2020

Common Error Flags in Automated Analyzers

The impact of spectrophotometry on the field of toxicology is indisputably significant. The introduction of the early commercially available ultraviolet-visible (UV-Vis) spectrophotometers laid the foundation for many toxicological analyses. In 1940, Arnold Beckman and colleagues at National Technologies Laboratories made their first laboratory spectrophotometer and later formed the Beckman Instrument Company. The Beckman Model DU Spectrophotometer was described by Nobel laureate Bruce Merrifield as "probably the most important instrument ever developed toward the advancement of bioscience" in early 1942.[1] In 1954, Bausch & Lomb introduced the Spectronic 20 UV-Vis spectrophotometer, and it became one of the most recognizable, mass-produced, low-cost UV-Vis spectrophotometers.[2]

The throughput of early spectrophotometers was limited by the configuration of a single reaction cell and a single display to output the absorbance or optical density value obtained. Because wavelength settings required manual adjustment to accommodate different analyses, the instruments were often used to perform one assay on a recurring basis. This began to change in 1973 with the advent and commercialization of the enzyme-multiplied immunoassay technique (EMIT®) by Syva Company for urine drugs of abuse testing. A variety of analyses could now be formulated using a standardized reaction cell and measured at common wavelengths based on a single enzyme–substrate reaction. This "open reagent" concept allowed instrument manufacturers to shift their focus from test development to automating instrument processes, such as sample pipetting, reagent addition, and reaction timing, to improve accuracy and throughput in their testing platforms.

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Among the earliest instruments to utilize open reagent systems were the Hitachi 704 and 705 automated analyzers. These analyzers were introduced in the early 1980s and featured automated pipetting, automated reagent addition, and multiple reaction cells that could be individually examined by a computer-controlled spectrophotometer at defined time intervals. This automation allowed for multiple tests to be run in a random access order on a single instrument platform under much more precisely controlled conditions. Later versions, such as the Hitachi 737 and the Olympus AU5000 family of instruments, performed so many tests simultaneously from such a wide variety of assays that operators could not effectively monitor all systems during instrument operation. To address the requirements of high-throughput instruments, manufacturers implemented on-board, real-time systems for monitoring instrument conditions during operation.

This monitoring is presented as "condition flags" or "error flags" that indicate the samples or tests that encountered a potential problem condition. During operation, error flags alert the operator to different types of error categories. These flags are, generally, categorized as "sampling" flags, "analysis" flags, "reagent" flags, and "communication" flags. Typically, these condition flags are assigned specific symbols with defined meanings by the manufacturer. When the error condition occurs, the associated symbolic flag is printed in the test result field for the analysis being performed, as exemplified below (**Figure 1**):

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S.ID
              032781
 Coc150     158:H ,r ,  ,  | Hydrc          :# ,? ,r ,  | THC50       -12:r ,  ,  ,  | 6AM        -0.1:r ,  ,  ,  |
 GenOx       -3:r ,  ,  ,  | AMP500         :/ ,  ,  ,  | pH             :# ,? ,r ,  | Oxycod       -5:r ,  ,  ,  |
 OPI2K         :/ ,  ,  ,  | Creat       38.7:r ,  ,  ,  | PCP            :/ ,  ,  ,  | MDMA           :/ ,  ,  ,  |
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Figure 1. Example Test Result Fields with Error Condition Flags

Manufacturers of automated clinical analyzers will provide some reference material to assist with the interpretation of these errors, and this information may be specific to individual models from the same manufacturer. The manufacturer or service contractor for the particular instrument is the recommended source for specific information pertaining to these types of flags. This article is intended to increase the reader's awareness about these types of errors and is not intended to serve as a replacement for manufacturer-specific information. **Note that the descriptions and flags presented are specific for the Beckman Coulter 5800 series of analyzers and its variants.**

Sampling-related flags provide indications of potential problems with the introduction of the sample into the reaction cuvette. The most obvious of these is the "insufficient sample volume" flag. The analyzer performs a liquid level detection by applying a weak electric charge to the sample probe and detecting the change in charge when the probe comes into contact with the liquid in the sample container. The error is generated when the liquid detection system indicates that there is inadequate sample in the container. This is indicated by the presence of a number sign, "#", in the test result field for the analysis in which the error occurred. Recommended corrective actions to resolve a "#" flag include verifying that the proper type of sample tube was used, verifying the amount of sample in the tube, and centrifuging the sample to remove air bubbles, followed by repeating all tests of the sample. Because "#" is an indication that sample was not added to the reaction environment, there may be additional flags linked to analysis-related errors. Given the limited report space available, these flags are often presented in a priority order established by the manufacturer. For example, on Beckman Coulter 5800 analyzers, a maximum of four flags can be displayed in a result field for a test.

