Universitat de Girona
Facultat de Medicina

Final Degree Project

Estimation of the Early Post-Mortem Interval:

A Multicentre Comparison of the Analysis of Vitreous Humour and the Henssge's Nomogram Method.

A Cross-sectional, Correlational Study

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1. SYMBOLS AND ABBREVIATIONS

ºC	*Degrees Celsius.*
CSF	*Cerebrospinal Fluid.*
dpm	*Days post-mortem.*
GS	*Gold Standard.*
hpm	*Hours post-mortem.*
Hx	*Hypoxanthine.*
IMLCFC	*Institut de Medicina Legal i Ciències Forenses de Catalunya.*
IQR	*Interquartile Range.*
K+	*Potassium*.
[K+]	*Potassium concentration.*
mpm	*Months post-mortem.*
Na+	*Sodium.*
PI	*Principal Investigator.*
PMI	*Post-Mortem Interval.*
SD	*Standard Deviation.*
TOD	*Time of Death.*
U	*Urea.*
VH	*Vitreous Humour*.
ypm	*Years post-mortem.*

2. ABSTRACT

Background: Estimating the Post-Mortem Interval (PMI) is a daily forensic task. It is especially important in those cases where a violent death has occurred in order to verify the suspects' alibis around this period of time. This estimation is also important for heritage purposes if victims are family related.

Nowadays, the medical examiner applies different PMI methods, such as body cooling or Rigor mortis, alongside witnesses' statements so as to increase the precision of death time estimation as much as possible.

So far, the only objective-quantitative method used in the regular forensic practice is the Henssge's Nomogram method which is based in the post-mortem body cooling. Academically, a promising objective-quantitative method has been studied: the PMI extrapolation from the analysis of the vitreous humour's potassium concentration.

Forensic field studies are needed in order to demonstrate the real applicability of a PMI method: to be precise, to be reliable and to give immediate results.

Objective: Our main goal is to assess whether the analysis of vitreous humour is more accurate than the Henssge's Nomogram method in estimating the early PMI in violent deaths occurred within the *Institut de Medicina Legal i Ciències Forenses de Catalunya*'s jurisdiction.

Method: We are going to carry out a multicentre cross-sectional, correlational study.

During the regular forensic practice, both methods will be applied to the same corpse at the same time. At the crime scene, the necessary data will be duly and systemically collected in order to calculate the early PMI subsequently. The early-PMI's estimations will be then compared with the exact PMI, the Gold Standard.

During 12 months, the data collection process will be done by forensic Doctors from every division of the *Institut de Medicina Legal i Ciències Forenses de Catalunya* alongside the Laboratory Personnel from the Biochemical Department of *Hospital Clínic (Barcelona)*.

Keywords: Post-Mortem Interval, time of death, vitreous humour, Henssge's nomogram, potassium concentration, rectal temperature.

3. INTRODUCTION

In this section, the fundamental theoretical concepts in which the project revolves will be described. First of all, the importance of estimating the Post-Mortem Interval (PMI) is described in ***section 3.1***. Afterwards, a general view of the available PMI Methods is defined in ***section 3.2***. Then, an introduction about the phenomenon in which one of the methods under discussion is based on and the method itself will be found in ***sections 3.3 and 3.4***, respectively. Finally, a similar thing happens with the second method in question, in ***sections 3.5 and 3.6***.

3.1. Estimating the PMI

The **Post-Mortem Interval (PMI)** is defined as the quantity of time that has passed since death occurred. This interval will comprehend two values: time 'A', the time when the victim was last seen alive, and time 'B', the time when the victim was found dead (1). We will always obtain an estimation of this value, except in those situations where death has been witnessed.

We can be interested in knowing the PMI in an **absolute way**, to just know the **time of death** (TOD); or in a **relative way**, to know the sequence of TOD when more than one decedent is involved, in other words, to establish which person has died first. The former approach will be essential in terms of police investigations, whereas the latter will be important for heritage purposes if both victims are family related (2).

According to B. Madea and C. Henssge, there are four objectives in estimating PMI (3). They are summarized in ***Table 1***.

Table 1 Objectives of Estimating the Time since Death. From (3) ***.***

Objectives of estimating the time since death
1. To give the police a preliminary idea on the time of an assault in criminal connotations.
2. To check whether the time since death is consistent with the alibi of a suspect.
3. When two deaths occur, especially of spouses or siblings, the order of deaths and hence survivorships.
4. For registration purposes: 'Enquire where, when and by what means a person came to death'.

Estimating time since death at the crime scene is a routine task for a medical examiner. He or she will give an approximate preliminary assumption to the police. Then, they are going to base their following investigation around this TOD. The witnesses' statements, the list of suspects and their alibis' assessments will depend on this estimation. Therefore, there is a huge responsibility on the forensic doctor (2–5).

Camps stated that in order to avoid miscarriage of justice we must provide a reasoned guess based on scientific evidence. Although estimating the TOD is often not an exact science, our goal should be reducing the margin of error inherent on the

measurement. He said *"It is better to prove without contradiction that death could have occurred at a time when a certain person was there, rather than it did occur at some exact moment"* (6). Providing an irrationally precise TOD is far much worse than giving a really wide range of time. In the latter scenario, there will still be methods to shorten that interval (3).

3.2. PMI Methods

There are two approaches to estimate the PMI. On one hand, there is the **evaluation of ante-mortem changes**, which comprises rough methods such as wound age or gastric content evaluation, and on the other hand, there is the **evaluation of post-mortem changes**, which comprises methods that are frequently evaluated at the crime scene. The latter approach is far much important than the former one (7).

A brief summary of the most widely known PMI methods is described in the ***ANNEX, section 15.1***. The ones regarding the present study are going to be discussed more deeply in ***sections 3.4 and 3.6***.

It is important to take into account that PMI methods are not equal in terms of scientific evidence (3). A classification regarding this aspect is represented in ***Table 2***.

Due to the fact that post-mortem changes follow a chronological progress until the body disintegrates, authors usually classify PMI methods in whether they can be applied in the **recent cadaver**, where putrefaction has not been established yet, or in the **ancient cadaver**, where putrefaction signs start to appear (2,4).

The majority of methods are useful within the first 24 – 48 hpm (hours post-mortem)(3).

Table 2 PMI Methods Classified according to their Scientific Value. Adapted from (3)**.**

Grade	Description	Method
1. Objective	Quantitative measurement. Mathematical description. Quantitative influencing factors. Declaration of precision. Proof of precision on independent material.	Algor mortis (the Nomogram method). Potassium in vitreous humour.
2. Subjective	Influencing factors are considered. Declaration of precision. Proof of precision on independent material.	Supravital reactions.
3. Subjective	Influencing factors are known 'in principle'. Empirical estimations.	Livor mortis. Rigor mortis.
4. Subjective	Analogous conclusions based on empiricism and assumptions.	Gastric contents.
5. Subjective	Velocity of progression of post-mortem changes entirely dependent on ambient factors. No sound empirical estimation possible.	Putrefaction.

Currently, forensic practitioners do not base their estimations on one method only. In order to increase the precision of death time estimation and, thus, minimize the error, several methods, such as Algor mortis, Livor mortis, Rigor mortis or putrefaction; alongside witnesses' statements are used at the crime scene. This is called the **compound method** (4,7). Until now, the Nomogram method developed by Henssge (8) has been proven to be the most accurate and reliable estimator of the PMI in comparison of other methods that are used in the regular forensic practice (7).

Because of the fact that conventional methods are frequently affected by external factors such as ambient temperature, new promising techniques based on biochemical changes have been ambitiously developed over the years. Academically, they appear to show precision in the estimation of the PMI and more resistance against external conditions. However, still nowadays, very few of them have been applied in the daily forensic casework (4,7).

In order for a PMI method to be incorporated in the regular forensic practice, it is essential to be **precise, reliable and to give immediate results**. For an evaluation of a method's applicability, field studies are needed. According to Madea and Henssge's knowledge, **field studies of biochemical methods** for estimating TOD **are missing in literature** (3,7).

3.3. Algor mortis

Algor mortis, also known as **the post-mortem body cooling**, is defined as the process when the core body temperature decreases until it reaches equilibrium with the ambient temperature (2). This decrease is due to 4 processes: conduction, convection, radiation and evaporation (7). Algor mortis is considered for many authors one of the most useful parameters to determine PMI (2,4).

Formerly, body cooling was roughly assessed by just palpating the cadaver. With the discovery of the thermometer, a period of developing body cooling curves and mathematical formulas initiated (2).

Although the core body temperature has been measured in different sites such as the abdominal skin surface, axilla, rectum, ear and nostril; **rectum** is the most common place (9).

Rainy (10) was the first to report the behaviour of the core body temperature throughout time. Its dropping is not linear; it follows a sigmoid shape curve and it is divided in three phases: there is an initial period after death in which temperature keeps constant generally for about half to 1 hour, it is called the **Plateau period**; then, temperature drops rapidly in a second phase; and finally, this falling is slower in a

third phase. By following Newton's law of cooling, second and third phases represent the exponential dropping. The body cooling curve is represented in ***Figure 1.***

After performing several body cooling experiments under **the standard conditions of cooling** ('naked body with dry surfaces, lying extended on a thermically indifferent base, in still air'), a formula that approximates the sigmoid shape body cooling curve was described by Marshall and Hoare (11). According to them, between 0 – 3 hpm, there is a loss of 0.55ºC/hpm and between 3 – 12 hpm, the loss is 1ºC/hpm. They also state that the slow rate in body cooling is due to metabolism and heat production and it is influenced by the body surface area and body mass.

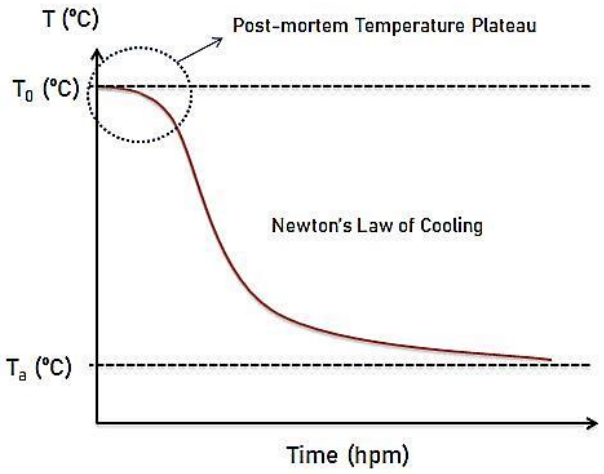


Figure 1 Sigmoidal Shape of the Body Cooling Curve. T_0 = Rectal Temperature at Death; T_a = Ambient Temperature. Adapted from (7,9)***.***

Henssge documented a **nomogram method** (8) which is based upon Marshall and Hoare's formula (11). The initial version was appropriate for ambient temperatures up to 23ºC. Then, a version for ambient temperatures above 23ºC was designed by the data reported by De Saram et al. (12). Both versions can be found in ***ANNEX, section 15.2***.

Algor mortis cannot be applied everywhere. For example, the rates of cooling established are not appropriate for hot summer seasons or tropical climates. What is more, some variables should be considered whenever Algor mortis is used in order to estimate PMI (3,4,7):

- Ambient conditions: temperature, wind, rain, humidity, snow…
- Weight of the body, mass/surface area ratio of the body.
- Body's posture (extended or thighs flexed on the abdomen).
- Presence of clothing and coverings.
- Hyperthermia or hypothermia cases.
- Place where the body remained after death.

Regarding the place where the body has died, we should be aware that the process of body cooling will be slightly different. In ***Table 3,*** it is represented the comparison of the evolution of body cooling between those subjects who have been submerged and those who have not (13).

Table 3 Comparison of the Evolution of Body Cooling between Submerged Cases and Non - Submerged Cases. Adapted from (13)**.**

Evolution	Submerged Cases	Non – Submerged Cases
hpm	°C lost / hour	°C lost / hour
0 – 12	1.6 in average	0.8 – 1.1
12 – 24	0.8 in average	0.4 – 0.5
	5 – 6 hpm the body is cold at palpation	10 – 12 hpm the body is cold at palpation
	8 – 10 hpm the body has cooled down.	20 – 24 hpm the body has cooled down.

3.4. Rectal Temperature in Estimating Post-Mortem Interval. The Henssge's Nomogram Method

A nomogram is defined as a two-dimensional diagram that is used for graphical representation of a mathematical formula (4). It consists of several lines connected from points of different scales whose intersection allows you obtain a new value (14).

Claus Henssge (8) developed a nomogram based on Marshall and Hoare's formula (11) in order to estimate PMI. It is known as **The Henssge's Nomogram Method** and its main variable is the rectal temperature's measurement alongside other processing variables, such as ambient temperature and body weight.

The Henssge's Nomogram takes into account the fact that the only factor influencing the exponential dropping of core body temperature is body weight. It was based upon **the standard conditions of cooling** established by Marshall and Hoare (11). After performing extensive body cooling experiments under different conditions, corrective factors for body weight were developed. Thus, the nomogram can also be used for **non – standard** cases (7). Those empirical **corrective factors** for a reference body weight of 70 Kg and their correspondent factor for the real body weight are represented in ***Table 4 and 5***, respectively.

