

December 2017 | Issue 17

**ASLM**  
AFRICAN SOCIETY FOR LABORATORY MEDICINE

# LAB CULTURE

ADVANCING THE LABORATORY PROFESSION AND NETWORKS IN AFRICA

## **Antimicrobial Resistance** The biggest threats to global health and how African laboratories can prepare

### **In this issue**

The science of sample collection

Genetic weapons against malaria

The journey to accreditation in Zimbabwe



December 2017 | Issue 17

# LAB CULTURE

ADVANCING THE LABORATORY PROFESSION AND NETWORKS IN AFRICA

## ANTIMICROBIAL RESISTANCE

**5** Africa CDC launches new plan to combat resistant microbes

By Jay Varma, Africa Centres for Disease Control and Prevention

**7** Strategies towards surveillance of antimicrobial resistance and antimicrobial stewardship

By Constance Schultsz, Department of Global Health

**9** Surveillance for antibiotic resistant pathogens

By Olumide Ajibola, Federal University Birnin Kebbi; Semeeh Omoleke, World Health Organization and Mari Mshelia, Federal University Birnin Kebbi

**11** Nature of the emerging threat of antimicrobial resistance with respect to tuberculosis

By Obert Kachuwaire, African Society for Laboratory Medicine

**14** Drug-resistant HIV on the rise in sub-Saharan Africa

By Raph Hamers, Seth Inzaule, Tobias Rinke de Wit, Amsterdam Institute for Global Health and Development

## DEPARTMENTS

**3** Letter from the CEO

**4** From the editor

## SPECIAL REPORT

**19** Proper patient preparation, specimen collection, and sample handling are critical to quality care

By MLO staff

## INDUSTRY WATCH

**23** Promising results for two genetic weapons against malaria

By MLO staff

## ACCREDITATION

**16** National Microbiology Reference Laboratory of Zimbabwe: The journey to accreditation

By Ellen Munemo, Charles Chiku, Patience Musasa and Raiva Simbi, National Microbiology Reference Laboratory, Zimbabwe

### Contribute to Lab Culture

ASLM is currently accepting article and photo submissions for upcoming issues of *Lab Culture*. We publish timely, informative, inspirational articles relevant to the unique challenges faced by laboratories in resource-limited settings. We are interested in articles on the critical aspects of laboratory medicine, best practices, success stories, leaders in the field, industry news, etc.

To submit articles, proposals, photos, etc., please contact the Editor at [newsletter@aslm.org](mailto:newsletter@aslm.org).

**Lab Culture**. Established along with ASLM in 2011 as a member newsletter, *Lab Culture* relaunched in 2017 as ASLM's magazine for laboratory medicine in Africa. Dedicated to bringing timely, informative articles relevant to the unique challenges faced by African laboratories, *Lab Culture* seeks to be Africa's premiere resource for laboratory professionals and other stakeholders working on with the continent. Published six times a year as a digital edition, *Lab Culture* includes features on critical aspects of laboratory medicine and best practices in resource-limited settings, success stories from the continent, industry news, and more.

## Dear ASLM members,

The end of a year is always a good time to take a step back, reflect on the past year and consider the next. As I think about 2017, I am proud to find an impressive list of accomplishments for ASLM as well as for laboratory medicine across Africa.

ASLM welcomed a number of new staff members in 2017. We strengthened the organisation by over tripling our staff, adding both in-country program personnel and support staff at headquarters in Addis Ababa. We also welcomed new members to the Board of Directors, Prof Amadou Sall (Senegal), Dr Donan Mmbando (Tanzania), Prof Oye-wale Tomori (Nigeria), Dr Philip Onyebujoh (World Health Organization (WHO) Regional Office for Africa), and Prof Rosanna Peeling (United Kingdom). In addition, a new Editor-in-Chief, Prof Iruka Okeke, took the helm of our scholarly journal, the *African Journal of Laboratory Medicine* (AJLM). Looking beyond ASLM, we collectively celebrated the appointment of Dr John Nkengasong as the Director of the new Africa Centres for Disease Control and Prevention (Africa CDC) and the election of Dr Tedros Adhanom Ghebreyesus as the Director General of the WHO.

2017 was also a year in which ASLM not only formed new partnerships but also renewed existing ones. This year saw the first joint technical staff meetings of personnel from ASLM and Africa CDC and the signing of a memorandum of understanding with the African Accreditation Cooperation (AFRAC). We also recommitted to relationships with International Association of National Public Health Institutes (IANPHI), WHO and others. Additionally, program staff participated in meetings across the continent and the world on topics ranging from scaling up point-of-care devices across Africa to preventing flu in Ghana

to the latest research on HIV/AIDS in Ethiopia and how to strengthen biosecurity in Tanzania.

This was also a great year for new initiatives and important milestones. The 2017 ASLM Strategic Planning Retreat was held in June. ASLM analysed our organizational objectives and explored opportunities for pan-African laboratory systems improvements. We released our first interactive mappings of SLIPTA laboratories and the locations of internationally accredited laboratories in Africa and launched the Laboratory Systems Strengthening Community of Practice. AJLM was accepted for inclusion in the US National Institutes of Health PubMed Central database, a validation of the journal's impact in the field. This year also saw a greater than three-fold increase in new African laboratories achieving international accreditation (see our listing of internationally accredited African laboratories now available on the ASLM website). Indeed, accreditation plays an extremely important role in raising medical laboratories reliability, credibility and ensuring better healthcare in Africa. In this issue, we share the accreditation story of a laboratory in Zimbabwe (p. 16).

It was a busy and productive year. Importantly, each of the new faces, partnerships, initiatives and achievements is a demonstration of the growing strength of ASLM and of laboratory medicine in Africa. We will carry that strength forward and build on it in 2018 and beyond and we hope that you will join us. In the meantime, from all of us at ASLM, we wish you a safe and joyous holiday season and healthy and prosperous new year!

Sincerely,  
Dr Ali Elbireer, CEO



Dr Ali Elbireer

**Lab Culture**  
EDITOR

**Ms Bethanie Rammer**  
brammer@aslm.org

**Lab Culture**  
ADVISORY BOARD

**Dr Ali Elbireer, MBA, PhD**  
Chief Executive Officer,  
ASLM

**Dr Pascale Ondo, MD, PhD**  
Director of Science and New  
Initiatives, ASLM

**Ms Mah-Sere Keita, MPH**  
Director of Global Health  
Security, ASLM

**Mr Nqobile Ndlovu, MPH**  
Project Director, ASLM

For submissions and  
inquiries, please contact:  
newsletter@aslm.org

**ASLM**  
Joseph Tito Street, Nega City  
Mall, Suite 800  
Kirkos Subcity, Kebele 08  
Addis Ababa, Ethiopia  
www.ASLM.org  
Newsletter@ASLM.org

*Lab Culture* is produced  
in partnership with NP  
Communications, LLC, publisher  
of *Medical Laboratory Observer*.



**Kristine Russell**  
Publisher/Exec Editor/President  
krussell@mlo-online.com

**Alan Lenhoff**  
Editor  
alenhoff@mlo-online.com

**Lisa Moynihan**  
Managing Editor  
lmoynihan@mlo-online.com

**Norma Machado**  
Design  
nmachado@npcomm.com

NP Communications  
2477 Stickney Point Rd., Suite 221B  
Sarasota, FL 34231  
www.mlo-online.com

***'If we fail to act, we are looking at an almost unthinkable scenario where antibiotics no longer work and we are cast back into the dark ages of medicine'***

**– David Cameron, former UK Prime Minister**

For a parent, it only takes a child being cured of an infection one time to truly appreciate the miracle of modern antibiotics. By that third or fourth dose of penicillin, when the sore throat or the sinus infection or the bronchitis starts losing its grip and your child is nearly back to his or her normal self, you breathe a sigh of relief.

These days, however, if you work in or around laboratories, you might also give a slight shudder as you try not to imagine what would happen if those 'dark ages' were to return. And yet, according to an increasing amount of evidence, unless we do something and soon, that seems to be where we are headed.

In its final report from 2016, the *Review on Antimicrobial Resistance* laid out some chilling projections: deaths attributable to antimicrobial resistance, around 700 000 per year today, by 2050 may be as high as 10 million per year. Nearly half of those deaths are projected to occur in Africa.

There are a number of factors contributing to the problem, which is not limited to drugs for childhood infections, but reaches into every aspect of human disease from HIV to malaria and even beyond to animal and environmental health. These include the lack of new antimicrobials in development pipelines, overuse of antimicrobials in both humans and animals, lack of rapid diagnostic tests, sanitation and hand-washing, and poor control of infection, among others.

But there is good news too.

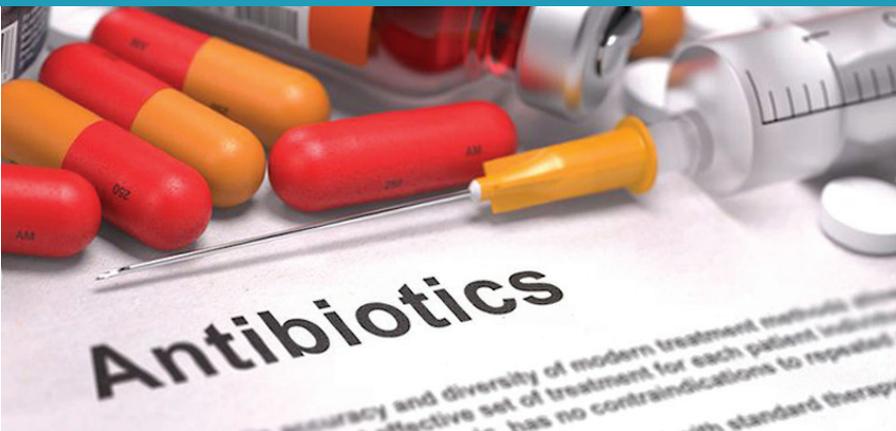
Those many factors translate into many fronts on which we can combat the problem. Laboratories will be critical to the success of many of those battles.

Thus, in this issue of *Lab Culture*, we bring you updates on surveillance of antimicrobial resistance, as well as disease-specific perspectives on what's coming and how African laboratories can best prepare. Global and regional surveillance is addressed in our first article from Dr Jay Varma on Africa CDC's 'Framework for Antimicrobial Resistance, 2018-2023', or AMRSNET, which provides a long-term strategy for the continent. A pair of articles then offer national and local perspectives. Dr Constance Schultsz offers 'smart strategies' to ramp up surveillance at the national level. Meanwhile, Dr Olumide Ajibola offers a local-level look at on-the-ground lessons from Nigeria. Finally, we also have detailed updates on what antimicrobial resistance means for two of Africa's most devastating diseases: HIV and tuberculosis. Dr Raph Hamers updates us on drug-resistant HIV, and Mr Obert Kachuwaire discusses the complexities of drug-resistant tuberculosis.

These articles barely begin to cover the many aspects of antimicrobial resistance. However, they demonstrate that the wheels are already in motion to prevent the 'dark ages of medicine' from returning. As a parent, that is enough, at least for now, to keep the shudders at bay.



Bethanie Rammer



## Africa CDC launches new plan to combat resistant microbes

### Plan seeks to monitor, prevent and mitigate harms

Antimicrobial-resistant organisms are increasing globally, threatening to render existing treatments ineffective against many infectious diseases.

