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Comparison of measurement accuracy between an ELISA reader and a UV-vis spectrophotometer

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Abstract

The study dealt with an extensive overview of the *Elisa* device, and what is the idea of its work, with a study of the *spectrophotometer* device, which is one of the classic devices that has a work idea close to the idea of *Elisa* work, and a comparison between the two devices. The techniques or strategies in which the two devices operate were studied and each type of which differed in the method of procedure and work steps as well as the accuracy of measurement. The safranin dye was used and the color intensity was measured, which is a reflection of the strength of the dye or color in each of them, and the data was tabulated and its results studied. The study showed that the *ELISA* reader was more accurate in the measurement process compared to the *spectrophotometer*. It was noticed that the *ELISA* technique is easy to measure, with the possibility of measuring a group of samples in one procedure instead of repeating the procedures with each sample as in the *spectrophotometer*, which could lead to contamination, especially when using sensitive samples or biological samples, noting that the time period needed by the *ELISA* reader is relatively less than the period required by the spectrometer device.

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مقارنة دقة القياس بين قارئ ELISA ومطياف الاشعة فوق البنفسجية والمرئية

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الملخص

تناولت الدراسة نبذة موسعة عن جهاز ELISA، وما هي فكرة عمله، مع دراسة جهاز الطيف الضوئي (spectrophotometer)، وهو من الأجهزة الكلاسيكية التي لها فكرة عمل قريبة من فكرة عمل ELISA، ومقارنة بين الجهازين. تمت دراسة التقنيات أو الاستراتيجيات التي يعمل بها الجهازين والتي يختلف كل نوع منها في طريقة الإجراء وخطوات العمل وكذلك دقة القياس. تم استخدام صبغة السفرانين وقياس شدة اللون وهو انعكاس لقوة الصبغة أو اللون في كل منهما، وتم جدولة البيانات ودراسة نتائجها. أظهرت الدراسة أن قارئ ELISA، كان أكثر دقة في عملية القياس مقارنة بالمقياس الضوئي. لوحظ أن تقنية ELISA، تتميز بسهولة القياس، مع إمكانية قياس مجموعة من العينات بإجراء واحد بدلاً من تكرار الإجراءات مع كل عينة كما في جهاز المطياف، مما قد يؤدي إلى التلوث، خاصة عند استخدام عينات حساسة أو بيولوجية. علماً بأن المدة الزمنية التي يحتاجها قارئ ELISA أقل نسبياً من المدة التي يحتاجها جهاز المطياف.

INTRODUCTION

Medical devices are a necessary health technology in order to provide health services in the field of disease prevention, diagnosis, treatment, rehabilitation of patients and providing them with complete health care. These devices are not medicines or vaccines, but are essential for detecting and diagnosing disease states. With the tremendous progress over the past few years, detecting the presence of any disease or damage in the body has become easy, by relying on a number of analyzes and medical tests and a number of modern devices that help ensure the presence or absence of the disease, as well as assisting in the correct diagnosis in order to prescribe the appropriate treatment for the patient (Organization 2017). And highlighting one of the most effective medical devices is the *Elisa* device, as it serves as the basis for clinical and routine tests. This widely applied technique provides specific detection for a variety of analyzes in different types of samples (Schuetz, Aujesky et al. 2015, Cawson and Odell 2017). Enzyme immunoassay (EIA) and enzyme-linked immunosorbent assay (Elisa) are widely used as basic diagnostic tools in medicine and as quality control procedures in various industries, they are also used as analytical tools in biomedical research to detect and identify specific antigens or antibodies in a given sample (Gan and Patel 2013). Doctors turn to the *Elisa* device in diagnosing and monitoring diseases that affect biological

