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# The Rate of Hemolysis in Samples Taken at the Mohammed VI University Hospital of Oujda (Morocco)

**Soumaia Farih[1,2,3], Oussama Rahhab[1,2,3], Oumaima Nassiri[1,2,3], Sabrina Belmahi[1,2,3], Noussaiba Benhamza[1,2,3], El-Houcine Sebbar[1,2,3], Mohammed Choukri[1,2,3]**

[1]Laboratory of Biochemistry Central Laboratory, Mohammed VI University Hospital, Oujda, Morocco
[2]Faculty of Medicine and Pharmacy of Oujda, Mohammed First University, Oujda, Morocco
[3]Central Laboratory, Mohammed VI University Hospital, Oujda, Morocco
Email: Farih01soumaia@gmail.com

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## Abstract

Hemolysis of biological samples has long been considered to impact the results of conventional biochemical examinations for various reasons. For some years now, suppliers of biochemistry automats and reagents have been providing thresholds or indexes for hemolysis, icterus and lactescence beyond which the results are no longer reliable. Hemolysis can interfere with assays performed in the laboratory. These interferences are due to the release in serum or plasma of constituents present in red blood cells. The present work aims to determine the rate of hemolyzed samples from the different departments of the Mohammed VI University Hospital of Oujda (Morocco) and to propose practical approaches to improve the accuracy and reliability of biochemical results.

## Subject Areas

Biochemistry

## Keywords

Hemolysis, Interference. Index, Biological Samples

## 1. Introduction

Hemolysis of biological samples has long been considered to impact the results of conventional biochemical examinations for various reasons. For some years now, suppliers of biochemistry automats and reagents have been providing

thresholds or indexes for hemolysis, icterus and lactescence beyond which the results are no longer reliable [1] [2]. Hemolysis becomes visible at free hemoglobin concentrations of 0.3 to 0.5 g/L, and the intensity of the red color of the serum or plasma increases further with increasing free serum hemoglobin concentration. Traditionally, biologists visually examine specimens for hemolysis by comparing the appearance of the serum/plasma with a color-based specimen integrity chart. This process is time-consuming and limited by subjectivity, low inter- and intra-observer variability, and difficulty in discerning subtle color differences between hemolyzed and iconic samples [3]. Several studies have shown that laboratory personnel are unable to accurately assess the degree of hemolysis in serum, even if they are well trained and use a color standard for comparison [4]. The present work aims to determine the rate of hemolyzed samples from the services of the Mohammed VI University Hospital of Oujda (Morocco) and to propose practical approaches to improve the accuracy and reliability of biochemical results.

## 2. Materials and Methods

This is a prospective study of blood samples taken on dry tubes without gel, received in the biochemistry laboratory of the central laboratory department and coming from five departments (Intensive care unit, Emergency, Nephrology, Pediatrics, Internal medicine) of the Mohammed VI University Hospital of Oujda (Morocco). The collection and analysis of samples was spread over a period of 70 days. We collected 787 samples from five hospital departments of the Mohammed VI University Hospital of Oujda (Morocco):

- Intensive care: 224 samples
- Emergency: 309 samples
- Nephrology: 75 samples
- Pediatrics: 80 samples
- Internal medicine: 99 samples

The serum hemolysis index was determined on the ABBOTT ARCHITECT ci8200 for each sample upon receipt in the laboratory to determine the incidence and degree of in vitro hemolysis in these departments. This index allows an estimation of the hemoglobin present in hemolyzed samples. The estimation is based on the measurement of the absorbance of the red color of hemoglobin. The interference index measurement is the process performed by the analyzer to measure hemolysis in a sample after it has been mixed with the reagent or saline solution. Samples containing interfering substances, such as hemoglobin, absorb at different wavelengths, as shown in the graph below. NADH is the reference absorbance peak. To measure the three interfering substances, the system measures the absorbance values of four wavelength pairs and applies, via the appropriate photometric readings [5].

