RESEARCH ON THE DEVELOPMENT OF AN IDENTIFICATION PROCESS FOR ANTIGENS A, Y, C, W_{135} IN MENINGOCOCCAL VACCINE BY AGGLUTINATION METHOD

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Abstract: Meningococcal disease, caused by the bacterium *Neisseria meningitidis* is a dangerous infectious disease that can be rapidly fatal. This study focuses on developing a procedure to identify serogroups A, Y, C, and W_{135} in meningococcal vaccines using the agglutination method, with the aim of improving vaccine testing efficiency in Vietnam. Through experiments with vaccine samples, the research team confirmed the high specificity of the agglutination method, ensuring its ability to accurately distinguish the target antigens. Compared to the ELISA method, the agglutination method offers significant advantages: a shorter testing time, lower cost, and no requirement for in-house reference standards from the manufacturer. Furthermore, this method can be widely applied to other vaccines containing similar antigen serotypes, creating a flexible and effective testing process. The results indicated that the optimal concentration for the test solution is 6,000 ng/ml, at which the agglutination reaction reaches a 4+ degree, signifying a strong and stable agglutination. These findings contribute to enhancing the quality of vaccine control in Vietnam, paving the way for the development of internal testing methods with high accuracy and optimal cost.

Keywords: *Meningococcal vaccine, Agglutination*

1. Introduction

Meningococcal disease is one of the leading causes of illness and death worldwide. The bacterium *Neisseria meningitidis* is an aerobic diplococcus from the family *Neisseriaceae*, often found in pairs or small clusters, and stains pink as Gram-negative bacteria. The bacteria have low resistance and are easily killed at 50°C in 5 minutes or 100°C in 30 seconds. Based on the structure of the capsular antigen, the bacteria are divided into groups such as A, B, C, D, W_{135}, X, Y, and Z. Among these, groups A, B, and C are the most common in Vietnam [1]. The bacteria are often found in the throat and nose of humans, especially in children under 5 years old. The disease spreads in the community through the respiratory tract and direct contact with saliva or secretions containing the bacteria. The disease can progress rapidly and lead to death within 24 hours [2,3]. Approximately 10-15% of patients still die despite antibiotic treatment [4].

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Anyone can get sick, but children under 5 years old are considered a high-risk group for meningococcal meningitis. Symptoms of the disease include high fever of 39-40°C, severe headache, nausea and vomiting, stiff neck, often a star-shaped petechial rash or sometimes blisters. Patients are often lethargic or comatose, and in some cases, they may experience sudden weakness, hemorrhagic patches, and shock [5,6].

According to statistics from the National Institute of Hygiene and Epidemiology, the incidence of meningococcal meningitis in Vietnam is about 2.3/100,000 people. This is one of the six infectious diseases with the highest mortality rate in the country, at 0.03/100,000 people [7].

Currently, the best way to prevent the disease is to proactively get vaccinated against meningococcal disease. A meningococcal vaccine is a general term referring to any vaccine used to prevent *Neisseria meningitidis* infection [8]. Different types of vaccines are effective against some or all types of meningococcal disease, including types A, B, C, W_{135}, and Y [8]. The first meningococcal vaccine became available in the 1970s and has been listed on the World Health Organization's list of essential medicines [9,10].

In Vietnam, several types of meningococcal vaccines are currently in circulation, such as the Menactra vaccine, VA-Mengoc-BC vaccine, and Bexsero vaccine. The Menactra conjugate vaccine (Sanofi Pasteur - France) prevents meningitis, septicemia, and pneumonia caused by meningococcal types A, C, Y, and W_{135}. The Menactra vaccine is a meningococcal conjugate vaccine containing polysaccharides from serogroups A, C, Y, and W_{135}, which are linked to a diphtheria toxoid protein carrier. The Menactra vaccine was first licensed in North America in 2005. The VA-Mengoc BC vaccine (Cuba) prevents meningitis caused by meningococcal types B and C [11]. The VA Mengoc BC vaccine was developed in Cuba in response to a major meningitis outbreak in the 1980s [12]. The VA Mengoc BC vaccine has two antigen types, B and C, which are adsorbed with an aluminum hydroxide adjuvant to enhance the immune response [13-15]. These two vaccines contain the types that are the focus of this study.

