Hematology Guidelines

Laboratory Quality Assurance Program
3475 Albert Street
Regina, Saskatchewan S4S 6X6

phone: (306) 787-8239
fax: (306) 787-7240

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Hematology

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Smudge Cells

Distinguished by their naked amorphous nuclear chromatin material, smudge cells were initially described as white blood cells with broken-down nuclei in patients with chronic lymphocytic leukemia. Subsequently, these nuclear shadows have most often been referred to as smudge cells, but the term basket cells is used synonymously.

The mechanism is often associated primarily with traumatic disruption of cells during blood film preparation. In the process, the cell membrane ruptures and when viewed under a microscope, what remains looks like a smudge, hence the term, smudge cells.

To ensure reliability of results, it is important to understand the effects of variables associated with smudge cell formation, particularly the blood film preparation. Thus, the angle and the degree of incline of the slide spreader, the type of slide spreader (sharp or smooth), the cleanliness of the slides, and the overall quality of the blood films cannot be overemphasized. For minimal morphologic alterations, blood films should be made within three hours and not more than twelve hours after collection.

It is recommended to include smudge cells in the differential as an absolute count, especially when the smudge cell numbers are noticeably increased. This identifies a more appropriate count because smudge cells are actually lymphocyte artifacts. It also avoids the need for repeating or verifying abnormal counts by the time - consuming albumin – treated method.

Education is needed (for the ordering physicians especially) to eliminate the risk of misinterpreting this smudge cell count as a new cell type.

Criteria for Reporting Smudge Cells

Absolute lymphocyte count should be greater than 5.0 x10^9/L.

Patient age should be more than 30 years*.

Smudge cells should be reported if greater than 10 per 100 leukocytes. Report smudge cells in absolute numbers.

* Although CLL is not often diagnosed in patients under the age of 40, patients over 30 years of age should be considered potentially at risk. CLL is rare in patients under 30 years of age.

3.2% Na Citrate anticoagulant recommended versus 3.8% for Coagulation studies

The International Standards Committee on Thrombosis and Hemostasis, and the NCCLS have recommended guidelines to standardize whole blood collection to 3.2% sodium citrate anticoagulant.

Several publications have indicated that the clotting times for PT and APTT are significantly shortened with the 3.2% NaCitrate. For this reason, exchange between the two concentrations is not acceptable. When selecting an anticoagulant for collection, it is important to note that 3.2% NaCitrate is used for ISI assignments for thromboplastin. For these reasons, it is important for laboratories to standardize the choice of anticoagulant for sample collection.

The anticoagulant used for coagulation assays should be 3.2% trisodium citrate. The proportion of blood to anticoagulant volume is 9:1. Inadequate filling of the collection device will decrease this ratio, and may lead to inaccurate results. The manufacturer recommendations should always be followed.

If the hematocrit is very high and the usual relative volumes of blood and citrate solution are mixed, a prolonged prothrombin time results from overcitration of the reduced proportion of plasma in the blood sample. This means the final citrate concentration in the blood should be adjusted in patients who have hematocrit (PCV) values above 0.55 (55%). Adjust the volume of NaCitrate in the draw tube by applying the calculation outlined in the NCCLS H21-A3. *Note: To maintain the vacuum in the NaCitrate collection tube, use a tuberculin syringe to draw out the anticoagulant.*

The anticoagulant volume may be calculated from the expression:

$$X = \frac{(100 - PCV)}{(595 - PCV)} \times \text{draw volume of tube (mLs)}$$

X = the volume of anticoagulant required to prepare volume of anticoagulated blood
PCV = packed cell volume (Hct) in %

For example: To determine the volume required for 5 mLs anticoagulated blood, calculate x 5 mLs

References:
H21-A3 NCCLS, BD Vacutainer Systems, Azko Nobel

Assessment of the influence of citrate concentration on the international Normalized Ratio (INR) determined with twelve reagent-instrument combinations. Chantarangkul, Tripodi, Clerici, Negri, Mannucci

Effect of concentration of trisodium Citrate Anticoagulant on Calculation of the international Normalised Ratio and the International Sensitivity Index of Thromboplastin, Duncan, Casey, Duncan, and Lloyd

The Prothrombin Time Test: Effect of Varying Citrate Concentration, Ingram, Hills.

