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A NEW LOW-COST AND PORTABLE ELISA READER BY USING A PHOTODIODE MATRIX AND ELECTROLUMINISCENT (EL) LAMPS

Beatriz García[1], Jesús A. Baro[2], Cristina de la Torre[1], Rocío Muñiz[1], Miguel A. Pérez[1]

Dpt. de Ingeniería Eléctrica, University of Oviedo. Gijón, Spain, maperezg@uniovi.es
Dpto, de Ciencias Agroforestales, University of Valladolid, Palencia, Spain, jbaro@arrakis.es

Abstract – The tool of immunological diagnosis denominated ELISA (Enzyme Linked Immuno-Sorbent Assay) it is probably the most used colorimetric test and it evaluates the compatibility antigen-antibody.

The development of a portable device and low cost that it is allows analyzing the ELISA plate ("in situ"). It will suppose one immediately identification of the disease and therefore a faster performance to palliate this disease. In addition to the being to low cost it will be more accessible for the population, especially of the underdeveloped countries population, where they have a depressed immunological systems as a result of the bad general condition of life; and this diseases will cause them serious injuries or even the death.

Another reason, but not less important it is that the presence of this disease cause great lost economic, with respect to a primary production sector like it is the farming sector, that produce a great social impact.

This paper presents a new portable instrument for read ELISA plates, based of the colour level (this measurement will take place in photodiodes) and using low-cost components.

Currently, plates are measured either photometrically which is costly and no portable or manually which is unacceptably subjective and dependent on training and illumination. This work shows a technique to improve the efficiency of clinical interpretation ELISA diagnostic plates.

Keywords: ELISA, clinical diagnosis, colorimetric measurement.

1. INTRODUCTION

Enzyme Linked Immuno-Sorbent Assay (ELISA), a useful tool used mainly in clinical immunology, is the first-choice test to detect the presence of certain pathogen in bodily fluids and it is used for routine screening of viral infections [1, 4, 5, 6, 7]. Based on the principle of specific antigen-antibody interaction, it makes feasible no use of toxic or radioactive substances, and its outcome lies in a change in colour. ELISA tests were performed taking several plates from routine monitoring in cattle of Brucellosis, red nose (Infectious Bovine Rhinotraqueitis, IBR) and Bovine Virus Diarrhoea (BVD) [2].

ELISA uses 80 x 120 mm polystyrene plates with an 8 x 12 matrix of 10 mm wells, 7 mm in diameter as is shown in Fig. 1. The plate wells are coated with pathogen antigens [1, 4]. Afterwards, each well is filled with a sample of suspected host fluids, then the plate is washed and all plate wells are filled with enzyme-conjugated anti-immunoglobulin. After a second wash step, a dye is added and a change in colour is produced due to the presence of the enzyme. This change in the colour will be produced in the well only if a specific link between the coating antigen and the anti-immunoglobulin was produced in the sample, due to the presence in the sample of an immunoglobulin specific for the antigen, which constitutes evidence of infection. The colour reaction is proportional to the concentration of immunoglobulin in the sample. The absorbance of the plate well is measured photometrically at a wavelength specific for the dye, usually TetraMethyl Benzidine (TMB) at 450 nm.

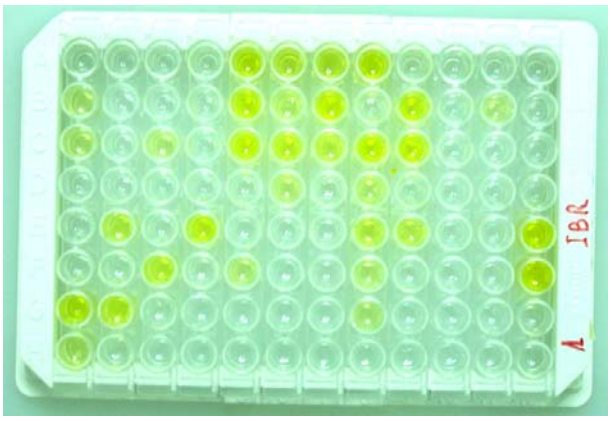

Fig. 1. Example of a ELISA plate for IBR diagnosis after processing. The plate is constituted by a matrix of 12x8 wells and the resultant colour informs about the presence of specific antibodies. In this case, the dye produces a scale of colour in yellow. The intensity of colour of each well can be determined by light absorption at 450 nm.

