CLINICAL LABORATORY TESTING IN OPTOMETRIC PRACTICE

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OUTLINE

The eye is *not* an isolated part of the body. It is only one part of a complicated, integrated total body unit, made up of a series of smaller systems. As doctors, we have to consider these systems when certain findings present ocularly. Laboratory testing can be very useful in helping to sort out a disease process. This paper attempts to familiarize the optometric doctor to commonly encountered laboratory tests. Within this paper is a detailed description of clinical laboratory tests that an optometrist may have reason to order, and when appropriate, the procedure is briefly described and a range of normal and abnormal is provided. Also included are those situations that will call for a systemic work-up and a list of the appropriate tests to order are listed by that disease.

Clinical laboratory testing in the optometric practice is utilized for a few main reasons. First, tests are ordered to aid in the diagnosis of a disease that cannot be otherwise established through routine testing. The testing is most useful if a clinician orders appropriate tests to differentially diagnosis an unknown or unclear disease process. Secondly, if a treatment is initiated, results can be periodically monitored throughout the course of treatment to see if a patient's condition is stabilized, worse, or improving. With some disease processes, it is helpful to determine the stage of activity of a disease as treatment varies with each individual stage. The third main reason is to evaluate the end result of a treatment or the final outcome.

It is in the best interest of a clinician to have a basic understanding of the commonly used lab tests used so as to assure that a patient who presents with an ocular manifestation of a systemic disease has the appropriate work up. Every patient is entitled to the highest standard of care. It is our duty to uphold that high standard.

MICROBIOLOGICAL TESTING

The object of collecting a microbiological sample is to see if growth can be duplicated onto an enriched medium. By knowing which organism is in the eye, treatment can become more specific than a broad-spectrum agent, which is often used as a "shotgun" approach. The shotgun approach may initially benefit the patient, but could ultimately lead to antibiotic resistance.

Ocular cultures are obtained from patients who are suspected of having a bacterial or fungal etiology. Viral infections are usually clinically evident or self-limiting. Specimens should be plated directly on agar plates so as to enable laboratory technicians to identify an organism's morphology more efficiently as potential growth will not be delayed. Solid media is superior to broth preparations for isolating and quantifying microorganisms. If a bacterial or fungal infection is suspected, it is recommended to use the first three solid media along with thioglycolate broth to aid in diagnosis of the etiology of the infection.

Blood Agar is a trypticase-soy agar with 5-10% sterile defibrinated—without fibrin, which is an essential clotting agent—sheep blood. Blood agar is the standard bacteriologic medium used for cultivating fastidious microorganisms and determining hemolytic reactions. Fastidious microorganisms require a complex nutritional and/or cultural medium for growth. This agar is useful for growing aerobic or anaerobic pathogens. The exceptions are Haemophilus, Neisseria, and Moraxella. (Bartlett 635)

Chocolate Agar is a polypeptone or beef infusion enriched with 2% hemoglobin released from defibrinated, heated rabbit or sheep's blood. Blood hemolysis creates the chocolate color. This agar is appropriate for cultivating Haemophilus, Neisseria, and Moraxella. These are the organisms that blood agar cannot isolate. (Bartlett 635)

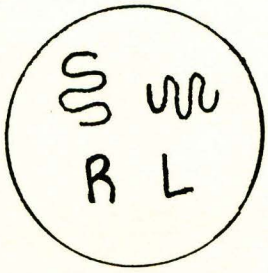
Sabouraud's Agar is a glucose-peptone combination with an adjusted pH of 6.7 to 7.1, which is helpful to encourage growth with opportunistic fungi. Antibiotics are added to prevent bacterial growth and to enhance fungal growth. (Bartlett 635)

Mannitol Salt Agar is a peptone-based agar containing mannitol with 7.5% sodium chloride and a phenol red indicator dye. This agar is used when Staphylococcus is suspected as it is selective for this alone. The salt inhibits most other bacteria. (Bartlett 635)

Thayer-Martin Medium is an enriched chocolate agar with vancomycin, colistin, trimethoprim, and nystatin added to inhibit growth of other bacteria and fungi. This medium is appropriate for growing Neisseria gonorrheae and Neisseria meningitidis. (Bartlett 635)

Procedure: The standard procedure for plating samples taken from patients is called the smear technique. It is important to wear latex gloves so as to prevent contamination of the cultures. While gathering samples, a cotton swab is moistened with sterile saline. It cannot be moistened with anesthetic due to the small antimicrobial properties in conjunction with the preservatives used. Use one swab and roll it across the entire conjunctival fornix of the right eye, taking care not to touch other structures. Repeat with a clean swab to the left eye. Next, obtain a culture from the right lid margin and repeat for the left eye. Plate as shown below, rolling the applicator along the surface. The applicator should not break the surface. (Bartlett 636)

Right conjunctiva

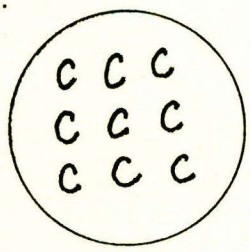


Left conjunctiva

Right eyelid

Left eyelid

Plating for corneal cultures is done using a Kimura platinum spatula. Place a drop of anesthetic in each eye. Sterilize the spatula with a flame. Focus the corneal ulcer with the slit lamp and place the spatula temporally and tangentially to the lesion. Always use a downward motion away from the eye. Take caution not to contact anything but the ulcer to prevent contamination. An eyelid speculum may be helpful in some cases. Inoculate the culture samples onto agar plates forming rows of "c's".