According to WHO recommendations, vaccine inspection refers to tests on Mennigo vaccine including: identification test, content determination, adsorption test,... [16]. According to Decision No. 1933/QD-BYT, which approves the guidelines for releasing vaccines and medical biological products, vaccines in circulation in Vietnam must be checked with all tests as registered for each production batch (for domestically produced vaccines) or imported batch (for imported vaccines).

For vaccines where the antigen is a polysaccharide or protein, identification is performed by detecting the antigen-antibody complex using biological techniques. Enzyme-Linked Immunosorbent Assay (ELISA) or SDS-polyacrylamide gel electrophoresis combined with Western blot (e.g., for pneumococcal, meningococcal, Hib vaccines) or agglutination methods like

for Hib vaccine… are used. These methods are all based on the principle of a specific antigen-antibody reaction [16]. Accordingly, the target antigen will only combine with its specific antibody according to the unique lock-and-key principle. Therefore, serological methods require an antibody specific to the antigen (vaccine) and a way to observe that combination. These methods are highly accurate because the specific antigen-antibody reaction is unique.

For meningococcal vaccine identification, the National Institute for Control of Vaccine and Biologicals (NICVB) currently uses the ELISA technique, which is based on the specific combination of an antigen and an enzyme-linked antibody. When an appropriate substrate is added, the enzyme hydrolyzes it into a colored substance, and the intensity of the color indicates the presence of the antigen or antibody. However, this method requires in-house reference standards for each type of vaccine, and the testing time is long and the cost of chemicals and equipment is high. It can take several months, sometimes up to a year, for in-house reference standards to be shipped from the manufacturer to the NICVB, leading to delays in vaccine inspection.

According to the latest version of WHO TRS (No. 962) for meningococcal vaccines, antigen identification is performed using serological assays with specific antibodies. WHO does not specify a particular or preferred method, meaning all methods are considered equivalent.

Therefore, the research team sought an alternative method: the agglutination method using the Neisseria Meningitidis antiserum kit produced by Difco. The kit is based on the principle that antibodies in the antiserum react and agglutinate with the corresponding bacterial antigens. The meningococcal agglutination antiserum in the kit is produced in rabbits and preserved with a 0.1% Sodium azide solution. This test is currently used on various clinical samples, such as bodily fluids, to diagnose diseases like meningococcal meningitis and purulent meningitis, by detecting the presence of corresponding antigens. This is a method recommended by the WHO in its rapid meningococcal disease testing guidelines [14]. The principle of identifying antigens A, C, Y, and W_{135} in the vaccine is the binding of specific (monoclonal) antibody molecules to the corresponding meningococcal antigens. Antigens in the sample bind to the exposed binding sites on the surface of the corresponding antibodies, forming cross-linked aggregates of antibodies and antigens. The antigen-antibody agglutination reaction can be observed with the naked eye [17].

2. Methodology

2.1. Research subjects

The research subjects were:

- Antigen types A, C, Y, W_{135} in the meningococcal vaccines Menactra and VA-Mengoc-BC.

- The meningococcal identification kits for types A, C, Y, and W_{135}: Neisseria Meningitidis antiserum groups A, C, Y, W_{135}, from Difco (item numbers 222281, 222301, 228811, 222531).

2.2. Research location and time

Research location: National Institute for Control of Vaccine and Biologicals.

Research time: From May 2024 to December 2024.

2.3. Research design

This was a descriptive, cross-sectional laboratory study with the following main steps:

The latex agglutination method was used to identify antigen types A, C, Y, and W_{135} in the meningococcal vaccine solution.

According to the kit's instructions for use:

- *Step 1: Sample preparation*

+ Cerebrospinal fluid samples were cultured on blood or chocolate agar media. Suspected colonies containing *N.meningitidis* antigens were selected and emulsified with physiological saline to create a smooth suspension.

