KOMPETENSI MALARIA

MAKMAL VEKTOR NEGERI PULAU PINANG

2019

PERANAN MAKMAL VEKTOR NEGERI PULAU PINANG

. Menerima dan memeriksa slaid – slaid malaria dan filaria yang dipungut dan dihantar oleh Pasukan Pelbagaiguna Daerah (MPT)dari pos-pos Active Case Detection(ACD: tapak binaan/kilang), Pos-pos Passive Case Detection (PCD: klinik kesihatan, hospital) dan hospital swasta (Pengesahan spesis sahaja)

. Melaksankan program kualiti dan latihan bagi meningkatkan kemahiran anggota makmal dari masa ke masa demi pendiagnosan yang tepat. (Latihan Ulangkaji/ kompetensi)

. Melaksanakan program kualiti dan latihan bagi meningkatkan kemahiran anggota kesihatan yang terlibat dalam pengambilan smear darah untuk malaria (BFMP) dan filaria (BFFP)

PENGENALAN

MALARIA

*Malaria adalah sejenis penyakit mudah berjangkit yang masih merupakan masalah kesihatan awam yang utama di kebanyakan tempat di dunia.

*Jangkitan penyakit ini adalah amat merbahaya dan boleh membawa maut jika tidak dirawat derngan segera.

*Pada asasnya jangkitan penyakit Malaria adalah melalui gigitan nyamuk.

*Nyamuk Anopheles betina bertangunggjawab memindahkan parasit Malaria daripada seorang pesakit kepada seorang yang sihat.

*Penyakit Malaria disebabkan oleh sejenis parasit darah yang dipanggil Plasmodium

*Parasit ini terlalu kecil dan hanya boleh dilihat melalui mikroskop.

FILARIA

- Penyakit Filariasis Limfatik juga dikenali sebagai elephantiasis (untut)
- 2 spesis cacing yang menyebabkan filariasis limfatik iaitu *Wuchereria brancrofti* dan *Brugia spp (B. malayi, B.timori, B.pahangi)*
- Dijangkiti melalui gigitan nyamuk *Anopheles* dan *Mansonia*
- Ia antara penyakit yng sangat sukar untuk sembuh sekiranya tidak ditangani dengan cepat selain mengakibatkan risiko cacat kekal terutama pada pembesaran kaki, lengan dan beberapa anggota badan yan lain.

Nyamuk *Anopheles* (menggigit antara waktu subuh dan senja)

TEORI PENGAMBILAN SLAID MALARIA & FILARIA

Unit Makmal Kawalan Penyakit
Bawaan Vektor Pulau Pinang

Sub topik

- Jenis Filem Darah
- Ciri-ciri Filem Darah
- Teknik Pengambilan Slaid Malaria & Filaria
- Kesilapan Dalam Penyediaan Filem Darah

Jenis Filem Darah

Malaria :

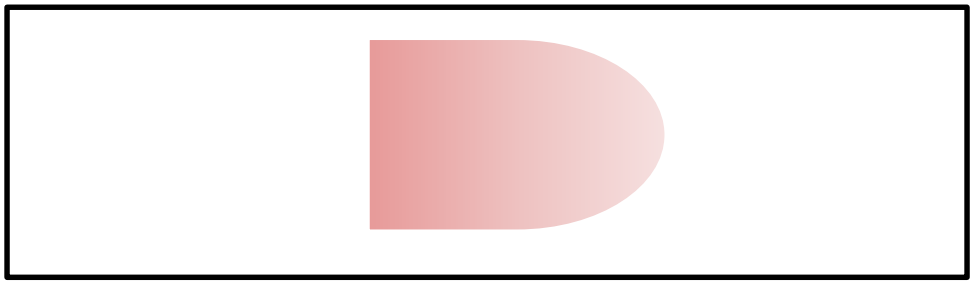
1) Filem Darah Tebal
2) Filem Darah Nipis

Filaria :

1) Filem Darah Tebal

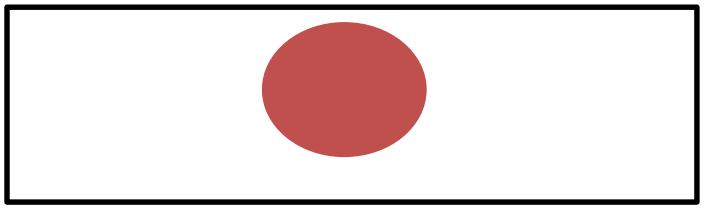
Ciri-ciri Filem Darah Nipis

1. Bentuk tebal, sederhana panjang dan membulat dihujung
2. Terdiri daripada satu lapisan sel-sel darah merah yang nipis dimana ciri-cirinya masih kekal dalam bentuk asal dan tersusun rapi.

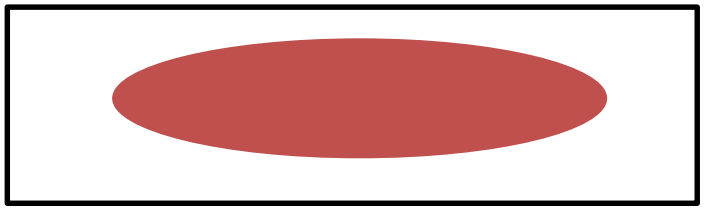


Ciri-ciri Filem Darah Tebal

1. Bagi malaria bentuk bulat dan saiznya lebih kurang 1cm (kecil sedikit dari syilling 5 sen)
2. Bagi filaria bentuk oval yang panjang, saiz 3cm x1cm
3. Disediakan sedikit tebal untuk mendapatkan sel darah merah yang banyak dan berlapis-lapis. Tujuannya supaya sebarang parasit yang hadir dapat dipadatkan dalam satu kawasan .
3. Selepas dicelup, proses hemolisis berlaku ke atas sel darah merah.



malaria



filaria

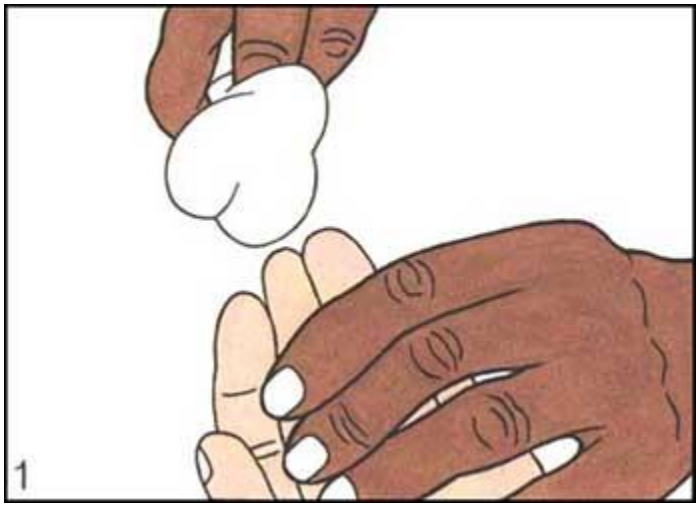
Pengambilan Slaid Malaria

Jenis sampel : Darah tusukan jari peri feri

Jenis filem darah : Filem darah tebal dan filem darah nipis

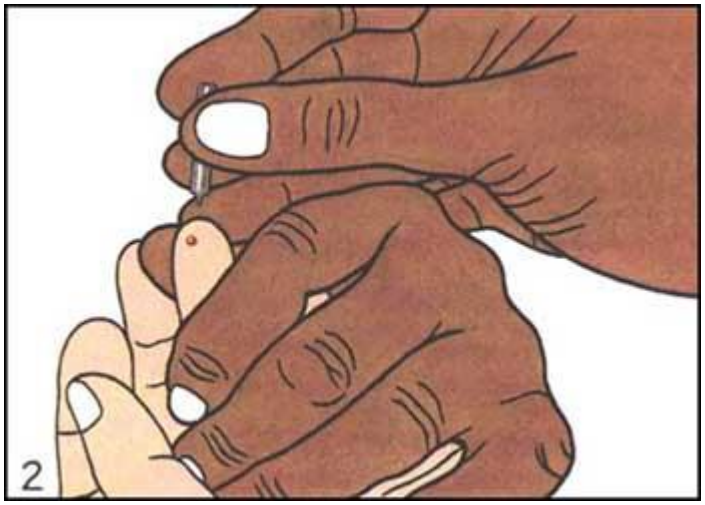
Peralatan:

1. Slaid kaca
2. Spreader
3. Sterile lancet
4. Alkohol swab
5. Kapas
6. Pensil



1

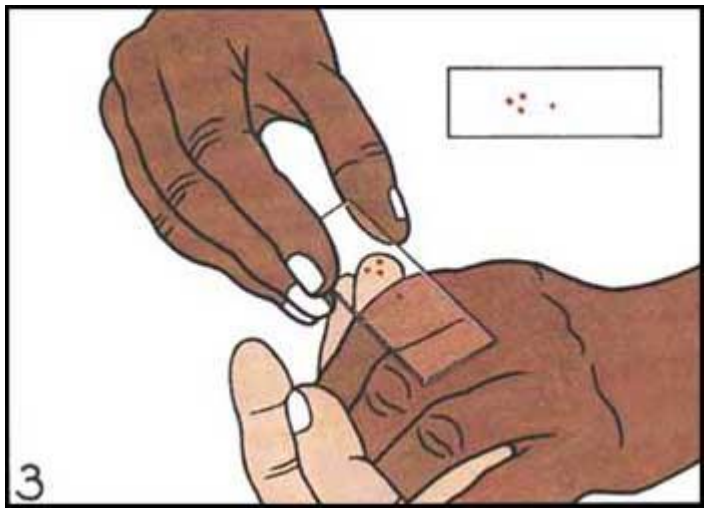
Sucihamakan bebola jari dengan menggunakan swab alkohol dan keringkan



2

Cucuk jari dengan sterile lancet dan tekan sehingga dapat setitik darah.

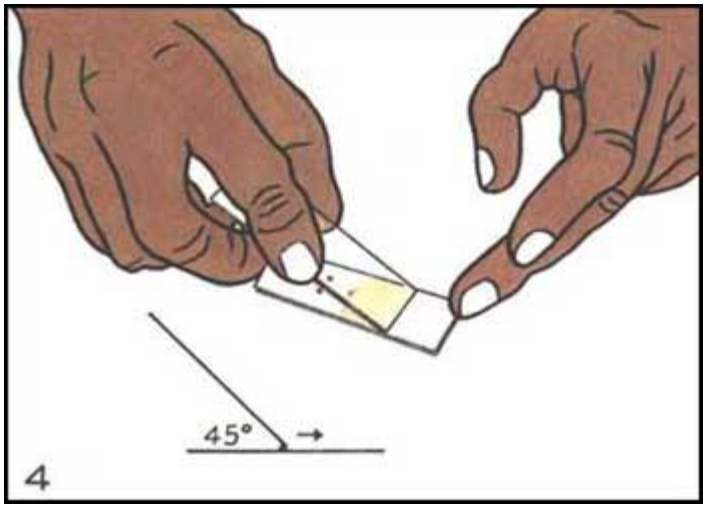
Titik darah pertama dibersihkan dan tekan perlahan-lahan untuk mendapat titik darah baru.



3

Sentuh permukaan slaid di atas titisan darah.

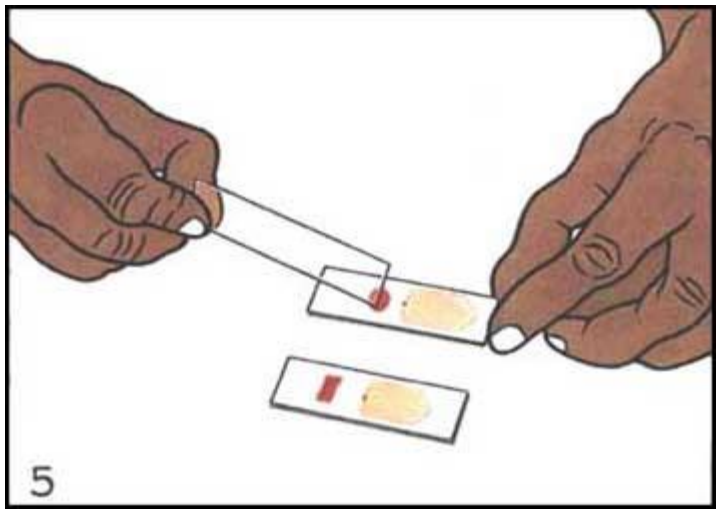
3 titik untuk FDT
1 titik untuk FDN



4

Untuk filem darah nipis, gunakan hujung spreader dan sentuh titisan darah pada sudut 45 darjah.

