Practical Exam "Animal Systematics,
Anatomy & Physiology"

Student Code:

IBO 2018
Tehran, Iran

29th International Biology Olympiad
July 15-22, 2018

Practical Exam

Animal Systematics, Anatomy & Physiology

Total Points: **100**
Duration: **90 minutes**

Animal systematics, anatomy and physiology lab

General information

Total points : 100
Task A : 36 points
Task B : 34 points
Task C : 30 points

Exam time : 90 minutes

Please check your student code in the box on the title page.

Use **answer sheet**, which is provided separately to answer all questions.

The answers written in the question paper **will not be evaluated**.

In order to use the flags (the signs on your desk) just put them in the **flag stand** located on the left wall of your desk.

Please ensure that all the materials and equipments are available to you. If anything is missing, put your yellow flag in the flag stand no later that **15 minutes** after the beginning of the exam. (Any complaints after 15 minutes will not be accepted)

In case of emergencies put your yellow flag in the flag stand.

The red flag is required for the experiment B, once you have finished the dissection.

No additional materials will be provided in any case of material loss during experiments.

We suggest you to read the entire protocol before starting the experiments which helps you with time management.

Stop answering and put down your pen **immediately** at the end of exam. Put the entire protocol with the answer sheet in the envelope. Our assistants will collect the envelopes.

Good luck

Write each indicated number in the cell next to it with your own handwriting.

1	
7	

THIS LAB CONSISTS OF THREE PARTS:

A- SYSTEMATICS AND TAXONOMY OF ACARI (25 minutes) (34 points)

B- ANATOMY OF LEECH Hirudo orientalis (20 minutes) (36 points)

C- Physiological changes during exercise (45 minutes) (30 points)

Materials and Equipment

Experiment A.

-A Box containing FOUR Acari specimens on microscopic slides marked by A,B,C,D.
-A compound microscope.

Experiment B:

-Mask (1 piece)
-Set of gloves (2 pairs in each of sizes).
-Tissue papers (1 box)
-Fine dissection scissors (1 pairs)
-Forceps (1 Piece)
-Plastic petri dish (1 piece)
-Leech in tube wrapped in wet tissue (1 specimen).
-Magnifier glass equipped with LED light (1 piece).
-Color-headed pins (6 piece).
-Pins (1 small box)
-Foam dissection board marked with student code (1 piece)
-Cotton wool tampon(5 pieces)
- NaCl drops (2 container)

Experiment C:

Microtubes A-B-C-D (1 each)
Empty microtubes (12 pieces)
Micropipettes and tips (0.5-10, 10-100, 100-1000 microliter)
Falcone containing NaOH
Microtube I (containing phenolphthalein)

EXPERIMENT A

SYSTEMATICS AND TAXONOMY OF ACARI

Acari (or Acarina) is a taxon of arachnids that contains mites and ticks. In most modern treatments, the Acari is considered a subclass of Arachnida and is composed of two superorders: **Acariformes** (or Actinotrichida), **Parasitiformes** (or Anactinotrichida). Acari are arachnids and, as such, evolved from a segmented body with the segments organised into two tagmata: a **gnathosoma** (including chelicerae and palps) and an **idiosoma** (remaining body). Internal transport and exchange of oxygen and carbon dioxide in some acarine taxa usually are mediated by a branched tracheal system that opens externally through spiracular ports or **stigmata**. Stigmata are placed on different parts of body in different orders of Acari and in some orders they are associated with peritremes (See Figs. 1 & 10).

This examination is composed of 2 tasks
You have four specimens of Acari (slides A-B-C-D).

TASK A.1: Based on shape of chelicerae and palps, please suggest the life mode of each specimen with "✔"in table A.1 of **the answer sheet.**

Soil-dwelling predator: Chelicera chelate and narrow (Figure 2). Palp simple, and five-segmented and sometimes with apotele (a thick seta on palpal tarsus with 2–3 distal branches) (Figure 7).

Free-living predator: chelicera blade-like (Figure 4). A large claw placed on palpal tibia distally and palpal tarsus placed on tibia laterally (thumb-claw process, Figure 8).

Parasite: Chelicera without fixed digit and pointed (Figure 5), palp five-segmented.

Saprophagous mite: Chelicera chelate and thick (Figure 3), palp two-segmented.

Phytophagous mite: Chelicera whip-like (stylet) (Figure 6), palp four-segmented.

