MEDLAB BASICS · STUDY SHEET

Immunology and Serology

Immunoglobulins, complement, antigen-antibody reactions and immunoassays, ANA patterns and autoantibodies, hypersensitivity, immunodeficiency, monoclonal proteins, hepatitis, HIV, EBV, syphilis, Lyme, IGRA and transplant immunology

54 sources + more detail

medlabbasics.com/sheets/lab-immunology-serology.html
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1 Immunoglobulins

Each immunoglobulin has two identical heavy chains and two identical light chains (kappa or lambda, never both on one molecule). The variable domains at the tips form the antigen-binding site from three CDRs on each chain; papain cuts IgG into two Fab fragments (bind antigen) and one Fc fragment (binds Fc receptors and C1q). The heavy-chain class sets the isotype.

Percentages from Schroeder and Cavacini 2010, Table 1.

CLASS	FORM	SHARE OF SERUM IG	WHAT TO REMEMBER
IgG	Monomer; subclasses IgG1 67%, IgG2 22%, IgG3 7%, IgG4 4%	About 75%	Most abundant, longest half-life; the only class carried across the placenta (by FcRn); C1q binding IgG3 > IgG1 > IgG2, IgG4 does not fix complement
IgM	Pentamer joined by a J chain	About 10%	The primary-response antibody; efficient at fixing complement and opsonizing
IgA	Monomer in serum; dimer with J chain and secretory component in secretions	About 15%	Mucosal defense (secretory IgA); over 90% of serum IgA is IgA1, IgA2 dominates many secretions
IgD	Monomer, mainly on the B-cell surface	Under 0.5%	Very low serum level, short half-life, function poorly understood
IgE	Monomer bound to FcεRI on mast cells and basophils	Under 0.01%	Lowest level and shortest half-life; allergy (type I) and helminths

EXAM TIP Primary vs secondary response: a first exposure makes IgM first; on re-exposure memory B cells give a faster IgG response. IgM above the cord-blood reference in a newborn points to an infection in utero, because maternal IgM does not reach the fetus.

2 Complement

PATHWAY	STARTED BY	C3 CONVERTASE	C5 CONVERTASE
Classical	C1 (C1q + C1r2C1s2) binding IgM or aggregated IgG on antigen; also CRP, polyanions, some gram-negative bacteria	C4b2a	C4b2a3b
Lectin	Mannose-binding lectin on microbial sugars (mannose, fucose, N-acetylglucosamine); no antibody	C4b2a	C4b2a3b
Alternative	Spontaneous low-level C3 hydrolysis, amplified on microbial surfaces (endotoxin) and by nephritic factor; stabilized by properdin, controlled by factor H and DAF	C3bBb	C3bBbC3b

- All pathways meet at C3: C3b is the main opsonin; C3a and C5a (C4a weakly) are anaphylatoxins; C5a also attracts neutrophils.
- C5b-9 is the membrane attack complex that lyses cells.
- Regulators: C1 inhibitor (classical), factor H and factor I with cofactors (inactivate C3b and C4b), DAF/CD55.
- Screening: CH50 tests the classical pathway and AH50 the alternative pathway; a low result does not name the missing component. C3, C4, C1q and C1 inhibitor level and function follow.

DEFICIENCY	RESULT
C1 or C4 (early classical; Merck also lists C5)	SLE-like immune complex disease
C1, C2 or C3	Recurrent bacterial infection (C3: the opsonin is missing)
Terminal components (C5 to C9)	Recurrent Neisseria infection, for example a second meningococcal episode
C1 inhibitor	Hereditary angioedema: recurrent swelling without hives
DAF on blood cells (PIGA mutation)	Paroxysmal nocturnal hemoglobinuria

SAFETY Complement is heat-labile and keeps activating in the tube. For CH50 and AH50 use serum (never EDTA plasma, which blocks the activity being measured), separate and process within about 1 hour on ice, and freeze at -80 °C (the strictest published guidance; laboratories set their own limits). Complement protein levels (C3, C4) by nephelometry survive storage, but hemolytic activity does not: an unspun tube left at room temperature for days can give an undetectable CH50 with normal C3 and C4. Reject and recollect per the procedure manual.

