Semiautomated Method for Microbiological Vitamin Assays

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A semiautomated method for microbiological vitamin assays is described, which includes separate automated systems for the preparation of the cultures and for the measurement of turbidity. In the dilution and dosage unit based on the continuous-flow principle, vitamin samples were diluted to two different dose levels at a rate of 40 per hr, mixed with the inoculated test broth, and dispensed into culture tubes. After incubation, racks with culture tubes were placed on the sampler of an automatic turbidimeter. This unit, based on the discrete-sample system, measured the turbidity and printed the extinction values at a rate of 300 per hr. Calculations were computerized and the results, including statistical data, are presented in an easily readable form. The automated method is in routine use for the assays of thiamine, riboflavin, pyridoxine, cyanocobalamin, calcium pantothenate, nicotinic acid, pantothenol, and folic acid. Identical vitamin solutions assayed on different days gave variation coefficients for the various vitamin assays of less than 10%.

The laborious nature of microbiological assays for antibiotics and vitamins has led to the development of more or less automated systems. These systems are of two general types: continuous flow and discrete-sample.

The first type of system has mainly found application in antibiotic assays (1, 5) where short incubation periods are required. In these assays, the production of carbon dioxide (3, 10) and bacterial growth (9) serve as parameters. Systems with longer incubation periods present problems in continuous-flow systems, requiring holding coils of considerable length in which the regular air-bubble pattern cannot be maintained because of the high pressure needed.

The first example of a discrete-sample system has been described by McMahan (8). In this apparatus, samples in tubes pass a number of stations where the various manipulations are performed mechanically. A discrete-sample system especially suitable for antibiotic assays has been described by Gualandi (4). Recently, Kuzel and Kavanagh (7) described an automated system consisting of a diluter and turbidity reader module. The system which is commercially available can be used for antibiotic and vitamin assays. An extensive survey of the theoretical and practical aspects of the design is given by the same authors (6).

In our laboratory, we aimed at the automation of the vitamin assays carried out as a modified two-point parallel line assay in which automation should include most of the laborious manipulations (i.e., the preparation of dilutions and of the test mixture in culture tubes, turbidity reading of the incubated cultures, and the evaluation of assay data). The system should be sufficiently flexible to perform alternately assays of all B vitamins, for example, thiamine, riboflavin, pyridoxine, cyanocobalamin, calcium pantothenate, nicotinic acid, pantothenol, and folic acid. Finally, the method should be able to cope with preparations of widely varying vitamin content.

To meet the latter requirement, automation was introduced from the point where prediluted vitamin samples could be treated identically. It proved to be impractical to include the rather long and variable incubation period in the automated system. Hence a semiautomated method consisting of two independent units was designed. A dilution and dosage unit based on the continuous-flow principle prepares suspensions containing vitamin solution and inoculated test broth in culture tubes ready for incubation. A discrete-sample
system performs the turbidity measurement of the cultures after incubation.

**MATERIALS AND METHODS**

Growth curves showed that the growth rate of the test organism is related to the concentration of the vitamin within the limits of the test. The growth was measured turbidimetrically after an incubation period of 16 to 18 hr. In the numerous vitamin assay carried out under standardized conditions, it was shown that the log dose response curves for all vitamins were steep and highly consistent between fixed concentration ratios. Hence a vitamin assay was designed based on the principle of the two-point parallel line assay according to Finney (2). In the two-point parallel line assay, preparations were diluted to two dose levels for which the responses lie on the steep slope of the log dose response curve.

In one assay, several preparations with an assumed vitamin content were assayed against the standard. One dilution series of each unknown preparation and 7 to 10 dilution series of the standard were used in one assay to attain maximum efficiency. Each dilution series consisted of two dilutions with the high and two dilutions with the low concentration. A test set-up was chosen for which the dilution series were prepared, and the turbidity of the series was measured in a random order.

In agreement with the design of a two-point parallel line assay, a statistical test was carried out to examine whether the regression coefficient of the log dose curve of each individual unknown preparation could be regarded as equal to the regression coefficient of the standard preparation. In case of an erroneous assumption of the potency of the unknown preparation or a dilution error, the test on equality of regression coefficients of unknown and standard (test on parallelity) is significant with a probability larger than 0.05. In the latter case, the unknown should be retested with a new assumption about its potency, if necessary.

