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Substitution of Diluent Solution in Semi-Quantitative Examination of C-Reactive Protein

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Abstract: The C-Reactive Protein (CRP) test using the agglutination method is performed through qualitative and semi-quantitative examinations. However, the diluent solution required for the semi-quantitative stage is not provided in the CRP reagent kit. The absence of this solution requires the laboratory to find an alternative diluent in order for the test to be conducted properly. This study aims to compare the effectiveness of 0.9% NaCl solution from table salt and 0.9% NaCl infusion solution with PBS as a diluent in semi-quantitative CRP examination and to provide an alternative diluent that is cheaper and easier to obtain. The study was conducted in the Immunology Laboratory, Health Polytechnic of the Ministry of Health Banjarmasin, using a true experimental design with a post-test only control group. Experimental groups included 0.9% NaCl from table salt and 0.9% NaCl infusion solution, while the control group used PBS. Nine positive CRP serum samples were used. Friedman test results showed no significant difference between the three diluent types (significance value 0.607 > α 0.05). It was concluded that 0.9% NaCl solution from infusion and table salt can be used as diluents for semi-quantitative CRP examination. Further research is recommended to test storage stability and application in similar tests, such as Anti-Streptolysin O and Rheumatoid Factor.

Keywords: Alternative diluent; C-reactive protein; phosphate-buffered saline; 0.9% sodium chloride.

INTRODUCTION

The immune system plays a vital role in protecting the human body by identifying and eliminating foreign substances and abnormal cells. The body's ability to resist and neutralize external or internal threats results in an immune response, which can be either specific or non-specific[1]. One of the body's non-specific immune responses is inflammation, which occurs as a reaction to tissue damage[2].

An important biomarker of inflammation in the body is the elevation of C-Reactive Protein (CRP) levels. CRP is an acute-phase protein produced by the liver in response to infection or inflammation[3]. Elevated CRP levels may indicate the presence of inflammatory processes such as hypertension, tuberculosis, diabetes mellitus[4,5,6], and the presence of CRP in bacterial infections, particularly in cases of sepsis[7].

CRP levels are influenced by various factors. For example, individuals with immune system disorders may exhibit higher CRP levels compared to those without such

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conditions. In other words, the more interfering factors present, the higher the baseline CRP value in relation to the likelihood of acute inflammation, and vice versa. Therefore, although CRP is a marker of inflammation, it remains challenging to determine whether elevated CRP levels reflect acute or chronic inflammation[8].

In clinical laboratories, CRP testing is typically performed manually using the agglutination method. This procedure consists of two stages: qualitative and semi-quantitative analysis. The semi-quantitative stage is essential for determining the CRP concentration in the sample. However, this diluent solution is not provided in the CRP reagent kit package. The absence of this diluent requires the laboratory to find and test a suitable alternative in order to ensure the test can still be performed properly.

This study tested two alternative diluents—0.9% NaCl prepared from table salt and 0.9% NaCl intravenous infusion solution—against the commonly used phosphate buffer saline (PBS). Previous studies have shown that 0.9% NaCl made from table salt can be used as an alternative reagent in ABO blood grouping tests[9], while 0.9% NaCl infusion solution has been used as a substitute for Hayem's solution in red blood cell counting[10].

Based on the above background, this study aims to evaluate the potential use of 0.9% NaCl made from table salt and IV solution as a more affordable and readily available alternative to PBS for CRP testing. The results are expected to support simple laboratories and be used as an alternative in certain serological tests.

MATERIALS AND METHODS

This study employed a true experimental design using a post-test only control group design. This approach allowed the researchers to measure the effect of the intervention (diluent substitution) by comparing the results between the experimental and control groups after treatment. The experimental groups were treated with two types of alternative diluents: 0.9% NaCl solution prepared from table salt commercial using Refined Salt Refina and 0.9% NaCl intravenous infusion solution from Braun Sodium Chloride Solution for infusion 0.9%. The control group was treated with phosphate buffer saline (PBS) solution, technical grade pH 7,2-7,4 as the standard diluent. The 0.9% NaCl solution from table salt was prepared by dissolving 9 grams of commercial table salt in 1000 mL of distilled water using Waterone from Onelab. The solution was homogenized and stored in a labeled bottle for use in testing.

This research was conducted at the Immunology Laboratory, Department of Medical Laboratory Technology, Health Polytechnic of the Ministry of Health, Banjarmasin, after obtaining approval from the Research Ethics Committee with number 167/KEPK-PKB/2025. The number of samples was determined using the Federer formula, where n is the number of samples required, and t is the number of treatment groups, namely three types of diluent solutions tested. The samples used consisted of nine C-Reactive Protein (CRP) positive serum specimens with the criteria of CRP positive serum in qualitative examination, non-hemolysis, and non-lipemic.

