INA-RESPOND

INDONESIA RESEARCH PARTNERSHIP ON INFECTIOUS DISEASE

KEMENTERIAN KESEHATAN REPUBLIK INDONESIA

NEWSLETTER

August 2019

Lifestyle and Sports
Exercising for A Better Sleep

Comic Corner
leaders Take The lead...
followers Have The Courage

Real-time implementation of
CDMS during Ebola Outbreak

FROM OUR LABORATORY:
NGS For Diagnosis
of Infection

TRIPOD and INA-PROACTIVE
Studies' Updates

NATIONAL INSTITUTE OF HEALTH RESEARCH AND DEVELOPMENT
MINISTRY OF HEALTH REPUBLIC OF INDONESIA
2019

NETWORK STEERING COMMITTEE MEETING

Jakarta, 5 August 2019

INA-RESPOND newsletter

content

August 2019 Edition | issue #71

FEATURES

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Badan Penelitian dan Pengembangan Kesehatan RI, Gedung 4, Lantai 5.
Jl. Percetakan Negara no.29,
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INA-RESPOND
Newsletter

TRIPOD & INA-PROACTIVE Study Updates

By: Eka Windari R., Lois E. Bang, Maria Intan Josi, M. Ikhsan Jufri, Venty Muliana Sari

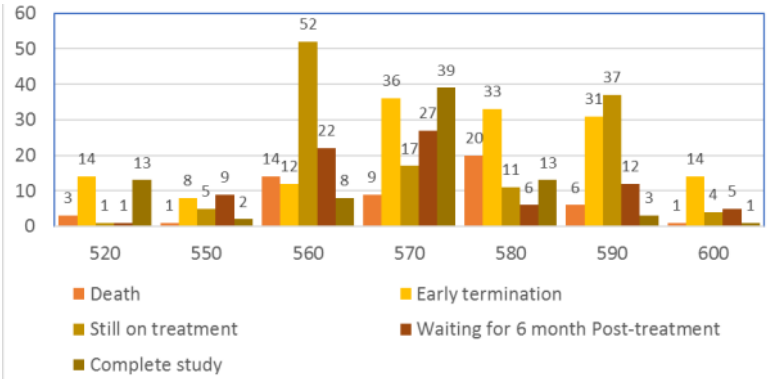


Figure 1.Participant status per site based on uploaded CRF per 31 July 2019

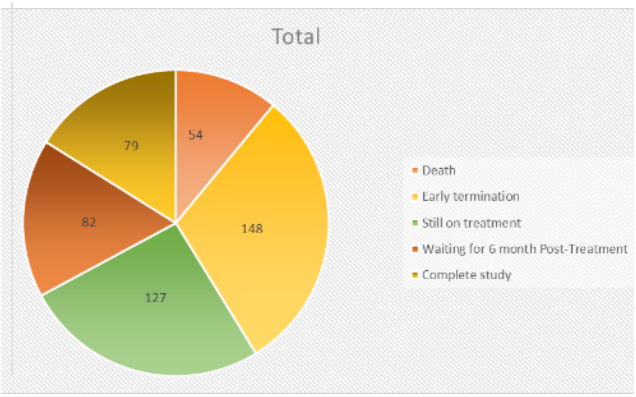


Figure 2. Total Participants Status based on uploaded CRF per 31July 2019

STUDY UPDATES

INA102

PARTICIPANT STATUS

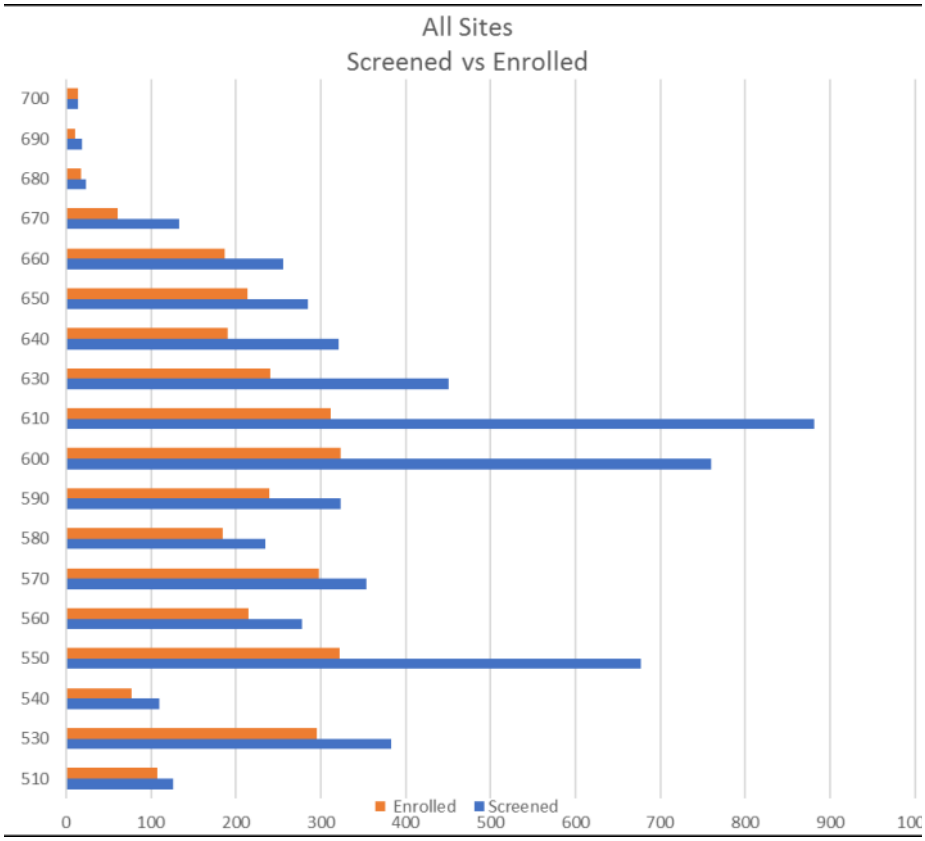
Per 31 July 2019, the total ongoing participants in TRIPOD study are 209 out of 490 enrolled participants. From those 209 current participants, 127 are still on TB treatment while 82 are waiting for 6-month post-treatment visit. Seventy-nine participants have completed the study while 202 participants are terminated early (including death). Therefore, there are still 42,6% of participants from the total enrolled participants in the follow-up status. From the uploaded CRFs, there are 2 participants from site 520 (RS Sanglah Denpasar) who still need to be followed up, 14 participants from site 550 (RSUP dr. Wahidin Sudirohusodo Makassar), 74 participants from site 560 (RSUP dr. Kariadi Semarang), 44 participants from site 570 (RSUD dr. Soetomo Surabaya), 17 participants from site 580 (RSUP dr. Sardjito Jogjakarta), 49 participants from site 590 (RSUP Persahabatan Jakarta), and 9 participants from site 600 (RSUP dr. Adam Malik Medan).

Site	Waiting for Baseline Study Culture Result	Waiting for Baseline DST Result
520 (n=32)	Complete	Complete
550 (n=25)	Complete	Complete
560 (n=108)	Complete	3
570 (n=128)	Complete	Complete
580 (n=83)	Complete	Complete
590 (n=89)	1	1
600 (n=25)	Complete	Complete

Figure 3.Culture and DST results up to 31 July 2019

