

Chapter 29

Non-cultural Methods

AGGLUTINATION TITRE: Antibody to antigen on surface of microorganism causes a visible precipitation of microorganism. Useful diagnostic alternative when direct demonstration unsuccessful or not possible. A 4-fold rise in titre between acute and convalescent sera is convincing evidence of recent infection (less may be due to inherent variation of test). A high titre in a single specimen may be significant.

ANTI-DEOXYRIBONUCLEASE B: Detects streptococcal infection. Levels of antibody are measured by visualising hydrolysis of DNA, which is linked to a colour reaction. It is consistently elevated with both rheumatic fever and glomerulonephritis, rises later than anti-streptolysin O titre, peaks at 4-6 w and remains elevated longer than anti-streptolysin O titre. The magnitude of response may be suppressed by antimicrobial therapy. Detergents, heavy metals, azide and other chemicals interfere with enzyme and colour reaction.

ANTI-STREPTOLYSIN O TEST: Antibody from patient's serum inhibits streptolysin O produced by *Streptococcus pyogenes* (lyses human erythrocytes). Normal in ? 20% of early rheumatic fever and 50% of glomerulonephritis (especially following skin infection). Peaks at 2-4 w. False positives due to activity of other substances neutralising haemolytic properties of streptolysin O (eg. serum ?-lipoproteins in liver disease and bacterial growth in serum).

COMPLEMENT FIXATION TESTS: Complement depleted (fixed) by combination with complex of microbial antigen and antibody. Rise within 2 w of onset, rarely remain raised longer than a few months.

COMPLEMENT LYSIS: Antibody to microbial surface antigen + complement lyses bacterium or enveloped virus. Used in research.

COUNTERIMMUNOELECTROPHORESIS: Detection time 30 minutes. Requires special equipment. Availability of quality sera is limited.

C-REACTIVE PROTEIN: Direct latex agglutination test positive in variety of inflammatory and necrotic processes.

DIRECT IMMUNOFLOUORESCENCE: Fluorescein-labelled antibody to antigen on microorganism or antigen formed in infected cell seen on microorganism or in infected cell by UV microscopy.

DYE TEST: Detects *Toxoplasma gondii*. Usually reliable, though status of disease activity may be uncertain.

ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA, EIA): antibody linked to enzyme reacts with microbial antigen. Specific binding revealed when enzyme causes colour change in substrate. Detection time 4 h. Requires special equipment. Very specific. Requires small sample. Long incubation. Time consuming procedure. Used for *Giardia*, herpes simplex virus, many other organisms.

GEL DIFFUSION: Antibody reacts with diffusible microbial antigen to form precipitation line in gel. Elek test is immunodiffusion test used to identify toxigenic strains of *Corynebacterium diphtheriae* and *Corynebacterium ulcerans*.

HAEMAGGLUTINATION INHIBITION ANTIBODY TECHNIQUE: Inhibition of erythrocyte agglutination by haemagglutinin forming part of surface of virus particle.

IMMOBILISATION TEST: Inhibition of motility by reaction with antigen on locomotor organ (flagellum, cilium).

IMMUNE ELECTRON MICROSCOPY: Virus particles clumped by reaction with antigen on surface of virus viewed by electron microscopy. Used in research.

LATEX AGGLUTINATION: Antibody to microbial antigen adsorbed to latex particles agglutinates latex particles. Detection time < 5 minutes. May require treating specimen to eliminate nonspecific agglutination. Used for *Cryptococcus* and many others.

MINI VIDAS: enzyme-linked fluorescent immunoassay performed in an automated instrument; provides assays for human immunodeficiency virus p24 antibody, human immunodeficiency virus anti-p24 antibody, hepatitis B core antigen IgM antibody, anti-hepatitis B surface antibody, hepatitis B surface antigen, hepatitis A IgM antibody, anti-hepatitis B core antigen antibody, cytomegalovirus IgG antibody, human immunodeficiency virus 1+2, mumps IgG antibody, measles IgG antibody, rubella IgG antibody, *Toxoplasma gondii* IgG antibody, *Toxoplasma gondii* IgM antibody, varicella-zoster IgG antibody, *Toxoplasma* competition assay, Lyme disease screen, cytomegalovirus IgM antibody,

rubella IgM antibody, rubella IgG antibody, *Chlamydia*, *Clostridium difficile* toxin A, herpes simplex, respiratory syncytial virus, *Campylobacter*, *Escherichia coli* O157, *Listeria*, *Salmonella* and *Staphylococcus* enterotoxin.

NEUTRALISATION ANTIBODY TITRE: Antibody reacting with viral surface antigen necessary for multiplication in experimental animal or cell culture inhibits multiplication and prevents pathological lesions, death or cell damage.

NEUTRALISATION TEST: Biological effect of bacterial toxin inhibited.

NUCLEIC ACID PROBES: Useful in detecting *Mycoplasma pneumoniae*, herpes simplex virus and others.

PASSIVE HAEMAGGLUTINATION: Antibody to microbial antigen absorbed to surface of erythrocyte agglutinates erythrocytes.

POLYMERASE CHAIN REACTION: Method of choice in detecting *Chlamydia*. Also useful for *Mycobacterium tuberculosis*, *M. avium*, *M. intracellulare*, hepatitis C virus, hepatitis B virus, gonorrhoea, human immunodeficiency virus, enterovirus, *Mycoplasma*, human herpesvirus 6, 7 and 8, parvovirus B19, *Bartonella henselae*, *Plasmodium*, enterohaemorrhagic *Escherichia coli* O111/O157, *Salmonella*, *Legionella*, *Entamoeba histolytica*, Ross River virus, dengue 1, 2, 3 and 4, Murray Valley encephalitis, Japanese encephalitis, flaviviruses, *Leptospira*

WHITE CELL COUNT: leucocytosis ($> 15\ 000/\text{mm}^3$) in hospital patients: 47% pneumonia, 38% physiological stress, 29% urinary tract infection, 16% soft tissue infection, 16% *Clostridium difficile* infection, 11% medications or drugs, 6% haematological disease, 6% necrosis or inflammation