CRP sample testing by Glory CRP latex reagent was performed qualitative and semi-quantitatively. Qualitative CRP sample testing was performed by bringing both the reagent and samples to room temperature. The latex reagent was homogenized before use. Positive control, negative control, and each sample (50 µL) were placed on separate circles on a black slide. One drop of latex reagent was added to each circle. The mixtures were evenly spread within the circles on the black slide. The slide was then rotated at 100

rpm for 2 minutes. Results were read under a lamp or bright light to observe the presence or absence of agglutination[11].

For semi-quantitative, using three different diluents: phosphate buffer saline (PBS), 0.9% NaCl infusion solution, and 0.9% NaCl solution prepared from table salt. The semi-quantitative procedure involved placing 50 µL of each diluent onto three separate circles on a test plate, ensuring that the diluent was not spread beyond the circles. Then, 50 µL of the sample was added to the first circle containing the diluent and mixed by gently aspirating and dispensing the liquid with a micropipette, taking care to avoid bubble formation. Next, 50 µL of the mixture was transferred from the first circle to the second, and then from the second circle to the third. After mixing in the third circle, 50 µL of the liquid was discarded. One drop of latex reagent was added to each circle, and the mixtures were spread evenly within the circles on a black slide. The slide was then rotated at 100 rpm for 2 minutes. The results were read under a lamp or bright light to observe the presence or absence of agglutination. The final agglutination result was multiplied by the minimum detectable value to determine the CRP concentration in mg/L, with the minimum detectable CRP level being 6 mg/L[11].

Statistical analysis using SPSS25 was carried out on the collected data. First, the normality of the data was tested using the Shapiro-Wilk test due to the small sample size ($n < 50$). As the data did not follow a normal distribution, non-parametric tests were applied. The Friedman test was used to determine whether there were significant differences among the three diluent groups. The Wilcoxon signed-rank test was subsequently used for pairwise comparisons between the groups to identify any statistically significant differences.

RESULTS AND DISCUSSION

Qualitative CRP Testing

All nine serum samples tested positive for CRP, as indicated by visible agglutination in the qualitative test. The results are shown in Table 1.

Table 1. Qualitative CRP Test Results

Sample	CRP Qualitative
1	+
2	+
3	+
4	+
5	+
6	+
7	+
8	+
9	+

Notes: "+" = presence of agglutination (positive reaction)
"–" = no agglutination (negative reaction).

Semi-Quantitative CRP Test Using Different Diluents

Tables 2, 3 and 4 present semi-quantitative CRP test using different diluents. Dilution (1/2, 1/4, 1/8) means the level of serum dilution with the diluent solution (1:2, 1:4,

1:8). Positive "+" = presence of agglutination (positive reaction) and negative "–" = no agglutination (negative reaction). The CRP level (mg/L) is determined based on the last dilution that still shows positive agglutination according to the kit standard. The CRP level result was multiplied by the minimum detectable value to determine the CRP concentration (6 mg/L).

Table 2. Semi-Quantitative CRP Test Results Using PBS Solution

Sample	Dilution			CRP Level using PBS Solution (mg/L)
	1/2	1/4	1/8	
1	+	+	–	24
2	–	–	–	6
3	+	+	–	24
4	–	–	–	6
5	+	+	+	48
6	+	+	–	24
7	–	–	–	6
8	+	+	+	48
9	+	–	–	12

Table 2 presents the semi-quantitative CRP test results using Phosphate Buffer Saline (PBS) solution as the diluent. Among the nine serum samples tested, samples number 2, 4, and 7 showed negative (–) results at all dilution levels, with a CRP concentration of 6 mg/L, indicating low CRP levels. Samples 1, 3, and 6 showed positive (+) results at 1/2 and 1/4 dilutions but negative at 1/8 dilution, with a CRP concentration of 24 mg/L. Samples 5 and 8 were positive at all dilution levels, indicating higher CRP concentrations of 48 mg/L. Sample 9 was positive only at the 1/2 dilution with a CRP level of 12 mg/L. These results demonstrate that PBS solution is effective in detecting CRP levels with variations in reactivity corresponding to sample concentrations.