The read-out of ELISA plate needs reference colours and some of wells are used for this purpose as positive and negative references (positive and negative controls), establishing the measurement scale for all wells. In addition, colour levels of controls are used to validate a plate [1]:

- Large differences between positive controls or between negative controls would indicate a defective plate. In that case, complete plate is

rejected and it becomes necessary to repeat the analysis with other plate.

- Similar values of average of positive controls and average of negative controls indicate a low-sensitivity plate, and causes a short measurement scale, thus, the identification process of positive and negative wells becomes very difficult. In that case, plate should be rejected.

Spectrophotometers are used to read-out ELISA plates [1, 4], but is a quite expensive piece of equipment. Other alternative such as subjective determination by human operators is generally unacceptable because of its low precision, ambient dependence, and lack of repeatability.

Image analysis applied to pictures of ELISA plates is an easy way to obtain excellent results in reading-out, providing back-up information (picture) and reliable results when a PDA and a commercial-grade camera is used [3] but it needs a training operator to take quality pictures without image aberrations and/or dark or defocused images.

In this paper, a new portable solution for reading-out ELISA plates is presented. This solution uses an electroluminescent (EL) lamp for excitation and a photodiode matrix for reading the colour level in each well, resulting in a compact, portable and low-cost reader, ready to use *in-situ* in most of cases. This fact becomes extremely important in the application of this system in areas where the access to analysis laboratories is not possible.

2. SYSTEM DESCRIPTION

The cross section of designed system shows the operating principle as it is shown in Fig. 2, where we can see the excitation light source and the photosensors. The produced light is modulated in intensity by the coloured liquid in the wells of ELISA plate and reaches the photosensor – a photodiode sensible to appropriate wavelength – carrying the information about the colour intensity and, so, the information of the presence of viral infection in the original fluid.

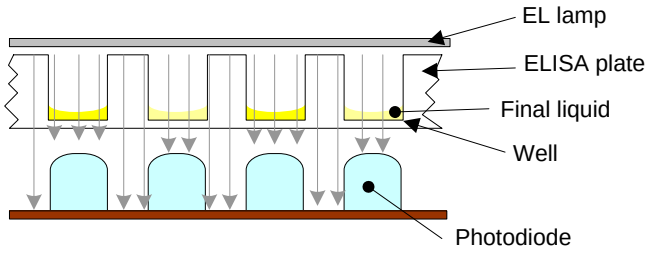

Fig. 2. Cross section of the ELISA reader showing the principle of operation, based on measured the light produced by an EL lamp, modulated by the coloured liquid in each well of plate. Thus, the light received by photodiode is depending on the antigen-antibody interaction.

The appropriate wavelength is depending on the type of plate and the involved fluids, but most of plates are read in 405, 450 nm (blue area) or 635 nm (red). Thus, EL lamp must produce enough light in those areas of spectrum without penalization of size and weight because is a thin film with less than 0.5 mm of thickness. EL lamp needs an AC voltage (100 V) at 400 Hz to obtain a flicker-free uniform illumination surface, able to cover the total area of plate. Fig. 3 shows the power supply of this special lamp, constituted by a rectifier stage and an inverter.

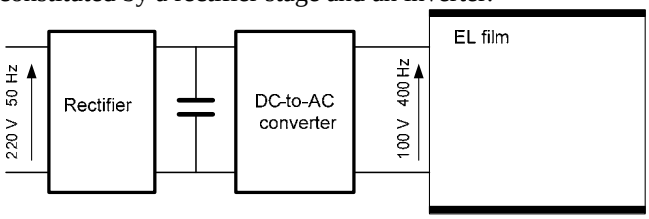

Fig. 3. Power supply for the EL lamp.

The emission colour of El lamp should be according to the type of ELISA plate under analysis, but a white EL lamp is used to design a universal analysis system, In that case, the wells are excited in a wide spectrum area, the photo-sensor receives all wavelengths and the processor will decide what is the appropriate channel for the best sensitivity of analysis.

In order to simplify the design, a white light EL lamp is used, able to produce enough light in blue, green and red wavelengths. Thus, all wells can be excited with the appropriate wavelength; the photodiode used is a triple RGB photodiode that provides independent photocurrents from the three fundamental colours.

The photocurrent produced by each channel of each photodiode would be processed by a current-to-voltage conditioning circuit but a simple operation will reconsider that choice: 8 rows x 12 columns x 3 channels = 288 circuits!! This result would cause a high volume, and a high cost solution, not valid for portable equipment.

A time-multiplexed circuit controlled by a MCU will reduce the size of complete processing system as is shown in Fig. 4.

