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CRIME SCENE INVESTIGATION

BRUSSELS

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Introduction

You are a team of scientists working for the special Crime Scene Investigation unit (CSI Brussels). You and your team are specialized in tracking down criminals and finding proof of their guilt. Time is a key factor in your work because the suspect could vanish soon before you have proof of his mischief. Therefore, there is a strict time schedule to be followed, it is provided further in this manual. Remember, you are racing against the clock for justice!
Today, an unsolved mystery is waiting for your expertise.

Mission objectives.

The Crime
Someone has broken into a highly automated factory of soft drinks undetected by the electronic alarm systems. The factory belongs to a well-known multinational soft drink company that was preparing to commercialize a new and special "Thai Mango" flavored drink. Preliminary analysis of some traces found on the floor of the factory indicates that the intruder was busy sabotaging the new product by adding extra phosphoric acid to some of the lots of the production, making the drink too acid. This would have been a major economic disaster for the company.
Before he could contaminate all the lots, a watchman spotted the intruder. To escape, the intruder killed the guard in a fight. Under the nails of the guard, your colleague, another crime scene investigator was able to recover some tissue belonging to the killer who escaped.
The police suspects 6 persons, possibly linked to some competing companies that could be involved in this attempt to sabotage the launch of the new drink.

<u>Mission I: Crime Scene Investigation - DNA fingerprinting.</u>

DNA was extracted from the sample isolated under the guard's nails. This belongs in all likelihood to the killer. Undercover police agents succeeded to obtain discretely some biological sample from the 6 suspects.
In all cases, the DNA was isolated by another crime scene investigator to allow your team to carry a DNA fingerprint analysis.

You, a Molecular Biologist working for the CSI unit, must analyze the different DNA samples and find if one of the suspects is responsible of the murder of the guard.

<u>Mission II: Crime Scene Investigation - Chemical analysis.</u>

An unknown amount of phosphoric acid was added to several lots of the new soft drink. In the normal production of these lots there is already a known and fixed amount of phosphoric acid (E338).
You, a Chemical Analyst of the Crime Scene Investigation special unit (CSI Brussels), has to find out quantitatively which lot(s) contain too much acid. A sample of five lots is provided. At least one of these lots was not contaminated by the intruder.

The additional questions you (the analyst) have to answer are:
1. What is the total amount of excess phosphoric acid that was added to the 5 lots of 100 000 liter each.
2. How to neutralize the excess of phosphoric acid
3. How much material is needed for the neutralization.

Warning: do not drink from the solutions

List of material provided by the headquarters of CSI Brussels

FOR DNA FINGERPRINTING:

- 2 glass micropipettes of 10 microliters (graduation of 1 microliter).
- 2 glass plates
- 1 gel electrophoresis comb
- 2 small bulldog clamps
- 3 small cards
- 2 plastic Pasteur pipettes
- One practice gel in a plastic Petri dish
- 15 empty "eppendorf" tubes (open) in a plastic bag
- some white stickers that you can put on your tubes to label them
- One rack containing the following tubes:
  - One red dot "eppendorf" tube containing 300 microliters of the practice orange solution
  - One blue dot "eppendorf" tube containing 50 microliters of the blue gel loading solution
  - One white dot "eppendorf" tube containing 20 microliter of the restriction buffer (5 times concentrated)
  - One yellow dot "eppendorf" tube containing 1 milliliter of water
  - One brown dot "eppendorf" tube containing 5 microliters of DNA from the Crime Scene (isolated from under the guard's nails)
  - Six "eppendorf" tubes, labeled 1 to 6, containing DNA from the 6 suspects.

- One green dot "eppendorf" tube containing the restriction enzyme is available in the ice bucket at the front of the room (see the CSI assistant)
- One blue-cap tube containing 25 milliliters of melted agarose is available at the front of the room (see the CSI assistant)
- Boxes of different sizes of gloves are available at the front of the room (see the CSI assistant)
- Tape is available by the assistants to stick your results in the answering sheets.
- Ruler
- Calculator
- Clock

FOR THE CHEMICAL ANALYSIS:

- 1 buret of 25 ml
- 1 pipet of 5 ml
- 3 erlenmeyers of 100 ml
- Solution of 0.100 M NaOH
- 1 beaker + 1 funnel
- 4 indicator solutions
- 5 unknown samples
- Detergents
- demineralised water
- Safety glasses
- Toilet paper

Materials that you are expected to bring:

- Writing material (pen, pencil, paper)
- Lab coat

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DNA FINGERPRINTING

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Mission I: Crime Scene Investigation - DNA fingerprinting.

