**B8R04653 Carolina Biological Presumptive Blood Test #84-0582**

## Perform the Kastle-Meyer Blood Test

1. Moisten a swab with water and touch it to a dried blood sample. You do not need to rub hard or coat the swab with the sample. You only need a small amount.
2. Add a drop or two of 70% ethanol to the swab. You don't need to soak the swab. The alcohol does not participate in the reaction, but it does serve to expose hemoglobin in blood so that it can react more fully, to increase the sensitivity of the test.
3. Add a drop or two of the Kastle-Meyer solution. This is a phenolphthalein solution, which should be colorless or pale yellow. If the solution is pink or if it turns pink when added to the swab, then the solution is old or oxidized and the test will not work! The swab should be uncolored or pale at this point. If it changed color, start again with fresh Kastle-Meyer solution.
4. Add a drop or two of hydrogen peroxide solution. If the swab turns pink *immediately*, this is a positive test for blood. If the color does not change, the sample does not contain a detectable amount of blood. Note that the swab will change color, turning pink, after about 30 seconds, even if no blood is present. This is a result of hydrogen peroxide oxidizing the phenolphthalein in the indicator solution.

## Alternate Method

Rather than wetting the swab with water, the test may be performed by moistening the swab with the alcohol solution. The remainder of the procedure remains the same. This is a nondestructive test, which leaves the sample in a condition such that it may be analyzed using other methods. In actual practice, it is more common to collect a fresh sample for additional testing.

## Sensitivity of the Test and Limitations

The Kastle-Meyer blood test is an extremely sensitive test, capable of detect blood dilutions as low as $1{:}10^7$. If the test result is negative, it is reasonable proof that heme is absent in the sample, however, the test will give a false positive result in the presence of any oxidizing agent in the sample. Examples include peroxidases naturally found in cauliflower or broccoli. Also, it is important to note that the test does not differentiate between heme molecules of different species. A separate test is required to determine whether blood is of human or animal origin.

## How the Kastle-Meyer Test Works

The Kastle-Meyer solution is a phenolphthalein indication solution which has been reduced, usually by reacting it with powdered zinc. The basis of the test is that the peroxidase-like activity of the hemoglobin in blood catalyzes the oxidation of the colorless reduced phenolphthalin into bright pink phenolphthalein.