Antimicrobial-resistant strains of bacteria, fungi, parasites and viruses prolong illness, increase case-fatality, facilitate transmission and increase treatment costs. To respond to this threat, the Africa Centres for Disease Control and Prevention (CDC) officially launched its “Framework for Antimicrobial Resistance, 2018-2023 (AMRSNET)” in October 2017.<sup>1</sup>

Established in January 2017, Africa CDC is a ‘specialised technical institution’ of the African Union responsible for strengthening capacity for surveillance and disease intelligence, emergency preparedness and response, laboratory systems, information systems and public health research and workforce

development in Africa. At a series of strategic planning meetings with public health, clinical and science experts in March 2017, Africa CDC identified antimicrobial resistance as a high-priority public health threat.

In accordance with the World Health Organization (WHO) Global Action Plan<sup>2</sup> and to meet needs specific to Africa, Africa CDC is establishing AMRSNET—a network of public health institutions and leaders from human and animal health sectors who will collaborate to measure, prevent and mitigate harms from antimicrobial-resistant organisms. Core members of AMRSNET will be derived from African national public health institutions and activities will be implemented by Africa CDC’s Regional Collaborating Centers in collaboration with Member States, non-governmental organisations, and existing stakeholders from WHO, Food

and Agriculture Organization, World Animal Health Organization and other relevant institutions. AMRSNET will work across the range of infectious pathogens (bacteria, viruses, parasites, fungi) and across both human and animal health.

AMRSNET’s four goals are to: (1) improve surveillance of antimicrobial-resistant organisms among humans and animals; (2) delay emergence of antimicrobial resistance; (3) limit transmission of antimicrobial resistance; (4) mitigate harm among patients infected with antimicrobial-resistant organisms. To achieve these goals, AMRSNET will conduct two essential cross-cutting activities: (1) advocate for policies and laws to enable long-term prevention and control of antimicrobial resistance, and (2) develop human resources.

Even though AMRSNET is a long-term strategy that involves partners from all across the



continent, laboratorians can start today on helping to achieve its goals by focusing on these four areas:

 Make sure your laboratory is following the most up-to-date standards for susceptibility testing. WHO's Global Laboratory Surveillance System recently consulted experts around the world and developed standards that you can use in your laboratory.<sup>3</sup> Using these standards will help make sure that your patients are receiving the highest quality diagnosis and also assist public health by making sure your results can be compared to other laboratories.

 Enroll in a quality assurance program. Even laboratories that follow standard operating procedures, such as those in the WHO's Global Antimicrobial Resistance Surveillance System,<sup>4</sup> need to make sure that they have

the systems in place to ensure quality results at all phases of testing.

 Work with clinicians and other members of your institution to establish a program for 'diagnostic stewardship'. Diagnostic stewardship involves making high-quality diagnostic tests readily available to clinicians, appropriately using these tests in your laboratory and helping clinicians apply test results to make patient care safer and more effective.

 Work with clinicians and other members of your institution to establish a program for 'antimicrobial stewardship'. Antimicrobial stewardship involves making sure that there are policies,

procedures and systems in place to ensure that antibiotics are prescribed only when necessary and, when prescribed, are narrowly tailored to treat the suspected infection.

#### RESOURCES

1. Press release. African countries launch framework to tackle the threat of antibiotic resistant infections. <https://au.int/en/pressreleases/20171107/african-countries-launch-framework-tackle-threat-antibiotic-resistant>.
2. World Health Organization. Global Action Plan on Antimicrobial Resistance. World Health Organization. 2015, Geneva, Switzerland.
3. Report of the meeting of the WHO Global Collaborating Centres to support AMR activities globally. 13-17 December 2016, WHO Headquarters, Geneva, Switzerland. World Health Organization. 2016, Geneva, Switzerland. <http://www.who.int/antimicrobial-resistance/AMR-Surveillance-QA-CCS-network-meeting/en/>.
4. Global Antimicrobial Resistance Surveillance System (GLASS). <http://www.who.int/antimicrobial-resistance/global-action-plan/surveillance/glass/en/>.

**Jay Varma, MD**  
Africa Centres for  
Disease Control  
and Prevention



# Strategies towards surveillance of antimicrobial resistance and antimicrobial stewardship

The lack of diagnostics for non-malarial febrile illness in many African countries hampers appropriate antimicrobial treatment and is a strong driver of inappropriate antibiotic usage, the main driver of antibiotic resistance. Point-of-care tests such as the measurement of C-reactive protein may aid in distinguishing between viral and bacterial infection and may thus, particularly when combined with rapid diagnostic tests for malaria, guide clinicians in their decision on whether or not to prescribe antibiotics. However, for appropriate empirical antibiotic treatment, knowledge of local antibiotic resistance prevalence is crucial.

The World Health Organization advocates laboratory-based surveillance as the first step for countries to initiate antimicrobial resistance surveillance within their national antimicrobial resistance program.<sup>1</sup> Laboratory-based surveillance relies on the availability of a functional clinical microbiology laboratory with the capacity to perform cultures of representative and relevant clinical samples and susceptibility testing of bacterial isolates. The development of clinical microbiology capacity requires allocation of sufficient financial and human resources and takes time. However, given the rapid increase in antimicrobial resistance reported from various regions around the globe, we do not have much time.

Smart strategies are urgently needed that allow for early data generation that provides clinicians with unbiased, population-specific information to guide empirical antibiotic treatment, in the short term. Such strategies should provide quality antimicrobial resistance prevalence data in a limited timeframe and at an affordable cost. Many efforts are now targeted towards (molecular) technologies that should provide rapid information about antimicrobial resistance profiles based on the absence or presence of resistance-encoding genes or mutations in bacterial isolates, or directly in clinical samples.<sup>2</sup> Unfortunately, these technologies do not yet perform well enough in predicting a susceptibility phenotype for microorganisms that are prone to horizontal gene transfer and frequent mutation, such as *Enterobacteriaceae* and other Gram-negative bacterial

species, and they come at a very high cost. Therefore, complementary alternative strategies are needed.

One good example of a smart strategy for antimicrobial resistance surveillance is lot quality assurance sampling. When applying this sampling methodology for antimicrobial resistance surveillance, a so-called lot (or batch) of the bacteria of interest, randomly collected from a specific patient population at a given hospital or within a region, is classified as having an *a priori*-defined high or low prevalence of antimicrobial resistance on the basis of the susceptibility test results of a small number of isolates from within the lot, whilst accepting a certain probability of misclassification. Lot quality assurance sampling-based surveillance substantially reduces required sample sizes and can give locally relevant population-based



Screen print of a telemicrobiology activity evaluating the quality of antimicrobial susceptibility testing.

information about antimicrobial resistance levels.<sup>3</sup> For example, resistance in *Escherichia coli* and *Klebsiella pneumoniae* against first-choice antibiotics used to treat urinary tract infections, could be classified as high (resistance prevalence >20%) or low (resistance prevalence ≤ 20%) with an acceptable probability of misclassification, on the basis of only 44 bacterial isolates, cultured from the urine of patients suspected of having a urinary tract infection. This number is substantially lower than the number required for precise prevalence estimates.<sup>3</sup>

Microbiology laboratories should join forces in their

efforts to generate reliable antimicrobial resistance data. Networking has been proposed as a means for laboratories to enhance critical mass and to strengthen and improve capacity.<sup>4</sup> Technological developments may aid in networking activities and in capacity building. Telemicrobiology is one such development, in which networks of laboratories communicate through freely available, internet-based applications, using high-quality digital imaging technology to share images of bacterial cultures and susceptibility tests using disc diffusion. Such an approach can be used for teaching purposes, as well as to

monitor the quality of surveillance cultures and susceptibility tests.<sup>5,6</sup>

Collaboration between public health and clinical laboratories and academic institutions can contribute to the design of strategies that may advance the fight against antimicrobial resistance.

#### RESOURCES

1. Perovic O., Schultsz C. Stepwise approach for implementation of antimicrobial resistance surveillance in Africa. *Afr J Lab Med.* 2016 Oct 31;5(3):482. doi: 10.4102/ajlm.v5i3.482.
2. Simner P. J., Miller S., Carroll K.C. Understanding the Promises and Hurdles of Metagenomic Next-Generation Sequencing as a Diagnostic Tool for Infectious Diseases. *Clin Infect Dis.* 2017 Oct 12. doi: 10.1093/cid/cix881. [Epub ahead of print]
3. van Leth F., den Heijer C., Beerepoot M., Stobberingh E., Geerlings S., Schultsz C. Rapid assessment of antimicrobial resistance prevalence using a Lot Quality Assurance sampling approach. *Future Microbiol.* 2017 Apr;12:369-377. doi: 10.2217/fmb-2016-0170.
4. Nkengasong J. N., Nsubuga P., Nwanyanwu O., Gersh-Damet G. M., Roscigno G., Bulterys M., Schoub B., DeCock K. M., Bix D. Laboratory systems and services are critical in global health: time to end the neglect? *Am J Clin Pathol.* 2010 Sep;134(3):368-73. doi: 10.1309/AJCPMSINQ9BRMU6.
5. Schultsz C., Lan N. P., Van Dung N., Visser C., Anh T. T., Bay P. V., Hong T. T., Brinke P., Hendriks W., Osinga T., van der Waals F., Botma J., Hien T. T., Farrar J. J., van Doorn H. R., Chau N.V., de Jong MD. Network building and knowledge exchange with telemicrobiology. *Lancet Glob Health.* 2014 Feb;2(2):e78. doi: 10.1016/S2214-109X(13)70112-8.
6. Sugianli A. K., Ginting F., Kusumawati R. L., Pranggono E. H., Pasaribu A. P., Gronthoud F., Geerlings S., Parwati I., De Jong M. D., Van Leth F., Schultsz C. Antimicrobial resistance in uropathogens and appropriateness of empirical treatment: a population-based surveillance study in Indonesia. *J Antimicrob Chemother.* 2017 May 1;72(5):1469-1477. doi: 10.1093/jac/dkw578.



**Constance Schultsz, MD, PhD**  
Department of Global Health, Academic Medical Centre - Amsterdam Institute for Global Health and Development, University of Amsterdam, Amsterdam, the Netherlands



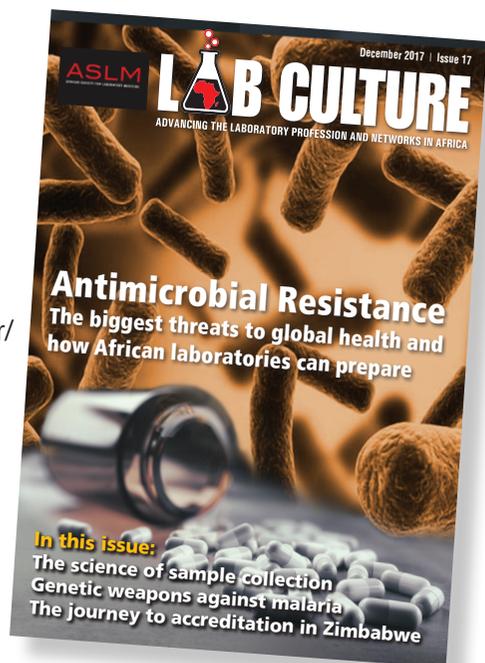
Pass this issue of **Lab Culture** to a colleague or go online

<http://www.aslm.org/stay-informed/press-room/lab-culture-newsletter/>



Become a member

<http://www.aslm.org/get-involved/become-a-member-2/>



# Surveillance for antibiotic resistant pathogens

From the inception of the first antibiotic (penicillin) in 1928, Alexander Fleming, its discoverer, had warned that it would not be difficult for microbes to develop resistance to penicillin.<sup>1</sup> Globally, it has been estimated that 700 000 people die annually from antibiotic-resistant infections, and it has been projected that this may rise to 10 million by 2050.<sup>2</sup> In developing countries, antibiotics can be readily purchased without any control. Such countries usually experience more cases of antibiotic resistance, which is in contrast to what occurs in developed nations where tight regulation of antibiotic use is in place. The purchase of antibiotics without a prescription, the preponderance of fake antibiotics, and the lack of antibiotic stewardship by healthcare practitioners make the situation worse.<sup>3</sup> In this article, our focus is on the need to enhance laboratory surveillance for antibiotic-resistant bacteria in Africa, with examples from Nigeria.