An indication of the precision of the assay is given by \( \lambda = a/b \), where \( s \) is the standard deviation of the mean extinction value of the standard, and \( b \) is the mean regression coefficient of the standard and of those unknowns that have not been rejected because they are not parallel.

The principal data on the assay method of the various vitamins have been compiled in Table 1. For all vitamin assay tests, commercially available media were used. Methods are also available for the preparation of alternative media (Table 2).

The dilution and dosage unit consisted of a main sampler containing the vitamin solutions under test; four auxiliary samplers delivering dilution fluid and test broth (in most cases already inoculated, see Table 1); a proportioning pump with a manifold in which dilution took place and in which two test solutions (one high and one low concentration) from each vitamin solution were prepared; and a combi-collector delivering identical amounts of the final mixture to culture tubes. All of these elements were linked to a control unit.

The main sampler (MS, Fig. 1) consisted of a tube-rack holder with a carriage carrying the sampling needle and a cup for rinsing fluid. The sampler had a capacity of eight racks each containing 20 tubes with a volume of 3 ml. The carriage automatically moved from one sample tube (and row) to another. With two small handles in the side panel of the carriage, the sampling needle could be moved to any position. Instead of the last rack, a reservoir with rinsing fluid can be placed in the rack holder. The sample tubes in the racks were always arranged in such a way that after the last vitamin sample solution the carriage arrived at the rinsing fluid reservoir. The sampling needle was adjusted to the required height by means of a micrometer.

The auxiliary samplers (Fig. 2) supplied either dilution fluid (H1, H2, and H4) or test broth (H3). In contrast to the main sampler in which rinsing fluid was drawn between the samples, the auxiliary samplers drew air when they were not sampling fluid. Hence, for the latter samplers, the cup for rinsing fluid was omitted. The construction of the auxiliary samplers was identical to the sampling device mounted on the main sampler.

The proportioning pump (Technicon Co., Inc., Tarrytown, N.Y.) has a capacity of 30 tygon tubes.

The manifold (Fig. 3) was built from tygon tubes with diameters of 0.110 inch (approximately 0.28 cm) and glass connections in accordance with common autoanalyzer systems.

Determination of the dosages of the final mixtures prepared in the manifold was performed by the combicollector (Fig. 4). The latter consisted of a conveyor belt above which were mounted two solenoid valves (Versa N.V., Apeldoorn, Holland) each provided with a stainless-steel outlet and a bypass. In the "on" position, the valves were open and connected via a bypass to the waste. When the valves were closed, the liquid passed to the outlets. Special racks containing two rows of six culture tubes were carried by the conveyor belt so that two tubes were centered below the two valve outlets. Each time the valves were opened, the next two culture tubes were moved to this position.

All of these elements were electrically linked to the control unit. In this unit (Fig. 5), adjustable electromechanical time clocks (Schleicher, W. Berlin, Germany) were grouped together to direct the operation of the various elements.

The sampling and rinsing times of the main sampler were controlled by the first pair of time clocks. Each operation of the other individual mechanical parts of the dilution and dosage unit was also controlled by a pair of time clocks. Four pairs of time clocks controlled the three auxiliary samplers (H1, H3, and H4) and the opening of the valves on the combicollector. The remaining two pairs of time clocks regulated the two operations performed by auxiliary sampler H2 (see below).

