## REFERENCES


1. Adams, E., and Hanson, R. P.
   1956. A Procedure for Adsorbing Virus Neutralizing Antibodies on Paper Discs, J. Bact., 72:572.

2. Anderson, R. I., Sadun, E. H., and Williams, J. S.
   1961. A Technique for the Use of Minute Amounts of Dried Blood in the Fluorescent Antibody Test for Schistosomiasis, Exp. Parasit., 11:111-116.

3. Banerjee, K.
   1965. Comparative Agglutinability of Erythrocytes of Different Species of Animals with Arboviruses, Ind. J. Med. Res., 53:199-203.

4. Bellanti, J. A.
   1971. Immunology, Asian Ed., W. B. Saunders Company, Tokyo, pp. 282-285.

5. Bellanti, J. A. and Artenstein, M. S.
   1964. Mechanisms of Immunity and Resistance to Virus Infections, Ped. Cli. North Amer., 11:549-561.

6. Brody, J. A.
   1963. Hemagglutination-Inhibition and Neutralization Tests Using Whole Blood Dried on Filter Paper Discs, Lancet, 2:616.

7. Brody, J. A., McAlister, R., Haseley, R., and Lee, P.
   1964. Use of Dried Whole Blood Collected on Filter Paper Disks in Adenovirus Complement Fixation and Measles Hemagglutination Inhibition Test, J. Immunol, 92:854-857.

8. Burrell, R. G., and Mascoli, C. C.
   1962. Experimental Immunology, 2 nd. Ed., Burgess Publishing Company, pp. 32-33.

9. Campbell, D. H., Garvey, J. S., Cremer, N. E., and Sussdort, D. H.
1964. Methods in Immunology, New York: W. A. Benjamin, Inc. pp. 149-155.

10. Casals, J., and Brown, L. V.
1954. Hemagglutination with Arthropod-borne Viruses, J. Exp. Med., 99:429-449.

11. Casey, H. L.
1970. Experience at the National Communicable Disease Center with Micro Serological Techniques for the Diagnosis of Viral Diseases, Hlth. Lab. Sc., 7:233-236.

12. Chanock, R. M., and Sabin, A. B.
1953 a. The Hemagglutinin of St. Louis Encephalitis Virus. I. Recovery of Stable Hemagglutinin from the Brains of Infected Mice, J. Immunol., 70:271-285.

13. Chanock, R. M., and Sabin, A. B.
1953 b. The Hemagglutinin of St. Louis Encephalitis Virus. II. Physico-Chemical Properties and Nature of Its Reaction with Erythrocytes, J. Immunol., 70:286-301.

14. Chanock, R. M., and Sabin, A. B.
1953 c. The Hemagglutinin of St. Louis Encephalitis Virus. III. Properties of Normal Inhibitors and Specific Antibody; Use of Hemagglutination for Diagnosis of Infection, J. Immunol., 70:302-316.

15. Clarke, D. H., and Casals, J.
1955. Improved Methods for Hemagglutination Studies with Arthropod-borne Viruses, Proc. Soc. Exp. Biol. Med., 88:96-99.

16. Clarke, D. H., and Casals, J.
1958. Techniques for Hemagglutination and Hemagglutination-Inhibition with Arthropod-borne Viruses, Am. J. Trop. Med. Hyg., 7: 561-573.

17. Conrath, T. B.
1972. Handbook of Microtiter® Procedures, Cooke Engineering Company, pp. 1-29, 394-398, 407-411, 1a-24a.

18. Davidsohn, I.
1966. The Blood. In: Clinical Diagnosis by Laboratory Method, 13 th ed., (Davidsohn, I. and Wells, B. B.) Philadelphia: W. B. Saunders Company, pp. 92-93.

19. DeSommer, P., and Prinzie, A.
1957. Poliomyelitis Virus Neutralizing Antibodies Determination by Filter Paper Discs on Solidified Bottle Cultures, Virology, 4: 387-388.

20. Dighero, M. W., Bradstreet, C. M. P., and Andrews, B. E.
1970. Dried Paper Discs for Serological Identification of Human Mycoplasma, J. Appl. Bact., 33:750-757.

