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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

Professional Challenges in Health care, Workforce and Education and Hope for the Future!

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

In 2019 in Wuhan China, a new coronavirus, SARS-CoV-2 or Covid-19, was identified as the cause of multiple cases of acute respiratory distress syndrome. With the advent of the pandemic, there has been a resurgence in awareness regarding the need for more laboratory science professionals worldwide. Although this is not a new challenge, it is now at the forefront of health care for several reasons. Many health care professionals chose to take early retirement or seek new career opportunities during the pandemic. Although the exit of professionals has slowed, it has not completely stopped. This is attributed to professional burnout and high workloads due to the increased workload and the decrease in the numbers of working professionals. It is essential that stakeholders, including administrators, educators and organizations come together to provide personal health and well being services to current and future staff. As the profession develops new and innovative ways to increase training opportunities, it is also essential that retention efforts of existing professionals are implemented. It is absolutely essential to maintain the working professionals to mentor and provide guidance to the new and developing professionals in order to maintain quality healthcare.

This edition of the journal provides significant insight into some of the professional challenges in the laboratory workforce in Australia. In addition, the laboratory spotlight highlights the significant efforts of the medical and laboratory staff at Sacre Coeur, in Guinea West Africa to provide medical laboratory services to the people!

It is the hope of the International Federation of Biomedical Laboratory Science (IFBLS) Executive Board and the International Journal of Biomedical Laboratory (IJBLS) Editorial Board that you find the information in the journal educational, interesting, and inspiring to continue to provide quality laboratory services worldwide.

Sincerely, IJBLS Editor in Chief,

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

Editorial

Leadership

Hassan A. Aziz PhD, FACSs, MLS(ASCP)^{cm}
Associate Editor of Education and Administration

As new graduates move from isolation to participation, they become part of the power structure, working alongside with administrators and other healthcare providers rather than under them. There are several reasons to cultivate leadership roles. One is to enhance the professional status of our profession. Other key elements in leadership are personal assessment, a supportive work environment, diverse strategies for influencing others, and planning for action. Personal assessment is important because each individual brings a unique combination of experiences, skills, and values to the leadership role. A supportive work environment plays a crucial role in leadership, which develops when a facility places a high focus on professional development, collegiality, autonomy, and open communication. Staff development can help laboratory professionals identify and develop the diverse strategies that will work best for them. With these preconditions in place, new graduates will be prepared to focus on a workable plan of action. On the other hand, offering laboratorians increased responsibility can have far reaching ramifications. Issues of structure, role of clarity and violation of norms create difficulties in implementing these new roles.

Throughout this process, the lab administrator's role is critical. Administrators serve as primary role models, teaching leadership through actions as well as words. They support the committed, encourage the reluctant, and pave the way by finding resources and removing barriers. They must function as one among equals, and the skills of team building, group process, and collaboration become more important than control and coordination. Administrators must also develop highly refined conflict management skills to keep the laboratory functioning smoothly.

Well, how should administrators and new graduates work out that kind of relationship? Administrators are usually concerned with protecting certain prerogatives, such as exercising the ability to make key decisions, representing the laboratory to the community, and knowing what activities were going on in the laboratory. For their part, graduates are concerned with maintaining relationships with peers, protecting their responsibility for working, and avoiding conflict with higher management. Both should utilize strategies to shape the relationship. For example, administrators can compliment employees, solicit their advice, and suggest tasks. On the other side, employees can use ways to assert their ideas without creating conflict. Overtime, however, the relationship should evolve from an emphasis on self-interest and interpersonal communication to symbiosis and a focus on the tasks to be accomplished. Resolving tensions and developing trust are essential first steps in developing productive partnership. A workplace should help both sides cultivate the skills needed for this task. Creating such a healthy relationship within the laboratory should maintain qualified laboratory practitioners in the profession

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Editorial

World Biomedical Laboratory Science Day 15th April
Guardians of Quality and Patient Safety: Biomedical Laboratory Scientists

Marie Culliton, MSc, MBA, FACSLM
President, International Federation of Biomedical Laboratory Science

The 15th of April, every year, is Biomedical Laboratory Science Day. The International Federation of Biomedical Laboratory Science (IFBLS) choses a theme to run for 2 years that highlights the role of Biomedical Laboratory Scientists in Healthcare.

This year the IFBLS Board has chosen the theme ***Guardians of Quality and Patient Safety: Biomedical Laboratory Scientists***.
This theme is central to everything a Biomedical Laboratory Scientist is and does. It compliments three recent position papers, approved by the members and published by IFBLS.

IFBLS Guidelines for Core Competence

Biomedical laboratory science combines knowledge, skills and abilities in medicine, physical science, technology, and statistics with emphasis on analysing specimens from patients to aid in diagnosing and treatment of disease.
The Biomedical Laboratory Scientist education and training make the profession unique compared to other professions in terms of knowledge of quality assurance, evaluation of pre-analytical conditions and assessment of their impact, validation of medical laboratory analysis, considerations of uncertainty of measurement and biological variability and understanding of post-analytical situations used for diagnosis of disease, monitoring of treatment and evaluation of health status.
The Core Competences for the Biomedical Laboratory Scientist include a thorough understanding of the fundamentals of scientific and technical biomedical laboratory processes and how these inform clinical decision-making. This includes development and validation of clinical laboratory methods and techniques, implementation and verification of new analytical testing methods, quality assurance of biomedical analysis, the end-to-end process from when an analyte is ordered, and the sample collection through to the validation of the test result, interpretation of the test result and potential clinical implication and communication of the test result.
The Core Competences for Biomedical Laboratory Scientists are built on scientific methods (evidence-based) and the ethics of patient care.
The Biomedical Laboratory Scientist is an important resource for other healthcare professionals and the public regarding the use of safe and appropriate laboratory diagnostic testing.

IFBLS Statement on the Role of the Biomedical Laboratory Scientist in the Delivery of Quality Healthcare

Biomedical Laboratory Scientists use scientific evidence in the provision of screening and diagnostic information; specifically, how a laboratory analytic procedure is selected, used and applied in clinical decisions for screening and diagnostic purposes.
Biomedical Laboratory Scientists are the appropriate professionals to provide diagnostic, screening and other analytical information because of their education and training in quality systems, quality

control, and quality improvement. Biomedical Laboratory Scientists have a specific expertise that is developed during education and clinical internship/experience.

IFBLS Position Paper on Patient Safety

The foundation of patient safety is 'First do no harm'. Improving patient safety requires preventing and reducing errors, and constantly mitigating and avoiding unsafe acts. Patient safety requires that health care delivery is safe, effective, patient-centred, timely, efficient and equitable. Safe healthcare delivery requires all healthcare professionals to assure quality processes and use evidence in their practice, continuously improve processes, incorporate current information technology, work in interprofessional teams to deliver patient (person)-centred care.

Biomedical Laboratory Scientists are responsible for ensuring patient safety by:

- Adhering to current high standards of practice and the application of quality assured protocols and governance;
- Focusing upon preventing errors in the entire laboratory testing process;
- Ensuring that laboratories conform with national and international standards of accreditation;
- Maintaining high standards for qualifications and continuing professional development;
- Including patient safety concepts and competencies into academic and continuing professional development requirements for Biomedical Laboratory Scientists;
- Improving laboratory testing services applying continuous quality improvement principles.
- Maintaining patient confidentiality throughout all phases of laboratory testing;
- Providing patients with information about laboratory testing prior to specimen collection in order to give informed consent;
- Providing test results and interpretation to inform the diagnosis;
- Focusing on improving patient outcomes using new technologies, such as personalized medicine by contributing our professional expertise.

Our poster celebrates biomedical laboratory science day. It encompasses the entire spectrum of a sample journey from the patient through the laboratory where it is the biomedical laboratory scientist who uses the best methods, with quality assurance, to ensure the correct result which is then interpreted correctly leading to treatment of the patient.Each of us knows that behind every sample we analyse is a person who must be cherished.

I am proud to call myself a biomedical laboratory scientist. I hope that you will join with me, and your colleagues, in celebrating biomedical laboratory science day.

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Obituary

Alba Marzo (1965 – 2023)

It is with deep sadness that we inform IFBLS members of the death of our former Board Member, colleague and friend from Italy, Dr Alba Marzo.

Alba was an enthusiastic proponent of the profession of Biomedical Laboratory Science in Italy, where she lectured Biomedical Laboratory Science students in the University of Florence.

She was a board member of IFBLS from 2014 – 2018, and chair of the IFBLS World Congress in Florence, Italy in 2018. I think it is safe to say there would not have been a World Congress in Florence without Alba. She worked very hard and the Congress was a great success. None of us present at the Congress in September 2018 will forget her warm enthusiasm for the profession, her pride in hosting the World Congress and our stay in the beautiful city of Florence.

In the IFBLS Board of Directors Alba was an enthusiastic board member, always well prepared, a constructive participants in discussions and always willing to try out new ideas. As chair of the Awards Committee, she worked very hard to judge applications for awards as well as organizing the huge task of poster judging at the World Congress in Kobe, Japan in 2016.

No one who knew Alba could miss her love for her students and our profession. She was so proud of them all, and I am sure her enthusiasm contributed to a good learning environment for her students.

Few who knew her will ever forget her keen intellect, radiant smile, sense of humor and love of fashion. We shall hold close our memories of her from the IFBLS Congress in her home city of Florence.

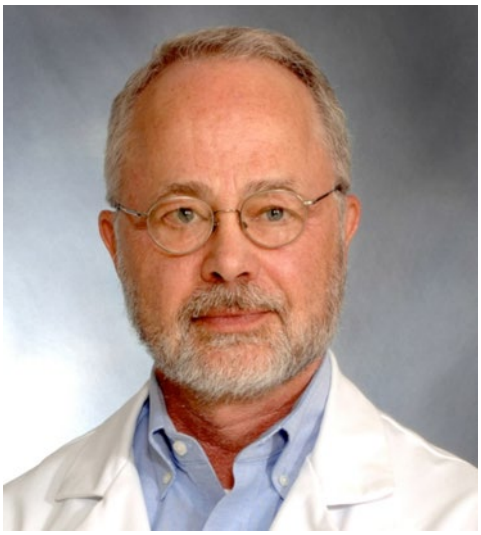
To her family, colleagues and friends IFBLS offers sincerest condolences.

On behalf of former and current IFBLS Board of Directors
Marie Nora Roald
IFBLS President 2016 – 2018

IFBLS Board meeting in Rome, April 2015.

Editorial: Laboratory spotlight

My Experience Helping a New Laboratory in Guinea West Africa



Joel E. Mortensen, Ph.D. FAAM, HCLD, AHI (AMT)

I am Dr. Joel Mortensen, the Director of the Diagnostic Infectious Diseases Testing Laboratory at Cincinnati Children's Hospital, in Cincinnati Ohio. I trained at The Ohio State University and Baylor College of Medicine. This is part of my professional story.

The Journey Begins

In 2020, during the time of CoVID, I received an email that had been sent to a group of Directors of Microbiology Laboratories from around the world. The email was sent by Dr. Chris Doern on behalf of two colleagues that he had helped train in the medical school in Dallas, TX. They were asking for help with several positions at a clinic that they were building in Guinea, West Africa. One of these positions was a clinical microbiologist. Response was a bit limited because of the need to spend several months a year in Guinea, the expense of travel to Guinea, and the need for "old school" expertise with basic microbiology and parasitology. Before I responded I headed to Google to look up Guinea.

- Guinea is on the west coast of Africa and facing the Atlantic Ocean. It is bordered by Guinea-Bissau, Senegal, Mali, Côte d'Ivoire (Ivory Coast), Liberia and Sierra Leone.
- Guinea is sometimes called Guinea-Conakry because of several other countries in Africa with Guinea in their name. Conakry is the capitol city.
- The country is one of the larger producers of the bauxite in the world.
- Additional exports include gold, diamonds, iron, oil, and other minerals.
- Over 20 languages are spoken in Guinea and are focused in tribal areas. The common language is French.
- French occupation of the region began in 1890. Guinea declared independence in 1958.
- The current regime is headed by the military following a coupe in the fall of 2021.
- Guinea was one of several West African countries involved in the Ebola outbreak in 2014.

While learning about Guinea, I found a few disturbing facts about healthcare in general, and pediatrics in particular, in Guinea. Mortality in children under 5 years is 96/ 1000, compared to 6/1,000 in the USA and 1.9/1,000 in Iceland. Neonatal mortality in Guinea is 62/1000 live births. Malaria is the primary cause of death (14.2%) in all age groups and a particular problem with children. The prevalence of diarrhea is 12.4% in children aged 0-59 months and

cholera has been endemic in the country since 2003. In Guinea, 31% of children are chronically malnourished and 14% are severely malnourished. This health care crisis is compounded by respiratory tract infections such as tuberculosis, and emerging and re-emerging diseases. [1,2]

I started an email dialogue with the founders of Sacre Coeur Pediatric Hospital. Sacre Coeur is a part of Hope Ignited, a non-profit charity with Adam and Rachel Jamison, MD, serving as Executive Director (Adam) and Medical Director (Rachel) of the center. Rachel is a board-certified pediatric cardiologist. They are supported by Courtney Baldridge, MD, a pediatrician and Aaron Baldridge, an educator. The two families have worked together since they moved to Paris several years ago to learn the French language. Following the year in France, both families moved to Guinea.

During CoVID, travel was limited and my responsibilities at Cincinnati Children's Hospital were all consuming, but as the pandemic quieted, I began to think about how I could best contribute to providing high quality microbiology in this resource limited setting. Although weekly calls were good, I needed a better understanding of what is really needed and what is really possible given the severely limited resources in Guinea. Construction plans for the housing units, the chapel and the hospital were finalized and construction began to develop the new pediatric health care center. The leadership team at Sacre Coeur also began to interview personnel for nursing, the pharmacy, the laboratory and local physicians. In 2022 it was time for me to go onsite and contribute to training of newly hired laboratory technologists and help refine the plans for the future of the laboratory services needed to support the hospital.

Getting ready to travel to West Africa was an event in and of itself. A current passport and entry visa into Guinea was reasonably easily accomplished. The visit to the travel clinic resulted in many vaccinations and discussions about CoVID, malaria, dengue, Ebola, diarrhea, mosquitoes and more. Soon enough I was ready to make the flight. I was lucky and only needed two flights; Cincinnati to de Gaulle Airport in Paris and de Gaulle to Conakry, Guinea. Each flight was about 8 hours long with 4 hours layover.

Guinea

Arriving at night, I did not fully appreciate the beauty, majesty, and abject poverty of the area – not to mention the incredible heat and humidity. The hospital and housing at Sacre Coeur consisted of four apartments – one for each of the families, Jamison's and Baldridge's; one that was shared by the Head Nurse, Pharmacist, Outreach Specialists and grade schoolteacher and a guest house. I was assigned to the guest house. It was bright and airy with a large living room/dining room, a kitchen and powder room, laundry room, along with a master bedroom and bath, and two bedrooms with a shared bathroom. All the rooms had fans and built-in air-conditioning units. Of course, air-conditioning requires electricity, but more on that later. The two-floor hospital had cement walls, tile floors, high ceiling and air conditioning units and were bright and clean throughout. There was space for examination rooms, radiology, pharmacy, laboratory, offices and meeting rooms. In addition, there was a large chapel beginning to take shape. Lots of workman and trucks gave the site a sense of energy and anticipation.

For me, the first step was to visit several laboratories in the capitol city to evaluate the current standard of care. The Sacre Coeur Pediatric Center is located just North of Conakry in a small town called Dubreka. It was about 25 miles to first of the Laboratories that our laboratory team was going to visit but because of the rough roads and slow and snarled traffic, we planned on a 2 ½ to 3 hours drive! During the day, we visited several laboratories, the first was new and bright at Centre de diagnostic de la CNSS Caisse. It had modern diagnostic equipment at a high level of sophistication. In microbiology, they had plated media (shipped in from France) and bioMerieux blood culture and identification and susceptibility testing platforms. Information seemed to be captured electronically, although ledgers seemed to be everywhere. Utilization of the equipment in microbiology was limited seemingly by the cost of importing the supplies. This large hospital only had 1 blood culture bottle in the incubator at the time we were there. I was told that the cost of a blood culture was equivalent to $50.00 US, pricing it beyond most patient's ability to pay.

Figure 1. Sacre Coeur Hospital.

The other sites that we visited were one room laboratories with simple bench top hematology and chemistry analyzers, and a microscope. Microbiology testing was limited to malaria smears and the direct examination of stool for parasites. Record keeping consisted of two large paper ledgers one for patient information and one for results. Patients were most often supplied with handwritten results in a small book that the patient carried with them from physician to physician. The smallest laboratory used a collection of home style glucose metering devices and a microscope. There, the technologists reused the slides and coverslips, and dried slides on the glass panels of the open window. The ceiling fan and single light bulb combined with the 95°F and 98% humidity created working conditions beyond anything I had experienced. Although the microscope had a light bulb, the technologists talked about needing to use their microscopes by the window reflecting the sunlight through the microscope mirror. Everyone seemed dedicated to their patients but with the exception of the first clinic, the terribly limited resources were striking, especially compared to my home laboratory at Cincinnati Children's Hospital.