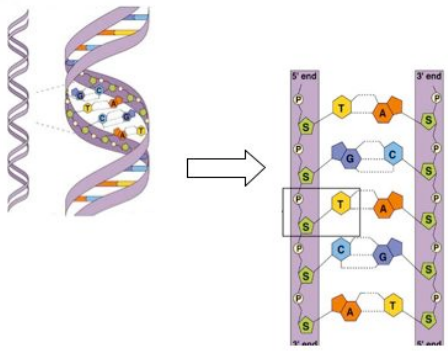
Background Briefing

The Structure of DNA

The characteristics of all living organisms, including humans, are largely determined by information contained within DNA that they inherit from their parents. The molecular structure of DNA can be imagined as a zipper with each tooth represented by one of four letters (A, C, G, or T), and with opposite teeth forming one of two pairs, either A-T or G-C (= a basepair). The letters A, C, G, and T stand for adenine, cytosine, guanine, and thymine, the basic building blocks of DNA. The information contained in DNA is determined primarily by the sequence of letters along the zipper. The traits of a human being are largely the result of information contained in the DNA code. Living organisms that look different or have different characteristics also have different DNA sequences. The more varied the organisms, the more varied the DNA sequences.

The DNA double helix can be represented as such:

```
(Top strand)                5'CCACTCATATTTCTTCAATGTCAA3'
(Complementary strand)      3'GGTGAGTATAAAAGAAGTTACAGTT5'
```



DNA Fingerprinting (DNA Profiling)

Like the fingerprints that came into use by detectives and police labs during the 1930s, each person has a unique DNA fingerprint. Unlike a conventional fingerprint that occurs only on the fingertips and can be altered by surgery, a DNA fingerprint is the same for every cell, tissue, and organ of a person. It cannot be altered by any known treatment. Consequently, DNA fingerprinting is rapidly becoming the primary method for identifying and distinguishing among individual human beings. DNA fingerprinting is a very quick way to compare the DNA sequences of any two living organisms.

DNA Fingerprinting makes use of segments of DNA that exhibit variability (caused by mutation) in the nucleotide base sequences from individual to individual. When these segments of DNA are cut using restriction enzymes DNA fragments of various lengths are produced. If the DNA of an individual has mutations within restriction sites the DNA will not be cut at those sites and that individual's DNA fragments will be different in length from another's DNA fragments produced by the same restriction enzymes.
When the DNA fragments of an individual are separated by electrophoresis, a unique pattern of bands is produced. These bands can be used to identify that individual.

Technical primer on making DNA Fingerprints

1: DNA structure.

Average molecular weight of a single nucleotide: 330 Daltons
1 pmol of a 1 kilobase pairs (=1000BP) DNA fragment (double stranded): 0,66µg
N_A= 6,02 x 10^{23} mol^{-1}

2: Cutting DNA.

Special enzymes called restriction enzymes are used to cut the DNA at specific places. A restriction enzyme recognizes and cuts DNA only at a particular sequence of nucleotides.
If this sequence occurs 3 times in a linear DNA molecule, treatment of this molecule with this enzyme will produce 4 DNA fragments, each with a precise length.
Almost all enzymes recognize palindromic sequences. A palindrome normally refers to word that reads the same forward and backwards (example: the name Leon Noel). In DNA a palindrome has a different meaning because genomic DNA is double stranded. For example the restriction site of another restriction enzyme *Eco*R1 is this palindrome:

```
5'GAATTC3'
3'CTTAAG5'
```

For a single strand, the 'reverse complement' of the sequence is the same as the sequence. Meaning the sequence of the complementary strand read backwards is the same as the top strand sequence.
*Afl*III, the enzyme that you will use for your analysis, also cuts a palindromic sequence:

```
5'ACRYGT3'
3'TGYRCA5'
```

R and Y mean that the enzyme recognizes multiple bases on that site: R can be either A or G, while Y can be either C or T

3: Sizing and sorting DNA: Gel electrophoresis.