Comparison of 3.2% vs. 3.8% Sodium Citrate Anticoagulant Collection Tubes for Coumadin, Heparin, Abnormal and Normal Specimens. Phillips, Sunnybrook ON

K_2EDTA recommended versus K_3EDTA for Hematology studies

In the clinical laboratory, plastic tubes containing dipotassium ethylene diamine tetraacetic acid (K_2EDTA) have emerged as an alternative to glass tubes containing tripotassium ethylene diamine tetraacetic acid (K_3EDTA).

The International Council for Standardization in Hematology and the NCCLS have recommended K_2EDTA as the anticoagulant of choice for blood cell counting and sizing for the following reasons:

1. *K_3EDTA causes greater RBC shrinkage with higher EDTA concentrations.*
2. *K_3EDTA produces a larger increase in RBC volume on standing of the specimen.*
3. *K_3EDTA leads to lower MCV values.*
4. *K_3EDTA is a liquid and therefore results in dilution of the specimen. All directly measured values (Hgb, RBC, WBC, and platelet counts) have been reported to be 1-2% lower than obtained with K_2EDTA.*
5. *With some instrument systems, K_3EDTA produces lower WBC when used in high concentrations (short vacutainer draws).*

Regardless of the form or type of EDTA salt used, **all tubes should be inverted 8 to 10 times to ensure thorough mixing and therefore, proper anticoagulation.**

The calibration of automated blood cell counters will depend on the anticoagulant used. This makes it essential to use the same anticoagulant for harmonization between counters.

Effective October 15, 2002 BD Vacutainer Systems has eliminated the use of K_3EDTA blood collection tubes. This vacutainer has been replaced by a plastic tube containing dried K_2EDTA anticoagulant.

References:

BD Preanalytical Solutions, NCCLS H1-A4

Comparing Hematology anticoagulants: K_2EDTA vs. K_3EDTA, Brunson, Smith, Bak, Przyk, Sheridan, Muncer

Recommendations of the International Council for Standardization in Haematology for Ethylenediaminetetraacetic Acid Anticoagulation for Blood for Blood Cell Counting and Sizing, International Council for Standardization in Haematology: Expert panel on Cytometry

$K_{2\ or}$ K_3EDTA: the anticoagulant of choice in routine haematology? Goossens, Van Duppen, Verwilghen

Performance of K_2EDTA vs. K_3EDTA collected blood specimens on various hematology analyzers, Phillips, Coiner, Smith, Becker, Leong

Vacutainer Brand PLUS Tubes with K_2EDTA: Comparison to Glass K_3EDTA Tubes for Blood Counts on the Coulter MAXM

MORPHOLOGY OF LYMPHOCYTES

BENIGN versus MALIGNANT

Reference: McTaggart Bill, SAIT Hematology Updating Correspondence Course, 5th Edition, 1993. pp. 10.

A variety of diseases and disorders may produce changes from the normal in numbers and/or morphology and functions of one or more of the leukocytes. The most important feature of variant lymphocyte morphology is the recognition of its benign nature. The pertinent fact is that these lymphocytes are normal cells that have been altered as the result of a normal response to stimulus. When changes in WBC's are produced by non-malignant disorders (e.g. infections), the cells are called atypical, a synonym for reactive or variant lymphocytes. When changes are produced by malignant disorders (leukemias, lymphomas, gammopathies) the cells are called abnormal.

References: Stiene-Martin, 1998, pp. 355-356, 484-485, 490, 507-508.
College of American Pathologists, Surveys, Hematology Glossary, 2001

In non-malignant disorders, the variant lymphocytes, reactive lymphocytes, atypical lymphocytes, virocytes, stress lymphocytes, Downey cells, transformed lymphocytes, transitional lymphocytes, and glandular fever cells, among others, are normal cells reacting to a stimulus, whether it be viral or other. The designation of reactive lymphocytes, atypical lymphocytes, variant lymphocytes are some proposed terms.

In Chronic Lymphocytic Leukemia, the lymphocytes are somewhat larger than normal, have nuclei with clumped or condensed chromatin, and may have prominent nucleoli. The cytoplasm may be abundant, nongranular and moderately basophilic, or it may be relatively scant.

In Prolymphocytic Leukemia, the prolymphocyte is a relatively large mononuclear lymphoid cell with an oval to round nucleus, coarse-appearing chromatin strands and one or two large vesicular nucleoli with perinuclear condensations of chromatin. The cytoplasm is abundant and usually granular and is basophilic with Romanowsky stains.