Table 4 Empirical Corrective Factors for a Reference Body Weight of 70 Kg. Adapted from *(7)***.**

DRY CLOTHING / COVERING	IN AIR	CORRECTIVE FACTOR
naked	moving	0.75
1-2 thin layers		0.9
naked	still	1.0
1-2 thin layers		1.1
2-3 thin layers		1.2
1-2 thicker layers	without influence	
3-4 thin layers		1.3

more thin / thicker layers			1.4
thick blanket			1.8
thick blanket + clothing combined			2.4
WET CLOTHING/COVERING	**IN AIR**	**IN WATER**	**CORRECTIVE FACTOR**
naked		flowing	0.35
naked		still	0.5
naked	moving		0.7
1-2 thin layers	moving		0.7
≥2 thicker layers	moving		0.9
2 thicker layers	still		1.1
>2 thicker layers	still		1.2

Table 5 Dependence of Corrective Factors on Body Weight. Adapted from (7).

	REAL BODY WEIGHT (Kg)																	
	4	6	8	10	20	30	40	50	60	70	80	90	100	110	120	130	140	150
CORRECTIVE FACTORS										1.3								
	1.6	1.6	1.6	1.6	1.5					1.4					1.3	1.2	1.2	1.2
	2.1	2.1	2.0	2.0	1.9	1.8				1.6				1.4	1.4	1.4	1.3	1.3
	2.7	2.7	2.6	2.5	2.3	2.2	2.1	2.0		1.8			1.6	1.6	1.6	1.5	1.4	1.4
	3.5	3.4	3.3	3.2	2.8	2.6	2.4	2.3		2.0		1.8	1.8	1.7	1.6	1.6	1.5	1.5
	4.5	4.3	4.1	3.9	3.4	3.0	2.8	2.6	2.4	2.2	2.1	2.0	1.9	1.8	1.7	1.7	1.6	1.6
	5.7	5.3	5.0	4.8	4.0	3.5	3.2	2.9	2.7	2.4	2.3	2.2	2.1	1.9	1.9	1.8	1.7	1.6
	7.1	6.6	6.2	5.8	4.7	4.0	3.6	3.2	2.9	2.6	2.5	2.3	2.2	2.1	2.0	1.9	1.8	1.7
	8.8	8.1	7.5	7.0	5.5	4.6	3.9	3.5	3.2	2.8	2.7	2.5	2.3	2.2	2.0	1.9	1.8	1.7
	10.9	9.8	8.9	8.3	6.2	5.1	4.3	3.8	3.4	3.0	2.8	2.6	2.4	2.3	2.1	2.0	1.9	1.8

According to Madea (7), the following steps must be taken in order to apply the Henssge's Nomogram:

1. **Inspection** of the crime scene, examination of the body, its posture, subject's clothing/covering, windows (e.g. are they open or closed?), radiators…
2. **Measurement of the ambient temperature** close to the body and at the same level (10 – 20 cm above the base). Determine whether thermic conditions have differed since the body was found.
3. **Measurement of the deep rectal temperature** (at least 8 cm within the anal sphincter) at the scene using a calibrated electronic thermometer.
4. **Estimation of body weight**. At the autopsy, determine if the estimation was correct.
5. **Evaluation of corrective factors**. For rectal temperature, the relevant factors are the ones concerning the lower trunk of the corpse.
6. Use the **Nomogram**:
    a. Connect the points on **the scales of rectal and ambient temperatures** by a straight line. See that it crosses the **diagonal line** of the nomogram.

b. Draw a second straight line (in red) that starts at the centre of **the circle** situated at the bottom left of the nomogram and crosses the point where the first line (in blue) intersects with the black diagonal line of the nomogram.
c. By looking body's coverings and the place where the body was found, select the appropriate corrective factor.
d. Multiply the victim's weight by the corrective factor. Round the result to the nearest value of the **weight's scale** of the nomogram if it is necessary.
e. Choose the weight's scale that is nearer to the second straight line.
f. Follow up or down the weight – value's column and choose the value where the second straight line is crossing. This value represents the **time since death**.
g. The curved scale from the outside of the nomogram represents the **standard deviation's values**. We will choose the value that has been crossed by the second straight line.

A graphical example (***see Figures 2 and 3***) will help the reader understand how the Henssge's Nomogram actually works.

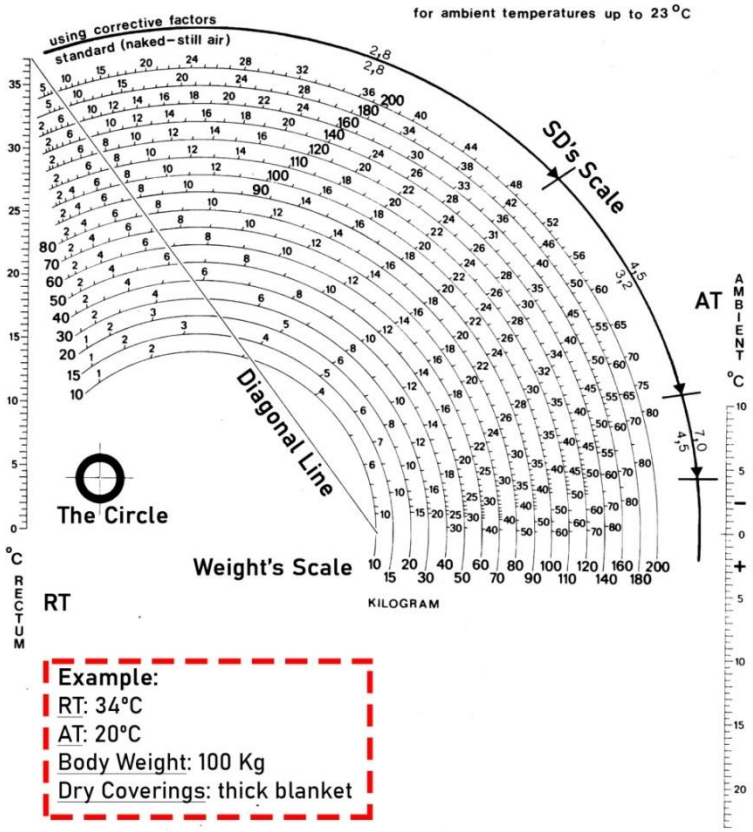


Figure 2 Application of the Henssge's Nomogram Method (Part 1). RT = Rectal Temperature; AT = Ambient Temperature; SD = Standard Deviation.

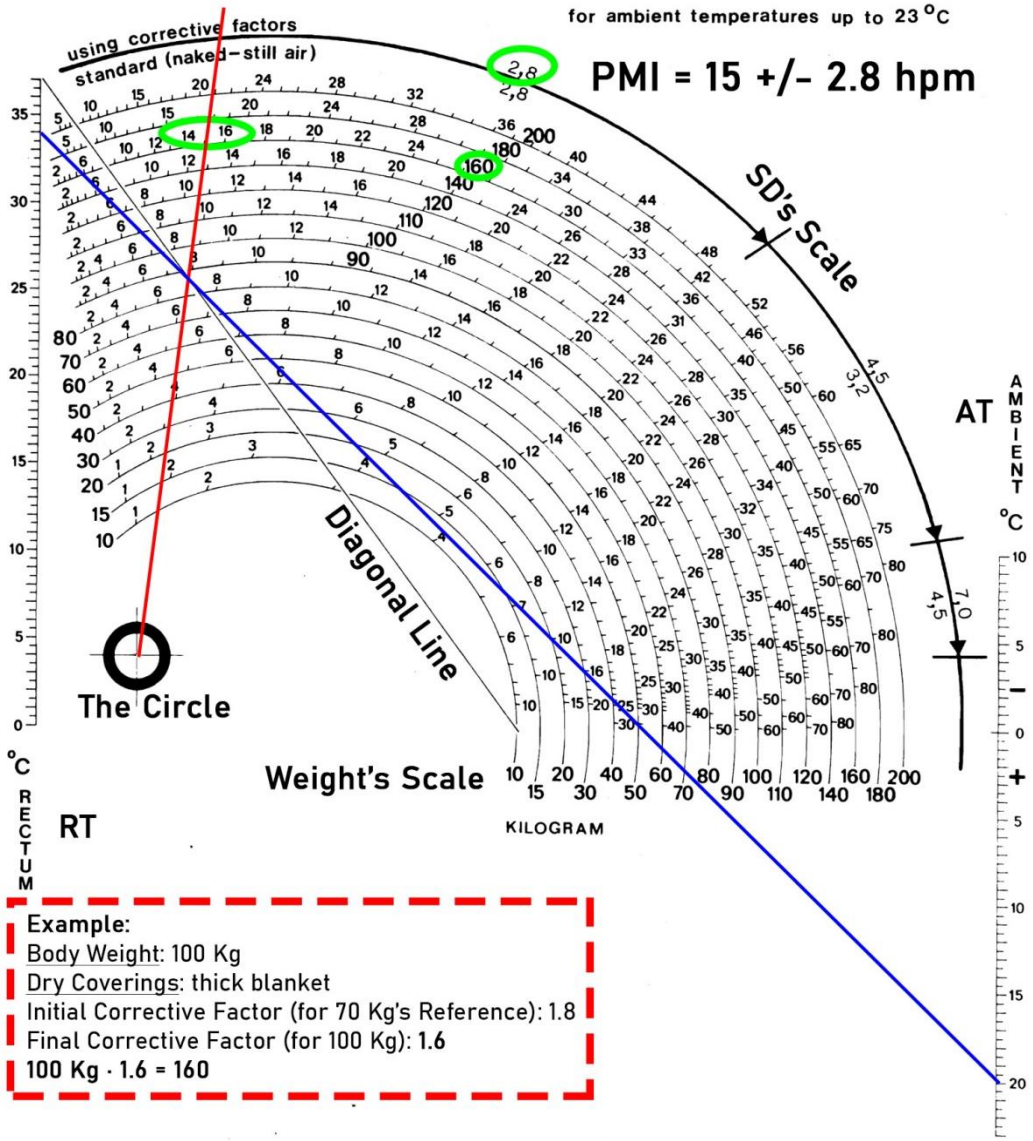


Figure 3 Application of the Henssge's Nomogram Method (Part 2). RT = Rectal Temperature; AT = Ambient Temperature; SD = Standard Deviation.

According to Henssge (8), there are 5 conditions where the nomogram must not be used:

1. Strong radiation.
2. Suspicion of general hypothermia.
3. The place where the body was found is not the same as the place of death.
4. Uncertain severe changes of the cooling conditions during the period between the TOD and the time of examination.
5. Unusual cooling conditions without any experience of a corrective factor.

3.5. Tanatochemistry: the Analysis of Vitreous Humour

Tanatochemistry or the 'chemistry of death', studies all the biochemical changes that occur after death (15).

Performing **objective-quantitative** determinations of a chemical component's concentration in a fluid compartment in a moment after death (when autolysis has already started) is the basis of biochemical methods in order to estimate the time since death (7).

So far, biochemical tests have had little influence in forensic pathology due to several reasons, such as the lack of trust in the available scientific literature on these topics or the medical examiner's ignorance about the potential possibilities that those methods can offer in order to solve some forensic questions (e.g. cause of death, TOD…) (16).

In a similar way to the **compound method**, biochemical methods to determine PMI should not be used alone in the regular forensic practice (16,17).

To pursue a biochemical method, the ease of access and the preservation from the action of post-mortem processes should be characteristics that must have the ideal fluid compartment that we are going to analyse (16). **Vitreous humour** (VH), a colourless transparent gel that occupies the vitreous chamber of the eyeball, is considered to have relatively isolation from bacterial contamination and general availability during post-mortem examination (15,18–20). In terms of accessibility, according to Luna and colleagues' experience (16), VH would be the easiest compartment to collect, whereas the cerebrospinal fluid (CSF) would be the most difficult.

Briefly, we can divide the eyeball in three fluid compartments: the anterior and posterior chambers which comprise the aqueous humour; and the vitreous chamber which comprises **the vitreous humour** (normal volume ≅ 4.5 mL) (21). An anatomical representation of the eyeball can be seen in ***Figure 4***.

Naumann (22) introduced for the first time the analysis of post-mortem chemical changes within the intraocular fluid compartment. Since then, many biochemical parameters of VH have been studied for different purposes. These post-mortem applications are shown in ***Table 6***.

Table 6 Post-mortem Applications of Different VH's Analytes. Hx = Hypoxanthine, Na+ = Sodium. Adapted from (20,23).