The DNA pieces obtained after restriction can be sorted according to size by a sieving technique called electrophoresis. The DNA pieces are passed through a gel made from seaweed agarose (a jelly-like product made from seaweed). The gel could be represented as a big sieve of which the pores get smaller towards the end. An electrical current is put on the gel that will drive the DNA fragments through it. This means that the smaller particles will migrate further through the gel than the bigger ones, they will be stuck at a certain position in the gel where they can't move through the pores of the sieve anymore.

After the running of the gel the DNA fragments can be visualized with a fluorescent dye that intercalates between the DNA bases. This dye is added in the gel before it is made and run, but can only be seen upon exposure to UV light. Therefore, after the running of the different samples on the gel, the gel will be put on a UV transilluminator and the different DNA fragments can be seen as bands on the gel. In this way, a restriction pattern of a certain DNA sample can be obtained.

Work organization and schedule.

Time table (min)	Actions
0 – 30'	Read the instructions and organize your team for efficient work. It is recommended that one member of your CSI unit may carry the chemical testing of the lots, one could start the DNA fingerprinting and the last member could coordinate the efforts and analyze the results.
30'- 45'	Train yourself to use the micropipette. Use the orange colored solution to pipette a few microliters and to deposit them at the bottom of one of the eppendorf tube. Repeat a number of times until you are able to manipulate the small volumes without creating air bubbles and add a few microliters together at the bottom of the tube. For example: can you add 4+3+2+1 microliters together? Check that the total is 10 microliters. (consult visual guide)
45'-1h15	Assemble the DNA restriction reactions in the tubes. See protocol 1.1.
1h15-2h	Place the 7 samples in the 37°C incubator. During these 45 minutes, the enzyme will restrict the DNA. During this incubation, you can pour the electrophoresis gel (warning: use gloves). See protocol 1.2. (consult visual guide) Train yourself to load the practice gel that was provided to you (read protocol 1.3). Use the orange solution. You should be able to load 10 microliters in a well of the gel (the density of the solution has been adjusted such that it will "fall" into the well, so just empty slowly the micropipette at the top or slightly above the well). Make sure that everything goes into the well and that you don't make bubbles.
2h-2h30	Prepare your samples and load the electrophoresis gel (warning: use gloves). See protocol 1.3 (consult visual guide)
2h30-3h00	Run the electrophoresis gel (this operation will be carried out for you by a specialized CSI assistant). While the gel is running you have the time to answer the questions.
3h00-3h15	Remove your gel from the electrophoresis box (warning: use gloves) and get a picture of your gel (this operation will be carried out for you by a specialized CSI assistant). Warning: put a facemask to visualize your gel with UV light.
3h30-4h	Analyze your results and complete the questions.

Protocol 1.1. Assembly of the DNA restriction reactions

For each DNA sample a restriction reaction must be assembled in the **following order** and must contain:

1) Water (x microliters to be calculated such that the final volume is 10 microliters)
2) Buffer (shoud be diluted 5 times in the total reaction volume)
3) DNA (use 2 microliters but remember to calculate the exact concentration later)
4) Restriction enzyme (use 2 microliters)

You can use one single micropipette but you should rinse in water the micropipette after each pipetting (just passing once up and down 10 microliter of water is enough).
Mix by pipetting delicately up and down (do not make bubbles).
Incubate the reactions by placing the rack in the 37°C incubator.

TIPS:

- put the water that you use for rinsing the micropipet in a separate tube so that you don't contaminate the water that you use for the restriction reaction
- Don't forget to close the tubes properly before you incubate them

Protocol 1.2. Pouring the electrophoresis gel (consult your visual guide)

Warning: the agarose gel and the electrophoresis buffer contain a very low dose of a mutagenic substance (Ethidium bromide). Wear gloves when manipulating them and wipe any stain or drop. In case of spill, immediately contact the closest CSI assistant.

