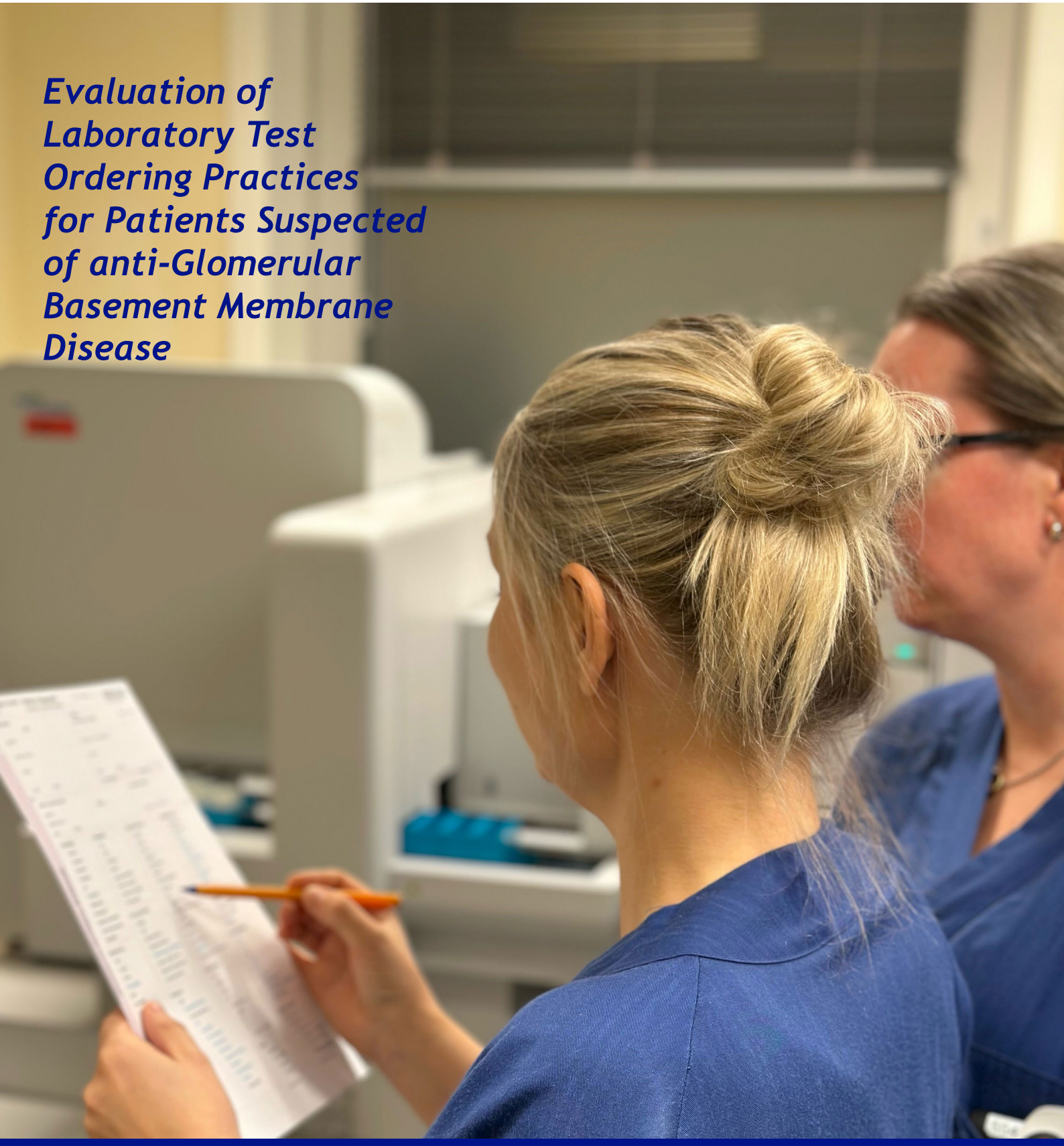
IFBLS

INTERNATIONAL JOURNAL OF BIOMEDICAL LABORATORY SCIENCE

Volume 12 • Number 2/2023 • Pages 59 - 113 • www.ijbls.org



Evaluation of Laboratory Test Ordering Practices for Patients Suspected of anti-Glomerular Basement Membrane Disease

IJBLS e-Journal Published by International Federation of Biomedical Laboratory Science
Online ISSN: 2308-7706

INTERNATIONAL JOURNAL OF BIOMEDICAL LABORATORY SCIENCE

Published by IFBLS
International Federation of Biomedical Laboratory Science

www.ifbls.org
communications@ifbls.org
Telephone: 001 905 667 8695
Fax: 001 905 528 4968
33 Wellington Street North
Hamilton, Ontario
L8R 1M7
CANADA

Online ISSN: 2308-7706

IJBLS is a free access on-line peer-reviewed journal, published bi-annually

Current issue: October 2023
Next issue: April 2024

Contents

INTERNATIONAL JOURNAL OF BIOMEDICAL LABORATORY SCIENCE

IJBLS seeking reviewers!

As part of the International Journal of Biomedical Sciences commitment to providing opportunities for all authors to publish and share information across the globe, the editorial board is dedicated to promoting mentorship of new authors and editors. We are always seeking reviewers for submitted works to the journal. If you are interested in serving as a reviewer, please send a short cover letter with your area of interest and a resume or curriculum vitae to the editor in chief, Dr. Pat Tille.

IJBLS active reviewers

In addition to the associate editors, IJBLS would also like to acknowledge those additional professionals who currently serve as active reviewers for the journal.

- Stephanie Jacobson DCLS, MLS (ASCP)CM, AHI(AMT)
- Erin Rumpke MS MLS(ASCP)CM, AHI(AMT)
- Elizabeth Warning MS MLS(ASCP)CM, AHI(AMT)
- Adrienne E. Davis Zapfe, EdD, MHA, MLS (ASCP)CM, AHI (AMT)
- Demetra Castillo M.Ed. MLS (ASCP) AHI(AMT)
- Cathy Otto, PhD, MBA, MLS(ASCP)

Patricia Tille
editor-in-chief@ijbls.org

IJBLS for Biomedical Laboratory Scientists

The International Journal of Biomedical Laboratory Science (IJBLS) is an on-line peer-reviewed journal published bi-annually by International Federation of Biomedical Laboratory Sciences (IFBLS).

The journal is intended to disseminate information and knowledge to the international laboratory community by accepting a variety of manuscripts for publication. Those manuscripts should be original research articles, literature or mini-reviews, case studies, brief communications and letters to the editor describing original investigations in all fields of biomedical laboratory sciences.

This journal is the ideal place for all Biomedical Laboratory Scientists, whether recognized experts in the field or starting their career, to publish their findings.

The Editor and Editorial Board are here to help you publish your work.

Editorial

Standardization and Advances in a Global Network! Looking for YOUR Ideas!

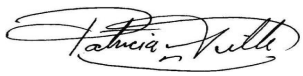
Patricia Tille Ph.D MLS(ASCP) AHI (AMT) FASCs
IJBLS Editor in Chief

There are numerous workforce and educational challenges worldwide when it comes to health care and in particular the laboratory workforce. Various groups and organizations are discussing how to standardize practice in order to improve global health care. But can it be done? I challenge you to consider the possibilities and if you have any insight, why not write an article for the International Journal of Biomedical Laboratory Science about your findings?

This edition of the journal provides an overview of peer review in laboratory science education and how it can improve student outcomes. Critical thinking and new experiences in how professionals are taught is important! Laboratory professionals are vital in many areas including clinical practice, education and research as noted by Dr. Tveten, in her editorial.

Also, in this issue you will see articles that highlight some of the technological advances that are evident in the use of molecular diagnostics and the challenges associated with standardizing common laboratory diagnostic tests. As laboratory medicine continues to evolve, consider submitting a narrative review, or even an editorial on a new technique.

Sincerely, IJBLS Editor in Chief,



Patricia Tille Ph.D. MLS(ASCP) AHI(AMT) FACSc

Editorial

Biomedical Laboratory Scientists in Cross Disciplinary Research

Ann-Kristin Tveten, Ph.D.

After graduation biomedical laboratory scientists have many choices when it comes to employment. Biomedical laboratory scientists (BLS) work in medical laboratories in hospitals, in various specialty laboratories, and some choose to continue with their education, or pursue research. While most BLS work in medical laboratories, they are highly attractive to other industries as well, like pharmaceutical laboratories, quality control in food production and research. BLS have many skills that are important to maintain high quality and reproducibility and include training in microbiology, pathology and clinical chemistry enables them to contribute to research and development in the perspective of "One health."

The World Health Organization (WHO) has defined the term "One health" as "an integrated, unifying approach to balance and optimize the health of people, animals and the environment." The goal is to raise awareness on how production of food and animal health impacts the human population, especially within infectious diseases and biosafety in food production. Biosafety in food production is highly regulated, and there is extensive on-going research to ensure a high level of biosafety, but also to provide high quality food products to consumers.

Among the various research and development projects we find projects that target novel approaches to disinfection strategies to prevent infectious diseases and reduce the use of antimicrobials in aquaculture and agriculture. The use of antimicrobials in aquaculture and agriculture contribute to antimicrobial resistance among food borne pathogens. The "one health" strategy is to reduce the use of antimicrobials in food production. The perspectives from BLS staff in these research projects link medical microbiology and health perspectivse with the knowledge from both veterinary medicine and industrial production, thus giving the project outcome a broader audience.

As a BLS researcher, I contribute to one of these projects called "RAS health" – a project that targets biosecurity in land-based aquaculture with the aim to improve water treatment methods using ozone (O3) and peracetic acid (PAA) and increase biosecurity by adopting disinfection strategies that consider the disease vectors and reservoirs of pathogens. This technology enables large-scale food production in an environment where biosafety can easily be monitored.

Norway is one of the largest producers of salmonid fish served in a global market. The use of antibiotics in Norwegian aquaculture are very low, but the global aquaculture industry consumes large quantities of antibiotics annually. New approaches to biosafety in food production will help fulfill the United Nations (2015) sustainability goal 2; End hunger, achieve food security and improved nutrition and promote sustainable agriculture.

The RAS health project is a cross-disciplinary research project and includes researchers from the Norwegian Food Research Institute (NOFIMA), and the universities, Norwegian University of Sciences and Technology, Norwegian University of Life Sciences and Danish Technical University. Three perspectives are included: animal health, chemical analysis, and microbiology. From the microbiology perspective both pathogen detection and microbiome analysis are used to study the role of commensal microbiome in pathogen outbreaks and understand how novel disinfection strategies affect microbiome resilience and pathogen dynamics. This is crucial when the microbiome serves a critical function in maintaining water quality and for the health and wellbeing of the animals. Biosafety and microbiome resilience has been investigated in two different settings, one in a high technology production system with modern sensor- and water disinfection systems. The other is aquaculture ponds, with low technology solutions. This is much like aquaculture sites in developing countries, which makes this study relevant to provide recommendation for biosafety strategies beyond high technology solutions. This could provide a long-term solution, enable the reduction of antimicrobials globally, and potentially provide solutions for safe food production on a small scale.

From a biomedical laboratory scientist perspective, I highly recommend other BLS with research interests to get involved in research projects that will put our BLS knowledge and expertise into new contexts. It is both challenging and educational to participate in cross-disciplinary research. Many topics that impact human health can benefit from the high standards of BLS knowledge and BLS have many skills needed to achieve the goals of “One health”.

Ann-Kristin Tveten Ph.D.
Associate professor
Department of Biological Sciences
Norwegian University of Science and Technology (NTNU), Aalesund.
Email: ann-kristin.tveten@ntnu.no

Research article

Evaluation of Laboratory Test Ordering Practices for Patients Suspected of anti-Glomerular Basement Membrane Disease

Clare Shea[1], Julie Soder[1], Juan U Rojo[1], Janet Enderle[1], Rajkumar Rajendran[1*]

Department of Clinical Laboratory Sciences, University of Texas Medical Branch, Galveston, Texas, USA[1]

Background: **The objective of this study was to evaluate the laboratory test ordering practices for patients suspected of anti-glomerular basement membrane (anti-GBM) disease at an academic teaching hospital.**

Methods: **A retrospective cross-sectional study was conducted using data from EPIC electronic medical records (EMR) system from January of 2013 to January of 2022 on patients suspected of anti-GBM disease. Data collected include patient demographics, medical history, and laboratory test results. Patient data was stratified and analyzed using SPSS statistical software version 28.**

Results: **From the total 110 patients analyzed in this study; 42.7% (n=47) patients did not have an anti-GBM test ordered appropriately. Analysis of patient demographics revealed most of the patients were female (54.5%, (n=60)) and white (73.6%, (n=81)) non-Hispanic or Latino (69.1%, n=76)). Regarding type of anti-GBM serology tests, in the appropriate group, 41.3% (n=26 out of 63) of patients had both an enzyme-linked immunosorbent assay (ELISA) and indirect fluorescent antibody (IFA) test performed, while the inappropriate group 57.4% (n=27 out of 47) of patients had only an ELISA test ordered. There was a significant difference observed in serum creatinine (p= 0.003) and estimated glomerular filtration rate (eGFR) (p=0.011) for patients who had an anti-GBM test ordered appropriately.**

Conclusions: **The opportunities for quality improvement identified in this study can be used to implement a test ordering algorithm for anti-GBM to eliminate unnecessary diagnostic procedures and reduce hospital costs to improve patient outcomes.**

Keywords: Anti-glomerular basement membrane (anti-GBM), ANCA (anti-neutrophil cytoplasmic antibodies), autoimmune, serology, laboratory testing

---

Accepted: August 6, 2023

*Corresponding author: Rajkumar Rajendran. E-mail: rarajend@utmb.edu

Introduction

Self-tolerance, which is the ability to differentiate self from non-self, is one of the most important characteristics of the immune system. Loss of self-tolerance can lead to autoimmune diseases which are characterized by the production of autoantibodies that bind to self-molecules leading to antigen-antibody deposits, cellular destruction, and tissue damage.[1] Anti-glomerular basement membrane (anti-GBM) disease, also referred to as Goodpasture syndrome, is an organ-specific autoimmune disorder marked by the production of autoantibodies against the glomerular and/or the alveolar basement membrane.[2] Specifically, the autoantibodies recognize and bind to the alpha-3 chain of type IV collagen, which activates the complement cascade and leads to tissue destruction.[3] Although basement membranes are found throughout the body, the basement membranes of the kidneys and lungs are predominately affected due to the structure of the alpha-3 collagen chains in the membranes are more exposed to antibodies.[2] Anti-GBM antibodies are not always associated with disease and can be present in healthy individuals. However, in patients with anti-GBM disease, the antibodies are potent and target two epitopes of type IV collagen leading to tissue destruction.[4]

Disease Incidence

Anti-GBM disease is considered rare with an incidence of 1-2 cases per million individuals.[4,5] A genetic predisposition for this disease is associated with human leukocyte antigen (HLA) allele, *HLA-DR15*, which is a common finding in other autoimmune diseases. Most patients with anti-GBM disease present with signs of progressive glomerulonephritis, in which most of the glomeruli have crescentic lesions.[6] Roughly, 40% to 60% of patients will also present with lung hemorrhage, and a small percentage will present with an isolated case of pulmonary disease.[7] Progressive glomerulonephritis in anti-GBM patients consists of renal damage, proteinuria, and glomerular hematuria. Lung hemorrhage or pulmonary disease presents as dyspnea or hemoptysis.[8]

Diagnosis of Anti-GBM Disease

Anti-GBM disease is primarily diagnosed by the detection of anti-GBM antibodies in serum or tissue.[7] Kidney biopsy is needed to confirm the diagnosis. However, biopsy is an invasive procedure and may not be possible in patients with severe cases of anti-GBM disease.[3] Guidelines published by Rovin et al, for the management of glomerular diseases conclude that treatment can start before biopsy, but biopsy confirms diagnosis.[9] In cases where kidney biopsy is not feasible, serum detection of anti-GBM antibodies is used. However, serological tests can produce false results and should only be ordered in patients with clinical suspicion of autoimmune disease.[10] Furthermore, due to difficulties in test result interpretation and insufficient knowledge among healthcare professionals regarding proper use of serology laboratory tests, autoantibody tests are often ordered unnecessarily.[11]

The prognosis of patients diagnosed with anti-GBM disease has improved over the last few years. However, most patients have limited renal survival and are dialysis dependent.[12] Thus, early diagnosis and treatment are essential for patients suspected of disease to prevent renal failure and death in severe cases.

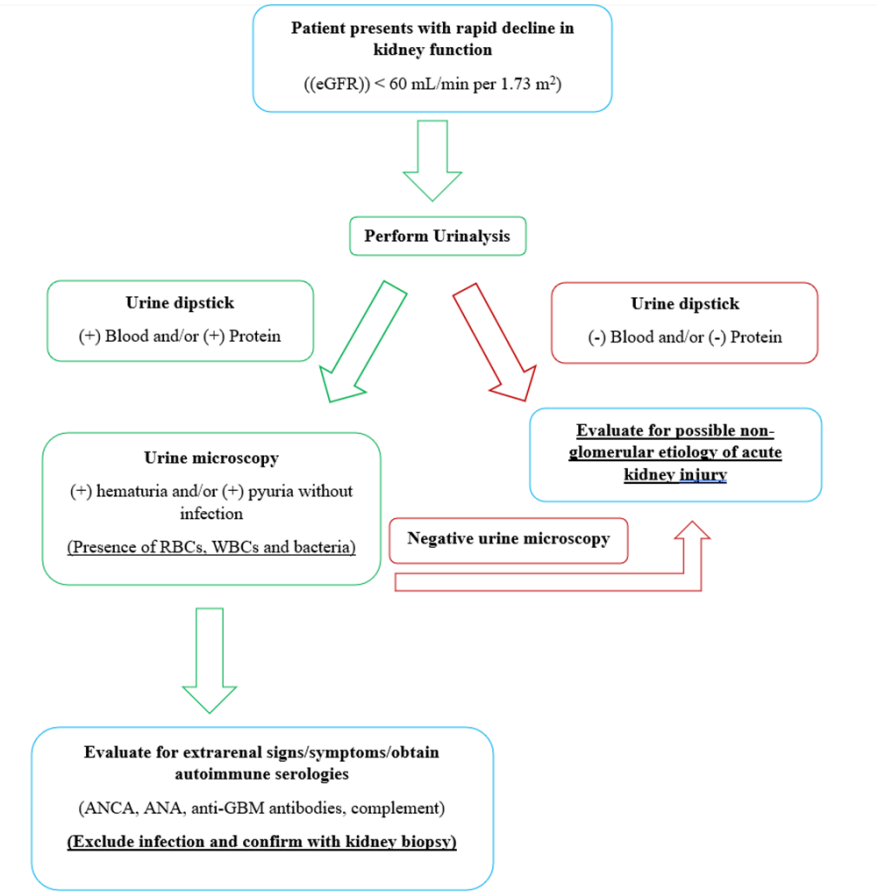
Purpose of Study

The lack of studies evaluating the efficient use of laboratory tests for patients suspected of anti-GBM disease led to the hypothesis that patients suspected of anti-GBM disease undergo unnecessary laboratory testing during the preliminary diagnostic process. In this study, individuals suspected of anti-GBM disease were defined as patients who presented clinically with glomerulonephritis and/or pulmonary hemorrhage. Laboratory test results investigated include serum albumin, creatinine, eGFR (estimated glomerular filtration rate), hemoglobin, hema-

tocrit, anti-GBM titer, antineutrophil cytoplasmic antibodies (ANCA), and urinalysis (urine dipstick and urine microscopy). The predictors of anti-GBM disease were identified using patient demographics, medical history, and laboratory test results. The objective of this project was to evaluate the current practices of test ordering for patients suspected of anti-GBM disease. The results from the study can aid in decreasing irrelevant testing, thus reducing costs for both the patient and the hospital.

Methods

This investigation consisted of a retrospective cross-sectional study using patients' EMR from January 2013 to January 2022 at an 800-bed academic teaching hospital. The study population included patients who clinically presented with glomerulonephritis and/or pulmonary hemorrhage suspected of having anti-GBM disease. Glomerulonephritis was defined by the International Classification of Diseases (ICD)10 and ICD9 codes, N00, N01, N02, N03, N04, N05, N06, N07, N08, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589. Pulmonary hemorrhage was defined by the ICD10 and ICD9 codes, R04, 786.3, 786.0. Anti-GBM disease was defined by ICD10 and ICD9 codes, M31.0 and 446.21. Analysis of the type of anti-GBM serology tests ordered included ELISA and IFA. Test results were interpreted as positive, negative, or indeterminate by the established reference standard of the specific test used. Patients less than 18 years of age, pregnant individuals, and prisoners were excluded from the study. The study was reviewed by the Institutional Review Board (IRB) and considered to be a quality assessment/quality improvement study that did not require approval or oversight.