In Waldenström's Macroglobulinemia, the abnormal B-lymphocytes involved are transitional cells. They have the ability to differentiate into large plasmacytoid lymphocytes and plasma cells. These malignant cells circulate in the peripheral blood only in the terminal stages.

In Lymphomas, peripheral blood involvement (i.e., abnormal circulating cells) is seen late in the disease. Lymphoma cells can exhibit a variety of appearances and the cellular morphology is variable and depends on the underlying type of lymphoma. These cells can exhibit variable size, shape, nuclear, and cytoplasmic characteristics. Lymphoma cells are usually round to oval, and can be irregular. Cell size ranges from 8 to 30 µm and the N-C ratio varies from 7:1 to 3:1. In diffuse small lymphocytic lymphoma (the tissue equivalent of chronic lymphocytic leukemia), the cells are generally small with round to oval nuclei, compact and coarse chromatin, and have a

scant amount of basophilic cytoplasm. They may be the same size as normal lymphocytes or may be slightly larger. Occasionally, the nuclei exhibit an angulated appearance with slightly more open chromatin. A small nuclear indentation may be present. Nucleoli are not seen. Scattered prolymphocytes, which are larger cells with a centrally placed nucleus, a prominent single nucleolus, and moderate basophilic cytoplasm, often are seen. In the small-cleaved cell lymphomas, the cells are slightly larger than normal lymphocytes and have an angulated appearance. The majority of nuclei have clefts, indentations, folds, convolutions, and may even be lobulated. The chromatin is moderately coarse and one or more nucleoli may be prominent. Their cytoplasm is scant to moderate and basophilic. The cells in small noncleaved lymphomas (Burkitt's lymphoma) appear similar to L3 lymphoblasts. These cells are generally moderate in size (10 to 25 µm) and have a round to oval nucleus with moderately coarse chromatin, and one or more prominent nucleoli. The cytoplasm is moderate, stains dark blue, and may contain numerous small vacuoles. Large cell lymphomas and immunoblastic lymphomas may exhibit some of the most blast-like and abnormal morphology. These cells are large (20 to 30 µm) and have scant to moderate amounts of deeply basophilic cytoplasm. The nuclei are generally round to oval, but may be angulated, folded, indented, or convoluted. Nucleoli are prominent and may be single or multiple. Vacuoles can occasionally be seen in the cytoplasm. These cells can be easily confused with blasts. T cell lymphomas can exhibit similar morphology to any of the above types of lymphomas. The typical appearance is a moderate-size cell with a markedly convoluted nucleus giving a cerebriform or grooved pattern. Their chromatin is moderately coarse and nucleoli are not apparent. The cytoplasm is generally scant and blue.

In Hairy Cell Leukemia, the abnormal lymphocytes (Hairy Cells) have scant to abundant, agranular, light grayish-blue cytoplasm. The plasma membrane appears irregular with hair-like or ruffled projections, which are seen more easily with phase microscopy. These cells often have a round or oval nucleus; sometimes, the nucleus appears folded or bilobed. The chromatin is loose and lacy, and one or two nucleoli are commonly seen.

In Sézary Syndrome, the abnormal lymphocyte is larger than normal with scanty cytoplasm, and the nucleus is large with clefting. Nuclear folding can be so extensive as to suggest an image of the brain, and these nuclei are thus described as cerebriform. The nuclear chromatin is fine with little condensation. There may or may not be visible nucleoli.

