Official Microbiological Assays

Official methods such as those of AOAC are important; they are used by regulatory analysts and generally have preferred status in courts. They are considered to be reliable enough to be used by those too inexperienced to judge the reliability of a method. They are expected to give reliable and precise results. Thus, AOAC methods should represent the state of the art in high quality assaying. Advances in physical and chemical methods are incorporated into official methods reasonably soon after their reliability has been established. The situation with microbiological assays for antibiotics and vitamins is quite different; official methods are essentially those available in 1945.

All antibiotic assays in the 12th edition of "Official Methods of Analysis of AOAC," as in the earlier editions, are by diffusion methods in petri dishes. Current practice in the United States is to assay as many antibiotics as possible by nonofficial turbidimetric methods, preferably automated ones. These methods are inherently more precise than diffusion methods, and even manual ones require much less work to obtain a more precise answer. However, the legally important sample must be assayed by the less desirable official methods. Does the analytical microbiologist, unlike the analytical chemist, need two distinct qualities of assays depending upon the legal importance of the sample? Should not official methods also be suitable for precise routine assays?

Official methods, in theory, specify exactly those operations essential to proper assaying, deviation from which can be claimed to reduce accuracy. If the unstated assumptions about operations are not examined too closely and if fairly wide limits are put on the answers, the methods seem dependable. However, in reality, the microbiological assays are of low accuracy and precision and are obsolete in concept and design. Lack of adequate specification of details of critical operations is one cause of poor interlaboratory agreement. Too many of the details given are not important to success, whereas those likely to cause significant problems are not mentioned. Among the latter are: dilution procedures and equipment; form and extent of calibration lines; prediffusion time and time schedule for heating to incubation temperature in diffusion assays; qualitative and quantitative identity of composition of standards and samples in many antibiotic assays; standards and samples in different menstrua; inadequate extracting solvent; excessive manipulation of samples; inaccurate measurement of volumes during sample preparation; exposure of photolabile substances to strong light; use of decayed standards; and the idea that the methods being biological are inherently inaccurate and that, consequently, care need not be exercised. The last may be the most damaging to high quality assaying.

The inherent accuracy of microbiological assays can be achieved only after the listed factors are recognized as important and are properly controlled. When these changes are made, as they can be with little cost and effort, microbiological assays can be as accurate, precise, and reliable as many chemical ones. Until these changes are made, "Methods" will be of more value to lawyers engaged in adversary proceedings than to analysts needing good methods of general applicability.

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