3 Antigen-antibody reactions and immunoassays

ZONE	RATIO	EFFECT
Prozone	Antibody excess	Too little lattice: false negative or falsely low titer in agglutination and flocculation (RPR, VDRL). Dilute the serum and retest.
Zone of equivalence	Balanced	Maximal cross-linking: visible precipitate, flocculation or agglutination
Postzone	Antigen excess	Lattice fails again; in a one-step sandwich immunoassay this is the high-dose hook effect (falsely low result)

METHOD	PRINCIPLE	EXAMPLES
Flocculation	Soluble lipid antigen particles clump with antibody	VDRL (read with a microscope), RPR (read by eye)
Particle agglutination	Antigen-coated particles clump with antibody	TP-PA (gelatin particles), latex RF
Single radial immunodiffusion (Mancini)	Monospecific antibody is mixed into agarose; antigen placed in a well diffuses out and forms a precipitin ring; the ring diameter squared is proportional to antigen concentration, read from a standard curve	Quantifying a single protein (for example an immunoglobulin or complement component) without an analyzer
Double immunodiffusion (Ouchterlony)	Antigen and antibody diffuse in gel; lines fuse (identity), fuse with a spur (partial identity) or cross (nonidentity)	Comparing antigens
Indirect ELISA	Antigen on the well, patient (primary) antibody, then enzyme-labeled secondary anti-human Ig; wash between steps; HRP or ALP converts a substrate (TMB, OPD) to color	The most common format for antibody detection

METHOD	PRINCIPLE	EXAMPLES
Sandwich (noncompetitive) immunoassay	Capture antibody, analyte, labeled detection antibody to a second epitope; signal rises with concentration	hCG, prolactin, thyroglobulin, calcitonin
Competitive immunoassay	Patient analyte and labeled analyte compete for limited antibody; signal falls as concentration rises	Small antigens or those with a single epitope
Chemiluminescence	Enzyme or label produces light (electrochemiluminescence uses a ruthenium label)	Large automated analyzers
Indirect immunofluorescence (IFA)	Patient antibody on a cell or tissue substrate, then fluorescent anti-human IgG; read pattern and titer	ANA on HEp-2 cells, FTA-ABS

INTERFERENCE	SANDWICH ASSAY	COMPETITIVE ASSAY	HOW TO SHOW IT
Hook effect (huge analyte)	Falsely low	Described for sandwich assays	Result rises on dilution (pituitary macroadenoma prolactin, molar pregnancy hCG)
Heterophile or HAMA antibodies	Usually falsely high (bridges the two antibodies)	Can be falsely high	Nonlinear dilution, blocking reagent, another platform; for hCG, serum positive with negative urine
Biotin (high-dose supplement)	Falsely low	Falsely high	Stop biotin 2 days and retest, or use a method without streptavidin-biotin
Macroprolactin	Falsely high prolactin	n/a	PEG precipitation, then measure monomeric prolactin

4 Autoantibodies

ANA screening by indirect immunofluorescence on HEp-2 cells is reported as titer plus pattern (ICAP AC codes). A titer above 1:80 is found in over 95% of SLE, but healthy people are positive too: about 30% at 1:40 and about 3% at 1:320 (Merck). Positive patterns go on to antigen-specific tests.

PATTERN (ICAP)	WHAT IT LOOKS LIKE	ANTIGENS	ASSOCIATIONS
Homogeneous (AC-1)	Even staining of the whole nucleus; mitotic chromatin stains strongly	dsDNA, nucleosomes, histones	SLE (follow with anti-dsDNA), drug-induced lupus (anti-histone; hydralazine, procainamide), autoimmune hepatitis, JIA
Dense fine speckled (AC-2)	Speckles of varying size and density; metaphase chromatin stains	DFS70 (LEDGF)	Often high titer in healthy people; argues against systemic autoimmune disease when it is the only antibody
Centromere (AC-3)	40-80 discrete coarse dots per nucleus, lined up on mitotic chromatin	CENP-A, B, C	Limited cutaneous systemic sclerosis (CREST); with Raynaud it predicts lcSSc; rarely PBC

