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The Crux

BIMONTHLY FORUM FOR THE LABORATORIANS

CONTENTS

Editorial

Eosinophilia is a condition in which the eosinophil count in the peripheral blood exceeds 4.5×10^{8}/L (450/µL). Eosinophils usually account for less than 7% of the circulating leukocytes. A marked increase in non-blood tissue eosinophil count noticed upon histopathologic examination is diagnostic for tissue eosinophilia.[1] Several causes are known, with the most common being some form of allergic reaction or parasitic infection. Diagnosis of eosinophilia is via a complete blood count (CBC), but diagnostic procedures directed at the underlying cause vary depending on the suspected condition(s). An absolute eosinophil count is not generally needed if the CBC shows marked eosinophilia. The location of the causal factor can be used to classify eosinophilia into two general types: extrinsic, in which the factor lies outside of the eosinophil cell lineage; and intrinsic eosinophilia, which denotes etiologies within the eosiniphil cell line. Specific treatments are dictated by the causative condition, though in idiopathic eosinophilia, the disease may be controlled with corticosteroids. Eosinophilia is not a disorder (rather, only a sign) unless it is idiopathic. The **"DISEASE DIAGNOSIS"** segment in this issue delves deep into all aspects of EOSINOPHILIA.

An important pre-analytical issue is **HEMOLYSIS** of blood before it is processed for testing. **"TROUBLE SHOOTING"** highlights all issues as related to this common problem encountered routinely.

Staying on with the liquid connective tissue of the body. Viz., BLOOD, we present to you basic aspects as related to **HEMOGLOBIN ELECTROPHORESIS** under the section – **"INTERPRETATION".**

Have we forgotten **"BOUQUET"**? Just peep inside and flip over !

DISEASE DIAGNOSIS

EOSINOPHILIA

Practice Essentials

In this article, the term eosinophilia is defined as an increase in peripheral blood eosinophilic leukocytes to more than 600 cells per microliter (µL) of blood. Emphasis is placed on the number of eosinophils circulating in the peripheral blood, although an increase in eosinophils can be observed in other body fluids (eg, cerebrospinal fluid [CSF], urine) and many body tissues (eg, skin, lung, heart, liver, intestine, bladder, bone marrow, muscle, nerve). Eosinophils are derived from hematopoietic stem cells initially committed to the myeloid line and then to the basophil-eosinophil granulocyte lineage. Nonpathologic functions of eosinophils and the cationic enzymes of their granules include mediating parasite defense reactions, allergic response, tissue inflammation, and immune modulation. Tissues of the pulmonary and gastrointestinal systems are the normal residence for eosinophils, but peripheral, or blood, eosinophilia (absolute eosinophil count [AEC] >600 cells/µL) indicates an eosinophilic disorder.Untreated, the eosinophilia can be categorized as mild (AEC 600-1500 cells/µL), moderate (AEC 1500-5000 cells/µL) or severe (AEC >5000 cells/µL). An increase in tissue eosinophilia may be seen with or without concurrent peripheral eosinophilia. A secondary or reactive increase in blood eosinophils, tissue eosinophils, or both is associated with a wide variety of conditions, as follows:

- Infections (especially helminthic parasites)
- Allergic responses
- Neoplasms
- Connective tissue disorders
- Medications
- Endocrinopathies

Primary eosinophilia is not a reactive phenomenon and can be described as either clonal or idiopathic in nature. If an underlying molecular or cytogenetic abnormality can be identified, the eosinophilia can be designated as a clonal disorder. If reactive causes are ruled out and no underlying clonal origin is proven, the eosinophilia is described as idiopathic. Given the broad spectrum of conditions linked to eosinophilia, this article emphasizes the diagnostic considerations that clinicians may want to focus on in patients with eosinophilia. The individual disease manifestations and therapies for the dozens of diseases associated with eosinophilia are not described in detail; other Medscape Reference articles specifically address these conditions, such as the following:

- Angiolymphoid Hyperplasia With Eosinophilia
- Dermatologic Manifestations of Eosinophilia-Myalgia Syndrome
- Hypereosinophilic Syndrome
- Loffler Syndrome
- Pulmonary Eosinophilia

Pathophysiology

Over the past 2 decades, substantial progress has been made in understanding the mechanisms of eosinophil production, eosinophil programmed cell death (apoptosis), and how eosinophil immunology contributes to both host defenses against infections and to tissue damage within the host in cases of allergic and autoimmune diseases. The primary stimuli for eosinophil production are interleukin (IL)-5, IL-3, and the granulocyte-macrophage colony-stimulating factor (GM-CSF). These cytokines are also the primary signals that inhibit eosinophil programmed cell death. Thus, eosinophilia can be triggered via these 3 eosinophilopoietic cytokines by increased eosinophil production, by eosinophil longevity, or by a combination of these. In addition, an evolving number of chemotactic cytokines (ie, chemokines) have been established as causing eosinophils to migrate from their site of production in the bone marrow into the blood and then into peripheral tissues. These chemokines include eotaxin-1, eotaxin-2, and RANTES (regulated on activation normal T cell expressed and secreted). Eosinophils are the source of a large number of cytokines, including the following:

- IL-2, IL-3, IL-4, IL-5, IL-7, IL-13, and IL-16
- Tumor necrosis factor–alpha (TNF-alpha),
- Transforming growth factor–beta (TGF-beta)
- RANTES

In addition to these cytokines, eosinophils are a source of several cationic proteins that also contribute to the immunologic responses against infectious disease agents and to tissue damage in allergic and autoimmune diseases. These cationic proteins include the following:

- Eosinophil cationic protein (ECP)
- Eosinophil peroxidase (EPO)
- Charcot-Leyden crystal lysophospholipase
- Major basic protein (MBP)
- Eosinophil-derived neurotoxin (EDN)

