benzein indicator and titrate with standard 0.1 *N* perchloric acid to a green end-point. Perform a blank determination and make any necessary corrections.

Recoveries were determined by first determining the purity of the crystalline salt by titration in triplicate with 0.1 *N* acetous perchloric acid, adding mercurous acetate prior to titration for the hydrochloride salts, and using *p*-naphtholbenzein indicator simultaneously with potentiometric determination of the end-point. An aqueous solution of the amine salt was then prepared at the same concentration as a commercial product and assayed in triplicate by the described method, and the recovery was determined. A commercial product was then assayed by the method; recovery in terms of percent of label claim was determined, both colorimetrically and simultaneously potentiometrically to show the effect of possible interferences with the indicator.

RESULTS

Results are summarized in Tables I and II.

DISCUSSION

Agreement between assays using potentiometric and indicator end-points is good, and all recoveries from a simple aqueous solution of the salt are good. The close agreement between potentiometric and indicator end-points for the pure compounds shows that *p*-naphtholbenzein is a proper indicator for the titration.

The results show the general utility of the method for assay of simple aqueous solutions and compounded injections of amine salts that meet the following criteria: (*a*) concentration of 20 mg./ml. or more, (*b*) amine base readily extractable into chloroform, and (*c*) amine base stable in chloroform.

Preliminary experiments showed that a large aqueous sample necessitated by low concentration led to erratic recoveries and that attempts with smaller samples using 0.01 *N* perchloric acid as the titrant also failed to yield reliably reproducible results.

Attention is called to the rapidity of the method, the low volume of extracting solvent used, and the complete freedom from emulsion formation as compared with methods involving solvent extraction.

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Generous donations of samples of official injections were received from the pharmaceutical manufacturers cited in the tables.

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TECHNICAL ARTICLES

Automated Turbidimetric Microbiological Assay Readout System▲

ROBERT A. RIPPERE and BERNARD ARRET

Abstract □ A new automated turbidimetric assay readout system utilizing commercially available off-the-shelf equipment is described. This system is capable of obtaining the absorbance values of over 400 individual tubes virtually unattended and is being used routinely in the certification of antibiotics with greater precision and speed than was previously attainable. Because the system involves no change in basic methodology from the current official procedure for antibiotic assay, no change in the "Code of Federal Regulations" is required.

Keyphrases □ Automated analysis—turbidimetric microbiological assay □ Microbiological assay—automated turbidimetric readout system □ Turbidimetric microbiological assay—automated readout system □ Antibiotics—automated bioassay readout

During the last several years the pharmaceutical industry has expended large amounts of time, energy, and resources in efforts to automate many of the more repetitive, time-consuming, and tedious aspects of microbiological assays of antibiotics and vitamins. Kuzel and Roudebush (1) described their experiences with several combinations of spectrophotometers and flow cells. Kuzel and Coffey (2) described a unique cell positioner which permitted two flow cells to be monitored by one spectrophotometer without large variations of blank readings due to changes of cell position in the spectrophotometer. Other investigators (3) used three matched flow cells to determine the transmittance readings of three-dose turbidimetric assays of vitamins, with one dose level always being read from the same flow cell to minimize carryover. This system employed a digital converter and a computer card punch on the output end.

Burns and Hansen (4) devised a system which accepted whole fermentation beers, filtered and diluted them, mixed the diluted sample with medium and inoculum, and dispensed it into a continuous chain of 2-ml. cups inside a 37° incubator. The chain was long enough so that when the desired period of incubation was com-