Specimen letter

Life mode	A	B	C	D
Soil-dwelling predator				
Free-living predator				
Parasite				
Saprophagous mite				
Phytophagous mite				

TASK A.2: Identification of Acari using a dichotomous key.

Use the dichotomous key below to identify the taxon to which each Acari belongs. Indicate your selections in the **answer sheet** by filling in the **most** appropriate boxes for each Acari. Both the figure and the table illustrate the same data.

1a. With 1–4 pairs of dorsolateral or ventrolateral stigmata posterior to coxae II (Figs. 1A, B, C, D) …… Superorder **Parasitiformes** ….....2

lb. Without visible stigmata posterior to coxae II..…… ……...……...….Superorder **Acariformes** ……..……5

2a. Body with 4 pairs of dorsolateral stigmata posterior to level of coxae III (Figure 1A)..…. Order **Opilioacarida**…..…….. Family **Opilioacaridae**

2b. Body with 1 pair of ventrolateral stigmata in region lateral to coxae II-IV or posterior to coxa IV (Figure1 B, C, D) ……..……..……. 3

3a. Stigmata without peritremes (Figure 1D) …Order **Ixodida** .....……..... 6

3b. Stigmata usually with peritremes (Figure 1B, C) ……..…….. 4

4a. Stigmata present between coxae II-III ….. Order **Holothyrida** …… 7

4b. Stigma present between coxae III-IV..... Order **Mesostigmata** ……. 8

5a. Tracheal system with 1 pair of stigmata opening between bases of chelicerae associated with peritremes dorsally on the cheliceral bases (Figure 1E) ……..…. Order **Trombidiformes** …....…. 9

5b. Tracheal system without stigmata, and peritremes never present between cheliceral bases …… Order **Sarcoptiformes** …....…… 10

6a. Paired spiracular plates situated dorsolaterally between coxae III-IV ……...……...… Family **Argasidae**

6b. Paired spiracular plates situated dorsolaterally posterior to coxa IV (Figure 1D)……. Family **Ixodidae**

7a. Corniculus (Figure 7) simple ……...……..…. Family **Allothyridae**

7b. Corniculus toothed …….…… Family **Holothyridae**

8a. Peritremes directly extended to level of anterior edge of coxae I ……...……. Family **Laelapidae**

8b. Peritremes short, looped medially or apically ..... Family **Varroidae**

9a. With 1 pair of stigmata opening between bases of chelicerae, palpal tarsus placed on tibia distally. ……..……..…… Family **Anystidae**

9b. With 1 pair of stigmata associated with peritremes dorsally on the cheliceral bases, palp with thumb-claw process (Figure 8) ……..……...…….. Family **Trombidiidae**

10a. Palps two-segmented, leg tarsi with one claw ……..… Family **Acaridae**

10b. Palps five-segmented, leg tarsi (plural of tarsus) with three claw ……..... Family **Pheroliodidae**

1a	With 1–4 pairs of dorsolateral or ventrolateral stigmata posterior to coxae II (Figs. 1A, B, C, D)	Superorder **Parasitiformes**	2
1b	Without visible stigmata posterior to coxae II	Superorder **Acariformes**	5
2a	Body with 4 pairs of dorsolateral stigmata posterior to level of coxae III (Figure 1A)	Order **Opilioacarida**-Family **Opilioacaridae**	-
2b	Body with 1 pair of ventrolateral stigmata in region lateral to coxae II-IV or posterior to coxa IV (Figure1 B, C, D)	-	3
3a	Stigmata without peritremes (Figure 1D)	Order **Ixodida**	6
3b	Stigmata usually with peritremes (Figure 1B, C)	-	4
4a	Stigmata present between coxae II-III	Order **Holothyrida**	7
4b	Stigma present between coxae III-IV	Order **Mesostigmata**	8
5a	Tracheal system with 1 pair of stigmata opening between bases of chelicerae associated with peritremes dorsally on the cheliceral bases (Figure 1E)	Order **Trombidiformes**	9
5b	Tracheal system without stigmata, and peritremes never present between cheliceral bases	Order **Sarcoptiformes**	10
6a	Paired spiracular plates situated dorsolaterally between coxae III-IV	Family **Argasidae**	-
6b	Paired spiracular plates situated dorsolaterally posterior to coxa IV (Figure 1D)	Family **Ixodidae**	-
7a	Corniculus (Figure 7) simple	Family **Allothyridae**	-
7b	Corniculus toothed	Family **Holothyridae**	-
8a	Peritremes directly extended to level of anterior edge of coxae I	Family **Laelapidae**	-
8b	Peritremes short, looped medially or apically	Family **Varroidae**	-
9a	With 1 pair of stigmata opening between bases of chelicerae, palpal tarsus placed on tibia distally	Family **Anystidae**	-
9b	With 1 pair of stigmata associated with peritremes dorsally on the cheliceral bases, palp with thumb-claw process (Figure 8)	Family **Trombidiidae**	-
10a	Palps two-segmented, leg tarsi with one claw	Family **Acaridae**	-
10b	Palps five-segmented, leg tarsi (plural of tarsus) with three claw	Family **Pheroliodidae**	-