Pastikan hujung spreader menyentuh sepenuhnya permukan slaid sewaktu rebakan darah, supaya FDN yang dihasilkan adalah rata.



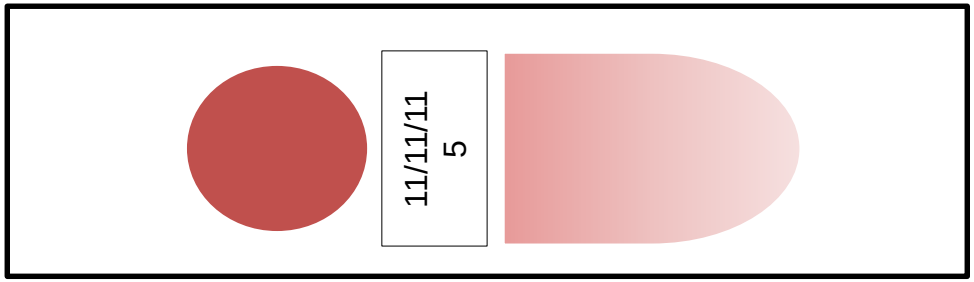
5

Satukan 3 titik darah FDT dengan menggunakan penjuru spreader sebesar 1cm untuk menyediakan satu filem darah tebal

6

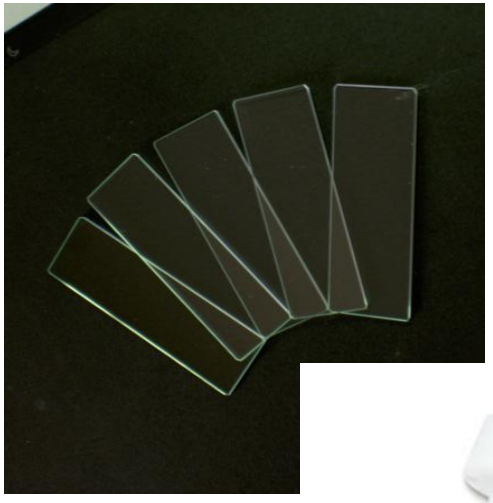
Tandakan tarikh, nama atau no pendaftaran pesakit dengan menggunakan pensil.

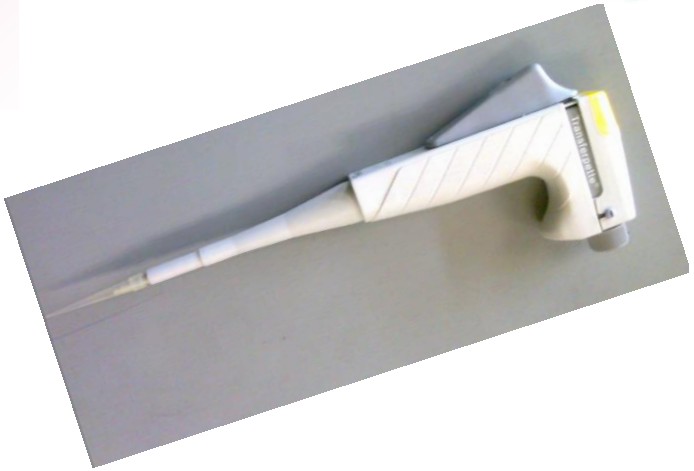
Filem darah hendaklah dikeringkan dalam keadaan mendatar, terutamanya untuk FDT supaya ianya kering pada ketebalan yang sama.



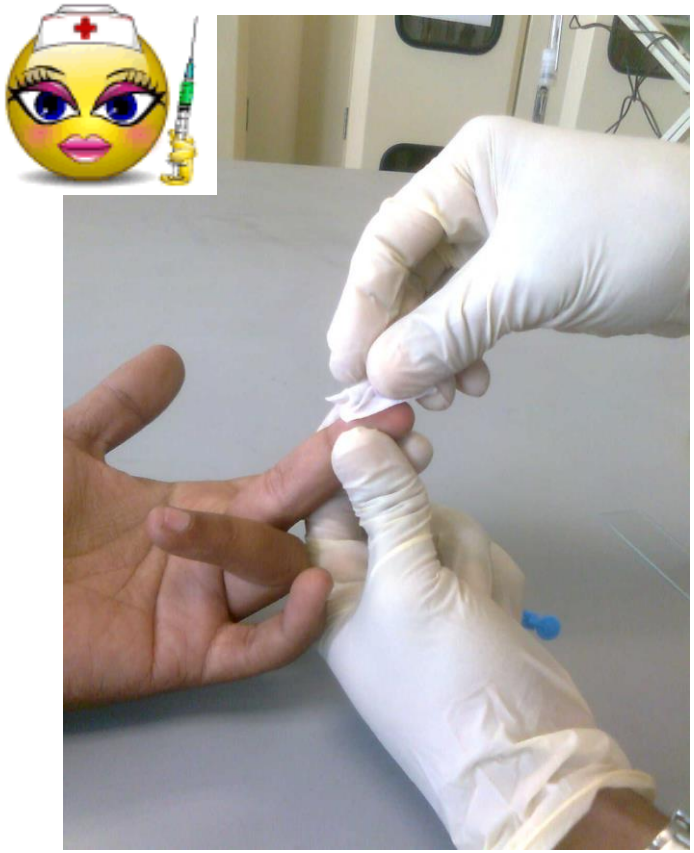
Peralatan

- Slaid
- Sterile lancet
- Pensel
- Mikropipet
- Alkohol swab



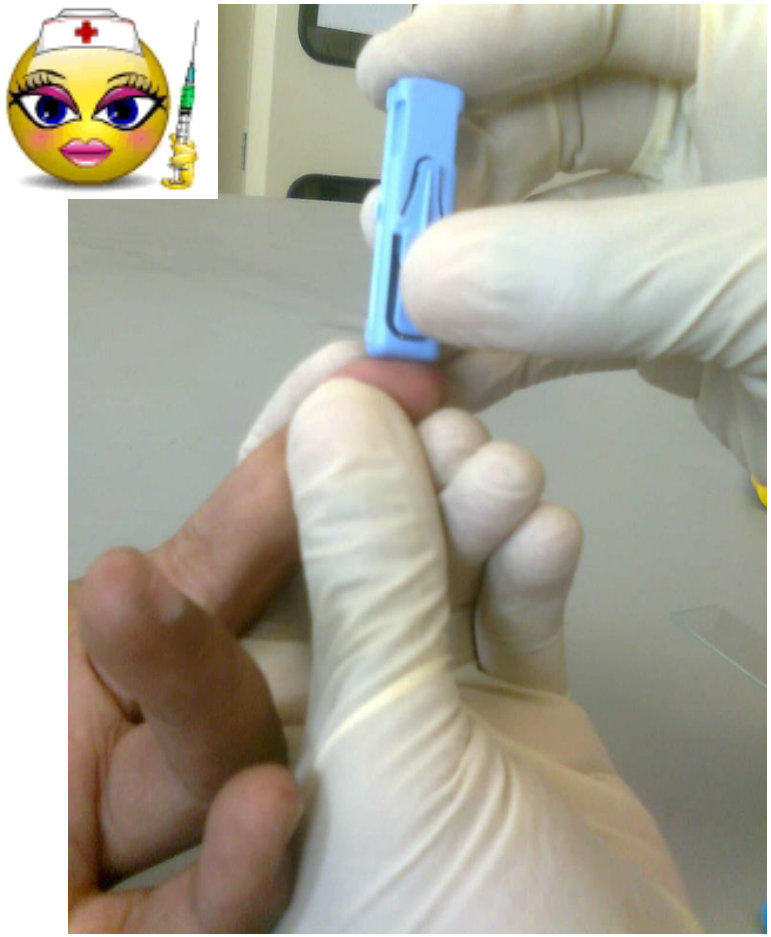


Cara Pengambilan Darah & Penyediaan FDT Untuk Filaria



1

Sucihamakan bebola jari dengan menggunakan swab alkohol dan keringkan



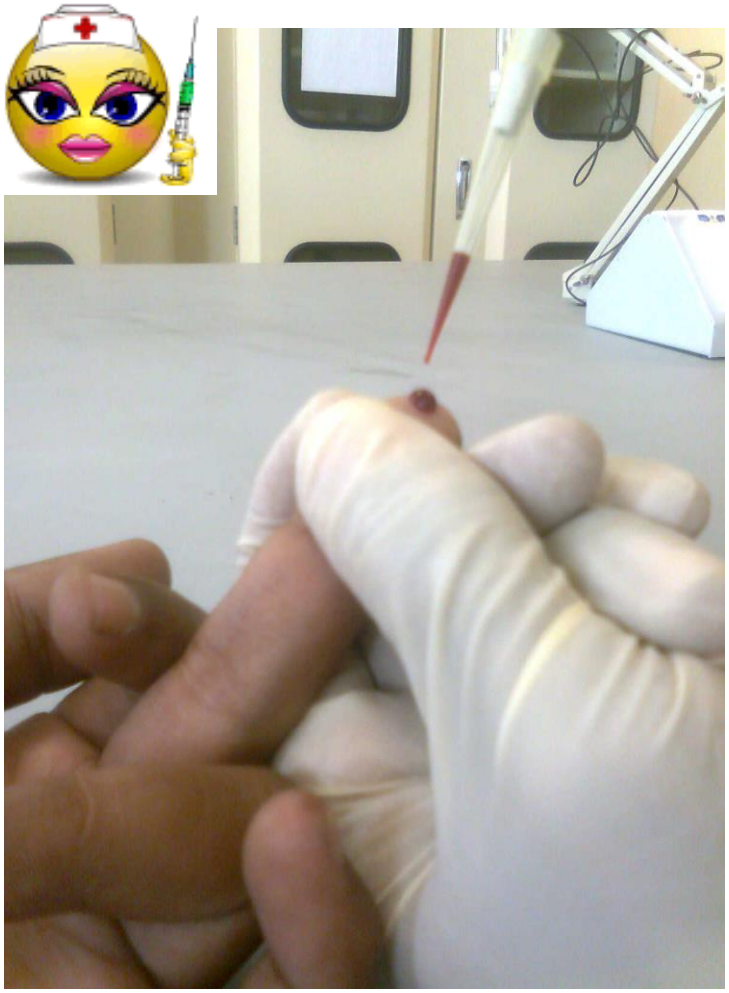
2

Cucuk jari dengan sterile lancet dan tekan sehingga dapat setitik darah.

Titik darah pertama dibersihkan dan tekan perlahan-lahan untuk mendapat titik darah baru.

