Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 500 - 514
Received 05 March 2021; Accepted 01 April 2021.

Assessment of Immune-Detection Capacity for Escherichia Coli Antigen in People at Risk in Holy Kerbala

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Abstract :

The objective of this experimental research was to determine the immunity detection capacity for *Escherichia coli* in people at risk in Kerbala Province, against a standardized *Escherichia coli* antigen under laboratory conditions. The antiserum was obtained by inoculation of the inactive antigen of bacterial suspension of *E. coli* to rabbits breed Iraq. The serum with antibodies was obtained 28 days after starting the antigen inoculation program, in the respective specimens. Verifying the presence of antibodies, by means of the agglutination reaction in the 10 dilutions (1:10 to 1: 5120), with the respective antigen. The confronted antigen, with the serum and blood of people, was obtained agglutination. Concluding that 91.7% of the people under study have generated antibodies to this isolation.

Keywords: Antiserum, antigen, *Escherichia coli*

1.0 Introduction

The immune system is a complex grouping of biochemical and cellular reactions that serve the purpose of defending the individual from foreign agents. Schematically and broadly, the immune system is divided into three: physical barriers, innate immunity, and adaptive immunity. In the human body, there are approximately ten times more bacterial cells than human cells, preferentially distributed in the skin and digestive tract [1]; man normally harbours around 400 species of microorganisms that exceed 1250 g, equivalent to the weight of the liver; of these, 80% (1000g) are found in the intestine, 20 g in the mouth, 30 g in the vagina and 200 g in the skin; each of these could invade tissues, causing disease and death, but thanks to the protective effect of the immune system, it makes most of these harmless or beneficial, recognizing harmful microorganisms and malignant cells, as entities strangers in the body, so they are rejected, allowing to preserve identity and integrity, otherwise the disease occurs [2] . By nature, different species of bacteria thrive in the large intestine of man, especially *Escherichia coli*, which corresponds to the group of non-photosynthetic Gram negative Eurobacteria; which is one of the most characteristic members of the normal intestinal flora of mammals and an important pathogen that causes intestinal and urinary tract diseases, which, being in minimal populations, do not show their virulence, until a disorder occurs fortuitous that sometimes cause infections, altering the immune physiology of the individual, leading to disease that can cause death [3]. *E. coli* infection can lead to the appearance of intestinal symptoms when the infection occurs due to the consumption of contaminated food or the increase in the amount of bacteria in the intestine due to changes in immunity, or urinary, being considered the main cause of urinary infection in women [4]. On

the other hand, the attacked individual can defend himself from the pathogen totally or partially, depending on the balance established between the pathogenicity of the bacterium and the efficiency of the natural, acquired, active or passive immune response of the host; evidenced through the presence of specific antibodies, being responsible for polymorphonuclear cells, monocytes, dendritic cells, lymphocytes and platelets, originating in the bone marrow from a pluripotential cell or basal cell, due to special influences, possibly of a hormonal type, received in its microenvironment [5].

Faced with the aforementioned problem, the present investigation was developed that allowed us to determine the immunity of people at risk to an isolation of *Escherichia coli*. [9] In Iraq, according to the report on potable water and sanitation services, it reports that 90% of the urban area and 61% have potable water; 81% of the urban area has sanitation while in the rural area it only has 36%, leading them preferentially to acquire gastrointestinal diseases, which over time possibly in them are acquiring immunity [6-7].

Description of the problem: Taking into account that the Kerbala Province population, in 100%, does not have optimal basic services, we are exposed to frequently acquire gastro-intestinal infections, which in most people present clinical manifestations and others do not, despite living conditions are unsanitary [8]. This characteristic led us to carry out the present investigation, in order to determine the existence or not of immunity through a known antigen.

Formulation of the problem: What is the immunity detection capacity for *Escherichia coli*, in people at risk from the Kerbala community [10], against a standardized *E. coli* antigen under laboratory conditions?