VH's Analytes	Post-mortem Applications
K+	Estimation of the PMI. [K+]>15 mmol/L suggests post-mortem decomposition
Hx	Estimation of the PMI
Ammonia	Estimation of the PMI
Na+	Salt water drowning, Dehydration, Hyper/Hyponatremia
Cl-	Salt water drowning, Dehydration
Creatinine	Renal failure, High protein intake, Large muscle mass, Heat shock
Glucose plus Ketones	Diabetic Ketoacidosis, Hyperglycaemic Hyperosmolar State, Stress response
Lactate	Interpret in conjunction with vitreous glucose
Urea nitrogen	Renal failure
Ethanol markers (ethyl glucuronide and ethyl sulphate)	Ante-mortem alcohol consumption
Cocaine	Drug related death

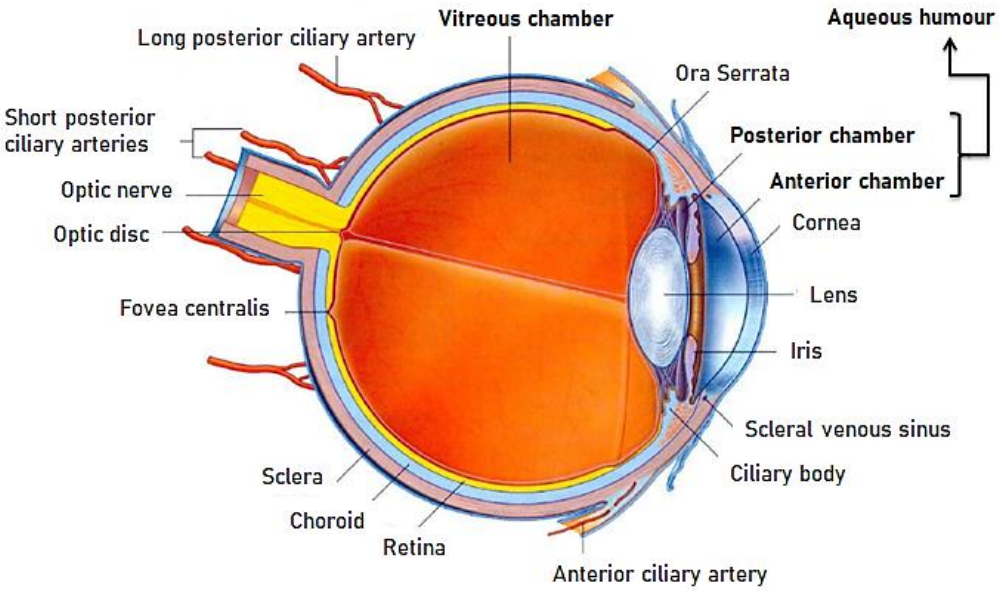


Figure 4 The Eyeball. Adapted from (21).

According to Coe (18), vitreous humour's samples must be obtained during the early post-mortem period, when its appearance is crystal-clear and colourless, before the onset of putrefaction, when VH adopts a cloudy and brownish appearance. The large-gauge needle combined with a small syringe should be gently inserted through the sclera, until it reaches the centre of the globe. Then, suction will be applied gradually and carefully avoiding blood or retinal cell contamination. About 2 – 3 mL/eye will be normally extracted in adults. All the available VH should be withdrawn due to the varying concentration throughout the globe of the eye.

3.6. Vitreous Humour's Potassium Concentration in Estimating Post-Mortem Interval

The **increase of potassium concentration** ([K+]) that occurs after death is the most deeply studied parameter for estimating the PMI (20). **Sturner** (24) was the first person to describe it and along with Gantner (25) were the first to discuss it. According to them, the majority of the [K+] existing in the vitreous chamber after death is the result of the diffusion of K+ from the retina cells, and in a minor extent, from the posterior capsule of the lens.

During life, an active transport mechanism keeps a balance between plasma potassium in the retinal vessels and the vitreous humour of the eye. Soon after death, the Na+/K+ ATPase pumps' breakdown along with the loss of cell-membranes' permeability lead to equilibrium of electrolytes' concentrations. Potassium exits from the cells to extracellular compartments (e.g. the **vitreous chamber**) (18,26).

By studying the behaviour of [K+] in the VH's compartment throughout time, many PMI formulae have been developed over the years (some of them are described in ***ANNEX, section 15.3***).

Many authors (25,27–35) concluded that [K+] follows **a linear rise** after a post-mortem period of a few days, in the early PMI. However, Zilg et al. (36) stated that this linear behaviour becomes non-linear, asymptotic, after 5 dpm.

PMI formulae have shown that potassium can become a useful parameter in terms of estimation of the early PMI; however, there is no consensus regarding the maximum PMI where the determination of VH's [K+] is still reliable. Few studies have assessed the analysis of VH in estimating the late PMI because the correlation gets weaker, probably due to putrefaction and volume changes at those times (36).

Characteristics of the two PMI formulas used in the present project are represented in ***Table 7***.

Table 7 Characteristics of the Two PMI Formulas used in the Present Research Project.

Muñoz et al. (30)	**Zilg et al.** (36)
Developed in Spain, in 2001.	Developed in Sweden, in 2015.
Only forensic cases were included.	A large sample's size (N= 462 cases).
Max PMI = 40 hpm	Max PMI = 409 hpm ≅ 17 dpm
There was a changing in variables: [K+] was considered the independent, and the PMI was considered the dependent variable. This change makes the estimated time of death more accurate.	The PMI formula was adjusted for variables 'Age of the deceased' and 'Ambient temperature'.

PMI Formula: $PMI = 3.92[K^+] - 19.04$	Due to the complexity of the proposed PMI formula, a website was created in order to facilitate the PMI calculation: https://slbd.shinyapps.io/pmiPredictor/

In addition to the PMI Formulas, Muñoz et al. (37) developed a PMI-Calculator, an R code-based software compatible with Windows, Mac and Linux operating systems. In order to run the PMI estimation, potassium (K), urea (U) and hypoxanthine (Hx) concentrations are needed. A screenshot of the software is represented in ***Figure 5***.

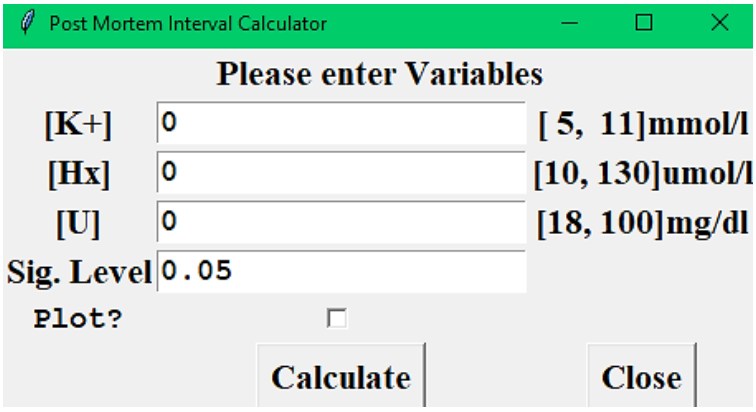


Figure 5 Screenshot of the PMI-Calculator, Developed by Muñoz et al. (37)

Different variables have been described by some authors as potential influential factors on the estimation of PMI by [K+] determination and, thus, they should be taken into account:

- Health conditions: renal failure, diabetes mellitus (36,38).
- Ambient temperature, morgue's room temperature – higher temperatures (34,36,38–42).
- Age of the deceased (31,36,43).
- The agonal period before death(36,38).
- Alcohol level at the time of death (38).
- Sampling method, instrumentation, pre-analytical, analytical and post-analytical treatment (32,44–47).

4. JUSTIFICATION

Estimating the PMI is a routine forensic task fundamentally transcendent for **violent deaths,** in particular the homicidal and accidental. In front of a homicide, a police investigation will take place having the estimated TOD as a reference for validation of witnesses' statements and the suspects' alibis. As for accidental cases involving various victims who are somewhat related, establishing the death sequence will be important for heritage purposes.

The present study is going to compare **two objective-quantitative-early-PMI estimators** between each other and with **the exact PMI**, the Gold Standard, which is going to be determined by knowing the subject's real TOD.

The **Henssge's Nomogram method** is based on the post-mortem body cooling. It requires knowing the corpse's **rectal temperature** mainly, along with other processing variables such as ambient temperature, body weight and body's coverings. It is considered to be the most accurate and reliable estimator of the PMI in comparison of other methods that are used in the regular forensic practice.

Academically, new promising **biochemical methods** have been studied. **VH's potassium concentration** has become the most studied parameter to estimate PMI by biochemical means. However, still now, it has not been incorporated in the regular forensic practice. In order to do so, **forensic field studies** are needed. That is the reason why **we want to compare both methods in a real forensic environment**.

PMI Formulas, which extrapolate the PMI from the analysis of the VH's potassium concentration, have been ambitiously developed over the years. Due to the fact that there is not a universal formula, we decided to choose the two best PMI formulas for our project, the one developed by Muñoz et al. (30) and the one developed by Zilg et al. (36).

Concerning the relevance on executing the present project, if results are positive in favour of the biochemical method, we strongly believe that we will put the analysis of vitreous humour into a new perspective and more efforts into this line of research will be made.

5. HYPOTHESES

- The analysis of vitreous humour and the Henssge's Nomogram method are accurate to determine the early Post-Mortem Interval in violent deaths occurred within the *Institut de Medicina Legal i Ciències Forenses de Catalunya'*s jurisdiction.

- The analysis of vitreous humour is more accurate than the Henssge's Nomogram method to determine the early Post-Mortem Interval in violent deaths occurred within the *Institut de Medicina Legal i Ciències Forenses de Catalunya'*s jurisdiction.

6. OBJECTIVES

- To evaluate the accuracy of the determination of potassium concentration of vitreous humour and the rectal temperature's measurement using the Henssge's Nomogram method in estimating the early PMI in violent deaths occurred within the IMLCFC's jurisdiction.

- To evaluate whether the determination of potassium concentration of vitreous humour is more accurate than the rectal temperature's measurement using the Henssge's Nomogram method in estimating the early PMI in violent deaths occurred within the IMLCFC's jurisdiction.

7. METHODOLOGY

7.1. Study design

A cross-sectional, correlational study design will be used.

7.2. Study population

This study is going to be conducted with violent deceased subjects admitted to any of the 5 divisions of the IMLCFC (Barcelona, Girona, Lleida, Tarragona, Terres de l'Ebre) during 12 months.

Violent death includes suicidal, homicidal and accidental cases. In order to avoid problems related with the N of the sample, suicidal cases will not be excluded from this project. However, we must take into consideration that a precise estimation of the PMI in this scenario is not as transcendent as in the two other types of violent death, homicidal and accidental cases.

Table 8 Inclusion and Exclusion Criteria.

Inclusion Criteria	Exclusion Criteria
TOD is exactly known. - The death was witnessed. - The victim's wrist-watch stopped ticking. - Video cameras recorded the moment of death. - Death occurred at the hospital.	Those cadavers with either damaged eyeballs or rectum tract.
	Cases of hypothermia/hyperthermia.
	Drowning cases.
Recent death (<24 hpm).	
Adults (≥18 years old).	
Ambient temperature between 0 and 35°C.	
Rectal temperature > Ambient temperature.	
Place where the body was found is the same where death occurred.	

7.3. Sampling

A consecutive sampling will be carried out.

7.3.1. Sample's size

According to Dr. Josep Ramis i Pujol's expertise, in about 10% of the autopsies done in the IMLCFC's division of Girona during a year correspond to cases where TOD is exactly known and with a high probability to fit the characteristics in order to be part of this project. Therefore, if we take into account the following:

- In Barcelona, 2200 autopsies are done in a year; a 10% of this value is 220 cases.
- In Girona, 550 autopsies are done in a year; a 10% of this value is 55 cases. For the rest of the divisions, a similar estimation can be approached.

Adding all the values from each division, we have a total of **440 cases** [220 + (4*55)].

This sample's size, N = 440, with a risk alpha of 5%, in a bilateral contrast, and assuming that the accuracy of both tests is moderately high (>0.7), the statistical power (1-β) is 98.73%.

Due to the fact that we have a really high statistical power and with just a minimum of 80% is required, in order to reduce costs, an N = 196 is calculated taking into account this new percentage. Approximately, there will be 40 cases per each division (196/5 ≅ 40).

Computations were carried out with the Prof. Dr. Marc Saez's software based on the library pwr of the free statistic environment R (version 3.6.2).

7.4. Variables

7.4.1. Main Variables

Exact PMI:

It is our Gold Standard (GS), our reference. We are going to calculate it by deducting from the exact TOD the time of the dead body's examination (t). Variable 't' is going to be established as the time the forensic doctor is about to start collecting rectal temperature and samples of vitreous humour.

$$\boldsymbol{GS = TOD - t}$$

Potassium concentration of vitreous humour:

We are going to extract samples of vitreous humour, of both eyes if possible, with a sterilized 10 mL syringe with 30-gauge needle (1 syringe-needle / eye). In order to determine [K+], an **Indirect Potentiometric determination** will be performed in the Department of Biochemistry of Hospital Clínic (Barcelona). The machine in order to perform the analyses is **A-LYTE Integrated Multi-Sensor (IMT Na K Cl),** with a sensitivity or limit of detection <1 mmol K+/L and its repeatability is less than 1%. A more detailed description of the machine can be found in ***ANNEX, section 15.4***.

Rectal temperature:

We are going to measure the deep rectal temperature, at least 8 cm within the anal sphincter, with a calibrated digital thermometer with a fixed stainless steel sensor probe. The chosen model is **Digital Thermometer With Probe Sensor TP3001**, with a range between -50ºC and 300ºC, a resolution of 0.1ºC and an accuracy ±1ºC from -50ºC to 200ºC and ±3ºC from 200ºC to 300ºC. A more detailed description of the rectal thermometer can be found in ***ANNEX, section 15.5***.

Early PMI:

It is going to be estimated by two different methods. On one hand, by using the **PMI Formula**, the one developed by Muñoz et al. (30) and the one developed by Zilg et al. (36), with the parameter "[K+] of vitreous humour"; and, on the other hand, by using the **Henssge's Nomogram method**, developed by Henssge (8), with the parameter "Rectal Temperature" alongside other processing variables which are detailed in ***section 7.5***.

Main variables are summarized in ***Table 9.***

Table 9 Main Variables.

