Dumbbell-Diffusion Screening for Carbon Monoxide, Volatile Alcohols, and Chlorinated Hydrocarbons in Blood

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Rapid screening tests for the detection of carbon monoxide, ethanol and other volatile alcohols, and chlorinated hydrocarbons in blood are presented. The dumbbell-diffusion cell used was found to be versatile in allowing reactions to be carried out at either room temperature or 100° and in acting as a support for pretreated paper discs.

The dumbbell-diffusion cell described by Nobel (1) is made from a ball and socket joint, each end of which is formed into a bell-shaped flask. This cell is applicable to rapid testing of blood for the presence or absence of various toxic constituents. It allows diffusion, absorption, and identification reactions to proceed concurrently under a variety of conditions (Table 1). Using this apparatus, carbon monoxide can be determined semiquantitatively in 10 min. at room temperature by placing a filter-paper disc, impregnated with palladium chloride, in the junction between the ball and socket members of the joint. To decrease the reaction time required for volatile alcohols and chlorinated hydrocarbons, the entire cell can be immersed in an oscillating water bath at 100°. The choice of chemical methods was based on previous work by Gettler and Freimuth (2) and by Feldstein and Klenshoj (3).

Materials and Methods

Reagents and Materials

Sulfuric acid, 10% (v/v) Slowly add 1 volume of 36 N sulfuric acid to 9 volumes of water.

Palladium chloride solution, 0.25% (w/v) in hydrochloric acid
Add 0.5 gm. palladium chloride to 0.5 ml. 12 N hydrochloric acid. Allow this mixture to stand overnight and then dilute it to 200 ml. with water.

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## Table 1. Outline of Dumbbell Diffusion Screening Methods

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Socket flask</th>
<th>Ball flask</th>
<th>Time (min.)</th>
<th>Temp. (°)</th>
<th>Oscillations (per min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon monoxide</td>
<td>0.2</td>
<td>0.8*</td>
<td>PdCl₂ paper disk</td>
<td>10</td>
<td>25</td>
</tr>
<tr>
<td>Volatile alcohols</td>
<td>0.5</td>
<td>0.2‡</td>
<td>0.3 ml. K₂Cr₂O₇ solution</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>Halogenated hydrocarbons</td>
<td>0.5</td>
<td>—</td>
<td>0.4 ml. pyridine</td>
<td>5</td>
<td>100</td>
</tr>
</tbody>
</table>

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| — | | | | | |

*H₂SO₄ solution.
‡K₂CO₃ solution.

**Potassium carbonate, saturated solution**

Potassium dichromate, 0.25% (w/v) in sulfuric acid. Dissolve 250 mg. of potassium dichromate in 100 ml. of 18 N sulfuric acid.

**Standard ethanol solution, 80 mg./100 ml.** Mix 0.010 ml. of ethyl alcohol with 10 ml. of blood. Alcohol-free blood (outdated blood-bank blood) can be used as blank and diluent for the standard ethanol solution.

**Pyridine, C. P.** Various sources of pyridine have a tendency to make high blanks with resulting difficulty in interpreting results. Analytical grade pyridine (Fisher) gave low blank values.

**Standard chloroform solution, 12 mg./100 ml.** Mix 0.001 ml. of chloroform with 10 ml. of blood. Normal blood serves as blank and diluent for the standard chloroform solution.

**Acetone, C. P.**

**Sodium hydroxide solution, 5 N**

**High-vacuum silicone grease**

**Filter-paper discs** S&S No. 740-E

**Ball and socket clamps** Sizes 18 and 18A (Fisher)

**Oscillatory hot plate** Fisher No. 11-492

**Dumbbell diffusion cells (1)**

### Procedures

**Carbon Monoxide**

A paper disc, moistened with 2 drops of the palladium chloride solution and blotted to remove excess solution, was centered on top of a ball flask. The disc was temporarily held in place by traces of high-vacuum silicone grease applied to the joint. Then, 0.2 ml. of blood and 0.8 ml. of the 10% sulfuric acid solution were placed in the socket flask. Without delay, the ball flask was inverted over the socket and clamped into position. Since the bottom of the cell is flat, it was found possible
to maintain vertical balance by using 2 clamps (size 18) placed opposite each other. The cell was mixed by swirling and kept at room temperature. After 10 min, the paper disc was removed and rinsed with water. It was then compared with standard black stains prepared by treatment of blood specimens of known carbon monoxide saturation under identical conditions.