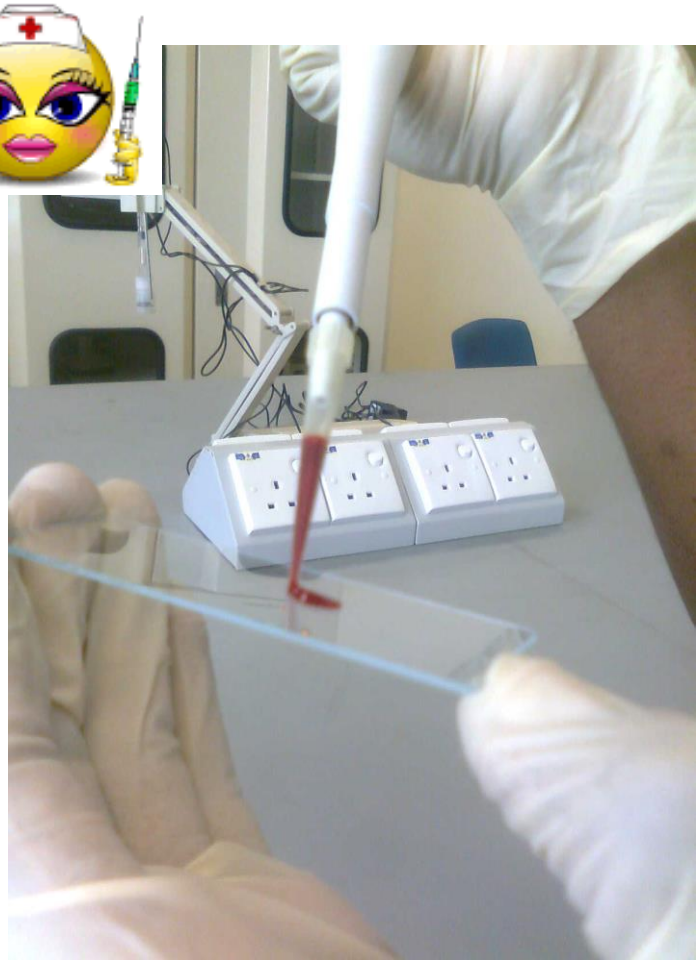
3

Jari diurut dan picit untuk mendapatkan titisan darah baru.



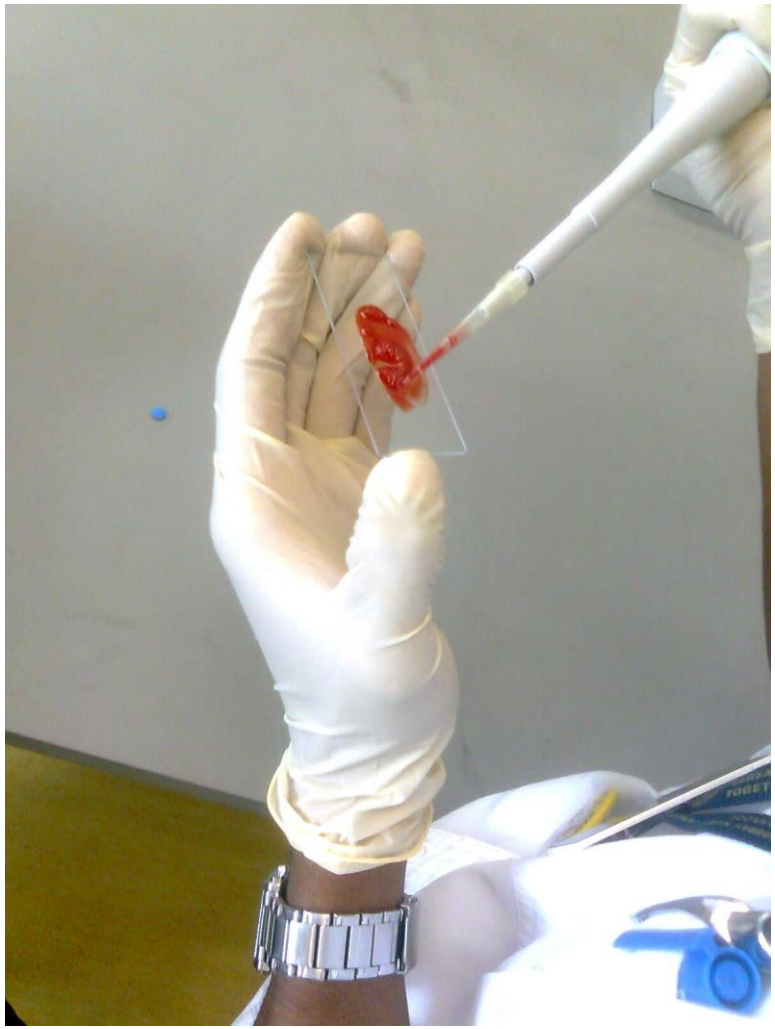
4

Darah diambil dengan menggunakan mikropipet yang telah di tetapkan isipadu 60 µl



5

Darah yang telah diambil diletakan diatas sekeping slaid kaca dan di sebarkan sebesar duit 20 sen (memanjang).

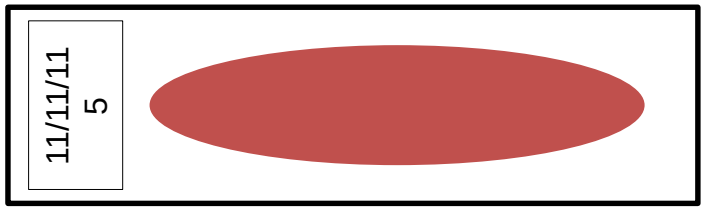


6

Seterusnya no slaid dan tarikh pengambilan darah dilabel.

Pengambilan Slaid Filaria

- ✓ Sampel : Darah periferi (tusukan jari) yang diambil pada waktu 8 malam hingga 12 malam
- ✓ Jenis filem darah : Filem Darah Tebal
- ✓ Isipadu darah : 60 µl

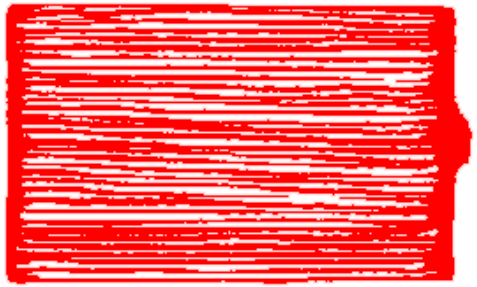


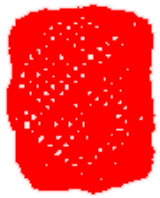
Selain daripada menggunakan mikropipet, boleh digunakan juga3 titik darah yang cukup untuk membuat smear dengan saiz dan bentuk yang betul.

Kesilapan Dalam Penyediaan Filem Darah

Darah terlalu banyak

- ❖Latar belakang terlalu biru
- ❖SDP banyak akan menutup parasit yang hadir
- ❖SDM berlapis-lapis





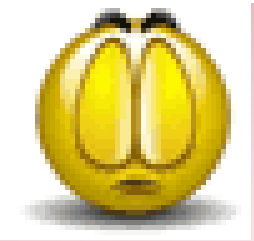
Darah terlalu sedikit

❖SDP terlalu sedikit dianggap tidak mengikut piawai yang memerlukan 200-500 SDP

Slaid berminyak

- Filem darah akan direbakan tidak rata diatas slaid yang berminyak
- Terdapat ruang kosong ditengah filem
- Sebahagian FDT tertanggal semasa pencelupan

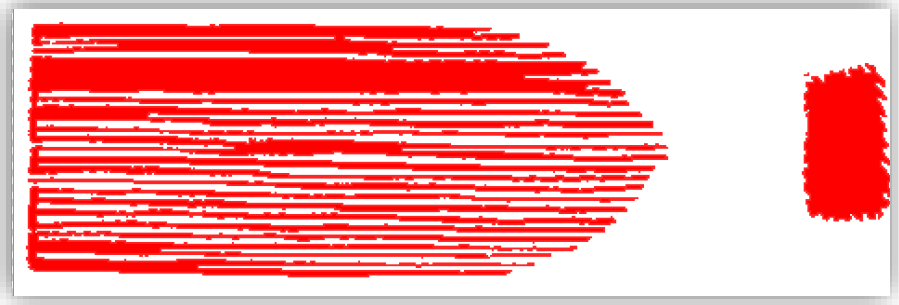
Hujung spreader sumbing

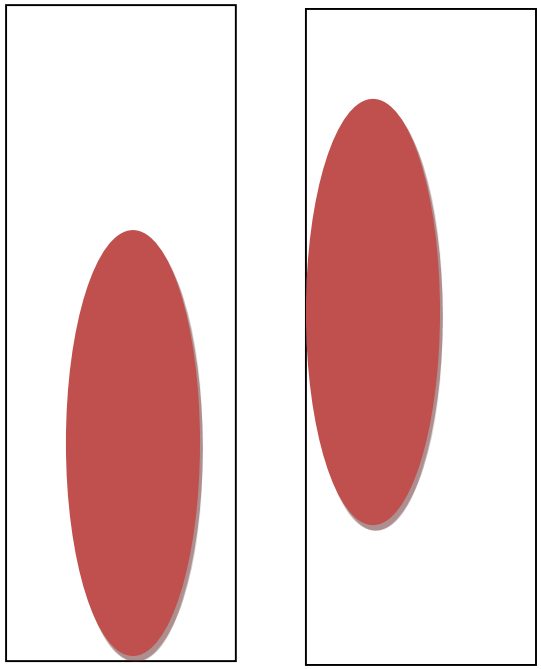


- ❖FDN yang dihasilkan tidak rata
- ❖Ekor FDN terlalu banyak .
- ❖Keadaan ini menyebabkan pemilihan kawasan sewaktu pemeriksaan adalah sukar

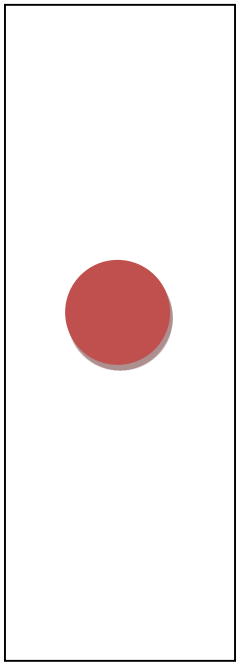
Kedudukan FDN dan FDT yang salah

- FDT yang disediakan berhampiran hujung slaid adalah tidak sesuai.
- FDN yang terlalu panjang memenuhi permukaan slaid.