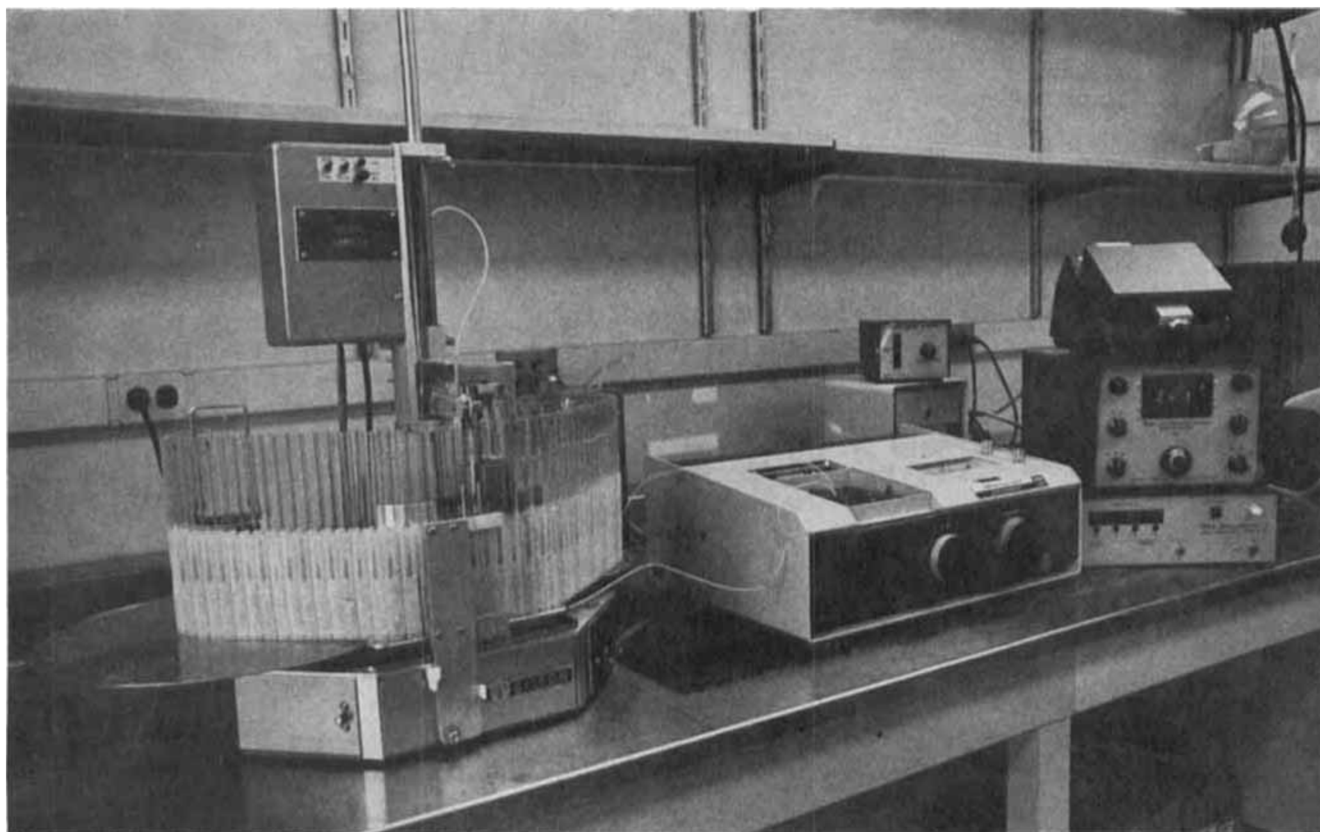

Figure 1—*The automated readout system showing (left to right) the sample changer and pipeter, the spectrophotometer and power supply, the sample identification repeater, and (from the bottom) the digital converter, concentration readout, and printout. The syringe pump is nearly hidden by the uncoiling spool of tubes. The pipeting probe is down in a sample tube, the contents of which are being withdrawn through the flow cell of the spectrophotometer.*

pleted, the cup was in position to be automatically sampled and read in a colorimeter connected on-line to a small computer. Kuzel *et al.* (5) published an exhaustive review of the developments in automated pharmaceutical analyses prior to August 1968.

One manufacturer has recently marketed a complete system (6, 7) for automated inoculation and readout of turbidimetric microbiological assays. This system requires an antibiotic sample to be manually dissolved and diluted to a concentration approximately 100 times the desired level in the assay tube. The sample then is withdrawn from a test tube by a sample probe and is automatically diluted to two different concentrations, with inoculated broth as the diluent, and is dispensed into four tubes in a rack. The rack is then manually placed in a water bath. After suitable incubation, the bacterial cultures are killed and the rack is returned to the machine, where a sample probe dips into the culture tube, removes an aliquot, passes the material through the flow cell of a spectrophotometer, and, at a specified point in the cycle, commands a recorder to print the spectrophotometer reading.

Because of the wide array of both antibiotic powders and dosage forms assayed in this laboratory on a daily basis, it was decided that the first procedures considered for automation should be the most repetitive and tedious. While those aspects of the assay procedure were being automated, any ability of the equipment under consideration to perform some additional functions currently being done either manually or by other machines would be an added advantage.