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Another commonly encountered sampling-related flag is the "clot detected" flag, which is indicated by the presence of a "%" symbol. This is a generalized flag indicating that the sample probe became blocked or partially blocked during sample aspiration. The blockage is detected by minute fluctuations in the system fluid pressure during sample aspiration by the sample syringe. In urine samples, this is not usually caused by the presence of a clotted sample but an apparent blockage related to viscous or fibrinous urine, sediment in the sample, or a potential malfunction of the sampling system. Because the blockage is detected by pressure fluctuations within the syringe pump plumbing, it is important to verify that the sample needle connections and the sample syringe connections are properly sealed. Other recommended corrective actions consist of a review of all other results on the same sample generated before the flag was initiated and centrifugation of the sample as needed to remove suspended material, followed by reanalysis of the sample for the affected tests. If the error reoccurs, it may be necessary to clean or replace the sample probe and then reanalyze the sample for the affected tests or to check tubing connections along the sample syringe tubing path. Partial blockage of the three-way valve on the wash syringe has also been linked with the "%" flag and may require a service call. As with other condition flags, the "#" and "%" flags may be displayed in a prioritized order on the instrument report. Typically, these are higher-priority flags and are listed early in the result field.[3]

Analysis-related flags are indicators of reaction characteristics that take place outside of a pre-defined set of reaction conditions. The activation of these flags is based upon the optical density (OD) measured by the spectrophotometer at different times from sample introduction through the completion of a specific test. Various flags can be set to monitor OD and alert the operator to potential errors. One of these condition flags is the "unable to calculate a result" flag indicated by the presence of a "?". Another related analysis flag is the "unable to calculate concentration" flag indicated by the presence of a "!". This is very similar to the "?" flag and may occur in conjunction with a sample volume error, indicated by "#". Because these errors are generated as a result of an absorbance abnormality, it is important to verify that conditions that may adversely affect the reaction environment are absent. Troubleshooting should include inspection of the cuvettes and the cuvette wheel for possible overflow or flooding. Other anomalies with the sample, such as very high analyte concentrations or hematuria, may also generate these analysis-related flags. It may also be beneficial to verify the validity and quality of the reagents used for the particular affected test. An accepted corrective action for these flags would be an investigation of the previously described causes followed by repeat analysis of the sample for the affected test(s) using both diluted and undiluted aliquots.

Other analysis flags that are often encountered are the "OD of reaction is higher than the maximum OD range" flag indicated by the presence of a "D" and the "OD is higher than 3.0" flag indicated by the presence of a "@" in the test result field. These flags are generated when the OD for the sample exceeds the test's setting for the upper OD limit as set in the Specific Test Parameters or the instrument's maximum OD limit of 3.0. The "D" flag is specific to RATE and FIXED reaction methods, and the "@" flag is specific to END and FIXED reaction methods. If the flags occur on only scattered samples, the concentration of the analyte in the sample may be too high. Reanalysis and/or reanalysis with dilution would be an acceptable corrective action. If the flags continue across multiple tests, the cuvettes should be checked for overflow or wet cuvette wheels. If there is no sign of flooding or overflow, it is advisable to perform a photo calibration to assess the condition of the lamp and/or replace the lamp, if needed. If the flag is recurring on only one test channel, the reagent(s) for that test should be checked for proper preparation and placement.

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The reagent-related conditional flags can be regarded as general informational flags to assist in maintaining reagent lot records and alerting the operator to reagents that are depleted or that expired during the course of the analysis. The following condition flags can be useful in assisting with these types of monitoring. The "reagent expired" error flag is indicated by the presence of "a" in the test result. This flag is generated when the reagent has either expired or has been on-board beyond the time period defined in the Specific Test Parameters page for that particular test. The proper corrective action for addressing this type of flag is to replace the existing bottle of reagent, perform a reagent check, and recalibrate the test if necessary.

