Sample and sampling for forensic alcohol analysis

Vitreous humor

- Fluid that occupies the space between the lens and the retina of the eye.
- It is colourless, transparent and gel-like, consisting of 99% water with small amounts of salts and mucoprotein.
- Vitreous humor is in a protected position behind the lens of the eye.
- Because of this protected position, it is isolated from putrefactive processes, from charring and from trauma.

Vitreous humor

- The time required for ethanol to enter the blood stream and penetrate eyes seems to be short.

- VH is useful for analysis of alcohol because:

  1- It has watery nature

  2- It is remote from the gut and less prone to contamination by spread of bacteria (important in decomposition and severe trauma)

  3- Ethanol and many abused drugs are stable in VH during prolonged period of storage at 4°C

Vitreous humor

- Vitreous humor can be obtained intact even if a corpse has been extensively burned or damaged.
- Blood is very susceptible to postmortem changes.
- Vitreous fluid is less susceptible to these effects, particularly because it is likely to be free from microorganisms.

Blood

- Peripheral blood (**femoral vein)** concentration have been shown to be more reliable for toxicological analysis than the conventional heart blood.

- ***Sodium fluoride*** protects blood from postmortem changes such as bacterial production of ethanol or other alcohols.

- It also helps to protect other labile drugs such as cocaine, nitrazepam and clonazepam from degradation.

Blood

- Many species of bacteria, yeast, and fungi have the ability to produce ethanol and other volatile organic compounds in postmortem specimens.
- The potential for postmortem ethanol formation complicates the interpretation of ethanol-positive results.
- The prevention of ethanol formation at all steps following specimen collection is a priority.
- **Sodium fluoride** is the most commonly used preservative for postmortem specimens.

Fluoride and enolase activity

- The fluoride ion is seemingly effective in inhibiting the activity of several kinds of enzymes, such as **enolase** a component in the glycolytic pathway.

- This enzyme is important for the action of yeasts, fungi and many micro-organisms responsible for fermentation.

Blood preservation

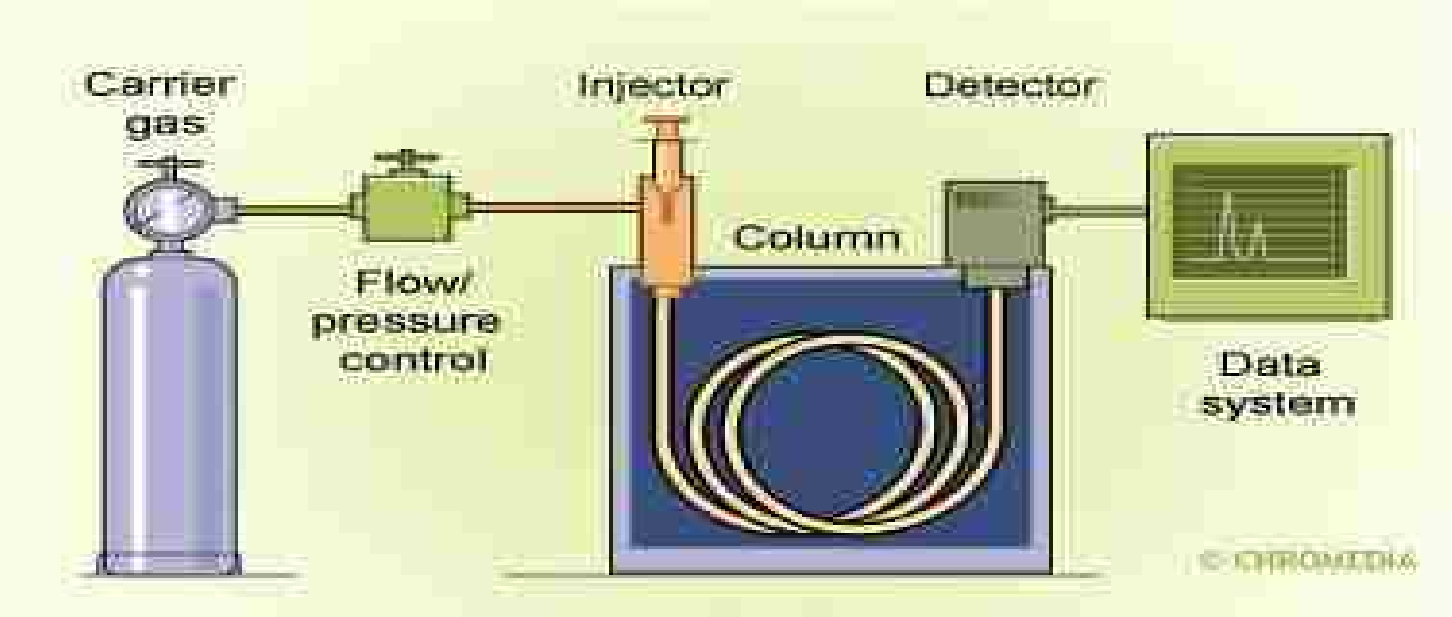
- Ethanol formation was virtually eliminated when specimens were mixed with 2% W/V sodium fluoride (NaF).
- There are published reports concluding that sodium fluoride may be ineffective for the prevention of ethanol formation in blood samples containing sufficiently high concentrations of Candida Albicans.

