

ผลของเฮเทอโรโลยีต่อโพลีโคลนอล และโมโนโคลนอลแอนติบอดี
ในการตรวจสอบแอมเฟตามีน เมทแอมเฟตามีน และอีเฟดรีนด้วยเมมเบรนอิมมูโนแอสเสย์



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EFFECT OF HETEROLOGY ON POLYCLONAL AND MONOCLONAL ANTIBODIES
IN THE DETECTION OF AMPHETAMINE, METHAMPHETAMINE AND EPHEDRINE
BY MEMBRANE IMMUNOASSAYS

Miss Waliluk Matapatara

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วลัยลักษณ์ เมธำภัทร : ผลของเฮเทอโรโลยีต่อโพลีโคลนอล และโมโนโคลนอลแอนติบอดี
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โดยอาศัยหลักการของเฮเทอโรโลยี โพลีโคลนอลแอนติบอดีต่ออนุพันธ์ของแอมเฟตามีน
เมทแอมเฟตามีน และอีเฟดรีน, N-(3-aminopropyl)amphetamine, N-(4-aminobutyl)ampheta
mine, N-(3-aminopropyl)methamphetamine, N-(4-aminobutyl)methamphetamine, N-(3-
aminopropyl)ephedrine และ N-(4-aminobutyl)ephedrine, ถูกนำมาใช้ศึกษาการตรวจสอบแอม
เฟตามีน เมทแอมเฟตามีน และอีเฟดรีนด้วยเอนไซม์อิมมูโนแอสเสย์ โดยเลือกเฉพาะคอมบิเนชันที่
ดีนำไปศึกษาต่อโดยใช้โมโนโคลนอลแอนติบอดี จากนั้นเลือกเอาคอมบิเนชันที่ดีที่สุดของทั้งหมดไป
ใช้ในศึกษาการตรวจสอบด้วยเทคนิคเมมเบรนอิมมูโนแอสเสย์ จากการศึกษาพบว่าเฮเทอโรโลยีมี
ผลต่อความไว และความจำเพาะของการใช้โพลีโคลนอลแอนติบอดีในการตรวจสอบเมทแอมเฟตา
มีนพร้อมกับอีเฟดรีน, อีเฟดรีน และแอมเฟตามีนด้วยอิมมูโนแอสเสย์ โมโนโคลนอลแอนติบอดีที่ได้
มีความไว และความจำเพาะของการตรวจสอบแอมเฟตามีน เมทแอมเฟตามีน และอีเฟดรีนน้อย
กว่าโพลีโคลนอลแอนติบอดี เนื่องจากโมโนโคลนอลแอนติบอดีที่ได้สามารถจับได้กับอนุพันธ์ที่ใช้
เตรียมเป็นอิมมูโนเจน การพัฒนาชุดตรวจสอบเมมเบรนอิมมูโนแอสเสย์โดยใช้เทคนิคของแลด
เทอร์รอลโพลด้วยโพลีโคลนอลแอนติบอดี พบว่าสามารถตรวจสอบเมทแอมเฟตามีน และอีเฟดรีนได้
ด้วยความไวของการตรวจสอบที่ความเข้มข้น 1000 และ 500 มิลลิกรัมต่อลิตร, ตามลำดับ ดังนั้น
โพลีโคลนอลแอนติบอดี จึงสามารถนำไปใช้ในการพัฒนาการตรวจสอบด้วยเทคนิคเมมเบรนอิมมูโน
แอสเสย์ได้อย่างมีประสิทธิภาพ สำหรับโมโนโคลนอลแอนติบอดี การเพิ่มความสำคัญให้กับการ
คัดเลือกเซลล์ที่ผลิตแอนติบอดีจะทำให้ได้แอนติบอดีที่เหมาะสมซึ่งสามารถนำไปใช้ในการพัฒนาการ
ตรวจสอบด้วยเทคนิคอิมมูโนแอสเสย์ อีกทั้งการใช้เมมเบรนอิมมูโนแอสเสย์นับเป็นทางเลือกหนึ่ง
ที่เหมาะสมกับการพัฒนาชุดตรวจสอบด้วยเทคนิคอิมมูโนแอสเสย์

สาขาวิชา เกษษเคมี และผลิตภัณฑ์ธรรมชาติ

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ลายมือชื่อผู้ผลิต

ลายมือชื่ออาจารย์ที่ปรึกษา

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม

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WALILUK MATAPATARA: EFFECT OF HETEROLOGY ON POLYCLONAL AND
 MONOCLONAL ANTIBODIES IN THE DETECTION OF AMPHETAMINE,
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The detection of amphetamine, methamphetamine and ephedrine by heterologous immunoassay was investigated through polyclonal antibodies raised from the derivatives of amphetamine, methamphetamine and ephedrine, namely N-(3-aminopropyl)amphetamine, N-(4-aminobutyl)amphetamine, N-(3-aminopropyl)methamphetamine, N-(4-aminobutyl)methamphetamine, N-(3-aminopropyl)ephedrine, and N-(4-aminobutyl)ephedrine. Only the selected antibody was transferred to heterologous immunoassay using monoclonal antibody. By comparison, only the selected immunoassay was developed to membrane immunoassay. These investigations confirmed the effect of heterologous immunoassay with polyclonal antibody on sensitivity and specificity of either amphetamine, methamphetamine or ephedrine detection. Three combinations of antiserum and enzyme-label showed significantly detection of methamphetamine with ephedrine, ephedrine and amphetamine. Monoclonal antibody raised showed less sensitivity and specificity for amphetamine, methamphetamine and ephedrine than polyclonal antibody due to the tight binding of immunogen hapten to monoclonal antibody. By lateral flow technique, membrane immunoassay using polyclonal antibody could be utilized for methamphetamine and ephedrine detection at the cut-off of 1,000 and 500 $\mu\text{g/L}$, respectively. It was therefore concluded that heterologous immunoassay using polyclonal antibody would be fruitful for membrane immunoassay development. For monoclonal antibody, the selection of the effective clone would be the main concerned for managing heterologous developed. The membrane immunoassay would possibly be further developed to practical immunoassay test-kit.