21. Farrell, L. N., and Reid, D. B. W.
1959. Disc-Plate Assay of Poliomyelitis Antibodies, Can. J. Pub. Hlth. 50:20-26.

22. Gaggero, A. C., and Sutmoller, P.
1965. The Use of Serum and Blood Dried on Blotting Paper in the Detection of Foot-and mouth Disease Antibody, Brit. Vet. J., 121:509-514.

23. Green, R. H., and Opton, E. M.
1960. A Micromethod for Determination of Poliovirus Antibody Suitable for Mass Surveys, Am. J. Hyg., 72:195-203.

24. Hierholzer, J. C., and Suggs, M. T.
1969. Standardized Viral Hemagglutination and Hemagglutination-Inhibition Tests. I. Standardization of Erythrocyte Suspensions, App. Micro., 18:816-823.

25. Hierholzer, J. C., Suggs, M. T., and Hall, E. C.
1969. Standardized Viral Hemagglutination and Hemagglutination-Inhibition Tests. II. Description and Statistical Evaluation, App. Micro., 18:824-833.

26. Kabat, E. A., and Mayer, M. M.
1967. Experimental Immunochemistry, 2 nd ed., Illinois: Charlese Thomas Publisher, Inc., pp. 634-643, 649.

27. Kalter, S. S.
1957. A Disc Method for Titration and Neutralization of Poliovirus, Fed. Proc., 16:419. (Abstract no. 1796)

28. Kalter, S. S.
1963. Disc Method for Identification and Titration of Cytopathic Viruses and Detection of Antibodies Resulting from Their Infection, J. Lab. Clin. Med., 62:525-534.

29. Karstad, L., Spalatin, J., and Hanson, R. P.
1957. Application of the Paper Disc Technique to the Collection of Whole Blood and Serum Samples in Studies on Eastern Equine Encephalomyelitis, J. Infect. Dis., 101:295-299.

30. Kolmer, J. A., and Boerner, F.
1945. Approved Laboratory Technic, 4th ed., New York: D. Appleton-Century Company, pp. 837-844.

31. Lynch, M. J., Raphael, S. S., Mellor, L. D., Spare, P. D., and Inwood, M. J. H.
1969. Medical Laboratory Technology, 2 nd ed., Philadelphia: W. B. Saunders Company, pp. 672-673.

32. Oppelaar, L.
1966. The Use of Filter Paper as a Transportmedium for Blood and Serum, Trop. Geogr. Med., 18:60-66.

33. Ouchterlony, O.
1967. Immunodiffusion and Immunoelectrophoresis. In: Handbook of Experimental Immunology (Weir, D. M. ed.) Blackwell Scientific Publications, pp. 688-704.

34. Porterfield, L. S.
1957. Use of Goose Cells in Haemagglutination Tests with Arthropod-borne Viruses, Nature, 180:1201-1202.

35. Rhodes, A. J., and van Rooyen, C. E.
1962. Text Book of Virology, 4th ed., Baltimore: The Williams & Wilkins Company, pp. 134-135.

36. Sabin, A. B., and Buescher, E. L.
1950. Unique Physico-Chemical Properties of Japanese B Encephalitis Virus Hemagglutinin, Proc. Soc. Exp. Biol. Med., 74: 222-230.

37. Sadun, E. H., Anderson, R. I., and Williams, J. S.
1961. Fluorescent Antibody Test for the Laboratory Diagnosis of Schistosomiasis in Humans by Using Dried Blood Smears on Filter Paper, Exp. Parasit., 11:117-120.

38. Schmidt, N. J.
1964. Tissue Culture Methods and Procedures for Diagnostic Virology. In: Diagnostic Procedures for Viral and Rickettsial Diseases (Lennette, E. H. and Schmidt, N. J., 3 rd ed.) New York: American Public Health Association, Inc., pp. 150-153.

39. Schmidt, N. J., and Lennette, E. H.
1965. Basic Technics for Virology. In: Viral and Rickettsial Infections of Man (Horsfall, F. L. and Tamm, I., 4th ed.) J. B. Lippincott Company, pp. 1211-1217.

