FACTORS AFFECTING THE PATHOGENESIS OF EIMERIA NECATRIX INFECTIONS IN CHICKENS

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APPENDIX VI

Acetate Buffer, 1.0 M, pH 5.0

141 gm

Sodium acetate

(C2H2O2na.3H2O)

Dissolve in 1000 ml distilled water.

Glycin - HC1, 0.1 M, pH 2.8

23.57 gm

Glycine

8.2 ml

Concentrated HC1

Dilute to 950.0 ml with distilled water.

Adjust pH to 2.8 with conc. HCl.

Add distilled water up to 1000 ml.

APPENDIX III

Phosphate Buffer, pH 7.2

8.0	gm	Sodium chloride
0.2	gm	Potassium chloride
1.15	gm	Anhydrous disodium
		hydrogen phosphate
0.2	gm	Potassium dihydrogen
		phosphate

Dissolve in one litre distilled water and autoclave at 121°C for 15 minutes.

Phosphate Buffer, pH 8.0

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27.80 gm Monobasic sodium phosphate

(NaH2PO4)

1000 ml Distilled water

Solution B

53.65 gm Dibasic sodium phsophate

(Na₂HPO₄·7H₂O)

1000 ml Distilled water

Mix 5.3 ml of Solution A with 94.7 ml of Solution B and bring up to 200 ml.

APPENDIX II

Sodium Acetate - Acetic Acid Buffer, 0.2 M, pH 3.6

- A) 1.15 ml acetic acid in 100 ml distilled water (Solution A)
- B) 2.82 gm Sodium acetate in 100 ml distilled water $(C_2H_2O_2\cdot 3H_2O)$ (Solution B)

To prepare pH 3.6 of this buffer, mix 1.63 ml of Solution A and 0.37 ml of Solution B.

Stain

1 gm Amido Black 10-B 1000 Sodium acetate - acetic acid buffer

Decolorizer 1 (25 minutes)

45 ml Methyl alcohol

10 ml Glacial acetic acid

50 ml Distilled water

Decolorizer 2 (25 minutes)

40 ml Absolute ethyl alcohol

10 ml Glacial acetic acid

50 ml Distilled water

APPENDIX I

Special Sporozoite Stain*

Intestinal tissues were fixed in Bouin's fixative. Paraffin waxembedded tissues were cut at a thickness of 3-5 um. Sections were stained as follows:

- 1 Stain nuclei in Ehrlish's hematoxylin for 10 minutes in water, differentiate in acid-alcohol, blue in lithium carbonate (few seconds) and wash in water (1 minute) followed by a rinse in distilled water.
- 2 Stain in 1% aqueous acid fuchsin for 5 minutes.
- 3 Wash in running tap water for 1 minute and leave in distilled water for 30 seconds.
- 4 Transfer to aniline blut-orange G mixture for 30 seconds.
- 5 Wash in running water to remove excess stain.
- 6 Dehydrate in 2 changes of iso-propyl alcohol.
- 7 Clear in xylol and mount in D.P.X.

^{*} Pierce, M.A. 1980. Parasitology 80: 551-554.

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