

Carmine staining methods

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Before the era of haematoxylin and synthesized aniline dyes as histological stains, the method of choice to stain tissue sections was one of the natural dyes such as carmine and saffron. G M SMITH (1915) reported that in the early days of histological staining H R GOEPPERT and F COHN (1849) and A CORTI (1851) have already used carmine for the study of cell contents. Yet, T HARTIG (1854) was given credit for discovering the process of tissue staining with carmine. A very valuable overview of the history of histological staining is given by G M SMITH (1915) which includes also some further developments of modern microtechnique.

Carmine is obtained from the bodies (female) of the insect *Dactylopius coccus cacti*. The active coloring agent in carmine is carminic acid (C.I. 75470, Natural red 4). For nuclear staining it is invariably used in conjunction with a mordant such as alum solution.

Today, carmine is quite rarely used for histological staining. Carmine staining, however, can be an alternative to haematoxylin counterstaining of immunostained tissue sections. A variety of carmine formulations exist for very clean and sharp nuclear staining. The most popular formulations are given here.*

Carmine, chrome alum (Fyg)

| Chemicals | Chemical solution |
|-------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Carminic acid (C.I. 75470) Chromium potassium sulfate (chrome alum) HCl (25%) Ethanol Distilled water | <ul style="list-style-type: none">• FYG carmine dye solution: 6.0 g chrome alum dissolved in 100 mL distilled water (heated), <i>add</i> 1.0 g carmine <p>the mixture is boiled for 15 min, then cooled down and filtered</p> <ul style="list-style-type: none">• HCl-ethanol solution: 97.5 mL 70% ethanol <i>plus</i> 2.5 mL HCl (25%) |
| Staining procedure Immuno-stained sections are passed through distilled water and stained: | |
| – FYG carmine dye solution | 1-6 hours |
| – distilled water | 5 x 1 min |
| – differentiate in HCl-ethanol solution | under microscopic control |

* Dyes and other chemicals in histological staining can be toxic. They must be handled with care

(only when section is overstained)

- distilled water 5 x 1 min

Slides are dehydrated in ascending series of ethanol and mounted under coverglass

Carmine, aluminium sulfate (Fyg)

| Chemicals | Chemical solution |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Carminic acid (C.I. 75470) Aluminum sulfate HCl (25%) Ethanol Distilled water | <ul style="list-style-type: none">• FYG carmine dye solution: 6.0 g aluminium sulfate dissolved in 100 mL distilled water (heated), <i>add</i> 1.0 g carmine <p>the mixture is boiled for 15 min, then cooled down and filtered</p> <ul style="list-style-type: none">• HCl-ethanol solution: 97.5 mL 70% ethanol <i>plus</i> 2.5 mL HCl (25%) |
| Staining procedure Immuno-stained sections are passed through distilled water and stained: <ul style="list-style-type: none">– FYG carmine dye solution 1-6 hours– distilled water 5 x 1 min– differentiate in HCl-ethanol solution under microscopic control (only when section is overstained)– distilled water 5 x 1 min Slides are dehydrated in ascending series of ethanol and mounted under coverglass | |

Carmine, ammonium alum (Grenacher)

| Chemicals | Chemical solution |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Carminic acid (C.I. 75470) Aluminium ammonium disulfate (ammonium alum) <i>alternatively</i> aluminium potassium sulfate (potassium alum) Formaldehyde (37%) Ethanol Distilled water | <ul style="list-style-type: none">• GRENACHER carmine dye solution: 3.0-5.0 g ammonium alum (or potassium alum) dissolved in 100 mL distilled water (heated), <i>add</i> 2.0 g carmine <p>the mixture is boiled for 60 min, then cooled down and filtered</p> <p>add 1.0 mL formaldehyde</p> |

Staining procedure

Immuno-stained sections are passed through distilled water and stained:

- GRENACHER carmine dye solution 1-6 hours
- distilled water several rinses
as long as dye veils are seen
- differentiate in HCl-ethanol solution under microscopic control
(only when section is overstained)
- 96% ethanol 2 x 1 min

Slides are dehydrated in ascending series of ethanol and mounted under coverglass