systems. It is considered an indispensable method in medical and research laboratories. It is a technique that enables detection of the interaction between Antigens and Antibody thanks to a colorimetric reaction (Poghossian and Schöning 2014, Nasseri, Soleimani et al. 2018). The basic idea is that the *Elisa* device works on the basis of the chromatic absorption of the rays that pass through the sample to be measured, which is similar to the work of the *Spectrophotometer* (Capitán-Vallvey, Lopez-Ruiz et al. 2015), and therefore the idea of the work of the two devices depends on shining a light beam on the sample to be worked with, and then the concentration of the test is estimated by measuring the intensity of the light beam, Then the electrons absorb it and move to the higher level because it becomes excited and then return again to the lower level and it emits energy in the form of light (Cassano, Mawatari et al. 2014). *Elisa* technology is one of the most sensitive immunoassays available, the typical detection range of *Elisa* technology is 0.01 ng to 0.1 ng (Su, Liang et al. 2021). In this paper, we will shed light on a comparison between the two *ELISA* devices and the *spectrophotometer*, while studing the advantages and disadvantages in the diagnostic and analytical performance of each, noting the specifications of each technique in terms of cost, speed, and bioanalysis capacity. Reliability, the most accurate.

THEORETICAL PRINCIPLES

Elisa

ELISA is short for enzyme-linked immunosorbent assay, which is a biochemical test that depend on the use of antibodies and color change to determine the presence of a specific substance in a sample. The idea of this test is based on an important piece of information in immunology, which is the idea of specialized immunity (Aydin 2015, Konstantinou 2017). When a foreign body enters the patient's body (antigens), the immune system produces antibodies against it, and these antibodies are permanent and remain in the patient's blood, which is called immunological memory, that is, if you are exposed again to this foreign body or virus, the immune system produces these antibodies immediately. Antigens aim to bind to and eliminate this antigen as much as possible (Jacofsky, Jacofsky et al. 2020).

Figure (1) shows a picture of the *Elisa* device

This indicates that any foreign particle that enters the vital body must have a change in the patient's blood and antibodies are formed in it, even if it was a minor injury, Hence came the idea of the *ELISA* test, which relies on detecting the presence of antibodies in the patient's blood, which are evidence of the presence of a virus or other foreign body in the body. This technology is designed to detect and quantify peptides, proteins, antibodies and hormones. This technique is widely used in medical laboratories to ascertain the presence of a specific antigen or antibody in the patient's blood (Aydin 2015, Chadha, Bhardwaj et al. 2022).

It is a plate-based assay technology designed to detect and quantify peptides, proteins, antibodies and hormones. In an enzyme-linked immunosorbent assay, the presence of *Elisa* reactants attached to the surface of the microplate makes it easy to separate unbound substances during the assay. This ability to specifically eliminate unrelated substances makes *Elisa* a powerful tool for measuring specific analytes within a crude preparation (TIP 2010, Morris 2015).

The technique can be applied in a number of ways, which allow it to work with a good degree of flexibility and can be modified based on the available antibodies, the desired results, or the complexity of the samples, and each of these methods has its advantages and disadvantages (Boonham, Kreuze et al. 2014).

1- Direct *Elisa*
2- Indirect *Elisa*
3- *Elisa* Sandwich
4- Competition *Elisa*

ELISA Equipment

Equipment for *ELISA* testing is widely available. Readers, washers and pipettes are available as manual or automated systems. Some of the factors affecting equipment selection are the number and types of tests and samples, technical training of staff and financial considerations. Below is a brief outline of some equipment available for performing *ELISA* testing (TIP 2010).

Washer Systems

• Manual systems that wash one row or column at a time

• Semiautomated systems that handle one strip or plate at a time

• Fully automated systems that can process multiple plates

***ELISA* Plate Readers**

• Manual readers that read one row or well at a time

• Semiautomated readers that read one plate at a time

• Fully automated systems that can process multiple plates simultaneously

Other

• Humidity chamber (not required for all *ELISAs*)

• Plate sealers for assays that have long incubation times

(to avoid evaporation)

• Incubator or plate shaker incubator (not required for all *ELISAs*)

Spectrophotometer

Spectroscopy represents the study of the interaction of light with matter, which includes some phenomena, including reflection, refraction, elastic, and inelastic scattering, absorption, and emission, at a given wavelength. The *spectrophotometer* is more specialized for measuring samples within the electromagnetic spectrum, as it deals with visible, near ultraviolet and near

infrared light (Sanda, Victor et al. 2012). There are two types of *spectrophotometers*: Single Beam and Double Beam, the first is more stable, but the second is higher in wavelengths (Kalinowski and Koronkiewicz 2017). The working principle of the *spectrophotometer* technique is the same as the Elisa is one of its main components (Upstone 2000).

