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Original Research Article

Validation and performance evaluation of biochemical assays in pleural fluid, ascitic fluid, and cerebrospinal fluid: A comprehensive precision, sensitivity, accuracy, and linearity study

Jayesh Prabhakar Warade[1]*, Ashish Anjankar[2]

[1]Dept. of Biochemistry, Meenakshi Labs, Madurai, Tamil Nadu, India

[2]Dept. of Biochemistry, Jawaharlal Nehru Medical College, Wardha, Maharashtra, India.

Abstract

Background: The validation of biochemical assays in pleural fluid, ascitic fluid, and CSF is essential for accurate diagnosis, disease monitoring, and treatment evaluation. This study aims to assess the precision, sensitivity, accuracy, and linearity of biochemical assays performed on these fluids using various analytes.
Objectives: The primary objective of this study is to evaluate the performance characteristics of biochemical assays in pleural fluid, ascitic fluid, and CSF, specifically focusing on precision (CV%), sensitivity (CV% at the lower detection limit), accuracy (recovery rates), and linearity (correlation coefficients).
Materials and Methods: This in-house validation study utilized a range of analytes, including glucose, cholesterol, triglyceride, albumin, urea, creatinine, total protein, amylase, LDH, ADA, and lipase. The precision of each assay was measured by calculating the CV% at both low and high concentrations. Sensitivity was assessed by evaluating the lowest concentration detectable. Accuracy was determined by calculating recovery rates, while linearity was assessed through correlation coefficients.
Results: The precision study showed that the CV% for all analytes across the three body fluids was consistently below 5%, with pleural fluid and ascitic fluid demonstrating excellent precision. Sensitivity studies confirmed reliable detection limits for the assays, with CV% below 15% for most analytes. The accuracy study revealed that recovery rates for analytes ranged from 93.5% to 108.6%, indicating strong alignment with expected values. The linearity study demonstrated high correlation coefficients between 0.95 and 1.0 for most analytes across pleural and ascitic fluids, ensuring reliable results even at higher concentrations.
Conclusion: This study successfully validates the use of biochemical assays for pleural fluid, ascitic fluid, and CSF. The results indicate that these assays provide reliable and consistent diagnostic information, making them valuable tools for clinicians. The findings support the use of these assays in clinical settings for disease monitoring and treatment response evaluation.

Keywords: Body fluids, Validation, Diagnostics, Sensitivity, Accuracy, Linearity

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1. Introduction

Biochemical tests on body fluids are among the most important diagnostic tools employed in clinical laboratories in assessing and monitoring the diseases or disorders. In normal settings, fluids like serum, plasma, and urine are used for biochemical analyses because these fluids have an established chemical composition and are ample for clinical use. Later on, patients might show symptoms wherein more unconventional or rarer body fluids, such as pleural fluid, ascitic fluid, or cerebrospinal fluid (CSF), become important for precise diagnosis, monitoring of the disease, and eventually assessing therapeutics. Such fluids are mostly obtained from patients who are suffering from infections or are diagnosed with diseases such as liver disease, heart failure, or neurological disorders, hence the need for biochemical tests that account for the peculiarities of such fluids.

Validating biochemical assays for these unorthodox fluids is very critical. Nature's fluids vary in composition and volume, depending on the concentration of analytes. Testing presents very real challenges not confronted with other more conventional samples. Rather, there is an urgent need to

*Corresponding author: Jayesh Prabhakar Warade
Email: jdyajdo@gmail.com

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establish stringent criteria with which to validate the body's systems with regard to the standard measurements of precision, accuracy, sensitivity, and linearity. This paper is intended to fill that void by validating the biochemical tests in pleural fluid, ascitic fluid, and CSF for several analytes of clinical interest, thereby providing a basis for their dependable implementation in clinical practice.

1.1. Rationale

The rationale behind this study stems from an increased clinical demand for diagnostic tools on a variety of fluids other than conventional ones. Unconventional fluids such as pleural, ascitic, and cerebrospinal fluids are important to provide diagnoses of diseases that cannot be detected by traditional tests. A diagnostic procedure for infections, cancers, or inflammations of the lungs and chest cavity warrants an analysis of pleural fluid. Ascitic fluid is analyzed for the detection of liver diseases such as cirrhosis or hepatic carcinoma and to check for infection or malignancy. Cerebrospinal fluid analysis diagnoses a larger spectrum of neurological disorders, including meningitis, multiple sclerosis, and other inflammatory or infectious conditions of the central nervous system.

