

CHAPTER 2

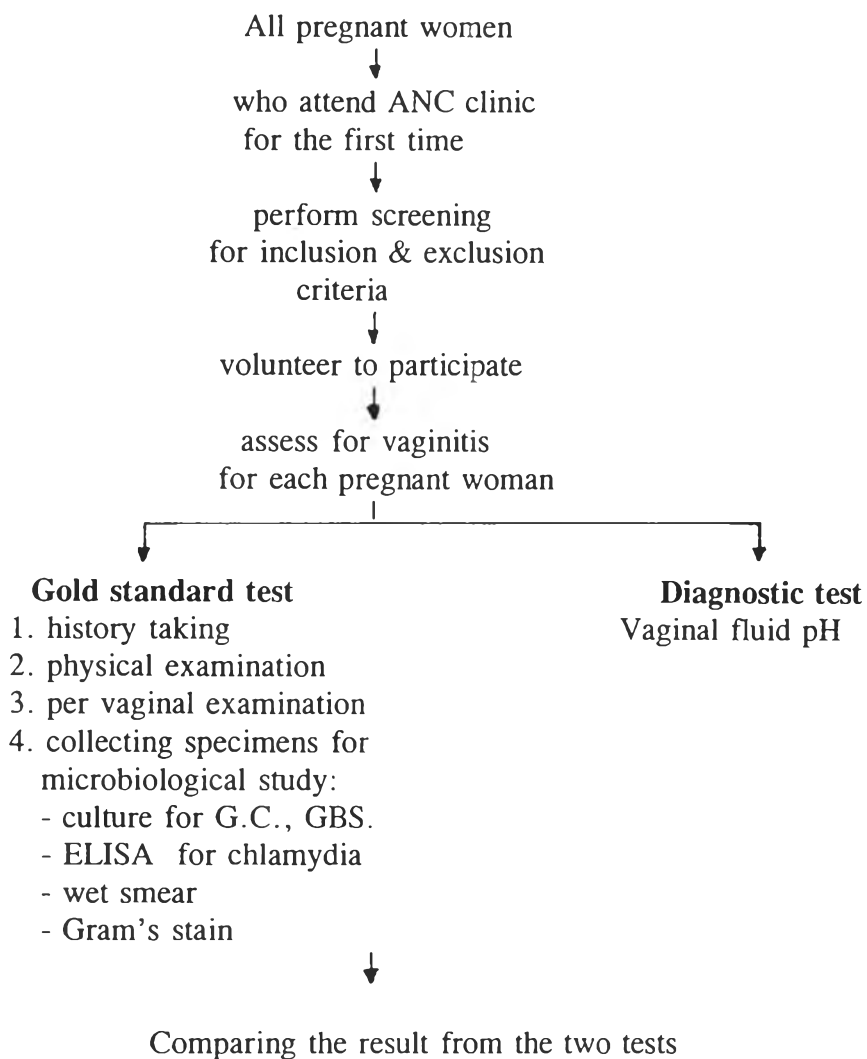
METHODOLOGY



Research design

This is a descriptive diagnostic test study. The study process is outlined in Figure 2.

Fig. 2 : Design overview



Note: *All subjects who have been identified as having any pathogenic organisms will be treated by their attending physicians.*



Population and Sampling

Population

All pregnant women attending antenatal care for the first time, during November, 1994 to February, 1995, at Srinagarind hospital were recruited into this study.

Sample size

A descriptive study to assess diagnostic performance of vaginal fluid pH to vaginal infection that estimated sensitivity of 80%.

From the formula ³² :	N	=	$Za^2 PQ / D^2$
When	N	=	number of sample
	Za	=	1.96 at 95% confident level
	P	=	sensitivity of test 80%
		=	0.8
	Q	=	1-P
		=	1-0.8
		=	0.2
	D	=	Acceptable error
		=	0.1
Then	N	=	$[(1.96)^2 \times 0.8 \times 0.2] / (0.1)^2$
		=	61.44

Thus the total number of cases required for illustrating the specified sensitivity of the test is at least 62.

The prevalence of vaginal infection in pregnant women is estimated to be 25% (prevalence of common vaginal pathogens in pregnant women varies from 10-50%³³). Therefore, the study should recruit at least 248 pregnant women.

Inclusion criteria

All pregnant women attending antenatal care clinic at Srinagarind hospital for the first time were recruited into this study.