Justification and importance: In the digestive tract of man, specifically in the large intestine, variants of the bacterium *Escherichia coli* thrive; whose populations in naturally normal quantities do not cause harm; except when physiological disorders occur, which allow to increase its population, causing intestinal infections; with symptoms of diarrhoea in the patient; they can also cause urinary tract infections, which under circumstances lead to death.

Technical-practical justification: Gastrointestinal diseases caused by *Escherichia coli* have become a public health problem due to their high incidence and prevalence, mainly in developing countries like ours. This type of infection is due to the lack of adequate crop management, food handling and basic sanitation; a condition that has resulted in the exposed population, in some cases having acquired immunity over time. Reason why the present investigation was organized and developed; whose objective was to verify the existence of immunity to a certain isolation of this pathogen.

Institutional and personal justification: Taking into account on the literature and scientific research at University of Kerbala, University in Kerbala, Iraq, results of different investigations are periodically reported; that are taken into account, to solve problems at the local, regional and national level. This research will serve as a source of information and a starting point for other research related to this one.

Research delimitation: This research was developed taking into account scientific information; The *Escherichia coli* strain was obtained from the Microbiology laboratory of the University of Kerbala, University in Kerbala, Iraq; Two rabbits (*Oryctolagus cuniculus*) Iraq breed were used; Informed consent was applied to people who voluntarily participated in the study; Non-maleficence was practiced; The research was self-financed.

Limitations: In Iraq, the issue of health for the inhabitants has a strong cultural component, particularly in Kerbala , where the population is characterized by being multi-diverse, in which the preconceptions in relation to undergoing diagnostic tests are limiting and negative, especially when it comes to invasive procedures such as blood collection. This situation is a great impediment to achieving their acceptance of participation as a study unit in any research work, since there is a belief that when small amounts of blood are extracted, they can harm them or whoever does the procedure can do it for commercial purposes. This is the case of the present study, in which there was an outright refusal to participate. Reason why informed consent was barely obtained from twelve people of legal age.

Research objective: To determine the immunity detection capacity for *E. coli* in people at risk in the Kerbala community, against a standardized *Escherichia coli* antigen under laboratory conditions.

Hypothesis and Variable Approach

2.1. Hypothesis: Alternative hypothesis. There is an immunity detection capacity for *E. coli* in people at risk in the Kerbala community, against a standardized *Escherichia coli* antigen under laboratory conditions.

Null hypothesis. There is no immunity detection capacity for *E. coli* in people at risk in the Kerbala community, against a standardized *Escherichia coli* antigen under laboratory conditions.

2.2. Variables / categories

- Independent variable: standardized *E. coli*, (antigen).
- Dependent variable: immunity to *E. coli*. (antibody)

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2.3. Operationalization and categorization of the components of the hypotheses

Variables	Categorization	**Operationalization**
Independent	Antigen: *Escherichia coli*	a) Isolation, purification, identification and multiplication of the *Escherichia coli* microorganism, in Mc culture medium. Conkey. b) Standardization and inactivation of *E. coli*. c) Inoculation of *E. coli* (antigen), to rabbits (Oryctolagus cuniculus), following an established program d) Titer test at different dilutions of serum with antibodies. e) Confrontation of antigen with antiserum in different dilutions
Dependent	Immunity to *E. coli*	The human immune system has the ability to detect molecules, microorganisms and virus particles, to generate antibodies as defense. This principle was determined through the present research work. Checking through the agglutination between human blood and the antigen constituted by standardized *E. coli* cells (antigen). a) Confrontation of antigen with human blood. b) Positive agglutination (+).

3.0 METHODOLOGICAL FRAMEWORK

3.1. **Geographic location:** The present investigation was developed in the Microbiology and Phytopathology laboratories of the University of Kerbala, Iraq, located on the university campus, located seven kilometres south of the city of Kerbala, in Kerbala Province.