Back at the Clinic, I began training the technologists. All the technologists had worked at other laboratories in the area, have college level education and are fluent in French. We started with "How to Wash our Hands" and what to wear in a laboratory (shoes were the biggest issue. The technologists know the basics; however, this was a chance to reinforce the basics, build from the ground up and create a shared culture. Then we moved on to basic

laboratory safety and personal protective equipment such as lab coats, gloves, goggles, and how to don and doff these items. The routine that we settled into was: discuss, observe, perform. As each person performed each basic task, they described what they were doing.

The next several days were spent balancing lectures and wet laboratory training. The first challenge was finding the space for the training laboratory and lecture space. Construction in the laboratory at the hospital was moving along but was not complete, so we adjusted by converting the living room and kitchen in my apartment to a laboratory and classroom space. I lectured in the living room, and we had teaching labs in the kitchen. We used a plastic wash pan for stain waste with an oven rack to hold the slides. The new Nikon microscope had a camera and is the most advanced scope they have ever seen. At night I adapted lectures that I brought with me from my Medical Laboratory Science teaching series to meet the needs of the clinic, and to match the knowledge and experience of the technologists. The entire process was a bit slow because I do not speak French and they do not speak English, so I had a translator with me all the time. Over the next 5 days we balanced general clinic orientation and microbiology lectures and labs including: Laboratory safety, Review of basic microscopy, the theory and application of the Giemsa and Gram stains, and basic direct stool microscopy. In addition, we covered the taxonomy, history, disease and laboratory identification of malaria, trematodes, nematodes and cestodes. All four technologists had been "taught" to identify malaria in the laboratory but they had little understanding of the life cycles or epidemiology of the infection. But all of them have had malaria. During one lecture session I was discussing systemic worms and one of the technologists called out that she had been infected as child with a worm that would emerge from her foot and her mother would try to grab it!

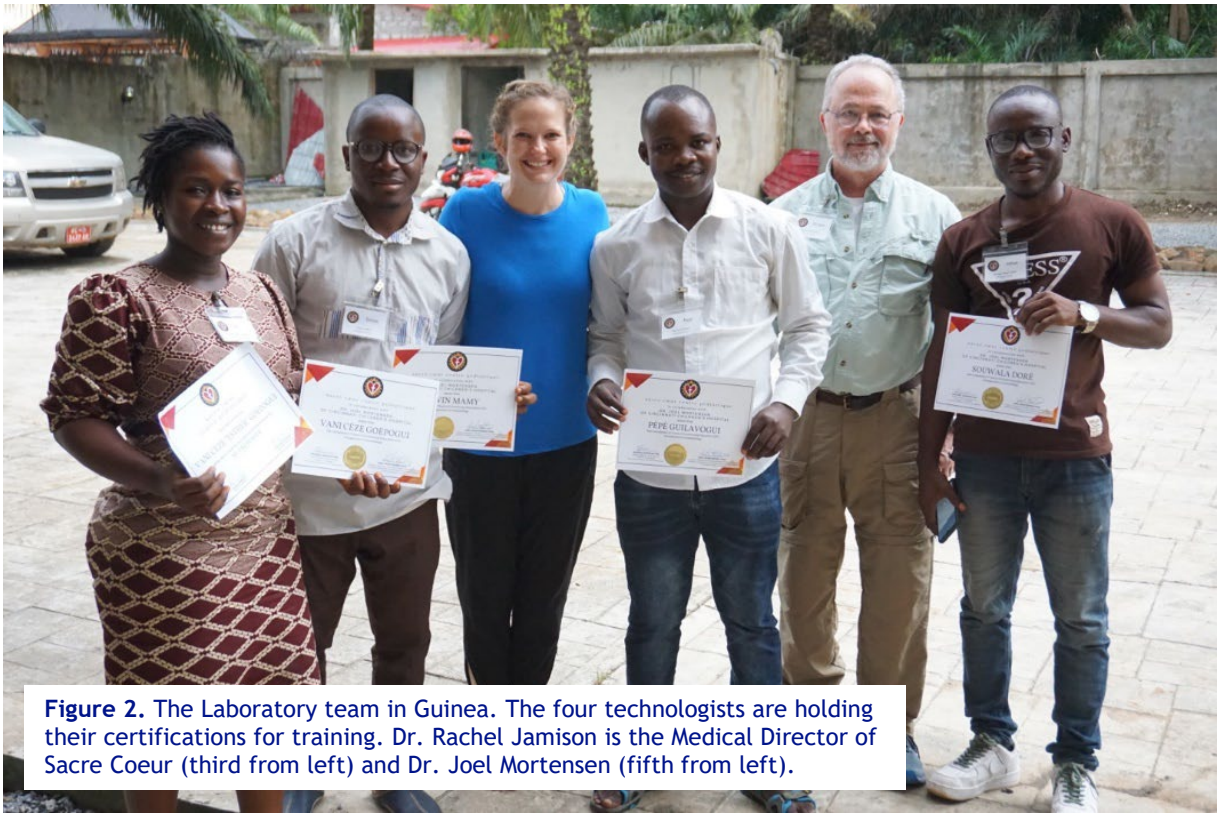


Figure 2. The Laboratory team in Guinea. The four technologists are holding their certifications for training. Dr. Rachel Jamison is the Medical Director of Sacre Coeur (third from left) and Dr. Joel Mortensen (fifth from left).

Daily Life

It is hard to adequately describe the impact of the weather on daily life. I was there in the beginning of November 2022. This was the beginning of the dry season so there was almost no rain and every day was sunny, hot (95 – 98°F) and humid (98 – 100%). Everything was green and plants grew everywhere – walls, walkways, drainage ditches, everywhere. There weren't many insects during my time there. I put mosquito repellent on every morning. Although early morning and dusk were times to watch out for mosquitoes. I had no problems with them.

Two other daily challenges were water and power, with power being an endless trial. Although my apartment had air conditioning, there was no power for four hours most every day. Other times, two dozen small voltage regulators struggled to balance out the continuously fluctuating city power or backup generators. The backup generators required constant attention and drank endless liters of gasoline. Cold showers were refreshing, but sometimes a little too refreshing. Water was usually available from a water tank in the compound, although without electricity the pumps didn't run. Water was filtered before consumption. The system of large filters in each apartment made filtering water easy.

I like to cook and enjoy good diverse food. However, I quickly found that the main staple in Guinea is rice with brown sauce. The sauce may contain chicken or fish or peanuts. There are certainly fruits and vegetables as well, but rice and sauce were the usual fare. Most evening meals were kindly provided by one of the family groups living at the center. Dinner was often American style food with a group "pizza and a movie" on Friday night. The families were all incredibly kind and inclusive.

My time in Guinea came to end much too quickly. The Medical Director and I gave each of the four technologists a training certificate for the 40 hours of contact time that they spent learning basic parasitology. I gave each of them my WhatsApp account and my email.

The 20 miles and two-and-a-half-hour trip back to the airport was punctuated by countless massive potholes, snarled traffic, and demands for bribes from the police and the military. The flight home on AirFrance was surreal compared to my experiences in the last two weeks. The champagne was chilled perfectly. The grilled vegetables and risotto were wonderful. There were two red and two white wines from which to choose. The baguette was warm and had just the right crunch. I reclined my seat/bed and tried to sleep. I wish someone had warned me how hard it was to reintegrate back into "normal" society. The resources and opportunities that I had taken for granted for years now screamed of excess and entitlement.

Home again

In two weeks, what had I learned? There were lots of lessons, but I came away with several key concepts for setting up a microbiology laboratory in a resource limited setting.

- Don't accept limitations – adapt.
    - Processes
    - Resources
    - Procedures

- Match the methods to the resources.
- Donations are wonderful but sustainability is the key.
- Long term commitment to the institution by foreign teaching/support staff.
- Develop the staff.

In addition to these concepts, I needed ongoing processes to keep the momentum and the reach these goals. To that end, we have set up weekly Zoom calls. There is always the cancelled calls due to lack of power or other crisis, but we are doing well. I am introducing the laboratory team and the rest of Sacre Coeur hospital leadership to what standard Policies and Procedures look like, and how to write them.

I have been trying to determine how to get basic quality control material for parasitology and bacteriology to the laboratory because there is no functional post office in the country. I took Gram-stain control slides and old CAP blood film slides with me. Stool parasite control material has been more of a challenge. I purchased several stool specimens in formalin with known intestinal parasites – 2 ml each (Tropical Biologicals). I had them shipped from Puerto Rico, USA to my house. Then I shipped them to Hope Ignited offices in Argyle Texas. Hope Ignited can find people travelling to Guinea to carry supplies in their luggage to Sacre Coeur.

The lectures are ongoing, and I have found some basic microbiology instructional material in French on YouTube. When I work with the team in Guinea over Zoom now, I make all my slides in English and French. Google translate isn't perfect, but it is usually adequate.

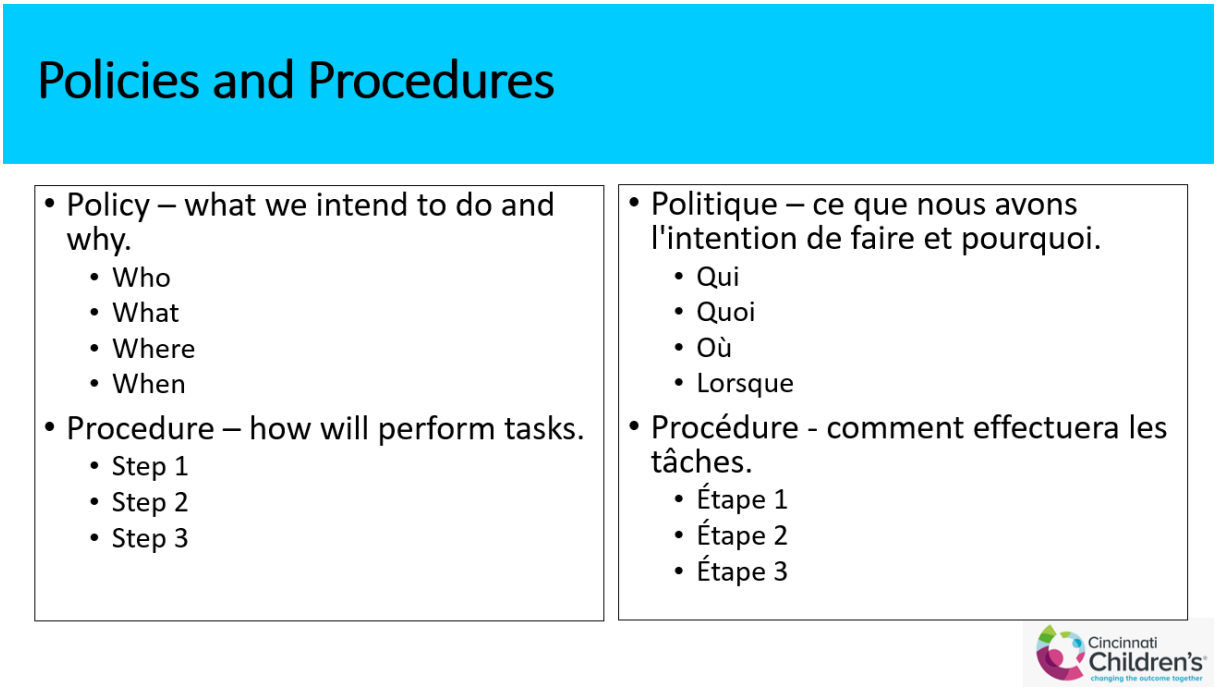


Figure 3. An example of a presentation slide in English and French

We use WhatsApp for communication. It seems to keep functioning no matter what and allows me to receive text on my computer and have Google translate it quickly. Below is a recent text from the two physicians at Sacre Coeur sent a couple of weeks after I returned.

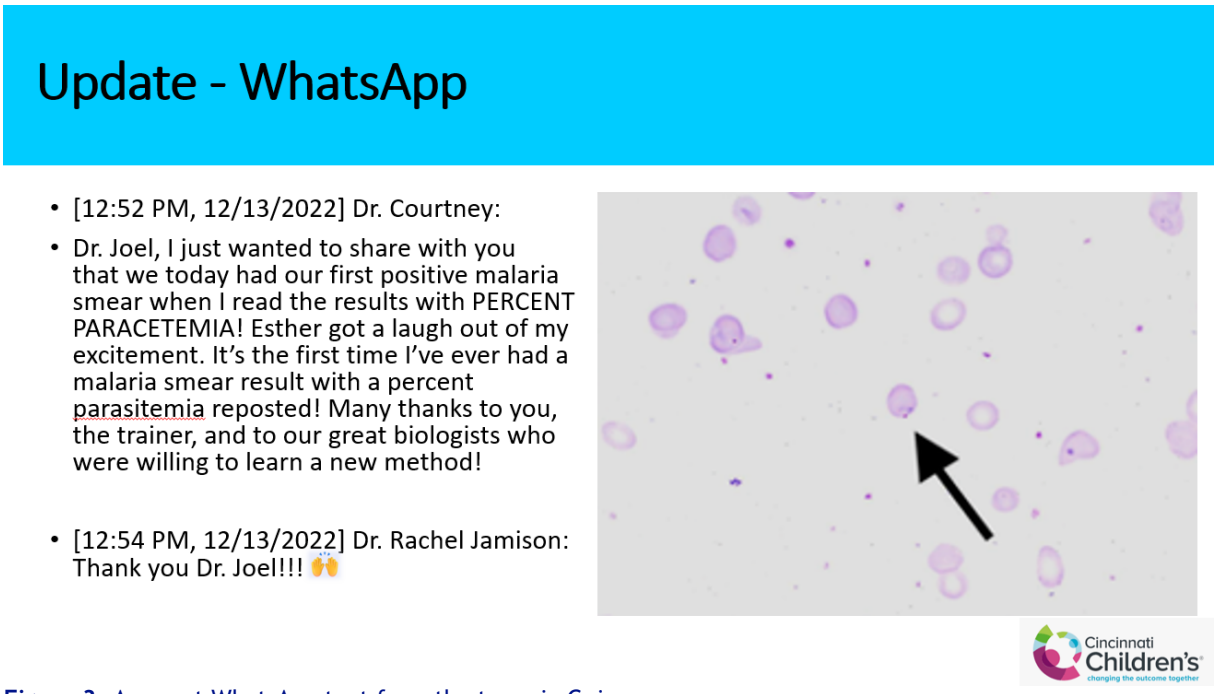


Figure 3. A recent WhatsApp text from the team in Guinea.

The week after that text, a small number of routine patients were seen at the hospital. One day, of the first 5 patients sent to the lab (still in the living room of the apartment!) for testing, 3 were positive for malaria, 2 *Plasmodium falciparum* and 1 *Plasmodium malariae*. The next day had 2 patients with malaria and one with *Hymenolepis nana*.

What is Next?

Together with the medical and laboratory staff at Sacre Coeur, the journey to bring state-of-the-art laboratory medicine to a resource limited setting has just begun. I will be going back.

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Brief Communication, Case Study

A Case Presentation of Urinary Tract Infection Due to *Staphylococcus lugdunensis*

Christina Seitopoulou[1], Nikoletta Renesi[1], Marilena Stamouli[2], Panagiotis Koumpouros[3] and Antonia Mourtzikou[3]

Public Health Center, Nikea, Pireus, Greece.[1] Department of Biochemistry, Naval and Veterans Hospital, Athens, Greece.[2] Department of Molecular Diagnostics, Nikea General Hospital "Agios Panteleimon", Piraeus, Greece[3]

Introduction

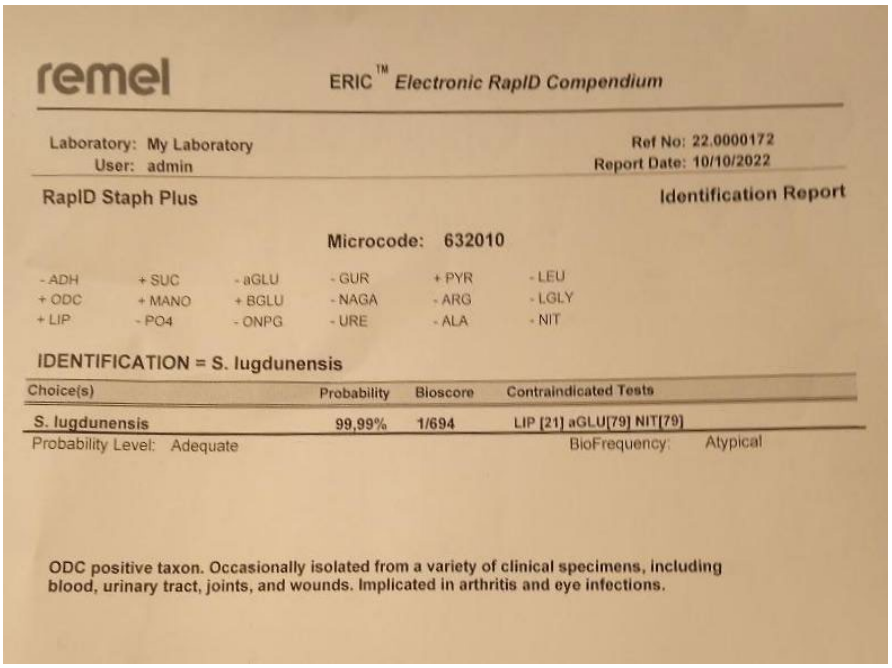
Urinary tract infections occur due to the colonization of the urinary tract by microorganisms and are considered one of the most common forms of infection in the community. Female gender, diabetes, and prior surgery of the urinary system are considered, among others, as high-risk factors for urinary tract infections.[1,2] The aim of this study is the presentation of a case of urinary tract infection caused by *Staphylococcus lugdunensis*.