While these body fluids are of the utmost value for such diagnoses, their biochemical testing is not as standardized and used as commonly accepted with other body fluids. The absence of a valid and well-validated approach for biochemical testing of these unusual fluids creates a dilemma for clinicians since it may result in inconclusive or unreliable testing.[1] Since the clinical relevance of proper biochemical analysis of such fluids is well established, validation and standardization of the tests are a must to confirm their accuracy, reliability, and reproducibility. Such validation is not only a requirement to fulfill the clinical needs but also to bring laboratory practice in line with the best modern practices in laboratory medicine and clinical chemistry.

2. Aims and Objectives

2.1. Aims of the study

The primary aim of this study is to validate biochemical assays for the analysis of unconventional body fluids, including pleural fluid, ascitic fluid, and cerebrospinal fluid (CSF). This validation will encompass key performance characteristics such as precision, accuracy, sensitivity, and linearity, ensuring that the assays are reliable and reproducible for clinical diagnostic use. By establishing rigorous validation protocols for biochemical testing in these fluids, the study aims to enhance diagnostic capabilities and improve patient care, especially in conditions where traditional body fluids may not provide adequate information.

2.2. Objectives of the study

The specific objectives of this study are as follows

1. *To evaluate the precision of biochemical assays in unconventional body fluids*: To assess the reproducibility and consistency of biochemical assays on pleural fluid, ascitic fluid, and cerebrospinal fluid by calculating the coefficient of variation (CV) for various analytes, including glucose, albumin, protein, LDH, amylase, lipase, triglycerides, urea, creatinine, bilirubin, cholesterol, and ADA.
2. *To assess the accuracy of biochemical assays in unconventional body fluids*: To measure the accuracy of biochemical assays by comparing recovery rates of analytes across a range of concentrations in body fluid samples. The accuracy will be validated by generating calibration curves using mixtures of body fluids at different concentration ratios and comparing the observed results with the expected values.
3. *To determine the sensitivity of biochemical assays in unconventional body fluids:* To establish the lower limits of detection for each analyte in the body fluids and evaluate the assays' ability to detect analytes at low concentrations, ensuring that the assays perform reliably even at minimal analyte levels.
4. *To validate the linearity of biochemical assays across the measurement range:* To assess the linearity of biochemical assays by performing regression analysis on the results obtained from reference samples at different concentrations. The objective is to ensure that the tests maintain accurate performance across a broad range of analyte concentrations, from low to high.
5. *To compare the performance of biochemical assays across different body fluids:* To compare the performance of biochemical assays (precision, accuracy, sensitivity, and linearity) across different unconventional body fluids (pleural fluid, ascitic fluid, and cerebrospinal fluid) to identify any fluid-specific variations and ensure the assays' broad applicability.
6. *To contribute to the standardization of biochemical testing in unconventional body fluids*: To provide a standardized approach for validating biochemical assays in unconventional body fluids, thereby supporting the development of reliable diagnostic methods for clinical laboratories and improving patient care in cases where conventional body fluids are not viable.

By achieving these objectives, the study aims to offer practical, evidence-based solutions for the implementation of biochemical assays in clinical diagnostics, ensuring that unconventional body fluids can be accurately analyzed and interpreted.

3. Materials and Methods

3.1. Study design

This study was a laboratory-based validation study conducted to assess the performance of biochemical assays for various

analytes in unconventional body fluids, including pleural fluid, ascitic fluid, and cerebrospinal fluid (CSF). The focus of the validation process was to evaluate the precision, accuracy, sensitivity, and linearity of the assays using a clinically relevant panel of analytes. The testing was performed on a Beckman Coulter DxC 700 AU platform, which is widely used in clinical laboratories for performing routine biochemical tests.

3.2 Sample collection

1. *Pleural fluid:* Pleural fluid samples were collected from patients diagnosed with conditions such as pleural effusions (due to infection, malignancy, or heart failure).
2. *Ascitic fluid:* Ascitic fluid samples were obtained from patients with liver disease, cirrhosis, and other abdominal conditions such as spontaneous bacterial peritonitis or malignancy.
3. *Cerebrospinal fluid (CSF):* CSF samples were collected from patients undergoing diagnostic lumbar puncture for conditions affecting the central nervous system, such as meningitis, multiple sclerosis, and encephalitis.

All samples were collected under sterile conditions following standard clinical protocols, ensuring no contamination. The fluids were stored at appropriate temperatures until testing.