No image info

Show serially your identification pathway **in table A.2 of the answer sheet**. For example, a pathway to the family Holothyridae is as follow:

Step / Specimen	1	2	3	4	5	6
Family Holothyridae	1a	2b	3b	4a	7b	-
Slide “A”						
Slide “B”						
Slide “C”						
Slide “D”						

EXPERIMENT B:

ANATOMY OF LEECH *Hirudo orientalis*

Introduction:

The Persian leech *Hirudo orientalis* Utevsky and Trontelj, 2005 is a clittellate annelid and belongs to family Hirudinidae. This was described from the Caspian region. It has been used for leech therapy (Hirudotherapy) from the time of Zoroastrian in the Persian traditional medicine. Avicenna, the great Persian philosopher and physician, used the leeches for treatment of different diseases. The cure was aided by injection of active compound of its saliva, while sucking blood, into the host bloodstream. Due to wide use of Hirudotherapy in the recent years the wild populations get close to extinction and therefore, School of Biology, University of Tehran has been working on this species life cycle for a decade, firstly to stop the pressure of leech caught from the natural ponds for conservation purposes and secondly to supply clean cultivated ones for leech therapy. The specimen on the table is a cultured one at indoor aquaculture facility at University of Tehran and was fed on pathogen free horse or camel blood.

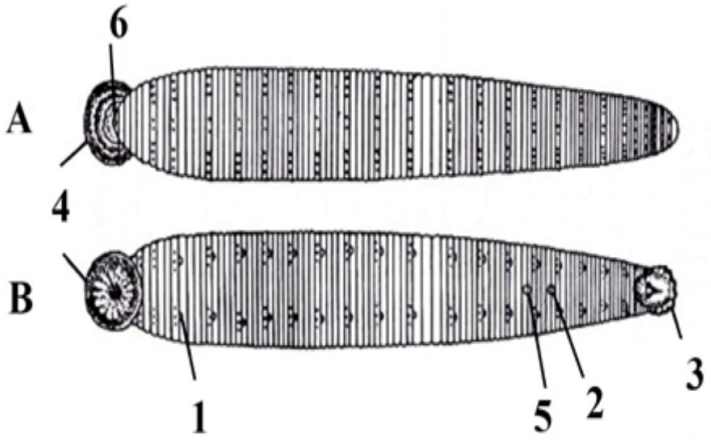
Task B.1 Identify the external structures of *Hirudo orientalis*.
Task B.2 Dissect and identify the internal structures of *Hirudo orientalis*.

Task B.1. Identify the external structure of *Hirudo orientalis*.

Use a hand magnifier glass to observe the anal pore, oral and rear suckers, nephridiopores, male and female genital pores in the provided specimen of *Hirudo orientalis*. Then, answer the following questions in the **ANSWER SHEET.**

Task B 1.1
Indicate If each of the following statements is true or false with a "✓" in the answer sheet.
Q.1 The clitellum is easily seen antero-dorsally on the provided specimen.
Q.2 The crawling of this species requires the use of three types of muscles, namely longitudinal, circular and diagonal.

Task B.1.2
According to the following figure match the correct number with its relevant characters in the following table **in your answer sheet.**



Characters	Oral sucker	Rear sucker	Anal pore	Male genital pore	Female genital pore	Nephridiopore
Number						

Task B.2.2. (18 points)

Dissection and identification of the internal structure of *Hirudo orientalis*.