Since all procedures that were planned to be used in this study are almost the same as routine standard procedures for the initial evaluation of pregnant women and have been recommended in current practice⁹. The major goals of such initial evaluation of pregnant women are to define the health status of the mother and fetus, to determine the gestational age of the fetus, and to initiate plan for continuing obstetrical care⁹. Additionally, pregnant women in any stages of pregnancy are considered to be screened for vaginal infection since they are all in the same risk status.

Exclusion criteria

Since some procedures for initial evaluation pregnant women may not be safe or even potentially harmful for some pregnant women. We excluded complicated pregnancy or any pregnant women that would be harmful by the procedure. Women with some activities and chemical exposure which could modify the vaginal pH level

were excluded from the study too. Therefore the exclusion criteria are the followings:

1. Sexual intercourse in the previous 24 hours.
2. Vaginal douche in the previous 24 hours.
3. Pregnancy complications including abortion, preterm labour, premature rupture of membranes, antepartum bleeding, cervical incompetence.
4. Antibiotic therapy in the previous 7 days.
5. Previous vaginal examination in the previous 24 hours.
6. In labour.

Diagnostic test being studied: The Vaginal fluid pH

There are no significant difference between the methods of using, pH test paper and electronic instrumentation for measuring the pH of vaginal secretions. A study by Thompson et al. (1990) showed that pH test paper was reliable for pH determination of vaginal secretions³¹. Therefore, in this study vaginal fluid pH will be measured by Phenolphthalein paper, which the pH value ranges from 0.5 to 5.5.

Vaginal fluid pH was measured by Phenolphthalein paper (Universal- and Special- Indicator- Paper). We can obtain the pH value directly from the paper with the pH range from 0.5 to 5.5. The pH paper was placed directly by the cotton swab containing vaginal fluid from the lateral and posterior fornices of the vagina. Alternatively, the pH paper can be placed on the surface of the speculum after it has been removed from the vagina. The cervical mucus have been avoided since it has higher pH (pH = 7.0) than the vaginal fluid²³. After about one minute, the colour of the strip was observed and compared with the colour scale. Cutoff point of 4.5 was

selected as the classification criteria, that is, one who have the pH of 4.5 or less would be classified as none vaginitis whereas who have the pH of more than 4.5 is classified as having vaginitis.

Gold standard for documented specific organisms involving vaginitis

The definitive criteria for vaginitis involves more than one criteria which need to be described some important information for each criteria. Then the gold standard criteria is described later at the end of this section.

1) Bacterial vaginosis (BV)

BV is caused by replacement of the normal lactobacillus predominant flora with high concentrations of *G. vaginalis*, anaerobes and *M. hominis*, and no single micro-organism defines the syndrome²¹.

Thus diagnostic tests based upon detection of any single group of bacteria are not useful. Additionally, many of these microorganisms are fastidious and very difficult to cultivate in the laboratory, which cause routine culture impractical and costly. The study, then, adopted the recommendation from Amsel et al.(1983) that the one who will be classified as having vaginitis based on the clinical diagnosis of bacterial vaginosis has to have at least three out of four of the following signs²²:

i) Thin, homogeneous vaginal discharge

The discharge is white, nonfloccular, and adherent to the walls of the vagina and is not markedly increased over that

normally seen.

ii) Vaginal fluid pH > 4.5

Vaginal pH is best determined by swabbing the lateral and posterior fornices of the vagina, and then placing the sample directly on the pH paper. Alternatively, the pH paper can be placed on the surface of the speculum after it has been removed from the vagina.

iii) Amine odour

Vaginal fluid release of a fish amine odour from vaginal fluid when mixed with 10% KOH.

iv) Presence of clue cells

These cells have a heavily stippled, granular appearance, in contrast to the more clear, translucent cytoplasm of normal cells. The border of clue cells tend to be obscured by the organisms. The presence of these cells on each of 10 separate low power microscopic fields, with or without accompanying normal exfoliated epithelial cells.

The presence of at least three of these four criteria has been used to define BV over the past 10 years. Recent studies have shown that *Gardnerella*, *Mobiluncus*, and other bacteria

may adhere to the vaginal epithelial cells. Lactobacillus may also bind to exfoliated epithelial cells, although they are seldom present in high enough concentrations to completely obscure the edges of the epithelial cell and with experience should not be confused with true clue cells. So some authors recommended that at least 20 % of vaginal epithelial cell present be noted as clue cells to establish the diagnosis of BV. However in their experience, even if only 1 to 20% of vaginal epithelial cells are noted to be clue cells by an experienced microscopist, this is highly correlated with Gram stain features of BV²³.

2) **Gonococcal cervicitis**

Isolation of *N. gonorrhoea* is the diagnostic standard for gonococcal infection, having diagnostic sensitivity of 80-95%²⁴. This organism is detected through culture. Details of the laboratory test is described later in Section 7: Specimen collection and microbiological studies.