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LIST OF ABBREVIATIONS

°C	centigrade degree
/	per
%	percent
>	more than
<	less than
=	equal to
Abs	absorbance
Ab	antibody
Au	gold
BSA	bovine serum albumin
CFA	complete Freund's adjuvant
cm	centimeter
cm ²	square centimeter
conc	concentration
CO ₂	carbon dioxide
DMSO	dimethyl sulfoxide
ELISA	enzyme-linked immunosorbent assay
EDCI	1-ethyl-3-(3-ethylaminopropyl)carbodiimide hydrochloride
g	Force of gravity
gm	gram
HAT medium	hypoxanthine-aminopterin-thymidine medium
HGPRT	hypoxanthine-guanine phosphoribosyl transferase
HT medium	hypoxanthine-thymidine medium
HRP	horseradish peroxidase
hr	hour
H ₂ O ₂	hydrogen peroxide
ICA	incomplete Freund's adjuvant

IP	intraperitoneal
kD	kilodalton
kg	kilogram
KLH	keyhole limpet hemocyanin
L	litre
M	molar
MAb	monoclonal antibody
mM	millimolar
mg	milligram
mm	millimeter
min	minute
ml	millilitre
MW	molecular weight
NaBH ₄	sodium borohydride
N	normality
No.	number
n	the number of sample
nm	nanometer
OPD	o-phenylenediamine
PAb	polyclonal antibody
PBS	phosphate buffer saline
PBS-T	phosphate buffer saline with tween 20
PEG	polyethylene glycol
ppm	part per million
r	correlation coefficient
rpm	revolutions per minute
RBCs	red blood cells
S.D.	standard deviation
Tris	Tris-(hydroxymethyl)aminomethane
TBS	Tris buffer saline
UV	ultraviolet

μg	microgram
μl	microlitre
v/v	volume by volume
w/v	weight by volume

Definition

Antiserum: the blood from an immunized host, the clotting proteins and RBCs have been removed.

Ascites/ascetic fluid: fluid product of intraperitoneal tumor growth induced in rodents after administration of pristane and hybridoma cells.

Bridge-hapten heterologous combinations: the combinations that the hapten for conjugated competitor and for inducing antibody not only different in the cross-linkers on the structure of compound but also different in the type of hapten used.

Bridge heterologous combinations: the combinations that the different cross-linkers of the haptens are used in immunization and conjugated competitor.

Clone: A group of cells derived from a single cell and therefore exhibiting genetic identity.

Cloning: the process of isolating single cell cultures.

Cross-reaction: Binding of an antibody to a molecule not present in the immunization mixture.

Enzyme-linked immunosorbent assay (ELISA): Chromogenic immunoassay in which the analyte is measured with an enzyme-conjugated antibody and the enzyme substrate.

Hapten: the small and non-immunogenic molecules such as drugs, simple sugars, amino acids, small peptides, phospholipids, or triglycerides when covalently attached to the large molecule such as bovine serum albumin, keyhole limpet hemocyanin (KLH) or other synthetic matrices, are term carriers, can stimulate the immune response.

Hapten heterologous combinations: the combinations that the different but related haptens are used for inducing antibody and competitor conjugation.

HAT medium/selection: Culture medium containing hypoxanthine, aminopterin and thymidine, in which myeloma cells in the enzyme HGPRT cannot survive. Hybridomas with one 'normal' parent inherit the ability to make this enzyme and so are 'selected' out of a cell mixture by their ability to survive.

Heterologous combinations: the combinations that the different but related haptens are use for inducing antibody and assay reagent such as for prepared coated plate or enzyme labeled.

Heterology: the structural difference between the hapten-protein conjugate used for immunization and assay reagent.

Homologous combinations: the combinations of antiserum and assay reagent that used the same hapten.

Hybridoma cell: a cell line produced *in vitro* by the fusion of a malignant and normal cell (i.e. myeloma).

Immunogen: Substance capable of inducing an immune response.

Monoclonal antibody: A homogeneous population of antibodies that can be raised by fusion of B lymphocytes with immortal cell cultures to produce hybridomas. Hybridomas will produce many copies of the exact same antibody.

Myeloma cell (plasmacytoma): Tumor of malignant plasma cells. Myeloma cells are immortalized cells that are cultured with 8-azaguanine to ensure their sensitivity to the hypoxanthine-aminopterin-thymidine (HAT) selection medium used after cell fusion.

Polyclonal antibody: the mixture of resulting antibodies that may recognize a variety of epitopes on the antigen.