- *Step 2: Sample application:*

+ Two separate drops were placed on a slide: the emulsified suspension and physiological saline. One drop of antibody from the kit was added to each drop and mixed thoroughly. The result of the agglutination reaction was observed after 3 minutes.

According to the kit's instructions, the sample used is a fresh bacterial sample after culturing, also known as a clinical specimen, which is antigenically similar to the *N.meningitidis* antigens contained in the vaccine. Furthermore, vaccine manufacturers (Heber Biotec, etc.) have used the agglutination method to identify Haemophilus influenzae type B. Therefore, the research team tested the kit with the vaccines currently in circulation and developed a procedure to identify antigens A, C, Y, and W_{135} in meningococcal vaccines using the agglutination method as follows:

- *Step 1: Prepare test samples, positive controls, and negative controls*

+ Draw the entire vaccine into a sterile 15ml plastic tube. Shake well with a voltex. Positive and negative control samples were brought to room temperature before testing.

- *Step 2: Sample application:*

+ Prepare a reaction card and place on a flat surface.

+ Add a drop (approximately 15*µ*l) of the Neisseria Meningitidis antiserum antibody solution for types A, C, Y, and W_{135} was placed in the center of the circles on the reaction card.

+ Add 15µl of the test sample *và* 15*µl* of physiological saline as a negative control, 15µl of the positive control sample to the corresponding circular centers where specific antibodies are added.

+ Quickly mix the 2 drops and gently swirl back and forth.

- *Step 3: Read results*

+ Observe the agglutination reaction with the naked eye after 3 minutes.

+ The identification test for antigen components A, C, Y, and W_{135} in the meningococcal vaccine is considered successful when agglutination appears in the test sample and positive control wells, and no agglutination appears in the negative control well.

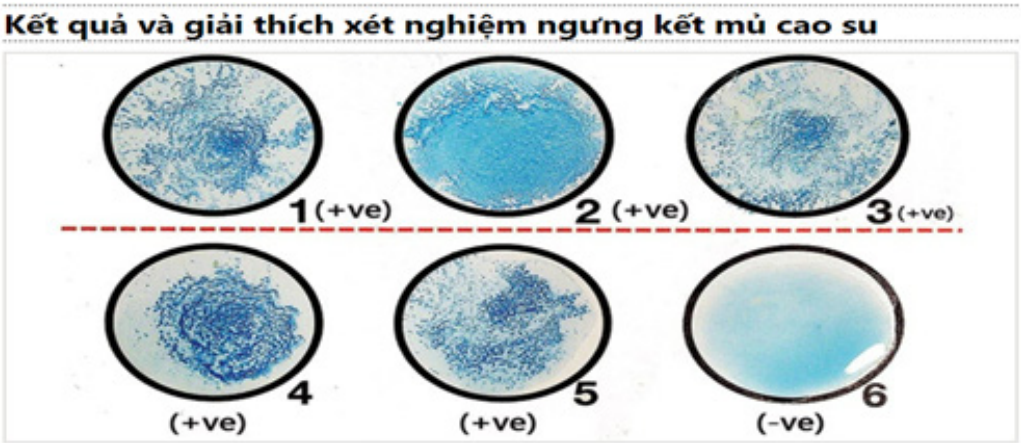

Figure 1. Results and interpretation of the latex agglutination test

Positive:

- The clumping of particles into clumps at any dilution is considered a positive result. This confirms that the sample contains the antigen. A positive reaction is graded on a scale from 1+ to 5+.

- Level 1+ has about 25% agglutination.
- Level 2+ has about 50% agglutination.
- Level 3+ has about 75% agglutination.
- Level 4+ has 100% agglutination.
- Level 5+ has 100% agglutination.

In which, levels 1+ and 2+ require a microscope to observe the agglutination reaction. Levels 3+, 4+, and 5+ can be observed with the naked eye. 4+ is the most visible and optimal reaction level.

Negative: No clumping or formation of clumps. The specific antigen is not present in the sample.

The study was designed to use 15 µl of a monoclonal antibody solution for each type (A, C, Y, W_{135}) specific to the corresponding meningococcal antigen for each test.

