MALARIAL PARASITES

GILLIAN ROZENBERG
TRAINING
WORKSHOPS

Geographical Areas of the World

Malaria occurs throughout most of the tropical areas of the world

- *Plasmodium falciparum* occurs mainly in Africa, New Guinea, Haiti and the Solomon Islands
- *Plasmodium vivax* is most commonly found in Central America and the Indian subcontinent
- *Plasmodium ovale* is found mainly in Sub-Saharan Africa

Geographical Areas of the World

- *Plasmodium malariae* is found throughout the endemic areas of the world, South America, Asia and Africa
- *Plasmodium knowlesi* is found in South East Asia

Clinical Symptoms

- Fever/rigors
- Night sweats
- Myalgia
- Headaches
- Vomiting and diarrhoea
- *P vivax* associated with splenomegaly
- *P falciparum* cerebral malaria, acute renal failure, severe anaemia
- *P malariae* nephrotic syndrome

Investigations

Full blood count

- Leucopenia
- Thrombocytopenia
- Anaemia in some cases

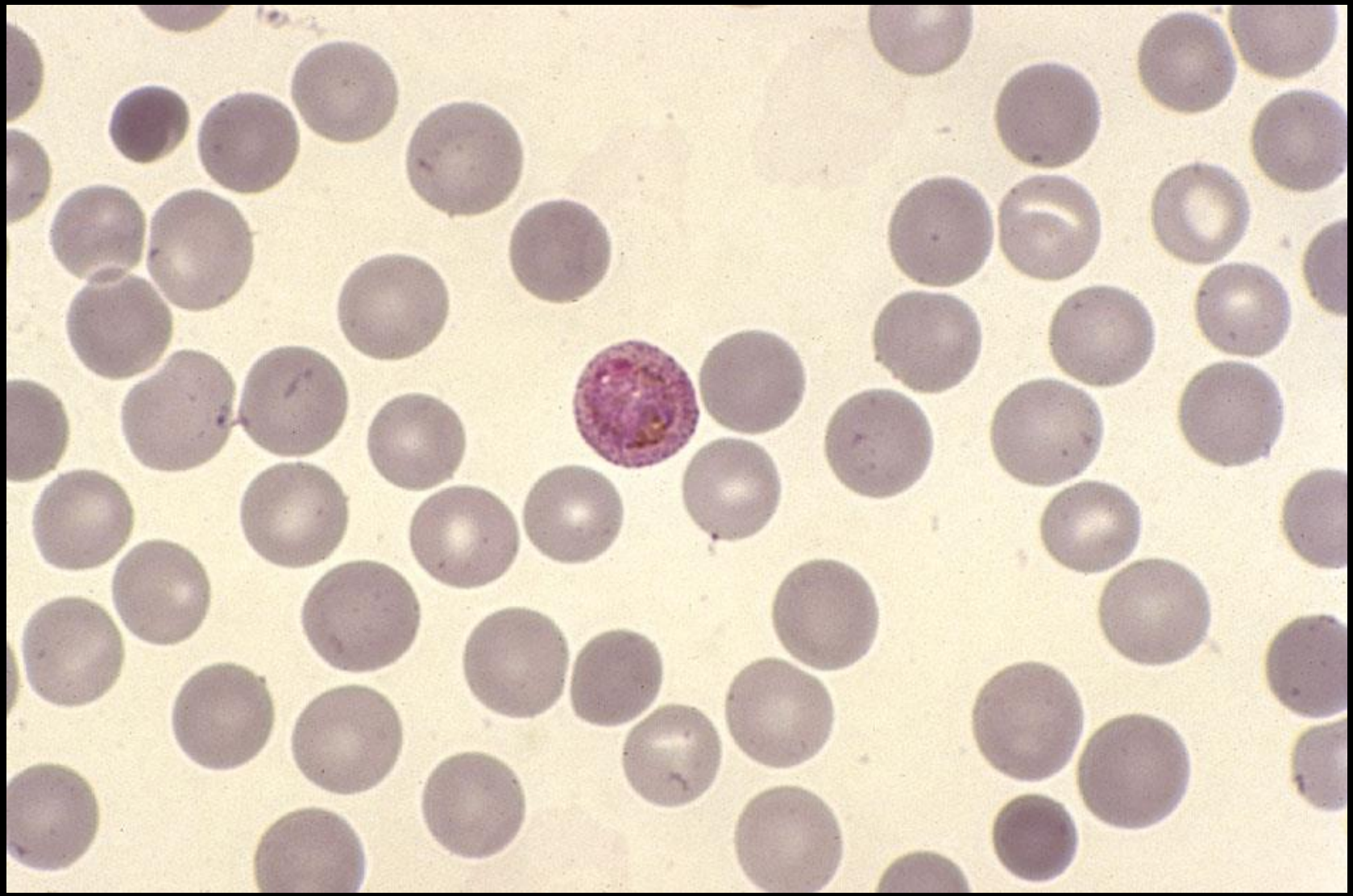
G6PD screen

Thin blood films

Two thin blood films made to assess parasite morphology and stage of development within the red cell

- Romanowsky stained film stained at pH 6.8
- To aid in the differential diagnosis of malaria, the second thin film stained pH 7.2 to stain Schüffner's dots present in *Plasmodium vivax* and *ovale* and Maurer's clefts present in *Plasmodium falciparum*

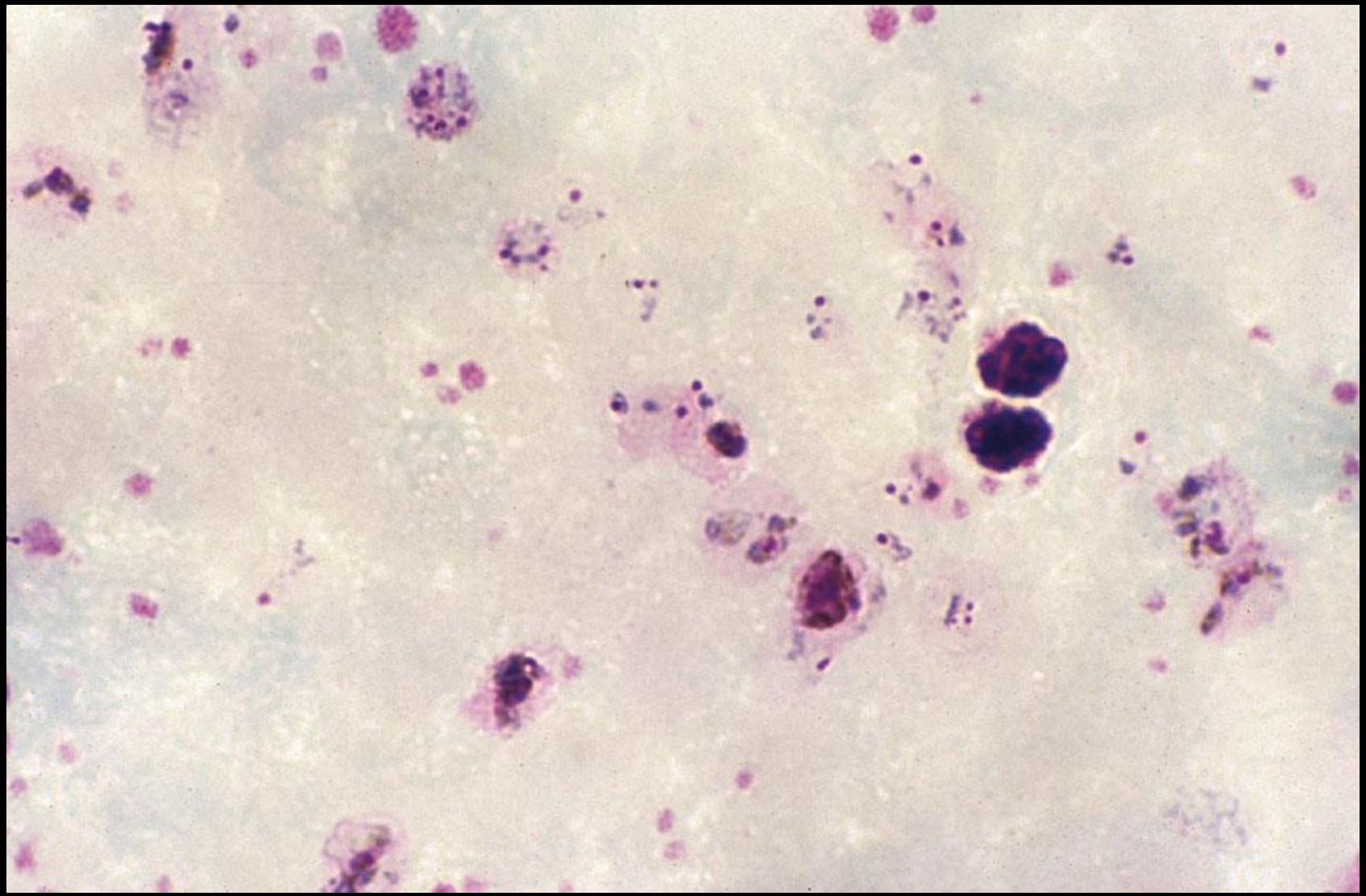
Thin film stained at pH 7.2



Thick blood films

Thick films concentrate parasites (low density)
Stain with Field's stain (commercial water based stain at pH 7.2)
Methylene blue (solution A) and eosin (solution B)
Dry slides for 10 minutes and place on top of heat block
Solution A 2 secs. rinse in buffered water
Solution B 4 secs. rinse in buffered water
Dry and examine under oil immersion

Thick film stained with Field's stain pH 7.2



Diagnostic Characteristics

Plasmodium falciparum

- Infected red cells not enlarged
- Only early trophozoites unless heavy density
- Early trophozoites thin / delicate cytoplasm, often 2 chromatin dots and Maurer's dots or clefts (pseudopodal attachment to red cell) with pH 7.2
- Multiple infection of red cells
- Accolé or marginal forms

Diagnostic Characteristics

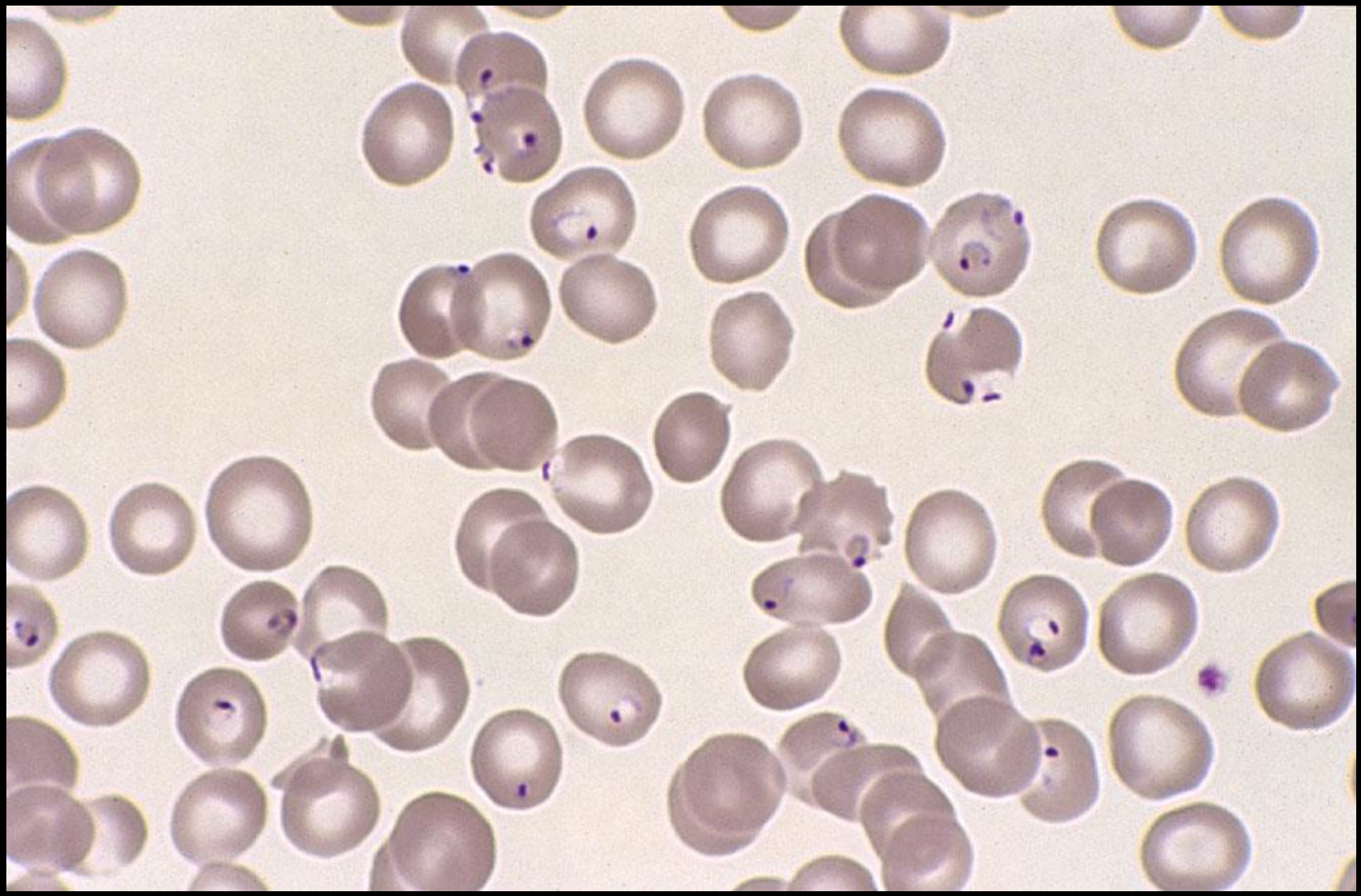
- Late trophozoites, rarely seen in PB; compact cytoplasm; dark pigment
- Schizonts contain between 16 and 36 merozoites (may be as few as 8 and as many as 40)
- Gametocytes crescent shaped with a central chromatin mass
- Male gametocytes pink; females blue

Case study
Plasmodium falciparum

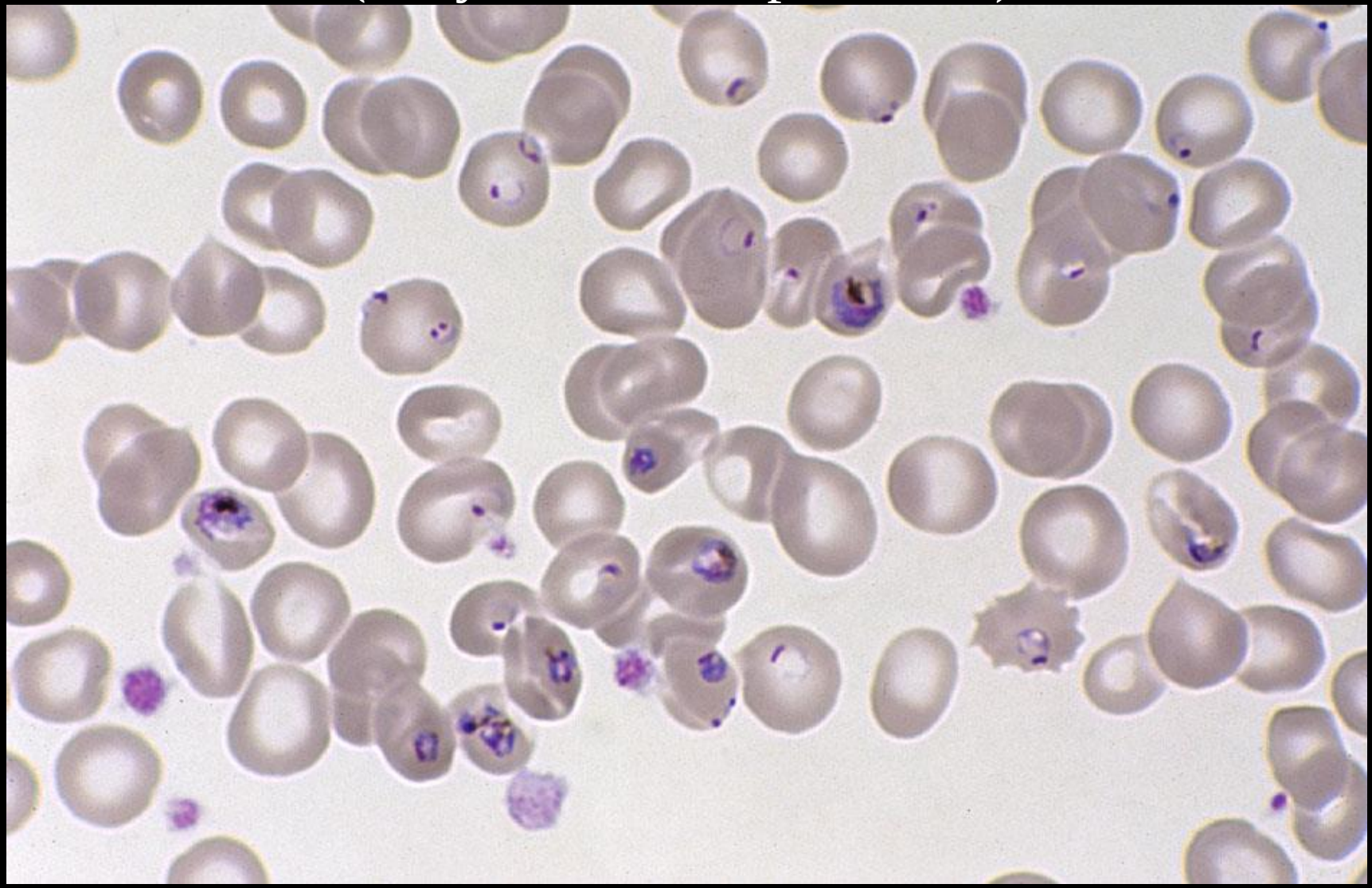
Male 35 years returns from the Solomon Islands with fever, rigors and confusion.
He was passing dark urine; he had cerebral malaria.

WCC	2.5 x 10^9/L	NR (3.5-11.0)
Hb	62 g/L	NR (130-180)
Platelets	33 x 10^9/L	NR (150-400)

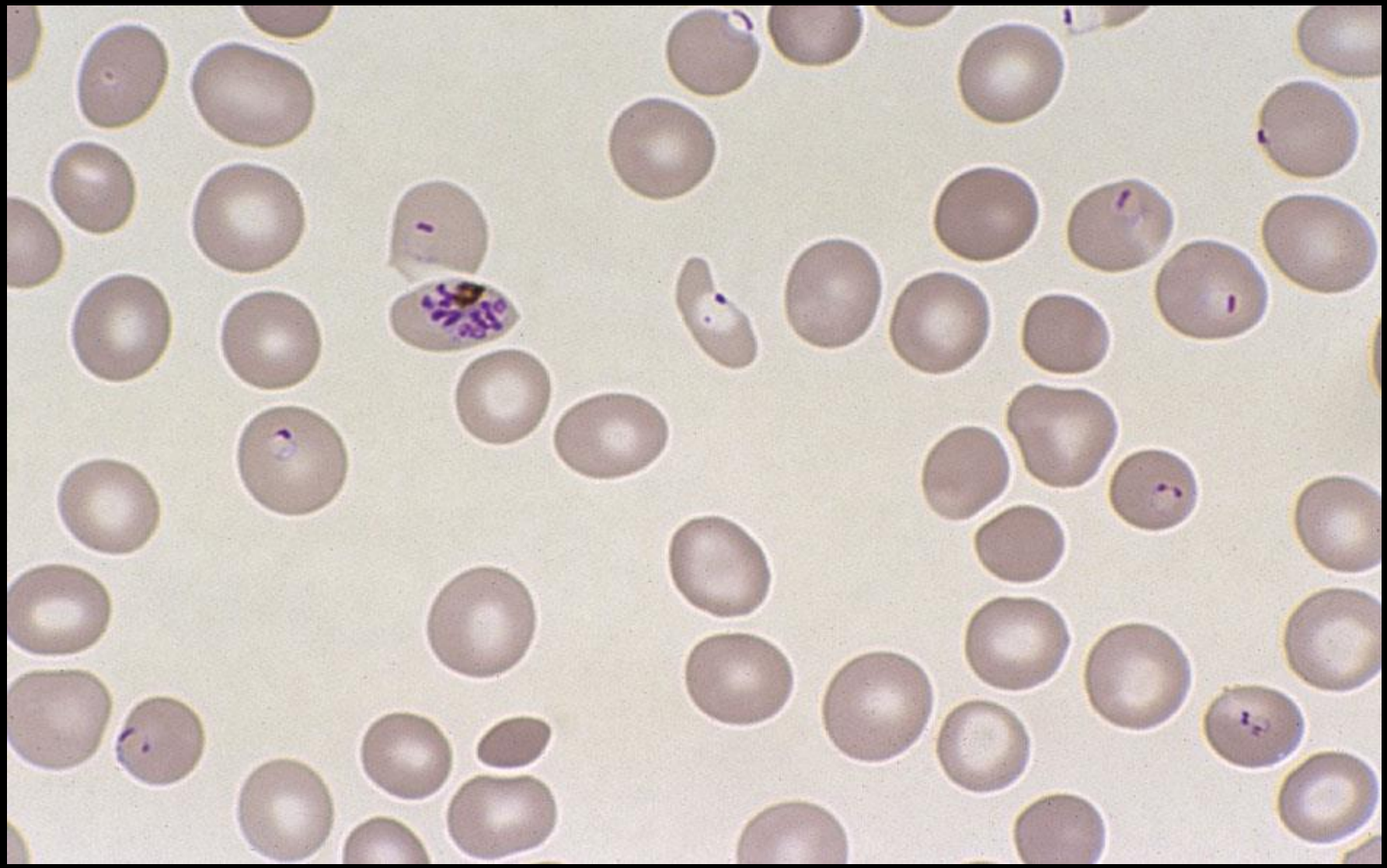
Plasmodium falciparum
(early trophozoites)



