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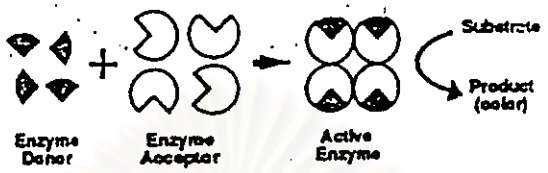
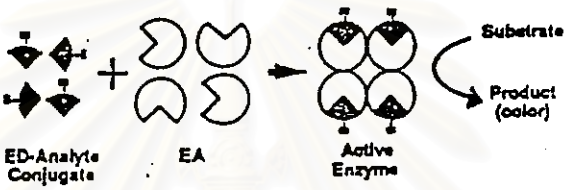
APPENDIX A

Figures about CEDIA®

CEDIA assays are based on the bacterial enzyme ß-galactosidase. The enzyme has been genetically engineered into two fragments; the Enzyme Donor (ED) and the Enzyme Acceptor (EA). ED and EA spontaneously reassociate to form fully active enzyme. In an assay format, the active enzyme cleaves a substrate generating a color change that can be measured on a spectrophotometric clinical chemistry analyzer.



In CEDIA assays, analyte is attached to ED in a way that does not interfere with this spontaneous reassociation.



Sample is mixed with EA and analyte-specific antibody. ED is then added. If analyte is present in the sample, antibody will bind to the analyte; ED will be free to form active enzyme with EA.

If there is no analyte present in the sample, the antibody will bind to ED and inhibit the reassociation of ED and EA. No active enzyme will be formed.

CEDIA assays are linear because the amount of enzyme formed is directly proportional to the amount of analyte present.

Figure A The mechanism CEDIA®

Contents of the kit	Cat. No. 1299930	
Bottle 1 Liquid	1 x 14 ml	**Enzyme Donor Reconstitution Buffer** MOPS 3-(N-morpholino-propanesulfonic acid buffer) , salts , and preservative.
Bottle 1a Lyophilisate	1 for14 ml	**Enzyme Donor Reagent** Enzyme donor (microbial) conjugated to phenobarbital, buffer salts, mouse monoclonal anti-phenobarbital antibody, Chlorophenol red-B-D galactopyranoside, carrier protein, stabilizers and preservative.
Bottle 2 Liquid	1 x 12 ml	**Enzyme Acceptor Reconstitution Buffer** MOPS 3-(N-morpholino-propansulfonic acid buffer), buffer salts and preservative.
Bottle 2a Lyophilisate	1 for 12 ml	**Enzyme Acceptor Reagent** Enzyme acceptor (microbial), goat anti-mouse antibodies, buffer salts, stabilizers and preservative.
Bottle 3 Liquid	1 x 4 ml	**Low Calibrator** Phosphate buffer with bovine serum albumin and preservative
Bottle 4 Liquid	1 x 4 ml	**High Calibrator** Phosphate buffer with bovine serum albumin, phenobarbital and preservative.

Figure B.1 The reagents information

Preparation and stability of the solution for

For stability of the unopened components, refer to the box label for the expiration date. DO NOT FREEZE.

Prepare the working solutions using cold reagents and buffers. Remove the kit from refrigerated storage (2-8 °C) immediately prior to preparation of the working solutions

NOTE : To ensure reconstituted EA reagent stability, protect from prolonged continuous exposure to bright light.

Prepare the working solutions in the following order to minimize possible contamination :

R1 Enzyme Donor Working Solution

Pour the entire contents of one Bottle 1 (ED Reconstitution Buffer) into one Bottle 1a (ED Reagent). Mix by gentle inversion. Allow to stand for 5 minutes at room temperature (20 - 25 °C), then mix again by gentle inversion. Avoid the formation of foam. Transfer R1 Working Solution back into Bottle 1. Place the bottle directly into the reagent compartment of the analyzer or into refrigerated storage (2 - 8 °C) and equilibrate for 30 minutes before use.

Stable for 30 days stored refrigerated on analyzer or at 2 - 8 °C. DO NOT FREEZE.

R2 Enzyme Acceptor Working Solution

Pour the entire contents of one Bottle 2 (EA Reconstitution Buffer) into one Bottle 2a (EA Reagent). Mix by gentle inversion. Allow to stand 5 minutes at room temperature (20 - 25 °C), then mix again by gentle inversion. Avoid the formation of foam. Transfer R2 Working Solution back into Bottle 2. Place the bottle directly into the reagent compartment of the analyzer or into refrigerated storage (2 - 8 °C) and equilibrate for 30 minutes before use.

Stable for 30 days stored refrigerated on analyzer or at 2 - 8 °C. DO NOT FREEZE

Low and High Calibrator

Ready for use. No preparation is required.

Stable for 30 days at 2 - 8 °C. DO NOT FREEZE.