The first time clock in such a pair controlled the lag time and the second controlled the actual operation period. The period between the receipt of the electrical signal and the commencement of the operation is defined as the lag time. Signals derived from the time clocks of the main sampler initiated the start of all lag times with the exception of the time
| Vitamin                  | Test organism                      | Stock stored in                  | Inoculum*          | Test Broth                          | Standard solution                                                                 | Extraction of samples | High dose level in final sample (µg/ml) | Incubation                  |
|-------------------------|------------------------------------|----------------------------------|--------------------|-------------------------------------|-----------------------------------------------------------------------------------|-----------------------|------------------------------------------|-----------------------------|
| Thiamine                | *Lactobacillus viridescens*        | ATCC 12706                       | Physiological saline | 24-hr culture in APT broth (Difco)  | Thiamine L.V. medium (Difco)                                                      | Used for 1 month; USP | 0.001 N HCl                              | Culture tubes autoclaved 10 min at 110°C; tube-wise | 16 hr at 30°C in water bath |
| Riboflavin              | *Lactobacillus casei*              | ATCC 7469                        | Physiological saline | 24-hr culture in lactobacilli broth AOAC (Difco) | Riboflavin assay medium (Difco)                                                   | Freshly prepared; USP | 4 M 25% acetic acid solution             | On dilution and dosage unit | 16 hr at 37°C in water bath          |
| Pyridoxine              | *Saccharomyces carlsbergensis*     | ATCC 9080                        | Lactobacilli broth AOAC | 24-hr culture in lactobacilli broth AOAC | Pyridoxine Y medium (Difco)                                                       | Used for 1 month; USP | 0.06 N HCl                              | Culture tubes autoclaved 10 min at 110°C; tube-wise | 16 hr at 30°C on shaker           |
| Cyanocobalamin          | *Lactobacillus leichmannii*        | ATCC 7830                        | Physiological saline | 24-hr culture in lactobacilli broth AOAC | B12 assay medium, (73.3 · g/750 ml) (Dano)                                         | Used for 1 month; USP | 0.0006% KCN                             | On dilution and dosage unit | 16 hr at 37°C in water bath          |
| Calcium pantothenate/nicotinic acid | *Lactobacillus arabinosus*              | ATCC 8014                        | Physiological saline | 24-hr culture in lactobacilli broth AOAC | Niacin assay medium or pantothenate medium USP (Difco)                             | Used for 1 month; USP | Distilled water                          | On dilution and dosage unit | 16 hr at 37°C in water bath          |
| Pantothenol             | *Acetabacter suboxydans*           | ATCC 6214                        | Lactobacilli broth AOAC | 24-hr culture in lactobacilli broth AOAC | Panthenol assay medium; panthenol supplement (Difco)                              | Freshly prepared; USP | 1.25 × 10⁻¹                             | Culture tubes autoclaved 10 min at 110°C; tube-wise | 16 hr at 30°C on shaker           |
| Folic acid              | *Lactobacillus casei*              | ATCC 7469                        | Physiological saline | 24-hr culture in lactobacilli broth AOAC | Folic acid assay medium (Dano)                                                    | Used for 1 month; USP | Distilled water                          | On dilution and dosage unit | 16 hr at 37°C in water bath          |

* For the preparation of the inoculum, the 24-hr cultures of the test organisms were washed three times with physiological saline solution.
### TABLE 2. Composition of basal media for assays of various vitamins (500 ml final volume)