Secondary eosinophilia is a reactive phenomenon driven by eosinophilopoietic cytokine release by nonmyeloid cells. Eosinophilic differentiation occurs in the bone marrow from myeloid progenitors through the actions of GM-CSF, IL-3, and IL-5. Mature eosinophils are released into the bloodstream where they migrate quickly to peripheral tissues of the bronchial and gastrointestinal mucosa and skin. Their survival is short, unless apoptosis is blocked by cytokines (GM-CSF, IL-3, and IL-5). Dysregulated production of these cytokines by various cell populations account for secondary hypereosinophilia such as seen in nonmyeloid malignancies (eg, Hodgkin lymphoma; transitional cell carcinoma [TCC] of the bladder; adenocarcinomas of the stomach, colon, and uterus; large cell undifferentiated lung carcinomas; and large cell cervical tumors), allergic reactions, parasitic infections, and other conditions. Primary eosinophilias include both clonal and idiopathic hypereosinophilic syndrome (HES). These disorders have very heterogeneous underlying pathophysiologies, not all of which are well-defined. They are by definition eosinophilia for longer than 6 months, without evidence of reactive cause and with signs and symptoms of organ involvement. In some neoplastic disorders, the hypereosinophilia is part of neoplastic clonal expansion affecting the myeloid lineage. This pathophysiology would describe the eosinophilia in the following disorders:

- Chronic myelogenous leukemia (CML), Philadelphia chromosome positive (Ph+) or *BCR-ABL* positive
- Acute myelogenous leukemia (AML), including inv(16), t(16;66)(p13;q22)
- Myeloproliferative diseases
- Myelodysplastic syndromes

A number of hypereosinophilic syndrome (HES) cases exhibit clonal expansion of abnormal lymphocytes. Immunophenotypically, they are characterized by aberrant and immature T cells, which exhibit abnormal cytokine production. T-cell receptor gene rearrangements are demonstrated in many. These T cells produce high levels of IL-5, thought to cause the hypereosinophilia. Eosinophilia is further classified as clonal or idiopathic, both clinically and pathologically. The World Health

Organization (WHO) proposed criteria to distinguish idiopathic hypereosinophilic syndrome (HES) from chronic eosinophil leukemia (CEL) with predominant eosinophilic differentiation. The diagnosis of CEL is made if (1) cytogenetic or molecular evidence of clonality is present, (2) an increase in peripheral blasts of more than 2% or marrow blasts of more than 5% but less than 19% occurs, and (3) other causes are excluded. The underlying chromosomal abnormalities leading to CEL have been described in some cases. A deletion on chromosome band 4q12 resulting in the *FIP1L1-PDGFRA* (*FIR1*- like-1–platelet-derived growth factor receptor–alpha) fusion gene causes an abnormal constitutively activated tyrosine kinase. These patients demonstrate *CHIC2* gene deletion in peripheral blood mononuclear cells as a result of this fusion gene. Another fusion gene involving *BCR-PDGFRA* has been seen in CML with marked eosinophilia. Mutations involving *PDGFRB* rearrangements have been described, as well as *FGFR1* (fibroblast growth factor receptor–1) fusions.Clinical features of eosinophil leukemia result from accumulation of leukemic cells in bone marrow, liver, and spleen.Inflammatory mediators from the eosinophils themselves cause tissue damage to the pericardium, myocardium, endocardium, and nervous system. In 38 patients with chronic eosinophilia studied by array comparative genomic hybridization (aCGH), Arefi et al found that aCGH revealed clonality in eosinophils in most patients with myeloproliferative neoplasias. These authors suggested that aCGH could be a useful technique for defining clonality in these diseases. Finally, idiopathic hypereosinophilic syndrome (HES) is the diagnosis of exclusion in patients with marked prolonged (>6 mo) eosinophilia with multiple organ involvement but without identifiable cytogenetic or molecular abnormalities. Organ damage occurs from release of the contents of eosinophilic granules. Some of these cases transform into identifiable entities.

Epidemiology

Frequency

International: Helminthic infections are the most common cause of eosinophilia worldwide due to the high prevalence of helminthic parasite infections, several of which are estimated to involve hundreds of millions of people.

Mortality/Morbidity

Patient mortality and morbidity depend on the individual disease associated with eosinophilia. Many helminthic infections develop into chronic diseases that cause morbidity but not mortality. Similarly, allergic reactions and conditions associated with eosinophilia usually do not cause mortality. Eosinophilia associated with nonmyeloid malignancies does not affect their individual prognosis or rates of mortality. The mortality and morbidity associated with clonal and idiopathic causes is associated with the degree of tissue involvement, damage, or both at diagnosis; how quickly therapy is implemented; and treatment responsiveness.

Race-, Sex-, and Age-related Demographics

No racial predilection exists for eosinophilia, although the occurrence of eosinophilia-associated helminthic parasitic infections is more common in certain geographic areas of the world. No male or female predilection exists in most subtypes of eosinophilia. However, there is a marked male predominance in clonal disorders involving the *PDGFRB* fusion gene and a small male predominance in clonal disorders of the *FGFR1* gene. People of all ages can be affected by eosinophilia.

History

- Obtaining a travel history is critical to assess whether a patient with eosinophilia has traveled to an area that is endemic for certain infections, including helminthic infections and coccidioidomycosis, which is the only fungal infection that is frequently associated with eosinophilia and is endemic in the southwestern United States and northern Mexico. In certain Gulf countries Schistosomiasiscan be the cause.
- Obtaining a medication and diet history is crucial to evaluate for allergic reactions associated with eosinophilia. Particularly the temporal relationship of medication changes to the onset of eosinophilia should be assessed. History of discontinued medications should also be obtained, as eosinophilia can persist long after cessation.
- Obtaining a history of symptoms associated with lymphoma, especially Hodgkin lymphoma, is important.
- A history that is suggestive of adrenal insufficiency, including the use and tapering of corticosteroid medications, can provide a clue that the observed eosinophilia is associated with adrenal insufficiency. Hypoadrenalism (ie, Addison disease) is the most common endocrine abnormality associated with eosinophilia.
- Symptom evaluation for respiratory symptoms, as well as cardiovascular symptoms including exertional dyspnea, fatigue, fever, muscle pain, rash, visual changes, and weakness, may indicate specific organ involvement.