You will have to pour around 9 milliliters of a hot agarose solution onto one of the glass plate where it will solidify. To achieve this:

1) Position the glass plate on a flat surface.
2) Fix the plastic comb to the two bulldog clamps and place it on top of the plate, parallel to the smaller side, about 8-10 mm from the edge. The comb must be perpendicular to the long edge. All 7 teeth of the comb must be above the glass plate. This will form 7 wells into which you will later load your DNA.
3) Insert the three small cards between the glass plate and the comb to create a small space between them.
4) Remove the card, leaving the assembly intact.
5) Put gloves on
6) Go get the agarose tube from the CSI assistant, it contains about 25 ml of hot molted agarose. Note: don't get the agarose too soon because it will solidify in about 5 minutes. Once you took the agarose out of the warm bath, you cannot bring it back.
7) Use a Pasteur pipette to aspirate the agarose from the tube and to dispense it on the glass plate. The agarose should not run off the plate. You should use 9 ml of agarose for one gel. To succeed, you need to dispense the agarose slowly with the pipette very close to the plate and move the pipette while dispensing. Don't make bubbles and be careful that you don't let "agarose drops" fall on the plate. Start next to the comb. The agarose must form a layer of rather uniform thickness (proceed relatively quickly). Do not put several layers of gel on top of each other.
8) Let the gel solidify. It takes about 10 minutes and the agarose gel turns translucent instead of transparent.
9) Before going to the electrophoresis box, remove the comb by gently pulling it upward.
10) If you move your gel (to go to the electrophoresis): leave it on the glass plate!

One gel is enough but you have been provided with enough agarose for one more gel in case of a problem with the first one. Note however that it will solidify in the tube.

OPTION:

- **If you have a problem pouring your gel you can ask one of the supervisors for a gel that is already made to make sure that you can finish up your experiment. Note however that this will cost you 16.6% of the marks !!!**
- **In case your agarose is already solidified and you want to try once more to make the gel yourself, you can also ask for a new tube of molted agarose. This will cost you 6.6% of the marks.**

Visual guide: see separate page that was put on your table

Picture 1: Use of micropipettes
Picture 2-4: making the electrophoresis gel
Picture 5: loading the gel

Protocol 1.3. Sample preparation and gel electrophoresis loading

After the 45 min. incubation, remove your samples from the incubator.
Add 1 microliter of the gel loading solution to each sample and mix it well.
Go with your samples and your gel on the glass plate (gloves!) to the electrophoresis table and ask the assistant for help.
The assistant will indicate in which gel box you should put your gel. Place your gel, together with the glass plate, perpendicular to the electrodes. The buffer should come a few millimeters above your gel and the wells.
Using the micropipette, load the samples.
From left to right, you must load in the following order: DNA from the crime scene then the DNA from the suspects 1 to 6.
Signal to the assistant that will start the electrophoresis for you.
Let the electrophoresis run until the blue stain reaches the end of the gel without running off of it. This shouldn't take more then 30 min.
When this point is reached, signal to the assistant and proceed to the UV station.
Slide your gel of the glass plate and place it on the UV transilluminator and place the facemask.
You can look at your gel briefly. The CSI assistant will take a Polaroid picture and give it to you.
Remove your gel and put it back on the glass plate (bring it back to your table and keep it there in case the picture didn't succeed).
Wait about 1 minute before "opening" the picture.

Warning: Be careful when you load your gel. You will have to share the gel box with another team. Your gel will be on one side of the box and the other team's gel on the other side. If you disturb the gel of the other team such that their work is compromised, your team will be automatically disqualified.

ANSWER FORM
Team ID:

Q1: In the following sequence: how many AflIII restriction sites can you find? Circle the sites on the sequence.
A1: Number of sites = _3_

5′GCAGAGAACATGTCGAAGCGGCTCCTCTGAATGTACACCCTGGGATGTACAGTCAGAAGGCGGC

TCGCCCGGCGCTGGAGGAGCGAGCTAAGAGCAGTGGGGACGCGTGTACAAGATCAAAGAGGAGCAC

TTAAGGGACACGTCGCAGACAGGGCAGCCACGCGTGAATTCTGCCGG 3′

Q2: In the sequence given above, how many different restriction enzymes other than AflIII cut the sequence? Consider only enzymes that recognize 6-basepairs palindromes. Underline all the sites found.
A2: Number of different restriction enzymes = _3_