| Stock solutions | Thiamine (ml) | Riboflavin (ml) | Pyridoxine (ml) | Cyanocobalamin (ml) | Calcium pantothenate (ml) | Nicotinic acid (ml) | Pantothenol (ml) | Folic acid (ml) |
|-----------------|--------------|----------------|----------------|---------------------|--------------------------|---------------------|-----------------|----------------|
| Thiamine·HCl (0.01%) in 0.2% HCl | 5 | 2 | 5 | 10 | 1 | 20 | 5 |
| Riboflavine-sodium phosphate (0.001%) in 0.1% CH₃COOH glacial | 50 | 1 | 50 | 100 | 20 | 150 | 50 |
| Nicotinic acid (0.01%) in distilled water | 10 | 10 | 2 | 10 | 10 | 20 | 10 |
| p-Aminobenzoic acid (0.01%) in 0.1% CH₃COOH glacial | 10 | 10 | 1 | 1 | 10 | 10 |
| Biotin (0.004%) in distilled water | 20 | 20 | 40 | 2 | 2 | 80 | 10 |
| Pyridoxine·HCl (0.01%) in distilled water | 20 | 20 | 20 | 20 | 4 | 10 | 20 |
| Calcium pantothenate (0.01%) in distilled water | 5 | 5 | 2 | 5 | 2 | 5 | 1.2 |
| Folic acid (0.002%) in 50% ethanol | 5 | 5 | 5 | 10 |
| Pyridoxal (0.01%) and pyridoxamine (0.002%) in distilled water | 20 | 20 | 20 |
| Xanthine (0.08%) in 0.6% aqueous ammonia | 10 | 10 | 10 |
| Cyanocobalamin (0.0001%) in distilled water | 2.5 | 2.5 | 2.5 |
| Cystine (0.4%) in 0.1 N HCl | 50 | 50 | 25 | 50 | 40 | 50 |
| L-Tryptophan (0.8%) in 0.1 N HCl | 25 | 2.5 | 2.5 | 12.5 | 12.5 | 25 |
| Asparaginase (1%) in distilled water | 10 | 10 | 10 | 10 |
| Adenine (0.075%), guanine (0.08%), and uracil (0.1%) in 0.3 N HCl | 10 | 10 | 10 | 5 | 10 |
| Polysorbate-80 (6%) in 96% ethanol | 10 | 10 | 10 | 10 |
| KH₂PO₄ (5%) and K₂HPO₄ (5%) | 10 | 10 | 10 | 10 | 10 |
| MgSO₄·7H₂O (2%), NaCl (0.1%), MnSO₄·4H₂O (0.1%), and FeSO₄·7H₂O (0.1%) in distilled water | 10 | 10 | 10 | 10 | 20 | 10 |
| KI (0.02%), (NH₄)₂Mo₇O₂₄·4H₂O (0.004%), CuSO₄·5H₂O (0.009%), ZnSO₄·7H₂O (0.008%), and FeSO₄·7H₂O (0.05%) in distilled water | 0.5 |
| L-Histidine (0.1%), dl-methionine (0.2%), l-isoleucine (0.1%), and l-valine (0.1%) in distilled water | 10 |
| CaCl₂ (1.25%) in distilled water | 10 |
| MgSO₄·7H₂O (1.25%) in distilled water | 10 |
| MnSO₄·4H₂O (0.1%) in distilled water | 2.5 |
| NaCl (25%), MgSO₄·7H₂O (4%), MnSO₄·7H₂O (0.7%), and FeSO₄·7H₂O (0.2%) in distilled water | 20 |
| Tomato juice B | 100 | 100 | 50 | 75 | 75 |
| Casein hydrolysate, vitamin free (10%) | 75 | 75 | 60 | 60 | 50 | 75 |
| Ascorbic acid | 2 | 2 | 2 |
| Glucose | 10 | 20 | 20 | 20 | 20 | 7.5 | 20 |
| CH₃COONa·3H₂O | 16.6 | 16.6 | 16.6 | 16.6 | 16.6 |
| Lactic acid | 0.3 |
| Glycerol | 50 |
| Sodium citrate, 5.5 aqueous (Baker) | 1 |
| α-Alanine | 1 |
| Boric acid | 100 |
| meso-Inositol | 2.5 |
| KH₂PO₄ | 1.5 |
| (NH₄)₂SO₄ | 2.5 |
| Tryptone (Difco) | 10 |
| K₂HPO₄ | 0.5 |
| Yeast extract, thiamine-free (10%) in 0.5 N NaOH | 100 |
| NaOH | 6.8 | 6.8 | 6.8 | 6.8 | 6.8 | 6.8 |

*Expressed in grams.

*Expressed in milligrams.
clock controlling the lag time for the second operation of auxiliary sampler H2. In this case, the signal was derived from the end of the first operation of the same auxiliary sampler. The starting signal for all time clocks was derived from the start or end of the sampling time of the main sampler. Signal lights on the panel showed which operations were taking place. The time clocks controlling the duration of the actual operations were fixed on constant time intervals (Fig. 5), whereas the lag time for each individual operation was adjustable. In this way, means were provided for synchronization of the various liquid streams in the manifold. In routine use, only minor adjustments of the lag times were necessary to ensure accurate synchronization.

The racks with tubes filled with the vitamin cultures on the combicollector were transported in special trays and incubated either in a thermostatically controlled water bath (Marius N.V., Utrecht, Holland) or on a shaker (type SL 69, Marius N.V.) in a thermostatically controlled cabinet. In the latter case, the racks were tilted on the shaker to increase aeration and agitation of the cultures in the tubes.

The turbidity measurement unit (Fig. 6) consisted of a modified automatic sample transfer apparatus (AST 100, Vitatron, Dieren, Holland) built over a transport system together with a Unicam SP 600 spectrophotometer (Philips N.V., Eindhoven, Holland) electrically connected via a digilog convertor (DRP 100, Vitatron, Holland) to a printer (Addo-X, Malmö, Sweden). A stirrer (Phillips N.V.) that operated as a sampling needle was mounted on the conveyor belt at a position six tubes before the point at which the sample was taken. The stirrer and the sampling needle were simultaneously introduced into the respective culture tubes. In this way, uniform cell suspensions were obtained before the turbidity measurement. The transport system of this unit was similar to that used in the combicollector. The culture tubes thus remained in the same racks throughout the assay. The sampler was connected to a 1-cm flow cell with a volume of 0.5 ml (type 186, Hellma GmbH, Mühlheim/Baden, Germany) mounted in the Unicam SP 600 spectrophotometer. In this system, a wavelength of 1000 nm proved to be optimal for
turbidity measurements. The spectrophotometer was modified to permit conversion of transmission into extinction values which were subsequently printed out via the digilog convertor on the Addo-X.