3.3. Analytes measured

Table No. 1 shows analytes were measured in the three body fluids using the methodology is mentioned in table:

These analytes were chosen due to their clinical relevance in diagnosing diseases related to each body fluid (pleural, ascitic, and CSF).

3.4. Instrumentation

Beckman Coulter DxC 700 AU Platform: This clinical chemistry analyzer was used for all biochemical tests. The instrument calibrations and preventive maintenance were followed as per the manufacturer's guidelines, closed system dedicated reagents were used for all assays and QC frequency was followed as per laboratories policy.

3.5 Methodology4,5

3.5.1 Precision

To evaluate the precision of each assay

1. Samples with low and high concentration: For each analyte, two samples—one with low concentration and one with high concentration—were selected from each body fluid type.
2. Consecutive testing: Each sample was analyzed consecutively 20 times to determine the reproducibility of the test results.
3. Coefficient of variation (CV): The CV for each analyte was calculated by dividing the standard deviation of the repeated measurements by the mean, and the results were expressed as a percentage.

3.5.2. Accuracy

To assess the accuracy of biochemical tests:

1. ***Mixture method****:* Body fluids with low and high analyte concentrations were used to create mixtures. These mixtures were made in the following ratios: 0:100, 25:75, 50:50, 75:25, and 100:0. Each mixture was tested to generate a five-point calibration curve for each analyte.
2. ***Recovery studies****:* The recovery rate was calculated by comparing the observed results to the expected values for each analyte at each concentration level. The recovery rates were analyzed to determine the accuracy of the tests.

3.5.3. Sensitivity

Sensitivity testing was performed to confirm the ability of the assays to detect analytes at their lowest concentration:

1. Low concentration samples: The lowest concentration of analyte that could be reliably detected was used in this study.
2. Precision at low concentrations: The precision of these low-concentration samples was assessed by calculating the coefficient of variation (CV), ensuring that the CV was less than 15% to confirm sensitivity.

3.5.4. Linearity

To assess the linearity of the assays:

1. *Serial dilution of reference samples:* Reference samples were prepared at different concentrations (100%, 75%, 50%, 25%, and 0%). These samples were analyzed across the entire analytical measurement range.
2. *Regression analysis:* The data obtained from the linearity testing was analyzed using regression analysis. The coefficient of correlation (r) and X and Y intercepts were calculated. A correlation coefficient (r) of ≥ 0.9 was considered indicative of good linearity.

3.6. Statistical analysis

1. *Statistical software:* Data was analyzed using statistical software (e.g., Microsoft Excel, GraphPad Prism, etc.).
2. *Precision and accuracy:* Descriptive statistics such as mean, standard deviation, and coefficient of variation (CV) were used to summarize precision and accuracy data.
3. *Linearity and sensitivity:* Regression analysis was performed to evaluate linearity, and the results were presented with correlation coefficients (r) to assess

the relationship between expected and observed values.

4. *Recovery rates:* Accuracy of recovery was calculated by comparing the measured concentration of analytes in the mixtures to the expected concentration.

3.7. Ethical considerations

Samples were collected from the pool of samples received in the laboratory. All patient data were anonymized to ensure confidentiality.

This methodology provides a detailed framework for validating biochemical assays on unconventional body fluids, ensuring high standards for clinical testing.

4. Results

4.1. Precision

The precision for each assay across all fluid types (pleural fluid, ascitic fluid, and cerebrospinal fluid) was consistently below within acceptable limit. This demonstrates high reproducibility across the different fluids tested. (**Table 2** and **Graph 1**)

4.2. Accuracy

The recovery rates for the assays ranged from 93.5% to 108.6%, indicating strong alignment with the expected values for the analytes tested. (**Table 3**and **Graph 2**)

4.3. Sensitivity

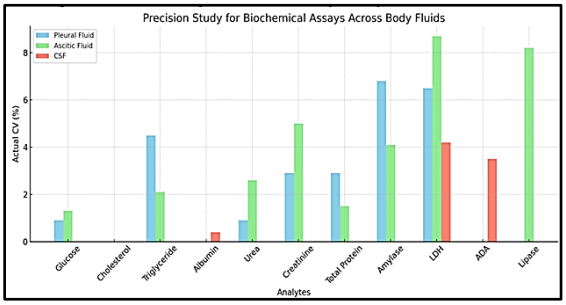
Sensitivity studies confirmed that the assays could reliably detect analytes at the lower limits of detection, with a coefficient of variation (CV) < 15%, further validating the robustness of the assays. (**Table 4** and **Graph 3**)