Thioglycolate Broth is a liquid enriched trypticase peptone containing glucose, hemin, and vitamin K. This broth is useful for growing a variety of fastidious aerobic or anaerobic microorganisms. (Bartlett 635)

Procedure: To collect test tube samples, remove the cap and sterilize the glass rim with a flame, then dip the cotton swab used to inoculate the agar plates into the broth and swirl the swab. Break off the end that was handled after insertion of the sample into the tube. (Cassar 205)
Interpretation: When growth on the culture medium is seen, the density, shape, and description of the colonies are important. Colonies are described looking at the size, pigmentation, shape, surfaces, odor, transparency, and consistency. Any of these characteristics can be graded 1+ to 4+. The growth can also be quantified from 1+ to 4+ or in colony-forming units (CFU's). If changes to the

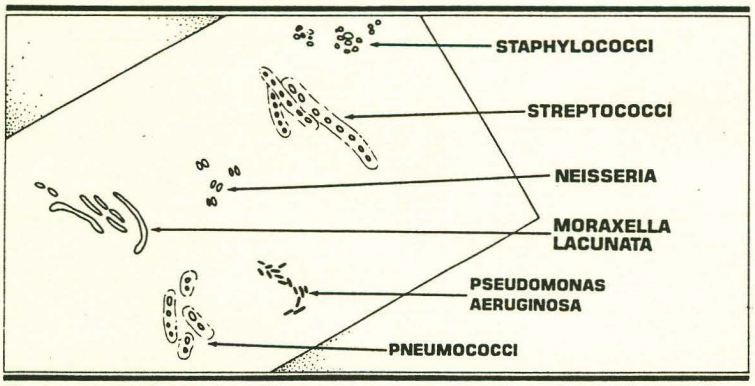
medium such as hemolysis occur, these need to be noted. From the description of the culture growth, a microorganism can be identified. Any growth away from a streak is viewed as contamination and not due to microorganisms. Usually, preliminary results are available in 24 hours, with final results in 48 to 72 hours. (Cassar 206)

CYTOLOGICAL TESTING

Cytological studies are useful in identifying epithelial cells, inflammatory cells, inclusion bodies, microorganisms, and other cellular elements. Samples of infected tissue are fixed onto a glass slide, treated with stain, and then inspected under a microscope.

Gram Stain This stain is used to differentiate bacterial microorganisms into two groups: gram positive (purple stain) and gram negative (pink stain), and to provide information regarding the morphology of the organism. (Cassar 210)

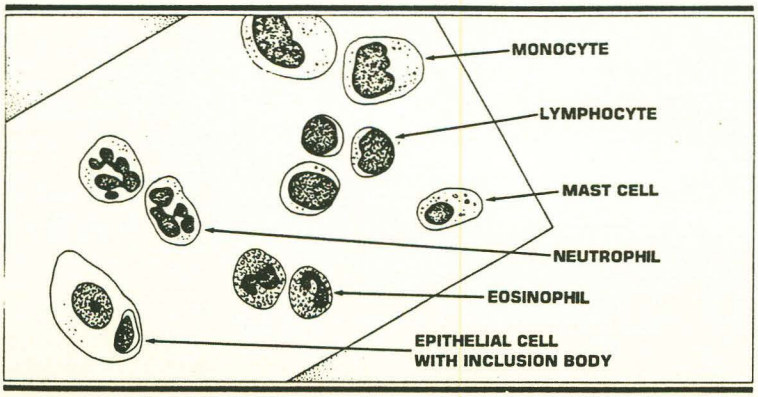
MORPHOLOGIC APPEARANCE USING GRAM STAIN



Giesma Stain The Giesma stain is useful to differentiate viral from bacterial from allergic response, especially for conjunctivitis. This stain contains methylene blue and violet, azure A and B, and eosin, which enables identification of the following by the stated appearance:

1) Lymphocytes and monocytes will appear as blue cytoplasm.
2) Eosinophils appear as pink to orange granules.
3) Neutrophils are pink to purple granules.
4) Basophils are dark blue cytoplasmic granules.
5) Red blood cells which stain pink.
6) This stain also outlines bacteria. (Eskridge 365)

INFLAMMATORY CELLS IDENTIFIED USING GIESMA STAIN



Diff-Quick is a solution that is stable over several weeks which allows the clinician to store this in-office more easily for a longer period of time. The following is a list of identifiable components:

1) Lymphocytes appear as a large round gray-blue nucleus.
2) Monocytes are large vacuolated cells with bluish bean-shaped nuclei.
3) Plasma cells are dark blue cytoplasm.
4) Eosinophils are pink staining granules. (Eskridge 365)

Wright stain. This stain's quality and shelf life make it less useful than the above.