Put on the gloves, take your alcohol treated leech wrapped in wet tissue from the provided tube using the forceps and place it inside the petri dish. For your convenience its gut blood is mostly ejected thorough a small horizontal cut into the body wall and gut, at the center of the body, after narcotization. But, there is always some blood in the gut. First, take the blue dissecting board (~15×20 cm), place the leech head forward (toward student code) and dorsal up. Fix the leech by inserting the provided ordinary pin obliquely into the anterior and posterior sucker on the foam dissecting board. Then, locate the anus and lift the dorsal cuticle using the forceps, about 2 cm away from the anus, anteriorly. Insert the tip of the scissors into the cuticle and make a small cut. The cut should be deep enough to reach the gut dorsally with the scissors. Continue to cut the dorsal cuticle and dorsal gut wall together toward the oral sucker. Clean the residual gut blood using cotton wool tampon and NaCl drops and throw away the unclean paper tissue to the small rubbish bin on your desk. Take the skin apart and pin it down using the ordinary pin, inserted obliquely. The reproductive, nervous, urinary systems are located ventrally under the gut wall and are easily visible by the naked eye or a magnifying glass.

Note that this species is a hermaphrodite and at any particular time, each individual can act as a potential male or female.

When dissection is finished, **mark the following organs using the provided colored pins.**

Organ	Pin color
Salivary gland	Pink
Vagina	Black
Testis	Yellow
Prostate	Green
Epididymis	White
Segmental ganglion (2 cm below the genital organs)	Blue

In accordance with the internal and external features of the dissected specimen, indicate if each of the following statements is true or false with a "✓" in the **answer sheet**.

Q.1 Gas exchange is cuticular.

Q.2 Potentially, each individual can mate with several potential females.

Q.3 Fertilization is internal.

Q.4 Individuals are capable of self-fertilization.

Q.5 The coelom is highly enlarged to support large amounts of blood storage in the gut.

Q.6 The gut wall bears lateral pouches to increase the intestinal surface.

Q.7 This species bears a proboscis.

Q.8 Analysis of the external rings and internal anatomy of the species shows 50 or more segments.

When the marking is finished, put your **RED** flag in flag stand and a laboratory assistant will take a photo of your dissection. He/she also will take your dissection board away. After this stage take your gloves out and put it in the rubbish bin.

EXPERIMENT C

Physiological changes of exercise.

In this part, we aim to evaluate physiological changes of exercise. To do so, we will go through the following steps:

Measuring QT interval in electrocardiogram (ECG), during exercise.
Indicating significant changes in ECG with statistical analysis.
Measuring post-exercise pH.

3.1. Measuring QT intervals

In this part, you will measure QT intervals in the following ECGs. We have provided four ECGs that belongs to the beginning, 1st, 2nd and 3rd minute of exercise. Based on the guide provided and using the grid, measure the QT interval of each ECG. (Note that in the ECGs, the smallest square equals to 0.04 seconds)

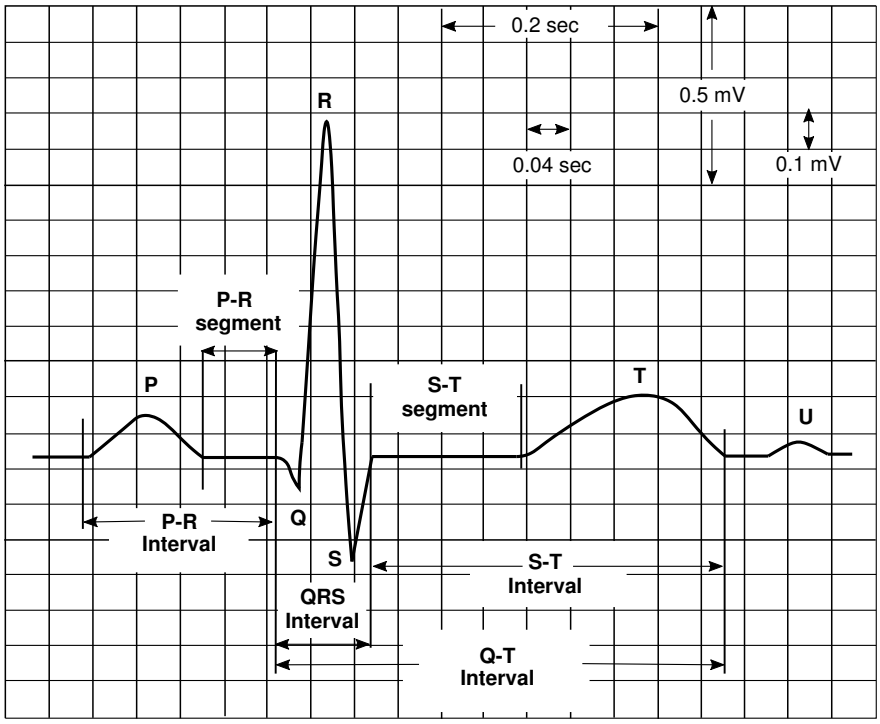


Figure 1. *ECG guide. Note that the QT interval is the exact time beginning at the start of Q wave until the end of the next T wave.*