Assay Methodologies

- Gas chromatography
- Enzymatic oxidation
- Chemical reaction
- Breath alcohol analysis

Gas chromatography (GC)

- **Gas chromatography (GC)**, is a common type of chromatography used in analytical chemistry for separating and analyzing compounds that can be vaporized without decomposition.



Gas Chromatography

- **Advantages:**
- Specificity for ethanol, methanol and other types of alcohol **identification** and **quantitation**.
- Enhanced with the use of multiple columns or varying chromatographic conditions.

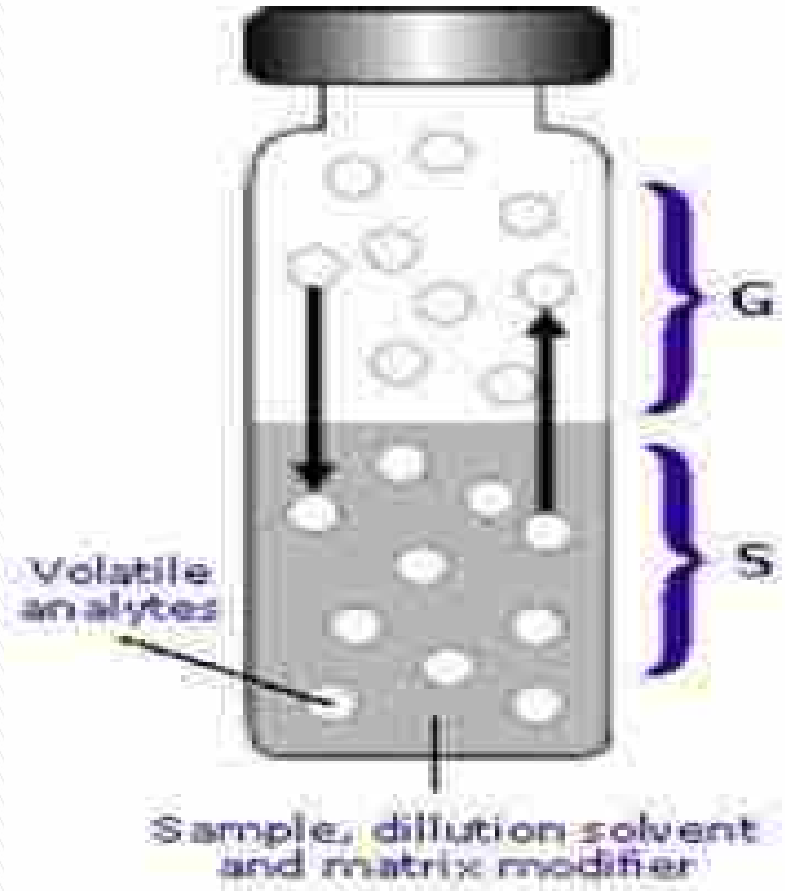
Gas Chromatography

- **Disadvantages:**
- Requires specialized instrumentation (gas chromatograph)
- Requires highly trained technical staff
- Analysis slower than enzymatic assay

A gas chromatograph with a headspace sampler

Headspace Gas Chromatography definition

- "Headspace" is the gas space above the sample in a chromatography vial.
- Volatile sample components diffuse into the gas phase, forming the headspace gas.
- Headspace analysis is therefore the analysis of the components present in that gas.