3) **Chlamydial infection**

The sensitivity and specificity of currently available isolation method for *C. trachomatis* from genital specimens are unknown, as the gold standard is not available²⁵.

Noncultural method for detection of *C. trachomatis* antigen in infected secretions eluted from swabs and measured by enzyme-linked immunosorbent assay (ELISA) methods will be used in this study.

4) Candidiasis

For candidiasis, lacking of specificity of symptoms and signs preclude a diagnosis which is based only on history and physical examination. Neither clinical signs and symptoms alone nor culture confirmation of the presence of candida should be regarded as a satisfactory basis for diagnosis²⁶. Therefore this study applied a combination between clinical findings, microscopic examination, and culture as the criteria for candidiasis.

5) Trichomoniasis

Symptoms and signs of increased vaginal discharge are noted by about 50-75% of women diagnosed in STD clinics as having trichomoniasis. Wet mount examination can identify trichomoniads in approximately 40-80% of cases. However, most studies suggested that in excess of 95% of women can be diagnosed by culture²⁷. Therefore, this study used clinical symptoms and signs combined with wet mount examination as a standard diagnosis of TV. This is also what we have done in the current practice.

6) Group B Streptococcal vaginitis

In general, patients with group B Streptococcal (GBS) colonization are entirely asymptomatic, cultures for this organism should be taken from multiple sites, including cervix, urethra, and anorectal area.

The diagnosis of group B Streptococcal infection in symptomatic adult patient with evidence of urethritis, vaginitis, cervicitis, or urinary tract infection is based on simple culture techniques²⁸.

Therefore the gold standard for diagnosing maternal genital colonization with GBS will be used in this study was culture which the specimen was taken from cervix.

7) Gram stain

Gram stain is a microbiological study which the positive result indicates of G.C. infection as well as presenting of clue cells and inflammatory reaction. Therefore, the positive gram stain referred to have vaginitis.

The positive for vaginitis based on the gold standard test means the pregnant women who have positive result of at least one of the above tests.

Specimen collection and microbiological studies

Specimens from vagina were taken with strictly aseptic precaution to avoid contamination and pregnancy complication. In order to collect specimens, a speculum was introduced into the vagina without lubricants. Specimens are then taken for Papanicolaou smear (Pap smear), culture for gonococci and group B Streptococci, ELISA for chlamydia, Gram staining, Wet mount preparation, and pH assessment. Detail for each technique was described as follows:

1) Papanicolaou smear

Smears are obtained prior to digital examination from the patient in lithotomy position. Additionally, the smear had performed without lubricants which can spoil the staining characteristics of the cells. Aside from that, douches can dilute and wet the cells, a satisfactory smear cannot be obtained within-12-24 hours after douching²⁹.

The spatula was used to scrape the portio circumferentially at the area of transformation zone. A slide was rapidly smeared and fixed by immersion into a small bottle containing fixative solution (95% alcohol). Fixation was completed within 15-30 minutes.

2) Culture for gonococci

A sampling swab was introduced into the cervical canal and moved from side to side before withdrawal after retaining for 10-30 seconds. Then it was inoculated directly on gonococcal culture media, after brought to room

temperature before being inoculated. The specimen swab was rolled over one-third to one-half of the agar surface after which the remainder surface was crossed streaked with a sterile bacteriologic loop. The agar plates are incubated within 20-30 minutes at 35-36 degree Celsius in an atmosphere of 4-6% CO₂. This atmosphere was most easily achieved with the candle extinction jar technique. A moistened blotting paper will be placed in the bottom of the jar to ensure high humidity. For maximum recovery and survival of gonococci, the plates will be incubated at 35-36 degree Celsius for 18-24 hours before transfer to the local laboratory³⁰.

3) **Culture for group B Streptococci**

The appropriate media for cultivation of group B streptococci was selective broth media, Todel-Hewitt broth, which contains gentamicin, colistin (or polymyxin B), and nalidixic acid. These antibiotics inhibit the growth of Gram negative enterobacteriaceae and other bacteria in the normal genital tract flora that could interfere with the recovery and identification of group B streptococci²⁸. The specimens will be taken from outer third of cervical canal after taking a swab for G.C.

4) **Chlamydia**

Chlamydia trachomatis antigen was detected in infected vaginal fluid eluted from swabs and tested by enzyme-link immunosorbent assay (ELISA) methods.