CLINICAL PRESENTATION

Physical

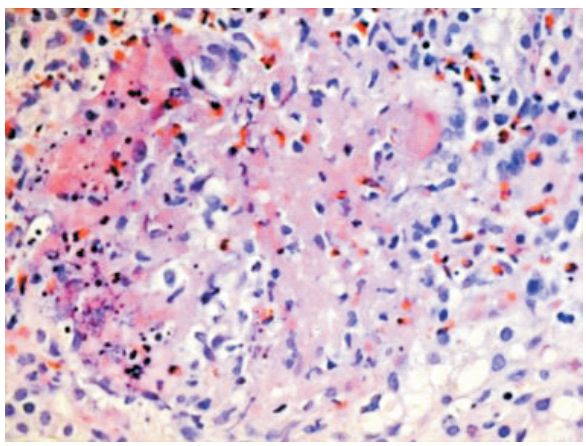
- A complete physical examination is required in patients, because diseases associated with eosinophilia can involve any part of the body, including the skin, brain, eyes, lymph nodes, lungs, heart, liver, spleen, intestine, bone, and nervous system.
- Cholesterol emboli due to atherosclerotic disease, with or without recent vascular catheterization, can present as eosinophilia and end-organ damage to the kidneys, skin, and lower extremities (causing blue/purple toes).

Causes

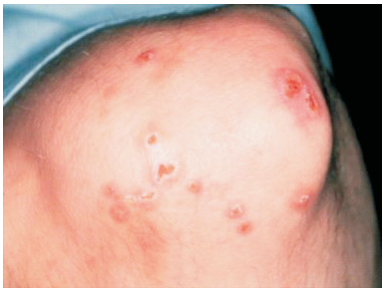
The mnemonic device CHINA (ie, connective tissue diseases, helminthic infections, idiopathic hypereosinophilic syndrome [HES], neoplasia, allergies) describes the categories of diseases that sometimes are associated with blood eosinophilia.

Connective tissue diseases include the following:

Churg-Strauss vasculitis

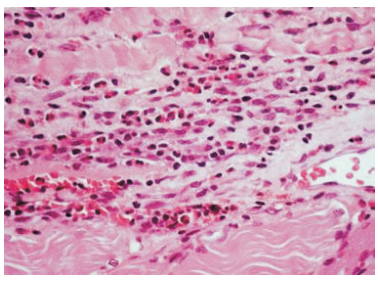


Granuloma with a central core of eosinophilic debris surrounded by a peripheral palisade of epithelioid histiocytes and eosinophils from a patient with Churg-Strauss syndrome (allergic granulomatosis).

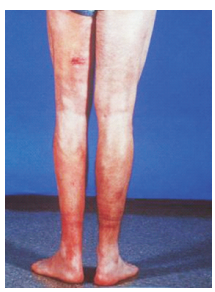


Magnified view of papules and nodules with central necrosis in a patient with Churg-Strauss syndrome (allergic granulomatosis).

- Rheumatoid arthritis
- Eosinophilic fasciitis



High-power photomicrograph of fascia shows heavy inflammatory infiltration with numerous eosinophils, lymphocytes, and occasional plasma cells in a patient with eosinophilic fasciitis.



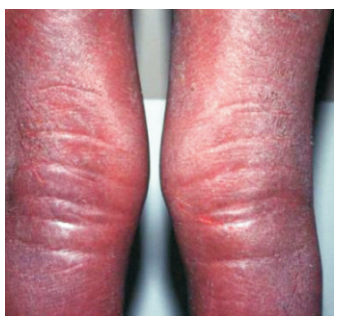
Lower back part of the legs in a patient with eosinophilic fasciitis shows hypopigmentation, induration, biopsy site, and asymmetric involvement.

- Eosinophilia-myalgia syndrome (due to tryptophan in the United States in 1989)
- Toxic-oil syndrome (due to contaminated rapeseed oil in Spain in 1981)
- Coccidioidomycosis

Helminthic (ie, worm) parasitic infections include the following:

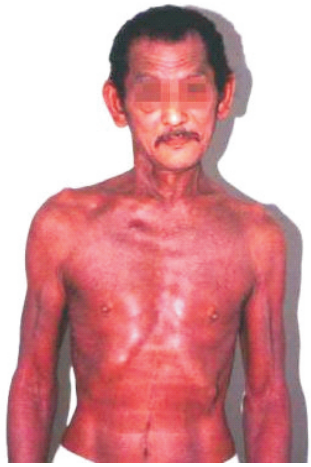
- Ascariasis
- Schistosomiasis
- Trichinosis
- Visceral larva migrans
- Gnathostomiasis
- Strongyloidiasis
- Fascioliasis
- Paragonimiasis

Idiopathic HES as shown in the images below:



Indurated edematous plaques of hypereosinophilic syndrome on a patient's legs.

Erythroderma in a patient with hypereosinophilic syndrome.



Neoplasias include the following:

- Lymphoma (eg, Hodgkin lymphoma, non-Hodgkin lymphoma)
- Human T-cell lymphotropic virus I (HTLV-I) infection
- Adult T-cell leukemia/lymphoma (ATLL)
- Eosinophilic leukemia (very rare)
- Gastric or lung carcinoma (ie, paraneoplastic eosinophilia)

Allergic/atopic diseases include the following:

- Asthma
- Allergic rhinitis

DIFFERENTIAL DIAGNOSIS

Diagnostic Considerations

The differential diagnosis of eosinophilia includes the following:

- Medication reactions
- Parasitic infestation
- Asthma/allergy
- Adrenal insufficiency due to critical illness
- Multiorgan autoimmune/idiopathic diseases (eg, eosinophilic granulomatosis with polyangiitis)
- Myeloid neoplasms with eosinophilia
- Secondary eosinophilia due to lymphoid or epithelial neoplasms
- Congenital syndromes (ie, hypereosinophilic syndrome, Omenn syndrome, familial eosinophilia)

WORKUP

Laboratory Studies

Laboratory studies begin with a complete blood cell (CBC) count with differential, to quantitate the percentage eosinophils and absolute number of eosinophils (AEC). Blood chemistries can indicate specific organ involvement (ie, liver, kidney). Spinal fluid examination can assess cerebrospinal fluid (CSF) eosinophilia due to the following:

- Worm infections (eg, *Angiostrongylus cantonensis*)
- Drug reactions (eg, phenytoin)
- Coccidioidomycosis fungal meningitis

