2400 form for guidance.

What Types of Cells are NOT Counted?

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

Practice Slide - Goat A

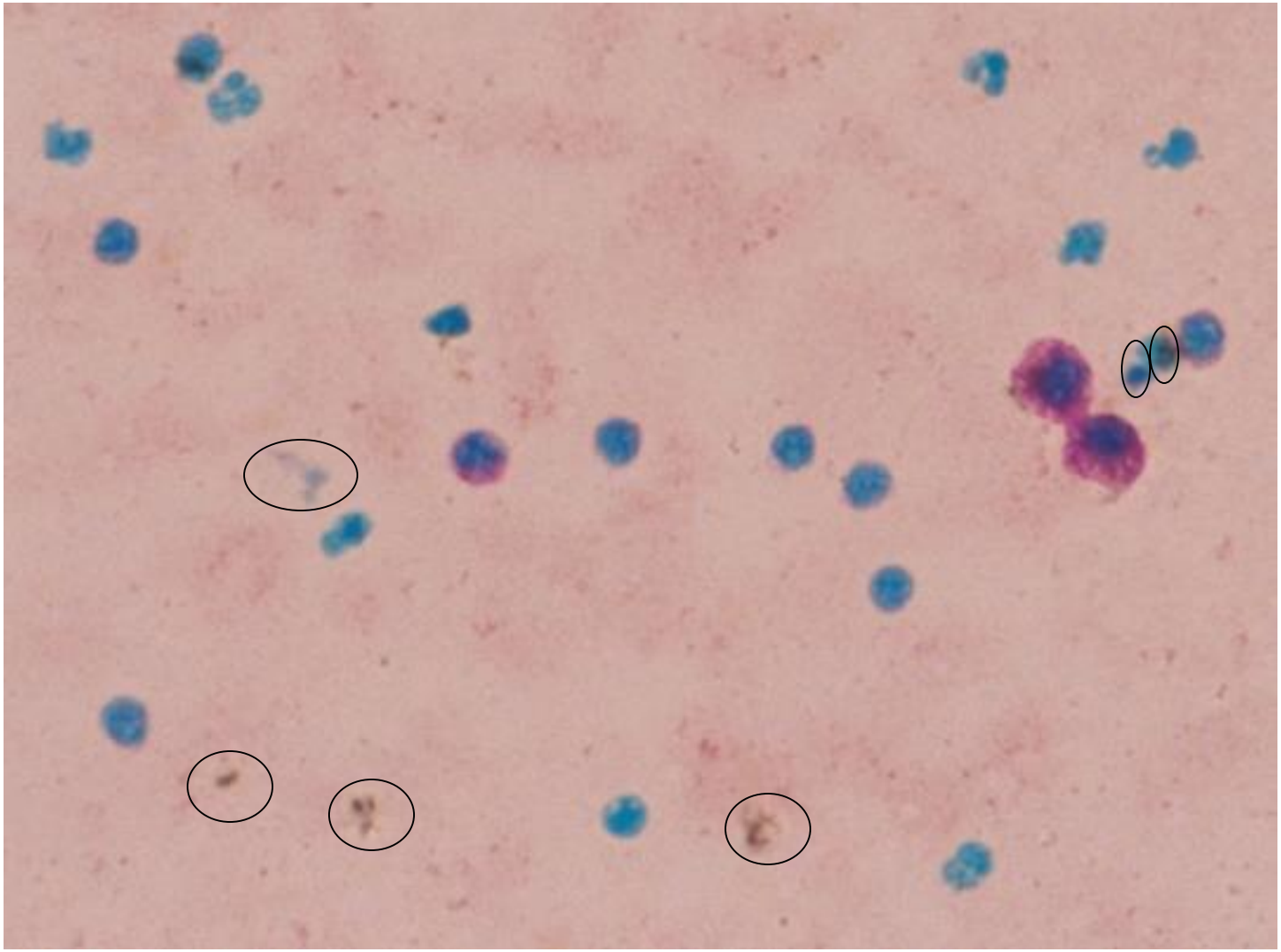
How many countable cells do you see?



