Rapid detection method of Skeletonema pseudocostatum and preparation of test strip

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Research Article

Keywords: Skeletonema pseudocostatum, HAB, GICG, Rapid detection method

Posted Date: March 15th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-194228/v1

Abstract

In eutrophic waters, harmful algal blooms(HAB) are particularly prone to occur, which will affect the ecological environment and public health and safety. How to quickly detect and monitor marine microalgae is the key to preventing and managing HAB. Our innovative application of colloidal gold immunochromatography(GICG) technology to detect the dominant species in red tide, *Skeletonema pseudocostatum*, to monitor the outbreak of red tide. The experimental results show that the method and the prepared test strips are extremely sensitive, and can specifically detect the presence of *Skeletonema pseudocostatum*. The approximate concentration of algae cells is judged by establishing a fitting relationship between the degree of color development and the concentration of algae cells. This test strip provides a quick and easy method for routine environmental monitoring, fishery water quality monitoring, and field testing of red tide monitoring. It effectively warns of the outbreak of red tides and also provides a new application direction for GICG technology.

1. Introduction

Harmful algal blooms are caused by the explosive growth of red tide algae in the seaweed family under specific environmental conditions, and *Skeletonema pseudocostatum* is one of the dominant species(Gu *et al.* 2012). The eutrophication of water environment causes frequent red tides, which seriously threaten human health and safety and the marine ecological environment(Lewitus *et al.* 2012). At present, the monitoring and early warning of phytoplankton and harmful algal blooms are mainly based on microscopic technology, chlorophyll content measurement or satellite remote sensing technology(Serodio *et al.* 1997; Rodriguez-Ramos *et al.* 2014; Zhao & Ghedira 2014). These technologies have their own constraints in terms of operation mode, timeliness and economic cost, making it impossible to be wide range of applications(Orozco & Medlin 2013; Ramaraj *et al.* 2013; Rodriguez-Ramos *et al.* 2014). The colloidal gold immunochromatography technique based on indirect competitive enzyme-linked immunosorbent assay may be the answer to this question.

Colloidal gold is composed of chloroauric acid in reducing agents (such as white phosphorus and ascorbic acid). Under the action of sodium citrate and citric acid, it polymerizes into gold particles of a certain size, and becomes a stable colloidal state due to static electricity, so it is called colloidal gold(Behra *et al.* 2015). Using colloidal gold in a negatively charged alkaline environment, The positively charged groups of protein molecules form strong bonds through electrostatic attraction. In addition to antibody proteins, colloidal gold can also bind to a variety of other biological macromolecules(Nakanishi *et al.* 1996). Various test strips made of colloidal gold immunochromatographic technology are widely used in the detection of toxic substances in food safety(Byzova *et al.* 2010; Li *et al.* 2013), as well as the detection of bacteria and viruses in clinical medicine and the prevention and control of epidemics(Ngom *et al.* 2010; Mertens *et al.* 2020). For the first time, we applied this technology to the detection of water environment. Its specificity for *Skeletonema pseudocostatum* can quickly and effectively detect and warn the outbreak of red tides. The highlight of this research is the short detection time (3-15 minutes), on-site

without special equipment, and the samples can be tested directly without any crushing treatment, which can meet the needs of various routine environmental monitoring and fishery aquaculture water testing.

2. Experimental Methods

The colloidal gold of 20~30 nm is prepared by the citric acid-trisodium citrate reduction method and stored at 4^{o}C. The growing cell culture solution was fixed overnight with 3% formaldehyde. Centrifuge for 10 min at 8000~12000 r/min to collect algal cell pellets. The algal cell pellet was washed once with distilled water and twice with PBS solution. The collected algal cells were resuspended in sterile 1ml PBS solution, mixed, and emulsified with an equal volume of Freund's incomplete adjuvant and Freund's complete adjuvant. The immunization dose was 2 multiply by 106 algal cells per animal, immunized four times, and whole blood was taken one week after the last immunization. The collected blood was allowed to stand at room temperature for 2 h, then centrifuged at 2000 to 6000 r/min for 5 min, and the serum was separated and frozen at -20 ^{o}C.