Patients with allergic symptoms should have a nasal smear for eosinophilia and Gram stain. Patients with asthma symptoms should have sputum examination for eosinophilia. In suspected cases of medication and some parasitic infections, evaluation of urine sediment may be helpful. Stool samples should be evaluated for ova and parasites if indicated by history. If reactive causes are unlikely, a bone marrow

biopsy should be done. Clues of clonality in peripheral blood include macrocytosis, thrombocytosis, left-shifted granulopoiesis and circulating blasts. In the bone marrow, myeloproliferation with dyshematopoiesis and reticulin fibrosis are suggestive of clonality. Staining for tryptase and immunophenotyping should be done. If primary eosinophilia is suspected, screening of peripheral blood with fluorescent in situ hybridization (FISH) or reverse transcriptase–polymerase chain reaction (RT-PCR) is peformed to detect fusion genes. FISH for the *CHIC2* gene deletion can indicate the presence of the *FIP1L1-PDGFRA* gene fusion, which places the disorder in the World Health Organization category of 'myeloid and lymphoid neoplasms with eosinophilia and abnormalities of *PDGFRA, PDGFRB*, or *FGFR1*; these conditions are exquisitely responsive to imatinib. T-cell receptor gene rearrangement can be evaluated by flow cytometry. Measurement for elevated serum levels of tryptase (seen in systemic mastocytosis [SM] and *FIP1L1-PDGFRA*-positive disease), interleukin-5 (common in clonal T-cell disorders), and IgE can also be peformed.

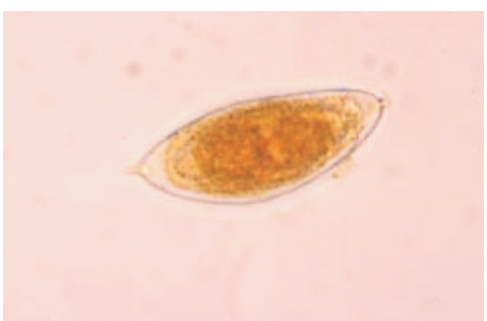
Imaging Studies

Computed tomography (CT) scanning may be used as follows:

- CT scans of the lungs, abdomen, pelvis, and brain evaluate for focal defects due to diverse causes of eosinophilia
- Worm infections of the liver (eg, *Fasciola hepatica*) can cause focal hepatic lesions
- Coccidioidomycosis can cause focal lesions in the lung, which are visible on a chest radiograph or CT scan
- Hodgkin or non-Hodgkin lymphoma can cause adenopathy in the abdomen, which is visible on a CT scan
- Echocardiography can be used to assess for thrombi (eg, mural, endocardial) due to hypereosinophilic syndrome (HES).

Procedures

- A bone marrow biopsy may be helpful (see Laboratory Studies).
- A lumbar puncture may be performed to evaluate spinal fluid for CSF eosinophilia. CSF eosinophilia may be due to worm infections (eg, *A ngiostrongylus cantonensis*), drug reactions, or coccidioidomycosis fungal meningitis.
- *Schistosoma hematobium* typically causes eosinophilia and hematuria due to infection of the bladder. All patients with blood eosinophilia who have lived or traveled in Africa and have either gross or microscopic hematuria should have their urine examined for the eggs of *S hematobium*. Cystoscopy can used for definitive diagnosis but is usually unnecessary to make the diagnosis, because the terminal-spined eggs of this species of schistosome can often be found in the urine if specifically sought. See the image below.



Egg of Schistosoma hematobium, with its typical terminal spine.

THERAPEUTICS

Medical Care

A detailed discussion of therapeutics for the many individual causes of eosinophilia, including parasitic and malignancy-associated forms, is beyond the scope of this article. General guidelines only are addressed here. Most cases of secondary eosinophilia are treated on the basis of their underlying causes. Allergic and connective tissue disorders may be amenable to corticosteroid treatment. Parasitic and fungal infections can be worsened or disseminated by use of steroids and should be ruled out if they are indicated by patient history. In patients with primary eosinophilia without organ involvement, no treatment may be necessary. Cardiac function should be evaluated at regular intervals, however, as peripheral eosinophilia does not necessarily correlate with organ involvement. Steroid responsiveness should be evaluated, both for prognosis (steroid-responsive patients do better) and to guide treatment when needed. Choices for systemic treatment of primary eosinophilia with organ involvement initially include corticosteroids, and interferon (IFN)-alpha for steroid-resistant disease. Other agents for steroid-resistant disease, which are usually given as long-term maintenance regimens to control organ involvement, include the following:

- Hydroxyurea
- Chlorambucil
- Vincristine
- Cytarabine
- 2-Chlorodeoxyadenosine (2-CdA)
- Etoposide
- Cyclosporine

In the presence of *PDGFRA* and *PDGFRB* mutations, imatinib has achieved complete and durable remissions and has become established as definitive first-line therapy. However, relapse may occur after discontinuation of imatinib. Hypereosinophilic syndrome (HES) patients with unknown or negative PDGFRA have a low response rate to imatinib.However, treatment with antibodies and antibody-based agents (eg, mepolizumab, alemtuzumab, brentuximab vedotin) directed against targets expressed on the surface of eosinophils has proved effective in some patients with HES. In refractory cases, many investigational combinations of chemotherapeutic agents, tyrosine kinase inhibitors (eg, imatinib), and monoclonal antibodies are being studied. Nonmyeloablative allogenic hematopoietic stem cell transplantation (HSCT) can also be considered in drug-refractory cases.

PROGNOSIS

- Patient prognosis depends on the associated condition. Many helminthic infections develop into chronic diseases that cause morbidity but not mortality. Similarly, many allergic reactions and conditions associated with eosinophilia usually do not cause mortality.
- The prognosis of primary eosinophilias is determined by their degree of organ involvement at diagnosis, the timeliness of treatment, responsiveness to treatment, and underlying cytogenetic and molecular pathophysiology.