The vitamin reference standards were weighed and dissolved in the appropriate solvent (Table 1) and diluted to a level of 2,000 times the concentration to be attained in the culture tube (high dose level). Products assayed for vitamin content consisted mainly of coated tablets, aqueous solutions, and freeze-dried preparations. Tablets were homogenized in the solvent by means of an ultrasonic device (S-75 Sonifier, Branson Sonic Power Co., Danbury, Conn). All solutions and freeze-dried preparations were diluted directly to a concentration approximating that of the standards. These prediluted vitamin standard and sample dilutions were prepared the day before performance of the assay and stored in the dark at 4°C overnight. The concentrated solutions of thiamine-HCl, pyridoxine-HCl, calcium pantothenate, nicotinic acid, and pantothenol can also be stored for longer periods without any detectable loss in activity. Before starting, the assay duplicate sample tubes were filled from each of these concentrated solutions together with a number of tubes with distilled water from which the inoculated blanks were derived. Following the statistical design of the assay, these pairs were randomized over the positions in the main sampler.

The dilution and dosage unit was a continuous-flow system capable of diluting 20 duplicate samples per hr. The main sampler drew sample and rinsing fluid (twice-distilled water containing 0.01% Triton X-405) during 60 and 30 sec, respectively. The auxiliary samplers also sampled liquid for 60 sec, thus providing fluid segments of equal length. By adjustment of the lag times for the individual samplers, the continuous-flow streams could be synchronized so that all liquid parts met precisely at the various intersections in the manifold (Fig. 3). At intersection I and II, the samples were diluted 20- and 50-fold with diluent from auxiliary samplers H1 and H2, respectively. After the second dilution step, the diluted sample was divided into two parts at intersection III in the manifold. One part (4 ml) went to intersection IV and was mixed with an equal volume of test broth from H3 to form the high concentration sample. The second part (2 ml) was mixed at intersection V with 2 ml of diluent (from H4) and with 4 ml of test broth (from H3), thus forming the low concentration sample.

Both samples were then dispensed into tubes by the combicollector as follows. The two ends of the tubing circuit were connected with the valves on the combicollector. These valves were controlled in such a way that from each liquid segment of approximately 8 ml the first 3 ml was discarded as waste. The valves were then closed for 30 sec, and approximately 4 ml from the central part of both liquid segments was dispensed into the culture tubes. The remainder of the liquid was led to the waste.

Rinsing fluid sampled by the main sampler served to avoid contamination of a sample segment with

Fig. 2. Auxiliary sampler.
the preceding one in the sample line up to intersection II. From that point on, the manifold was rinsed with dilution fluid from auxiliary sampler H2. For this purpose, the latter sampler was programmed to sample dilution liquid during a period of 15 sec between its main sampling periods. To limit carry-over between two consecutive vitamin samples, care was taken to build a circuit with minimal total length. After each assay run, the manifold was rinsed with 5% aqueous formaldehyde solution and subsequently with 0.2% aqueous sodium lauryl sulfate solution. Every fortnight, an additional rinse with 2 N sulfuric acid was carried out. Every two months, the tubes of the manifold were renewed.

After each row of culture tubes was filled, the six tubes were covered with one stainless-steel cap. To avoid premature growth in the culture tubes, the racks were placed in cold water (0°C) immediately after filling.

The assay procedure continued for the various vitamins as described in Table 1.

After an overnight incubation period, growth was terminated by immersion of the racks in cold water (0°C).

On the turbidity measurement unit, the cultures were mechanically stirred and the turbidity was read at a rate of 300 tubes per hr. On both conveyor belts of the combicollector and the turbidity measurement unit, the tubes were handled in a similar sequence. From each of the racks
containing 12 tubes, first the front row of tubes (6) was filled or sampled; then all of the racks were turned, and the other row of tubes was handled.

All data required for calculation of the vitamin content of the products were transferred to data sheets. These were transcribed on punch cards which were fed into an IBM 360/50 or a PDP 15 computer, programmed to calculate the vitamin content of the samples expressed in terms of the vitamin reference standard. The computer supplied all input data, statistical data on the assay (Fig. 7a), and the calculated vitamin contents (Fig. 7b, c) of the products together with 95% confidence limits in an easily readable form.