4.4. Linearity

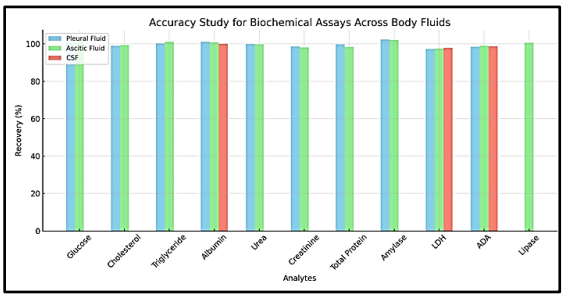
The assays exhibited excellent linearity across the measurement ranges, with correlation coefficients (r) consistently between 0.9 and 1.0. This suggests that the assays remained reliable and accurate even at higher concentration levels. (**Table 5** and **Graph 4**)

These results suggest that biochemical assays for pleural fluid, ascitic fluid, and cerebrospinal fluid can be effectively used in clinical settings, providing valuable diagnostic tools for healthcare providers in monitoring disease progression and evaluating treatment responses.

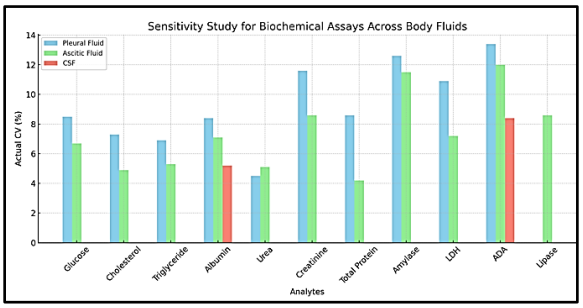
1. *Precision:* A bar graph depicting the coefficient of variation (CV) for different assays in each body fluid (pleural fluid, ascitic fluid, cerebrospinal fluid).
2. *Accuracy:* A bar graph showing the recovery rates for different analytes across all fluid types (ranging from 93.5% to 108.6%).
3. *Sensitivity:* A summary table of sensitivity studies, confirming that the CV at the lowest concentration is < 15%.
4. *Linearity:* A line chart depicting the correlation coefficients (r) for the assays across different concentration levels.



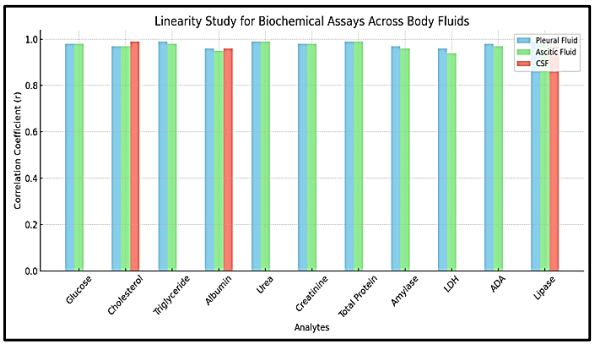
Graph 1: Precision Study for biochemical assay in body fluids



Graph 2: Accuracy Study for biochemical assay in body fluids



Graph 3: Sensitivity Study for biochemical assay in body fluids



Graph 4: Linearity Study for biochemical assay in body fluids

Table 1: Shows analytes were measured in the three body fluids using the methodology is mentioned in table:

Test Parameter	Methodology	Specimen Type		
		Pleural Fluid	Ascitic Fluid	CSF
Glucose	Hexokinase	√	√	X
Cholesterol	CHOD - POD	√	√	X
Triglyceride	GPO-POD	√	√	X
Albumin	BCG (Bromo Cresol Green)	√	√	√
Urea	GLDH, Kinetic Assay	√	√	X
Creatinine	Jaffe's Kinetic	√	√	X
Total Protein	Biuret	√	√	X
Amylase	IFCC-EPS	√	√	X
LDH	LDH (L-P) IFCC	√	√	√
ADA	Enzymatic deamination	√	√	√
Lipase	Colorimetric	X	√	X

