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## การศึกษาเชื้อจุลินทรีย์ในยาเตรียมชนิดน้ำ แพนิจจขัยนสำหรับแก้ไอ

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ปฐมนิพนธ์  
ORIGINAL ARTICLE

## การศึกษาเชื้อจุลินทรีย์ในยาเตรียมชนิดน้ำ แผนบ่อจุ่มสำหรับแก้ไอ

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### บทคัดย่อ

การตรวจหาเชื้อจุลินทรีย์ในยาน้ำแก้ไอซึ่งผลิตจากโรงงานในประเทศ 79 ตัวอย่าง และที่เป็นยานำเข้าจากต่างประเทศ 38 ตัวอย่าง ตามวิธีใน U.S.P. XIX ทำโดยการสุ่มตัวอย่างยาจากร้านขายยาต่างๆ ในกรุงเทพมหานครและเขตจังหวัดใกล้เคียง ผลปรากฏว่า ยาจากต่างประเทศทั้ง 38 ตัวอย่างอยู่ในกำหนดมาตรฐาน แต่ยาเตรียมชนิดน้ำจากโรงงานผลิตยาในประเทศพบว่า มีอยู่ 5 ตัวอย่างที่ไม่เข้าตามกำหนดมาตรฐานดังกล่าว เพราะมีเชื้อ *Staphylococcus aureus* ปนอยู่ด้วย และอีก 1 ตัวอย่าง มีจำนวนแบคทีเรียทั้งหมดเกินกว่า 10,000 ตัวต่อมิลลิลิตรของน้ำยา ซึ่งนำมาใช้ทดสอบ.

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## Study on Microorganisms in Modern Liquid Preparations of Cough Mixture

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### Abstract

The test for microbial contamination in cough mixtures comprising 79 samples of local preparations and 38 samples of imported mixtures were performed by the method described in USP XIX. The Samplings were done from the preparations sold in various drug stores in Bangkok and provincial areas. The results showed that all 38 imported mixtures met the standard requirements. Among those preparations from local pharmaceutical plants, there were 5 samples which do not conform to the standard limits because of the contamination with *Staphylococcus aureus*. There was also one sample found to contain total bacterial counts greater than 10,000 per millilitre of the test mixture.

### Introduction

The modern liquid preparations for therapeutic administration are used world wide. The medicines are of several types such as anti-pyretic, anti-diarrheal, anti-histaminic, analgesic and anti-tussive or cough mixture etc. The active ingredients are either solid or liquid in which the vehicle, diluent and/or suspending or flavoring agents are used. The simplest mixture that could be made consisting of a solution or suspension of a solid drug in water, known as aqueous preparation. Many other preparations of drug used to make medicines include syrup, spirit or elixir. These preparations are usually resistant to microbial growth, either because of their ethanol content in spirit or elixir or because of their osmotic pressure due to their sucrose concentration in syrup. However, there is evidence of microbial contamination in various preparations. *Pseudomonas aeruginosa*, *Aspergillus niger*, *Penicillium notatum* and *Candida albicans* were found to grow in various preparations consisting of sugar, glycerol and/or propylene glycol. The esterases production from these organisms will split the ester linkages of surface active molecules, which

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inturn, to adversely affects the physical properties of the products. The presence of a preservative in sweetened aqueous preparation is then essential, unless the mixtures are made with pure syrup, or contain very high concentrations of such esters. Moreover, some suspending or emulsifying agents themselves such as acacia and tragacanth are of carbohydrates in nature which are liable to microbial attack. Methyl cellulose and other various cellulose derivatives used as suspending or emulsifying agent were found to be attacked by microorganisms under certain conditions and formed complexes with a number of common preservatives, notably the parabens. Another factor that must be taken into account is the toxicity of the substance. All useful antimicrobial agents are toxic substances. For maximum protection of the consumer, the concentration of the preservative shown to be effective in the final packaged product should be considerably below the concentrations of the preservative that may be toxic to human beings.

#### **Purpose and scope of investigation**

The purpose and scope of this study is to investigate the microbial contamination of various modern cough mixtures, not only the preparations formulated in this country, but also those (preparations which are imported) from other countries.

The total amount and the presence of various kinds of microorganisms in the preparations will inturn implicate various different conditions where the constituents come from, the storage condition, handling and compounding techniques, as well as (those in) mixing of filling machines and containers. These will help to contribute to the knowledge for controlling and standardization of the production and qualification of the local pharmaceutical preparations in Thailand. It is also a potentially useful measure to improve the standard of the manufacturing plants to meet international standard. However the scope of investigation of this study is confined to only preparations obtained from various drug stores in Bangkok and territorial areas.

#### **Methods and Materials**

##### **1. Samples**

Cough mixtures of various different constituents, diluents, with or without preservative (s) are obtained from various different drug stores in Bangkok and territorial areas. These are of different companies in different patent names from both local pharmaceutical plants and imported materials.