Figure B.2 The reagent preparation and stability

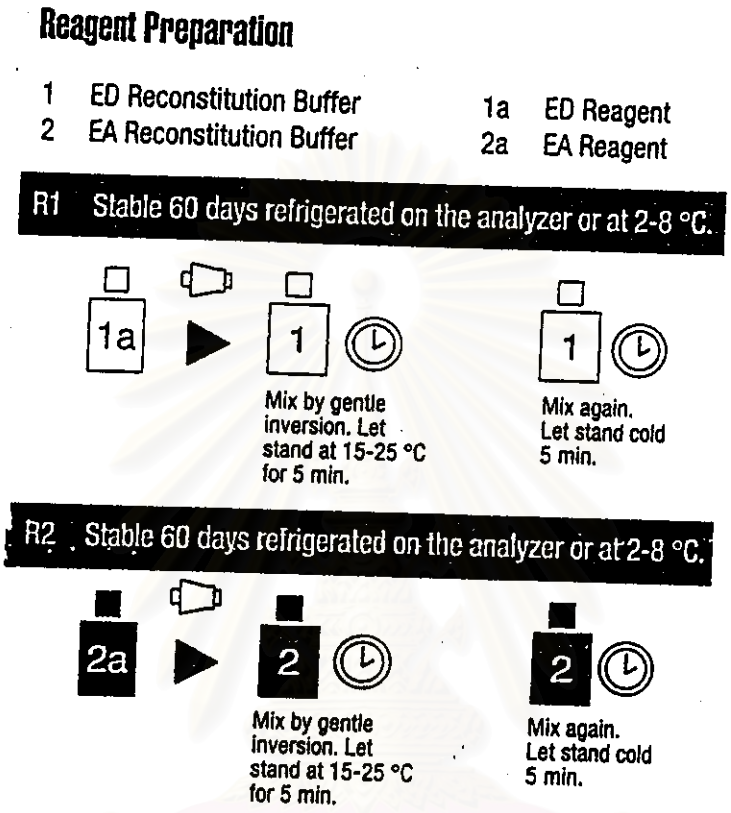


Figure B3 : Reagent Preparation diagram

Cross - Reactivity

Compound	Cross-reactivity (%)
Amytryptyline	< 1%
Carbamazepine	< 1%
Chlorazepate	< 1%
Chlorpromazine	< 1%
Diazepam	< 1%
Ethotoin	< 1%
Ethosuximide	< 1%
5-Ethyl-5-phenylhydantoin	< 1%
Imipramine	< 1%
Mephenytoin	< 1%
Methosuximide	< 1%
2-Phenyl-2-ethylmalonamide	< 1%
Phenytoin	< 1%
Primidone	< 1%
Promethazine	< 1%
Sulthiame	< 1%
Valproic Acid	< 1%
p-Hydroxyphenobarbital	< 1%
Aprobarbital	< 1%
Butabarbital	< 1%
1, 3-Dimethylbarbituric acid	< 1%
Secobarbital	< 1%
Pentobarbital	< 1%
Barbital	< 1%

Amobarbital (>20 %) and mephobarbital (> 100%) show significant interference with the CEDIA Phenobarbital Assay.

Figure C. % Cross reactivity of phenobarbital to other medicines.

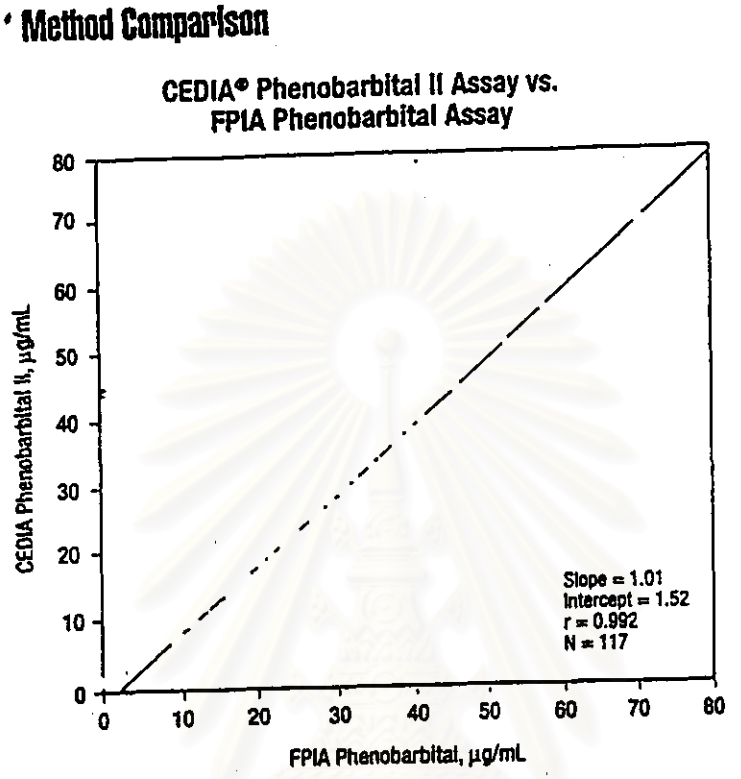


Figure D. Method comparison between CEDIA® and FPIA

VITAE

Lt. Jg. Oranee Ungphakorn was born on April 1, 1971, in Bangkok, Thailand. She graduated with a Bachelor Degree in Pharmaceutical Science in 1993 from the Faculty of Pharmaceutical Science, Chulalongkorn University, Bangkok, Thailand. Her current position is a staff in Department of Pharmacy, Somdej Phranangchao Sirikit Hospital, Sattahip, Chonburi, Thailand