Note. eGFR = Estimated glomerular filtration rate; RBC = Red blood cells; WBC = White blood cells; ANCA = Antineutrophil cytoplasmic antibodies; ANA = Antinuclear antibody

Figure 1. Determination of Appropriate Utilization of Laboratory Tests. Algorithm adapted from Rovin BH, Adler SG, Barratt J, et al. Executive summary of the KDIGO 2021 Guideline for the Management of Glomerular Diseases. Kidney Int. Oct 2021;100(4):753-779. doi:10.1016/j.kint.2021.05.015

During the chart review process, data collected included patient demographics (age, sex, ethnicity, race), patient medical history for pre-existing comorbidities (diabetes, dyslipidemia, smoking, hypertension), laboratory test results (urinalysis, creatinine, hematocrit, hemoglobin, eGFR) and type of anti-GBM test ordered (ELISA, IFA, or both). Assessment of appropriate laboratory test utilization in the anti-GBM disease diagnosis was accomplished by evaluating patient charts using the algorithm outlined in Figure 1.[9] Patients included in the study were divided into two groups, appropriate or inappropriate, based on the results of the algorithm evaluation.

Statistical Analysis

The statistical analyses were performed using IBM SPSS software version 28. Descriptive statistics were used to provide an overview of the patient population. Frequencies were determined for categorical variables including race/ethnicity and gender. Mean, median, and standard deviation were determined for continuous variables including age and laboratory test results. A Mann-Whitney *U* test was performed to compare laboratory test results between patients in the appropriate group and inappropriate group. *P* values < 0.05 were considered statistically significant.

Results

Between January 2013 and January 2022, 110 patients were evaluated for anti-GBM disease. Patients assessed for anti-GBM disease were primarily female (54.5%, (n=60)), white (73.6%, (n=81)), non-Hispanic or Latino (69.1%, n=76)) with a mean age of 52 years as seen in Table 1. Regarding frequency of comorbidities, hypertension (50.9%, (n=56)) was the most common condition, followed by smoking (37.3%, (n=41)) as listed in Table 2.

Following Rovin et al. algorithm, of the 110 patients suspected of anti-GBM disease, 63 (57.3%) patients had an anti-GBM test ordered appropriately, while 47 (42.7%) patients had tests ordered inappropriately as seen in Table 3.

Table 1. Frequency of sex, ethnicity, and race for patients suspected of anti-GBM disease.

Demographic	N (%)[n=110]
Age	
≥ 40 years	78 (70.9)
≤40 years	32 (29.1)
Sex	
Male	50 (45.5)
Female	60 (54.5)
Ethnicity	
Not Hispanic or Latino	76 (69.1)
Hispanic or Latino	33 (30)
Unknown	1 (0.9)
Race	
White	81 (73.6)
Black or African American	24 (21.8)
Asian	4 (3.6)
American Indian or Alaskan Native	1 (0.9)

Table 2. Frequency of comorbidities for patients suspected of anti-GBM disease.

Condition	N (%)[n=110]
Diabetes	
Present	23 (20.9)
Absent	87 (79.1)
Dyslipidemia	
Present	9 (8.2)
Absent	101 (91.8)
Smoking	
Present	41 (37.3)
Absent	69 (62.7)
Hypertension	
Present	56 (50.9)
Absent	54 (49.1)

Table 3. Frequency of appropriate and inappropriate ordering for anti-GBM tests.

Appropriate	N (%)[n=110]
Yes	63 (57.3)
No, No UA done	9 (8.2)
No, UA neg for protein	11 (10)
No, UA neg for blood	12 (10.9)
No UA neg for protein and blood	15 (13.6)

Note. UA = urinalysis; neg = negative

When assessing the frequency of the type of anti-GBM test ordered, the study identified, in the appropriate group, 26 of 63 (41.3%), had both an ELISA and an IFA test ordered. In the inappropriate group, 27 of 47 (57.4%), had only an ELISA test ordered as listed in Table 4.

Table 4. Type of anti-GBM serology test ordered.

Serology Test Ordered	Group	
	Appropriate Group *(n=63)*	Inappropriate Group *(n=47)*
Only ELISA	23	27
Only IFA	14	11
Both ELISA and IFA	26	9

Note. ELISA = Enzyme-linked immunosorbent assay; IFA = Indirect fluorescent antibody

When comparing laboratory tests between appropriate and inappropriate patient groups, the study found a statistically significant difference for serum creatinine (p = 0.003) and eGFR rate (p = 0.011). No statistically significant difference was seen for hematocrit (p = 0.059), hemoglobin (p = 0.67) and albumin (p = 0.131) as listed in Table 5.

Table 5. Comparison of laboratory tests between patients who had an anti-GBM test ordered appropriately and patients who had an anti-GBM test ordered inappropriately.

Variable	Mean for Appropriate Group	Mean for Inappropriate Group	p-Value
Creatinine (mg/dL)	4.41 *(n=66)*	2.81 *(n=37)*	0.003*
Hematocrit (%)	34 *(n=63)*	33.7 *(n=30)*	0.059
Albumin (g/dL)	3.47 *(n=62)*	3.69 *(n=29)*	0.131
eGFR (mL/min/1,73m^2)	3.34 *(n=57)*	58.7 *(n=34)*	0.011*
Hemoglobin (g/dL)	10.1 *(n=63)*	11.1 *(n=30)*	0.67

Note. eGFR = Estimated glomerular filtration rate

Discussion

The findings of this study indicate that 42.7% (n = 47) of patients suspected of having anti-GBM disease have inappropriate laboratory tests ordered according to the guidelines published by Rovin and colleagues.[9] The analysis of laboratory tests ordered (Table 3) showed that providers order anti-GBM serology tests without fully utilizing the results from patient's urinalysis. Unnecessary testing can be harmful to patients since it leads to diagnostic errors associated with inappropriate test results.[10,13] In patients suspected of anti-GMB disease, serology tests should only be ordered after assessment of clinical symptoms, comorbidities, and preliminary laboratory tests such as albumin, creatinine, eGFR, hemoglobin, hematocrit, and urinalysis.

Although, the patients included in the study were not diagnosed with anti-GBM disease, the demographics of the patients were found to be similar to other documented studies in which most patients were female, white not Hispanic or Latino.[7,14] Most patients in this study were female, but the study by Shen and others observed a male predominance in anti-GBM patients.[15]

In this study, the dominant comorbidity seen in patients was hypertension followed by smoking. This is in agreement with several other studies in which 34% of anti-GBM patients had hypertension or a history of hypertension and 58.3% of anti-GBM patients had hypertension or a history of hypertension.[12,14] Hypertension is the most common comorbidity that is associated with anti-GBM disease. This can be the result of early glomerular lesions associated with fibrin deposition and formation of epithelial crescents that narrow the blood vessels leading to kidney damage presenting initially as hypertension. Also, environmental factors, mainly smoking, increases the risk of developing anti-GBM disease as smoke damage to the pulmonary membranes leads to exposure of the alveolar capillaries to anti-GBM antibodies.[2] It is important to note that anti-GBM tests performed in this study's population (n=110) were all negative and anti-GBM disease was not the final diagnosis. However, when considering initial evaluation of a patient suspected of anti-GBM, patient demographics and comorbidities identified are variables that should be considered. For example, a multicenter French study that had a total of 201 patients diagnosed with anti-GBM disease identified 57% of the study population was male and the two common comorbidities were chronic arterial hypertension and tobacco use.[16] Patient demographics and more importantly, existing comorbidities, can be informative to healthcare professionals in the initial evaluation of patients suspected of anti-GBM disease.

In terms of laboratory testing, ELISA and IFA were the two types of anti-GBM tests utilized and these tests were ordered either as a panel or individually. The anti-GBM tests were performed by a reference laboratory, it is possible that ordering providers overlooked Rovin et al testing algorithm in order to attempt faster turnaround time on test results.[9] This practice could have contributed to the inappropriate test ordering for the 47 patients in this study. Additionally, because the tests are offered as a panel, ordering providers could be prompted to order the panel rather than individual tests, especially if the provider is unfamiliar with the listed test.

Furthermore, when the test order recommendations listed in the reference laboratory website were reviewed, the guidelines for ordering a type of anti-GBM serology test (ELISA, IFA, or both) was unclear. For example, for the anti-GBM IFA tests, the reference laboratory recommends that this test may be useful in detecting GBM antibodies. However, the anti-GBM ELISA and IFA combo is listed as the preferred panel for detecting GBM antibodies in suspected or established anti-GBM disease. Interestingly, the result interpretations provided by the reference laboratory regarding anti-GBM IFA or ELISA and combo, are identical. This confusion could be a reason providers decide to order both tests. There was not a clear ordering pattern for disease diagnosis as indicated by the variation in ELISA and IFA test ordering for patients in both the appropriate group and the inappropriate as seen in Table 4. Errors in test ordering could be related to the ambiguous information presented in the reference laboratory website, especially if the result interpretation provided is similar for all anti-GBM test types.

The comparison of laboratory tests completed between patients in the appropriate group and patients in the inappropriate group demonstrated a significant difference for creatinine and eGFR. The average creatinine for patients in the appropriate group was 4.41 mg/dL, while the average creatinine for patients in the inappropriate group was 2.81 mg/dL (Table 6). The average eGFR for patients in the appropriate group was 34.3 mL/min/1.73m^2, while the average eGFR for patients in the inappropriate group was 58.7 mL/min/1.73m^2 (Table 6). The average results for creatinine and eGFR for patients in the appropriate group and patients in the inappropriate group were abnormal. However, patients in the appropriate group had drastic abnormal results than patients in the inappropriate group. This is significant, as anti-GBM disease is associated with severe kidney injury as many patients with delayed diagnosis require permanent renal replacement therapy.[17]

The findings from the study indicate that patients who had an anti-GBM test ordered appropriately were experiencing more severe kidney disease as observed by the mean eGFR which would place them in moderate (3b) to severe chronic kidney disease (CKD) stage. Patients who had an anti-GBM test ordered inappropriately had an eGFR mean that would indicate mild to moderate (3a) CKD stage. These results may be useful in establishing cut off values for anti-GBM test ordering, as patients who had more abnormal values for creatinine and eGFR had an anti-GBM test ordered appropriately.

The contribution of this study notes a high volume of tests ordered inappropriately for patients suspected of anti-GBM disease despite a low prevalence of disease. Leaf et al, evaluated 4,903 patients with 5,731 acute kidney injury (AKI) episodes and identified that anti-GBM antibodies were tested in 1% of AKI episodes and all were found to be negative.[18] Since the prevalence for anti-GBM disease is low, greater emphasis should be placed on patient signs and symptoms, comorbidities, and routine laboratory tests such as urinalysis, creatinine and eGFR before performing autoantibody testing.

While the preferred testing for initial diagnosis in patients suspected of anti-GMB disease include GBM antibody testing by IgG by multiplex bead assay and immunofluore-

scence, it should be noted that other laboratory tests along with renal biopsy must be included for proper diagnosis. This is of particular interest as extremely rare cases of anti-GBM can present with seronegative anti-GBM antibodies.[19] Hospitals should consider newly developed methodologies, such as anti-GBM IgG chemiluminescence immunoassay as part of the anti-GBM workflow. When implemented appropriately, these assays have shown increased detection of GBM antibodies in addition to traditional ELISA testing.[20] Additionally, novel interventions could include educational seminars, built in test ordering sets within EMR, and dissemination of educational pamphlets detailing anti-GBM laboratory workup along with test costs to various departments of a hospital. Lastly, as part of on-going quality improvement, institutions should evaluate implemented interventions with a follow-up period of 3-6 months to assess the effectiveness of an intervention and modify as needed to continually improve patient outcomes while reducing hospital costs.

Conclusion

There is no evident diagnostic algorithm for anti-GBM that is available to providers. This may have contributed to improper test ordering. The results from this study should encourage institutions to evaluate the current practices in the diagnosis of anti-GBM testing and implement evidence-based diagnostic algorithms that can aid providers with laboratory test ordering for improved patient outcomes.

Acknowledgments

The authors would like to thank the entire Clinical Laboratory Sciences department at University of Texas Medical Branch in Galveston, TX for their continued support and dedication.

Ethical Approval

This study was reviewed the IRB and considered it to be a quality assessment/quality improvement study that did not require IRB approval or oversight.

References

1. Khalid N, Aeddula NR. Antinuclear Cytoplasmic Antibody. Text. 2022/08/08 2022;doi:https://www.ncbi.nlm.nih.gov/books/NBK562339/
2. Greco A, Rizzo MI, De Virgilio A, et al. Goodpasture's syndrome: a clinical update. Autoimmun Rev. Mar 2015;14(3):246-53. doi:10.1016/j.autrev.2014.11.006
3. Shiroshita A, Oda Y, Takenouchi S, Hagino N, Kataoka Y. Accuracy of Anti-GBM Antibodies in Diagnosing Anti-Glomerular Basement Membrane Disease: A Systematic Review and Meta-Analysis. Am J Nephrol. 2021;52(7):531-538. doi:10.1159/000518362
4. Hellmark T, Segelmark M. Diagnosis and classification of Goodpasture's disease (anti-GBM). J Autoimmun. Feb-Mar 2014;48-49:108-12. doi:10.1016/j.jaut.2014.01.024
5. Segelmark M, Hellmark T. Anti-glomerular basement membrane disease: an update on subgroups, pathogenesis and therapies. Nephrol Dial Transplant. Nov 1 2019;34(11):1826-1832. doi:10.1093/ndt/gfy327
6. Weiner M, Segelmark M. The clinical presentation and therapy of diseases related to anti-neutrophil cytoplasmic antibodies (ANCA). Autoimmun Rev. Oct 2016;15(10):978-82. doi:10.1016/j.autrev.2016.07.016
7. McAdoo SP, Pusey CD. Anti-Glomerular Basement Membrane Disease. Clin J Am Soc Nephrol. Jul 7 2017;12(7):1162-1172. doi:10.2215/cjn.01380217
8. Akhtar M, Taha NM, Asim M. Anti-glomerular Basement Membrane Disease: What Have We Learned? Adv Anat Pathol. Jan 2021;28(1):59-65. doi:10.1097/pap.0000000000000280

9. Rovin BH, Adler SG, Barratt J, et al. Executive summary of the KDIGO 2021 Guideline for the Management of Glomerular Diseases. Kidney Int. Oct 2021;100(4):753-779. doi:10.1016/j.kint.2021.05.015
10. Watad A, Bragazzi NL, Sharif K, et al. Anti-Glomerular Basement Membrane Antibody Diagnostics in a Large Cohort Tertiary Center: Should We Trust Serological Findings? Isr Med Assoc J. Jul 2017;19(7):424-428.
11. Man A, Shojania K, Phoon C, et al. An evaluation of autoimmune antibody testing patterns in a Canadian health region and an evaluation of a laboratory algorithm aimed at reducing unnecessary testing. Clin Rheumatol. May 2013;32(5):601-8. doi:10.1007/s10067-012-2141-y
12. Zahir Z, Wani AS, Prasad N, Jain M. Clinicopathological characteristics and predictors of poor outcome in anti-glomerular basement membrane disease - a fifteen year single center experience. Ren Fail. Dec 2021;43(1):79-89. doi:10.1080/0886022x.2020.1854301
13. Vrijsen BEL, Naaktgeboren CA, Vos LM, van Solinge WW, Kaasjager HAH, Ten Berg MJ. Inappropriate laboratory testing in internal medicine inpatients: Prevalence, causes and interventions. Ann Med Surg (Lond). Mar 2020;51:48-53. doi:10.1016/j.amsu.2020.02.002
14. Marques C, Carvelli J, Biard L, et al. Prognostic Factors in Anti-glomerular Basement Membrane Disease: A Multicenter Study of 119 Patients. Front Immunol. 2019;10:1665. doi:10.3389/fimmu.2019.01665
15. Shen CR, Jia XY, Cui Z, Yu XJ, Zhao MH. Clinical-Pathological Features and Outcome of Atypical Anti-glomerular Basement Membrane Disease in a Large Single Cohort. Front Immunol. 2020;11:2035. doi:10.3389/fimmu.2020.02035
16. Caillard P, Vigneau C, Halimi J-M, et al. Severe Infection in Anti-Glomerular Basement Membrane Disease: A Retrospective Multicenter French Study. Journal of Clinical Medicine. 2020;9(3). doi:10.3390/jcm9030698
17. Henderson SR, Salama AD. Diagnostic and management challenges in Goodpasture's (anti-glomerular basement membrane) disease. Nephrol Dial Transplant. Feb 1 2018;33(2):196-202. doi:10.1093/ndt/gfx057
18. Leaf DE, Srivastava A, Zeng X, et al. Excessive diagnostic testing in acute kidney injury. BMC Nephrol. Jan 15 2016;17:9. doi:10.1186/s12882-016-0224-8
19. Zhong Z, Tan J, Tang Y, Li Z, Qin W. Goodpasture syndrome manifesting as nephrotic-range proteinuria with anti-glomerular basement membrane antibody seronegativity: A case report. Medicine (Baltimore). Sep 25 2020;99(39):e22341. doi:10.1097/md.0000000000022341
20. Kühnl A, Hartwig L, Dähnrich C, Schlumberger W. Serodiagnosis of Anti-glomerular Basement Membrane Disease Using a Newly Developed Chemiluminescence Immunoassay. Front Med (Lausanne). 2022;9:915754. doi:10.3389/fmed.2022.915754

Review article

The Clinical Utility of CYP2D6 Molecular Methods for Personalized Pain Management

Madisson Hall[1*], Stephanie Jacobson[1] and Patricia Tille[1]

Medical Laboratory Sciences Program, College of Allied Health Science, University of Cincinnati, Cincinnati, Ohio, USA[1]

As the world faces the persisting opioid epidemic ravaging the globe, one of the ways to combat the overuse and misuse of opioids will be through a personalized approach to pain management. The clinical implementation of pharmacogenetic testing is essential to creating patient-centered drug therapies for the safe and effective treatment of pain. Opioids are used for pain management, but the analgesic affect is different on every individual due to genetic variation and the ability to metabolize the drugs. The highly polymorphic CYP2D6 gene is responsible for the metabolism of the commonly prescribed opioids. Genotype and copy number variation of CYP2D6 play a critical role in how affectively the body metabolizes opioids. A wide variety of molecular methodologies are available for the genotype and copy number analysis of CYP2D6 including real-time polymerase chain reaction, sequencing, microarray, and matrix-assisted laser desorption/ionization-time of flight mass spectroscopy. While these technologies have advanced significantly, there are still limitations and challenges associated with integrating pharmacogenetic testing into routine clinical practice. Further research is needed to establish standardized pharmaceutical recommendation guidelines based on CYP2D6 analysis. However, there is compelling evidence that suggests CYP2D6 testing is useful when considering prescribing opioids for pain management. Implementing pharmacogenetic testing for CYP2D6 may reduce aggressive prescription practices of opioids which, in time, will diminish the devastating effects of the opioid crisis.

Keywords: Opioid, CYP2D6, pharmacogenetics, pain management

Accepted: May 13, 2023

*Corresponding author: Madisson Hall. E-mail: hall3mp@mail.uc.edu

Introduction

With recent advancements in genetics, medicine is shifting from a "one size fits all" approach to a customized method based on each individual patient's genetic profile. This emerging practice, called precision medicine, is also known as personalized medicine.[1] One of the ways clinicians can personalize medical treatment is through the use of pharmacogenetic testing. Pharmacogenetic testing allows medical providers to make informed decisions about drug therapies based on how an individual patient will metabolize the medication.

Genetics play a crucial role in how the body metabolizes medications. Variations in metabolism can result in a drug having a wide range of different effects on people. This is particularly important within the context of prescribing medications to control pain management. There are specific genes that play a particularly significant role in the drug metabolism process. Cytochrome P450, also referred to as CYP, represents a large heme-containing enzyme superfamily.[1] CYP enzymes are abundant in the liver and are responsible for the metabolism of various molecules such as drugs, chemicals, and fatty acids.[1] Among the several genes that encode these CYP members is the highly polymorphic CYP2D6 gene located on chromosome 22q13.1.[2] It is estimated that CYP2D6 actively metabolizes around 25 percent of all drugs including antidepressants, antiarrhythmics, antipsychotics, ß-blockers, and opioid analgesics used to treat pain.[1,3]

Due to the variation in CYP2D6, individuals metabolize opioids at different rates. Identification of the genetic variation in the CYP2D6 of each patient by implementing pharmacogenetic testing in routine clinical practice allows providers to take a personalized approach to pain management, leading to safer and more effective treatment. In addition, the clinical practice of genotyping CYP2D6 may reduce the over prescribing of opioids and indirectly assist in the management and reduction of the opioid crisis worldwide.