MAIN VARIABLES		
Variable	**Type**	**Units**
Exact PMI	Quantitative Continuous	hpm
[K+] of Vitreous Humour		mmol/L
Rectal Temperature		ºC
Early PMI		hpm

7.4.2. Covariables

Age:

It is going to be determined either because relatives or police investigators tell you so or because you possess his or her ID card *(Documento Nacional de Identidad, DNI).*

This quantitative variable is going to be categorized using quartiles (P25, P50 and P75).

Sex: determine the sex of the cadaver.

Centre of origin: determine the division of the IMLCFC where the cadaver belongs to.

Covariables are summarized in ***Table 10.***

Table 10 Covariables.

COVARIABLES		
Variable	**Type**	**Units**
Age	Quantitative Continuous / Qualitative Polytomous Ordinal	Years old / Age groups
Sex	Qualitative Dichotomous Nominal	Male / Female
Centre of origin	Qualitative Polytomous Nominal	IMLCFC (Barcelona) IMLCFC Divisió de Girona IMLCFC Divisió de Lleida IMLCFC Divisió de Tarragona IMLCFC Divisió de Terres de l'Ebre

7.5. Process of data collection

A common scenario is going to be cases where a temptation to perpetration of a violent type of death has taken place. Eventually, the subject dies at the hospital and, consequently, the **exact TOD is known**. The following process of data collection is considering those cases as an example, nevertheless in every other situation where TOD is exactly known, the same steps could be perfectly applied.

When death occurs at the hospital, the Judge of their jurisdiction is the first person the patient's medical doctor contacts. Frequently, in those cases, the funeral home transfers the corpse to the IMLCFC directly, although, theoretically, in front of any violent death, the Judge must call the medical examiner on duty first in order to go to the place where death has occurred and have a preliminary view of what has happened. Taking into account that those are potentially cases for our study, a coordination meeting with all the Senior Judges of each Judicial Party of Catalonia will take place.

When the forensic doctor on duty (who will be known, from now on, as **'Forensic Doctor A'**) arrives at the crime scene (in this case the hospital) will make a preliminary assessment of the body to decide whether it can be a subject for our study or not (see ***Inclusion and Exclusion criteria*** from ***section 7.2***).

Forensic Doctor A will be responsible to fill all the required data which has been systematically detailed in **Data Collection Sheet 1** (see ***ANNEX, section 15.7***).

He or she will firstly record the ambient temperature near the corpse and at the same level with a common Weather Station (a more detailed description of the device can be found in ***ANNEX, section 15.6***), a processing variable needed for the Henssge's Nomogram. Then, a **Nomogram Corrective Factor** will be chosen from the table provided in the Data Collection Sheet 1 taking into account **the body lower trunk's coverings** and its **weight** (an estimation will be used provided that the weight's value is not available; later on, during the autopsy, this estimation will be verified).

Then, he or she will calculate the **Exact PMI** by deducting from the exact TOD the time of the dead body's examination. Soon after, he or she will record the **deep rectal temperature** by placing the calibrated digital rectal thermometer at least 8 cm within the anal sphincter. In order to avoid miscalculations, three measurements in a row will be done and their arithmetical mean will be the value filled in the correspondent blank of the Data Collection Sheet. Immediately afterwards, Forensic Doctor A will gently extract a **sample of vitreous humour** of the eye through its sclera (from both eyes if possible) with a sterilised 10 mL syringe with 30-gauge needle (the standard size used by ophthalmologists for intravitreal injections). The syringes used will have a plug to facilitate its storage and transportation. Vitreous humour's samples

will be correctly labelled (main items: 'subject's personal data', 'a [K+] determination is needed', 'results must be send to Forensic Doctor A – contact information') and stored at -20ºC within a maximum of 30 days. They will be stored alongside other biological samples from other autopsies in order to be sent to the Laboratory of the IMLCFC (Barcelona). There, the ones regarding a biochemical analysis will be transported to the Biochemical Department of Hospital Clínic (Barcelona).

The moment the Forensic Doctor A has duly completed Data Collection Sheet 1, he or she will hand it to the Sub-director of the Division who will keep it safely in a separated box. Then the Sub-director will momentarily hand **Data Collection Sheet 2** (see ***ANNEX, section 15.8***) to Forensic Doctor A in order to fill a few repeated items from Data Collection Sheet 1.

When the results of [K+] determination arrive (normally, after 2 weeks, although each indirect potentiometric determination lasts 60 minutes), Forensic Doctor A will hand them to the Sub-director who will submit this recent data to the correspondent Data Collection Sheet 2. Afterwards, this Data Collection Sheet will be handed to another forensic doctor (who will be known as '**Forensic Doctor B**').

Forensic Doctor B will be blinded from the Gold Standard, in our case, the Exact PMI. He or she will have to determine the **Early PMI** by applying the PMI Formulas on one hand and the Henssge's Nomogram method on the other. As for the **PMI Formula**, for the one designed by **Muñoz** et al. (30) there will be a mathematical calculation, and for the one designed by **Zilg** et al. (36) the required values ([K+], age of the subject, average ambient temperature) will be entered into the Zilg's website that was created in order to facilitate the calculation. An exhaustive explanation of how the **Henssge's Nomogram** works has already been described in the ***Introduction, section 3.4***.

Forensic Doctor B, after duly completing Data Collection Sheet 2, will hand it to the Sub-director who will keep it safely in a separated box alongside the correspondent Data Collection Sheet 1.

In order to coordinate all those instructions, a meeting with the Director and all the Sub-directors of each division of the IMLCFC will take place. Emphasis on having periodic calibration of thermometers will be done.

The Principal Investigator (PI) and the Research team will be always available in order to solve any doubt regarding the Data Collection Process. A contact number and email will be given to all the divisions of the IMLCFC.

At the end of the Data Collection Process, all Data Collection Sheets will be revised and collected by the PI and the Research team. They will send them to a Qualified Statistician in order to pursue the appropriate statistical analyses.

8. STATISTICAL ANALYSES

8.1. Descriptive analysis

For **main variables** and **covariable 'Age',** results will be summarized in means and SD or in medians and IQR, depending on the distribution's symmetry. Either histograms or box plots are going to be used to represent the values graphically.

For the **rest of covariables,** which are qualitative, results will be expressed in proportions and in percentages. Either a sector graph or a bar graph will be used to represent the values.

A **scatterplot** for each pair, GS|T1, GS|T2, GS|T3, T1|T2, T1|T3, T2|T3; will be represented. GS (the Gold Standard) corresponds to the variable 'Exact PMI'; T1 (Test 1) corresponds to the variable 'Early PMI determined by [K+] of vitreous humour with the PMI Formula designed by Muñoz et al. (30)'; T2 (Test 2) corresponds to the variable 'Early PMI determined by [K+] of vitreous humour with the PMI Formula designed by Zilg et al. (36)'; and T3 (Test 3) corresponds to the variable 'Early PMI determined by rectal temperature'.

Pearson's Correlation Coefficient (r) will be calculated for each pair. A value near 1 means there is a perfect correlation between variables; whereas a value near 0 means there is no correlation.

8.2. Bivariate analysis

For each pair, GS|T1, GS|T2, GS|T3, T1|T2, T1|T3, T2|T3; having a null hypothesis of r = 0 and an alternative hypothesis of r = 1, a **Chi-squared test (χ2)** will be performed.

We will stratify for covariables, age group, sex and centre of origin; to control for confounding.

8.3. Multivariate analysis

First of all, 6 variables of difference are going to be created:

- DIF1 = GS – T1
- DIF2 = GS – T2
- DIF3 = GS – T3
- DIF4 = T1 – T2
- DIF5 = T1 – T3
- DIF6 = T2 – T3

Using each of the 6 differences as dependent variables and taking into account that they are all quantitative continuous, we are going to adjust them in a **Lineal Regression Model**, controlling for covariables to avoid confounding.

If the difference between each test with the Gold Standard and between each other were only caused by randomness, none of the estimators of the regression coefficients would be significant; they would not be different from 0.

The adjusted correlation is going to be derived from this lineal regression.

9. ETHICAL ASPECTS

The present study will be presented to *Comissió de Docència i Investigació de l'IMLCFC* in order to request and obtain authorization to pursue the research.

The present study complies with the principles of the Declaration of Helsinki of the World Medical Association (WMA) on human research requirements.

In terms of Personal Data Protection, according to Article 3 of *Ley Orgánica 3/2018, 5 diciembre, de Protección de Datos Personales, garantía de los derechos digitales (BOE núm. 294, de 6 de diciembre de 2018);* relatives of the deceased as well as the respective heirs/heiresses can request access to his or her personal data, except those who the deceased has expressively declined it to.

Informed consent will not be requested to any of the subject's relatives, as all the processes belong to the regular clinical forensic practice.

All data of every subject participating in this project will be treated as any other corpse admitted in any of the IMLCFC's divisions.

10. STUDY LIMITATIONS

The goal of this project is to compare two methods. Because of the fact that we are applying them into the same corpse, the required **inclusion-exclusion criteria** imply items that affect both methods. This might seem a limitation in our study in terms of the sample size. However, the major proportion of the N are going to be cases that end up dying at the hospital which are the ones that with a very high probability will meet the criteria in order to be part of this project. Consequently, a meeting with all the Senior Judges of each Judicial Party of Catalonia will be fundamental in order to avoid losses of potential cases due to the fact that in the real forensic practice they are frequently transferred to the IMLCFC directly.

Due to variations on the body cooling process, **submerged cases** were decided not to be included.

As we want to develop a field study, in other words, we want to apply both methods during the regular forensic practice in order to be as nearer to real conditions as possible, a **consecutive sampling** is needed. By performing this kind of sampling we might not obtain a representative sample of the population. However, by executing a multicentre study, we will diminish this limitation.

Although we initially had an **N** of 440 cases / year with a statistical power of 98,73%, in order to minimize costs, we recalculated the N taking into account the minimum statistical power required (80%). This new N of 196 cases / year will be distributed in the different divisions of the IMLCFC. So, having reduced the expected N for one year, we strongly believe that the established **duration of the data collection process** will be optimal.

Assessing **interactions** in a study implies doubling the N for each interaction and, consequently, increasing costs. Although we think it could be interesting to know the **influence of covariables** as sex, age, body weight or cause of death (e.g. hanging, traffic accidents, fallings or intoxications), we have not included them as our secondary objectives. In this project, we want to focus on assess the correlation between both methods against the Gold Standard first, before doing anything else.

Guidelines of studies that evaluate the accuracy of diagnostic tests emphases on the fact that the investigator who knows the result of the Gold Standard has to be different from the one who will apply the tests under discussion. In the present project, this aspect will be reflected on the **blinding** of Forensic Doctor B **from the Exact PMI**.

We are aware that almost every medical examiner possesses in his or her equipment a thermometer. However, in order to avoid **systematic errors** in rectal and ambient temperatures' measurements, every division of the IMLCFC will have the

same digital thermometer and weather station. What is more, during the meeting with the Director and Sub-directors, both emphasis on periodical calibration of the devices and training on how to perform those measurements including the extraction of samples of vitreous humour, the application of the Henssge's nomogram and the PMI formulas will be done. Every step that a forensic doctor will have to take during the Data Collection Process will be accurately explained to the Director and Sub-directors and it will be documented in order to be available for any forensic doctor who wants to revise them. Obviously, the PI and the Research team will always be available if there is any doubt.

In terms of [K+] determination in VH's samples, it remains unclear which the best analytical instrument is. At the Biochemical Department of Hospital Clínic (Barcelona), the multi-sensor used for **indirect potentiometric determinations** is specially designed for plasma, serum or urine samples. However, the Laboratory Personnel recommended this option as the best one for our study.

Unfortunately, nowadays there is not a universal **PMI formula regarding [K+]** yet. Out of the many formulae available, for this project we have selected the two formulas that give quite reliable results in Europe: the one designed in Spain in 2001 by Muñoz et al. with an N of 133 forensic cases only (30); and the one designed in Sweden in 2015 by Zilg et al. with an N of 462 cases and it was adjusted for variables of age and ambient temperature (36).

Possible **confounding** will be taken into account during the statistical analyses.

Because of the fact that in order to **use a method in the regular forensic practice** has to accomplish three basic principles, to be precise, to be reliable and to give immediate results; specially for the latter one, we are aware that even if we success in showing that the analysis of vitreous humour is more precise than the Henssge's nomogram method, at this precise moment we will not be able to apply the new method in a daily basis. This project aims to put the analysis of vitreous humour into perspective. If the results of this project are positive in favour of the biochemical method, we strongly believe that efforts should be made into this line of research. and, luckily, in a near future, there is a device that allows you to get instant analyses of [K+] of vitreous humour, as it has already been invented a device that gets instant [Fe^{2+}] of vitreous humour (48).

11. WORK PLAN

Stage 0: Study Design. 6 months (20th November 2019 – 30th April 2020).

Task 1: Literature Research and Protocol Design. 3 months (from 20th November 2019 till 27th January 2020). It has been done by the PI and the Research Team.

Task 2: Operative Protocol Design. 3 months (from 1st February 2020 till 30th April 2020). It will be done by the PI and the Research Team.

Stage 1: Ethical Approval. 1 month (1st May 2020 – 31st May 2020).

Task 3: Ethical Assessment of the Protocol. It will be done by *Comissió de Docència i Investigació de l'IMLCFC*.

