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Rapid bio-test strips reader with image processing technology

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Abstract: We present an algorithm of automatic vision inspection to solve the problems in a rapid bio-test strips reader. The image inspection area of a rapid bio-test strip includes control line testing zone, test line testing zone, and the assist-measuring zone of reference. By determining the central line of control line testing zone, we can calculate the central line of test line testing zone, and can calculate the characteristics of control line, test line and the reference light-field out further. We set different threshold values for each division for the different light-field to identify whether the reaction has occurred or not. The number of pixels in positive condition is usually less than that in negative condition for the divisions in the testing zone. In this bio-strips reader we can acquire the result within 0.3 seconds computer judgment after specimen rose to test line in 10–15 minutes.

Key words: Bio-test strips reader – image inspection – threshold value

1. Introduction

Automatic vision inspection system of many rapid test strips (fig. 1) is getting important with the development in biotechnology. In this paper we present an automatic read-out of information from bio-test stripes and use Hepatitis C bio-test stripes as one example of applications. Hepatitis C is a kind of liver inflammation caused by Hepatitis C Virus (HCV), and its major route of infection is through the infected HCV blood or body fluid that gets into the inner body through skin or mucosa and enters into blood last. HCV, makes chronic Hepatitis with unobvious symptoms and is usually unveiled by regular health inspection. Hepatitis C can lead to a series of complications; the infected patient often dies from cirrhosis or hepatocellular carcinoma in 15–20 years. The human body can produce antibody (Anti-HCV) to resist the intrusive HCV, therefore, Anti-HCV is the main inspection target for automatic bio-inspection system [1–3].

This rapid bio-test strip system makes use of immunoassay technology and chromatographic method [1]. It is to make the HCV's recombination protein (NS3 · NS4 · NS5 · core protein) on the fixed test line position and to exam the Anti-HCV antibody (IgG · IgM · IgA) by the color displayer combined with Protein G. This technology would be able to detect HCV in blood, and help people and doctors make an early diagnosis. The test stripe we adapted is a standardized one with well defined test patterns. Furthermore, there are three conditions of HCV test strip inspection.

1. Positive reaction:
   When the test result shows color in the control line, and if the Test-Zone turns up color at the same time or individually, the specimen already has Anti-HCV antibody. Different levels of the color can indicate the levels of antibody titer.
2. Negative reaction:
   When the test result presents color in the control line only, the specimen doesn't have Anti-HCV antibody.
3. Invalid:
   If the test result doesn't show any colors or even nothing in the control line, it means that the test strip is invalid, expired, or improperly operated. The test has to be repeated again.

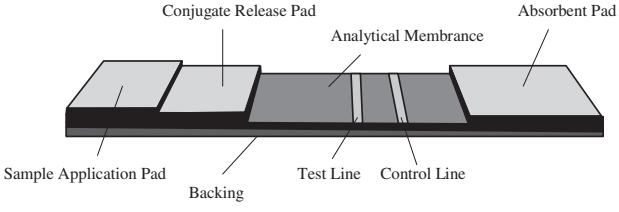

Fig. 1. The rapid bio-test strip structure.

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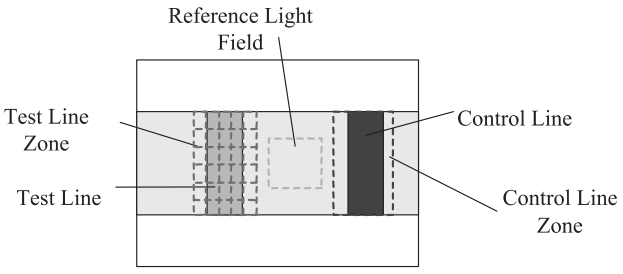


Fig. 2. The scope of control line, test line and the reference light-field.

After drawing blood with centrifugation handling, we take serum or plasma as specimen to examine right away. For short term reservation, the specimen has to be kept at 2–8 °C; for long term reservation, it should be preserved at −20 °C.

When the bio-test strip is inspected by visual method, the inspectors easily depart with subjective judgments. In general, the bio-test strip is a colored pattern with very low contrast on a white/gray background. The test line is usually made by a roller to smear it on the rapid bio-test strip, but the biological reagent is not smeared very symmetrically.