Answer Slide - Goat A

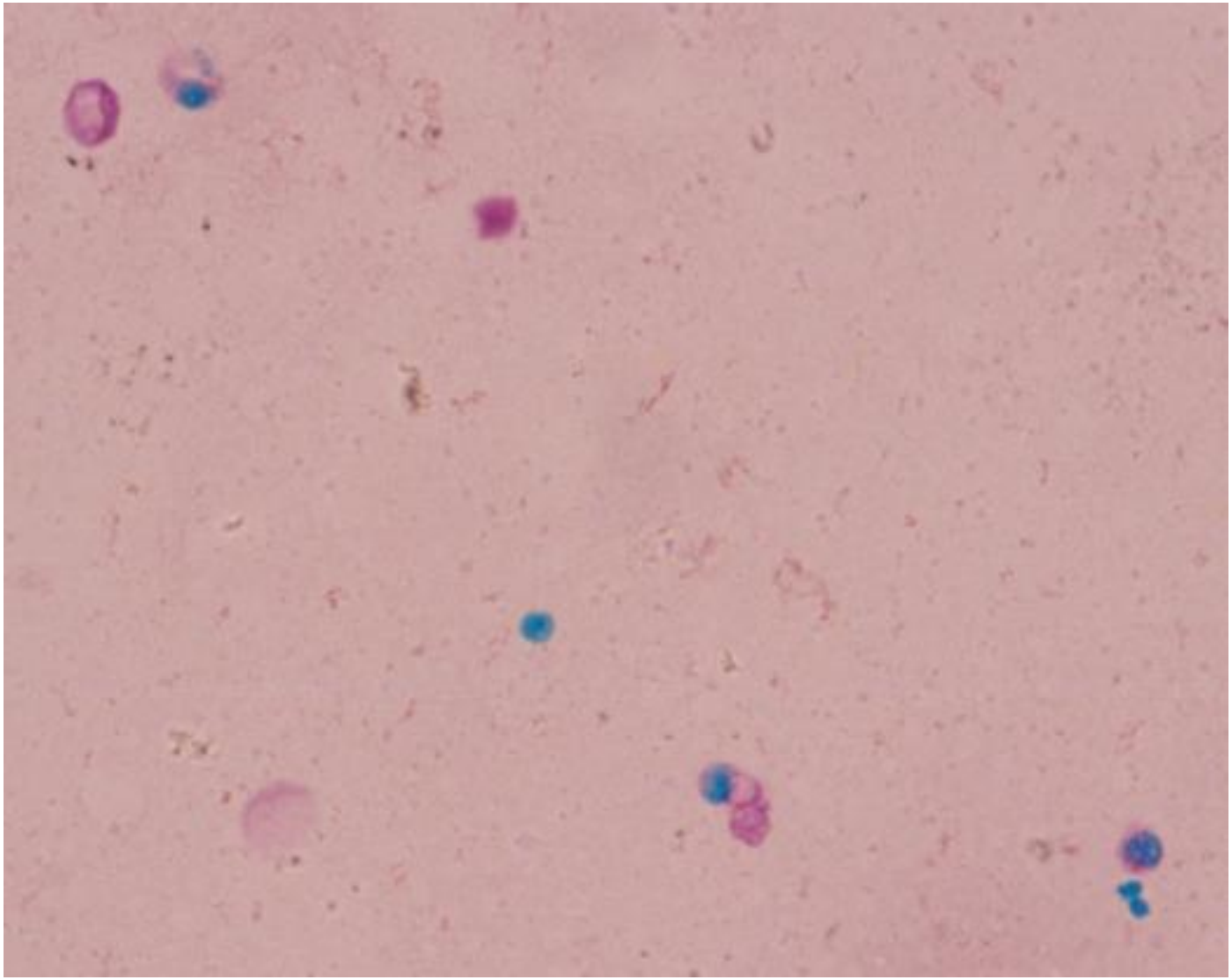
19 countable cells

Do not count the circled objects.