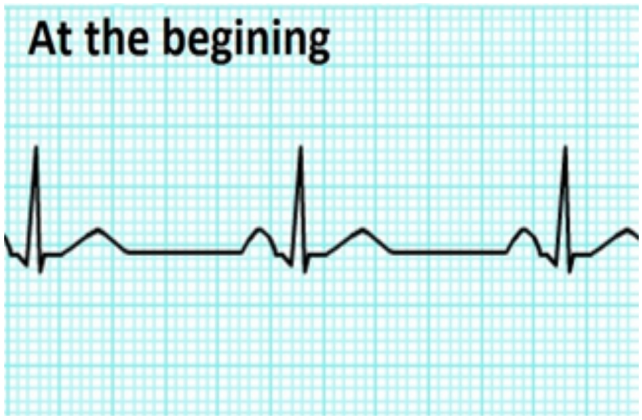


Figure 2. *A part of ECG at the beginning of exercise.*

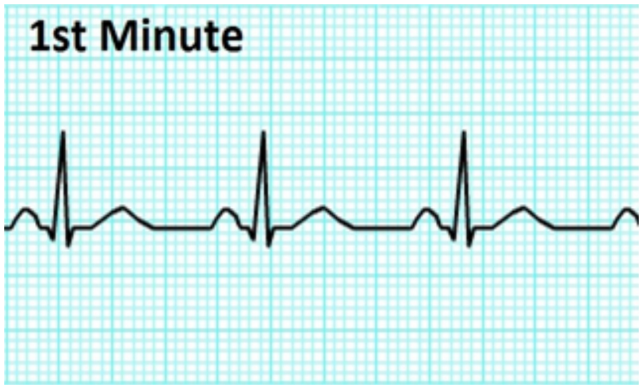


Figure 3. *A part of ECG in the 1st minute of exercise.*

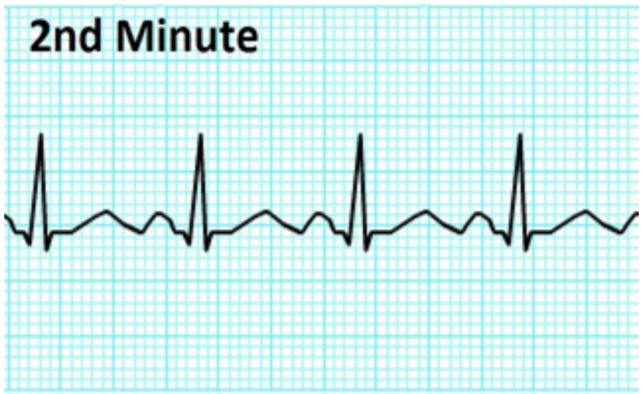


Figure 4. *A part of ECG in the 2nd minute of exercise.*

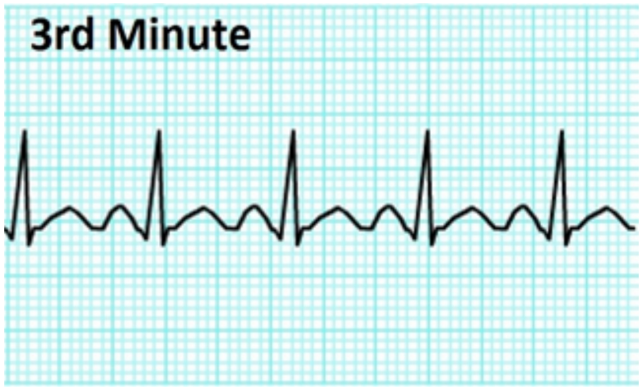


Figure 5. *A part of ECG in the 3rd minute of exercise*

Task 3.1. Write your answers in seconds in the **Answer sheet (rounded to two decimal places)** . (0.5 point each)

	QT interval
At the beginning	
1st minute	
2nd minute	
3rd minute	

3.2. Statistical analysis of QT intervals

We have measured QT intervals in three different subjects during exercise. In **3.2.A.,** you will perform analysis of variance (ANOVA) to test for significant changes of QT interval during exercise in healthy individuals. In **3.2.B.,** the same analysis were performed on healthy individuals during pacing stress testing, instead of measuring QT interval during exercise. Finally, you will interpret your results and make the conclusion.