40. Sever, J. L.
1962. Application of a Microtechnique to Viral Serological Investigations, J. Immunol., 88:320-329.

41. Tuchinda, P.
1971. Immunochemistry, Ann. Report. Virus. Res. Ins. (Thailand) 7:30-31.

42. Tuchinda, P.
1971. Studies of Arboviruses, Ann. Report. Virus. Res. Ins. (Thailand), 2:10-20.

43. Vaisman, A., Hamelin, A., and Guthe, T.
1963. La Technique des Anticorps Fluorescents Pratiqueé sur sang Desséché et Elué, Bull. Wld. Hlth. Org., 29:1-6. (Summary pp. 6)

44. Van Thiel, P. H., Van Der Hoeven, J. A., and Couve'e, L. M. J.
1963. Leptospirosis in the Highlands of West New Guinea - A Survey with Paper-Dried Blood Samples, Trop. Geogr. Med., 15: 70-75.

45. Williams, C. A., and Chase, M. W.
1967. Methods in Immunology and Immunochemistry, Vol 1, New York: Academic Press, Inc. pp. 114.

46. Williams, C. A., and Chase, M. W.
1968. Methods in Immunology and Immunochemistry, Vol. II, New York: Academic Press, Inc., pp. 25-37.

47. Work, T. H.
1964. Isolation and Identification of Arthropod-borne Viruses. In: Diagnostic Procedures for Viral and Rickettsial Diseases (Lennette, E. H. and Schmidt, N. J., 3 rd ed.) New York: American Public Health Association, Inc., pp. 334-338.

48. Work, T. H., and Hammon, W. McD.
1964. Arbovirus Infection in Man: In: Diagnostic Procedures for Viral and Reckettsial Diseases (Lennette, E. H. and Schmidt, N. J., 3 rd. ed.) New York: American Public Health Association, Inc., pp. 268-279, 286-290, 309-310.

49. Worth, R. M.
1964. The Filter Disc Blood Collection Method Adapted for a Mumps HAI Serologic Survey, Am. J. Hyg., 79:245-249.

# APPENDIX

# APPENDIX A

## Acid-Citrate-Dextrose Solution (ACD) (17, 47)

| | | |
|---|---|---|
| Sodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$) | 11.26 | g. |
| Citric acid ($H_3C_6H_5O_7 \cdot 2H_2O$) | 4.00 | g. |
| Dextrose | 11.00 | g. |
| Distilled water, q.s. | 500 | ml |

Sterilize by autoclaving 10 min. at 10 lb/in$^2$ pressure or by filtration

## Acid-Washed Kaolin, 25 % (17, 48)

| | | |
|---|---|---|
| Acid-washed kaolin powder | 25 | g. |
| Borate saline solution pH 9.0 | 100 | ml |

Constant shaking for maximal suspension to obtain a slurry.
This mixture can be held indefinitely at 4° C.

## 2 % Agar

| | | |
|---|---|---|
| Special noble agar (Difco) | 2 | g. |
| Distilled water, q.s. | 100 | ml |

Boiled this mixture until the agar is dissolved.

## 1 % Agar in 0.05 % Sodium barbital buffer, pH 8.2

| | | |
|---|---|---|
| 2 % Agar | 50 | ml |
| 0.05 % Barbital buffer | 50 | ml |

## Amido Black Dye (Modified from 9 and 33)

| | | |
|---|---|---|
| Amido black 10 B (Merck) | 0.6 | g. |
| Methyl alcohol | 45 | ml |
| Glacial acetic acid | 10 | ml |
| Distilled water, q.s. | 100 | ml |

## Bovalbumin-Borate Saline Solution (BABS), 0.4%, pH 9.0 (17, 47)

a. 1.5 M NaCl

| | | |
|---|---|---|
| NaCl | 87.675 | g |
| Distilled water, q.s. | 1,000 | ml |

b. 0.5 M Boric acid

| | | |
|---|---|---|
| $H_3BO_3$ | 30.92 | g |
| Hot distilled water (dissolve, then cool) | 700 | ml |
| Distilled water, q. s. | 1,000 | ml |

c. Borate saline solution, pH 9.0

| | | |
|---|---|---|
| 1.5 M NaCl | 80 | ml |
| 0.5 M $H_3BO_3$ | 100 | ml |
| 1.0 N NaOH | 24 | ml |
| Distilled water q.s. | 1,000 | ml |

Check pH in pH meter (Corning model 10)

d. 4% Bovalbumin (Fraction V), pH 9.0

| | | |
|---|---|---|
| Bovalbumin | 4 | g |
| Borate saline solution, pH 9.0 | 90 | ml |
| (adjust to pH 9.0 with 2 N NaOH) | | |
| Borate saline solution, pH 9.0, q.s. | 100 | ml |

