

Antibody to Hepatitis C in Serum- Human serum or plasma is diluted in specimen diluent and incubated with a polystyrene bead coated with recombinant HCV antigen. Following a 1-hour incubation, the beads are washed to remove unbound material. A peroxidase-conjugated antibody directed against human IgG is added to each bead. Following a 30-min incubation, the beads are washed again to remove unbound material. The beads are then transferred to 10- x 75-mm polystyrene tubes, and a peroxidase-specific chromogenic substrate solution is added to each tube. The substrate solution consists of hydrogen peroxide and o-phenylenediamine (OPD) in a citrate buffer. Following a 30-min incubation at 20-25 C, 1 N sulfuric acid is added to stop the enzyme-substrate reaction. Anti-HCV antibody will bind to the HCV antigen on the bead. Subsequently, the conjugate binds to that antibody. The reaction of the conjugate with the substrate solution results in the generation of an orange color. Absence of color indicates the absence of anti-HCV in the sample. The intensity of the color generated is measured spectrophotometrically at 492 nm. The instrument used to measure the test results is equipped with software that calculates a cutoff value. The cutoff calculation is based upon values obtained from control reagents included with each testing series. Results are expressed as "positive" or "negative" for anti-HCV. Positive specimens are repeated in duplicate according to the same procedure. Repeatedly positive specimens are supplementally tested using the MATRIX instrument (Abbott Laboratories). Matrix is an unlicensed technology that is in use by the HRL under an Investigational New Drug (IND) agreement. This is an FDA-licensed method commercially obtained in kit form (1-3). The literature and instructions in each kit constitute the standard operating procedure (SOP) for the method. Its diagnostic utility lies in its capacity to determine whether an individual has been infected with hepatitis C.

1. Abbott Laboratories, Diagnostics Division. Abbott HCV EIA directional literature included with each assay kit: Abbott document #83-7499/R7, North Chicago (IL):Abbott Laboratories,1992.
2. Bradley DW. The agents of non-A, non-B hepatitis. J Virol Meth 1985;10:307-319.
3. Aach RD, Stevens CE, Hollinger B, et al. Hepatitis C virus infection in post-transfusion hepatitis: an analysis with first and second generation assays. N Eng J Med 1991;325:1325-9.

-from *Laboratory Procedures Used for the Third National Health and Nutrition Examination Survey (NHANES III) 1988-1994*
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