CATACHEM

METHANOL FasTox® TEST KIT
PRODUCT NO. C604-0B
[IN THE USA, FOR RESEARCH USE ONLY AND NOT FOR USE IN DIAGNOSTIC PROCEDURES]

Intended Use
For ***in vitro*** diagnostic use in the qualitative determination of Methanol in serum or plasma.

Clinical Significance (1-4)
An important toxicological problem in clinical diagnosis is Methanol poisoning. Methanol ingestion produces a severe intoxication that may be fatal in many cases. Methanol results in central nervous system depression. The metabolism of methanol proceeds through the formation of formaldehyde, which is rapidly converted to formic acid or reacts with serum proteins and is not easily detected in the blood of methanol intoxicated patients or animals.

Method Principle (5-6)
The Catachem Methanol procedure is based on the affinity of the enzyme Alcohol Oxidase (EC 1.1.3.13) from a specific bacteria to catalyze the oxidation of Methanol to Formaldehyde and H2O2, the Formaldehyde thus produced is subsequently converted to formic acid by the action of Formaldehyde Dehydrogenase (EC1.2.1.46) in the presence of NAD. This two point kinetic procedure is read at 340nm and the increase in absorbance is directly proportional to the concentration of Methanol in the serum sample.

$$\text{Methanol} + O_2 \xrightarrow{\text{Alcohol Oxidase}} HCHO + H_2O_2$$

$$HCHO + H_2O + NAD \xrightarrow{\text{Formaldehyde-DH}} HCOOH + NADH + H^+$$

ENZYME REAGENT
Each liter contains:

Buffer	
Alcohol Oxidase	≥4000 Units
Formaldehyde Dehydrogenase	≥2000 Units
NAD	≥1.5 mmol
Stabilizer and nonreactive ingredients	

ENZYME REAGENT DILUENT
Surfactant
Stabilizer and nonreactive ingredients

CONTENTS

Each kit, Product Number C604-0B, contains all of the materials needed to perform three patient tests:

C604-03 Methanol Reagent (3 x 4 mL) (dry powder to be reconstituted).

C604-04 Methanol Diluent (3 x 4 mL Buffer solution)

C604-13 "Negative" Control (Blue Vial) (3 x 100 µL) (liquid) at a level of 5 mg/dL (50 mg/L) (1.58 mmol/L).

C604-14 "Positive" Control (Red Vial) (3 x 100 µL) (liquid) at a level of 50 mg/dL (500 mg/L) (15.8 mmol/L).

Each vial of reagent and control is for *a single use only*. Discard opened vials after use.

TO PERFORM THE TEST

Reconstitute a Methanol Reagent vial (C604-03) by adding the contents of a Methanol Diluent vial (C604-04) to one of the three Methanol Reagent vials. Mix gently until powder has visibly dissolved and solution is clear.

With a volumetric pipette, dispense 1.0 mL of this solution into each of three labeled cuvettes, for the negative control, positive control and patient sample.

Read the INITIAL OD at 340 nm of each cuvette at Room Temperature or 37°C if preferred, and record in the chart below. (Note that all tests should be run and all readings taken at the same temperature whether this is Room Temperature or 37°C.)

At approximately 15 second intervals, add 10 µL of "Negative" Control to the first cuvette, 10 µL of "Positive" Control to the second cuvette and 10 µL of the Patient Sample to the third cuvette. Gently mix all cuvettes.

Incubate for 5 minutes. Read the FINAL OD at 340 nm for each cuvette, using the same approximate 15 second intervals and record in the chart below.

RECORD YOUR RESULTS:

Sample	Column A Initial OD at 340 nm	Column B Final OD at 340 nm	Difference in OD (OD in B minus OD in A) (Final OD minus Initial OD)
Negative Control			
Positive Control			
Patient Sample			

Results/Analysis

1. Is the difference in OD for the Patient sample cuvette greater than the difference in OD for the "Negative" control cuvette? **Circle one**: YES NO

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2. Is the difference in OD in the "Positive" control cuvette noticeably higher than the difference in OD for the "Negative" control? **Circle one**: YES NO
3. If BOTH answers are YES, then there is a strong likelihood that the patient HAS ingested methanol.
4. If the answer to Question #1 is NO and the answer to Question #2 is YES, then there is a strong likelihood that the patient has NOT ingested methanol.
5. If any other results are observed, please repeat the test.

Reagent Indications of Deterioration
- Turbidity
- Absorbance > 0.8 OD, 1 cm light path, 340nm
- Incorrect results on the provided kit controls as determined under results section in Attachment A.

If these reagent characteristics are observed, contact Catachem Technical Service at service@catacheminc.com

Specimen Collection and Stability [4, 6]
To maintain sample integrity and avoid changes in Methanol concentrations, venous specimens should be collected without the use of a tourniquet or immediately after a tourniquet has been applied.
Serum specimens should be collected in plain tubes or in silicone barrier collection tubes.
Plasma specimens should be collected in tubes with EDTA, lithium heparin, sodium fluoride, citrate or oxalate as anticoagulants. Separate immediately from the cells and analyze promptly or store, well-sealed at 2-8°C for up to 24 hours until analysis can be performed.

Materials Required But Not Provided
A spectrophotometer equipped with 340nm wavelength, volumetric pipettes and a timer.

Calibration
The manner in which this test is run eliminates the need for further calibration.

Reference Values [2,7-11]
Any noticeably detectable level of methanol in a sample is not normal. As this format of the test is a qualitative format and not designed for quantitative measurement, the reference values noted below are provided for informational purposes only.

None detected	≤ 5 mg/dL
Potentially Toxic	≥ 25 mg/dL

Interfering Substances (5)
The following substances have no significant effect on the accuracy of this Methanol procedure at the concentrations stated.

• Acetone	≤2000mg/dL
• Butanediol	≤100mg/dL
• Bilirubin	≤ 30 mg/dL
• Ethanol	≤ 500mg/dL
• Ethylene Glycol	≤ 300 mg/dL
• Hemoglobin	≤ 1000 mg/dL
• Isopropanol	≤ 900 mg/dL
• Polyethylene Glycol	≤ 2000 mg/dL
• Propylene Glycol	≤ 2000 mg/dL
• Triglycerides	≤ 1000 mg/dL

Other substances and certain drugs are also known to influence the Methanol values (1-2).

Method Performance Characteristics
Sensitivity: Using a path length of 1 cm, a Δ-absorbance of 0.01-0.015 per mg/dL should be obtained.
Linearity: This procedure is linear over the range of 0-200 mg/dL (0-2,000 mg/L)(63.2 mmol/L) as determined by quantitative measurements.

Accuracy
Correlation studies were carried out between a Catachem enzymatic automated method (Kit number C604-0A) (Y) and this method. Thirty (30) Serum samples were divided into three categories <50 mg/L; 51-500 mg/L and >500 mg/L. 100% correlation was obtained when results and therefore decision points, were correlated.

Bibliography
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