It may be necessary to filter this solution to avoid growth of contaminants on long storage as a stock solution (4° C).

e. 0.4% Bovalbumin-borate saline solution (BABS), pH 9.0

| | | |
|---|---|---|
| 4% Bovalbumin, pH 9.0 | 100 | ml |
| Borate saline solution, pH 9.0 | 900 | ml |

Store at 4° C

## Dextrose-Gelatin-Veronal Buffer (DGV), pH 7.3 (17, 47)

| | | |
|---|---|---|
| Veronal (Barbital) | 0.58 | g |
| Gelatin | 0.60 | g |
| Sodium veronal (Sodium barbital) | 0.38 | g |
| Calcium chloride (anhydrous) ($CaCl_2$) | 0.02 | g |

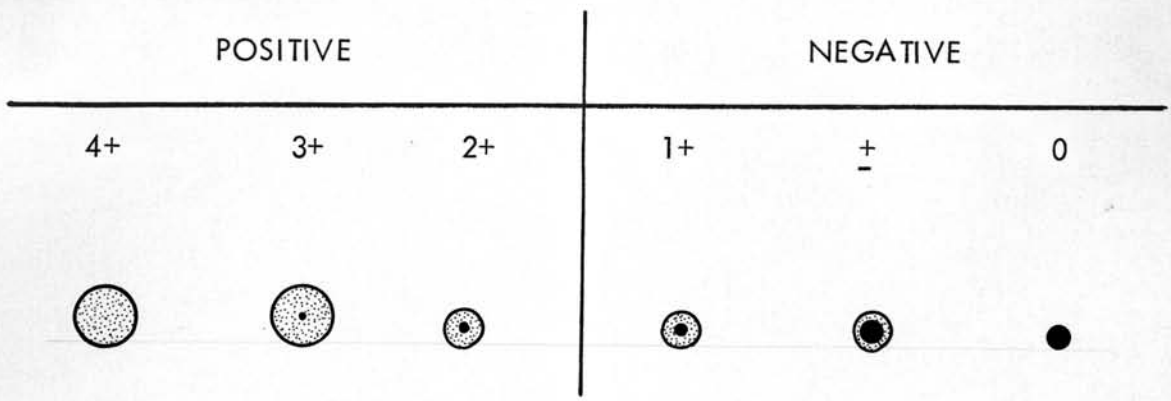
| | | |
|---|---|---|
| Magnesium sulphate ($MgSO_4 \cdot 7H_2O$) | 0.12 | g |
| Sodium chloride (NaCl) | 8.50 | g |
| Dextrose | 10.00 | g |
| Distilled water, q.s. | 1,000 | ml |

The veronal and geletin are dissolved in 250 ml of water by heating. This solution is combined with the other reagents. Check pH in pH meter (Corning model 10). Sterilize by autoclaving 10 min. at 10 lb/in$^2$ pressure. Store at 4°C. The solution is usuable for up to 2 months if it remain sterile.

Haemagglutination Reactions (17) (V-plate)

Score the degree of reactivity on a scale of negative to 4 + as follow:

4+ = smooth mat of cells covering the entire bottom of well,
3+ = smooth mat of cells covering less area of well with very small red button,
2+, 1+ = smooth mat of cells covering least area of well with bigger red button,
$\pm$ = nearly definite red button in center of well,
0 = definite compact button in center of well.




1N HCl

| | | |
|---|---|---|
| HCl (conc.) (36%, 12N) | 10 | ml |
| $H_2O$ | 110 | ml |

Phenol Red, 1% (38)

1. A 1.0 N NaOH solution is prepared by mixing 10 ml of saturated, concentrated NaOH with 90 ml of distilled water.
2. 10 g of alcohol-soluble phenol red (Hartman-Leddon Company, Phila.) are placed in a 100 ml beaker and approximately 20 ml of the NaOH solution are added, mixed, and allowed to stand for a few minutes.
3. The dissolved dye is transferred to a 1,000 ml volumetric flask.
4. Additional 10 ml amounts of the NaOH solution are added to the beaker and the dissolved material is added to the volumetric flask. No more than a total of 70 ml of the NaOH solution should be used.
5. The solution is brought to a final volume of 1,000 ml with distilled water and store at room temperature.