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4 Autoantibodies CONTINUED

PATTERN (ICAP)	WHAT IT LOOKS LIKE	ANTIGENS	ASSOCIATIONS
Fine speckled (AC-4)	Fine speckles; mitotic chromatin does not stain	SSA/Ro, SSB/La	Sjogren (SSA about 75%, SSB about 60%); also SLE and scleroderma
Coarse speckled (AC-5)	Coarse speckles; mitotic chromatin does not stain	Sm, U1-RNP, RNA polymerase III	Sm: specific for SLE; U1-RNP: mixed connective tissue disease (95-100%)
Nucleolar (AC-8, 9, 10)	Nucleoli stain (homogeneous, clumpy or punctate)	PM-Scl, Th/To, fibrillarin, RNA polymerase I	Systemic sclerosis (ANA in at least 90% of SSc, often nucleolar); PM-Scl with SSc-myositis overlap
Topo I-like (AC-29)	Fine speckled nucleus plus nucleolar organizer staining	Topoisomerase I (Scl-70)	Highly specific for systemic sclerosis, mainly diffuse cutaneous and more aggressive disease

- Anti-dsDNA and anti-Sm are highly specific for SLE but not sensitive. Low C3 and C4 track active disease, lowest in nephritis.
- Anti-Jo-1: polymyositis (about 30%), interstitial lung disease.
- Rheumatoid factor (antibody to human IgG): about 70% of RA, but also Sjogren, SLE, granulomatous disease, chronic infection, cancer, about 3% of the general population and 20% of older adults; titer does not follow disease activity. Anti-CCP: specificity about 90%, sensitivity about 77-86%, and absent in hepatitis C, which can give a positive RF with joint swelling.
- Thyroid: Hashimoto has high-titer anti-TPO (antithyroglobulin less often). Graves has TSH-receptor stimulating antibody (TSI) with suppressed TSH and high free T4 or T3; most Graves patients also have anti-TPO.

5 Hypersensitivity (Gell and Coombs)

TYPE	MECHANISM	TIMING	EXAMPLES	LAB CLUE
I	Allergen cross-links IgE on mast cells and basophils; histamine, leukotrienes	Under 1 hour	Anaphylaxis, allergic asthma, rhinitis, urticaria	Allergen-specific IgE; serum tryptase within 2 hours of onset
II	Antibody to cell-surface or cell-bound antigens; complement, ADCC	Varies	Coombs-positive hemolytic anemia, anti-GBM disease, Hashimoto thyroiditis, hyperacute graft rejection	DAT positive in immune hemolysis
III	Circulating immune complexes deposit in vessels and tissue and activate complement	Hours, days or weeks	Serum sickness, SLE, RA, leukocytoclastic vasculitis, cryoglobulinemia, several glomerulonephritides	Complement consumption (low C3 and C4 in active SLE)
IV	Sensitized T cells, no antibody	Delayed, read at 48 and 72-96 hours	Contact dermatitis (nickel), tuberculin response, allograft rejection, many drug reactions	Patch test that grows between readings; IGRA measures T-cell IFN-gamma

6 Hepatitis serology

CDC recommends the triple panel (HBsAg, anti-HBs, total anti-HBc) at least once for all adults aged 18 and over.

HBSAG	TOTAL ANTI-HBC	IGM ANTI-HBC	ANTI-HBS	INTERPRETATION (CDC)
+	+	+	-	Acute infection
+	+	-	-	Chronic infection
-	+	not needed	+	Resolved infection (immune; can reactivate)
-	-	not needed	+	Vaccinated (immune if over 10 mIU/mL after a complete series)
-	+	not needed	-	Isolated anti-HBc: waned anti-HBs after past infection, occult infection, passive transfer to an infant, false positive (susceptible) or an HBsAg mutant
-	-	not needed	-	Susceptible, never infected

- HBsAg means the person is infectious, except that it can be transiently positive within 30 days of a hepatitis B vaccine dose.
- HBeAg marks viral replication and high infectivity; anti-HBe goes with lower viral load. HBV DNA is the viral load.
- IgM anti-HBc means infection within about 6 months, but it can reappear in flares of chronic infection. Total anti-HBc persists for life and never follows vaccination.
- Hepatitis A: IgM anti-HAV from 5-10 days before symptoms to about 6 months = acute; total anti-HAV positive with IgM negative = past infection or vaccine. Test IgM only in symptomatic patients.
- Hepatitis C: anti-HCV first; a reactive result reflexes to HCV RNA on the same specimen. Reactive + RNA detected = current infection; reactive + RNA not detected = resolved infection or false positive. Antibody takes 8-11 weeks; RNA appears in 1-2 weeks, so test RNA after a recent exposure or in an immunocompromised patient.