Carmine, acetic acid (Schneider)

| Chemicals | Chemical solution |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Carminic acid (C.I. 75470) Glacial acetic acid Ethanol Distilled water | <ul style="list-style-type: none">• SCHNEIDER carmine dye solution: (carmine acetic acid) 55.0 mL distilled water <i>plus</i> 45.0 mL glacial acetic acid <i>plus</i> 1.0 g carmine <p>the mixture is heated to boil weakly for about 30-60 min using a reverse cooling system</p> <p>when cooled down the stain is filtered</p> |
| Staining procedure Immuno-stained sections are passed through distilled water and stained by covering the section with drops of the dye solution: | |
| <ul style="list-style-type: none">- SCHNEIDER carmine dye solution 1-2 min glass slide is slightly heated by a Bunsen flame from below and then transferred to the cold- distilled water rinse (2 x)- 96% ethanol 2 x 1 min | |
| Slides are dehydrated in absolute ethanol and mounted under coverglass | |

Carmine, alum (Mayer)

| Chemicals | Chemical solution |
|--------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Carminic acid (C.I. 75470) Potassium aluminium sulfate (potassium alum) Formaldehyde (37%) | <ul style="list-style-type: none">• MAYER carmalum dye solution: 10.0 g potassium alum dissolved in 200.0 mL distilled water (heated) <i>plus</i> 1.0 g carmine |

| | |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Ethanol Distilled water | <p>the mixture is cooled down and filtered</p> <p>add 1.0 mL 10% formaldehyde solution</p> <ul style="list-style-type: none"> potassium alum solution: 0.5 g potassium alum dissolved in 100.0 mL distilled water |
| <p>Staining procedure</p> <p>Immuno-stained sections are passed through distilled water and stained:</p> <ul style="list-style-type: none"> – MAYER carmalum dye solution 15 min – distilled water 2 x 1 min – differentiate in potassium alum solution 1-2 min – distilled water 5 x 1 min – 96% ethanol 2 x 1 min <p>Slides are dehydrated in absolute ethanol and mounted under coverglass</p> | |

Carmin, aluminium chloride - calcium chloride (Mayer)

| Chemicals | Chemical solution |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Carminic acid (C.I. 75470) Aluminium chloride Calcium chloride Glacial acetic acid Ethanol Distilled water | <ul style="list-style-type: none"> MAYER paracarmin dye solution: 0.5 g aluminium chloride dissolved in 100.0 mL 70% ethanol (heated) <i>plus</i> 4.0 g calcium chloride <i>plus</i> 1.0 g carmine <p>the mixture is cooled down and filtered</p> <ul style="list-style-type: none"> ethanol-acetic acid solution: 98 mL 70% ethanol <i>plus</i> 2.0 mL glacial acetic acid |
| <p>Staining procedure</p> <p>Immuno-stained sections are passed through distilled water and stained:</p> <ul style="list-style-type: none"> – MAYER paracarmin dye solution 15-30 min – differentiate in ethanol-acetic acid under microscopic control – 70% ethanol rinse – 70% ethanol 3 x 1 min – 96% ethanol 3 x 1 min <p>Slides are dehydrated in absolute ethanol and mounted under coverglass</p> | |

Carminic acid, lithium carbonate (Orth)

| Chemicals | Chemical solution |
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| Carminic acid (C.I. 75470) Lithium carbonate Ethanol HCl (25%) Distilled water | <ul style="list-style-type: none"> • Lithium carbonate stock solution: add as much lithium carbonate to distilled water to give an aqueous saturated solution • ORTH lithium carmine dye solution: 2.5 g carmine added to 100.0 mL lithium carbonate stock mixture is heated in a boiling water bath (for 10-15 min) solution is then cooled down and filtered prior to use • HCl-ethanol solution: 97.5 mL 70% ethanol <i>plus</i> 2.5 mL HCl (25%) |
| <p>Staining procedure</p> <p>Immuno-stained sections are passed through distilled water and stained:</p> <ul style="list-style-type: none"> – ORTH lithium carmine dye solution 2-5 min – differentiate in HCl-ethanol solution under microscopic control – distilled water 5 x 1 min – 70% ethanol rinse – 70% ethanol 3 x 1 min – 96% ethanol 3 x 1 min <p>Slides are dehydrated in absolute ethanol and mounted under coverglass</p> | |

References for further readings

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 Corti A (1851)
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