First, the research team checked the compatibility of the kit's antibodies with positive controls, negative controls, and two different vaccine samples: VA Mengoc BC and Menactra. The negative control used here was physiological saline. Positive controls for the other types (e.g., for the type A kit, positive controls for types C, Y, and W_{135} were used as negative controls, and so on for the other kits) were also used as negative controls. A positive reaction occurred between the corresponding sample and the positive control with the kit's antibody, and no agglutination reaction occurred between the negative control sample and the kit's antibody.

The next step was to select the optimal concentration for the procedure:

Table 1. Concentration of types in meningococcal vaccines currently in circulation worldwide

No.	Vaccine name	Manufacturer	Concentration
1	Nimenrix	Pfizer	Types A,C,Y,W_{135}: 10.000ng/ml
2	Menquadfi	Sanofi Pasteur	Týe A,C,Y,W_{135}: 20.000ng/ml
3	Menveo	GlaxoSmithKline	Type A: 20.000ng/ml, Types C,Y,W_{135}: 10.000ng/ml
4	Va Mengoc BC	Finlay Institute	Type C: 100.000ng/ml
5	Menactra	Sanofi Pasteur	Types A,C,Y,W_{135}: 8.000ng/ml
6	Menjugate	GlaxoSmithKline	Type C: 20.000ng/ml
7	Neisvac-C	Pfizer	Type C: 20.000ng/ml
8	Quadri Meningo	Bio-Med	Types A,C,Y,W_{135}: 100.000ng/ml
9	Penbraya	Pfizer	Types A,C,Y,W_{135}: 10.000ng/ml

Among the vaccines listed, the research team noted that the Menactra vaccine has the lowest concentration for each type (8000 ng/ml). Therefore, we proceeded with optimization on this vaccine to find the minimum optimal concentration.

The research team tested the agglutination reaction of the kit's antibody solution with samples at three concentrations: 8000 ng/ml, 4000 ng/ml, and 2000 ng/ml. From these three concentrations, we identified the concentration that yielded a 4+ agglutination result. Once the reaction range was determined, we performed a narrower search to find the optimal concentration for a 4+ result.

+ Develop a procedure to apply at that optimal concentration:

All tests were run in parallel with positive and negative control solutions. If the positive control wells showed a positive result and the negative control wells showed a negative result, the test was considered valid. If at least one of these three results was incorrect, the test was considered invalid. Tests with samples at each concentration were repeated 3 times on 3 different days. The final result was based on a synthesis of the results from the 3 independent tests.

2.3. Materials and Equipment

Table 2. List of materials and equipment

No.	Material name	Estimated quantity	Item number	Expiration date
1	Meningo identification kit type A: Neisseria Meningitidis antiserum groups A, Difco	01 kit	222281	2 years from opening date (19/02/2024)
2	Meningo identification kit type C: Neisseria Meningitidis antiserum groups C, Difco	01 kit	222301	2 years from opening date (19/02/2024)

3	Meningo identification kit type Y: Neisseria Meningitidis antiserum groups Y, Difco	01 kit	228811	2 years from opening date (19/02/2024)
4	Meningo identification kit type W_{135}: Neisseria Meningitidis antiserum groups W_{135}, Difco	01 kit	222531	2 years from opening date (19/02/2024)
5	Positive control types A, C, Y, W_{135}	01	NIBSC 13/246 NIBSC 20/314 NIBSC 16/206 NIBSC 16/152	
6	VA MENGOC BC	21 vials	Lô 443M	31/07/2027
7	Menactra	24 vials	U8412AA	04/06/2026
8	15ml tube	24	430052	
9	Physiological saline	1 liter	NaCl 12/24	Within expiry date
10	100µl manual pipette	02 units	MP1 007-VK	23/11/2024
11	1000 ml sterile glass beaker with spout	01 unit	Schott Duran	
12	1000µl manual pipette and corresponding tips	01 unit + 01 box	MP1 001-VK	23/11/2024
13	Timer	01 unit	Vietnam	

