We standardized a serologic enzyme immunoassay (EIA) for human immunoglobulin G and M antibodies against Haemophilus ducreyi. We evaluated the performance of this test with respect to the time from acute chancroid and coinfection with human immunodeficiency virus (HIV). Antibody to a crude, soluble bacterial antigen of one H. ducreyi strain was detected in a panel of serum samples from clinically and microbiologically confirmed cases of chancroid and from controls. Test interpretation was standardized for optimal sensitivity and specificity. Performance of the EIA was enhanced in the period of early convalescence from acute primary chancroid and was not diminished in the presence of HIV coinfection. The EIA performed adequately as a serologic screening test for field evaluation and epidemiologic application in conjunction with sexually transmitted disease and HIV detection and control efforts.