CHAPTER 3

Research Methodology

3.1 Research Question:

Is diagnostic value of typhi dot test accurate (sensitivity 95% and specificity 90%) for diagnosis of typhoid fever in Vietnamese patients?

3.2 Research objective

3.2.1.To determine the diagnostic value of typhi dot test in diagnosing typhoid fever in Vietnamese patients

- Determine the sensitivity and specificity
- Determine the positive and negative predictive value
- Determine the accuracy

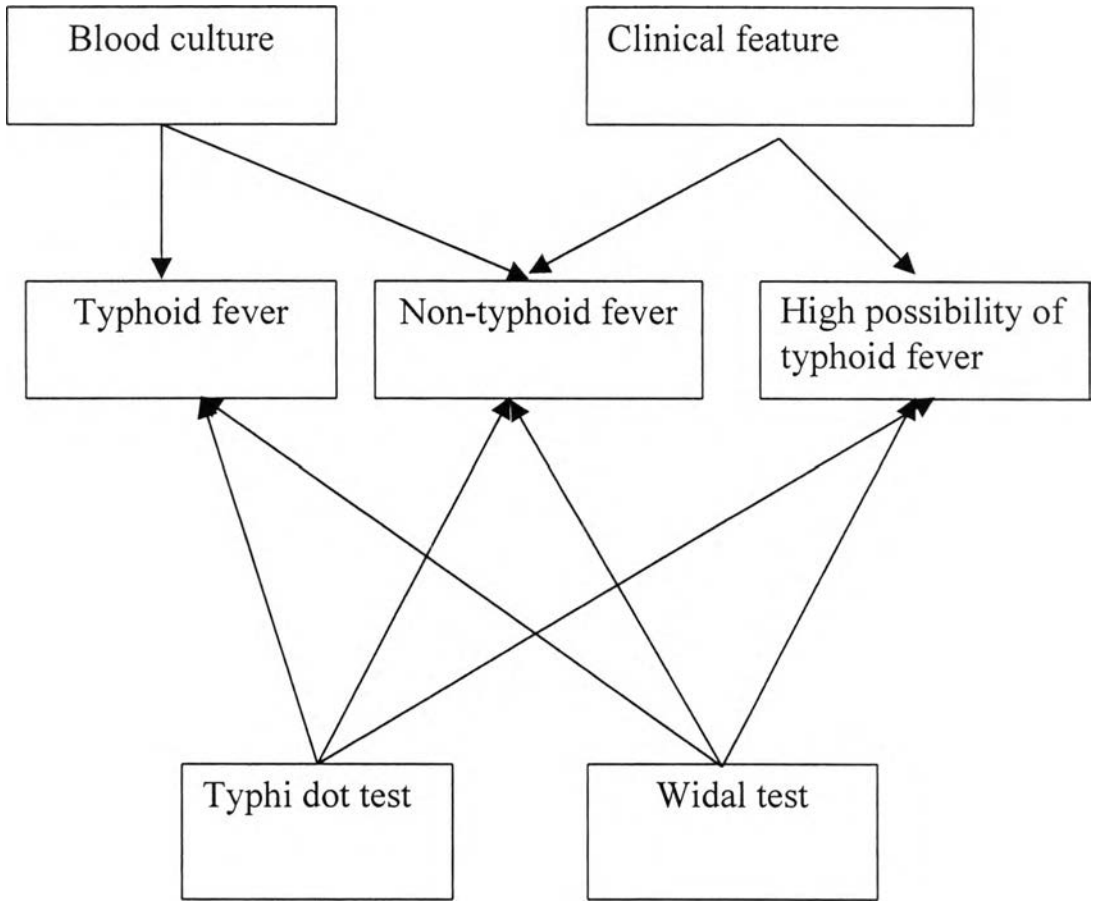
3.2.2.To compare the sensitivity and specificity of typhi dot to Widal test in diagnosing typhoid fever in Vietnamese patients.

3.3. Hypothesis:

Typhi dot test is accurate and more accurate than Widal test in diagnosing typhoid fever in Vietnamese patients.

Conceptual Frame Work.