INTERPRETATION

Hemoglobin Electrophoresis

Background

Hemoglobin electrophoresis is used as a screening test to evaluate for and identify variant and abnormal hemoglobins. Alkaline and/or citrate agar electrophoresis is the commonly used method. Separation of hemoglobins is based on variable rates of migration of charged hemoglobin molecules in an electrical field. Hemoglobin is a tetramer with two pairs of globin chains, each containing an identical heme group. Normal adult hemoglobin (HbA) has two α- and two β-globin chains (α_2 β_2). Fetal hemoglobin (HbF) has two α- and two γ-globin chains (α_2 γ_2). Minor adult hemoglobin (HbA_2) is made of two α- and two δ-globin chains (α_2 δ_2). Hemoglobin electrophoresis has been superseded by more rapid, sensitive, and quantitative methods of hemoglobin separation. Cellulose acetate (CA) electrophoresis at pH 8.2-8.6 can be used to resolve common variants such as HbS and HbC, but it cannot be used to distinguish between HbS and HbDand HbG.Citrate agar electrophoresis at pH 6.0-6.2 provides better resolution for different hemoglobin variants. Murine monoclonal antibodies against human normal and variant hemoglobins may be used for identification and/or quantification of hemoglobins, such as HbF measurement with radial immunodiffusion or HbF-containing erythrocytes (F-cells) with flow cytometry. Mass spectrometry, a newer analytical technology, may be used to identify highly unstable hemoglobins that may manifest clinically as hemolytic anemia or thalassemia.Small amounts of these variant hemoglobins might not be detected by diagnostic techniques used in most clinical laboratories. Additionally, mass spectrometry may provide information on posttranslational modifications, such as oxidation and glycation. Capillary electrophoresis (CE) is comparable to CA electrophoresis for reliable measurement of Hb fractions. It is suitable for screening of hemoglobinopathies in many clinical laboratories.

Indications/Applications

Indications and applications of hemoglobin electrophoresis include the following:

- Evaluation of unexplained hemolytic anemia
- Microcytic anemia unrelated to iron deficiency, chronic disease, or lead toxicity
- A peripheral smear with abnormal red cell features (eg, target cells or sickle cells)
- Positive family history of hemoglobinopathy
- Positive neonatal screen results
- Positive results on sickle cell or solubility test

Considerations

Evaluation of a suspected hemoglobinopathy should include electrophoresis of a hemolysate to detect abnormal hemoglobins and quantification of HbA_2 and HbF with column chromatography. If HbS is detected, a solubility test should be performed. Interpretation of hemoglobin electrophoresis results should be placed in the clinical context, including the family history and results of serum iron studies, red cell morphology, hemoglobin, hematocrit, and red cell indices (eg, mean corpuscular volume). Molecular testing aids in genetic counseling of patients with thalassemia and combined hemoglobinopathies. Automated high-pressure liquid chromatography instruments are proving to be useful alternative methods for hemoglobinopathy screening.

Reference Range

Hemoglobin electrophoresis is used as a screening test to identify variant and abnormal hemoglobins, including hemoglobin A_1 (HbA_1), hemoglobin A_2 (HbA_2), hemoglobin F (HbF; fetal hemoglobin), hemoglobin C (HbC), and hemoglobin S (HbS).

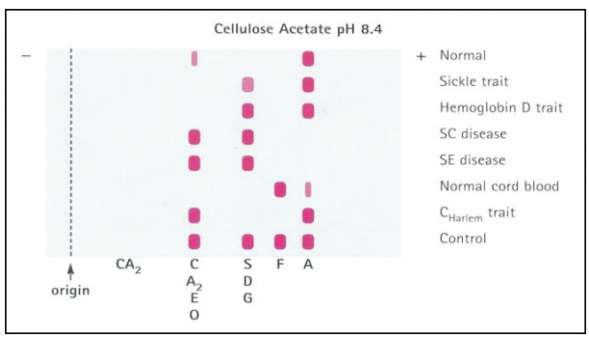
The reference ranges are as follows:

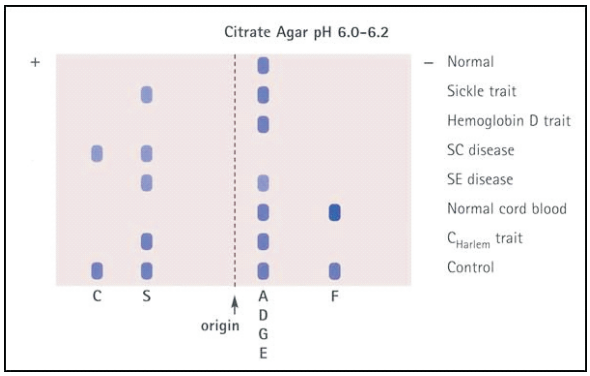
- HbA_1: 95%-98%
- HbA_2: 1.5%-3.5%
- HbF: < 2% (age-dependent)
- HbC: Absent
- HbS: Absent

Interpretation

Results indicate the following:

- Presence of HbS, but with a higher proportion of HbA than HbS: Sickle cell trait (HbAS) or sickle α-thalassemia
  Presence of HbS and HbF, but no HbA: Sickle cell anemia (HbSS), sickle beta-thalassemia (hereditary persistence of fetal hemoglobin [HPFH]), or sickle–HPFH
- Overall higher proportion of HbS than HbA and HbF: Sickle beta -thalassemia (most likely)
- Presence of HbC, but with a higher proportion of HbA than HbC: HbC trait (HbAC)
- Presence of HbC and HbF, but no HbA: HbC disease (HbCC), HbC –beta -thalassemia (HbC-HPFH)
- A higher proportion of HbC than HbA: HbC beta -thalassemia
- Presence of HbS and HbC: HbSC disease
- Presence of HbH: HbH disease
- Increased HbA_2: Beta-thalassemia minor
- Increased HbF: Hereditary persistence of fetal hemoglobin, sickle cell anemia, beta-thalassemia, HbC disease, HbE disease





Collection and Panels

Specimen: Blood
Container: Lavender-, green-, or blue-top vacuum tube
All samples must be sent in a sealed, leak-proof container marked with a biohazard sticker to comply with Occupational Safety and Health Administration (OSHA) safety standards.

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BOUQUET

Brain Teasers

1. **Which of the following is not a postanalytical error?**
   A. Bubbles in wells
   B. Calculation mistakes
   C. Choosing a wrong graph scale
   D. Comparison of result with inappropriate reference interval.