Red Blood Cell Morphology Reporting Guideline

Red Blood Cells/100x oif	Abnormality to be Reported	Implications for Diagnosis
(200 RBC field) x 10 fields Any Present	Schistocytes / Helmet Cells	Thrombotic thrombocytopenia purpura (TTP), RBC fragmentation syndromes such as hemolytic uremic syndrome, DIC, microangiopathic, hemolysis, malignant hypertension, eclampsia, Cardiac valve hemolysis, Some renal vascular diseases
	Echinocytes / Burr Cells	Kidney Disease
	Bite Cells	Drug or chemical induced oxidative damage, Unstable hemoglobins
	Sickle Cells	Sickle Cell Anemia, Hemoglobin SC/SD Disease
	Basophilic Stippling	Lead Poisoning, Thalassemia, Sideroblastic & Megaloblastic Anemia, Sickle Cell Anemia
	Howell Jolly	Megaloblastic anemia, Post-splenectomy state
	Dimorphic	Hemorrhage, Response to treatment, Myelodysplastic, Post-Transfusion
	RBC Rouleaux (5 cells stacked)	Paraproteinemia, Increased Fibrinogen, Inflammatory Disorders
	RBC Aggutination	Autoimmune Hemolytic Anemia (Cold)
	Parasites	Identify specific forms
	Nucleated RBC	Severe hemolysis, Part of Leukoerythroblastic picture, Bone marrow stress
>5	Polychromasia	Response to treatment, Blood loss, Hemolysis
	Oval Macrocytes	Megaloblastic state, Aplastic Anemia, Myelodysplastic Syndrome
	Target Cells	Liver Disease, Post-splenectomy/hyposplenism, Hemoglobinopathy, Thalassemia
	Tear Drops	Myelofibrosis, Pernicious Anemia
	Spherocytes	Hereditary Spherocytosis, Auto Immune Hemolytic Anemia
	Pappenheimer Bodies	Sideroblastic Anemia, Chronic hemolysis, Liver Disease
>10	Round Macrocytes	Liver Disease, Alcoholism
	Elliptocytes	Hereditary elliptocytosis
	Acanthocytes / Spur Cells	Post-splenectomy state, Liver Disease, Abetalipoproteinemia
	Stomatocytes	Liver Disease
	Hypochromic Cells	Iron Deficiency, Thalassemias, Treated Polycythemia
Avoid using the terms anisocytosis and/or poikilocytosis since they convey no specific meaning.		

The numeric value is meant for internal use to indicate a significant abnormality presence. No numeric value is reported, just the abnormality.

Blood Film	Reference Range	Perform a Differential or Scan on first occurance or significant change	Referral
WBC Count	4.0 - 11.0 x 10^9/L		
Lower referral range		<1.5 x 10^9/L	<1.0 x 10^9/L
Upper referral range		>20.0 x 10^9/L	>20.0 x 10^9/L
Absolute Neutrophils	1.5 - 7.5 x 10^9/L	<1.5 x 10^9/L	<1.0 x 10^9/L
Absolute Granulocytes	1.5 - 7.5 x 10^9/L	<1.5 x 10^9/L	<1.0 x 10^9/L
Absolute Eosinophils	0.0 - 0.6 x 10^9/L	>1.0 x 10^9/L	>2.0 x 10^9/L
Absolute Basophils	0.0 - 0.2 x 10^9/L	>0.3 x 10^9/L	>0.5 x 10^9/L
Absolute Lymphs	1.1 - 4.4 x 10^9/L		
adults		>5.0 x 10^9/L	>7.0 x 10^9/L
children (0-14 years)		>7.0 x 10^9/L	>10.0 x 10^9/L
Absolute Monocytes	0.2 - 0.8 x 10^9/L	>1.0 x 10^9/L	>1.5 x 10^9/L
Hemoglobin			
Lower referral range			
adult female	115 - 160 g/L	<100 g/L	<100 g/L
adult male	135 - 180 g/L	<120 g/L	<120 g/L
Upper referral range			
adult female	115 - 160 g/L	>160 g/L	>160 g/L
adult male	135 - 180 g/L	>180 g/L	>180 g/L
Peadiatric ranges			
Neonatal ICU	135 - 195 g/L	>210 g/L	>210 g/L
newborn (0-1 month)	135 - 195 g/L	<160 g/L	<135 g/L
children (1month-14 years)	105 - 145 g/L	<100 g/L	<100 g/L
HCT	0.37 - 0.50 L/L	none	0.65 L/L
MCV			
Lower referral range			
0 - 3 months	98 - 114 fL	< 97 fL	< 90 fL
> 3 months	87 - 103 fL	< 80 fL	< 80 fL
Upper referral range			
0 - 3 months	98 - 114 fL	>115 fL	none
> 3 months	87 - 103 fL	>100 fL	>110 fL
MCH	27 - 32 pg	none	none