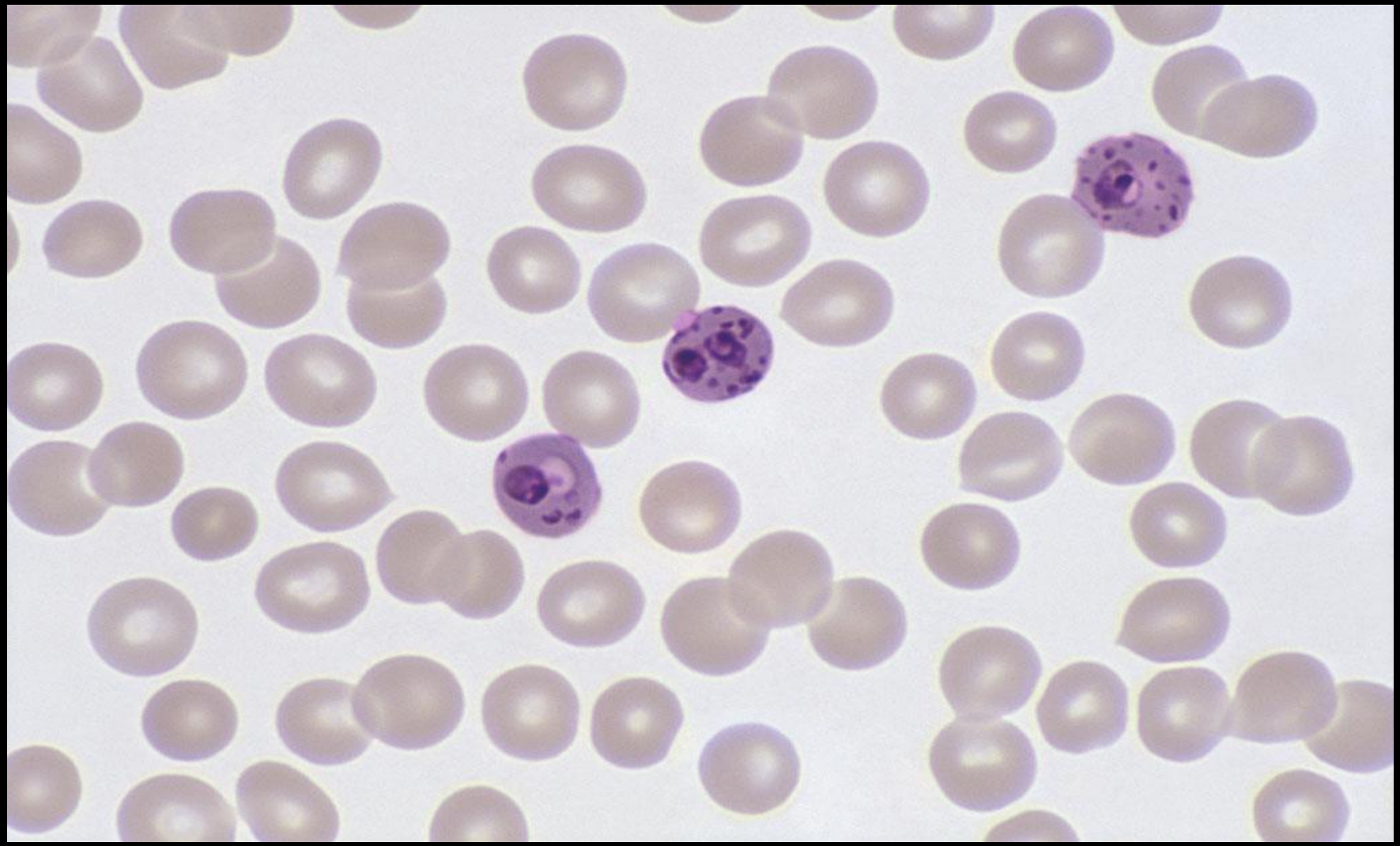
Plasmodium falciparum
(early and late trophozoites)



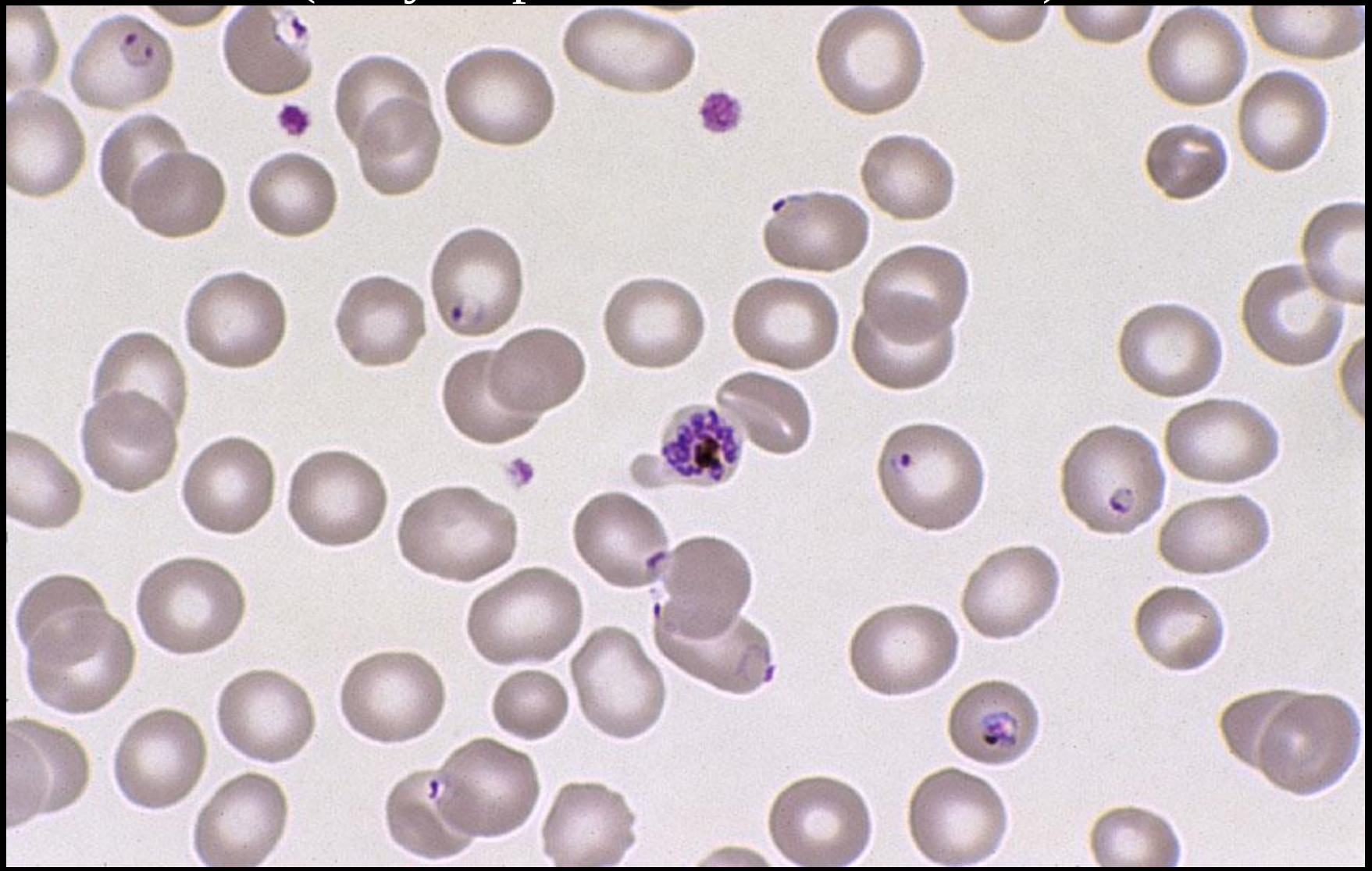
Plasmodium falciparum
(early trophozoites and schizont)



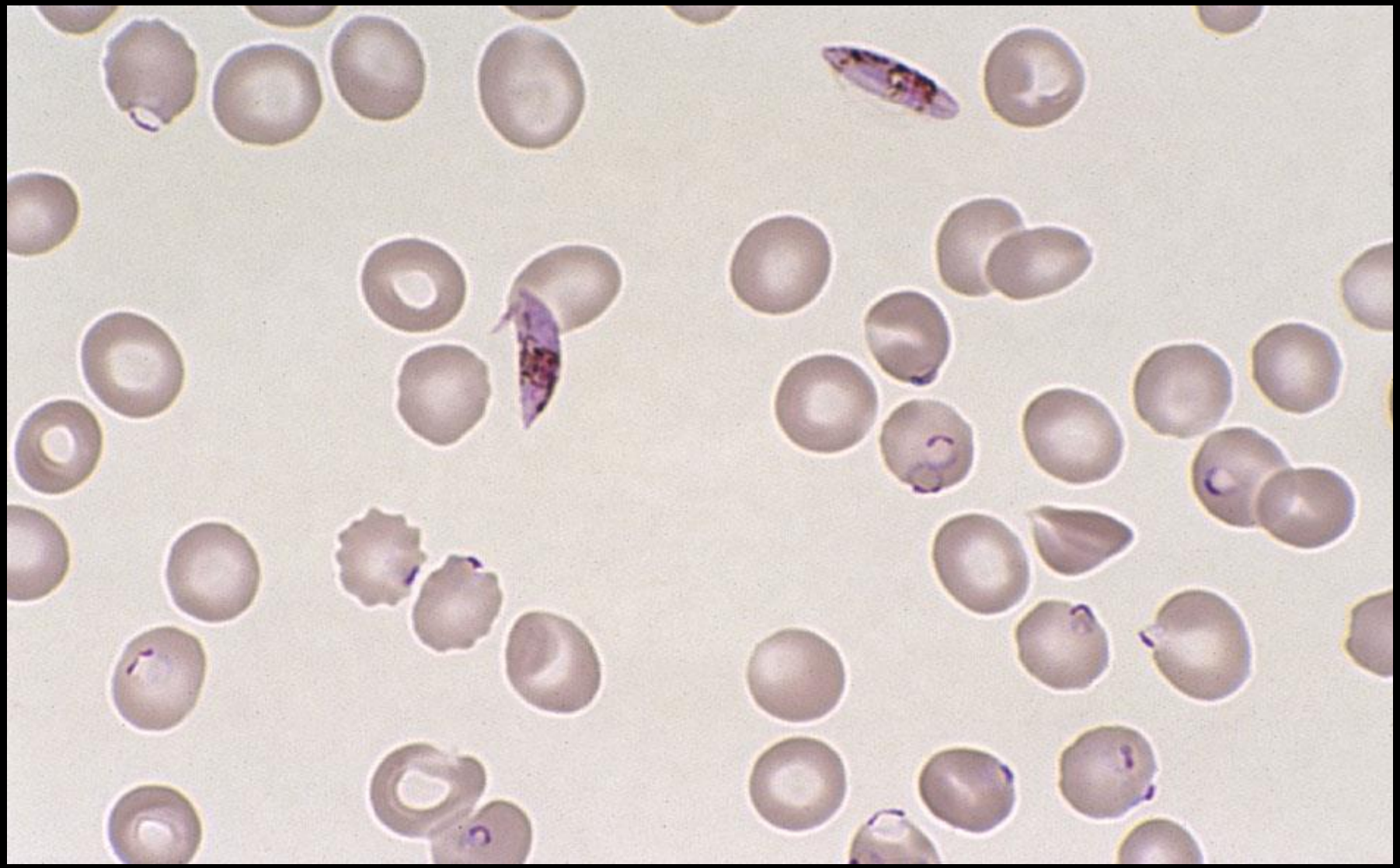
Plasmodium falciparum
(Maurer's clefts pH 7.2)



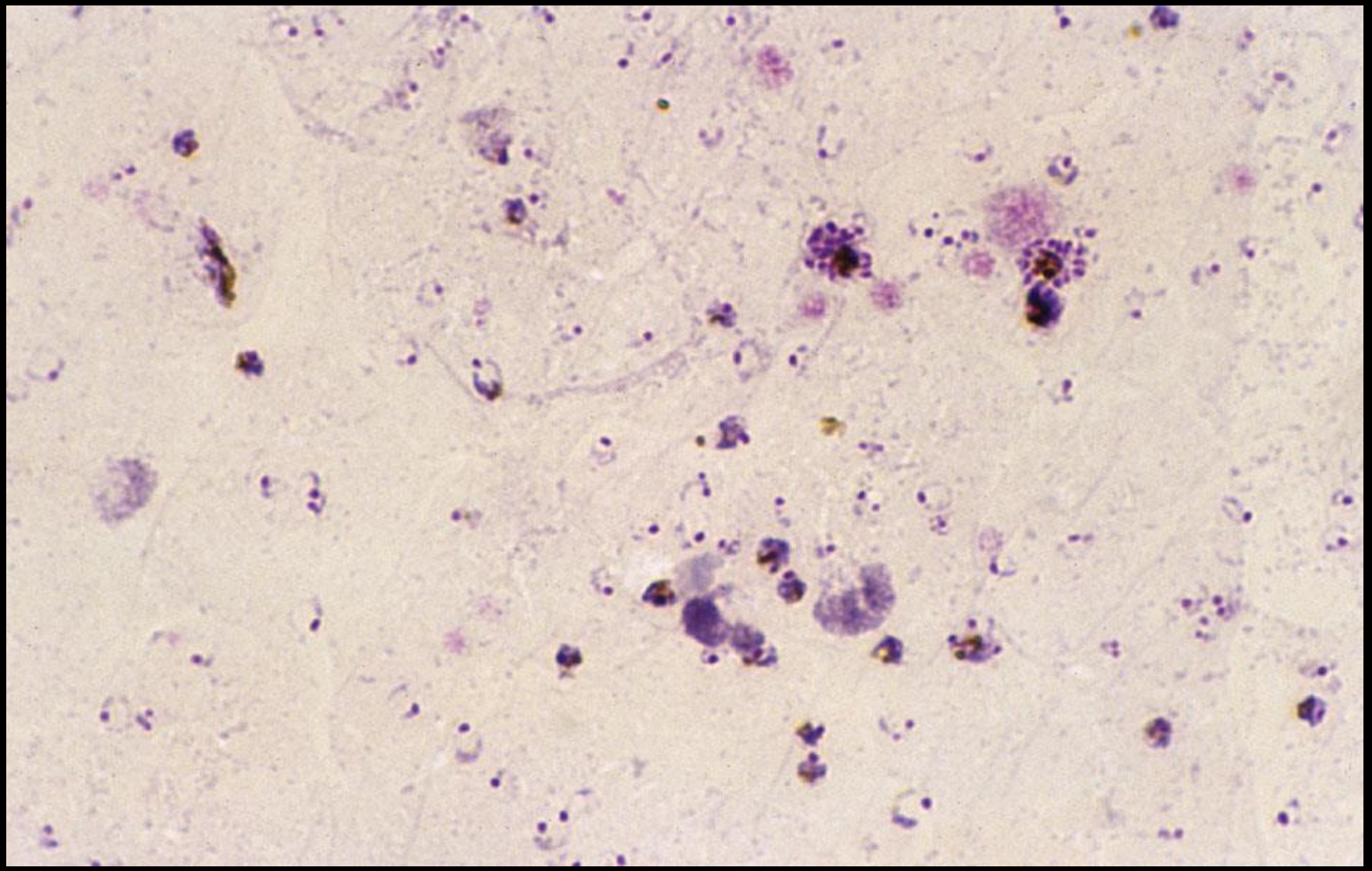
Plasmodium falciparum
(early trophozoites and schizont)



Plasmodium falciparum
(early trophozoites and gametocytes)



Plasmodium falciparum
(early and late trophozoites/schizonts/gametocytes)
Field's stain pH 7.2



Diagnostic Characteristics

Plasmodium vivax

- Infected red cells enlarged
- Parasites at all stages of maturation
- Early trophozoites, cytoplasm larger and thicker, chromatin dot larger than their *falciparum* counterparts
- Rarely have 2 chromatin dots
- Multiple infection of red cells only when parasite density is high
- Late trophozoites, cytoplasm irregular in outline, amoeboid, occupying most of the red cell

Diagnostic Characteristics

- Schüffner's dots present when stained with pH 7.2 (nature of these dots is unknown)

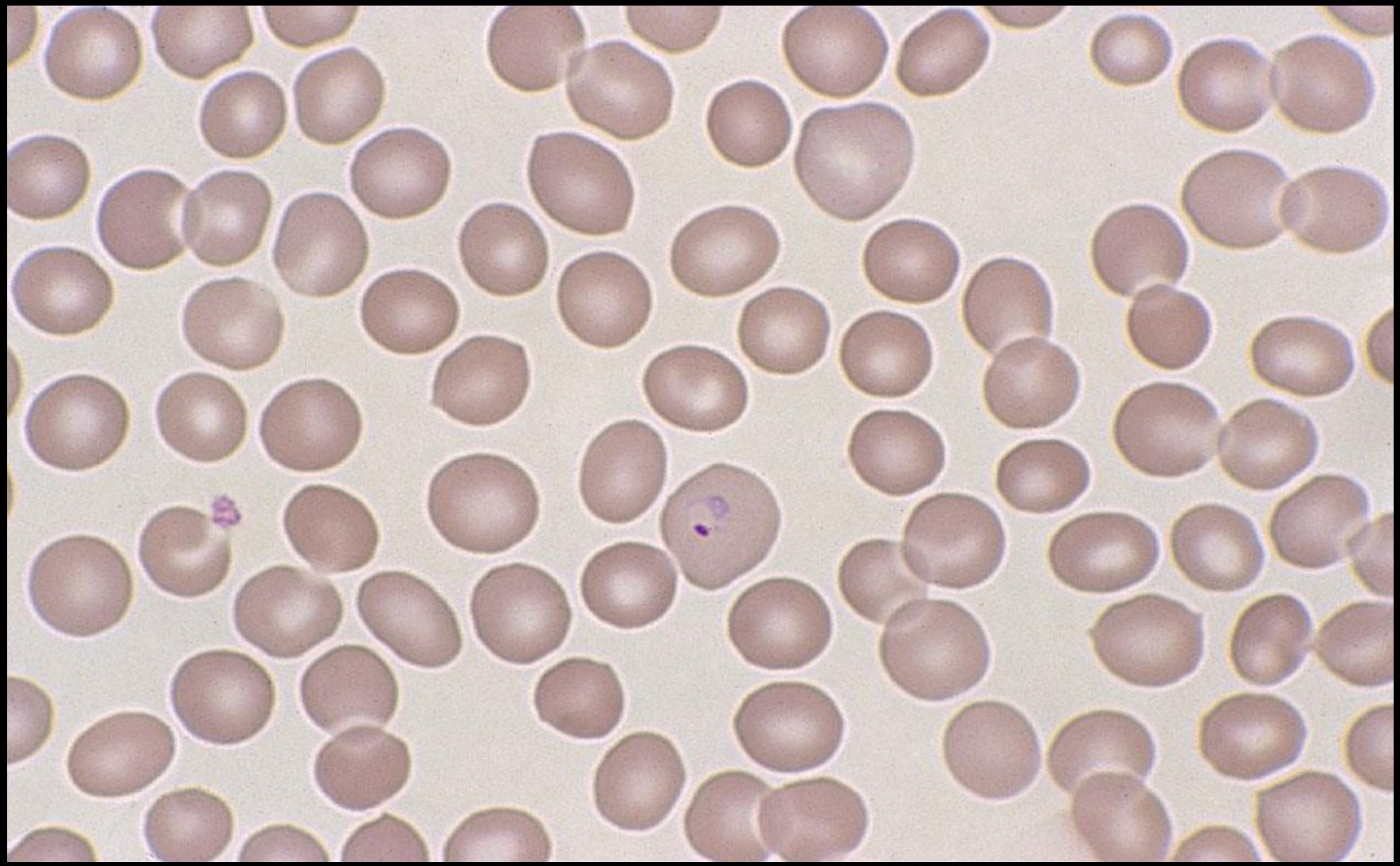
  Schüffner believed them to be related to changes in the host cells making them susceptible to eosin in the Romanowsky stain
- Schizonts contain 12 to 24 merozoites
- Gametocytes are round and fill the red cell