Procedure: The procedure for swabbing is simply done by directly placing the swab/curette on to a glass slide. Scraping is done with a cooled platinum spatula. Apply anesthetic first, then scrape with the motion repeated in the same direction. Make sure to collect enough sample and spread evenly before fixing as directed.
Interpretation: The gram stain is used to divide bacteria into two groups: those retaining (Gram positive) or losing (Gram negative) the primary crystal violet stain when treated with 95% methanol. Gram-negative organisms do not retain the primary stain and are subsequently counterstained with safranin, appearing red under the microscope. The makeup of the cell wall determines if the stain is preserved. The Gram stain is also useful in describing the morphologic appearance of the microorganisms. Three shapes (cocci, rods, and bacilli) and four organizational patters (single, pairs, clusters, or chains) can be identified. Not all shapes occur for all organized patterns. Organisms having a similar morphologic appearance with Gram's stain may be definitively diagnosed with a culture. (Cassar 214)

HEMATOLOGICAL TESTING

Complete Blood Count with Leukocyte Differential (CBC with Diff) This is probably the most common clinical laboratory test obtained in medical practice. This provides an initial point of entry into the differential diagnosis of a number of

disorders. Components include red blood cell, white blood cell, and platelet evaluation. Below is listed a more detailed description of included tests. (Alexander 76)

RBC—A count of red blood cells, used to detect anemias and polycythemias.
Hgb/Hb—Hemoglobin, the concentration of oxygen-carrying protein. Depression of this indicates the possibility of decreased oxygen to peripheral tissues.
Hct—Hematocrit, the percentage of total blood volume compromised of cells. This is used to establish presence and severity of anemia. It is elevated in polycythemias, depressed in anemias.
MCV—Mean corpuscular volume is the measure of relative cell size—normocytic, macrocytic, microcytic. This is used to evaluate the type of anemia.
MCH—Mean corpuscular hemoglobin measures the amount of hemoglobin in the average RBC—normochromic, hyperchromic, and hypochromic.
MCHC—Mean corpuscular hemoglobin concentration is a measure of chromicity or a percentage of the average RBC volume is Hgb.
Reticulocye count—A count of immature RBC's which is an indication of RBC production.
Platetet count—A count of total platetets.
WBC—A total count of all white blood cells.
Differential—A breakdown in percentages of the types of white blood cells.

Table 1: Normal Ranges for CBC with Differential Values

TEST	MALES	FEMALES
RBC	4.6-6.2 million/µL	4.2-5.4 million/µL
Hgb/Hb	14-18 g/dL	12-16 g/dL
Hct	42-52%	37-47%
MCV	80-94 µm3	87-99µm3
MCH	27-31 pg	Same
MCHC	32-36%	Same
Reticulocyte count	0.5-1.5%	Same
Platelet count	140,000-440,000/uL	Same
Total WBC		
Children	4,500-13,500/µL	Same
Adults	4,500-11,000/µL	Same
Differential Values		
Seg neutrophils	56% of total WBC's	Same
Band neutrophils	3% of total WBC's	Same
Lymphocytes	21-35% of total WBC's	Same
Monocytes	4% of total WBC's	Same
Eosinophils	2.7% of total WBC's	Same
Basophils	0.3% of total WBC's	Same

Source: Alexander 78

Erythrocyte Sedimentation Rate (ESR or sed rate)

Erythrocytes normally settle slowly. If cells become aggregated, they sediment rapidly because of the proportional increase in their total mass exceeds the proportional increase in their volume. This is a nonspecific test that measures the settling of RBC's. In the modified Westergren method anticoagulated venous blood is diluted 4:1 with edetic acid (EDTA) and put in a 200 mm glass tube with a 2.5 mm internal diameter. At one hour, the distance from the meniscus to the top of the column of erythrocytes is recorded as the ESR. The ESR becomes elevated in any inflammatory, infectious, or neoplastic disorder. The ESR is higher in females and increases with age.
(Sox 111-113)

Table 2: The Upper Limit of Normal for ESR in Men and Women

	Upper Limit of Normal
	mm/h
Age < 50 years	
Male	15
Female	20
Age > 50 years	
Male	20
Female	30

Source: Sox 114 (From Bottiger and Svedberg)

Clotting Indices and Platelet Evaluation

There are many tests available to access blood coagulation. These tests are used for four common clinical situations:

1) To screen preoperative patients to reduce the risk of hemorrhage postoperatively.
2) To screen non-surgical patients for coagulation and liver disorders.
3) To evaluate abnormal bleeding.
4) To monitor treatment with anticoagulants.

The following two tests are often used for their accuracy and low cost.
(Sox 156)

Activated Partial Thromboplastin Time (APTT)

This test allows evaluation of clotting factors of the intrinsic pathway (except Factor VII and Factor XIII) of the coagulation system by measuring the time required for formation of a fibrin clot after the addition of calcium and phospholipid emulsion to a plasma sample, which is citrated and platelet-poor. The result is compared to a distribution of normal values. APTT is valuable to screen for preoperative bleeding tendencies and it is the test of choice for monitoring heparin therapy. (Loeb 10)
Procedure: Blood sample drawn.
Normal value: A fibrin clot forms 25-36 seconds after the addition of a reagent.

