PROFICIENCY TESTING SERVICE
AMERICAN ASSOCIATION OF BIOANALYSTS
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PARTICIPANT STATISTICS | **CELL IDENTIFICATION** | **FIRST QUADRIMESTER 2019**

Q1-2019 Pernicious Anemia

A 63 year-old man with a 20-year history of non-insulin dependent diabetes, presents with recent 25 pound weight loss. He complains of a sore tongue and paresthesis. His lab values are as follows: WBC-3.8, RBC-2.59, Hgb-10.4, Hct-31.4, MCV-121, MCH- 37.8, platelet count -138,000, RDW-19.5.

Review of the CBC results indicates that the patient has macrocytic anemic. Additional laboratory testing determined that he suffered from pernicious anemia.
Although the term "pernicious anemia" is both outdated and descriptively vague, it is still used to identify the megaloblastic anemia that develops as a results of vitamin B12 deficiency due to lack of production of intrinsic factor (IF) in the stomach.

Human beings cannot manufacturer vitamin B12. Vitamin B12 is created by special microorganisms and is found almost exclusively in animal products – such as fish, meat, dairy and eggs. Plant-based produce, which are the sources for most of the other vitamins we need, contain almost no B12 – whether fruit, vegetables, nuts or seeds. Only fermented plant foods, such as sauerkraut and beer, as well as algae, called chlorella, contain small amounts of the vitamin.

Vitamin B12, also called cobalamin, is an essential water-soluble vitamin. It is the largest and most chemically complex of all the vitamins. Vitamin B12 is a cofactor in DNA synthesis, and in both fatty acid and amino acid metabolism. It is particularly important in the normal functioning of the nervous system via its role in the synthesis of myelin and in the maturation of developing red blood cells in the bone marrow.

Vitamin B12 absorption is a complex, multistep process. Once food containing vitamin B12 is ingested, pepsin and acid pH in the stomach will degrade these food proteins and release vitamin B12. Vitamin B12 is structurally very sensitive to the hydrochloric acid found in the stomach secretions and, without some kind of protection, would denature in that environment before it had a chance to be absorbed by the small intestine. Therefore, when initially released from the protein binding in food, vitamin B12 then binds to a different, carrier protein, R protein, which protects the vitamin B12 as it moves through the stomach and into the duodenum. On entering the duodenum B12 releases from the R protein by action of the pancreatic enzymes. The released B12 then binds to intrinsic factor (IF). IF is secreted by the parietal cells of the stomach lining. This B12-IF complex then travels through the small bowel until it reaches the ileum, the terminal tertiary portion of the small intestine. The ileum is the longest of all portions of the small intestine, and has on its surface specialized receptors, that identify the B12-IF complexes. Via endocytosis mediated absorption, the B12-IF complexes are and taken into the circulation. After absorption, Vitamin B12 then is released from the B12- IF complex and enters the portal vein where it next attaches, in different proportions, to one of three proteins, transcobalamin I, II or III. The main transport protein of vitamin B12, transcobalamin II, moves a portion of the B12 to the liver for storage and the remainder to the bone marrow and other tissues for DNA synthesis.

Most omnivorous people in developed countries obtain enough vitamin B12 from consuming animal products including meat, dairy, eggs, and fish. The average, adult daily diet contains 5-30 ug of vitamin B12. The recommended adult daily dietary intake of vitamin B12 is 5 ug/day, however no more than about 2 µg of vitamin B12 can be ingested per meal. Body storage of vitamin B12 is between 1 and 5 mg, about half of which is stored in the liver. Vitamin B12 requirements increases in pregnancy, infancy, during growth spurts and in the elderly. Because there are no reliable vegetable sources of the vitamin, vegans must use a supplement or fortified foods for B12 intake or risk serious health consequences.

There are five basic mechanisms that can lead to vitamin B12 deficiency,
1) inadequate intake, 2) increased requirement, 3) malabsorption, 4) defective transport, and 5) disorders of B12 metabolism.

