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Yale - Aspartate Amino Transferase

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Protocol status: Working
We use this protocol and it's working

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Abstract

Summary:

Procedure used to measure the activity of Aspartate Amino Transferase (AST). AST activity is measured by the enzymatically coupled reactions of AST (to form oxaloacetate from aspartate and α-ketoglutarate) and malate dehydrogenase (conversion of oxaloacetate to malate with oxdiation of NADH to NAD). The rate of NAD formation is monitored by the change in absorbance at 340 nm.

Materials

MATERIALS

- Aspartate Amino Transferase Reagent **Prolabs(cliniqa) Catalog #**R85121
- Assayed Control Serum 1 **Prolabs(cliniqa) Catalog #**R83082
- Assayed Control Serum 2 **Prolabs(cliniqa) Catalog #**R83083

Reagent Preparation:

Aspartate Amino Transferase Reagent: Add the appropriate amount of water (6.5mL) to the reagent bottle. Invert to mix, allowing 15 minutes for the reagent to settle.

Assayed Control Serum 1: Add the appropriate amount of water (6.5mL) to the chemical control bottle. Invert to mix, allowing 15 minutes for the reagent to settle.

Assayed Control Serum 2: Add the appropriate amount of water (6.5mL) to the chemical control bottle. Invert to mix, allowing 15 minutes for the reagent to settle.

Troubleshooting

Before start

Analysis by automated system Cobas Mira Plus.

1 Calibrate Cobas for Aspartate Amine Transferase Activity analysis by running twoassayed control serum.

2 Sample handling as performed by the Cobas Mira Plus.
a) Pipette 16 µL of sample into a cuvette slot.
b) Add 145 µL of Aspartate Amino Transferase Reagent.
c) Mixture is incubated at 37˚C and spun for 10 minutes.
d) Absorbance is measured at 340 nm.