Prothrombin Time (PT)

Also sometimes called pro time, this test measures the amount of time required for a fibrin clot to form in a citrated plasma sample after addition of calcium ions and tissue thromboplastin. It is then compared to the fibrin clotting time in a control plasma sample. It can detect an isolated deficiency of Factor VII, which APTT can not do. PT measures prothrombin indirectly and is an excellent screening for evaluation of the extrinsic coagulation sequence. This includes Factors V, VII, and X. (Loeb 384)

Procedure: Blood sample drawn.

Normal value: Can vary, but in general--Men: 9.6 to 11.8 seconds
Women: 9.5 to 11.3 seconds

SERUM AND BLOOD CHEMISTRY TESTING

Serum/Blood chemistries provide information concerning numerous chemical constituents in the blood or serum. Often, more than one test will be abnormal in a disease process so more than one test is routinely ordered. Standard batteries/ profiles/ packages are available in the interest of disease diagnosis as well as cost savings to the patient. Individual labs will provide manuals on their particular combinations for the panals. (Marks 12)

BLOOD GLUCOSE

This has been the cornerstone for diagnosis of diabetes as well as monitoring the control of diabetes for years. Listed below are the specific tests utilized for these purposes.

Fasting Blood Glucose/ Fasting Blood Serum (FBS) is currently the standard for the diagnosis of both Type I and Type II diabetes mellitus. It is not that effective for assessing the efficacy of control.

Procedure: Venous draw after a 12- hour overnight fast.

Abnormal: A reading over 140 mg/dL on two separate occasions is diagnostic of diabetes.

2-Hour Postprandial Test is a random sample of glucose.

Procedure: Venous draw or finger prick two hours after eating.

Abnormal: A well-controlled patient with diabetes should never be >200mg/dL.

Oral Glucose Tolerance Test (OGTT) is a test that is crucial for the diagnosis of hypoglycemia.

Procedure: The patient is placed on an unrestricted diet and physical activity for three days prior to testing. They fast for 10 hours overnight. In the morning, they are given a 75-gram glucose load. Blood is drawn on a regular basis and levels accessed.

Normal: Fasting—less than 115 mg/dL
2-Hour—less than 140 mg/dL
Never exceeding 200 mg/dL during the testing

Glycosylated Hemoglobin Level Test (HgbA1c) has become the standard to assess the level of blood glucose control over time. The test is a

measure of the degree of glycemic control over the past 60 days and is expressed as a percentage. It will not allow the opportunity of cheating a couple of days before the visit for an improved fasting glucose level. The glycosylation of the hemoglobin decreases the oxygen-carrying capacity and represents a situation of hypoxia. Increased HgbA1c correlates with increased incidence of severe non-proliferative and proliferative retinopathy. Values over 11% are related to incidences of retinopathy.

Fructosamine Level is an evaluation of blood glucose levels over the past week.

Gestational Oral Glucose Tolerance Test is recommended for all pregnant women between the 24th and the 28th week as a screening for gestational diabetes. This test is different from the OGTT in that after a 10 hour fast, a 100-mg glucose load is given. Two of the below outside of the normal range will be indicative of gestational diabetes.

Abnormal: Fasting—greater than105mg/dL
1-Hour—greater than 190 mg/dL
2-Hour—greater than 165 mg/dL
3-Hour—greater than 145 mg/dL

BLOOD LIPIDS

Lipids are necessary to the body because the serve as a source of energy for metabolism, building blocks in the construction of cell membranes, precursors of steroid hormones and bile acids, and a transport medium for essential vitamins. Altered lipids can increase the risk of coronary heart disease, which is the leading cause of death in the United States. (Alexander 81) A lipid profile is used to diagnose, manage, and treat lipid disorders and their sequelae.

Cholesterol fractionation tests use ultracentrifugation or electrophosesis to isolate and measure the cholesterol in LDL's and HDL's. Ultracentrifugation is the separation and sedimentation of the molecules of a substance by an exceedingly high rate of rotation. Electrophosesis is the separation of ionic solutes based on differences in their rates of migration in an applied electric field. It has been shown that elevated HDL's lower the incidence of CAD but elevated LDL's increase the risk and incidence of CAD. HDL's act to offset the negative effects of the other lipid constituents. A cardiovascular risk ratio may be generated using HDL compared to LDL, which is supposed to be below 3 to 1.

Low Density Lipoproteins (LDL's) LDL's are a class of lipoproteins responsible for the transport of cholesterol to extrahepatic tissue.

Table 3: LDL cholesterol is directly related to risk of coronary heart disease.

Low risk (desirable)	<130 mg/dL
Borderline elevation	130-159 mg/dL
Elevated	160 + mg/dL

Source: Wallach 408

High Density Lipoproteins (HDL's) HDL's are a class of lipoproteins which promote transport of cholesterol from extrahepatic tissue to the liver for excretion into the bile.

Table 4: HDL is inversely related to risk of coronary heart disease.