Case study
Plasmodium vivax

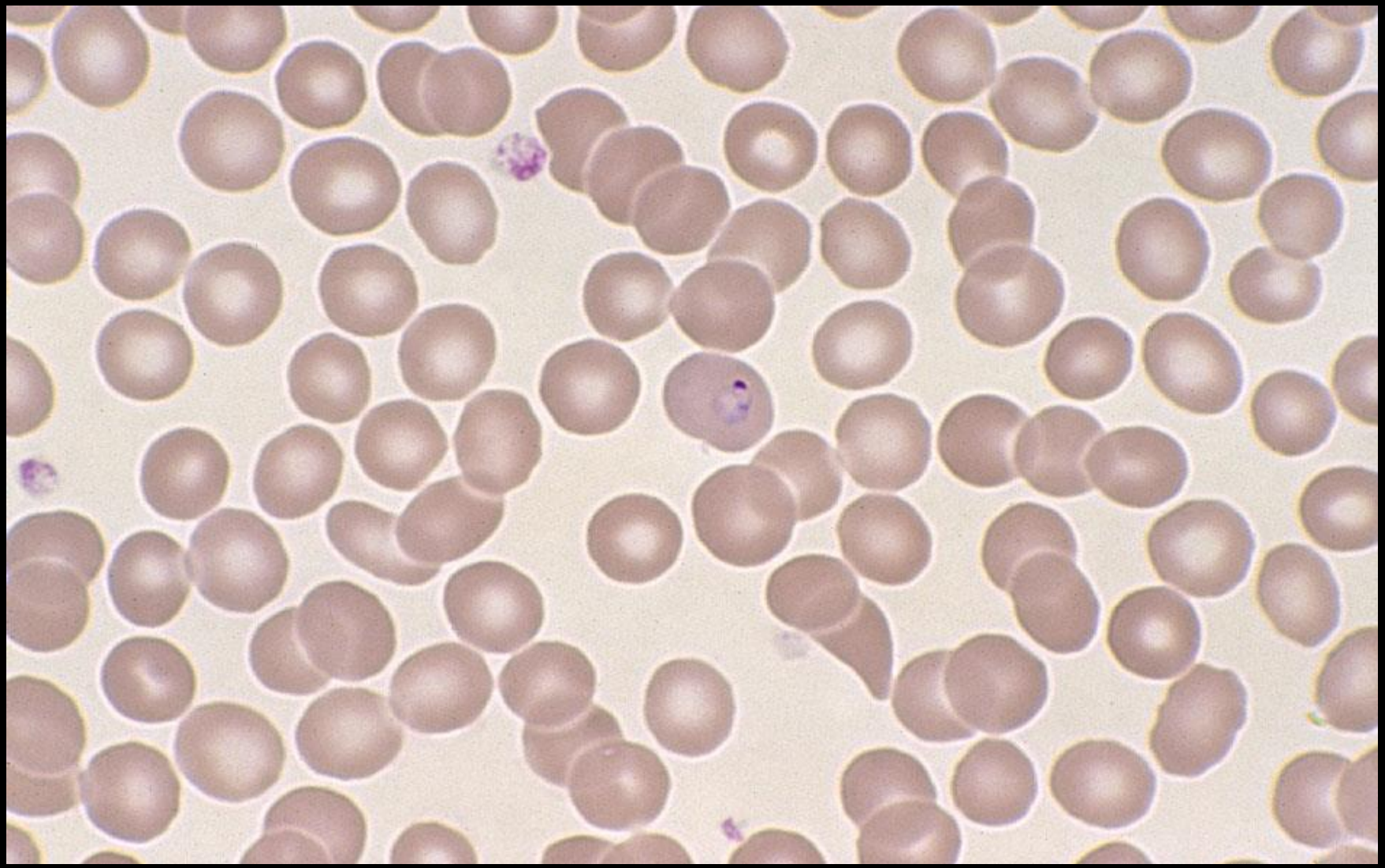
Male 23 years returns from New Guinea with lassitude and fever

WCC	4.6 x 10^9/L	NR (3.5-11.0)
Hb	98 g/L	NR (130-180)
Platelets	69 x 10^9/L	NR (150-400)

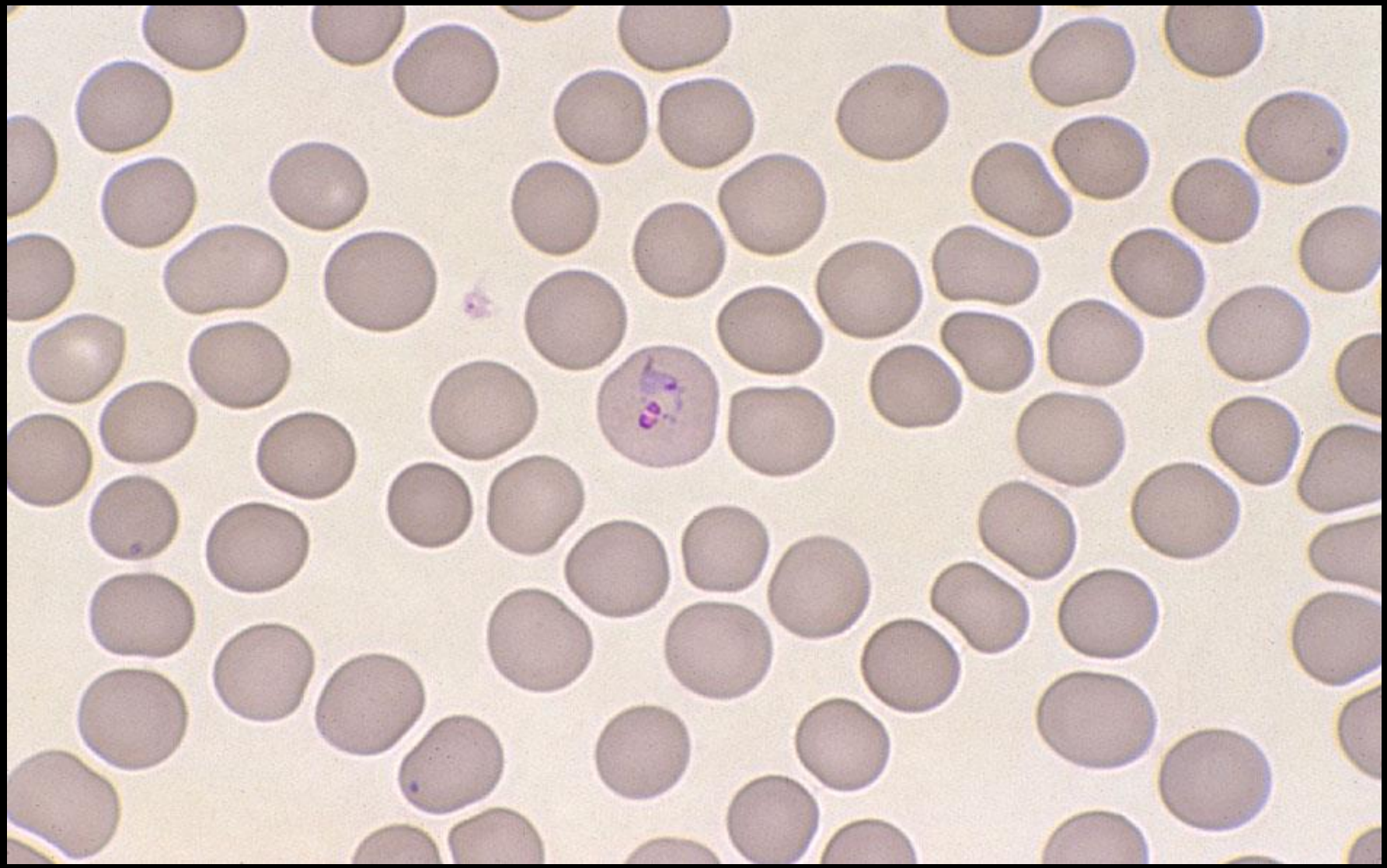
Plasmodium vivax
(early trophozoite)



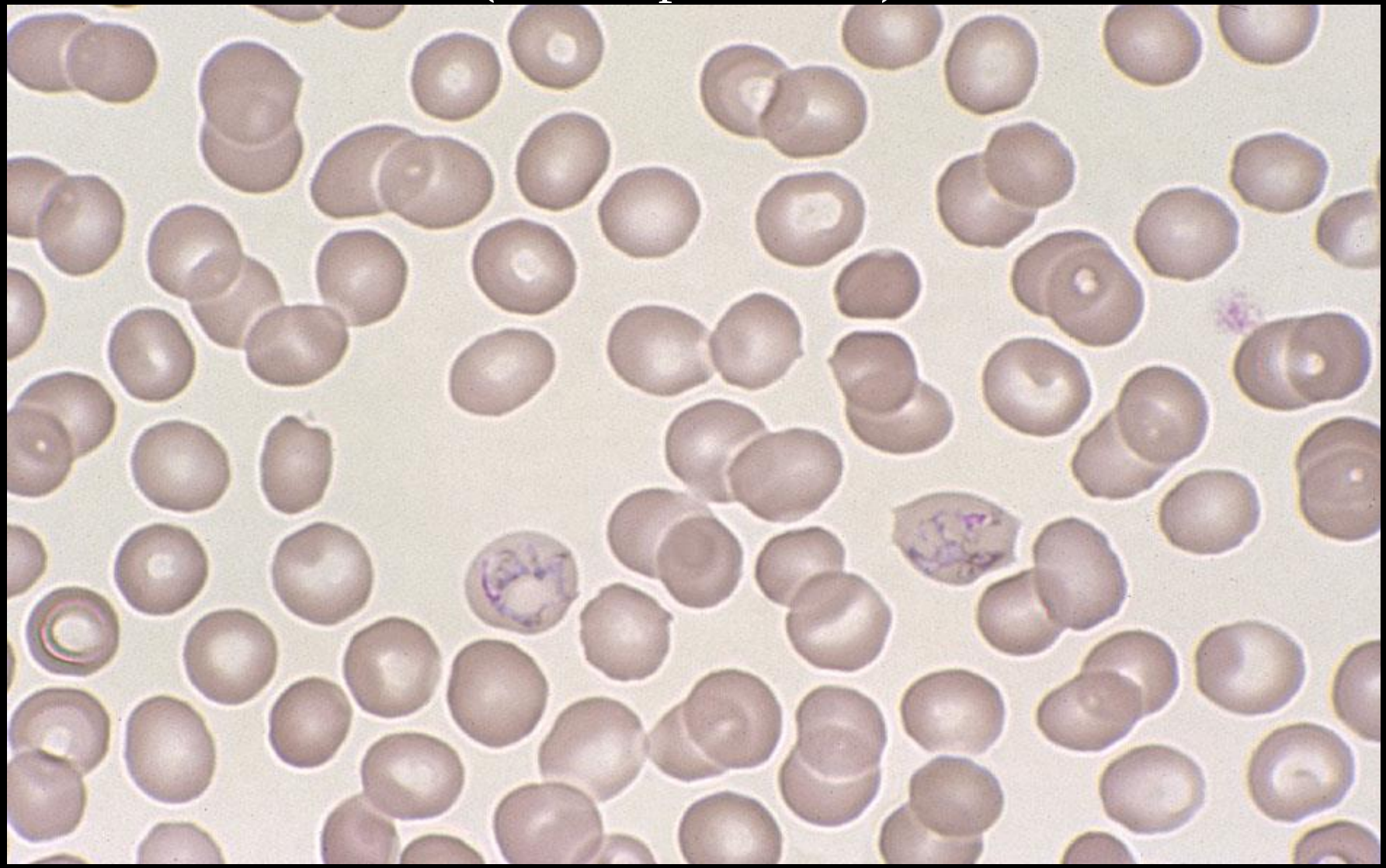
Plasmodium vivax
(early trophozoite)



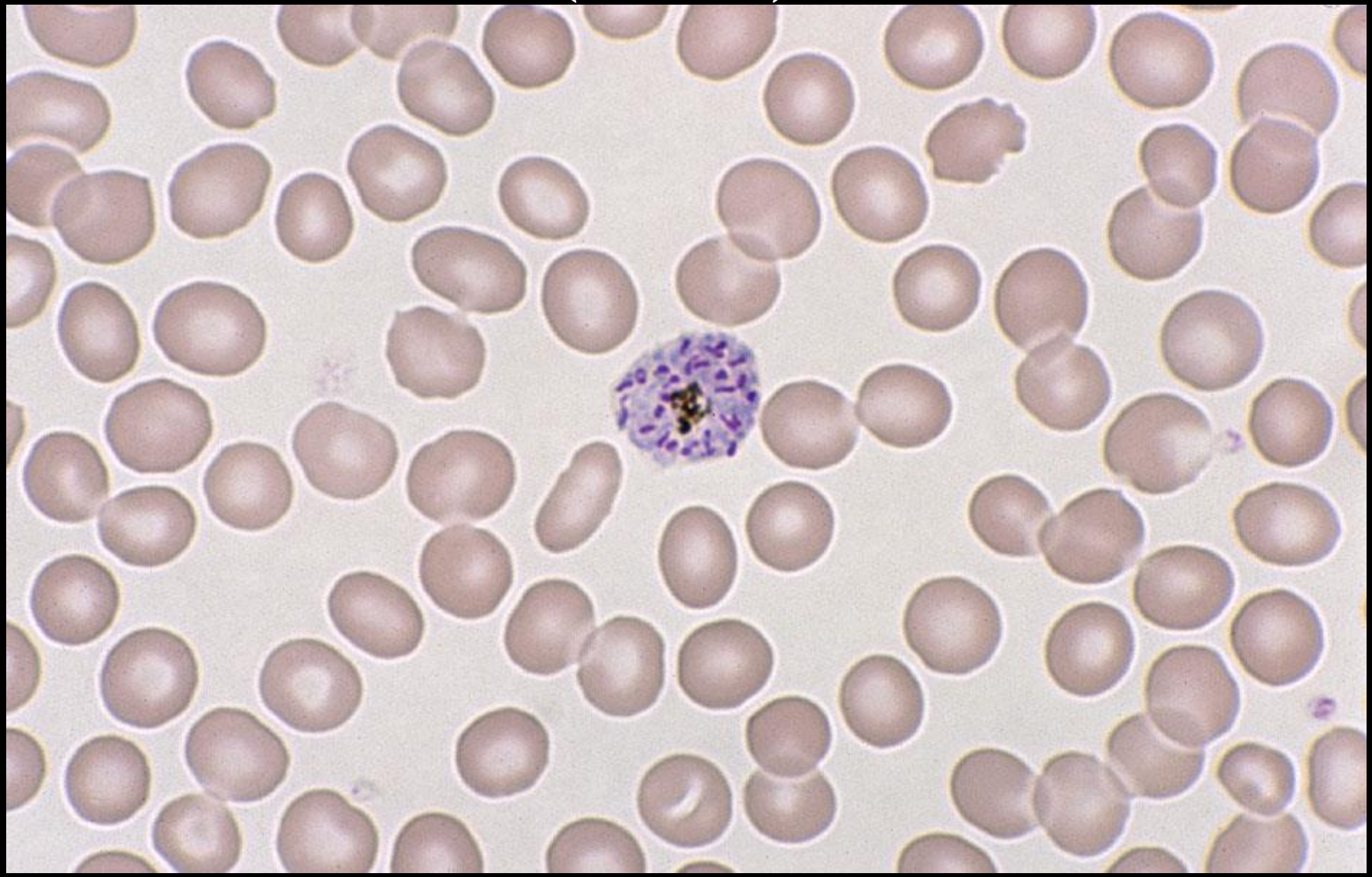
Plasmodium vivax
(late trophozoites)



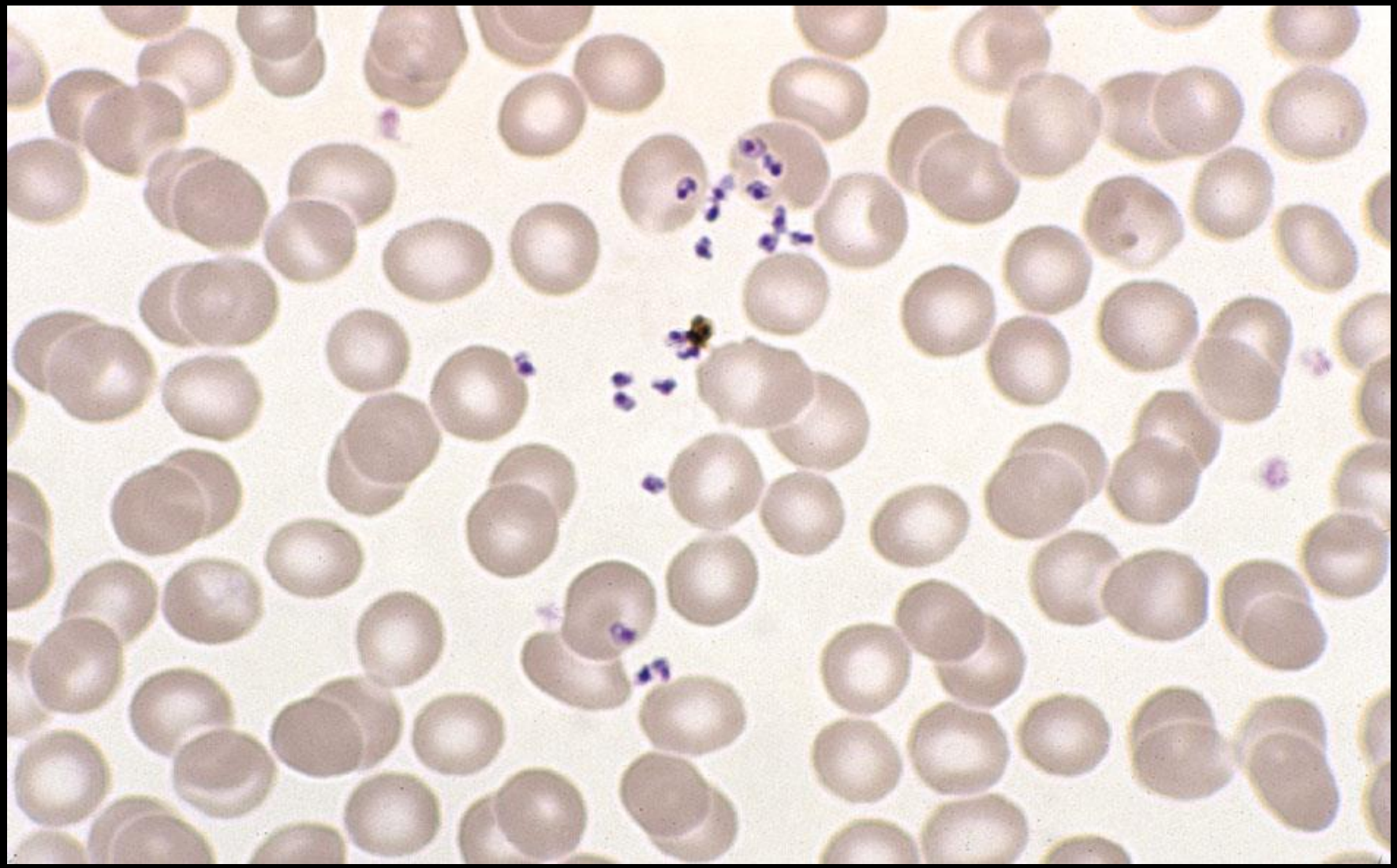
Plasmodium vivax
(late trophozoites)



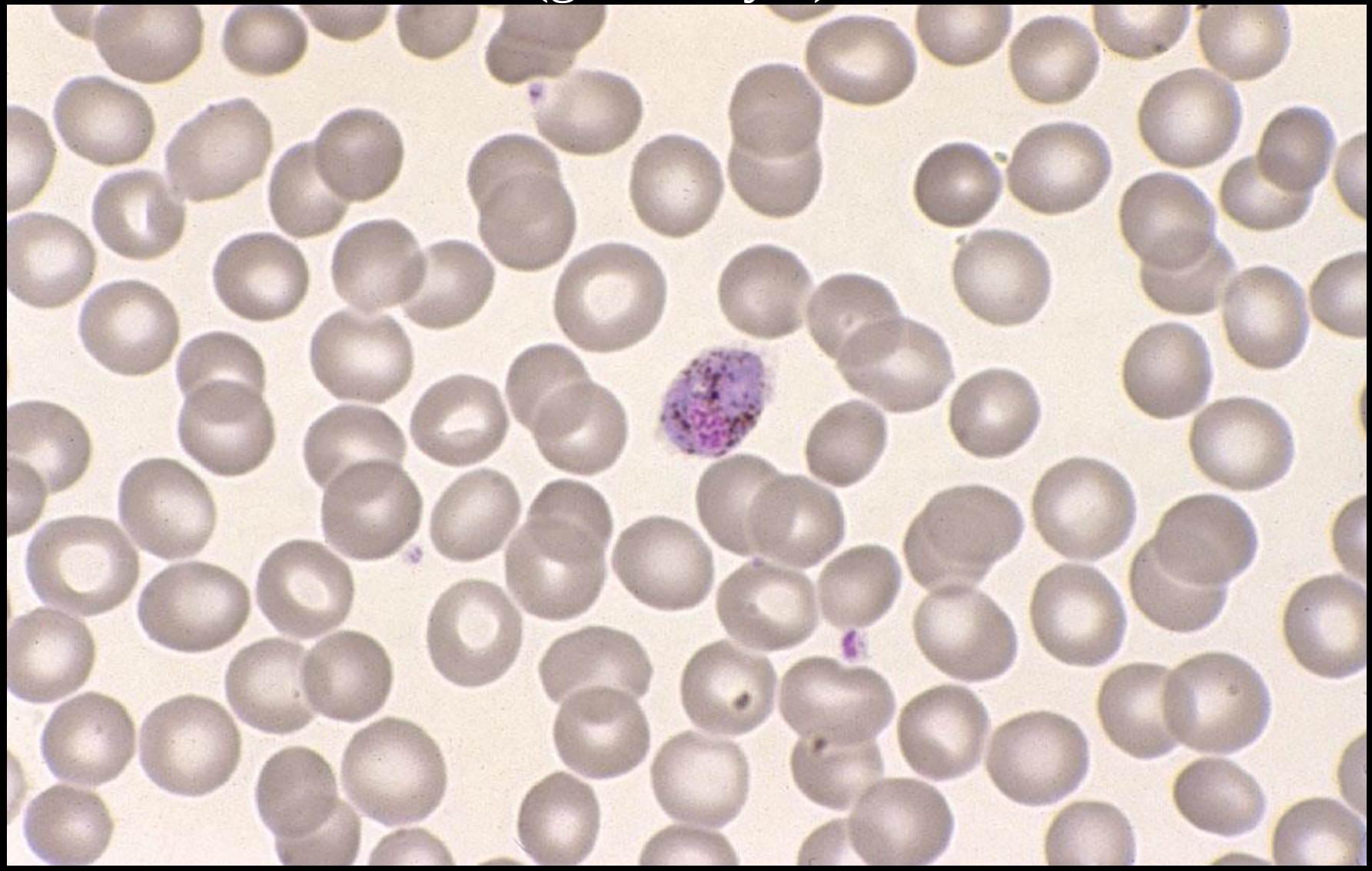
Plasmodium vivax (schizont)