The spent time in reading process is not a problem due to the steady state conditions of measurement and the total number of circuits is reduced to 12 columns x 3 channels = 36 analog multiplexer (8:1) and three current-to-voltage converter – one for each colour channel – achieving a high reduction of size and complexity from initial solution. However, the area restrictions imposed by the size of ELISA plate force to use a multilayer PCB to find a cost-effective solution for the system.

Each RGB photodiode (3 photodiodes) will produce three photocurrents depending on received light in each band of spectrum. When a photodiode is selected by means of three lines (row selection) and 12 lines that control the inhibition input of multiplexer (column selection), these three photocurrents are converted to voltage, read by A/D, and averaged to obtain three values of received light. This process continues with each RBG photodiode until complete the matrix. Thus, the MCU has three values of light for each well of ELISA plate.

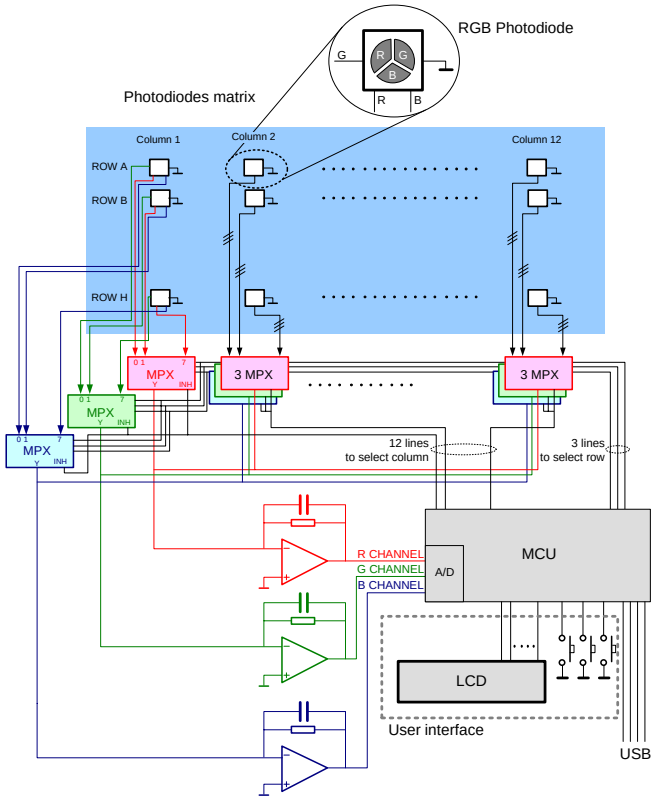

Fig. 4. Time-multiplexed system controlled by means of a MCU to read the photocurrent of 8×12 RGB photodiodes matrix with one I/V converter for each colour channel. System also includes a user interface for introducing/displaying data and an USB port.

The analysis process begins after this point. User must introduce some information about the plate under test by means a simple user interface:

- Coordinates of positive and negative well controls. There is not standardization about position and number of controls and they can be place anywhere, depending on technician. It is usual to put two, three or four positive and negative controls.
- Threshold percentage L_H to consider positive or negative each well. The threshold can vary from 20% to 80% of measurement scale depending on type of disease and plate manufacturer. So, user must introduce this value to complete the analysis. If user does not provide this value to MCU, final output will contain only a percentage value for each well.

There are two options for intensity analysis: the user can select a specific channel by indicating if the analysis must be carried-out in red, green or blue channel or, the system can determine what the better choice for that analysis is. In this case, the three values of positive and negative controls (R, G and B), calculating what is the channel with maximum colour sensitivity.

Then, the plate is statistically validated in the selected colour channel guaranteeing: first, positive controls have similar values, second, negative controls have similar values and, finally, the difference between positive and negative controls is significant enough to establish a measurement scale. This step provides the values of maximum, C_P, minimum, C_N of measurement scale:

$$C_P = \frac{\sum_i C_{Pi}}{P} \qquad C_N = \frac{\sum_i C_{Ni}}{N} \tag{1}$$

where each C_{Pi} is the value of colour in the selected channel for each of P positive control wells and C_{Ni} is the value of colour in the selected channel for each of N negative control wells.

To complete the analysis, each colour level for each well, C_{well} in the selected colour channel is transformed to a percentage L_{well} by means the scale established by positive and negative controls:

$$L_{well} = \frac{C_{well} - C_{Ni}}{C_{Pi} - C_{Ni}} \times 100 \tag{2}$$

Finally, each well is classified as "POSITIVE" or "NEGATIVE" taking into account its value, L_{well} and the threshold, L_H provided by user (see Fig. 5). Results are displayed for user information and/or transmitted by USB.