Table 3. Semi-Quantitative CRP Test Results Using 0.9% NaCl Infusion Solution

Sample	Dilution			CRP Level using 0.9% NaCl Infusion (mg/L)
	1/2	1/4	1/8	
1	+	+	–	24
2	–	–	–	6
3	+	+	+	48
4	–	–	–	6
5	+	+	+	48
6	+	+	–	24
7	–	–	–	6
8	+	+	+	48
9	+	–	–	12

Table 3 displays the semi-quantitative CRP test results using 0.9% NaCl infusion solution as the diluent. Samples 2, 4, and 7 consistently showed negative results across all dilution levels with CRP concentrations of 6 mg/L, reflecting stable detection at low CRP levels. Samples 1, 3, 5, 6, and 8 showed positive results at most or all dilutions.

Notably, samples 3, 5, and 8 remained positive up to the 1/8 dilution with CRP levels reaching 48 mg/L, indicating that the 0.9% NaCl infusion solution has high sensitivity in detecting elevated CRP concentrations. Sample 9 was positive only at the 1/2 dilution with a CRP level of 12 mg/L. Overall, the NaCl infusion solution showed stable and sensitive results across various CRP concentrations.

Table 4. Semi-Quantitative CRP Test Results Using 0.9% NaCl Table Salt Solution

Sample	Dilution			CRP Level using 0.9% NaCl Table Salt Solution (mg/L)
	1/2	1/4	1/8	
1	+	+	–	24
2	–	–	–	6
3	+	+	+	48
4	–	–	–	6
5	+	+	–	24
6	+	+	–	24
7	–	–	–	6
8	+	+	+	48
9	+	–	–	12

Table 4 shows the semi-quantitative CRP test results using 0.9% NaCl solution prepared from table salt as the diluent. Samples 2, 4, and 7 exhibited negative results at all dilution levels with CRP concentrations of 6 mg/L, indicating the solution's ability to detect low CRP levels. Samples 1, 3, 6, and 8 showed positive reactivity at some or all dilutions. However, sample 5 was positive only up to the 1/4 dilution, unlike PBS and NaCl infusion solutions that remained positive up to the 1/8 dilution, resulting in a lower detected CRP concentration (24 mg/L compared to 48 mg/L). Sample 9 showed positive results only at the 1/2 dilution with a CRP level of 12 mg/L, consistent with the other diluents. These findings suggest that the NaCl solution from table salt can be used as a diluent, although its sensitivity is slightly lower at higher CRP concentrations compared to PBS and NaCl infusion solutions.

Overall, the results from Tables 2, 3, and 4 show that the three diluents produce fairly consistent results. For samples with low CRP levels, such as samples 2, 4, and 7, all three diluents showed negative results at all dilution levels, with a CRP value of 6 mg/L. Similarly, for samples with high CRP levels, like sample number 8, all three diluents showed positive results at all dilutions, with a CRP value of 48 mg/L. Other samples, such as numbers 1, 6, and 9, also showed similar results across all three diluents.

However, some differences were observed in samples with moderate CRP levels in Tables 2, 3, and 4. For example, in sample number 3, the NaCl infusion solution and NaCl from table salt remained positive up to the 1/8 dilution (48 mg/L), while PBS was only positive up to the 1/4 dilution (24 mg/L). This suggests that the NaCl solutions are slightly more sensitive in detecting CRP levels in this sample. A similar difference was seen in sample number 5, where PBS and NaCl infusion solution were positive at all dilutions with a CRP value of 48 mg/L, but the NaCl from table salt was only positive up to the 1/4 dilution with a CRP value of 24 mg/L.

Statistical Analysis of CRP Levels Using Various Diluents

The Shapiro-Wilk test was used to assess normality. The significance values were: PBS = 0.045; NaCl infusion = 0.023; and table salt NaCl = 0.045. All were below 0.05, indicating non-normal distribution.

The Friedman test was then used to determine whether there were any statistically significant differences between the three groups. The test showed a significance value of 0.607 ($p > 0.05$), indicating no significant difference in CRP levels between the diluent groups. See Table 5.

Table 5. Friedman Test Results

Test Statistic	Value
N	9
Chi-Square	1.000
Df	2
Asymp. Sig. (p-value)	0.607

Pairwise comparisons were performed using the Wilcoxon signed-rank test, which revealed no significant differences between PBS and 0.9% NaCl infusion ($p = 0.317$), PBS and 0.9% NaCl from table salt ($p = 1.000$), and between 0.9% NaCl infusion and 0.9% NaCl from table salt ($p = 0.317$). These results indicate that all three diluents produced statistically similar CRP values (Table 6).