3.2. **Research Methods:** In the present investigation the analytical and deductive methods were used; because they are based on experimentation and observation of the phenomena under study

3.2.1. **Feeding rabbits:** The specimens, before being exposed to the antigen inoculation program (dead cells of *E. coli*) were fed with rabbit for 20 days; increasing its weight in 102 and 120 g respectively. From the first day of the antigen inoculation program until its completion, the animals had the same diet.

3.2.2. Obtaining control serum: 10 ml of blood was drawn from each rabbit; the same one that was arranged in two tubes, with their respective identification. After 20 minutes of rest of the blood tissue, the serum was extracted, placing them in two other tubes, keeping them refrigerated at the pole; the same that was later used as a means of verification.

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Figure 1. Blood tissue and serum suspension, from rabbits

3.2.3. Obtaining the antigen.

I. **Isolation of *E. coli***, the sample of *E. coli* was obtained from the Microbiology laboratory of the University of Kerbala, Iraq, a strain verified through Gram staining, and the respective biochemical tests.

II. **Preservation of *E. coli***, in order to obtain young *E. coli* cells, to be conserved in sterile distilled water. The original culture medium was seeded in two new Petri dishes with Mac Conkey medium, incubated at 30°C for 24 hours; part of these colonies were placed in two "screw cap" test tubes containing sterile distilled water, kept refrigerated at 8 ° C.

III. **Preparation of the suspension** *E. coli*, as antigen, the other part of new colonies were extracted, with a seeding loop and placed in two test tubes, containing 10 cc of the saline solution at 0.85%; immediately after leaving the first portion of the bacterial sign, in the saline solution, it was stirred, with an electric stirrer; observing the respective turbidity; This process was carried out three times, until obtaining a bacterial suspension of $1x10^8$ to $1x10^9$, per cm^3; Verifying the turbidity through the coloration of the McFarlan scale [12].

IV. **Preparation of the antigen,** the suspensions of the *E. coli* bacterial cells, to be used as antigen, were inactivated, subjecting them to a stable temperature of 70 º C, for two hours, in a "water bath". The verification of death of these cells was verified, after having carried out seeding in Mac Conkey medium, that after 24 hours of incubation at 30ºC, there was no presence of colony growth.

3.2.4. **Obtaining antibodies, originated by the inoculation of dead *E. coli* cells, in rabbits [13]:** The suspension of dead cells of *E. coli* was inoculated in the outer vein of the ear of each rabbit, according to the program in Table 1.

On the first and third day, 0.1 ml was injected; on the sixth day 0.3 ml; on the eighth and tenth day 0.5 ml; at fourteen and seventeen days 1 ml; at twenty-twenty-three 2 ml; performing the titration test at 28 days, in order to determine the presence of antibodies formed by the induction of an *E. coli* isolation, in the blood tissue of these animals; the same one that consisted of extracting 10 ml of blood from each rabbit; obtaining the serum, to be confronted with the antigen (suspension of dead *E. coli*), in dilutions 1:20; 1:40; 1:80 and 1:

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160. Procedure that was based on the scientific foundation that all antigens (dead cells, proteins, polysaccharides, lipids, nucleic acids that are injected into a warm-blooded animal induce the formation of antibodies, causing immunity; in most cases, the level The maximum number of antibodies in the blood is detected between 3 and 5 weeks after the beginning of the infection, where the antibody specifically binds to the antigen that has given rise to the immune response; because the structures of both molecules complement each other as a key to the lock, as mentioned by Parker (2012).

Table 1. Program for inoculation of the antigen (*E. coli*) to rabbits, to obtain the antiserum or serum with antibodies.

Day	Volume (mL)	Via
1	0.1	Intravenous
3	0.1	
6	0.3	
8	0.5	
10	0.5	
14	1	
17	1	
20	2	
23	2	
28	Degree test	

3.2.5. Titer test [15]: Once the intravenous inoculation of the antigen (dead *E. coli* cells) into the rabbits was concluded, the "titer test" was carried out, using certain dilutions (1:10; 1:20; 1:40; 1:80; 1: 160; 1: 320 and 1: 640) of the serum with antibody, these, mixed with 0.5 ml of antigen, gave a positive reaction (+), forming a milky precipitate at the bottom of the tube. With this evidence, the blood was extracted from the specimens, to obtain the serum to be used in the antigen mixture and to determine until which dilution the presence of antibodies induced by *E. coli* was evidenced.