Pain Management and the Opioid Crisis

Safe and effective pain therapies require a personalized approach to treatment. Pain is subjective and can be difficult to treat. Individual patients can have a wide range of responses to pain medications based on their genetic profiles. Implementing pharmacogenetic testing in clinical pain management and the prescribing of narcotics is particularly important.

Narcotics or opioids are a class of drugs commonly prescribed for long- and short-term pain management. As a result, opioid addiction has become an increasingly prevalent issue which has led to the opioid epidemic or opioid crisis. In the United States, opioid use has risen by 10 to 14 times in the past two decades.[4] The rate of opioid prescription more than doubled worldwide between 2001 and 2013, largely affecting the United States, Canada, Australia, and the United Kingdom.[5] The increase in opioid prescriptions, misuse and abuse of the drugs has led to the subsequent rise in opioid-related overdoses and deaths. This is attributed to multiple factors including the increased use of prescription opioids by the growing geriatric population as well as efforts to resolve the previous under-treatment of chronic pain.[5] However, evidence suggests that the main contributor to the opioid crisis is the overaggressive prescription practices encouraged by pharmaceutical manufacturers.[5,6,7]

Opioids

Opioids are a class of medication prescribed for the treatment and management of pain. Opioids create an analgesic effect which simply refers to pain relief.[8] The commonly used opioids include oxycodone, hydrocodone, methadone, morphine, codeine, tramadol, and the synthetic opioid, fentanyl.[9]

Opioids produce analgesic effects by acting on the presynaptic and postsynaptic terminals of the body's neurons by binding to cell membrane receptors. The presynaptic binding blocks calcium channels to prevent the release of neurotransmitters that contribute to nociception or the sensation of pain.[8] The postsynaptic binding of opioids opens

potassium channels, increasing the required action potential to generate nociceptive transmission.[8] The released neurotransmitters stifle the sensation of pain and create a sense of wellbeing. When the effects of the drug wear off, the body can crave the same feeling of pleasure and wellbeing, generating the potential for addiction.[10]

Pharmacogenetic Testing

Clinically integrating pharmacogenetic testing into pain management practices may help to combat the current opioid crisis by reducing the over-prescription and misuse of opioids. Pharmacogenetics investigates how a specific gene influences the body's response to a given drug. Genetic polymorphisms contribute to the high variability in pharmacokinetics and pharmacodynamics which describe how the body processes and responds to various drugs.[11] Pharmacogenetic testing involves assessing an individual's genetic profile to determine how the individual will respond to drug therapy.

CYP2D6

The Role of CYP2D6 in Drug Metabolism

CYP2D6 is one of the most important pharmacogenes. Cytochrome P450 2D6 is an enzyme encoded by the CYP2D6 gene sequence which is primarily expressed in the liver.[3] CYP enzymes facilitate reactions during phase I metabolism, the process of adding or exposing a polar functional group such as -NH^2 or -OH on a lipophilic drug to increase the hydrophilic nature of the molecule.[12] The reaction creates metabolites transforming a prodrug into an active therapeutic form.[12] If the CYP450 system is inhibited in any way it will lead to a decrease in metabolism and an increase in the drug level, whereas if the system is induced, it will cause metabolism to increase and the drug level to decrease.[12]

Significant variability in interindividual CYP2D6 metabolism occurs because the gene is highly polymorphic. More than one hundred CYP2D6 genetic variants have been identified resulting from point mutations, duplications, insertions, and deletions.[1] The genetic variants contribute to the high variability in opioid metabolism by different individuals. Determining one's genotype for CYP2D6 would insure prescribing the correct dose and type of opioid for effective pain management.

CYP2D6 Genotypes and Metabolizer Phenotypes

Determining an individual's CYP2D6 genotype enables healthcare providers to make informed decisions regarding drug choices and doses based on the patient's CYP2D6 metabolizer phenotype. CYP2D6 allelic variants correspond with specific phenotypes categorized as either a poor metabolizer (PM), intermediate metabolizer (IM), ultrarapid metabolizer (UM), or normal metabolizer (NM). NM and IM are the most common phenotypes comprising an estimated 43% to 67% and 10% to 44% of the general population, respectively.[3] NMs provide the baseline for how most individuals are able to process compounds metabolized by CYP2D6 enzymes. IMs may express slightly less CYP2D6 metabolism than NMs but are not considered to be at substantial risk for adverse reactions or failed treatments when prescribed a compound that is metabolized by CYP2D6. In contrast, PMs are typically at higher risk for failed treatments and adverse drug reactions because these individuals exhibit no CYP2D6 enzyme activity and are therefore more likely to experience diminished analgesic effects from opioids due to the inhibited metabolism of the drug.[3,13,14] UMs are often at high risk of adverse reactions when prescribed compounds metabolized by CYP2D6 because these individuals exhibit increased CYP2D6 enzyme activity which can lead to toxic concentrations of the drug even at low doses.[14,15] While PMs and UMs are less common than NMs and IMs, drug choices and doses for these individuals must be carefully considered to minimize risks of dangerous or ineffective treatments.

The CYP2D6 metabolizer phenotype is determined based on the individual's activity score calculated by adding assigned values of each of the alleles.[3] There are five functional allele types for CYP2D6: normal (also known as wild type), decreased function, severely decreased function, no function, and increased

function. Normal function alleles include **1, *2,* and **35* which are all assigned an activity score of 1.[3,15] Decreased function alleles include **9, *17, *29,* and **41* and are assigned a value of 0.5.[3] The Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines also group *CYP2D6*10* in with the decreased function allele type. It is the only decreased function allele with an activity score of 0.25 rather than 0.5.[15] Due to this distinction, the allele can be categorized separately from the rest of the decreased function alleles to highlight the difference in activity values. CYP2D6 alleles that are considered to have no function are given a score of 0 and these include **3, *4, *5, *6,* and **40*.[3,16] Increased function alleles, which are **1x2* and **2x2,* are assigned twice the value of a normal allele, contributing a value of 2 to the activity score.[3] An activity score of 0 indicates a PM phenotype, 0.25 to 1 indicates an IM, 1.25 to 2.25 indicates a NM, and activity scores greater than 2.25 indicate a UM phenotype.[3,15] For example, an individual with a *CYP2D6*1/*2* diplotype has an activity score or 2 making them a NM, whereas an individual with a *CYP2D6*4/*5* diplotype has an activity score of 0 making them a PM.

The frequencies of the alleles and phenotypes vary by population and ethnic groups. No function *CYP2D6*3, *4, *5,* and **6* alleles are common among individuals of European descent.[3] Compared to other biogeographical groups, Europeans exhibit the highest frequency of poor metabolizers.[16] In contrast, Sub-Saharan African populations have the highest prevalence of activity scores of 3.0 and higher.[16] The severely decreased function *CYP2D6*10* allele is common among Asian populations. East Asian populations have the highest rate of 0.25 activity scores relative to other biogeographical groups.[3,16] The frequency of the alleles in a given population affects the chances of an individual in that population being a PM or UM for CYP2D6.

CYP2D6 Copy Number Variation

In addition to genotype, copy number variation (CNV) in CYP2D6 resulting from gene duplications or deletions can also influence metabolism. Two copies of an allele are normally inherited, one from the mother and one from the father.[17] In cases where CNV occurs, two or more copies of a gene may be inherited from one of the parents, or the gene may be deleted altogether.[17] When there is a duplication of the CYP2D6 gene, the additional copy is also factored into the activity score. Therefore, the more copies of CYP2D6 present, the more likely that individual will have increased CYP2D6 function.[3,17] The increased CYP2D6 function causes the individual to metabolize compounds much faster than a person with only one copy of the gene.[17] In contrast, reduced CYP2D6 function resulting from a deletion will inhibit the individual's ability to metabolize the compound, which will reduce or eliminate the intended analgesic effect of the opioid.

Over 12 percent of the United States population has a CNV in the CYP2D6 gene.[17] The total copy number of CYP2D6 can range from zero copies to as high as ten copies.[17] This additional factor of genetic variation can significantly influence the expected metabolism type hindering the process of translating data into patient-specific outcomes. Scientists have previously overlooked the impact of CNV on drug response.[18] Additionally, some researchers have criticized the under-utilization of CYP2D6 CNV testing, particularly in laboratory-developed tests, stating that many of these tests only identify the single nucleotide polymorphisms and do not account for CYP2D6 CNVs.[18] The prevalence of CYP2D6 CNVs combined with the significant contribution to metabolizer phenotypes suggests that CYP2D6 CNV testing should be used alongside genotype analysis for the most accurate and clinically useful results in pharmacogenetic testing.[18]

CYP2D6 Molecular Methods

There are a variety of molecular test methods used for CYP2D6 testing. The methods include real-time polymerase chain reaction (PCR), sequencing, microarrays, and matrix-assisted

laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS). Each of the methods come with benefits and limitations when implementing them in a clinical setting.

Real-time PCR

PCR is a commonly used method for CYP2D6 genotype analysis in clinical laboratories. There are a variety of PCR tests available for genotyping CYP2D6, however, only a couple are Food and Drug Administration (FDA) approved.[19] The quality and usefulness of CYP2D6 PCR assays depend heavily on the design of the PCR primers specific to the intended target.[20]

A wide variety of PCR chemistries use a direct detection method for genotyping CYP2D6, one being TaqMan chemistry. TaqMan chemistry utilizes two oligonucleotide probes with a fluorescent molecule attached at the 5' end and a quencher located on the 3' end. In TaqMan assays designed for the detection of SNPs, one allele is detected by the fluorescein adamites (FAM) probe and the other by the aequorea victoria (VIC) probe.[21,22] The primers, probes, and master mix containing DNA polymerase, deoxynucleotide triphosphates (dNTPs), cofactor, and buffer are combined with the sample.[23] Then a thermal cycling protocol is performed on a PCR detection instrument such as the Applied Biosystems 7500 where the DNA is amplified and the fluorescence is measured.[24] The endpoint read of the fluorescence is used for genotype analysis.[24] Software programs such as *TaqMan Genotyper Software* analyze the amplification to generate allele plots in which distinct clusters should form.[24] One axis of the allele plot represents the allele detected by FAM and the other axis represents the allele detected by VIC. Clusters that are formed along the FAM axis represent the samples that are homozygous for the FAM-detected allele and those along the VIC axis are homozygous for the VIC-detected allele. A cluster that forms in the middle of the plot equidistant from both axes marks the samples that are heterozygous, meaning both alleles were detected in the samples.[24] TaqMan PCR is a fast and reliable method for CYP2D6 genotyping that is simple to perform compared to other methods.[20]

In addition to genotyping, PCR tests are also available for CNV identification for CYP2D6. The assays typically use TaqMan chemistry with real-time quantitative PCR methods.[19] In TaqMan CNV assays for CYP2D6, one probe labeled with FAM targets the CYP2D6 sequence while another probe labeled with VIC targets a reference gene with a known copy number, such as Ribonuclease P.[24] Both sequences are amplified in the same well simultaneously, and at the end of the PCR, a cycle threshold (Ct) value is determined for both targets. The Ct value is the number of PCR cycles it takes for the target to cross the threshold where it eventually exceeds background amplification.[25]

To determine the number of copies of CYP2D6 in a sample, the difference between the FAM and VIC Ct values are measured for the tested sample and compared to the difference in Ct values measured in a calibrator sample with a known copy number for CYP2D6. Special software automatically performs the calculations and determines a predicted copy number using a data file exported from the PCR instrument. An example of the technology is the *CopyCaller 2.1* software created by Thermo Fisher Scientific (Waltham, MA).[24] The software also provides statistics to assess the confidence of each copy number call and assigns each sample a score. One of the limitations of the technology is that it requires a minimum of seven samples with the same predicted copy number in a single PCR run in order to accurately generate confidence scores for each sample's predicted copy number.[24]

Sequencing

Various sequencing techniques are available for CYP2D6 analysis. In fact, the use of Sanger sequencing actually led to the discovery of the CYP2D6 gene and pseudogenes.[2] Eventually, long-range PCR enabled Sanger sequencing of targeted exons across full-length CYP2D6 amplicons, leading to the identification of the

initial CYP2D6 star alleles.[2] As innovative technologies emerge, Sanger sequencing remains the gold-standard for many molecular methods including genotyping applications. However, it is rapidly being replaced in clinical laboratories with higher throughput methods such as targeted next generation sequencing (NGS).[2]

Targeted NGS includes a step where specific gene regions are selectively amplified through PCR using a gene panel. A gene panel is a pool of oligonucleotide primer pairs used to amplify the target region during PCR, creating a sequencing-ready library of DNA amplicons.[26] The DNA is denatured and added to a small plate, sometimes referred to as a flow cell containing oligonucleotides that match the adapter sequences of the library. The adapter sequences on the DNA fragments hybridize with the targets on the plate. PCR synthesizes a complementary DNA (cDNA) sequence. Fragments that fail to hybridize to a complementary sequence are removed in a subsequent washing. The free end of the cDNA sequence then hybridizes to a secondary oligonucleotide on the plate in a process known as bridge building.[27] The bridge is amplified and denatured again, and the process is repeated, creating multiple copies of forward and reverse strands. A primer then binds to the oligonucleotide to start the sequencing process using fluorescently labeled nucleotides. As the nucleotides bind to the sequence, they are excited by a laser to obtain a color-coded signal until the sequencing is complete, generating millions of reads in the process.[27]

While NGS offers a rapid and reliable method for detecting SNPs in CYP2D6, the massive amount of data produced by the millions of reads requires extraordinarily complex bioinformatic systems to analyze the results. Another limitation of this method for CYP2D6 testing is the challenge associated with detection of CNVs which typically requires the use of quantitative real-time PCR.[2,28] The challenges are caused by the variances in coverage depth often associated with NGS methods. The variances are due to the amount of GC content in the target regions and biochemical properties of the kits used in the initial enrichment steps prior to sequencing.[28]

Microarrays

A substantial portion of all pharmacogenomic tests use microarray method. Microarrays use a grid that contains small wells, each of which contains multiple copies of a probe fixed to a solid surface in the well. Each well represents a different gene or region of interest. The sample DNA is denatured and cut into smaller, more manageable fragments.[29] The small fragments are labeled by attaching a fluorescent dye. The labeled sample DNA is inserted into the wells where it hybridizes with complementary probes.[29] Any unbound DNA is then washed away, and the bound DNA fluoresces resulting in the identification of a specific gene arrangement or variation.

Microarrays are commonly used for pharmacogenomic applications because the technology can analyze thousands of genetic variants simultaneously, including CNV.[30] Other advantages of microarray technology include rapid output, affordability, and availability of the technology as well as high accuracy and relatively simple analysis and variant calling compared to other methods.[31 - 33] Microarray panels can be customized to include specific genes of interest to create pharmacogenomic panels to identify the variants important in pain management, cardiac, and psych disorders.[31,33] The main limitation associated with microarray technology is that it cannot detect novel variants. However, genome-wide array technology can detect virtually all SNPs of known clinical importance making it suitable for clinical applications.[31] In fact, multiple assays that detect drug metabolizing enzymes that have been granted FDA approval utilize microarray technology.[19]

MALDI-TOF Mass Spectrometry

One of the less commonly used methodologies for CYP2D6 genotyping is MALDI-TOF MS. In this method, forward and reverse primers are created for the target SNP and a PCR step is

performed to amplify the region of DNA containing the SNPs.[34,35] An extension step is then performed during which an extension primer anneals to the polymorphic base and a terminator extends the fragment by a single additional base.[34] The product of the reaction is added to a chip containing matrix solution. The matrix assists in the ionization process by absorbing energy from a laser.[34,35] Electrostatic potential accelerates the ionized DNA molecules through a tube toward a detector which measures the relative time of flight of each molecule.[34] The mass of the DNA fragment is calculated. The modified terminator bases enable detection of mass differences between fragments differing by only one base.[34] This method is used to detect SNPs based on the mass of the variant sequence.[34 - 36]

Tests using MALDI-TOF MS are proving to be a competitive analytical method due to the many benefits including rapid high throughput, ability to customize, relatively easy setup protocols, and low cost per test.[35,36] Additionally, MALDI-TOF MS technology has demonstrated high accuracy, sensitivity, and specificity for pharmacogenetic testing.[35,36] There is currently only one MALDI-TOF MS-based genotyping assay approved for clinical use in the United States, however it is not for CYP2D6 genotyping.[19]

Discussion

Pharmacogenetic testing for CYP2D6 is essential to determine a patient's ability to metabolize a compound appropriately and predict the response to CYP2D6-metabolized drugs such as opioids. Both genotype and copy number variation analyses provide critical information that can help health care providers prescribe appropriate treatments and dosages based on the genetic profile of the patient.

Understanding the clinical utility of CYP2D6 testing for personalized pain management is more important than ever given the current opioid crisis. Opioid use has increased by more than 10 times in the last 20 years, and it has been predicted that 480,000 people in the United States could die from opioid overdose in the next 10 years.[5,37] The recent COVID-19 pandemic may have contributed to the severity of the worldwide opioid problem due to intense social isolation, increased experiences of grief and trauma, and limited access to an already low number of in-person treatment centers for people struggling with opioid abuse.[37]

Also contributing to the high use of opioids is the prevalence of opioids like oxycodone as a postoperative treatment for pain. Of the 3.9 million surgeries undergone by children each year in the United States, oxycodone is prescribed for 2,116 out of every 100,000 patients.[38] Despite the high variability in response to the medication and the increased risk for opioid dependence, oxycodone is the most commonly prescribed oral opioid for children.[38]

The immense threat that the overuse and misuse of opioids poses to the population highlights the urgent need for an increased awareness and understanding of the utilization of CYP2D6 testing to aid in prescribing safe and effective treatment for pain. Evidence suggests that in addition to oxycodone dosage requirements, CYP2D6 genotypes may also affect a patient's risk for opioid side-effects like respiratory depression.[38] This contradicts current information provided by the CPIC which stated that "there is insufficient evidence and confidence to provide a recommendation to guide clinical practice at this time for oxycodone" based on CYP2D6 genotype.[15]

While the current research available may be "insufficient" at this time, there is enough substantial evidence to warrant further investigation and consideration for clinical implementation. Studies have shown that genotype availability and guidance in a clinical setting influences postoperative prescriptions of opioids. These effects include a decrease in the prescription of a medication due to high variability in response to the drug as well as a

lower rate of oxycodone prescriptions for PMs and UMs.[38,39]

One of the major challenges of integrating CYP2D6 testing into routine clinical practice is the lack of standardization of activity scores, associated phenotypes, and the clinical recommendations for each phenotype. Translating CYP2D6 genotype to phenotype has posed a significant challenge to the scientific community.[40] CPIC guidelines on converting CYP2D6 activity scores to metabolizer phenotype have been modified in recent years, primarily changing the activity score ranges that constitute an IM, NM, and UM.[41,42] A recent study found that the metabolic ratio (the ratio of parent drug to metabolite after 3 hours) of three different CYP2D6 genotype groups was significantly different, even though all three groups would be classified as NMs according to CPIC guidelines.[40] The clinical implications of these differences need to be carefully considered when establishing or modifying the activity score translation system. To produce sufficient comparable data to aid in establishing guidelines for prescription recommendations for each phenotype, it would be beneficial to adopt one standard CYP2D6 genotype-to-phenotype translation system.

One of the factors that is improving the ability to implement pharmacogenetic testing in clinical laboratories is that the same molecular methodologies used for CYP2D6 can also be used for a variety of other important pharmacogenes, including other members of the Cytochrome P450 superfamily such as CYP2C9 and CYP2C19.[43] Once a molecular method for a gene such as CYP2D6 has been implemented, it can be a relatively straightforward process to continue implementing additional assays to monitor other genes, as most principles and procedures are transferable to other pharmacogenes, apart from the unique primer designs.

There are a variety of molecular methodologies currently available for pharmacogenetic testing including PCR, sequencing, microarray, and MALDI-TOF MS. While molecular methods for genetic testing have improved, leading to more sensitive and less labor-intensive tests, there are still limitations associated with these methods that remain a challenge for clinical laboratories. One of the current limitations of copy number analysis is that most CNV assays do not identify which alleles have multiple copies.[15] The main challenge associated with genotype analysis across all molecular methods is the risk of incorrect variant calling.[44] For instance, rare and de novo variants will not be detected by most assays and may be falsely reported as a functional allele by default.[15] Regardless of the diagnostic tool being used, laboratorians should be especially cautious when analyzing variants in homologous regions to reduce the chance of an incorrect call.[44] In some cases, incorrect variant calls can in turn be translated into incorrect phenotypes, which may have significant negative outcomes for the patient. Ambiguous results and variants in highly homologous regions require confirmation with an independent methodology to help avoid this risk.