7 HIV laboratory algorithm (CDC/APHL)

STEP	TEST	RESULT AND MEANING
1	HIV-1/2 antigen/antibody combination immunoassay (4th generation: antibodies plus p24 antigen)	Nonreactive: negative, stop. Reactive: go to step 2
2	HIV-1/HIV-2 antibody differentiation immunoassay	Reactive: HIV-1, HIV-2, or HIV antibodies undifferentiated. Nonreactive or indeterminate: go to step 3
3	HIV-1 nucleic acid test (NAT)	NAT reactive + step 2 nonreactive: acute HIV-1 infection. NAT reactive + step 2 indeterminate: HIV-1 infection. NAT negative: the step 1 result was a false positive

EXAM TIP The Western blot is no longer used: it turns positive later than current immunoassays (missing acute infection, when viral RNA is at very high levels) and misclassifies most HIV-2 infections as HIV-1. A reactive step 1 with a nonreactive step 2 in a patient with fever, rash and pharyngitis is classic acute HIV: the NAT decides.

8 Syphilis, Lyme and TB

TEST TYPE	EXAMPLES	USE
Nontreponemal (cardiolipin-lecithin-cholesterol antigen)	RPR, VDRL	Screening (traditional algorithm) and monitoring treatment by quantitative titer; can be falsely positive; usually fall after treatment
Treponemal	TP-PA, FTA-ABS, EIA, CIA, immunoblot	Confirmation (traditional) or screening (reverse); stay reactive for life in most patients; not used to judge treatment

REVERSE SEQUENCE RESULT	NEXT STEP AND MEANING
EIA/CIA nonreactive	No serologic evidence of syphilis
EIA/CIA reactive, RPR reactive	Syphilis, current or past: report the RPR titer
EIA/CIA reactive, RPR nonreactive	Run a second, different treponemal test (TP-PA preferred)
... second treponemal test reactive	Past treated syphilis, or untreated (late latent or unknown duration) if never treated
... second treponemal test nonreactive	Likely false-positive screen; in a low-risk patient no further evaluation is needed

- A clinically meaningful change is fourfold (two dilutions), on the same test, preferably at the same laboratory. RPR and VDRL titers cannot be compared with each other (RPR runs slightly higher).
- Lyme: two tiers on one specimen; stop if tier 1 is negative. Standard (STTT): EIA, then Western blot (IgM positive with 2 of 3 bands: 24, 39, 41 kDa; IgG positive with 5 of 10 bands). Modified (MTTT): EIA then a second EIA. Disregard a positive IgM if illness has lasted more than 30 days. Serology can be negative in the first 4-6 weeks.
- IGRA (QuantiFERON-TB Gold Plus, T-SPOT.TB): measures IFN-gamma from T cells stimulated by ESAT-6 and CFP-10, antigens absent from BCG and most NTM. QFT-Plus (Nil, TB1, TB2 and Mitogen tubes; QIAGEN instructions for use, 2025): positive when TB1 or TB2 minus Nil is at least 0.35 IU/mL and at least 25% of Nil (both from the same tube); negative when neither TB tube meets that and Mitogen minus Nil is at least 0.50 IU/mL; indeterminate when Nil is above 8.0 IU/mL, or when neither TB tube is positive and Mitogen minus Nil is under 0.50. A positive TB response stands even with a low mitogen. Preferred after BCG and for people unlikely to return for a skin test reading. Neither IGRA nor TST separates latent infection from TB disease.

EXAM TIP MLT note: the MLT outline drops Treponema and Borrelia stages, TB/IGRA and cytokine testing; RPR and treponemal test principles stay.

MORE Monoclonal gammopathies

TEST	WHAT IT SHOWS
Serum protein electrophoresis (SPEP)	A narrow M-spike, usually in the gamma region; finds M-protein in about 80-90% of myeloma
Immunofixation (serum and urine)	Names the heavy and light chain of the band (for example IgG kappa); do it even with a normal SPEP if myeloma is suspected
Serum free light chains	Kappa/lambda ratio about 0.26-1.65 with normal kidney function; the key marker in light-chain-only, oligosecretory and nonsecretory myeloma and in AL amyloidosis; kidney disease raises both chains and can shift the ratio
Urine Bence Jones protein	Free light chains in urine; over 200 mg/24 h supports myeloma when serum M-protein is absent

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