NRL WORKSHOP ABSTRACTS

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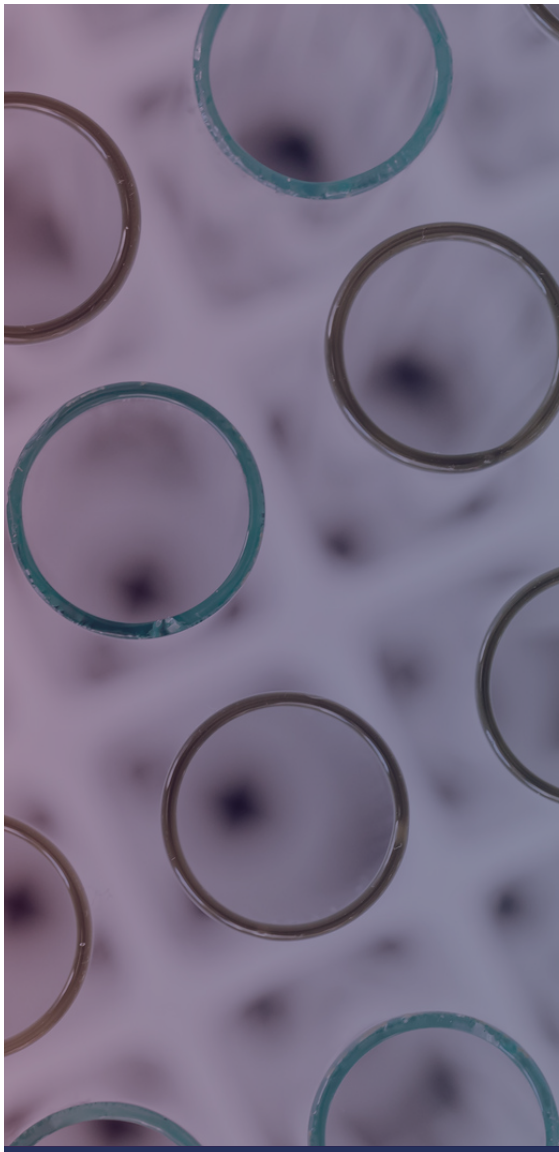

Title: Post-pandemic laboratory capacity strengthening in low- and middle-income countries – our story.

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Abstract

Quality laboratory systems are critical for prevention, diagnosis, case management, monitoring and treatment of diseases. The COVID-19 pandemic highlighted the role that good quality laboratories and diagnostic systems play in contributing towards effective disease surveillance and prevention; disease screening to allow timely contact tracing and interventions; and disease monitoring to allow easing of pandemic restrictions. Increased global travel and trade and climate changes being experienced across the globe are a fertile breeding ground for the next pandemic. However, as demonstrated during the COVID-19 pandemic, many countries in LMIC do not have the laboratory capacity to allow effective response to the next pandemic.
The NRL Scientific Consulting and Training Team contributes to the development and improvement of laboratory capacity for quality infectious diseases testing in LMIC across the globe. Between 2021 – 2023 we have been involved in seven laboratory capacity improvement projects covering countries in South-East Asia, Indo-Pacific and African regions. In this talk we
detail how we use the lessons learnt from our projects and the COVID-19 pandemic to design and continually improve our laboratory capacity improvement projects. Effective laboratory capacity support requires programs that are designed to ensure transfer of knowledge and technical expertise to allow sustainability of activities beyond the project and funding cycles.

Title: Post-market Evaluation of COVID-19 Rapid Antigen Test Kits for the TGA

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Introduction: All SARS-CoV-2 (COVID-19) rapid antigen tests (RATs) must be included in the Australian Register of Therapeutic Goods (ARTG) for use in Australia. The Therapeutic Goods Administration (TGA) oversees the regulation and release of IVDs through pre-assessment based on manufacturers' evidence and post-market monitoring. As different SARS-CoV-2 variants arise, manufacturers are required to undertake an analysis, to verify that their tests continue to perform as intended, and that any adverse impacts are identified and communicated.

In addition to this, in 2022, the TGA commissioned the Peter Doherty Institute for Infection and Immunity (Doherty Institute), in collaboration with NRL, to determine if the ARTG listed COVID-19 RAT kits had been adversely impacted in detecting the emerging SARS-CoV-2 Delta and Omicron variants.

