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Chemical Principles and Performance Aspects of Dry Phase Seralyzer® Reagents

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Dry reagent chemistry refers to the reagent format -- unitized reagents with a ready to use, convenient instrument system to provide quantitative results. Common to all types of unitized reagents are the features of: minimum user preparation, long shelf life before first use (some systems require refrigerated or frozen reagent storage), clinically useful results, and basic technologies new to clinical chemistry, but well accepted in other branches of analytical chemistry.

Systems based on reflectance spectroscopy at their simplest have three zones: a reagent, a reflective, and a support layer (3). The ordering varies among the systems. In the Ames SERALYZER system, the support layer is the backing with the reflective coating between the reagent and the support layers. The reagent layer receives the sample directly, and the reflectance is read through the specimen. In both the Eastman Kodak DT-60™ and Boehringer Mannheim REFLOTRON® systems, the sample is applied to or above the reflective layer. The reagent zone is between the reflective layer and the transparent support layer. The reagent layer is read through the support layer.

These three dry reagent systems also occupy the same place in the analytic cycle for clinical chemistry. This cycle consists of: a physician deciding to order laboratory tests based on patient status; obtaining the specimen at the proper time; preparing the plasma, serum or keeping the blood well mixed without lysis; performing the assays with the instrument/reagent system; reporting the results; making decisions on therapy by the physician; and lastly informing the patient. Between the ordering of the tests and therapy decisions, the clinical chemistry professionals make many decisions. The successful implementation of decentralized testing has in many cases depended on how the decisions made routinely in central laboratories are effectively transferred to the remote site. The design of decentralized testing systems certainly helps in making the assays easy to perform, but cannot provide replacement for the judgment by the laboratory or medical professional.

The design of the Ames SERALYZER system is both flexible (to permit easy addition of new analytes) and consistent in use. One simple manual protocol is needed for all tests. Each test comes with a plug-in instrument module and strip with a bar code for identification. The color coding on the reagent packaging with the same coding on the dilution system helps to prepare the specimens. Live calibration was chosen to provide the longest reagent utility under any laboratory environment. Live calibration uses calibration fluids to reduce the small bias differences from preparing dilutions and specimens, instrument to instrument differences, and reagent storage at different sites. Alternative calibration systems can either reduce the variations between instruments or reduce the reagent variability by restrictions on reagent storage or use. Room temperature reagent stability permits the system to be a fast STAT system and requires the least auxiliary refrigeration. Because of the fast assay time for each test, there is no benefit by storing samples for batch testing. The longest tests are four minutes (CK, ASAT), the shortest is 30 seconds, and the average is two minutes per result. By keeping the mechanical complexity low, the system can be made low cost and highly reliable.

There are five main system components. The reflectance photometer provides timed reflectance measurements, thermal control, calculation of results, and storage of calibrations for twenty-one different assays. For each assay, there are the reagent strips and a test module containing the optical interference filter and a read only computer memory. The system calibrators consist of two lyophilized materials for all the metabolites, enzymes and electrolytes; synthetic analytes in liquid form for bilirubin and hemoglobin; and liquid, serum-based calibrators with gravimetric levels of the therapeutic drugs. The dilution system has two pipets and two dispensers each coded to match the reagents.

The instrument reads the reagents by diffusely illuminating the inoculated reagent in an integrating sphere (4). The light reflected perpendicular to the reagent pad is detected by the sample photodiode. Light is both scattered and absorbed by the reacting material in the pad. The light that reaches the sample photodiode contains the concentration information. A reference photodetector monitors the flash intensity of light at the same wavelength for each lamp flash. The light source provides an intense, wide spectrum flash to allow measurements in the ultraviolet to near infrared regions. Approximately 28% of the pad is viewed directly. Reflections and scattering internal to the pad occur, modifying the intensity of the reflected light. Thus a greater portion of the pad than viewed directly contributes to the reflectance measured. The instrument also features dynamic temperature control that permits system use

in a wide range of ambient conditions with the same degree of temperature stability and reproducibility as the larger liquid analyzers.