As a regular check, the dose response curves for all the vitamins were determined every three months.

RESULTS AND DISCUSSION

Routine use of this semiautomated method provided reliable results. Table 3 shows the coefficients of variation and the mean values calculated from the vitamin contents of identical vitamin standard solutions assayed on different days. The low coefficients of variation calculated and the mean vitamin contents determined for thiamine, riboflavin, pyridoxine, cyanocobalamin, calcium pantothenate, nicotinic acid, and pantothenol demonstrated the good reproducibility and high precision of the method. Data for the folic acid assay were not included in Table 3 since only a few assays of this vitamin were performed. Preliminary experience indicated that folic acid could also be assayed automatically if Triton X-405 was omitted from the dilution and rinsing fluids.

Riboflavin, cyanocobalamin, calcium pantothenate, and nicotinic acid were assayed without special care to avoid contamination.

The test media used for the assay of thiamine and pantothenol were very susceptible to contamination. In these assays, individual inoculation of each tube was necessary after sterilization of the tubes containing the vitamin test medium mixture in an autoclave for 10 min at 110 C. Better assays of pyridoxine were obtained when the tubes were inoculated individually immediately prior to incubation.

Flow birefringence of the rod-shaped bacteria caused no problems in the turbidity measurement. In the turbidity measurement unit, one suspension of Lactobacillus leichmannii divided over a great number of tubes was read. The standard deviation in extinction units proved to be so small that this error could be neglected.

The system combined the advantages of both continuous-flow and discrete-sample systems. The continuous-flow system with synchronization is eminently suitable for the performance of the large dilution step of all vitamin samples with a high degree of reproducibility. On the other hand, the discrete-sample system is more suitable for the turbidity measurement of large numbers of cultures in a short period (11).

With two dilution and dosage units and one turbidity measurement unit, four different vitamin assays could be performed daily. Every assay included 20 to 25 individual unknown vitamin sample solutions yielding a test capacity of 300 to 400 samples per week. Turbidity measurement for four assays took about 4 hr which left the automatic turbidimeter available for other purposes for the remainder of the day.
FIG. 5. Control unit with time clocks. First pair of time clocks controls main sampler (upper clock rinsing time: 30 sec fixed; lower clock sampling time: 60 sec fixed). Time clocks pairs 2 to 5 control auxiliary samplers H1–H4 (upper clocks lag time: 0 to 30 sec adjustable; lower clocks operation time: 60 sec fixed). Sixth pair of time clocks controls the extra rinse cycles performed by auxiliary sampler H2 (upper clock lag time: 0 to 30 sec adjustable; lower clock operation time: 10 sec fixed). Seventh pair of time clocks controls the combi-collector (upper clock lag time: 0 to 60 sec adjustable; lower clock operation time: 30 sec fixed).
Fig. 6. Turbidity measurement unit consisting of sampling and stirring devices built onto a conveyor belt, a spectrophotometer, a digilog converter, and a printer.
### MICROBIOLOGICAL TWO POINT PARALLEL LINE ASSAY

**BASIC DATA STAND-7**

| Subsance | CYANOCOBALAMIN |
|-----------|-----------------|
| DATE      | 10- 1-71        |
| ABBF NUMBER | 10              |
| NUMBER STANDARD SETS | 1              |
| ACTUAL NUMBER OF STANDARD SETS | 1              |
| NUMBER UNKNOWNS | 10             |
| ACTUAL NUMBER OF UNKNOWNS | 10             |
| CONCENTRATION RATIO | 8.88           |
| ML QUANTITY mL | 548.90         |
| DILUTION (%) | 10.00           |
| DIFF. MAX | 100.00          |

**STANDARD SETS**

| NUMBER | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|--------|---|---|---|---|---|---|---|---|---|
| CONC. MN | 7 | 11 | 15 | 19 | 23 | 27 | 31 | 35 | 39 |
| ML | 461 | 363 | 265 | 167 | 79 | 37 | 27 | 17 | 8 |
| TURBIDITY | 461 | 363 | 265 | 167 | 79 | 37 | 27 | 17 | 8 |
| MEAN | 461 | 363 | 265 | 167 | 79 | 37 | 27 | 17 | 8 |