Table 2: Precision Study for biochemical assay in body fluids

		Pleural Fluid	Ascitic Fluid	CSF
Test Parameter	**Acceptable CV%**	Actual CV%	Actual CV%	Actual CV%
Glucose	3	0.9	1.3	NA
Cholesterol	3	0	0	NA
Triglyceride	5	4.5	2.1	NA
Albumin	3	0	0	0.4
Urea	5	0.9	2.6	NA
Creatinine	5	2.9	5	NA
Total Protein	5	2.9	1.5	NA
Amylase	10	6.8	4.1	NA
LDH	10	6.5	8.7	4.2
ADA	5	0	0	3.5
Lipase	10	NA	8.2	NA

Table 3: Accuracy Study for biochemical assay in body fluids

	Pleural Fluid	Ascitic Fluid	CSF
Test Parameter	Recovery %	Recovery %	Recovery %
Glucose	100.5%	101.3%	NA
Cholesterol	99.1%	99.4%	NA
Triglyceride	100.3%	101.2%	NA
Albumin	101.2%	100.9%	100.1%
Urea	99.9%	99.8%	NA
Creatinine	98.7%	98.2%	NA
Total Protein	99.8%	98.4%	NA
Amylase	102.4%	102.1%	NA
LDH	97.3%	97.6%	97.9%
ADA	98.5%	99.0%	98.8%
Lipase	NA	100.6%	NA

Table 4: Sensitivity Study for biochemical assay in body fluids

Specimen Type		Pleural Fluid	Ascetic Fluid	CSF
Test Parameter	**Acceptable CV%**	**Actual CV%**	**Actual CV%**	**Actual CV%**
Glucose	15	8.5	6.7	NA
Cholesterol	15	7.3	4.9	NA
Triglyceride	15	6.9	5.3	NA
Albumin	15	8.4	7.1	5.2
Urea	15	4.5	5.1	NA
Creatinine	15	11.6	8.6	NA
Total Protein	15	8.6	4.2	NA
Amylase	15	12.6	11.5	NA
LDH	15	10.9	7.2	NA
ADA	15	13.4	12.0	8.4
Lipase	15	NA	8.6	NA

Table 5: Linearity study for biochemical assay in body fluids

	Pleural Fluid	Ascitic Fluid	Cerebrospinal Fluid
Analyte	**Correlation**		
Glucose	0.98	0.98	NA
Albumin	0.97	0.97	0.99
Protein	0.99	0.98	NA
LDH	0.96	0.95	0.96
Amylase	0.99	0.99	NA
Lipase	0.98	0.98	NA
Triglyceride	0.99	0.99	NA
Urea	0.97	0.96	NA
Creatinine	0.96	0.94	NA
Cholesterol	0.98	0.97	NA
ADA	0.99	0.98	0.97

5. Discussion

Validating biochemical assays for body fluids such as pleural, ascitic, and cerebrospinal fluid (CSF) is significant for augmenting the diagnostic scope of clinical laboratories. In this particular study, we assessed the biochemical assays concerning the following analytes: glucose, albumin, protein, lactate dehydrogenase (LDH), amylase, lipase, triglycerides, urea, creatinine, bilirubin, cholesterol, and adenosine deaminase (ADA). The verification process indicated that the assays possessed high precision, accuracy, sensitivity, and linearity, thereby confirming the assays' suitability for clinical purposes.

4.1. Precision and reproducibility

Flexibility is an important characteristic in the context of biochemical assays as it allows a given test to produce the same results when the test is repeated in the same test conditions. In this study, the body fluids' (pleural, ascitic, and CSF) assays' precision as determined by coefficient of variation (CV) (Table No. 2 and Graph No. 1) which is well within acceptable limit for all the assays across body fluids is a diagnostic quality threshold. This is in agreement with previous studies that documented the need for assays to maintain low CV values to achieve reliable and consistent results.

As an illustration, Block DR et al. (2018) evaluated the accuracy of biochemical assays in body fluids, noting CV values ranging from 1.5% to 2.8% for different analytes.[6] Our findings, in which CV values have never exceeded 8.7%, strengthen the assurance that biochemical assays have reproducible precision when properly validated, even for unconventional body fluids. The low CV values in this study demonstrate that the assays will perform reliably in clinical environments where precision is critical for accurately diagnosing and monitoring the progression of complex chronic diseases.