2.5. Research Methods

a. Compatibility check

Prepare test solutions from the two test vaccines, VA Mengoc BC and Menactra, both positive and negative controls. Add 15**µ**l of antigens from the two test vaccines, VA Mengoc BC and Menactra, on a card, along with 15**µ**l of the kit›s antibody, and mixed well. Observe the agglutination after 3 minutes. Record the results. The test was repeated 6 times on 6 different days by the same group of people, with the same equipment, in the same laboratory.

b. Optimal concentration selection

To select the optimal concentration for the procedure, the research team prepared test solutions of the Menactra vaccine sample at concentrations of 8,000ng/ml - 4,000ng/ml - 2,000ng/ml. Add 15 **µ**l of the antibody solution on a card, add 15 **µ**l of the sample and mixed well. Observe the agglutination after 3 minutes. Record the results. Selection of the optimal sample concentration gives the agglutination reaction at level 4+. The procedure was performed 3 times for each concentration. The same was done for the other sample solutions at different concentrations and for the positive and negative controls.

c. Specific research tools

- Sample application and mixing techniques for the agglutination method.

- Technique for observing agglutination within a specific time frame.

2.6. Data processing method

The results were analyzed based on the repeatability of the 3 observed results, checking if they had the same degree of agglutination or if they were consistent with the sample.

3. Research results

3.1. Evaluation of Kit Compatibility with Samples

Table 3. Results for determining the compatibility of the procedure "identification of antigens A, C, Y, W_{135} in meningococcal vaccine by agglutination method"

Identification kit	Test run	Positive control	Negative control	Mengoc BC test sample	Menactra test sample
Type A	01	+	(-)	(-)	+
	02	+	(-)	(-)	+
	03	+	(-)	(-)	+
	04	+	(-)	(-)	+
	05	+	(-)	(-)	+
	06	+	(-)	(-)	+
Type C	01	+	(-)	+	+
	02	+	(-)	+	+
	03	+	(-)	+	+
	04	+	(-)	+	+
	05	+	(-)	+	+
	06	+	(-)	+	+
Type Y	01	+	(-)	(-)	+
	02	+	(-)	(-)	+
	03	+	(-)	(-)	+
	04	+	(-)	(-)	+
	05	+	(-)	(-)	+
	06	+	(-)	(-)	+
Type W_{135}	01	+	(-)	(-)	+
	02	+	(-)	(-)	+
	03	+	(-)	(-)	+
	04	+	(-)	(-)	+
	05	+	(-)	(-)	+
	06	+	(-)	(-)	+

Note: +: Positive reaction result.

(-): Negative reaction result.

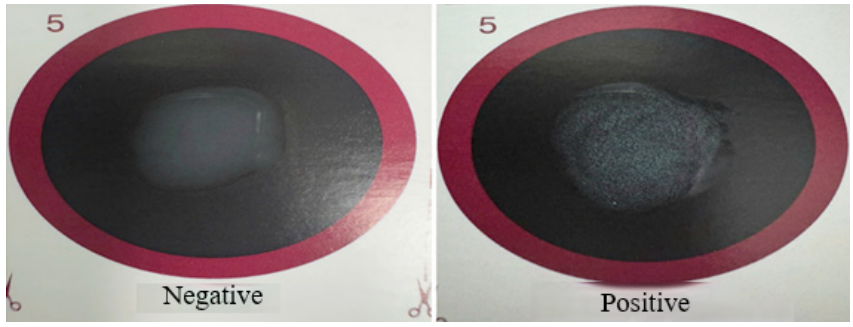

Figure 2. Different reaction results between negative and positive

Figure 2 clearly shows the difference between a negative and a positive reaction. In a positive reaction, antigens in the sample link with the corresponding binding sites on the antibodies, forming visible clumps on the surface, while in a negative reaction, these clumps do not appear. The table shows that all tests were valid because all positive control wells gave a positive reaction. The negative control used was physiological saline, which gave a negative result, and we also used positive control samples from the other types as negative controls, which also gave negative results. All wells for the four types gave positive results with their respective positive controls. The Mengoc BC sample well showed a negative reaction for types A, Y, and W_{135}, and a positive reaction for type C. The Menactra sample well showed a positive reaction for all four types: A, C, Y, and W_{135}. Thus, the procedure for *"identifying antigens A, C, Y, W_{135} in meningococcal vaccine by the agglutination method"* met the compatibility requirements, and the kit used in the study has high specificity.