Blood Film	Reference Range	Differential/Scan	Referral
MCHC **Upper referral range**	310 - 360 g/L	 > 360 g/L	 > 365 g/L
RDW	11.5 - 14.5 %	none	none
RBC Count adult female adult men	 3.2 - 5.4 x 10^{12}/L 4.6 - 6.2 x 10^{12}/L	>6.5 x 10^{12}/L	>6.5 x 10^{12}/L
MPV **Lower referral range** **Upper referral range**	7.4 - 10.4 fL	none	 < 6.0 fL > 14.0 fL
Platelet Count **Lower referral range** **Upper referral range**	150 - 400 x 10^{9}/L	 <100 x 10^{9}/L >600 x 10^{9}/L	 <100 x 10^{9}/L >600 x 10^{9}/L
WBC Morphology			> 10 % Atypical lymphs Pelger-Huet anomaly Hypogranulated neutrophils Hairy Cells Blasts / Immature cells
Nucleated RBCs			> 5 NRBC / 100 WBC
RBC Morphology			RBC inclusions -Pappenheimer, Howell-Jolly or Heinz Body Presence of schistocytes, echinocytes, bite cells, sickle cells, basophilic stippling, marked rouleaux, autoagglutination, significant polychromasia, oval or round macrocytes, target cells, tear drops, spherocytes, elliptocytes, acanthocytes, stomatocytes, hypochromic cells Dimorphic picture Parasites - Malaria
Platelet Morphology			none
Other Criteria Specified Instrument Flags Ordered by Physician Technologist discretion		 When indicated Physician request Technologist initiated	 Physician request Technologist initiated - if suspicious cells are present refer to a pathologist.

VITAMIN B_{12} & FOLATE

Background: The normal proliferation of cells depends on adequate folate and vitamin B_{12}. Folate is necessary for efficient thymidylate synthesis and production of DNA. B_{12} is needed to successfully incorporate circulating folic acid into developing RBCs retaining folate in the RBC.

✷Measuring both B_{12} and folate levels is not necessary in all patients.✷

Serum B_{12}: The clinical indications for ordering serum B_{12} include:
1) Evaluation of patients with **MACROCYTIC** (high MCV in the CBC) anemia and the clinical information suggesting possible B_{12} deficiency;
2) Evaluation of patients with psychiatric and neurologic impairment (symptoms of subacute combined degeneration of spinal cord).

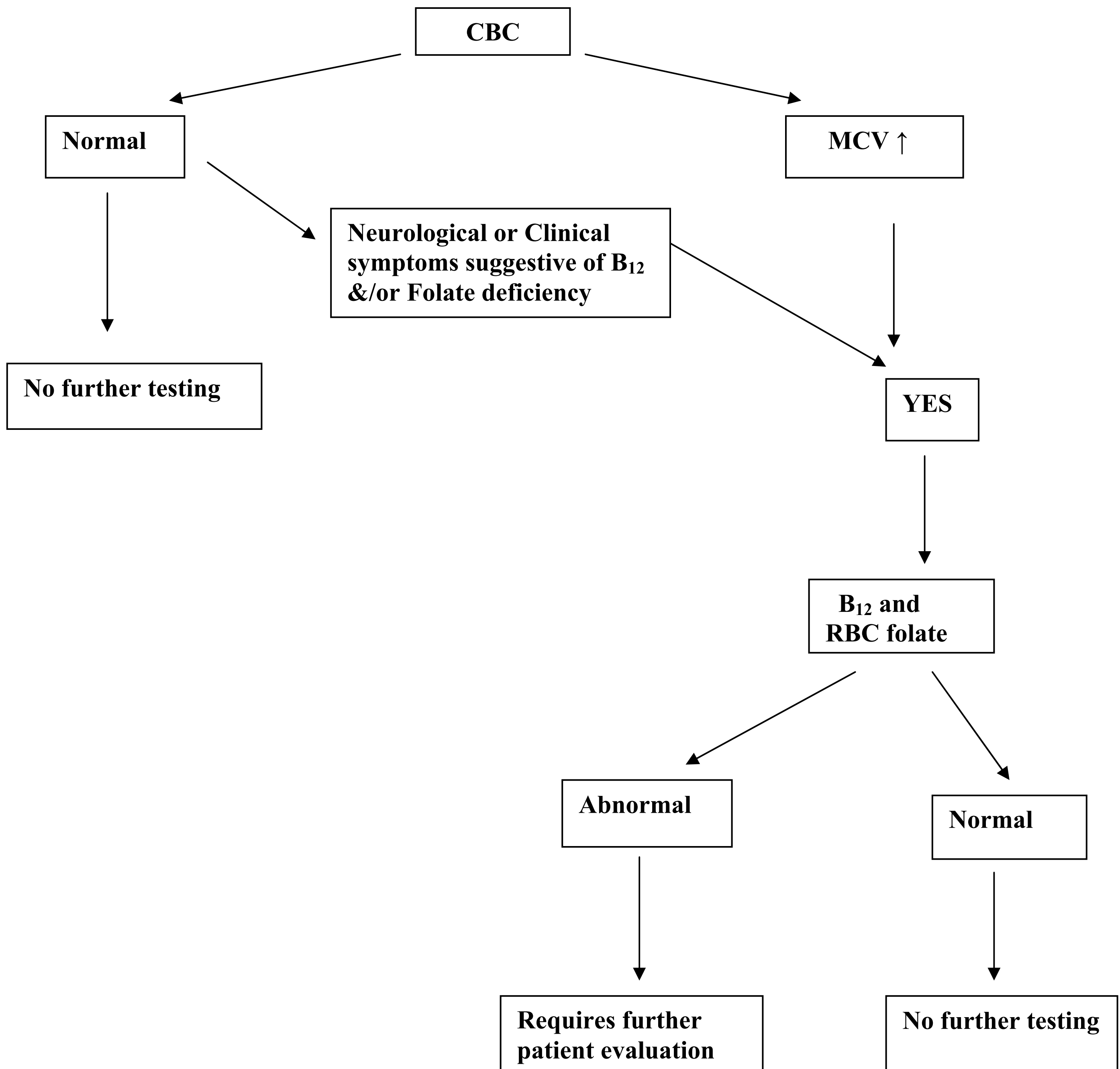
Red Cell Folate: Red cell folate is ordered as it is an indication of the folate status over a longer period of time (several months) as opposed to serum folate that reflects levels over the last few days. Population studies have shown that dietary supplements have increased average folate levels and therefore folate deficiency is much rarer. The clinical indications for ordering red cell folate include the evaluation of **MACROCYTIC** (high MCV in the CBC) anemia and the clinical information suggesting possible folate deficiency.