Low risk (desirable)	>60 mg/dL
Moderate risk	35-60 mg/dL
High risk	<35 mg/dL

Source: Wallach 408

Very Low Density Lipoproteins (VLDL's) VLDL's are a class of lipoproteins that transport triglycerides from the intestine and liver to adipose and muscle tissue. The liver synthesizes them and they contain primarily triglycerides in their lipid cores.

Triglycerides These are neutral fats synthesized from carbohydrates for storage in adipose cells. Upon enzyme hydrolysis, it releases free fatty acids into the blood.

Table 5: Normal Triglyceride Values

Ages 0-29	30-140 mg/dL
Ages 30-39	35-150 mg/dL
Ages 40-49	40-160 mg/dL
Ages 50-59	45-190 mg/dL

Source: Loeb 465

ANGIOTENSION CONVERTING ENZYME (ACE) helps to regulate arterial pressure by converting angiotensin I to angiotensin II, which is a powerful vasoconstrictor. Measurement of serum ACE levels helps to diagnose sarcoid, which is associated with high levels of ACE. This serum test can monitor therapy and is also used to confirm the diagnosis of leprosy. It is assumed that increased serum levels reflect macrophage activity. (Loeb 33)

Procedure: Blood sample drawn. A patient being tested must fast twelve hours before the test. Failure to do this will contaminate the results with significant lipemia.

Normal: > or = to 20 years of age—18-67 U/L

Abnormal: Above 67 U/L with correlation of the patient's clinical condition.

ENDOCRINE TESTING

Endocrine tests are ordered if thyroid disease is suspected. The following tests are often included in the standard profile.

Triiodothyronine (T_3) is a highly specific test that measures total (bound and free) serum content of T_3. T_3 is a potent thyroid hormone and derived mainly from thyroxine (T_4). Like T_4 secretion, T_3 secretion occurs in response to TSH

released by the pituitary and secondarily, to thyrotropin-releasing hormone from the hyopthalmus through a complex negative feedback mechanism. (Loeb 466)
Procedure: Blood sample.
Normal: Serum T_3 levels are usually 150-250 ng/dL.

Triiodothyronine Resin Uptake (T_3RU) is a good ancillary test to assess thyroid dysfunction. It indirectly measures free thyroxine (FT_4) levels by demonstrating the availability of serum protein-binding sites for thyroxine (T_4). In this test, a known amount of radioactive T_3* and a resin are added to a serum sample. The amount of T_3* added exceeds the capacity of thyroxine-binding globulin (TBG) to bind to it. The radioactive hormone combines with unoccupied sites on TBG and any leftover hormone remains free and available for binding to the resin particles. The resin is separated from the serum and the amount of radioactivity left on the TBG or bound to resin is expressed as a percentage of the total amount of T_3* initially added. (Loeb 467)
Procedure: Blood sample drawn.
Normal: 25-35% of T_3* binds to the resin.
Abnormal: A high resin uptake percentage with elevated T_4 levels indicates hyperthyroidism.
A low resin uptake percentage with low T_4 levels indicates hypo.

Serum Thyroxine (T_4). Thyroxine is an amine secreted by the thyroid in response to TSH from the pituitary and indirectly from the hypothalamus. Only a fraction of T_4 (0.3%) circulates freely in the blood. The rest binds strongly to plasma proteins. The above small fraction is responsible for the clinical effects of thyroid hormone on body cells and tissues. This radioimmunoassay measures the total circulating T_4 level. Free T_4 can also be measured. (Loeb 456)
Procedure: Blood sample drawn.
Normal: Total T_4 levels 4-11 µg/dl
Free T_4 levels 0.8-2.8 ng/dl
Abnormal: Increased T_4 indicates primary or secondary hyperthyroidism.
Decreased T_4 indicates primary or secondary hypothyroidism.

Free Thyroxine Index (FTI) measures the minute portions of T_3 and T_4 not bound to thyroxine-binding globulin (TBG) and other serum proteins. As the active components of T_3 and T_4, these unbound hormones enter target cells and are responsible for the thyroid's effects on cellular metabolism. Circulating FT_3 and FT_4 levels are regulated by a feedback mechanism that compensates for adjusting total hormone levels. (Loeb 457)
Procedure: Blood sample drawn.
Normal: FT_3—0.2 to 0.6 ng/dl
FT_4—1 to 3 ng/dl
Abnormal: Increased levels of both indicate hyperthyroidism.
Increased FT_3 and normal or low FT_4 indicate a distinct form of hyperthyroidism—toxicosis.
Decreased FT_4 levels indicate hypothyroidism.

Thyroid Stimulating Hormone (TSH) is a test to access thyroid gland function. TSH stimulates an increase in size, number, and secretory activity of thyroid cells. It also stimulates the release of T_3 and T_4, hormones that exert a generalized effect on total body metabolism and are essential for normal growth and development. (Loeb 453)
Procedure: Blood sample drawn.
Normal: Standard assay: 0-6 μU/ ml
Hypersensitive: 0.25-3.5 μU/ ml
Abnormal: > 10μU/ mL indicates primary hypothyroidism.
Decreased or undetectable levels indicates secondary hypothyroidism or Grave's disease.