A system has been developed and used satisfactorily for all of the turbidimetric microbiological assays for more than a year. On a typical day the absorbance values of 800–1000 tubes are read in this laboratory. As many as 14 different antibiotics are assayed. The sample solutions are prepared by from two to six analysts with varying degrees of experience and mechanical ability. Accordingly, and in view of the laboratory's regulatory nature, the primary considerations in making a final choice of equipment were: (*a*) mechanical and electronic reliability and precision, (*b*) stability of the equipment during prolonged use, (*c*) operator-free capability, (*d*) capacity, (*e*) compatibility among the various instruments, (*f*) ease of operation, (*g*) safety, (*h*) mechanical simplicity, and (*i*) speed.

Before combining instruments to create the automated system, various types of fraction collectors or sample changers and several spectrophotometers of different manufacturers were investigated. A Gilson Escargot sample changer was selected because it alone met all of the criteria. It has a minimum of moving parts. One or two switches, depending on the last previous use of the machine, are flipped to start it. One incubation unit consists of up to 438 tubes, thus permitting unattended running time of up to 80 min. All of the electrical relays are fail-safe: if the tubes do not advance properly or the probe is driven down outside a

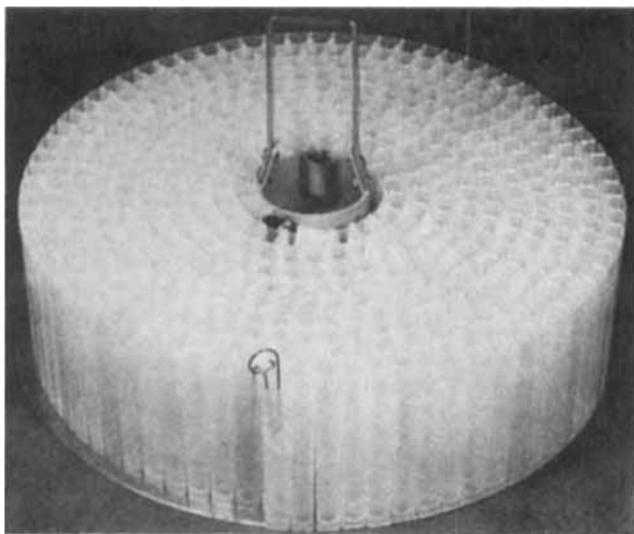

Figure 2—*A spool of carrier tubes showing the elongated handle. The darker tubes on the sides of the spool locate the position of the first culture tube for each antibiotic solution. The first nine tubes to the right of the clamp are for culture tubes containing reference concentration antibiotic standard solution to be used to check the rate of growth of the test organism during incubation.*

tube, the machine simply shuts off. One unit has been in satisfactory use for more than a year; during that time the machine has not caused itself to shut off and no known errors have occurred. Maintenance has been minimal; so far, one shaft has once required lubrication and the plunger of the pumping syringe has required four new rubber gaskets.

EXPERIMENTAL

Equipment—Figure 1 shows the assembled sampling system in operation. This system consists of an extensively modified Escargot sample changer (model SC-15)[1] with a pipeting unit (model FD-6)[1] and programmable syringe pump[1]. Polypropylene "snap tubes"[1], 15-ml. capacity, purchased without bottoms, serve as carriers for disposable 13 × 150-mm. glass culture tubes[2] on stainless steel spools[1] (Fig. 2). The syringe pump (Fig. 3) consists basically of a variable speed motor and a series of indented rings (Fig. 4) which revolve and close circuits in sequence to perform the various operations of the system. The spectrophotometer (Coleman model 111)[3], visible range only, is fitted with a 9095-T20 flow cell adapter[4] and a 9120-N05 flow cell[4] with 0.25-ml. volume and 10-mm. light path. The voltage output from the spectrophotometer is digitized in a digital converter (model C-8)[4], displayed on a concentration readout (model 8)[4], and printed, with the sample identification number, on paper tape. A 9096-J70 sample identification repeater[4] provides three (or any integral divisor of 12) readings of one sample number.

Assay Procedure—All antibiotics that are assayed with a common inoculum and incubation period are routinely assayed in a single spool, organized so that the 15 tubes for a standard curve (three tubes of each of five concentrations) are to be read first, followed by not more than 20 samples. A sufficient number of standard curve tubes are then placed at 20-sample intervals to read the number of samples being assayed on a given day.

