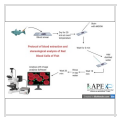

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Staining of fish Red Blood Cells

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1 *Works for me* dx.doi.org/10.17504/protocols.io.bd9yi97w

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ABSTRACT

This is a technique to extract blood and prepare blood films of fish to make stereological analysis of blood cells. This is a modification of Rosenfeld (1947) technique by Tavares-Dias and Moraes (2006) using common staining techniques and is called **May-Grünwald-Giemsa-Wright**.

Rosenfeld, G. (1947) Corante pancrômico para hematologia e citologia clínica. Nova combinação dos componentes do May-Grünwald e do Giemsa num só corante de emprego rápido. Memórias do Instituto Butantan 20:329-334

Tavares-Dias, M., & de Moraes, F. R. (2006). Caracteristicas hematologicas da Tilapia rendalli Boulenger, 1896 (Osteichthyes: Cichlidae) capturada em "pesque-pague" de Franca, Sao Paulo, Brasil. Bioscience Journal, 19(1): 107-114.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Martins, B. O.; Franco-Belussi, L.; Siqueira, M. S.; Fernandes, C. E. S.; Provete, D. B. (2020): The evolution of red blood cell shape in a continental radiation of fishes.

MATERIALS

NAME	CATALOG #	VENDOR
Methanol	PA-33900HPLCCS4L	P212121
100ml Giemsa Stain Stock Solution	786-1065	G-Biosciences
20 mg Eugenol	orb104769	biorbyt
Wright's stain (Eosin methyl blue)	WB0989.SIZE.25g	Bio Basic Inc.
1 ml syringes (or U-100 Insulin Syringe)	329461	BD Biosciences
EDTA	17892	Thermo Fisher
Shandon™ Wright-Giemsa Stain Kit, Wright-Giemsa solution	9990710	Thermo Fisher

STEPS MATERIALS

NAME	CATALOG #	VENDOR
Ethylenediaminetetraacetic acid	E9884	Sigma – Aldrich

MATERIALS TEXT

You can make your own MGGW stain solution or buy ready-to-use kits.

SAFETY WARNINGS

Citation: Robson Andrade Rodrigues, Mayara Schueroff Siqueira, Brenda Oliveira Martins, Carlos Eurico Fernandes, Lilian Franco-Belussi, Diogo Provete (04/02/2020). Staining of fish Red Blood Cells. https://dx.doi.org/10.17504/protocols.io.bd9yi97w

Methanol is volatile, so use a fume hood.

BEFORE STARTING

Before collecting blood samples, you need to anaesthetize fish using a eugenol solution (**50 mg** /L)

20 mg Eugenol
by biorbyt
Catalog #: orb104769

.

Blood extraction

1 Prepare a **1 ml** syringe with **3 % volume** EDTA.

Syringe spec is 24 G x 3/4” (20 mm x 0.55 mm)

Ethylenediaminetetraacetic acid
by Sigma – Aldrich
Catalog #: E9884

1.1 Dilute **3 g** of EDTA powder in **100 ml** of distilled water

2 Extract blood from the *vena caudalis* or by cardiac puncture (for large fish specimens), or by decaptation (for small specimens)

If you have small fish specimens, you don't need the syringe. You just have to use 1 µL pippete to get one drop of blood from the head of fish.

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Cardiac puncture of small fish specimens