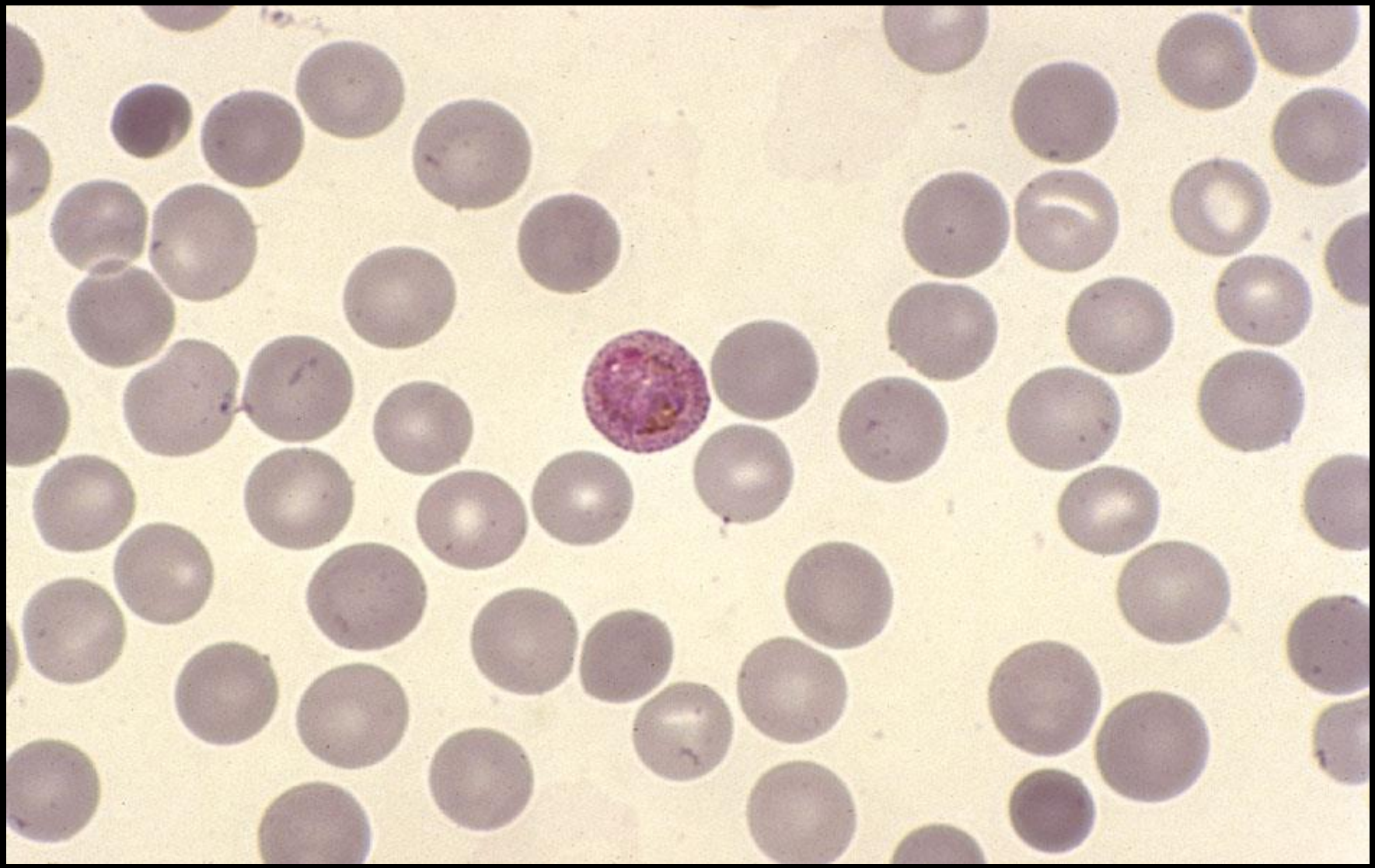
Plasmodium vivax (merozoites)



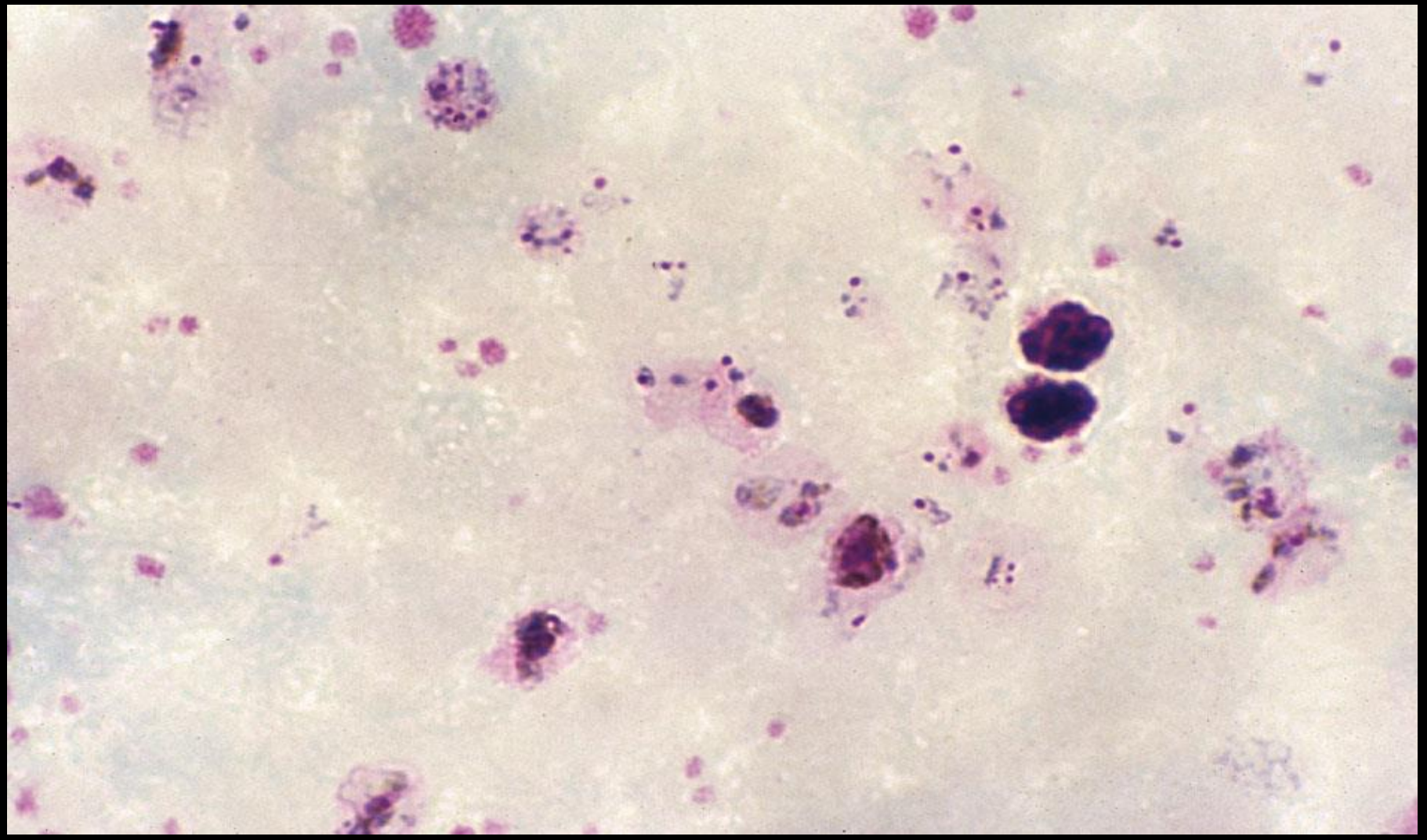
Plasmodium vivax (gametocyte)



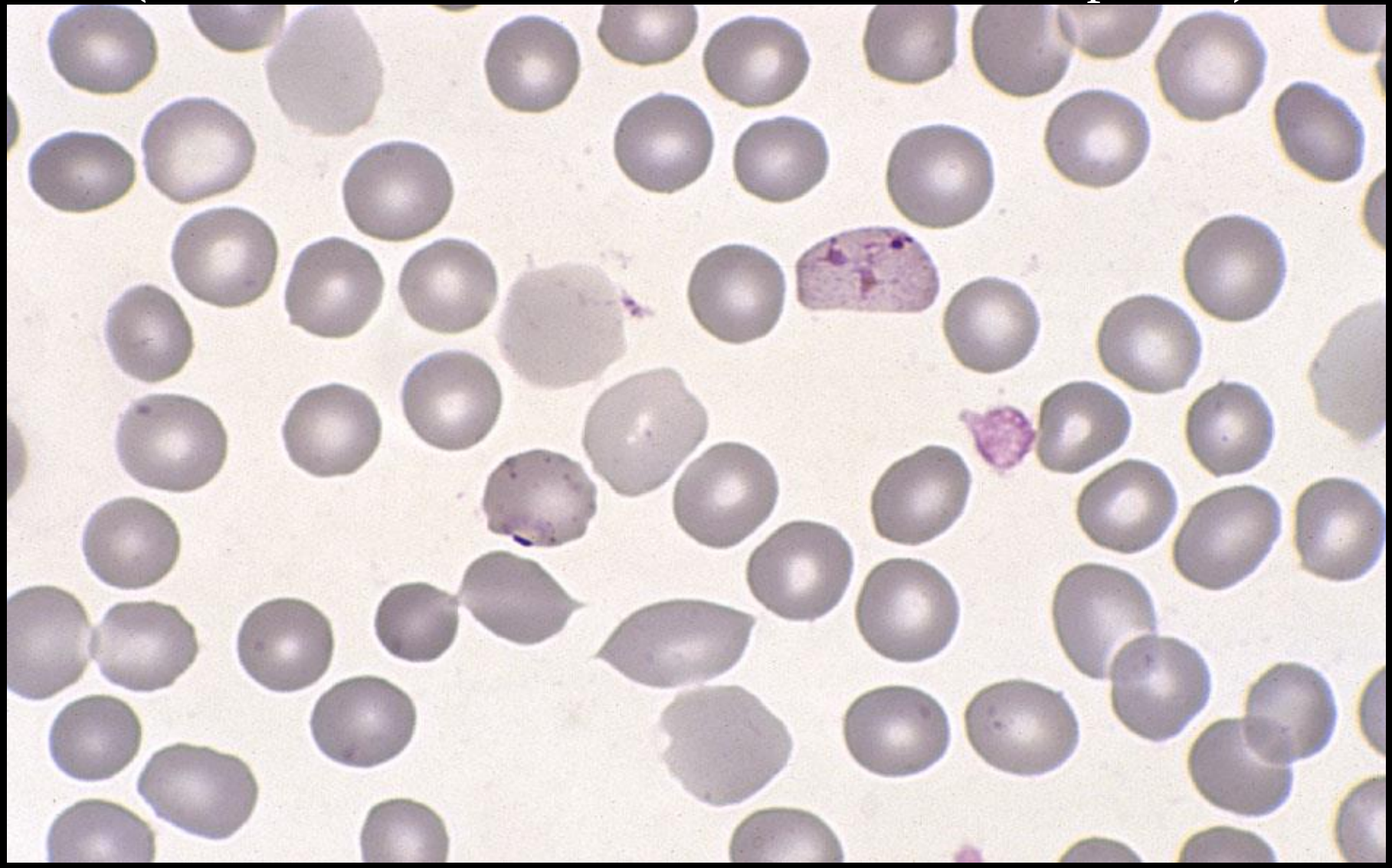
Plasmodium vivax
(gametocyte pH 7.2)



Plasmodium vivax
(early and late trophozoites/schizont/gametocytes Field's stain pH 7.2)



Mixed infection
(Plasmodium vivax and Plasmodium falciparum)



Diagnostic Characteristics

Plasmodium ovale

- This parasite closely resembles *Plasmodium vivax*
- Infected red cells enlarged but not to the same degree as in *vivax*
- Parasites at all stages of maturation
- Early trophozoites are approximately the same size as their *vivax* counterparts
- Multiple infection of red cells rarely occurs
- Late trophozoites are compact and occupy about two thirds of the red cell; fimbriated forms

Diagnostic Characteristics

Plasmodium ovale

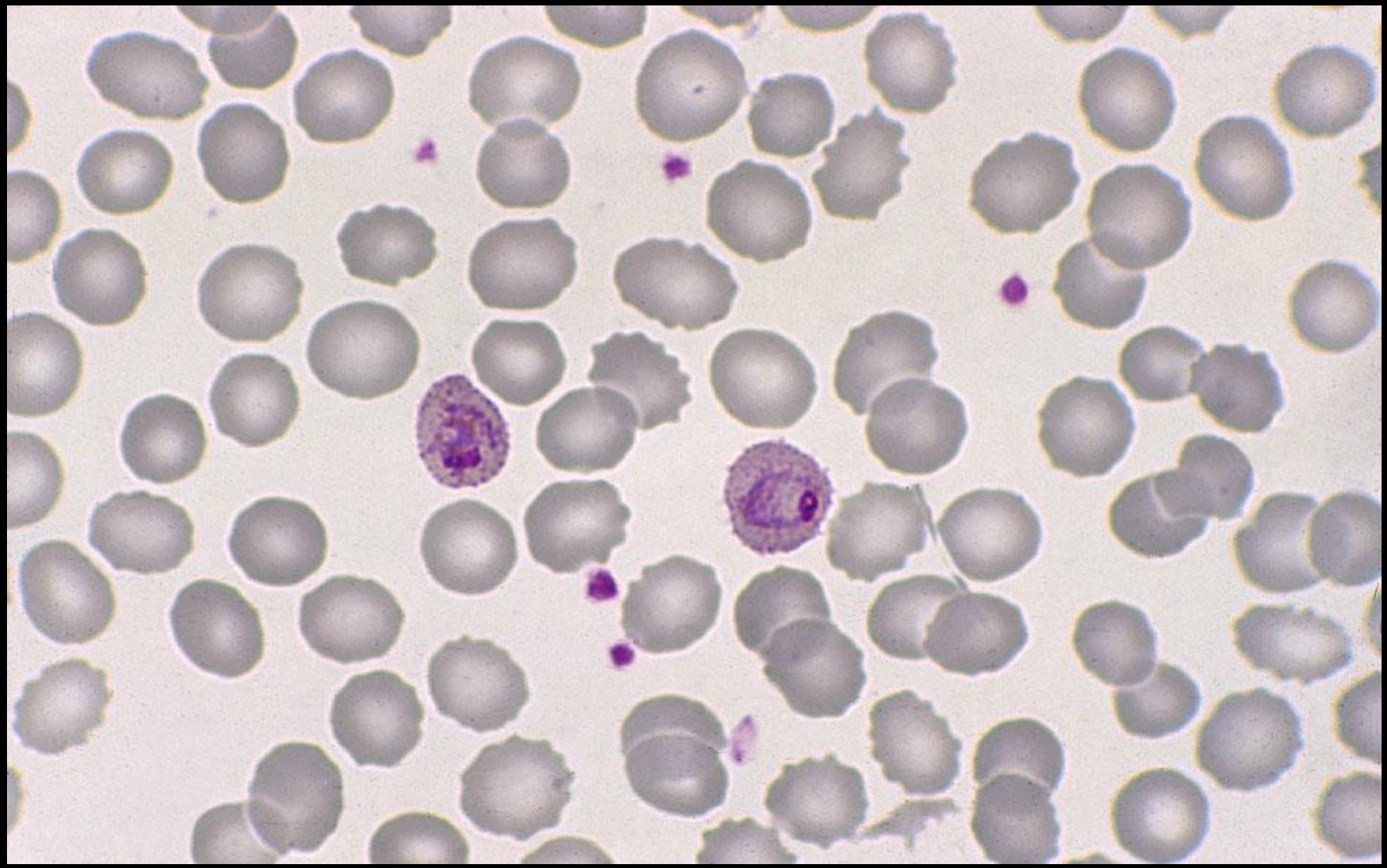
- Schüffner's dots present at pH 7.2
- They stain more intensely than *P* vivax
- Schizonts contain between 6 and 18 merozoites
- Gametocytes similar to those of *P. vivax* except they are more compact and do not fill the entire red cell

Case study
Plasmodium ovale

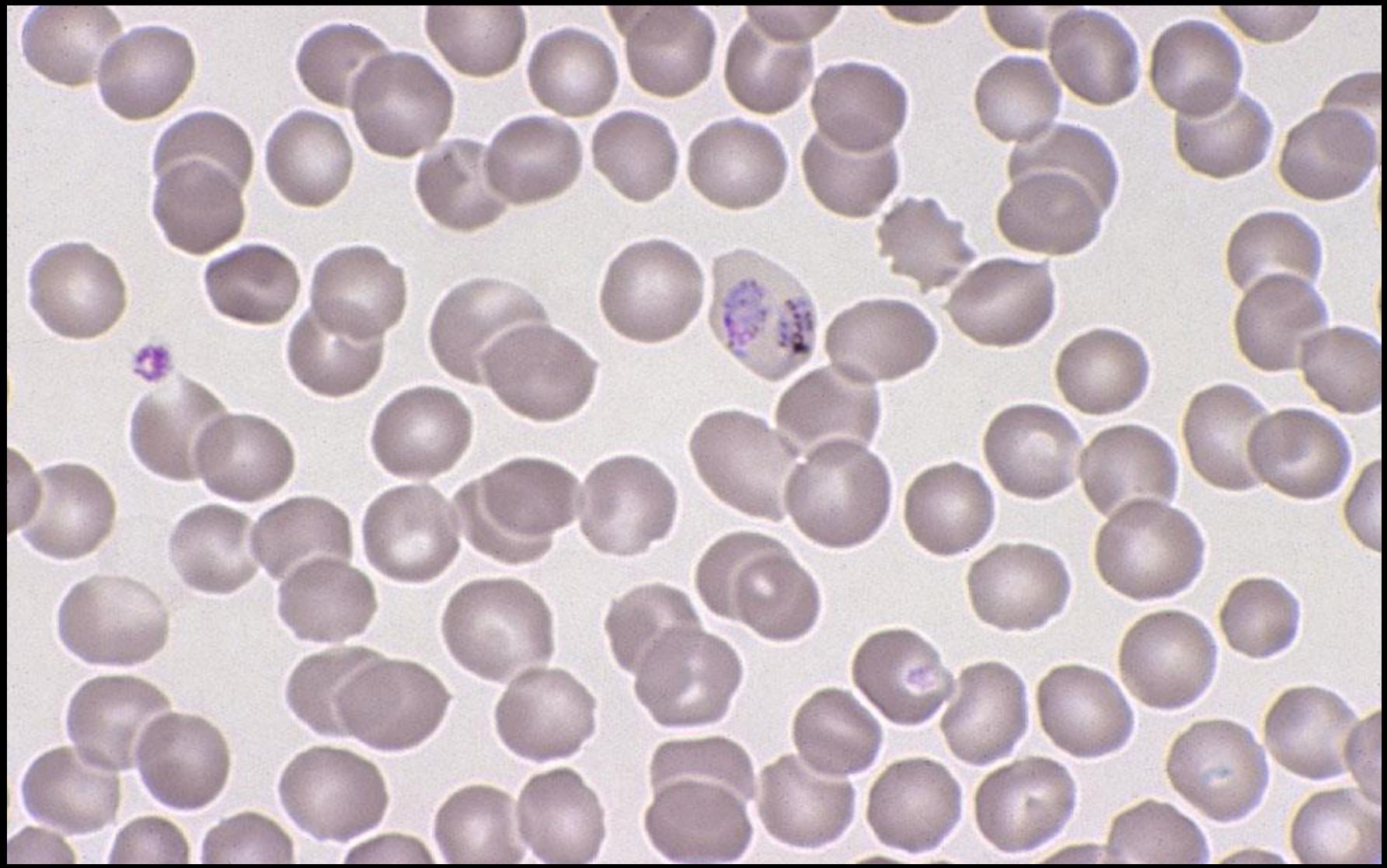
A 23 year old female returns from 3 months in Ghana with myalgia and night sweats

WCC	4.8 x 10^9/L	NR (3.5-11.0)
Hb	103 g/L	NR (115-165)
Platelets	102 x 10^9/L	NR (150-400)