One of the key challenges faced by laboratories in sub-Saharan Africa is a lack of trained laboratory scientists who have the requisite skills and expertise to engage in surveillance. For example, in a state in northern Nigeria with a population of 4.5 million people, the state government has approximately 40 laboratory scientists in its employ.<sup>3</sup> With the paucity of trained medical laboratory scientists, medical laboratory technicians take on the role of laboratory scientists, and they are usually overwhelmed by the volume of work they have to do. Medical laboratory scientists have undergone a 5-year training program and obtained a Bachelor's degree and professional certification, whereas medical laboratory technicians have undergone a 2-year training and

obtained a diploma and have passed professional exams to practice; medical laboratory technicians should always work under the supervision of a medical laboratory scientist. Unskilled technicians who lack training on antibiotic resistance surveillance may miss important details potentially present in patients' laboratory culture results.

Second, there is the challenge of sub-optimal laboratories (Figure 1). Most government-owned laboratories in hospitals do not have the resources to carry out blood culture assays to isolate pathogens. Nor do most government hospital laboratories in Nigeria have access to automatic blood culture systems for isolating pathogens from blood. For a robust surveillance system in any developing country, there must be an effective laboratory system that ensures that pathogens from clinical samples submitted by patients are not missed.

Third, most laboratories in Nigeria do not follow Clinical and Laboratory Standards Institute (CLSI) or similar guidelines for carrying out susceptibility testing of pathogens isolated from clinical samples.<sup>4</sup> Instead, they employ a vague scoring approach based on one, two or three pluses (one or two pluses equals intermediate, three pluses equals susceptible, less than one equals resistant). These vague estimations are based on visual examination of the culture plates without following any clearly defined protocol or measurement of zones of inhibition for each bacterium being tested against antibiotics. In addition to poor susceptibility testing practices in laboratories is the use of sub-standard antibiotic discs. Such discs usually contain excess or fewer concentrations of the antibiotics, which could result in bacteria that



**Olumide Ajibola,**  
BSc, MSc, MRes,  
PhD Department of  
Microbiology, Federal  
University Birnin Kebbi,  
Kebbi State, Nigeria



**Semeeh Omoleke,**  
MBBS, MPH  
Immunisation, Vaccines  
and Emergencies, World  
Health Organisation,  
Kebbi State Field Office,  
Nigeria



**Mari Mshelia,**  
BSc, MSc Department  
of Microbiology, Federal  
University Birnin Kebbi,  
Kebbi State, Nigeria

should normally give a resistant reading wrongly interpreted as intermediate or vice-versa.

In the short term, governments in sub-Saharan Africa need to focus on certain key areas in order to improve antibiotic surveillance approaches: increase funding to revamp laboratories, improve training of laboratory scientists on antibiotic resistance surveillance with standardized protocols, identify diseases that are a priority for antibiotic resistance surveillance, improve infrastructure, provide consumables to laboratories, build a database for disease notification of antibiotic-resistant pathogens collected from each district and submitted to a national database, and collaborate with technical partners to build a robust infectious disease and antibiotic resistance surveillance system.<sup>5</sup> Developing countries also need to embrace molecular approaches for the characterization and identification of antibiotic-resistant strains that might be in circulation in humans, animals, and environmental samples.

Future plans should focus on development of rapid diagnostic test kits for bacterial infections that are

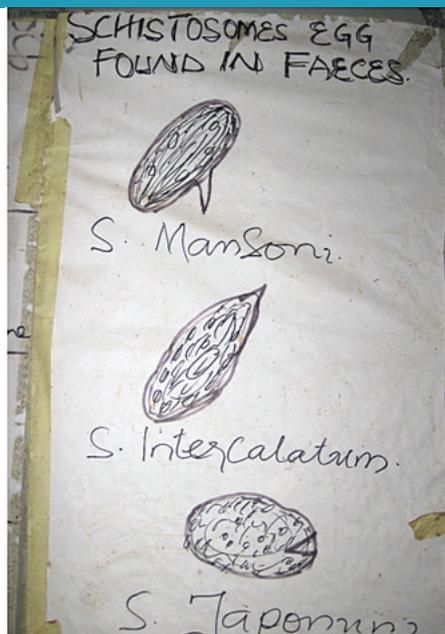


Figure 1. Example of poorly equipped laboratory. A hand-drawn image of *Schistosoma* ova is used for identification of parasites in patient samples in a hospital laboratory in sub-Saharan Africa.

prevalent in sub-Saharan Africa. Such test kits should have the capacity to indicate whether a pathogen might be resistant to antibiotics. In addition, there needs to be investment in building zonal or central laboratories equipped with state-of-the-art molecular tools for continuous surveillance of antibiotic-resistant bacteria in the population, and a national policy/

framework for antibiotic resistance surveillance in developing countries.<sup>6</sup>

#### RESOURCES

1. Nobel Lecture by Alexander Fleming. (1945). Available at: [https://www.nobelprize.org/nobel\\_prizes/medicine/laureates/1945/fleming-lecture.pdf](https://www.nobelprize.org/nobel_prizes/medicine/laureates/1945/fleming-lecture.pdf).
2. Williams, D. N. Antimicrobial resistance: are we at the dawn of the post-antibiotic era? *J. R. Coll. Physicians Edinb.* 46, 150–156 (2016).
3. Medical Laboratory Science Council of Nigeria (MLSCN) Practitioners' register, MLSCN Headquarters, Abuja Nigeria.
4. CLSI Standards Center: Our Standards Help Foster Health Care Excellence. (2017). Available at: <https://clsi.org/standards/>.
5. Ayuokebong, J. A., Ntemgwa, M., Atabe, A. N. The threat of antimicrobial resistance in developing countries: causes and control strategies. *Antimicrob. Resist. Infect. Control.* 6, 47 (2017).
6. Antimicrobial resistance in the African Region: Issues, challenges and actions proposed. (2017). Available at: <https://www.who.int/en/ahm/issue/16/reports/antimicrobial-resistance-African-region-issues-challenges-and-actions-proposed>.

## ASLM Laboratory Community of Practice (LabCoP)

- 1) Identify best practices, innovations, and implementation solutions;
- 2) Disseminate best practices through hubs and spokes;
- 3) Implement a monitoring and evaluation framework to measure adoption of best practices and impact of their implementation; and
- 4) Develop country investment cases and advising on specific interventions.

### How to Join

Countries should form a multidisciplinary team involving representatives from laboratories, clinical teams and civil society.

Submit an official Letter of Interest from country Ministry of Health to Dr Legese Mekuria (LMekuria@asm.org) or Dr Charles Kiyaga (Ckiyaga@asm.org) at ASLM.



Share your expertise and success:

Participate in LabCoP!

### ASLM Experts Database

ASLM needs your expertise to implement our projects and advance laboratory medicine in Africa.

If you would like to contribute to our work, we kindly ask you to register with our laboratory community database and share your profile with us. We will then be able to contact you whenever one of our activities falls within your areas of expertise.

More information available at: <https://asmexpertdb.org/guest.php?m=guest&c1=login>





Kenya National Tuberculosis Reference Laboratory in-charge explaining resistance surveillance procedures during WHO mid-term review (2014)



Conventional drug-susceptibility testing on Lowenstein-Jensen media



Xpert Ultra Cartridge, Cepheid

## Nature of the emerging threat of antimicrobial resistance with respect to tuberculosis

Antimicrobial resistance to tuberculosis is a major public health threat, posing potential negative ramifications to global health and security. First documented in the late 1940s, following the introduction of antibiotics for tuberculosis therapy, drug-resistant tuberculosis gained global attention in the 1990s. This period recorded the emergence of outbreaks of multidrug-resistant tuberculosis, which were notably associated with high patient mortality.<sup>1</sup> Globally in 2016, there were 600 000 new cases of tuberculosis that were resistant to rifampicin, one of the most effective first-line drugs. Of those cases, 490 000 had multidrug-resistant tuberculosis.<sup>2</sup>

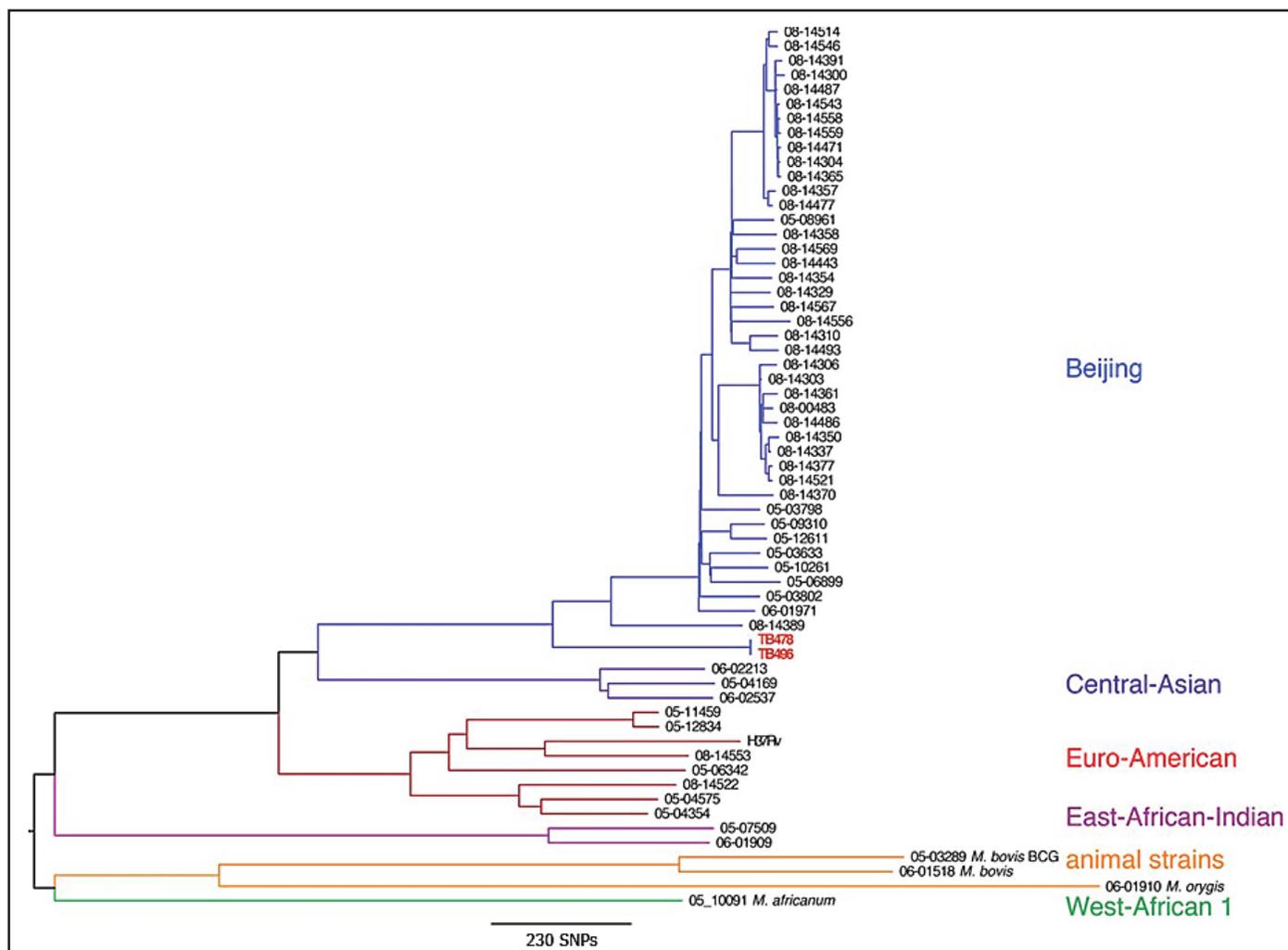
Drug-resistant tuberculosis presents in a number of categories including: mono-resistant, poly-resistant, rifampicin-resistant, multidrug-resistant, and extremely drug-resistant tuberculosis. Mono-resistant tuberculosis is resistant to one first-line anti-tuberculosis drug only. Poly-resistant tuberculosis is resistant to more than one first-line anti-tuberculosis drug, excluding both isoniazid and rifampicin.