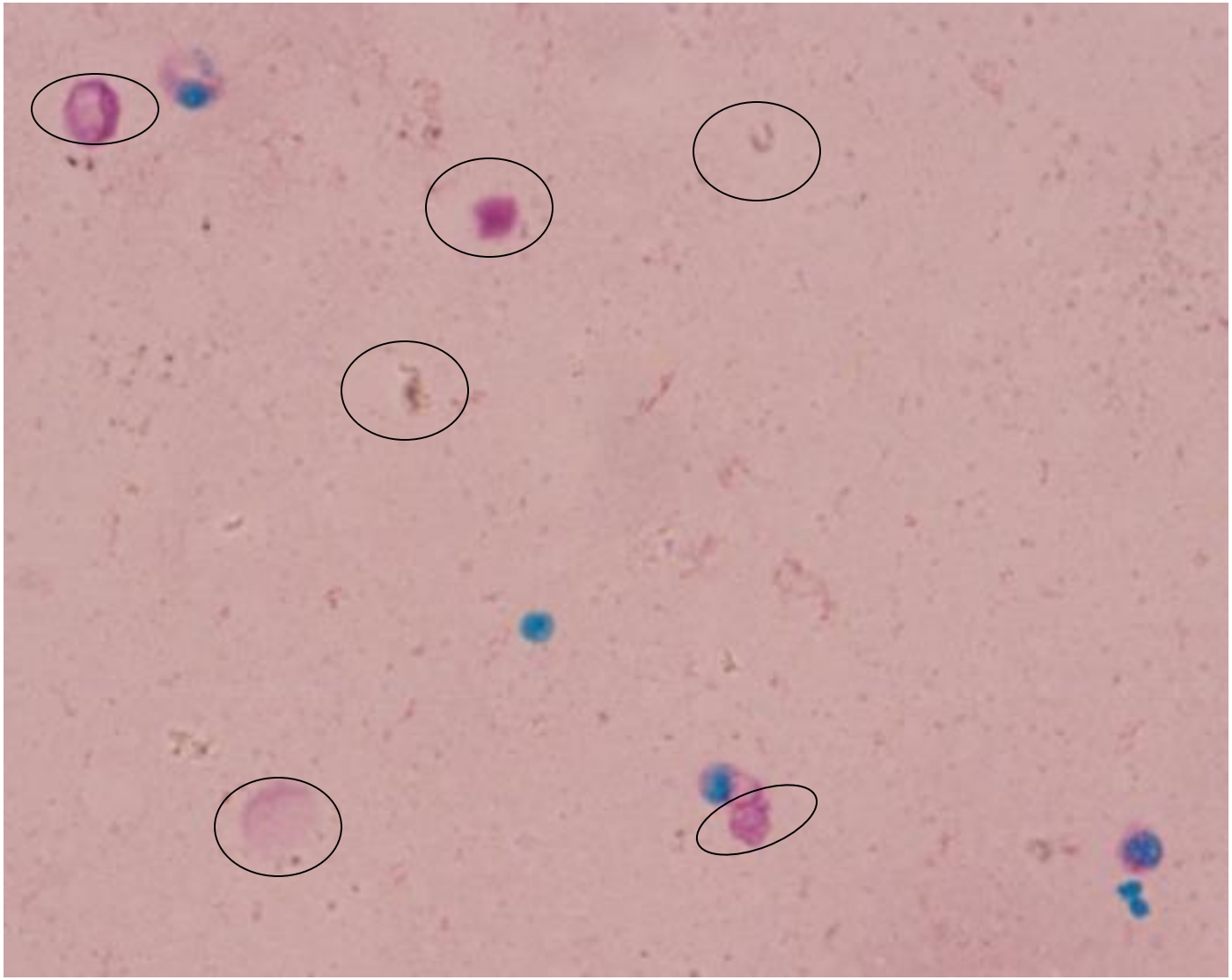


Practice Slide - Goat B

How many countable cells do you see?