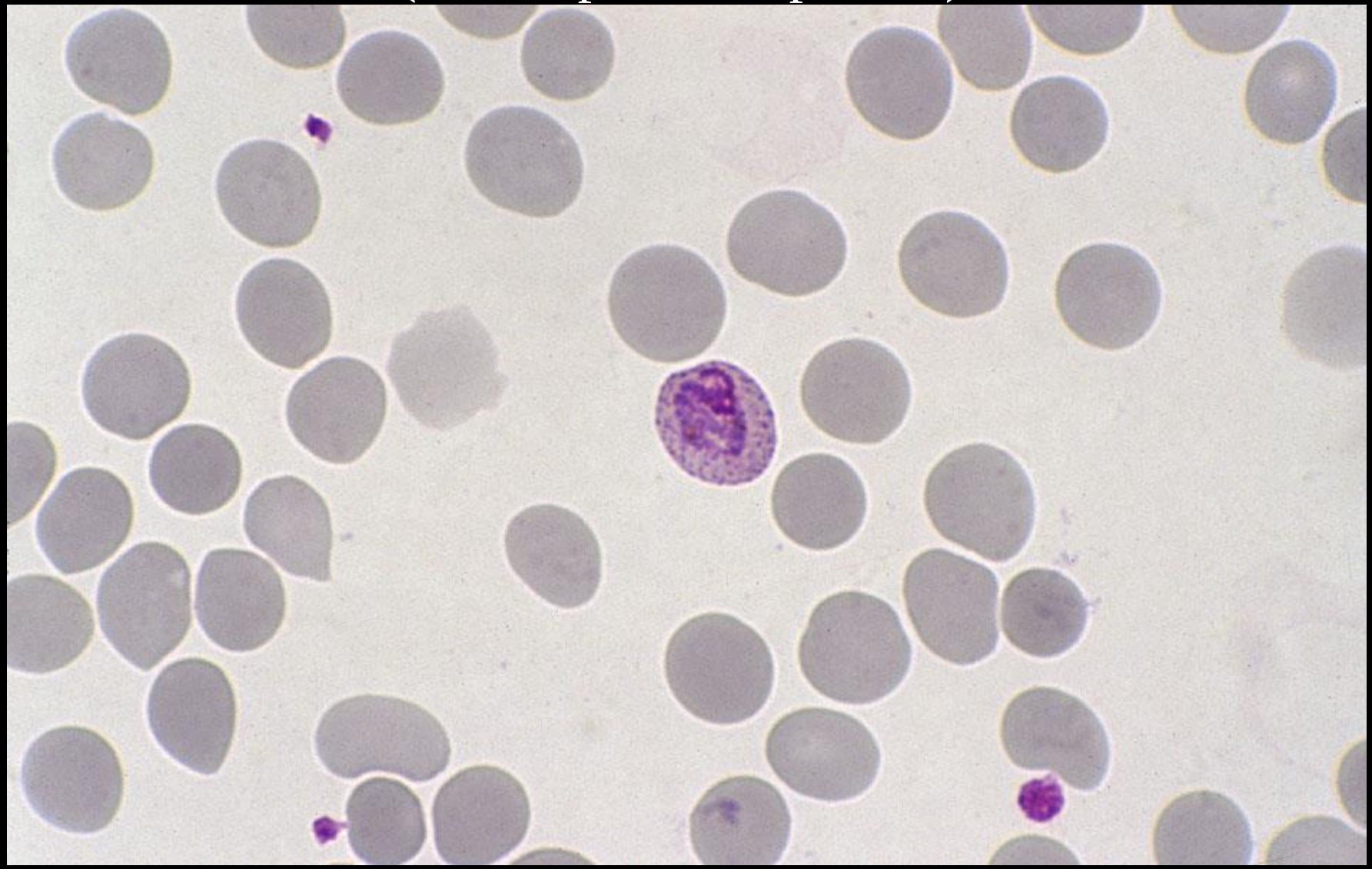
Plasmodium ovale
(early and late trophozoites pH 7.2)



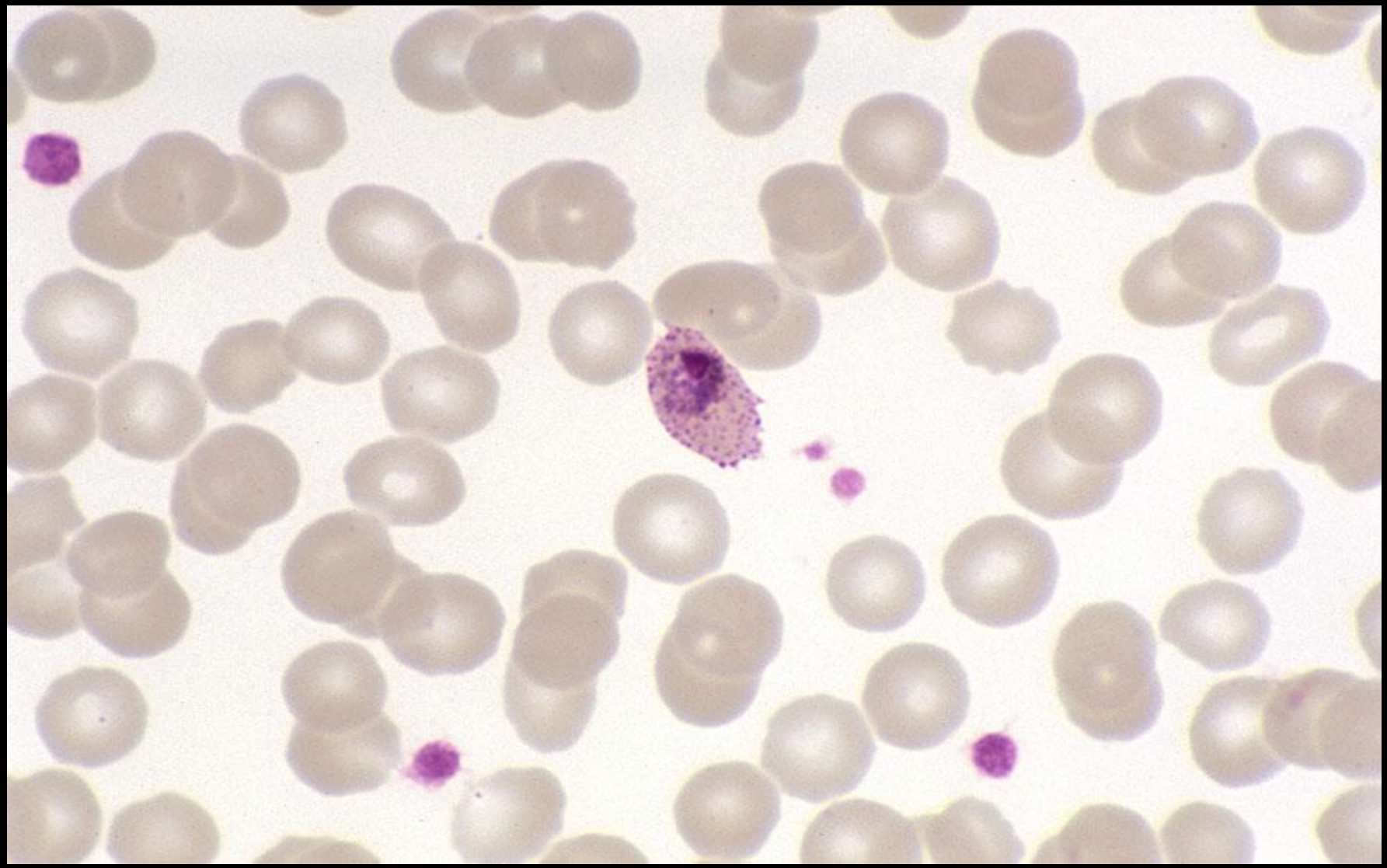
Plasmodium ovale
(late trophozoite)



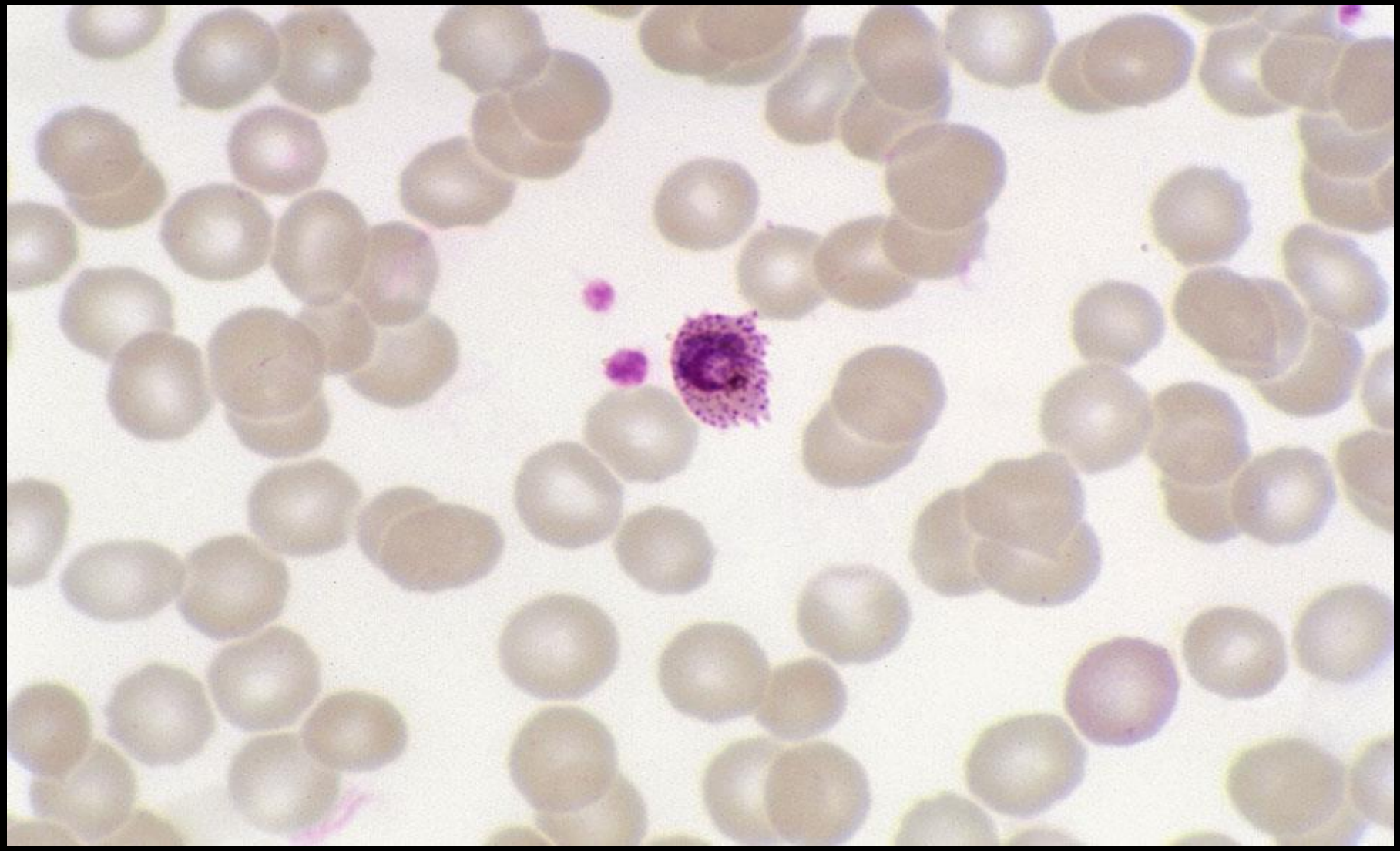
Plasmodium ovale
(late trophozoite pH 7.2)



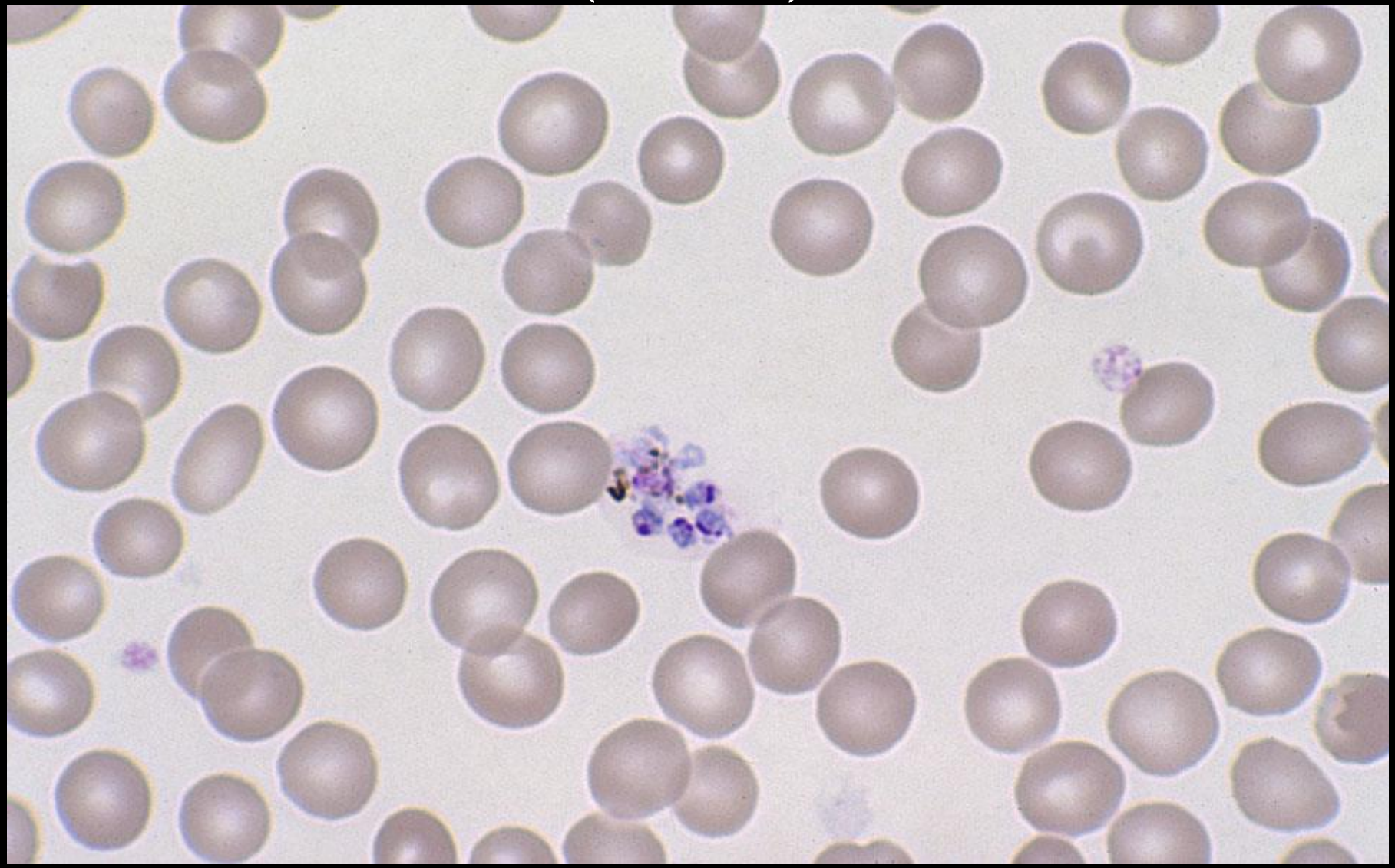
Plasmodium ovale
(late trophozoite pH 7.2)



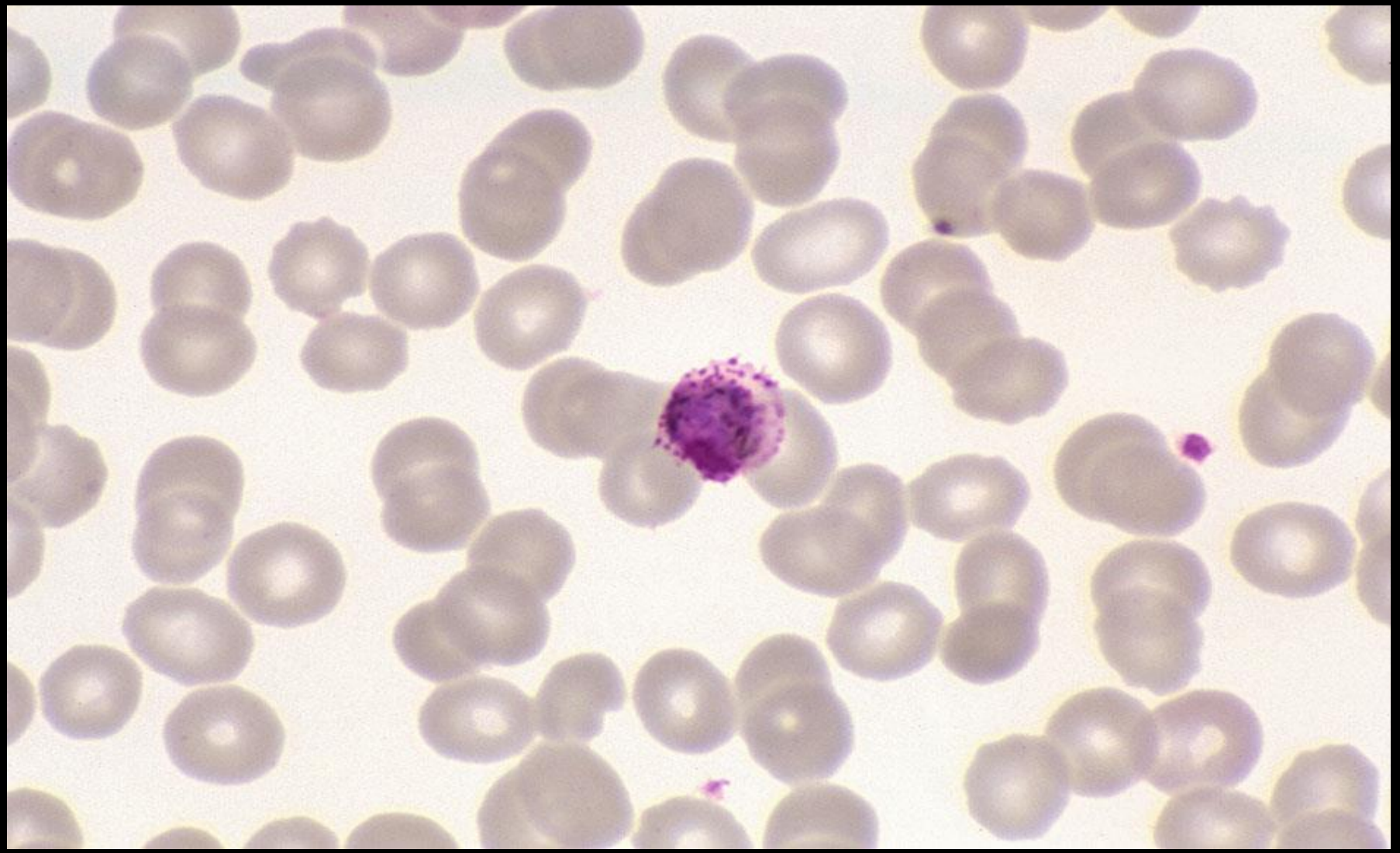
Plasmodium ovale
(late trophozoite, fimbriated form pH 7.2)



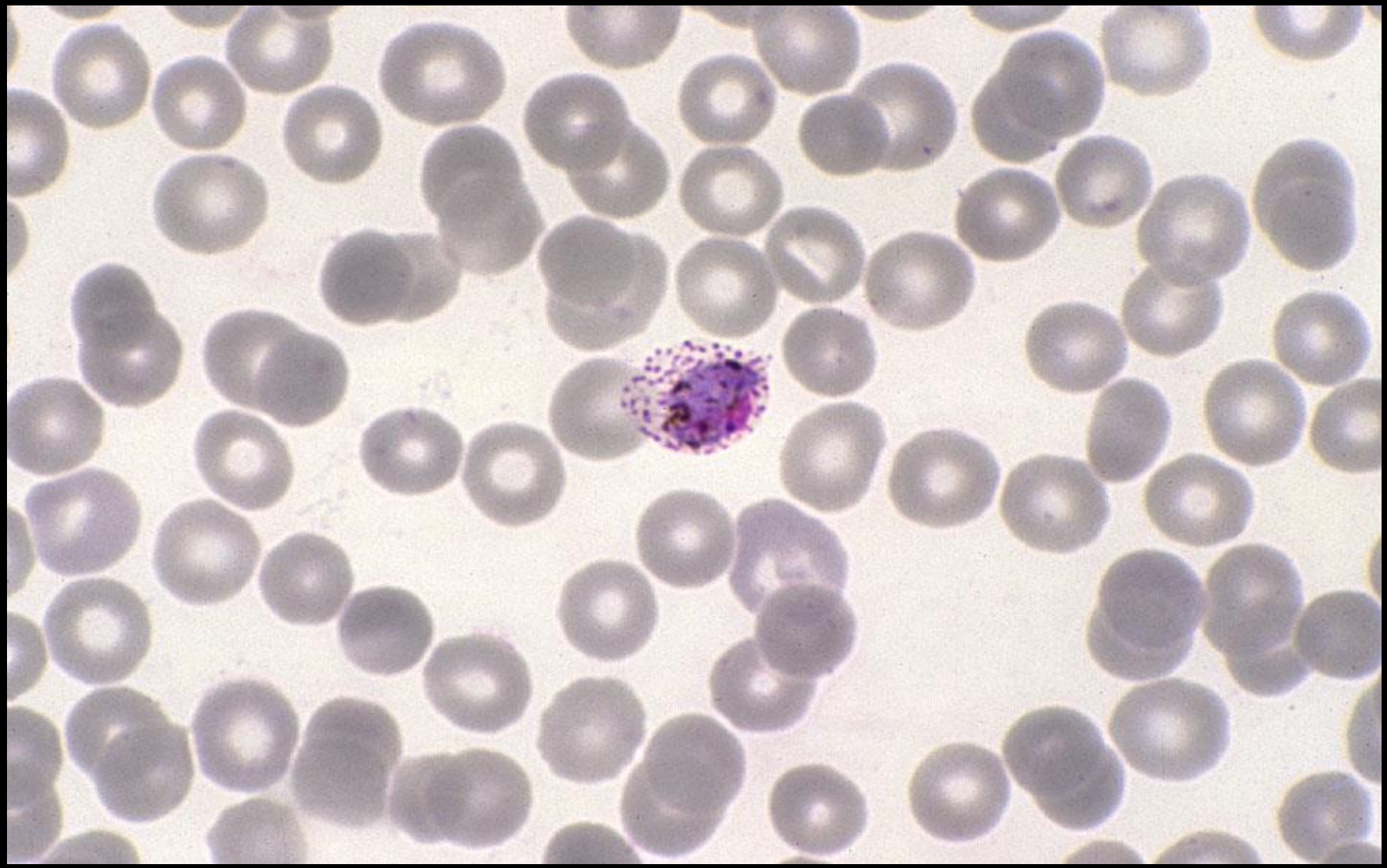
Plasmodium ovale (schizont)