Multidrug-resistant tuberculosis is resistant to both rifampicin and isoniazid, the two most powerful anti-tuberculosis drugs; it requires treatment with a second-line drug regimen. Rifampicin-resistant tuberculosis derives its definition from the now widespread use of the Xpert MTB/RIF assay for the concurrent detection of tuberculosis and rifampicin resistance without further testing for isoniazid resistance. Extremely drug-resistant tuberculosis is a form of the pathogen that is resistant to the two most important classes of anti-tuberculosis drugs in a multidrug-resistant regimen. It comprises resistance to isoniazid and rifampicin, in addition to resistance to any of the fluoroquinolones (such as ofloxacin or moxifloxacin) and to at least one of three injectable second-line drugs (amikacin, capreomycin or kanamycin).<sup>3</sup>

Antimicrobial resistance to tuberculosis presents a daunting barrier to effective tuberculosis care and prevention globally. Acquisition of drug-resistant tuberculosis occurs in two modes.

First, tuberculosis patients acquire drug-resistant tuberculosis when their anti-tuberculosis treatment is subpar. A number of causative factors contribute to this. Patients may fail to adhere to an appropriate and complete treatment regime. Or, the cause may be due to incorrect prescription of tuberculosis medicines or administration of low-quality tuberculosis drugs for therapy. Second, resistance arises when a patient with a resistant strain infects another person; this is known as transmitted or primary drug-resistant tuberculosis. Thus, antimicrobial resistance arises in areas with weak tuberculosis control programmes. Resistance has the effect of making case management difficult, with potentially life-threatening results and imposes catastrophic economic and social costs on patients while seeking care and treatment.<sup>4</sup>

The first pillar of the World Health Organization (WHO) End TB Strategy emphasises integrated, patient-centered care and prevention. One of its major components is early diagnosis of tuberculosis, including universal



Whole-genome sequencing for investigation of suspected tuberculosis outbreak. [Courtesy of JCM]

drug-susceptibility testing and systematic screening of contacts and high-risk groups. Bacteriological detection of tuberculosis facilitates the correct diagnosis and identification of the most effective treatment regimen that a patient should be started on in as short a time as possible and is critical for infection control.<sup>2</sup> Drug-susceptibility testing for at least rifampicin for all tuberculosis cases, plus drug-susceptibility testing for at least fluoroquinolones and second-line injectable agents among all tuberculosis cases with rifampicin resistance, are a priority. Drug-susceptibility testing methods include both phenotypic (conventional) and genotypic (molecular) assays. Conventional drug susceptibility testing is time-

consuming and characterized by procedures that are difficult to standardize, and proficiency in performing these tests requires an understanding of many elements.<sup>6</sup>

Molecular methods detect the mutations associated with resistance with the advantage of rapid turn-around times. However, disadvantages, including cost, adequate and appropriate laboratory infrastructure and equipment and limited drugs that can be tested, do exist. Current molecular drug-susceptibility tests recommended by WHO include line probe assays and the Xpert MTB/RIF assay.

The WHO Global Tuberculosis Report shows that, although there has been an increase in

notifications of multidrug-resistant and rifampicin-resistant tuberculosis in recent years, progress in closing detection and treatment gaps remains lethargic and fraught with large gaps.<sup>2</sup> In most resource-limited countries affected by a high burden of tuberculosis and multidrug-resistant tuberculosis, this is due to insufficient laboratory capacity and infrastructure, as evidenced by weak specimen and result transport systems, data collection and management.<sup>1</sup> In addition to the limited use of these technologies for detection and therapy guidance, surveillance of drug resistance is hampered by similar factors.

Treatment of drug-resistant tuberculosis relies on the use of second-line drugs that are costlier

and have adverse side effects. These medications are administered over lengthy periods of up to two years; however, promising shorter treatment regimens with fluoroquinolones and pyrazinamide are being evaluated and could become the cornerstones of tuberculosis treatment in the future. Two new drugs for the treatment of tuberculosis, bedaquiline and delamanid, became available in 2013 and 2014, respectively. They have recently been approved for the treatment of multidrug-resistant tuberculosis and are already being used in several countries. Rapid development of technologies to monitor the acquisition of resistance to these new drugs in patients with multidrug-resistant tuberculosis and the transmission of resistance in the community are keys to preserving the effectiveness of these new agents.<sup>1</sup>

## RESOURCES

1. Matteo Zignol, M.D., Anna S. Dean, Ph.D., Dennis Falzon, M.D. et al; Twenty Years of Global Surveillance of Antituberculosis-Drug Resistance 2016; *N Engl J Med.* 375;11.
2. Global Tuberculosis Report 2017, Geneva, World Health Organisation 2017; [http://www.who.int/tb/publications/global\\_report/en/](http://www.who.int/tb/publications/global_report/en/).
3. Companion handbook to the WHO guidelines for the programmatic management of drug-resistant tuberculosis Geneva, World Health Organisation 2014; [http://www.who.int/tb/publications/pmdt\\_companionhandbook/en/](http://www.who.int/tb/publications/pmdt_companionhandbook/en/).
4. <http://www.who.int/tb/areas-of-work/drug-resistant-tb/types/en/>.
5. [https://www.cdc.gov/tb/topic/laboratory/drug\\_testing.htm](https://www.cdc.gov/tb/topic/laboratory/drug_testing.htm).
6. <https://www.tbfacts.org/treatment-of-drug-resistant-tb/>.
7. Abstract extract from Chakravorty S., Simmons A. M., Rownecki M., Parmar H., Cao Y., Ryan J., Banada P. P., Deshpande S., Shenai S., Gall A., Glass J., Krieswirth B., Schumacher S. G., Nabeta P., Tukvadze N., Rodrigues C., Skrahina A., Tagliani E., Cirillo D. M., Davidow A., Denking C. M., Persing D., Kwiatkowski R., Jones M., Alland D. 2017. The new Xpert MTB/RIF Ultra: improving detection of *Mycobacterium tuberculosis* and resistance to rifampin in an assay suitable for point-of-care testing. *mBio* 8: e00812-17. <https://doi.org/10.1128/mBio.00812>

**Obert Kachuwaire, MPH**

African Society for  
Laboratory Medicine



## Promising Results

Gene Xpert, a technology recommended by the WHO in 2010, utilizes the Xpert MTB/RIF assay (Cepheid, United States). Since then, 23 million Xpert tests have been procured in 130 countries. Although Xpert showed high overall sensitivity and specificity with pulmonary samples, its sensitivity has been lower with smear-negative pulmonary samples and extrapulmonary samples. In addition, the prediction of rifampin resistance in paucibacillary samples and for a few *rpoB* mutations has resulted in both false-positive and false-negative results. A collaborative study involving Cepheid, FIND Diagnostics and partners has demonstrated the design features and operational characteristics of an improved Xpert Ultra assay. This study showed that the Ultra format overcomes many of the known shortcomings of Xpert. The new assay is supposed to significantly improve tuberculosis detection, especially among patients with paucibacillary disease, and provide more reliable detection of rifampicin resistance.<sup>7</sup>

## Looking Ahead

In order to meet future drug-susceptibility testing needs for detection, monitoring of trends in drug resistance and detecting outbreaks and hotspot regions, African laboratories should consider the following:



Routine testing of all patients with tuberculosis is widely recognized as the most appropriate approach. Laboratories will have to adopt algorithms that prioritize WHO recommended diagnostic molecular tests as the initial tests for all presumptive tuberculosis cases. This can be aided by:



Developing strong integrated tuberculosis diagnostic networks for cost-effective utilization of available technologies that provide rifampicin-resistance testing through strengthening all core capacities that comprise laboratory networks in an integrated system. (African Society for Laboratory Medicine is currently either leading or collaborating in the following efforts towards this end: Laboratory network tool development and assessments, Laboratory mapping tools, Continuous Quality Improvement and e-tools for management of laboratory proficiency testing, which are all pivotal in functional networks.)



Implementing connectivity solutions for improved equipment and supply management, bridging the laboratory-clinical interface and supporting strong surveillance systems that take advantage of connectivity solutions linked to national health management information system and international antimicrobial resistance networks.



Future developments point to the likelihood of molecular technologies replacing conventional phenotypic testing in drug resistance. High-throughput sequencing-based technologies are expected to become standard tools for surveillance with anticipated cost reductions in equipment and tests. These tests will be pivotal in providing further understanding of the clinical significance of mutations in the tuberculosis genome.<sup>1</sup>

# Drug-resistant HIV on the rise in sub-Saharan Africa

To curb the emerging threat of drug-resistant HIV in sub-Saharan Africa, multi-faceted interventions are warranted to improve the functioning of antiretroviral therapy programmes and enhance laboratory-based resistance surveillance.

In sub-Saharan Africa, over 14 million HIV-positive people are currently receiving antiretroviral therapy (ART)—about 70% of the global total.<sup>1</sup> The United Nations has committed to the goals of ensuring that by 2020, 90% of people with HIV know their diagnosis, 90% of those infected are receiving ART, and 90% of those receiving ART have sustained viral suppression.<sup>1</sup> The third goal is critically important to maximize the health and survival of HIV-positive persons and reduce HIV transmission.

According to the World Health Organization (WHO) HIV Drug Resistance Report 2017, evidence is growing that following the rollout of ART, HIV variants that are resistant to non-nucleoside reverse transcriptase inhibitor (NNRTI) drugs are on the rise in many low- and middle-income countries.<sup>2</sup> NNRTIs are the core drugs in first-line regimens in most such countries. In 6 of 11 countries surveyed (Argentina, Guatemala, Namibia, Nicaragua, Uganda and Zimbabwe), pre-treatment drug resistance was present in more than 1 in 10 persons before they started on ART, and in more than 1 in 5 persons with prior antiretroviral drug use (those restarting ART or women with past use of peri-partum prophylaxis) (Figure 1).<sup>2</sup> Drug-resistant HIV has also been reported in up to one-third of children starting first-line ART, even among those with no history of using perinatal prophylaxis.<sup>3</sup> Pre-treatment NNRTI resistance impacts the effectiveness of subsequent first-line NNRTI-based ART in terms of increased risk of virological failure, impaired immune recovery, treatment switch and death, as well as augmenting programmatic costs.<sup>2,4,5</sup>

HIV drug resistance is a marker of failure of ART programmes. The 2016 WHO report of the so-called Early Warning Indicators for HIV drug

resistance found that 1 in 3 ART programmes had at least one drug stock-out in the year reported and that 1 in 5 patients were not retained in care beyond the first year after they began ART. These findings emphasize the need to strengthen systems for both drug supply and patient retention.<sup>6</sup>

There is increasing recognition that HIV drug resistance is an emerging threat to epidemic control.<sup>7</sup> Therefore, national and global policies need to shift towards sustaining viral suppression to reduce HIV transmission and prevent large-scale HIV drug resistance. To support this, the WHO has launched the Global Action Plan for HIV Drug Resistance (2016-2021).<sup>8</sup>

Accelerated efforts to achieve universal access to routine viral load monitoring of patients on ART are ongoing.<sup>9</sup> Viral load monitoring permits early detection of ART failure and thus mitigates the emergence and transmission of drug-resistant HIV.