A related flag that is often encountered in larger volume laboratories is the "insufficient reagent" flag indicated by the presence of "R" in the test result. This flag is triggered by the reagent liquid level-sensing module associated with the reagent probe. The reagent probe detects the level of liquid in the reagent bottle and determines the amount of reagent left in the container based on a capacity algorithm associated with the on-board container. The volume in the reagent bottles is recalculated, and the remaining number of tests or "shots" left for each reagent is updated after the completion of the reagent check dialog routine for the particular test channels specified in the reagent check dialog box. It is important to note that the software is limited to bottles made by the instrument manufacturer. The use of aftermarket bottles can cause erroneous calculations of remaining reagent volume. If the flag occurs in the presence of sufficient reagent, the neck of the reagent bottle should be checked for bubbles or droplets. After bubbles and droplets have been removed, the reagent check should be repeated. Because this error is generated by liquid level detection, it is important to verify that the reagent probe is properly installed and connected. Any sample with an "R" condition flag should be reanalyzed because of the possibility of false values based on reagent shortage.

Another often encountered flag that may be associated with a reagent is the "y" flag or the "reagent blank or routine (patient) OD at first photometric point high" flag. This flag is activated when the first photometric point OD of the reagent blank or the OD at "Point 0" (P0) of normal analysis is higher than the reagent OD limit defined in the Specific Test Parameters page. This flag is more correctly categorized as an analysis-related issue, but if it occurs routinely throughout an assay, it can be an indication of a problem with the associated reagent. Typically, a reagent is improperly placed in the reagent compartment, is contaminated, or was prepared incorrectly. The corrective action for this flag would be to inspect the reagent expiration and on-board expiration dates. The reagent should be checked for proper preparation and correct positioning in the reagent compartment, followed by reanalysis for the affected test(s). It is important to remember that this flag is an analysis-related flag and may be triggered by a characteristic of an individual sample. A thorough and attentive investigation is often required to determine the source of this flag.

The final condition flags being discussed are those linked to communications of test order information or result information between the instrument and the laboratory information system (LIS). One commonly encountered communication flag is the "/", which is translated as a "test pending or not analyzed" condition. The full interpretation of this flag is that the instrument has received the order for the test, but the testing is still in process. This is typically associated with either a sample insufficiency (#) or possibly a reagent shortage flag (R), and the testing is not completed. The corrective action associated with this flag would be to resolve the sample insufficiency or reagent shortage and reanalyze the sample. The original testing of the sample may have been completed for some of the ordered tests. The presence of a lower case

"r" indicates that the result has been transferred or "reported" to the LIS through the instrument's online communication. In some cases, the result that has been reported may have another error flag, such as a "?". In this type of scenario, the sample would need to be reanalyzed for the test(s) with a "?", as this flag indicates a potential analytical error.

These flags are intended to inform the instrument operator of deviations from the expected performance of any test for any sample submitted to the analyzer. The complete listing of flags is a vital source of information to any laboratory using this type of automated chemistry analyzer and can be obtained from the instrument manufacturer. The flags facilitate monitoring of sample-related events, analysis-related events, and information on instrument status. Many of the flags will be best addressed by repeat analysis of the sample for the affected test, either undiluted or with an appropriate dilution. These types of flags and on-board monitoring systems are of critical importance to assure analytical integrity in automated analyzers as they continue to strengthen the role of spectrophotometry in the field of toxicology.

This article highlights commonly encountered flags utilized by the Beckman Coulter AU5800 series of instruments and is based on product literature available online at the time of writing.[3] **Appendix 1** at the end of this newsletter provides the flags and the manufacturer's recommended corrective action for each[3] and is intended to serve as a ready reference guide for users of AU5800 instruments. **Figure 2** provides an example flowchart showing the process from observing the flag (in this example, "@") and identifying the error to troubleshooting and taking appropriate corrective action.

