P5.2 Analytical Procedures

The analytical procedures of **VIVERE CREAM** complied with in-house methods and BP2022, Aciclovir Cream as attached in the following pages.

Specification and Control Method of Product

1. **Appearance:** Test by visual inspection, the appearance must conform **"**A smooth odourless white cream".

2. **Identification:** The retention time of the major peak of the Sample solution corresponds to that of the Standard solution, as obtained in the Assay.

3. **Assay: Acyclovir (5 g/100 g) by LC method**

Mobile phase: 0.02 M acetic acid (0.02M GAA = 1.15 ml of GAA + Water to 1000 mL)

System suitability solution A: Dissolve accurately weighed quantities of standard Acyclovir and guanine in 0.1 N NaOH, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution having a known concentration of about 0.1 mg of each per ml, as the instruction below.

(Acyclovir 20 mg + Guanine 20 mg + 20 ml 0.1 N NaOH --- H_2O --- > 200 mL)

System suitability solution B: Dissolve accurately weighed quantity of guanine in 0.1 N NaOH, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution having a known concentration of about 0.002 mg of each per ml, as the instruction below.

(Guanine 20 mg --- 0.1 N NaOH --- >100 ml, 2 ml --- H_2O --- > 200 mL)

Standard preparation: Dissolve accurately weighed quantities of standard Acyclovir in 0.1 N NaOH, and dilute, quantitatively and stepwise if necessary, with water to obtain a solution having a known concentration of about 0.1 mg per ml, as the instruction below.

(Acyclovir 100 mg --- 0.1N NaOH --- > 100 ml, 5 ml --- H_2O --- > 50 ml)

Sample solution: Nominally 0.1 mg/mL of acyclovir prepared as follows. Weigh, accurately, equivalent about 10 mg of Acyclovir (about 0.2 g of Vilerm cream) and transfer into a 100 ml volumetric flask, add about 10 ml of 0.1N NaOH, stir for about 15 minutes, adjust with water to volume, and mix. Adjust dilution if necessary.

(~0.2 g Vilerm cream+ 10 ml 0.1 N NaOH ---H_2O---> 100 ml)

Chromatographic system:

Mode: HPLC

Detector: UV 254 nm

Column: 4.6 x 250 mm, 5 µm packing L1

Column temperature: 40°C

Flow rate: 1.5 ml/minute (The flow rate could be adjusted between the ranges 1.50-4.50 ml/minute to achieve the suitability of the system.)

Injection volume: 20 µL

System suitability:

Samples: System suitability solution A and System suitability solution B

[Note– The relative retention time for guanine and Acyclovir are 0.6 and 1, respectively.in System suitability solution 1]

System suitability requirements:

Resolution: NLT 2.0 between guanine and acyclovir, System suitability solution A

Relative standard deviation: NMT 2.0% for the acyclovir peak, System suitability solution A

NMT 2.0%, System suitability solution B

Procedure: Separately inject equal volume of all preparations into the chromatograph, record the chromatograms and measure the responses for major peaks. Calculated the %LA of Acyclovir in Vilerm cream by the formula:

$$\%LA = \frac{Au}{As} \times \frac{Ws}{100} \times \frac{5}{50} \times \frac{100}{Wu} \times \frac{100}{5} \times \frac{\%Std(\text{on as is basis})}{100} \times 100$$

Where

A_U = peak area of Acyclovir obtained from the sample solution

A_S = average peak area of Acyclovir obtained from the standard solution

W_S = the weight in mg of standard Acyclovir use in standard solution

W_U = the weight in mg of sample

Limit: 95.0 – 105.0 %LA of Acyclovir

4. Minimum fill

Procedure: Sampling 10 units of sample, weigh individually accurately (W1). Remove all content from each unit, weigh the empty containers individually (W2). Calculate the weight of content of each unit (W1-W2). The average weight of content of 10 units is not less than the labeled weight, and the content of any unit is not less than 90% of the labeled weight. If this requirement is not met, determine the content of 20 additional units. The average content of the 30 units is not less than the labeled weight, and the net content of not more than 1 of the 30 containers is less than 90% of the labeled amount.

5. pH: Measure directly by pH meter, the result must between the pH ranges of 5.0-7.0

Specification and Control Method of Product

6. Benzyl alcohol content (1 g/100 g) by LC method

Buffer solution: Dissolve 13.6086 g of KH_2PO_4 in 1000 mL of water, adjust pH of this solution to 2.5 with Phosphoric acid.

Standard solution: Accurately weighed about 50 mg of Benzyl alcohol standard into a 200-mL volumetric flask, dissolve and dilute to volume with water and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dissolve and dilute to volume with water and mix, to obtain a solution having a known concentration of about 25 µg/mL of Benzyl alcohol.

(Standard 50 mg ---Water-----> 200 mL, 10 mL ---Water-----> 100 mL) (25 µg/mL)

Sample solution: To a weighed quantity about 0.25 g of Vilerm cream (equivalent to 2.5 mg of Benzyl alcohol) to a 100-mL volumetric flask, add 70% of volume with water and stir for 10 min and dilute to volume with water and mix.

(Sample: Vilerm cream 0.25 g (eq.to 2.5 mg benzyl alc.) ---Water-----> 100 mL) (25 µg/mL)

Mobile phase: Gradient, See table 1

Table 1

Time (minutes)	Buffer solution (%v/v)	Acetonitrile (%v/v)
0	77	23
15	77	23
16	70	30
20	70	30
21	77	23
30	77	23

Chromatographic system:

Mode: Liquid chromatograph (LC)

Detector: UV 210 nm

Column: 4.6 × 250-mm; 5.0-µm, packing L7, or equivalent is suitable

Column temperature: 35 °C

Sample temperature: 20 °C

Flow rate: 1.0 mL/min

Injection volume: 20 µL

System suitability:

Relative standard deviation (5 replicate injections): NMT 2.0%

Analysis: Calculate the percentage of Benzyl alcohol in the portion of Cream taken:

$$\%LA. = \frac{A_U}{A_S} \times \frac{W_S}{200} \times \frac{10}{100} \times \frac{100}{W_U} \times \frac{100}{1} \times \frac{\%\text{Potency std(on as is basis)}}{100} \times 100$$

Where

A_U = peak response from the Sample solution

A_S = average peak response from the Standard solution

W_S = Weight in mg of Benzyl alcohol standard in Standard solution

W_U = Weight in mg of Vilerm cream in Sample solution

Limit: 80.0 – 120.0%LA of Benzyl alcohol

7. Related substances by LC method

Solution A: 1 volume of dimethyl sulfoxide and 4 volumes of water.

Phosphate buffer solution pH 3.1: Dissolve 3.84 g of dipotassium hydrogen orthophosphate in 1000 mL of water and adjust to pH 3.1 with orthophosphoric acid.

Phosphate buffer solution pH 2.5: Dissolve 3.84 g of dipotassium hydrogen orthophosphate in 1000 mL of water and adjust to pH 2.5 with orthophosphoric acid.

Mobile phase A: 1 volume of acetonitrile and 99 volumes of phosphate buffer solution pH 3.1.

Mobile phase B: 50 volumes of acetonitrile and 50 volumes of phosphate buffer solution pH 2.5.

Mobile phase: See table1

Table 1

Time (min)	Mobile phase A (%v/v)	Mobile phase B (%v/v)	Comment
0-5	100	0	Isocratic
5-27	100 ---> 80	0 ---> 20	Linear gradient
27-40	80	20	Isocratic
40-46	80 ---> 100	20 ---> 0	Linear gradient

Specification and Control Method of Product

(1) Test solution: Mix with the aid of ultrasound a quantity of the well-mixed cream containing 25 mg of Acyclovir in 10 mL of dimethyl sulfoxide dilute to 25 mL with solution A and filter through a 0.2-µm nylon filter.

(Vilerm cream 0.5 g + dimethyl sulfoxide 10 mL ----solution A----> 25 mL)

(2) Dilute test solution: Dilute 1 volume of solution (1) to 100 volumes with solution A and dilute 1 volume of this solution to 5 volumes with solution A.

(1 mL of solution (1) ---solution A----> 100 mL, 1 mL ----solution A ----> 5 mL)

(3) System suitability solution A: Dissolve 5 mg of acyclovir for system suitability A EPCRS in 1 mL of dimethyl sulfoxide and dilute to 5 mL with water.

(Acyclovir for system suitability A EPCRS 5 mg + dimethyl sulfoxide 1 mL ----water---> 5 mL)

(4) System suitability solution C: Dissolve the contents of a vial of acyclovir for impurity C identification EPCRS in 200 µL of dimethyl sulfoxide and dilute to 1 mL with water. Prepare the solution immediately before use.

(5) System suitability solution G: Dissolve the contents of a vial of acyclovir for impurity G identification EPCRS in 1 mL of solution (3)

Chromatographic system:

Mode: Liquid chromatograph (LC)

Detector: UV 254 nm

Column: 4.6 × 250-mm; 5.0-µm, packing L1 (Octadecylsilys silica gel for chromatography) Supelcosil LC-18-DB or equivalent is suitable

Column temperature: use an ambient temperature

Flow rate: 1.0 mL/min

Injection volume: 10 µL

System suitability:

Resolution: NLT 1.5 between the peak due to impurity C and acyclovir.

NLT 1.5 between the peaks due to impurity K and impurity G.

Analysis: Calculate the percentage of related substance in the portion of Cream taken:

$$\%. = \frac{R_U}{R_S} \times \frac{1}{100} \times \frac{1}{5} \times 100$$

Where

R_U = peak response from the Test solution (1)

R_S = peak response from the dilute test solution (2)

Specification and Control Method of Product

Limit:

In the chromatogram obtained with Solution (1):

- The area of any peak corresponding to impurity B is not greater than 5 times the area of the principal peak in the chromatogram obtained with solution (2) (1.0%)
- The area of any other secondary peak is not greater than the area of the principal peak in the chromatogram obtained with solution (2) (0.2%)
- The sum of the areas of any secondary peaks is not greater than 10 times the area of the principal peak in the chromatogram obtained with solution (2) (2.0%)
- Disregard any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with solution (2) (0.1%)

  [Note : Identify any peak in solution (1) corresponding to impurity C using the chromatogram obtained with solution (4) and multiply the area of this peak by a correction factor of 2.2]

Vilerm Cream

Analytical Method for Microbial limit Test

Method : USP42 ,Chapter <61> ,<62>

Limit for Microbial contamination

Total aerobic microbial count (TAMC)

: NMT 10^2 cfu /g (maximum acceptable count = 200)

Total combined yeasts and molds count (TYMC)

: NMT 10^1 cfu /g (maximum acceptable count = 20)

Absence of *Staphylococcus aureus* per g

Absence of *Pseudomonas aeruginosa* per g

Test for Total aerobic microbial count (TAMC) and **Total combined yeasts and molds count (TYMC)**

Enumeration method : Plate Count method

Sample preparation : Transfer 10 g of sample to 90 ml diluent = Dilution 1 in 10

Diluents : Casein Peptone Lecithin Polysorbate Broth

Media for TAMC : Tryptic Soy Agar

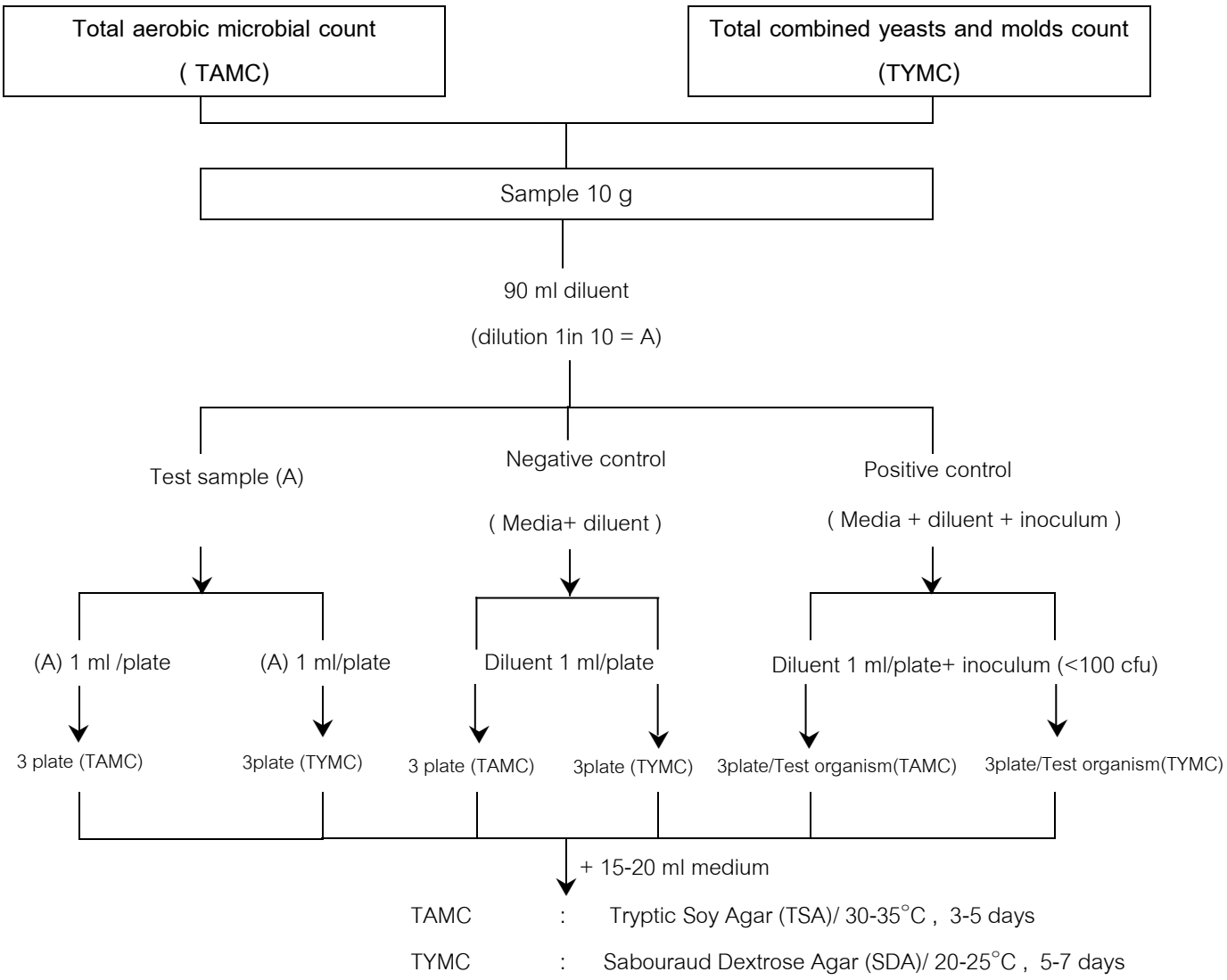
Media for TYMC : Sabouraud Dextrose Agar

Test for *Staphylococcus aureus & Pseudomonas aeruginosa*

Sample preparation : Transfer 10 ml from Dilution 1 in 10 (eq.to sample 1 g) to 100 ml TSB
= Dilution 1:100

Vilerm Cream

Part I : Microbial Enumeration Tests <61> Pour Plate Method



TAMC : Tryptic Soy Agar (TSA)/ 30-35°C , 3-5 days

TYMC : Sabouraud Dextrose Agar (SDA)/ 20-25°C , 5-7 days

Test sample: Select the plates corresponding to a dilution that showing the highest number of colonies less than 250 for TAMC and 50 for TYMC .Take the arithmetic mean per culture medium of the counts , and calculate the number of cfu per g.

Criteria of Negative Control : No growth from all plates

Criteria Positive control : Growth obtained must not less than 70% from the calculated value of inoculum

Note : Test strains of inoculum for Positive control are

S. aureus ATCC6538

Ps. Aeruginosa ATCC9027

B. subtilis ATCC6633

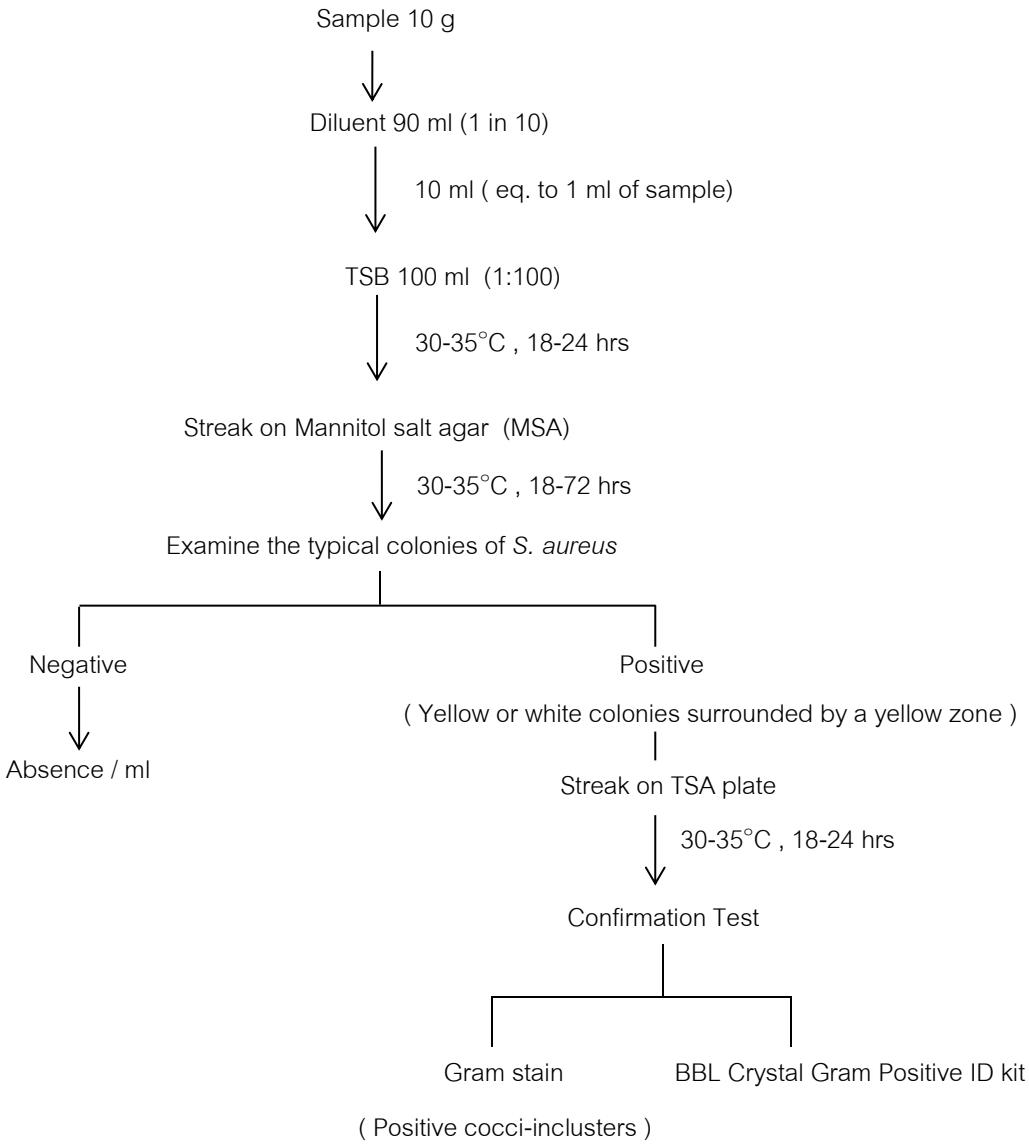
C. albicans ATCC10231

A. brasilliensis ATCC16404

Vilerm Cream

Part II <62> Test for specified microorganisms

Detection of *Staphylococcus aureus*

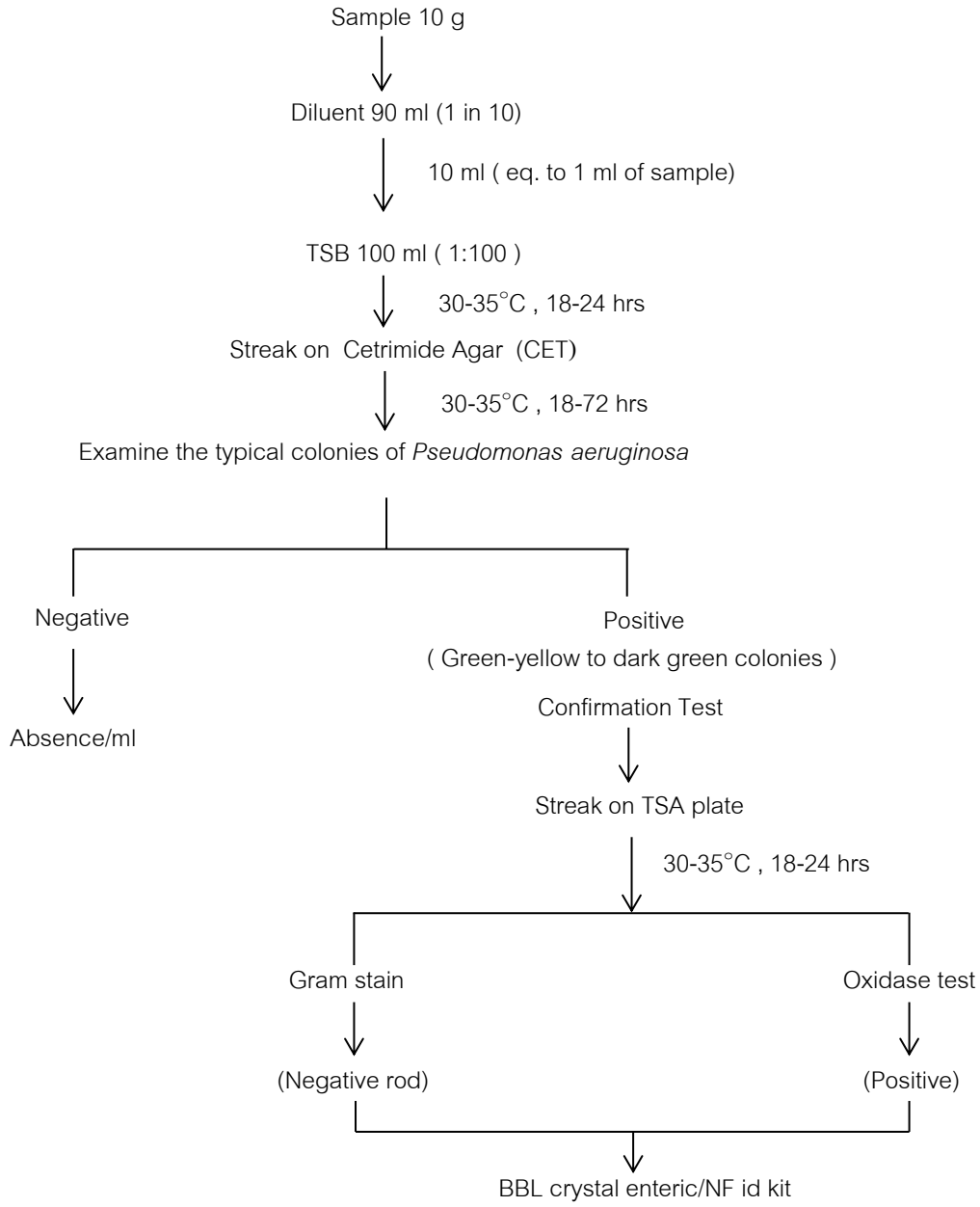


The product complies with the test if colonies are not present or if confirmatory identification tests are negative

Vilerm Cream

Part II <62> Test for specified microorganisms

Detection of *Pseudomonas aeruginosa*



The product complies with the test if colonies are not present or if confirmatory identification tests are negative