5) Wet mount examination for clue cells²³

Clue cells are squamous vaginal epithelial cells which are covered with many vaginal bacteria, causing them stippled or granular appearance. The bordered are obscured or fuzzy owing to adherence of small rods or cocci.

Clue cells can be found by obtaining a sample of vaginal fluid with a swab and mixing with a drop of normal saline on a glass slide. A cover slip should be placed over the suspension. This suspension can also be used for detection of motile trichomonads, and intracellular gram negative diplococci(G.C.).

6) Odour or Whiff's test²³

A drop of vaginal fluid will be placed on a glass slide and a drop of 10% KOH was added. If the amines are present, the odour dissipate quickly. Then a cover slip will be placed over this preparation for microscopic exam for hyphae forms associated with candidiasis.

7) Vaginal fluid pH test

Vaginal fluid was swabbed at the lateral and posterior fornices of the vagina. The fluid which was taken for gram stain was also placed directly on the pH paper. Alternatively, the pH paper can be placed on the surface of the speculum after it has been removed from the vagina. The cervical mucus must be avoided since it has higher pH (pH = 7.0) than the vaginal fluid²³.

Study variables

The data was collected in data collection form (see Appendix 3).

The study variables includes :

1. Baseline characteristics

- Age
- Race
- Religion
- Educational Attainment
- Occupation of the pregnant women
- Occupation of the husband
- Weight
- Height

2. Obstetrical & Gynecological history

- Age of menarche
- Gravidity (total number of pregnancy)
- Parity (total number of delivery)
- Outcomes of previous pregnancy
- Previous experience of vaginal infection
 - . Seeking for treatment
 - . Duration of treatment

- Previous experience of sexually transmitted diseases
 - . Seeking for treatment
 - . Duration of treatment
- Husband's sexually transmitted disease history
 - . Seeking for treatment
 - . Duration of treatment

3. Current pregnancy status

- Order of the current pregnancy
- Last menstrual period (LMP)
- Gestational age
- Total number of previous antenatal care attendance
- Complaint of obstetrical or gynecological problem

4. Risk behavior

- Smoking
- Alcoholic drinking

5. Per vaginal examination

- External genitalia appearance
- Vaginal condition (mucosa, discharge)
- Cervical condition
- Uterus

- Adnexal condition
- Cul-de-sac condition

6. Microbiological studies

- Papanicolaou smear (Pap smear)
- Culture for G.C., GBS., fungus
- ELISA for chlamydia
- Gram stain
- Amine odour (Whiff's test)
- Wet mount examination

7. Vaginal fluid pH

Measurement of outcomes

There are two major outcomes of interest, that is vaginal fluid pH and a series of microbiological tests as the gold standard test as mentioned earlier. The result of vaginal fluid pH is determined at the same time of obtaining data collection form as in Appendix 3. Then the Identification number (ID number) which corresponds to the number specified in the questionnaire was recorded in another separated form showing only the ID number and series of microbiological test. In other words, the vaginal fluid pH result was not appear in this form. The result of microbiological test is obtained from the other observers rather than the one who knows the vaginal pH result of a certain patient. Once the result was available, it was recorded in the form

mentioned above. Then the investigator merged the two parts of the information for further analysis. This is to ensure blinding of outcome measurement.

Data processing and analysis

After the data collection forms were filled completely, the researcher assign the code corresponding to each question according to the coding manual. Then the data was enter twice by the two independent key operators, to the computer using Epi Info computer program. The two data files were then validated to check if there was any inconsistency between them. Correction was made accordingly.

For data analysis, STATA statistical package was used. Descriptive statistics, including mean, median, standard deviation and proportion, was applied for continuous and categorical variables respectively. Finally the sensitivity, the specificity, and the predictive value of the test were estimated.

Ethical consideration

This type of study carries minimal risk to study subjects. The study involves no intervention rather than asking questions, carrying out medical examinations and laboratory tests. In current practice, it is recommended that vaginal examination should be performed to identify cytological abnormalities and to detect some common pathogenic organisms such as gonorrhoea, trichomonas, candida⁹ in the first ANC visit. So the procedures to be performed in this study will be no more harm than current practice.

All subjects will also be treated in accordance with four basic ethical principles namely respect for persons, beneficence, non-maleficence and justice³⁴. After being personally informed (Appendix 1) about the study, all subjects will make their own decision to participate and can withdraw their participation at any time without any effect on the care that they should receive.

Aside from that, the subjects may also have benefit if their test result is abnormal. The physicians who take care of the subjects would know the result and would provide the appropriate treatment for the subjects.

All subject's medical information will be kept strictly confidentially. No one can be identified individually in any published reports. Only the researchers will have access to the information.