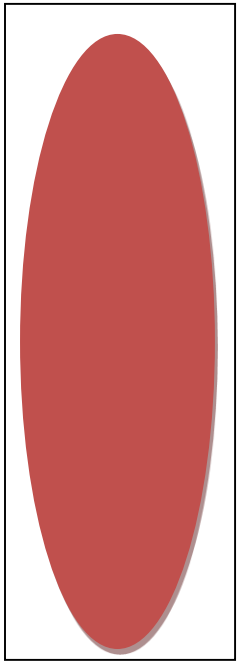




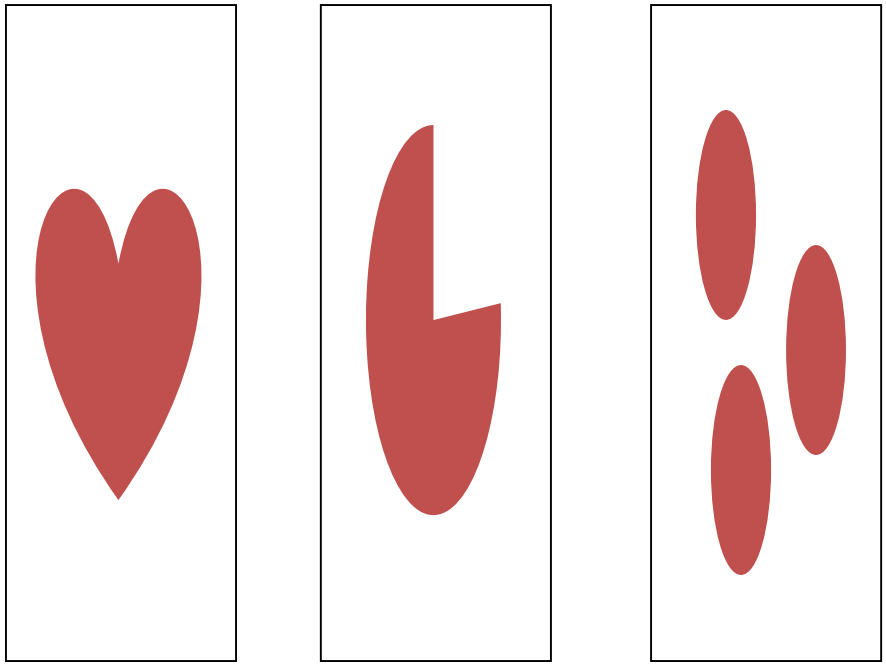
Smear terlalu
ke tepi



Smear sedikit, tidak mencukupi 60 µl

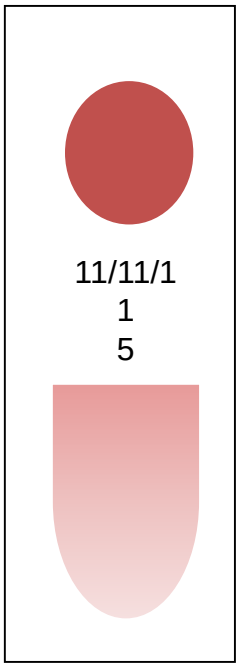


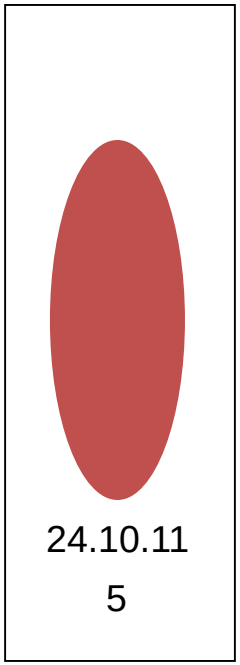
Smear terlalu besar

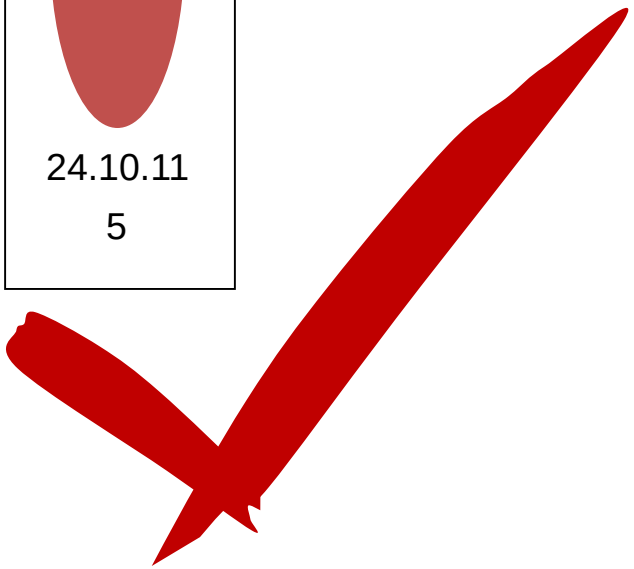


Bentuk smear yang tidak sesuai

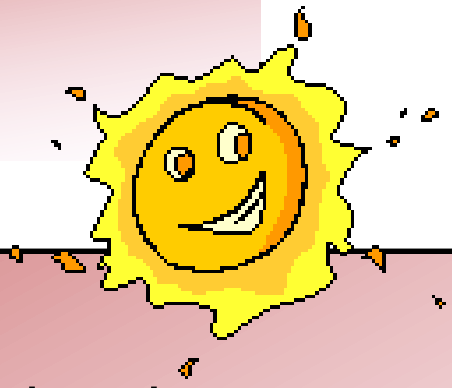
Smear Malaria & Filaria yang betul







Kesilapan Lain:



Filem-filem darah dibiarkan kering dibawah cahaya matahari atau tempat yang panas sehingga ‘autofixation’ berlaku.

Slaid-slaid dibungkus sebelum ianya betul-betul kering dan menyebabkannya melekat antara satu sama lain.

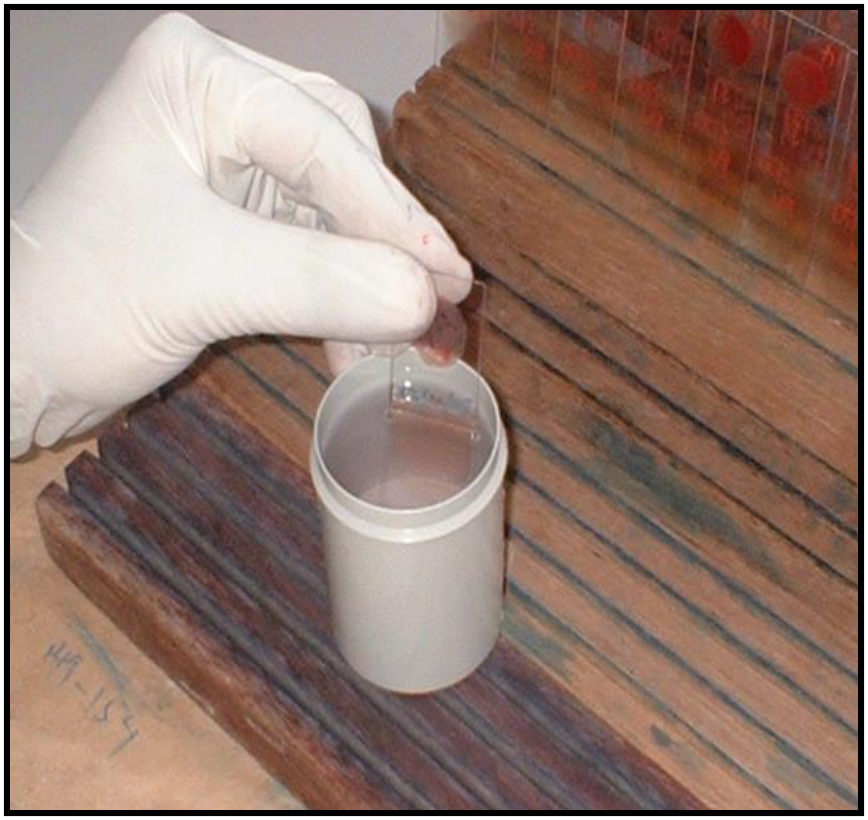
Filem darah yang dikeringkan ditempat yang terbiar menyebabkan ianya dimakan oleh serangga seperti lipas, semut dan lain-lain.

Slaid yang dikeringkan secara menegak menyebabkan FDT kering tidak rata.

Label yang dilekatkan menutupi kawasan filem darah, ini menghalang cahaya menembusi filem darah.

PENCELUPAN SLAID MALARIA DAN FILARIA

1.Slaid di'fiks' dengan 'methanol'



Kira 1, 2, 3, 4, 5 **ambil keluar!**

2.Slaid dikeringkan selepas fiks

3.Slaid disusun didalam ‘staining jar’

4.Slaid-slaid yang siap disusun

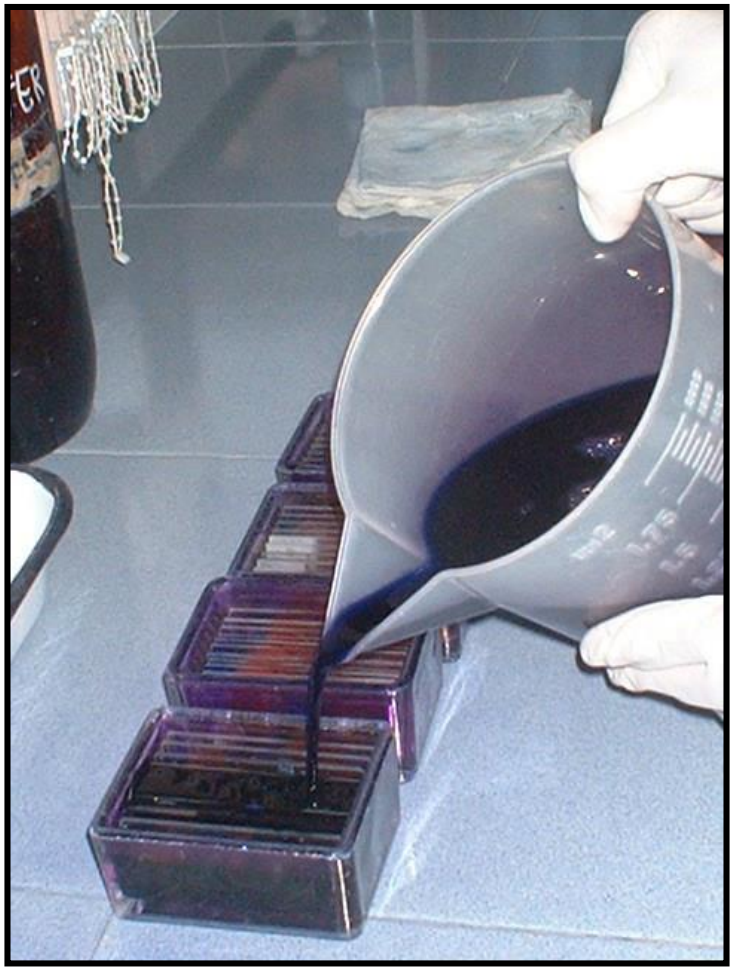
5.Menyukat stok Giemsa

6.Menyukat larutan Buffer pH 7.2

7.Stok Giemsa dan larutan Buffer dicampurkan

8.Larutan dikacau sebati

9.Tuang larutan Giemsa dalam ‘staining jar’

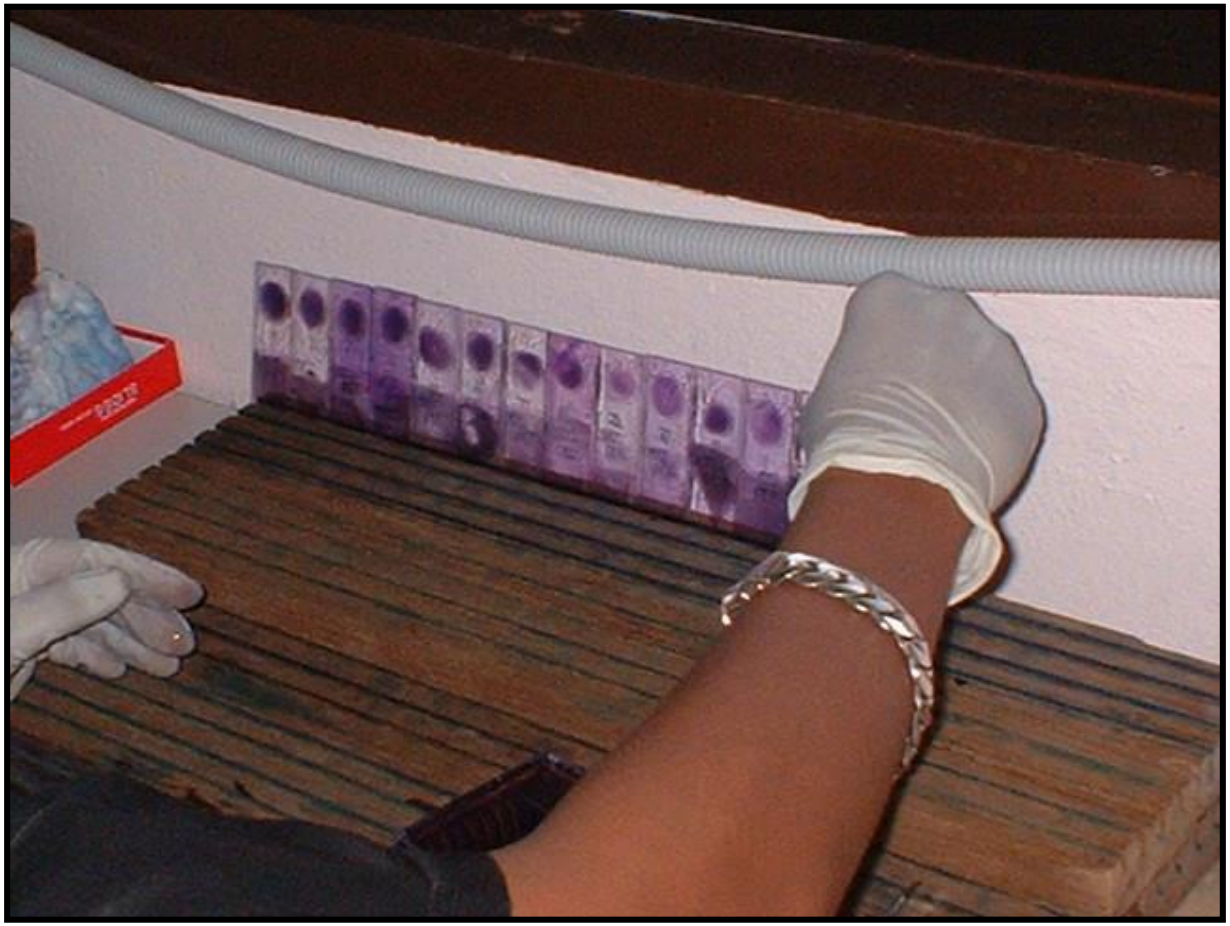


10. Slaid dicelup selama 45 minit

11.Slaid dibilas dengan air yang mengalir

12.Slaid yang telah dibilas

13.Slaid dikeringkan diatas rak kayu



PENCELUPAN (MALARIA)

PENCELUPAN STANDARD

- 3% kepekatan larutan kerja.
- Giemsa Ambil 3 ml stok giemsa pekat + 97 ml larutan buffer pH 7.2
- Celup selama 30 – 45 minit
- Untuk slaid lebih dari 10
- Sesuai untuk makmal vektor.