There are always some hazy places and bad symmetrization in the edge of strip. Fig. 2 shows our new method of the image inspection area diagram including control line testing zone, test line testing zone, and the assist-measuring zone of reference light-field. It's a remodeling structure of a rapid bio-test strip in fig. 1. The illuminating light should be incident obliquely on the test line to highlight the test zone. This will lead to the gradual layers variation problem of image inspection. We have to set the reference light-field measuring zone by the light intensity for the reference signal. For combining the specimen and strip well, we usually dye the test image on the motto paper or blotting paper, that will cause the noises. Although there are two spray guns in the fixed range spraying the reagent on control line testing zones and test line testing zone, the breadth will not be fixed due to the different spraying pressure. These problems will influence the test precision by using mechanical vision technology to identify the bio-test stripe, and it will lead to an improper judgment. That's the reason why we prompt the automatic vision inspection algorithm to solve these problems by determining the central line of control line testing zone to calculate the central line of test line testing zone, and can figure the scope of control line, test line and the reference light-field out further. The wavelength of light source is 530 nm, so we can use gray scale processing of green channel in test and control line image.

2. Algorithm for rapid bio-test strips reader

Below is the algorithm to calculate the test line testing zone, control line, test line and the reference light-field:

1. Filter out the picture noises
   Two strategies for noises filtering [4]:
   (1) Choose average pieces of continuous images. Assuming that the noises are randomly generated, and then it can average the data to restrain the noises.

   $$\mathrm{Gray}_i(X,Y) = S(X,Y) + N_i(X,Y)\,.$$

   Set $S(X,Y)$ as the signal value of image, $N_i(X,Y)$ as the noise value for the ith test. If the total noise value was randomly generated in the ith test, the noise value of total image is,

   $$\sqrt{\varepsilon\{N_i^2(X,Y)\}}\,.$$

   The noise ratio of image is given in the following:

   $$\mathrm{SNR} = \text{signal value of image/image impurity value}$$

   $$= S(X,Y)/\sqrt{\varepsilon\{N_i^2(X,Y)\}}\,.$$

   Take M times image and then ask for the average value

   $$\overline{\mathrm{Gray}}(X,Y) = \frac{1}{M}\sum_{i=1}^{M}\mathrm{Gray_i}(X,Y)\,.$$

   $\mathrm{Gray}_i\,(X, Y)$ is the gray value for a single pixel of each image; M is the number of images. It can be inferred and defined by

   $$SNR' = \sqrt{M}\times SNR\,.$$

   It is proved that the noise ratio of image rose $\sqrt{M}$ times and the noises were restrained after taking M times image.
   (2) Use 2×2 mask filtering to filter the non-random noises (fig. 3). A 2×2 Mask operation filter will smooth the single variation by removing high band single and re-sampling. The noise strength will reduce 4 times and the image sharpness will debase after every wave filtering. In this research, we can have the balance between filtering non-random noises and maintaining the features of high band singles after using 22 even wave-filtering mask for 3 times.
   Assuming image $f(x,y)$ contain impurity $n(x,y)$

   $$f(x,y) = f'(x,y) + n(x,y) \qquad (1)$$

   $f'(x,y)$ is the image before filtering. After 2×2-mask filtering

1/4	1/4
1/4	1/4

Fig. 3. The 2×2-filtering mask.

$$g(x,y) = \frac{1}{4}\sum_{k=0}^{1}\sum_{l=0}^{1} f'(x-k,y-l) + \frac{1}{4}\sum_{k=0}^{1}\sum_{l=0}^{1} n(x-k,y-l) \quad (2)$$

$g(x,y$ is the image after filtering.

2. Examine if the control line is normal in the control line inspection zone.
3. Calculate the geometry center position of the control line and estimate the position of test line and the reference light-field:
   If the geometry center position of test line is P_t, position of control line is P_c, position of reference-light field is P_l, the distance between control line and the reference light-field is Δd. Then