Headspace suitability

- Headspace gas chromatography is most suited to the analysis of the very light volatiles in samples that can be efficiently partitioned into the headspace gas volume from the liquid or solid matrix sample.
- Complex sample matrices, which may be difficult to analyse directly or would otherwise require sample extraction or preparation, are ideal candidates for headspace since they can be placed directly in a vial with little or no preparation.

HSGC in forensic toxicology

- The technique of static headspace gas chromatography has great acceptance in the forensic field, especially for the determination of alcohols in biological samples, so most forensic laboratories in the world have this equipment and perform this analysis on a routine basis.

Enzymatic Oxidation Assay

- Most of the commercial kits use alcohol dehydrogenase (ADH):

- $C_2H_5OH + NAD^+ \xrightarrow{ADH} CH_3CHO + NADH + H^+$

- The reaction is monitored following the absorbance of NADH at 340 nm or that of a color product at a higher (visible) wavelength formed by reacting NADH with a dye.

Enzymatic Oxidation Assay

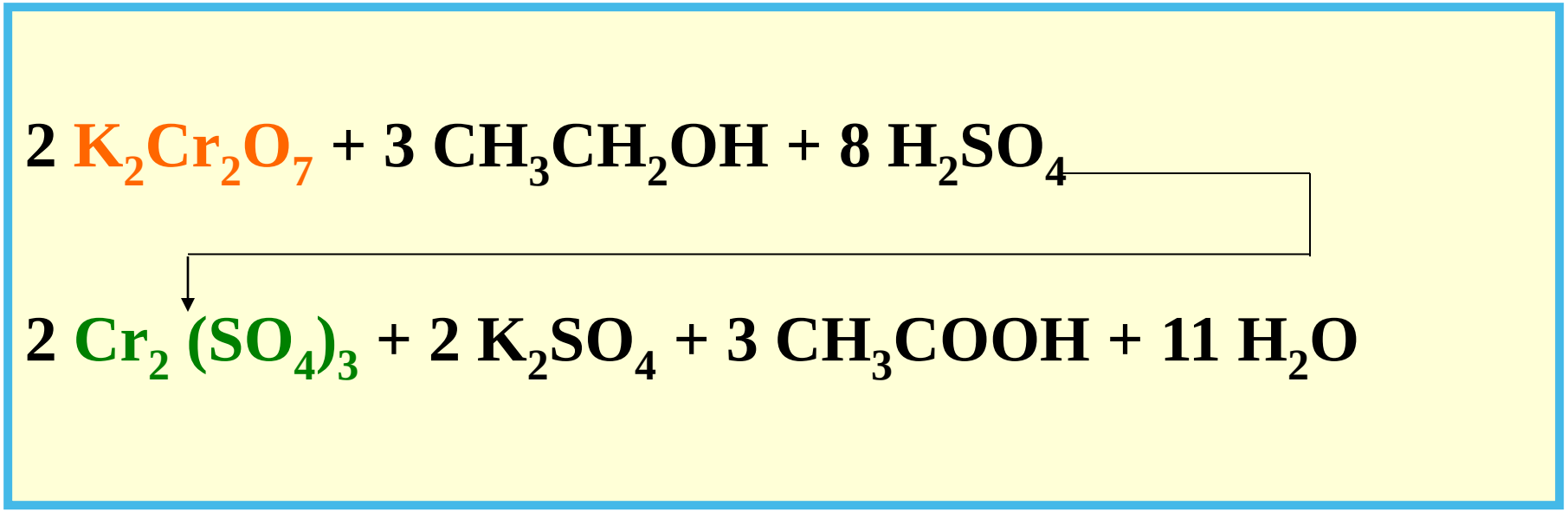
- **Advantages**:
- Rapid, easy to use kits are widely available
- This allows the smallest of clinical laboratories to perform stat quantitative alcohol test
- **Disadvantages**:
- Not specific for ethanol. Other alcohols can interfere at high concentrations
- Will miss methanol and isopropanol overdose