Phosphate Buffer Saline, pH 7.4 (38)

Solution A (10 x stock solution):

| | | |
|---|---|---|
| NaCl | 80.00 | g |
| KCl | 2.00 | g |
| $CaCl_2$ | 1.00 | g |
| $MgCl_2 \cdot 6H_2O$ | 1.00 | g |
| Distilled water, q.s. | 1,000 | ml |

Solution B (10 x stock solution):

| | | |
|---|---|---|
| $Na_2HPO_4 \cdot 12H_2O$ | 28.98 | g |
| $KH_2PO_4$ | 2.00 | g |
| Distilled water, q.s. | 1,000 | ml |

To dilute to 1x, 100 ml of Solution A are added to 800 ml of distilled water and 100 ml of solution B are added to this mixture.
The solution is sterilized by filtration through a Seitz-type pad, and added 1% phenol red as an indicator.

Phosphate Buffers for Arbovirus Haemagglutination Tests (17, 47)

| | | | |
|---|---|---|---|
| a. | 1.5 M NaCl | | |
| | NaCl | 87.675 | g |
| | Distilled water, q.s. | 1,000 | ml |
| b. | 2.0 M Dibasic sodium phosphate | | |
| | $NaHPO_4$ (anhydrous) | 283.96 | g |
| | Distilled water, q.s. | 1,000 | ml |
| c. | 2.0 M Monobasic sodium phosphate | | |
| | $NaH_2PO_4 \cdot H_2O$ | 276.02 | g |
| | Distilled water, q.s. | 1,000 | ml |
| d. | Solution A (0.15 M NaCl, 0.2 M $Na_2HPO_4$) | | |
| | 1.5 M NaCl | 100 | ml |
| | 2.0 M $Na_2HPO_4$ | 100 | ml |
| | Distilled water | 800 | ml |
| e. | Solution B (0.15 M NaCl - 0.2 M $NaH_2PO_4$) | | |
| | 1.5 M NaCl | 100 | ml |
| | 2.0 M $NaH_2PO_4$ | 100 | ml |
| | Distilled water | 800 | ml |

f. 0.2 M Phosphate Buffers or Virus-adjusting Diluent (VAD) (for final dilution of goose erythrocytes).

Combine solution A and solution B according to the table below to obtain buffer of the desired pH.

VAD Combination for Required pH values:

| Desired pH in Haemagglutination Test | Solution A (%) | Solution B (%) |
|---|---|---|
| 5.75 | 3.0 | 97.0 |
| 6.0 | 12.5 | 87.5 |
| 6.2 | 22.0 | 78.0 |
| 6.4 | 32.0 | 68.0 |
| 6.6 | 45.0 | 55.0 |
| 6.8 | 55.0 | 45.0 |
| 7.0 | 64.0 | 36.0 |
| 7.2 | 72.0 | 28.0 |
| 7.4 | 79.0 | 21.0 |

To check the pH of these buffer mixtures, mix equal parts of the 0.2 M phosphate buffer with 0.4% bovalbumin-borate saline solution, pH 9.0. This pH indicates that pH which is obtained after the erythrocytes are diluted in the phosphate buffer and added to a test well which contains 0.4 % bovalbumin-borate saline solution, pH 9.0.

0.05% Sodium barbital buffer, pH 8.2 (Modified from 9)

| | | |
|---|---|---|
| Sodium barbiturate (Merck) | 47.6 | g |
| HCl 1N | 69 | ml |
| 10 % $NaN_3$ (Merck) | 42 | ml |
| Distilled water to | 4,200 | ml |

0.9% Sodium Chloride (17)

Dissolve 9.0 g of sodium chloride in one litre of distilled water.

10% Sodium nitride ($NaN_3$)

| | | |
|---|---|---|
| $NaN_3$ | 10 | g |
| Distilled water to | 100 | ml |

# BIOGRAPHY

| | |
|---|---|
| NAME | Miss Suchada Kaolaead |
| DATE OF BIRTH | November 25, 1949 |
| PLACE OF BIRTH | Chiengmai, Thailand |
| DEGREE | B. Sc. (Botany) |
| INSTITUTION | Faculty of Science, Chulalongkorn University Bangkok, Thailand |
| DATE GRANTED | July, 1970 |
| FELLOWSHIP | Chulalongkorn University Fellowship in Medical Science; August 1972-July 1973. |