PENCELUPAN RAPID

- 10% kepekatan larutan kerja Giemsa.
- Ambil 10 ml stok giemsa pekat + 90 ml larutan buffer pH 7.2
- Celup selama 10 – 15 minit
- Untuk slaid kurang dari 10
- Sesuai untuk anggota hospital , klinik

PENCELUPAN (FILARIA)

- **3%** kepekatan larutan kerja.
- Giemsa Ambil 3 ml stok giemsa pekat + 97 ml larutan buffer pH 7.2
- Celup selama **1 JAM**

PEMERIKSAAN SLAID MALARIA DAN FILARIA

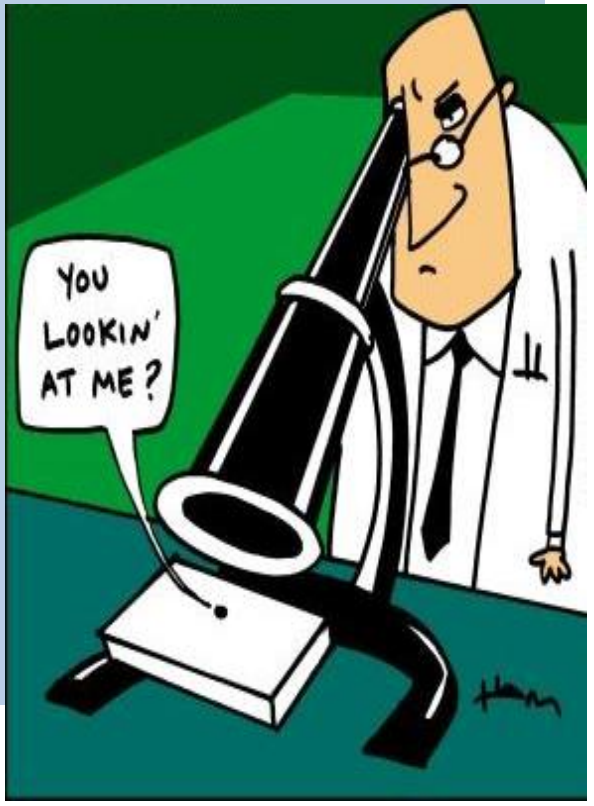
Tujuan mikroskopik adalah untuk melihat kehadiran parasit, mengenalpasti spesis dan pembilangan parasit

Spesis Malaria :

1) *Plasmodium falciparum*
2) *Plasmodium vivax*
3) *Plasmodium malariae*
4) *Plasmodium knowlesi*
5) *Plasmodium ovale*

Spesis Filaria :

1) *Wuchereria brancofti*
2) *Brugia malayi*
3) Dan lain-lain



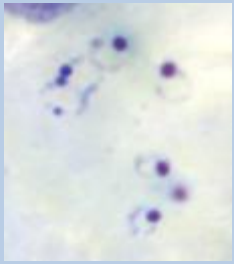
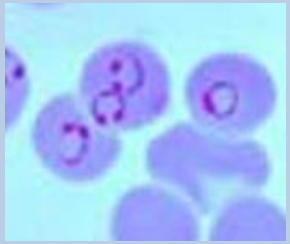
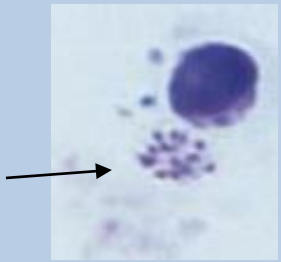
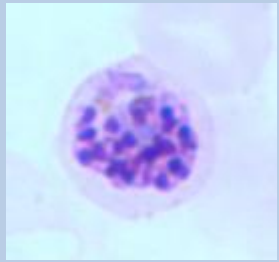
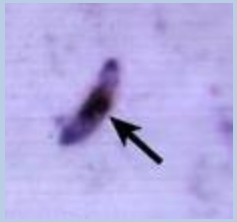
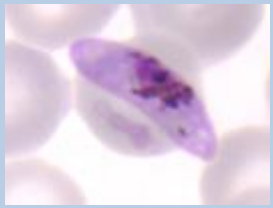
Tujuan menyaring filem darah tebal

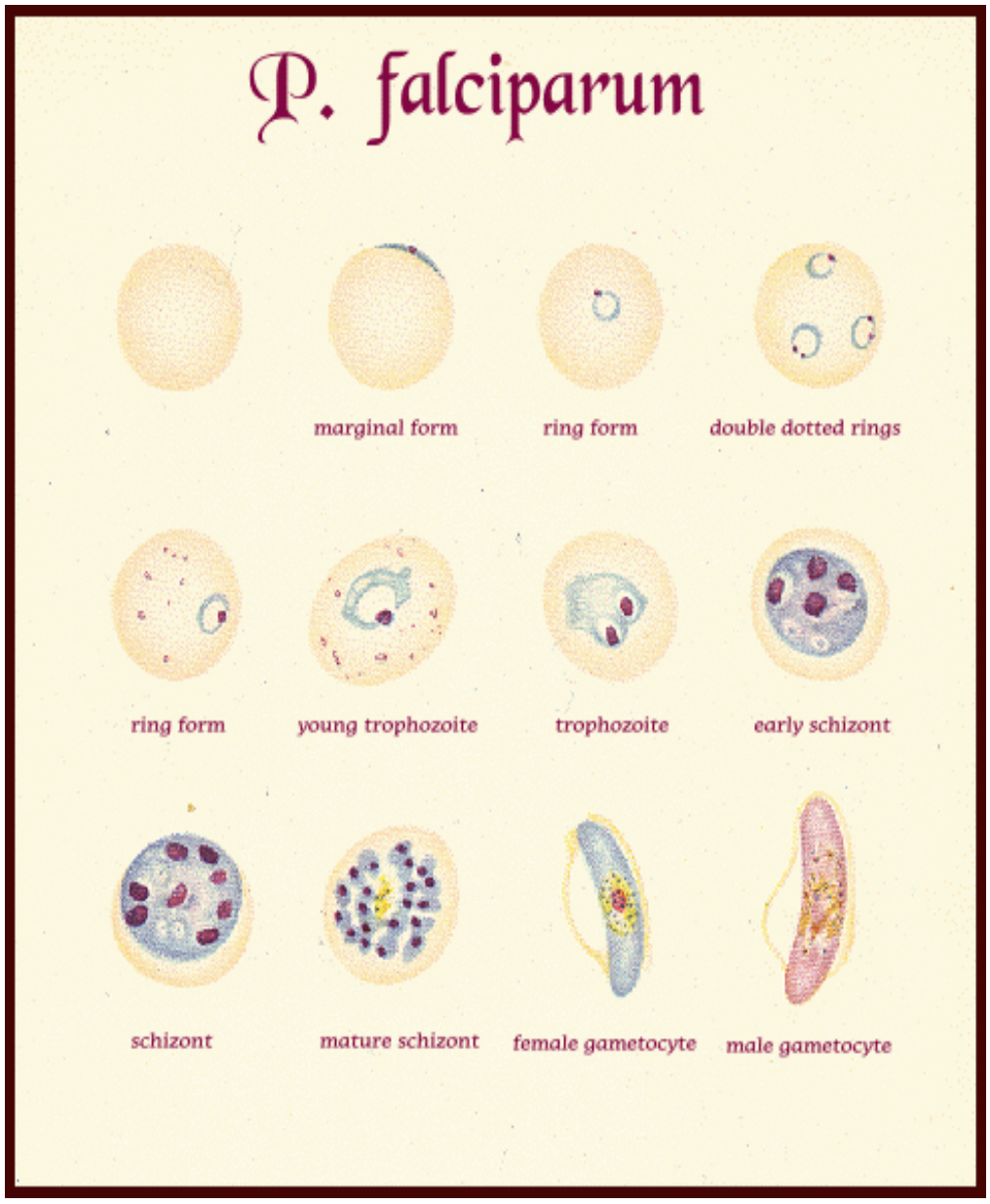
- ❖Mencari parasit
- ❖Melihat keterukan infeksi
- ❖Pembilangan parasit

Tujuan menyaring filem darah nipis

- ❖Menentukan spesis
- ❖Melihat perubahan morfologi SDM

Morfologi *Plasmodium Falciparum* x100

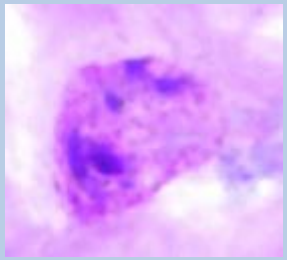
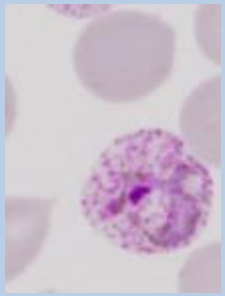
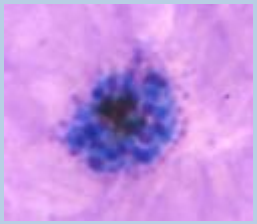
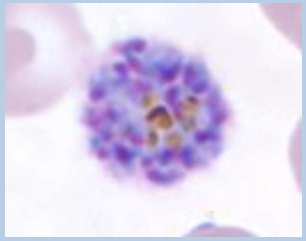
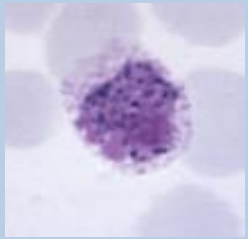
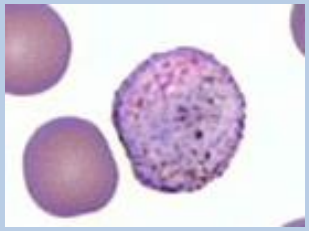
Filem Darah Tebal	Filem Darah Nipis	Huraian
Trofozoit 		•Saiz 1/5 daripada SDM •Double chromatin •SDM tidak membesar • terletak bahagian pinggir SDM (Marginal Form) •Mengandungi Maurer's Cleft
Skizon 		•Jarang terdapat dalam darah periferi. •Saiz hampir memenuhi SDM •SDM tidak membesar •Bentuk padat •Kromatin 8-30 (min 24) •Pigmen perang bertaburan
Gamet 		•Saiz lebih besar dari saiz SDM •Bentuk pisang /ginjal •Pigmen warna coklat hitam •Kromatin padat ditengah •Sitoplasma biru gelap