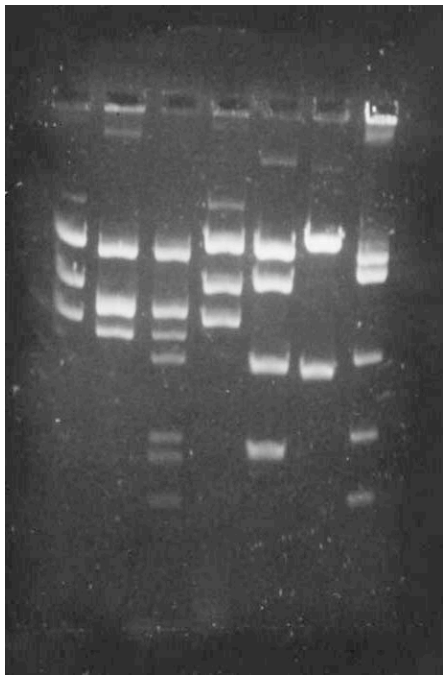
Q3: During the electrophoresis, the DNA migrates towards which electrode ? (circle the good answer(s))
A3: + electrode OR – electrode

Q4: Why does the DNA migrate toward that electrode ? (circle the good answer(s))
A4:
- It is a base
- It is an acid
- It is a complex biological molecule
- It is positively charged
- It is negatively charged
- It is a lipid
- It is a very long polymer

Q5: Who was present on the crime scene ? (circle the good answer(s))
A5:
- Suspect 1
- Suspect 2
- Suspect 3
- Suspect 4
- Suspect 5
- Suspect 6
- None of them

ANSWER FORM
Team ID:

Q6: Paste your picture of the gel into the frame and write down the number of bands you see for each sample.



A6:

Sample	Number of bands
DNA Crime scene	
Suspect 1	
Suspect 2	
Suspect 3	
Suspect 4	
Suspect 5	
Suspect 6	

The accepted number of bands was counted based on any visible band.

Q7: Fill in the table below and indicate the number of microliters you used for setting up the reactions.
A7:

DNA	2
Buffer	2
Water	4
Enzyme	2
Total	10

Q8: Because of the urgency of the mission you didn't have time to calculate precisely the amount of DNA to use in the reaction. Please calculate now the following:

A8: Fill in in the table how many micrograms of DNA you used for the different digestion reactions of the suspect DNA samples knowing that the average length of 1 DNA molecule was 10.000 basepairs and that the concentrations of the different samples are:

Sample	**1**	**2**	**3**	**4**	**5**	**6**
Concentration	500 ng/µl	6,49 x 10^{-8} M	7,57 x 10^{-2} pmole/µl	6,01 x 10^{-10} kg/µl	0,42 g/l	81,4 nM
Amount (µg)	1	0,857	0,999	1,202	0,84	1,075

ANSWER FORM
Team ID:

Q9: How many DNA molecules was present in your restriction digestion, knowing that the DNA from the Crime Scene had a concentration of 0,5 micrograms per microliters and that the length was 10724 basepairs

A9 = ____8,48 x 10^{10} _____ DNA molecules

Q9: Knowing that the concentration of the restriction enzyme AflIII that you used is 1.5 units per microliter, that 1 unit correspond to the amount of enzyme required to cut once 1 microgram of DNA in one hour and that you put 1,0 microgram of linear DNA from the crime scene, what is the minimal number of microliters of enzyme that you could have used to be sure that the DNA was fully cut.

A9 = ___1,78µl ____ microliter

CHEMICAL ANALYSIS

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Mission II: Crime Scene Investigation - Chemical analysis.

1. !!! Warning: do not drink from the solutions

2. Method

The concentration of phosphoric acid in each sample has to be determined by titration with 0.100 M NaOH solutions. Several pH indicators are available.

2.1 Preliminary work

In order to select a suitable indicator the pH values of the equivalent points of the titration curve (see figure 1) have to be calculated.