Stage 2: Coordination Meetings and Acquisition of the Necessary Resources. 1 month (1st June 2020 – 30th June 2020).

Task 4: Coordination Meeting with the Director and Sub-directors of each division of IMLCFC. 1 day. It will be conducted by the PI alongside the Research Team.

Task 5: Coordination Meeting with the Senior Judges of each Judicial Party of Catalonia. 1 day. It will be conducted by the PI alongside the Research Team.

Task 6: Acquisition of the Resources (see ***Section 13, Material Expenses***) that will be necessary in order to do the proper Data Collection Process. 28 days. It will be directed by the PI.

Stage 3: Data Collection Process. 12 months (1st July 2020 – 30th June 2021).

Task 7: Filling the two Data Collection Sheets. It will be done by Forensic Doctors from IMLCFC.

Task 8: Laboratory Analyses. It will be done by the Laboratory Personnel from the Biochemical Department of Hospital Clínic (Barcelona).

Stage 4: Statistical Analyses and Interpretation of the Results. 2 months (1st July 2021 – 31st August 2021).

Task 9: Data Processing and Statistical Analyses. 1 month and 2 weeks (from 1st July 2021 – 15th August 2021). It will be done by a Qualified Statistician.

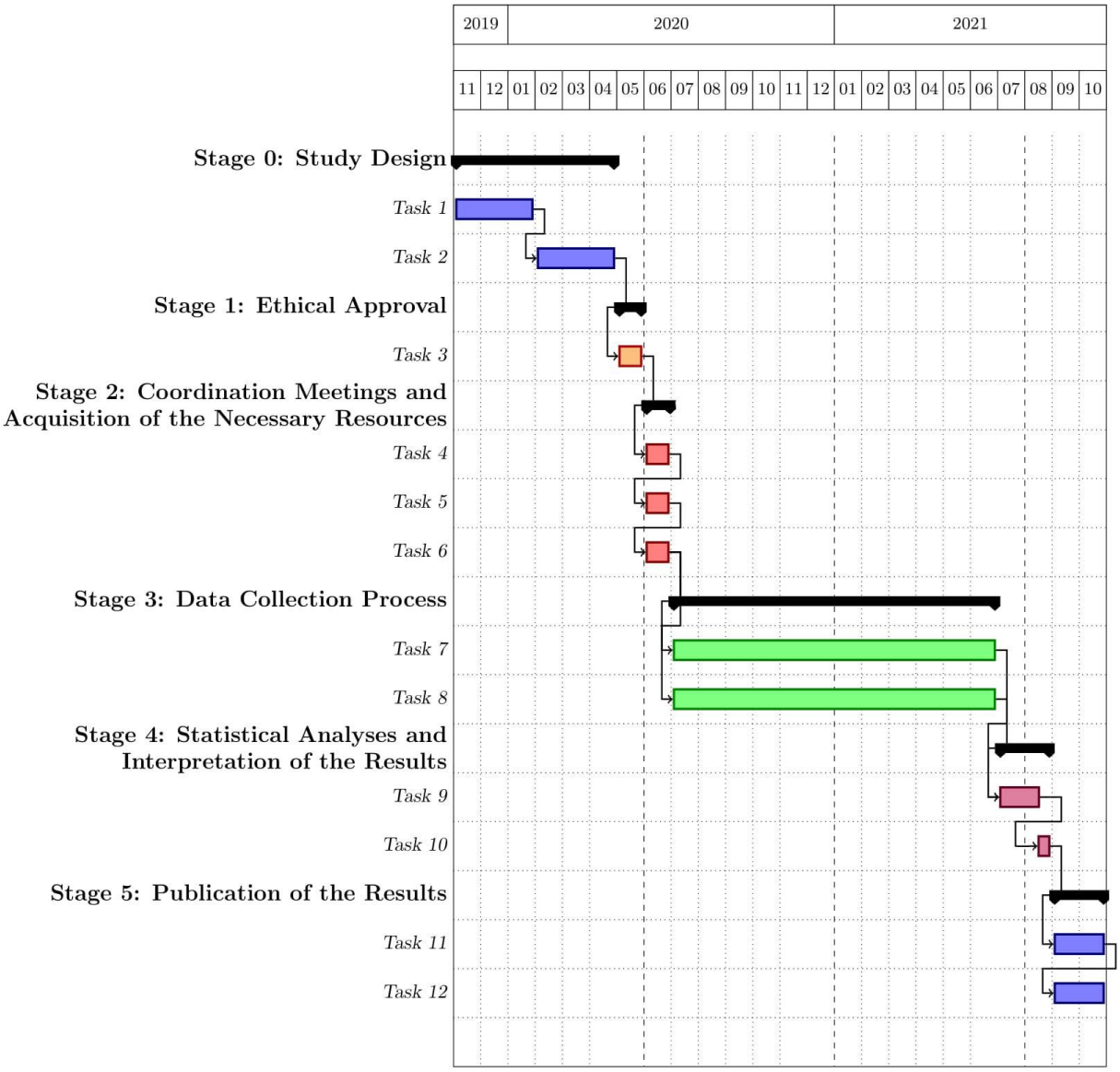
Task 10: Report Elaboration of the Statistical Results. 2 weeks (from 16th August 2021 – 31st August 2021). It will be done by a Qualified Statistician alongside the PI and the Research Team.

Stage 5: Publication of the Results. 2 months (1st September 2021 – 31th October 2021).

Task 11. Elaboration of the Final Report. It will be done by the PI and the Research Team.

Task 12. Submitting the Final Report into Forensic Journals.

12. CHRONOGRAM



13. BUDGET

PERSONNEL EXPENSES	Time Worked (h)	€/h		Total (€)
Qualified Statistician	30	30		900
MEETINGS	**Number of Attendants**	**€/Transport/Person**	**€/Menu/Person**	**Total (€)**
Coordination Meeting with the Director and Sub-directors of each division of IMLCFC (Barcelona city, Barcelona region, Girona, Lleida, Tarragona, Terres de l'Ebre).	7	80	20	700
Coordination Meeting with the Senior Judges of each Judicial Party of Catalonia.	49			4.900
MATERIAL EXPENSES	**Description**	**Units**	**€/Unit**	**Total (€)**
Digital Thermometer With Probe Sensor TP3001	Fixed probe made of stainless steel, 145 mm long. Temperature unit °C/°F. Range: -50 +300°C (-58 + 572°F), resolution 0.1°C/°F and accuracy ±1°C from -50°C to 200°C and ±3°C from 200°C to 300°C. Includes PVC protection cover.	4 (Barcelona) 1 (Girona) 1 (Lleida) 1 (Tarragona) 1 (Terres de l'Ebre)	10,56	84,48
Colored Weather Station WS6812 WHITE BLUE	La Crosse Technology. INDOOR TEMPERATURE: Units: °C or °F. From 0°C to 50°C (32°F to 122°F). Indoor temperature trend indicator. Interval: every 30 seconds. OUTDOOR TEMPERATURE: Units: °C or °F. From -40°C to 60°C (-40°F to 140°F). Outdoor temperature trend indicator. Interval: every 50 seconds.	4 (Barcelona) 1 (Girona) 1 (Lleida) 1 (Tarragona) 1 (Terres de l'Ebre)	30	240
Sterilized 10 mL Syringe with 30-	Syringe 3c Emerald 10ml	400	10,30 (100 units)	41,2

gauge Needle	Needle Sterican 30G x1/2" 0,3x12mm	400	4,62 (100 units)	18,48
LABORATORY ANALYSES	**Number of [K+] Determinations**	**€ / [K+] Determination**		**Total (€)**
LYTE Integrated Multi-Sensor Analyses	400	4		1.600
PUBLICATION EXPENSES				**Total (€)**
Scientific Publication				1.500
TOTAL BUDGET (€)				**9.984,16**

14. REFERENCES

1. Prahlow J. Postmortem Changes and Time of Death. In: Prahlow J, editor. Forensic Pathology for Police, Death Investigators, Attorneys, and Forensic Scientists [Internet]. Totowa, NJ: Humana Press; 2010. p. 163–84. Available from: http://link.springer.com/10.1007/978-1-59745-404-9_8

2. Somoza Castro O. Capítulo IV. Data de la muerte i fenómenos cadavéricos. In: Somoza Castro O, editor. La muerte violenta Inspección ocular i cuerpo del delito Las decisivas primeras 24 horas. Madrid: LA LEY; 2004. p. 81–101.

3. Madea B, Henssge C. General remarks on estimating the time since death. In: Madea B, editor. Estimation of the time since death. 3rd ed. CRC Press; 2015. p. 1–6.

4. Mathur A, Agrawal YK. An overview of methods used for estimation of time since death. Aust J Forensic Sci [Internet]. 2011;43(4):275–85. Available from: http://www.tandfonline.com/doi/abs/10.1080/00450618.2011.568970

5. Hilal M, El-sayed W, Said A, Magdy A. Updates In Estimating Postmortem Interval. Sohag Med J [Internet]. 2017;21(3):171–4. Available from: http://smj.journals.ekb.eg/article_36979.html

6. Camps F. Establishment of the time of death—a critical assessment. J Forensic Sci. 1959;4:73–82.

7. Madea B. Methods for determining time of death. Forensic Sci Med Pathol [Internet]. 2016;12(4):451–85. Available from: http://link.springer.com/10.1007/s12024-016-9776-y

8. Henssge C. Death time estimation in case work. I. The rectal temperature time of death nomogram. Forensic Sci Int. 1988;38(3):209–36.

9. Henßge C, Madea B. Estimation of the time since death in the early post-mortem period. Forensic Sci Int. 2004;144:167–75.

10. Rainy H. On the Cooling of Dead Bodies as Indicating the Length of Time That Has Elapsed Since Death. Glasgow Med J [Internet]. 1869 May;1(3):323–30. Available from: http://www.ncbi.nlm.nih.gov/pubmed/30432687

11. Marshall T, Hoare F. I Estimating the time of death. The rectal cooling after death and its mathematical expression. II The use of the cooling formula in the study of postmortem body cooling. III The use of the body temperature in estimating the time of death. J Forensic Sci. 1962;7:56–81, 189–210, 211–21.

12. de Saram GSW, Webster G, Kathirgamatamby N. Post-Mortem Temperature and the Time of Death. J Crim Law Criminol Police Sci. 1955;46(4):562–77.

13. Vergara López C. Medicina Forense y Criminalística. Los fenómenos cadavéricos que nos ayudan a datar la hora de la muerte en cadáveres recientes

y sus posibles modificaciones en relación al entorno y la causa de la muerte. [Internet]. Barcelona: CFEC, Centro de Formación Estudio Criminal; 2015. Available from: https://www.estudiocriminal.eu/wp-content/uploads/2017/02/Medicina-Forense-y-Criminalistica-Casandra-Vergara-Lopez.pdf

14. Nomogram | Definition of Nomogram [Internet]. Merriam-Webster; 2019. Available from: https://www.merriam-webster.com/dictionary/nomogram

15. Madea B. Is there recent progress in the estimation of the postmortem interval by means of thanatochemistry? Forensic Sci Int [Internet]. 2005;151(2–3):139–49. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0379073805000472

16. Luna A. Is postmortem biochemistry really useful? Why is it not widely used in forensic pathology? Leg Med. 2009;11:S27–30.

17. Vass AA. The elusive universal post-mortem interval formula. Forensic Sci Int [Internet]. 2011;204(1):34–40. Available from: https://www.sciencedirect.com/science/article/pii/S037907381000232X?via%3Dihub

18. Coe JI. Postmortem chemistry update. Emphasis on forensic application. Am J Forensic Med Pathol [Internet]. 1993;14(2):91–117. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8328447

19. Madea B, Musshoff F. Postmortem biochemistry. Forensic Sci Int [Internet]. 2007;165(2–3):165–71. Available from: https://www.sciencedirect.com/science/article/pii/S0379073806003161?via%3Dihub

20. Baniak N, Campos-Baniak G, Mulla A, Kalra J. Vitreous Humor: A Short Review on Post-mortem Applications. J Clin Exp Pathol [Internet]. 2015;4(6):1–7. Available from: https://www.omicsonline.org/open-access/vitreous-humor-a-short-review-on-postmortem-applications-2161-0681.1000199.php?aid=37331

21. Drake RL, Vogl W, Mitchell AWM. Cabeza y Cuello. In: Gray, Anatomía para Estudiantes. Elsevier Inc.; 2005. p. 850–1.

22. Naumann HN. Postmortem Chemistry of the Vitreous Body in Man. Arch Ophthalmol [Internet]. 1959;62(3):356–63. Available from: http://archopht.jamanetwork.com/article.aspx?articleid=625937

23. Belsey SL, Flanagan RJ. Postmortem biochemistry: Current applications. J Forensic Leg Med. 2016;41:49–57.

24. Sturner WQ. The Vitreous Humour: Postmortem Potassium Changes. Lancet [Internet]. 1963;281:807–8. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0140673663915095

25. Sturner WQ, Gantner GE. The Postmortem Interval. Am J Clin Pathol [Internet].

1964;42(2):137–44. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14202146

26. Lange N, Swearer S, Sturner WQ. Human postmortem interval estimation from vitreous potassium: an analysis of original data from six different studies. Forensic Sci Int. 1994;66(3):159–74.