3.2.A. Conducting ANOVA on QT intervals data.

3.2.A.1. In the following table, we have measured QT intervals (in milliseconds) of three different subjects.

	Subject 1 QT interval	Subject 2 QT interval	Subject 3 QT interval	Mean
1st minute	360	340	347	349
3rd minute	320	312	325	319
10th minute	310	298	307	305
20th minute	298	280	295	291
Mean	322	307.5	318.5	Total mean (M_T) = 316

Task 3.2.A.2. Calculate the "total sum of squares" (**SS_T or SS_{total}**), using the following formula. **Write in Table 3.2.A of the answer sheet (rounded to one decimal place).**

$$SS_T = \sum (X - M_T)^2$$

X: individual value for each subject for each time-point, $\mathbf{M_T}$: total mean,

Task 3.2.A.3 Calculate the "between group sum of squares" ($\mathbf{SS_{between}}$ **or** $\mathbf{SS_B}$), using the following formula. **Write in the Table 3.2.A of the answer sheet (rounded to one decimal place).**

$$SS_B = n \sum (M_G - M_T)^2$$

$\mathbf{M_G}$: mean for each group compared, $\mathbf{M_T}$: total mean, **n**: the number of observations in each group.

Task 3.2.A.4 Based on the following table, fill the blank cells in the summary of ANOVA for QT intervals. **Write in the Table 3.2.A of the answer sheet. (rounded to one decimal place)**

Summary of ANOVA for QT intervals:

Source	Sum of Squares	Degrees of Freedom	Variance Estimate (MS)	F Ratio
Between	SS_B	K - 1	$MS_B = \frac{SS_B}{K - 1}$	$\frac{MS_B}{MS_w}$
Within	$SS_W = SS_T - SS_B$	N - K	$MS_w = \frac{SS_w}{N - K}$	
Total	SS_T	N - 1		

K is the number of time-points and N is the total number of observations.

Task 3.2.A.5. Using the table below, estimate the upper threshold for the P value corresponding to the F ratio you have obtained. **Write in the answer sheet. (1 point)**

F ratio	P value
0.45	0.5
2.92	0.1
5.42	0.025
7.59	0.01
9.60	0.005
15.83	0.001

P value	

3.2.B. In the second experiment, similar data was obtained from three subjects who underwent pacing stress testing during cardiac catheterization. In this experiment, heart rate was elevated gradually during the experiment using a cardiac catheter, instead of the elevated heart rate being observed during exercise, which was done in the previous experiment. The results are presented in the table below.

	Subject 1 QT interval	Subject 2 QT interval	Subject 3 QT interval
1st minute	310	315	310
3rd minute	310	310	310
10th minute	310	305	310
20th minute	310	305	305

Then again, we have performed ANOVA and **F ratio of 2.2 was obtained.** Using the table below, estimate the upper threshold for the P value corresponding to the F ratio given above. **Write in the answer sheet. (1 point)**

F ratio	P value
0.45	0.5
2.92	0.1
5.42	0.025
7.59	0.01
9.60	0.005
15.83	0.001

P value	

Task 3.2.C.1. Considering a P value less than 0.05 to be statistically significant, are the effects of exercise and pacing different? (Indicate the correct answer with "✓" in the answer sheet.) (2 points)

Yes	
No	

Task 3.2.C.2. Based on your physiology knowledge and the results of this study, indicate if each of the following statements is true of false. (Indicate the correct answer with "✓" in the answer sheet.) (1 point each)

statement	**True**	**False**
1. Opening and closure of the aortic valve happen in the QT interval in healthy subjects.		
2. Based on the results from the first study, cardiac output would decrease between 1st and 20th minute of exercise.		
3. Results from the second study indicate that sympathetic activation is probably the main factor behind QT interval changes.		

Task 3.3: Measuring urine and blood plasma pH after exercise.