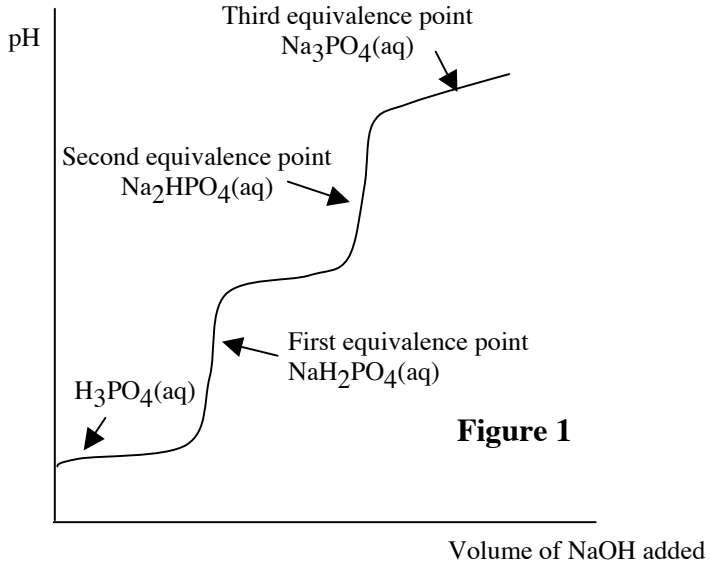

Figure 1

The pH of the equivalence point can be estimated from the acid dissociation constants. These constants are the equilibrium constants for the dissociation reaction of the acid in aqueous solutions:

$$HA(aq) + H_2O(l) \leftrightarrows H_3O^+(aq) + A^-(aq)$$

Here HA is the acid and A^- is its conjugated base. The acid dissociation constant K_a of this reaction is

$$K_a = \frac{[H_3O^+]\,[A^-]}{[HA]}$$

One can also define a base-protonation constant K_b of a weak base A^- as follows

$A^-(aq) + H_2O(l) \leftrightarrows OH^-(aq) + HA(aq)$

$$K_b = \frac{[OH^-]\,[AH]}{[A^-]}$$

Here A^- is the base and HA its conjugated acid.

The ion-product constant K_w of water is defined by the following equilibrium (auto-protonation of water) as follows

$H_2O(l) + H_2O(l) \leftrightarrows H_3O^+(aq) + OH^-(aq)$

$K_W = [H_3O^+]\,[OH^-] = 10^{-14}$

Table 1 gives the K_a, pK_a , K_b and pK_b values for phosphoric acid ($pK = -\log_{10} K$)

Acid	**K_a**	**pK_a**	**Conjugated base**	**K_b**	**pK_b**
H_3PO_4	5.93×10^{-3}	2.23	$H_2PO_4^-$	1.69×10^{-12}	11.77
$H_2PO_4^-$	6.32×10^{-8}	7.20	HPO_4^{2-}	1.58×10^{-7}	6.80
HPO_4^{2-}	4.84×10^{-13}	12.32	PO_4^{3-}	2.07×10^{-2}	1.68

Table 1

This implies that the $H_2PO_4^-$ anion is acidic, while the HPO_4^{2-} and PO_4^{3-} ions are basic.

Note that phosphoric acid is a triprotic acid with successive acid dissociation constants about 10^{-5} times the value of the preceding one. Therefore the second and third dissociations of H_3PO_4 (and the autoprotonation of H_2O) can be neglected in the pH calculation of the first equivalence point with the acidic $H_2PO_4^-$ anion. For the pH calculation of the second and the third equivalence points with the basic anions HPO_4^{2-} and PO_4^{3-} respectively the first and third and first and second dissociations of H_3PO_4 can be neglected.

At the equivalence points you have solutions of respectively NaH_2PO_4, Na_2HPO_4 and Na_3PO_4.

As examples of pH calculations of acidic and basic ions the following two pH calculations are given.

Example 1 : pH calculation of an 0.05 M aqueous solution of hypochlorous acid (a weak acid).

The value of K_a for an aqueous solution of hypochlorous acid, HClO(aq), is 3.0×10^{-8} M. The equilibrium equation is

$HClO(aq) + H_2O(l) \leftrightarrows H_3O^+(aq) + ClO^- (aq)$

The only other source of $H_3O^+(aq)$ is from the autoprotonation reaction of $H_2O(l)$.
But, because $K_a \gg K_w$, we can ignore in this case that source of $H_3O^+(aq)$ and set op the following concentration table for initial and equilibrium concentrations of the species in solution :

Concentration	**HClO(aq)**	**+**	**$H_3O(l)$**	**$\leftrightarrows$**	**$H_3O^+(aq)$**	**+**	**$ClO^-(aq)$**
Initial	0.050 M		-		0		0
Equilibrium	0.050 M - $[ClO^-]$		-		$[H_3O^+]$		$[ClO^-]$
Equilibrium (substituting $[H_3O^+]$ for $[ClO^-]$)	0.050 M - $[H_3O^+]$		-		$[H_3O^+]$		$[H_3O^+]$