INA104

Screening and enrollment activities at all 18 sites are ongoing. As of Aug 11, a total of 3,310 subjects were enrolled, which consist of 3,3167 adults and 143 pediatrics from a total of 5,630 subjects screened. The enrollment rate was 58.79% from total screening. Details are shown in Figure 1 on the right.



The enrollment failure rate was 41.21% from total screening, information on the reason for failures are shown in Figure 2 below.

Several site visits for INA-PROACTIVE study has been conducted within this latest one month, the details are:

- 4th Site Monitoring Visit to RS Kabupaten Tanggerang on 15,16,17, & 23 July 2019
- 3rd Site Monitoring Visit to RSUP dr. Adam Malik, Medan on 16-18 July 2019
- 3rd Site Monitoring Visit to RSUD dr. Soetomo, Surabaya on 29-31 July 2019
- 1st Site Monitoring Visit to RSUD TC Hillers, Maumere on 13-14 August 2019
- 1st Site Monitoring Visit to RSUD Abepura, Jayapura on 14-16 August 2019

INA-RESPOND 2019 2nd NSC meeting was held on 5th August 2019 and there where some significant decision was agreed, i.e. propose Sanglah Hospital, Bali as the 19th site for INA-PROACTIVE studies and time frame of enrollment staggering termination for sites that have reached the minimum target based on study projection. The acknowledgment letter has been distributed to sites study team via email. In the NSC meeting forum also announced sites ranked based on performance indicator. The top 3 best sites performance are:

- Site 630: Moch. Ansari Saleh Hospital, Banjarmasin.
- Site 640: St. Carolus hospitals, Jakarta.
- Site 650: Budi Kemuliaan hospital, Batam.

Reason for failures	510	530	540	550	560	570	580	590	600	610	630	640	650	660	670	680	690	700	Total
1. Suspect HIV	1	0	0	16	0	0	0	0	2	11	9	0	0	7	10	0	0	0	56
2. Refuse to consent	3	12	2	6	9	0	0	7	1	5	0	0	7	2	2	0	0	0	56
3. Unwilling to comply with the study procedures	0	25	4	2	18	4	10	0	28	26	0	17	6	2	0	0	1	0	143
4. Plans to move away	2	10	1	11	7	0	4	8	13	6	14	12	6	3	2	0	5	0	104
A. No show	5	33	18	116	5	15	1	21	357	323	79	26	6	13	25	3	0	0	1046
B. Busy (in a hurry)	1	6	7	36	7	14	9	5	11	33	6	63	28	27	10	1	0	0	264
C. Has been enrolled	6	0	0	157	17	20	4	42	24	139	96	13	17	15	22	1	1	0	574
D. Participated in other CT	0	0	0	0	0	0	14	0	0	0	0	0	0	0	0	0	0	0	14
E. Hospitalized or unwell	0	0	0	10	0	0	0	2	0	2	0	0	1	0	1	0	0	0	16
F. Others (e.g. no referral letter from other health facility, equipment trouble)	0	2	1	1	0	3	8	0	1	24	6	0	0	0	0	0	1	0	47
Grand Total	18	88	33	355	63	56	50	85	437	569	210	131	71	69	72	5	8	0	2320