Table 6: Indications of Abnormal Thyroid Test Results

Hyperthyroidism	Hypothyroidism
Increased T_3	Decreased T_3
Increased T_3RU	Decreased T_3RU
Increased T_4	Decreased T_4
Increased FTI	Decreased FTI
Depressed TSH	Elevated TSH

SEROLOGICAL TESTING

Serological tests look for the presence of immunologically active proteins—antibodies, immunoglobulins, and antigens.

Microhemagglutination (MHA) tests are used to detect the antibody-antigen reaction by the clumping of sheep erythrocytes. This test is used in the determination of the patient's immunoglobulin G (Ig G) antitreponemal antibody and is useful in the diagnosis of syphilis. This is a treponemal test.

Fluorescent Treponemal Antibody Absorption Test (FTA-ABS) detects the antibodies to *Treponema pallidum*, the spirochete that causes syphilis. In this test, the patient's serum is combined with an absorbed preparation of Reiter treponema and added to prepared *T. pallidum* that's been fixed onto a slide. If syphilitic antibodies exist in the test serum, they will coat the treponemal organisms and, after being stained with fluorescein-labeled antiglobulin, will fluoresce when viewed with a microscope under ultraviolet light. The FTA-ABS test is done to detect primary or secondary syphilis.
Procedure: Blood sample drawn.
Normal: No fluorescence or a negative reaction indicates the absence of treponemal antibodies.
Abnormal: Fluorescence of the treponemal antibodies in the serum suggests syphilis.(Loeb 222)
Venereal Disease Research Laboratory (VDRL) This is used to detect a non-specific rise in patients with high reagin levels in conjunction with acute

inflammation. It is a non-treponemal test used for the screening of syphilis and uses flocculation to demonstrate the presence of reagin, an antibody specific for the spirochete *Treponema pallidum*. Flocculation is a colloid phenomenon in which the disperse phase separates in discrete, usually visible, particles rather than in a continuous mass, as seen in coagulation. (Loeb 505)
Procedure: Blood sample drawn.
Normal: The serum shows no flocculation and is reported as nonreactive.
Abnormal: Definite flocculation represents a reactive test. A reactive test occurs in about half of the patients with primary syphilis and nearly all patients with secondary syphilis.

Rapid Plasma Reagin (RPR) is correlated with VDRL in that it is a screening test for syphilis. These tests are used to cover the possibility of activation when the FTA-ABS or MHA test negative but syphilis is still suspected.

Direct Flourscent Antibody (DFA) detects the presence of an antigen in a patient's blood sample, whereas the indirect fluorescent antibody test detects the presence of antibodies occurring as the result of an antigen. In either case the method of detection depends of fluorescence. The direct test is often used for chlamydia and herpes. (Alexander 83)

Antinuclear Antibody (ANA) This test is a screening for certain collagen vascular diseases, such as systemic lupus erythematosus (SLE), but is not specific enough to be completely diagnostic because of the multiorgan involvement (Alexander 83). It is a useful screening test for SLE but can only partially confirm clinical evidence. Indirect immunofluorescence is used to measure the relative concentration of antinuclear antibodies in a serum sample. Serial dilution of the serum is mixed with rat cell nuclei. If the serum contains ANA, it will form an antigen-antibody complex with the cell nuclei. This preparation is then mixed with fluorescein-labeled antihuman serum and examined under a microscope. If ANA is present, then the nuclei will fluoresce.
Procedure: blood sample drawn. (Loeb 44)
Normal: The ANA is negative at a titer of 1:32 or below.
Abnormal: The higher the titer, the more specific the test is for SLE. The pattern of nuclear fluorescence helps to identify the type of immune disease present.

Rheumatoid Factor (RF) is the most useful immunologic test for confirming rheumatoid arthritis. In this disease, "renegade" IgG or IgM antibodies (which are produced by lymphocytes in the synovial joints), react with IgG to produce immune complexes, complement activation, and tissue destruction. The IgG or IgM molecules that react with altered IgG are called rheumatoid factor. Immune complexes can migrate from the synovial fluid to other areas of the body, causing vasculitis, subcutaneous nodules, or lymphadenopathy. Agglutination and flocculation tests can detect RF. Agglutination is best for diagnosis where flocculation is used best for screening. (Loeb 419)

Procedure: blood sample drawn.
Normal: RF is < 1:20 and nonreactive.
Abnormal: Titers above 1:80 tend to be associated with rheumatoid arthritis. It is difficult to interpret titers between 1:20 and 1:80 because this occurs in many other diseases.

Human Leukocyte Antigen Typing (HLA-B27) The human leukocyte antigen consists of two main classes. Class I antigens exist on the surface of all nucleated cells. Class II antigens exist on B-lymphocytes, macrophages, monocytes, endothelial cells, and on some activated T lymphocytes. The immune system uses these antigens to facilitate interactions between various cells. Matching these antigens determines histocompatibility typing in organ transplantation. Many diseases have a strong association with certain types of HLA, such as HLA-B27 being associated with ankylosing spondylitis.
Procedure: Blood sample drawn. (Loeb 269)
Normal: The results will be expressed to indicate the numerical designation of the antigens found. Each person can have two antigens detected at each loci.
Abnormal: HLA typing may reveal various degrees of matching between a potential donor and recipient. HLA typing can also help to determine paternity.