2. **Which of the following can induce matrix effects?**
   A. Complement
   B. Albumin
   C. Rheumatoid factors
   D. All of the above.

3. **"Chemiluminescence" is defined as the production of electromagnetic radiation (as a result of a chemical reaction).**
   A. Ultraviolet
   B. Visible
   C. Near-infrared
   D. Any of the above.

4. **Of the following which substance is usually used for signal and light generation in chemiluminescence?**
   A. Luminol
   B. 1, 2 Dioxetanes
   C. Acridinium ester
   D. Ruthenium salts.

ANSWER: 1. A, 2. D, 3. D, 4. A

BOUQUET

In Lighter Vein

Vodka + Water = Injures Kidney

Rum + Water = Injures Liver

Whiskey + Water = Injures Heart

Gin + Water = Injures Brain .

I think there is something wrong in water!!

What's winning attitude?

3 ants saw an elephant coming.

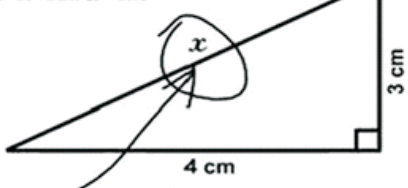
Ant1: We will kill him

Ant2: We will break his legs.

Ant3: Forgive him guys, he is alone and we are 3

Maths question for engineers

3. Find x.

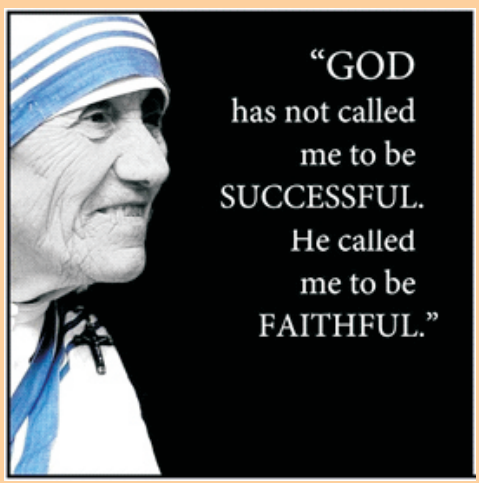


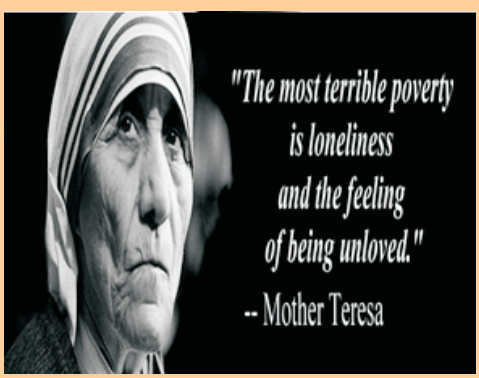
Here it is

Question By A STUDENT !!

If A Single Teacher Can't Teach Us All The Subjects, Then.. How Could You Expect A Single Student To Learn All Subjects??

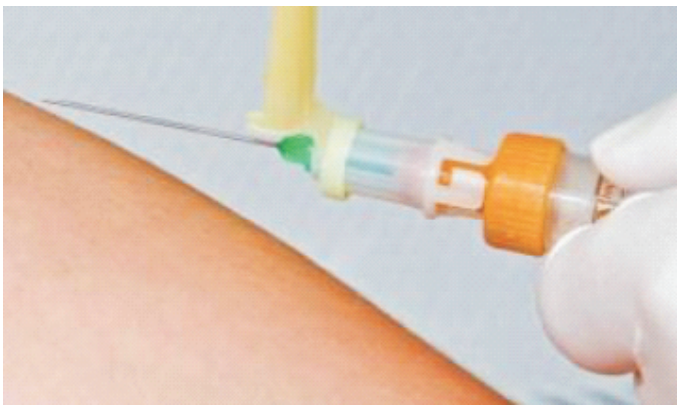
Wisdom Whispers





TROUBLESHOOTING

Haemolysis: a major challenge in the lab



Haemolysis is defined as the release of haemoglobin and other intracellular components from erythrocytes, thrombocytes and leukocytes into the extracellular fluid i.e. the plasma or serum, following damage or disruption of cell membranes. Haemolysed specimen is the most common reason for rejection of specimens in the lab. Studies have shown that out of the total specimens received in the lab, 3.3% are found to be haemolysed, and out of the total rejected; 40-70% has been identified to be due to haemolysis. The American Society of Clinical Pathology has established a 2%, or lower, benchmark for haemolysis rates amongst laboratory blood samples. Haemolysis may occur either *in vivo* or *in vitro*, and is a most undesirable condition that influences the accuracy and reliability of laboratory testing. It has been observed that 3.2% of all detectable haemolysis is *in vivo*. *In vivo* haemolysis can originate from hereditary, acquired and iatrogenic conditions, such as autoimmune haemolytic anaemia, severe infections, intravascular disseminated coagulation, haemoglobinopathies, drug transfusion reactions etc. *In vivo* haemolysis does not depend on the techniques of the healthcare provider and is virtually unavoidable and cannot be resolved. *In vitro* haemolysis occurs more often and is caused by improper sample drawing, improper sample processing and improper sample transport (*see figure 1*).

Figure 1. In Vitro Hemolysis

IMPROPER SAMPLE DRAWING
Improper selection of the venepuncture site
Prolonged tourniquet time
Continuous clenching of the fist
Improper drying of the alcohol at venepuncture site
Needle guage
Tube under filling
Syringe Transfer
Catheter IV collection
Traumatic draw
Capillary collection

SPECIMEN PROCESSING
Excessive mixing or vigorous shaking of the tube
Not allowing clotting for sufficient time
Applicator sticks
Time delay before centrifugation
Centrifuge conditions, Speed, time and temperature

TRANSPORT
Mechanical trauma drug transport
Temperature humidity at the time of transport
Length, speed and number of times the sample is transported.