3.2.6. Process to determine antibodies, originated by *E. coli*, in the blood of rabbits: To find out until which dilution of the antiserum, it is feasible to determine antibodies by agglutination, we experimented with 10 dilutions of antiserum, from 1:10 to the 1: 10250 dilution, for this, 10 test tubes were used, with 0.85% saline solution.

- 0.9 ml of saline solution was placed in the first tube and 0.5 ml in the remaining 9 ml of antiserum (serum with antibodies) was added to the first tube, shaken and left to rest, this dilution corresponds to 1:10; 0.5 ml of this was extracted and added to the second tube obtaining the 1:20 dilution, shaking and extracting 0.5 ml, to add to the third tube, obtaining the 1:40 dilution. This procedure is repeated until the tenth tube obtaining the 1: 5120 dilution of the antiserum.
- Subsequently, 0.5 ml of antigen (dead *E. coli* cells) is added to the tubes containing the diluted antiserum (serum with antibodies), shaken and left to rest; When the

reaction is positive (+), a white precipitate or agglutination will be observed at the bottom of the tube and if it is negative (-), there is no agglutination.

3.2.7. Reaction; antigen – antibody: To determine the presence of antibodies, in the blood plasma of people, that coincide with the antigen that is the reason for this investigation; The antigen (*E. coli*) confrontation was carried out with the blood of 12 people at risk. The test consisted of obtaining a drop of blood from each of the 12 people, arranged on object slides, to which a drop of the conserved antigen (dead cells of *E. coli*) was added, making the respective mixing.

3.3. Design of the investigation: The study was experimental, descriptive, qualitative, because the *Escherichia coli* bacterium was isolated and recognized, which when inoculated intravenously to rabbits, the formation of antibodies to this isolate was obtained, which was verified by confronting the antigen with the antibodies generated through the formation of precipitates in different dilutions from 1:10 to 1: 5120

3.4. Population, sample, unit of analysis and units of observation

3.4.1. Population and sample: It was made up of 12 people.

3.4.2. Inclusion criteria

- Public cleaning workers exposed to acquiring gastrointestinal diseases.
- People who gave their informed consent.

The reasons that explain the small number of the study sample have been explained in the category of limitations, also adding that these workers can be considered high risk since the handling of solid waste from the municipal service garbage collection, performed without the basic protective implements for this type of work, and therefore would be more likely to be affected by pathogenic microorganisms, in this case by *E. coli*, a situation that is aggravated by the omission of hygiene measures, especially the hand washing.

3.4.3. Analysis unit: Each blood sample obtained from the 12 people who authorized the extraction of the same.

3.5. Techniques for the processing and analysis of information: It was made use of; the documentary technique which allowed us to collect the necessary information in the present investigation that sustains the phenomena and processes; the field technique that allowed the observation of the experiment to contrast theory with practice in the search for objective truth.

3.6. Biological material

a) *Escherichia coli* strain, the same one that was purified in the Microbiology Laboratory and multiplied in the Plant Pathology Laboratory.
b) Two Iraq white rabbits weighing 4,280 and 4,766 kg.
c) Twelve volunteers, exposed to the handling of solid waste from the city of Kerbala.

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3.7. Equipment, materials and supplies, etc.

3.7.1. Laboratory equipment

- Optical equipment: stereoscope, microscope, photographic camera.
- Sterilization and asepsis equipment: laminar flow chamber, incubator, autoclave, stove, Bunsen burner, alcohol burner, alcohol spray.
- Blood collection equipment: brand PHLEGM SUCTION 7E-A.