Conclusion

In order to continue developing and refining strategies for personalized medical treatment it is necessary to assess the clinical utility and challenges associated with current practices. Pharmacogenetic testing is a valuable tool in personalized medicine due to the influence that genetic variation has on the diversity of drug metabolism within and between populations. In addition to pain management, pharmacogenetic testing has other useful clinical applications. Gene sequences are grouped to create different test panels including cardiovascular, psychiatric, cancer and immunosuppression, and endocrine and metabolic panels.

CYP2D6 testing has proven to be a valuable way to predict an individual's response to opioids as well as other CYP2D6-metabolized drugs. Aside from pain medications, CYP2D6 testing may be clinically useful for the

prescription of antidepressants, antiarrhythmics, antipsychotics, and ß-blockers. In the new era of personalized medicine, CYP2D6 testing will play a pivotal role in determining an individual's best drug treatment options.

References

1. Ahmed S, Zhou Z, Zhou J, Chen SQ. Pharmacogenomics of Drug Metabolizing Enzymes and Transporters: Relevance to Precision Medicine [published correction appears in Genomics Proteomics Bioinformatics. 2018 Apr 21]. Genomics Proteomics Bioinformatics. 2016;14(5):298-313. doi:10.1016/j.gpb.2016.03.008
2. Yang Y, Botton MR, Scott ER, Scott SA. Sequencing the CYP2D6 gene: from variant allele discovery to clinical pharmacogenetic testing. Pharmacogenomics. 2017;18(7):673-685. doi:10.2217/pgs-2017-0033
3. Kane M. CYP2D6 overview: allele and phenotype frequencies. Med Genet Sum [Internet]. 2021 Oct [cited 2023 Apr 10]. Available from: https://www.ncbi.nlm.nih.gov /books/NBK574601/.
4. U.S. Food & Drug Administration [Internet]. Silver Spring: U.S. Food and Drug Administration; c2018 [cited 2023 Apr 10]. Precision Medicine; Available from: https://www.fda.gov/medical-devices/in-vitro-diagnostics/precision-medicine#:~:text=Precision%20medicine%2C%20sometimes%20known%20as,genes%2C%20environments%2C%20and%20lifestyles.
5. Shipton EA, Shipton EE, Shipton AJ. A Review of the Opioid Epidemic: What Do We Do About It?. Pain Ther. 2018;7(1):23-36. doi:10.1007/s40122-018-0096-7
6. Robert M, Jouanjus E, Khouri C, Fouilhé Sam-Laï N, Revol B. The opioid epidemic: A worldwide exploratory study using the WHO pharmacovigilance database. Addiction. 2023 Apr;118(4):771-775. doi: 10.1111/add.16081. Epub 2022 Nov 22. PMID: 36331523.
7. Celentano D. The Worldwide Opioid Pandemic: Epidemiologic Perspectives. Epidemiol Rev. 2020;42(1):1-3. doi:10.1093/epirev/mxaa012
8. Cohen B, Ruth LJ, Preuss CV. Opioid Analgesics. In: StatPearls. Treasure Island (FL): StatPearls Publishing; October 10, 2022.
9. Murphy DL, Lebin JA, Severtson SG, Olsen HA, Dasgupta N, Dart RC. Comparative Rates of Mortality and Serious Adverse Effects Among Commonly Prescribed Opioid Analgesics. Drug Saf. 2018;41(8):787-795. doi:10.1007/s40264-018-0660-4.
10. Mayo Clinic Staff, editors. How opioid addiction occurs [Internet]. Rochester: Mayo Foundation for Medical Education and Research; 2022 [cited 2023 Apr 10]. Available from: https://www.mayoclinic.org/diseases-conditions/prescription-drug-abuse/in-depth/how-opioid-addiction-occurs/art-20360372.
11. Bishop JR. Pharmacogenetics. Handb Clin Neurol [Internet]. 2018 Jan [cited 2023 Apr 10]; 147(1). Available from: https://doi.org/10.1016/B978-0-444-63233-3.00006-3.
12. Ahmed S, Zhou Z, Zhou J, Chen SQ. Pharmacogenomics of Drug Metabolizing Enzymes and Transporters: Relevance to Precision Medicine [published correction appears in Genomics Proteomics Bioinformatics. 2018 Apr 21]. Genomics Proteomics Bioinformatics. 2016;14(5):298-313. doi:10.1016/j.gpb.2016.03.008
13. Yang Y, Botton MR, Scott ER, Scott SA. Sequencing the CYP2D6 gene: from variant allele discovery to clinical pharmacogenetic testing. Pharmacogenomics. 2017;18(7):673-685. doi:10.2217/pgs-2017-0033
14. Kane M. CYP2D6 overview: allele and phenotype frequencies. Med Genet Sum [Internet]. 2021 Oct [cited 2023 Apr 10]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK574601/.

15. Phang-Lyn S, Llerena VA. Biochemistry, Biotransformation. In: StatPearls. Treasure Island (FL): StatPearls Publishing; August 22, 2022.
16. T Miotto K, Cho AK, Khalil MA, Blanco K, Sasaki JD, Rawson R. Trends in Tramadol: Pharmacology, Metabolism, and Misuse. Anesth Analg. 2017;124(1):44-51. doi:10.1213/ANE.0000000000001683.
17. Zhu Y, Lopes GS, Bielinski SJ, et al. Impact of Pharmacogenomic Information on Values of Care and Quality of Life Associated with Codeine and Tramadol-Related Adverse Drug Events. Mayo Clin Proc Innov Qual Outcomes. 2021;5(1):35-45. Published 2021 Jan 13. doi:10.1016/j.mayocpiqo.2020.08.009.
18. Crews KR, Monte AA, Huddart R, et al. Clinical Pharmacogenetics Implementation Consortium Guideline for CYP2D6, OPRM1, and COMT Genotypes and Select Opioid Therapy. Clin Pharmacol Ther. 2021;110(4):888-896. doi:10.1002/cpt.2149.
19. PharmGKB [Internet]. Stanford: PharmGKB; c2001-2023 [cited 2023 Apr 10]. Gene-specific Information Tables for CYP2D6. Available from: https://www.pharmgkb.org/page/cyp2d6RefMaterials.
20. Jarvis JP, Peter AP, Shaman JA. Consequences of CYP2D6 Copy-Number Variation for Pharmacogenomics in Psychiatry. Front Psychiatry. 2019;10:432. Published 2019 Jun 20. doi:10.3389/fpsyt.2019.00432
21. Beoris M, Amos Wilson J, Garces JA, Lukowiak AA. CYP2D6 copy number distribution in the US population. Pharmacogenet Genomics. 2016;26(2):96-99. doi:10.1097/FPC.0000000000000188.
22. U.S. Food & Drug Administration [Internet]. Silver Spring: U.S. Food and Drug Administration; c2023 [cited 2023 Apr 10]. Nucleic Acid Based Tests. Available from: https://www.fda.gov/medical-devices/in-vitro-diagnostics/nucleic-acid-based-tests.
23. Larsen JB, Rasmussen JB. Pharmacogenetic testing revisited: 5' nuclease real-time polymerase chain reaction test panels for genotyping CYP2D6 and CYP2C19. Pharmgenomics Pers Med. 2017;10:115-128. Published 2017 Apr 18. doi:10.2147/PGPM.S131580.
24. Ye H, Lu Q, Duan N, Wang Z. GO-amplified fluorescence polarization assay for high-sensitivity detection of aflatoxin B1 with low dosage aptamer probe. Anal Bioanal Chem. 2019;411(5):1107-1115. doi:10.1007/s00216-018-1540-6.
25. Lambert GG, Depernet H, Gotthard G, et al. Aequorea's secrets revealed: New fluorescent proteins with unique properties for bioimaging and biosensing. PLoS Biol. 2020;18(11):e3000936. Published 2020 Nov 2. doi:10.1371/journal.pbio.3000936.
26. MilliporeSigma [Internet]. Darmstadt: MilliporeSigma; c2023 [cited 2023 Apr 10]. PCR Master Mix. Available from: https://www.sigmaaldrich.com/US/en/technical-documents/technical-article/genomics/pcr/pcr-master-mix.
27. ThermoFisher Scientific, editor. Pharmacogenomics Experiments Application Guide [Internet]. Waltham: ThermoFisher Scientific; 2022 [cited 2023 Apr 10]. Avalable from: https://www.thermofisher.com/document-connect/document-connect.html?url=https%3A%2F%2Fassets.thermofisher.com%2FTFS-Assets%2FLSG%2Fmanuals%2FMAN0009612_Pharamacogenomics_UG.pdf&title=QXBwbGljYXRpb24gR3VpZGU6IFBoYXJtYWNvZ2Vub21pY3MgRXhwZXJpbWVudHM=.
28. ThermoFisher Scientific, editor. Data Analysis on the ABI PRISM® 7700 Sequence Detection System: Setting Baselines and Thresholds [Internet]. Waltham: ThermoFisher Scientific; 2022 [cited 2023 Apr 10]. Available from: https://www.thermofisher.com/document-connect/document-connect.html?url=https%3A%2F%2Fassets.thermofisher.com%2FTFS-Assets%2FLSG%2FApplication-Notes%2Fcms_042502.pdf&title=QXBwbGljYXRpb24gTm90ZXMgJmFtcDsgVHV0b3JpYWxzOiBEYXRhIEFuYWx5c2lzIG9uIHRoZSBBQkkgUFJJU00mcmVnOyA3NzAwIFNlcXVlbmNlIERldGVjdGlvbiBTeXN0ZW06IFNldHRpbmcgQmFz

ZWxpbmVzIGFuZCBUaHJlc2hvbGRzOiBHdWlkZTogUmV2IEE=.
29. Kim MJ, editor. Complete Guide to Ion Ampliseq Technology – How Does Ion Ampliseq Technology Work? [Internet]. Waltham: ThermoFisher Scientific; 2017 [cited 2023 Apr 10]. Available from: https://www.thermofisher.com/blog/behindthebench/complete-guide-to-ion-ampliseq-technology-how-does-ion-ampliseq-technology-work/.
30. Illumina, Inc. An Introduction to Next-Generation Sequencing Technology [Internet]. San Diego: Illumina, Inc.; 2017 [cited 2023 Apr 10]. Available from: https://www.illumina.com/content/dam/illumina-marketing/documents/products/illumina_sequencing_introduction.pdf.
31. May V, Koch L, Fischer-Zirnsak B, et al. ClearCNV: CNV calling from NGS panel data in the presence of ambiguity and noise. Bioinformatics. 2022;38(16):3871-3876. doi:10.1093/bioinformatics/btac418.
32. National Human Genome Research Institute [Internet]. Bethesda: National Institutes of Health; c2020 [cited 2023 Apr 10]. DNA Microarray Technology Fact Sheet. Available from: https://www.genome.gov/about-genomics/fact-sheets/DNA-Microarray-Technology.
33. Mou X, Ali Z, Li B, Li T, Yi H, Dong H, He N, Deng Y, Zeng X. Multiple genotyping based on multiplex PCR and microarray. Chin Chem Let [Internet]. 2016;27(11):1661-1665. Published 2016 Nov. Available from: https://www.sciencedirect.com/science/article/abs/pii/S100184171630078X.
34. Shahandeh A, Johnstone DM, Atkins JR, et al. Advantages of Array-Based Technologies for Pre-Emptive Pharmacogenomics Testing. Microarrays (Basel). 2016;5(2):12. Published 2016 May 28. doi:10.3390/microarrays5020012.
35. Krebs K, Milani L. Translating pharmacogenomics into clinical decisions: do not let the perfect be the enemy of the good. Hum Genomics. 2019;13(1):39. Published 2019 Aug 27. doi:10.1186/s40246-019-0229-z.
36. Medwid S, Kim RB. Implementation of pharmacogenomics: Where are we now? [published online ahead of print, 2022 Nov 10]. Br J Clin Pharmacol. 2022;10.1111/bcp.15591. doi:10.1111/bcp.15591.
37. Ellis JA, Ong B. The MassARRAY® System for Targeted SNP Genotyping. Methods Mol Biol. 2017;1492:77-94. doi:10.1007/978-1-4939-6442-0_5.
38. Li D, Yi J, Han G, Qiao L. MALDI-TOF Mass Spectrometry in Clinical Analysis and Research. J Am Chem Soc [Internet]. 2022 Aug [cited 2023 Apr 10];2(5). Available from: https://pubs.acs.org/doi/full/10.1021/acsmeasuresciau.2c00019.
39. Cui JJ, Wang LY, Tan ZR, Zhou HH, Zhan X, Yin JY. MASS SPECTROMETRY-BASED PERSONALIZED DRUG THERAPY. Mass Spectrom Rev. 2020;39(5-6):523-552. doi:10.1002/mas.21620.
40. Hornberger J, Chhatwal J. Opioid Misuse: A Global Crisis. Value Health. 2021;24(2):145-146. doi:10.1016/j.jval.2020.12.003.
41. Cavallari LH, Cicali E, Wiisanen K, et al. Implementing a pragmatic clinical trial to tailor opioids for acute pain on behalf of the IGNITE ADOPT PGx investigators. Clin Transl Sci. 2022;15(10):2479-2492. doi:10.1111/cts.13376.
42. Merchant S, Prows CA, Yang F, et al. Association of CYP2D6 genotype predicted phenotypes with oxycodone requirements and side effects in children undergoing surgery. Ann Transl Med. 2022;10(23):1262. doi:10.21037/atm-2022-58.
43. Jin Y, Zhang S, Hu P, et al. The impact of CYP2D6*41 on CYP2D6 enzyme activity using phenotyping methods in urine, plasma, and saliva. Front Pharmacol. 2022;13:940510. Published 2022 Aug 30. doi:10.3389/fphar.2022.940510.
44. Hicks JK, Sangkuhl K, Swen JJ, et al. Clinical pharmacogenetics implementation consortium guideline (CPIC) for CYP2D6 and CYP2C19 genotypes and dosing of tricyclic antidepressants: 2016 update. Clin Pharmacol

Ther. 2017;102(1):37-44. doi:10.1002/cpt.597.
45. Caudle KE, Sangkuhl K, Whirl-Carrillo M, et al. Standardizing CYP2D6 Genotype to Phenotype Translation: Consensus Recommendations from the Clinical Pharmacogenetics Implementation Consortium and Dutch Pharmacogenetics Working Group. Clin Transl Sci. 2020;13(1):116-124. doi:10.1111/cts.12692.
46. Gaedigk A, Ingelman-Sundberg M, Miller NA, et al. The Pharmacogene Variation (PharmVar) Consortium: Incorporation of the Human Cytochrome P450 (CYP) Allele Nomenclature Database. Clin Pharmacol Ther. 2018;103(3):399-401. doi:10.1002/cpt.910.
47. Nfonsam L, Ordorica S, Ghani M, et al. Leveraging the power of new molecular technologies in the clinical setting requires unprecedented awareness of limitations and drawbacks: experience of one diagnostic laboratory. J Med Genet. 2019;56(6):408-412. doi:10.1136/jmedgenet-2018-105443.

Review article

Harmonization of Thyroid Hormone Testing; A Complex Challenge to Improve Patient Outcomes

David Hamilton[1], Demetra Castillo[1], and Patricia Tille[1*]

Medical Laboratory Sciences Program, College of Allied Health Science, University of Cincinnati, Cincinnati, Ohio, USA[1]

Thyroid diseases are extraordinarily complex endocrine conditions. Hyperthyroidism, hypothyroidism, and thyroid cancer is diagnosed and monitored using thyroid hormone (TH) laboratory testing. Treatment decisions adhere to published clinical practice guidelines that rely heavily upon laboratory measurements such as thyroid stimulating hormone (TSH), thyroxine (T4), free thyroxine (FT4), and thyroglobulin (TG). The immunoassays for TH testing utilize various methodologies dependent upon specific reagent manufacturers. This presents a challenge for laboratories to provide TH measurements that are dependable and consistent. This challenge has led to initiatives for the standardizations and harmonization of TH testing. TH immunoassays are complex methods that are highly susceptible to interferences such as heterophile antibodies, binding proteins, and anti-reagent antibodies. In addition, there is a lack of appropriately established reference intervals (RI) for TH laboratory values. Factors such as pregnancy, age, sex, and geographical location complicate the standardization of TH RIs. Harmonization and standardization for TH testing is challenging, however, it is clear that patients with thyroid disease would benefit from these initiatives.

Keywords: Thyroid hormones; Thyroid testing; Harmonization; Standardization

---

Accepted: June 13, 2023

*Corresponding author: Patricia Tille. E-mail: tillepm@ucmail.uc.edu

Introduction

Thyroid testing is among the highest volume assays utilized across the global healthcare network.[1] Medicare costs for thyroid stimulating hormone (TSH) have been reported at $469 million per year. Approximately 59 million TSH and 18 million free thyroxine (FT4) tests performed are performed annually with TSH among the top 25 laboratory tests performed in four out of five hospitals.[1,2] FT4, TSH, along with thyroglobulin (TG) immune-assays play an important role in the diagnosis and management of thyroid diseases.[3] The availability of numerous testing platforms and diagnostic methods for TH testing creates a complex challenge in establishing harmonization across manufacturer's and standardizing reference intervals. In laboratory medicine, harmonization of laboratory testing refers to achieving equivalent results with the same interpretation irrespective of the procedure used, the unit or reference interval applied, and when and/or where a measurement is made.[4] Additionally, standardization involves the attainment of harmonization through traceable methods of laboratory values to primary reference material.[2]

Over the last decade, organizations such as the American Association for Clinical Chemistry (AACC) and the College of American Pathologists (CAP) have led the effort to harmonize laboratory results by establishing the International Consortium for Harmonization of Clinical Laboratory Results (ICHCLR).[4] In addition to the work for ICHCLR, thyroid-specific organizations such as the Partnership for the Accurate Testing of Hormones (PATH) are attempting to improve standardization and harmonization among thyroid immunoassays.[5] The lack of standardization and harmonization prevents clinical laboratories from assessing the accuracy and reliability of reference materials.[5] Additionally, systemic bias or disparity in interferences among the different immunoassays has been observed when a single patient is analyzed with multiple methodologies leading to misinterpretations.[6] Thyroid immunoassays are among the many laboratory tests that are lacking uniformity through standardization and harmonization. Harmonizing results for thyroid tests would lead to the development of standardized treatment guidelines, improve the accuracy of clinical diagnostics, and reduce the number of medical errors improving patient outcomes.

Thyroid Hormones and Disease

THs regulate cellular differentiation, growth, and metabolism in every tissue in the body.[7] The hypothalamic-pituitary-thyroid axis regulates thyroid hormone levels. The pituitary gland produces TSH, which stimulates the production of T4 and 3,3'5-triiodothyronine (T3).[7,8] Given the importance of THs in cellular growth and metabolism, TH testing, specifically TSH, FT4, and TG levels are important for the diagnosis and management of thyroid diseases such as hyperthyroidism (excessive thyroid hormone production), hypothyroidism (reduction in thyroid hormone production), and thyroid cancer. Studies have shown 4.6% of individuals in the United State (U.S.) aged 12 years or older have hypothyroidism while 1.2 % have hyperthyroidism.[9] Despite the prevalence of thyroid disease, the U.S. Preventative Task Force recommends thyroid testing only in the presence of symptoms and risk factors and advises against using it as a screening method.[10]

Patient's presenting with symptoms of hypothyroidism include hair loss, weight gain, dry skin, constipation, fatigue, and depression.[11] Hashimoto's thyroiditis is an autoimmune form of hypothyroidism that restricts FT4 production, resulting in an increased production of TSH.[10,11] Hyperthyroidism symptoms include weight loss, palpitations, heat intolerance, fatigue, tremors, and exophthalmos.[12] Graves' disease (GD), toxic multinodular goiter (TMNG), and toxic adenoma (TA) are examples of hyperthyroidism. The excess production of thyroid hormone inhibits the release of TSH.[10] It is also important to note that conditions such as GD produce autoantibodies that can complicate the

interpretation of TH values. Symptoms associated with hyperthyroidism and hypothyroidism mimic other conditions resulting in patients failing to recognize the symptoms of thyroid disease. This can delay the diagnosis and treatment for serious conditions such as thyroid cancer.

