

Presumptive Testing to Detect Blood and Freezing Temperatures

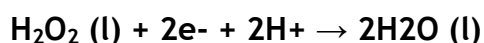
Amy Du Beau, PhD | 2019

It was a dark and stormy night in Alaska....is it blood? Detection of dried bloodstains starts with using a presumptive chemical screening test followed by a confirmatory test. Importantly, presumptive tests are indicative of the presence of blood but are not wholly definitive. If basic physical conditions of the test are not met, the test result is invalid. Hint: Winter in Alaska!

Presumptive tests for the detection of blood include phenolphthalein, o-tolidine, leucomalachite green and tetramethylbenzidine. (The benzidine test is virtually no longer used in modern forensic settings since benzidine is recognized as carcinogenic).

How do presumptive tests detect blood? The indicator reagent, phenolphthalein for this example, reacts with the addition of hydrogen peroxide (H₂O₂) in the presence of blood. This test is positive (basic pH) for the presence of blood if the swab turns magenta pink and negative (neutral to slightly acidic pH) if the swab remains colorless. This catalytic reaction is quick, occurring within seconds, making it handy for crime scene analysis.

Blood is mostly composed of water. Blood plasma constitutes about 55% of blood by volume, and plasma is greater than 91% water. Hemoglobin is a molecular protein contained within red blood cells. Iron-based hemoglobin accounts for why our blood is red. (Incidentally, lobster blood is blue because it is copper-based). By catalytic action, H₂O₂ reacts with hemoglobin in the presence of an indicator (e.g., phenolphthalein), which supplies electrons (e⁻). The catalytic reaction:



H₂O₂ = hydrogen peroxide

e⁻ = electron

H⁺ = hydrogen ion

H₂O = water

l = liquid (physical state of matter, fluid)

Positive test for blood
Anchorage, Alaska | January 2019



So what can go wrong? Alaskan winters are cold with temperatures usually well below freezing. This catalytic reaction that is the presumptive test only occurs when the molecules involved are in a fluidic (liquid) state. Under freezing conditions, all constituent molecules are solid, rendering this reaction impossible. Hemoglobin is physically bound inside red blood cells, unreactive when blood is solid.

Blood freezes at $\approx -2^{\circ}\text{C}$ ($= 16.6^{\circ}\text{F}$). H_2O_2 freezes at -0.4°C ($= 31.2^{\circ}\text{F}$) and H_2O freezes at 0°C ($= 32^{\circ}\text{F}$). Such physical constants are irrefutable facts. Chemical reactions are constrained by such thermodynamic constants.

Ice melt | During wintertime in Anchorage, ice melt (KCl) is commonly applied to slippery public walkways. Chemically, KCl is a reducing agent, adding electrons to the catalytic chemical reaction. Presumptive tests can yield a false negative result in the presence of such reducing agents. Be aware of this potential confounding factor.

Special medical considerations | Blood external to the body comes from breached vasculature. The body's first physiological response to such a wound is to stop the blood loss by vasoconstriction, fluid retention and recruiting platelets. Resultantly, body temperature acutely drops as homeostatic control is lost. Left untreated, hypotensive shock ensues with unconsciousness. For context, for an adult weighing 150 pounds, a loss of about 1.5 liters of blood within an hour results in hypotensive shock and this physiological process is greatly exacerbated by freezing environmental temperatures. Alaskans: be aware that fatal blood loss can be less than expected during freezing wintry conditions.

Advice | Does your presumptive test result seem fishy? As a scientist, I will offer you my advice. Physical evidence does not lie. Presumptive tests are a powerful tool to detect blood provided the proper conditions are met. Any test is fallible. Importantly, if any given test result defies reality, make note of the questionable result and collect an evidentiary sample for a confirmatory test. Police officers are not scientists, of course (and vice versa), but critical thinking skills may be your best investigative tool. Troubleshoot questionable results and countermand as necessary.

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