Table 2

If we combine the entries in this table with the expression for K_a, then we have

$$K_a = \frac{[H_3O^+][ClO^-]}{HClO} = \frac{[H_3O^+]^2}{0{,}050\ M - [H_3O^+]} = 3{,}0 \times 10^{-8} M$$

We use the method of successive approximations to solve this equation. Neglecting $[H_3O^+]$ relative to 0.050 M, we obtain the first approximate solution :

$$[H_3O^+] = \sqrt{(0{,}050\ M)(3{,}0 \times 10^{-8} M)} = 3{,}9 \times 10^{-5} M$$

Because $[H_3O^+]$ is very small relative to 0.050 M, the second approximation yields the same value of $[H_3O^+]$ as the first approximation : namely

$$\frac{[H_3O^+]^2}{0{,}050M - 3{,}9 \times 10^{-5} M} = 3{,}0 \times 10^{-8} M$$

And

$$[H_3O^+] = \sqrt{(0{,}050M - 3{,}9 \times 10^{-5} M)\ (3{,}0 \times 10^{-8} M)} = 3{,}9 \times 10^{-5}\ M$$

The pH of the solution is
$pH = - \log [H_3O^+] = - \log (3.9 \times 10^{-5}) = 4.41$

Example 2 : pH calculation of an 0.05 M aqueous solution of $NaCH_3COO$.
When $NaCH_3COO(s)$ is dissolved in water, solvated sodium ions and acetate ions are produced

$$NaCH_3COO(s) \xrightarrow[H_2O(l)]{} Na^+ (aq) + CH_3COO^- (aq)$$

The sodium ions are neutral, so they do not react with $H_2O(l)$; but he acetate ions, being conjugate bases of a weak acid, are basic and undergo the reaction

$CH_3COO^-(aq) + H_2O(l) \leftrightarrows CH_3COOH(aq) + OH^-(aq)$

$K_b = 5.75 \times 10^{-10}$ M for this equation. Because $K_a \gg K_w$, we ignore in this case the $H_3O^+(aq)$ from the autoprotonation reaction of water and write

Concentration	**$CH_3COO^-(aq)$ +**	**$H_2O(l)$**	**$\leftrightarrows$**	**$CH_3COOH(aq)$ +**	**$OH^-(aq)$**
Initial	0.050 M	-		0	□ 0
Equilibrium	0.050 M - $[CH_3COOH]$	-		$[CH_3COOH]$	$[OH^-]$
Equilibrium (substituting $[OH^-]$ for $[CH_3COOH]$)	0.050 M - $[OH^-]$	-		$[H_3O^+]$	$[OH^-]$

Table 3

The expression for K_b is

$$5{,}75 \times 10^{-10}\text{M} = \frac{[OH^-]^2}{0{,}050\text{M} - [OH^-]}$$

Neglecting $[OH^-]$ relative to 0.050 M, we obtain

$[OH^-] = (0.050 \text{ M} \times 5.75 \times 10^{-10} \text{ M})^{1/2} = 5.4 \times 10^{-6}$ M

Which is negligible compared with 0.050 M, as confirmed by successive approximation. The value of pOH can be obtained from the value of $[OH^-]$:

$pOH = - \log[OH^-] = - \log(5.4 \times 10^{-6}) = 5.27$

The pH of the solution is

$pH = 14.00 - pOH = 8.73$

Task 1
Calculate the pH of the 3 equivalent points of the titration of 0.100 M phosphoric acid with 0.100 M NaOH.

Task 2
Selection of an indicator. There are 4 indicators at your disposal. The pH range of color-change and the color change is given in table 4.

Indicator	pH range/Color-change	Color change
1) Methyl orange (M.O.)	3.1 – 4.4	red to orange
2) Methyl red (M.R.)	4.4 – 6.2	red to yellow
3) Bromthymol blue (B.T.B.)	6.2 – 7.6	yellow to blue
4) Phenolphthalein (f.f)	8.0 – 10.0	colorless to red

Table 4

Which of these will you select for the first and second equivalence points? Report the indicator number in the answer form.
In order to determine quantitatively the concentration of acid you have to make a choice of which equivalence point you will use.