Thyroid cancer prevalence has increased substantially accounting for approximately 2.1% of all cancer diagnoses and ranked as the 9th leading cancer in 2020, worldwide.[13,14] Common types of thyroid cancer include papillary, follicular, and medullary, with mortality rates at 20 years averaging 1% - 2%, 10% - 20%, and 25% - 50% respectively.[15] Over 90% of endocrine malignancies include a thyroid cancer diagnosis.[16] Thyroid cancer is the most common diagnosis among adolescents and adults, and is the seventh most common in females.[16] Studies have shown an increased risk of thyroid cancer in individuals who are diagnosed with benign thyroid nodules, adenoma, and goiter.[17] While TSH and FT4 immunoassays provide information for diagnosing hyperthyroidism and hypothyroidism, thyroid cancers are detected through imaging of suspected nodules or goiters and confirmed with fine needle aspiration cytology.[13] TG is secreted in small amounts by the thyroid gland in healthy individuals but is often elevated in papillary and follicular thyroid cancer.[16] TG immunoassays become an important piece for monitoring and treating thyroid cancer. With the increasing incidence of thyroid cancer, it is important to educate patients on the importance of routine preventative care, which includes routine TH testing.

Routine care and treatment for thyroid disease is important because TH regulates the metabolism throughout the body. Treatment of hypothyroidism has historically been through prescribing the synthetically produced exogenous form of T4, levothyroxine.[10] While the U.S. Food and Drug Administration has approved the use of generic versions of levothyroxine, the brand-name drug is the treatment of choice for endocrinologists and professional organizations.[10] Hyperthyroidism disorders such as GD, TMNG, and TA can lead to a condition called thyrotoxicosis. Treatment for each of the disorders is dependent on the diagnosis and include thyroidectomy, ß-blockers, radioactive iodine, or antithyroid drugs.[18] Thyroid cancer treatment for most low-risk cancers involves surgical removal including lobectomy and total thyroidectomy.[15] Active surveillance is a viable alternative to traditional surgical treatment options for low-risk differentiated thyroid cancer.[16]

Monitoring thyroid disease is difficult and often specific to the patient or the stage of the disease. Factors such as, sex, and pregnancy can dictate need and frequency for thyroid laboratory testing. For example, monitoring non-pregnant patients with hyperthyroidism requires testing TSH levels at intervals of six to eight weeks until within the reference range, then every six to twelve months, pending no change in clinical status.[18,19] This is significantly different than monitoring thyroid cancer.

Thyroid cancer, like other cancers, has a possibility of recurrence. Monitoring the disease is an essential step for managing patient care. Imaging and laboratory testing methods are used to monitor disease following partial or complete removal of the thyroid. The presence of or increase in TG post-surgery may be evidence of the recurrence of thyroid cancer.[20] To reduce recurrence and improve monitoring of TG levels, post-surgical radioactive iodine ablation of residual thyroid tissue is an option.[20] Regardless of what TH is measured, the monitoring of each analyte plays a significant role in determining the type of patient care required.

Clinical Utility of Thyroid Immunoassays

TSH Immunoassays

TSH immunoassays are extremely sensitive and specific, making it the most utilized laboratory test for the initial diagnosis of thyroid disease.[23] TSH immunoassays are classified as first to third generation according to the limit

of detection or improvement in the sensitivity.[8,24] Historically, competitive, and non-competitive immunoassays are utilized for TSH. Competitive first-generation immunoassays use polyclonal antibodies while non-competitive second and third-generation immunoassays utilize monoclonal antibodies.[25] The third-generation immunoassays are available on various automated laboratory analyzers. The automated methods are primarily two-site sandwich immunoassays that detect labeled antibodies specific to TSH epitopes.[26] In addition to improving sensitivity (0.01–0.02 μIU/mL) for TSH testing, the evolution to third-generation immunoassays can differentiate hyperthyroid, euthyroid, and hypothyroid conditions.[24-26]

Despite the progress made with improving sensitivities in newer-generation testing, comparability between different manufacturer methods exist. The International Federation of Clinical Chemistry (IFCC) Working Group on Thyroid hormones demonstrated that method-related variations exist in thyroid immunoassays.[27] The variations indicate potential issues with RIs in TSH testing. As defined by the Clinical Laboratory Standards Institute (CLSI), RIs are ranges derived from healthy individuals within a definitive percentage measurement of 95%. The RIs for TSH are calculated within a percentage of measurements from between the 2.5th - 97.5th percentile.[28,29] Most laboratories follow this recommendation for all diagnostic assays.

While following CLSI recommendations for establishing RIs through analysis of healthy individuals, there are additional factors to consider. Factors such as additional patient conditions, age, sex, ethnicity, and regional iodine intake should all be included when establishing valid RIs for thyroid testing.[30] For example, the 2017 American Thyroid Association (ATA) guidelines recommend using RIs of population and trimester-specific TSH based on a population without known thyroid disease and in an area with premium iodine distribution.[31] More recent developments in establishing RIs utilize a patient-personalized approach. Personalized RIs are established by comparing the patient's laboratory test results with their individual RIs as opposed to population-based methods.[32] Regardless of the method utilized, the need for consistent and dependable TSH results and RIs remains to accurately diagnose and treat thyroid disease.

In addition to the RI challenges, limitations associated with TSH immunoassay methodologies include non-analyte-specific interferences and analyte-specific interferences.[33] Non-analyte interferences include heterophile antibodies (HAb), anti-reagent antibodies, and streptavidin-biotin.[33,34] HAb present in individuals can falsely elevate TSH values. Anti-reagent antibodies (ARA) and streptavidin-biotin found in patient serum can block the functionality of assay-specific reagents, interfering with TSH results. Analyte interferences include TSH autoantibodies which can falsely elevate test results and TSH variants, including nine separate variants detected by TSH immunoassays.[34] Additional limitations include medications such as steroids, the timing of administering levothyroxine as well as season and diurnal TSH variations.[33] Immunoassay manufacturers continue to develop methods to limit the effect of interfering substances and understand the fluctuation in hormones that can alter the accuracy of TSH results.

FT4 Immunoassays

In addition to TSH, FT4 laboratory testing plays a significant role in the diagnosis and treatment of thyroid disease. Approximately 98.98% of T4 is protein bound, leaving 0.02% of FT4 for detection and quantification in immunoassays.[35] The testing methods for FT4 have similar characteristics as TSH immunoassays. Diagnostic screening for thyroid disease commonly includes TSH and FT4 in tandem, contributing to laboratory test utilization problems. FT4 testing is recommended only as a follow-up to abnormal TSH values.

FT4 testing methods also vary in design. While direct method liquid chromatography-tandem mass spectrometry (LC-MS/MS) assays

are available, automated indirect competitive immunoassays are the most popular for measuring FT4.[35] These methods deploy either one-step, labeled antibody, or two-step principles. One-step methods are easily automated and are competitive immunoassays using labeled hormone analogs. This limits interaction with binding protein thyroid hormones and hormones found in a patient's sample for a solid-phase anti-hormone antibody. The FT4 in a patient's sample competes with solid-phase hormone for the labeled antibody creating a measurement from a function of the fractional occupancy of hormone-antibody binding sites in the reaction mixture. A final washing step in the procedure creates an inversely proportional measurement of the FT4.· The two-step method, also known as back-titration, harnesses immobilized T4 to isolate a portion of total T4 from a diluted patient sample followed by a washing step, allowing for an inversely proportional calculation of FT4.[34,36]

Like TSH, RIs for FT4 are method dependent because of calibration biases and rely heavily on population-specific statistics such as patient conditions, ethnicity, age, and sex.[30,35] Guidelines for RI calculation methods are published by international organizations such as the ATA. However, data indicates that most laboratories do not follow the recommenddations.[31]

FT4 immunoassays are affected by non-analyte-specific limitations in testing which include protein interferences, thyroxine-binding globulin (TBG) excess or deficiency, pregnancy, familial dysalbuminemic hypothyroxinemia (FDH), transthyretin-associate hypothyroxinemia (TAH), HAb, and ARA. Protein interferences include the presence of paraproteins and abnormal immunoglobulins. Congenital TBG excess or deficient quantities can also cause interference with FT4 immunoassays.[34]

FT4 is monitored closely in patients who are pregnant due to the prevalence of thyroid disease. Evidence has shown that the thyroid gland becomes stressed during pregnancy. Thyroid disease, because of pregnancy, is incredibly more complicated than hypo or hyperthyroidism. Pregnancy interferences are method-related due to existing standardization differences and method sensitivity to the decreasing amounts of albumin present during gestation.[34] However, protein-specific FT4 immunoassay interferences are not unique to patients who are pregnant.

Additional interfering proteins include binding proteins thyroxine-binding globulin, transthyretin, and serum albumin. Each of the binding proteins are important in the proper storage and transport of TH. Presence of these proteins maintains appropriate levels of available TH to prevent a deficiency in the accessible substrate, loss of iodine, and other thyroid-related clinical factors.[37] Additionally, the autosomal mutation caused by FDH and TAH alters the structure of the binding proteins resulting in falsely elevated FT4.[34] This is due to the immunoassay's reliance upon the binding proteins present in human serum. Comparably, FT4 immunoassays also depend upon antibodies to perform measurements.

HAb, specifically autoimmune antibodies such as rheumatoid factor can interfere with FT4 immunoassays. Capture antibodies for measuring FT4 are unsuccessful because the assay is unable to differentiate them from HAb. This type of interference is reduced from 2 – 5 % by adding a HAb blocker reagent.[34] While this reduction improves performance, interference from HAb remains clinically significant.

In addition to HAb, human serum can also contain interfering ARA that target frequently used FT4 immunoassay reagents such as ruthenium or streptavidin.[34] For example, exposure to the bacterium *Streptomyces avidinii* produces an antibody that competes with streptavidin reagent, leading to falsely elevated FT4 levels.[38] There is a likelihood that human serum contains interfering ARA not yet discovered. These unknown factors complicate the diagnosis of thyroid diseases.

TG Immunoassays

TG immunoassays have evolved much like the methodologies used for detecting TSH and FT4. Improving sensitivity for TG is of particular

importance for the treatment and monitoring of thyroid cancer in patients following a thyroidectomy. The quest for sensitive detection methods with limited interferences for TG is essential. This has led to multiple testing methodologies that include chemiluminescent immunoassay (CLMIA), radioimmunoassay (RIA), and immunometric (IMA) technology.[39]

CLMIA methodologies utilize a luminescent labeled molecule that produces detectable radiation of light. This method performs measurements on analytes such as albumin and TG.[40] TG CLMIA methodologies are available in clinical laboratories.

RIA utilizes radioisotope-labeled antigens that compete with TG in a patient's sample for binding to a high-affinity TG antibody.[14] Although TG-RIA are standardized against BCR®457 certified reference material (Merck KGaA, Darmstadt Germany) from the European Commission Institute for Reference Materials, the method demonstrates unacceptable sensitivity. TG-RIA does however offer improved performance in the presence of interfering polyclonal antibodies.[39]

Similar to TSH and FT4 testing, first-generation LC-MS/MS provides reliable and consistent test results but due to expensive equipment accompanied by the need for highly trained laboratory personnel has limited availability.[39] In comparison, IMA is a sandwich or two-site methodology that uses two binding antibodies termed capture and detection antibodies. First, the highly specific binding antibodies are added to the patient sample, attaching to TG epitopes, followed by the detection antibodies to form a 'sandwich' where the automated process can detect and measure TG.[41] This methodology is highly popular internationally among testing laboratories as a tool for monitoring patients with thyroid cancer.

TG is primarily monitored as a tumor marker for differentiated thyroid cancer patients post-thyroidectomy, making the process for establishing RIs different from other THs.[42] As previously indicated, the production of TG occurs in response to the stimulation of the TSH receptor by TSH.[14] After two years of age, TG levels fall in the same range as adults but because most testing occurs post thyroidectomy or lobectomy, RIs become irrelevant. Individualized RIs based on thyroidectomy versus lobectomy procedures are preferred for determining appropriate TG levels.[34]

Immunoassays for TG measurements present several limitations which include heterophile and autoantibody interference. As previously mentioned with TSH and FT4 immunoassays, heterophile antibodies cause interferences, even in the presence of a low quantity of heterophile antibodies. The prevalence of autoantibodies represents a significant amount of the interferences associated with TG immunoassays. Most interestingly, elevated levels of autoantibodies may not interfere while low levels have a remarkable effect.[34,39] To limit interferences caused by autoantibodies, testing laboratories implement tandem testing to include TG antibody (TGAb) immunoassay. For example, in some laboratories, TG testing begins with an assay for TGAb via IMA. When the TGAb measurement is below the detectable limit, then TG testing is performed by a sensitive second-generation IMA. If TGAb is detected, specimens are assayed by RIA or LC-MS/MS because each is resistant to TGAb. However, some studies indicate that there is no diagnostic advantage to using TG LC-MS/MS versus an immunoassay methodology.[34]

Harmonization and Standardization Challenges

Improving and providing high quality patient care is essential for healthcare organizations. Laboratory services are essential to the delivery of high-quality patient care. There is an overwhelming consensus that the harmonization of laboratory results can play a significant role in improving patient outcomes.[4] The prevalence of thyroid disease is significant and oftentimes is accompanied by signs and symptoms that can be difficult to

detect.[21] Laboratory testing utilized by physicians and endocrinologists for the detection and management of thyroid disease should be accurate and reliable.[4] In the presence of harmonization, clinical practice guidelines would become more uniform, allowing for more consistent and appropriate healthcare decisions for the treatment of patients with thyroid disease.[4]

An additional consideration that affects the harmonization of a laboratory test is the variability of reference intervals (RIs) which are impacted by the patient population, test methodology, and the laboratory performing the assay. Clinical laboratories establish RIs for each thyroid immunoassay. However, there are circumstances where laboratories solely depend on manufacturer RIs. Manufacturer RIs are established by gathering data on populations either regionally or nationally.[22] This poses a problem for providers because of the variability that exists between patient populations in distinct locations. This can compromise a health care provider's ability to make sound clinical decisions regarding a patient's diagnosis and treatment options.

When considering the massive effort required to accomplish standardization and harmonization, the implications for manufacturers, governing bodies, and clinical laboratories are significant. For example, before implementation of standardization in laboratory practice, considerations are necessary to meet applicable regulatory requirements.[35] Thyroid disease is prevalent worldwide, and many countries have clinical practice standards for disease diagnosis and management. Standardization will require newly published guidelines and provider buy-in for thyroid hormone testing. International laboratory organizations, general and specialty healthcare providers, laboratory professionals, nursing professionals, and patient-centered organizations will require substantial educational time to prepare for any standardization efforts.[35]

Large-scale initiatives for change require a tremendous amount of time for planning before implementation. Standardization and harmonization initiatives are designed to improve patient outcomes but there are concerns about the potential increased risks for patient safety during a change in laboratory test values and RIs.[35] Laboratory reports are a vital resource for assisting providers with interpreting laboratory values. During the transition to standardized thyroid immunoassays, laboratory administrators in the clinical laboratories performing tests will need to design laboratory reports in a manner that prevents confusion. For example, administrators could provide customized patient reports including pre- and post-standardization results and RIs.[35] Quality management systems in the laboratory are designed to assess pre-analytic, analytic, and post-analytic processes in the laboratory, including changes in laboratory operations that may lead to non-conforming events.[43] Like other healthcare services, clinical laboratories are looking for ways to mitigate costs in the services provided. Standardization and harmonization for each clinical laboratory must be evaluated to determine the most cost-effective way to implement the processes. Keeping in mind not only reagent costs but also the time contributed to the planning, implementation, and monitoring of the change.

Discussion

Thyroid diseases are complex conditions that require a tremendous amount of endocrine scientific expertise to treat patients. This expertise includes interpreting TH laboratory test values. What is clear about TSH, FTA and TG immunoassays is that there is a multitude of methods commercially available. For example, RIA and IMA for TG are performed in clinical laboratories worldwide. The development of numerous different testing methodologies has improved analytical sensitivities and limited interferences. While developing new methods provides accurate and reliable laboratory values, there are additional implications to consider.

The overwhelming consensus suggests that TH testing lack of standardization and harmonization of the immunoassays contributes to inconsistencies in the diagnosis and monitoring of thyroid disease.[44] It is important to understand that while standardization and harmonization terms are often used interchangeably, each is uniquely different. It is not always the case that standardizing thyroid immunoassays will lead to harmonized thyroid test results and standardized reference methods are not always necessary for harmonization of thyroid test results.[45] The paramount objective in thyroid immunoassay testing standardization is the harmonization of test results so laboratory values from the same sample are interchangeable no matter the testing personnel, laboratory, or methodology.[45,46] Experts have recommended three steps for the standardization and harmonization to achieve equivalent results between methods including 1) the use of reference methods and materials for creating a reference system, 2) utilizing the reference system for the development of calibrating measurement procedures, and 3) evaluate the correlation of laboratory values throughout each method to verify the uniformity of patient results from patient care and research settings.[47] Overall benefits for reaching this objective include improved monitoring of disease progression, proper utilization of TH testing, and development of evidence-based practice guidelines.[46]

There are limitations associated with achieving standardization and harmonization in the laboratory. First, standards with known International System of Units (SI units) are not available for TSH and FT4 immunoassays. The IFCC approved an international conventional reference measurement procedure for FT4. However, because of the intricacies associated with TSH immunoassays, reference measurement procedures have not been developed.[44] This further emphasizes the need to harmonize thyroid hormone testing.

Second, establishing standardized RIs for FT4 and TSH immunoassays is a complex issue. As mentioned previously, laboratories are inconsistent in establishing dependable RIs, making it extremely difficult for providers to correctly interpret laboratory values. While an FT4 RI procedure exists, an attempt to establish a procedure for TSH is seen as unlikely.[5] The IFCC Committee for Standardization of Thyroid Function Tests (C-STFT) considered the more logical approach to harmonization as opposed to standardization. The C-STFT follows the International Organization for Standardization process for traceability. The C-STFT completed a multi-assay comparison with untreated and clinical specimens concluding that harmonization was feasible.[5] Harmonization is possible when immunoassay manufacturers are allowed to individually adjust calibrators using previously established target means from another method of comparison with similar sample types. The C-STFT believes this may allow manufacturers the ability to develop consistent RIs.[5]

Third, there is a wide variety of interferences associated with TH immunoassays including autoantibodies, reagent antibodies, and binding proteins. For example, due to the high prevalence of autoantibodies present in patients treated for differentiated thyroid cancer, experts recommend that all TG testing be performed with an TG antibody measurement.[14] Considering these interferences, along with the absence of known SI units and the complexity with establishing RIs, it is understandable why initiatives for reaching harmonization have yet to be accomplished.

Conclusion

Thyroid disease is prevalent worldwide. Hyperthyroidism, hypothyroidism, and thyroid cancer diagnosis relies heavily upon TH testing that is sensitive, accurate and precise. Accuracy or trueness of laboratory values means results produced are close to values derived from referenced methods. Precision or repeatability means the degree to which a laboratory can produce the same values.[46] Considering the definitions along with the wide variety of

methodologies available for TH testing emphasizes the importance of standardization and harmonization. RIA, IMA, and LC-MS/MS methods are all subject to interferences that can be further complicated by the nature of the thyroid disease.

Professional groups such as the ATA and C-TFT recognize the limitations associated with thyroid testing and are guiding efforts for standardization and harmonization.[5,31] While there are organized efforts to produce comparable results and appropriate RIs, achievement of these efforts remain unsuccessful. Endocrine specialists, clinical laboratories, and assay manufacturers must collaborate to successfully implement standardization and harmonization. Harmonizing thyroid laboratory test results is important to assist health care providers in making the best treatment decisions to improve patient care.