One-milliliter aliquots of each manually diluted antibiotic standard or sample solution are dispensed to each of three 13 × 150-mm. culture tubes on a spool by means of a Cornwall syringe[5]. To each tube is added 9 ml. of broth inoculated with the appropriate organism for the antibiotic under test [as specified by the "Code of Federal Regulations," §141.104(a) and 141.111(b)](8) with a Brewer automatic pipeter[6].

A study was made of the feasibility of utilizing the tube advance mechanism of the sample changer in conjunction with an inoculated broth dispenser for inoculating the assay tubes. Time studies of the manual procedures, which involved moving the dispensing port from one tube to the next, indicated that this technique was considerably more rapid than an inoculation rate based on the advancing of tubes under a dispensing port on the sample changer. Using a Brewer automatic pipeter and manually transferring the dispensing port permit an inoculation rate of 40 tubes/min., approximately twice the rate attainable with these machines performing the entire operation.

After suitable incubation in a 37° circulating water bath, the spool of assay tubes is removed and further growth is stopped by the rapid addition to the tubes of 12% (w/v) formaldehyde solution in water with a Cornwall continuous pipeter[5]. The spool of assay tubes is then placed on the sample changer.

The first plastic tube on the assay spool is fastened to a lead tube attached to the core of an empty spool by a vinyl tape, and the first assay tube is positioned directly under the stainless steel sample probe. The chain of polypropylene tubes is then clamped against a sprocketed drive wheel. The clamping of the tubes also closes a microswitch, initiating the readout procedure. The growth of the test organisms used in this laboratory in liquid media is such that the contents of tubes do not have to be shaken just before reading. Other test cultures could require postincubation agitation.

Automated Cycle—The syringe pump is programmed by the indented rings (Fig. 4) to perform the following cycle:

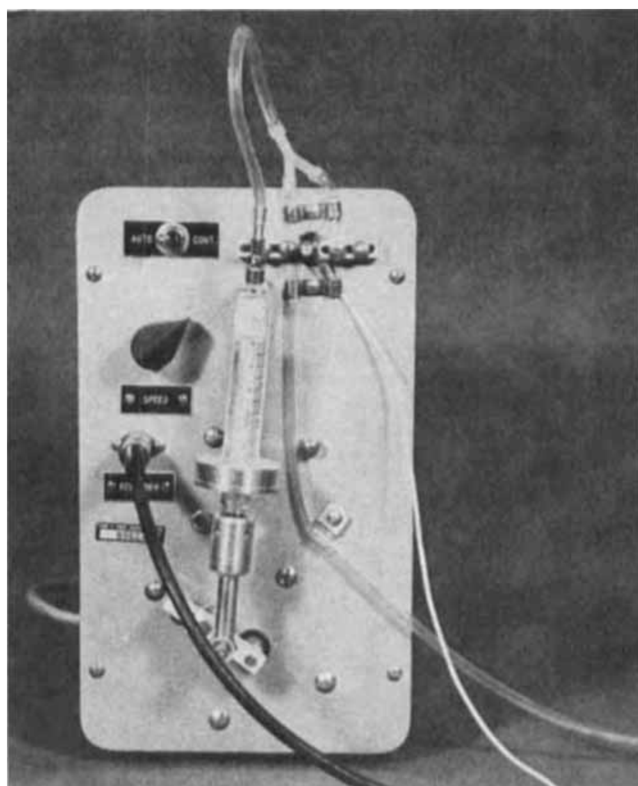

Figure 3—*Exterior view of the syringe pump. The narrow-bore tubing is connected to the outlet from the flow cell. The wide-bore tubing is the discharge line. The electric cord transmits the print command to the digital converter. The "Auto-Cont" switch permits operation of the syringe without lowering the sample probe to enable an analyst to check the growth in reference concentration standard solution tubes.*

[1] Gilson Medical Electronics, Inc., Middleton, WI 53562
[2] Rochester Scientific Co., Rochester, NY 14624, or Demuth Glass Division, Brockway Glass Co., Inc., Parkersburg, W. Va.
[3] Perkin-Elmer Corp., Norwalk, CT 06852
[4] Arthur H. Thomas Co., Philadelphia, PA 19105
[5] Becton, Dickinson and Co., Rutherford, NJ 07070
[6] Baltimore Biological Laboratories, Baltimore, Md., Catalog No. 60453.