Chemical Reaction (Widmark method)

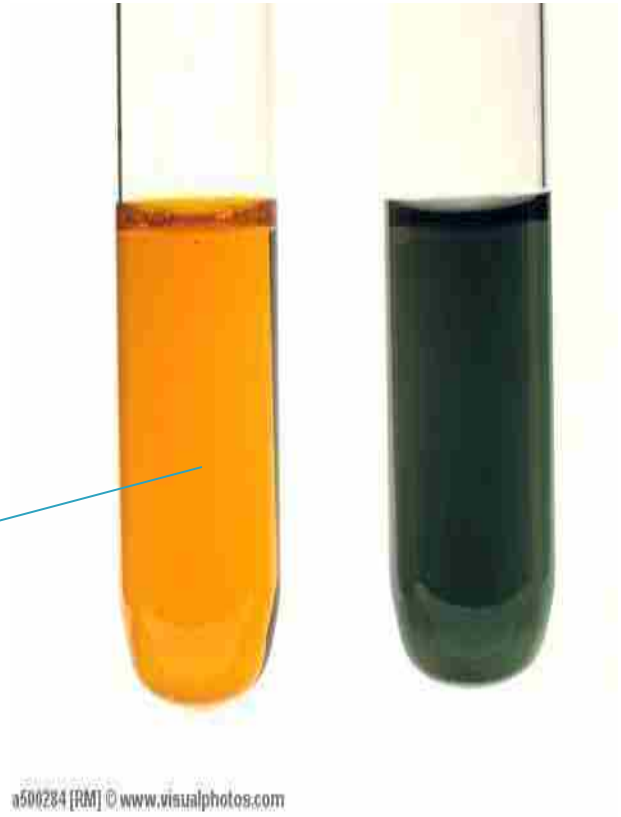
- this is a method for quantifying alcohol based on the oxidation of potassium dichromate in the presence of sulphuric acid, followed by a titrimetric analysis.

- It is non-specific, as alcohols other than ethanol (eg. methanol) and related compounds such as acetone and ether can all be involved in the oxidation reaction.

Chemical Reaction (Widmark method)