References

1. Kluesner JK, Beckman DJ, Tate JM, et al. Analysis of current thyroid function test ordering practices. J Eval Clin Pract. 2018; 24:347–352. https://doi.org/10.1111/jep.12846
2. Miller WG, Greenberg N. Harmonization, and standardization: where are we now? The J of Applied Lab Med. 2021; 6:510-521.
3. Faix JD, Miller WG. Progress in standardizing and harmonizing thyroid function tests. Am J Clin Nutr. 2016 Sep;104)3):913S-7S. doi: 10.3945/ajcn.115.110379. PubMed PMID: 27534642; PubMed Central PMCID: PMC5004503.
4. The need to harmonize clinical laboratory test results-white paper [Internet]. Washington (DC): American Association for Clinical Chemistry; 2015. Available from: https://www.harmonization.net/media/1bpacy14/aacc_harmonization_white_paper_2015.pdf
5. Thienpont LM, Faix JD, Beastall G, et al. Standardization of FT4 and harmonization of TSH measurements — a request for input from endocrinologists and other physicians [Opinion]. Endocrine Journal. 2015; 62:855-856.
6. Padoan A, Clerico A, Zaninotto M, et al. Percentile transformation and recalibration functions allow harmonization of thyroid-stimulating hormone (TSH) immunoassay results. Clinical Chem and Lab Med. 2020; 58:1663-1672.
7. Jongejan R, Klein T, Meima M, et al. A mass spectrometry-based panel of nine thyroid hormone metabolites in human serum. Clinical Chemistry. 2020; 66:556-566.
8. Singh RJ, Kaur P. Thyroid hormone testing in the 21st century. Clinical Biochemistry. 2016; 49:843-845.
9. Alyas T, Hamid M, Alissa K, Faiz T, Tabassum N, Ahmad A. Empirical method for thyroid disease classification using a machine learning approach. BioMed Research International. 2022; 2022:1-10.
10. Davis, M.G. and Phillippi, J.C. Hypothyroidism: diagnosis and evidence-based treatment. Journal of Midwifery & Women's Health, 2022; 67: 394-397.
11. Birtwhistle R, Morissette K, Dickinson JA, et al. Recommendation on screening adults for asymptomatic thyroid dysfunction in primary care. Canadian Medical Association Journal. 2019;191: E1274-E1280.
12. Akhavan NN, Maska E. Graves' disease presenting as a unilateral breast mass. Case Reports in Medicine. December 2022:1-3. doi:10.1155/2022/6641661
13. Kitahara CM, Körmendiné Farkas D, Jørgensen JO, Cronin-Fenton D, Sørensen HT. Benign thyroid diseases and risk of thyroid cancer: a nationwide cohort study. The Journal of Clinical Endocrinology & Metabolism. 2018 Jun;103(6):2216-24.
14. Li S, Ren C, Gong Y, Ye F, Tang Y, Xu J, Guo C, Huang J. The Role of Thyroglobulin

in Preoperative and Postoperative Evaluation of Patients With Differentiated Thyroid Cancer. Front Endocrinol (Lausanne). 2022 Jun 2;13:872527. doi: 10.3389/fendo.2022.872527. PMID: 35721746; PMCID: PMC9200986.
15. Tufano, R. P., Noureldine, S. I., & Angelos, P. Incidental thyroid nodules and thyroid cancer: considerations before determining management. JAMA Otolaryngology-- Head & Neck Surgery, 2015; 141(6), 566–572.
16. Evans C, Tennant S, Perros P. Serum thyroglobulin in the monitoring of differentiated thyroid cancer. Scandinavian Journal of Clinical & Laboratory Investigation. Supplement. 2016;76: S119-S123
17. Chou R, Dana T, Haymart M, et al. Active surveillance versus thyroid surgery for differentiated thyroid cancer: a systematic review. Thyroid (New York, N.Y.). 2022; 32:351-367.
18. Ross DS, Burch HB, Cooper DS, et al. 2016 American Thyroid Association guidelines for diagnosis and management of hyperthyroidism and other causes of thyrotoxicosis. Thyroid. 2016; 26:1343-1421.
19. Wilson SA, Stem LA, Bruehlman RD. Hypothyroidism: diagnosis and treatment. American Family Physician. 2021; 103:605-613.
20. Peiris AN, Medlock D, Gavin M. Thyroglobulin for monitoring for thyroid cancer recurrence. JAMA. 2019;321(12):1228. doi:10.1001/jama.2019.0803
21. Li Z, Yu B, Wang J, Yang Q, Ming J, Tang Y. Reference intervals for thyroid-stimulating hormone and thyroid hormones using the access TSH 3rd IS method in China. Journal of Clinical Lab Analysis. 2020;34: e23197-n/a.
22. Ribera A, Dabbs-Brown A, Poynter K, et al. CDC clinical standardization programs (CSP) for free thyroxine (FT4) to improve the accuracy and reliability of FT4 measurements in patient care and clinical research. Journal of the Endocrine Society. 2021;5: A826-A826.
23. Henze M, Brown SJ, Hadlow NC, Walsh JP. Rationalizing thyroid function testing: which TSH cutoffs are optimal for testing free T4? The J of Clinical Endocrinology and Metabolism. 2017; 102:4235-4241.
24. Çalcı E, Doğan HO, Sağlam F, Turhan T, Berker D. Comparison of the performance of second (fast TSH) and third (HYPERsensitive TSH) generation automated TSH immunoassays in healthy euthyroid subjects. Erciyes Medical Journal. 2019; 41:46-49.
25. Mohapatra S, Chakraborty S. Analytical variation between two different TSH reagents from the same manufacturer. Indian J of Clinical Biochemistry. 2023;2021; 38:132-135.
26. Nerenz R. Thyroid function tests. Clinical Chemistry Training Council; 2017 April 24; AACC; 2017.
27. Barth JH, Luvai A, Jassam N, et al. Comparison of method-related reference intervals for thyroid hormones: studies from a prospective reference population and a literature review. Annals of Clinical Biochemistry. 2018; 55:107-112.
28. Daly CH, Higgins V, Adeli K, Grey VL, Hamid JS. Reference interval estimation: Methodological comparison using extensive simulations and empirical data. Clinical Biochemistry. 2017; 50:1145-1158.
29. Jonklaas J, Razvi S. Reference intervals in the diagnosis of thyroid dysfunction: treating patients not numbers. The Lancet. Diabetes & Endocrinology. 2019; 7:473-483.
30. Mirjanic-Azaric B, Jerin A, Radic Z. Thyroid stimulating hormone values of clinical decisions of hypothyroidism measurement by three different automated immunoassays. Scandinavian J of Clinical and Laboratory Investigation. 2020; 80:151-155.
31. Osinga JAJ, Derakhshan A, Palomaki GE, et al. TSH and FT4 reference intervals in pregnancy: a systematic review and individual participant data meta-analysis. The J of Clinical Endocrinology and Metabolism. 2022; 107:2925-2933.
32. Coşkun A, Sandberg S, Unsal I, et al. Personalized reference intervals in laboratory

medicine: a new model based on within-subject biological variation. Clinical Chemistry. 2021; 67:374-384.
33. Ling C, Sun Q, Khang J, Lastarria MF, Strong J, Stolze B, et al. Does TSH reliably detect hypothyroid patients? Annals of Thyroid Research. 2018;4(1):122.
34. Spencer CA. Assay of thyroid hormones and related substances. Endotext [Internet]. 2017 Feb [cited 2023 March 3]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK279113/
35. Midgley JE. Global FT4 immunoassay standardization. Response to: Kratzsch J et al. Global FT4 immunoassay standardization: an expert opinion review. Clinical Chemistry and Laboratory Medicine. 2021; 59:223-224.
36. van Deventer HE, Soldin SJ. The expanding role of tandem mass spectrometry in optimizing diagnosis and treatment of thyroid disease. In: Makowski G, ed. Advances in Clinical Chemistry. Vol 61. SAN DIEGO: Elsevier Science & Technology; 2013. p. 127-152.
37. Mimoto MS, Refetoff S. Clinical recognition and evaluation of patients with inherited serum thyroid hormone-binding protein mutations. Journal of Endocrinological Investigation. 2020; 43:31-41.
38. Favresse J, Burlacu M, Maiter D, Gruson D. Interferences with thyroid function immunoassays: clinical implications and detection algorithm. Endocrine Reviews. 2018; 39:830-850.
39. Kitamura Y, Narita S, Kuroda Y, Yagi S, Aoyagi K. A novel thyroglobulin immunoassay using the specimen-pretreatment process improves the accuracy of thyroglobulin measurements in anti-thyroglobulin positive specimens. The J of Applied Laboratory Medicine. 2021; 6:1463-1475.
40. Cinquanta L, Fontana DE, Bizzaro N. Chemiluminescent immunoassay technology: what does it change in autoantibody detection? Auto Immun Highlights. 2017 Dec;8(1):9. doi: 10.1007/s13317-017-0097-2. Epub 2017 Jun 24. PMID: 28647912; PMCID: PMC5483212.
41. Algeciras-Schimnich A. Thyroglobulin measurement in the management of patients with differentiated thyroid cancer. Critical Reviews in Clinical Laboratory Sciences. 2018; 55:205-218.
42. Gholve C, Kumarasamy J, Damle A, et al. Comparison of serum thyroglobulin levels in differentiated thyroid cancer patients using in-house developed radioimmunoassay and immunoradiometric procedures. Indian Journal of Clinical Biochemistry. 2019; 34:465-471.
43. Pillai S, Calvert J, Fox E. Practical considerations for laboratories: Implementing a holistic quality management system. Frontiers in Bioengineering and Biotechnology. 2022; 10:1040103-1040103.
44. Padoan A, Clerico A, Zaninotto M, et al. Percentile transformation and recalibration functions allow harmonization of thyroid-stimulating hormone (TSH) immunoassay results. Clinical Chemistry and Laboratory Medicine. 2020; 58:1663-1672.
45. George G Klee, Harmonization, and standardization of thyroid function tests. Clinical Chemistry. 2010;56(6):879–880.
46. Laboratory Services Committee of the American Thyroid Association. Standardization and Harmonization. Alexandria (VA): American Thyroid Association; 2019.
47. Vesper HW, Myers GL, Miller WG. Current practices and challenges in the standardization and harmonization of clinical laboratory tests1–3. The American Journal of Clinical Nutrition.2016;104: S907-S912.

Review article

Benefits of Implementing Oral Fluid Testing for Opioid Pain Management Compliance

Lorie Minton[1*], Stephanie Jacobson[1] and Patricia Tille[1]

Medical Laboratory Sciences Program, College of Allied Health Science, University of Cincinnati, Cincinnati, Ohio, USA[1]

An increasing number of adults are treated for chronic pain, making the risk of opioid misuse much greater. One of the primary compliance strategies in pain management is drug testing. Drug testing ensures that patients properly take prescribed medications and can identify aberrant behaviors such as illicit drug use. Urine is the preferred matrix for drug testing in pain management compliance but has many drawbacks. Patient care is often negatively impacted due to the collection process and the difficulties that can occur in the elderly and disabled. Although new test methods for urine drug testing have advanced, preparation methods can still be lengthy, and sample tampering is a common element that continues to affect the accuracy of results.

Oral fluid testing is a viable method with several advantages when compared to urine testing. Results are easier to interpret, collection methods remove barriers and avoid sample tampering, and technical procedures are less cumbersome. Despite the few limitations associated with oral fluid testing, laboratories implementing oral fluid testing can offer better results using a streamlined preparation method, with the most significant impact being the elimination of sample tampering. Implementing oral fluid testing can be considered a positive contribution to compliance monitoring in pain management.

Keywords: Pain management, compliance, oral fluid, opioids.

Accepted: August 26, 2023

*Corresponding author: Lorie Minton. E-mail: mintonlc@mail.uc.edu

Introduction

Pain management compliance measures have increased exponential concurrently with the global opioid crisis. According to the Centers for Disease Control and Prevention (CDC) an increasing number of adults are being treated for chronic pain.[1] Common opioids prescribed for chronic pain include morphine, hydromorphone, hydrocodone, oxycodone, methadone, fentanyl, and buprenorphine. The misuse of these opioids is more prevalent in chronic pain patients putting them at an increased risk of developing opioid use disorder (OUD).[2, 3] Drug testing is a valuable component for monitoring the compliance of prescribed medications. While drug testing supports compliance measures, it can also identify therapeutic failures and detect potential drug interactions.[4] Since patients are at high risk of misuse, illicit drugs such as heroin, methamphetamine, cocaine, and ecstasy are often considered for testing.

Traditionally, urine has been the matrix of choice for detecting opioids and illicit drugs. However, oral fluid has emerged as an alternative matrix. Physiological differences between urine and saliva allow laboratories to improve efficiency using preparation methods requiring a small sample size.[5] In addition, oral fluid presents a practical option for streamlining the laboratory's drug testing workflow.[6, 7] Oral fluid provides an additional advantage by solving the challenge of adulteration commonly seen with urine samples. Compared to urine, oral fluid offers better patient care by providing a safer and less invasive collection method. Implementing oral fluid to detect opioids for pain management compliance improves sample integrity, simplifies workflow, and improves patient care.

Urine Testing

Hepatic and Renal Physiology in Drug Metabolism

Drug metabolism and excretion occur primarily in the liver and kidneys. There are two general ways drugs are metabolized and excreted. One way is the excretion of the drug in the intact form, and another is metabolism by biotransformation, followed by excretion.[8] The method of metabolizing drugs depends on whether the drug is hydrophilic or hydrophobic. Hydrophilic drugs are directly excreted through the renal pathway, while hydrophobic drugs must undergo metabolic modification through the liver before excretion.[8, 9]

The opioids commonly prescribed for pain are a combination of hydrophilic and hydrophobic drugs, including morphine, hydromorphone, hydrocodone, oxycodone, methadone, fentanyl, and buprenorphine. While the opioids are tested for compliance, illicit drugs such as 6-acetylmorphine, *d*-methamphetamine, cocaine, and 3, 4-Methylenedioxy-methamphetamine are often tested since chronic pain patients have been known to use them in conjunction with prescribed medication.[2] In addition to detecting the parent drug, drug metabolites are highly concentrated in the urine and are often measured to ensure the ingestion of the appropriate dose of medication.[10] Table 1 summarizes the parent drugs, associated metabolites, and commonly tested illicit drugs. When including the detection for metabolites, the half-life of most opioids is one to four days, except buprenorphine, which can be detected up to 10 days.[11]

Table 1. Commonly Prescribed Opioids and Associated Metabolites

Drug	*Metabolites*
Morphine	Hydromorphone
Hydrocodone	Hydromorphone, Norhydrocodone & Dihydrocodeine
Oxycodone	Noroxycodone & Oxymorphone
Methadone	EDDP
Fentanyl	Norfentanyl & Hydroxynorfentanyl
Buprenorphine	Nobuprenorphine
6-MAM	Morphine
Methamphetamine	None
Cocaine	Benzoylecgonine
MDMA	MDA

Sample Collection, Transport, and Storage

Sample collection, transport and storage requirements are essential components for the proper detection of opioids and drug metabolites. The collection of the urine sample does not require a unique device. Urine samples are typically collected in a single-use plastic container with the option of a temperature gauge on the outside of the cup or container.[12, 13] The minimum sample volume can be up to 30mL and stored for a limited time, depending on the laboratory's established stability requirements.[13] The collection is generally unobserved in a restroom facility within the clinic, and can create additional patient challenges. Challenges for urine collection are notable in elderly or disabled patients. Physical and mental disabilities should be considered when initiating collection from patients with cognitive impairment who may be at risk for falling due to gait instability.[14, 15]

Instrumentation

Gas chromatography-mass spectrometry (GC-MS) or liquid chromatography-mass spectrometry (LC-MS) are commonly used for urine confirmation testing.[7, 10] Both instruments separate and identify molecules based on the structure and chemical properties.[10, 16] GC separates the molecules in the gas or vapor phase. LC separates molecules based on affinity, absorption, partition, ion exchange, or size exclusion while in solution.[12] Although GC-MS is considered the gold standard in confirmatory testing, LC-MS is typically preferred due to its high selectivity, sensitivity, and decreased drug interferences.[10, 17]

Despite the preferred qualities of LC-MS, sample hydrolysis pretreatment is required to remove matrix interferences and extract drug targets.[17] Hydrolysis of the sample breaks-down drug-glucuronide conjugates, extending the detection window of quickly metabolized drugs.[18] Hydrophobic opioids, such as morphine, undergo glucuronidation. As such, this is an essential step in identifying the metabolism of the parent compound. Quality control is required to verify the hydrolysis activity using control samples containing known amounts of drug-glucuronide conjugates. Laboratories can produce a control material by purchasing the drug-glucuronide conjugates, morphine-6ß-D-glucuronide, and buprenorphine-3ß-D-glucuronide from manufacturers such as Cerilliant (Round Rock, TX) or Lipomed (Cambridge, MA). These standards come in either 1.0 mg/mL or 100 µg/mL. Each standard requires dilution with a certified negative urine matrix until the desired target concentration is achieved. Laboratories may also purchase control material from vendors such as Utak (Valencia, CA). Custom-made control material can be designed to meet a desired concentration and comes ready to use.

Additional considerations in confirmatory urine testing includes limitations and interferences that can complicate the interpretation of results. The variation in the enzyme activity of cytochrome P450 (CYP450) in some patients may reduce or increase drug metabolism.[8] Since CYP450 enzymes are heavily responsible for metabolizing opioids, gene mutations or drug interferences may prohibit accurate interpretations. One example of drug interference is the commonly prescribed anti-depressant fluoxetine. Fluoxetine inhibits CYP450 enzymes responsible for the metabolic process of opioids.[19]

Workflow

Although the laboratory may select a robust LC-MS system, the turn-around time of the testing largely depends on the steps in sample preparation and the LC-MS method design to achieve sensitivity and good peak performance. Glucuronide metabolism requires enzymatic hydrolysis in the sample preparation affecting time from sample collection to result or turn-around time. There are different ways to hydrolyze the sample. One way is to purchase a genetically modified enzyme, such as IMCSzyme® (IMCS, Inc., Irmo, SC), marketed to hydrolyze the sample faster than traditional methods. Laboratories may hydrolyze the sample using ß-glucuronidase from different sources such as *Patella vulgate, Helix pomatia,*

Escherichia coli, bovine liver, or abalone.[20] In addition to the hydrolysis method, different extraction techniques in sample preparation are also available, such as solid-phase extraction (SPE) or liquid-to-liquid extraction (LLE). SPE is considered a simple process with a short extraction time and uses less solvent, while LLE demonstrates a high recovery rate for opioids such as buprenorphine.[17]

Validity Testing

In addition to sample hydrolysis and sample preparation methods in mind, the laboratory must consider the validity of the sample. Patients use adulteration techniques to conceal aberrant medication-taking behaviors.[2, 21] There are various sample tampering methods, such as diluting, and substituting using manufactured and household products. "Spiking" is another form of adulteration in which individuals dissolve prescribed medication into the sample to simulate a positive result.[22] Dilution is accomplished by overhydrating to reduce the chance of detecting targeted drugs, while substitution replaces the sample with synthetic urine or one obtained from another person.[21, 23, 24] Although some patients aim to alter the sample to hide drug misuse, some patients may unintentionally dilute the urine by drinking excessive water to stimulate urination. The chemicals from manufactured and household products used to adulterate the sample interfere with drug detection and are commonly made up of acids, alkalis, oxidizing agents, or surfactants.[24]

Automated chemistry methods are used to determine the validity of urine specimens. Validity tests can include urine creatinine, specific gravity, and pH. Assays that detect invalidity are assessed by observing the acceptability criteria expected in human urine. Urine creatinine concentrations should be between 80-200 mg/dL, specific gravity in the range of 1.003-1.035, and pH within 4.7-7.8.[25] Validity assay methods use colorimetric reactions measured by absorbance.

Validity results indicate if a urine sample is clean or adulterated. The Substance Abuse and Mental Health Services Administration (SAMHSA) offers guidelines for acceptability parameters for validity testing based on creatinine and specific gravity, as summarized in Table 2. SAMHSA is a sector of the U.S. Department of Health and Human Services that develops measures to support overdose prevention. Forensic laboratories use SAMHSA's acceptability parameters for validity testing and can be implemented in clinical laboratories.

Table 2. SAMHSA Classifications of Validity

Classification	Creatinine	Specific Gravity
Dilute	≥ 2 and < 20	> 1.0010 and <1.0030
Substituted	< 2	≤ 1.0010
Substituted	< 2	≥ 1.020
Invalid	< 2	> 1.0010 and < 1.020
Invalid	≥ 2	≤ 1.0010

An additional measure to aid in determining validity or to rule out "spiking" is to observe the drug metabolites. For example, the confirmatory results of patients taking buprenorphine as prescribed should indicate hepatic metabolism from the parent drug, buprenorphine, to the metabolite, norbuprenorphine. The presence of norbuprenorphine suggests that the patient did not "spike" the sample with the medication at collection. Alternatively, the metabolite would be absent if the patient did "spike" the sample. Therefore, it can be helpful to monitor the concentration of norbuprenorphine in patients prescribed buprenorphine.[22, 26]

Further, observing the presence of naloxone has been used to confirm the ingestion of buprenorphine. Some buprenorphine formulas, such as the medication branded Suboxone® (Indivior, Inc., North Chesterfield, VA) or Zubsolv® (Orexo US, Inc., Morristown, NJ), contain naloxone. Naloxone is an opioid antagonist used to reverse opioid overdose. Naloxone could be considered a validity measure that ensures the patient has ingested the medication as directed. Although this may be a helpful aid in validating the sample, it is not reliable. Other drugs, such as naloxegol (brand

name Movantik™), commonly prescribed for opioid-induced constipation, may cause a false positive. A false positive could occur since naloxol is a derivative of naloxone, and the drug's manufacturing process can leave impurities.[27]

When considering validity testing of urine samples, the cost per test increases. While the cost for urine confirmation testing may be similar to the testing of other matrixes, validity testing should be added to the total cost per test.