Table I—Summary of Absorbance Readings of Replicate Tubes of Pooled and Divided *K. pneumoniae* (ATCC 10031)

Day	Number of Tubes	Coleman 111 Mean	Coleman 111 Range	Coleman 111 σ	Spectronic 20 Mean	Spectronic 20 Range	Spectronic 20 σ
1	10	0.456	0.032	0.0104	0.510	0.074	0.0240
2	58	0.386	0.034	0.0089	0.433	0.026	0.0069
3	60	0.436	0.005	0.0011	0.412	0.060	0.0130

1. The stainless steel sample probe is driven vertically into the assay tube.
2. The intake line to the syringe opens, simultaneously clamping shut the discharge (to waste) line from the syringe.
3. The syringe pulls bacterial culture through polyethylene tubing and the spectrophotometer flow cell at a rate of approximately 1.1 ml./sec.
4. After 5.3 sec. of flow, an impulse is sent from the syringe pump to the digital converter, commanding the latter to print the absorbance value and sample number on paper tape.
5. The discharge line from the syringe is opened, clamping off the intake line at the apex of the syringe cycle.
6. The sample probe is withdrawn from the culture tube.
7. The drive wheel at the base of the sample changer advances one position, bringing the next tube under the probe.

When the equipment is in operation (Fig. 1), the syringe pump runs continuously, causing an air-free intermittent flow pattern through the spectrophotometer. A cycle is completed in 11.5 sec., thus permitting the reading of approximately 5 tubes/min. or 313 tubes/hr. The variable speed motor and the adjustable volume drawn by the syringe permit both somewhat slower and faster sampling rates for other applications.

RESULTS

The "Code of Federal Regulations" [§141.111(c)] (8) prescribes that the absorbance value of each tube be determined in a suitable photoelectric colorimeter. Prior to assaying and reading samples in the new equipment, replicate tube studies were undertaken to compare the uniformity of readings obtained with a pooled suspension of *Klebsiella pneumoniae* (ATCC 10031). A large number of tubes containing 1 ml. of a 30-mcg./ml. solution of dihydrostreptomycin standard were inoculated with broth containing the test organism. After incubation and treatment with formaldehyde, the contents of the tubes were pooled, thoroughly mixed, and redistributed into clean tubes. The absorbance values were determined manually for half of the tubes with a Bausch & Lomb Spectronic 20 and automatically for the other half of the tubes with the Coleman 111. The results, summarized in Table I, show that automated readings from the Coleman instrument were at least as uniform as those read manually with the Spectronic 20.

Inasmuch as manual reading in a spectrophotometer such as the Spectronic 20 is an officially recognized procedure and has been this laboratory's procedure for several years, it also was necessary to demonstrate that results obtained automatically for all dosage forms of all turbidimetrically assayed antibiotics did not differ significantly from results obtained by the manual procedure. Accordingly, analyses of variance were performed for the results obtained with the Coleman 111 compared to those obtained with the Spectronic 20 for each antibiotic over a minimum of 2 days for each dosage form available. Aliquots of the same solutions of standard material and of samples were dispensed into three replicate tubes to be read in each spectrophotometer, so that theoretically any difference in the results would be due to the reading system, and sample preparation would be excluded as a variable.

The results, summarized in Table II, show that in no instance was the observed difference statistically significant at the 95% level of confidence.

The Spectronic 20 instruments in this laboratory are fitted with apertures to intensify the differences in absorbance values due to changes in concentration of the antibiotic. Such a device is not practical in the Coleman 111 because of the size and openness of the sampling chamber. Very little difference between the spectrophotometers was found in absorbance readings of any particular assay tube containing *K. pneumoniae*, *Escherichia coli* (ATCC 10536), or *Streptococcus faecalis* (ATCC 10541). However, a *Staphylococcus aureus* (ATCC 6538P) suspension that gave an absorbance reading of approximately 0.4 in the Spectronic 20 yielded an absorbance of only about 0.3 in the Coleman 111. On the basis of this information, it was believed that no change in inoculum

Table II—Comparison of Potencies Obtained with a Coleman 111 Spectrophotometer and with a Bausch & Lomb Spectronic 20

Antibiotic	Number of Comparisons	Average Percent Difference[a]	Calculated F	Significance Level (α)
Tetracycline	95	−1.27	0.1077	0.75
Oxytetracycline	33	+0.62	0.2120	0.67
Doxycycline	18	+1.97	0.4476	0.51
Chlortetracycline	41	−0.77	0.0566	0.82
Demeclocycline	27	−0.65	0.0054	0.94
Gramicidin	38	+0.76	0.0494	0.84
Dihydrostreptomycin	52	−2.45	3.60	0.07
Troleandomycin	14	+1.10	0.0708	0.81
Chloramphenicol	30	+0.38	0.0142	0.90

[a] Results were obtained with the Coleman 111 and are expressed in terms of the results of the same sample solutions as read from the Spectronic 20.