The primary aim of the evaluation was to verify the test kit manufacturers' claim of analytical sensitivity – Limit of Detection (LOD), for Wild type, Delta and Omicron variants. All RATs were required to meet the sensitivity recommendations as prescribed by the WHO, which was a LOD no higher than 1,000 TCID50/mL (tissue culture infectious dose – TCID).

Method: Three SARS-CoV-2 isolates – Wild type, Delta and Omicron of known quantification (TCID50/mL and RNA copies/mL) were serially diluted in viral transport media at various concentrations to create a 210 member LOD testing panel. Dilutions were verified by gravimetric measurement. The LOD panel was tested on each RAT as per the manufacturer's instructions for use (IFU) and results analysed by PROBIT analysis – as part of the Analyse-it statistical software package. Each RAT LOD was reported as both TCID50/mL and RNA copies/mL.

Results: Of 93 RAT kits received, 85 were able to be evaluated for LOD. Eight RATs could not be evaluated as they demonstrated reactivity to the panel sample diluent or there were inconsistencies with the IFU and kit components supplied. In total, 79/85 RATs were found to be compliant with TGA's requirements, as they had acceptable product quality (the number of invalid results being less than 5%, appropriate labelling and instructions for testing) and analytical sensitivity was less than 1,000 TCID50/mL for Wild type, Delta, and Omicron variants. Six RATs were found to be non-compliant and were cancelled from the ARTG, initiated either by the sponsor or the TGA, and are no longer supplied in Australia. The results of the evaluation are published on the TGA website.

Conclusion: This study demonstrated the importance and need for post-market monitoring of SARS-CoV-2 RATs (particularly given emerging variants of concern) to ensure tests remain fit for purpose and maintain public health safety.

Title: U = U

Author: Lara Vojnov

The World Health Organization recently released new WHO guidance and an accompanying Lancet systematic review that describe the role of HIV viral suppression and undetectable levels of virus in both improving individual health and halting onward HIV transmission. The guidance describes key HIV viral load thresholds and the approaches to measure levels of virus against these thresholds, helping ensure all people living with HIV have access to viral load testing. People living with HIV who achieve an undetectable level of virus by consistent use of antiretroviral therapy, have zero risk of transmitting HIV to their sexual partner(s). The evidence also indicates that there is negligible, or almost zero, risk of transmitting HIV when a person has a HIV viral load measurement that is detectable but less than or equal to 1000 copies per mL, also commonly referred to as having a suppressed viral load. This session will present the evidence from the systematic review and key messages from the policy brief. Two well-known and prominent panelists will join the session to provide their valuable and thoughtful perspectives.

Title: How much is too much? Cost-effective biosecurity for Indonesian feedlots

Author: **Emma Zalcman**

Foot and Mouth disease is a highly contagious disease of cloven hoofed animals that has severe economic consequences, especially in intensively raised livestock. The mortality of animals infected with foot and mouth disease is low but the morbidity is high and the production impacts are significant. Until 2022, Indonesia had been free of FMD since the late 1980s. However, the disease entered in early 2022, along with Lumpy Skin Disease, another infectious disease of cattle that has never been seen in Indonesia.

Both these diseases have had severe consequence for the Indonesian feedlot industry who import live Australian feeder cattle originating from extensive properties in Northern Australia. The industry is both critical to Northern Australian economies and to food security within Indonesia. Roughly 30% of Indonesian beef comes from Australia and the industry is worth approximately A $1.1 billion to the Australian economy.

To protect Australian animals that arrive into Indonesian feedlots from these infectious diseases, businesses have poured millions of rupiah into biosecurity, including vaccination. Biosecurity measures were implemented hurriedly as disease spread rapidly across Indonesia. There were big and rapid spends on biosecurity-related infrastructure in the early months and in some cases, a large amount of money is spent weekly on things like disinfection and disposable personal protective equipment for staff.

This talk is about making biosecurity cost-effective for the long term by systematically identifying high risk areas and removing practices with limited evidence-base or efficacy.