3.7.2. Materials

- Glassware: beakers, erlemeyers of different capacities, Petri dishes, 15 cc screw-cap test tubes and thermometer.
- Inputs MacConkey culture medium: for 1000cc of medium was used:
    - 0.0005 ml Crystal Violet 7.5 g Agar
    - 10 g of Peptone 5 g of D-Sorbitol
    - 0.75 g bile salt 2.5 g sodium chloride
    - 0.015 ml neutral red
- Reagents: to perform the Gram staining of *E. coli*, through the process modified by Nyfel, the following were used:
    - Crystal violet solution:
    - Violet crystal 0.5 g
    - Phenol 2.5 g
    - Ethyl alcohol 96 - 97% 20.0 ml
    - Glycerin 80.0 ml
    - Distilled H_2O 100.0 ml
    - Lugol's solution:
    - Iodine 1.0 g
    - KI 2.0 g
    - Distilled H_2O 100.0 ml
    - Safranin solution: 1% safranin aqueous solution 0.85% sodium chloride solution

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3.8. Methodological consistency matrix

Problem formulation	**Objectives**	**Hypothesis**	**Variables**	**Indicators / qualities**	**Source or data collection instrument**	**Methodology**	**Population and sample**
What is the detection capacity of immunity against *Escherichia coli*, of a standardized *E. coli* antigen under laboratory conditions in people at risk in the city of Kerbala 2018?	To determine the capacity to detect immunity against *Escherichia coli* of a standardized *E. coli* antigen under laboratory conditions in people at risk in the Kerbala community. 2018.	**Ha.** There is a capacity to detect immunity against *Escherichia coli*, a standardized *E. coli* antigen under laboratory conditions in people at risk in the city of Kerbala . 2018. **Ho.** There is no detection capacity of immunity against *Escherichia coli*, of a standardized *E. coli* antigen under laboratory conditions in people at risk in the city of Kerbala .	**Independent Antigen:** Standardized *Escherichia coli***.** **Dependent** Detecting capacity of immunity to *Escherichia coli.*	Obtaining rabbit serum with antibodies from *E. coli* isolate Positive agglutination reaction	Obtaining rabbit serum without antibodies. Obtaining the serum with antibodies to different solutions from serum with antibodies. Agglutination reaction of serum with antibodies with human blood	a) Isolation, purification, identification and multiplication of the *Escherichia coli* microorganism, in Mc.Conkey culture medium. b) Standardization and inactivation of *E. coli*. c) Inoculation of *E. coli* (antigen), to rabbits (Oryctolagus cuniculus), following an established program d) Titer test at	**Population** 12 people at risk Sample 100% of the population

		2018?				different dilutions of serum with antibodies. e) Confrontation of antigen with antiserum in different dilutions	

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4.0 RESULTS AND DISCUSSION

4.1. Results presentation

4.1.1. Checking for *E. coli*, the sample was found to:

It is a facultative anaerobic bacterium Reaction to negative Gram stain (-) Reaction to catalase positive (+); Negative oxidase reaction (+)

These results are consistent with studies conducted by Brock.

4.1.2. Reaction of antiserum with antigen

4.2. Analysis, interpretation and discussion of results

Table 2. Positive reaction (+), of the antigen (dead cells of *Escherichia coli*), in 10 dilutions of antiserum (serum with *E. coli* antibodies).

Tube number	**NaCl 0.85 %**	Antiserum mL	**Dilution**	**Antigen-mL**	**Final dilution**	**Agglutination**
1	0.9	0.1	01:10	0.5	01:20	+
2	0.5	0.5	01:20	0.5	01:40	+
3	0.5	0.5	01:40	0.5	0.097222	+
4	0.5	0.5	0.097222	0.5	0.152778	+
5	0.5	0.5	0.152778	0.5	0.263889	+
6	0.5	0.5	0.263889	0.5	0.486111	+
7	0.5	0.5	0.486111	0.5	0.930556	+
8	0.5	0.5	0.930556	0.5	1.819444	+
9	0.5	0.5	1.819444	0.5	3.597222	+
10	0.5	0.5	3.597222	0.5	1:10250	+