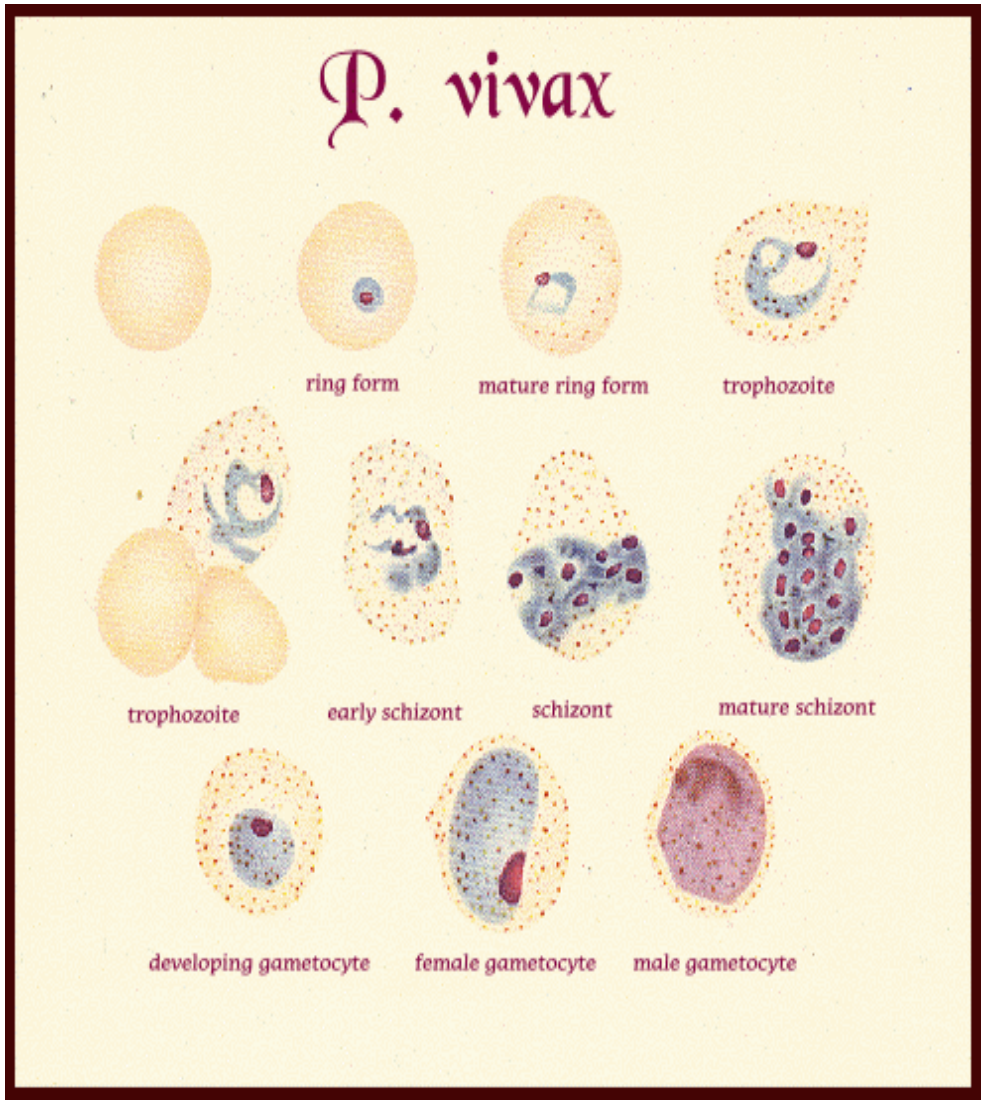


Diagnostic Points

1.Red Cells are not enlarged.
2.Rings appear fine and delicate and there may be several in one cell.
3.Some rings may have two chromatin dots.
4.Presence of marginal forms.
5.It is unusual to see developing forms in peripheral blood films.
6.Gametocytes have a characteristic crescent shape appearance. However, they do not usually appear in the blood for the first four weeks of infection.
7.Maurer's dots may be present.

Morfologi *Plasmodium Vivax* x100

Filem Darah Tebal	Filem Darah Nipis	Huraian
Trofozoit 		•Bentuk cincin •Saiz 1/3 daripada SDM •SDM membesar •Sitoplasma bentuk ameboid dengan vakuol yang jelas kelihatan •Terdapat pigmen halus (bintik Schuffner)
Skizon 		•Bentuk ameboid •Pigmen bertaburan •SDM membesar •Jumlah merozoit 14-24
Gamet 		•Memenuhi SDM •Bentuk bulat padat •Sitoplasma biru gelap •Kromatin padat.



Diagnostic Points

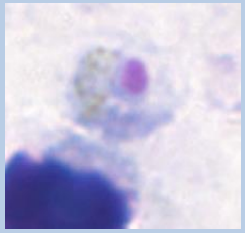
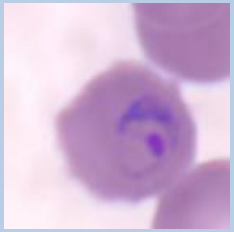
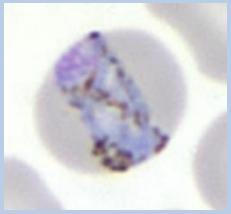
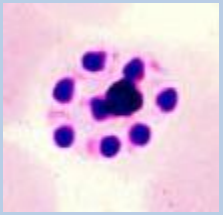
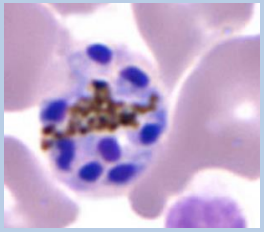
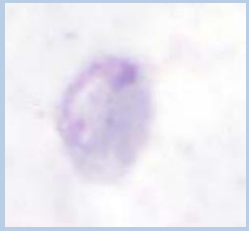
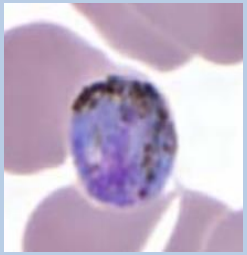
1.Red cells containing parasites are usually enlarged.

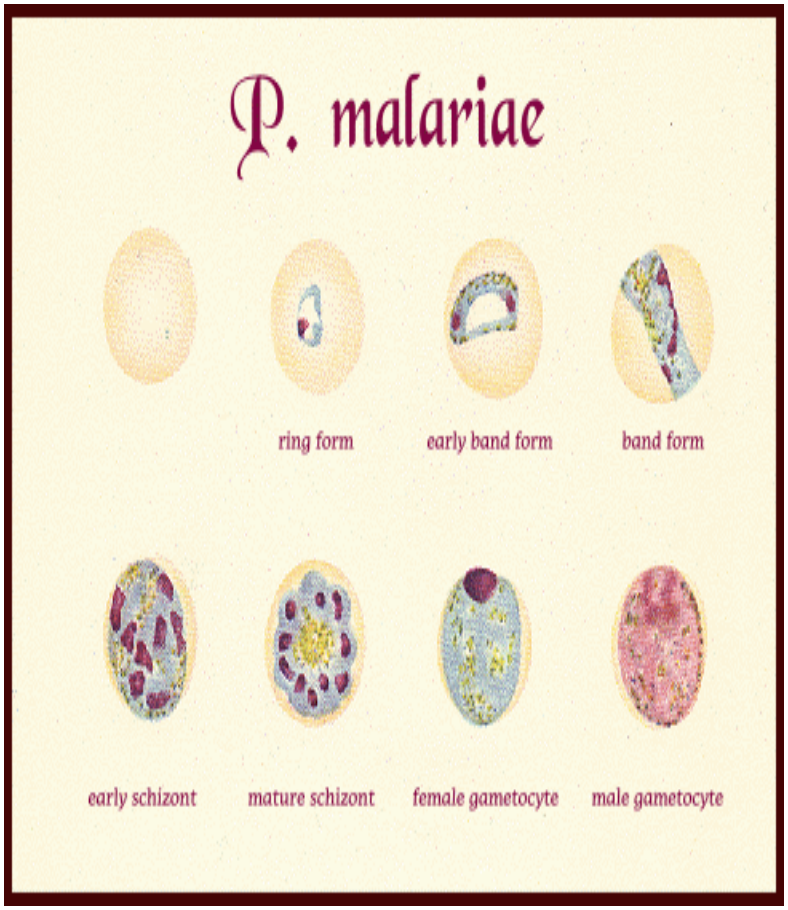
2.Schuffner's dots are frequently present in the red cells as shown above.

3.The mature ring forms tend to be large and coarse.

4.Developing forms are frequently present

Morfologi *Plasmodium Malarae* x100

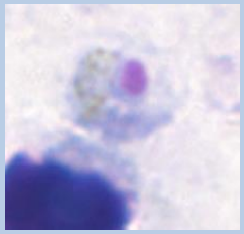
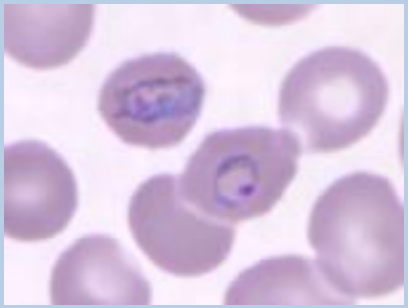
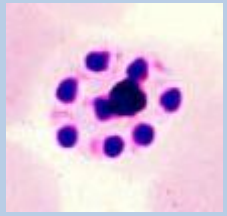
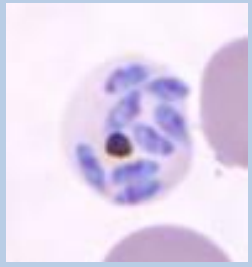
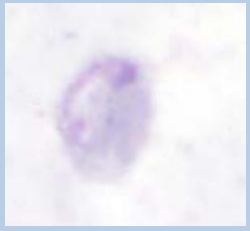
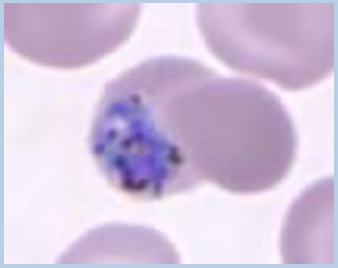
Filem Darah Tebal	Filem Darah Nipis	Huraian
Trofozoit 	 	•Bentuk cincin •Saiz 1/3 daripada SDM •SDM tidak membesar •Trofozoit awal berbentuk Bird Eye •Trofozoit matang berbentuk padat berjalur. •Pigmen kasar bertabur (Ziemann's Stipling) dalam bentuk rod •Kromatin berbintik/berfibril
Skizon 		•SDM tidak membesar •Memenuhi SDM •Bentuk bersegmen (daisy head) •Pigmen perang bertaburan
Gamet 		•SDM tidak membesar •Saiz lebih kecil daripada SDM •Bentuk bulat dan padat



Diagnostic Points

1.Ring forms may have a squarish appearance.
2.Band forms are a characteristic of this species.
3.Mature schizonts may have a typical daisy head appearance with up to ten merozoites.
4.Red cells are **not** enlarged.
5.Chromatin dot may be on the inner surface of the ring

Morfologi *Plasmodium Knowlesi* x100

Filem Darah Tebal	Filem Darah Nipis	Huraian
Trofozoit 		Ciri-ciri seperti *Plasmodium Malarae* PCR dilakukan untuk membezakan antaranya. Parasit ini diperolehi daripada Monyet – Manusia sahaja.
Skizon 		
Gamet 		