3.2. Optimal concentration selection

Table 4. Research results for selecting the optimal concentration for the procedure "identification of antigens A, C, Y, W_{135} in Menactra meningococcal vaccine by agglutination method"

Identification kit	Result at concentration	Run	Positive control	Negative control	Sample
Type A	2.000ng/ml	01	+	(-)	2+
		02	+	(-)	2+
		03	+	(-)	2+
	4.000ng/ml	01	+	(-)	3+
		02	+	(-)	3+
		03	+	(-)	3+
	8.000ng/ml	01	+	(-)	4+
		02	+	(-)	4+
		03	+	(-)	4+

Type C	2.000ng/ml	01	+	**(-)**	**2+**
		02	+	**(-)**	**2+**
		03	+	**(-)**	**2+**
	4.000ng/ml	01	+	**(-)**	**3+**
		02	+	**(-)**	**3+**
		03	+	**(-)**	**3+**
	8.000ng/ml	01	+	**(-)**	**4+**
		02	+	**(-)**	**4+**
		03	+	**(-)**	**4+**
Type Y	2.000ng/ml	01	+	**(-)**	**2+**
		02	+	**(-)**	**2+**
		03	+	**(-)**	**2+**
	4.000ng/ml	01	+	**(-)**	**3+**
		02	+	**(-)**	**3+**
		03	+	**(-)**	**3+**
	8.000ng/ml	01	+	**(-)**	**4+**
		02	+	**(-)**	**4+**
		03	+	**(-)**	**4+**
Type W_{135}	2.000ng/ml	01	+	**(-)**	**2+**
		02	+	**(-)**	**2+**
		03	+	**(-)**	**2+**
	4.000ng/ml	01	+	**(-)**	**3+**
		02	+	**(-)**	**3+**
		03	+	**(-)**	**3+**
	8.000ng/ml	01	+	**(-)**	**4+**
		02	+	**(-)**	**4+**
		03	+	**(-)**	**4+**

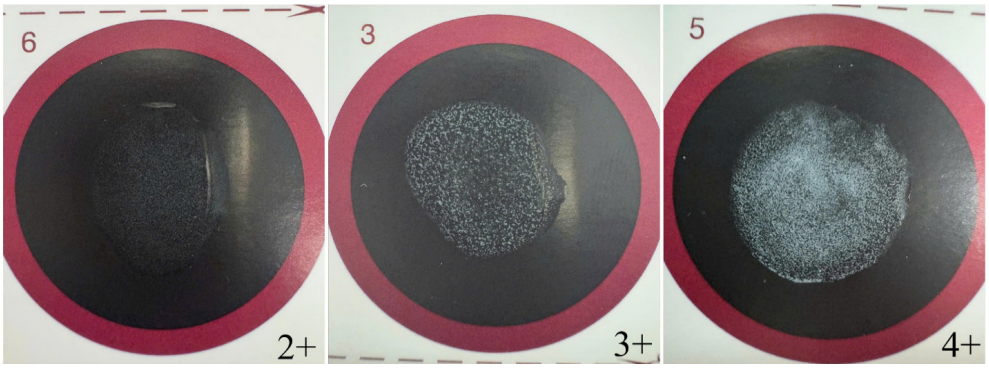

Figure 3. Positive reaction results at levels 2+, 3+, 4+

The results show that all tests were valid because the positive control samples gave positive results and the negative control samples gave negative results. At a sample concentration of 2,000ng/ml, the result was a 2+ positive, while a concentration of 4,000ng/ml resulted in a 3+ positive, and a concentration of 8,000ng/ml resulted in

a 4+ positive. This shows that the optimal concentration is within the range of 4,000-8,000ng/ml. We continued to test at concentrations of 5,000ng/ml, 6,000ng/ml, and 7,000ng/ml to find the optimal concentration for this procedure. The results are shown in the table below:

Table 5. Research results for the next concentrations to optimize the procedure "identification of antigens A, C, Y, W_{135} in Menactra meningococcal vaccine by agglutination method"

Identification kit	Result at concentration	Run	Positive control	Negative control	Sample
Type A	5.000ng/ml	01	+	(-)	3+
		02	+	(-)	3+
		03	+	(-)	3+
	6.000ng/ml	01	+	(-)	4+
		02	+	(-)	4+
		03	+	(-)	4+
	7.000ng/ml	01	+	(-)	4+
		02	+	(-)	4+
		03	+	(-)	4+
Type C	5.000ng/ml	01	+	(-)	3+
		02	+	(-)	3+
		03	+	(-)	3+
	6.000ng/ml	01	+	(-)	4+
		02	+	(-)	4+
		03	+	(-)	4+
	7.000ng/ml	01	+	(-)	4+
		02	+	(-)	4+
		03	+	(-)	4+
Type Y	5.000ng/ml	01	+	(-)	3+
		02	+	(-)	3+
		03	+	(-)	3+
	6.000ng/ml	01	+	(-)	4+
		02	+	(-)	4+
		03	+	(-)	4+
	7.000ng/ml	01	+	(-)	4+
		02	+	(-)	4+
		03	+	(-)	4+
Type W_{135}	5.000ng/ml	01	+	(-)	3+
		02	+	(-)	3+
		03	+	(-)	3+
	6.000ng/ml	01	+	(-)	4+
		02	+	(-)	4+
		03	+	(-)	4+
	7.000ng/ml	01	+	(-)	4+
		02	+	(-)	4+
		03	+	(-)	4+

The results show that all tests were valid because the positive control samples gave positive results and the negative control samples gave negative results. At a sample concentration of 5,000ng/ml, the result was a 3+ positive, while at 6,000ng/ml and 7,000ng/ml, the result was a 4+ positive. Thus, the optimal concentration for this procedure is to use a test solution at 6,000 ng/ml. The research team has developed a procedure for identifying antigens A, C, Y, and W_{135} in meningococcal vaccines using the agglutination method.

4. Discussion

4.1. Discussion of kit compatibility with samples

The research results for identifying antigens A, C, Y, and W_{135} confirmed the effectiveness and feasibility of applying the agglutination method to meningococcal vaccines. Compared to other identification methods like ELISA or UPLC, the agglutination method has several notable advantages: it is less expensive, the testing time is shorter (an ELISA test takes about 2 days, while agglutination only takes 1-2 hours to prepare and costs less)… The feasibility of commercial kits not depending on the manufacturer's in-house standard sample has been discussed above. Compared with the method currently being implemented at the institute, the Elisa method, the agglutination method has the disadvantage of not being able to quantify the antigen content, but for identification testing, the agglutination method has the advantages of high accuracy, stability, cost savings, time, and it is specific to each antigen type, the antigen content of bacteria detected by the agglutination process has been proven to be as low as 0.1 ng/mL. Furthermore, the technical steps of the agglutination method are simple, the procedure has fewer steps, and there are fewer influencing factors, resulting in lower error rates, making it suitable for vaccine identification tests.

The materials needed for the latex agglutination test are quite simple, including the Neisseria Meningitidis antiserum kits for groups A, C, Y, and W135, each containing antibodies specific to each type, mixing sticks, a reaction card (black), and droppers. In this study, we used the meningo type A identification kit: Neisseria Meningitidis antiserum group A (Difco), item number 222281; meningo type C identification kit: Neisseria Meningitidis antiserum group C (Difco), item number 222301; meningo type Y identification kit: Neisseria Meningitidis antiserum group Y (Difco), item number 228811; and meningo type W135 identification kit: Neisseria Meningitidis antiserum group W135 (Difco), item number 222531.

The materials needed for the latex agglutination test are quite simple, including *Neisseria Meningitidis* antiserum groups A, C, Y, and W_{135} kits containing the antibodies for each type, mixing sticks, a reaction card (black), and droppers. In this research, we used the meningo type A identification kit: Neisseria Meningitidis antiserum groups A (Difco), item number 222281; meningo type C identification kit: Neisseria Meningitidis antiserum groups C (Difco), item number 222301; meningo type

Y identification kit: Neisseria Meningitidis antiserum groups Y (Difco), item number 228811; meningo type W_{135} identification kit: Neisseria Meningitidis antiserum groups W_{135} (Difco), item number 222531.