Case presentation

A 68-year-old woman presented to a primary care physician at the Nikea Health Center, Pireus, Greece, reportıng urinary frequency, urinary urgency and dysuria. Patient history reveals hypercholesterolemia, hypertriglyceridemia, cyctocele grade 3 (severe) operated before decade, urethrocele operated before 5 years, arterial hypertension, and diabetes type II. History also reveals recurrent urinary tract infections, usually caused by Escherichia coli (>10^5CFU/ml). She had no fever and her pelvic examination was normal, without evidence of abnormal vaginal or cervical discharge or inflammation. Urinalysis showed proteinuria, intense pyuria, traces of blood and absence of nitrites (Multistix 10 SG Reagent Strips, Siemens Healthineers). A urine culture grew monomicrobial *Staphylococcus lugdunensis* (>10^5CFU/ml). The bacterium was identified by the RapID REMEL identification system (Thermo Fisher Scientific). (Figure 1) Antimicrobial susceptibility testing revealed susceptibility to Erythromycin, Ceftriaxone, Clindamycin, Vancomycin, Levofloxacin, Oxacillin, Cefuroxime, Cefotaxime, Ampicillin, Nitrofurantoin, Moxifloxacin, Cofrimoxazole, Amikacin, Ciprofloxacin, Norfloxacin, Tetracycline (Kirby-Bauer Disk Diffusion Susceptibility Test Protocol). The patient received treatment with Cefuroxime and her clinical status improved significantly. The follow-up urine culture which was performed after 10 days of antibiotic therapy was negative.

Discussion

Staphylococcus lugdunensis is a Gram-positive cocci, nonsporulating, nonmotile, facultatively anaerobic, catalase-positive, coagulase-negative, oxidase-negative, delta-hemolytic organism, which is commonly part of the human skin flora.[3,4] It can also be found in the axilla, the nasal cavity, the perineal region, and the lower extremities. According to the literature it can also cause bone and joint infections, native and prosthetic valve endocarditis with significant mortality rates and bacteremia.[5-7] The frequency of S. lugdunensis in urine cultures is not known, but there is growing evidence that it is an infrequent cause of urinary tract infection.[8,9] Since it is associated with a high level of virulence and the capacity to produce a broad range of infections, its occurrence is subject of the attention of the scientific community.



remel ERIC™ *Electronic RapID Compendium*

Laboratory: My Laboratory
User: admin

Ref No: 22.0000172
Report Date: 10/10/2022

RapID Staph Plus — Identification Report

Microcode: 632010

- ADH	+ SUC	- aGLU	- GUR	+ PYR	- LEU
+ ODC	+ MANO	+ BGLU	- NAGA	- ARG	- LGLY
+ LIP	- PO4	- ONPG	- URE	- ALA	- NIT

IDENTIFICATION = S. lugdunensis

Choice(s)	Probability	Bioscore	Contraindicated Tests
S. lugdunensis	99,99%	1/694	LIP [21] aGLU[79] NIT[79]

Probability Level: Adequate — BioFrequency: Atypical

ODC positive taxon. Occasionally isolated from a variety of clinical specimens, including blood, urinary tract, joints, and wounds. Implicated in arthritis and eye infections.

Figure 1: Remel Rapid Identification of urinary isolate of *Staphylococcus lugdunensis* demonstrating a typical biotype with a probability of identification at 99.99%

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Review article

A Narrative Review of the Clinical Utility of Next-Generation Sequencing and the Diagnosis of Acute Myeloid Leukemia

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Acute myeloid leukemia (AML) is a cancer affecting the blood and bone marrow progenitors due to heterogeneous genetic aberrations. The standard diagnostic testing regimen for AML includes cytologic and molecular techniques to determine the presence of abnormal myeloid cells and identify the genomic anomalies contributing to disease. These methods are beneficial for diagnosing AML but possess drawbacks in recognizing specific mutations within individual patient samples. AML mutations are being revealed with recent advancements in next-generation sequencing (NGS). Combining NGS-based approaches with standard hematopathology methods could significantly benefit physicians and patients by expediting AML diagnoses, contributing information about the disease, and supporting the development of tailored treatment.

Key words: Next Generation Sequencing, Acute Myeloid Leukemia, Molecular Diagnostics

Introduction

Clinical Overview of Acute Myeloid Leukemia

Acute myeloid leukemia (AML) is a bone marrow and blood disease that affects hematopoietic stem cells, or the precursors of blood cells including red blood cells, white blood cells, and platelets.[1,2] When bone marrow progenitor cells develop chromosomal changes or genetic alterations in the genes controlling cell division and cell death, the cells become leukemic myeloblasts which multiply rapidly and do not function properly.[3] Myeloblasts limit the number of circulating healthy blood cells, which can present phenotypically in the patient as easy bruising or unusual bleeding, persistent fever, abnormal cell counts in blood, and frequent infections.[3] Signs and symptoms of AML include bone pain, clotting problems, and abdominal swelling.[3] Factors associated with an increased risk of developing AML include age, history of other blood cancers, previous cancer treatments, genetic disorders, smoking, and gender.[3] Upon experiencing symptoms, patients may seek medical attention. AML cases are usually discovered following physician evaluation but may also be detected during routine blood work for asymptomatic patients.[4]

AML is primarily characterized by genetic mutations and epigenetic changes, for which

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most cases do not have clear etiology. Disease severity is determined through hemato-pathology examination of blood and bone marrow specimens.[1] Considering that AML is defined through the discovery of genetic anomalies, it is critical that physicians have access to a standardized classification system to characterize and understand the severity of disease for each patient to specifically define the diagnosis, aid in treatment plan development, and provide prognostic clarity. Evidence-based guidelines have been developped by the World Health Organization (WHO) to explicate cancer grading.[5] The latest WHO classification system categorizes AML into two major groups: AML with defining genetic abnormalities and AML defined by differentiation.[5] The category for AML with defining genetic abnormalities maintains the established AML diagnostic criteria outlined in previous WHO update versions and encompasses disease with recognizable genetic alterations.[5] The differentiation category includes cases that are not defined by genetic abnormalities to allow for classification of rare or novel disease presentations.[5] These groups simplify the classification structure for AML and encompass the six subgroups, while still allowing for prognostic risk group placement as favorable, intermediate, or adverse based on molecular and cytogenetic anomalies.[1,5]

The prognostic group assignment, which considers a patient's specific genotypic abnormalities, can be used to predict the patient's response to standard therapeutics, assist primary care providers to decide if clinical trial enrollment could benefit a patient on a case-by-case basis, and determine the likelihood of remission and overall survival.[1,5] A core set of mutations has been identified as precursors for AML with direct diagnostic, therapeutic, and prognostic significance. The most common genes identified in this group are *FLT3*, *NPM1*, *CEBPA*, *IHD1/2*, *DNMT3A,* and *TET2*, which produce functionally diverse proteins involved in signal transduction, ribosomal biogenesis, transcription, and DNA methylation.[2,6]

In AML, genetic mutations and epigenetic modulations are important differences to identify when building patient diagnoses. Genetic mutations are often easier to distinguish when comparing diseased and normal specimen. Epigenetic modifications are heterogeneous, have fewer mutations, and affect gene expression by interrupting DNA modulation and disrupting cellular differentiation.[5] AML cases with epigenetic abnormalities are more difficult to identify and study than cases with mutations in commonly identified genes.[5] By understanding the mutagenic basis of AML, important prognostic information can be used to determine targeted therapeutic approaches for patients.

Mutations in the *FLT3*, *NPM1*, and *DNMT3A* genes make up around 27% of identified mutations in AML patients. When treated with therapies targeting specific mutations, like midostaurin for *FLT3* and crenolanib for *DNMT3A,* patients show significant improvement in clinical response and overall survival.[2,7,8] *TET2* mutations have been identified in 20-25% of AML cases, and patients with these mutations are predicted to have favorable clinical outcomes with high response to standard chemotherapy combined with hypomethylating agents like azacitidine.[7,9,10] *CEBPA* and *IHD* mutations are less common and have been identified in 6-10% of AML cases, but typically predict good outcomes with chemotherapy.[2,10] The clinical implication for AML patients with these genetic abnormalities and the groundbreaking research which led to the novel therapeutic regimens would not be possible without the development and improvement of cytogenetic methods and advancements in molecular testing, like NGS.

Next-Generation Sequencing (NGS)

NGS, also referred to as massively parallel sequencing, is a nucleic acid sequencing technology which involves extracting deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) from a patient specimen. The nucleic acids are fragmented into smaller portions and amplified to make millions of

copies in a library. The fragment library is sequenced, and the data is analyzed using bioinformatic workflows to identify the differences in patient samples compared to normal controls.[11]

Several sequencing methods can be classified as NGS, including whole exome sequencing to look for point mutations commonly found in AML samples and transcriptome sequencing to identify sequence mutations and fusion genes.[11] NGS is becoming an increasingly important tool for effectively pinpointing mutational differences in AML and other myeloid cancers. Within the last 10 years, NGS has been utilized to elucidate the specific genetic abnormalities associated with AML.[12] Through these advancements, a multitude of NGS arrays have been developed and optimized for nucleic acid library construction depending on the input material of the specimen. Standardized kits from companies like New England Biolabs, Illumina, Qiagen, and more are commercially available for both investigational and diagnostic use.[13]

NGS can provide detailed qualitative information for a patient's unique mutations which may influence how AML treatments are prescribed on a patient-specific level.[13] Since NGS can simultaneously detect more than 100 genes in a single assay, the approach enables the recognition of targeted aberrations.[7,14] NGS-based methods can also identify mutations in less common leukemogenic genes, providing further insight into the mutational effects of AML.[2]

Background

Standard AML Diagnostic Strategies

The leading method for screening and diagnosing AML is through evaluation of patient specimen. Blood samples, biopsies and fluid aspirations are critical to accurately diagnosing AML. Specimens provide critical information including the presence of genetic variations, the stage or severity of the cancer diagnosis, and other prognostic information.[15] Hematopathology specimens can be obtained through fine needle aspirations from the bone marrow or from bodily fluids like blood or cerebrospinal fluid.[15]

After sample collection, specimens are initially processed and analyzed in the clinical laboratory. Specimen preparation for biopsies involves stabilizing the structural integrity of the sample by chemical fixation followed by paraffin wax embedding.[15] The paraffin-embedded tissue sample undergoes histologic examination. The tissue is cut into microscopically thin sections, placed onto microscope slides, and stained with dyes that selectively intercalate into specific tissue structures and cellular components.[15] Blood smears are subject to similar staining methods, but do not require paraffin embedding or cutting. The prepared slides are evaluated first by medical laboratory professionals in the clinical laboratory to confirm the presence of aberrant cells or tumor markers, and abnormal specimens are referred to a pathologist for the final evaluation.[3,15]A pathology report is generated that includes a visual description of the specimen, a microscopic evaluation, and the final diagnosis.[15]

Clinical diagnostic laboratories have established molecular testing protocols to identify relevant genetic variations in patient samples, including Sanger sequencing, polymerase chain reaction (PCR) fragment analysis, and fluorescence *in situ* hybridization (FISH).[2,16] The molecular-based methods can provide insight into the genetic factors involved in a patient's disease. Molecular testing is valuable in diagnosing AML. Combining molecular techniques with hematopathology-based methods like cytogenetic testing, immunehistochemistry, and flow cytometry to identify the presence of AML-specific markers are important to develop a thorough diagnostic report.[2,6,16] While current testing platforms are essential for establishing AML diagnoses, the limitations of the approaches hinder the specificity of the data provided by testing. Recent advancements in whole-genome sequencing approaches, like NGS, can further elucidate genomic variants and provide greater

detail for diagnosing a patient's specific cancer subtype.

Diagnostic Implications for Treatment

Though chemotherapy and stem cell transplantation remain the best treatment regimens for AML patients, a "one-size-fits-all" approach for treating AML does not ensure success. This is due to the variable nature of genetic mutations and the inconsistency of abnormal genes identified in AML patients.[1] AML is considered a medical emergency for which the genetic factors associated with the disease must be identified as soon as possible to begin proper treatment regimens to improve patient outcomes.[4] Studies have shown that although the time from diagnosis to treatment does not have a negative effect on overall survival, the sooner that leukemic blasts can be eliminated in the patient the better chances a patient will have to reach a minimal measurable residual disease (MRD) or remission status.[2,4] NGS could be a beneficial tool for obtaining quick, qualitative diagnoses and determining the most applicable and tailored treatment regimen on a case-by-case basis.

Next-Generation Sequencing for Cancer Diagnostics

The recent development of NGS has drastically improved the speed and efficiency of diagnosing cancer. NGS methods have been used to identify cancerous tumors and annotate germline mutations, providing valuable information to guide molecular-targeted treatment plans for patients.[14] Various panel-based NGS tests have been approved for implementation, including the National Cancer Center Oncopanel test which recognizes 114 tumor-associated genes in patient specimens and can detect actionable gene mutations for therapeutic targets. Another diagnostic device, the ClonoSEQ manufactured by Adaptive Biotechnologies, quantifies MRD by differentiating normal and malignant cells.[14,17] By identifying gene mutations through NGS and mapping the frequency of mutations to specific cancer subtypes, drug developments and therapeutic clinical trials could benefit patients.[18] Individuals who have histologically-confirmed metastatic cancer are treated with broad chemotherapy regimens and generally have poor quality of life and prognoses.[18] NGS studies allow for deeper genomic investigation for diagnoses. Site-specific and targeted therapies based on tumor molecular profiles may be identified, providing patients with precision medicine when standard treatments are not beneficial.[18]

Next-Generation Sequencing in Standard Testing

While traditional hematopathology-based methods are the gold standard for identifying and diagnosing AML, NGS-based approaches may support and improve these testing techniques. NGS could become a high-quality diagnostic tool for accurately determining the genetic basis of AML on a patient-specific level. Implementing NGS-based approaches may enhance the results obtained with hematopathology-based and molecular diagnostic methods to better understand genetic mutations in AML.

Standard Diagnostic Techniques

Hematopathology- and molecular-based diagnostic tests are used to identify and diagnose AML in patient specimens. By taking a multidisciplinary approach to characterize the severity of a patient's disease through cytomorphology, immunohistochemistry, and molecular methods, patient care teams can provide a definitive diagnosis and an optimal treatment plan for disease management.[19] Cellular abnormalities are identified by hematopathology, and clinically relevant gene mutations associated with AML have been historically identified through molecular biology techniques.[2]

Benefits of Hematopathology Techniques

One major advantage of the traditional routine diagnostic workup for AML is the fast turnaround time. Results from bone marrow aspirates and blood smears can be obtained from the laboratory within hours of receiving

specimens.[20] The speed of results from these methods is important for the initial disease diagnosis. While stable patients can wait longer for more detailed genetic testing results, a short duration from sample collection to disease determination is crucial for clinically unstable patients to begin chemotherapy or other interventional therapies. Physicians recommend starting treatment within 48 hours of diagnosis in severe circumstances for optimal clinical outcomes.[4,20]

To perform AML diagnostic techniques in the clinical laboratory, laboratories do not require additional equipment to perform testing. This should be considered a significant benefit in favor of hematopathology-based methods. Most laboratories perform automated differential blood counts to measure the percentage of each cell type in specimens, and the equipment in a clinical diagnostic laboratory can be used for more applications than AML testing alone. Protocols and reagents can be also used for evaluating bone marrow and blood samples for other diseases, improving the utility of the reagents in the laboratory. While these testing methods are beneficial for diagnosing AML, there are drawbacks to be considered.

Limitations of Hematopathology Methods

Even though hematopathology approaches are widely used for elucidating the recurrent phenotypic abnormalities in AML testing, these methods have weaknesses. AML is characterized by the presence of greater than or equal to 20% leukemic myeloblasts in the bone marrow.[1,5,23] Normal myeloblast counts are usually less than 5% in bone marrow, and no myeloblasts should be visible in the blood.[4,15] Considering the WHO diagnostic criteria of defining AML with defining genetic abnormalities or lacking genetic abnormalities, hematopathology approaches do not provide information to characterize AML entities into classifying groups. While hematopathology provides fundamental information for diagnosing AML, results should be supported by a multidisciplinary testing approach including molecular-based testing methods.[4]

While the utility of hematopathology in a clinical laboratory is valuable for AML testing, not all clinical laboratories are equipped to analyze specimens using the complex hemopathology methods required for a comprehensive AML diagnosis. Bone marrow and blood samples identified as abnormal by automated testing may need to be sent to larger institutions for testing. By sending out plausible AML specimens to other laboratories the time from sample intake to result reporting increases, delaying diagnoses.