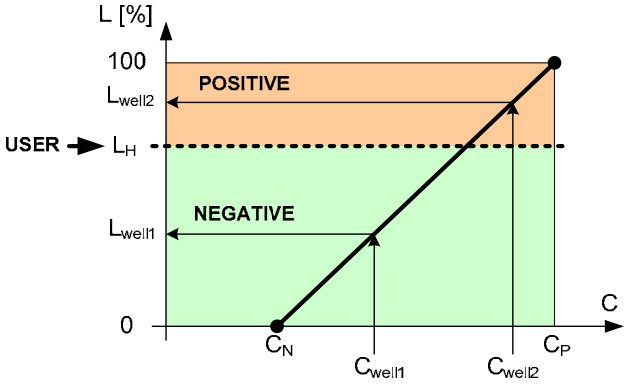

Fig. 5. Classification of wells in an ELISA plate with the calibration curve provided by positive and negative controls.

In some cases, ELISA plate manufacturer provides two threshold to refine the classification of wells in three categories: positive, negative and doubtful (see Fig. 6).

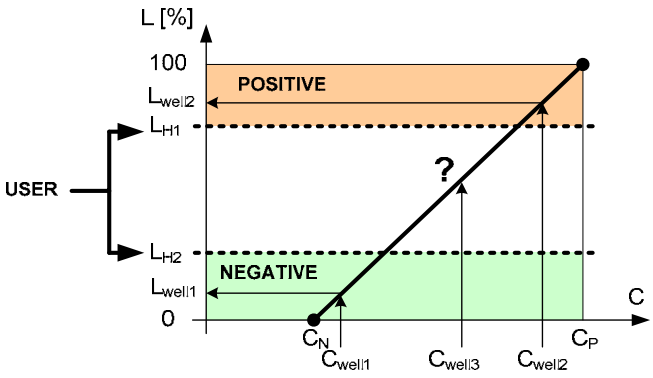

Fig. 6. A refined classification of wells in an ELISA plate in three areas: y positive, negative and doubtful. In this case, the user must provide two levels for identification of limits.

However, a simple classification uses to be enough because ELISA constitutes a first screening analysis and, in

case of positive, additional clinical analysis will be carried out to obtain a better diagnose.

The calibration curve of Fig. 5 is the usual curve for ELISA reverse plates, but there are other ELISA plates (direct plates) with negative slope in the calibration curve, that is, positive controls have the minimum value and negative controls, the maximum. The proposed system is valid for both cases because the system determines the calibration curve for each plate and the slope is indifferent in this analysis procedure.

3. EXPERIMENTAL RESULTS AND DISCUSSION

Several experimental results are carried out to verify the operation of the proposed system for veterinary diagnosis of IBR and BVD; in all cases, ELISA plates has been read-out with a commercial equipment based on spectrophotometer (standard method) and with the proposed system.

In all cases, we have not found significant differences between quantitative results; in addition, the classification of each well as positive or negative has matched the classification obtained from standard method. Fig. 7 presents a comparison between both cases for two types of ELISA plates, for Infectious Bovine Rhinotraqueitis (IBR), and Bovine Virus Diarrhoea (BVD), after determining the calibration curve with positive and negative control wells.

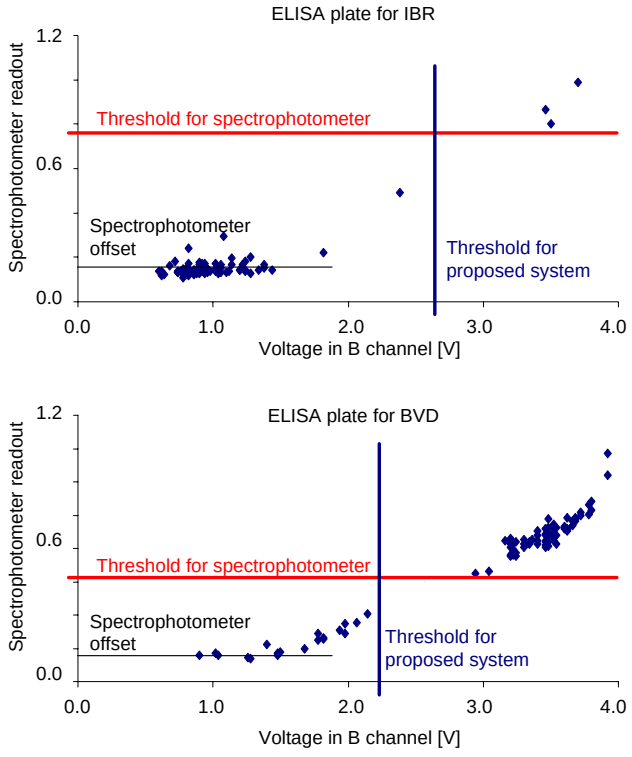

Fig. 7. Comparison of standard ELISA reader and the proposed system for two plates, for IBR and BVD. A full concordance of well classification is obtained in despite of limitations of standard method in low values: all positive and negatives cases from standard method are correctly identified for the proposed system.

