Lab Exercise 7

B12 and Folate

(115 points)

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Note: Clinical values are reported to **one decimal place**; please report your final calculations accordingly. Must show calculations and units for credit. Please put answers in the space provided.

(1) You are monitoring a 62 yrs. old male who began taking a drug with known Folate antagonist ("antifolate") properties 9 weeks ago and is now half way through his treatment regimen. His current lab values are: (14 points)

MCH (pg)	RBC folate (nmol/L)	RBC# (x 10^{12})	MCV [± SD] (fL)	Serum Folate (nmol/L)
28.3	202.2	3.89	104.8 [16.0]	6.1

Based on these values, fill in the tables below:

	Calculation	Answer (with units)
Hct	$104.8 = x/(3.89) = .4077 \times 100$	41%
HgB	$28.3=(x/10)/(3.89) = 11.0087$	11 g/dL
MCHC	$28.3/104.8 = .2700$	27.00 g/dL
RDW	$(16.0 \times 100)/104.8 = 15.26717$	15%

	Answer
Based on these values, is he anemic? (Yes / No)	**Yes**
Based on: Low Hct / Low HgB / Both Low / Both Normal (choose one)	**Low HgB**

Describe his current RBC Morphology (circle all that apply):

microcytic	normocytic	**macrocytic**
hypochromic	normochromic	hyperchromic

*Use MCHC when determining morphology

Is there evidence that he has been compliant in taking the medication with anti-folate properties? (Yes / No)	**No**

Provide brief rationale for your answer:

Serum folate and RBC folate are heavily deficient still

If he was indeed compliant, which parameter would you expect to change first when he starts taking the medication?	**Serum folate**

Provide brief rationale for your answer:

It reacts immediately after folate enters the system but it is still extremely low.

Which parameter would you expect to take the longest to change?	RBC folate

Provide brief rationale for your answer:

RBC cell life is around 120 days so it will take time to see an adjustment to his RBC folate value even if he is taking the medicine as required.

(2) Just over 2 weeks ago (15 days ago), a 38 yr. old male subject presented with complaints of "tiredness" and that he "fatigues easily" with any simple exertion. At the time a blood sample was taken his CBC values were: (15 pts)

RBCs = 4.22×10^{12} cells/L, Hct = 43.0 %, HgB = 11.5 g/dL.

	Answer
Based on these values, was he anemic? (Yes / No)	**Yes**
Based on: Low Hct / Low HgB / Both Low / Both Normal (choose one)	**Low HgB**
If you answered yes, what was the severity?	**Mild**

Using the values listed above, calculate the following:

	Calculation	Answer (with units)
MCV	.43/(4.22)= .1018957	102 fL/cell
MCH	11.5/4.22 = 2.7255118	27 pg/cell
MCHC	11.5/.43 =26.744	26.7 g/dL

Describe his current RBC Morphology (**circle ALL that apply**):

Microcytic   normocytic   **macrocytic**

| **hypochromic** | normochromic | hyperchromic |

*Use MCHC when determining morphology

In reviewing his health history, you learn that he is a habitual 3 pack/day smoker. Which of the indices could be affected by this?   **(Circle ALL that apply)**

| Hct | HgB | MCV |

| MCH | MCHC | **All could be affected** |

He also reported that he started on a new diet about 2 months ago. Additional blood work just completed provided the following values:

Serum Folate (nmol/L)	RBC folate (nmol/L)	Serum MMA (pmol/L)
6.0	258	280

Based on <u>all</u> the information you have, describe and discuss his likely current folate and B12 status – list three considerations that influence your interpretation.

	Distinct considerations
1	His serum folate and RBC folate value points to being deficient in Folate (not B12).
2	His serum MMA is high and that relates to his B12 value. B12 deficiency is reflected in his results.
3	His chronic smoking can be attributed to his folate deficiency.

 (3)   Data sets for 3 adult non-pregnant females are presented below. Match the data set to the most logical corresponding nutritional situation. (9 points)

Situation **A**	Situation **B**	Situation **C**	Situation **D**
Early B$_{12}$ Depletion	Early Folate Deficiency	Recovering from Folate Deficiency Anemia	Early B12 Deficiency

Data set 1:

MCV	96.9 fL	**HgB**	11.7 g/dL	**MMA**	301 pmol/L	**RBC Folate**	253 nmol/L
HoloTC	29 pmol/L	**Serum Folate**	9.9 nmol/L	**Hcy**	18 µmol/L	**Situation**	**D**

Data set 2:

MCV	97.6 fL	HgB	11.8 g/dL	MMA	71 pmol/L	RBC Folate	256 nmol/L
HoloTC	43 pmol/L	Serum Folate	5.9 nmol/L	Hcy	17 µmol/L	Situation	**B**