Answer Slide - Goat B

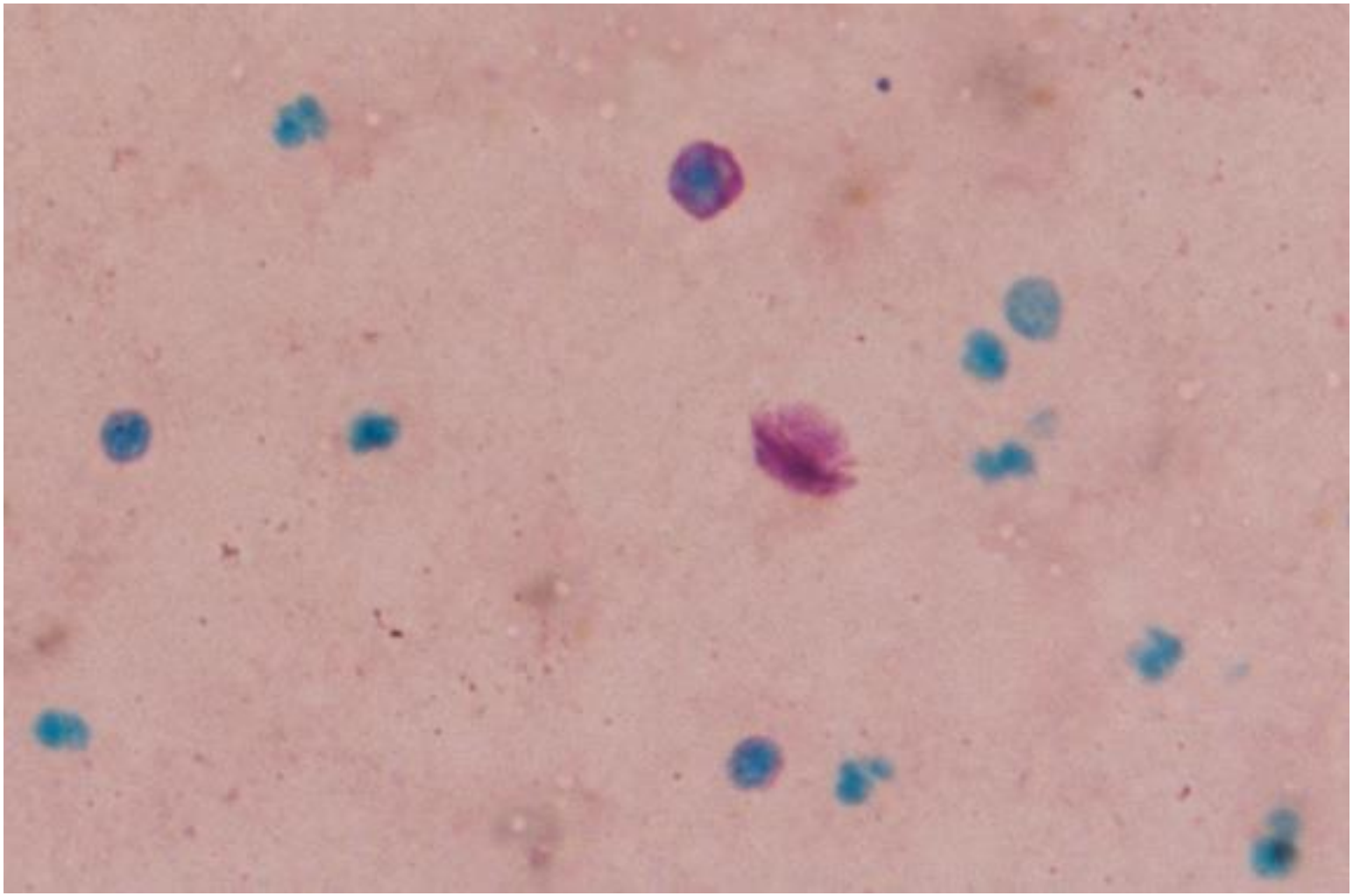


5 countable cells

Do not count the circled objects.