*Standardization* Natelson's procedure (4) served as a guide. A 10-ml blood specimen from a healthy nonsmoking individual was obtained with the aim of observing a negative blank. The 100% carbon monoxide saturated blood was prepared by slowly bubbling carbon monoxide through 5 ml of blood for 30 min. The untreated portion of the blood specimen was used as a diluent in the scheme outlined below. Carbon monoxide was generated by dropping formic acid on concentrated sulfuric acid and drying the liberated gas by passage over calcium chloride. An adequate apparatus was constructed by properly assembling a dropping funnel, one-hole rubber stopper, suction flask, drying tube, and appropriate tubing. A preliminary gas-generation period of 10 min was allowed to displace the air in the apparatus with carbon monoxide. This entire operation was carried out in a well-ventilated hood.

Mix 1.0, 0.9, 0.8, 0.6, and 0.4 ml of untreated blood with 0.0, 0.1, 0.2, 0.4, and 0.6 ml of the saturated blood, respectively. This results in samples that have values of 0%, 10%, 20%, 40%, and 60% saturation. The 0.1- and 0.2-ml volumes were pipetted with corresponding 0.1 and 0.2 ml Mohr-type pipets graduated above the tip. The standard discs obtained by the above procedure may be photographed with the aid of a yellow filter. The use of photographs to serve as a reference is recommended since the black-density values of the original standard discs do not remain constant more than 2 months even if protected from fumes, heat, and direct sunlight (2, 4).

*Volatile Alcohols*

To each of 3 ball flasks representing the blank, ethanol standard, and unknown, 0.3 ml potassium dichromate reagent was added. Then add 0.2 ml of potassium carbonate solution and 0.5 ml of the appropriate blood specimen to the socket flasks. Each cell consisting of a ball and socket flask was immediately clamped in a horizontal position with a screw-tension clamp (size 18A) and placed, with the aid of tongs, in a 400-ml beaker containing approximately 150 ml of boiling water which is on an oscillating hot plate. After 10 min, the cells were removed and compared visually. The blank should be orange and the 80 mg./100 ml. standard, greenish.
Halogenated Hydrocarbons

To each of 3 ball flasks representing the blank, chloroform standard, and unknown, 0.4 ml. of pyridine, 0.2 ml. of 5 N sodium hydroxide, and 0.1 ml. acetone were added. This was followed by adding 0.5 ml. of the appropriate blood specimen to the socket flasks. The cells were immediately clamped in a horizontal position with a screw-tension clamp (size 18A) and each cell placed in a 400-ml. beaker containing approximately 150 ml. of boiling water which is on an oscillating hot plate. The chloroform standard gave a strong rose color within 1 min. Similar concentrations of chloral hydrate and carbon tetrachloride required 5 min. before a distinct rose color appeared.

Discussion

These 10-min. qualitative screening tests were developed to aid the clinical chemist in rapidly determining whether a toxicologic problem exists in a particular analytic area. Thus, when indicated, valuable time may be devoted to further analysis with more specific and refined methods. The design of the dumbbell-diffusion cell has permitted adaptation of both the flange aeration and Conway methods. The time, space, and labor required to set up an aeration train has been eliminated and the time factor in Conway methods has been accelerated by working at 100°, a condition for which the Conway cell is poorly suited.

Limitations of these qualitative methods deserve mention. A specimen contaminated by bacteria may contain products which reduce palladium chloride. Anemic bloods will introduce an appreciable error due to the difference in hemoglobin concentration between the blood used for standardization and that of the patient, since carbon monoxide values are based on % saturation of total hemoglobin. Values as high as 7% saturation have been reported for heavy smokers (5). As a guide to interpreting values, Curry (6) has found that in the majority of cases, 25% saturation marks a division between minor and the beginning of major symptoms of poisoning. In view of the above uncertainties, it is suggested that a screening value of 10% saturation or higher should be followed by a quantitative method (4).

The test for chlorinated hydrocarbons detects compounds containing 3 halogen atoms on a single carbon, and further analysis is required if the specific identity of the compound involved is required (7). Chloroform, for which this test is highly sensitive, has been found to cause light surgical anesthesia in man at a blood level of 7 mg./100 ml. (8).

The dichromate-reduction test for volatile alcohols is only a presumptive test for ethyl alcohol whose forensically critical range is 100–200
mg./100 ml. of blood (9). A positive test may be due to the presence of one or more alcohols or carbonyl compounds (3), and other tests (9) are required to ascertain the identity and concentration of the compound(s) involved.

References