The method of operation is simple:

1. Place the test module in the instrument port.
2. Prepare the needed dilution using the pipet and distilled water dispenser.
3. Place the reagent strip under the clamps on the feed table.
4. Deliver 30 µL of well mixed specimen to the pad.
5. Press the Start key and push feed table in.

When the timed flash sequence is completed, the reflectance measurements are converted to the clinical value, and the results are displayed until the next assay is done. Calibration requires only the additional step of using the key pad to provide the values for each of the two calibrators. Once a test is calibrated, only the proper test module need be inserted to run any assay on any specimen. There is no need to batch all assays of one type.

Quality control of decentralized systems is important for many reasons. The SERALYZER system is compatible with many commercial controls besides those available from Ames. Users are encouraged, if not required by regulatory agencies, to participate in proficiency programs. In the U.S.A., Ames provides a free quarterly service of specimens, results analysis, and consultation through Customer Service. Over 2000 customers participate in this program. Quality is often monitored by between lab agreement using proficiency specimens. Equally important are two other aspects of quality control. These are good correlation with a wide variety of field methods and ability to handle properly the unusual specimens. The SERALYZER instrument checks the quality of the calibration, the match-up between the test strip and the module, the proper operation during each light flash throughout the assay, the consistency of the temperature control, and the conformity of the assay in progress to a well behaved profile. In each case, failure to meet assay criteria will result in an aborted assay and an error code to the operator. The error code leads to a suggested method to correct the problem. No clinical result can be obtained until a corrective action is done. Specimens with analyte levels above the assay range can be reanalyzed with a further dilution. The instrument provides the correctly calculated value in the display.

The reagents for the SERALYZER system are in five categories: metabolites, enzymes, therapeutic drugs, electrolytes, and whole blood hemoglobin. The assays for cholesterol, triglycerides, uric acid, glucose, CK, and LD are

based on conventional enzymatic methods. The methods for total bilirubin and hemoglobin are based on the standard inorganic chemistries. For several analytes, special chemistry methods were needed to prepare homogeneous reagents. For urea, an adaptation of the ortho-phthalaldehyde method is used; for creatinine, the Benedict-Behre procedure permits a safe substitution for picric acid. For the transaminase enzymes, a method to generate a visible color change was incorporated into a unitized format. The method uses oxaloacetate decarboxylase, pyruvate oxidase, and peroxidase with indicator to generate color in proportion to the rate of formation of oxaloacetate by ASAT. The method detects and corrects for elevated levels of endogenous pyruvate. A similar scheme is used for ALAT. For the therapeutic drugs, theophylline, phenytoin, and phenobarbital, the Ames ARIS™ (apo-enzyme reactivation immunoassay system) technology was used (2). Competition between the drug and drug labeled with FAD for the antibody provides a controlled way to reactivate apo-glucose oxidase by FAD. Glucose oxidase and peroxidase convert glucose to colored indicator. The rate of color formation is proportional to the level of drug in the specimen. The assay is complete in less than two minutes. The ability to perform electrolyte measurements in a stable, colorimetric format is a second breakthrough methodology (1). The potassium assay is based on an ionophore-mediated, cation-proton exchange reaction. When a potassium ion is transferred to the organic phase, the deprotonation of an indicator dye, also in the organic phase, maintains charge neutrality and gives color proportional to the potassium level. Additional tests will be added to the system using the methods outlined above. This has been a brief overview of the system. Dr. Holmgård will present his experiences with the SERALYZER system in his paper.

References:

1. Charlton, S. C., Fleming, R. L. & Zipp, A.: Solid-Phase Colorimetric Determination of Potassium. Clin Chem 28:1857-1861, 1982.
2. Rupchock, P., Sommer, R., Greenquist, A., Tyhach, R., Walter, B. & Zipp, A.: Dry Reagent Strips Used for Determination of Theophylline in Serum. Clin Chem 31:737-740, 1985.
3. Walter, B.: Dry Reagent Chemistries in Clinical Analysis. Anal Chem 55: 498A-514A, 1983.
4. Zipp, A.: Development of dry reagent chemistry for the clinical laboratory, J Auto Chem 3:71-75, 1981.