$$P_l = P_c + \frac{\Delta d}{2}; \quad P_t = P_c + \Delta d\,. \quad (3)$$

4. Light-field measurement:
   To ensure the consistency of outside environmental factor and reducing the interference inaccuracy in every test, light-field strength in some special places must be taken to control the variation of the test environment. Set the average gray degree in the light-field as $\text{Gray}_{\text{standard}}$, which is the basic of the middle threshold value in the test line.
5. The threshold value adjustment by the variation of light-field:
   In theory, the intensity of light-field should be a constant. But the inaccuracy of lighting strength from the lighting system and the color shifting of light-intensity chip on the camera will cause some deviation in the colors from the image. There are almost fixed lighting intensity variation between each division on the test line zone, as shown in fig. 4. We can adjust the threshold value for each division to reduce the inaccuracy caused by the lighting source by the variation of light-field [5–7]. The average gray degree of the light-field is $\text{Gray}_{\text{standard}}$.
   The difference in lighting intensity between light-field and each division is ω_n,

$$n = 1 \sim 30\,.$$

$$T_n = \text{Gray}_{\text{standard}} + \omega_n\,, \quad n = 1 \sim 30\,. \quad (4)$$

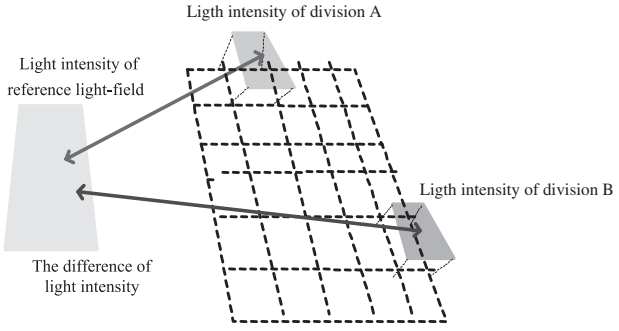


Fig. 4. Light-field location chart.

6. The test line examination:
   There are 30 small divisions in the test line zone. We set different threshold values for each division for the different light-field to identify whether the reaction has occurred or not. The number of dark pixels in the positive condition (PC, equivalent with positive reaction) is usually less than that in the negative condition (NC, equivalent with negative reaction) for the divisions in the testing zone. To prevent the average gray degree being mistaken as negative reaction, we increase the weighted pixels gray degree value to enhance the accuracy in the probable positive cases. The threshold value in the zone is T_n, $n = 1 \sim 30$. The average gray degree in the zone is Gray_{ave}. The pixel gray degree value which is lower than the threshold value in the area is $\text{Gray}_{\text{low}}(x,y)$. The pixel number which is lower than the threshold value in the area is Num_{low}. The weighted pixel gray degree which is less than the threshold value in the area is ω. The pixel gray degree value which is higher than the threshold value in the area is $\text{Gray}_{\text{high}}(x,y)$. The pixel number which is higher than the threshold value in the area is Num_{high}

$$\text{Gray}_{\text{ave}} = \frac{\sum \text{Gray}_{\text{low}}(x,y) \times \omega + \sum \text{Gray}_{\text{high}}(x,y)}{\text{Num}_{\text{low}} + \text{Num}_{\text{high}}} \quad (5)$$

$$\text{Gray}_{\text{low}}(x,y) \le T_n < \text{Gray}_{\text{high}}(x,y)\,.$$

7. Judgment of testing result:
   When the Gray_{ave} of the reacting color in any division zone is less than the threshold value in the area T_n, $n = 1 \sim 30$, it presents the positive reaction. We can also calculate the average color gray degree, Gray_P, in all the positive reaction area and define the degree of biochemical reaction consistency by the average gray degree. If there was no reaction in the test zone, it presented the negative reaction.
   (1) Positive reaction:
   Let us assume that there are Num_p divisions representing positive reaction. The reacting color in the division zone which is represents positive reaction is Gray_{ave}.
   The lighting strength difference between reference light-field and division zone is ω_n, $n = 1 \sim 30$. The average color gray degree in all the positive reaction division zone is Gray_p. The average gray degree in the reference light-field is $\text{Gray}_{\text{standard}}$. The degree of biochemical reaction consistency is Level. The degree of interval is n

$$\text{Gray}_p = \frac{\sum \|\text{Gray}_{\text{ave}} - \omega_n\|}{\text{Num}_p}\,, \quad (6)$$

$$\text{Num}_p > 0 \text{ and } \text{Gray}_{\text{ave}} < T_n$$

$$\text{Level} = \frac{\|\text{Gray}_p - \text{Gray}_{\text{standard}}\|}{n}\,. \quad (7)$$