The comparison can establish a linear concordance between both systems except for a small offset in standard ELISA reader. However, this effect does not affect to the classification of wells. As we can see, there is a full concordance between both methods.

Additional experimental results with more than 20 ELISA plates (around 1800 cases of blood analysis) have validated this proposed method, showing similar concordance that expressed in Fig. 7. In Fig.8 we can se a comparison of results provided by reference method and the proposed system.

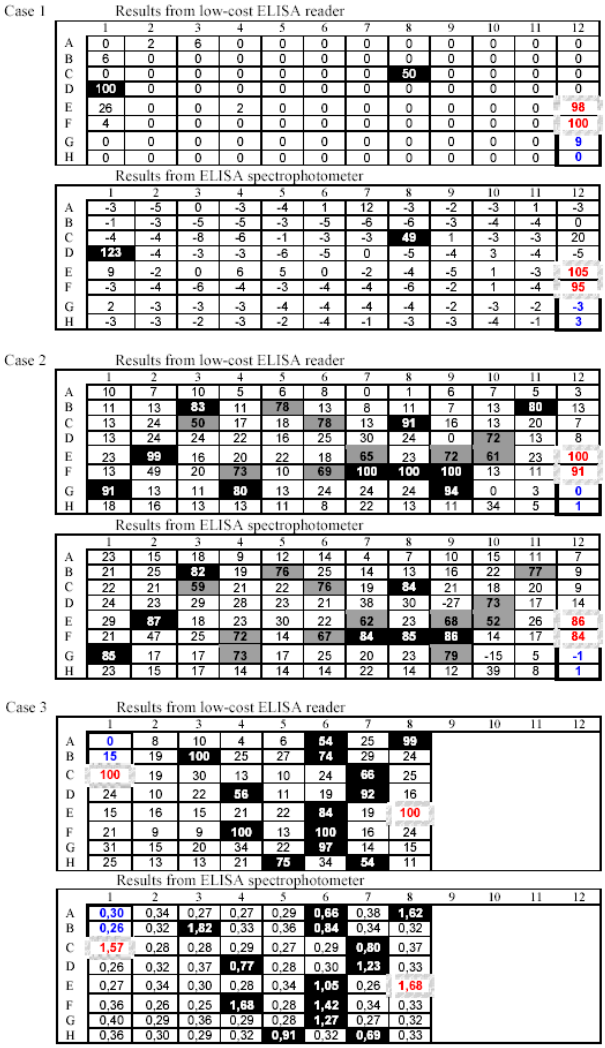

Fig. 8. Experimental comparison between results provided by proposed method (above) and results from standard equipment for ELISA reading-out (bottom) for three cases of ELISA plates. Positive references are E12, F12, negative references are G12, H12 for Cases 1 and 2 and positive references are C1, E8 wells, and negative references, A1 and B1 for Case 3; highlighted (by reverse colour) wells in both tables denotes a positive well, grey denotes indecisive wells and white, negative well in all cases. As it can be seen, there is a high concordance between both methods.

A full comparison of the system proposed in this paper with standard method and PoketELISA – based on image analysis in PDA [3] – should include additional

considerations such as spent time to results, portability, cost, etc.

The proposed method is ready to use anywhere, achieving full portability, it has low volume and weight, quite similar to PocketELISA, but its cost is lower. The standard system is a laboratory level procedure that involves transportation, increasing costs, time to results and causing readout errors due to the progressive decay of well colour. So, the proposed method constitutes a good solution for ELISA reader in relation to the present possibilities.

4. CONCLUSIONS

This paper has proposed a new method to read ELISA test plates automatically. The system estimates the concentration of specific immune products in the sample used to measure the degree of exposition to the pathogenic agent by means a colour intensity level. Overall, the method achieves very low costs, a short time to results, full portability, and it is extremely easy to use, because they can select automatically the better wavelength to carry out the analysis and the type of ELISA plate (direct or reverse).

An extensive experimental verification returned a complete concordance in well classification such as positive or negative between results provided by standard equipment (and high-cost) ELISA reader and results from proposed system for several cases and diseases.

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