27. Coe JI. Postmortem Chemistries on Human Vitreous Humor. Am J Clin Pathol [Internet]. 1969;51(6):741–50. Available from: https://academic.oup.com/ajcp/article-lookup/doi/10.1093/ajcp/51.6.741

28. Adjutantis G, Coutselinis A. Estimation of the time of death by potassium levels in the vitreous humour. Forensic Sci [Internet]. 1972;1(1):55–60. Available from: https://linkinghub.elsevier.com/retrieve/pii/0300943272901471

29. James RA, Hoadley PA, Sampson BG. Determination of postmortem interval by sampling vitreous humour. Am J Forensic Med Pathol. 1997;18(2):158–62.

30. Muñoz JI, Suárez-Peñaranda JM, Otero XL, Rodríguez-Calvo MS, Costas E, Miguéns X, et al. A new perspective in the estimation of postmortem interval (PMI) based on vitreous [K+]. J Forensic Sci [Internet]. 2001;46(2):209–14. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11305419

31. Jashnani KD, Kale SA, Rupani AB. Vitreous Humor: Biochemical Constituents in Estimation of Postmortem Interval*,†. J Forensic Sci [Internet]. 2010;55(6):1523–7. Available from: http://doi.wiley.com/10.1111/j.1556-4029.2010.01501.x

32. Bortolotti F, Pascali JP, Davis GG, Smith FP, Brissie RM, Tagliaro F. Study of vitreous potassium correlation with time since death in the postmortem range from 2 to 110 hours using capillary ion analysis. Med Sci Law [Internet]. 2011;51:20–3. Available from: http://journals.sagepub.com/doi/10.1258/msl.2010.010063

33. Mihailovic Z, Atanasijevic T, Popovic V, Milosevic MB, Sperhake JP. Estimation of the Postmortem Interval by Analyzing Potassium in the Vitreous Humor. Could Repetitive Sampling Enhance Accuracy? Am J Forensic Med Pathol [Internet]. 2012;33(4):400–3. Available from: https://insights.ovid.com/crossref?an=00000433-201212000-00028

34. Siddamsetty AK, Verma SK, Kohli A, Puri D, Singh A. Estimation of time since death from electrolyte, glucose and calcium analysis of postmortem vitreous humour in semi-arid climate. Med Sci Law [Internet]. 2013;54(3):158–66. Available from: http://journals.sagepub.com/doi/10.1177/0025802413506424

35. Ortmann J, Markwerth P, Madea B. Precision of estimating the time since death by vitreous potassium—Comparison of 5 different equations. Forensic Sci Int [Internet]. 2016;269:1–7. Available from: https://www.sciencedirect.com/science/article/pii/S037907381630442X?via%3Dihub

36. Zilg B, Bernard S, Alkass K, Berg S, Druid H. A new model for the estimation of time of death from vitreous potassium levels corrected for age and temperature. Forensic Sci Int [Internet]. 2015;254:158–66. Available from: https://www.sciencedirect.com/science/article/pii/S0379073815002984?via%3Dihub

37. Muñoz-Barús JI, Rodríguez-Calvo MS, Suárez-Peñaranda JM, Vieira DN, Cadarso-Suárez C, Febrero-Bande M. PMICALC: An R code-based software for estimating post-mortem interval (PMI) compatible with Windows, Mac and Linux operating systems. Forensic Sci Int [Internet]. 2010;194(1–3):49–52. Available from: https://www.sciencedirect.com/science/article/pii/S0379073809004174?via%3Dihub

38. Madea B, Henssge C, Hönig W, Gerbracht A. References for determining the time of death by potassium in vitreous humor. Forensic Sci Int [Internet]. 1989;40(3):231–43. Available from: https://www.sciencedirect.com/science/article/pii/0379073889901813

39. Rognum TO, Hauge S, Øyasaeter S, Saugstad OD. A new biochemical method for estimation of postmortem time. Forensic Sci Int. 1991;51(1):139–46.

40. Komura S, Oshiro S. Potassium Levels in the Aqueous and Vitreous Humor after Death. Tohoku J Exp Med [Internet]. 1977;122(1):65–8. Available from: http://joi.jlc.jst.go.jp/JST.Journalarchive/tjem1920/122.65?from=CrossRef

41. Bray M. The Effect of Chilling, Freezing, and Rewarming on the Postmortem Chemistry of Vitreous Humor. J Forensic Sci. 1984;29(2):404–11.

42. Bray M. The Eye as a Chemical Indicator of Environmental Temperature at the Time of Death. J Forensic Sci. 1984;29(2):396–403.

43. Coe JI. Vitreous potassium as a measure of the postmortem interval: An historical review and critical evaluation. Forensic Sci Int [Internet]. 1989;42(3):201–13. Available from: https://www.sciencedirect.com/science/article/pii/037907388990087X

44. Zhou B, Zhang L, Zhang G, Zhang X, Jiang X. The determination of potassium concentration in vitreous humor by low pressure ion chromatography and its application in the estimation of postmortem interval. J Chromatogr B [Internet]. 2007;852(1–2):278–81. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1570023207000712

45. Honey D, Caylor C, Luthi R, Kerrigan S. Comparative alcohol concentrations in blood and vitreous fluid with illustrative case studies. J Anal Toxicol. 2005;29(5):365–9.

46. Blana SA, Mußhoff F, Hoeller T, Fimmers R, Madea B. Variations in vitreous humor chemical values as a result of pre-analytical treatment. Forensic Sci Int.

2011;210(1):263–70.

47. Coe JI, Apple FS. Variations in Vitreous Humor Chemical Values As A Result of Instrumentation. J Forensic Sci. 1985;30(3):828–35.

48. Garcia PT, Gabriel EFM, Pessôa GS, Santos Júnior JC, Mollo Filho PC, Guidugli RBF, et al. Paper-based microfluidic devices on the crime scene: A simple tool for rapid estimation of post-mortem interval using vitreous humour. Anal Chim Acta. 2017;974:69–74.

15. ANNEXES

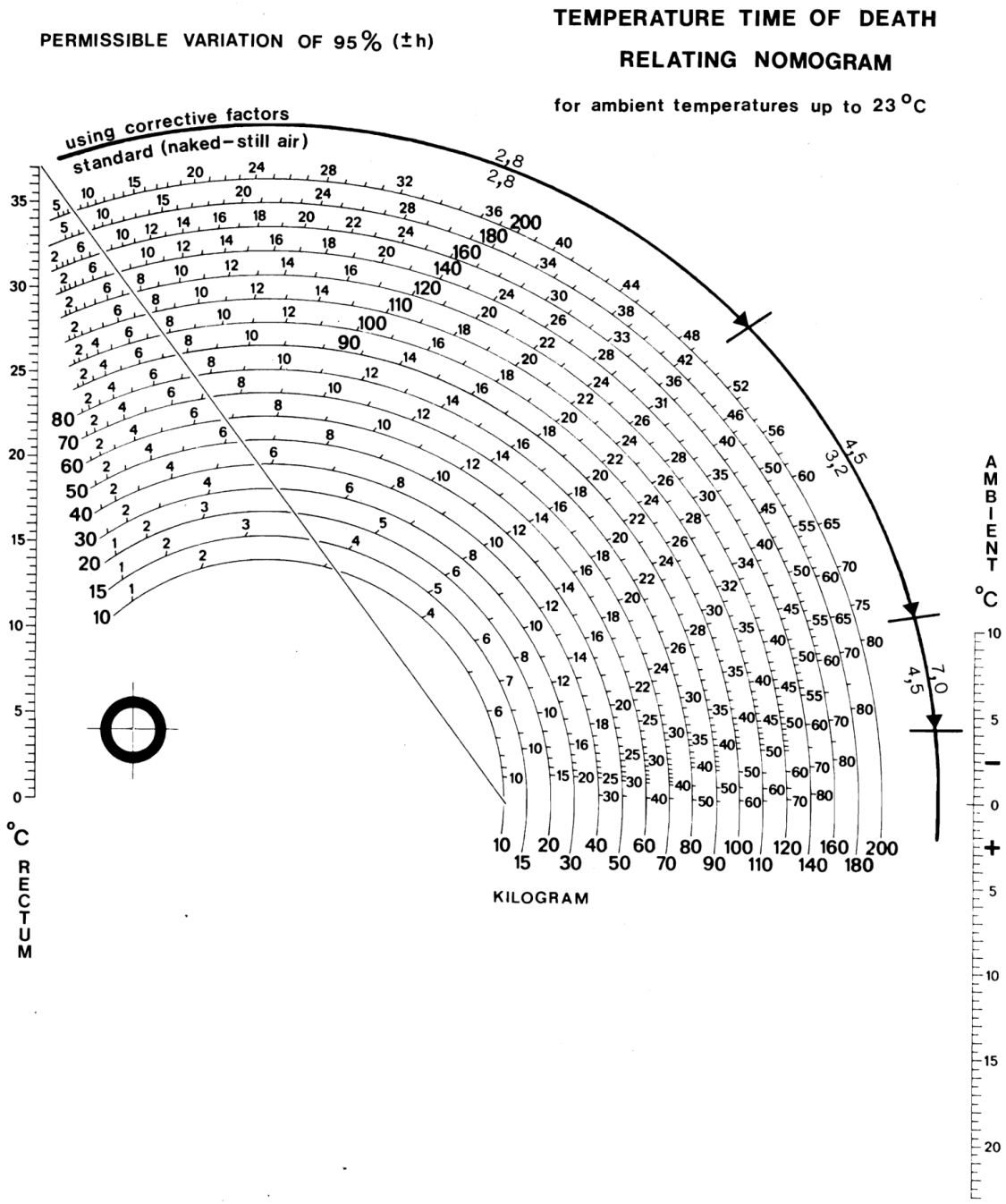
15.1. PMI Methods

References: (2,4,5,7,13) **. *CSF = Cerebrospinal Fluid***

PHYSICAL METHODS		
Method	**Description**	**Evolution (hpm)**
Algor mortis	Post-mortem body cooling. Core body temperature decreases until it reaches equilibrium with the ambient temperature.	In general, according to Marshall and Hoare (11): 0 - 3 hpm: there is a loss of 0.55°C/hpm. 3 – 12 hpm: there is a loss of 1°C/hpm. 12 - 24 hpm: the loss of temperature is slower (0.25 – 0.75°C/hpm) until it reaches equilibrium (13).
Livor mortis	Synonym: Post-mortem lividity. It is considered the earliest post-mortem change. After the irreversible circulatory arrest, there is a loss of hydrostatic pressure that leads to a settling of blood in lower parts of the body, simply by the action of gravity. Consequently, there is a pink discolouration of the skin which progressively gets darker because of the consumption of oxygen. Depending on the position of the cadaver, livor mortis will develop in different areas. For example, in supine position, there will be a posterior distribution of livor mortis. Lividity does not appear in body areas that are in contact with another surface.	1 – 4 hpm: becomes perceptible. 7 – 8 hpm: well developed. 8 – 12 hpm: reaches its maximum degree. First 12 hpm: if we turn the cadaver into a new position, new lividity will develop while the former will disappear. 12 – 24 hpm: if we turn the cadaver into a new position, new lividity will develop while the former will remain fixed. >24 hpm: the former lividity will remain fixed. No more lividity will develop.
Other Methods	Supravital reactions.	
PHYSICOCHEMICAL METHODS		
Method	**Description**	**Evolution (hpm)**
Rigor mortis	After the irreversible circulatory arrest, a Primary Muscular Flaccidity develops. When levels of ATP are <85% of the initial value, actin and myosin filaments contract, macroscopically resulting in a stiffening of the body known as Rigor mortis. After a period of time, a	2 – 4 hpm: starts to develop (in normal ambient temperature). 6 – 12 hpm: fully developed. 12 – 72 hpm: gradually dissipates.

	Secondary Muscular Flaccidity will gradually appear. When death occurs in the supine position, according to Nysten's rule (1811), rigor gradually appears: eyelids/jaw > neck > trunk > limbs.	- Between 24 – 36 hpm, it is invincible.
BIOCHEMICAL METHODS (Tanatochemistry)		
Method	**Description**	
Vitreous Humour Analysis	It has become the most studied material for estimating time since death by biochemical means. The most studied parameter is potassium. Because of the fact that vitreous humour is topographically isolated and well protected, autolytic changes occur more slowly than other liquids, such as blood or CSF.	
Other Methods	- Blood analysis. - CSF analysis. - Pericardial liquid analysis. - Protein degradation. - Degradation of DNA and RNA.	
BIOLOGICAL METHODS		
Method	**Description**	
Autolysis	Endogenous hydrolytic enzymes destroy the cell.	
Method	**Description**	**Evolution (dpm/mpm/ypm)**
Putrefaction	Synonym: Post-mortem decomposition. Tissues degrade into gases, liquids and salts by the action of human-associated microbiota. This process is influenced by many environmental factors, especially ambient temperature.	10 dpm: chromatic period. - After 1 – 2 dpm, a green discoloration of abdominal wall appears. 1st mpm: emphysematous period. 9 – 15 mpm: liquefaction period. 2 – 5 ypm: skeletal period.
ENTOMOLOGY		
The study of the cycle of life of cadaveric fauna.		