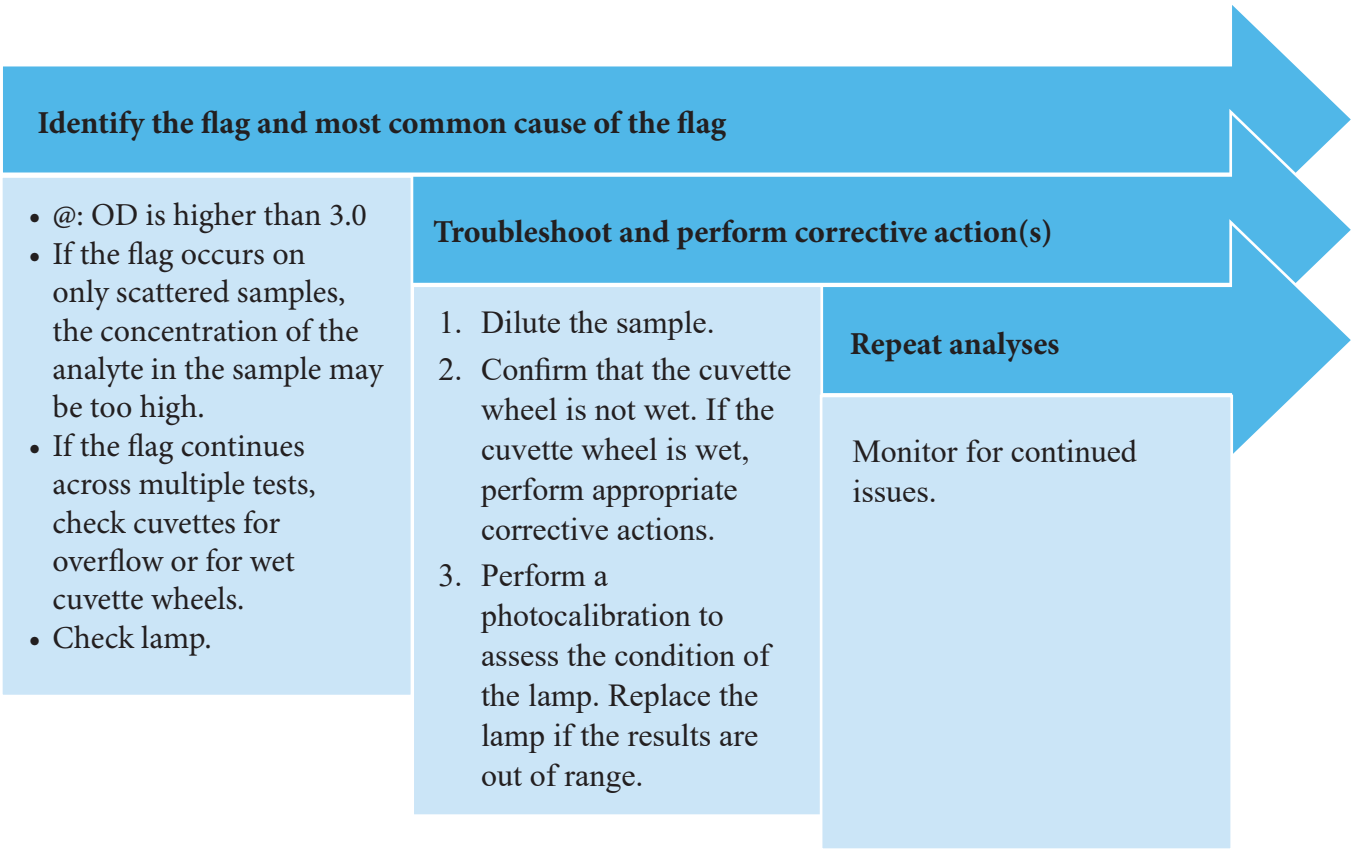

Figure 2. Example Flowchart to Address an Analysis Flag

References

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Randal Clouette has over 30 years of experience in analytical toxicology including veterinary diagnostic and post-mortem toxicology, human therapeutic drug monitoring, and workplace drug testing in urine, oral fluid, and hair. He has served as a Responsible Person for a U.S. Department of Health and Human Services (HHS)-certified laboratory, and is a current NLCP inspector. Mr. Clouette is a registered Toxicological Chemist and a Diplomate of the American Board of Forensic Toxicology. He is currently the manager of analytical toxicology for Clinical Reference Laboratory (CRL). Prior to joining CRL, Mr. Clouette worked as the Director of Esoteric Testing for Quest Diagnostics, Inc., performing anabolic steroid analysis in urine and drugs of abuse testing in hair. He also has an extensive background in chromatography and mass spectrometry.

