in this example), and elongation at 72°C for 2 minutes. Add drops of mineral oil or suitable equivalent as needed during the reaction to prevent evaporation.

**5. Agarose gel electrophoresis**

1) Mix 10 μL of each of the first stage and second stage PCR products with 2 μL of an appropriate dye as a migration marker, and perform 1% agarose gel electrophoresis.

2) Stain the gel with ethidium bromide and take a photograph under UV irradiation.

3) The test is judged to be positive if a DNA band is detected.

**[An Example of Primer]**

For mycoplasma detection

Outer primer

F1:5′-ACACCATGGGAG(C/T)TGGTAAT-3′

R1:5′-CTTC(A/T)TCGACTT(C/T)CAGACCCAAGG-CAT-3′

Inner primer

F2:5′-GTG(G/C)GG(A/C)TGGATCACCTCCT-3′

R2:5′-GCATCCACCA(A/T)A(A/T)AC(C/T)CTT-3′

( ) indicates a mixture.

**[PCR reaction solution]**

| | [First stage] | [Second stage] |
|---|---|---|
| dNTP solution (each 1.25 mol) | 16 μL | 16 μL |
| Primer (10 pmol/μL) | F1 2 μL | F2 2 μL |
| Primer (10 pmol/μL) | R1 2 μL | R2 2 μL |
| Heat-resistant DNA polymerase (1 U/μL) | 2 μL | 2 μL |
| Reaction buffer solution | 68 μL | 77 μL |
| 25 mmol/L magnesium chloride hexahydrate | 8 μL | 8 μL |
| 10-fold buffer solution* | 10 μL | 10 μL |
| Sterile distilled water | 50 μL | 59 μL |

*Composition of 10-fold buffer solution

| | |
|---|---|
| 2-amino-2-hydroxymethyl-1,3-propanediol-hydrochloric acid (pH 8.4) | 100 mmol/L |
| Potassium chloride | 500 mmol/L |
| Magnesium chloride hexahydrate | 20 mmol/L |
| Gelatin | 0.1 g/L |

**[Method of cultivating mycoplasma within Vero cells]**

1) Use at least two cell culture dishes for each of the test sample, positive control and negative control.

2) Into each cell culture dish (diameter 35 mm), inoculate 2 mL of the Vero cell suspension ($1 \times 10^4$ cells per 1 mL) in Eagle's minimum essential medium containing 10 percent bovine calf serum (tested in advance using the PCR method to verify that it does not contain any detectable mycoplasma DNA). Incubate the cultures at 36 ± 1°C in an atmosphere of air containing 5 percent carbon dioxide for one day.

3) Replace the culture media with fresh media, and add 0.5 mL of the test sample (cell culture supernatant) to each of two or more Vero cell culture dishes. Perform the same procedure for the positive (such as 100 CFU or less *M. hyorhinis*) and negative controls.

4) Incubate the Vero cell culture dishes for the test sample, positive and negative controls for 3 to 6 days at 36 ± 1°C in an atmosphere of air containing 5 percent carbon dioxide.

# 10. pH Test for Gastrointestinal Medicine

In this test, medicine for the stomach and bowels, which is said to control stomach acid, is stirred in a fixed amount of the 0.1 mol/L hydrochloric acid for a fixed duration, and the pH value of this solution is obtained. The pH value of a stomach medicine will be based on the dose and the dosage of the medicine (when the dosage varies, a minimum dosage is used) and expressed in the pH value obtained from the test performed by the following procedure.

**Preparation of Sample**

Solid medicine which conforms to the general regulations for medicine (the powdered medicine section) can be used as a sample. When the medicine is in separate packages, the content of 20 or more packages is accurately weighed to calculate the average mass for one dose and mixed evenly to make a sample. For granules and similar types in separate packages, among the solid medicine which does not conform to the general regulations for medicine (the powdered medicine section), the content of 20 or more packages is accurately weighed to calculate the average mass for one dose and is then powdered to make sample. For granules and similar types not in separate packages, among solid medicine which does not conform to the general regulations for medicine (the powdered medicine section), 20 doses or more are powdered to make a sample. For capsules and tablets, 20 doses or more are weighed accurately to calculate the average mass for one dose or average mass and then powdered to make a sample.

Liquid medicine is generously mixed to make a sample.

**Procedure**

Put 50 mL of the 0.1 mol/L hydrochloric acid with the molarity coefficient adjusted to 1.000, or equivalent 0.1 mol/L hydrochloric acid with its volume accurately measured in a 100-mL beaker. Stir this solution with a magnetic stirrer and a magnetic stirrer rotator (35 mm length, 8 mm diameter) at the speed of about 300 revolutions per minute. While stirring, add the accurately weighed one-dose sample. After 10 minutes, measure the pH value of the solution using the pH Determination. The solution temperature should be maintained at 37 ± 2°C throughout this operation.

# 11. Plastic Containers for Pharmaceutical Products

Various kinds of plastics are used in the manufacture of containers for pharmaceutical products. Such plastics should not alter the efficacy, safety or stability of the pharmaceutical products. In selecting a suitable plastic container, it is desirable to have full information on the manufacturing processes of the plastic container including the substances added. Since each plastic has specific properties and a wide variety of pharmaceutical products may be stored in containers made from it, the compatibility of plastic containers with pharmaceutical products should be judged for each combination of container and the specific pharmaceutical product to be contained therein. This judgement should be