To find a solution to rising levels of pre-treatment resistance, WHO recently issued interim ART guidelines.<sup>10</sup> These guidelines recommend using high resistance-barrier drugs, i.e., dolutegravir, in first-line ART, in populations with high levels of pre-treatment NNRTI resistance and in persons with prior antiretroviral drug use. Some countries (e.g., Botswana, Kenya, Uganda, Nigeria) have already started implementing dolutegravir-based first-line ART. Further operational research is needed to monitor the impact of the dolutegravir rollout in terms of resistance and outcomes. Pre-treatment drug resistance

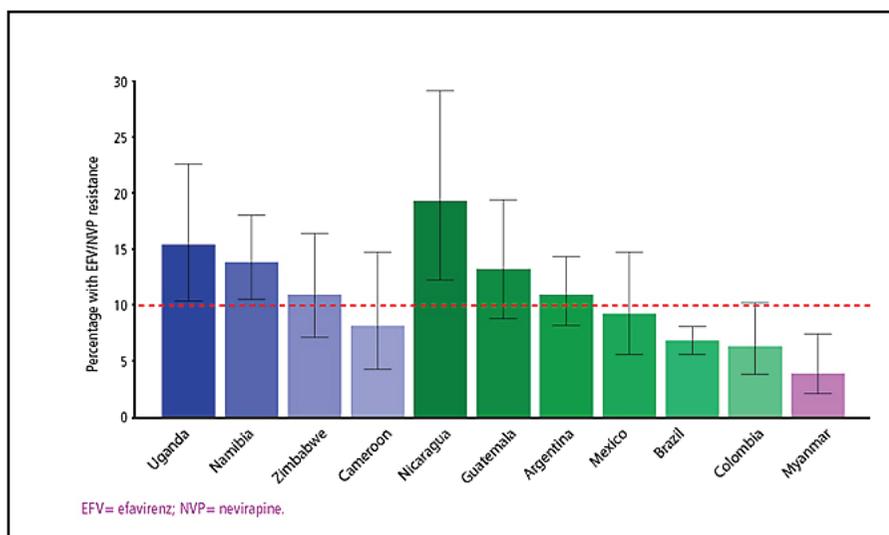


Figure 1. Resistance to non-nucleoside reverse transcriptase inhibitors. (Source: HIV drug resistance report 2017. Geneva: World Health Organization; 2017. Licence: CC BY-NC-SA 3.0 IGO.)

in infants (after failure of perinatal antiretroviral prophylaxis) can be mitigated by adopting closer viral load monitoring of HIV-positive women during pregnancy and breast-feeding and more robust ART regimens for perinatal prophylaxis. The ongoing scale-up of targeted use of pre-exposure prophylaxis (PrEP) for high-risk populations to enhance primary HIV prevention brings a need to closely monitor the emergence of PrEP-associated resistance in breakthrough infections, especially given that the core drug tenofovir is also used in first-line ART.

Nonetheless, as ART scale-up matures, the need for second and further lines of therapies will grow. The number of people receiving second-line ART in the region is forecasted to grow from less than 1 million now to around 4–6 million people by 2030, comprising up to 20% of all people on ART.<sup>11</sup> These developments will lead to increasingly complex clinical management, with emerging needs in terms of advanced skills and training of ART providers, as well as laboratory capacity to perform individualised drug resistance testing. Resistance testing could be beneficial in two ways: prevention of premature switches to more costly regimens (if resistant HIV variants are not detected) and improved virological outcomes through selection of optimal drug combinations. Feasible and affordable technologies are being developed, such as low-cost Sanger sequencing, next-generation sequencing, point mutation assays and genotype-free machine-learning approaches.<sup>12</sup> Further clinical evaluations are needed to demonstrate their utility and cost-effectiveness, prioritizing WHO-recommended, population-based surveillance and management of complex patient categories, such as patients failing second-line ART.

Lastly, there is a need to step up the implementation of the laboratory-based

resistance surveillance framework, as an integral component of national ART programs, to continuously monitor resistance at the population level and adapt ART guidelines as necessary.

In conclusion, given current limitations in available antiretroviral drug options, diagnostics, and clinical expertise in the African region, there is no room for complacency. To curb drug-resistant HIV and achieve the third UNAIDS goal,<sup>1</sup> a coordinated and resourced response is needed.

#### RESOURCES

1. UNAIDS. Ending AIDS. Progress towards the 90–90–90 Targets; 2017. [http://www.unaids.org/sites/default/files/media\\_asset/Global\\_AIDS\\_update\\_2017\\_en.pdf](http://www.unaids.org/sites/default/files/media_asset/Global_AIDS_update_2017_en.pdf).
2. World Health Organisation. HIV Drug Resistance Report 2017. Geneva; 2017. <http://apps.who.int/iris/bitstream/10665/255896/1/9789241512831-eng.pdf?ua=1>.
3. Boerma R. S., Sigaloff K. C. E., Akanmu A.S., et al. Alarming increase in pretreatment HIV drug resistance in children living in sub-Saharan Africa: a systematic review and meta-analysis. *J Antimicrob Chemother*. 2017;72(2):365–371. doi:10.1093/jac/dkw463.
4. Boender T. S., Hoenderboom B. M., Sigaloff K. C. E., et al. Pretreatment HIV drug resistance increases regimen switches in sub-Saharan Africa. *Clin Infect Dis*. 2015;61(11):1749–1758. doi:10.1093/cid/civ656.
5. Hamers R. L., Schuurman R., Sigaloff K. C. E., et al. Effect of pretreatment HIV-1 drug resistance on immunological, virological, and drug-resistance outcomes of first-line antiretroviral treatment in sub-Saharan Africa: A multicentre cohort study. *Lancet Infect Dis*. 2012;12(4):307–317. doi:10.1016/S1473-3099(11)70255-9.
6. World Health Organisation. Global Report on Early Warning Indicators of HIV Drug Resistance. Technical Report. Geneva; 2016. <http://apps.who.int/iris/bitstream/10665/246219/1/9789241511179-eng.pdf?ua=1>.
7. Beyrer C, Pozniak A. HIV Drug Resistance - An Emerging Threat to Epidemic Control. *N Engl J Med*. 2017;377(17):1605–1607. doi:10.1056/NEJMp1710608.
8. World Health Organisation. Global action plan for HIV drug resistance 2016–2021. WHO Geneva. 2016;(February):1–2. [http://apps.who.int/iris/bitstream/10665/204518/1/WHO\\_HIV\\_2016.02\\_eng.pdf](http://apps.who.int/iris/bitstream/10665/204518/1/WHO_HIV_2016.02_eng.pdf).
9. Lecher S., Williams J., Fonjungo P. N., et al. Progress with Scale-Up of HIV Viral Load Monitoring — Seven Sub-Saharan African Countries, January 2015–June 2016. *MMWR Morb Mortal Wkly Rep*. 2016;65(47):1332–1335. doi:10.15585/mmwr.mm6547a2.
10. World Health Organisation. Guidelines on the Public Health Response to Pretreatment HIV Drug Resistance. Geneva; 2017. <http://apps.who.int/iris/bitstream/10665/255880/1/9789241550055-eng.pdf?ua=1>.
11. Estill J., Ford N., Salazar-vizcaya L., et al. The need for second-line antiretroviral therapy in adults in sub-Saharan Africa up to 2030: a mathematical modelling study. *Lancet HIV*. 2016;3(18):16–3. doi:10.1016/S2352-3018(16)00016-3.
12. Inzaule S. C., Ondo P., Peter T., et al. Affordable HIV drug resistance testing for monitoring antiretroviral therapy in sub-Saharan Africa: perspectives and challenges. *Lancet Infect Dis*. 2016;In press.

The **Joep Lange Institute** was founded to continue the legacy of Prof Joep Lange and Jacqueline van Tongeren after the MH17 shootdown. The Institute promotes an innovation agenda in global health, based on digital technology, behavior change, better policy and innovative finance. With its partners, the JLI tests innovations in practice and advocates to scale those that work. HIV drug resistance is of particular interest to the Institute not only because of Prof Lange's international leadership in HIV advocacy and research, but also because it requires innovation to bridge the gap between policy and practice.

#### About the authors



**Raph Hamers, MD, PhD**, Amsterdam Institute for Global Health and Development; Department of Global Health, Academic Medical Center of the University of Amsterdam, Amsterdam, The Netherlands; Eijkman-Oxford Clinical Research Unit, Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, Jakarta, Indonesia



**Seth Inzaule, MSc, PhD** Amsterdam Institute for Global Health and Development; Department of Global Health, Academic Medical Center of the University of Amsterdam; and Joep Lange Institute, Amsterdam, The Netherlands



**Tobias Rinke de Wit, PhD** Amsterdam Institute for Global Health and Development; Department of Global Health, Academic Medical Center of the University of Amsterdam; and Joep Lange Institute, Amsterdam, The Netherlands

# National Microbiology Reference Laboratory of Zimbabwe: The journey to accreditation

The National Microbiology Reference Laboratory (NMRL) in Harare, Zimbabwe achieved ISO 15189 accreditation, which was awarded by the Southern African Development Community Accreditation Service for HIV TNA-PCR, viral load and HIV ELISA antibody testing in September 2017. As one writer once said, 'Quality is an attitude'. It's the state of mind that dictates how well one must do a job. Involving both personal and corporate integrity, it's an attitude that says, 'We do things right around here because that's our policy and because it's the right thing to do'. The quality of health care service is of paramount importance in the modern epoch and implementing ISO 15189 is an important pillar in laboratory strengthening to ensure quality healthcare. However, the implementation of an ISO 15189 quality management system (QMS) is the biggest challenge that is faced by many laboratories.

The NMRL embarked on our journey to attain accreditation through undertaking a process of implementing a QMS towards ISO 15189:2012. Plans for developing a QMS were in place as early as 2005 with consultants who were contracted to assist with document development. Unfortunately, despite the documents being put in place, there was no progress on the implementation of the QMS for the first three years.

In 2009, the NMRL was identified by the Southern African Development Community as a laboratory with the potential to be a supranational reference laboratory for HIV. At that time, in order to receive supranational reference laboratory status, a laboratory

was required to obtain accreditation to the ISO 15189 standard. This marked the true beginning of our journey to implement our QMS. However, when the Southern African Development Community project ended and our laboratory failed to achieve accreditation by end of 2015, the question was, 'Where are we going wrong?'

It was then that we evaluated our own QMS and realized the missing links. First among these, was that the system was known by only a few individuals – those who mainly worked on the QMS documents. The rest of the staff members were not part of the system. Second, the management was detached from the staff; thus, there was no team work. Finally, there were three groups of people working in silos: management, quality personnel and staff. For us to achieve accreditation, we needed to change the way we were operating.