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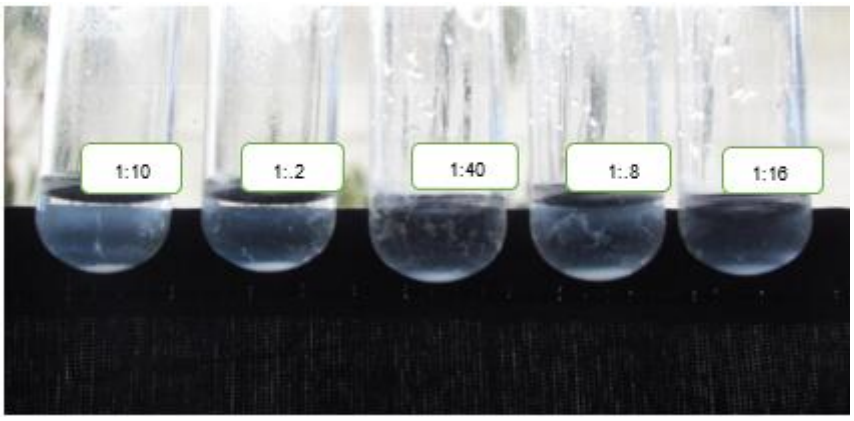

Figure 2. Agglutination reaction of five dilutions (1:10, 1:20, 1: 40,1: 80 and 1: 160) of the antibody with the antigen (dead cells of *E. coli*)

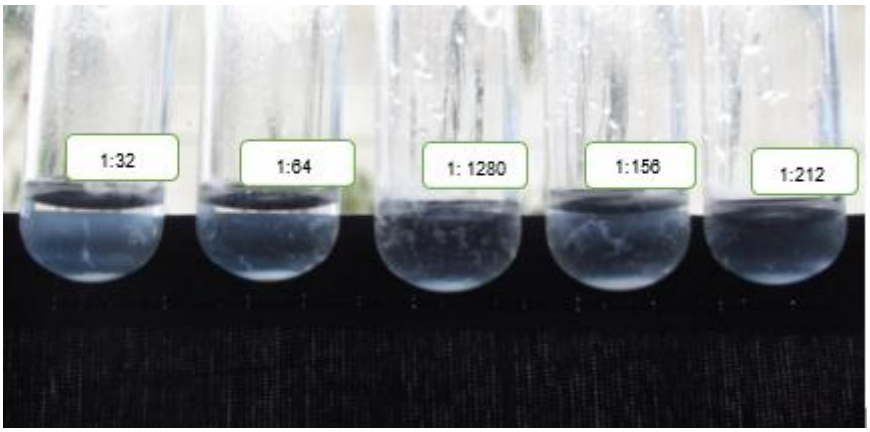

Figure 3. Agglutination reaction of five dilutions (1: 320, 1: 640, 1: 1280, 1: 15600 and 1: 2120) of the antibody with the antigen (dead cells of *E. coli*)