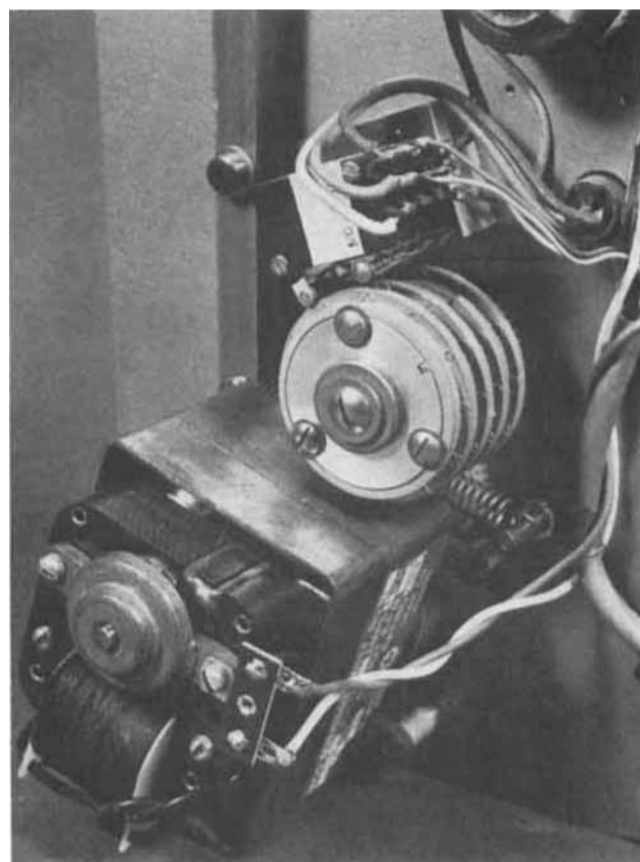

Figure 4—*A view of the interior of the syringe pump showing (top to bottom) the series of microswitches which control the operation of the equipment, the plastic rings which, respectively, open and close the microswitches by revolving, and the variable speed motor. The third ring shows an indentation which causes its microswitch to close, initiating one action in the readout cycle.*

Table III—Raw Data Obtained from a Randomized Three-Dose Assay of a Proposed Rolitetracycline Reference Standard

Tube Number	Absorbance	Tube Number	Absorbance	Tube Number	Absorbance
1	0.371	11	0.278	21	0.365
2	0.318	12	0.364	22	0.273
3	0.275	13	0.365	23	0.361
4	0.280	14	0.274	24	0.365
5	0.317	15	0.360	25	0.282
6	0.327	16	0.318	26	0.361
7	0.369	17	0.276	27	0.321
8	0.328	18	0.268	28	0.330
9	0.320	19	0.318	29	0.276
10	0.323	20	0.369	30	0.280

Table IV—Decoded Data from Table III[a]

Absorbance					
S_1	S_2	S_3	U_1	U_2	U_3
0.275	0.318	0.365	0.280	0.327	0.371
0.274	0.317	0.360	0.278	0.328	0.369
0.273	0.320	0.365	0.276	0.323	0.364
0.268	0.318	0.361	0.282	0.318	0.369
0.276	0.321	0.361	0.280	0.330	0.365
Range					
0.008	0.004	0.005	0.006	0.012	0.007

[a] *S* is the working standard; *U* is the proposed reference standard. The numerical subscripts refer to the three dose levels.

or incubation conditions need be considered for any turbidimetric assay other than possibly the *S. aureus* procedures. Growth curves were, therefore, run with *S. aureus* in the presence of tetracyclines to determine the optimal combination of inoculum and incubation period. It was found that, regardless of the inoculum level, a greater dose response was obtained with 5 hr. of incubation than with any shorter period. All of the growth curves, however, were linear for the concentrations of antibiotic used; it was decided to retain the 4-hr. incubation period routinely employed, with a midpoint absorbance reading of approximately 0.3.