##### **2. Diluent and Buffer Solution**

Purified water and phosphate buffer solution pH 7.2.

##### **3. Media**

3.1 Media used for isolation and identification of *Staphylococcus aureus* and *Pseudomonas aeruginosa* :

- Soybean-Casein Digest Broth

- Vogel-Johnson Agar, Mannitol-Salt Agar, and Baird-Parker Agar Media.
- Cetrimide Agar Medium, Pseudomonas Agar Medium for Detection of Fluorescin and Pseudomonas Agar Medium for Detection fo Pyocyanin.

### 3.2 Media used for isolation and identification of *Escherichia coli* and *Salmonella* Species :

- Fluid Lactose Medium
- Fluid Selenite-Cystine Medium and Fluid Tetrathionate Medium
- Brilliant Green Agar, Xylose-Lysine-Desoxycholate Agar, and Bismuth sulfite Agar Media
- Triple Sugar Iron Agar
- MacConkey Agar and Levine Eosin-Methylene blue Agar Media

## 4. Procedure

The investigation of total amount, isolation, and identification of microorganism were carried out *in vitro* by plate methods described in the Microbial Limit Tests of the Pharmacopeia of the United States. The preparatory testing and total viable counts of both pathogenic and non-pathogenic microorganisms were performed.

### 4.1 Preparatory Testing

#### 4.1.1 Test for Absence of Inhibitory Property.

Separately dilute 10 ml of each sample in 90 ml of Fluid Soybean-Casein Digest Medium and Fluid Lactose Medium to make two  $10^{-1}$  dilutions of a sample in two different media. Use these two dilutions of each sample for one test cultures. The separate viable broth cultures used are of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella* species. Add into the dilutions of each sample, 1 ml of not less than  $10^{-3}$  dilution in phosphate buffer pH 7.2 of a 24-hours broth culture of each organism as mentioned, then follow the test procedure.

The test samples to which they are applied must not, of themselves, inhibit the multiplication, under the test conditions, of microorganisms that may be present. However, if they appear to alter the number of microorganisms originally present, the procedure in 4.1.2 of this section must be carried out.

#### 4.1.2 Treatment of Inhibitory Property

Failure of the organism (s) to grow in the relevant medium invalidates that portion of the examination. Therefore, it is necessary to treat the test sample before application by either one of these three procedures:

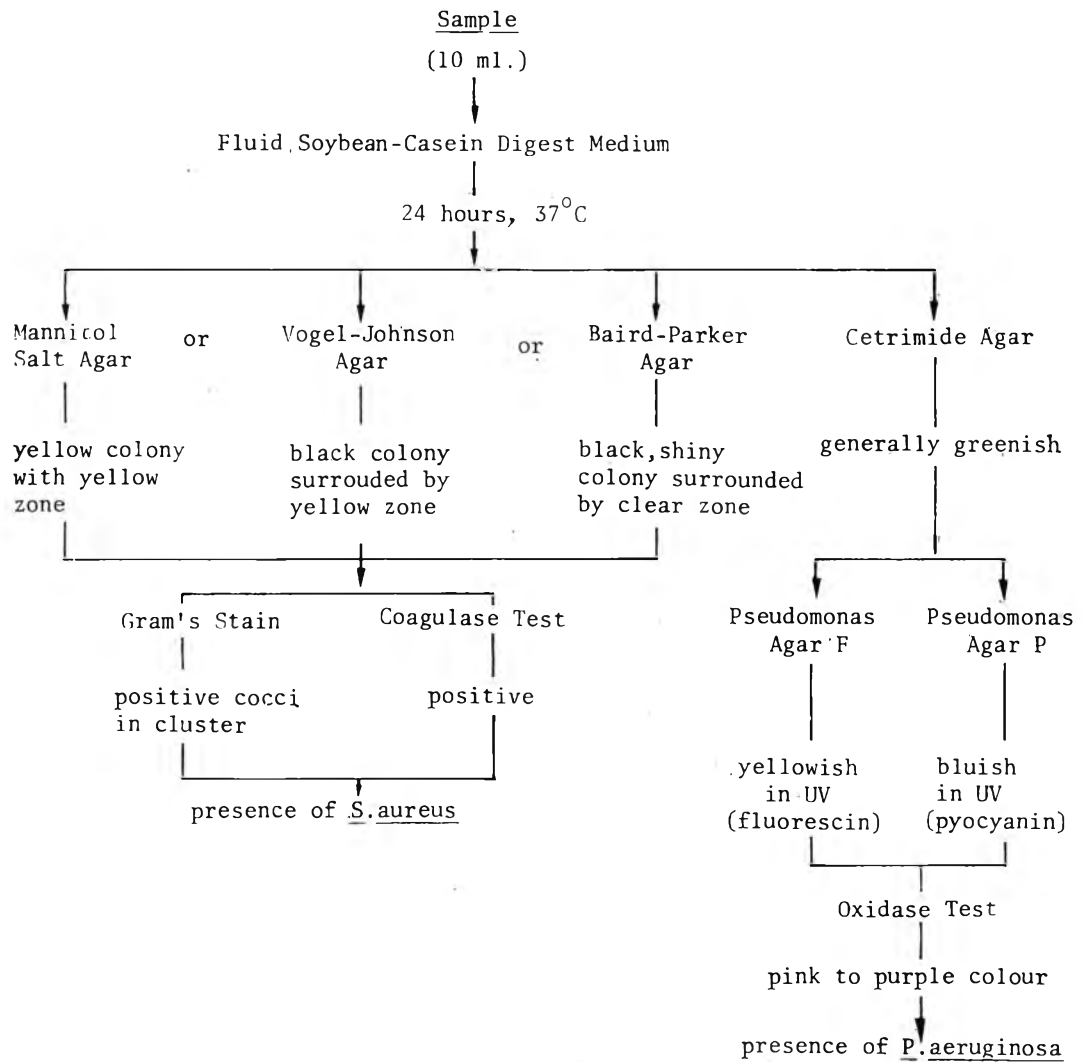
- (1) By increasing the volume of diluent, while the quantity of test material remaining the same, or
- (2) by the incorporation of a sufficient quantity of suitable inactivating agent(s), e.g. 0.5 % of soy lecithin or 4.0 % of polysorbate 20, in the diluents, or
- (3) by an appropriate combination of modifications (1) and (2), so as to permit growth of inocula.