Title: Flavivirus infection in the eye and brain and induction of inflammatory responses

Authors: Evangeline Cowell, Amy L Green, Aidan J Norbury, Luke P Kris and Jillian M Carr*

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Neurotropic flaviviruses such as Japanese encephalitis virus (JEV) and West Nile Virus (WNV) can have devastating effects on the brain and the eye. Historically, dengue virus (DENV) is not considered a neurotropic or encephalitic flavivirus, but we have used a brain replication model to study host innate responses since DENV doesn't replicate systemically in immunocompetent mice. Our studies have shown that DENV can infect the brain and eye and move locally from the brain to the eye. Although these tissues are sites of immune privilege, substantial host inflammatory responses are still induced, although traditional antivirals such as viperin, are not as important for controlling infection. Comparison of DENV with Zika virus (ZIKV), which is tropic for the developing brain and eye, highlights differences in induction of key components of the complement system. Our current goal is 3D CT-imaging of the developing ZIKV-infected brain and eye, to align morphological changes with expression of the complement system – which has known roles in neurodevelopment and pathogen control. Outcomes would hope to modulate pathogenic complement responses and prevent immune-mediated tissue damage. Defining these processes might also reflect on neuronal repair following flavivirus encephalitis in an adult brain.

While neurovirulence of flaviviruses is well known, there are no current treatments for flavivirus-induced encephalitis. As we see further incursions of pathogens such as JEV and recurrent emergence of viruses such as Murray Valley Encephalitis Virus, understanding the mechanisms driving serious and life impacting effects of flaviviruses on the brain and eye takes on a new importance for study.

Title: Complex entanglements for Neglect of NTDs

Author: Maxine Whittaker

This paper will explore the many interlocked factors that contribute to the continued neglect of neglected tropical diseases in global, national and local health policy and funding agendas.

Working through the characteristics of the NTDs themselves, programmes developed and implemented to address them, knowledge and research gaps , and sociocultural factors, Maxine will illustrate how NTDs present a complicated situation to be addressed, which makes them a target for neglect, but also what can and should be done to reduce the burden of NTDS globally, often inequitably a burden shared by the poor and disenfranchised.

Title: Regional Public Health Laboratory Network: Regional approach model of Laboratory Systems Strengthening

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The Regional Public Health Laboratory Network (RPHL Network), housed within the Department of Medical Sciences, Thailand Ministry of Public Health, was established in 2019 under the Global Health Security Agenda (GHSA), funded by USAID. The foundational aim was to provide support to the GHSA Member Countries in Asia Pacific region to strengthen national laboratory systems for timely detection of pathogens with potential infectious disease outbreaks and for improved routine diagnostics services. Current membership includes 14 members countries in Asia Pacific (Cambodia, Indonesia, Malaysia, Myanmar, Laos, Brunei Darussalam, Philippines, Singapore, Thailand, Vietnam, Nepal, Timor Leste, Papua New Guinea and Pakistan).

Currently FIND, the global alliance for diagnostics is partnering with RPHL Network with the objectives to formulate the 5 -year Strategic Workplan [2023-2027] to strengthen the secretariat, foster the network membership, develop capacity budling development informed by a regional situational assessment, and to collaboratively mobilise resources.

Three approaches have been adopted for implementation of the capacity building development workplan including regional capacity building/training, direct technical assistance to requested countries, and south -south knowledge sharing.

FIND will continue to partner with RPHL Network to collaboratively build regional capacity within identified key thematic areas including Quality Management, Diagnostic Network Optimisation, Data Management Systems, Disease Surveillance/Sequencing and Governance of laboratory programs within key identified needs of members.

Title: E, the forgotten letter in hepatitis infections

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Hepatitis E virus (HEV) is a leading cause of acute hepatitis worldwide, particularly affecting individuals with weakened immune systems, hematological disorders, and pregnant women. HEV is often underdiagnosed, potentially due to its similarity to other forms of hepatitis but moreover, due to its limited awareness among healthcare providers. This can lead to missed cases and inadequate management of the disease. The use of optimal technologies enhances the ability to diagnose Hepatitis E accurately and promptly, leading to improved patient outcomes.

Title: EXTERNAL QUALITY ASSURANCE FOR HIV TESTING IN THE PHILIPPINES

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External Quality Assurance Program (EQA) is a monitoring tool given by EQA provider to testing facilities that perform HIV testing. Unknown or blinded samples are sent to test the participant's diagnostic capability while assessing its QC methods and procedures from pre-analytical to post-analytical. The NRL STD-AIDS Cooperative Central Laboratory (NRL-SACCL) pilots 3 EQA methods. Proficiency testing is the main EQA activity where panels are sent to multiple sites. The NRL also does periodic site visits especially to participants with fair EQA ratings. The re-testing method is being employed specifically for CrCLs wherein these facilities send monthly samples to the NRL for validation. Year by year, number of laboratories participating in the EQA program for HIV is increasing.

The flow of EQA process starts as the participants test the EQA samples. They will then submit the results online and the NRL will evaluate the results and provide feedback to participants.

For the preparation of samples, NRL gets EQA panels from blood bank facilities here in the Philippines. All panels received are characterized using different HIV and STI serological assays. It includes luminescence assays, rapid test kits, agglutination assays, neutralization, and Western blot. All results are recorded in the main database.

The NRL follows a criteria for grading of results. If a participant obtained 100% correct results, an EXCELLENT rating is given. SATISFACTORY rating for a participant having 1 aberrant result, presence of outliers, or encoding error. If there are 2 or more aberrant results, no result submitted, or using an unregistered kit, the participant is given an UNSATISFACTORY rating.

Results

During the 2022 EQA event for HIV, a total of 1,344 laboratories participated and 95% of them were able to submit results.

Most HIV testing facilities are from municipal health centers (26%). Seventy eight percent (78%) of participants use rapid HIV test kits compared to only 22% who use immunoassay (machine-based). The latest EQA event also shows 37 out of 1,344 (2.9%) participants getting aberrant results while 36 facilities (2.8%) have outliers. They are given post EQA notices by the NRL and are required to submit corrective action report using that will be monitored in the upcoming EQA events.

Common errors include clerical, wrong sample tested, use of unregistered kits, unsubmitted results before the deadline, and lack of equipment calibration which caused outliers.

Future plans include increasing panels in the sample bank by partnering with more blood bank facilities and joining EQA programs with other NRLs in the country so that one over-all system will be used (from registration to releasing of certificates). In addition, the NRL plans to develop more post EQA activities like surveys and re-testing of panels from sites.

Title: Assessing the suitability of finger-prick blood collection devices for HTLV-1 serology and molecular testing in remote and or resource limited communities.

Authors: Fabian Busby, Adwoa Agyapomaa, Nicholas Vandegraaff

Abstract: The detection and quantification of HTLV-1 genomic DNA within leukocytes isolated from blood is an important complement to serology testing for the diagnosis and management of HTLV-1 infection. However, HTLV-1 infection is most prevalent in remote and resource limited communities in Australia and worldwide, presenting significant logistical challenges for specimen collection, stability and transport. Molecular testing also requires concentrated leukocyte genomic DNA, which is not currently collected routinely for HTLV-1 testing.

A range of finger-prick blood collection devices are available on the market that have been developed for the isolation of plasma from blood for the purposes of assessing antibodies or whole virus plasma. Consequently, most are designed only for plasma elution and not intended to stabilise or recover genomic DNA from the residual leukocyte fraction.

The objective of this project was to evaluate finger-prick blood collection systems for their suitability for both HTLV-1 serology and molecular testing. Suitability was assessed based on the ability to collect both plasma and leukocyte fractions, sample input quantity; sample stability, recovery of genomic DNA; and ease of use. This project focused on evaluating three devices with the most potential: the Whatman 903 Proteinsaver Card (Cytiva), the VLPlasma Separation Device (BioPoint) and the cobas® Plasma Separation Card (Roche).

Title: Evaluation of the Diagnostic Capability of two SARS-CoV-2 Rapid Antigen Tests using their cycle threshold values by GeneXpert real time RT-PCR; and How WHO ACT-A project on External Quality Assessment for COVID-19 RDTs improves clinical management of covid-like cases presented to remote healthcare facilities in Samoa.

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The use of antigen rapid diagnostic tests for the detection of SARS-CoV-2 in suspected covid-19 cases is vital for effective patient management and public health mitigative measures. However there has been debate in the literature about the ability of rapid antigen tests to detect SARS-CoV-2 in a number of suspected cases, in which findings of this study strongly correlates with.

This is a sub-study of the WHO ACT-A project, which was coordinated by NRL Australia, in which the main objective is to assess the quality of rapid tests used in low- and middle-income countries during the covid19 pandemic. The sub-study compares the diagnostic capability of the two RDTs SARS-CoV-2 Antigen Rapid Test Kit (Colloidal Gold Immunochromatography) and Sure Status COVID-19 Antigen Card Test used in remote healthcare facilities all over the two most populated islands of Samoa, against their corresponding cycle threshold values by GeneXpert RT-PCR.

A total of 50 nasopharyngeal samples from clinically suspected cases in eight of the twelve remote healthcare facilities which also participated in the ACT-A EQA for COVID19 project, were submitted to the main laboratory for confirmation using GeneXpert RT-PCR. Thirteen of these samples were recorded to be equally positive on both Lepu and Sure Status RDTs, while 37 were equally negative on both.

The verification testing on GeneXpert showed that all 50 samples were positive with lower CT values (E:14-19; N2:15-21) for those positive on RDTs, while higher CTs (E:20-41; N2:21-42) for those negative.

We compared these to the CTs (E:22 average; N:24 average) of the 6 positive NRL-EQA samples that were verified earlier on GeneXpert and realized that the average CTs {E:16.5(14-19); N:18(15-21)} of the thirteen RDT-positive samples are much lower than those of the positive EQA samples.

This concludes that the capability of these rapid diagnostic tests to detect SARS-CoV-2 is very low, and clinical decisions should not be entirely dependent on their results especially during a post-peak period. These findings have been conveyed and discussed with clinical staff in charge of the respective healthcare facilities. Henceforth these medical centres have improved their practice by collecting a second nasopharyngeal sample for PCR confirmation, whenever covid19 is strongly suspected in a RDT- negative sample.

Keywords:

Antigen RDTs, SARS-CoV-2, GeneXpert PCR, Cycle threshold, Diagnostic capability, WHO ACT-A Project, External Quality Assessment

Title: Validation Study of Alternative Instrument-free Rapid HIV Diagnostic Algorithm (rHIVda) in the Philippines

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HIV testing in the Philippines is mostly facility-based with only one National Reference Laboratory (NRL-SLH/SACCL) in the country offering HIV confirmatory testing. This makes it challenging to achieve same-day HIV diagnosis and treatment. To address this issue, the Philippines has been implementing the World Health Organization's (WHO) three-assay HIV testing strategy since 2018, aiming to decentralize confirmatory testing.

The validated rapid HIV diagnostics algorithm (rHIVda) used in the Philippines consists of SD Bioline HIV 1/2 as Test 1 (T1), Determine HIV 1/2 as Test 2 (T2), and Geenius HIV 1/2 as Test 3 (T3). However, the Geenius HIV 1/2 test requires an expensive automated reader, making it difficult to roll out nationwide. To overcome this challenge and improve accessibility and turnaround time, the country plans to validate alternative instrument-free HIV diagnostic algorithms.

The proposed algorithms achieved the WHO-recommended threshold of 99% positive predictive value (PPV). Two algorithms showed no shared false reactivity, while the other two had low shared false reactivity and can be used as backup options. Based on the results, HIV 1/2 STAT-PAK was recommended as T2 due to its high specificity, and QuickProfile can be used as T3.

The validation study used remnant plasma specimens that were confirmed as reactive or nonreactive by NRL-SLH/SACCL's reference gold standard. HIV tests previously approved by the Philippine FDA and NRL-SLH/SACCL will be selected for evaluation. A combination of rapid diagnostic tests (RDTs) and enzyme immunoassays (EIAs) were assessed to ensure scalability and leverage existing laboratory capacity. The tests with the lowest rate of false-reactivity will be chosen for the three-test HIV testing algorithms, following WHO recommendations of using the most sensitive tests as T1 and the most specific tests as T2 and T3.

Title: Is kissing safe? The evidence for oral transmission of Treponema pallidum

Author: Janet Towns[1]

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Syphilis is a stigmatized infectious disease, with anthropological evidence of its emergence dating back millennia. Globally there are 10.6 million incident syphilis cases annually, predominantly in heterosexuals, but in high-income countries men who have sex with men are the primary risk group, with worsening epidemics since 2000 in many global settings. Congenital syphilis continues to be problematic in low-income countries and is re-emerging in high-income countries, including Australia. Syphilis is a complex sexually transmitted disease that has a highly variable clinical course through a number of distinct stages, and can invade and disseminate throughout all body compartments, including across the blood-brain-barrier, within days of inoculation. The evidence for oral transmission of syphilis can be gleaned from historical case studies, epidemiological evidence, and recent studies using modern molecular diagnostics. Six recent studies using Treponema pallidum polymerase chain reaction diagnostics are examined in detail, to gain new perspectives on oral transmission, and on potential future public health recommendations.