The implementation of a QMS is very challenging as it introduces processes that affect the way the laboratory has been conducting all of its activities. Therefore, there is a need for strong management, leadership and organizational skills to facilitate the coordination of the new processes being implemented, so that the change is achieved effectively and efficiently. To ensure this, the first major step we put in place was the appointment of a full time Quality Officer who would guide the organization through implementing the QMS.

Antoine de Saint Exupéry said, 'If you want to build a ship, don't drum up people together to collect wood and don't assign them tasks and work, but



**Ellen Munemo**, DMLT, SDMLT, MSc Medical Micro, EMBA, Acting Coordinator, NMRL, Harare, Zimbabwe



**Charles Chiku**, HBMLS, MPH, Quality Manager, NMRL, Harare, Zimbabwe



**Patience Musasa**, HBMLS, MSc Medical Micro, Dip M&E, PT Coordinator, NMRL, Zimbabwe



**Raiva Simbi**, HBMLS, MCLin Epi, Deputy Director – Laboratory services

rather teach them to long for the sea'. We took this concept and started working to make sure that we had a team that knew what the ultimate goal was and how to attain it. They had to long for quality in everything they did. As part of ensuring staff commitment and compliance to NMRL QMS requirements, it was noted that training in the ISO 15189:2012 standard was required; this was done through consultants and in-house trainings. In addition, because we were a fragmented team, we started working on team building activities based on the illustration in Figure 1.

A team that works well together is more effective, more productive, and more successful — not to mention happier and more fun to work with!

The following best practices were realised as we worked on the implementation of our QMS.

 The commitment of laboratory management to ensuring the **availability of funding** for service, maintenance and calibration of all equipment and maintenance of the QMS was critical. Without a funded budget, accreditation can be only a dream. Reliable funding ensures the smooth flow of work in the laboratory and uninterrupted service delivery; it also motivated our staff.

 The appointment of a competent and dedicated full-time Quality Officer also contributed to ensuring the implementation of the QMS. A Quality Officer drives the system and ensures the accountability of all involved. Having a quality officer on duty full time helped us to address QMS issues at all times. We also appointed a Deputy Quality Officer, who worked together with the Quality Officer ensure implementation of QMS. The deputy Quality Officer was appointed a year after the appointment of the Quality Officer. This was done to assist the departments in the implementation of QMS as they both mentored departments. It also helped in making sure that they were complementing each other in their QMS duties.

 **Engagement and involvement of staff at all stages** ensured commitment to our common goal. The efforts of management to make sure that staff were motivated and inspired at all times made staff members feel that they were part of the bigger picture. This made staff want to do more as they felt their services were accepted and their voices heard.



Figure 1

 **Communication** was also an important factor in the implementation of our QMS. This pertains to both internal and external customers. Internal customers (staff) want to be heard and to contribute to the organisation's wellbeing, while external customers (clinicians, patients, principal investigators of projects and study and program officers) help us improve the way we do things.

 **Training and mentorship of all staff members**, from laboratory managers to the lowest level technician was needed in order for all to understand their role in the implementation of the QMS. Failure of any cadre causes a weak link in the system and results in poor implementation of the QMS. Trainings were offered by both internal and external experts. External experts were beneficial as the team got to hear about QMS from someone outside the organisation. Internally, the Quality Officer and Deputy Quality Officer conducted mentorship activities within the different sections of the laboratory.

 Implementation of a QMS increases staff workload, as documentation is needed for all laboratory processes. Laboratory management continuously reassured staff and motivated them to always appreciate quality service. It was not easy when we started, as staff members were not used to documentation. However, **continued follow up and reassurance** resulted in staff appreciating its importance.

 Management should make sure that **corrective actions and action items are closed out on time**. To achieve this, constant follow up is needed. Staff

members must account for each responsibility given to them and make sure that relevant documentation is in place.

 Laboratory management implemented **change management**

**skills** so as to transition the NMRL staff to understand the bigger vision of where the laboratory was headed. This was not easy as we were introducing a new way of doing processes. A number of challenges were encountered but as management continued to refocus the staff on what was at stake, change was achieved.

 **Employee attitude** is very important in implementation of QMS. Tactics that create a favourable employee attitude should be used. For example, always consult staff on proposed changes, as they often have helpful suggestions on what is needed. It is important for managers to arouse enthusiasm by appealing to the values, ideals and aspirations of the organization and providing logical

*'Coming together is a beginning.  
Keeping together is a process.  
Working together is success.'*

**Henry Ford**

arguments and factual evidence for changes being implemented.

In conclusion, laboratory management is the key to achieving quality in the laboratory.

Management must personally pledge their support for the QMS to staff.

This helps in moving the vision of the organisation forward. We recommend that laboratory managers consider focusing their efforts on the following three aspects to achieve a common goal with their team:

- How to achieve the task (quality)
- How to manage the team (focus on the goal)
- How to manage individuals (attitude change)

Putting all of this together resulted in the Zimbabwe NMRL attaining ISO 15189:2012 accreditation. The implementation of ISO 15189 remains an important pillar in the strengthening of our laboratory services and ensures quality healthcare for all.



## AJLM Update

### RECENT PAPERS

- Editorial: Honing in on disease etiology**  
Iruka N. Okeke, Editor-in-Chief
- Real-time PCR assay for Shiga toxin-producing E. coli**  
JB Kalulu, et al.
- Mother-to-child transmission of HIV in Cameroon**  
VN Fonoh, et al.
- Use of MALDI-TOF in a developing country**  
A Bulane, et al.
- Point-of-care rotavirus diagnosis in Nigerian infants**  
SE Nnukwu, et al.

### Submit to AJLM in four easy steps!

1. Read AJLM's instructions to authors page to determine the best article type.
2. Format your manuscript according to the instructions.
3. Create an author account, if you do not already have one.
4. Click on the "Submit Online" link on the right-hand side of the page and follow the instructions.

**AJLM Special Issue on African laboratories in antimicrobial resistance surveillance**

**Submission deadline: January 31, 2018**

The submission deadline for AJLM's special issue on the role of African laboratories in antimicrobial resistance (AMR) surveillance is approaching fast! Don't miss the opportunity for your work to be featured in this high-profile issue. Read the full Call for Papers on the AJLM website.

*AJLM is ASLM's official, peer-reviewed, open-access, scholarly journal. All AJLM articles are available to read online for free, and the journal currently charges no fees to authors.*

# Proper patient preparation, specimen collection, and sample handling are critical to quality care

Garbage In, Garbage Out (GIGO), as the saying goes. This adage has been applied in a universal manner in addressing human errors. It certainly applies to establishing laboratory procedures that ensure care in managing the pre-analytical phase of laboratory testing. Years ago, many common laboratory tests were performed manually, and thus were prone to inaccuracy and analytical mistakes. Today's advanced technology places laboratory science in a highly automated and quality-focused environment that ensures accurate testing processes.

## Total Testing Process (TTP)

Medical errors are a leading cause of death in healthcare institutions worldwide. The laboratory's contribution to this major healthcare concern is only 0.33%.<sup>1</sup> While this number appears small, laboratory errors do occur, not always resulting in death, but nevertheless having an important impact on patient care. As clinical laboratory scientists, we must make every effort to produce accurate test results.

One of the first efforts (1947) in laboratory quality management involved the collection of survey results where six common analyte samples were shared with a number of laboratories. The results pointed to wide and significant variation.<sup>2</sup> In later years (1997), the concept of 'brain-to-brain turn-around time' was introduced by George Lundberg and later modified to 'brain-to-brain loop'.<sup>3</sup> This process included nine steps involving laboratory testing: 1) ordering, 2) collection, 3) identification, 4) transportation, 5) separation, 6) analysis, 7) reporting, 8) interpretation, and 9) action.<sup>1</sup> This became the basis for the concept of the Total Testing Process (TTP)

to include pre-analytic, analytic, and post-analytic phases of testing. Over the past decades, as a result of intense quality management programs, laboratory errors have been reduced by as much as 75%.<sup>1</sup>

While initial efforts were focused on the analytical part of laboratory testing, it became clear that the pre-analytical and post-analytical phases of testing were just as, if not more, important in providing quality services. A number of studies have looked at the accuracy of laboratory testing. In general, error rates for each phase range from 46% to 68% for pre-analytical, 7% to 13% for analytical, and 18% to 47% for the post-analytical phase.<sup>4</sup> Since almost 70% of all laboratory errors reside within the pre-analytical phase, laboratories began to focus on preemptive practices that would minimize, and hopefully avoid, common errors.

## Pre-pre-analytic and pre-analytic phases

The pre-pre-analytic phase has been added as part of the initial patient encounter process.<sup>5,6</sup> As noted, this is where most laboratory errors occur. Table 1 identifies some of the most common errors. These errors may cost a moderate-sized hospital significant amounts of money in quality assurance investigations, blood redraws, repeat testing, and management oversight.<sup>7</sup> In African labs, this may also contribute to negative perceptions of lab results among clinicians.

One of the most common errors is ordering the wrong test.<sup>5,8</sup> With the ever-expanding clinical laboratory test menu, ensuring that the right test is ordered can be somewhat daunting.

Pre-pre-analytical	Pre-analytical	Analytical	Post-analytical	Post-post-analytical
Wrong test ordered	Improper sorting, aliquoting, labeling, centrifugation	Poor quality control	Reporting errors	Incorrect interpretation of laboratory test
Error in order entry		Instrument malfunction	Increased turn-around times	Delay in looking at results
Wrong patient identification		Interference (lipemia, hemolysis, etc.)	Transcription errors	No follow-up testing
Collection error			Failure in critical value reporting	No consultation
Improper handling & transportation				

Table 1: Examples of Errors in Each Laboratory Testing Phase<sup>6</sup>

Some examples: factor V and factor V Leiden; 25-hydroxyvitamin and 1,25-dihydroxyvitamin D; and thyroid tests. With a huge influx of information overload, the addition of new tests, the pressure to see more patients, and the expanding use of molecular diagnostics create numerous opportunities to make mistakes in laboratory test orders.

### Other pre-analytic variables

One of the least invasive procedures a patient may endure is the phlebotomy, yet it may be a significant source of poor patient care.<sup>8-13</sup>

- Proper patient identification is mandatory. It is recommended that at least two patient identifiers be obtained, usually spelling of their name and their date of birth.
- Proper labeling of the specimen is also critical (patient's name, hospital number or date of birth, time and date of collection, phlebotomist's name).
- Fasting status, especially prolonged fasting, may cause increases in amino acids, bilirubin, ketones, growth hormones, fatty acids, and triglycerides. Decreases may be observed in assays for glucose, high density lipoprotein (HDL), insulin, triiodothyronine (T3), and lactate dehydrogenase (LD). Fatty meals may increase potassium, triglycerides, alkaline phosphatase, and 5-hydroxyindoleacetic acid (5-HIAA). Meat, fish, iron, horseradish, and some bismuth-based antacids may render a false positive in stool occult blood tests.
- Patient diagnosis. It is also useful to understand the patient's health status, which may have an influence on test results.
- Correct test ordered.

### Physiologic factors and diet

- Vegetarians, especially long-term vegetarians, may show decreased levels of low density lipoprotein (LDL), very low density lipoprotein (VLDL), phospholipids, cholesterol, triglycerides, and vitamin B12.
- High protein/meat diets show increased serum urea, ammonia, and urate levels.
- High protein/low carbohydrate diets can result in increased urine ketones and serum blood urea nitrogen (BUN).
- Alcohol intake can increase triglycerides, lipoprotein, B12, lactate, and liver enzymes. Decreased levels of glucose, prolactin, and cortisol may also occur. Alcohol abuse can reflect elevation of HDL,  $\gamma$ -glutamyl transferase (GGT), urate, and mean corpuscular volume (MCV).
- Coffee/caffeine may increase glucose, plasma rennin, free fatty acids, and catecholamine concentrations. Increased levels of non-esterified fatty acids

may interfere with albumin-bound drug and hormone measurements. Studies suggest that caffeine may decrease platelet aggregation and increase vitamin D levels.<sup>14,15</sup>

- Bananas, pineapples, tomatoes, and avocados may elevate urine 5-HIAA. Cholesterol, triglycerides, and apoB lipoproteins may be altered in obese patients.