Data set 3:

MCV	103.4 fL	HgB	11.8 g/dL	MMA	71 pmol/L	RBC Folate	378 nmol/L
HoloTC	43 pmol/L	Serum Folate	14.5 nmol/L	Hcy	11 µmol/L	Situation	**C**

(4) 3 non-pregnant 33 yr.old female subjects have the following identical blood data: (9 pts)

MCV (fL)	HgB (g/dL)	RBC# (x 10^{12})	HCT (%)	RDW (%)
85	13.1	4.00	38	11.8

Unique aspects for each subject are:

Subject	Serum folate (nmol/L)	RBC folate (nmol/L)	Additional information
A	10-12	380	Takes asthma medication
B	11-14	420	Kidney disease
C	7-8	340	(+) Antibodies against IF; smokes

All 3 are about to start an experimental Folate deficient diet. Given the above information, answer and briefly discuss the following questions:

Which participant would you expect to display abnormal RBC indices and/or display clinical Anemia first? (**circle one**):

A   **B**   C

Provide three different reasons about that participant describing why you believe that person will display the clinical symptoms first.

Rationale
- Decreased erythropoiesis (stimulates RBC production) - Large Serum folate value but low RBC folate value. - Cannot take up folate during erythropoiesis nor store it efficiently

(5) Data for 3 patients is presented below: (7 pts)

Patient	Serum folate (nmol/L)	RBC folate (nmol/L)	Homocys (μmol/L)	HgB (g/dL)	MCV (fL)	HoloTC (pmol/L)	Serum B_{12} (pmol/L)	Serum MMA (pmol/L)	RDW (%)
Bill (M)	16.8	353	8.4	10.4	103.2	44	298	86	19.2
Anne (F)	12.5	368	18.3	8.2	108.6	29	123	284	13.4
Jane(preg)	13.2	398	7.6	8.6	84.5	42	322	124	12.1

Possible diagnoses:

Early depletion/depletion stage (Folate)   Early depletion/depletion stage (B_{12})

Early Deficiency stage (Folate)   Early Deficiency stage (B_{12})

Anemia from Folate deficiency   Anemia from B_{12} deficiency

Recovering from Folate Deficiency Anemia   Recovering from B_{12} Deficiency Anemia

Moderately Anemic (but NOT from either a Folate or B_{12} deficiency)

Based on all the above data, write the "most consistent" diagnosis (choose from diagnoses listed above) with respect to their lab values next to the patient name. Also, indicate whether each patient should have the following tests done.

Patient	Diagnosis (from choices above)	Test for Intrinsic-Factor Antibodies (IF-Abs) Yes or No
Bill (M)	Anemia from Folate Deficiency	No
Anne (F)	Anemia from B12 deficiency	Yes
Jane(Preg)	Moderately Anemic but not from folate or b12 deficiency	No
One of the 3 patients is vitamin B_{12} deficient. Who is it?	Anne	

(6) You are monitoring the progress of 3 patients (John, Pat, and Mary). Mary is a 37 yr old non-preg female suffering from a chronic folate deficiency anemia, John (33 yr old male) is recovering from surgery after a traumatic motorcycle accident, while Pat (40 yr old male) is being monitored following the accidental, prolonged ingestion (over a 5-week period) of a potent Folate antagonist, but this was almost 2 months ago. (27 pts)

You have the data sets below. Unfortunately, the patient codes were not attached.   Based on these data sets, deduce which describes the condition and thus identify the likely patient for that data set. *(note that in a real clinical setting you would NOT take a guess!)*

Data set A:

HgB (g/dL)	RBC# (x 10¹²)	HCT (%)	RDW (%)
10.8	3.90	32.0	12.7

Based on the above information, fill out the tables below:

	Calculation	Answer (with units)
MCV	.3200/3.90=0.820512	82 fL/cell
MCH	(10.8 x 10)/3.90 = 27.6923	**28 pg/cell**
MCHC	10.8/.32=33.75	34 g/dL

	Answer
Based on these values, is the patient anemic? (Yes / No)	**Yes**
Based on: Low Hct / Low HgB / Both Low / Both Normal (choose one)	**Both low**

Describe his/her current RBC Morphology (circle all that apply):

microcytic   **<u>normocytic</u>**   macrocytic

hypochromic   **<u>normochromic</u>**   hyperchromic

*Use MCHC when determining morphology

Serum folate (nmol/L)	RBC folate (nmol/L)	Serum MMA (pmol/L)	Serum Hcy (µmol/L)
15.3	457	71	7.4

Based on your answers and the additional results shown above, write the probable patient in the white box.	John