Plasmodium ovale (gametocyte pH 7.2)



Plasmodium ovale
(gametocyte pH 7.2)



Diagnostic Characteristics

Plasmodium malariae

- Infected red cells are not enlarged
- Parasites at all stages of maturation
- Early trophozoites are approximately the same size as their *vivax* counterparts and have single chromatin dot
- Multiple infection of red cells rarely occurs
- Late trophozoites are compact and heavily pigmented

Diagnostic Characteristics

Plasmodium malariae

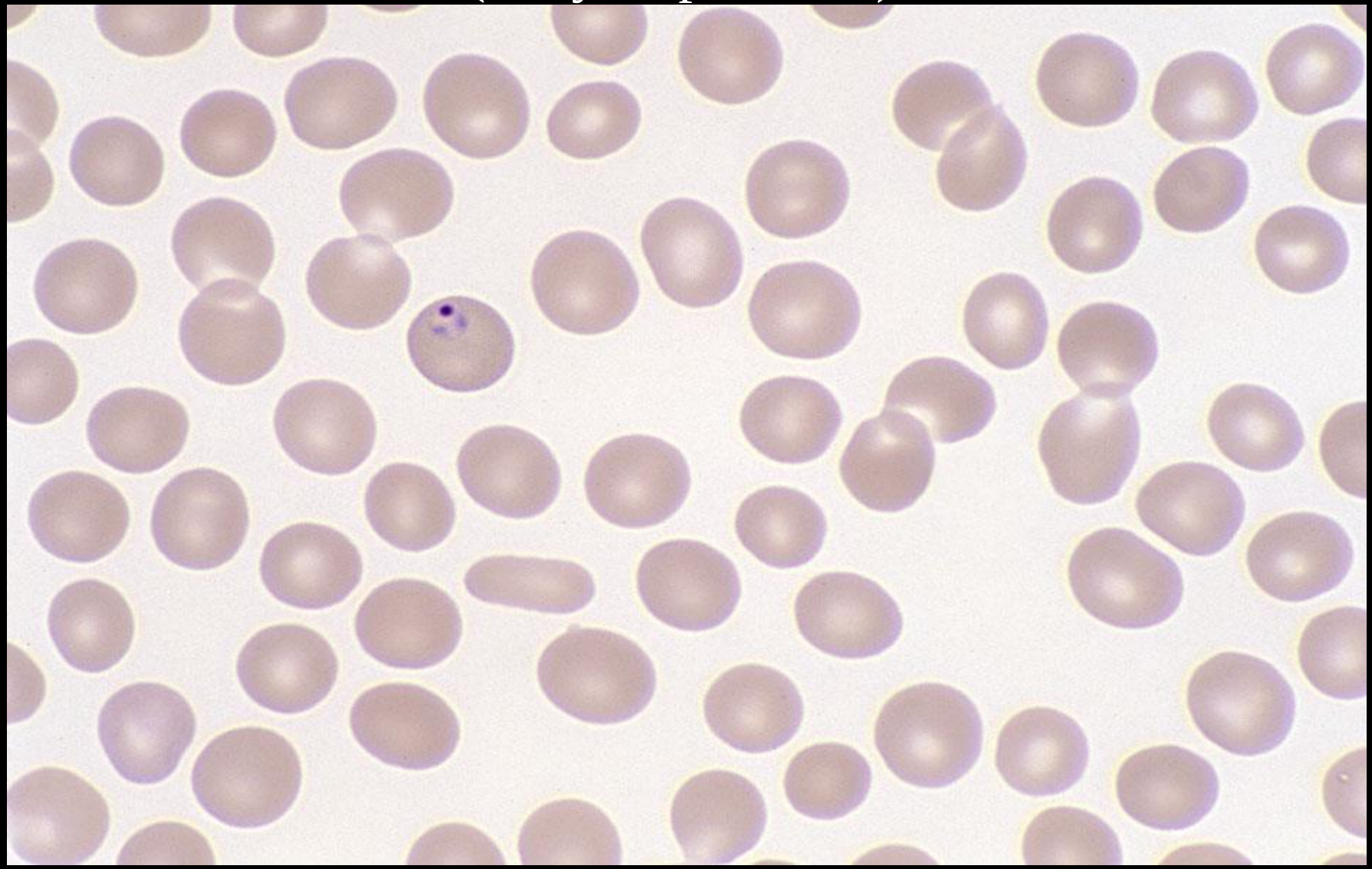
- Band form trophozoites are common
- Schizonts contain between 6 and 12 merozoites; occasional rosette or ‘daisy’ head forms
- Gametocytes are compact and fill the red cell and are similar to *vivax* gametocytes

Case study
Plasmodium malariae

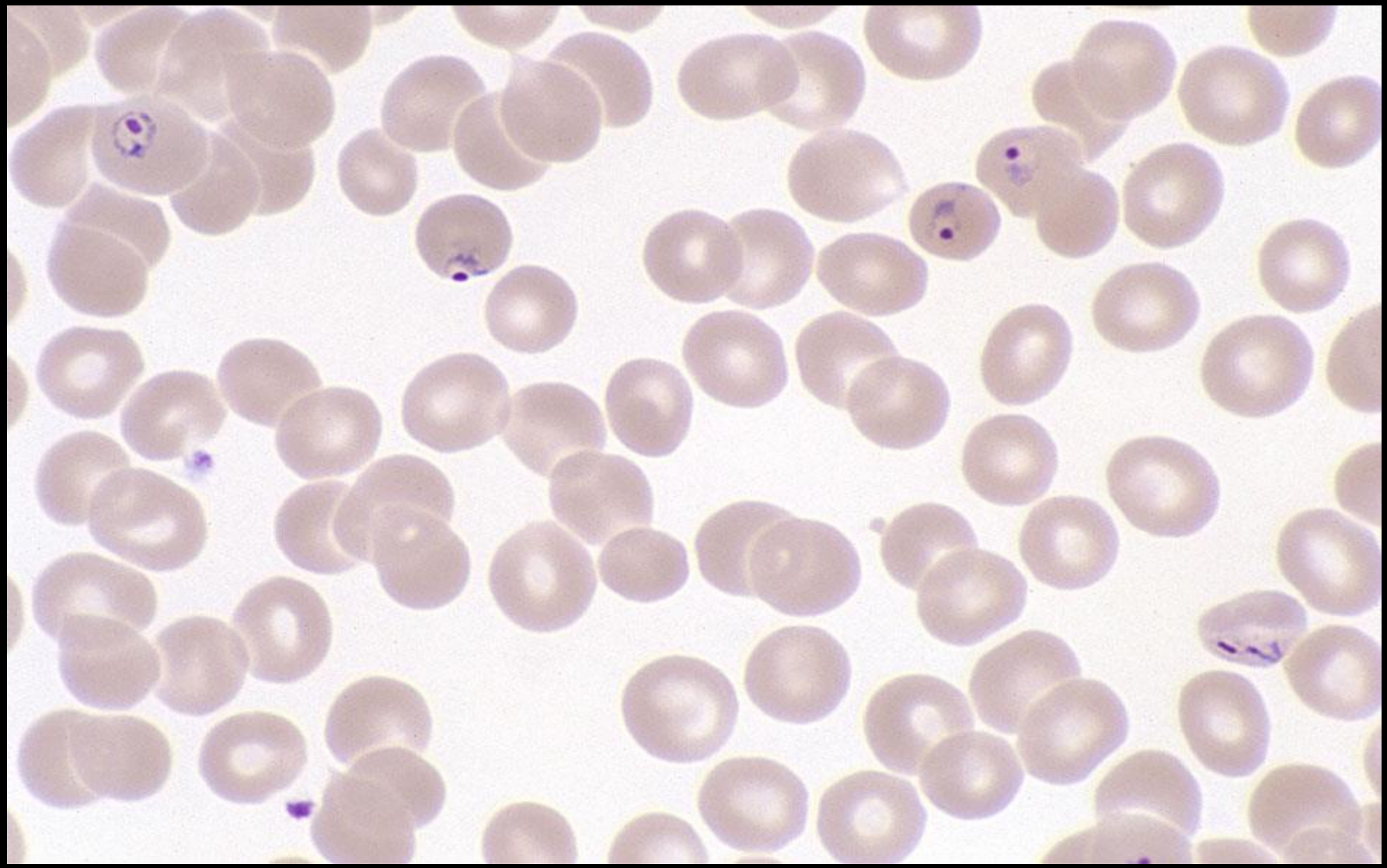
A 49 year old male returns from living in South America for 2 years. He had a history of CLL and had been having night sweats for several months

WCC	7.6 x 10^9/L	NR (3.5-11.0)
Hb	124 g/L	NR (130-180)
Platelets	155 x 10^9/L	NR (150-400)

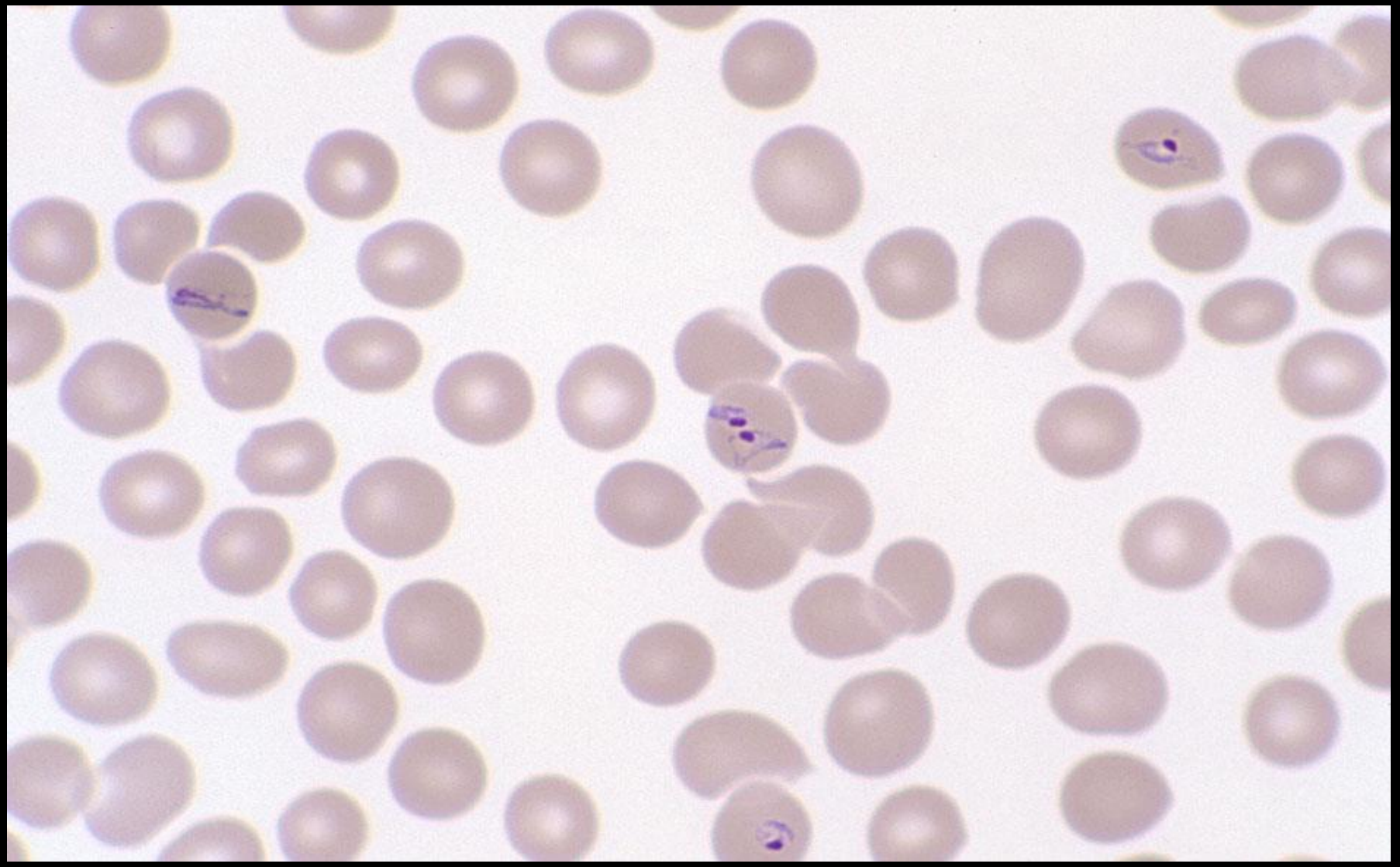
Plasmodium malariae (early trophozoite)



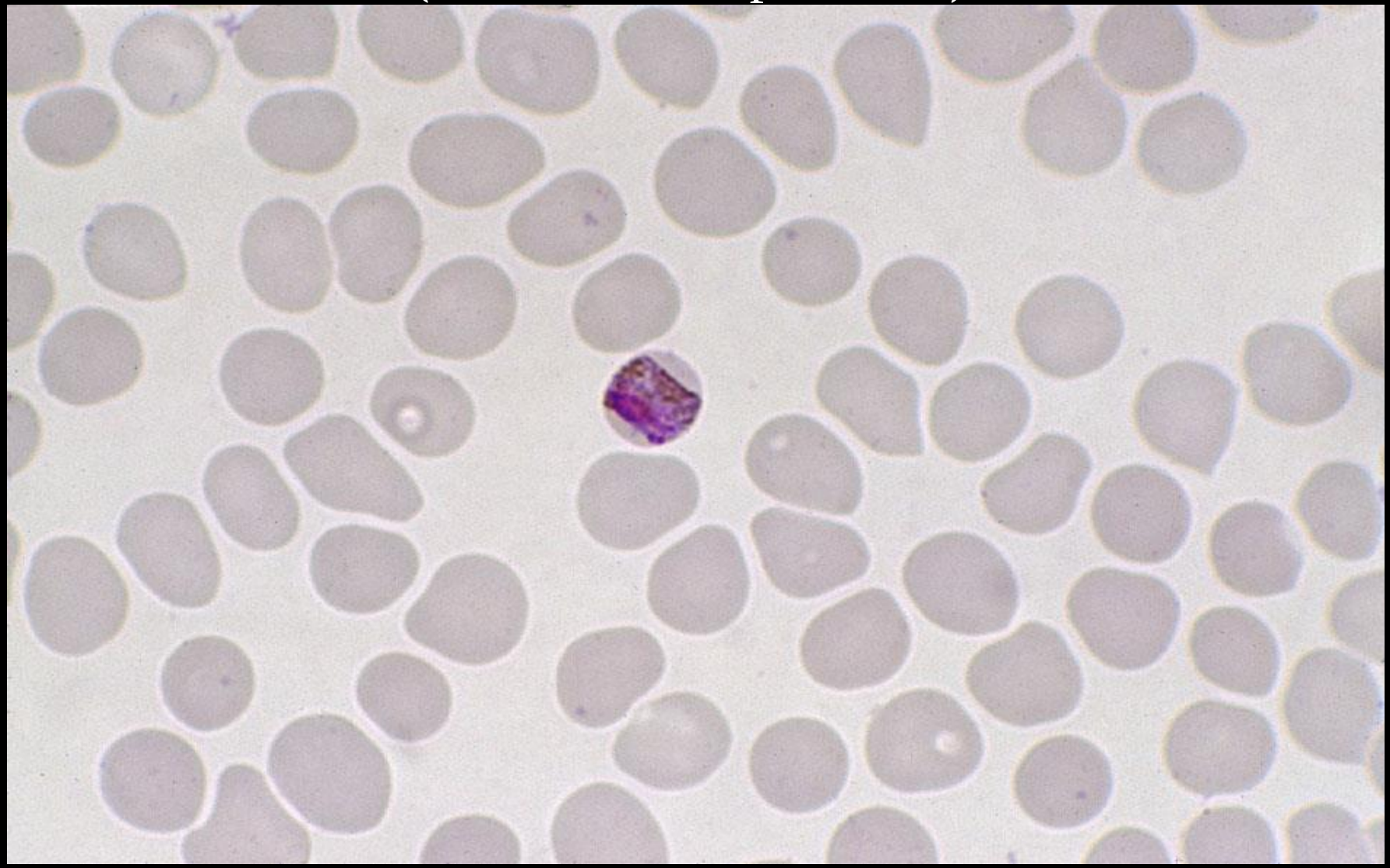
Plasmodium malariae
(early trophozoites)



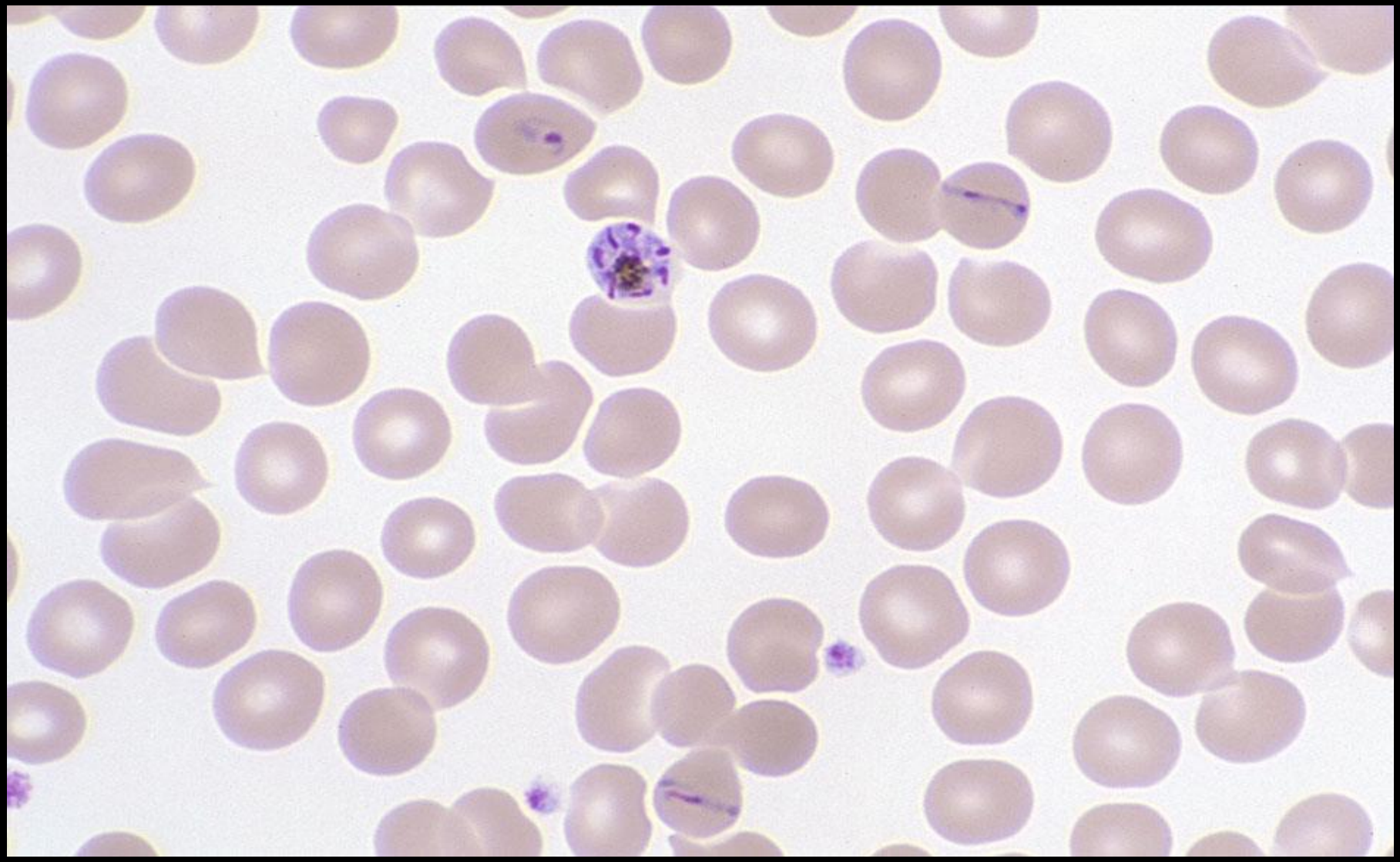
Plasmodium malariae
(early, late and band form trophozoites)