Another disadvantage of hematopathology is that the technique does not provide patient-specific genetic information. Without knowledge of a patient's present genetic mutation or pattern, inadequate treatment decisions may be made to combat a patient's specific AML.[23] The limitations of hematopathology-based methods can be overcome by incorporating molecular methods, like sequencing and FISH, to better understand the diagnosis.

Benefits of Molecular Diagnostic Techniques

Molecular testing methods provide information about genetic abnormalities that cannot be determined through hematopathology approaches. These methods generate comprehensive genetic data, allowing for better understanding of a patient's diagnosis. Cytogenetic and molecular methods include karyotype testing to indicate chromosomal changes, FISH for visualizing genetic mutations, PCR-based assays for amplifying genetic regions to identify abnormalities, and Sanger sequencing to determine the full sequence of targeted genomic regions.

Chromosome evaluation through cytogenetic testing is beneficial for identifying abnormalities in the chromosomes of leukemic cells by microscopy. Conventional cytogenetic analysis by karyotyping is recommended for all AML cases.[5,16] This method is useful for determining if AML leukemic cells have complex genetic changes at the chromosomal level including

translocations, inversions, deletions, additions, and duplications.[15] Determining anomalous chromosomes can help classify the severity of AML, providing valuable diagnostic and prognostic information. Cytogenetic testing results can be used to influence treatment decisions, assess disease response to treatments, and aid in monitoring disease progression and kinetics.[19]

FISH is useful for identifying changes in cellular DNA, similarly to cytogenetic karyotype testing, but is better for visualizing genetic mutations. FISH involves small fragments of DNA conjugated to fluorophores, termed probes, designed to bind to short genomic DNA sequences.[16] When probes bind to targeted genomic DNA in patient specimens the fluorophores can be visualized by fluorescence microscopy. Since FISH is a targeted analysis approach, the method can be advantageous to cytogenetic testing by identifying genetic changes with higher sensitivity and specificity.[15,16] FISH can detect smaller chromosomal abnormalities with higher analytical resolution. Cytogenetic testing can resolve structural abnormalities to 2000 kilobases and resolution of FISH is achieved down to 100 kilobases.[16]

PCR-based assays involve amplification of targeted DNA to generate more copies. Like the binding of FISH probes, short oligonucleotide fragments called primers bind to complementary sequences in the genomic DNA. These primers are significantly shorter than FISH probes, so specific DNA changes can be identified with even higher resolution.[16] The resolution threshold of PCR assays is significantly lower than both cytogenetic testing and FISH at 75-100 base pairs.[16] Most PCR-based assays are single-gene assays, where one specific gene in the patient genome is being evaluated for mutations. For AML, PCR assays focus on more commonly identified genes, such as *FLT3* alterations, as *FLT3* mutation is associated with severe prognostic implications but can be treated with targeted therapies.[13,16,24] This technique is helpful for finding genetic abnormalities in small numbers of cells, which is important for early detection and diagnosis of AML.

Sanger sequencing is widely considered traditional sequencing and can be used to assess the severity of AML diagnoses.[16] The method is similar to PCR-based testing methods, where DNA is amplified, and the nucleotide sequence is determined by tracking fluorophore emission. However, Sanger sequencing uses one primer to identify the full sequence of the target DNA in a linear fashion, where PCR uses a forward and reverse primer to create exponential copies of the DNA template.[16,19] Sanger sequencing is most frequently used in AML testing to detect *CEBPA* mutations, as they are difficult to detect through other molecular methods like FISH or karyotyping.[21]

Despite the benefits of incorporating molecular methods to understand genetic abnormalities in AML, these methods also have limitations. Molecular testing approaches have associated pitfalls, including limited results in cytogenetic testing, the requirement of locus-specific probe design for successful FISH assays, and false negatives in PCR- and Sanger sequencing methods.[16] Understanding the drawbacks of molecular methods is critical when determining which tests are best for diagnosing AML.

Limitations of Molecular Diagnostic Techniques

Cytogenetic testing and karyotyping rely on the generation of metaphase chromosomes for successful analysis. Cells must be grown in the laboratory for one to three weeks before microscopy to visualize the chromosomes.[15,21] If the cells in a patient specimen do not proliferate the chromosomes cannot be identified, which will not produce results.[16] Another caveat of this method is that abnormalities may involve small regions of the chromosome beyond the scope of visual recognition, which would require detection by alternative methods like FISH.[16]

For FISH assays, probes are designed to match specific regions in the patient DNA. The

design and optimization process for accurate FISH probes can be time-consuming and costly, and mutations will not be properly identified if the designed probes are not specific to the affected genes.[16] While probes can determine if specific mutations are present in a patient specimen, chromosomal abnormalities outside the genomic region targeted by the designed probe will not be identified.[21]

A limitation of PCR-based molecular testing is the identification of complex genetic variants. For example, translocations have multiple break points where chromosomes recombine.[16] Multiple primers spanning the break points must be designed to correctly amplify translocations, making PCR less practical for detecting these variants.[15,16] The phenomenon of allele drop-out is a common issue in PCR-based assays. Allele drop-out occurs when mutations are located within the primer binding site, leading to poor primer annealing and false negative results due to failed amplification.[25] Another common issue in PCR-based techniques is the potential for polymerase artifacts that result from the numerous amplification events for the method, which may also lead to false positive readouts.[25] The limitations of PCR-based testing assays can be resolved through careful primer design.

Sanger sequencing is limited by sequencing capacity. While Sanger sequencing analyzers may have multiple channels to sequence more than one sample at a time, this method is not capable of multiplexing, reducing the rate by which data is collected.[19] Sanger sequencing has the additional drawback of limited sensitivity. If the mutation of interest in a sample is found in less than 15% of the sample, the sequencing method will not be able to detect it which can be deciphered as a false-negative result.[16]

Improvements in testing efficiency for molecular methodologies are critical for combatting the shortcomings of each technique. Standard hematopathology and molecular testing methods limit the capacity and specificity of results necessary for diagnosing AML. Multidisciplinary approaches combining more than one method are important when diagnosing patients with AML. These challenges may be overcome with the consideration and addition of recent technological improvements and a, like NGS, to the AML diagnostic testing regimen.

NGS Diagnostic Utility

NGS platforms are becoming routinely used for identifying clinically critical malignancies for cancer diagnostics.[25,26] NGS is still considered a new technological advancement for molecular biology and diagnostics, as this method emerged within the last decade.[24] Despite the novelty of this method, major advantages can be identified when considering if or when to implement NGS into the standard diagnostic workflow for AML testing.

Advantages of Next-Generation Sequencing

Sanger sequencing and NGS are similar, as both methods amplify specific target fragments and determine the sequence of the DNA template strand.[19] Unlike Sanger sequencing, NGS has the capability for massive parallelization. Where Sanger sequencing is limited, NGS can sequence thousands of genetic regions simultaneously.[19] This allows for comprehensive genomic coverage and more data produced to establish diagnoses.

A considerable advantage for NGS is that the data generated by the method can be contributory for diagnosing clinically difficult neoplasms that emerge in AML. The classification and mutation profiling information provided by NGS may otherwise be missed when building a diagnosis.[26] NGS can simultaneously detect numerous mutations in multiple patient samples at once, which could improve the efficiency of AML screening if the method was integrated into routine clinical testing.

Genetic anomalies can appear anywhere in the genome of AML patients, leading to structurally diverse genomic abnormalities from patient to patient.[7,27] One of the biggest challenges identified when studying the genetic mutations of AML is the complexity and

specificity of mutations that can occur, including single nucleotide polymorphisms, insertions/deletions, copy number variants, chromosomal rearrangements, and fusion genes.[7,26] NGS can detect AML mutational discrepancies with 85-99.9% efficiency depending on which mutation combinations are present in patient specimens.[12,26,27] NGS-based testing is also significantly more specific than traditional Sanger sequencing. NGS reaches less than 5% specificity and Sanger cannot detect gene mutations with less than 15% specificity, which is important for accurate detection of mutant alleles in patient specimens.[16] This technological improvement can provide more detailed information to patients and physicians in less time.[25,26]

A revolutionary advantage of NGS in AML diagnostics is the potential to match patients with specific mutations to clinical trials that may provide significant clinical benefit.[2] With the advancement of molecular technology, novel molecular-targeted therapeutic strategies are being developed and entering clinical trial testing for AML and other clinically complex. NGS mapping has identified "drug-gable" genetic mutations that can be targeted using small molecule inhibitors, which provides options for patients who have previously undergone chemotherapy regimens with little to no clinical resolution or who have relapsed following remission after chemotherapy.[14,19] The information generated through NGS allows the patient care team to make informed decisions for a patient's medical treatment based specifically on the individual's mutational profile, ultimately improving the standard of care for the patient. As with the other testing methods discussed, NGS has limitations which must also be examined when determining the clinical utility of this approach.

Disadvantages of Next-Generation Sequencing

Although NGS is a promising resource for improving early detection and treatment for patients, more research needs to be performed before the method can be implemented in AML diagnostic testing.[25,27,28] One major downside to NGS for AML diagnostics is that there is no one consensus list of biomarkers for AML. The Association for Molecular Pathology leukemia focus group has published a definitive list of the minimum genes required for testing for chronic myeloid neoplasms, but no list currently guides which genes should be tested when diagnosing AML.[26] Fortunately, the understanding of AML, the genetic mutations involved, and novel therapeutic targets is also rapidly improving.

Another disadvantage of NGS compared to hematopathology and other molecular-based methods is while NGS is more specific and sensitive than traditional PCR approaches, NGS is still vulnerable to the shortcomings of PCR-based molecular methods previously addressed.[25] NGS assays are often multiplexed so that libraries can be generated and sequenced from more than one patient samples in a single run. Multiplexed sequencing reactions are susceptible to primer errors which lead to inaccurate results. The likelihood of errors increases when the number of primers and amplicons in a reaction are increased. Annealing temperature variation, the number of cycles needed for sufficient amplification, and extension times can vary.[25] These drawbacks can usually be mitigated through careful assay design.[25]

NGS is becoming a cost-effective method to obtain comprehensive genomic data for multiple patients in a single run. However, the upfront cost associated with NGS may not be feasible for laboratories operating on tight budgets or those that do not have a high enough sample volume to invest in the equipment and consumables needed. Studies have found that the advancement of the technology has improved the operational costs of NGS. The cost per sample tested by NGS is comparable to or lower than the expenses required for standard cytological and molecular analyses.[6,29] Even with the price reduction of NGS testing, the financial burden of purchasing the sequencing reagents,

training personnel to perform the complex sequencing protocols, the costs of performing the sequencing reactions, and deciphering the large datasets produced may be difficult for some clinical laboratories to justify.[13,29]

An additional caveat hindering the motivation to implement NGS in clinical laboratories is the time it takes to get results back to physicians. NGS runtimes vary based on the sequencing platform used and the workflow required for the system. Sequencing can take between three to five days to complete from library preparation to analysis.[6,13] This does not include the time to analyze the data produced and provide patients with a definitive genetic conclusion, which can take up to two weeks.[20] FISH, PCR-based testing, cytogenetic testing, and Sanger sequencing have turnaround times of roughly 72 hours or three days.[21]Although the genetic information obtained by NGS is highly specific, the prolonged timeframe is a limitation compared to Sanger sequencing and other conventional testing methods.

Considering the benefits and shortcomings of diagnostic approaches like NGS, the impact on early diagnoses and precision treatment options will continue to advance. These improvements will positively impact patient care and quality of life. Being aware of the potential pitfalls of this technology is critical for avoiding erroneous reporting in clinical diagnostics and practice.[25]

Discussion

A major strength of NGS is the sensitivity of the method, which allows for the detection of atypical variations that standard hemato-pathology and molecular methods are likely to miss in routine diagnostics. NGS has signify-cantly improved the understanding of AML pathogenesis and the genetic mutations which serve as prognostic indicators for patient survival and outcome.[30,31] Conventional AML testing only detects chromosomal abnor-malities in 50% of patients, so including NGS in the testing algorithm can dramatically improve genomic risk factor assessments and determine if targeted therapies will have a better clinical impact on patients compared to current chemotherapy regimens.[31] Considering that new therapies are being researched and tested in clinical trials, the breadth of knowledge for treating AML is rapidly expanding to positively impact patient care. With advancements in NGS technologies, NGS-based approaches for diagnosing AML may provide beneficial information on patient-specific basis.[30,31]

Despite the potential advantages of incorporating NGS-based approaches to the AML diagnostic methods, there are still shortcomings that need to be addressed before the technology can be implemented into clinical laboratory workflows. Some genes are difficult to identify with NGS-based methods, and current NGS technologies are still prone to the relatively high error rates that challenge traditional PCR-based methods.[30] Other pitfalls of the approach include long turnaround times from specimen collection to result reporting to physicians and the up-front costs of imple-menting NGS testing. The disadvantages must be considered and further optimized before NGS can be fully integrated into the daily clinical workflow for AML diagnostics.

While the argument can be made for and against the addition of NGS in the initial AML diagnostic workup, there are a significant number of cases in which patients do not respond to therapeutic interventions, or there are no effective drugs or applicable clinical trials available for patient-specific mutations. One main uncertainty of NGS implementation is the frequency by which patients should undergo genomic NGS testing to track genetic changes, proliferation, and MRD monitoring.[31] There are currently no standards in place for retesting patient specimens with NGS when patients fail to reach remission with traditional treatment methods. Important standards that must be defined before NGS can be used in daily clinical practice are the timepoints for NGS analysis during treatment and which genes should be included in NGS methods for clinical testing.[31] With further work, NGS-based

approaches could be combined with standard hematopathology to improve AML diagnostics.

The impacts of implementing NGS-based approaches concurrently with standard techniques for AML diagnostics include, but are not limited to, improving the depth of understanding of AML, providing qualitative results to physicians and patients, developing a less-invasive yet descriptive method for monitoring MRD, and bridging the gap in knowledge of treating remission and clinically complex cases through the advancement of precision medicine and patient-centric treatment plans. Precision oncology is a cancer treatment approach with the goal to identify and execute the exact therapeutic strategy for every patient at the right time to successfully reach and maintain remission.[32,33] Recent studies have shown that using NGS methods for genomic profiling-directed therapies correlate with increased survival rates and ultimately better outcomes.[34]

By elucidating the genetic underpinnings of patients' unique diagnoses, optimal therapies can be implemented to maximize survival outcomes and reduce the potential for relapsing post-remission, allowing patients to see beyond the cancer diagnosis. Fast-paced, groundbreaking research is being performed to discover druggable targets and novel compounds to challenge cancer progression and revolutionize the way cancers are diagnosed and treated.

Conclusion

The genetic variability of AML makes diagnosing and treating the heterogeneous disease incredibly difficult. It is of critical necessity to understand AML mutations on a case-by-case basis. Standardizing NGS approaches for classifying the genomic and molecular basis of disease on a patient-specific level will have diagnostic, prognostic, and predictive relevance for AML assessment.[31] Traditional hematopathology methods can be used to obtain rapid diagnostic results for urgent treatment interventions, while implementation of NGS methods provides qualitative results for clinically relevant genetic mutations improving overall patient care and outcomes. With further research, NGS has the potential to become a standard diagnostic technology for diagnosing AML.

All authors have no conflict of interest to declare.

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Research article

Phenotypic Detection and Antimicrobial Profile of Metallo-Beta-Lactamase Producing *Pseudomonas aeruginosa* from Gunshot Wounds of In-Patients in Tertiary Hospitals in Maiduguri, Nigeria

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Background: *Pseudomonas aeruginosa* (*P. aeruginosa*) is the most common and significantly drug resistant bacteria isolated from all categories of in-patient wounds. The resistance is attributed to the production of metallo-ß-lactamases (MßLs) responsible for high morbidity and mortality.

Methods: *P. aeruginosa* was isolated and identified from wound samples using Bergey's manual of systemic bacteriology. Each wound swab was used to inoculate MacConkey agar and Cetrimide agar. The ethylenediaminetetraacetic acid (EDTA) disk testing method and modified Kirby-Bauer disc diffusion method was conducted to determine the phenotypic characterization and antimicrobial profile of MßL-producing *P. aeruginosa*.

