Thesis Title : Dot-immunobinding assay for monoclonal anti-A and anti-B

Name : Witoon Arunosisakul

Degree : Master of science (Clinical Pathology)

Thesis Supervisor Committee : Pimol Chiewsilp, M.D.
Bencha Petchclai, M.D.
Pimolparn Ratanasirivanich, B.Sc.(Pharm) M.Sc.

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Abstract

In ABO blood grouping, the main source of anti-A and anti-B reagents are human sera. There are few people whose sera are suitable for standard reagent and the quality of plasma derived reagents are varied from lot to lot. These lead to the attempts to find the other sources, e.g. lectin, albumin gland extract of snail but there are some limitation of their application in routine laboratories. Until recently monoclonal anti-A and anti-B have been produced to obtain the high quality reagents in term of potency and specificity as well as large quantities. Screening assay is one of the most important part of monoclonal antibody production. If the technique used for screening assay is sensitive, certain steps of procedure can be reduced, such as rapid detection of cell line to reduce time consuming and labour-intensive of production. The objective of the study was applied avidin-biotin DIA to the sensitive technique for screening assay. Red cell ghost dotted onto nitrocellulose filter disc as solid phase was incubated

with antibody or hybridoma supernates for 3 hours and washed. Anti-mouse IgM conjugated to biotin was added, incubated for 1 hour and washed. Then avidin-peroxidase was added, incubated for 1 hour and washed. Enzyme substrate, 4-choro-1-napthol was added and left for 15 minutes. The enzyme reaction was stopped with washing and the disc was dried for reading by nake eye. For comparison of IgM antibody detection, avidin-biotin DIA technique was more sensitive about 16-32 times than haemagglutination technique and it was useful for screening hybridoma producing antibody.