The following mathematical formulas are used to determine the relative concentration of interfering substances:

$$\text{Hemolysis} = M\left(a_{05} \times A_1 + a_{06} \times A_2 + a_{07} \times A_3 + a_{08} \times A_4\right)$$

where:

- $M$ = is the sample dilution correction (Reagent volume + sample volume)/ (sample volume).
- $a_{05}$, $a_{06}$, $a_{07}$ and $a_{08}$ = Constants specific to each interferer are used by the system to calculate the interference indices of the samples. These constants cannot be defined by the user.
- Absorbance level measured at different wavelength pairs:

- $A_1$ (500 nm/52 nm)
- $A_2$ (572 nm/604nm)
- $A_3$ (628 nm/660nm)
- $A_4$ (524 nm/804nm)

The analyzer calculates the interfering substance on a semi-quantitative scale in one of six categories: normal, 1+, 2+, 3+, 4+ and 5+. Table 1 summarizes the analyzer's semi-quantitative indices and their corresponding approximate free hemoglobin concentration [6]. Statistical analysis was performed by SPSS software.

## 3. Results

In the nephrology department, the percentage of hemolyzed tubes received in the biochemistry laboratory of the central laboratory of the Mohammed VI University Hospital in Oujda (Morocco) is estimated at 19%, followed by the intensive care unit at 17%, then the emergency department at 14%, then the pediatrics department at 13% and finally the internal medicine department, which accounts for the 8% of hemolyzed samples (Table 2). The prevalence of hemolyzed samples received was 14.6%.

Degrees of hemolysis for each service:

✓ No hemolyzed tube has a hemolysis index of 1+ or 2+ in any department.
✓ Intensive care unit the hemolysis index measurement found that 65% were hemolyzed at 3+ and 24% at 4+ while 12% showed hemolysis at 5+.
✓ Emergency Hemolysis index measurement finds that 28% of tubes are hemolyzed at 3+ while 47% measure 4+ and 26% measure 5+.

Table 1. Semi-quantitative values of the hemolysis index and their corresponding free hemoglobin concentration.

| Semi-quantitative value | Free hemoglobin (mg/dl) |
|---|---|
| Normal | <50 |
| 1+ | 50 - 99 |
| 2+ | 100 - 199 |
| 3+ | 200 - 299 |
| 4+ | 300 - 500 |
| 5+ | >500 |

**Table 2.** Percentage of hemolyzed samples from each department.

| Department | Number of samples | Number of hemolyzed samples | Percentage of hemolysis index | | | | | Percentage of hemolyzed samples |
|---|---|---|---|---|---|---|---|---|
| | | | 1+ | 2+ | 3+ | 4+ | 5+ | |
| **Intensive care unit** | 224 | 39 | 0% | 0% | 65% | 24% | 12% | 17% |
| **Emergency** | 309 | 44 | 0% | 0% | 28% | 47% | 26% | 14% |
| **Nephrology** | 75 | 14 | 0% | 0% | 71% | 21% | 7% | 19% |
| **Pediatrics** | 80 | 10 | 0% | 0% | 70% | 30% | 0% | 13% |
| **Internal medicine** | 99 | 8 | 0% | 0% | 87% | 13% | 0% | 8% |

- ✓ Nephrology: The majority of hemolyzed tubes are measured at 3+ for a percentage of 71%, hemolysis at 4+ represents 21% which leaves 7% for hemolysis at 5+.
- ✓ Pediatrics: 70% of hemolyzed tubes were measured at 3+ and 30% at 4+. No tube expressed hemolysis at 5+.
- ✓ Internal medicine: the majority of tubes are hemolyzed at 3+ for a percentage of 87% while the remaining 13% express 4+ hemolysis. Despite the fact that the nephrology department is distinguished by the highest percentage among the departments concerned, high degrees of hemolysis (4+ and 5+) remain more predominant in the emergency and intensive care departments.

## 4. Discussion

The preanalytical phase constitutes the first step of the macro process of the core business of the laboratory "to carry out a medical biology examination", because of the crucial role of the preanalytical phase and the direct relation of this one with the quality and the reliability of the results of the biochemical examinations, the central laboratory of the Mohammed VI University Hospital in Oujda (Morocco) attached a great importance to the control of all the steps of this process, The prescription connected to the central laboratory service of the Mohammed VI University Hospital of Oujda is done via the HIS (HOSIX) hospital computer system, thus simplifying and securing the entire preanalytical process and easily meeting the requirements of international standards. Each doctor has his own session and each prescription form specifies the identity of the prescriber, the date and time of the prescription, the patient's IP, the gender, the date of birth, the age, the list and the nature of the examination requested, the type of insurance, the clinical and therapeutic information useful for the proper interpretation of the results, so any prescription form that has not been duly completed is not validated. According to the ISO 15189 standard, it is the biologist who is responsible for the pre-analytical phase. Therefore, and especially at the hospital level, the laboratory should manage this phase and ensure the awareness and continuous training of all personnel in charge of blood sampling. Dialogue with the care services and reminders of good sampling practices are essential elements that allow the frequency of hemolyzed samples to be reduced. For these