Results: Out of the 100 wound swabs examined, 24 (24.0%) of the isolates were identified as *P. aeruginosa*. Among the 24 isolates identified as *P. aeruginosa*, 8/24 (33.3%) of those isolates were MßL-producing. Male in-patients had a higher prevalence of 19 (79.2%) for *P. aeruginosa* than females with 5 (20.8%) (P< 0.05). MßL-producing *P. aeruginosa* was of a higher prevalence in males with 7 (87.5%) than in females with 1 (12.5%) (P<0.05). Based on wound location, the lower limbs had a higher prevalence of 18 (75%) than those on the upper limbs 3 (12.5%), head 2 (8.3%) and abdomen 1 (4.2%) (P<0.05) for *P. aeruginosa*. MßL-producing *P. aeruginosa* also had higher prevalence for wounds on the lower limbs 7 (87.5%) compared with those on the upper limb 1 (12.5%), head and abdomen each with 0(0%) (P<0.05). Antimicrobial profiles of *P. aeruginosa* isolates indicated a 100% resistance against cefepime 30 µg and ceftazidime 30 µg and highest level of susceptibility (20.8%) for meropenem 10 µg, imipenem 10 µg and gentamicin 10 µg. While all the MßL-producing *P. aeruginosa* isolates recorded a 100% resistance against all the antibiotics used.

Conclusion and Recommendation: These results demonstrate that spread as well as the rate of drug resistance among the MßL-producing *P. aeruginosa* is concerning. Imipenem and meropenem are potential drugs of choice for treatment of infections caused by MßL-producing *P. aeruginosa*.

Key words: Metallo-Beta-Lactamases, *Pseudomonas aeruginosa,* Gunshot-wounds, In-patients, Antibiotic resistance, Maiduguri-Nigeria

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Introduction

Gunshot wounds are one of the most common cause of trauma worldwide and contribute significantly to economic burden, death and disability.[1,2] The wound is usually contaminated with foreign materials such as bullets and thus, promotes pathogenic colonization and breakdown of defense mechanisms.[3,4] The presence of colonizing microorganisms continue to provoke the immune system and interfere with normal healing process.[5] Moreover, antibiotic resistance in wound pathogens reduces the efficacy of the antibiotic treatment protocol.[4]

Pseudomonas aeruginosa (*P. aeruginosa*) is regarded as the most common agent of wound infection especially in developing countries.[6,7,8,9] The organism is intrinsically resistant to multiple antibiotics including ß-lactams (carbapenems), aminoglycosides, fluoroquinolones, and polymyxin B.[10,11] The prevalence of carbapenem resistance has been increasingly reported in several countries.[10,12]

In 2017, the World Health Organization (WHO) ranked carbapenem-resistant-*P. aeruginosa* as the second most critical-priority bacterium among 20 antimicrobial-resistant bacterial species.[13] This resistance is attributed to the production of metallo-ß-lactamases (MßLs) which cleave the amide bond of the ß-lactone ring, impermeability or loss of porin OprD, or increased expression of an efflux pump.[14,15] MBL–mediated resistance has been responsible for high morbidity and mortality among in-patients worldwide.[10,12,18,16,17,18] It is imperative to conduct the rapid detection of MßL-producing *P. aeruginosa* amongst gunshot in-patients in Maiduguri as an aid to management and empirical therapy.

Study area

The study was conducted at the University of Maiduguri Teaching Hospital and Nigeria Army 7 Division Medical Services and Hospital, Maiduguri Nigeria from February 2022 to June 2022. Maiduguri, the capital of Bono State in Nigeria, is located in North-Eastern Nigeria and lies within latitude 11.15°N and longitude 30.05°E in the sudano-sahelian savanna zone.[19] The state has an area of 71.210sq km with the population of 4,151,193 according to National census conducted in 2006.[20]

Methodology

Samples population, collection, and processing

A total of one hundred (male: 76 and female: 24) wound swabs were aseptically collected using convenience purposive sampling from gunshot in– patients at the University of Maiduguri Teaching Hospital and the Nigerian army 7 Division medical services and Hospital Maiduguri. The samples were presserved on ice for investigation at the department of microbiology laboratory, University of Maiduguri. Informed consent for each sample collected was obtained and included the age of the patient, gender, and wound location.

Isolation and identification of Pseudomonas aeruginosa

Isolation and identification of the bacteria was conducted as described in Bergey's manual of systemic bacteriology.[21] Each wound swab was inoculated by spread plate on MacConkey agar and Cetrimide agar and incubated aerobically at 37°C for 24hrs. Isolates were identified using morphological and biochemical characteristics.[22]

Phenotypic test for detection of MßL-producing Pseudomonas aeruginosa

The phenotypic detection of MßL-producing *P. aeruginosa* isolates was conducted by EDTA disk testing. An overnight culture of the isolates was prepared in 2 mL of Mueller-Hinton broth (MHB), with turbidity 0.5 McFarland Standard (which is approximately 10^8 CFU/ml). The bacterial suspension was streaked evenly onto a 150-mm-diameter plate containing Muller Hinton Agar (Oxoid Ltd., Basingstoke, Hampshire, England). Two disks of imipenem 10mg, one of which was impregnated with 5 µL of 0.5 EDTA (Sigma, USA) solution were placed 10 mm apart from edge to edge on the surface of the same Muller Hinton Agar plates for 24 hours of incubation at 37°C. A difference of

≥7mm in diameter between the zones of inhibition for the EDTA impregnated imipenem disk and imipenem disk alone indicated the presence of MßL-producing *P. aeruginosa*.[23,24]

Antimicrobial susceptibility profile

The antibiotic susceptibility profile of the *P. aeruginosa* and MßL-producing *P. aeruginosa* isolates was evaluated using the Kirby-Bauer disk diffusion method as recommended by the Clinical Laboratory Standard Institute (CSLI) guidelines.[25,26] Bacterial suspensions were prepared in 2 mL of Mueller-Hinton broth (MHB), with turbidity 0.5 McFarland Standard (which is approximately 10^8 CFU/ml). The bacterial suspension was streaked evenly onto a 150-mm-diameter plate containing Muller Hinton Agar (Oxoid Ltd., Basingstoke, Hampshire, England). The anti-biogram was determined by comparing the zone of inhibition with the CSLI interpretative chart using meropenem 10 µg, imipenem 10 µg, ceftazidime 30 µg, cefepime 30 µg, gentamicin 10 µg and ciprofloxacin 5 µg.

Statistical analysis

Data obtained were presented as percentage prevalence and statistical significance was determined using the student "T" test to compare the prevalence of *P. aeruginosa* isolates and MßL-producing *P. aeruginosa* isolates from gunshot wounds of in-patients with respect to location of the wounds and gender of the patients. P –values equal to or less than 0.05 are regarded as significant.

Results and Discussion

ß-lactams, including carbepenems are considered the most potent agents for treatment of infections caused by *P. aeruginosa* .[27] Resistance to MßL among *P. aeruginosa* is increasing and has been reported in several countries.[28,29]

In this study, out of the total of one hundred (100) (male: 76 and female: 24) gunshot wound samples collected, a prevalence of 24 (24%) were recorded for *P. aeruginosa*. Of these, 8 (33.3%) were recorded as MßL-producing *P. aeruginosa* by imipenem-impregnated EDTA test. (Figures 1,2) However, the result revealed that male in-patients had a higher prevalence rate of 19 (79.2%) for *P. aeruginosa* than females with 5 (20.8%), while male had a higher prevalence rate of 7(87.5%) for MßL-producing *P. aeruginosa* than females with 1(12.5%). (Figures 3, 4) Based on wound location, the lower limbs had a higher prevalence rate of 75% (18/24) for *P. aeruginosa* than the upper limbs 12.5% (3/24), head 8.3% (2/24) and abdomen 4.2% (1/24). (Figure 5) The lower limbs had a higher prevalence rate of 87.5% (7/8) for MßL-producing *P. aeruginosa* than the upper limb 12.5% (1/8), head 0% (0/8) and abdomen 0% (0/8). (Figure 6) The variation in the patient's gender and wound location in relation to the prevalence of *P. aeruginosa* and MßL-producing *P. aeruginosa* infection was statistically significant (P<0,05).

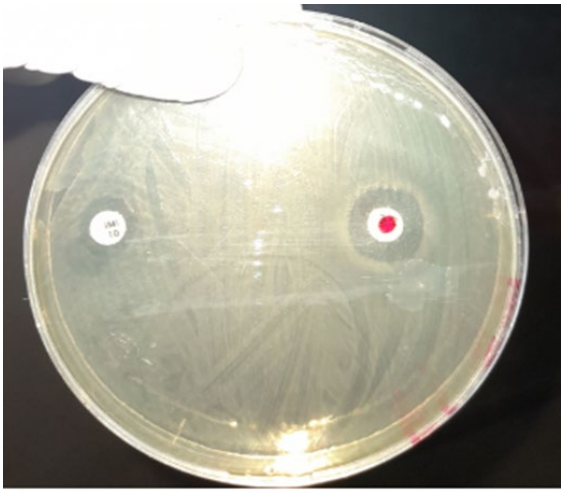

Figure 1. EDTA Disk Testing method showing enhanced inhibition zone of >7mm around IPM + EDTA disc indicating MßLs positivity.

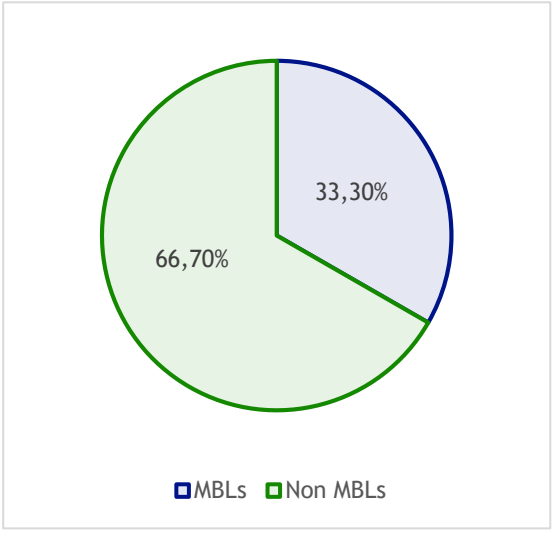

Figure 2: Percentage prevalence of metallo-ß lactamase (MBL) producing *P. aeruginosa*

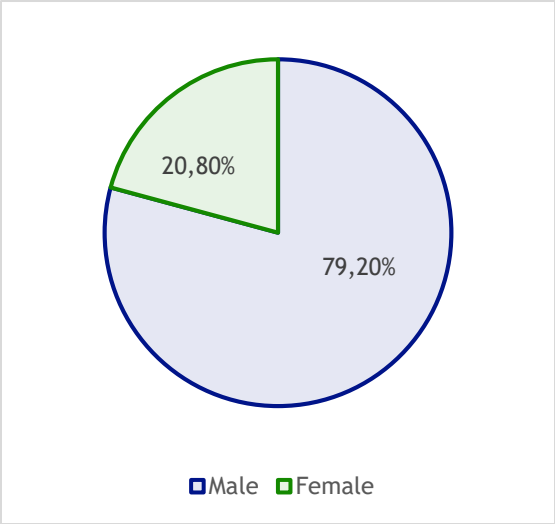

Figure 3: Percentage prevalence of *P. aeruginosa* based on gender of in-patients

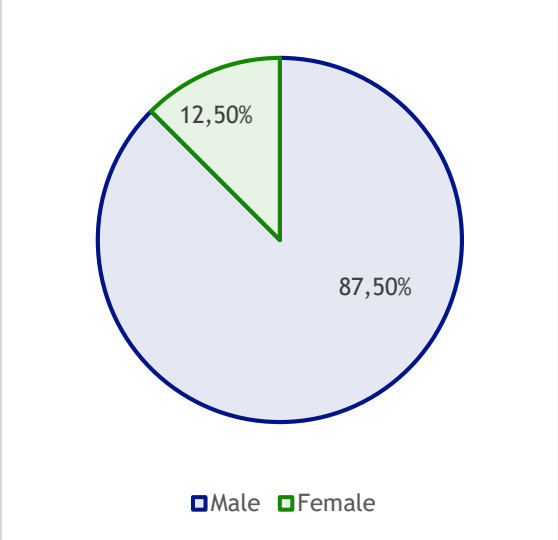

Figure 4: Percentage prevalence of metallo-ß lactamase producing *P. aeruginosa* based on gender of patients

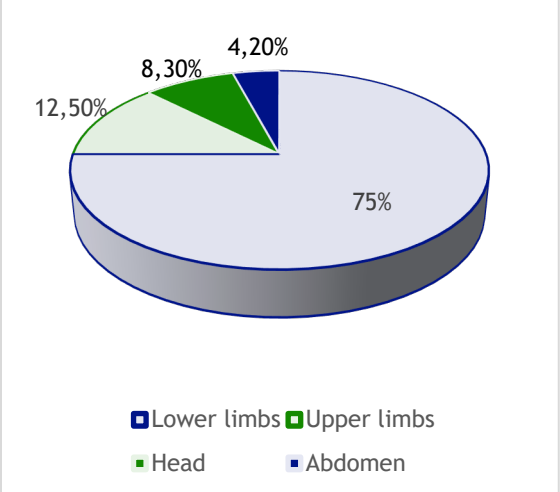

Figure 5: Percentage prevalence of *P. aeruginosa* based on wound locations.

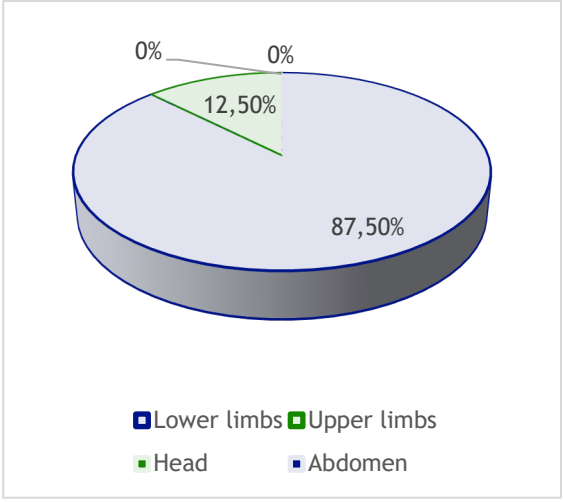

Figure 6: Percentage prevalence of metallo-ß lactamase producing *P. aruginosa* based on wound location.

Though, the prevalence rates in this study are low, MßLs among *P. aeruginosa* have been increasingly recognized from clinical isolates worldwide. Ettu *et al.* (2021) in Nigeria reported 81.5%; 37.6% in West of Iran, Hamadan by Arash *et al.*, (2022); 35.1% in Egypt by Rehab *et al.*, (2021); 34.2% in Ghana by Hayford *et al.*, (2021); 18.95% in India by Kunachgi *et al.* (2015); 24.2% in Korea by Nam *et al.*, (2010) and 15% in Nepal by Reshma *et al.* (2020).[30,31,32,33,34,35,36] The findings in this study, however are substantially higher than the reports from Europe and other developed nations where infection rates of 2.3% (23/996) in 2004 and 2.1% (21/992) in 2006 across clinical specimens were reported in Japan.[37] In Italy and Spain, the MßL prevalence in *P. aeruginosa* was reported as 1.3% and 0.1% respectively.[38,39]

The incidence of MßL-producing *P. aeruginosa* is due to intrinsic resistance of the organism,[40] and its associated risk factors, both of which led to spread of the bacteria. It has been emphasized that the detection of MßLs among *P. aeruginosa* is crucial for optimal treatment of patients due to the increase in ß-lactam usage and emergence of resistant bacteria under antibiotic pressure.[41]

Figure 7 and 8 show the antimicrobial susceptibility profile of *P. aeruginosa* isolates and MßL-producing *P. aeruginosa* isolates denoting the resistance, intermediate and

susceptibility patterns. The *P. aeruginosa* isolates demonstrated a 100% resistance to cefepime 30 µg and ceftazidime 30 µg, while the highest susceptibility was 20.8% each for meropenem 10 µg, imipenem 10 µg, and gentamicin 10 µg. This result indicates that the resistance levels were high across all the antibiotics tested. Such resistance has been previously reported and are due to the coexistence of genes encoding drug resistance to other antibiotics on the plasmids the isolates are harboring.[42.] However, because of the very low permeability of the cell wall, *P. aeruginosa* is naturally resistant to ß-lactams including broad spectrum cephalosporins and quinolones.[43,44,45]

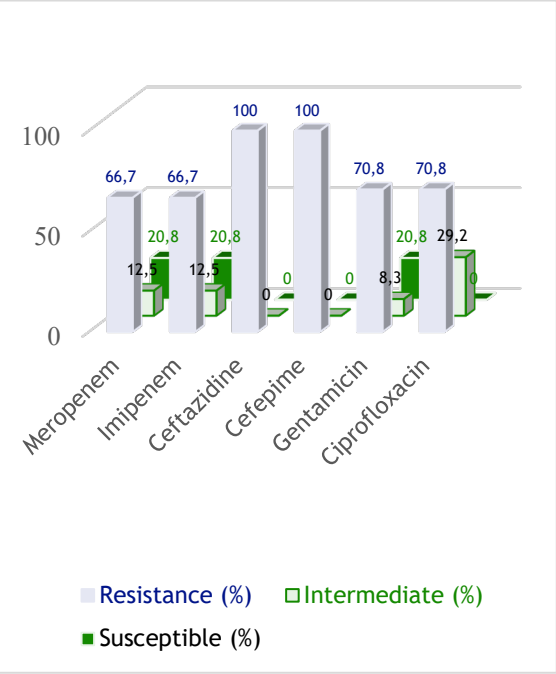

Figure 7: Antimicrobial profile *P. aeruginosa* isolate to ß-lactam antibiotics and other non-ß-lactam antibiotics