The water absorbing layer of the glass fiber membrane is glued on the right end of the nitrocellulose membrane detecting layer. The nitrite membrane detecting layer is disposed in the middle of the plastic carrier sheet, and is disposed at a boundary between the detecting layer and the glass fiber membrane sample layer. A glass fiber membrane antibody labeling layer carrying a colloidal gold-labeled *P. striata* antibody was placed between it and the glass fiber membrane sample layer. One end of the layer is placed on the glass fiber membrane sample layer and glued to the nitrocellulose membrane detection layer.

The concentration of the captured antigen required for detecting the algae cell sample is determined by the test of the sample concentration of the spirulina platensis. The detection line is drawn on the membrane to obtain an immunochromato graphic test strip for the analysis of *Skeletonema pseudocostatum*.

Take 80~100μL of the sample solution, directly drop it on the test strip and wait for 3~15 min. After the color development is stable, observe the experimental results.

3. Results And Discussion

3.1 Structure of test paper

As shown in Fig.1 and Fig. 2, the immunochromato graphic test paper for the analysis of *Skeletonema pseudocostatum* is composed of a plastic carrier plate (1), a glass fiber membrane sample layer (2), a glass fiber membrane antibody label layer (3), a nitrocellulose membrane detection layer (4), and The glass fiber membrane water absorbing layer (5). The glass fiber membrane sample layer, the glass fiber membrane antibody labeling layer, the nitrocellulose membrane detecting layer as well as the glass fiber membrane water absorbing layer are sequentially glued and fixed to one side of the plastic carrier sheet. The nitrocellulose membrane detection layer has a detection line (6) and a secondary resistance control

line (7). The glass fiber membrane water absorbing layer is glued to the right end of the nitrocellulose membrane detecting layer. The middle of the plastic carrier plate has a nitrocellulose membrane detection layer. A glass fiber membrane antibody labeling layer carrying a colloidal gold-labeled *P. striata* antibody was placed between it and the glass fiber membrane sample layer. One end of the glass fiber membrane antibody labeling layer is placed under the glass fiber membrane sample layer and glued to the plastic carrier sheet. The other end of the glass fiber membrane antibody labeling layer was placed on the nitrocellulose membrane detecting layer and glued to the nitrocellulose membrane detecting layer.

3.2 Antibody sensitivity detection

Mixing the *Skeletonema pseudocostatum* solution (about 108 cells/ml) and water into 7 proportion of 1:2, 1:128, 1:256, 1:512, 1:1024, 1:2048 and 1:4096. They were added dropwise in squares which are 2 μL per cell. In the last square, 2 μL of a PBS solution was added dropwise instead of the *P. striata* solution as a control group. Observing the coloration results, the presence of chromogenic spots indicates that the antibody can detect the antigen at certain concentration. If no color is developed, the antibody cannot detect the antigen at the concentration. The experimental results are shown in Figure 3. From the results of the reaction, the antibody was able to detect a 2048-fold diluted solution of *Skeletonema pseudocostatum* (concentration: 1.2×10^5 cells/ml). Moreover, the spots displayed by the respective concentrations of antigens in the figure have obvious depth and darkness, and have a corresponding relationship with the concentration gradient. It could explain the detection limit of the method for the lowest concentration.

3.3 Specific detection of antibodies

Six kinds of common red tide algae were selected and reacted with the prepared antibody by dot hybridization in the specific experimental test. Based on the experimental color rendering results, the specificity of the antibody to *Skeletonema pseudocostatum* was determined. Six kinds of algal stock solutions (about 10^6 cells/ml) were separately added to the squares, 2 μL per grid. Each algae cell was used as an experimental group, and eight parallel samples were set for each group. Observing the coloration results, the chromogenic spots indicated that the antibody could detect the antigen. If no color was developed, the antibody could not detect the antigen, thereby verifying the specificity of the antibody to *Skeletonema pseudocostatum*. The experimental results show that the antibody can only detect the antigen of *Skeletonema pseudocostatum*. This illustrates the specificity of the antibody of *Skeletonema pseudocostatum* as shown in Fig. 4.

