How could a seemingly simple series of tests for human blood identification have been misconstrued in forensic casework as revealed by attorney query, review, audits and investigation? This is a common source of error in forensic biology and two examples are given here for quality control issues essential for the correct interpretation of forensic blood identification.

The Difference Between Presumptive and Confirmatory Blood Identification

IDENTACODE NEWSLETTER

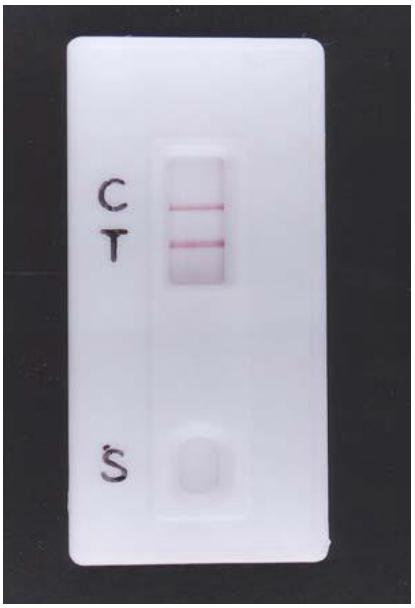
Quality assurance refers to an overall program at a forensic science laboratory that accurately identifies key points in evidentiary processing that are critical to maintaining the scientific integrity of the system (example – auditing). Quality control refers to the step by step process that insures the validity of the scientific results in any given method or procedure (example- use of positive and negative controls).

Presumptive and Confirmatory Blood Identification

The word presumptive means that a test result can be read as "could be blood." What is the purpose of performing a test that is not specific for human blood? In forensic science, hundreds of samples are collected in casework and a rapid, sensitive assay to allow for quick screening for possible blood at the crime scene or in the laboratory is critical for maintaining effective work flow. However, scientific accuracy is required for legal purposes so a second confirmatory test specific for human blood is performed that may be less sensitive that the first assay but is more specific to human blood. The two tests combined and when performed properly give highly sensitive and specific results for human blood identification. Forensic laboratory procedure needs to be tightly monitored and protocols need to be evaluated to insure that scientifically accurate results are being obtained. Although many blood identification tests exist, two commonly used tests are shown here:

Phenolphthalein – also known as Kastle-Meyer reagent, this test is a presumptive blood identification test for detection of "possible" blood, human or otherwise, so the test lacks human specificity. The basic test involves taking a trace amount of sample and hydrating with a drop (20ul) of sterile water or phosphate buffered saline for 10 seconds. A drop of Kastle-Meyer reagent (20ul), a pH indicator, is added to the hydrated sample. After 20 seconds, the sample is checked to insure that no color change has occurred as at this point, the sample should be colorless or the original color. With the addition of a third reagent, hydrogen peroxide (20ul), a rapid color change to pink should occur with 30 seconds. This indicates a "possible" presence of blood and confirmatory testing should be subsequently performed. Quality control for this test includes the following: (1) a known blood sample (positive control) and a negative substrate sample (negative control) need to be tested prior to use of the reagents on the evidence or the test results are invalid, (2) any color change at the Kastle-Meyer step should be recorded by time and test result to establish the test result as inconclusive and (c) if the test is not timed correctly after addition of hydrogen peroxide, a positive color reaction will occur anyway, so the test result needs to be read at the 30 second point or the result will be a false positive. Other samples that yield a false positive with this test are plant peroxidases and heavy metals from soils, for example.

HemaTrace – This is an immunochromatographic test that is based on antigen-antibody interaction for detection and confirmation of human blood in crime scene samples. The antigen or target substance for detection is human hemoglobin giving human specificity to the test. The detection method is by human antibody recognition of a target region of human hemoglobin; the antibody is conjugated to a chromagen (color change indicator on binding to antigen). A small amount of sample is hydrated in the buffer provided with the test cartridge to solubilize the human hemoglobin. The liquid sample is loaded into the test well (s) and the sample migrates up the embedded test strip to the second well. Two antibodies are present in this test cartridge, one that is mobile and migrates along with the test sample; the other is fixed in the read region of the cartridge, the (C) and (T) wells. How does this work? The mobile antibody binds to the soluble hemoglobin and migrates to the upper portion of the strip. As the hemoglobin-antibody complex migrates, it is captured by the second antibody which is fixed to the strip and a color change to red is interpreted as a positive confirmation of human blood being present. Quality control for this test is the following: (1) sufficient hemoglobin must be solubilized for the test to detect human blood (incubation is typically 5 minutes in buffer), (2) sufficient liquid must be added to the test cartridge for the sample to migrate up the test strip (20-100ul), and (3) sample must not be too concentrated or the (C) line will not turn the expected positive red, indicating the binding to the second antibody has been blocked by excessive hemoglobin protein in the sample (high dose hook effect)-all of these circumstances will give a false negative reading for the confirmatory test for human blood samples.



This example shows a positive confirmatory test for human hemoglobin which meets the legal standard for testimony for a sample containing human blood. If the test (T) line was absent and the control (C) line present, then the test result would be read as negative for the presence of human blood regardless of whether the presumptive test was positive. The confirmatory test is the most specific test available for human blood identification in casework samples. DNA tests do not replace human blood identification tests as the target substance is a different molecule. Human hemoglobin identification is specific to the substance we know as blood and the only manner in which blood can meet the legal standard for identification in forensic science.