All eight (8) of the MßL-producing *P. aeruginosa* isolates recorded a 100% resistance against all the antibiotics examined in this study. A particularly important feature is that the MBL producers were resistant to all the ß-lactam antibiotics and the non-ß-lactam antibiotics. This however indicates the multi-drug resistant attribute of MßL-producing *P. aeruginosa* isolates and supports the claim that these 'superbugs' are minimally susceptible to empirical therapy.[41] This was comparable to reports from other parts of the world, which

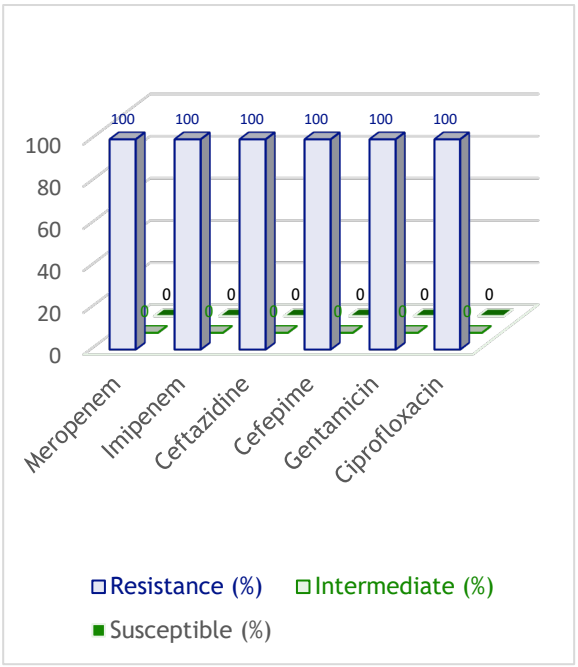

Figure 8: Antimicrobial profile of the metallo-ß lactamase producing *P. aeruginosa* isolates to ß-lactam antibiotics and other non-ß-lactam antibiotics

also revealed multiple drug resistance among *P. aeruginosa* and the limited treatment options for wounds.[14] Moreover, there are reports of growing concern of the MßL-producing *P. aeruginosa* showing cross resistance to non-ß-lactam antibiotics.[46] Kateete *et al.* and Horieh *et al.* reported that resistance in *P. aeruginosa* was mainly due to the production of MßLs and other factors such as genetic mutations in over expression of the ampC gene, increased expression of the efflux pumps, decreased expression of proteins, impermeability through alteration or loss of the porin OprD, reduced outer membrane permeability, alteration in the target sites of antibiotics and less possibly involved are the increased activity of chromosomal cephalosporinases.[15,41]

This study also demonstrated significant differences in the susceptibility profiles between MßL-producing and non MßL-producing *P. aeruginosa* for meropenem, imipenem and gentamicin, except for ceftazidime, cefepime and ciprofloxacin where no significant difference was observed in the susceptibility patterns of both organisms. This indicates that MßL-producing strains of *P. aeruginosa* are

more likely to have low susceptibility to non ß-lactam antibiotics compared with non MßL-producing isolates of *P. aeruginosa*. In this regard, the hospitals should formulate an effective antibiotic policy.

Limitations

In this study, the great proportion of the population are male in- patients. All patients were determined to have wound infections resulting from the gunshot injuries during counter-insurgency/counter-terrorism operations in Bono State, Nigeria. Hence, lack of a comparison group is a definite limitation of the study. Clinical details of patients such as history of antibiotic use are not included due to insufficient information. Also, molecular epidemiologic analysis and characterization to determine the MßL types and resistant genes were not carried out due to funding constraint.

Conclusion

This study revealed the presence and prevalence of MßLs in *P. aeruginosa* of gunshot wound patients. The isolates showed >60% resistance to ß-lactam antibiotics including carbapenems and cephems and other non-ß-lactam antibiotics, including gentamicin and ciprofloxacin. This high level of resistance may put the afflicted patient at an increased risk of developing a multidrug resistant infection. However, accurate identification and reporting of MBL-producing *P. aeruginosa* will aid in limiting the spread of the organism.

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Ethical Approval

The study was approved by the Research Ethics Committee of the University of Maiduguri Teaching Hospital Maiduguri, Nigeria and Nigeria Army 7 Division Medical Services and Hospital, Maiduguri Nigeria (decision number: UMTH/REC/21/949 and 7 DMSH/G1/300/26 respectively).

Informed Consent

The written informed consent was obtained from the study participants.

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Research article

Introducing Digital Pathology in Fast-Frozen Section by Validating the Whole Slide Imaging Scanner Slideview VS200 Research Slide Scanner

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Background: The implementation and usage of digital pathology has undergone a huge development in recent years, However, the area of intraoperative consultation has not yet become digitized or fully investigated. The aim of this study was to explore the possibilities to digitize this area and evaluate the consistency between the diagnoses based on the digital slides versus the traditional microscopic review. The whole slide image scanner Olympus VS200 STL proclaimed that it would be able to scan glass slides with wet mounting glue.
Method: To find the optimal scanning profile a field of four different scan profiles were tested in different contexts. The chosen profile was used to generate 126 digital slides from 60 cases. These slides were assessed on three parameters; compliance between diagnosis of the digital and traditional method, compliance with the visual quality and could the scan time including operating time be completed within 180 seconds per slide.
Results: The overall result showed no deviation between diagnoses made with conventional microscope and the digital slide in 83% of the cases and the average operation time was 92.5 seconds.
Conclusion: Olympus VS200 STL has the potential to become implemented in a clinical pathology department for use in intraoperative diagnostics without affecting workflow, diagnostic accuracy, and demonstrates an acceptable time for review (180 seconds per slide).

Key words: Digital pathology, imaging, microscopy, pathology, whole slide image, fast-frozen section

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Introduction

Digital pathology has experienced a tremendous development in the last decade. Based on this evolution many pathology laboratories have or are in the process of exchanging traditional microscopes with whole slide imaging (WSI) scanners and digital microscopy. The labs are on the way to total digitization.[1-5] One area of pathology, intraoperative consultation (fast-frozen section), has the potential of becoming digitalized but this has not yet been fully investigated or well documented in the literature.[6-9] It has previously been shown that slides from frozen sections, can be scanned successfully and a correct diagnosis can be made from the scans.[6-9] However, only very few studies are based on glass slides where the mounting glue still is wet (wet slides) or within a time frame of minutes, which is the most realistic condition for working with fast-frozen sections in a clinical pathology setting. Therefore, there is little to no documentation to the best of our knowledge, on how WSI scanners handle the emergency function fast-frozen section, which can pose a problem as many pathology laboratories are going digital.

The fast-frozen section procedure is often required in many major hospitals which perform an extensive number of cancer-related surgeries. The surgeons need the diagnostic answer, presence of malignant cells or not, as fast as possible because the patient is still under anesthesia. Thus, the surgery can continue as soon as the diagnostic answer from the pathology department is available. Some of the reasons why the frozen section has not yet become digitized and implemented in the clinical pathology workflow are due to the WSI scanners available on the market. The scanners have not demonstrated the ability to perform successful scans on wet slides or perform the scan fast enough with satisfying results. Time and digital quality are of the essence in a clinical pathology department when working with fast-frozen sections, and a slide scan is an extra step in the workflow. Therefore, the operation of the WSI scanner must be easy and manageable, which also has been a challenge with the WSI scanners. The scanners have often been too complicated to use when a quick turnaround time is required for results.

The issue of digital quality, operation and scanning time in terms of being able to scan wet slides, is probably the most interesting for laboratories that perform many fast-frozen sections. This validation study was completed in the department of Pathology at Rigshospitalet, Copenhagen in 2020. The department performs about 75 to 80 urgent cryoslides per day and thereby this function constitutes a large part of the laboratory workload. In order to meet the urgent need for obtaining results, the maximum whole slide operation and scan time of a single wet slide should take no more than 180 seconds (sec.) per slide. This limit was set by experienced clinical staff at the pathology department at Rigshospitalet.

Therefore, it's important how these issues can be addressed and solved so that fast-frozen section can become digitized without compromising the diagnostic quality. Not only to achieve the obvious reasons by becoming digital, such as telemedicine and less transportation time but for the sole reason of becoming digital so future tools like artificial intelligence (AI) and machine learning (ML) can be used. However, pathology departments have been prevented from implementing digitization in this frozen section area, as there are very few validation studies on how to digitize a fast-frozen section laboratory. On the marked a new WSI scanner from Olympus; Slideview VS200 Research Slide Scanner (VS200 STL) holds potential and may fulfil the department's requirements.[10]

The aim of the current study was threefold. First, is the WSI scanner SLIDEVIEW VS200 Research Slide Scanner capable of scanning wet slides. Second, is the total operation and scan time within the limit of 180 sec. per slide, and finally, is the scanning quality high enough to contribute to a correct and credible diagnosis?

Materials and Methods

To ensure the most standardized and valid method on how to validate WSI systems for diagnostic purposes in pathology the guidelines *Validating Slide Imaging for Diagnostic Purposes in Pathology: Guideline from the College of American Pathologists (CAP), Pathology and Laboratory Quality Center* from 2013 were utilized.[11] The study still fulfils the guideline recommendations from 2022.[12] It was also sought in the project that the virtual rendering of the digital slides mimicked what one might expect in the traditional microscope. It was also emphasized that assessments and decisions based on subjective judgement were made based on experience and professional knowledge.

In the first part of the validation of the slide scanner, it was essential to explore the best settings for the scanning profile, where magnification/resolution, autodetection and number of focus points were included. It was important that the process was fast and that the digital slides had the same morphological quality one could expect in the traditional microscope. A x20 magnification was used, which is the usual magnification used in the pathology department at Rigshospitalet. This magnification is also supported by Borowsky et al. as suitable.[2] Furthermore, the pre-program scan settings on autodetecting tissue on the VS200 STL were utilized. The selection included the amount of focus points needed for the best scan profile after a test of the four predefined settings by the company: *LOW, NORMAL, HIGH* and *EXTRA HIGH.*

The four options were tested on 26 randomly selected cryoslides, where 20 of them were wet slides. The slides were obtained from the workflow of the fast-frozen section laboratory at Rigshospitalet, where the freezing method used was Prestochill.[13] A quadviewer (Olyvia 3.1) was used for the histological assessment of the four possible focus point settings. The rating was blinded to avoid bias and performed by experienced laboratory personnel.

In the second part of the validation of the slide scanner, the slide scan time composed of the scanner operating and scanning time was evaluated, to assess the 180 sec. time limit per slide.

Measurements of the operating time were performed 20 times with two cryoslides at the same time. The operating time was defined as the time from mounting the glass cover to beginning the scanning of the slides. This part of the validation study aimed to investigate whether waiting time could occur and thereby create a potential possible bottleneck for the pathology department.

In the third part of the validation of the slide scanner, how the scanner handled different types of tissue was evaluated and if there would be any variation in the diagnoses made using a conventional microscope compared with diagnosis made using a digital scanning. The way in which it was investigated whether there was a discrepancy between the diagnoses (malignant or not), was performed independently by two senior consultant pathologists experienced in a clinical pathology laboratory setting performing diagnostics on fast frozen tissue for more than 20 years within gastrointestinal pathology, head and neck pathology and breast pathology, respectively. The samples were first assessed by using conventional microscope of the fast frozen slide, and after a washout period of more than 14 days. The pathologists reviewed the same slides again based on the digital scans. In fast frozen sectioning assessment, the pathologists distinguish tumors from other lesions and distinguish malignant from benign tumors as these can directly affect the patient's treatment decision. The diagnosis is primarily based on whether malignant cells are present or not.

Fresh tissue samples from breast, gastroenterological and otolaryngology surgery were included in the study. The tissue types had different textures, shapes, and sizes, but were not larger than 20x60mm. Samples originated from 60 different patients with a roughly equal distribution between men and

women. The age range was from 39 to 91 years. The data collection and storage were performed in compliance with the Danish Data Protection Agency.

By using different types of tissue samples, it was possible to explore how different pathology subspecialties will be affected by replacing the traditional microscope with WSI and whether there are differences between them. This also allowed the laboratory to get an indication of how tissue samples from e.g., otolaryngology, which receives a large proportion of freezing samples, could be affected by a possible WSI implementation. The type of tissue, number of cases and slides are listed in Table 1.

Table 1. Table of the tissue types within the three pathology subspecialties, as well as number of cases and number of slides.

Tissue type	Number of cases	Number of slides
Breast tissue		
- Sentinel lymph node	20	40
Gastrointestinal tissue		
- Esophagus	3	6
- Pancreas	5	12
- Peritoneum	3	6
- Ductus hepaticus	4	8
Liver	2	4
- Other types of tissues from gastroenterology	3	7
Otolaryngology		
- Tonsil	5	12
- Tongue	2	4
- Plica aryepiglottica	2	4
- Other types of tissues from Otolaryngology	11	23
Total	60	126

The total amount of 126 cryoslides fulfil the College of American Pathology (CAP) guideline recommendations.[11,12] The diagnostic results are obtained by comparing the original fast-frozen diagnosis (conventional microscopy) to the diagnoses based on the WSI. If discrepancies arose between the two diagnoses, this would either be confirmed or not by means of the verification slide, which was considered as the true value of the diagnosis. A formalin-fixed paraffin-embedded (FFPE) slide, made from the tissue, post the fast-frozen workflow, was used as the verification slide (golden standard) in this study.

There was a distinction between major and minor discrepancies. Major discrepancies were defined as where the discrepancy in the diagnosis would be significant for the sample's further examination in the pathology department or whether the patient should undergo alternative analyzes, examinations or treatments. Minor discrepancies would not cause a change for the sample's further examination in the pathology department or changed patient treatments if the alternative diagnosis had been made e.g., identification of inflammation. This way of assessing minor or possibly major inconsistencies has been used before in other studies.[2,6]

Results

The results of the assessment of the four possible scan profiles *LOW* and *EXTRA HIGH* were opted out either because of demonstrated low morphological quality or unacceptable scanning time extension in relation to morphological quality achieved. An illustration of how the quadviewer presented the four scan profiles (*LOW, NORMAL, HIGHT* and *EXTRA HIGH)* is shown in Figure 1. Based on the results, the best scan profile was chosen to be the focus point setting *HIGH*. This profile had an average scan time of 95.5 sec. per slide (the shortest scan time was 46 sec. per slide, and the longest scan time was 189 sec. per slide), based on 20 wet slides and therefore there was a presumption that the time limit of 180 sec. per slide could be met. Furthermore, the *HIGH* profile showed no area where the slides were out of focus or blurred compared to using both *LOW, NORMAL* and *EXTRA HIGH* profile.

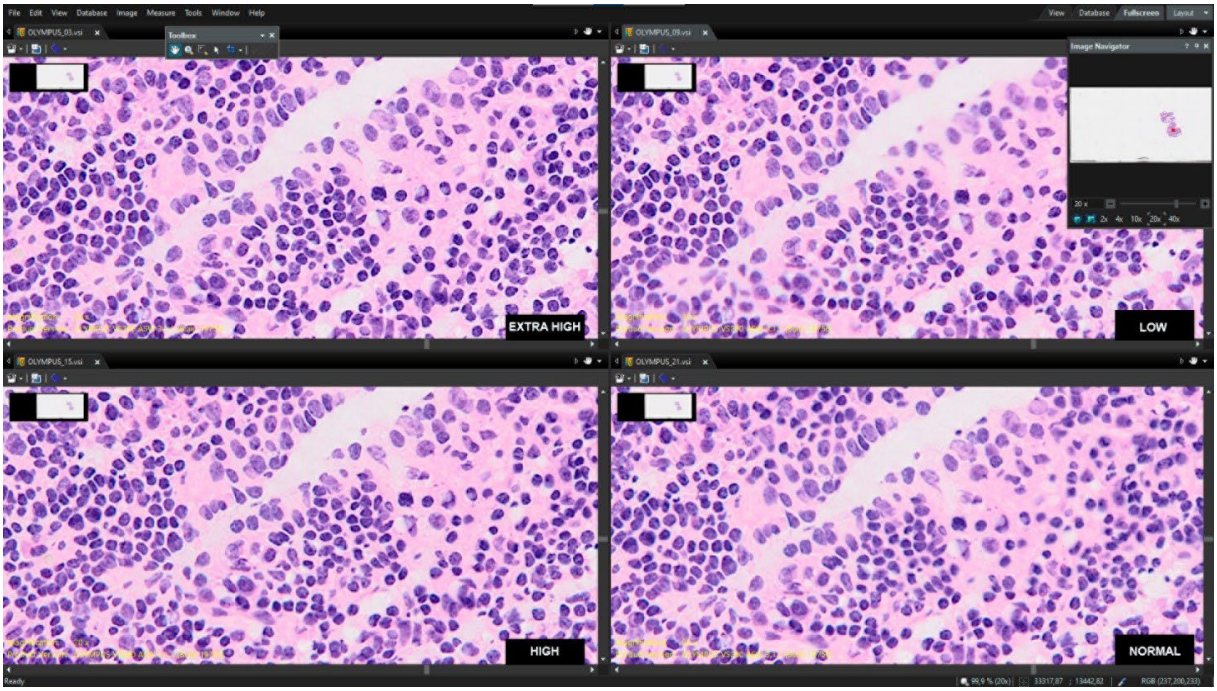

Figure 1: Random placement of the four scan options (LOW, NORMAL, HIGH and EXTRA HIGH) in the quadviewer from Olyvia 3.1 to illustrate how the assessment was performed.