Practice Slide - Goat C

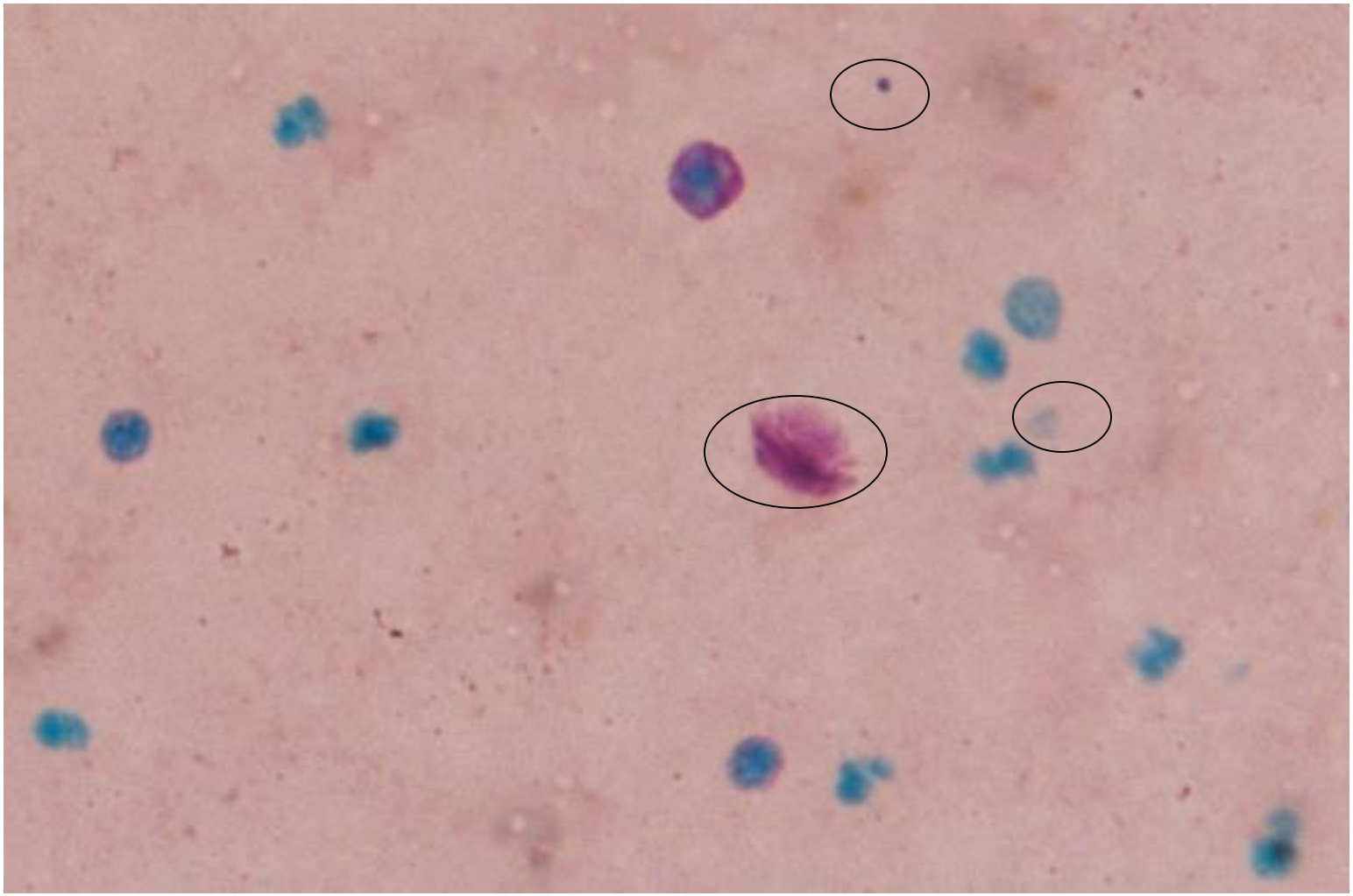
How many countable cells do you see?