- Images showing agglutination reaction of the antigen with the antibody

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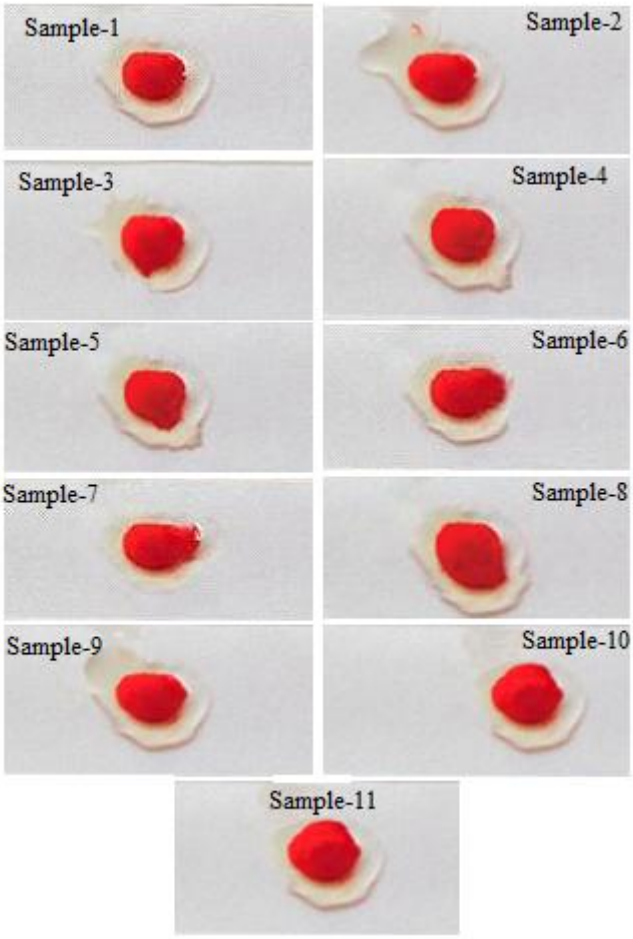

The positive reaction (+) of the antigen with the antiserum (serum with antibodies), shows us that the dead cells of *E. coli*, injected into the rabbits, were mobilized through the blood tissue of the animal and that by physiological nature they were arranged in lymph and lymphoid organs such as the spleen; in these organs the cells responsible for the formation of antibodies were activated; as it was possible to verify when carrying out the assay for the presence of antibodies under *in vitro* conditions, as shown in Table 2 and Figures 2 and 3.

Table 2 shows the positive reaction (+), of the standardized antigen (dead *Escherichia coli* cells), in 10 antiserum dilutions (serum with *E. coli* antibodies); result considered as a primary response, which counteracts the infection of the isolation of this *E. coli*. These results endorsed by Plückthun (1990) who affirms that, when harmful microorganisms enter the human body; the cells responsible for immunity, which are the T lymphocytes, intervene by activating the B lymphocytes, to generate the formation of the respective antibodies.

The results of the previous test; where the presence of antibodies in the blood tissue of rabbits, caused by the presence of the antigen, constituted by the dilution of cells from an *E. coli* isolate, was demonstrated, led us to continue the research, using blood from volunteers; whose way of working, in most of them, is the collection of solid waste, from the city of Kerbala.

To check the presence of antibodies in humans, as a protection measure, against a future infection, by an isolation identical to the isolation of *E. coli*, which we use as an antigen; we

determined that, when facing a drop of blood from people at risk, with an aliquot of the antigen (known *E. coli*), we obtained agglutination reaction or positive reaction in 11 people; of the 12 that made up the study sample, which percentage equates to 91.7%, a result that coincides with Abram et al (1998) [17], who states that agglutination occurs when an antibody meets an antigen that is part of a cell or an insoluble particle and that serves for the identification of pathogens and their products. Also endorsing this research, there is the report by Plückthun (1994) [18] who affirm that glutination is positive when a suspension of the microorganism that we want to diagnose is used as antigen and confronts serum. In this case, the positive result (+), of the serum of the people at risk, indicates the existence of antibodies, because the immunoglobulins, against the antigen, reacted with agglutination.

The positive reaction determined that the immunoglobulins (Ig) or antibody and the molecules of the major compatibility complex (MHC), which are in the blood of people, responded by recognizing the standardized antigen, through agglutination; result that coincide with the report by Walle, et al.,(2010).

4.3. Hypothesis testing

If there is a detection capacity of immunity against *Escherichia coli*, of a standardized *E. coli* antigen under laboratory conditions in people at risk in the city of Kerbala. 2018.

5.0 CONCLUSION

The *E. coli* antigen standardized under laboratory conditions at the University of Kerbala,Iraq has the ability to detect immunity against *Escherichia coli* in 91.7% of people at risk studied.

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