Data set B:

HgB (g/dL)	RBC# (x 10¹²)	HCT (%)	RDW (%)
11.9	4.03	41.2	18.6

Based on the above information, fill out the tables below:

	Calculation	Answer (with units)
MCV	.412/4.03=.102233	102 fL/cell
MCH	11.9/4.03=2.952	**30 pg/cell**
MCHC	11.9/.412=28.883	29 g/dL

	Answer
Based on these values, is the patient anemic? (Yes / No)	**Yes**
Based on: Low Hct / Low HgB / Both Low / Both Normal (choose one)	**Low HgB**

Describe his/her current RBC Morphology (circle all that apply):

microcytic   normocytic   **macrocytic**

hypochromic   normochromic   hyperchromic

*Use MCHC when determining morphology

Serum folate (nmol/L)	RBC folate (nmol/L)	Serum MMA (pmol/L)	Serum Hcy (µmol/L)
13.3	337	87	12.7

Based on your answers and the additional results shown above, write the probable patient in the white box.	Pat

Data set C:

HgB (g/dL)	RBC# (x 10¹²)	HCT (%)	RDW (%)
9.0	2.90	31.4	15.3

Based on the above information, fill out the tables below:

	Calculation	Answer (with units)
MCV	.314/2.90 = .1082758	108 fL/cell
MCH	9.0/2.90=3.103	**31 pg/cell**
MCHC	9.0/.314=28.662	29 g/dL

	Answer
Based on these values, is the patient anemic? (Yes / No)	**Yes**
Based on: Low Hct / Low HgB / Both Low / Both Normal (choose one)	**Both low**

Describe his/her current RBC Morphology (circle all that apply):

microcytic   normocytic   **macrocytic**

hypochromic   normochromic   hyperchromic

*Use MCHC when determining morphology

Serum folate (nmol/L)	RBC folate (nmol/L)	Serum MMA (pmol/L)	Serum Hcy (µmol/L)
4.7	194	180	19.9

Based on your answers and the additional results shown above, write the probable patient in the white box.	Mary

(7) You are evaluating a 65 yr. old female patient who presented 2 weeks ago with complaints symptomatic for anemia. At that time, blood tests were done as indicated in the table below. Current, follow-up blood test data is also presented. Use the blood index data in the table below to answer the questions that follow. (24 points)

Index	2 wks ago	Current
RBC # (x10^{12}/L)	3.28	3.34
HgB (g/dL)	9.5	9.3
Hct (%)	33.8	33.2

RDW (%)	16.4	16.5
MCV (fL)	103.0	99.4
MCH (pg)	29.0	27.8
MCHC (g/dL)	28.1	28.0

Two weeks ago	Answer
Based on these values, was the patient anemic? (Yes / No)	**Yes**
Based on: Low Hct / Low HgB / Both Low / Both Normal (choose one)	**Both low**

Describe her RBC Morphology <u>two weeks ago</u> **(circle all that apply):**

microcytic   normocytic   **macrocytic**

hypochromic   normochromic   hyperchromic

*Use MCHC when determining morphology

Currently	Answer
Based on these values, is the patient anemic? (Yes / No)	**Yes**
Based on: Low Hct / Low HgB / Both Low / Both Normal (choose one)	**Both low**

Describe her RBC Morphology <u>currently</u> **(circle all that apply):**

microcytic   **normocytic**   macrocytic

hypochromic   normochromic   hyperchromic

*Use MCHC when determining morphology

Blood tests evaluating Folate and vit.B_{12} were done 2 weeks ago.   At that time, she was prescribed daily, oral tablets for BOTH Folate and vit. B_{12}.   She is adamant that she has been taking the daily supplements exactly as prescribed.   Her 2 week old and current Folate and vit.B_{12} data are provided below. Fill-in the evaluative data and answer the questions that follow.

Test	2 wks ago	Status	Current	Status
Ser. Folate (nmol/L)	10.5	**Depletion**	18.2	**Normal**
RBC Folate (nmol/L)	205	**Deficient**	222	**Early deficiency**
Serum Hcy (µmol/L)	17.7	**Deficient**	16.5	**Deficient**
Ser. B$_{12}$ (pmol/L)	124	**Deficient**	127	**Deficient**

In 1-2 sentences, what is the evidence that she has been compliant in taking the supplements (as she claims)? Include two specific indices to justify your answer.

All of her values have improved but in the slightest number values. She is still deficient in Hcy, B12, and has slightly improved her RBC folate. Her serum folate values are now normal.