Answer Slide - Goat C

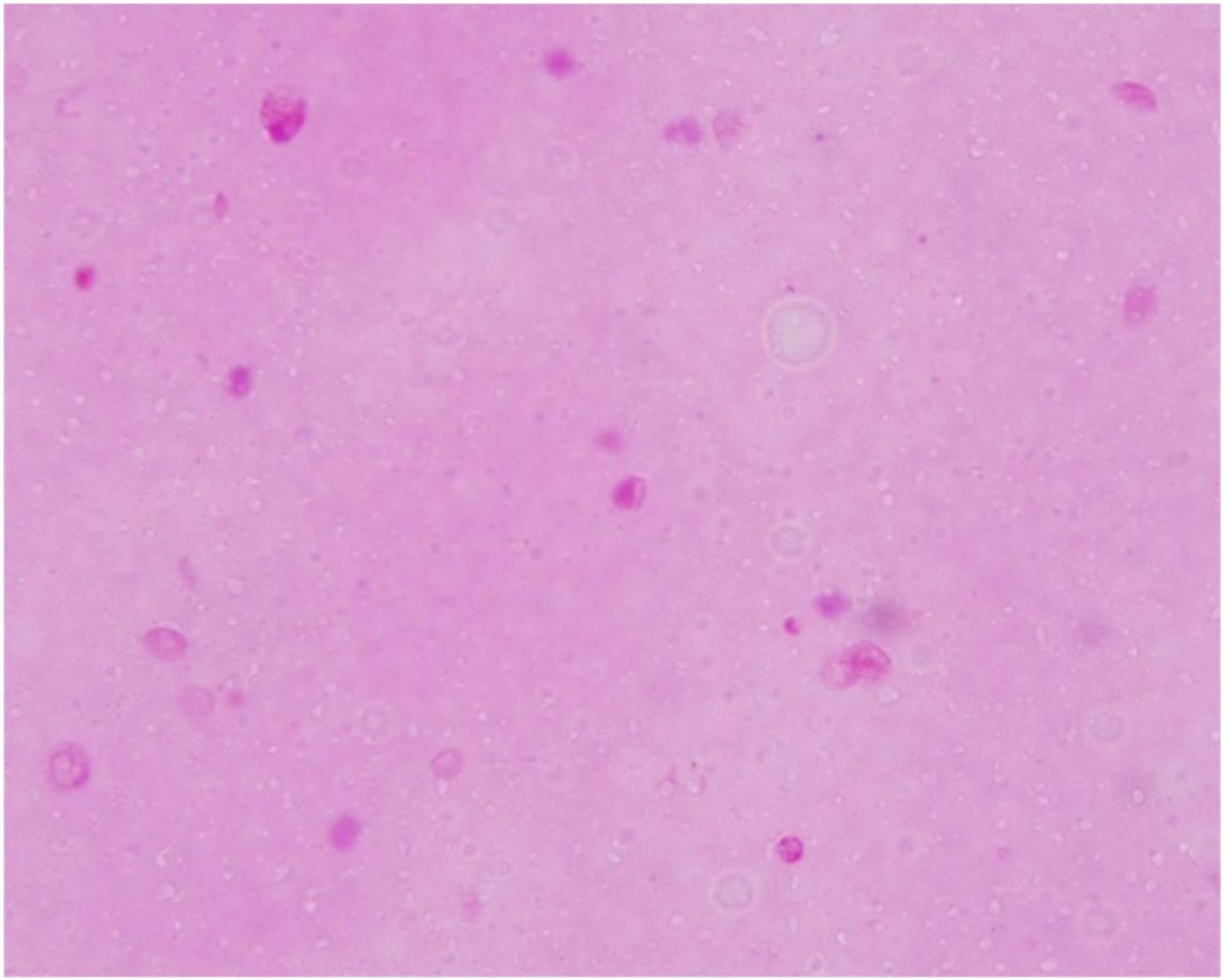
12 countable cells

Do not count the circled objects.