Table 2. The average scanning time distributed within the three subspecialties. The calculated average total slide scan time/slide is also included.

Tissue type	Number of slides	Average scanning time/slide	Average operation time/slide	Average total slide scanning time/slide
Breast tissue	40	170.6 sec	26.4 sec	197.0 sec
Gastroenterology	43	146.5 sec		172.9 sec
Otolaryngology	43	100.0 sec		126.4 sec
All three tissue types	126	137.8 sec		164.2 sec

This assumption was confirmed as the average scanning time for all the 126 cryoslides was 137.8 sec. per slide (Table 2). The result of the additional task in the validation study, about how long it took to operate VS200 STL had an average time of 26.4 sec. per slide.

Based on golden standard assessment of the cases, the total count of 19 malignant cases and 41 benign cases with an overall total of 60 cases. Diagnosis with either minor or major deviations was compared to the diagnosis made from the FFPE tissue slide using conventional microscopy (the verification slide). The overall result showed no major discrepancies between the two diagnoses made by using the conventional microscope compared by using the digital slide in 50 out of 60 cases, this corresponds to 83% of the cases. An overview of the results can be seen in Table 3.

In total 7 minor diagnostic deviations were identified. Of minor diagnostic deviations, 1 out of 7 made by the conventional microscope was not in alignment with the verification slide. The 6 minor diagnostic deviations were due to additional findings of ulcerations and acute/chronic inflammation in the digital slide.

In total 3 major deviations were identified. Of major diagnostic deviations, 2 out of 3 made by the conventional microscope were not in alignment with the verification slide due to the finding of 1) ulceration and 2) adenocarcinoma (digital microscopy) versus the finding of 1) radiation damages and 2) no sign of squamous

Table 3. Description of the five types of discrepancies that could be judged between the traditional microscope and the digital slides. The number of cases is stated as well as in percentage.

Description of compliance between the fast frozen diagnosis using a traditional microscope and the digital slide	Cases	Percentage
No deviation between the two diagnostic methods	50	83%
Minor deviation and in accordance with the verification slide	1	2%
Minor deviation which is not seen in the verification slide	6	10%
Large deviation and in accordance with the verification slide	2	3%
Large deviation which is not seen in the verification slide	1	2%

squamous cell carcinoma (digital microscopy) versus the finding of a suspect lymphoma (conventional microscopy).

Discussion

The overall conclusion of the results is that there will be no change in the treatment of the patient in 98% (59:60) of the cases, where the diagnosis is made on a digital slide instead and compared with the verification slide.

The expectation for the validation of slide scanner VS200 STL was that with only small variations it would be possible to make the same diagnosis on the digital slides that had previously been made by using the traditional microscope. In a study by Bauer et. al a validation of a WSI scanner for use in the fast-frozen section had set a limit of 4% for large discrepancies in the variations between the diagnosis.[3] However, a final limit on how much discrepancy can be accepted between the two diagnostic methods is difficult to decide. The difference may be due to intra- and interpersonal variations and variations may also occur in different tissue types and specialties. As a result of this study, the 4% limit was also accepted as it is supported by the study from Borowsky et.al.[2]

In addition, the 126 cryoslides were dry when scanned. This prevented disrupting the routine procedure in the fast-frozen section laboratory because patients were in real time surgeries and there were surgeons waiting for diagnostic responses (presence of malignancy or not). Therefore, the process could not be delayed in any way, by scanning the slides before the diagnosis has taken place. One potential method to scan the 126 cryoslides without delaying the diagnostic work could have been to cut an extra slide on the specific tissue sample. This could have been problematic when the two diagnostic answers had to be compared as the extra slide would not have been 100% identical to the first diagnostic slide. It was also impossible to scan the 126 cryoslides after the diagnosis was released as the mounting glue would have been completely dry at this point. The second reason for not using wet slides, was the results obtained during the preliminary testing were completed to find the most optimal settings for the Scanning profile. During this testing both wet and dry slides were used. In these tests, the wet slides were presented as superior in quality to the dry slides. The reason for this was that no glue artefacts were present in the slides until the slides dried. As for the expectation of messy work with having wet glue in a scanner, this did not seem to be the case with this scanner. With the slides being locked in position the provided racks, and the racks always staying in a horizontal position, the scanner was never actually in contact with any of the slides and therefore the wet glue.

The study hypothesis was that it would be possible to scan slides and then diagnose subsequently, but it was unknown at what speed or what image quality would be possible. It is a matter of finding the point where the image quality is adequate and maintain the time frame of 180 sec. per slide. The possibility of this improvement could be achieved by examining the VS200 STL autodetection mechanism in more detail.

The diagnostic results obtained in the study on the digital slides had great compliance with the diagnoses obtained by the traditional method and even though the approach was

very close to how the clinical work usually proceeds, the limitation of the study removed the psychological pressure from the pathologists during the process. In this study, the pathologists could review and determine a diagnosis from the digital slides without time pressure and with the knowledge that the patient was already in treatment, so the results were without consequences. According to the CAP guidelines, the optimal validation, conventional versus digital assessment should have been performed in random order.[11,12] However, this was not possible as surgeons were waiting for a diagnostic answer.

The way the verification slide was used in the study was not optimal. It was only used when there was a discrepancy between the two diagnoses from the fast-frozen section made using digital slide and traditional microscopy. It could be interesting also to examine the verification slide when the two diagnoses were consistent. Since this has not been investigated, there is no information as to whether there were any undetected irregularities or if there would have been a different result. Another possible shortcoming was that gynecology was not included in the validation study, although Borowsky et.al. included it, where it was referred to that it can be complicated and with major deviations to place diagnoses on gynecology. However, the tissue types included in this study were a good representation of what is being treated and examined in a pathology department. Although the tissue samples from breast surgery were too monotonous, it was the fact that only the sentinel node was included and no other type of breast tissue. A reason for this was that the cases were not specifically selected for the study. Instead, a date was chosen, further back than the necessary washout period, and from there the last 20 cases that were handled by the participating pathologists were selected within the chosen subspecialties. Tissues from neurology were unfortunately not included. It would have been interesting to include to see if it would be as easy to diagnose as the other tissue types included in the validation study.

Conclusions

The results of this study, within the set limits, demonstrates that wet slides from fast-frozen sections could be handled (scanner's operation time) and scanned within a time limit of 180 sec. It was also demonstrated that reliable and credible diagnoses (95 % sensitivity) could be achieved. It was also demonstrated that reliable and credible diagnoses based on whether malignant cells were present or not could be achieved as well on the digital slides as by using the traditional microscope. Overall, the study showed that implementation of a whole slide imaging scanner (e.g., Slideview VS200 research Slide Scanner, Olympus) has the potential of being implemented in a clinical pathology department for use in the fast-frozen intraoperative diagnostic section without affecting the laboratory workflow and maintaining the diagnostic accuracy.

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Narrative review

A Review of the Current Medical Science Career Framework in Australia and Recommendations for the Future

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Australian pathology laboratories are governed by a pathologist registered by the Royal College of Pathologists, Australasia (RCPA) and accredited by the National Association of Testing Authorities (NATA). It would seem that neither body support professional registration through the Australian Health Practitioner Regulation Agency (AHPRA) but have funded an independent program of certification for medical scientists.

This program aims to provide solutions to many issues within the profession, but it is entirely voluntary in nature. This means that it will not have the ability to sanction misadventure and with no universal mechanism to recognize the professional status of laboratory staff. This review intends to look at the current framework and identify deficiencies while making suggestions to address the concerns.

A three-pronged approach would seem most beneficial with the first being a revision of the National Accreditation Advisory Council occupational definitions. Secondly, the Australian Institute of Medical Scientists (AIMS) should provide relevant education for supervisory staff and discipline- specific fellowship pathways for laboratory management and clinical scientists and finally, the certification program should be scrapped as it currently has only 314 certified scientists, less than 1% of the approximated workforce.

Replaced with an employer financed, blockchain based credentialing framework which would provide accurate workplace data, a secure opensource framework and legitimate oversight of the competency and education of practitioners. There is a chronic under-recognition and lack of ongoing development of medical laboratory scientists within the Australian healthcare community which considering the importance placed on the accuracy of results during the pandemic needs to be addressed.

Key words: Registration, blockchain, credentialing

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Introduction

The International Organization for Standardization (ISO) was founded in 1947 to promote conformity in worldwide standards.[1] It is a union of two earlier organizations: the International Federation of the National Standardizing Associations (ISA) and the United Nations Standards Coordinating Committee (UNSCC).

The ISA was established in New York in 1926 but was based in Switzerland and its standards were adopted by many European countries that used the metric system. However, the ISA ceased operations at the beginning of hostilities in Europe. In contrast, the UNSCC was adopted by those countries that used the imperial system such as America, Canada, and the United Kingdom. The UNSCC was established as a branch of the International Electrotechnical Commission (IEC) since 1944 to aid the reconstruction efforts following the Second World War.[1]

In 1987, the ISO published its first quality management systems standard, ISO 9001, which described the fundamental principles of quality management. This standard has become one of the most popular management tools used today. In 1999, the ISO published the *General requirements for the competence of testing and calibration laboratories* as document ISO/IEC 17025:1999, which is used to assess the competence of most laboratories. Finally in 2003, the first edition of ISO 15189 *Medical Laboratories – Requirements for quality and competence* was released which provides specific advice for pathology testing.[2] These documents have formed the basis for standardization of clinical laboratories worldwide; and have been adopted by all 162 of the ISO member nations including Australia, wherefrom the National Australian Testing Authority (NATA) was formed in 1947.

Initially established to ensure the standard of munitions produced in Australia, NATA eventually expanded to provide services for a third of all chemical and mechanical laboratories in Australia by the end of the 1970s and began accrediting medical facilities in 1983. In 1988, NATA signed a Memorandum of Understanding with the Commonwealth Government of Australia to provide accreditation services across Australia, which would allow accredited facilities to claim Medicare benefits. Today, any Pathology laboratory in Australia must be inspected biennially to ensure that they hold to the standard required in order to practice.[3]

The National Pathology Accreditation Advisory Council (NPAAC) is a government appointed body charged with ensuring that laboratory staff are "*appropriately qualified, competent and have a relevant scope of practice and accountable for the testing performed.*"[4] This is done through the *"Requirements for Medical Pathology Services"* and "*Requirements for Supervision in the Clinical Governance of Medical Pathology Laboratories."*[5,6] Within the scope of these documents, their importance is described as providing standards for good medical pathology practice. Describing the categories of pathology laboratories, roles of key staff, including, Pathologists, Clinical Scientists, Scientists and Technical Officers, and ensuring that all tests are supervised by competent persons who are working within their Scope of Practice.

The Australian Institute of Medical Scientists (AIMS) is considered the largest professional body representing medical scientists in Australia. It provides various services that adhere to the NATA and NPAAC requirements for Australian laboratories. Along with the Australian Association of Clinical Biochemists, it commissioned the National Certification for Medical Laboratory Scientists and Technicians using funding from the Australian Government's Quality use of Pathology program (QUPP).

All of these important bodies have significant roles in defining the pathology service in Australia. With the imminent implementation of the certification scheme, it would seem prudent to review these documents to assess their impact on the disciplines as a whole. Where possible, it

would be valuable to recommend improvements to ensure that the new framework is relevant and meaningful to the Pathology Medical service in the future.

Australian Pathology Occupational Definitions

Internationally, the medical science profession is controlled by a governmental registration body and a professional society. The former has legal authority to apply sanctions to practitioners, when and if required, while the latter acts as a credentialing body and ensures the highest level of professional practice through continuing professional development (CPD). In Australia, medical science is not recognized as a profession and laboratories are controlled by a registered Pathologist and industry accreditation.

NPAAC has provided the standards of practice for the pathology services within Australia since 1999 with the first edition of the "*Requirements for Supervision in the Clinical Governance of Medical Pathology Laboratories*" revised and reprinted in 2018.[6] This document includes definitions of the roles and functions of a pathology laboratory, and guidelines to ensure legislative compliance. All laboratories must comply in order to receive compensation through the Medicare fund.

To this end NPAAC has defined four grades of pathology laboratory workers in Australia. These grades are based on education and experience and encompass the role description Technical Officer through to Clinical Scientist.

1) A Technical Officer is someone who has completed a 2-year certificate or diploma level qualification in the field of pathology. These qualifications match those required of a Medical Laboratory Technician which is a classification provided under the Australian and New Zealand Standard Classification of Occupations (ANZSCO) 311213.[7] The question arises as to why this level of practitioner carries two different titles; one assigned by the NPAAC, and the other by ANZSCO.

2) A Scientist requires:

a) a degree at Australian Qualifications Framework (AQF) level 7 (Bachelor) with subjects relevant to the field of pathology, as determined by the person responsible for the scientific management of the laboratory and/or person responsible for the clinical governance of the laboratory, awarded from a university in Australia; or

b) a degree at Australian Qualifications Framework level 7 (Bachelor) with subjects relevant to the field of pathology awarded by an overseas tertiary institution if the qualification is assessed as equivalent to a degree accredited by the Australian Institute of Medical Scientists (AIMS), according to their authority approved by Australian Education International via the National Office of Overseas Skills Recognition (AEI−NOOSR); or

c) An associate qualification conferred by the Australian Institute of Medical Technologists before 1 December 1973.

In practice, however, there are only two pathways to employment as a medical scientist in clinical laboratories in Australia, as the third is historic. The first pathway suffers from the problems of relevance and responsibility. In the context of relevance, what subjects are considered relevant to pathology?

Any life science graduate could be considered to have the requisite background to fulfil this criterion. But they will not have any understanding of test validations, quality control metrics or proficiency testing requirements. The net is therefore cast very wide and lacks any appreciation of vocational training. The issue of responsibility lies with the person determining the relevance of degrees, which appears to lie with either the scientific or clinical lead of an individual laboratory. Critically, the second pathway includes an objective body (i.e., AIMS) assessing the relevance of degrees, with universal responsibility for that assessment; however, this independent assessment only has authority over foreign qualifications.

The requirements to work as a medical scientist in Australia have evolved since the

2007 edition of the NPAAC "Requirements For The Supervision Of Pathology Laboratories" when a medical scientist needed an Australian qualification "*that provides for direct entry or following examination to a professional class of membership of the AACB, AIMS, Australian Society of Microbiology (ASM), Australian Society of Cytology (ASC),or the Human Genetics Society of Australasia (HGSA).*"[8] Therefore, in 2007 the NPAAC acknowledged the professional societies as appropriate credentialing bodies with the understanding that the education would allow membership. Thus, by allowing domestic applicants to bypass the only credible assessment of their education, the NPAAC has effectively negated the need for vocational degrees in Australia and any universal oversight of the relevance of Australian degrees.

Internationally, professional societies such as AIMS are used to assess the relevance of higher education degrees for working in pathology laboratories. For example in the UK a Biomedical Scientist must hold a BSc (Hons) degree in biomedical science accredited by the Institute of Biomedical Science (IBMS).[9] In New Zealand a graduate must hold a Bachelor of Medical Laboratory Science or a Graduate diploma in Medical Laboratory Science to be registered and to work in a pathology laboratory.[10] In Canada no one is considered for registration without graduating from a degree program approved by the Canadian Society for Medical Laboratory Science (CSMLS) programs and then passing a certification exam.[11]

The "*Requirements for the Supervision in the Clinical Governance of Medical Pathology Laboratories*" [6] issued by NPAAC defines a "credentialing body" as a; "*formally constituted committee of practitioners and managers who collectively analyze and verify the information submitted by an applicant.*" This definition allows the management of each individual laboratory in Australia to decide whether the applicant's qualifications are adequate for employment. Suggesting that, in the case of medical scientists, anyone who is working in the field is capable of providing primary source verification of every domestic qualification available within Australia and its *"subjects relevant to the field of pathology"*.

One of the main directives of NPAAC that offers an extremely important layer of security for the public, is to ensure a consistent and transparent application of occupational definitions within the pathology environment. This can only be achieved if a single body is responsible for it and as AIMS is already providing the service for international applications. It is the author's view that professional society's involvement is required as a gateway for all graduates.