Because this laboratory's procedures are based on a linear dose-response line between the concentrations of antibiotics used in the standard response lines, it was necessary to determine that the dose responses obtained with the Coleman 111 were linear. Analyses of regression and linearity were performed on the standard response lines obtained for each antibiotic for a minimum of 2 days. A large majority of the responses analyzed proved to be linear for each antibiotic.

The equipment has been used in collaborative studies with other institutions to determine the potency of proposed antibiotic reference standards. The data obtained from one test for rolitetracycline is presented in Table III, which shows the absorbance values obtained for randomly placed tubes of the three dose levels of the working standard and the proposed reference standard. The absorbances of the decoded tubes are shown in Table IV. It can be seen that for the replicate tubes of any solution the range of readings is very narrow. In only one instance was the range of observed absorbances greater than $\pm 1.5\%$, and in only one instance was any single observation more than $\pm 2.0\%$ from the mean.

DISCUSSION

The original Gilson model VFC fraction collector was modified in many ways, both by the manufacturer and by this laboratory. The manufacturer made several design changes. The microswitch at the base of the sample changer, which drives the equipment as long as plastic tubes are present, was raised and extended so that the apparatus operates until the last glass tube moves away from the plane of the sample probe. A foot switch was added to enable an analyst to dispense diluted solutions to the tubes in a spool as they pass in front of him. This switch bypasses the syringe pump. A switch was installed on the syringe pump to permit the pump to operate without lowering the sample probe or moving the driving wheel on the sample changer. Therefore, an analyst can obtain a preliminary absorbance reading of an assay tube of diluted standard material prior to killing the samples still being incubated. To reduce corrosion, a stainless steel supporting base was supplied with each spool instead of the standard aluminum bases. The spools were supplied with longer handles to permit carrying with the glass tubes.

Convenience modifications to the purchased equipment were made in the FDA Instrument Shop or by the authors. One-fourth-inch (0.63-cm.) holes were drilled at 1-in. (2.54-cm.) intervals in the stainless steel spool bases to eliminate some weight and to facilitate draining of water from the plastic tubes after they were removed from the water bath. Gilson's standard indented toe plate on the clamp of the sample changer, which was shaped to accommodate their volumetric fraction collecting equipment, was replaced with a solid plate to keep the polypropylene carrier tubes from snagging. To overcome the maintenance problems encountered with the rubber gaskets on the pumping syringe, the plastic syringe was replaced with a 10-ml. glass syringe[5]. Fiberglas tape was wound around the vinyl lead tapes to prevent crimping and separation of the plastic tubes next to the cores of the spools.

If reliable, drift-free performance could be achieved without using a double-beam spectrophotometer, economics dictated the use of a single-beam instrument. Several were tried, with success ranging from very little to excellent. A Coleman Hitachi model 111 spectrophotometer gave excellent results. This instrument maintained its calibrations over a period of weeks, and the indicator needle and output responded quickly and completely to changes in absorbance from one tube to the next. The Coleman model 111 was chosen after thorough evaluation because of its superior monochromator, its narrower bandpass, and its improved drift-free specifications.

During the preliminary evaluation of the integrated system, results of the same inoculated and incubated antibiotic solutions were compared when the absorbance values were obtained both from flowing samples and from the same tubes as settling suspensions. The *K. pneumoniae* and *E. coli* cultures demonstrated the flow birefringence phenomenon described by Kuzel and Roudebush (1). However, no significant difference was found in the actual potencies of the individual samples as determined by the two methods of reading, since all of the tubes of both standard material and samples demonstrated the phenomenon. Because of slightly greater uniformity of absorbance readings between replicate tubes, and as a matter of preference, absorbance values of the flowing samples are recorded in this laboratory.

Cocci demonstrate only a negligible change in absorbance values between a flowing culture and the same suspension in a settling condition. *Strep. faecalis* cultures show a shift in absorbances of <0.005 between the two states, whereas this variation is routinely <0.003 absorbance unit with staphylococcal suspensions.

Earlier publications (3, 4) indicated that cultures need to be agitated in the tubes just before reading. Two methods for mixing the contents of a tube are the introduction of a slight flow of compressed air by a probe into the tube and the use of a mechanical stirrer. To determine the necessity of installing such a device, several replicate tubes were removed from their plastic carrier tubes and vigorously shaken less than 1 min. before the contents were drawn through the flow cell. There was no difference in absorbance values between a series of shaken tubes and a similar set of parallel undisturbed tubes.