Oral Fluid

Oral Physiology and Drug Metabolism

Saliva is a filtrate of plasma by way of diffusion. Saliva contains cellular debris, secretions, and other residues expressed from the salivary glands in the oral cavity.[28] The passive diffusion of drugs from the blood through the salivary glands depends mainly on the pH of the saliva. Other factors include whether the drug is lipid-soluble, the percentage of the bound proteins, and the method by which the drug is administered.[6, 28] Opioids are weakly basic in pKa and have a low percentage of bound protein. Opioids also have a lower molecular weight which causes the parent drug to be present at higher concentrations in the oral fluid. Since oral fluid does not require the drug to undergo metabolism before excretion, the detection window or half-life is shorter, ranging from less than 1 hour to 48 hours.[5] The sample collection time should be relatively close to when the patient was administered the drug.

Sample Collection, Transport, and Storage

The collection of oral fluid can be performed by passive drool, expectoration, and commercial devices.[28, 29] The passive drool collection technique is the non-stimulated pooling of saliva collected into a container. Expectoration is a collection method in which the patient spits into the container. The passive drool and expectoration collection methods provide an initial also referred to as a neat sample free of diluents.[28] One benefit of collecting neat fluid is the ability to split a single collection into two samples if needed.[5] Commercial devices generally use a swab to absorb the saliva from the oral cavity, and a transport container with a buffer that stabilizes and preserves the sample for testing. Swab with transport containers are available from many manufacturers and are more popular with patients and collectors due to the ease of handling. Commercial swabs are more sanitary than passive and expectoration collection methods.

One of the main advantages of oral fluid is the ease of collection and provides improved patient care. All patients have a safer and more private experience and significantly easier for the elderly and disabled populations. Medical personnel collect the oral fluid in a safe, controlled environment such as the exam room or designated collection area. Furthermore, the sample volume requirement is typically only 1 mL, adding to the benefits of an oral collection.[28] The small sample volume requirement is beneficial for patients with kidney dysfunction that are not unable to produce a sample size to meet that of urine.

Although there are benefits to oral collection, there are limitations for some patients. An attempt to collect saliva may be difficult for those suffering from conditions causing xerostomia.[29] Xerostomia, or hyposalivation or "dry mouth," is when the salivary gland fails to produce adequate saliva. The condition is common in patients with autoimmune disorders such as Sjögren's syndrome. Additionally, medications and anxiety can also cause hyposalivation.[30] Though the sample volume required is low, patients with the condition may still have difficulty collecting the minimum saliva volume.

Collecting the sample is vital to achieving accurate results. With commercial devices, the procedure must be performed according to the manufacturer's instructions. Pre-analytical failures such as not allowing the volume-adequacy indicator to change color or insuring the patient's mouth is free of foreign debris can contribute to test and result interferences.[28]

Instrumentation

The instrumentation for oral fluid testing is GC-MS or LC-MS.[7, 10] Oral fluid requires high-sensitivity instrumentation to detect low concentrations making the LC-MS the preferred instrumentation. Compared to GC-MS, LC-MS methods have high specificity and the robustness necessary for oral fluid testing.[28] When developing a preparation method, pre-treatment of the sample may be necessary for commercial devices that contain a buffer. However, since oral fluid captures larger concentrations of the parent drug in the free fraction form, the sample does not require a hydrolysis phase eliminating the need for quality control materials containing drug-glucuronide conjugates.

Despite the robustness associated with GC-MS, some limitations and interferences remain including the pH of patient saliva, improper collection procedures, and environmental exposures. The normal range of pH in saliva is between 5.8 and 6.8.[31] Patients with increased saliva pH due to stimulation of the salivary flow can decrease the drug concentrations.[7, 32] One of the methods utilized to stimulate salivary flow is sucking on citric-acid candy. This method can increase the salivary pH more than other methods, such as chewing on paraffin. Since there is no consensus on whether pH can be normalized, paraffin is preferred if salivary flow must be stimulated for collection.

Workflow

An additional pre-analytical consideration is the sample preparation method. While SPE or LLE methods are successful preparation techniques for oral fluid testing, laboratory workflow can be reduced with an effective dilute-and-shoot (DnS) sample preparation method. The DnS approach is a simple dilution of the oral fluid by adding LC/MS-grade water (1:4, *v:v*) before injecting it into the instrument for analysis.[33, 34]

Another consideration in the workflow and the absence of extensive drug metabolism in oral fluid is that many metabolites may not be required for testing. Since the parent drug is most concentrated in oral fluid, laboratories can consider eliminating metabolite testing from the method. With fewer drug analytes requiring analysis, run time is reduced, decreasing the turn-around time for the method.

Validity Testing

Validity testing is traditionally a consideration in drug testing but is not necessary when testing oral fluid for the clinical setting. SAMHSA recognizes Immunoglobulin G (IgG) and albumin as validity test markers. The antibody, IgG, and albumin, a polypeptide, are present in normal human saliva. The normal range for IgG is 0.1-1.0 mg/L, and the normal range for albumin is 0.2-0.3 mg/mL.[32, 35] If the concentrations fall below the laboratory's established limit of detection or are absent, it is an invalid sample.[12] In addition, collections are performed by clinical staff and observed and it is unlikely the sample can be manipulated or adulterated.

Another consideration is the utilization of metabolites and other compounds to support medication compliance. As with buprenorphine-prescribed patients, the parent drug can be observed independently to monitor patient compliance and adherence to the prescribed medication regime. The observation of norbuprenorphine or naloxone is unnecessary.

Discussion

The use of oral fluid in clinical toxicology is gaining momentum. In comparing oral fluid and urine testing, oral fluid is more useful to laboratories and provides a better resource for providers to treat chronic pain patients. The comparison of the physiological characteristics of urine and oral fluid is important. In oral fluid, the passive diffusion of lower-weighted opioid molecules allows the observation of the parent drug to determine drug compliance. Oral fluid is optimal for chronic pain patients because they are typically in a steady state of prescribed medications.[2] Consistent capture of the parent drug from saliva is a strong determinant of compliance.

Although the lower weight of opioid molecules improves parent drug detection, oral fluid still requires a high-sensitive instrument to measure small concentrations. GC-MS and LC-MS can be used, but LC-MS is most prevalent due to the robustness, sensitivity, and specificity required for oral fluid.[28] In addition, oral fluid does not require a hydrolysis step, a DnS preparation is the ideal method. A quick preparation method such as DnS decreases laboratory turn-around time, reducing the amount of solvents and technician time needed compared to SPE or LLE.

Despite the preferred qualities of LC-MS and effective sample preparation methods, the most significant impact of oral fluid testing is the improvement in the integrity of the sample due to preanalytical processes. Urine can be easily adulterated, but oral fluid collection ensures that the sample presented for testing is without impairment. The collection of oral fluid can be observed without interfering with a patient's privacy and significantly reduces or eliminates adulteration. It also removes the limitations associated with collecting urine specimens for patients with physical disabilities. Oral fluid does not need a particular collection environment and can be obtained using a manufactured swab and transport device.

Patient care is improved by testing oral fluid because providers are able to interpret the results more easily. The provider's ability to correctly interpret the results is essential in determining compliance. Whether providers can accurately interpret results, especially those exhibiting adulterated characteristics, is questionable. Twenty-eight percent of providers report contrasting interpretations to the laboratory.[36] Providers may assume aberrant medication-taking behaviors if the metabolite is missing and fail to consider the possibility of a CYP450 gene mutation that reduces or increases drug metabolism. The characteristics of oral fluid and the ability to eliminate adulteryation also removes providers' errors in interpretation. Oral fluid identifies the parent drug and indicates to the providers that a patient is taking the medication as prescribed.

Despite the many benefits of oral fluid testing, some limitations should be highlighted. Although determining compliance based on the parent drug is easier to interpret, it could result in the provider overlooking the potential identification of a CYP450 mutation. If the provider is knowledgeable about pharmacogenomics, the routine absence of the drug metabolite in urine would lead to further clinical diagnostics and potentially alter the patient's treatment plan. A second limitation of oral fluid testing is the collection from patients with xerostomia. Patients unable to produce saliva may have difficulty producing the minimum volume needed for testing. Salivary stimulation techniques can affect the saliva's pH, which may impact the test results.

Conclusion

The misuse of opioids is prevalent in chronic pain patients and has increased pain management compliance measures. A compliance measure such as drug testing is an element necessary in proper pain management. Oral fluid testing improves patient care due to the ability to capture the presence of prescribed, non-prescribed, and illicit drugs, and potentially aiding in reducing opioid use disorder. Oral fluid testing could be useful in testing other classes of medications, such as benzodiazepines, that are commonly prescribed in chronic pain patients. More research is needed to determine whether screening an oral fluid sample would be beneficial before definitive drug testing is required. The need for pain management compliance continues to grow, and implementing oral fluid testing provides better sample integrity, streamlined testing, and better patient care.

References

1. Rikard SM, Strahan AE, Schmit KM, Guy GP Jr.. Chronic Pain Among Adults — United States, 2019–2021. MMWR Morb Mortal Wkly Rep 2023;72:379–385. DOI http://dx.doi.org/10.15585/mmwr.mm7215a1
2. Shaparin N, Mehta N, Kunkel F, Stripp R, Borg D, Kolb E. A Novel Chronic Opioid Monitoring Tool to Assess Prescription Drug Steady State Levels in Oral Fluid. Pain Med. 2017;18(11):2162-9. doi: 10.1093/pm/pnw335. PubMed PMID: 28339737; PubMed Central PMCID: PMC5914322.
3. Bonnie RJ, Kesselheim AS, Clark DJ. Both Urgency and Balance Needed in Addressing Opioid Epidemic: A Report From the National Academies of Sciences, Engineering, and Medicine. Jama. 2017;318(5):423-4. doi: 10.1001/jama.2017.10046. PubMed PMID: 28715551.
4. Florete OG Jr. Urinary Drug Testing in Pain Management. Practical Pain Management. 2018;5(3).
5. Grabenauer M, Moore KN, Bynum ND, White RM, Mitchell JM, Hayes ED, et al. Development of a Quantitative LC–MS-MS Assay for Codeine, Morphine, 6-Acetyl-morphine, Hydrocodone, Hydromorphone, Oxycodone and Oxymorphone in Neat Oral Fluid. Journal of Analytical Toxicology. 2018;42(6):392-9. doi: 10.1093/jat/bky021.
6. Mahowald GK, Khaliq TP, Griggs D, O M, Flood JG, Uljon S. Comparison of Oral Fluid and Urine for Detection of Fentanyl Use Using Liquid Chromatography with Tandem Mass Spectrometry. J Appl Lab Med. 2021;6(6):1533-40. doi: 10.1093/jalm/jfab068. PubMed PMID: 34327523.
7. de Campos EG, da Costa BRB, Dos Santos FS, Monedeiro F, Alves MNR, Santos Junior WJR, et al. Alternative matrices in forensic toxicology: a critical review. Forensic Toxicol. 2022;40(1):1-18. Epub 20210819. doi: 10.1007/s11419-021-00596-5. PubMed PMID: 36454488; PubMed Central PMCID: PMC9715501.
8. Garza AZ, Park SB, Kocz R. Drug Elimination. [Updated 2022 Jul 11]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK547662/.
9. Koning MV, Klimek M, Rijs K, Stolker RJ, Heesen MA. Intrathecal hydrophilic opioids for abdominal surgery: a meta-analysis, meta-regression, and trial sequential analysis. Br J Anaesth. 2020;125(3):358-72. Epub 20200711. doi: 10.1016/j.bja.2020.05.061. PubMed PMID: 32660719; PubMed Central PMCID: PMC7497029.
10. Arthur JA. Urine Drug Testing in Cancer Pain Management. Oncologist. 2020;25(2):99-104. Epub 20191011. doi: 10.1634/theoncologist.2019-0525. PubMed PMID: 32043770; PubMed Central PMCID: PMC7011652.
11. Knezevic NN, Khan OM, Beiranvand A, Candido KD. Repeated Quantitative Urine Toxicology Analysis May Improve Chronic Pain Patient Compliance with Opioid Therapy. Pain Physician. 2017;20(2s):S135-s45. PubMed PMID: 28226335.
12. Substance Abuse and Mental Health Administration [Internet].Rockville: 2022 Medical Review Officer Case Studies; c2022 [cited 2023 July 27] U.S. Department of Health & Human Services. Available from: https://www.samhsa.gov/workplace/drug-testing-resources#specimen.
13. Raouf M, Bettinger JJ, Fudin J. A Practical Guide to Urine Drug Monitoring. Fed Pract. 2018;35(4):38-44. PubMed PMID: 30766353; PubMed Central PMCID: PMC6368048.
14. Kuan YC, Huang LK, Wang YH, Hu CJ, Tseng IJ, Chen HC, et al. Balance and gait performance in older adults with early-stage cognitive impairment. Eur J Phys Rehabil Med. 2021;57(4):560-7. Epub 20201201. doi:

10.23736/s1973-9087.20.06550-8. PubMed PMID: 33258361.
15. Bahureksa L, Najafi B, Saleh A, Sabbagh M, Coon D, Mohler MJ, et al. The Impact of Mild Cognitive Impairment on Gait and Balance: A Systematic Review and Meta-Analysis of Studies Using Instrumented Assessment. Gerontology. 2017;63(1):67-83. Epub 20160513. doi: 10.1159/000445831. PubMed PMID: 27172932; PubMed Central PMCID: PMC5107359.
16. Whitehead HD, Hayes KL, Swartz JA, Prete E, Robison-Taylor L, Ellen Mackesy-Amiti M, et al. Validated method for the analysis of 22 illicit drugs and their metabolites via liquid chromatography tandem mass spectrometry (LC-MS/MS) in illicit drug samples collected in Chicago, IL. Forensic Chemistry. 2023;33:100475. doi: https://doi.org/10.1016/j.forc.2023.100475.
17. Shan X, Cao C, Yang B. Analytical Approaches for the Determination of Buprenorphine, Methadone and Their Metabolites in Biological Matrices. Molecules. 2022;27(16):5211. PubMed PMID: doi:10.3390/molecules27165211.
18. Dahlin JL, Palte MJ, LaMacchia J, Petrides AK. A Rapid Dilute-and-Shoot UPLC-MS/MS Assay to Simultaneously Measure 37 Drugs and Related Metabolites in Human Urine for Use in Clinical Pain Management. The Journal of Applied Laboratory Medicine. 2019;3(6):974-92. doi: 10.1373/jalm.2018.027342.
19. Deodhar M, Rihani SBA, Darakjian L, Turgeon J, Michaud V. Assessing the Mechanism of Fluoxetine-Mediated CYP2D6 Inhibition. Pharmaceutics. 2021;13(2). Epub 20210123. doi: 10.3390/pharmaceutics 13020148. PubMed PMID: 33498694; PubMed Central PMCID: PMC7912198.
20. Algethami FA. Assessing and Optimising Several Enzymes Used for Drug Analysis in Urine. Multi-Knowledge Electronic Comprehensive Hournal For Education And Science Publications. 2019(56).
21. Goggin MM, Tann C-M, Miller A, Nguyen A, Janis GC. Catching Fakes: New Markers of Urine Sample Validity and Invalidity. Journal of Analytical Toxicology. 2017;41(2):121-6. doi: 10.1093/jat/bkw119.
22. Suzuki J, Zinser J, Issa M, Rodriguez C. Quantitative testing of buprenorphine and norbuprenorphine to identify urine sample spiking during office-based opioid treatment. Substance abuse. 2017;38(4):504-7.
23. Vikingsson S, Krauss ST, Winecker RE, Flegel RR, Hayes ED. Update on Urine Adulterants and Synthetic Urine Samples to Subvert Urine Drug Testing. Journal of Analytical Toxicology. 2022;46(7):697-704. doi: 10.1093/jat/bkac029.
24. Rajšić I, Javorac D, Tatović S, Repić A, Đukić-Ćosić D, Đorđević S, et al. Effect of urine adulterants on commercial drug abuse screening test strip results. Arh Hig Rada Toksikol. 2020;71(1):87-93. doi: 10.2478/aiht-2020-71-3315. PubMed PMID: 32597134; PubMed Central PMCID: PMC7837240.
25. Microgenics Corporation. DRI Creatinine-Detect Test. Fremont (CA): Thermo Scientific; 2021 May.
26. Waters RC, Perez M. How do I interpret and use quantitative buprenorphine and norbuprenorphine urine levels? Cleveland Clinic Journal of Medicine. 2022;89(10):557-60. doi: 10.3949/ccjm.89a.21019.
27. Haidari M, Mansani S, Ponds D, Romero L, Alsaab S. Consumption of Movantik™ (Naloxegol) results in detection of naloxone in the patient's urine evaluated by confirmatory urine drug testing. Clinical Biochemistry. 2019;67:48-53. doi: https://doi.org/10.1016/j.clinbiochem.2019.03.006.
28. Lee LA, McGee AC, Sitasuwan P, Tomashek JJ, Riley C, Muñoz-Muñoz AC, et al. Factors Compromising Glucuronidase Performance in Urine Drug Testing Potentially Resulting in False Negatives. J Anal Toxicol. 2022;46(6):689-96. doi: 10.1093/jat/bkab090. PubMed PMID: 34401904; PubMed Central PMCID: PMC9282255.
29. Zheng Y, Sparve E, Sparring S, Bergström M. Detection of Drugs in Oral Fluid Samples Using a Commercially Available

Collection Device: Agreement with Urine Testing and Evaluation of A and B Samples Obtained from Employees at Different Workplace Settings with Uncontrolled Sampling Procedures. Journal of Analytical Toxicology. 2021;44(9):1004-11. doi: 10.1093/jat/bkaa024.
30. Dreyer NS, Lynggaard CD, Jakobsen KK, Pedersen AML, von Buchwald C, Grønhøj C. [Xerostomia]. Ugeskr Laeger. 2021;183(27). PubMed PMID: 34219641.
31. Malaca S, Busardò FP, Gottardi M, Pichini S, Marchei E. Dilute and shoot ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC–MS/MS) analysis of psychoactive drugs in oral fluid. Journal of Pharmaceutical and Biomedical Analysis. 2019;170:63-7. doi: https://doi.org/10.1016/j.jpba.2019.02.039.
32. Desrosiers NA, Huestis MA. Oral Fluid Drug Testing: Analytical Approaches, Issues and Interpretation of Results. J Anal Toxicol. 2019;43(6):415-43. doi: 10.1093/jat/bkz048. PubMed PMID: 31263897.
33. Greer B, Chevallier O, Quinn B, Botana LM, Elliott CT. Redefining dilute and shoot: The evolution of the technique and its application in the analysis of foods and biological matrices by liquid chromatography mass spectrometry. TrAC Trends in Analytical Chemistry. 2021;141:116284. doi: https://doi.org/10.1016/j.trac.2021.116284.
34. Bassotti E MG, D'Urso A, Savini F, Locatelli M, Tartaglia A, Dossetto P, D'Ovidio C, de Grazia U. A new LC-MS/MS confirmation method for the determination of 17 drugs of abuse in oral fluid and its application to real samples. Forensic Science International. 2020;312(110330).
35. Ngamchuea K, Chaisiwamongkhol K, Batchelor-McAuley C, Compton RG. Chemical analysis in saliva and the search for salivary biomarkers - a tutorial review. Analyst. 2017;143(1):81-99. doi: 10.1039/c7an01571b. PubMed PMID: 29149225.
36. Chua I, Petrides AK, Schiff GD, Ransohoff JR, Kantartjis M, Streid J, et al. Provider Misinterpretation, Documentation, and Follow-Up of Definitive Urine Drug Testing Results. J Gen Intern Med. 2020;35(1):283-90. Epub 20191111. doi: 10.1007/s11606-019-05514-5. PubMed PMID: 31713040; PubMed Central PMCID: PMC6957646.