IMPROPER SAMPLE DRAWING

One of the most important reasons for haemolysis is improper selection of the venipuncture site. Drawing blood from distal arm rather than anticubital fossa can result in haemolysis. A study done by Edward et al clearly shows that the anticubital fossa may be more favourable than the distal arm because of the faster flow of blood due increased diameter of the vein and reduced resistance. Difficulty to locate easy venous access, small or fragile veins (alternate sites to the anticubital area, such as small hand veins, are fragile and easily traumatised) unsatisfactory attempts, and missing veins can all lead to haemolysis. An improper venipuncture is indicated by a slow blood flow due to the lumen of the needle not centred in the vein, meaning the bevel opening of the needle is partly in the vein and partly in the vein wall, leading to an increasing aspiration force and rupture of the RBC. The Clinical and Laboratory Standards Institute (CLSI) recommends the use of a tourniquet for localising suitable veins for $\leqslant$ 60 sec. Prolonged tourniquet time, or continuous clenching the fist, results in rupture of the RBC. Venous stasis greater than one minute affects potassium, calcium and albumin by 2.8%, 1.6% and 3.5% respectively, and venous stasis greater than three minutes affects potassium, calcium and albumin by 4.8%, 3.6% and 8.6% respectively. Cleaning the vein puncture site with alcohol and not allowing the site to air dry results in transfer of alcohol from the skin to the blood specimen resulting in haemolysis. The choice of needle gauge size is very important. The use of large bore needles may result in a much faster and more forceful flow of blood through the needles resulting in haemolysis. Using a smaller gauge needle results in a large vacuum force applied to the blood and may cause sheer stress on the RBC, causing them to rupture. The ideal gauge of needle for venipuncture is usually 20-22. Haemolysis from excessive aspiration force is relatively frequent, mainly in cases of small or superficial veins. Pulling the syringe plunger back too fast or forcefully can result in haemolysis. Forcefully expelling the blood from the syringe into the blood tubes can also rupture the RBC. A study done by Edward et al shows that under-filling tubes with blood will cause improper blood-to-additive ratios (especially EDTA), resulting in haemolysis. Haemolysis can be avoided by filling the tubes to the required level. When blood is drawn from a peripheral IV catheter, a higher incidence of haemolysis occurs due to frothing of the blood from a loose connection of the blood collection assemblies.

SPECIMEN PROCESSING

Excessive mixing or shaking of the blood after collection, not allowing to clot for recommended amount of time (30 mins), use of applicator sticks to dislodge the fibrin, prolonged contact of serum or plasma with cells, failure to separate serum from red cells within 60 minutes of venipuncture, exposure to excessive heat or cold, can all cause RBC rupture and haemolysis. Centrifugation at too high a speed frequently compromises the integrity of the blood samples. Re-centrifugation (re-spin) of tubes with gel separators (the gel barrier may open and allow any supernatant that has been in contact with erythrocytes, to mix the supernatant previously above the separator).

TRANSPORTATION

Mechanical trauma during transport may occur, resulting in haemolysis. Variable factors such as temperature, humidity, length, speed, and number of times the specimen is transported, as well as the number of angles or turns the system uses also affects the integrity of the sample. Placing ice or frozen gel packs directly on tubes of blood can result in haemolysis.

HAEMOLYSIS INDEX/SERUM INDEX

The index aids in evaluating sample integrity by determining the level of haemoglobin in serum or plasma. It improves the quality of reported results, in almost no time, with minimal cost and also improved handling of paediatric samples.

The extent of haemolysis can be detected in two ways:

1. Visual haemolysis detection
2. Automated Serum Index.

Visual haemolysis detection Visually, haemolysis is defined as free haemoglobin concentration >30-50mg/dL conferring detectable pink/red hue to serum or plasma. It becomes clearly visible in specimens containing as low as 0.5% lysed erythrocytes. It is based on comparing patient samples with photographs of samples containing various concentrations of haemoglobin. Drawbacks of the visualising method are that it is unreliable since it may over and underestimate the actual prevalence of haemolysed serum specimens (i.e., trained observers are unable to accurately rank the degree of interference in serum). Elevated concentration of bilirubin may further impair the ability to detect haemolysis by visual inspection and therefore lead to serious underestimation of haemolysis in neonatal samples, where elevated bilirubin concentration is commonplace.

Automated Serum Index This is by directly measuring haemoglobin concentration photometrically followed by comparing it with the serum index values for haemolysis that have been determined by the instrument vendor. (These values represent the levels at which the haemoglobin significantly interferes with the analyte testing.) The advantage of an Automated Serum Index is its consistency, reproducibility and improvement in detection of haemolysis.

Notable examples of tests affected by hemolysis are found in the table below.

Fig. 2 : Effect of Hemolysis on some Clinical Biochemistry and Hematology Test Results

Degree of change in analyte	Test result increased by hemolysis	Test result decreased by hemolysis	Test result increased or decreased by hemolysis
Slight change	Phosphate, Total Protein, Albumin, Magnesium, Calcium, Alkaline Phosphatase	Haptoglobin, Bilirubin	
Noticeable change	Alanine Amino Transferase, Creatinine Kinase, Iron, Coagulation tests		
Significant change	Potassium, Lactate Dehydrogenase, Aspartate Amino Transferase	Troponin T	Hemoglobin, Red Blood Cells, MCHC, Platelet Count

Note: If the specimen is grossly hemolyzed, a recollected specimen will be requested. If the recollected specimen is also grossly hemolyzed, it will be processed and a comment added.

INFLUENCE OF HAEMOLYSIS ON ROUTINE CLINICAL TESTING

- Leakage of haemoglobin and other intracellular components into the surrounding fluid may induces false elevation of some analytes or dilution effect. If the analyte in question is present in a higher concentration in blood cells than in plasma, then the analytical value will be increased. Conversely, if the concentration in blood cells is lower, the plasma becomes diluted, and the analytical result is too low. Caraway reported that erythrocytes contain 160-fold as much lactate dehydrogenase, 68-fold as much acid phosphatase, 40-fold as much aspartate aminotransferase, and 6.7-fold as much alanine aminotransferase as does plasma
- Haemoglobin absorbs light strongly at 415, 540 and 570nm. Haemolysis therefore increases absorption in this wavelength range and causes apparent increase in the concentration of analytes measured in this range
- In addition to haemoglobin, erythrocytes also contain proteins, enzymes, lipids and carbohydrates and many of these may also interact or compete with the assay reagents. E.g. free haemoglobin with its pseudo-peroxidase activity interferes in the bilirubin procedure by inhibiting the diazonium colour formation and thereby resulting in low values. Increased concentration of CK is most likely due to analytical interference, due to release of intracellular adenylate kinase, which is not completely inhibited under operating conditions
- Release of intracellular and thromboplastic substances from either leukocytes or platelets, results in prolongations in prothrombin time and dimerised plasmin fragments D(D-dimer) whereas shortening of activated partial thromboplastin time and decrease in fibrinogen values.