Inadequate intake is uncommon primarily because normal body storage of vitamin B12 is between 1 and 5 mg. About half is stored in the liver. Because normal the daily requirement is only 1-3 ug/day but the amount stored in the body is 1-5 mg, , it would takes several years for a person to develop B12 deficiency as a result of inadequate intake. However, a diet that does not include food products of animal origin, or where meat is avoided, would lead to a higher risk of vitamin B12 deficiency. Infants of vegetarian/vegan mothers are also in danger of developing vitamin B12deficiency, even though their mothers may not suffer from disorders of B12 absorption and do not show any deficiency symptoms.

Increased requirement for vitamin B12 does occur in pregnancy, infancy, during growth spurts and in the elderly. But again, even with increased utilization, except in the infants who have yet to build up adequate body stores, generally there is sufficient vitamin B12 stores to meet most sort term and even many long term increased demands for vitamin B12.

By far, the most common mechanism for vitamin B12 deficiency is malabsorption due to a number of gastrointestinal conditions. Vitamin B12 malabsorption can result from autoimmune disorders, old age, gastritis, or certain medications. Pernicious anemia is an autoimmune disorder which prevents the body from manufacturing or accessing intrinsic factor correctly, resulting in severe vitamin B12 deficiency. Sometimes, pernicious anemia is caused by gastrointestinal damage (atrophic gastritis) from Crohn's disease or ulcerative colitis. In true pernicious anemia where there is an autoimmune component, there are three different types of antibodies that could be the cause. Those which bind to the intrinsic factor-vitamin B12 complex preventing uptake, antibodies which bind to intrinsic factor itself preventing binding with vitamin B12, and antibodies to gastric parietal cells preventing the production of intrinsic factor.

Old age is another risk factor for vitamin B12 malabsorption, as elderly individuals often do not make enough digestive enzymes to break down vitamin B12. Surgeries involving removal of the ileum, such as gastric bypass surgery, also impair the body's ability to absorb sufficient vitamin B12. Certain medications, for example protein pump inhibitors (PPI) for GERD or metformin for diabetes, interfere with vitamin B12 absorption. Vitamin B12 malabsorption is also one of many effects of alcoholism.

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Defective DNA synthesis affects all proliferating cells making the onset of pernicious anemia insidious and vague. Some patients may present with the classic triad of weakness, sore tongue and paresthesias,. However, much more frequently patients present with symptoms initially suggestive of any one of the following system disorders: cardiac, renal , genitourinary , gastrointestinal, infectious , mental or neurological disorders. Often, it is during the investigation of the presenting symptom that it is discovered that the patient has a megaloblastic pernicious anemia.

About half of patients have a smooth tongue with the loss of papillae. The tongue may be “beefy red” and quite painful. These patients will often complain of changes in taste or appetite. Patients may report either constipation or having semi solid bowel movements daily. These symptoms have been attributed to megaloblastic changes in the cells of the intestinal mucosa. Nonspecific GI symptoms are not unusual and include anorexia, nausea, vomiting, heartburn, flatulence and a sense of fullness.

Neurologic symptoms are frequently identified in patients with pernicious anemia. The most common are paresthesias, weakness, clumsiness and an unsteady gait. These symptoms are due to myelin degeneration and loss of nerve fibers in areas of both the spinal cord and cerebral cortex. Older patients may present with symptoms suggesting senile dementia or Alzheimer’s. Memory loss, irritability and personality changes are commonplace. In the most dramatic cases patients may even exhibit delusions, hallucinations outbursts and paranoid schizophrenic ideation. Significant reversal of these symptoms and findings can occur with vitamin B12 administration. .

The laboratory workup for the patient suspected of having pernicious anemia should include the following: CBC, peripheral blood smear examination, indirect bilirubing, LDH, serum vitamin B12, folic acid, iron, iron binding capacity, methylmalonic acid and homocysteine. Intrinsic factor (IF) antibodies, type 1 and type 2, occur in 50% of patients with pernicious anemia and are specific for this disorder. Parietal cell antibodies occurs in 90% of patients with pernicious anemia.