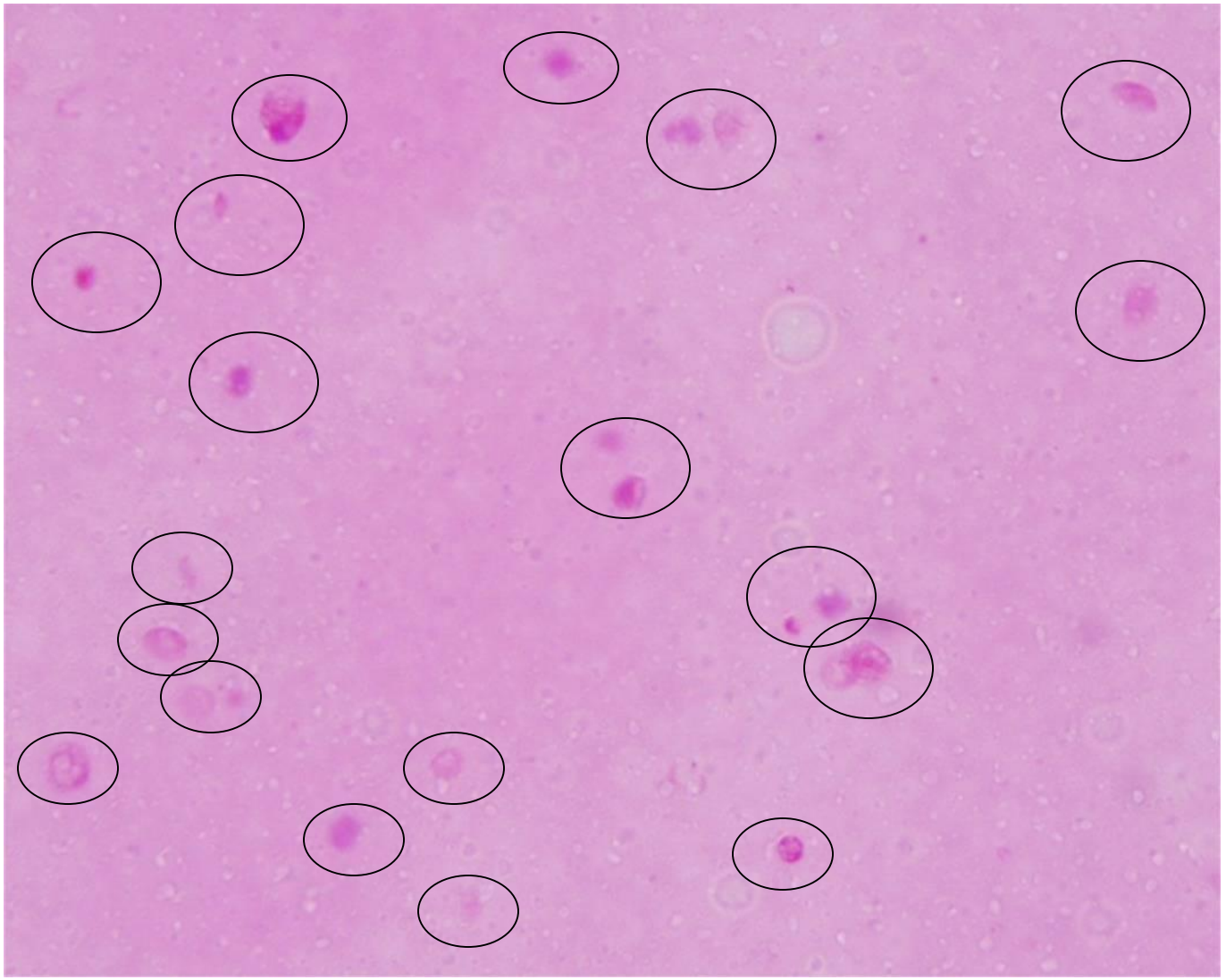


Practice Slide - Sheep A

How many countable cells do you see?



Answer Slide - Sheep A



0 countable cells

Do not count the circled objects.