#### 4.2 Total Aerobic Microbial Count by plate Method

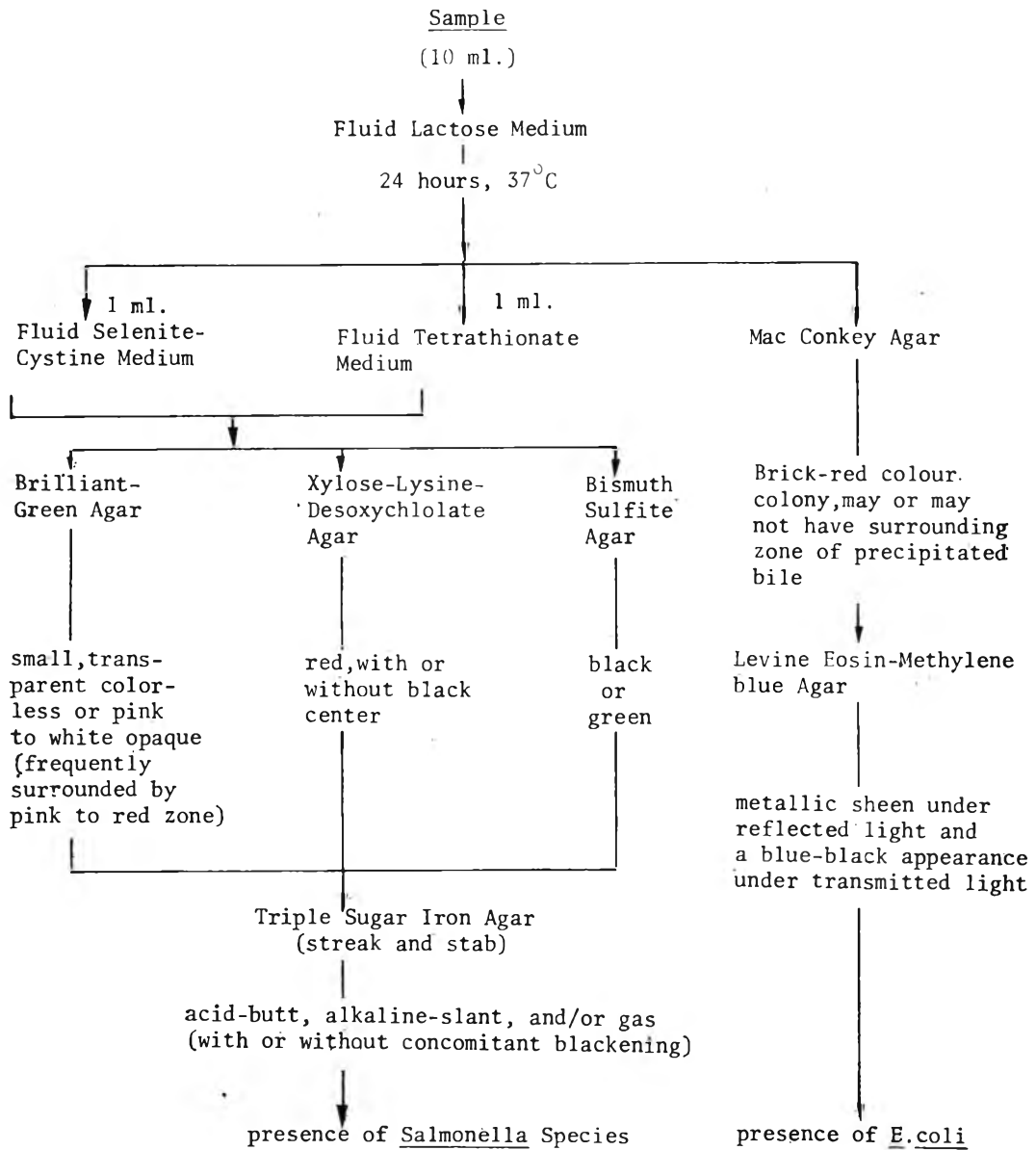
Pipet 1 ml of  $10^{-1}$  dilution of each sample onto each of two sterile Petri dishes. Promptly add to each dish 20 ml of Soybean-Casein Digest Agar Medium that previously has been melted and cooled to approximately  $45^{\circ}\text{C}$ . Cover the Petri dishes, mix the sample with the agar by tilting or rotating the dishes, and allow the contents to solidify at room temperature. Invert the Petri dishes, and incubate for 48 to 72 hours. Following incubation, examine the plates for growth, count the number of colonies, and express the average for the two plates in terms of the number of microorganisms per ml of sample.

### 4.3 Stepwise Diagram for Isolation and Identification Methods

#### 4.3.1 Isolation and Identification of *Staphylococcus aureus* and *Pseudomonas aeruginosa*



4.3.2 Isolation and Identification of *Salmonella* Species and *Escherichia coli*





## Result

The results obtained from 79 samples of local preparation and 38 samples of imported preparation of cough mixture are shown in the following tables.

**Table 1** Number of samples showing bacterial counts per ml

Range of number of bacteria (count/ml)	Number of sample	
	Local preparation	Imported preparation
0-9	23	8
10-49	37	15
50-99	9	8
100-199	8	5
200-499	1	2
500-10,000	0	0
> 10,000	1	0
Total	79	38

**Table 2** Number of samples showing kind (s) of microorganism present

kind of Microorganism	Number of sample	
	Local preparation	Imported preparation
<i>S. aureus</i>	5	0
<i>Salmonella</i> spp.	0	0
<i>P. aeruginosa</i>	0	0
<i>E. Coli</i>	0	0
Aerobic spore forming bacilli	10	6
Total number of tested sample	79	38

## Discussion

From the results, Table 1 showed that the majority of both local and imported preparation have bacterial counts per ml in between 0-49 counts and few in 50-500 counts. There is non greater than 500 counts, except one local pharmaceutical mixture. Among 79 tested samples from local products, it seems to be accidental that only one was found to contain bacterial counts per ml greater than 10,000 while none was found in the range of 500 to 100,000 counts.

It should also be noted that the bacterial contaminants among those positive samples of both imported and of local preparations were shown to be the aerobic spore-forming bacilli. Six out of 38 samples (15.8%) taken from imported products while 10 out of 79 samples (12.7%) from the local preparations were isolated. In our opinion, the presence of the spore-forming bacilli, may adversely affected physical and/or chemical properties of the products and do harm to the consumers. They may also, in optimum conditions, germinate to be vegetative form and increase the number of counts of the contaminants.

No pathogenic bacteria was found in 38 imported preparations, while 5 out of 79 samples from local products were found to be contaminated with *Staphylococcus aureus* (Table 2). The presence of such organism in the preparations may indicate sub-standard conditions of the pharmaceutical plants.

Except the presence of such pathogenic contaminant as mentioned, and the one sample from the local pharmaceutical plant in which the bacterial counts per ml was found to be greater than 10,000, the distribution of bacterial counts showed no statistical significant difference in cough mixtures obtained from both local and imported products.

### Conclusion

In conclusion, the result indicate that some of the cough mixtures from local pharmaceutical plants are not safe for the consumer. Some preparations were found to contain pathogenic microorganism. These preparations must be controlled to ensure that no pathogenic organism is present in all preparations, and to conform to the Microbial Limit Tests of the Pharmacopeia of the United States of America. For the conformity to the tests of the stated Pharmacopeia, the absence of the following microorganisms are limited to *Pseudomonas aeruginosa*, *Salmonella* spp., *Escherichia coli*, and *Staphylococcus aureus*.

Eventhough, this work has been done in limited time, budget, and small number of samples of cough mixtures as mentioned, it appears to necessitate, to some extent, more stringent control of manufacturing pharmaceutical plants.

The tests for fungal contaminants and the identification of the aerobic spore-forming bacilli, whether or not they are pathogenic, as well as increasing the number of sample are recommended for more details and accurate results.

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