3.4. Conceptual framework of the study (figure2).



3.5.Operational definition

Group 1:

Definite typhoid fever

Blood culture is positive for Salmonella typhi

Group 2:

High possibility of typhoid fever

When blood culture is negative but the clinical feature meet all criteria below:

- Has fever >= 7 days with documentation of high fever >=39^0C
- And has diarrhea >=2 times/day, splenomegaly, hepatomegaly, rose spots, abdominal ileus, abdominal pain, relative bradycardia, mental confusion, lower GI bleeding, intestinal perforation. A patient must at least has four among them
- And afebrile between the first and fifth day of treatment with Ciprofloxacine for adult and Cephalosporin (with third generation) for children
- And has no specific clinical symptoms of other diseases and no tests suggesting other diseases.

Group3:

Non-typhoid fever

Blood culture is positive for other organisms.

Or blood culture is negative and clinical feature does not meet the criteria of group 2.

3.6. Research design:

Observational cross-sectional study.

3.7. Target population

Vietnamese patients who are suspected of having typhoid fever.

3.8. Study population

Vietnamese patients who are suspected of having typhoid fever and admitted in Long Xuyen and Cai lay hospitals from 7/1999 to 15/5/2000.

3.9. Sample size:

The formula used to calculate the sample size is:

$$N = \frac{Z^2.P.Q}{D^2}$$

Estimated sensitivity of typhi dot test is 95%

Estimated specificity of typhi dot test is 90%

D = 5%.

For sensitivity of typhi dot test

$$N = \frac{(1.96)^2.(0.05).(0.95)}{(0.05)^2} = 73$$

P = 0.95

Q = 0.05

Z = 1.96

For specificity of typhi dot test

$$N = \frac{(1.96)^2.(0.9).(0.1)}{(0.05)^2} = 139$$

The estimated prevalence of typhoid fever cases among the clinically susspected typhoid fever in Tien Giang and An Giang province was 18-25%. So with 420 typhoid fever suspected cases we ensure that they will cover the required number of typhoid fever and non-typhoid fever patients.

3.10. Inclusion criteria

The eligible patients must meet the following criteria

- Inpatients
- Do not have specific clinical symptoms of other disease at the time admitted to the hospitals.
- Have fever >=37.8^{0}C for >= 5 days.

3.11. Exclusion criteria

- The patients are not willing to participate in the study.

3.12. Data collection

Eligible patients were admitted to the LongXuyen and Cailay hospitals. All information of medical history and physical examination were recorded in the medical record by physicians.

Blood specimen with the patients' code number and with different codes: A for blood sample of typhi dot test and B for Widal test.

The results of blood culture and widal tests were recorded by physicians in medical record.

The results of typhi dot test were recorded by a technician in special record (see appendix).

At the end of every month, data collection and entry were done.

All clinical symptoms were noted by the physicians.

3.13. Measurement

All information of medical history and clinical symptoms were noted and followed-up by the physicians.

Blood specimen was drawn at once for culture (before antibiotic administration) and after that for typhi dot and Widal test. Blood culture, typhi dot test, Widal test were done independently by different group of experienced technicians.

3.13.1 *Blood culture*

Blood cultures were done twice. The second blood culture was done after the first one for 30 minutes. Blood samples were incubated in brain heart infusion broth (BHI) for 10 days at 37^0C. Subculture on blood agar were made on day 1, 3, 5, 7, 9. If characteristic non-lactose-fermenting colonies of Salmonella appeared, the organism was more specifically identified on the basis of biochemical reaction and agglutination with specific antiserum.

3.13.2. *Widal test*

The Widal test was performed with suspension of O and H antigens from Salmonella typhi, H antigen from Salmonella paratyphi A, and H antigen from Salmonella paratyphi B. All antigen were obtained from Pasteur Institute of Ho Chi Minh City.