SKIN TESTING

Kveim-Siltzbach This skin test is used for sarcoid diagnosis. (Panzer 292)
Procedure: an intradermal injection of human sarcoid tissue is given and the injection site is examined in 4-6 weeks for induration.
Abnormal: Induration at site.

Purified Protein Derivative (PPD) This tuberculin skin test looks for a hypersensitivity reaction (wheal) on the forearm skin to PPD of the mycobacterium tuberculosis. This test is often combined with the chest x-ray in the differential diagnosis of granulomatous anterior uveitis. (Marks 14)
Procedure: an intradermal injection is given and 48 hours later the site is inspected for induration.
Abnormal: A positive result (10mm or more) indicates exposure only and must be followed by a chest x-ray to determine if active disease is present.

MISCELLANEOUS TESTING

Enzyme-linked Immunosorbent Assay (ELISA) This test is used to detect the presence of HIV antibody. In this test color change (versus fluorescence, such as in the direct and indirect antibody tests) is used in the detection of an abnormal result. ELISA assists in the differential diagnosis of many of the same conditions as the fluorescent antibody test—Lyme disease, toxoplasmosis, toxocara, and so on. (Alexander 83)
Procedure: Blood sample.
Normal: Serum is negative for HIV antibodies.
Abnormal: Positive results may indicate HIV infection but is confirmed with the Western Blot test.

Cerebrospinal Fluid Testing (CSF) A lumbar puncture allows evaluation of CSF, which look at the color, protein, glucose, cells, and immunoglobulins. It also measures intracranial pressure in mm of H_2O.

Radiological Studies Body tissue causes x-rays to be scattered and absorbed in predictable patterns which are recognizable as human anatomy. Disease states may obscure normally visualized anatomic structures by silhouetting their outline. These are typically ordered for cases which may involve intraocular or orbital foreign body or trauma to the orbit and in sinus disease. It is also indicated for a blowout fracture or when ankylosing spondylitis or Reiter's syndrome are suspected. Chest films are indicated for anterior and posterior uveitis, as well as Horner's syndrome to asses the lung for apical lesions.

Sphygomomanometry This is the method of measuring blood pressure indirectly with a blood pressure cuff and a stethoscope. Primary indications for sphygomomanometry in the optometric office include screening for undiagnosed or uncontrolled hypertension, as well as confirming patient compliance with known hypertension. (Casser 254)

Classification of Hypertension in Adults Aged 18 to 74 Years

Diastolic Blood Pressure in mmHg	**Blood Pressure Category**
< 85	Normal
85-89	High Normal
90-104	Mild Hypertension
105-114	Moderate Hypertension
> or = 115	Severe Hypertension
Systolic Blood Pressure (Diastolic < 90 mmHg)	**Blood Pressure Category**
< 140	Normal
140-159	Borderline Isolated Systolic Htn
> or = 160	Isolated Systolic Hypertension

Source: Walling's Cardiovascular Disease Lecture Notes

Computed Tomography (CT Scan) This is an x-ray procedure that allows for the examination of a single layer or plane of tissue. A computer performs rapid calculations to eliminate the multiple x-rays not absorbed by the plane of tissue in focus. CT may be employed for viewing any part of the body, but is especially useful for visualizing the brain and chest. (Marks 15) The entire process takes approximately ten minutes. It is less expensive than magnetic resonance imaging. It is used to aid in the diagnosis of Grave's disease which will show as an enlargement of the extraocular muscles. It is also used for unilateral disc swelling, orbital masses, and visual field defects.

Magnetic Resonance Imaging (MRI) MRI images soft tissue and therefore is used in the evaluation of small infarcts and neurogenic tumors. This is the test of choice for imaging lesions of the posterior visual system and the brain stem. This test is indicated for intracranial tumors or when suspecting multiple sclerosis as it shows white plaques within gray matter. (Walling/Diagnostic Imaging Methods)

Carotid Auscultation Bruits are rushing sounds heard over medium and large arteries caused by vibrations of the blood vessel walls induced by turbulent blood flow. This blood flow turbulence may be caused by partial lumen occlusion from atherosclerotic plaque formation. Although generally not audible until the vessel is 50% occluded, bruits are detected by ausculating ("listening to") the affected artery with a stethoscope. (Casser 258)

Carotid Duplex Scan This test, also called Doppler ultrasonography, evaluates the blood flow in the neck. It is a noninvasive procedure, which means that nothing enters the body. This test can accurately detect artery and vein disease that reduces blood flow by at least 50%. This test takes about twenty minutes and does not involve any risk or discomfort. Abnormal results include a reduced blood flow velocity signal, indicating arterial narrowing or blockage. At the lesion, the signal is high-pitched and occasionally, turbulent. The doctor uses this test to assess where a blockage is and how badly circulation is compromised. (Cahill 139-40)

Echocardiogram This noninvasive test examines the size, shape, and motion of cardiac structures. A transducer is placed at an acoustic window. An acoustic window is an area free of bone and lung tissue. The transducer picks up echoes and converts them to electrical impulses relaying them to an echocardiography machine for display and recording. Abnormal results can include mitral valve stenosis, mitral valve prolapse, valvar disorders, congestive heart failure, and more. This test is sometimes ordered for certain retinal vascular diseases. (Loeb 186)

This concludes the descriptions of some of the most common laboratory tests an optometric doctor may have need to order or interpret. The following pages will show the appropriate work-ups for a number of ocular conditions.