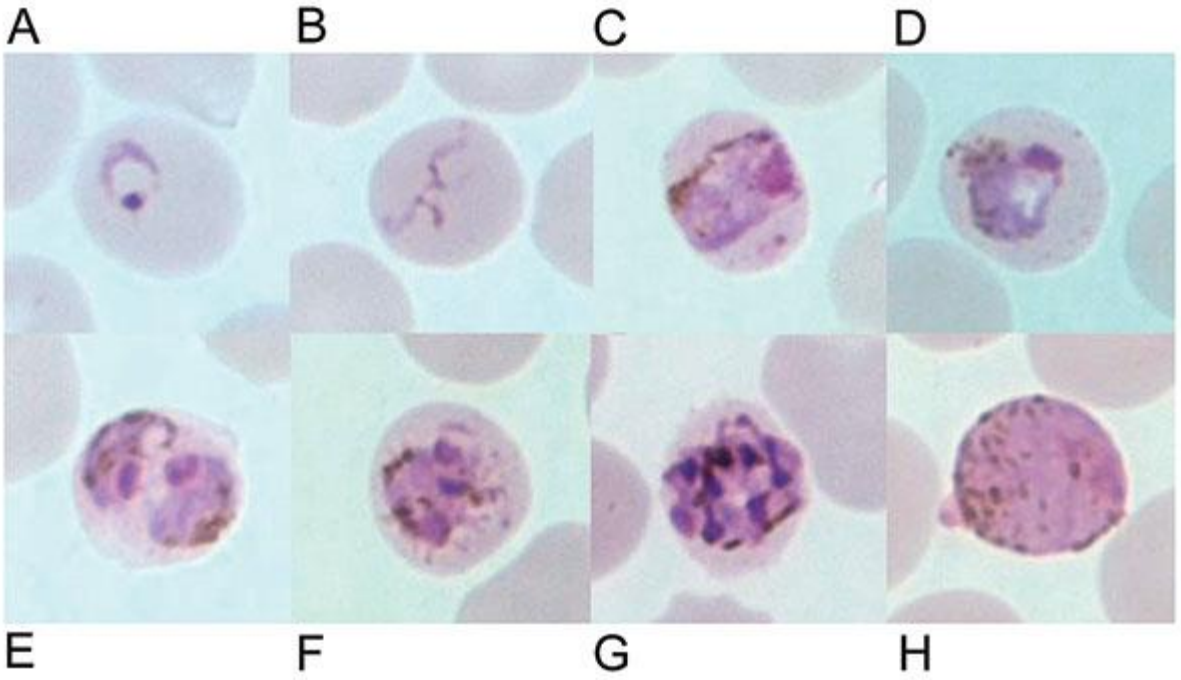
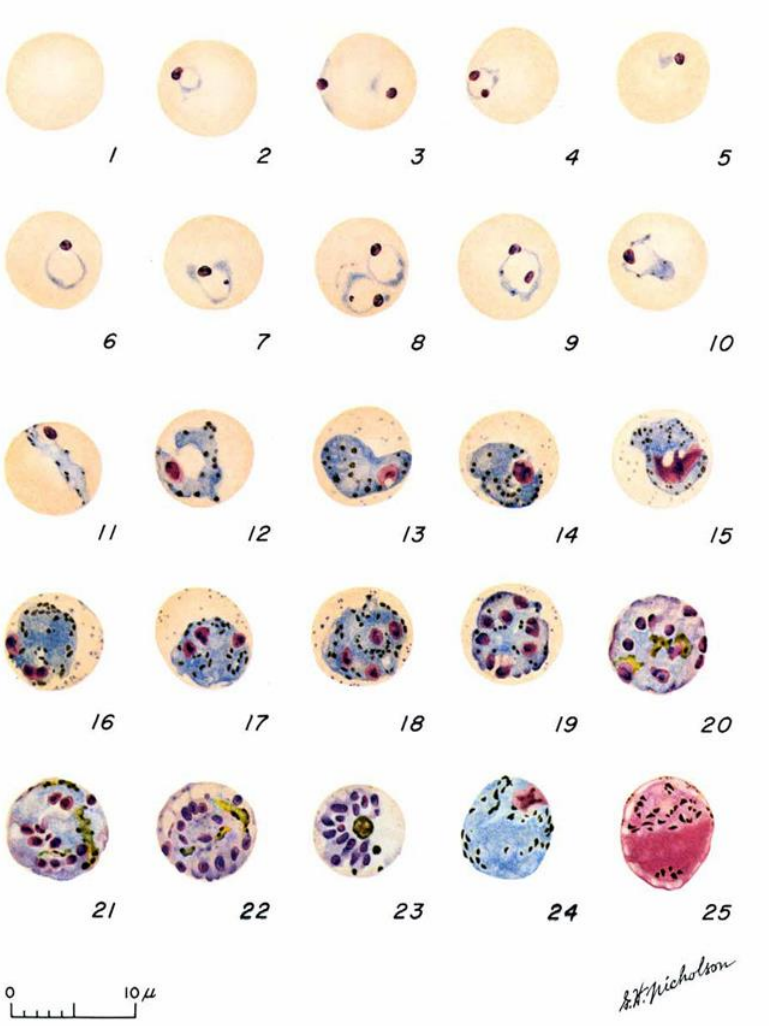


Figure 1. Giemsa-stained thin blood films depicting A) ring stage, B) tenue form of young trophozoite, C) band-shaped growing trophozoite, D) growing trophozoite with little or no amoeboid activity, E) double growing trophozoites, F) early schizont, G) late schizont in an erythrocyte with fimbriated margins, and H) mature macrogametocyte. Discernible Sinton and Mulligan stippling is in C, D, and F



PLASMODIUM KNOWLESI

PENGIRAAN DENSITI PARASIT MALARIA DAN FILARIA

Thick and Thin Films

- **THICK FILM**
  - lysed RBCs
  - larger volume
  - positive or negative
  - parasite density
  - more difficult to diagnose species

- **THIN FILM**
  - fixed RBCs, single layer
  - smaller volume
  - good species differentiation
  - requires more time to read
  - low density infections can be missed
  - More 80,000 parasite per µL or ≥100 parasite in 1 fields thick smear

Examination of Blood film

Thick blood film to determine if *Plasmodium* is present

If thick film is positive , then examine the thin blood film to determine the species (single or multiple) present.

In Malaria Microscopy

Three key questions need to be answered during the observation / screening to ascertain malaria infection:

1. Are malaria parasites present in the blood smear? Yes or No……
2. If yes, which species and stages do they belong to?
3. If yes, how many of them are present per µl of blood?

Criteria for examination

Thick blood film

Reading using immersion oil, 100 X

Thin Blood Film – 100 X

- Read area of blood film where red blood cells are evenly distributed and no overlapping

Materials you require:

- A microscope
- two tally counters (one to count parasites and the other to count leukocytes)
- immersion oil
- a simple electronic calculator.
- lens paper

Determination of Presence and absence of malaria infection

- Examine under X 100 magnification and look for malaria parasite.
- If no parasite seen, then move until 200 microscopic fields, it will be considered as negative for malaria parasite infection.
- It will then be reported as “no malaria parasite seen in 200 thick film microscopic fields.

Calculating Parasite Density – Thick blood film

- Count the number of asexual stages (rings, trophozoites and schizont) on one hand
- Count sexual stage (gametocyte), separately (record in a note book)
- Count white blood cells in the same fields, (count ≥ 200 WBCs) on another hand,

- If, ≥ 200 white blood cells **have** been counted, 100 or more parasites are found, Then record your parasite density.

- If, ≥ 200 white blood cells have been counted, the number of parasites is 99 or fewer, counting should be continued up to 500 white blood cells.

- Some parasitaemias are so heavy that hundreds of parasites are counted per oil immersion field.

- In this situation, counting up to 100 white blood cells or the total number in about five oil-immersion fields (assuming about 15 white blood cells per thick-film field) recommended.

Thick film.

- The number of parasites is counted in relation to a standard number of leukocytes.
- The most accurate count is obtained when the patient's true white cell count is known, this is usually not possible if you are working in the field.
- The number of leukocytes used is **8,000** assuming an average normal total leukocytes count in human
- It is arbitrary, with wide variations among individuals, but the figure is accepted as reasonably accurate.

The parasitemia per ul of blood is calculated by using the formula

$$= \frac{\text{number of asexual stages}}{\text{number of leukocytes counted}} \times 8000^{*}$$

And

$$= \frac{\text{number of sexual stages}}{\text{number of leukocytes counted}} \times 8000^{*}$$

* 8000 = assuming an average normal total leukocytes

Example of calculation

number of asexual stage = 120

number of sexual stage = 4

number of leukocytes = 200

Therefore ,

Asexual stage density = (120 ÷ 200) X 8000 = 4800

Sexual stage density = (4 ÷ 200) X 8000 = 160

The result is reported as parasite species 4800 / 160 per μl of blood

If no gametocyte stage presence the result is reported as Parasite species 4800 / 0 per μl of blood

The blood film is considered negative for malaria parasite only when there is no parasite seen in 200 microscopic fields

The parasitemia per ul of blood is calculated by using the formula – if more than one species you may do the following,

Parasite A and B individually

$$= \frac{\text{number of asexual stages} \times 8000^*}{\text{number of leukocytes counted}}$$

$$= \frac{\text{number of sexual stages} \times 8000^*}{\text{number of leukocytes counted}}$$

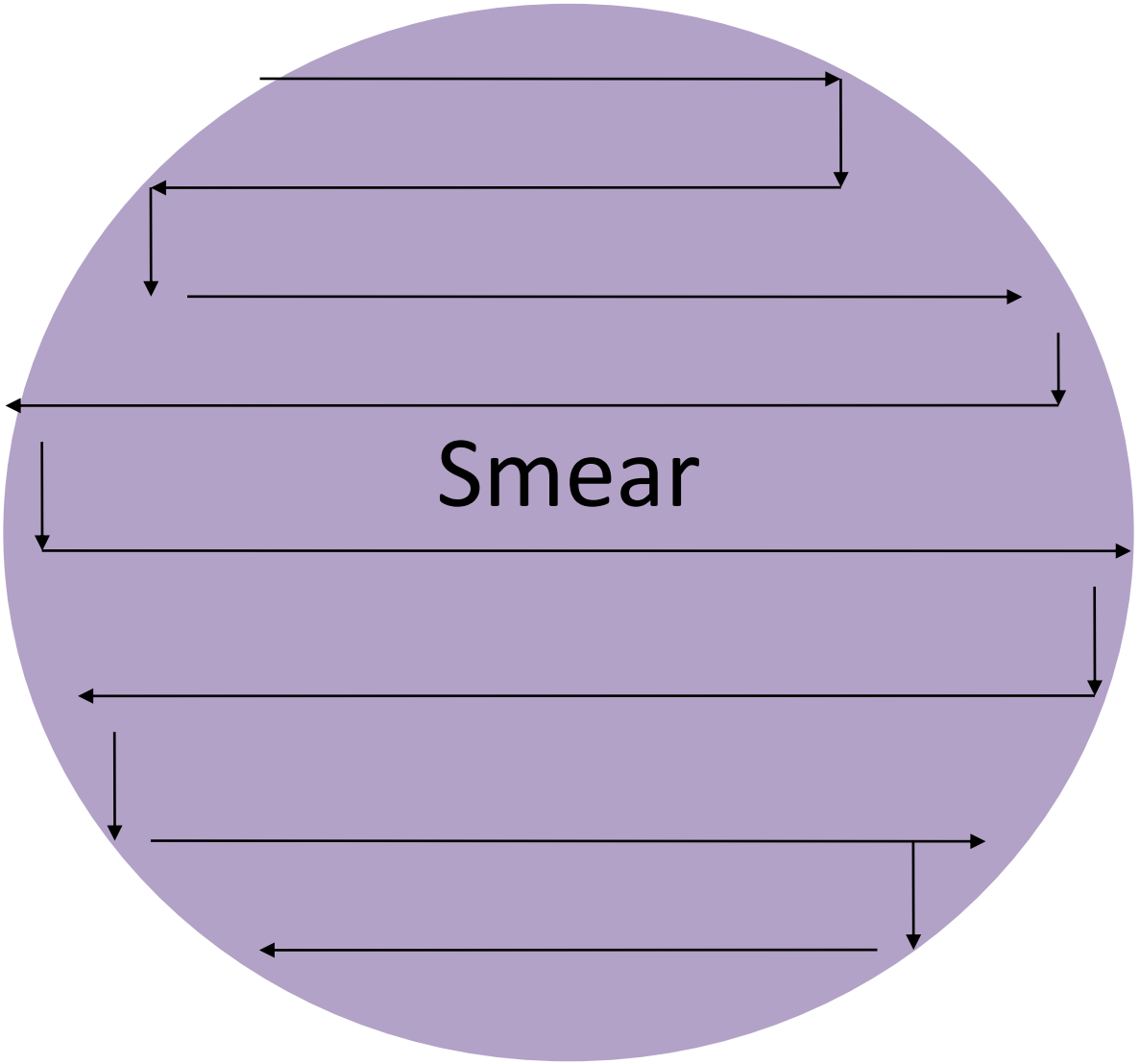
The result is reported as :

parasite A asexual / gametocyte per μl of blood

parasite B asexual / gametocyte per μl of blood

- Even if only one parasite is found after screening in only one microscopic field, it is considered as presence of malaria infection. The report will be reflected in the calculation of parasite density.
- Then proceeds to identification of the parasite using thin blood film.