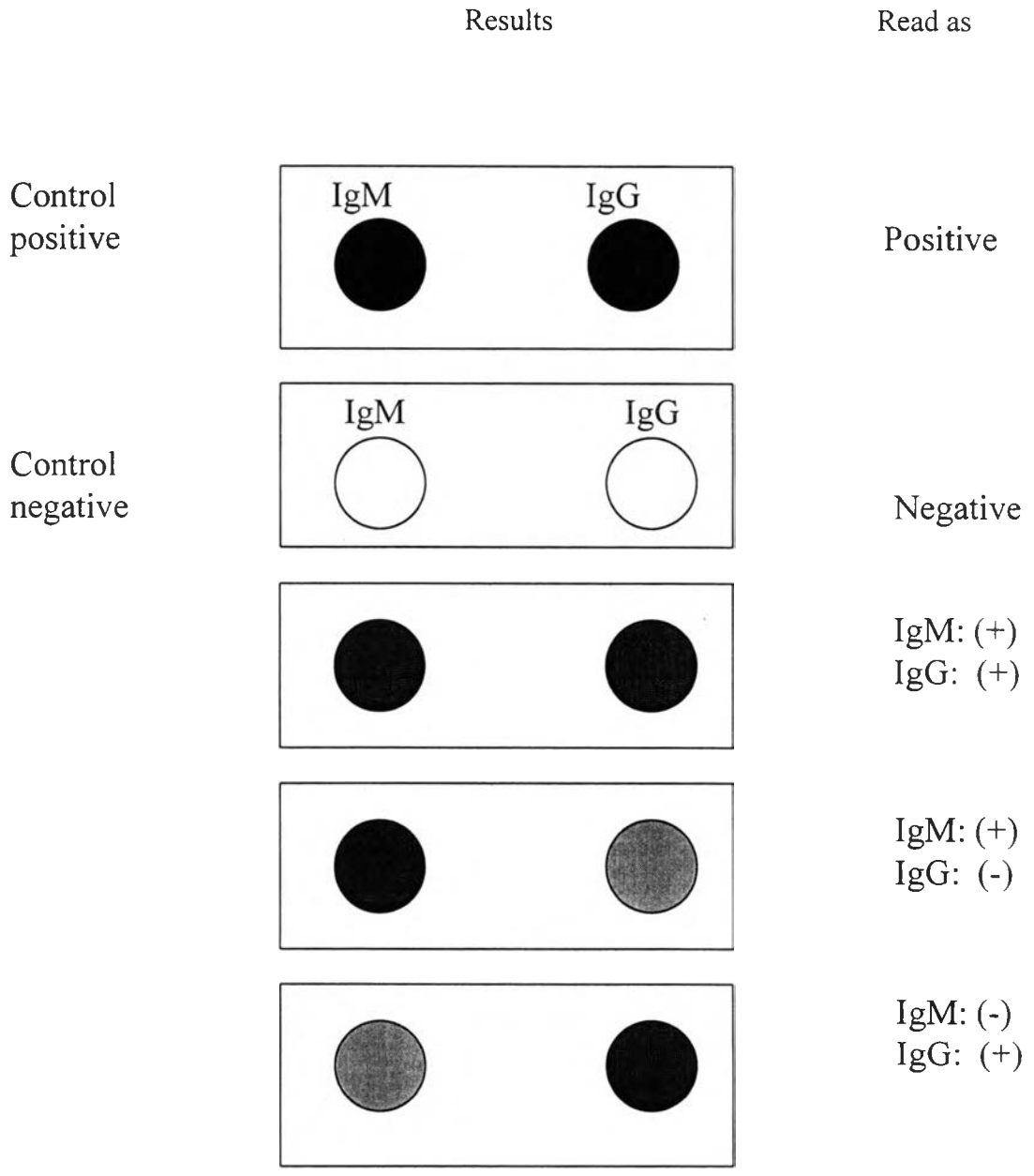
Diluted serum with different titers: 1:80, 1:100, 1:160, 1:200, 1:300...with the presentation of antigen O and H. The reaction was kept at 37^0C for 24 hours and the result can be interpreted after.

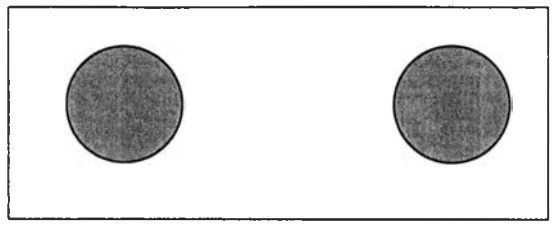
Widal test was positive if the result of the second sample has 4-fold rise when compared to the first sample or it is considered positive when the H/or O antibody titer is 1:100 or more.

3.13.3. *Typhi dot test*

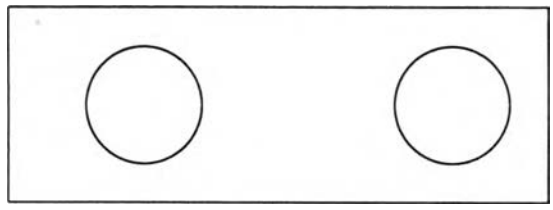
1 µL containing 0.3 µg of the 50 KD protein was dotted on to nitrocellulose strip. After probing with a 1:100 dilution serum, the strip was washed, and antigen – antibody complexes were visualized one hour after the addition of horseradish peroxidase –

conjugated antiserum to human IgM or IgG. A substrate, 4-chloro-1-naphthol, was added for color development. Serum containing either IgM or IgG antibodies to specific antigen gave a blue color as intense as or more intense than the color of the positive control. The EIA was considered positive when the IgM titer and/or IgG titer was >=1:100.