If the diagnosis remains unclear then a clinical trial of vitamin B12 may be indicated. Finally, if the diagnosis need additional confirmation a bone marrow aspiration and biopsy may be necessary.

Cell Identification

Specimen 1

Result	No.	Flag
Macrocytic	197	
Elliptocyte/Ovalocyte	4	
Abnormal RBC, would refer	2	
Segmented Neutrophil (PMN, poly	1	***
Dimorphic RBC	1	
Spherocyte	1	***
Polychromatophilic RBC	1	***
Segmented Neutrophil (PMN, poly	1	***
Hypersegmentated Neutrophi	1	***

Total Population 209
Intended result: Macrocytic

Specimen 2

Result	No.	Flag
Platelet, normal	205	
Platelet, giant	3	***
Abnormal Platelet, would refer	1	***

Total Population 209
Intended result: Platelet, normal

Specimen 3

Result	No.	Flag
Teardrop Cell (dacrocyte)	206	
Abnormal RBC, would refer	2	
Elliptocyte/Ovalocyte	1	***

Total Population 209
Intended result: Teardrop Cell (dacrocyte)

Specimen 4

Result	No.	Flag
Segmented Neutrophil (PMN, poly)	135	
Hypersegmentated Neutrophil	61	
Segmented Neutrophil (PMN, poly)	9	
PMN with Toxic Granulation/Vacuolization	2	***
Eosinophil, any stage	1	***
PMN with Dohle Bodies	1	***

Total Population 209
Intended result: Segmented Neutrophil

Specimen 5

Result	No.	Flag
Schistocyte (bite, blister, helmet)	127	
Poikilocytosis	30	
Acanthocytes (spur)	28	
RBC Fragments	8	***
Abnormal RBC, would refer	7	
Hypochromic	3	***
Abnormal, would refer	1	
Dimorphic RBC	1	***
Microcytic	1	
Sickle Cell (drepanocyte)	1	***
Stomatocyte	1	***
Teardrop Cell (dacrocyte)	1	***

Total Population 209
Intended result: Schistocyte

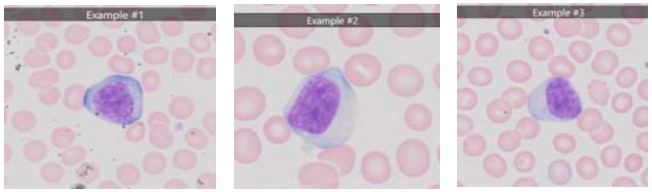
Correct responses are defined as those reflecting agreement among 80% or more of all participants or referees. Unacceptable responses are indicated by "*****" on the Flagging line of each specimen.

Cell Identification - Educational Challenge

Specimen 1

	No.
Lymphocyte, reactive	55
Lymphocyte; atypical, Downey,	41
Lymphocyte, abnormal/atypical	14
Monocyte, any stage	10
Lymphocyte, normal	9
Lymphocyte, normal	6
Abnormal Lymphocyte, would refer	5
Monocyte, normal	3
Blast, undifferentiated	2
Abnormal, would refer	1
Immature WBC, would refer	1

Total Population: **147**
Intended result: Lymphocyte,

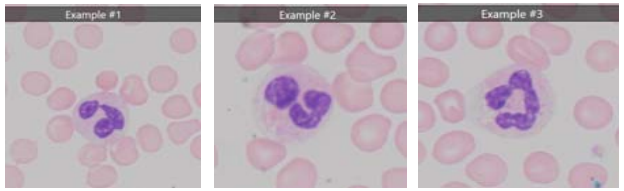


*To see the original fullsized immages, please sign on to your data entry sheet at http://www.aab-pts.org

Specimen 2

Segmented Neutrophil (PMN, poly	96	**96**
Segmented Neutrophil (PMN,	33	33
PMN with Pelger-Huet Nucleus	6	6
PMN with Toxic Granulation/Vacu	5	5
Immature Neutrophil	4	4
Abnormal Granulocyte, would	1	1
PMN with Degenerated Nucleus	1	1

Total Population: **146**
Intended result: Segmented Neutrophil



Sample 19Q1 - Clinical Discussion

Pending