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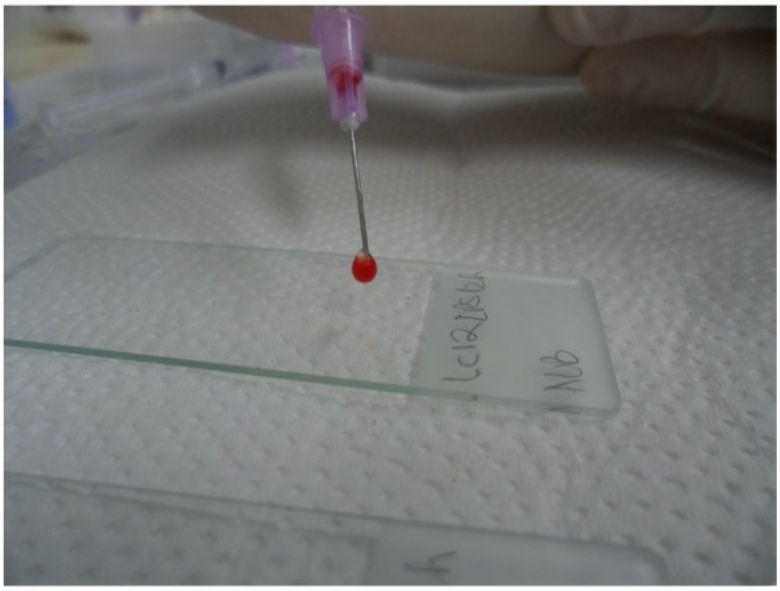
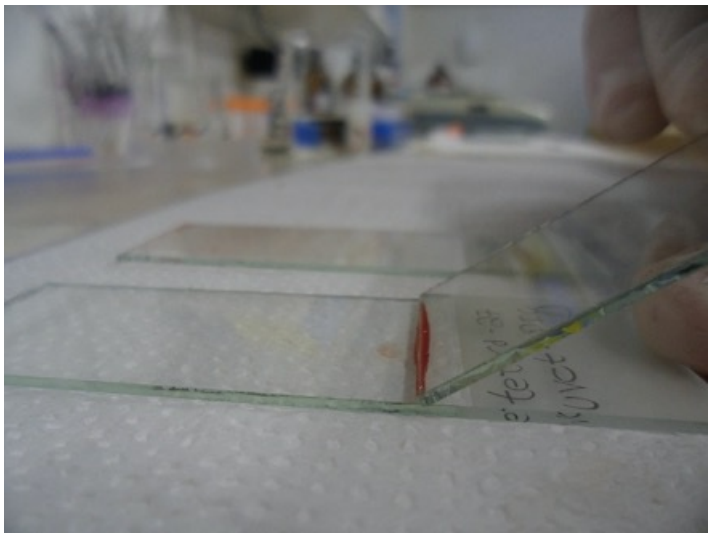
Preparing blood film

3 Place one blood drop on a microscope slide



Placing blood drop on the slide

4 Take an extra microscope slide, touch the blood drop at 45º and then slide it until the drop is fully spread



Begining of the procedure showing how to position the extra slide

5 Let the slide dry **00:20:00** 20m

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Staining

6 Take about **300 µl** of the MGGW stain solution and drop it on the slide so all the slide is covered.

The stock solution of the May-Grünwald-Giemsa-Wright (MGGW) stain is made with **1 g** of eosin methyl blue of May-Grünwald, **1 g** of eosin methyl blue of Giemsa, and **1 g** eosin methyl blue of Wright, diluted in **1 L** of methanol. The stock solution should be kept at **25 °C** for at least 1 week before use. Then, you should filter it and it's ready to be used.

Methanol
by P212121
Catalog #: PA-33900HPLCCS4L

500ml Giemsa Stain Stock Solution
by G-Biosciences
Catalog #: 786-1066

Wright's stain (Eosin methyl blue)
by Bio Basic Inc.
Catalog #: WB0989.SIZE.25g

7 Wait for **00:03:00** 3m

8 Add drops of distiled water to the slide with stain to dilute the stain.

you can use a plastic straw or a pasteur pipette to homogeneize the solution on the slide.

A fine, shiny layer of stain precipitate will appear on the slide. This is because the stain does not dilute in methanol.

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9 Wait the stain dry for **00:10:00** 10m

10 Wash slides in tap water **00:00:15** 10s

11 Wait slides dry **00:20:00** 20m

12 Analyse cells in image analysis software, such as ImageJ or Motic Image Plus

FIJI (Image J)
by NIH

Motic Images Plus 2.0
Windows and macOS
by Motic

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