Fill out the following table based on the above values:

	Answers
What is your overall interpretation of her condition?	B12 deficient
What is your plan for further tests?	Test with IF antibodies
What is your next course of action in terms of treatment?	Supplements or lifestyle change

(8) In evaluating values and indices during the progression leading to folate deficiency anemia in an adult male, which of the following sequences is/are correct? (circle the correct answer)

a) low folate intake → decrease serum folate → decreased RBC folate → an RDW = 17.7%

b) a prolonged RBC folate concentration of 260 nmol/L would lead to an elevated MCV

c) acute renal failure as well as liver disease would result in further decreases in serum folate

d) all of the above

e) a and b only

(9) Which of the following is/are consistent with Folate deficiency anemia in an adult female? (circle the correct answer) (2 pts)

    a) an MCV = 106 fL and a Hct = 30%

    b) an MCV = 108 fL and a HgB value = 11.2 g/dL

    c) a serum folate concentration = 5.2 nmol/L and an RBC Folate conc. = 213 nmol/L

    d) all of the above

    e) a and b only

(10) Results from a blood smear analysis state a finding of anisocytosis with hypochromacia. Based on these observations, which of the following indice values would you expect? (circle the correct answer) (2 pts)

    a) an MCV = 78 fL and HgB = 10 g/dL

    b) an RDW of 25.2% with an MCHC = 38 g/dL

    c) an RDW of 20.4% with an MCHC = 27 g/dl

    d) a and b only

    e) **none** of the above

(11) Folate/folic acid deficiency anemia can be characterized by (circle the correct answer): (2 pt)

    a) normal MCV and low MCHC

    b) Low MCH and high Hct

    c) High MCV and normal MCHC

    d) a and b only

    e) none of the above

(12) Concerning the inter-relationships and distinctions between folate and vitamin B12, which of the following statements is/are true? (circle the correct answer) (2 pts)

    a) a lack or low amount of B_{12} can result in a functional folate deficiency because the folate becomes metabolically "trapped" in an inactive form

    b) the anemia blood cell indices resulting from either a folate or B_{12} deficiency can present identically

    c) measurement of methylmalonic acid can establish that B_{12} is the main (primary) nutrient underlying the deficiency if the MMA level is <100 pmol/L

    d) all of the above

    e) a and b only

LAB 5 – APPENDIX

Biomarker values at STAGES in DEFICIENCY PROGRESSION

Indice / Biomarker	NORMAL / ADEQUATE	DEPLETION	EARLY DEFICIENCY	DEFICIENCY
Serum Folate (nmol/L)	≥ 11.3	< 11.3	≤ 6.8	≤ 6.8
RBC Folate (nmol/L)	> 450	< 360	< 320	< 227
Homocysteine [Hcy] (µmol/L)	< 10	10 - 13	> 13	> 13
MMA (pmol/L)	< 210	210 - 270	> 270	> 270
Total ser B_{12} (pmol/L)	> 221	221 - 149	221 - 149	≤ 148
*holo*TC (pmol/L)	> 40	40 - 35	40 - 35	≤ 30
*holo*HC (pmol/L)	> 170	170 - 110	170 - 110	< 110
RDW (%)	< 14	≤ 14	> 14	> 14
MCV (fL)	80 - 100	80 - 100	80 - 100	> 100
MCHC (g/dL)	32 - 36	32 - 36	*May* be outside normal range	outside normal range
Hematocrit [Hct] (%)	normal	normal	normal	↓ or normal
Hemoglobin [HgB] (g/dL)	normal	normal	normal	↓ or normal

Values for Assessing RISK of DEFICIENCY

FOLATE biomarker	ACCEPTABLE [Adequate]	BORDERLINE [MARGINAL]	DEFICIENT
Serum Folate (nmol/L)	> 11.3	11.3 - 6.8	< 6.8
RBC Folate (nmol/L)	≥ 450	360 - 320	< 320
Vit. B_{12} biomarker			

Total serum B$_{12}$ (pmol/L)	> 221	221 - 149	≤ 148
*holo*TC (pmol/L)	> 40	40 - 30	< 30
*holo*HC (pmol/L)	> 170	170 - 110	< 110
MMA (Methylmalonic acid) (nmol/L)			> 270

RDW $=$ (Std.Dev. MCV $\times$ 100) $\div$ MCV [$> 14\%$ = abnormal]

MCV $=$ (Hct *10) $\div$ RBC conc. ($\times$ 10^{12} / L)   Normocytic = 80–100fL

MCH $=$ (HgB (g/dL) *10) $\div$ RBC conc. ($\times$ 10^{12} / L)   Normochromic = 26–34 pg

MCHC $=$ HgB (g/dL) $\div$ Hct   <u>or</u>   MCH (pg) $\div$ MCV (fL)   Normochromic = 32–36 g/dL