

Field's stain A & Field's stain B

Product No. A6592; A6600

Description

Field's stain is a histological method for staining of blood smears. The definitive diagnosis of malaria infection and other parasites is still based on finding such organisms in blood films. In thin films the red blood cells are fixed and parasitized cells can be identified by their morphology.

Field's stain A Product No. A6592

Order No.	Quantity
A6592,0005	5 g
A6592,0025	25 g

Specification

λ_1 max. (1 cm; H₂O) approx. 660 nm
 λ_2 max. (1 cm; H₂O) approx. 290 nm

Field's stain B Product No. A6600

Order No.	Quantity
A6600,0005	5 g
A6600,0025	25 g

Specification

λ_1 max. (1 cm; H₂O) approx. 520 nm
 λ_2 max. (1 cm; H₂O) approx. 305 nm
 λ_3 max. (1 cm; H₂O) approx. 255 nm

Storage: RT, protect from light.

Preparation of Field's Solutions

Field's Solution A:

- (a) Dissolve in 1 L of distilled Water
- | | |
|---|-------|
| Field's stain A (A6592, Methylene blue/Azure) | 2.6 g |
| Disodium hydrogen phosphate anhydrous | 2.6 g |
| Potassium dihydrogen phosphate | 2.6 g |
- (b) Heat solution in a warm water bath (max 60°C) for 30 min.
(c) Leave to stand 24 hours at room temperature.
(d) Filter the solution!

Field's Solution B:

- (a) Dissolve in 1 L of distilled Water
- | | |
|---------------------------------------|-------|
| Field's stain B (A6600, Eosin) | 2.0 g |
| Disodium hydrogen phosphate anhydrous | 2.6 g |
| Potassium dihydrogen phosphate | 2.6 g |
- (b) Filter the solution!

Protocols*

Staining methods for the identification of parasites

1. Field's Stain Method for Thick Blood Films

Red blood cells are lysed during this procedure. Diagnosis is based on the appearance of the parasite. The parasites appear more concentrated (denser under the microscope) in thick blood films as compared to thin blood films.

Instructions

Prepare Field's Solutions A & B as described on page 1.

1. Place a drop of blood on a microscope slide and spread to make an area of approximately 1 cm².
(The film should be spread thin enough so that it appears transparent)
2. Air dry the film.
Do not fix in methanol.
3. Stain the slide into **Field's stain A** for 3 seconds.
4. Wash the slide in water for 3 seconds; agitate gently.
5. Stain the slide into **Field's stain B** for 3 seconds and wash gently in tap water for a few seconds to remove excess stain.
6. Drain the slide and air dry the slide.

Results

Microscopic features of Malaria parasites in Field's stained thick blood film.

Note: Only experienced personnel should carry out the interpretation of stained malaria parasite.

Caution should be exercised when examining thick blood films as clotted cells and blood platelets may be confused with malaria parasites.

Preferentially examine the edges of the dried blood film. The edges of the film will often be better than the centre, where the film may be too thick or cracked.

Examine 200 microscopic fields of a stained thick film (approx. 10 min), before declaring the slide negative

Malaria parasites: deep red chromatin and pale blue cytoplasm.

Leucocyte: purple nuclei and pale blue background.

Red cells are lysed: only background stroma remains.

Note: lysis of red cells may occasionally fail.

White cells, platelets and malaria pigment can also be seen on a thick film.

Schizonts and gametocytes, if present, are also easily recognizable.

2. Rapid Field's Stain Method for Thin Blood Films

This is a modification of the original Field's stain to enable rapid staining of fixed thin films. It is a useful method for rapid presumptive species identification of malarial parasites. This method is suitable for malaria parasites, *Babesia sp.*, *Borrelia sp.* and *Leishmania sp.*

Malaria parasites: It shows adequate staining of all stages of development. Malaria species identification can be made, based upon the size and shape of the various stages of the parasite and the presence of stippling (i.e. bright red dots) and fimbriation (i.e. ragged ends). However, malaria parasites may be missed on a thin blood film when there is a low parasitaemia. Therefore, examination of a thick blood film is recommended. With a thick blood film, the red cells are approximately 6-20 layers thick which results in a larger volume of blood being examined.

However, staining with Giemsa is always the method of choice for definitive species differentiation.

Instructions

1. Air-dry the film.
2. Fix in methanol for 1 minute.
3. Flood the slide with 1 ml of **Field's stain B**, diluted 1 in 4 with distilled water.
4. Immediately, add an equal volume of undiluted **Field's stain A**, mix well and allow to stain for 1 minute.
5. Rinse well in tap water and drain dry.

Note: Only experienced personnel should carry out the interpretation of stained malaria parasite!

When examining thin blood films for malaria you must look at the infected red blood cells and the parasites inside the cells!

Literature

* **Protocols** are adapted from

<http://www.southampton.ac.uk/~ceb/Diagnosis/Vol1.htm>

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