A group of researchers wanted to measure urine and blood plasma pH 30 minutes after exercise, so they have collected urine and blood plasma samples from three vessels of a monkey with ventricular septum defect (VSD) 30 minutes after exercise. The defect in the ventricular septum allows blood to leak from the left ventricle to the right ventricle.

They used chromatography technique to eliminate the effect of proteins on titration of samples by separating proteins of **"Original samples"**.

In the second step, researchers wanted to remove the effect of other buffers in their sample, so they have added HCl to the collected samples based on their routine protocols.
The "**Treated samples"** from four different sources are provided in four microtubes (A-D).

To find the source from which each of the samples were collected, you have to measure the pH of microtubes (A-D) using the following protocol:

1. Start with choosing one of your samples (A-D) and adding 100 microliters of that sample to an empty microtube.

2. Using your micropipettes, add 10 microliters of phenolphthalein indicator (microtubes “I”) to the micrutube containing 100 microliters of the sample. (The indicator is colorless in the acidic solutions and pink in basic solutions.)

3. Start the primary titration of the solution you have prepared through last two steps by adding 100 microliters of **NaOH (0.01M) solution**.

4. Do the previous step (Adding 100 microliters of NaOH) again until the point that your indicator turns faint pink and the pink color does not disappear by pipetting. (At pH of 7, the indicator turns faint pink and it is the end point of titration.)

5. Now to do a more accurate titration of the chosen sample, throw away the microtube of the titrated solution (solution that turned faint pink in the step 4) and prepare another solution in another empty microtube for the chosen sample based on steps 1 and 2.

6. Add NaOH to the solution of step 5 until you have 100 microliters left to reach the end point volume you have reached at step 4.

7. Add 10 microliters of NaOH to the solution of step 6.

8. Do the previous step (Adding 10 microliters of NaOH) again until the point that your indicator turns faint pink and you reach the end point. Write down the total volume of NaOH you used to reach the end point. The NaOH volume you reached in this step is more accurate than the estimated volume of step 4.

9. Choose another sample (A-D) and do steps 1 to 8 for that sample to find out the NaOH volume you need to reach the end point of titration.

Task 3.3.1. Based on the volume of NaOH (0.01M) solution you have used to reach the end point of titration of each sample, calculate the pH of each **“Treated sample”**. **(rounded to three decimal places).**

sample	A	B	C	D
volume of NaOH added (microliters)				
pH of **“Treated sample”**				

Task 3.3.2. The protocol that researchers used to diminish the buffers of original samples, has provided a formula (find below) to calculate the pH of “Original Sample” based on the results of titration of “Treated Sample”.

$$\text{pH of Original Sample} = 6.37 + \log \frac{10^4 - V}{\alpha}$$

V: Volume of NaOH used to reach the end point of titration of **“Treated Sample” (microliters)**

α: It is an index corresponding to buffer content of the “Original Sample”. The α index of each sample is provided in the table below.

Sample	A	B	C	D
α Index	6420	902	1111	709

Considering the provided formula, the α index and the NaOH volume used to reach the end point of titration, calculate the pH of **"Original Samples"** and write them in the answer sheet. **(rounded to three decimal places).**

Sample	A	B	C	D
pH of **"Original Sample"**				

Task 3.3.3 Based on the calculated pH of the original samples, determine the source of each sample and write its name (A-D) under its source in the answer sheet.

Source	Inferior vena cava	Pulmonary artery	Pulmonary vein	Urine
Sample name (A-D)				

Task 3.4 Determine whether each of the following statements is true or false and check your answer in the answer sheet (indicate your answer with "✓" in the related box).

statement	**True**	**False**
1. Partial pressure of O_2 (PO_2) in right atrium is higher than that of pulmonary vein.		
2. Partial pressure of CO_2 (PCO_2) in pulmonary artery is higher than that of pulmonary vein.		
3. In a healthy subject with hyperventilation that has led to decreased PCO_2 in the blood, the kidney tries to increase the amount of bicarbonate ions in inferior vena cava.		
4. During fasting that leads to increased amount of lactic acid in blood, the kidney tries to decrease the amount of hydrogen ions in urine.		
5. In a subject with normal respiration and without hyperventilation, if the hydrogen ion pumps of the nephrons get inhibited and hydrogen ion secretion into the tubular fluid gets decreased, the amount of bicarbonate ions in pulmonary artery would be less than normal.		