Practice Tips:
1. Persons on B_{12}/folate supplements or other multivitamins do not require testing.
2. Lab tests are used to confirm a specific diagnosis and not as a fishing expedition.
3. Marginal B_{12} deficiency in any elderly patient with dementia, peripheral neurological symptoms or impaired immunity should be taken seriously.
4. B_{12} should not be done on patients on oral contraceptives.

Prepared by Dr. A. Saxena,
Department of Laboratory Medicine,
Saskatoon Health Region

Current Indications for B_{12} and Folate Investigation

Measuring both B_{12} and Folate levels is not necessary in all patients.



Notes:

**B_{12} tests should not be done if the person is on B_{12}*
**False low B_{12} in pregnant women and women on oral contraceptives*
Serum folates are not a useful screening tool
Folic Acid Deficiency is rare due to folate fortification in food
RBC folate is more indicative of tissue folate levels
Folate tests will not be done if the person is on folate

D-dimer

D-dimer is a test that is ordered, along with other laboratory tests and imaging scans to help rule out, diagnose and monitor diseases and conditions that cause hypercoagulability, a tendency to clot inappropriately.

The D-dimer assay is the evaluation of fibrinolytic activity, which measures a specific fragment arising from fibrin degradation problems. Because it measures fibrinolysis the presence of D-dimer is specific evidence of a physiologic response to intravascular fibrin formation.

Elevated D-dimer is found in Deep Vein Thrombosis (DVT), Pulmonary Embolism (PE), Disseminated Intravascular Coagulation (DIC) and Pre-eclampsia. D-dimer is also increased after surgery or major trauma, in inflammatory arthritis, cancer, and infection in advancing age.

The diagnostic performance of the D-dimer is dependent on the method used. Different assays have different cut off points and therefore the diagnosis should always be included with the request.

There are basically two methods to assay D-dimer: Enzyme-immunoassay (EIA) and latex bead assay. The EIA is useful when levels of D-dimer are low and a very sensitive assay is required. It does, however, require a special technique and is expensive and time-consuming. The latex bead assays that start at >500ng/ml are for DIC only and are not sensitive enough to exclude Venous Thromboembolism (VTE) indications.

Sensitive, quantitative EIA D-dimer assays that give precise results below 500ng/ml may be used to exclude venous thromboembolism in suitably selected ambulatory populations. High probability patients need definitive testing which include Diagnostic Imaging. D-dimers should NOT be used in that setting. Even if the result is negative, there is still a high enough possibility of VTE that further testing is required. In low and moderate probability patients, a negative EIA D-dimer result is sufficient to rule out VTE, and no further testing is required.

Following are the Guidelines for The Diagnosis and Treatment of Pulmonary Embolism as provided in a recent newsletter for physicians.