Figure (٢) shows the picture of the *spectrophotometer*

Main differences between the two technologies

As we mentioned that the two technologies have the same working principle, but there are some differences between them, the most important of which is that: the *Elisa* device, is used a plate often with 96 well into which the samples to be measured are placed, unlike the *spectrophotometer* uses a glass tube from the sample to be measured called a cuvette (a special test tube made of glass, plastic, or quartz) (Long, Woodburn et al. 2017). The plate helps to measure multiple samples and replicate different concentrations simultaneously, unlike a *spectrophotometer* which needs to change the sample at each measurement (Lim, Goh et al. 2017, Cao 2021). The *Elisa* device consumes a very short time compared to that of the *Spectrophotometer*. The *Elisa* device gives the required data at one specific wavelength that is fixed before the measurement, while the *Spectrophotometer* gives the required data over a range of wavelengths that must be fixed before the measurement (Thiha and Ibrahim 2015).

As well as being we need a very small amount of samples to be measured using the *Elisa* device, while in the *Spectrophotometer* we need a large amount of the sample to be measured (Christodouleas, Nemiroski et al. 2015).

MATERIALS AND METHODS

1- Sample preparation

The safranin dye (which is a vital dye used in histology and cell science, it is used as a dye for discrimination and contrast in some detection processes, for example in detecting types of bacteria, as it dyes the cell nucleus red) with a wavelength 490nm, was used in This research is for color discrimination, (This wavelength was adopted because it is often used to evaluate the bacterial content), as show in figure (٣).

There are several steps that were followed in the preparation of samples, which included the following:

1- Safranin dye was used at a concentration of 0.5 as the reference concentration (Control).

2- Dilutions of the dye were made in a ratio of 1:1 of the dye and distilled water, which distinguishes the water in red.

3- The solution was diluted in seven stages to be seven different concentrations and graduated in color.

4-The concentration of each solution includes half of the concentration that precedes it.

5- These dilutions were used for the two measurements in *Elisa* and *Spectrophotometer* devices.

Figure (٣) A picture of samples of different dye concentrations

2- *Elisa* measurement method

The color intensity or the amount of absorbance of the color used (the red color of safranin dye) was measured using the *Elisa* device absorbance values for all samples of the pallet or sheet with its replicates at the same time, as there is a feature to read the absorbance of all wells (96 wells) (Crowther 2009).

The measurement process was carried out as follows:

1- The pallet designated for the *Elisa* device was used, which consists of 96 wells, numbered from (1-12) for the horizontal arrangement that represents the types of concentrations or samples, and from (A-H) for the vertical arrangement that includes repeats for concentrations.
2- Seven different concentrations were used with five replications for the same sample.
3- Distilled water was added to the pits of the first column, which is the reference value for the rest of the samples or concentrations to which the dye was added.
4- Distribute the first concentration that was previously prepared to the second column, so that each column adds the same type of dilution and the same amount, which are considered replicates for one sample or one concentration of the dye.
5- Thus, the rest of the other diluted concentrations were distributed in a sequence from the sample with the highest concentration to the lowest, as shown in the figure (٤).

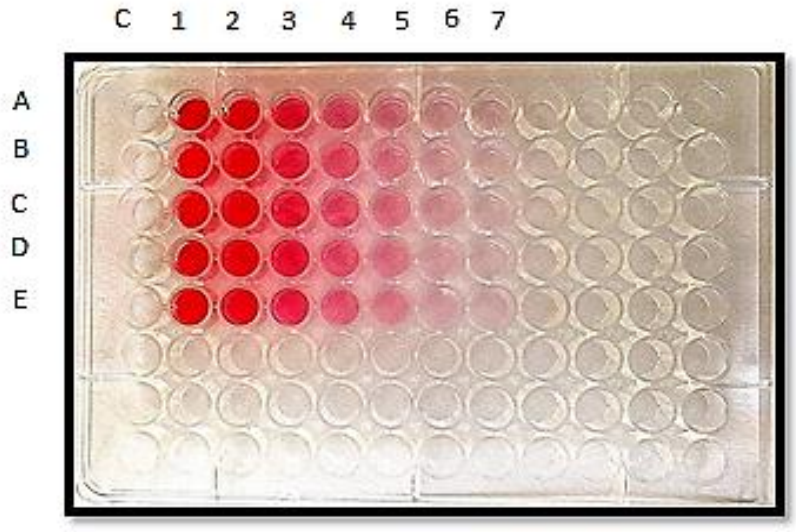

Figure (4): Image of samples in the plate

experiment steps

The measurement method takes place in several steps, including:

1- After the device was turned on, the plate was placed in the location designated for it.
2- The wavelength (490nm) has been fixed on the device screen.
3- After giving the measurement instructions, a table appears on the device screen showing the absorbance values for all the well in the plate.
4- It is possible to photograph the values from the screen, or extract the data using a printer of the device.
5- The data results were fixed in a table and compared.

3- Measurement method using a *spectrophotometer*

Measuring absorbance with a *spectrophotometer* is the classic method for measuring absorbance.

A device (UV-Vis 1800, USA) was used in which each sample is tested separately and compared to the value of Control. The first test tube (Cuvette) containing the Control sample is installed and compared with each sample (Morris 2015).

experiment steps

1- The measurement process takes place in a number of steps, as follows:
2- The test tube (Cuvete) was cleaned well before starting to add the dye.
3- After operating the *spectrophotometer*, the wavelength value of the dye was fixed, as the wavelength 490nm of the safranin dye was chosen.
4- Distilled water was added to the first test tube (Cuvete 1), which is the reference value (Control).
5- The first concentration of the dye was added to the second test tube (Cuvete 2), which is the absorption value of the sample of the first concentration of the dye.
6- The control tube was placed, the device was reset, then the first sample of the dye was added in the second location, designated for the sample to be measured inside the device, and the absorption value was read on the screen of the device and record it.
7- Replace the first tube with the second tube for measurement and read it in the same way as reading the first sample with the control sample installed.
8- In this way, five samples were measured with different dye concentrations.

RESULTS AND DISCUSSION

Elisa results

After obtaining the absorbance values, the average values for all replicates were taken to obtain the average values, as shown in the table (1):

Table (١): Absorbance using the *Elisa* device

sample	concentration	absorbance rate
١	٠,٢٥	٢,٤٥
٢	٠,١٢٥	١,٧٥
٣	٠,٠٦٢	٠,٩٠
٤	٠,٠٣١	٠,٤١
٥	٠,٠١٥	٠,٢٢
٦	٠,٠٠٧٨	٠,١١
٧	٠,٠٠٣٩	٠,٠٧

Spectrophotometer results

After obtaining the absorbance values using a *spectrophotometer*, the average values were taken, as shown in the table (2):

Table (٢): Absorbance by *spectrophotometer*

sample	Concentration g/l	absorbance average
١	٠,٢٥	٤,٠
٢	٠,١٢٥	٣,٤١
٣	٠,٠٦٢٥	١,٧
٤	٠,٠٣١٢٥	٠,٨٥
٥	٠,٠١٥٦	٠,٤٤

٦	٠,٠٠٧٨	٠,٢٧
٧	٠,٠٠٣٩	٠,١٦

The readings were represented in the graph as in Figure (5), in order to obtain the form of the relationship between concentration and absorbance.