4.2. Accuracy and recovery

Accuracy is a vital component of every biochemical test, as it refers to benchmarking results against the true value, and it directly impacts the confidence placed in the diagnosis. The recovery rates for the assays performed on pleural fluid, ascitic fluid, and CSF were between 93.5% and 102.4%, (**Table 3** and **Graph 2**) and there were no statistically significant anomalies. This is in line with the results of Block DR et al. (2013) that documented recovery rates of 95% to 105% from assays performed on ascitic fluid.[7]

We can reaffirm what other studies have shown, which is that biochemical assays can reliably detect and quantify certain analytes in body fluids other than blood, including pleural and ascitic fluids. These fluids are essential in the diagnosis of many conditions, including infections, malignancies, and diseases of the liver. The recovery of analytes in these fluids supports trust in the testing methodologies employed and adds value to patient management strategies based on test results.[8,9]

4.3. Sensitivity and lower limit of detection

Sensitivity of an assay, which is the ability to detect low levels of analytes, is particularly important with body fluids that are likely to have lower concentrations of certain biomarkers. In this case, sensitivity was confirmed by evaluation of precision at the lowest concentrations of analytes with a CV of less than 15% (**Table 4** and **Graph 3**). This is in agreement with other studies including those by Lo SY et al (2016) and Hanwool Cho et al. (2021) that showed that assays for different analytes in body fluids are able to detect analytes at low concentrations reliable.[10,11]

The capacity of ADA assays in CSF to discern even minute ADA concentrations is vital for diagnosing certain neurological disorders such as meningitis and encephalitis. The reliability of ADA detection in CSF fluid was separately analyzed in a study conducted by Raviraj et al (2017) in which the authors concluded that ADA levels are a significant and reliable diagnostic marker for tuberculous meningitis.[12]

4.4. Linearity of the assays

Linearity is another important criteria of validation of an assay. It should be ascertained that the assay does not deviate from its accuracy claim within a broad range of test concentrations. In our study, the assays demonstrated remarkable linearity with all correlation coefficients (r) exceeding 0.9 and reaching 1.0 at the highest concentrations (**Table 5** and **Graph 4**). This is in compliance with the results of Arrigo C et al. (2023) who showed that for assays of some analytes like glucose and albumin, their use in some pleural and ascitic fluids did not exhibit loss of linearity even at high concentrations.[13]

The importance of high linearity is particularly noteworthy for our study given the various factors that can shift the concentrations of different analytes within the unusual fluids of analytes. Take pleural fluid for instance: it may contain much higher or lower concentration of protein depending on whether the fluid is a transudate or exudate—a state that is often linked with infections or malignancies.[14,15] In our study, the high correlation coefficients make it possible for the assays to be used reliably for diagnosis and monitoring and with varying analyte concentrations, multidisciplinary use is possible.

4.5. Clinical relevance and implications

The unconventional body fluids, such as pleural, ascitic, and CSF fluids, used for the biochemical diagnosis of diseases have high importance clinically. In the case of pleural fluid, its analysis is important for the diagnosis of infectious diseases, heart failure, and some cancers. Likewise, the analysis of ascitic fluid is important for liver disease and some infections. Finally, a CSF analysis is essential for infections and some neurodegenerative diseases like meningitis or multiple sclerosis.

Because these body fluids are less common, it is essential to verify that the assays for measuring analyte concentrations are precise and trustworthy. Our research adds to the existing knowledge on the value of biochemical assays for evaluation of unusual body fluids, offering clinicians dependable resources for performing accurate diagnoses, monitoring the disease progress, and evaluating treatment response.

4.6. Limitations and future directions

In my opinion, the validation of biochemical assays for body fluids that are not conventionally used for testing is an essential research focus, but it does have some limitations. One such limitation is that not all types of body fluids are represented within the sample population. Studies with an increased sample population will be more beneficial especially for these fluids that are affected by rare diseases. Moreover, although in this study we paid attention to particular analytes, it would be interesting to expand the range of biochemical examined biomarkers in order to evaluate the range of biochemical testing done on these body fluids.

There is also the possibility that only one used testing method, the Beckman Coulter DxC 700 AU, would not be acceptable as all other platforms. While it is relative common, it would be interesting to correlate how these biochemical assays would work when tested on other common platforms used in other laboratory settings.

6. Conclusion

This study successfully validated biochemical assays for various analytes in unconventional body fluids, including pleural fluid, ascitic fluid, and CSF. The assays demonstrated high precision, accuracy, sensitivity, and linearity, making them reliable tools for clinical use. These findings contribute to the growing body of literature on the use of body fluids other than blood for diagnostic testing, highlighting the importance of assay validation in improving patient care and expanding the diagnostic capabilities of clinical laboratories.

7. Source of Funding

None.

8. Conflict of Interest

None.

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