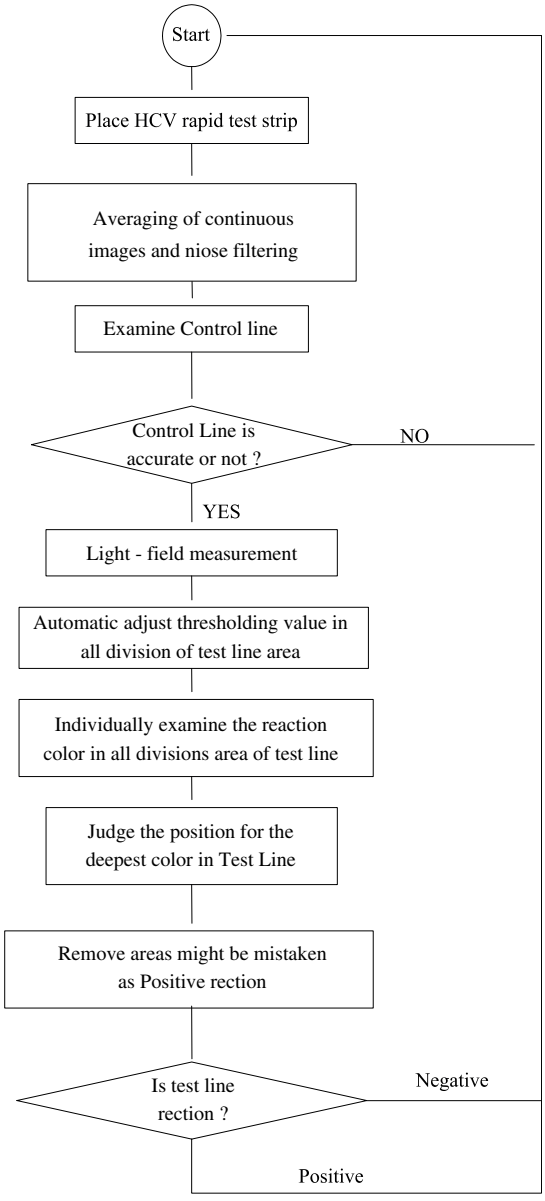


Fig. 5. The procedures of the rapid test strips reader.

(2) Negative reaction:
If the average gray degree is higher than the area's threshold value in all the division zone, it presented (NC). The procedures of the rapid test strips reader is shown in fig. 5.

3. Experimental Result

For examining the rapid test stripe, the test line is usually made by a roller to smear it on the rapid test strip and is usually not smeared very symmetrically. There are often some hazy places and different thicknesses in the edge of the test stripe. In order to combine the specimen and the material quality of the test stripe firmly, they are usually generated with dyeing effect on the motto paper or blotting paper, that will cause noises. These problems will affect the precision in recognizing the test stripe's result by mechanical vision technology and even lead to wrong judgment. Therefore, the following two difficulties need to be overcome.

1. The hardware has to overemphasize the features of the rapid test stripe, especially where they are hard to recognize by eyes.
2. Efficiently filter out the image noises.

The image has to be taken by a mega pixels PC camera with USB 2.0 interface (Aperture: F 2.8; Focal length: 8.76 mm; Magnification: 0.94 X) whose dynamic noise is far less than an analog camera. In order to ask for a stable light source and stressing the features of the rapid test strip, light has to be incident obliquely from two yellow back light module after continuous tests.

For software, the noise interference has to be reduced by the self-material as motto paper or blotting paper of the test stripe. Taking two strategy such as average pieces of continuous image and even wave-filtering are improved the noises interference a lot.

The following discussion is the actual practice efficiency on the rapid test strip's inspection. We experiment the test strip with NC, PC concentration level 10, PC concentration level 25, PC concentration level 50, PC concentration level 75, and PC concentration level 100. At first, drop the reagent is applied to the test strip and then the specimen will rise up to test line position after 5 minutes (fig. 6). In 15 minutes, the test strip figures are reduced separately from the noises not filtered and the filtered situation (fig. 7). As shown in fig. 7b, it is not recognizable any test line position in a HCV rapid test strip with PC concentration level 10. And we can also find in fig. 7c that it is still hardly visible in a HCV rapid test strip with PC concentration level 25. The test results will be confused for NC and PC concentration level 10 of HCV rapid test strip if noises are not filtered (table 1). Using 6 scanned lines

Fig. 6. Drop the liquid specimen into the test stripe.