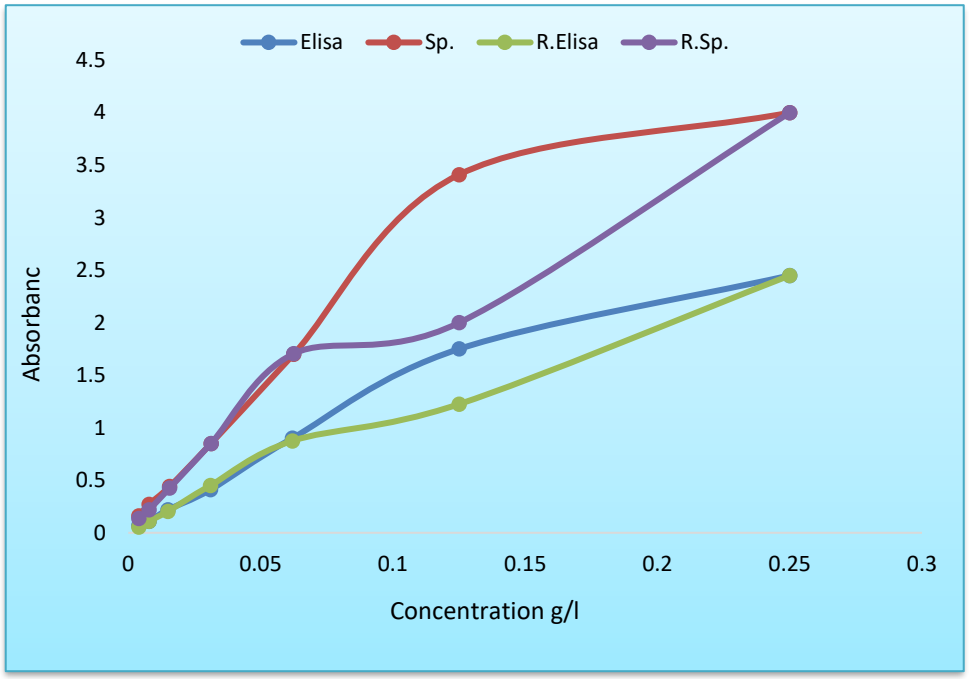

Figure (5): shows the relationship between concentration and amount of absorption.

After confirming the results for both *Elisa* and *Spectrophotometer* devices, several things were observed and confirmed theoretically, the most important of which are:

The measurement with the *Elisa* device is characterized by a kind of ease and flexibility compared to the work with the *spectrophotometer*, with which the measurement process was somewhat tiring, bearing in mind that there are no duplicates for the sample, but only one value is measured and relied upon. In the *spectrophotometer*, the outer surface must be cleaned continuously with each sample being changed in order to avoid any defect in the reading.

Through the results of the tables obtained and Figure (5), it was noted that the measurement using the *ELISA* device was more accurate and closer to correct

than the readings obtained using the *spectrophotometer*, with relative differences in the values of the two devices.

After diluting the samples (dye) by half or by a ratio of 1:1 of the dye and distilled water, it is expected that the absorbance ratio will decrease to half continuously with increasing dilution and according to the law of dilution (Johari and Paduano 1997). which states that:

$$M_1 V_1 = M_2 V_2$$

M_1: Initial solution concentration (before dilution)
M_2: Final solution concentration (after dilution)
V_1: Initial solution volume (before dilution)
V_2: Final solution volume (after dilution)

And this is what was observed from the measurement with the *Elisa* device. As for the *spectrophotometer*, the values began to decrease to half of the third section, approximately, Therefore, a curve of reference values was attached to each curve of the results of any device (R. Sp. , R. Elisa) to determine the amount of halving the values.

The *ELISA* device has a high sensitivity, Since the technology is intended for the detection of specific antibodies or a specific target compound. so, a high sensitivity can be achieved in the tests used for this technique. This means that lower concentrations of a target compound can be detected more accurately which is usually of a specific color and concentration (Li, Nie et al. 2010).

Also, the technique is more selective, as the *ELISA* technique is considered more selective compared to the *spectrophotometer*. Antibodies that interact specifically with the target compound are used, thus avoiding interference from other compounds. In addition to having multiple analysis, it is an technique that allows analysis of multiple reads to be performed at the same time, which increases the efficiency of the process and reduces the time taken (Weber, Raymond et al. 2006, Xing, Meng et al. 2012).

CONCLUSION

A comparison has been made between the ELISA reader and the spectrophotometer using the color dye (safranin), showed that the *Elisa* device is more accurate and better than the *spectrophotometer*. It was noted that the *Elisa* technique is characterized by softness, ease of visualization of results, and a high

level of accuracy. Its tests are very specific and sensitive, as well as the possibility of conducting a large number of tests that can be performed by with a small number of employees. And the opposite of what is in the *spectrophotometer*, which requires a relatively large time.

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