Appendix 1: Beckman Coulter AU5800 Series Flags and Recommended Corrective Actions

Flag	Definition	Suggested Corrective Actions
#	Insufficient sample detected	1. Review all other results for the same sample before the flag to confirm validity and consistency (no extremely low or high values). 2. Confirm that there is enough sample in the cup or tube. Repeat analysis using a valid cup or tube with sufficient sample volume. ***Note:*** *if the flag is present upon reanalysis, continue with actions described below.* 3. Wipe the probe with an alcohol prep pad (70% isopropyl alcohol) and inspect the probe to confirm that it is installed and connected correctly. 4. Replace the sample probe. 5. Confirm that the correct sample cup or tube is in use.
%	Clot detected	1. Review all other results for the same sample before the flag to confirm validity and consistency (no extremely low or high values). 2. Confirm that the sample is free of clots or fibrinous material. If necessary, centrifuge the sample and repeat analysis. 3. If the flag is present upon reanalysis, clean or replace the sample probe.
?	Unable to calculate a result	1. High concentration of drug or hematuria may affect the test. Dilute the sample and repeat analysis. ***Note:*** *if the flag is present upon reanalysis, continue with actions described below.* 2. Confirm the reagent condition. 3. Review the reaction data including the reaction data processed immediately before and after the flagged result. • In the presence of any abnormality with the reaction data, inspect the cuvettes and cuvette wheel for a possible overflow. • If the cuvette wheel is wet, perform appropriate corrective actions.

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Flag	Definition	Suggested Corrective Actions
!	Unable to calculate concentration	1. If the flag is for a single sample, reanalyze and/or dilute and reanalyze if necessary. 2. If multiple samples are affected, review all operating parameters, such as: • Reagent quality • Calibration • Sample integrity • General system issues. 3. Review the reaction data including the reaction data of those samples processed immediately before and after the flagged result. • In the presence of any abnormality, inspect the cuvettes and cuvette wheel for a possible overflow. • If the cuvette wheel is wet, perform appropriate corrective actions. 4. Confirm that all tubing is connected correctly.
D	OD of reaction is higher than the maximum OD range	1. If this flag is only generated on one or a few samples, inspect the sample quality: • If the sample has a high OD, the sample may be severely lipemic, icteric, or hemolytic or may contain an excessive concentration of the analyte being tested. Dilute the sample and repeat analysis. • If the sample has a low concentration, no reaction will occur in the cuvette. Confirm that there is enough sample volume in the tube. 2. If this flag is only generated on one reagent, inspect the reagent quality and for reagent contamination: • Inspect the reagent expiration, on-board expiration, and reagent blank expiration. • Confirm that the reagent was prepared correctly. • Confirm that fixed reagents are in the correct position. 3. If this flag is generated randomly on several samples and several different tests: • Confirm that the cuvette wheel is not wet. If the cuvette wheel is wet, perform appropriate corrective actions. • Perform a photocalibration to assess the condition of the lamp. Replace the lamp if the results are out of range.
@	OD is higher than 3.0	1. Dilute the sample and repeat analysis. 2. Confirm that the cuvette wheel is not wet. If the cuvette wheel is wet, perform appropriate corrective actions. 3. Perform a photocalibration to assess the condition of the lamp. Replace the lamp if the results are out of range.

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Flag	Definition	Suggested Corrective Actions
a	Reagent expired	Replace the reagents and perform a reagent check and a calibration if necessary.
R	Insufficient reagent detected	1. Review all other results for the same sample before the flag to confirm validity and consistency (no extremely low or high values). 2. Place new reagent onto the system and repeat analysis. • Confirm that the reagent is placed securely and correctly on the reagent tray and that the partitions and adapters hold the reagent in the correct position for level sensing. 3. If the flag occurs even though there is sufficient reagent, the reagent bottle may contain bubbles. Remove the bubbles and perform another reagent check. 4. Dry the reagent bottle opening if it is wet. 5. Inspect the reagent probe and clean or replace as required. 6. Confirm that the reagent probe is correctly installed and connected.
y	Reagent blank or routine (patient) OD at first photometric point high	1. Inspect the reagent expiration and on-board expiration date. 2. Confirm that the reagent was prepared correctly. 3. Confirm the Reagent OD Limit range programmed in Specific Test Parameters is correct. 4. Replace the reagent and repeat analysis.
/	Test pending or not analyzed	Perform analysis as needed.
r	Result has been transferred to the laboratory information system (LIS) through online communication	No additional action required.