**VARIANCE (RES/COEFF)** 1340.3646

**MEAN STANDARD** 461.57

**UNWON PREPARATIONS OF ABBF NUMBER 1 IN**

| Number | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|--------|---|---|---|---|---|---|---|---|---|
| CONCE. MN | 7 | 11 | 15 | 19 | 23 | 27 | 31 | 35 | 39 |
| PREPARED | STAND | PREPARED | PREPARED | PREPARED | PREPARED | PREPARED | PREPARED | PREPARED | PREPARED |
| TUBE A | TUBE B | TUBE A | TUBE B | TUBE A | TUBE B | TUBE A | TUBE B | TUBE A | TUBE B |
| MATCHED | UNKNOWNS | COAT TUBE | COAT TUBE | COAT TUBE | COAT TUBE | COAT TUBE | COAT TUBE | COAT TUBE | COAT TUBE |
| QUANTITY | 1.64 MCG | 1.00 CRY | 1.86 CRY | 1.00 CRY | 1.00 CRY | 1.00 CRY | 1.00 CRY | 1.00 CRY | 1.00 CRY |
| ML | 461 | 363 | 265 | 167 | 79 | 37 | 27 | 17 | 8 |
| TURBIDITY | 461 | 363 | 265 | 167 | 79 | 37 | 27 | 17 | 8 |
| MEAN | 461 | 363 | 265 | 167 | 79 | 37 | 27 | 17 | 8 |

**RES/COEFF** 442.97

**TEST ON**

| N | 461 | 461 | 461 | 461 | 461 | 461 | 461 | 461 | 461 |
|---|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| ML | 461 | 363 | 265 | 167 | 79 | 37 | 27 | 17 | 8 |
| TUBE A | TUBE B | TUBE A | TUBE B | TUBE A | TUBE B | TUBE A | TUBE B | TUBE A | TUBE B |
| POTENCY | 1.64 MCG | 1.00 CRY | 1.86 CRY | 1.00 CRY | 1.00 CRY | 1.00 CRY | 1.00 CRY | 1.00 CRY | 1.00 CRY |

**PREPARATION NO.**

| C | STANDARD | PREPARED | TUBE A | TUBE B | PREPARED | TUBE A | TUBE B |
|---|----------|----------|--------|--------|----------|--------|--------|
| 1 | 1.00 ( | 0.28 - 1.00 ) | 2.00 | 2.00 | 2.00 | | |

**MEAN** 1.81 MCG / 1.00 MCG

**Fig. 7.** Standard computer printout of a cyanocobalamin assay. (7a) Basic data of the assay and standard sets; (7b) complete data about preparations; (7c) assay results arranged according to preparation.
Table 3. Mean vitamin content and the coefficient of variation of the various vitamin reference standard solutions determined by the automatic method in a number of vitamin assays performed at different days

| Vitamin       | No. of assays | Vitamin content (%) of the reference standard | Coefficient* of variation (%) |
|---------------|---------------|-----------------------------------------------|-------------------------------|
|               |               | Mean  | Low-  | High- |               |                  |
| Thiamine      | 19            | 99.9  | 93    | 111   | 9.2           |                  |
| Riboflavine   | 18            | 99.6  | 95    | 102   | 3.9           |                  |
| Pyridoxine    | 16            | 101.8 | 96    | 108   | 7.2           |                  |
| Cyanocoba-    | 45            | 100.7 | 92    | 107   | 6.3           |                  |
| min           |               |       |       |       |               |                  |
| Calcium panto- | 14            | 100.6 | 96    | 103   | 4.5           |                  |
| theenate      |               |       |       |       |               |                  |
| Nicotinic acid| 16            | 102.3 | 100   | 106   | 4.9           |                  |
| Pantothenol   | 10            | 99.5  | 96    | 104   | 5.0           |                  |

*Coefficient of variation of a random variable is defined by \( s(y)/E(y) \), where \( s(y) \) is the standard deviation of \( y \) and \( E(y) \) is the estimation of \( y \).

To assay 300 unknown sample solutions per week, four technicians were necessary, three of whom were required for preparation of the prediluted vitamin samples and the test media while one operated the automated equipment.

Use of the computer has greatly added to the assay procedure. The only manual transfer of data was in entering the pertinent data of the assay, including the turbidity measurements, onto the computer input data sheets.

On a separate sheet (Fig. 7c), the assay results were reported in such a way that the printout can be used as data sheet for the Quality Control Department.

Although in our laboratory the above automated system has only been used for vitamin assays, it should also be applicable to automation of turbidimetric antibiotic assays.

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