3.4 Test paper test for samples of each period

Fig. 5 shows the results of a paper strip test for the experiment of *Skeletonema pseudocostatum* in each period(1: The logarithmic phase of Skeletonema pseudocostatum was untreated; 2: The logarithmic phase of Skeletonema pseudocostatum was crush. 3: The adaptation period and the platform stage were not treated with the ribbed algae mixture; 4: The adaptation period and the platform period were planned. The ribs and algae mixed and crushed; 5: the adaptation period, the logarithmic stage, the platform stage,

and the ribbed algae mixed untreated; 6: the adaptation period, the logarithmic stage, the platform stage, and the Skeletonema pseudocostatum mixed crushing treatment.) . Using the prepared test papers to test the *Skeletonema pseudocostatum* in each growth period. A total of three growth stages or algal cells of different growth stages are used as antigens. Each antigen is further divided into a crushing treatment and an unbroken treatmen. The results of the implementation showed that the antigens could be detected by the test strips in each period, and the color was detected on both the detection line and the quality control line. The seawater without the ribbed algae is only displayed on the quality control line, and the detection line cannot be detected.

3.5 Sensitivity test results of test strips

The algae solution (3.20×10^6 cells/ml) was subjected to gradient dilution, and the cells were spotted on the test strip without any treatment. And the color development result was observed after about 5 minutes. It was found that the positive detection line gradually weakened as the concentration decreased. The lowest concentration that can be detected by this method is 3.20×10^4 cells/ml. In actual operation, if there is no red tide, the concentration of *Skeletonema pseudocostatum* is often lower than this. Therefore, this test paper has the function of warning the occurrence of red tide. And the test paper takes very short time in use, which is very suitable for on-site operation.

In order to confirm the concentration of *Skeletonema pseudocostatum* by test strips, we used Elisa to determine the relationship between absorbance and algae concentration. Monoclonal antibody concentration is 5 μg per ml for the plate concentration. Horseradish Peroxidase diluted 20,000 times. Results are shown in Figure 6. The algae concentration can be well fitted to its absorbance. The fitting formula is shown in Figure 7. Therefore, simply measure the absorbance of the test strip or compare it with the stain to know the approximate concentration of algae.

3.6 Test strip specific test result

The method for using the test strip of *Skeletonema pseudocostatum* is as follows. Take 80~100 microliters of the sample solution to be tested, and drop directly on the test strip sample without any treatment. The fastest is 3 minutes. The average result is about 15 minutes. After the color development is stable, the colorimetric plate is used for comparison, and the algae cell concentration can be finally estimated to achieve semi-quantitative.

Several other algae were tested separately with *Skeletonema pseudocostatum* to prove the specificity of the test strip. The results showed that only *Skeletonema pseudocostatum* had a positive reaction, and the other algae were negative. This indicates the specificity of the antibody and was not destroyed by the preparation of the test strip. The test strip can distinguish between *Skeletonema pseudocostatum* and other algae.

4. Conclusions

Compared with the previous research, the result has the following outstanding technical effects. Immunological techniques are used to immunize experimental animals with algal cells to prepare monoclonal antibodies to planktonic algae, and to use antibodies to establish an immunoassay for GICA. The colorimetric results of GICA were compared by colorimetric plates to estimate the concentration of red tide algae in the water. No need for experimental equipment, simple operation, rapid detection, and special treatment. Such as no need to break the sample, directly add the water sample containing algae to the test strip to obtain the result positive, and can be given according to the strength of the reaction. The approximate numerical results of algal cell concentration. Although *Skeletonema* is non-toxic, it is still one of the important algae that cause red tide in the southeast coast. At present, the application of immunological methods for related technology detection is only ELISA method (long time, about 4h), while the rapid detection of the present invention Colloidal gold techniques and methods (generally available in 3 to 15 minutes) and related products have not been reported. This monoantibody and detection method will apply for anlysis the relative protein function in diatom.

Declarations

Ethics approval and consent to participate:

All methods were carried out in accordance with relevant guidelines and regulations.All experimental protocols were approved by the Ethic committee of Xiamen University of China.