OVERCOMING THE CHALLENGE OF HAEMOLYSIS

There should be proper laboratory guidelines and recommendations for the management of haemolysed samples. Proper training and knowledge of the factors that can influence laboratory results, along with appropriate training of the phlebotomists, are essential prerequisites to minimise errors. Every laboratory personnel should be trained properly. Standardised blood collection and handling procedures should be followed. Collection from a haematoma site and prolonged tourniquet time should be avoided. Equipment and connections that may lead to turbulent blood flow leading to haemolysis should be avoided. Vigorous mixing of the specimens after collection should be prevented and appropriate conditions of temperature and humidity should be maintained. Standardised practices for sample transportation and storage should be observed. The blood specimens should be centrifuged within a suitable time of collection, with appropriate conditions of centrifugation (force, spin, time and temperature), supernatant (serum or plasma) timely separated from the blood cells unless the primary tube is provided with a gel separator. There has always been a debate as to whether we should or should not process the haemolysed samples. Basically when a haemolysed sample reaches the laboratory, we can; **1.** Reject the sample for analysis and ask for re-collection **2.** Perform the analysis and report the results with a comment **3.** When a haemolysed sample is referred to the laboratory, the personnel should always ask for new sample(s). In case new sample(s) cannot be obtained, it is the responsibility of the laboratory specialist to communicate the problem to the concerned physician and seek for the best solution for the best of the patient care. It is pointed out by the authors of the recommendations that it is always better not to report the result rather than producing spurious data on unsuitable samples.

New

CounCell-23 Plus V2

Fully Automatic Hematology Analyzer

Coral Clinical Systems

TECHNICAL SPECIFICATIONS

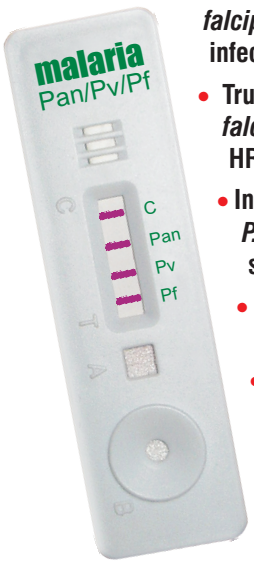
Principle:	Electrical impedance method (WBC/RBC/PLT) and cyanide free (HGB) method.
Reagent Used:	Diluent & Lyse.
Reportable Parameters:	3 Part Differential, 21 Parameters - WBC, Lym%, Mid#, Gran#, Lym%, Mid%, Gran%, RBC, HGB, HCT, MCV, MCH, MCHC, RDW-CV, RDW-SD, PLT, PDW, MPV, PCT,P-LCC , P-LCR, 3 histograms for RBC, WBC and PLT, alarm for abnormal erythrocytes, leukocytes and platelet.
Throughput:	Up to 60 samples per hour
Sample Volume:	Minimum volume 9 µl.
Power Requirement:	100 V - 240V (A.C), 50/60 Hz , ≤ 300 VA
Calibration:	Three types of calibration Manual calibration, Auto calibration with calibrators and auto calibration using fresh sample.
Quality Control:	2 QC Method; L-J and X-B with appropriate QC charts
Data Storage Capacity:	Internal memory can store 50,000 Test values with parameters, Histograms and patient information.
Communication:	Communication: Four USB ports and Bi-directional LIS Connection.
Operating Environment:	Temperature :15°C - 30°C, Relative Humidity : 30% - 85%
Dimension & Weight :	410 mm (L) x 353 mm (W) x 471mm (H) Weight: 25 kg

Let The Counting Begin...

paramax-3

RDT for detection of *P. falciparum*, *P. vivax* & other malarial species.

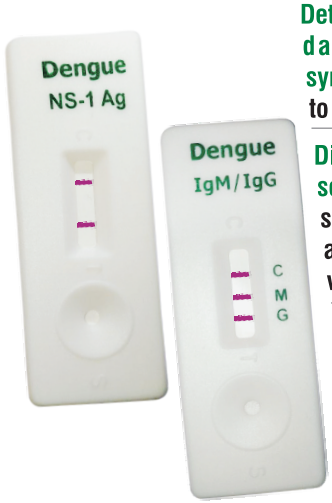
Zephyr Biomedicals



- Detection and differentiation of *P. falciparum*, *P. vivax* , *P. ovale* & *P. malariae* infection.
- True speciation of the "Big Two" i.e. *P. falciparum* & *P. vivax* through specific Pf HRP 2 & Pv - pLDH Bands.
- Indirect speciation between *P. v.* and *P.o.*, *P.m.* infection through Pv pLDH and Pan specific pLDH band.
- Detection of mixed infection of *P. falciparum* & *P. vivax.*
- Sensitivity and specificity in excess of 98% for *P. falciparum* & *P. vivax* and other malaria species.
- Useful for monitoring successful therapy through vivax specific and Pan specific pLDH bands.

Dengucheck™ Combo

Rapid test system for the detection of Dengue NS-1 antigen and IgG/IgM antibodies to Dengue virus in human serum/plasma.



Detects dengue infection on the first day of appearance of clinical symptoms – Test system designed to detect dengue NS 1 antigen.

Differentiates between primary & secondary infection – Combined test system for detection of NS1 antigen and IgG/IgM antibodies to dengue virus.

Choice of testing either NS1 antigen or antibodies to dengue – Separate test pouches for NS 1 antigen & IgG/IgM test.

Reliable performance – Excellent correlation with standard NS1 detection and dengue antibody detection test.

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