Task 3
Choice of the equivalence point and motivation (select the answer in the answer form).

2.2. Titrations
Materials at your disposal:

- 1 buret of 25 ml
- 1 pipet of 5 ml
- 3 erlenmeyers of 100 ml
- Solution of 0.100 M NaOH
- 1 beaker + 1 funnel
- 4 indicator solutions
- 5 unknown samples
- Detergents
- demineralised water
- Safety glasses
- Toilet paper

Task 4
Determine the concentration in phosphoric acid of the 5 unknown solutions. Perform 3 titrations for each unknown. Report in the answer form for each unknown solution the following:

- volume of unknown solution used
- volume of NaOH added (3 times and average)
- concentration of phosphoric acid

Task 5
Identify the solution(s) to which the intruder added phosphoric acid. How much acid (in kg) was added?

Task 6

Which one of the following solutions would you use to make the lots drinkable again: NH_3, $CaCO_3$, NaOH, KOH or $NaHCO_3$?

Report the chemical formula of the neutralizing agent, its concentration and total amount in kg of the neutralizing agent for each lot. The volume of neutralizing solution must be 10 % v/v of the volume of the lot.

ANSWER FORM
Team ID:

Task 1: pH of the 3 equivalent points

1. Equivalence point 1 pH: .....
   Equivalence point 2 pH: .....
   Equivalence point 3 pH: .....

Task 2: Choice of indicator

2. Equivalence point 1 indicator n° .....
   Equivalence point 2 indicator n° .....

Task 3: Choice of the equivalence point

3.1 Equivalence point
- _ first
- _ second
- _ third

Motivation

3.2
- _ The smaller volume of titration agent (NaOH) is better
- _ The larger volume of titration agent is better
- _ A better color change can be observed

Task 4: Titrations

Unknown solution	Volume unknown used		Volume NaOH added			Concentration of phosphoric acid	
1	.....	ml	1	.....	ml		
	.....	ml	2	.....	ml		
	.....	ml	3	.....	ml		
			average	.....	ml	.....	M
2	.....	ml	1	.....	ml		
	.....	ml	2	.....	ml		
	.....	ml	3	.....	ml		
			average	.....	ml	.....	M
3	.....	ml	1	.....	ml		
	.....	ml	2	.....	ml		
	.....	ml	3	.....	ml		
			average	.....	ml	.....	M
4	.....	ml	1	.....	ml		
	.....	ml	2	.....	ml		
	.....	ml	3	.....	ml		
			average	.....	ml	.....	M
5	.....	ml	1	.....	ml		
	.....	ml	2	.....	ml		
	.....	ml	3	.....	ml		
			average	.....	ml	.....	M

ANSWER FORM
Team ID:

Task 5: Identification of the lot to which phosphoric acid was added

5. Lot n°	Phosphoric acid was added	Total mass of acid added
1	_	..... kg
2	_	..... kg
3	_	..... kg
4	_	..... kg
5	_	..... kg

Task 6: Neutralization of the excess phosphoric acid

6. Lot n°	Neutralizing agent	Concentration	Total mass of neutralizing agent
1		..... M	..... kg
2		..... M	..... kg
3		..... M	..... kg
4		..... M	..... kg
5		..... M	..... kg

Time table (min)	Actions
0 – 30'	Read the instructions and organize your team for efficient work. It is recommended that one member of your CSI unit may carry the chemical testing of the lots, one could start the DNA fingerprinting and the last member could coordinate the efforts and analyze the results.
30'- 1h00	Calculation of the pH of the equivalent points and choice of the indicator and equivalence point. If you cannot find the answers you can obtain them after 1h00, however your score for task 1 will be zero (0). Training of the use of the titration instruments. A preliminary titration for the determination of the color change at the equivalence point is recommended.
1h00'-3h30	Titrations of the 5 unknown solutions, about 30 minutes per solution is needed.
1h30-3h45	Calculation of the concentrations of the unknown solutions, the total mass of acid added to the lots and total mass of neutralizing agent. Perform this calculation for each solution immediately after the titrations.
3h30-4h00	Report the results and complete the answer form.