### Other factors

- Herbal supplements: Herbal supplements are used globally for a variety of ailments. Numerous studies have shown that some supplements can interfere with therapeutic drugs as well as laboratory testing. Various Chinese herbs have been shown to affect some laboratory test values. In addition, heavy metal poisoning has been reported based on elevated lead and zinc protoporphyrin levels. Table 2 lists some common supplements.<sup>11,12</sup>
- Exercise: Exercise may increase free fatty acids, lactate, creatine phosphokinase (CK), creatinine, pyruvate kinase, aspartate aminotransferase (AST), lactate dehydrogenase (LD), aldosterone, cortisol, HDL, prolactin, uric acid, bilirubin, platelets, white blood cells (WBC), and ammonia. Cholesterol and triglycerides may decrease.
- Posture: Plasma rennin activity and calcium may be lower when supine. Changing from supine to upright can alter haemoglobin, hematocrit, red blood cells (RBC), WBC, calcium, protein, and lipid levels.
- Stress: Hormones tend to increase with stress. Total cholesterol may increase, while HDL may decrease.

### Blood collection

- Tourniquet: Prolonged tourniquet application (haemoconcentration > one minute) may affect potassium, magnesium, albumin, protein, serum enzymes, coagulation factors, iron, ammonia, calcium, cholesterol, and triglyceride levels.
- Decontamination: Alcohol that has not dried (it needs 30 to 60 seconds) can destroy RBC. Iodine may elevate phosphate, uric acid, and potassium.
- Order of draw: First to draw are blood cultures, followed by light blue tubes (Na citrate), red SST (serum separator tube), green (heparin), lavender (versene), yellow ACD (Anticoagulant Citrate Dextrose), and finally gray (K oxalate/Na fluoride) tubes.
- Wrong tubes: Using the wrong anticoagulant in collection can alter test results.
- Improper mixing: Gentle mixing, using complete 180° inversions, is critical. For serum separation tubes (SST), inverting five times, allowing to sit 30 minutes, and then centrifuging (1,000-1,300 Relative Centrifugal Force or RCF) in a swing bucket for 10 minutes provides a proper specimen for analysis.<sup>16</sup>

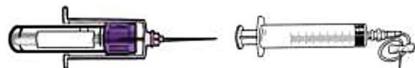
## How to safely collect blood samples by phlebotomy

Before entering patient room, assemble equipment for collecting blood.

- Laboratory sample tubes for blood collection (sterile glass or plastic tubes with rubber caps, vacuum-extraction blood tubes, or glass tubes with screw caps). EDTA tubes are preferred.



- Blood sampling systems (needle and syringe system, vacuum extraction system with holder, winged butterfly system (vacuum extraction) or winged butterfly system (syringe)).



- Tourniquet (single-use)



- Skin antiseptic solution: 70% isopropyl alcohol



- Gauze pads



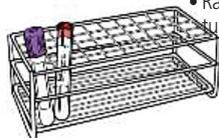
- Adhesive bandage



- Tray for assembling blood collection tools



- Rack for holding blood tubes



- Durable marker for writing on laboratory samples



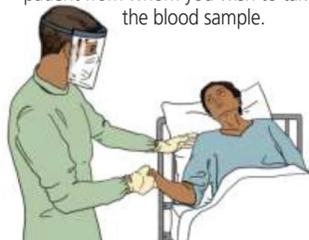
### Before entering patient room, fill out patient documentation

- Label blood collection tubes with date of collection, patient name, and his/her identifier number.
- Do NOT forget to fill out necessary laboratory form and epidemiological questionnaire.
- If several patients have to be sampled in the same place or during the same investigation, create a list. One patient per line. This list should include: patient name, identifier number, sex, age (birth date), clinical information: symptoms, date of onset, date specimen was collected, type of sample taken.



### Identify and prepare the patient

- Introduce yourself to the patient and explain what you will do with the blood sample and why.
- Make sure that this is the correct patient from whom you wish to take the blood sample.



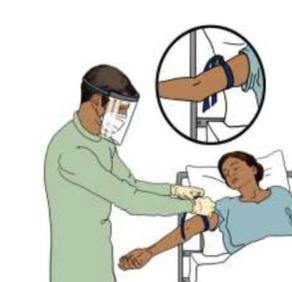
### Select the site, preferably at the bend of the elbow

- Palpate the area; locate a vein of good size that is visible, straight and clear.
- The vein should be visible without applying a tourniquet.



### Apply a tourniquet around the arm

- Tie approximately 4-5 finger widths above the selected site.



### Ask the patient to form a fist so that the veins are more prominent



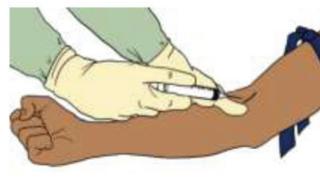
### Disinfect the area where you will put the needle

- Use 70% isopropyl alcohol
- Wait 30 seconds for the alcohol to dry
- DO NOT touch the site once disinfected.



### Anchor the vein by holding the patient's arm and placing a thumb BELOW the place where you want to place the needle.

- DO NOT touch the disinfected site
- DO NOT place a finger over the vein to guide the needle.

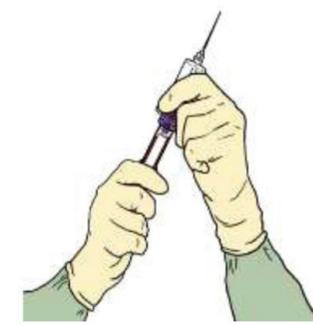


### Perform the blood draw

- Enter the vein swiftly at a 30 degree angle.



### Remove blood collector tube from holder and put in rack.



### Put needle into leak-proof and puncture resistant sharps container.

#### If the sharps container DOES NOT HAVE a needle remover:

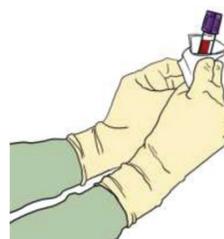
- Put the needle and holder into a sharps container
- Do not remove the needle from the holder
- Do not reuse the needle.

#### If the sharps container DOES HAVE a needle remover:

- Remove the needle following instructions on the sharps container
- Put the holder into the infectious waste bag for disinfection.



### Protect the sample from breaking or leaking during transport by wrapping the tube of blood in a paper towel.



### Have the gloved assistant tightly close the top of the plastic leak-proof packaging container.

- Disinfect the outer side of the plastic leak-proof packaging container with a disinfectant.



Herbal Supplement	Possible Impact on Lab Tests
Black Cohosh, Chaparral, Comfrey, Germander, Kava-kava, Mistletoe	Liver toxicity; may see altered liver enzyme tests (ALT, AST, Alkaline phosphatase, GGT, Bilirubin)
Licorice	Hypokalemia, CK, Aldosterone, Cortisol, Renin Activity
Kelp	Thyroid tests
Lab Testing for Therapeutic Drug Interference	
Chan Su, Dan Shen, Siberian ginseng	Digoxin
St. John's Wort	Digoxin, Theophylline, Cyclosporin
Borage oil (Starflower), Evening primrose, Shankhpushpi	Anticonvulsants
Angelica Root, CoQ10, Cranberry, Devils Claw, Feverfew, Garlic, Ginger, Ginko, Licorice, Saw Palmetto	Warfarin
Chromium, Garlic, Melatonin	Glucose
Goldenseal	Antipsychotic drugs

Table 2. Effect on Laboratory Tests by a Few Herbal Supplements <sup>11,12,18</sup>

## Interfering substances

- Tobacco: Haemoglobin, carboxyhaemoglobin, catecholamines, glucose, lactate, growth hormone, cholesterol, triglycerides, LDL, cortisol, WBC, RBC, and MCV may be elevated with tobacco use. Decreased levels of vitamin B12, HDL, IgA, IgG, IgM, and sperm counts/motility are also seen.
- Haemolysis: As RBC break apart, potassium, phosphorus, ALT, LD, CK, coagulation factors, magnesium, iron, sodium, HDL, triglycerides, and cholesterol levels may be altered.
- Lipemia: When triglycerides exceed 300 mg/dL, lipemia occurs, causing difficulties in measuring hemoglobin, white blood cells, and platelets. Any assay using spectral analysis may be affected by the abnormal light scatter caused by lipemic particles.<sup>16</sup>

## Knowing the variables

Proper preparation of the patient regarding fasting needs as well as careful phlebotomy procedures are essential to ensuring accurate laboratory testing results. General practice is to have the patient fast 12 hours prior to blood draw, especially when testing for a lipid profile and glucose tests. There is also evidence that fasting for creatinine levels should be considered, especially when high protein/meat diets are consumed.<sup>17</sup> In addition, patients should avoid alcohol, tobacco, physical stress, and caffeine. Having the patient sit for 15 minutes prior to the blood draw and performing it in the morning (7-11 AM) is ideal.<sup>13</sup>

While all pre-analytic variables cannot be eliminated, phlebotomists and technical staff need to be made aware of the many variables that can impact laboratory testing accuracy. Up-to-date blood collection procedures should provide guidance in addressing potential critical outcomes in the pre-analytical phase of testing. Establishing quality assurance monitors for selected possible pre-analytic errors can help identify opportunities that can be addressed, thus minimizing the risk of adverse outcomes.

## RESOURCES

1. Ghaedi M, El-Khoury J. The leading cause of error in laboratory medicine. *Clin Lab News*. 2016. <https://www.aacc.org/publications/clin/articles/2016/july/preanalytical-variation-the-leading-cause-of-error-in-laboratory-medicine>.
2. Belk WP, Sunderman FW. A survey of the accuracy of chemical analysis in clinical laboratories. *Am J Clin Pathol*. 1947;17(11):853-861.
3. Lundberg GD. Acting on significant laboratory results. *JAMA*. 1981;245:1762-1763.
4. Hammerling JA. A review of medical errors in laboratory diagnostics and where we are today. *Lab Med*. 2012;43(2):41-44.
5. Laposata M, Dighe A. "Pre-pre" and "post-post" analytical error: high-incidence patient safety hazards involving the clinical laboratory. *Clin Chem Lab Med*. 2007;45(6):712-719.
6. Hawkins R. Managing the pre- and post-analytical phases of the total testing process. *Ann Lab Med*. 2012;32(1):5-16.
7. Kaushik N, Green S. Pre-analytical errors: their impact and how to minimize them. *MLO*. 2014;46(5):22-26.
8. Lifshitz MS. Preanalysis. Henry's Clinical Diagnosis and Management by Laboratory Methods, 23rd ed. McPherson & Pincus, eds. 2016; pp. 20-32.
9. Finnagan K. Pre-analytical variables and patient safety. ASCP presentation, San Francisco. April 28, 2015.
10. Young DS, Bermes EW, Haverstick DM. Specimen collection and other preanalytic variables. *Tietz: Fundamentals of Clinical Chemistry*, 6th ed. Burtis, Ashwood, Bruns, eds. 2008; pp 42-62.
11. Dasgupta A, Bernard DW. Herbal remedies: effects on clinical laboratory tests. *Arch Pathol Lab Med*. 2006;130(4):521-528.
12. Dasgupta A. Review of abnormal laboratory test results and toxic effects due to use of herbal medicines. *Am J Clin Pathol*. 2003;120(1):127-137.
13. Irjala KM, Gronroos PE. Preanalytical and analytical factors affecting laboratory results. *Ann Med*. 1998;30(3):267-272.
14. Natella F, Nardini M, Beilelli F, et al. Effect of coffee drinking on platelets: inhibition of aggregation and phenols incorporation. *Br J Nutri*. 2008;100:1276-1282.
15. Al-Othman A, Al-Musharaf S, Al-Daghri NM, et al. Tea and coffee consumption in relation to vitamin D and calcium levels in Saudi adolescents. *Nutrition*. 2012;11:56.
16. Kurec AS. Can the type of blood collection tubes used be a source of lab error? *MLO*. 2016;48(10):18.
17. Kurec AS. Answering your questions. Lipemia and hyperleukocytosis can lead to CBC errors. *MLO*. 2016;48(7):64.
18. Lab Tests Online. [www.labtestsonline.org](http://www.labtestsonline.org).
19. Anderson L. 18 Herbal supplements with risky drug interactions. *Drugs.com*. 2014; <https://www.drugs.com/slideshow/herb-drug-interactions-1069>.