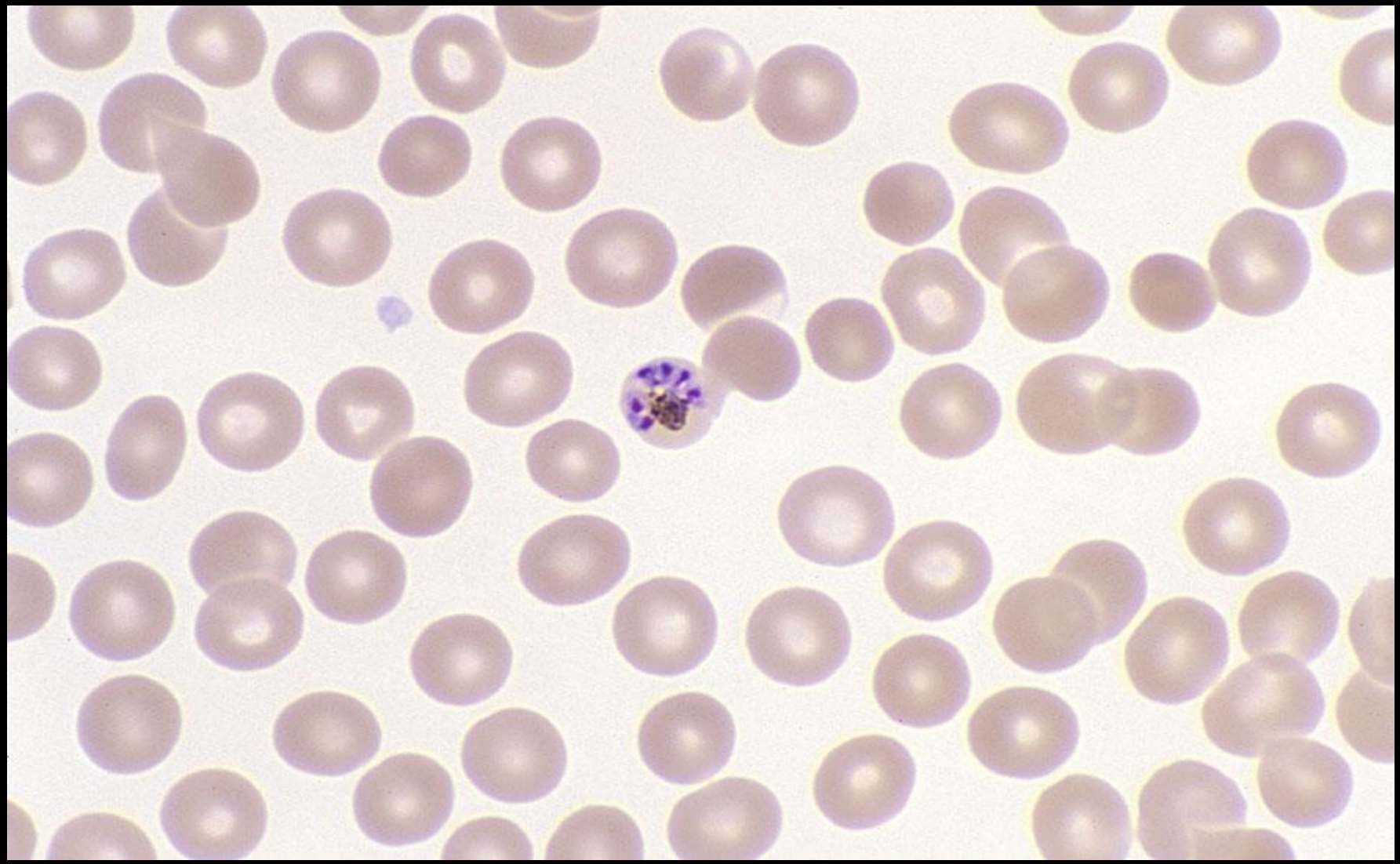
Plasmodium malariae
(band form trophozoite)



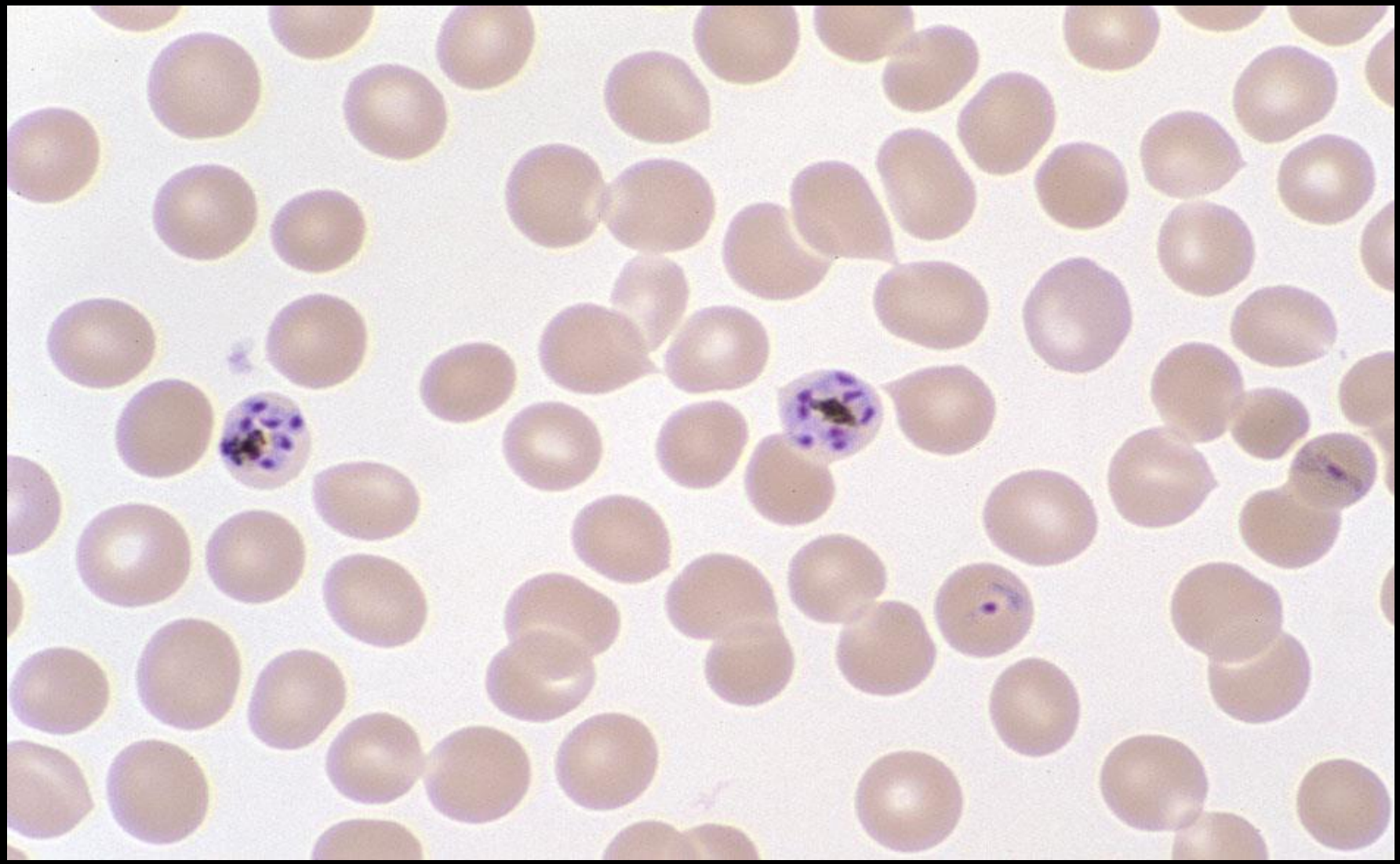
Plasmodium malariae
(early trophozoites, bands forms and schizont)



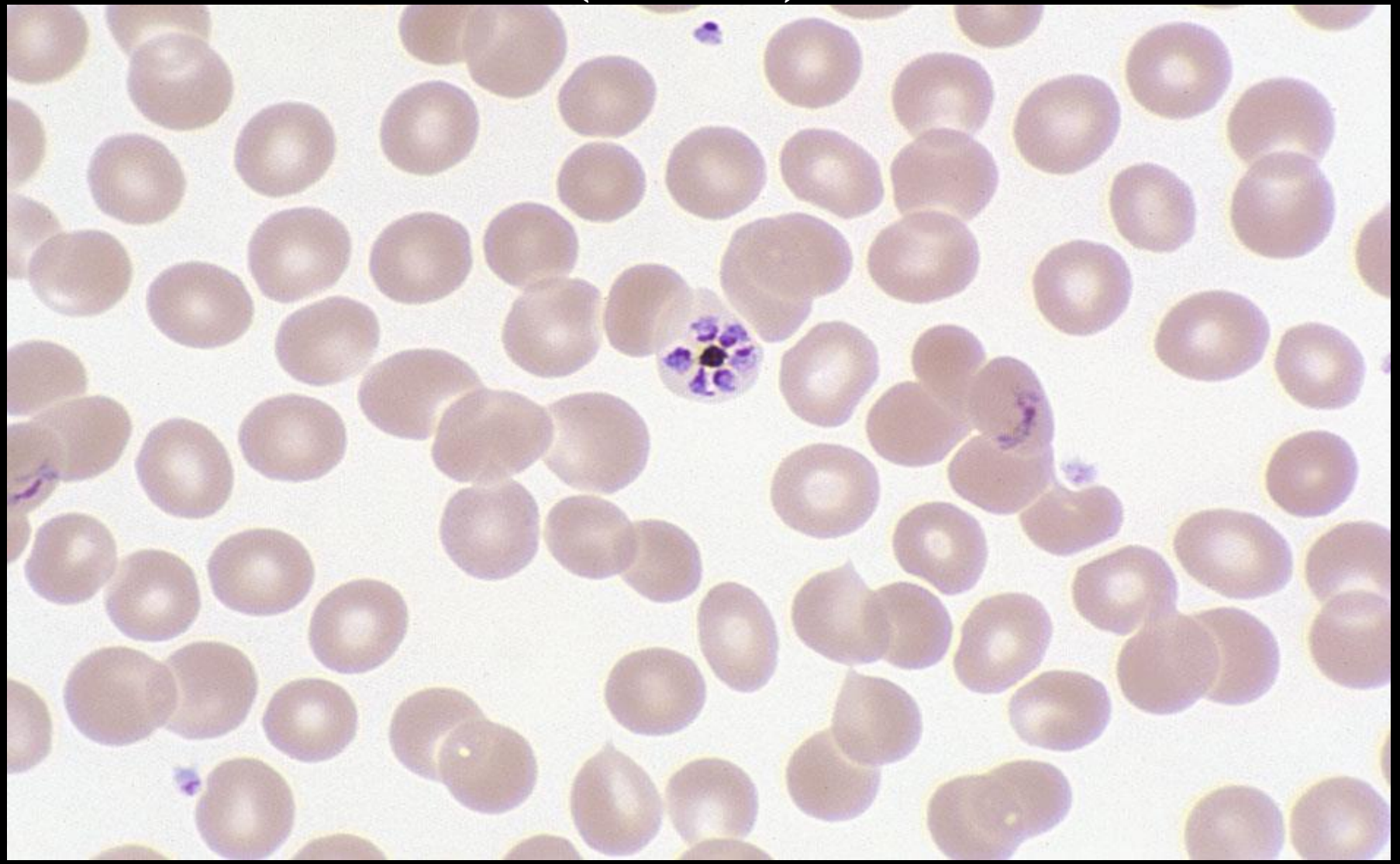
Plasmodium malariae (schizont)



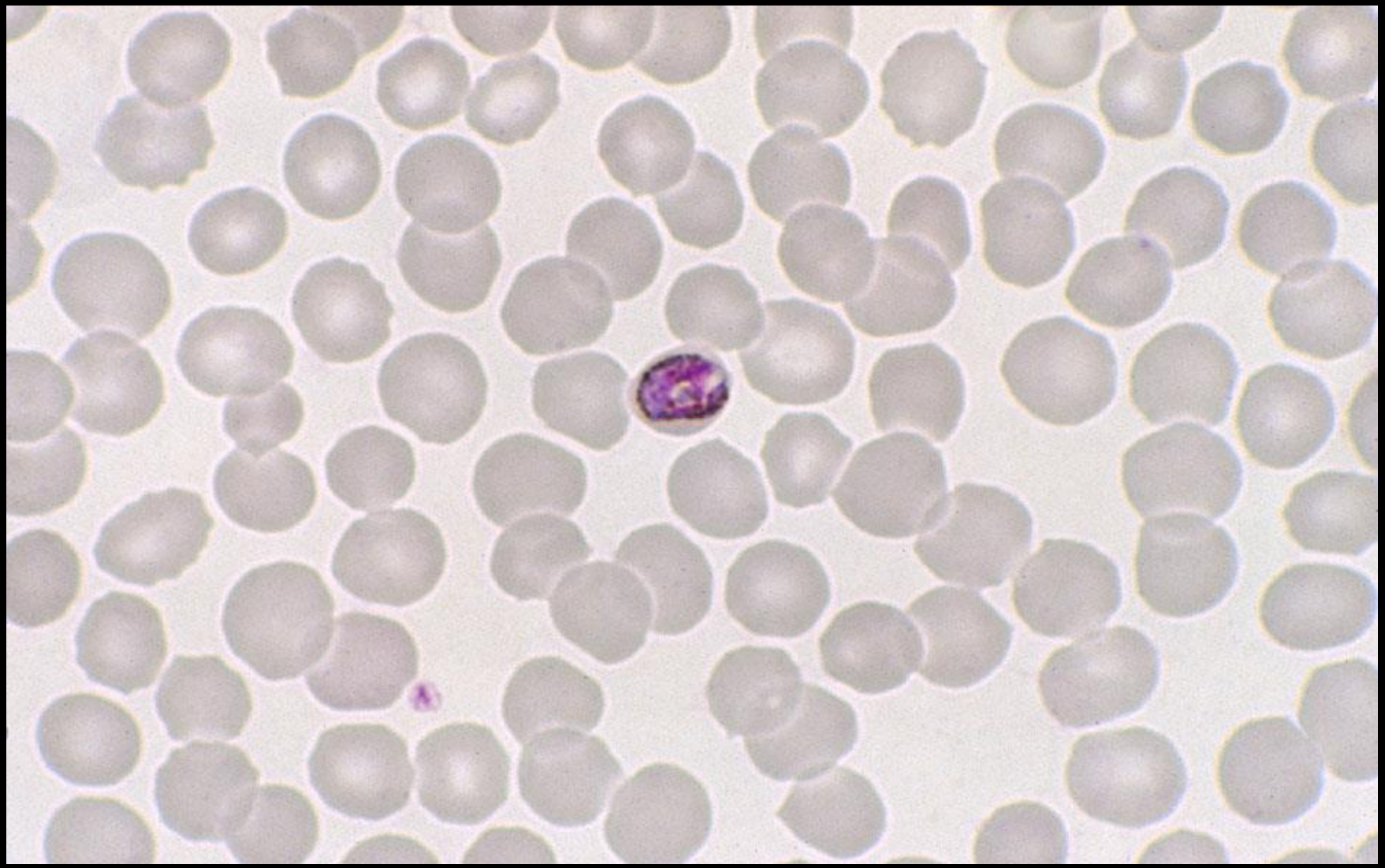
Plasmodium malariae (schizonts)



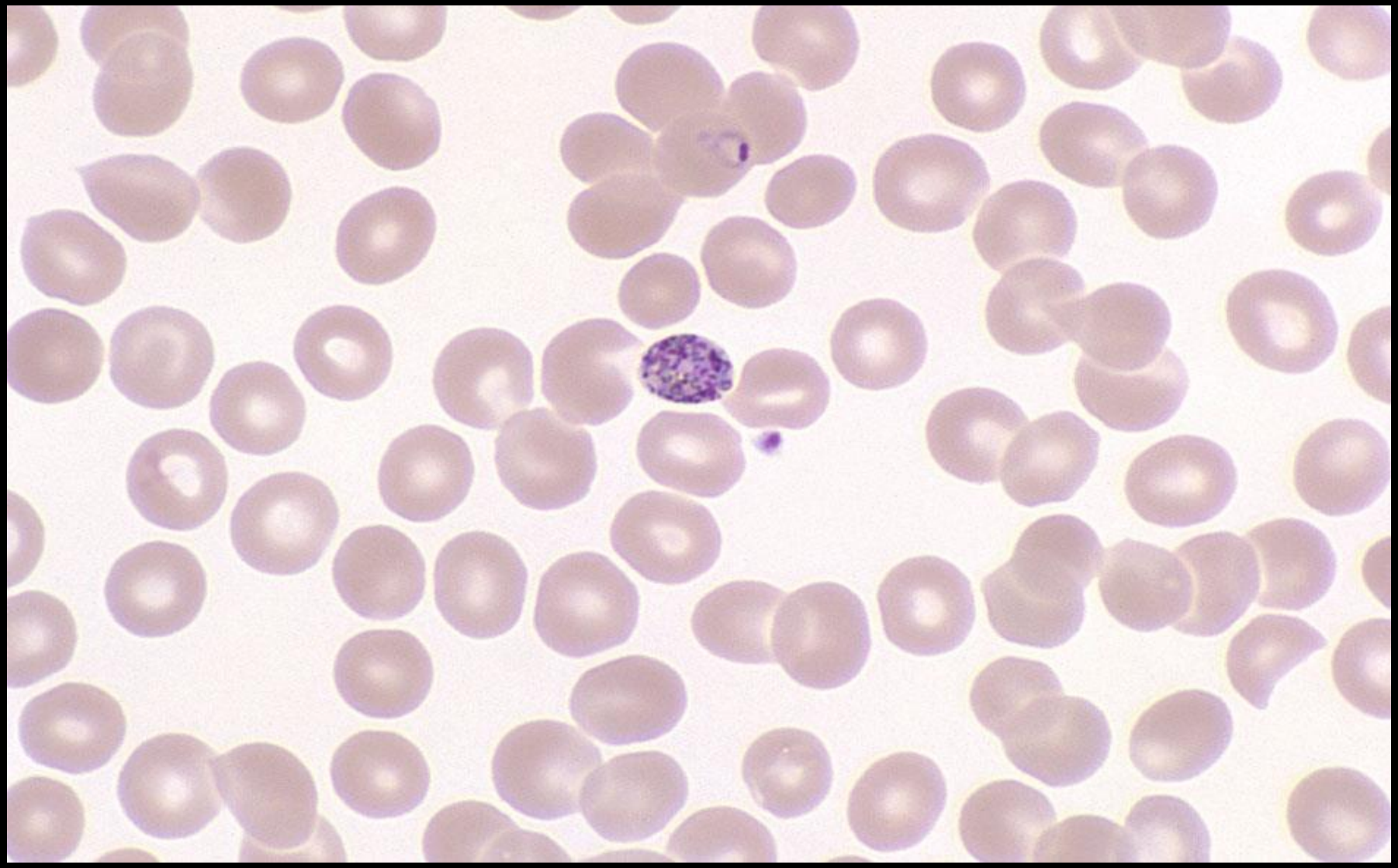
Plasmodium malariae (schizont)



Plasmodium malariae (gametocyte)



Plasmodium malariae (gametocyte)



Fifth species of human malaria

Plasmodium knowlesi, known to infect long-tailed macaques found in the rainforests of South East Asia is now recognised as a fifth species of malaria infecting humans.

Plasmodium knowlesi

- Using DNA-based technology, professors Janet Cox-Singh and Balbir Singh, noted that more than one in four patients in Sarawak, Malaysian Borneo, were infected with *P Knowlesi.*
- *P Knowlesi* is unprecedented among malaria parasites in that it reproduces every 24 hours. Infected patients have a high number of infected red cells.

Plasmodium knowlesi

- Even a short delay in ‘accurate’ diagnosis and treatment could lead to the rapid onset of complications, including liver and kidney failure, and death.