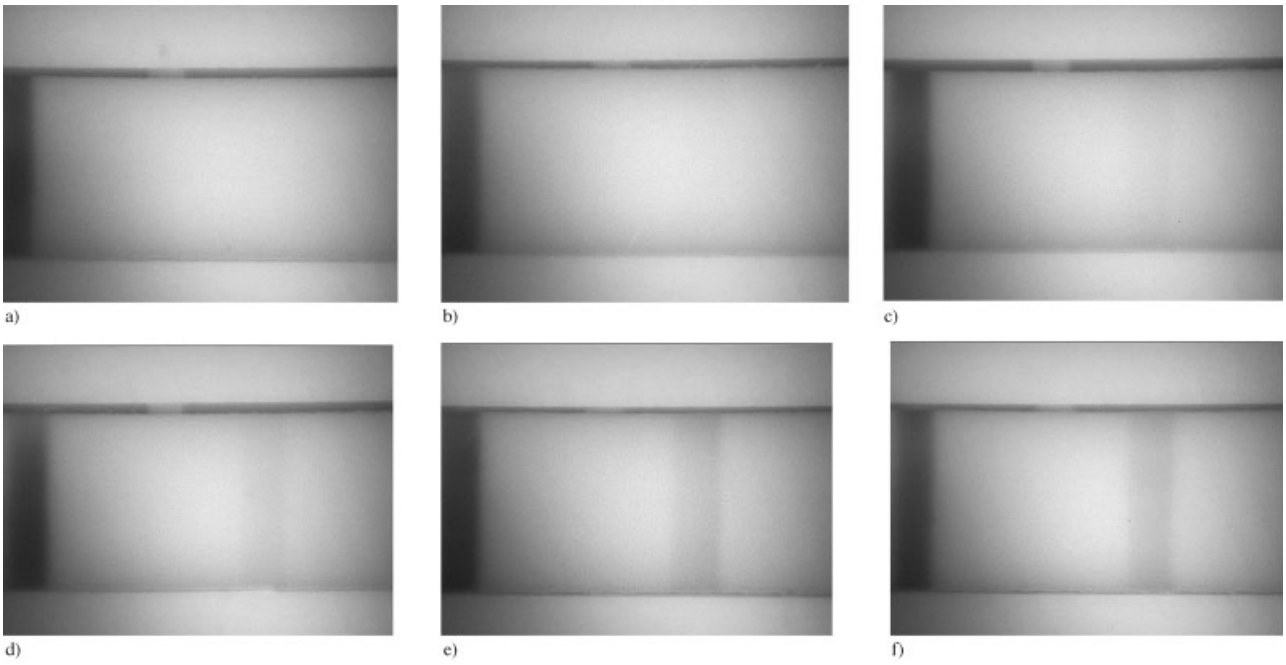


Fig. 7. NC of HCV rapid test strip a). HCV rapid test strip with PC concentration level: b) 10; c) 25; d) 50; e) 75; and f) 100.

Table 1. a) The test result for NC of HCV rapid test strip (noises are not filtered); b) The gray-level distribution graph.

a)

Control Line	Work				
Test Line					
	N	N	N	N	N
	N	N	N	P	P
	N	N	P	P	P
	N	P	N	P	P
	N	N	P	N	P
	N	N	N	N	P
Reactions	Positive Reactions				
Level	10				
Difference from NC	2				

b)

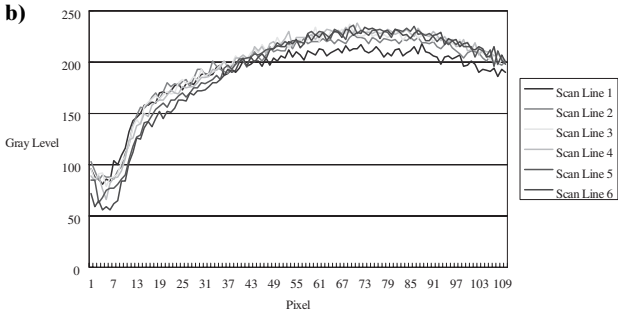


Table 2. a) The test result for NC of HCV rapid test strip (noises are filtered); b) The gray-level image distribution graph.

a)

Control Line	Work				
Test Line					
	N	N	N	N	N
	N	N	N	N	N
	N	N	N	N	N
	N	N	N	N	N
	N	N	N	N	N
	N	N	N	N	N
Reactions	Negative Reactions				
Level	NC				
Difference from NC	0				

b)