**Anthony Kurec, MS, H(ASCP) DLM**, is Clinical Associate Professor, Emeritus, at SUNY Upstate Medical University in Syracuse, New York. He is also a member of the MLO Editorial Advisory Board.

# Promising results for two genetic weapons against malaria

Two new papers by researchers at the Johns Hopkins Bloomberg School of Public Health's Malaria Research Institute in Baltimore, Maryland, report successes for highly promising strategies against malaria, a disease that kills more than 400 000 people each year, mostly children age five and younger in sub-Saharan Africa.

The two studies discovered different ways by which resistance to the malaria parasite can spread into a mosquito population, potentially opening the way for the development of self-propagating malaria control strategies. The advantage of this feature is less need to continuously apply malaria control measures such as insecticides and bed nets.

One team of researchers discovered a strain of bacteria that can spread rapidly and persist long-term among malaria-carrying mosquitoes. A genetically modified version of the bacterial strain strongly suppresses development of the malaria parasite, making the mosquitoes much less likely to transmit these parasites to humans.

A second research team discovered that a genetic modification that boosted the immune system of malaria-carrying mosquitoes not only suppresses malaria parasites in the insects but also can spread quickly in a test population, by changing the mosquitoes' mating preferences.

Malaria is spread by female *Anopheles* mosquitoes carrying the malaria parasite. One promising way to prevent malaria, in addition to traditional approaches such as bed nets and insecticides, is to modify the mosquitoes so they are no longer capable of spreading the parasite to humans.

These new findings could lead to developing bacteria and mosquitoes that would be released into mosquito populations in the wild, and would propagate on their own to reduce malaria transmission to humans in endemic areas. These strategies are designed to be complementary and would be used in conjunction with things like bed nets and insecticides to diminish the transmission of disease.

The two papers appear in the September 29 issue of *Science*.

## Bacteria vs. parasites

The discovery of the new mosquito-infecting bacterial strain was a chance event. 'We were working with a different bacterium when a researcher on the project happened to find evidence of a bacterial colony in our mosquitoes' ovaries,' says senior author Marcelo

Jacobs-Lorena, a professor in the Bloomberg School's Department of Molecular Microbiology and Immunology and a member of its Malaria Research Institute (JHMRI). 'That was unusual—normally we find bacteria only in the mosquito gut.'

His team soon characterized these odd microbes as a strain of *Serratia* bacteria, and dubbed them *Serratia* AS1.

Jacobs-Lorena and other researchers have been developing genetically engineered bacteria that can infect mosquito populations and kill the malaria parasites the mosquitoes harbor, without harming the mosquitoes themselves. Getting such bacteria to spread efficiently has been a key challenge, but experiments revealed *Serratia* AS1 to be almost perfect for the task. Jacobs-Lorena and colleagues found that, unlike other mosquito-infecting bacteria, *Serratia* AS1 are easily transmitted from males to females during mating, and from female mosquitoes to their offspring. The bacteria also stably colonize the mosquito gut, where malaria parasites develop.



In one experiment, the scientists used *Serratia* AS1-laden sugar bait to infect males and virgin females representing just 5% of a mosquito test population, and found that in the next generation the bacterial strain was present in 100% of the larvae and adult mosquitoes. The researchers followed this mosquito population for two more generations—about a month's time—and found that the bacteria remained ubiquitous. The results suggest that *Serratia* AS1 bacteria are likely to spread and persist long-term in wild mosquito populations.

The scientists modified *Serratia* AS1 by adding genes for five potent antimalarial proteins that were developed in the laboratory. Powered by these antimalarial proteins, the bacteria strongly inhibited malaria development in

colonized mosquitoes, reducing the levels of an early stage form of the parasite (oocytes) by more than 90% compared to mosquitoes that didn't contain the modified bacteria.

Further experiments showed that the modified *Serratia* AS1 bacteria don't have a significant effect on mosquito lifespan or fertility. The modified bacteria also appear able to colonize the most common species of malaria-transmitting mosquitoes, while the antimalarial proteins secreted by the bacteria can suppress the most common species of malaria parasites.

'So far all indications are that these anti-malarial proteins are universally effective against malaria parasites, and the *Serratia* AS1 bacteria that carry them can go into any malaria-carrying mosquito species,' Jacobs-Lorena says.

He and his colleagues now plan to follow their small-scale laboratory experiments with larger-scale experiments at the Malaria Research Institute's 'Mosquito House' research facility in Macha, Zambia. After that, the scientists hope to be able to release *Serratia* AS1 bacteria in an isolated, island-like environment to study how the microbes spread among wild mosquitoes.

## Antimalarial genes alter mate preference to spread quickly in mosquito populations

In the second study, a team led by George Dimopoulos, professor in the Bloomberg School's Department of Molecular Microbiology and Immunology in Baltimore, Maryland, made small modifications to the DNA of malaria-transmitting *Anopheles* mosquitoes to boost the activity of immune genes in the insects. The enhanced immunity made the mosquitoes more resistant to infection by malaria parasites, and thus less likely to transmit the parasites to humans.

That result was expected. What wasn't expected was the unusually high efficiency with which the modified mosquitoes spread their genetic modification to ensuing generations in a mixed population of modified and unmodified, wild-type mosquitoes.

Investigating this surprising result, Dimopoulos and colleagues found that boosting the mosquitoes' immune genes also boosted their defenses against bacteria, reducing the normal bacterial load and altering the normal mix of bacterial species in the mosquito intestine and reproductive organs. This change in the insect 'microbiota' in turn led to a change in mating preferences, such that modified male mosquitoes began to prefer unmodified, wild-type females, while wild-type males began to prefer modified females.

'We believe that by changing the microbiota we're changing the scent of modified mosquitoes—which in

turn alters mating preference,' says Dimopoulos. 'It's the perfect change in mating preference in this case, because it maximizes the chances of producing genetically modified offspring when mosquitoes compete for mates.'

It's important to note that the DNA modifications only involved an alteration of the mosquito's own gene activity, and not the introduction of foreign genes. Other laboratories are developing a different method to release mosquito genetic modifications into test populations using complex 'gene drive' DNA modifications. These essentially override the normal dynamics of inheritance to force new genes into nearly 100% of the offspring of mating mosquitoes. While potentially very promising, gene drives are still controversial because of their artificiality, complexity, and potential for long-term instability.

The findings from Dimopoulos and his colleagues show that even a subtle genetic modification that merely boosts the activity of existing genes can spread quickly into a mosquito population—without the need for a complex gene drive system. However, the two systems could also be combined to maximize the spread of malaria-resistant mosquitoes.

Dimopoulos's modified mosquito population has now been living in a colony in his laboratory for more than seven years, and has retained its high level of resistance to malaria for all that time, without any apparent adverse side effects from the genetic modification. 'These mosquitoes haven't changed in terms of feeding behavior or any other things that could be of concern,' Dimopoulos says.

Dimopoulos and his colleagues now plan to study the effects of their genetic modifications in larger settings, such as the Institute's Mosquito House in Zambia.

'Driving mosquito refractoriness to the malaria parasite with engineered symbiotic bacteria' was written by Sibao Wang, André L. A. Dos-Santos, Wei Huang, Kun Connie Liu, Mohammad Ali Oshaghi, Ge Wei, Peter Agre, and Marcelo Jacobs-Lorena. The study was supported by grants from the National Institute of Allergy and Infectious Diseases (AI 031478), the Strategic Priority Research Program of Chinese Academy of Sciences (XDB11010500), and the National Nature Science Foundation of China (31472044).

'Changes in the microbiota cause genetically modified *Anopheles* to spread in a population,' was written by Andrew Pike, Yuemei Dong, Nahid Borhani Dizaji, Anthony Gacita, Emmanuel F. Mongodin, and George Dimopoulos. The study was supported by a grant from the National Institute of Allergy and Infectious Diseases (AI061576).

The research was also supported by Bloomberg Philanthropies.



On the **WHO's list**  
of prequalified  
In Vitro Diagnostic  
products



## AQUIOS CL FLOW CYTOMETER

### Fast, Compact, Fully Automated CD4 Monitoring

The Aquios CL flow cytometer was accepted by the World Health Organization (WHO) Prequalification of In Vitro Diagnostics Programme in 2015. It can be used specifically for the immunologic assessment of patients having, or suspected of having, immune deficiency.

The World Health Organization (WHO) Prequalification of In Vitro Diagnostics (IVDs) Programme aims to promote and facilitate access to safe, appropriate and affordable in vitro diagnostics of good quality in an equitable manner. Focus is placed on in vitro diagnostics for priority diseases and their suitability for use in resource-limited settings.

The Prequalification IVD Programme has accepted the Aquios CL cytometer together with the Aquios Tetra-1 Panel (CD45-FITC/CD4-RD1/CD8- ECD/CD3-PC5) and Aquios Immuno-Trol/Immuno-Trol low controls. They are intended for use with in-vitro diagnostic flow cytometric applications, involving four fluorescent detection channels using a blue (488 nm) laser, two light scatter detection channels and electronic volume (EV).\*\*

Fast and easy to use, the Aquios is the first authentic "LOAD & GO" cytometry system and designed specifically to streamline workflow and reduce backlogs when handling applications, such as immunophenotyping. It is suitable for clinical labs of all sizes and requires minimal training to operate.

Discover more at [www.AQUIOSCL.com](http://www.AQUIOSCL.com)

\*\*These reagents provide identification and enumeration of the following lymphocyte subset populations: total CD3+, CD3+CD4+, CD3+CD8+, CD3+CD4+/CD3+CD8+ (ratio only) lymphocyte percentages and absolute counts; CD45+ absolute count; and CD45+ Low SS (lymphocytes) percentage and absolute count.



© 2017 Beckman Coulter Life Sciences. All rights reserved. AQUIOS CL with AQUIOS Tetra-1 and Tetra-2+ is for In Vitro Diagnostic Use. AQUIOS CL with Tetra-1 has been accepted for the WHO list of prequalified in vitro diagnostic products. Class 1 Laser Product. AQUIOS, AQUIOS Tetra, and Load & Go are trademarks of Beckman Coulter, Inc. Beckman Coulter and the stylized logo are registered in the USPTO. All other trademarks are the property of their respective owners.

For Beckman Coulter's worldwide office locations and phone numbers, please visit "Contact Us" at [beckman.com](http://beckman.com)

CM0019-0111