Table 6. Wilcoxon Signed-Rank Test Results

Comparison	Z Value	Asymp. Sig. (2-tailed)
Infusion vs. PBS	-1.000	0.317
Table Salt vs. PBS	0.000	1.000
Table Salt vs. Infusion	-1.000	0.317

The findings of this study indicate that there was no significant difference in CRP levels when using phosphate buffer saline (PBS), 0.9% NaCl intravenous infusion, or 0.9% NaCl solution prepared from table salt as diluents in semi-quantitative CRP testing. This result suggests that all three solutions exhibit comparable isotonic properties, allowing them to function effectively as diluents in CRP semi-quantitative tests.

Phosphate buffer saline (PBS) has properties comparable to those of 0.9% NaCl solution. PBS contains sodium chloride, potassium chloride, sodium dihydrogen phosphate, and potassium dihydrogen phosphate. Being isotonic and non-toxic to cells, PBS helps maintain osmolarity. Both PBS and 0.9% NaCl solutions have similar osmotic pressure and isotonic characteristics, which do not significantly affect laboratory test results[12,13]. Studies by Alifia et al. (2023) and Edijanto et al. (2024) demonstrated no differences between 0.9% NaCl infusion solution and PBS in erythrocyte sedimentation rate (ESR) testing12,13. However, it should be noted that the function of diluents differs between ESR and CRP tests. In ESR, the diluent plays a role in maintaining red blood cell suspension and viscosity to optimize the sedimentation process. In contrast, in CRP testing, the diluent serves as a medium supporting the antigen-antibody reaction without interfering with the agglutination process.

Other studies have also demonstrated that 0.9% NaCl infusion solution can be used as an alternative to Hayem's solution in erythrocyte counting tests10. These findings support that 0.9% NaCl infusion solution possesses sufficiently stable physical and chemical characteristics and is compatible with various hematological applications. Therefore, there is potential for using 0.9% NaCl infusion solution as an alternative medium in a range of laboratory tests.

Sodium chloride solutions in concentrations of 0.85–0.9% are generally considered isotonic and resemble the osmotic pressure of human blood. These isotonic solutions are widely used in clinical laboratories and medical procedures due to their ability to maintain cellular stability[14,15].

The use of 0.9% NaCl solution prepared from table salt as an alternative diluent in CRP testing is also supported by previous studies. Ammariah et al. (2022) reported no significant difference in the degree of agglutination in ABO blood grouping tests (serum grouping tube test) between commercially packaged 0.9% NaCl solution and 0.9% NaCl prepared from table salt[9]. It should be noted that ABO blood grouping and CRP testing involve different antigen-antibody interactions and may have different sensitivities to the composition of the diluent. In ABO blood grouping, the diluent mainly functions to maintain the isotonicity of red blood cells to ensure clear agglutination, whereas in CRP testing, the diluent serves to support the antigen-antibody reaction without interfering with the agglutination process. Nonetheless, these findings indicate that table salt-based NaCl solution can be used as an alternative substitute in certain serological tests.

Table salt-based NaCl solutions can be easily prepared in the laboratory, reducing dependency on commercially available reagents. They can also be freshly prepared to avoid deterioration during storage or transportation and eliminate the need for preservatives[16]. Practically, the use of 0.9% NaCl infusion solution is easy to implement in laboratories, as it is commercially available and can be easily obtained from pharmacies in sterile packaging. Meanwhile, a solution made from table salt can serve as a low-cost and easily prepared alternative, especially in laboratories with limited logistical resources. However, it is important to note that the salt used should be branded and of reliable quality (food grade), and the preparation of the solution must be carried out in a sterile and homogeneous manner to avoid affecting the test results. Thus, this study demonstrates that both 0.9% NaCl infusion solution and 0.9% NaCl solution prepared from table salt can be used as alternative diluents in CRP testing.

This study has several limitations that should be considered. The relatively small sample size and lack of repeat testing limited the statistical power of the analysis. Additionally, this study was limited to using only one type of commercial CRP latex reagent kit. Nevertheless, this research can serve as a backup solution for alternative diluents in serological testing in the laboratory.

CONCLUSION

Based on the study, the use of 0.9% NaCl solution from infusion and 0.9% NaCl solution made from table salt can be used as diluents for semi-quantitative CRP level examination. Further research is recommended to test and strengthen the evidence and ensure practical application with more specific directions. Future studies should use larger sample sizes with varied CRP levels and include stability testing of the NaCl solutions over time at room temperature. Additionally, evaluating their effectiveness in other

agglutination tests, such as Anti-Streptolysin O and Rheumatoid Factor, would broaden their clinical utility.

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CONFLICT OF INTEREST

In this study there is no conflict of interest.

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