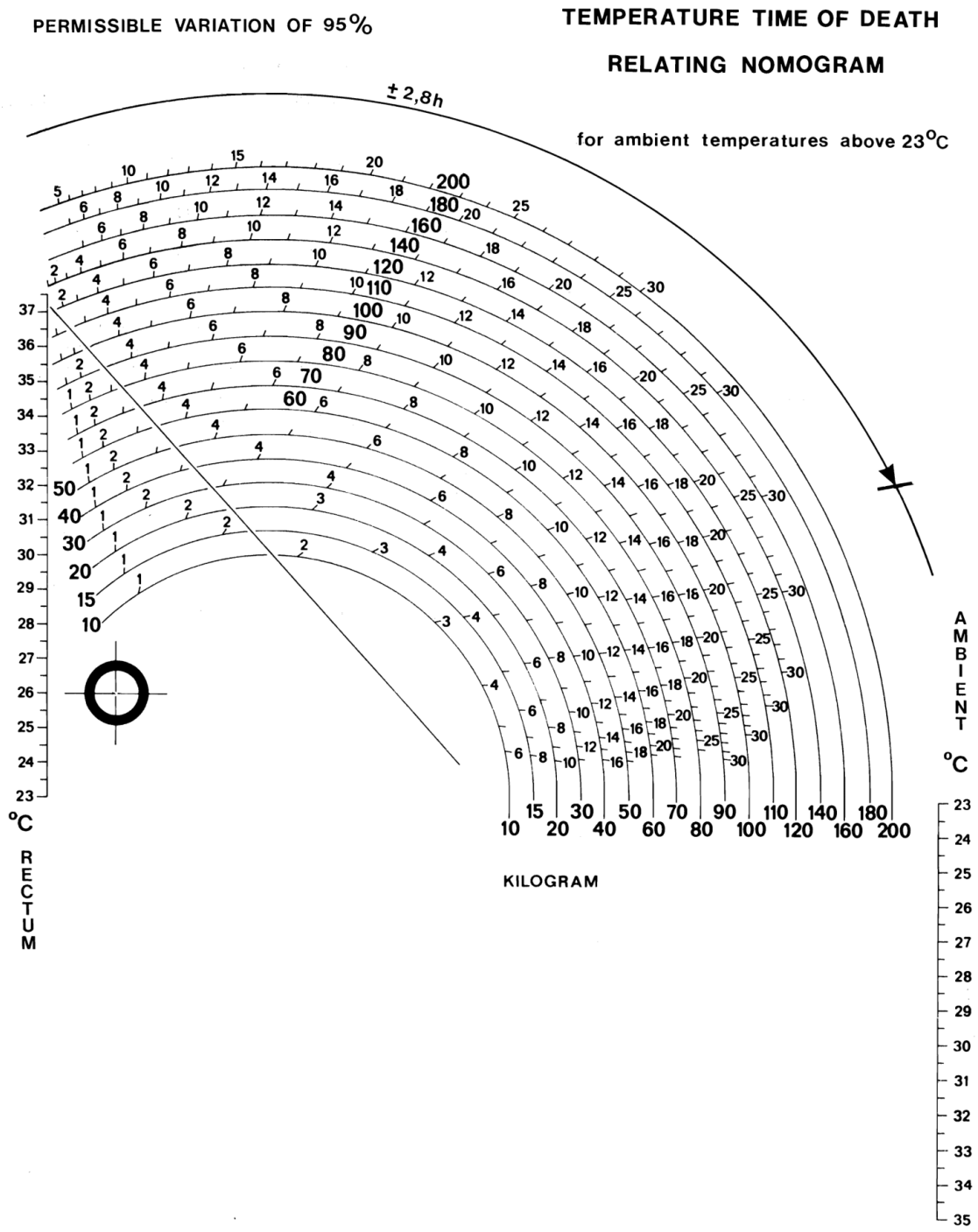
15.2. Henssge's Nomogram



The nomogram expresses the death-time (t) by:

$$\frac{T_{rectum} - T_{ambient}}{37.2 - T_{ambient}} = 1.25 \exp(B \times t) - .25 \exp(5 \times B \times t);\ B = -1{,}2815\ (kg^{-.625}) + .0284$$

The nomogram is related to the chosen standard i.e. naked body extended lying in still air. Cooling conditions differing from the chosen standard may be proportionally adjusted by corrective factors of the real body weight, giving the corrected body weight by which the death-time is to be read off. Factors above 1.0 may correct thermal isolation conditions and factors below 1.0 may correct conditions accelerating the heat loss of a body.



The nomogram expresses the death-time (t) by:

$$\frac{T_{rectum} - T_{ambient}}{37.2 - T_{ambient}} = 1.11 \exp(B\ t) - .11 \exp(10\ B\ t); \quad B = -1.2815\ (kg^{-.625}) + .0284$$

15.3. PMI Formulas

Adapted from (7,35,36)**.**

Year	Author	PMI Formula	Regression	Max PMI (hpm)	n	Country	Comments
1963	Sturner and Gantner (25)	$PMI = 7.14[K^+] - 39.1$	$Y = 0.14x + 5.6$	104	125	USA	The slope of their Regression line is the flattest reported in the literature. As a consequence, there is a systematic over-estimation of the time since death. This formula may be advantageous in a climate zone with low ambient temperature.
1969	Coe (27)	$PMI = 6.15[K^+] - 38.1$	$Y = 4.99x + 0.332$ (first 6h) $Y = 6.19x + 0.1625$ (over 6 h)	100	145	USA	Relationship between [K+] and the PMI: it is not a straight line but is biphasic with a steeper slope in the first 6 h than for prolonged times. The results by this formula are most satisfactory when the ambient temperature in which the body has lain is below 10°C. However, even under these conditions, the results may be quite inexact.
1997	James et al. (29)	$PMI = 4.32[K^+] - 18.35$	$Y = 0.23x + 4.2$	80	100	Australia	Also included Hypoxanthine.
2001	Muñoz et al. (30)	$PMI = 3.92[K^+] - 19.04$	$Y = 0.17x + 5.60$	40	133	Spain	For Central Europe, this formula gives quite reliable results. Only non-hospital cases were examined. It considers [K+] as the independent variable and PMI as the dependent. This change in variables is necessary in forensic work and it also leads to a higher accuracy of death time estimation.

2010	Jashnani et al. (31)	$PMI = 1.076[K^+] - 2.815$	$Y = 0.929x + 2.616$	50	120	India	The slope of their Regression line is very steep. As a consequence, there is a systematic underestimation of the time since death. This formula may be advantageous in a climate zone with high ambient temperature. Mostly included cases involving sepsis or tuberculosis.
2011	Bortolotti et al.(32)	$PMI = 5.77[K^+] - 13.28$	$Y = 0.1733x + 2.3008$	110	164	USA and Italy	----------
2012	Mihailovic et al.(33)	$PMI = 2.479[K^+] - 11.98$		30	32	Serbia	Repetitive sampling.
2015	Zilg et al. (36)	$PMI = \frac{\ln\left(\frac{M - C_0}{M - [K^+]}\right)}{L_0 + m_A A + m_t T}$ M is the steady state concentration of K+ (mmol/L); L is the K+ membrane crossing term (in 10^{-3}/day); C_0 is [K+] at death (mmol/L); A is age; T is temperature and mA and mT are their associated coefficients respectively. PMI is only defined when $[K+] < M$ and $[K+] \geq C_0$.	Unknown	409	462	Sweden	This study has found that the post-mortem rise in vitreous potassium is non-linear and that decedent age and the ambient temperature significantly influence the rise. The higher the average ambient temperature, the steeper the increase in potassium. The younger the subject, the faster the increase in potassium. No cases were excluded. To simplify the calculation process for the user, they have created a web application where the potassium concentration, decedent age, and ambient temperature (if available) can be entered into a form and the PMI with confidence intervals will be calculated: https://slbd.shinyapps.io/pmiPredictor/
2016	Madea et al. (35)	$PMI = 3.92[K^+] - 30.9$	$Y = 0.19x + 5.88$	120	170	Germany	For Central Europe, this formula gives quite reliable results.

15.4. LYTE Integrated Multi-Sensor Datasheet

Atellica™ CH
Analyzer

SIEMENS Healthineers

Multisensor Integrado A-LYTE (IMT Na K Cl)

Revisión y fecha actual[a]	Rev. 01, 2017-05	
Nombre de producto	A-LYTE Integrated Multisensor (IMT Na K Cl)	REF 11099315 (60.000 pruebas)
Nombre de producto abreviado	IMT Na K Cl	
Nombre/ID de la prueba	Na K Cl	
Sistemas	Atellica CH Analyzer	
Materiales necesarios pero no suministrados	A-LYTE IMT Standard A	REF 11099304
	A-LYTE IMT Standard B + Salt Bridge	REF 11099306
	A-LYTE IMT Diluent	REF 11099305
	A-LYTE IMT Dilution Check	REF 11099325
Tipos de muestra	Suero, plasma (heparina de litio) y orina	
Volumen de muestra	25 µl	
Intervalo de medición	**Sodio** Suero/plasma: 50–200 mmol/l (mEq/l) Orina: 10–300 mmol/l (mEq/l)	
	Potasio Suero/plasma: 1–10 mmol/l (mEq/l) Orina: 2–300 mmol/l (mEq/l)	
	Cloruro Suero/plasma: 50–200 mmol/l (mEq/l) Orina: 20–330 mmol/l (mEq/l)	

[a] Una barra vertical en el margen de la página indica contenido técnico que difiere de la versión anterior.

CE

11109447_ES Rev. 01, 2017-05 1 / 20

15.5. Digital Thermometer With Probe Sensor TP3001 Datasheet

labbox

Date: 30/04/2019

Product data sheet

GENERAL INFORMATION

Product: Digital thermometer with probe sensor TP3001

Description: Fixed probe made of stainless steel, 145 mm long. Temperature unit ºC/ºF. Range: -50 300 ºC (-58 572 ºF), resolution 0.1 ºC/ºF and accuracy ±1 ºC from -50 ºC to 200 ºC y ±3 ºC from 200 ºC to 300 ºC. Hold function included (temperature hold in display). Power supply: 1 battery cell G13. Dimensions: 235 x 22 mm, weight 25 g. Includes PVC protection cover

SPECIFICATIONS

Reference	uds/ box	range	type	Stabilization time
THER-D30-001	1	-50 +300 ºC	forme droite	10 s

PACKING

Type: Carboard box
Label:

labbox

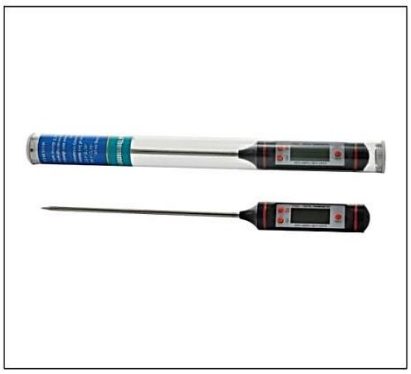
THER-D30-001

Digital thermometer with probe, straight, -50 +300 ºC

Termómetro digital con sonda, forma recta, -50 +300 ºC

Thermomètre numérique avec sonde, -50 + 300ºC

Batch nº.: xxxx



MATERIAL

Acrylobutadiene-styrene copolymer (ABS)
Tolerated temperature range in normal use: from -40 ºC to +85 (100) ºC

15.6. Colored Weather Station WS6812 WHITE BLUE

COLORED WEATHER STATION WS6812 WHITE BLUE. DETAILED DESCRIPTION

LA CROSSE® TECHNOLOGY

HOUR DATE
Manual Time Setting
Time format : 12H or 24H
Calendar: month / day / date
Alarm with Snooze (10 mn)

INDOOR TEMPERATURE
Units : °C or °F
From 0°C to 50°C (32°F to 122°F)
Daily Min/Max records
Indoor temperature trend indicator
Indoor temperature alerts
Interval : every 30 seconds

INDOOR HUMIDITY
Unit : %RH

OUTDOOR TEMPERATURE
Units : °C or °F
From -40°C to 60°C (-40 °F to 140 °F)
Daily Min/Max records
Outdoor temperature trend indicator
Outdoor temperature alerts
Interval : every 50 seconds

OUTDOOR HUMIDITY
Unit : %RH

TRANSMISSION
Signal strength icon for sensor transmission
Wireless transmission until 90 meters (300 ft.) in open field
Frequency : 433 MHz

MOON PHASES
12 icons

POINT DE ROSÉE
Units : °C or °F

POWER SUPPLY
Power supply of the base : 2 x AA LR6 1.5V
(Batteries excluded)
Power supply of the sensor : 2 x AA LR6 1.5V
(Batteries excluded)

DIMENSIONS
Dimensions of the base : 137.2 x 111.8 x 42.8 mm
Dimensions of the sensor : 40 x 20 x 130 mm

NET WEIGHT (KG)
0.35

FEATURES
Selection of the day week language : French, English, German, Dutch, Italian, Spanish & Danish
Low battery indicators
Table standing

15.7. Data Collection Sheet 1

Universitat de Girona
Facultat de Medicina

DATA COLLECTION SHEET 1

INSTITUT MEDICINA LEGAL IMLC CATALUNYA

PROJECT: *Estimation of the Early Post-Mortem Interval: A Multicentre Comparison of the Analysis of Vitreous Humour and the Henssge's Nomogram Method.*

Date and Time of Data Collection: [_ _ / _ _ / _ _ _ _] (dd/mm/yyyy) [_ _ : _ _] h

Case number: [ ].

Sex of the deceased:

- □ Female
- □ Male

Age of the deceased: [ ] years old.