Consent for publication:

Not applicable

Availability of data and materials:

Not applicable.

Competing interests:

The authors declare that they have no competing interests.

Funding:

This study was supported by the National Key Research and Development Program of China 2016YFC0502901 and by the National Key Research and Development Program of China 2017YFC0506103.

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Jingfeng Chen:Investigation

Jingli Li:Investigation

Xinyi Wang:Investigation

Yingzi Wang:Investigation

Mingyang Li:Investigation

Chuang Chen:Investigation

Kefu Zhou:Conceptualization,Methodology,Resources,Investigation,Supervision,Project

administration,Funding acquisition

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Figures

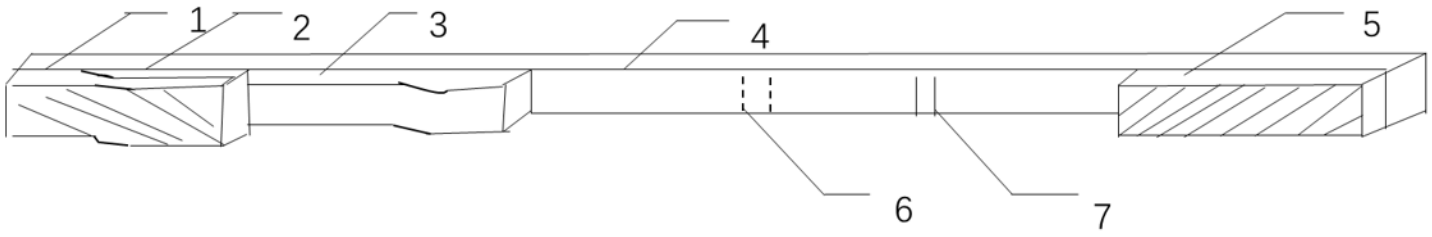

Figure 1

Schematic diagram of the test paper (1: Plastic carrier plate; 2: glass fiber membrane sample layer; 3: B. sphaeroides antibody glass fiber membrane antibody labeling layer; 4: antibody gold label, nitrocellulose membrane detecting layer; 5: glass fiber membrane water absorbing layer; 6: Antigen detection line; 7: secondary resistance quality control line.)

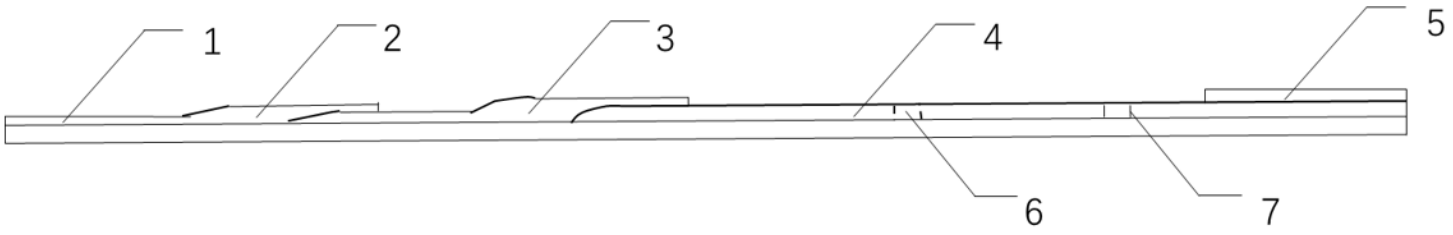

Figure 2

Side view of the test paper (1: Plastic carrier plate; 2: glass fiber membrane sample layer; 3: B. sphaeroides antibody glass fiber membrane antibody labeling layer; 4: with antibody gold label, nitrocellulose membrane detecting layer; 5: glass fiber membrane water absorbing layer; 6: Antigen detection line; 7: secondary resistance quality control line.)