3) The next recognized level of appointment available to a medical scientist is described as the "Onsite Manager of a Category B or branch laboratory." This role is defined as a scientist with at least two years relevant experience in a larger laboratory. A subset of this role is the Quality Manager which is described *"as a member of staff appointed with delegated authority to ensure that processes needed for the Quality System (QS) are established, implemented and maintained."* Therefore, in a branch laboratory, only the onsite manager may be responsible for this role which adds a large level of complexity to an already demanding role.

In order to effectively manage a profitable and compliant laboratory of any size it would be prudent to have some managerial training, financial education and functional appreciation of human resources concepts. In the UK a Laboratory Manager (Training, Quality or Operational) has a very specific set of well-defined responsibilities to ensure these important, and largely non-clinical obligations support the laboratory. With none of these roles defined in the Australian pathology workforce it would seem beneficial to establish definitions for the future of the service and its workers.

There is an opportunity for the Australian professional societies to demonstrate servant leadership, using the fellowship program as a

vehicle for the role of Laboratory Manager. Fellowship is a well-respected professional qualification around the world and a valuable addition to any resume. It should be used to provide professional recognition of the role of manager in a pathology laboratory.

The requirements for Laboratory Managers should include a Masters' level qualification coupled with a professional fellowship. Currently, fellowship is only available in a clinical discipline and in some instances, it is too specific for many of the regions in Australia. For example, the AIMS Anatomical Pathology Fellowship requires examination in Electron Microscopy. A valuable alternative for many experienced scientists would be the development of a general management fellowship with management, financial and human resources components. This suggested structure is shown in Figure 1.

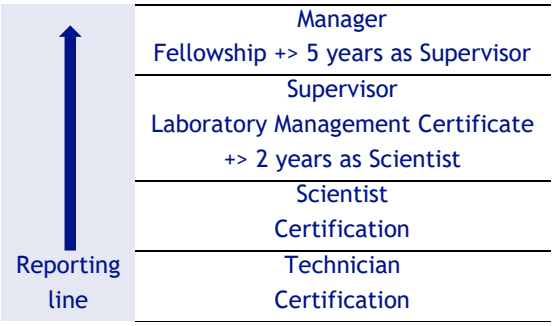

Figure 1: Laboratory Managerial structure

The final level of promotion available to medical scientists is that of a Clinical Scientist which is defined as a scientist who has five years laboratory experience. They must also be in possession of a Doctor of Philosophy or a Fellowship from AIMS, AACB, ASM, HGSA, ASC or Royal College of Pathologists of Australasia (RCPA).

Clinical Scientists hold important positions in a pathology laboratory. Their advanced education is valuable in the clinical setting and provide clinical assistance to Pathologists. Their career pathway is prescribed by the Royal College of Pathologists (UK) and provides a valuable alternative for interested and experienced medical scientists. Medical scientists in Australia can also train under a Pathologist in order to gain a Fellowship of the Faculty of Science of the RCPA.

However, the current definition does not consider PhD graduates of non-clinical subjects such as education or management. It would seem imprudent to label these members as clinical scientists in view of their specialist subject.

Figure 2: Clinical Scientist pathway

Scope of Practice

Any occupation's "Scope of Practice" is a foundational document commonly used to "*describe the procedures, actions and processes that a healthcare practitioner is permitted to undertake in keeping with the terms of their professional license.*"[12] NPAAC has provided a definition of the scope of practice for any pathology worker in the "Requirements for Supervision in the Clinical Governance of Medical Pathology Laboratories" which states.

"*The discipline and/or areas of testing in which a person has been trained and successfully examined or assessed as competent by the relevant College, professional society, or credentialing body and in which they have met current Continuing Professional Development and recency of practice requirements.*" [6]

Ironically this definition is more applicable to those countries that require registration of their laboratory workers but seems at odds with the current environment in Australia. The second part of the phrase which mentions competency assessment and CPD requirements, stipulating that this can only be done by "*the relevant College, professional society, or credentialing body*" is difficult for medical

scientists to comply with. Given that there is no relevant college governing medical scientists in Australia, and while a number of professional societies do exist, membership is not mandatory and it has been transferred to NATA to determine competency as part of the laboratory accreditation process in Australia.

However, as NATA does not provide practicing licenses to individuals and is only required to ensure that staff are competent during inspection. The individual laboratories are left to ensure CPD, and that training and competency requirements are met. As the laboratory management can be defines as a credentialing body, can impose any scope of practice that they deem appropriate on their staff. This leads to an inconsistency across the entire pathology service, which can only be addressed through standardization.

The current guidance provided by the Pathology Associations Council in the "*Competency-based Standards for Medical Scientists,*" released in 2009, in consultation with each of the professional societies seems to be the recognized scope of practice.[13] However, the AACB released a *"Scope of Practice of the Scientific Workforce of the Pathology Laboratory"* in 2011.[14] While these documents seem to complement each other there is confusion over which is the official version and who it would apply to within the workforce.

Due to the fact that there is no relevant college or over-arching professional society these documents need to be reviewed with respect to the recently released NPAAC document as the definitions are now out of date. However, as noted, it is of little consequence as the new definition means that it need not be applied unless the laboratory chooses to and this will not be resolved until the NPAAC revises its own definitions.

The role of the certification scheme

AIMS and AACB have proposed a certification scheme for Medical Laboratory Scientists to be implemented in 2020. This project is backed by funding from the Quality Use of Pathology Program (QUPP) which is a national program for promoting initiatives within the pathology services. Some may question why the Ministry of Health recognized that there is enough cause to fund a project of this nature and not use the funds to inject medical scientists into the existing AHPRA framework.

In principle, this project has many redeeming qualities and addresses many of the deficiencies currently facing the profession in Australia. However, it also has identifiable flaws in its own execution; most notably its voluntary nature and inability to sanction practitioners for misadventure. Both of these failings would be addressed by the recognition of medical science as a profession within the AHPRA framework.

Over the course of the last two years there have been a number of stakeholder meetings and the latest update, released in December 2018 contains multiple proposals.[15] Beginning with a lax stance on participation, which highlights important benefits for the public and employees, but fails to press the issue with employers. This approach stands in stark contrast to other health-related professions where participation in a certification scheme is mandated by the Government.

The proposed position for the initial level of certification lowers the required education level for scientists from an AQF Level 8 (Bachelor's degree with Honors) to AQF Level 7 (Bachelors). There is no appreciable reason for this as internationally, an honor's degree is required by the IBMS for employment in the UK, or in New Zealand by the NZIMLS.[9,16] This would mean that domestic applicants would not be required to achieve the same qualifications as their colleagues overseas.

When it comes to entry requirements there is an error in the initial definition of a scientist in Australia. The certification document states, *"The current NPAAC definition of "scientist" has for many years included a requirement of 2 years' professional practice in an accredited laboratory before that role definition can be applied."* [15] In fact, the NPAAC definition of scientist does not prescribe a period of time, this is only

mentioned in the definition of an Onsite Manager of a Category B laboratory. [6] It is beneficial to require a level of professional experience and this should be in recognition of the qualification gained e.g., BSc (Hons) = automatic certification vs BSc + 2 years' post-graduate experience.

On the topic of competency-based certification, much of the discussion seems to be around ownership of the medical scientist's scope of practice. After review of the current document, it is the view of the author that the document is adequate but requires up-dating to reflect modern laboratory practices developed in the last decade. However, it does need to be adopted nationally, because the current NPAAC document allows for too many interpretations of an important function.

The proposal goes on to suggest a framework for CPD and recertification, which is a very important part of the scheme. CPD is critical for providing a quality service, which is a particular failing of the current system as evidenced by the SA Pathology incident of 2016. [17] AIMS provide a robust CPD framework with its Australasian Professional Acknowledgement of Continuing Education (APACE) which would be sufficient if it were to become mandatory for all laboratory workers.

It is the voluntary nature of the proposal that lets it down again with the ability to punish misadventure. This important protection mechanism cannot be applied indiscriminately to different members of the laboratory community based on a non-mandatory requirement. This would be remarkably unfair and the only realistic method by which to achieve this important public security is for mandatory governmental regulation by admission to AHPRA.

In the proposal document, the financial projections are based on 9-year-old survey for the Department of Health and Ageing. [18] From this publication they have estimated a workforce consisting of (senior) scientists and technicians of approx. 14,000. However, the estimated phlebotomy workforce in the survey is 4,083 not the 1,800 that is quoted. The cost of around $300 for certification is reasonable but given the fact that membership is not mandatory nor recognized by employers then certification will pose no value for laboratory workers.

When it comes to ownership and governance, is there any need to create a new body to manage the scheme when the mechanisms already available from the existing professional societies. If we consider that AIMS already has the framework in place to move from a member-benefit professional association to a certifying body if the other societies support its oversight of the program.

A New Fellowship Model

The current fellowship model used by the Australian professional societies for medical scientists is very different for each group.

- The AACB require two written and an oral exam
- The ASM has a 3-part process with an exam, 3 written essays and 5-10 high impact journal publications.
- The ASC require a written, and oral exam, and a 5000-word literature review
- AIMS require 4 written exams, a viva and a doctoral thesis.
- The HGSA send their applicants to the RCPA Faculty of Science program.

The proposed certification scheme is striving to provide a standardized structure for the profession, then a standardized fellowship would also be beneficial. It would seem an unnecessary expense for each society to offer a unique pathway when the entry requirements could be standardized across the profession. The common feature of all of the current models is an oral exam and this should continue to be conducted by experienced members of each distinct society.

The IBMS offer many educational opportunities for scientists, but I would like to highlight the certificates in extended practice that they offer.[9] A recent graduate in any laboratory who has completed an AIMS accredited degree will have enough clinical training to work and will necessarily develop

their skills on the job. If they are considered by NPAAC to be eligible for supervisory positions after two years of employment the professional society should provide easily accessible, basic managerial education specific to their role.

An online offering would be the easiest to develop and maintain and should become a mandatory requirement for prospective Supervisors together with topics in employee relations and financial responsibility. A second offering for Quality managers/officers would be beneficial providing training in health and safety, document management and risk management. With a third for Training managers/officers developed for training and education concepts and competencies. The completion of one of these courses would be the initial step toward a professional fellowship.

A Doctor of Philosophy (PhD) is the highest level of qualification offered by a university for very good reason, it's extremely difficult to achieve. When you consider the time that it takes and the fees that may be imposed it is very hard for anyone working full-time and supporting a mortgage and a family in Australia today. An M-level or Masters' degree is a much more achievable goal and the management structure of a laboratory needs to recognize this, with the PhD being the province of the clinical scientist.

There are many taught Masters' courses, such as a Master of Science (MSc) or Master of Business Administration (MBA), currently available from a number of respected Australian universities, all which would be appropriate for a laboratory manager to have. These could be either science or management based as either would develop skills and education required to be proficient and successful in the role. There is a third avenue, through the Research Training Program available from the Department of Education and Training.[19]

This is a program which essentially removes any fee burden and is provided for research only degrees. This includes the Master of Philosophy (MPhil) program, which requires a shorter dissertation than a PhD, or by publication, which means four journal articles, which could promote the society's journal. This degree can be done remotely and therefore is an accessible route for any regional employee who doesn't live close to a major university and can be on any topic which is relevant to the individual's practice.

Many of the current offerings do not offer the same flexibility, but the main benefit to the societies is that all of the expenses are borne by the universities. The online management programs only need minimal oversight and maintenance which is centralized to ensure relevancy and reduced costs. They only need to conduct the oral exam for prospective fellows who have completed the pre-requisites.

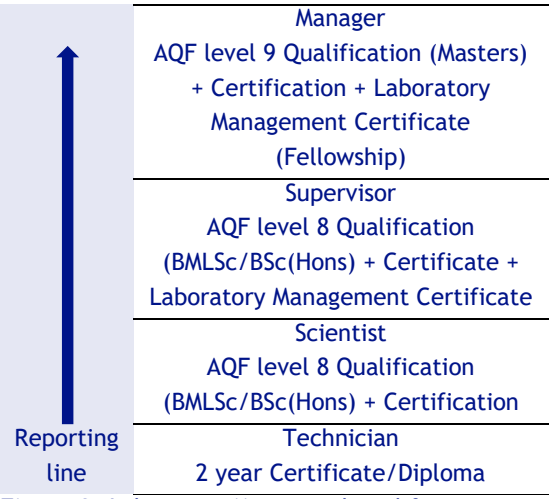

Figure 3: Laboratory Managerial qualifications

Conclusions

In order to appreciate the current career pathway for medical scientists in Australia it is necessary to review the foundational documents governing the occupation. Two documents have been published by the National Pathology Accreditation Advisory Council (NPAAC), whose members are appointed by the Minister of Health to advise on best practice accreditation of the Pathology service in Australia. The two critical documents *"Requirements for Medical Pathology Services"* and *"Requirements for Supervision in the Clinical Governance of Medical*

Pathology Laboratories" and have been reviewed recently to improve clarity regarding the governing principles of the occupation.[5,6]

During this critical evaluation a number of discrepancies were identified that have shown an erosion of the role of the professional society's role in governing the profession. The definitions of the occupational titles have changed between the 3rd and 4th editions, with the professional bodies initially providing credentialing expertise which is now only required for overseas qualifications. The latest edition allows the appropriateness of a domestic qualification and the scope of practice for any Australian medical scientist to be determined by the individual laboratory management.

These fundamental definitions need to be reviewed by NPAAC to allow appropriate control to be returned to the professional societies. This is important for the successful implementation of the proposed certification framework next year. Which is a process that is critical to legitimizing a profession in desperate need of recognition.

In the author's opinion the best way to ensure recognition is by placing authority to govern all aspects of the regulation of medical scientists under the umbrella of the Australian Institute of Medical Scientists (AIMS), not inventing a new entity responsible for administering it. Internationally, a single body is responsible for overall governance of the profession i.e., IBMS in the UK or NZIMLS in New Zealand, in Australia the obvious choice is AIMS. The smaller groups have a place in providing discipline-specific expertise in the awarding of a fellowship.

NPAAC must empower AIMS to provide primary source verification of all degrees domestic and international alike, which allows a standardized application of their scope of practice. The granting of certification following graduation can be applied with respect to degree e.g., BSc (Hons) = automatic certification vs BSc + 2 years' post-graduation.

There is a professional responsibility for experienced scientists to tutor younger members of a society. AIMS should develop online learning tools specifically for prospective laboratory supervisors. These certificates are among a number provided by the IBMS as continuing professional education. The CPD component of professional certification is a critical property that is insufficiently enforced in the Australian medical science profession. AIMS already has a vehicle to provide this with its APACE program.[20]

The profession must also provide transparency for its members regarding promotion requirements, which is currently lacking, as the only provision for supervision of a branch laboratory involves 2 years of working in a general laboratory. The UK model for promotion within the profession provides a useful guide and large number of supportive documents. The most important change from a scientist to a supervisor is the need for some basic managerial education, whether that is operational or as a training or quality officer.

This would ideally be provided online to allow access to medical scientists across Australia. The online format would allow easy maintenance and ensure that the information is relevant to the role. It would only require the development of three distinct certifications i.e., operations, training and quality, to cover any laboratory educational needs.

Currently each of the professional societies in Australia provide different pathways to gain a fellowship which is unnecessarily confusing within the occupation. If the profession is looking to gain respect through professionalism, then this needs to be streamlined. I would suggest that a fellowship for any of the disciplines should consist of a three-part process following initial certification.

First, the scientist must pass the industry manager's certification required for supervisors and, secondly, pursue a Masters' program either MSc, MBA or MPhil as appropriate. Finally, the prospective fellow should undergo an oral examination conducted by experts in their field of medical science. This would substantially simplify the current

process, reduce costs to the societies by pushing the bulk of the work back to universities as education providers and remove a majority of the administration needs from the society's executive.

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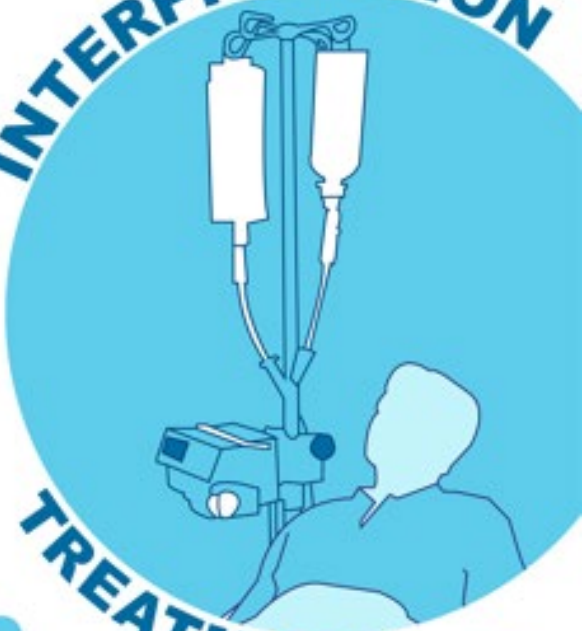
PATIENT

METHOD

RESULT

QUALITY ASSURANCE

INTERPRETATION



TREATMENT

GUARDIANS OF QUALITY AND PATIENT SAFETY: BIOMEDICAL LABORATORY SCIENTISTS

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