Intended Use
For use in the automated, quantitative, determination of **Methano**l 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 maybe 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 bacteria to catalyze the oxidation of Methanol to Formaldehyde and H_2O_2, the Formaldehyde thus produced is subsequently converted to formic acid 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.

$$\text{Methanol} + O2 \xrightarrow{\text{Alcohol Oxidase}} \text{HCHO} + \text{H2O2}$$

$$\text{HCHO} + \text{H2O} + \text{NAD} \xrightarrow{\text{Formaldehyde-DH}} \text{HCOOH} + \text{NADH} + \text{H+}$$

ENZYME REAGENT
Each liter contains:

Buffer	
Alcohol Oxidase	≥4000 Units
Formaldehyde Dehydrogenase	≥2000 Units
NAD	≥1.8 mmol
Stabilizer and nonreactive ingredients	

ENZYME REAGENT DILUENT
Surfactant
Stabilizer and nonreactive ingredients

Precautions
Handle this reagent with good laboratory practice. **DO NOT PIPETTE REAGENT BY MOUTH.** Avoid contact with skin and eyes. If contact occurs, wash affected area with plenty of cold water. Clean spills immediately.

Reagent Storage and Stability
Store the Methanol reagents at 2-8°C. When stored as directed, the reagents are stable until expiration date stated on the label.

Working Reagent Preparation
Reconstitute the required number of vials of Methanol Enzyme Reagent with the volume of Enzyme Reagent Diluent indicated on the label of each reagent vial. Mix reconstituted reagent by inversion, gently to prevent foaming. Store the Working Reagents at 2-8°C. When stored and prepared as directed the Methanol Working Reagent is stable for 15 days.

Reagent Indications of Deterioration
- Turbidity
- Absorbance > 0.5 OD, 1 Cm light path, 340nm
- Quality control values out of assigned ranges

If these reagent characteristics are observed, call Catachem technical service.

Specimen Collection and Stability
To maintain sample integrity and avoid changes in Methanol concentrations care should be taken to collect the sample specimens:

Venous specimens should be collected without the use of a tourniquet or immediately after a tourniquet has been applied.

Plasma specimens should be collected in tubes with heparin, sodium fluoride EDTA, Citrate and oxalate as anticoagulants. Separate immediately from the cells and analyze promptly or store at 2-8°C.

Procedure
These instructions are outlined for performing the Catachem Methanol assay using a manual procedure.

Materials Provided
Catachem Methanol Reagent
Catachem Methanol Calibrator material with assigned value
Catachem Methanol Quality Control material with assigned values

Materials Required but Not Provided
Spectrophotometer equipped with 340nm wavelength

Calibration
Catachem protein-based Calibrator which contains a known Methanol value is recommended.

Quality Control
To monitor the quality performance of the procedure used, Catachem Methanol Control Level I and Control Level II should be included in the assay procedure as follows:

- Daily
- Replacement of reagent lot numbers
- Analyzer service maintenance

Directions For Use
The Catachem Methanol method requires one Single Reagent.

Analytical Parameters

Wavelength	340nm
Temperature	37˚C
Pathlength	1 cm
Reaction Mode	End Point
Reaction Time	5-6 minutes
Regent Volume	0.500 ml
Sample Volume	0.0025 ml
Total Volume	0.5025 ml
Sample-to-reagent ratio	1:200

Assay Procedure

1. Bring the Methanol Working Reagent to room temperature.
2. Set spectrophotometer wavelength at 340nm and zero the instrument with the cuvette containing water.
3. Pipette 0.5 ml of Working Reagent into each of four cuvettes marked: “Sample” “Calibrator” and “Controls”.
4. Incubate cuvettes for 3.0 minutes at 37˚C.
5. Read the cuvettes reagent absorbance.
6. Pipette 0.0025 ml of calibrator, controls, and sample(s) into their respective cuvettes. Mix all cuvettes well.
7. Continuously monitor the change in absorbance for at least 5 minutes.
8. Read absorbances.
9. Calculate the Methanol concentration (mg/dL) in the sample(s), as shown in calculations and results.

Calculations And Results

$$\text{Ethylene Glycol (mg/dL)} = \frac{\text{Sample (A2 - A1)}}{\text{Calibrator (A2 - A1)}} \times \text{Calibrator (mg/dL)}$$

Example:

	A1	A2
Sample	0.20	0.2
Calibrator	0.20	0.5

Calibrator = 100 mg/dL

$$\text{Methanol (mg/dL)} = \frac{0.3 - 0.2}{0.5 - 0.2} \times 100 \text{ mg/dL}$$

$$= 33.3 \text{ mg/dL}$$

Procedure Limitations

Samples with Methanol values greater than 200 mg/dL should be diluted 1:2 with physiological saline and re-assayed. Multiply results obtained by 2 to adjust for the sample dilution.

Reference Values (5)

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.

- Bilirubin ≤ 30 mg/dL
- Ethanol ≤ 690 mg/dL
- Ethylene Glycol ≤ 300 mg/dL
- Hemoglobin ≤ 1000 mg/dL
- Isopropanol ≤ 900 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 pathlength 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 0-200mg/dL.

Precision: Precision data was obtained using three levels of protein based controls and following the NCCLS EP5-T2 procedure **(7)**. The following results were observed:

Precision

Methanol	Within-Run Precision		Total Precision	
Mean	SD	CV	SD	CV
mg/dL	mg/dL	%	mg/dL	%
10	0.788	8.033	0.593	5.941
50	1.064	2.095	1.330	2.610
150	3.154	2.099	3.267	2.182
200	2.896	1.479	6.408	3.219

Accuracy

Correlation studies were carried out between Catachem automated method (Y) and a manual, reference Gas Chromatography method (X). Serum samples were assayed and the results compared by the least squares regression. The following statistics were observed:

N = 34
Range = 0.6-155 mg/dL
Mean X = 48.515
Mean Y = 50.00
Y = 0.9692X– 5.299
Sy.x = 10.641
r = 0.9738

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