Figure 3

The results of the lowest detection concentration (The ratio of antigen to water from left to right: 1:2, 1:128, 1:256, 1:512, 1:1024, 1:2048, 1:4096 and PBS only)

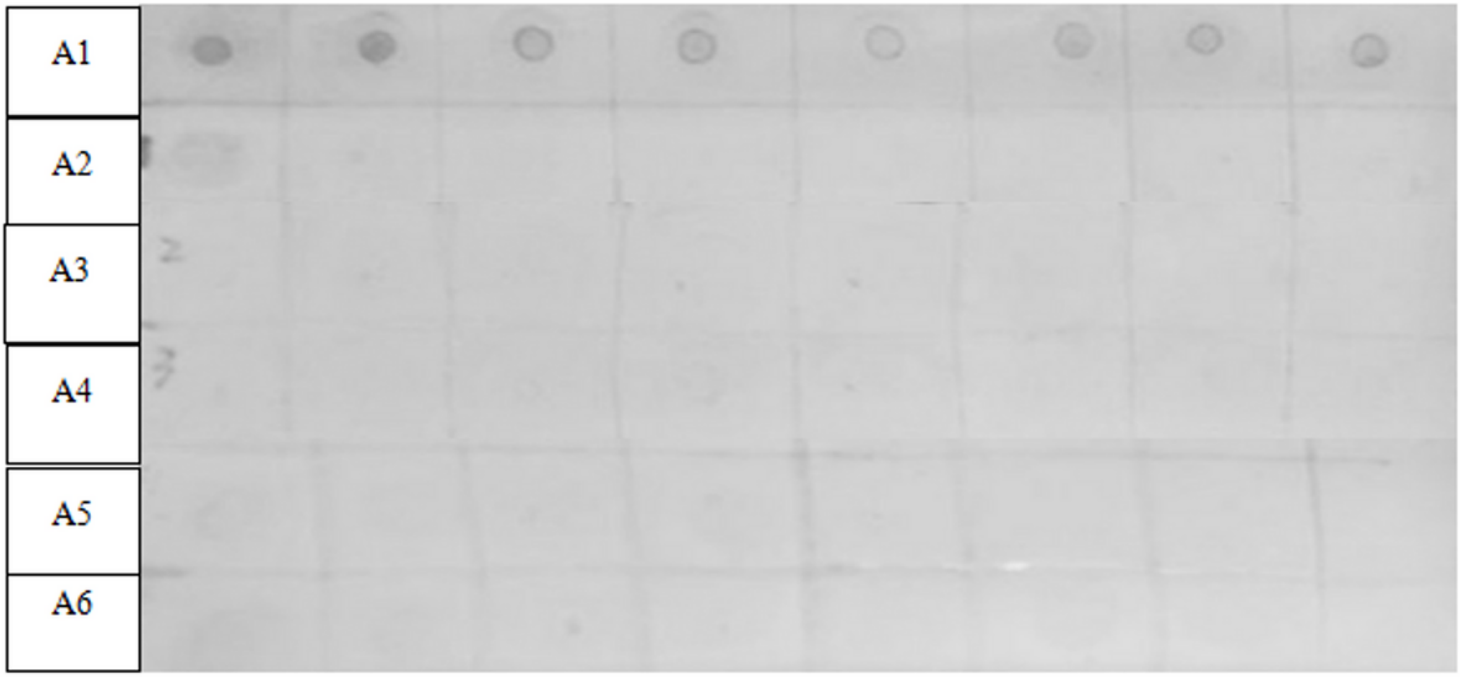

Figure 4

The result of specific detection. (A1: Skeletonema pseudocostatum; A2: chlorella; A3: Trichosanthes aeruginosa; A4: Phyllostachys pubescens; A5: Prototheca platensis; A6: Isochrysis galbana

Figure 5

Results with Skeletonema pseudocostatum in various periods (1: The logarithmic phase of Skeletonema pseudocostatum was untreated; 2: The logarithmic phase of Skeletonema pseudocostatum was crush. 3: The adaptation period and the platform stage were not treated with the ribbed algae mixture; 4: The adaptation period and the platform period were planned. The ribs and algae mixed and crushed; 5: the adaptation period, the logarithmic stage, the platform stage, and the ribbed algae mixed untreated; 6: the adaptation period, the logarithmic stage, the platform stage, and the Skeletonema pseudocostatum mixed crushing treatment.)