Once laboratory results are in from the ordered work-up, inform your patients of their results in a manner in which they can easily understand. Make sure that you thoroughly understand the results yourself before attempting to council the patient. Know what you want to say before you say it. Know how to deliver bad news as well as good news. Practice if you have to and be prepared to answer questions. Finally, remember that it is important to document the discussion in the file.

INFLAMMATORY DISEASE

This includes anterior and posterior uveitis, recurrent episcleritis, scleritis, and vitritis. For these clinical signs, you must be suspicious of an underlying cause. The tests listed below will help to determine the cause.

Blood work:

1) CBC with Differential—non-specific.
2) ESR—also non-specific.
3) ANA—to rule out autoimmune or connective tissue disorders.
4) ACE—to rule out sarcoid.
5) RPR—to screen for syphilis.
6) FTA-ABS—to confirm syphilis.
7) RF—to rule out rheumatoid arthritis.
8) HLA typing—to rule out ankylosing spondylitis and Reiter's syndrome.
9) Lyme ELISA/ IFA—to rule out Lyme disease.

Other tests:

1) PPD—to rule out tuberculosis
2) Chest X-ray—to rule out tuberculosis and sarcoid.
3) Sacro-Iliac joint X-ray—to rule out ankylosing spondylitis and Reiter's.

OPTIC DISC DISEASE

This includes disc edema, pallor/ atrophy, shunt vessels, and neovascularization of the disc.

Blood work:

1) CBC with Differential—non-specific.
2) ESR—to rule out Giant Cell Arteritis.
3) RPR—to screen for syphilis.
4) FTA-ABS—to confirm syphilis.
5) Lyme ELISA/ IFA—to rule out Lyme disease.

Other tests:

1) Blood pressure—to rule out malignant hypertension.

2) CSF—to rule out increased intracranial pressure.
3) CT of the head and orbits—to rule out space occupying lesions.
4) MRI of the head and orbits—to rule out space occupying lesions and demyelinating plaques.
5) Chest X-ray—to rule out primary tumors, tuberculosis, and sarcoid.

RETINAL VASCULAR DISEASE

This includes occlusions, emboli, vasculitis, hemorrhages, exudates, cotton wool spots, and neovascularization.

Blood work:

1) CBC with Differential—non-specific.
2) ESR—to rule out Giant Cell Arteritis.
3) RPR—to screen for syphilis.
4) FTA-ABS—to confirm syphilis.
5) ANA—to rule out autoimmune or connective tissue disorders.
6) Clotting times: PT and PTT—to rule out bleeding disorders.
7) Serum protein electrophoresis*
8) Hemoglobin electrophoresis—to rule out sickle cell.*

Other tests:

1) Blood pressure—to rule out hypertension.
2) Chest X-ray—to rule out tuberculosis and sarcoid.
3) Carotid auscultation—to rule out carotid occlusive disease.
4) Carotid duplex scan—to rule out carotid occlusive disease.
5) Echocardiogram—to rule out valvular disease.
6) Holter monitor—to rule out cardiac arrhythmias.*

*These tests are not defined in this paper, but are included in the work-up for completeness.

ORBITAL DISEASE

This includes proptosis, exophthalmos, cellulitis, and pseudotumor.

Blood work:

1) T_3
2) T_3RU
3) T_4
4) FT_4
5) TSH

Other tests:

1) Exophthalmometry.
2) Orbital auscultation.
3) Orbital ultrasound.
4) Forced ductions.
5) CT of the orbits to rule out space occupying lesions.
6) MRI of the orbits to rule out space occupying lesions.

RED EYES

If you suspect a complicated bacterial or fungal conjunctivitis, the list below will assist in isolating the organism. Corneal ulcers are routinely cultured due to the potentially serious visual consequence that may occur.

1) Blood agar.
2) Chocolate agar.
3) Sabouraud's agar.
4) Thioglycolate broth.

As this paper shows, the eye can give us clues to a systemic disease process of the body. We must be aware of the ocular manifestations that call for a more thorough investigation. Laboratory tests are useful in helping to sort out a suspected diagnosis or to evaluate a treatment regimen.

Seek out the laboratory services that are available to you in your community. Evaluate the lab for accuracy, efficiency, and cost. Develop a good working relationship with your lab. Use their services to make your job easier and to enhance the total patient care in your practice.

Patient care is the utmost of importance and we must do our best to serve *all* of our patient's needs. As part of the medical community, it is our responsibility to care for a patient's total health. We must be able to refer appropriately for systemic problems, not just ocular problems. Make an effort to find the best internist, cardiovascular specialist, rheumatologist, neurologist, oncologist, etc. in your area. As primary care doctors, we *will* be making referrals to these doctors and together we will provide the highest standard of care to our patients.

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