Regarding effectiveness, the results showed the kit's compatibility with the test samples. When testing with positive controls, negative controls, and test samples using the kits containing specific antibodies for the four types (A, C, Y, W_{135}), the results showed that the specific positive control for each type only showed a positive reaction with its corresponding antibody in all 6 test runs. While the negative control used was physiological saline which did not have agglutination and the negative control was the positive control of the remaining types, no agglutination reaction appeared: the positive control of types Y, C, W_{135} did not have a reaction with the type A kit and similarly with the remaining type kits, showing that the kit has high specificity for each different type. For the vaccine test samples, the study used two types: Menactra and VA Mengoc BC. The results showed that agglutination occurred in the Menactra samples for types A, C, Y, and W_{135}, and in the VA Mengoc BC sample for type C, while the VA Mengoc BC samples for types A, Y, and W_{135} were negative. This is because the VA Mengoc BC vaccine does not contain antigens for types A, Y, and W_{135}, so no agglutination reaction occurred with the three identification kit antibodies, further proving the kit's compatibility with the samples and its specificity. This result proves that the kit has very high compatibility and meets inspection requirements.

4.2. Discussion on optimal concentration

After confirming compatibility, the research team proceeded to evaluate the optimal concentration for the test. The tests were conducted with concentrations of 2000 ng/ml, 4000 ng/ml, and 8000 ng/ml. The results showed that the 2000 ng/ml concentration gave a 2+ positive result, the 4000 ng/ml concentration gave a 3+ positive result, and the 8000 ng/ml concentration showed an optimal 4+ positive result. The results showed that in all 4 positive types there was a linear increase of 2+; 3+; 4+ according to the concentration of the tested sample. With the probe range, we found that at 8000 ng/ml, we received a positive result of 4+, so the research team continued to probe from the range of 3+ to 4+, which is 5000ng/ml, 6000ng/ml and 7000ng/ml, giving results of 3+; 4+ in all 3 tests. Thus, the study determined the optimal concentration of the test solution to be 6000 ng/ml with agglutination level at 4+. Optimization not only ensures the accuracy of the test but also increases the consistency of the results when put into actual testing. Although the method has outstanding advantages, the qualitative method is only applicable to identification testing, not quantitative, so it cannot be used in testing to determine the content of each vaccine type; this test is suitable for the identification of vaccine batches during semi-finished product inspection. It can be combined with other methods for quantification when needed.

For routine inspection, this method has the potential to be widely applied in routine inspection at NICVB, especially

in the context of limited supply of internal reference materials from manufacturers. Using commercial kits helps solve problems related to transportation and storage, while reducing dependence on foreign suppliers.

Thus, the agglutination method has proven to be an effective, simple, and economical solution for identifying antigens A, C, Y, and W_{135} in meningococcal vaccines. Applying this method in practice will help enhance domestic inspection capacity, ensure vaccine quality, and improve public health. However, further validation research is needed to expand the application scope and increase the accuracy of the procedure.

5. Conclusion

The project *"Research on the development of an identification process for antigens A, Y, C, W_{135} in meningococcal vaccine by the agglutination method"* conducted from May 2024 to December 2024, confirmed the compatibility of the kit with antigens A, C, Y, and W_{135} in the test samples.

The optimal concentration of the test sample with the kit's antibody was determined to be 6000 ng/ml. Therefore, the procedure "identification of antigens A, C, Y, W_{135} in meningococcal vaccine by the agglutination method" is performed with an optimal vaccine test concentration of 6000ng/ml.The optimal concentration of the test sample with the kit's antibody was determined to be 6000 ng/ml. Therefore, the procedure *"Identification of antigens A, C, Y, W_{135} in meningococcal vaccine by the agglutination method"* is conducted with an optimal vaccine test concentration of 6000ng/ml.

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