Figure 6

Sensitivity test results of test strips (C1: 3.20×106 cell/ml; C2: 1.60×106 cell/ml; C3: 0.80×106 cell/ml; C4: 3.20×105 cell/ml; C5: 3.20×104 cell/ml; C6: 1.60×104 cell/ml.)

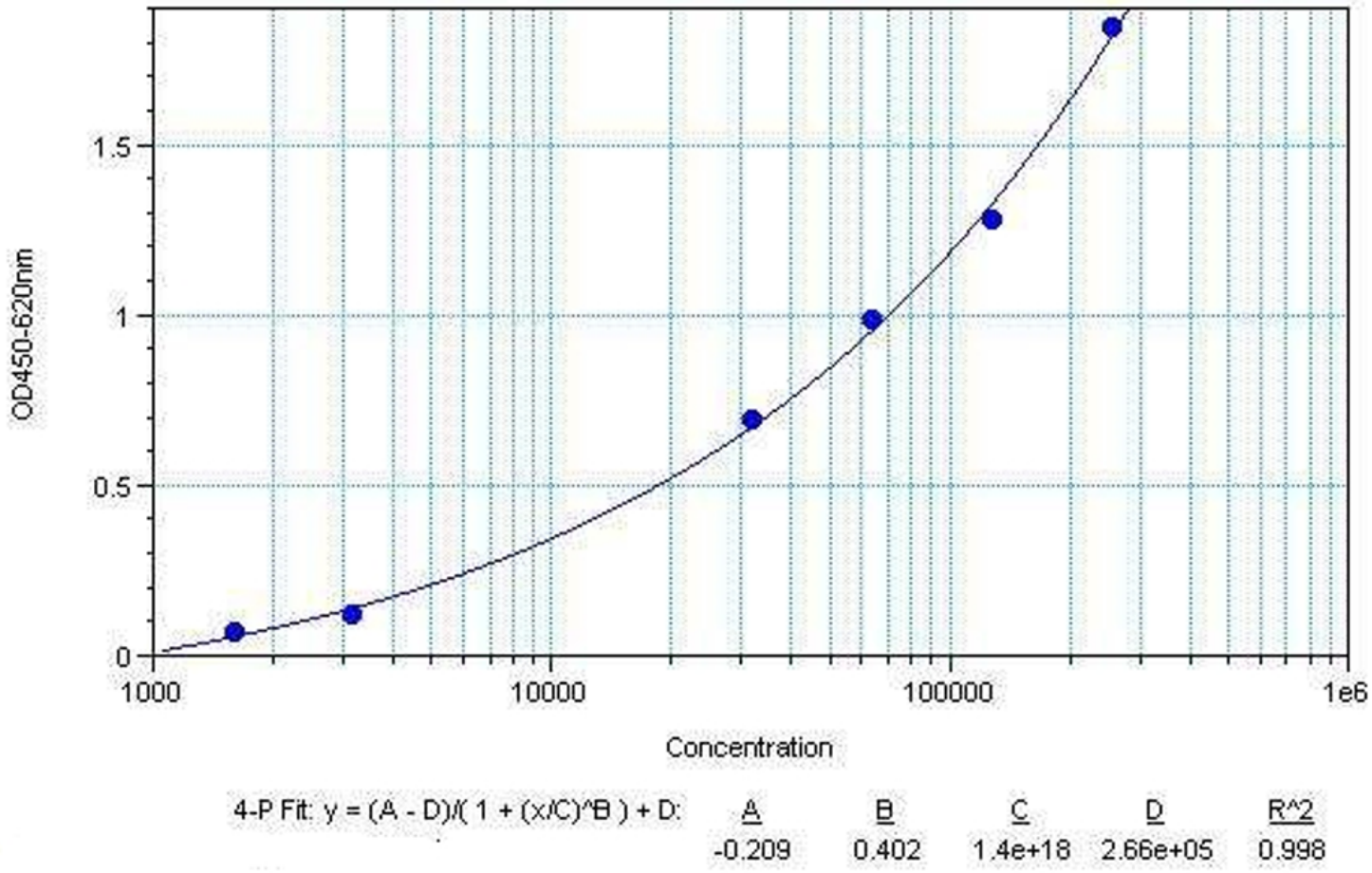

Figure 7

Concentration standard curve of Skeletonema pseudocostatum

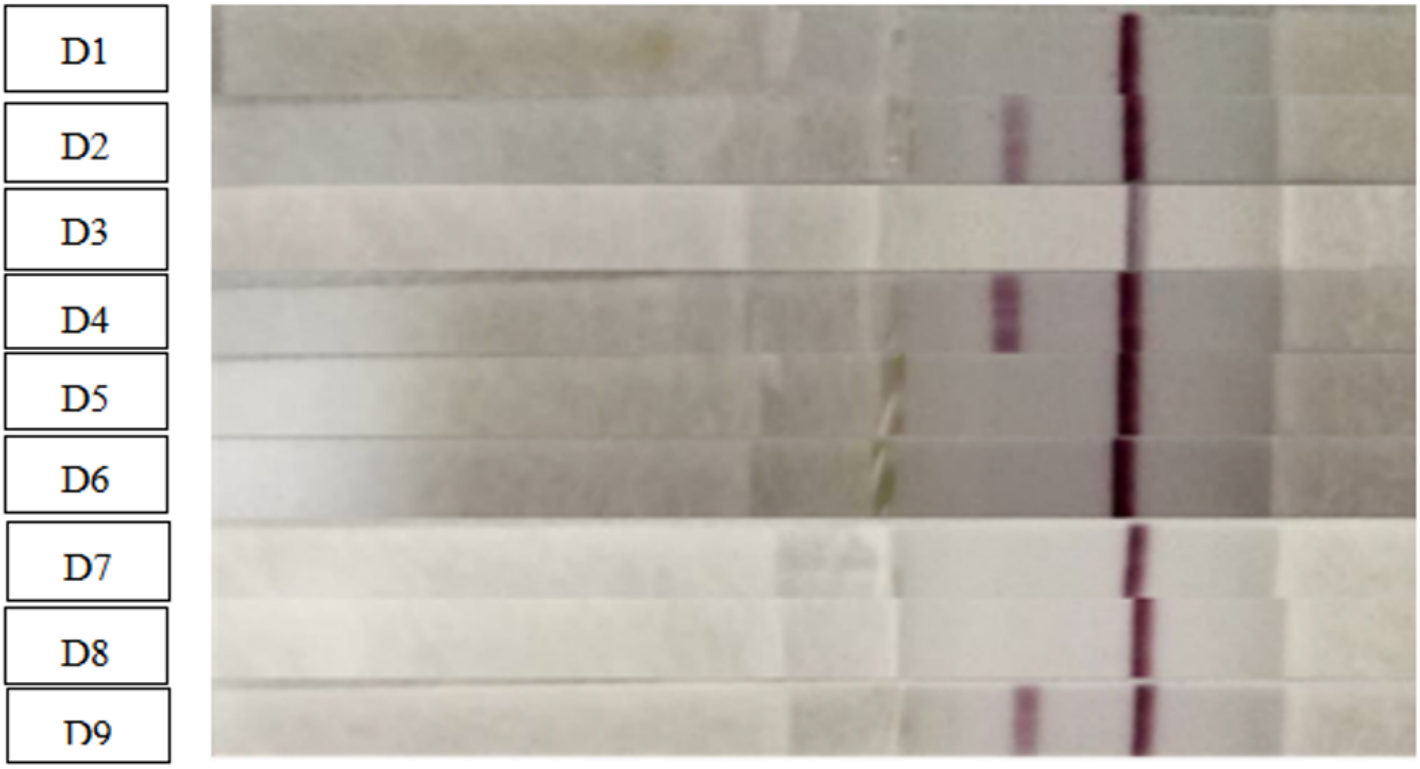

Figure 8

The results of the test strip specificity (D1: Skeletonema costatum; D2: Skeletonema pseudocostatum; D3: Skeletonema variabilis; D4: Skeletonema platensis, B. sphaeroides and Skeletonema variabilis; D5: Phaeodactylum tricornutum; D6 : Chlorella; D7: R. crassifolia; D8: B. serrata; D9: 7 kinds of algae mixture.)