Incubation studies comparing the amount of growth obtained with *S. aureus* in the presence of tetracycline in tubes in tightly coiled spools with the same antibiotic solution, inoculum, and culture tubes in a standard wire rack indicated that growth in all tubes in a spool is at least as uniform as it is from one tube to another in a rack. The cause of this uniformity is the fact that, although the square polypropylene tubes are situated in close proximity to each other, the glass tubes, which fit only 50 mm. down into the carriers, are sufficiently far apart to permit adequate circulation of water around them.

The automated equipment yielded several immediate advantages to the laboratory. The manual readout procedure required two analysts: one to add samples and to read aloud the spectrophotometric readings and another to record the values and to calculate the results. This operation required approximately 6 man-hours a day for the current workload. With the automated readout, the

tubes are sampled and recorded at the rate of one every 11.5 sec., which is more rapid than was possible manually, particularly for the bacillary cultures. Only one analyst is required intermittently for a period of less than 3 hr. a day to place fresh spools on the sample changer, average the three readings per sample, and calculate the results. The resulting saving in time of approximately 2.2 man-hours per day permits the equipment to pay for itself in 2.7 years.

The system also eliminates normal human bias from the reading of borderline samples, as well as the fatigue, eyestrain, and consequent loss of precision in determining the exact position of the spectrophotometer needle.

The sensitivity of the spectrophotometer and the thorough washing of the flow cell of the previous tube's contents with those of the current tube combine to give precise absorbance readings. Experience has indicated that the laboratory obtains greater uniformity of results between samples of a batch of antibiotic than was formerly obtained.

The equipment has demonstrated particular value in research. A complete series of growth curves can be inoculated, incubated, and read as one incubation unit. Instead of being required to read, record, and calculate all of his results, the researcher, after stopping growth in his assay unit, flips one or two switches, returns to his bench to pursue other activities, and comes back sometime later to pick up his results for calculation. Increased precision is significant also for new assay methods and for assays that are to be subjected to statistical analyses.

The complete readout assembly requires only 6 linear feet of bench space. The Escargot spool permits a vastly greater number of samples to be assayed as one unit and, consequently, requires much less water bath space for incubation of samples and their associated standard response concentrations. With the assays of many different antibiotics being inoculated, incubated, and killed concurrently, standard metal racks required large amounts of bench and water bath area. One spool can replace three 144-tube racks; it occupies less than 2 sq. ft. of space; and when completely full of inoculated tubes, it weighs only 27 lb. This capacity and the saving of space, along with the compactness of the sampling and readout equipment, will enable the laboratory to meet the demands of assaying new antibiotics and using new assay procedures within its present space limitations.

The day's run of all antibiotics that require both the same inoculum and the same incubation period is routinely placed in one spool, with the tubes for the standard response levels for each antibiotic preceding those for the samples. There are no spaces between antibiotics; in this way, the machine runs continuously until the last tube has been recorded.

To raise the temperature in the culture tubes to the proper incubation temperature as rapidly as possible, it is suggested that the spool of inoculated tubes be lowered into and raised from the water bath two or three times at the start of the incubation period.

The system is easily cleaned. Three tubes of distilled water are placed after the last sample tube in a spool. They then are cycled under the probe and through the tubing, flow cell, and syringe.

There has been no change in the basic procedure of a turbidimetric microbiological assay. Operations such as sample and inoculum preparation have not been altered at all. A more sophisticated photoelectric colorimeter is being used, and the contents of assay tubes are being introduced into the spectrophotometer mechanically instead of manually. Because there has been no basic change in the assay procedure, no change in the "Code of Federal Regulations" was required in order to accept the system. Its use in this or other laboratories is thus assured.

At present, the automated readout system ends when the absorbance value of each tube is printed on paper tape, along with the sample number. However, if the using laboratory has access to a computer, the data can be fed, either on-line or on a delayed basis, into the computer which can be programmed to perform all necessary calculations of results.

SUMMARY

A highly reliable and accurate automated readout system for turbidimetric microbiological assays of antibiotics was developed. This system utilizes only commercially available equipment. It has increased the uniformity of tube-to-tube readings and the precision of microbiological assays. A minimum of mechanical and electronic problems have been experienced with the apparatus. The laboratory's work capacity has been increased by the speed of operation and the compactness of the equipment. No change in the "Code of Federal Regulations" is required to accommodate this system.

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