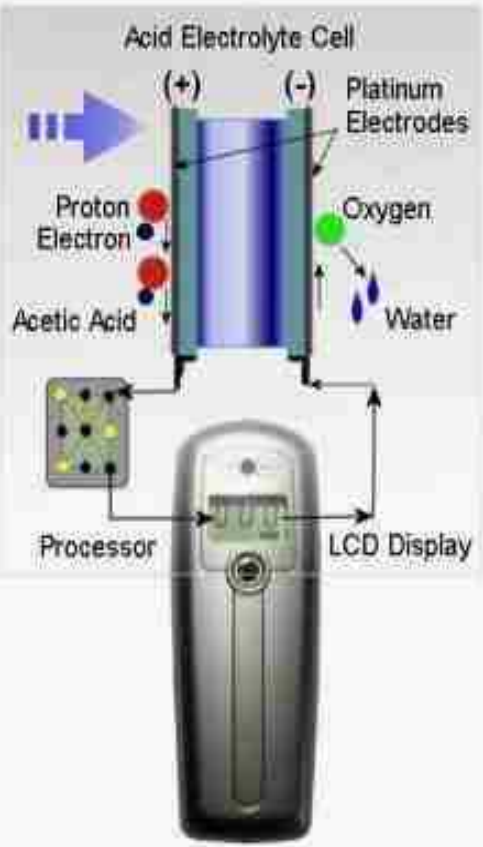


Potassium dichromate
conversion to Chromium sulfate



Breath alcohol analyser

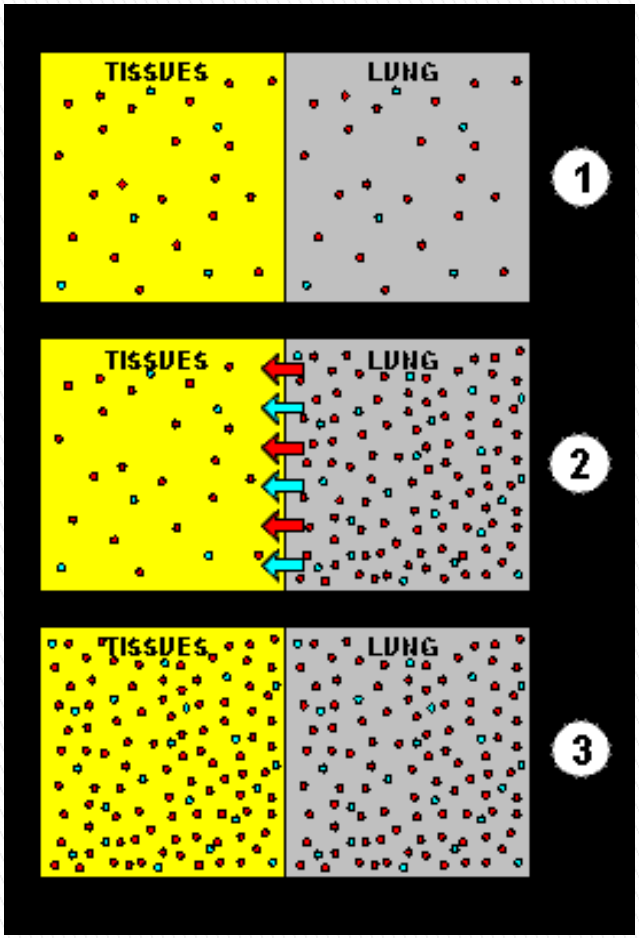
Fuel Cell Detectors



- Apparatus consists of two platinum electrodes with acidic electrolyte material between them
- Ethanol in breath **oxidized** at surface of **anode** to give acetic acid, protons, and electrons
- Atmospheric oxygen **reduced** at **cathode** to give two oxygen atoms
- Protons and electrons from anode travel to the cathode and combine with oxygen to form **water**
- Movement of electrons produces a **current** that is proportional to the amount of alcohol in the breath sample
- Microprocessor measures the current and calculates **BAC**

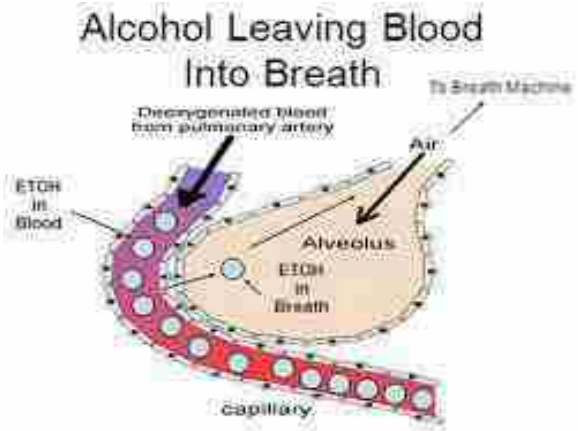
Henry's law and breath alcohol test

- Solubility of gas in a liquid is proportional to the partial pressure of gas over liquid in a *closed system* under *constant temperature*.



Basic Principle of Breath Alcohol Testing

- Following oral consumption, alcohol is absorbed from the gastro-intestinal tract and distributed throughout the body by the circulatory system.
- Alcohol diffuses freely and is found in relative concentrations according to the water content of the various tissues.
- Alcohol conc. in end-expiratory breath (BrAC) is proportional to alcohol conc. in the blood (BAC) suffusing the alveolar bed.



Breath Alcohol Concentration (BrAC) Measurement

- Advantages:

•Breath collection is *noninvasive*

•Collection does not require phlebotomist; can be performed by many more people

•Instrument designed for portability and *easy* breath collection; onsite testing

•Collection and test can be done *simultaneously* with immediate result

Interfering compounds

- Dieters and diabetics may have acetone levels hundreds or even thousands of times higher than those in others.
- Acetone is one of the many substances that can be falsely identified as ethyl alcohol by some breath machines.
- However, *fuel cell* based systems are non-responsive to substances like acetone.