Review article

A Systematic Review of Peer Feedback in Biomedical Laboratory Science Education: An Effective Tool for Growth, Collaboration and Professional Development

Filis Necip[1*] and Camilla Christine Qvist[1]

Department of Pathology, Rigshospitalet, Denmark[1]

Introduction: **Peer feedback is widely recognized and an effective pedagogical approach that promotes active learning, student engagement and develops analytical and communication skills. This can provide value in biomedical laboratory science education where the teaching and training of students in laboratory techniques, research methodologies, and scientific principles foster professional development. The objective of this systematic review was to examine feasible utilization, effectiveness, and quality of peer feedback in biomedical laboratory science education.**

Methods: **To guide the systematic approach conducting this review the PRISMA statement for reporting was used. Cochrane PICO (patient, population, or problem) method was used to support the comprehensive search strategy to identify relevant studies. The data extraction process was conducted by one reviewer and verified by a second to ensure accuracy and consistency. The quality and risk of bias was assessed using the Cochrane Risk of Bias Tool for randomized controlled trials. This assessment provided an evaluation of the methodological rigor and potential sources of bias. Thematic analysis was performed to identify common themes and patterns.**

Results: **The final review included 6 studies. Oral and written peer feedback were the most common evaluated. Several studies did not provide detailed description of the introduction of the peer feedback activities for the student as well as the frameset, criteria, or assessment focus. All articles had full focus on the outcomes, effects, or the students' opinion of the conducted peer feedback activity. No studies assessed the quality of the peer feedback.**

Conclusion: **Peer feedback in biomedical laboratory science education holds significant potential for enhancing student learning outcomes, professional development, and preparation for real-world practice. Through an iterative feedback loop, students develop a deeper understanding of laboratory techniques, scientific reasoning, and critical thinking skills.**

Keywords: biomedical laboratory science, education, peer feedback, learning outcomes

---

Accepted: September 9, 2023

*Corresponding author: Filis Necip. E-mail: Filis.necip@regionh.dk

Introduction

Peer feedback is a widely recognized and effective pedagogical approach that promotes active learning and student engagement in various educational contexts.[1-3] In the field of biomedical laboratory science education, where practical skills and critical thinking are paramount, the use of peer feedback has gained increasing attention to enhance learning outcomes and professional development.[4] This systematic review aims to examine the existing literature on peer feedback in biomedical laboratory science education or other related areas, synthesizing the findings to gain a comprehensive understanding of the impact, implementation strategies, and associated benefits and challenges.

Biomedical laboratory science education encompasses the teaching and training of students in laboratory techniques, research methodologies, and scientific principles relevant to the biomedical laboratory sciences. It plays a crucial role in preparing students for careers in clinical laboratories, research institutions, and healthcare settings. Traditionally, biomedical laboratory science education has relied heavily on instructor-led assessments and feedback. However, peer feedback introduces a collaborative and interactive dimension to the learning process, allowing students to provide feedback to peers, learn from each other's experiences, and develop analytical and communication skills. [5]

The use of peer feedback in biomedical laboratory science education aligns with the principles of constructivist learning theory, which posits that knowledge is actively constructed through social interactions and collaboration. By engaging in the process of peer feedback, students become active participants in learning, analyzing, and evaluating their peers' work, and reflecting on individual practices.[6] Through this iterative feedback loop, students develop a deeper understanding of laboratory techniques, scientific reasoning, and critical thinking skills.

While peer feedback has been widely studied in various educational domains, the specific application and impact in biomedical laboratory science education requires further investigation. This systematic review aims to fill this gap by synthesizing the existing literature and exploring the research questions related to peer feedback in an educational context. The review addresses the following key aspects:

- Impact on learning outcomes: Examine the effects of peer feedback on student learning outcomes in biomedical laboratory science education and explore the extent to which peer feedback contributes to knowledge acquisition, skill development, and critical thinking abilities.
- Implementation strategies: Analyze the different approaches and strategies employed to implement peer feedback in biomedical laboratory science education including the examination of the methods used to structure feedback sessions, establish assessment criteria, and facilitate student engagement.
- Benefits and challenges: Identify the benefits and challenges associated with the use of peer feedback in biomedical laboratory science education and explore the advantages of peer feedback, such as promoting student engagement, fostering a collaborative learning environment, and preparing students for teamwork and professional practice. Additionally, evaluate the challenges related to variability in student expertise, biases in feedback provision, time constraints, and emotional impact.

Systematically synthesizing the existing literature through evidence-based practices provides educators, researchers, and policymakers with a comprehensive understanding of the role and effectiveness that peer feedback can enhance and improve learning strategies in biomedical laboratory science education.

Methods

Data Sources and Search Strategy

A comprehensive search strategy was developed to identify relevant studies. Electronic databases such as PubMed, Electronic Registration Information Center (ERIC), and Google Scholar were searched using a combination of keywords related to peer feedback, biomedical laboratory science education, and related terms using Cochrane PICO.[7]

Combination of keywords, use of Boolean operators and truncation (*): "biomedic* laboratory science" OR bioanal* OR medic* laboratory students OR "health education" OR "clinical education" AND Peer feedback OR peer assessment AND "biomedical laboratory science education" OR "Collaborative learning" OR "peer assessment" AND "biomedical education." The search was limited to articles published in English and Danish.

Inclusion and Exclusion Criteria

Inclusion criteria for the studies was established based on the research question and the scope of the review. The primary focus was on empirical research studies investigating the impact of peer feedback on learning outcomes in biomedical laboratory science education. Studies involving undergraduate or graduate students, as well as conducted in different educational settings (e.g., universities, colleges, training programs) and non-peer reviewed publications were considered. Studies exploring the implementation strategies, benefits, challenges, and student perspectives related to peer feedback were also included. Only research articles were included.

Study Selection

Two reviewers screened the titles and abstracts of the identified articles to determine the relevance to the research question using the Preferred Reporting Items for Systematic Reviews and Meta-Analysis Statement (PRISMA). [8] Full-text articles meeting the inclusion criteria were retrieved for further evaluation. Any disagreements between the reviewers were resolved through discussion and consensus. A flowchart was created to illustrate the study selection process. (Figure 1).

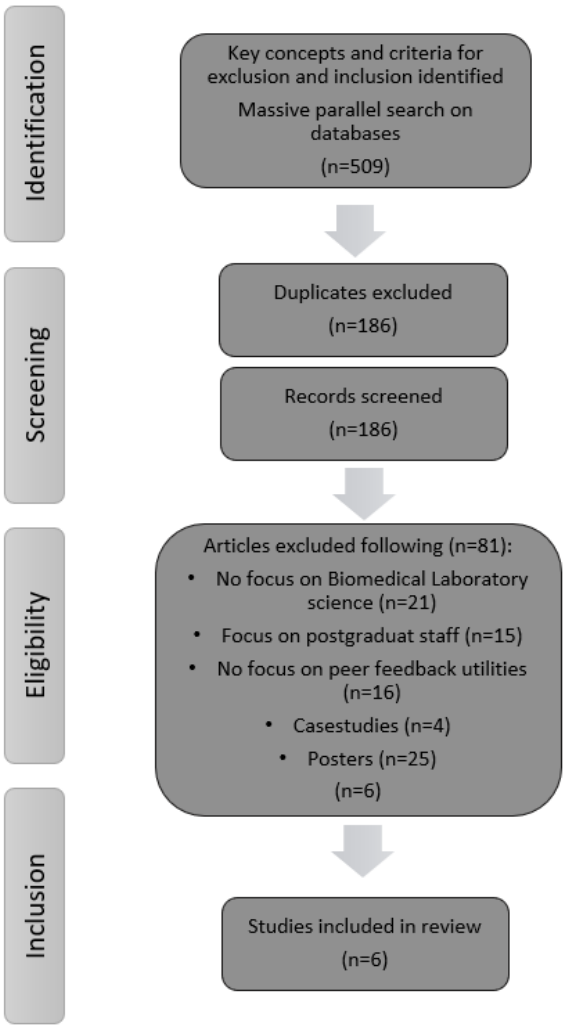


Figure 1. Flowchart of study selection process with number of search results (n) presented as a decreasing selection process.

Data Extraction and quality assessment

Data extraction involved systematically extracting relevant information from the studies. A standardized data extraction form was developed, including fields such as study characteristics (e.g., authors, publication year, study design), participant characteristics, intervention details (e.g., type of peer feedback, assessment criteria), outcome measures, and key findings. The data extraction process was conducted by one

reviewer and verified by a second to ensure accuracy and consistency.

The quality and risk of bias of the studies was assessed using the Cochrane Risk of Bias Tool for randomized controlled trials. [9] The assessment provides an evaluation of the methodological rigor and potential sources of bias.

Data Synthesis and Analysis

A narrative synthesis was conducted to summarize and analyze the findings from the studies. The synthesis involved thematic analysis, identification of common themes and patterns across the studies. Quantitative data, such as effect sizes or statistical outcomes, was summarized and assessed. A meta-analysis was conducted to provide a summary of the overall effects of peer feedback.

Limitations

The limitations of the studies, such as sample size, study design, and potential biases, are acknowledged.

Results

The majority of the studies included were conducted in the Nordics countries. Other studies are from Singapore and Australia. The sample size of the studies ranged from 77-575 students. All studies included undergraduate student and bachelor level programs in biomedical or health education. One study also included 43 instructors or educators. The research methodology of peer feedback assessment included 1 quantitative, 1 qualitative and 4 mixed methods. The quantitative methodology included questionnaires or grading whereas the qualitative data conducted narrative comments, focus groups interviews, semi structured interviews, and open discussions. Several studies did not provide a detailed description of the peer feedback process for the student or the peer feedback frameset, criteria, and assessment focus. Two studies provided a detailed description of the peer feedback process to the students, the course/feedback setup, and criteria. No studies assessed the quality of the peer feedback. All articles included outcomes, effects, or the students' opinion of the peer feedback activity (Table 1).

Discussion

Peer feedback in biomedical science education is a valuable tool for enhancing student learning, promoting critical thinking, and fostering collaboration within the field. [10] One of the prominent findings across the studies is that peer feedback has a positive impact on student learning outcomes.[11-14] By engaging in the process of providing and receiving feedback from peers, students gain multiple perspectives on their performance, leading to a deeper understanding of the subject matter. Through this iterative feedback loop, students identify areas for improvement, refine experimental methodologies, and enhance the quality of research findings.[10] This aligns with the constructivist approach to learning, where students actively participate in knowledge construction through social interactions and engagement with their peers.[6]

Peer feedback plays a significant role in the development of critical thinking skills in biomedical laboratory science education.[4,11,14-15] Through the process of analyzing and evaluating a peers' work, students are exposed to diverse research approaches, methodlogies, and scientific reasoning.[10] The exposure broadens a student's perspectives and challenges assumptions, fostering a more robust and analytical mindset. Furthermore, by providing constructive criticism and suggestions for improvement, students refine the ability to evaluate scientific work objectively and communicate ideas effectively.[14] The development of critical thinking skills is essential for success in the biomedical laboratory science field, where evidence-based decision-making and problem-solving are paramount.

Collaboration is another key aspect that emerges regarding effective peer feedback. In the clinical setting, collaboration is integral to the health professions.[13] Peer feedback facilitates collaboration among students and prepares them for collaborative work. Through

Table 1. Student peer feedback and outcomes in different context of course and feedback activity.

Author (year)	Country	Type of course	Partici-pants	Sample size	Type of peer feedback activity	Outcome of peer feedback evaluation	Coch-rane Risk of Bias Tool
Colt-horpe (2014)[14]	Australia	Molecular and cellular physiology	Bachelor of Science Students	77 students	Written (anonymous) peer feedback and feedback from academics	Students give extensive, rich, and detailed feedback. Improvement of student learning outcome was greater with peer feedback than with feedback from academics alone.	2
Elle-gaard (2022)[10]	Denmark, Finland, Sweden	Didactics, Physics, Microbio-logy, Urban develop-ment, Science projects, Teachers	Under-graduate Post-graduate	575 students	Written through electronic platform, oral or combination of written and oral peer feedback (Both anonymous and not)	Placing students as both receivers and givers of feedback results in high student activity. Using feedback as a process where effect and output is returned to modify next step (feedback loop) can support students to drive their own learning process.	1
Jacob-sen (2017)[11]	Denmark	Molecular Biology and genetic analysis	Biomedical Laboratory Science students	224 students	Individual written feedback in portfolio and general plenum feedback from teachers	Peer feedback supports students learning and enhances the student independency, hours used studying, professionally challenged, and combining theory and practice. Highlights the significance of thorough introduction and guidance implementation peer feedback, clear frameset of the feedback and focus on establishing formative feedback. Yet, the students demand more individual feedback from teacher.	2
Liika-nen (2018)[15]	Denmark, Finland	All the biomedical laboratory science courses	Biomedical Laboratory Science students	142 students 43 teachers	Peer feedback through information and communication technology	Use information and communication technology results in more prompt and timely feedback. The agency supports the peer feedback by document sharing and voice comments as feedback option	2
Yoong (2023)[13]	Singapore	Not described	Nursing students	164 first year students 69 senior students	Video and verbal, peer and faculty feedback, peer tutors	Improvement of student reflective abilities and clinical competence in technical nursing skill when using video and verbal peer feedback compared to control group with only faculty feedback. Peer video feedback can be time-consuming and stressful to the students. An increase in sense of empowerment was shown. Peer feedback was beneficial for both first year and senior students.	2

peer feedback, students learn to communicate ideas, provide constructive feedback, and work collectively towards shared goals. The collaborative learning environment created by peer feedback nurtures teamwork skills, interpersonal communication, and the ability to engage in scientific discourse.[13] These skills are crucial for biomedical laboratory scientists who often work in interdisciplinary teams to tackle complex scientific challenges.

The effectiveness of peer feedback in biomedical laboratory science education is contingent upon several factors. Clear guidelines and assessment criteria provided by instructors is essential for ensuring the quality and relevance of feedback.[11, 14-15] Guidelines help students provide specific, constructive, and actionable feedback supporting the growth and improvement of their peers.[10-11] Moreover, a supportive and respectful learning environment is crucial for effective peer feedback. Students should feel comfortable offering and receiving feedback, and instructors play a vital role in fostering this atmosphere. Regular monitoring and feedback from instructors ensures the accuracy and effectiveness of peer feedback, providing guidance and direction to students as they navigate the process. [10]

While peer feedback offers numerous benefits, it is important to acknowledge the limitations. Variability in student expertise and experience impacts the quality and depth of the feedback. Instructors should guide students in providing feedback that is both helpful and meaningful. Additionally, time constraints and workload considerations pose challenges to the implementation of peer feedback, especially in large laboratory science classes.[11] Balancing the workload and ensuring sufficient time for students to provide thoughtful feedback is crucial to maintain the effectiveness of the process. Some of the key limitations to consider include:

- Variability in expertise and knowledge: Students in biomedical laboratory science education may have different levels of knowledge and expertise. This variability impacts the quality and depth of the feedback. Students with limited understanding of the subject matter may struggle to provide insightful feedback, while those with greater expertise may find it challenging to provide feedback at an appropriate level.[13] Instructors must be mindful of the differences and provide support and guidance to ensure that feedback is meaningful and helpful.
- Lack of training: Students may not have received specific training on how to provide effective feedback. Without proper training and guidance, students may struggle to deliver feedback that is constructive, specific, and actionable.[10,11] This should be considered when incorporating training sessions or workshops to provide the students with the skills necessary for giving and receiving feedback effectively.[11]
- Potential for bias: Peer feedback is subject to biases, both conscious and unconscious. Students may have personal biases, such as favoritism or prejudice, that can influence the feedback they provide.[6] Biases can undermine the objectivity and fairness of the feedback process. Instructors should be aware of this potential bias and monitor the feedback process to ensure its integrity.
- Time constraints: Implementing peer feedback requires additional time and resources.[11] In busy laboratory science courses, time constraints make it challenging to allocate sufficient time for students to provide thoughtful feedback.[4] Students have commitments that compete for their time and attention, making it difficult to dedicate the necessary effort to provide comprehensive feedback.[13] Strategies to manage time effectively and strike a balance between the benefits of peer feedback and the demands of the curriculum should be considered.
- Emotional impact: Receiving feedback, particularly constructive criticism, can have an emotional impact on students. Some students may feel discouraged or

demotivated by feedback that highlights areas for improvement.[13] It is crucial for instructors to create a supportive and safe learning environment where students feel comfortable receiving feedback and are encouraged to use it as an opportunity for growth and development.[3,6]

- Reliability and consistency: Ensuring the reliability and consistency of peer feedback can be challenging. Different students may interpret assessment criteria differently, leading to inconsistencies in the feedback.[3,4,10,11] It is important for educators to establish clear assessment criteria and guidelines to minimize subjectivity and promote consistency in the feedback.
- Limited perspectives: Peer feedback provides insights from the perspective of fellow students but may lack the expertise and experience of instructors or educational professionals in the field.[10] While peer feedback can be valuable, it should be supplemented with input from instructors who can provide expert guidance and ensure the accuracy and depth of feedback.

Addressing the limitations requires careful planning, training, and ongoing evaluation of the peer feedback process. Instructors must provide clear guidelines, training, and support to students, monitor the feedback process for fairness and objectivity, and ensure a supportive learning environment where feedback is viewed as a constructive tool for growth.

Conclusion

Peer feedback in biomedical laboratory science education, specifically in the context of clinical education, holds significant potential for enhancing student learning outcomes, professional development, and preparation for real-world practice.[4] Despite the potential benefits of peer feedback in clinical education within the field of biomedical laboratory science, there is a notable lack of research specifically focused on this area. While peer feedback has been widely studied in other educational contexts, such as general healthcare education or medical education, limited attention has been given to the application and effectiveness in the context of biomedical laboratory science clinical education.

The lack of research can be attributed to several factors. Clinical education in biomedical laboratory science often receives less emphasis compared to other healthcare professions, such as medicine or nursing. As a result, research funding and resources may be directed towards other areas, leading to a dearth of studies specifically investigating the use of peer feedback in clinical education within the field. Given the limited research conducted on peer feedback in clinical education within biomedical laboratory science, there is a need for further investigation to explore the potential benefits and challenges.

Collaboration between instructors, educators, and teaching practitioners from the clinical environment and the universities is essential to address the research gap in this area.[4] By conducting rigorous studies and sharing best practices, the biomedical laboratory science community can generate evidence to inform educational strategies and optimize the integration of peer feedback in clinical education. Such research endeavors will contribute to enhancing the quality of biomedical laboratory science education and preparing students for successful careers in the field.

Acknowledgements

This work was fully supported and funded by the Department of Pathology at Rigshospitalet, Denmark. We would also like to thank Magdalena Kersting and Marianne Achiam from University of Copenhagen, Denmark for valuable peer feedback on the manuscript.

Funding

Funded by the Department of Pathology, Rigshospitalet, Denmark

References

1. Hattie J, Timperley H. The Power of Feedback. Rev Educ Res. 2007;77(1):81–112.
2. Black P, Harrison C, Lee C, Marshall B, Wiliam D. Working Inside the Black Box: Assessment for Learning in the Classroom. Phi Delta Kappan. 2004;86(1):8–21.
3. Dolin J, Black P, Harlen W, Tiberghien A. Exploring Relations Between Formative and Summative Assessment. 2018. p. 53–80.
4. Necip F, Qvist C, Jacobsen D, Ellegaard M. Didaktisk samarbejde mellem campus og klinik udvikler peer feedback-kulturen i undervisningen. Danske Bioanalytikere. 2023;2:30–2.
5. København Professionshøjskole. Om peer feedback - Peer feedback [Internet]. 2023 [cited 2023 Jun 6]. Available from: https://peerfeedback.dk/om-peer-feedback/
6. Lauvås P, Bruun J. Ren formativ evaluering i skolen. 1st ed. Aarhus: Klim; 2021.
7. Cochrane PICO search | Cochrane Library [Internet]. [cited 2023 Jun 7]. Available from: https://www.cochranelibrary.com/ about/ pico-search
8. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. International Journal of Surgery [Internet]. 2010;8(5):336–41. Available from: https://www.sciencedirect.com/science/article/pii/S1743919110000403
9. RoB 2: A revised Cochrane risk-of-bias tool for randomized trials | Cochrane Bias [Internet]. [cited 2023 Jun 7]. Available from: https://methods.cochrane.org/bias/resources/rob-2-revised-cochrane-risk-bias-tool-randomized-trials
10. Ellegaard M, Niss M, Bruun J, Lämsä J, Voetman Christiansen F, Green Linell G, et al. Unfolding principles for student peer feedback: A comparative analysis across higher education contexts. Högre utbildning. 2022;12(2):53–77.
11. Jacobsen D, Bahrenscheer JG. Digital portfolio og peer to peer feedback - skaber transfer og engagerede studerende. Tidsskriftet Læring og Medier (LOM). 2017 May 17;10(17 SE-Artikler inden for tema).
12. Thomé G, Hovenberg H, Edgren G. Portfolio as a method for continuous assessment in an undergraduate health education programme. Med Teach. 2006 Jan 1;28(6):e171–6.
13. Yoong SQ, Wang W, Chao FFT, Dong Y, Goh SH, Chan YS, et al. Using peer feedback to enhance nursing students' reflective abilities, clinical competencies, and sense of empowerment: A mixed-methods study. Nurse Educ Pract. 2023;69:103623.
14. Colthorpe K, Chen X, Zimbardi K. Peer feedback enhances a "journal club" for undergraduate science students that develops oral communication and critical evaluation skills. Journal of Learning Design. 2014 Sep 9;7.
15. Liikanen E, Björn M, Nielsen M. Use of information and communications technology by teachers and students in biomedical laboratory science educations in the Nordic countries. Educ Inf Technol (Dordr). 2018;23(6):2867–78.