TITLE: Analytical Validation of Body Fluid Testing

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Regulatory agencies such as CAP require analytical validation for performing body fluid testing in the clinical chemistry lab. The extent of the validation depends on whether the test is FDA-approved for a particular body fluid. Unfortunately, there are very few tests that are FDA-approved, with the exceptions of CSF protein and glucose and pleural fluid pH on some blood gas analyzers. The remaining body fluid tests that labs perform are considered FDA-modified by virtue of the fact that a specimen other than serum or plasma is being tested. As a result, we must perform a full validation of these body fluid tests.

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The standard required experiments to satisfy most regulatory agencies include accuracy, precision, reportable range, reference interval, analytical sensitivity and analytical specificity. In addition, specimen stability, clinical sensitivity and clinical specificity are not required but may be equally as useful.

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The recommended place to start the analytical body fluid validation is by establishing accuracy or the ability to measure the true concentration or activity of an analyte. The goal is to confirm that an analyte in a body fluid matrix can be measured accurately with instruments and reagents that are FDA-approved for serum or plasma.

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The predominant issue that contributes to potential interference when testing body fluids is the impact that an alternate matrix has on accuracy. Matrix interference is caused by any variation in the composition of the sample that influences the ability to accurately measure an analyte.

There are multiple contributors including properties such as pH and ionic strength that may affect enzymatic rate reactions to falsely increase or decrease a result, such as measuring amylase in a gastric fluid of pH 4. If the result is undetectable, you wouldn't know if it is due to the absence of amylase activity in the fluid or because amylase activity is inhibited at low pH. Additionally, body fluid samples can have increased viscosity or surface tension that can give rise to sampling errors and short samples. You may observe irreproducibility in repeat measurements when such sampling errors occur. The protein and lipid content may also vary in a body fluid matrix, which can influence the solubility of the analyte of interest or other components of the reaction, thus potentially impacting accuracy.

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The recommended approach to establishing accuracy in a body fluid validation experiment is to perform two experiments: spiked recovery in a low sample and dilution of a high sample. These experiments should be performed for multiple sample types, chosen to reflect those received most frequently for the test being validated. Each fluid should be tested in triplicate at minimum to verify reproducibility. Calculate % recovery using the ratio of measured over expected. The acceptance criteria for recovery and slope will be influenced by method performance specifications for plasma/serum, and/or where there is a potential to impact the clinical interpretation of the results. For instance, if you determine that lipase under recovers in pancreatic cyst fluid by 10%, but the test is "positive" if lipase is 10-fold greater than serum lipase, the analytical bias observed does not impact the clinical interpretation of the result. Additional questions to consider include choice in material to spike with and how much should be added. For most applications, using a high calibrator, control, or serum sample is appropriate when the amount added is kept to a minimum of less than 10% volume change. When choosing a diluent, there are a few options which include manufacturer-recommended diluent or a matrix similar choice, such as 7% bovine serum albumin solution. The accuracy experiments are important to perform first and perform well because they form the basis for the choices made throughout the rest of the analytical validation.

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Precision experiments are performed to demonstrate reproducibility of the method. When designing the precision experiments, it is important to consider which and how many body fluid types to include. This is where the hard work in the accuracy experiments pays off because it would be appropriate to choose one or two representative body fluids to perform intra- and inter-assay precision experimentally similar to plasma or serum validation measuring 20 replicates and calculating %CV. Once again, the acceptance criteria may be based on serum or plasma performance or where clinical interpretation is impacted.

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Reportable range experiments are performed to demonstrate the range of concentrations which the analyte can be accurately measured on a sample prior to dilution or concentration. This may be chosen to reflect the serum or plasma analytical measuring range; however, this is not mandatory.

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The reportable range is established experimentally by performing a mixing study of high and low samples chosen to encompass the intended AMR. When mixed according to the scheme shown in 1:1 ratios, 5 equally spaced samples are created which span the AMR. Acceptance criteria may be set according to serum or plasma specifications or to meet clinical needs. One last thing to keep in mind is semi-annual calibration verification and changes that may occur with new lots or reagent reformulations when these experiments may need to be repeated following initial validation.

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Reference intervals are important to guide proper interpretation of test results; however, for body fluid testing this is challenging at best, and nearly impossible at worst. Normal healthy body fluid donors do not exist and always require an invasive procedure to collect the specimen. A handful of literature references do exist; however, most are older and used methods that may be outdated in today's lab.

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The goal is to establish normal ranges for clinical decision limits for a given analyte in a given fluid. For the small number of FDA-approved body fluid tests, the package insert values can be used. Be aware that in vitro diagnostic manufacturers are unlikely to seek approval for new body fluid specific tests in the future because they face the same challenges laboratories do and therefore, are unlikely to invest the time and resources, at least for the foreseeable future. For non-FDA-approved tests, a thorough literature review of clinical utility and provision of interpretive information with the sample report that is test- and fluid-specific is an option. For many tests, it may be sufficient to recommend interpreting the result in conjunction with a paired serum or plasma sample collected at the same time as the body fluid.

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The limit of quantitation is a determination of acceptable precision at or below the lowest reportable range. The experiment is similar to precision. Keep in mind that low end precision may not impact the utility of the test; however, it is reassuring to confirm that the assay has similar precision to serum or plasma at the low end in a body fluid matrix.

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Analytical specificity is tested by determining the impact that specimen handling and common interferences have on accuracy. Experimentally, samples are tested before and after treatment such as with hyaluronidase to decrease sample viscosity, or spiking with an interferrent such as hemoglobin or bilirubin at increasing concentrations. Analysis includes calculating % difference and comparing to your preset acceptance criteria. For example, +/– 10% difference might be used to set a threshold for degree of hemolysis tolerance.

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We will wrap up our discussion by considering some final useful pieces of information. Body fluid sample stability is important to measure when determining appropriate pre-analytical transport conditions. In addition, determining appropriate storage conditions is important when offering to perform additional tests or rechecking samples in the case of QC failures. The last piece of advice involves handling odd requests, such as total protein in a nasal secretion. Contact the provider to ask whether they meant to order the test. Sometimes a test is ordered accidentally or they could use some guidance in choosing the appropriate test. If they insist the odd request is still desired, ask how they will interpret the result. If they are unsure, it is appropriate to suggest they cancel the order. If they have researched the test utility in this fluid and can provide some rationale for performing the test, the laboratory director or their surrogate can decide whether to approve the test. When considering approving a test, it will be important to verify accurate results are produced, which can be accomplished by performing additional dilutions or recovery depending on the neat result.

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In conclusion, by following these guidelines, you will have completed a regulatory compliant validation as well as improved patient care because your laboratory will now be offering tests that have proven accuracy and interpretive information.

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