IMLCFC Division:

- □ Barcelona
- □ Girona
- □ Lleida
- □ Tarragona
- □ Terres de l'Ebre

VARIABLES

Exact PMI = Exact Time of Death – Time of Data Collection

Exact PMI = [ ] - [ ] = [ ] hours post-mortem.

Samples of Vitreous Humour:

Number of Samples:

- □ 1 (from 1 eyeball)
- □ 2 (1 from each eyeball)

Quantity from each eyeball:

[ ] mL (Right Eye)

[ ] mL (Left Eye)

Submit samples to Laboratori de l'IMLCFC (Barcelona) in order to determine [K+].

Rectal Temperature: [ ] ºC.

Ambient Temperature: [ ] ºC.

Weight of the deceased: [ ] kg.

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INSTITUT MEDICINA LEGAL CATALUNYA IMLC

DATA COLLECTION SHEET 1

Nomogram Corrective Factor: [ ].

Reference: 70 kg

DRY CLOTHING / COVERING	IN AIR	CORRECTIVE FACTOR
naked	moving	0.75
1-2 thin layers		0.9
naked	still	1.0
1-2 thin layers		1.1
2-3 thin layers		1.2
1-2 thicker layers	without influence	
3-4 thin layers		1.3
more thin / thicker layers		1.4
thick blanket		1.8
thick blanket + clothing combined		2.4

Real Body Weight [kg]

4	6	8	10	20	30	40	50	60	70	80	90	100	110	120	130	140	150
									1.3								
1.6	1.6	1.6	1.6	1.5					1.4					1.3	1.2	1.2	1.2
2.1	2.1	2.0	2.0	1.9	1.8				1.6				1.4	1.4	1.4	1.3	1.3
2.7	2.7	2.6	2.5	2.3	2.2	2.1	2.0		1.8			1.6	1.6	1.6	1.5	1.4	1.4
3.5	3.4	3.3	3.2	2.8	2.6	2.4	2.3		2.0		1.8	1.8	1.7	1.6	1.6	1.5	1.5
4.5	4.3	4.1	3.9	3.4	3.0	2.8	2.6	2.4	2.2	2.1	2.0	1.9	1.8	1.7	1.7	1.6	1.6
5.7	5.3	5.0	4.8	4.0	3.5	3.2	2.9	2.7	2.4	2.3	2.2	2.1	1.9	1.9	1.8	1.7	1.6
7.1	6.6	6.2	5.8	4.7	4.0	3.6	3.2	2.9	2.6	2.5	2.3	2.2	2.1	2.0	1.9	1.8	1.7
8.8	8.1	7.5	7.0	5.5	4.6	3.9	3.5	3.2	2.8	2.7	2.5	2.3	2.2	2.0	1.9	1.8	1.7
10.9	9.8	8.9	8.3	6.2	5.1	4.3	3.8	3.4	3.0	2.8	2.6	2.4	2.3	2.1	2.0	1.9	1.8

Example: Real body weight 90 kg. Chosen corrective factor (reference 70 kg) 2.0. Use a corrective factor of 1.8.

Forensic Doctor A's Name:

Signature:

15.8. Data Collection Sheet 2

Universitat de Girona
Facultat de Medicina

DATA COLLECTION SHEET 2

INSTITUT MEDICINA LEGAL IMLC CATALUNYA

PROJECT: *Estimation of the Early Post-Mortem Interval: A Multicentre Comparison of the Analysis of Vitreous Humour and the Henssge's Nomogram Method.*

'Case number'; 'Sex of the deceased'; 'Age of the deceased'; 'IMLCFC Division'; 'Number of Samples of Vitreous Humour'; 'Rectal Temperature'; 'Ambient Temperature'; 'Weight of the deceased'; 'Nomogram Corrective Factor' *must be filled by the same forensic doctor who filled 'Data Collection Sheet 1' [FORENSIC DOCTOR A] before handing the present 'Data Collection Sheet 2' to another forensic doctor [FORENSIC DOCTOR B].*

Case number: [ ].

Sex of the deceased:

- □ Female
- □ Male

Age of the deceased: [ ] years old.

IMLCFC Division:

- □ Barcelona
- □ Girona
- □ Lleida
- □ Tarragona
- □ Terres de l'Ebre

VARIABLES

Samples of Vitreous Humour:

Number of Samples:

- □ 1 (from 1 eyeball)
- □ 2 (1 from each eyeball)

[K+] from each eyeball*:

[ ] mmol/L (Right Eye)

[ ] mmol/L (Left Eye)

**Data received from Laboratori de l'IMLCFC (Barcelona).*

Rectal Temperature: [ ] ºC.

Ambient Temperature: [ ] ºC.

Weight of the deceased: [ ] kg.

Nomogram Corrective Factor: [ ].

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Facultat de Medicina

INSTITUT MEDICINA LEGAL CATALUNYA IMLC

DATA COLLECTION SHEET 2

Early PMI 1 [K+]: ☐ **hours post-mortem.**

PMI Formula designed by Muñoz et al. $\mathrm{PMI} = 3.92[\mathrm{K}^+] - 19.04$

If two values of [K+] are available, their arithmetical mean will be used.

Early PMI 2 [K+]: ☐ **hours post-mortem.**

PMI Formula designed by Zilg et al. PMI Predictor = https://slbd.shinyapps.io/pmiPredictor/

If two values of [K+] are available, their arithmetical mean will be used.

Early PMI 3 [Rectal Temperature]: ☐ **hours post-mortem.**

Henssge's Nomogram Method. *Use the more suitable version according to the ambient temperature. Version 1 (see page 3) for ambient temperatures up to 23ºC; Version 2 (see page 4) for ambient temperatures above 23ºC.*

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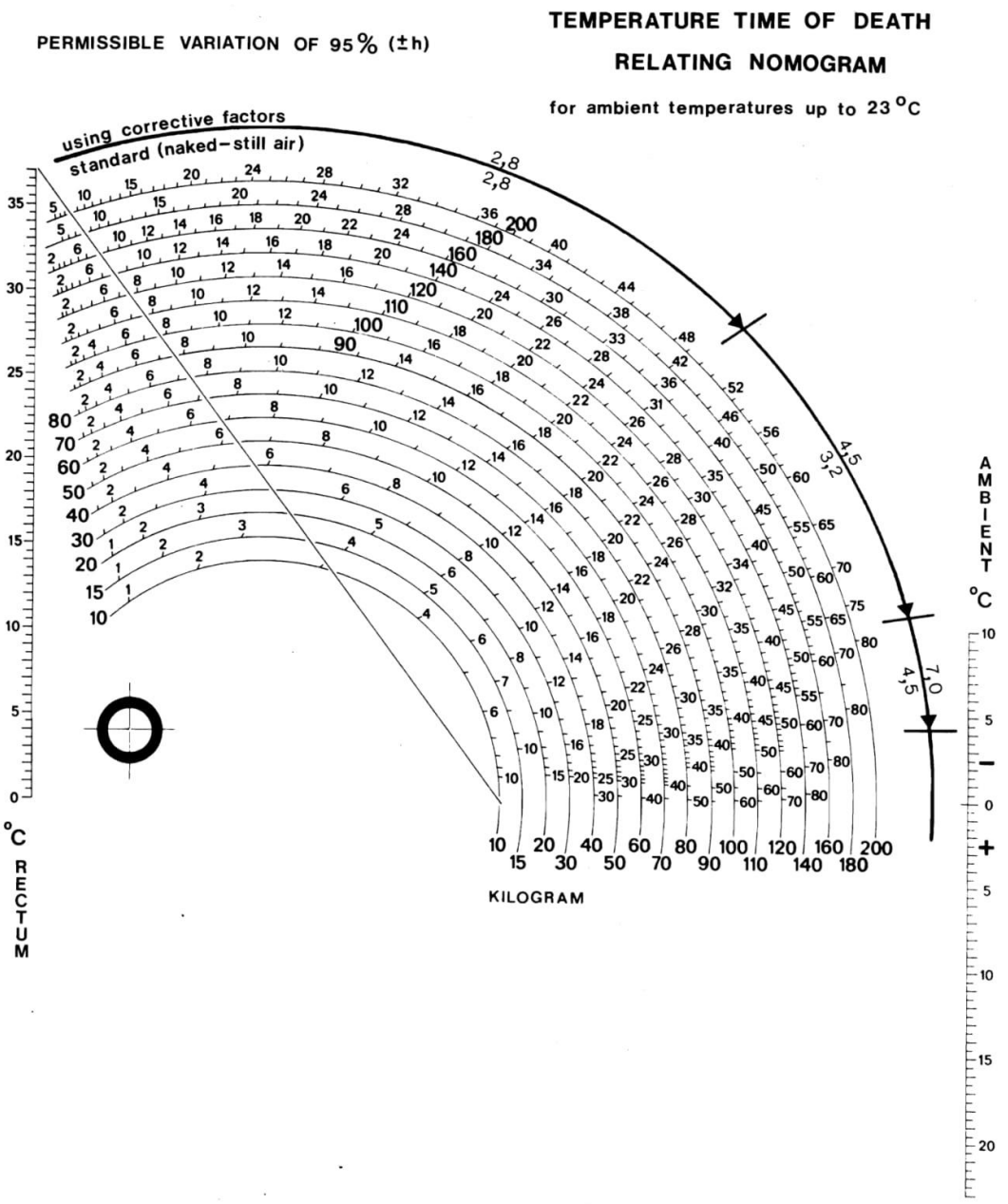
INSTITUT MEDICINA LEGAL CATALUNYA IMLC

DATA COLLECTION SHEET 2



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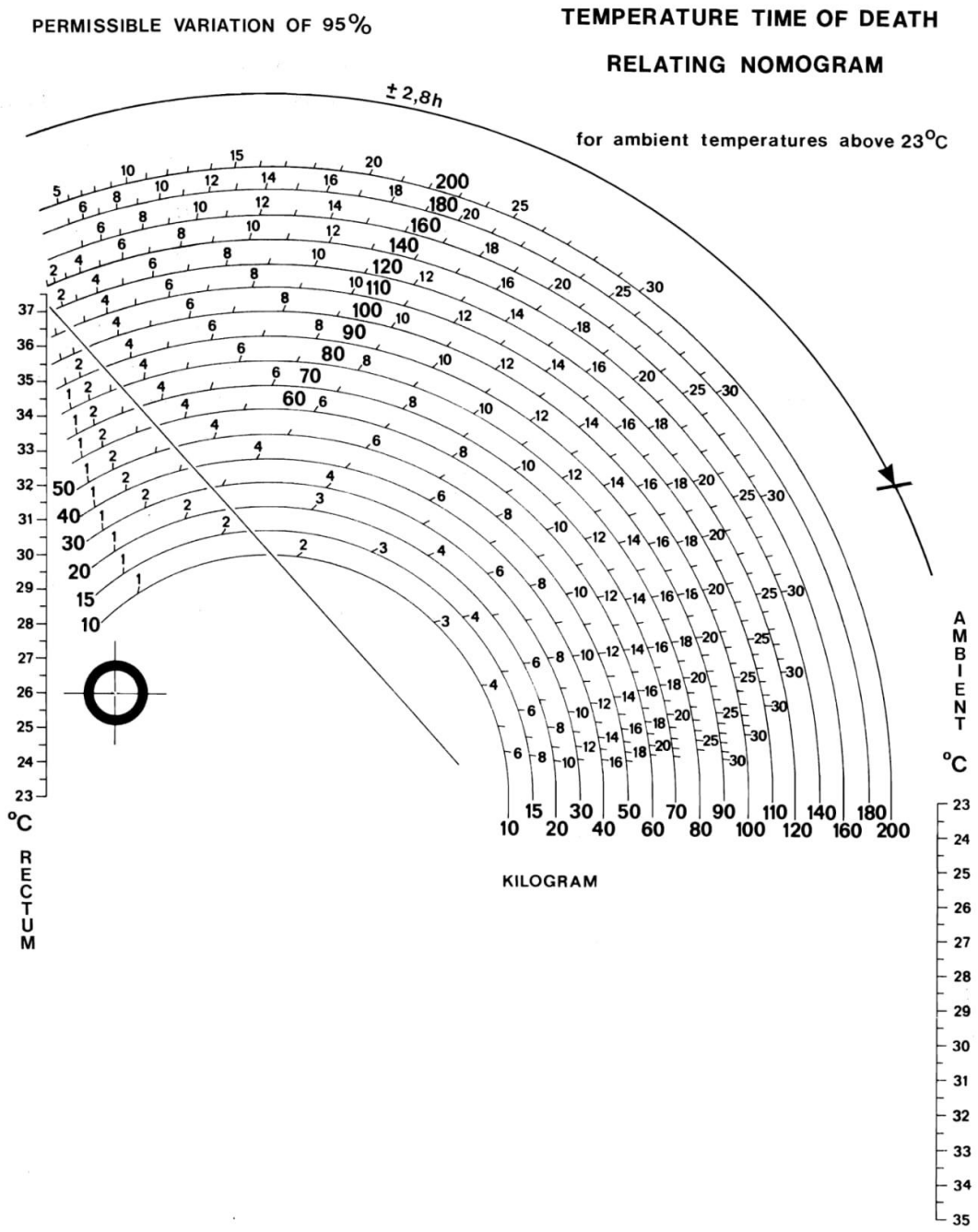

Forensic Doctor B's Name:

Signature: