medicines and medical devices. These are methods used (a) to determine the potency or activity of antimicrobial chemicals, e.g. antibiotics, preservatives and disinfectants, and (b) as part of the microbiological quality control of manufactured sterile and non-sterile products. The chapter describes the experimental procedures that are unique or particularly relevant to pharmacy, rather than those that are common to microbiology as a whole. In the latter category, for example, are procedures used to identify and enumerate microorganisms. These, together with staining and microscopical techniques, are described in Chapter 13.

Several of the methods and tests discussed here are the subject of monographs or appendices in pharmacopoeias or they are described in national and international standards or other recognized reference works. It is not the intention to reproduce these official testing procedures in detail but rather to explain the principles of the tests, to draw attention to difficult or important aspects, and to indicate the advantages, problems or shortcomings of the various methods.

Measurement of antimicrobial activity

In most of the methods used to assess the activity of antimicrobial chemicals, an inoculum of the test organism is added to a solution of the chemical under test, samples are removed over a period of time, the chemical is inactivated and the proportion of surviving cells determined. Alternatively, culture medium is present together with the chemical and the degree of inhibition of growth of the test organism is measured. In each case it is necessary to standardize and control such factors as the concentration of the test organism, its origin, i.e. the species and strain employed, together with the culture medium in which it was grown, the phase of growth from which the cells were taken, and the temperature and time of incubation of the cells after exposure to the chemical. Because such considerations are common to several of the procedures described here, e.g. antibiotic assays, preservative efficacy (challenge) tests and determinations of minimum inhibitory concentration (MIC), it is appropriate that they should be considered first, both to emphasize their importance and to avoid repetition.

Factors to be controlled in the measurement of antimicrobial activity

Origin of the test organism

Although two cultures may bear the same generic and specific name, i.e. they may both be called *Escherichia coli*, this does not mean that they are identical. Certainly, they would normally be similar in many respects, e.g. morphology (appearance), cultural requirements and biochemical characteristics, but they may exhibit slight variations in some of these properties; such variants are described as strains of *E. coli*. A variety of strains of a single species may normally be obtained from a culture collection, e.g. the National Collection of Industrial and Marine Bacteria or the National Collection of Type Cultures. Different strains may also occur in hospital pathology laboratories by isolation from swabs taken from infected patients or by isolation from contaminated food, cosmetic or pharmaceutical products, and from many other sources. Strains obtained in these ways are likely to exhibit variations in resistance to antimicrobial chemicals. Strains from human or animal infections are frequently more resistant to antimicrobial chemicals, particularly antibiotics, than those from other sources. Similarly, strains derived from contaminated medicines may be more resistant to preservative chemicals than those obtained from culture collections. Therefore, in order to achieve results that are reproducible by a variety of laboratories, it is necessary to specify the strain of the organism used for the determination.

It is becoming increasingly common, too, for official testing methods to limit the number of times the culture collection specimen may be re-grown in fresh medium (called the number of subcultures or passages) before it must be replaced. This is because the characteristics of the organism (including its resistance to antimicrobial chemicals) may progressively change as a result of mutation and natural selection through the many generations that might arise during months or years of laboratory cultivation.

Composition and pH of the culture medium

There are several methods of assessing antimicrobial activity which all have in common the measurement