Cross-sectional Method



Cross -sectional method

- Start the count at top left hand corner of smear
- Focus on a field , count accurately WBC and / or MP
- Continue counting , about 5 fields apart , with or without WBC and / or MP

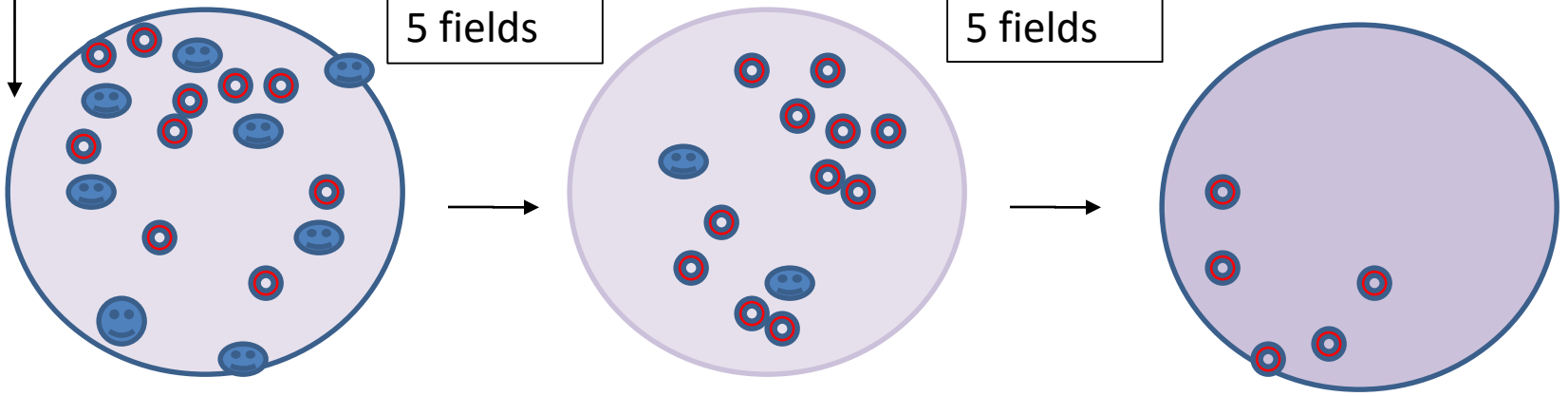
Thick Film : x100 , oil immersion count

Top left hand area – start to count here

Separate by about 5 fields

5 fields

5 fields



Do not count exactly 5 fields apart

Counting each field accurately

- Scan whole field
- Don't scan just the centre of field
- Look at the edge of field
- Scan before counting
- Divide field into quadrants
- Only count WBC or MP that can be clearly seen
- Do not move the stage

Reasons for high & low counts

- High counts – counting too many MP or not enough WBC
- Low counts – counting too many WBC or not enough MP
- SLOW DOWN !!!!
- No special technique

Estimating Parasite Density using the plus system

The ‘plus system’ is an old method, less accurate, unreliability, no longer accurate

,

+	1-10 parasites per 100 oil immersion thick film
++	11-100 parasites per 100 oil immersion thick film
+++	1-10 parasites per each oil immersion thick film
++++	> 10 parasites per each oil immersion thick film

Depending on your SOP - not encourage to use the plus system in malaria diagnosis

Calculating Parasite Density – Thin blood film

- Select one microscopic field
- Count the number of parasitized (on one hand) and nonparasitized red blood cells (RBCs) (on another hand)
- Count asexual stages separately from gametocytes
- Count 500-2000 RBCs (fewer RBCs if parasitemia is high)
- The calculate:

$$\% \text{ parasitemia} = \frac{\text{\# parasitized RBCs}}{\text{total \# of RBCs}} \times 100$$

Parasite count

The parasite density of a positive blood film must be known because:

- The clinician needs to know the severity of the infection.
- The clinician needs to know how the infection is responding to treatment.

Sources of errors

- Procedure

- Wrong method - Not examining enough fields / WBC

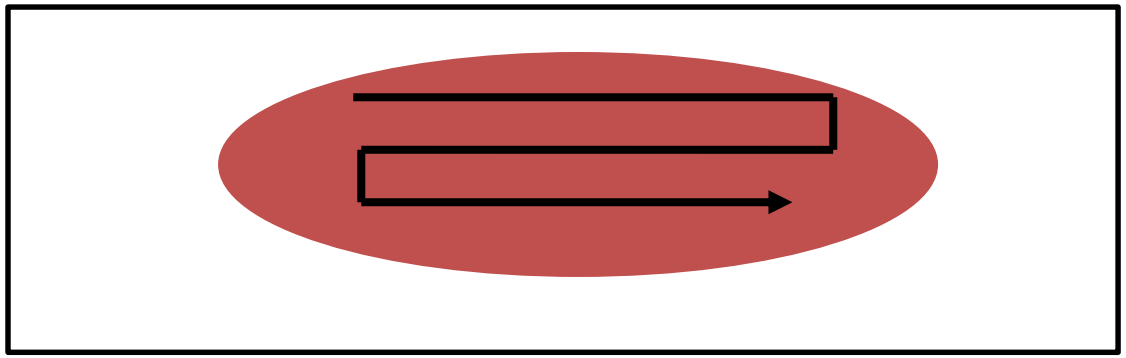
- Mechanical

- Tally counter - Calculator

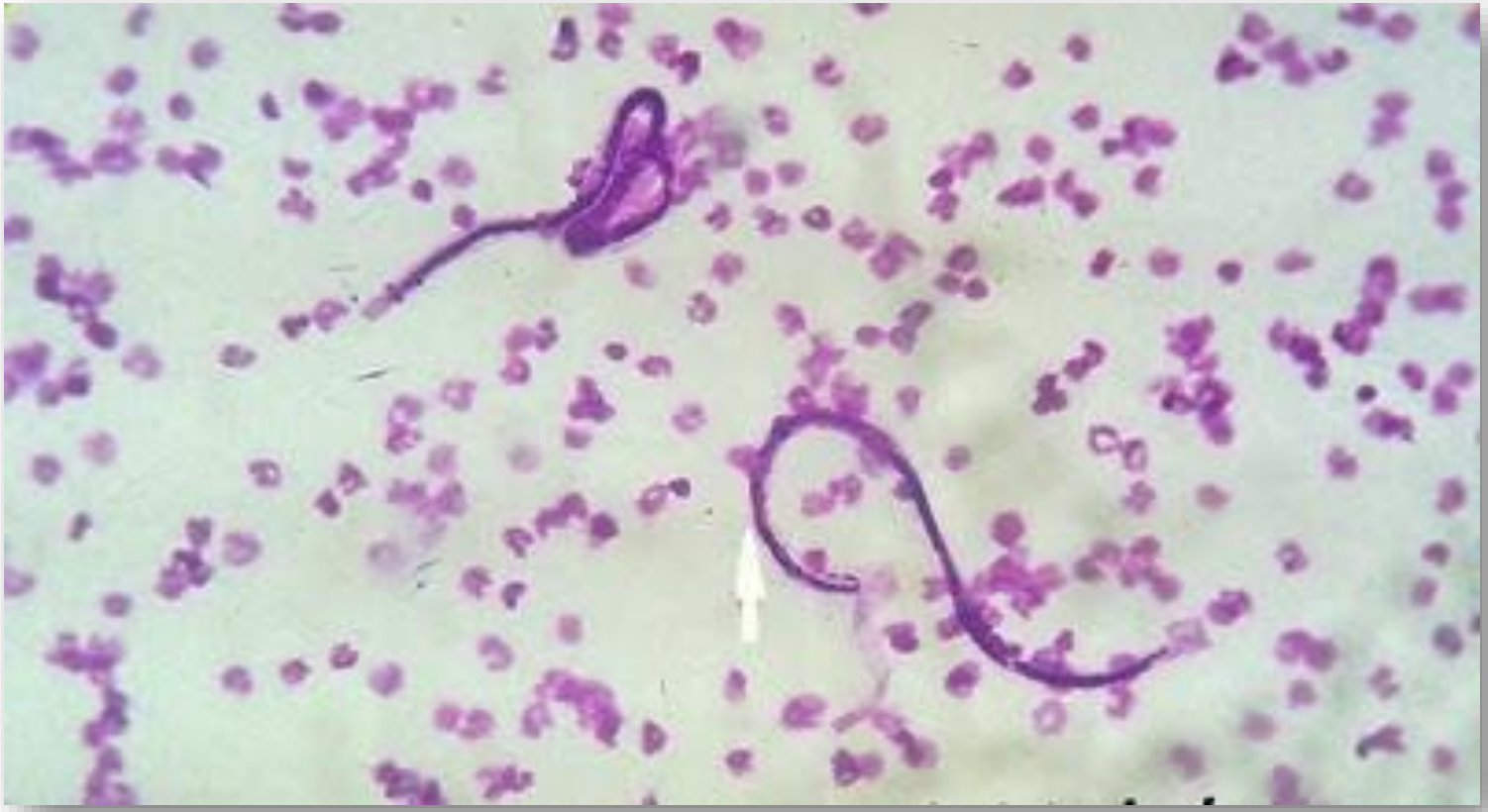
- Human

- Tired - Concentration

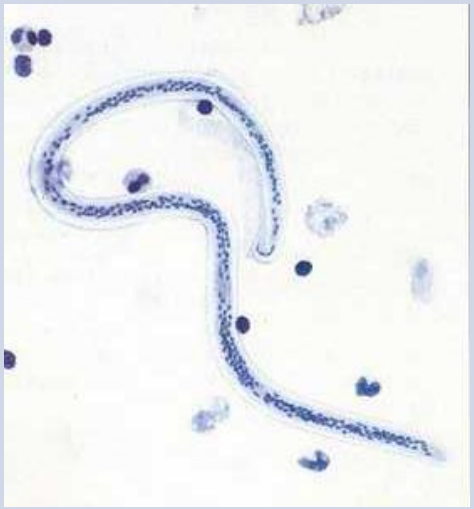
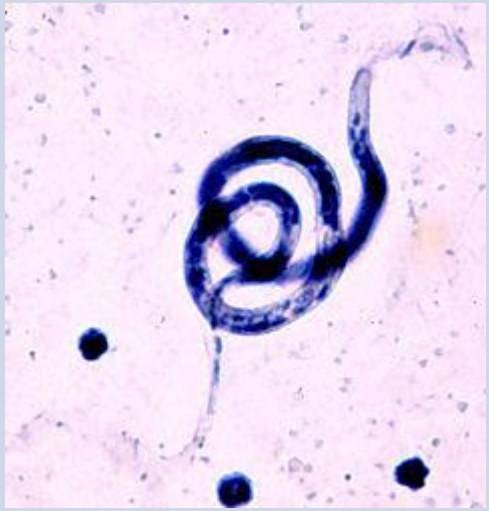
Pembilangan filaria dilakukan pada keseluruhan smear. Penyaringan dilakukan secara vertical



Morfologi mikrofilaria x10



Morfologi mikrofilaria x100

Wuchereria brancofti	*Brugia malayi*
	
Sarung tidak diwarnakan Tidak berbelit Nukleus tersusun	Sarung bewarna merah ungu Berbelit-belit Nukleus bertindih

SOALAN ?

TERIMA KASIH