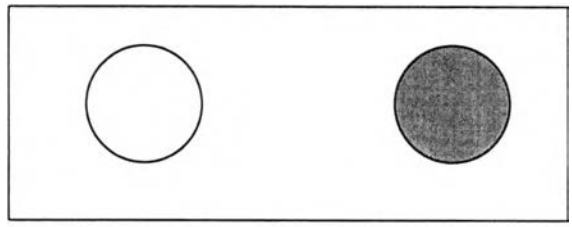




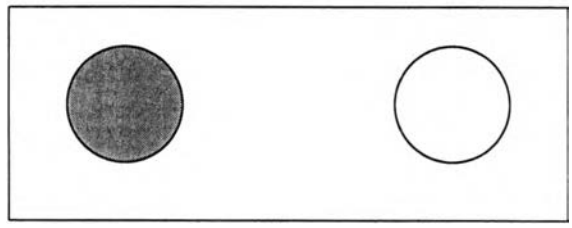
IgM: (-)
IgG: (-)



IgM: (-)
IgG: (-)



IgM: (-)
IgG: (-)



IgM: (-)
IgG: (-)

The typhi dot test is repeated if the results are inconclusive:

- If the color intensity of the negative control is similar to the positive of the control.
- If the color intensity of the test serum is high but not equal to the positive of the control.

The results of typhi dot test are interpreted as:

IgM (+), IgG (-): Acute typhoid fever.

IgM (+), IgG (+): Acute typhoid fever.

IgM (-), IgG (-): Non-typhoid fever

IgM (-), IgG (+): Possibilities include:

- Previous successfully treated case of typhoid fever. Now has another disease
- Re-infection of typhoid fever.
- Person who has been immunized with typhoid vaccine.
- Typhoid carrier. Now has another disease.

The validity of typhi dot test is evaluated by determining the sensitivity, specificity, predictive value, accuracy. Widal test is reevaluated and compared to typhi dot test.

$$\text{Sensitivity} = \frac{\text{Diseased with positive test}}{\text{All diseased}}$$

$$\text{Specificity} = \frac{\text{Disease-free with negative test}}{\text{All disease-free}}$$

$$\text{Positive predictive value} = \frac{\text{Diseased with positive test}}{\text{All with positive test}}$$

$$\text{False positive rate} = \frac{\text{Disease-free with positive test}}{\text{All disease-free}}$$

$$\text{Negative predictive value} = \frac{\text{Disease-free with negative test}}{\text{All with negative test}}$$

$$\text{False negative rate} = \frac{\text{Diseased with negative test}}{\text{All diseased}}$$

$$\text{Accuracy} = \frac{\text{Diseased with positive test + disease-free with negative test}}{\text{All diseased + disease free}}$$

$$\text{Prevalence} = \frac{\text{Number with disease}}{\text{Total number of individuals in the study}}$$

3.14. *Content validity of criteria of group 2.*

The criteria of this group is completely based on clinical symptoms. So the validity in determining typhoid fever of this criteria need to be tested.

These criteria were sent to four experts of infectious diseases. They were asked to give their opinion for these criteria by scoring.(see appendix)

1 = relatively valid criteria

0 = Not sure

-1 = relatively irrelevant criteria.

The criteria correlation is calculated by using the formula:

$IC = \sum R / N$

R: Total score of the criteria

N: Number of experts

If IC is more than 0.5 that criteria will be accepted.

Results of score from 4 experts:

- 4 experts gave score: 1

So the criteria of group 2 were accepted. It means that any patients who meet the criteria of group 2 were accepted to high possibility of typhoid fever.

3.15. Data analysis:

3.15.1. The validity of typhi dot and Widal test were analyzed based on : sensitivity, specificity, predictive value, false positive and false negative rate, accuracy.

3.15.2 Statistics:

The difference between sensitivity, specificity of typhi dot and Widal test were tested by Mc Nemar chi square test.