reasons, the laboratory must take the initiative to apply standard operating procedures that are easy to understand. The central laboratory of the Mohammed VI University Hospital in Oujda (Morocco) has proceeded via national experts to the realization of several Audits on the pre-analytical phase particularly at the level of the emergency services, pediatrics, internal medicine and visceral surgery. The results have raised several non-conformities and non-compliance with the requirements by some services, including:

- Prolonged tourniquet application.
- Lack of hand washing and use of gloves.
- Failure to follow the order of sampling.
- Labeling of tubes before collection.
- Low tube filling rate.

Consequently, the central laboratory service has taken corrective and preventive measures and actions to improve the sampling process, namely awareness raising and theoretical and practical training on the conditions of sampling, the commitment of the central laboratory service and the management of the CHU in coordination with the pharmacy to provide adequate material of a suitable calibre for sampling and meeting the requirements of the standards and to use syringes only for injections and not for sampling (21G green sampling needle, 22G black sampling needle, Vacuum system: vacuum tube + body).

In our study, the prevalence of hemolyzed specimens was 14.61%, which is higher than that of M. Benlakehal *et al.* [7] and Carraro *et al.* [8] who reported a prevalence of 10% and 3.3% respectively.

The degrees of hemolysis were 1+ and 2+ during the determination of the hemolyzed tubes, which can be explained by the impact of agitation of the pneumatic system used for the transport of the totality of the tubes, in association with the stagnation of tubes at the level of the services and samples of the fragile patients, thus promoting the occurrence of hemolysis. The incidence of hemolyzed specimens was higher in the nephrology department with a percentage of 19%, mostly composed of hemolysis index 3+. This can be explained by the progressive deterioration of red blood cells due to mechanical stress (chronic hemolysis) that is inevitable during treatments that involve extracorporeal blood circulation, such as hemodialysis [9]. When blood is forced to flow through a narrow orifice or channel, the formed elements are subjected to severe turbulence and immense shear stress. As a result, red blood cells can be damaged and fragmented, a phenomenon known as red blood cell fragmentation syndrome. [10] These damaged cells are highly susceptible to lysis.

In our study, the prevalence of hemolyzed specimens was 14%, which is consistent with the literature that has shown that hospital emergency departments have been identified as the major source of hemolysis. Two studies conducted in hospital emergency departments found hemolysis rates of more than 30% [11] [12], while many other studies have observed rates (ranging from 6.8% to 19.8%) [13] [14]. Several other studies [11] [15] identified rates of hemolysis in the Emergency that were significantly higher than in other hospital departments.

Given that these services hospitalize fragile patients predisposed to hemolysis on the one hand, and on the other hand that these patients require follow-up and monitoring with several check-ups per day, rigorous attention to sampling conditions must be given to these patients in order to minimize the occurrence of hemolysis and its impact on the parameters studied. We believe that the sample is representative, but other studies must be conducted in our structure to detect other anomalies or dysfunctions and perfect the preventive and corrective improvement system. We also recommend that other studies cover the other services that were not studied in our study.

## 5. Conclusion

The medical biology act is part of a preventive, diagnostic, prognostic and therapeutic approach. The biologist is responsible for this procedure, which includes the entire macro-process with all the pre-analytical, analytical and post-analytical stages, from the prescription to the validation and transmission of results. The NF EN ISO 15189 and NF EN ISO/CEI 17025 standards define the general requirements concerning the quality and competence of medical biology laboratories and testing laboratories. This is why the quality search must be an essential and constant preoccupation of the biologist and of all the laboratory personnel. Therefore, quality control requires the commitment of all the personnel, rigorous daily work, the follow-up and the monitoring of all the components of this process, plus the continuous training and the maintenance of skills.

## Conflicts of Interest

The authors declare no conflicts of interest.

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