Diagnostic Characteristics

Plasmodium knowlesi

- Infected red cells are not enlarged
- Parasites at all stages of maturation maybe present within the red cell
- Early trophozoites are approximately the same size as their *P malariae* counterparts
- Early trophozoites may have 2 chromatin dots; occasional band forms

Diagnostic Characteristics

Plasmodium knowlesi

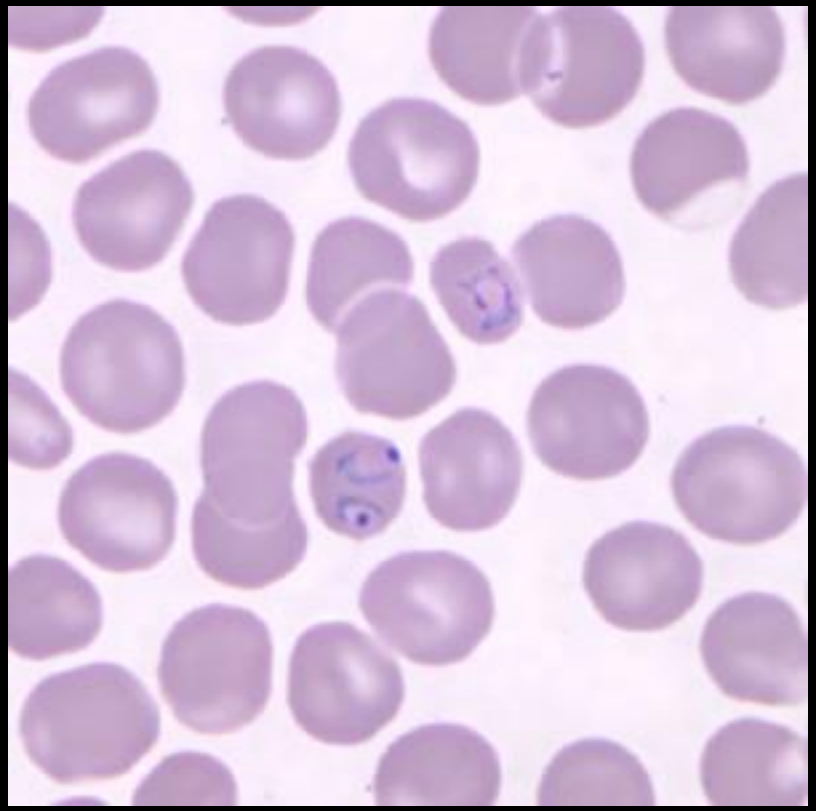
- Multiple infection of red cells not uncommon
- Late trophozoites are compact and heavily pigmented
- Schizonts contain between 8 and 10 merozoites; occasional rosettes; mature merozoites appear segmented
- Gametocytes are compact and fill the red cell, similar to the gametocytes of *P malariae*

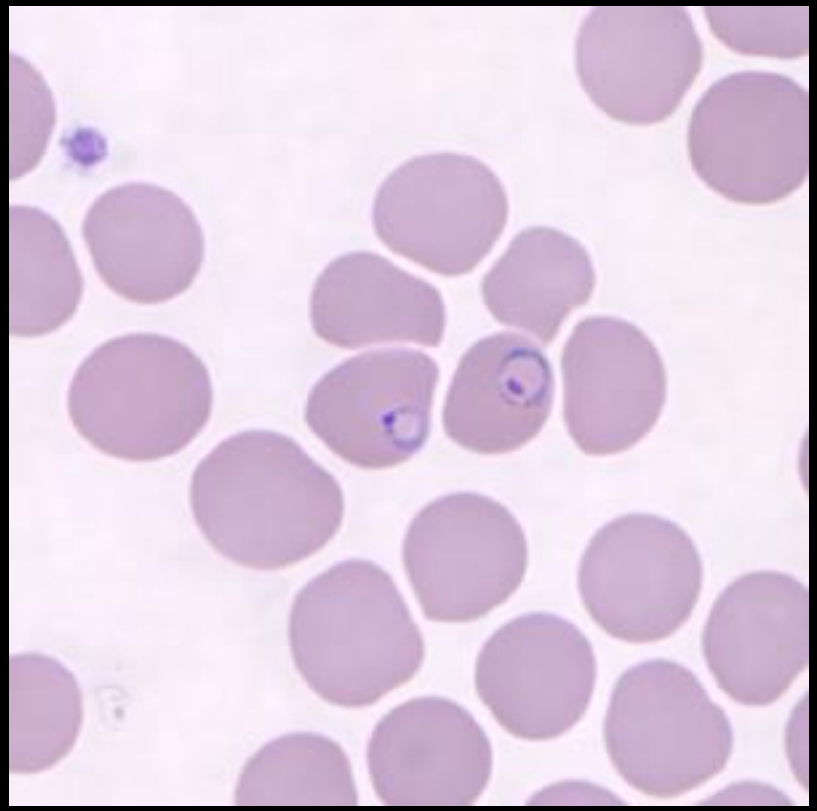
Case study
Plasmodium knowlesi

A 34 year old male returned from working in the forests of Borneo with low grade fevers over a period of two to three weeks

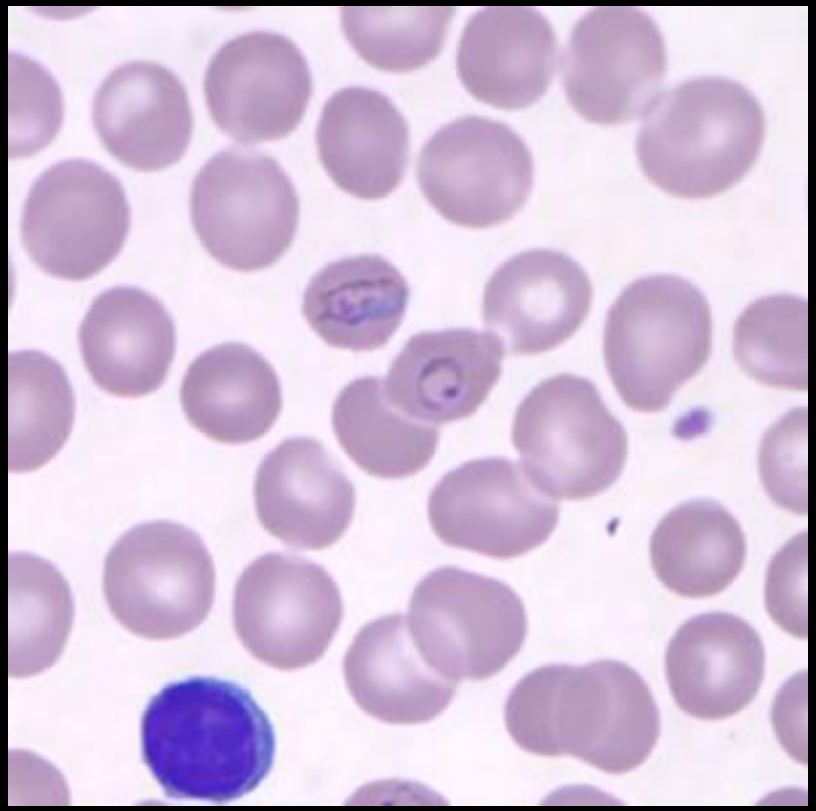
WCC	3.4 x 10^9/L	NR (3.5-11.0)
Hb	122 g/L	NR (130-180)
Platelets	97 x 10^9/L	NR (150-400)

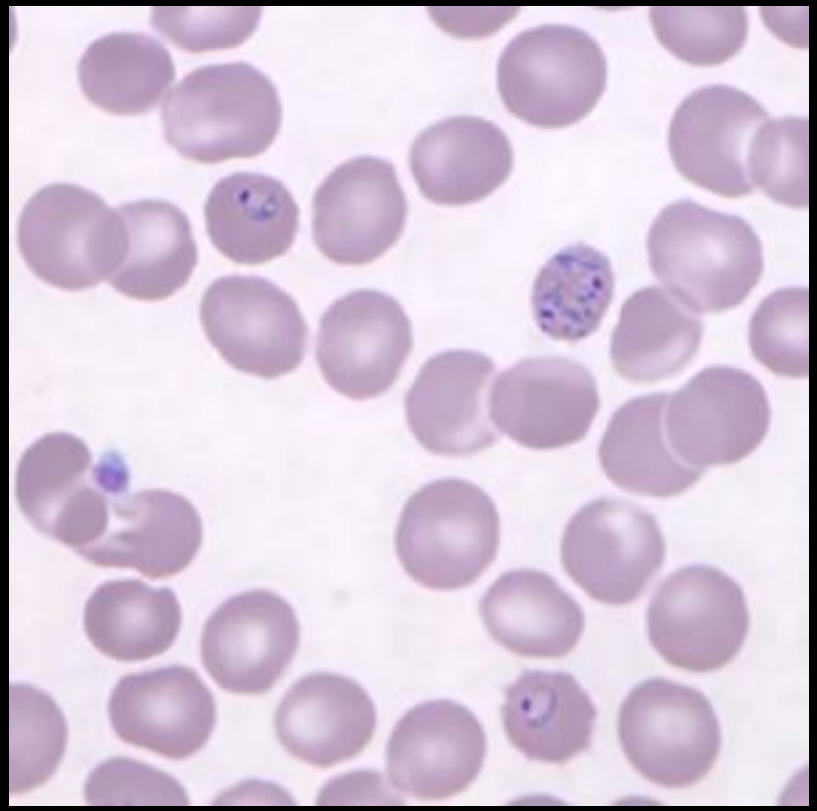
Plasmodium knowlesi
(early trophozoites)
Professor Singh



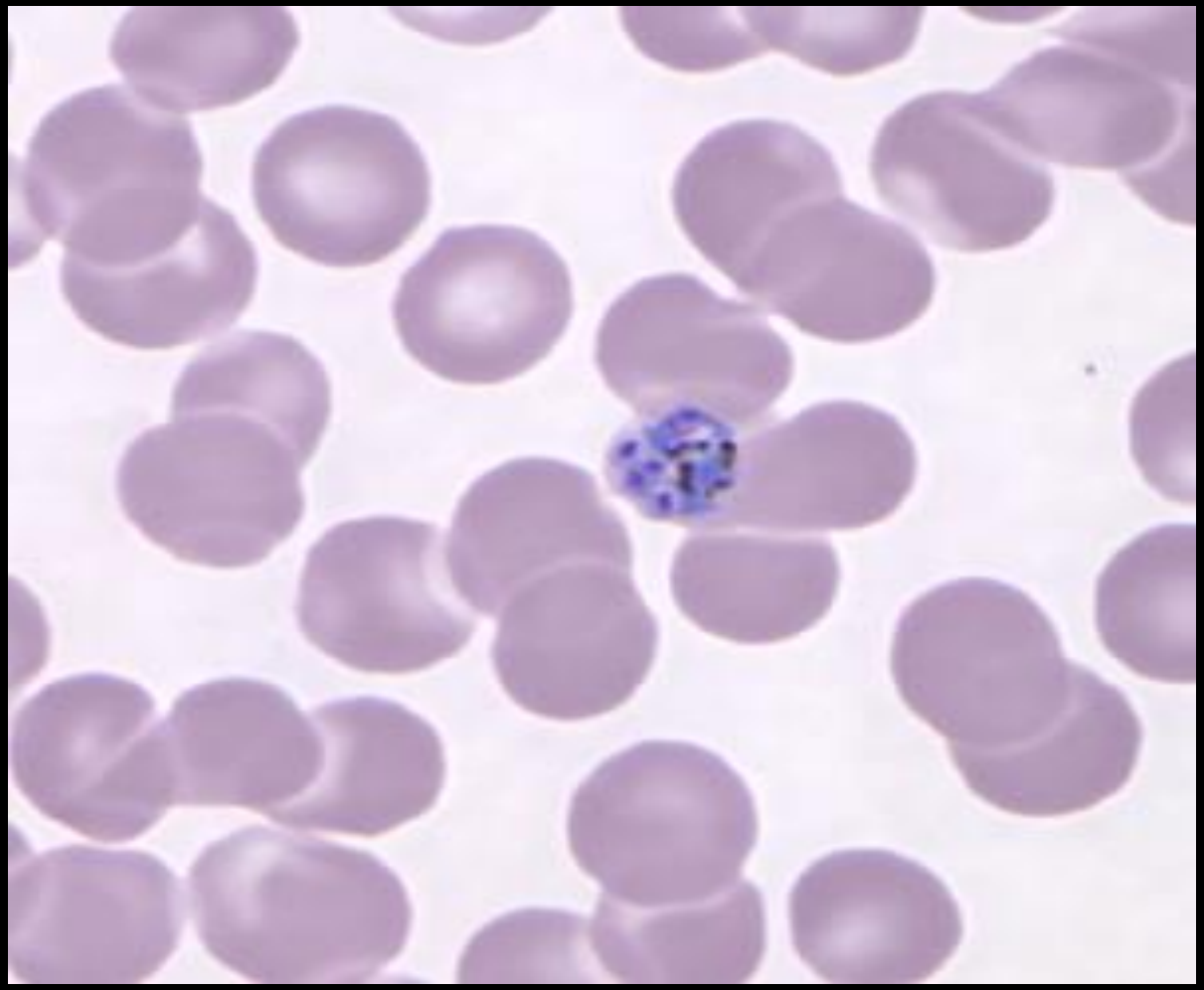


Plasmodium knowlesi (early trophozoite, band form and developing trophozoite)

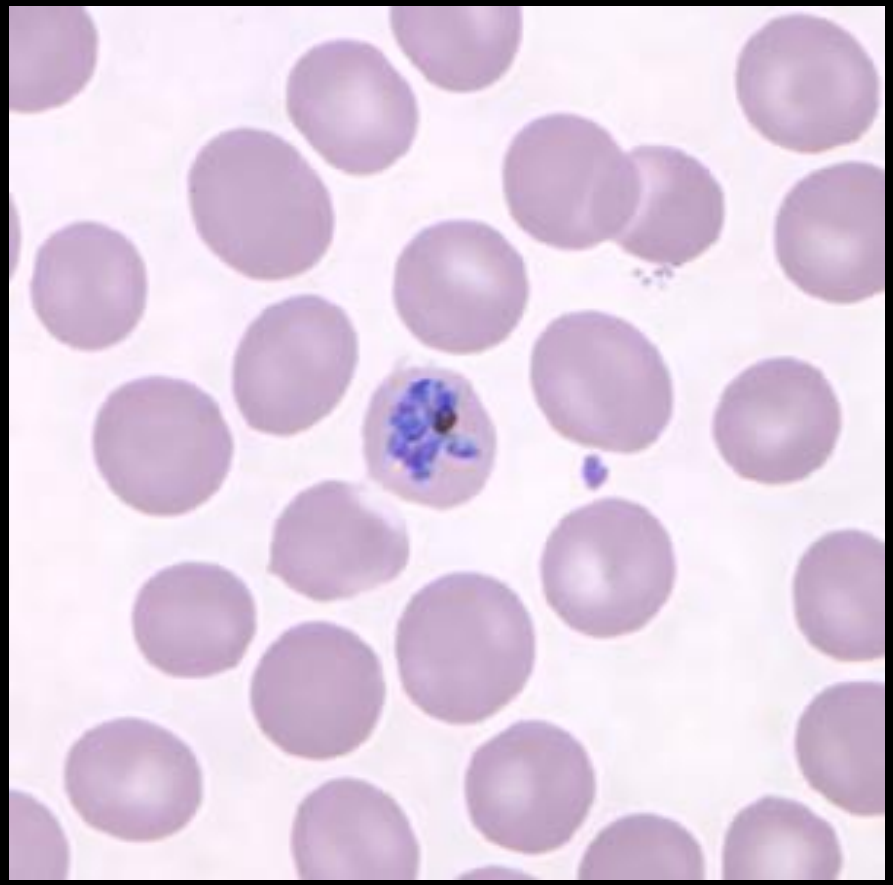


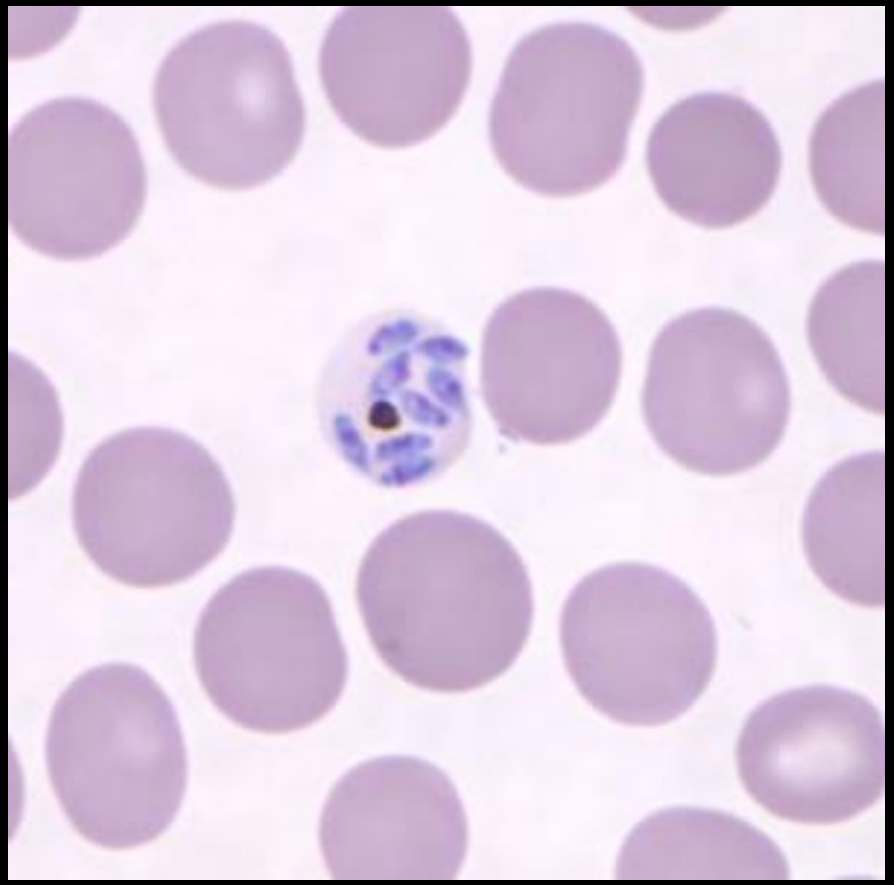


Plasmodium knowlesi (schizont)

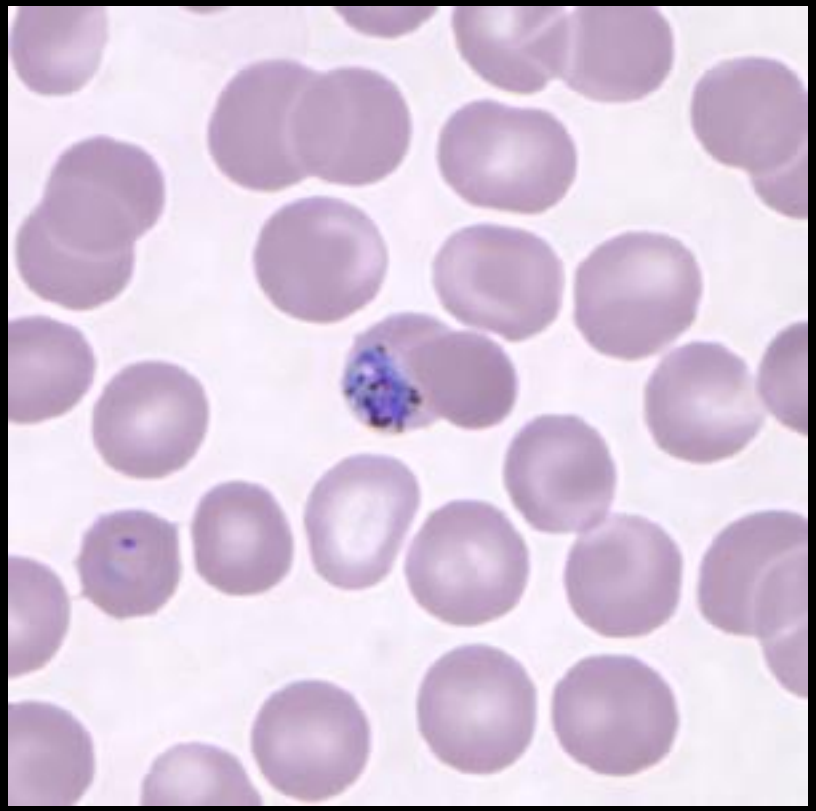


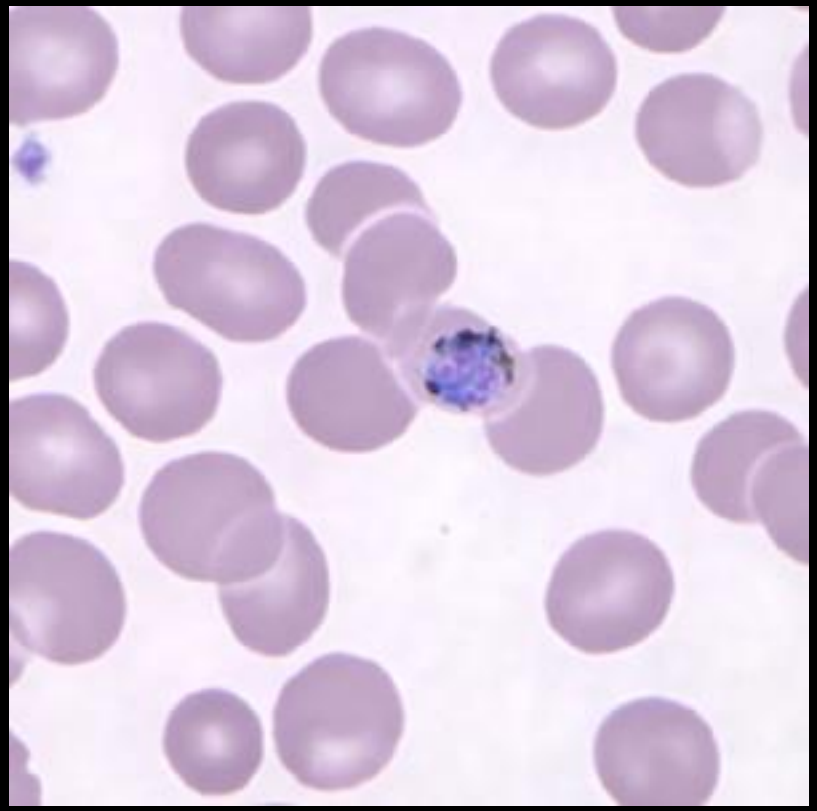
Plasmodium knowlesi (schizont)





Plasmodium knowlesi (gametocytes)





Summation by Professor Singh

"I believe if we look at malaria infections in South East Asia more carefully, we will find that this potentially fatal type of the disease is more widespread than is currently thought.
Given the evident severity of the illness that it causes, I would recommend that doctors treating patients with a laboratory diagnosis of *P malariae* remain alert to the possibility that they may be dealing with the potentially more aggressive *P knowlesi*.

This would be particularly important in patients who have spent time in the forest fringe areas of South East Asia where the non-human primate host exists.”

Parasite Density Counts

- Parasite density is part of the microscopic diagnosis of *P. falciparum* and is used to assess the effect of specific treatment
- *P knowlesi* density counts
- Compare number of parasites with number of white cells on a thick film
- Count 100 white cells in consecutive fields; then count the number of parasites occurring in those same fields
- Number of parasites expressed x 10^9/L

Parasite Density Counts

$\frac{\text{Number of parasites}}{\text{100 white cells}} \times \text{WCC} = \text{parasite density} \times 10^9/\text{L}$

Relating parasite numbers to red cells is not recommended since anaemia and multiple infections will introduce errors.
However, most treating physicians request the number of red cells infected.
It is thus also suggested the parasite density be expressed x 10^{12}/L for the number of red cells infected.

Immunochromatographic tests (ICT)

These are rapid diagnostic tests developed to detect circulating malarial antigens and are said to improve the time-consuming task, sensitivity and objectivity of malarial diagnosis

“Evaluation of the ICT Malaria P.f/P.v and the OptiMal Rapid Diagnostic Tests for Malaria in Febrile Returned Travellers” has been published in the Journal of Clinical Microbiology, Nov 2002, p.4166-4171 by E. Geoffrey Playford and John Walker

Differential diagnosis

Travel related:

- Dengue fever
- Enteric fever: typhoid, paratyphoid
- Leptospirosis
- Scrub typhus
- HIV

Dengue fever
Türk cells