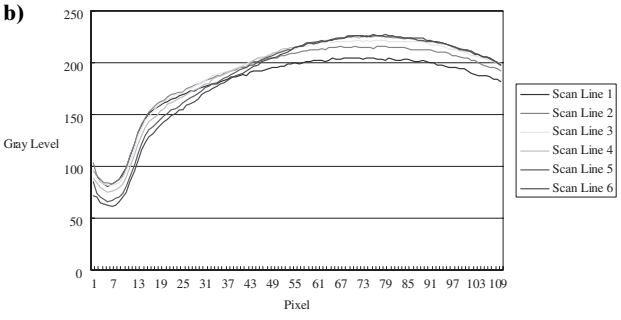


Table 3. a) The test result for HCV rapid test strip with PC concentration level 10 (noises are filtered); b) The gray-level image distribution graph.

a)

Control Line	Work				
Test Line					
	N	N	N	N	N
	P	P	N	N	N
	N	N	P	N	N
	N	N	N	N	N
	N	N	N	N	N
	P	P	N	N	N
Reactions	Positive Reactions				
Level	10				
Difference from NC	2				

b)

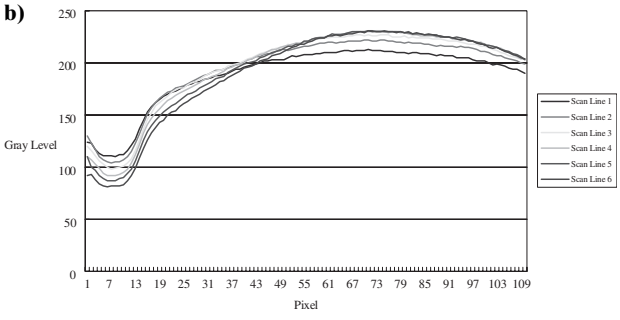
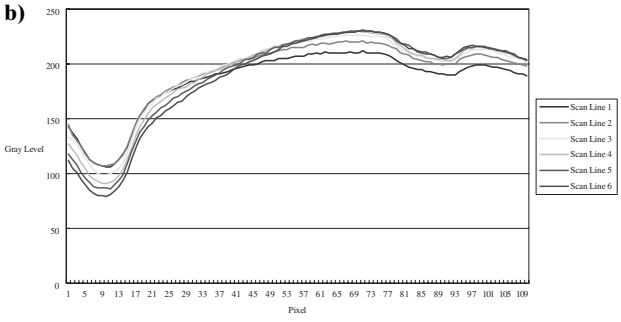


Table 4. a) The test result for HCV rapid test strip with PC concentration level 100 (noises are filtered); b) The gray-level image distribution graph.

a)

Control Line	Work				
Test Line					
	P	P	P	N	N
	P	P	P	N	N
	P	P	P	N	N
	P	P	P	N	N
	P	P	P	N	N
	P	P	P	N	N
Reactions	Positive Reactions				
Level	100				
Difference from NC	19				

b)



at the image gray level distribution graph, the hollow zone of gray level graph was separated for control line and test line (tables 2–4). When the source light density is adjusted and the system is calibrated, the judgment error for the on line inspection is retained to within 0.1%.

From above test figures, we understood that the noise that are not filtered would seriously affect to mechanical vision system on the test strips' judgment. However, the noises can be removed after filtering, and even the sensitivity of system identifying is quite high that could recognize the very light color's test stripe.

4. Conclusion

In this research, we present a low cost automatic vision inspection system for the rapid test strip reading. Hepatitis bio-test stripes is only one example of applications and the important of this paper is the method of the automatic read-out of information from bio-test stripes. A new method of the image inspection divide an image to control line testing zone, test line testing zone, and the assist-measuring zone of reference light-field. In the test line examination, 30 small divisions in the test line zone with different threshold values for the different light-field can identify the reaction. This rapid test strip read-out system has the following characters:

1. Quick inspection: Acquire the result within 0.3 seconds computer processing time after specimen rose to test line in 10–15 minutes.
2. High sensitivity.
3. Use vision inspection system on color reading is easy.
4. Simple and convenient operation.
5. Efficient data analysis.

We believe this work can significantly help researchers working in bio-test.

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