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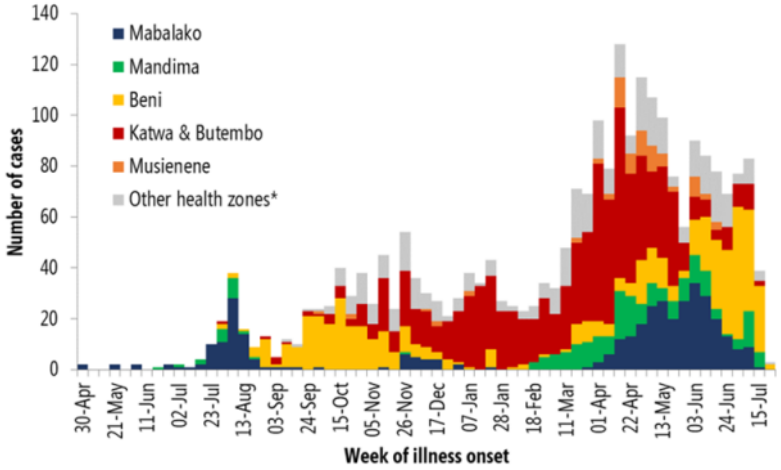
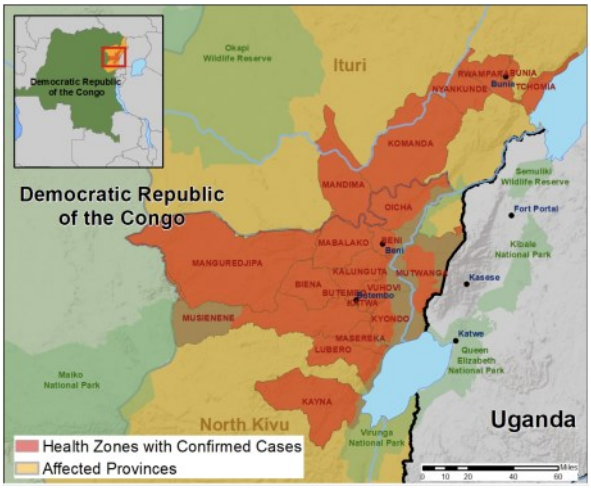
REAL-TIME IMPLEMENTATION OF CDMS DURING EBOLA OUTBREAK

By: Michael Duvenhage [C]





Confirmed and probable Ebola virus disease cases by week of illness onset by health zone. Graph data as of 23 Jul 2019

FROM OUR SPONSOR

The 2018–19 Kivu Ebola epidemic began on 1 August 2018, when it was confirmed that four cases had tested positive for Ebola virus disease (EVD) in the eastern region of Kivu in the Democratic Republic of the Congo (DRC). The Kivu outbreak includes Ituri Province after the first case was confirmed on 13 August, and as of June 2019, the virus spread to Uganda, having infected a 5-year-old Congolese boy who entered Uganda with his family. In November 2018, the outbreak became the biggest in the DRC's history. It has become the second-largest outbreak in recorded history, behind only the 2013–2016 West Africa epidemic.

As of 23 July 2019, a total of 2612 EVD cases were reported, including 2518 confirmed and 94 probable cases, of which 1756 cases died (overall case fatality ratio 67%). Of the total confirmed and probable cases, 56% (1470) were female, and 29% (744) were children aged less than 18 years.

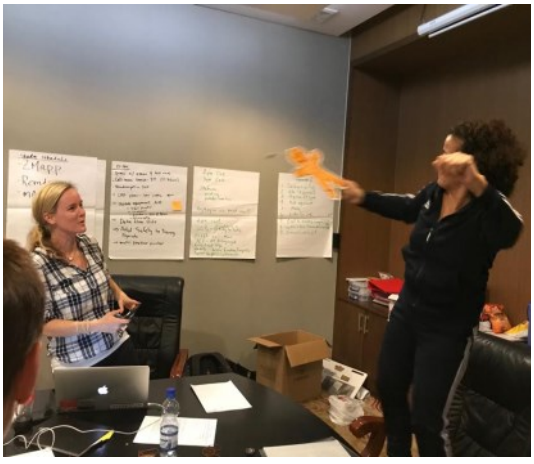
The PALM protocol, which has been named from the Swahili words "Pamoja Tulinde Maisha" (meaning "Together, Save Lives") enrolled the first EVD-positive participant on 20 Nov 2018 at the Beni Ebola Treatment Centre. The PALM protocol was implemented by the INRB (Institut National pour la Recherche Biomedicale), and sponsored by the NIAID (National Institute of Allergy and Infectious Disease).

The primary objective of the PALM protocol is to compare 28-day mortality in EVD patients who receive investigational therapeutics relative to Zmapp. The PALM protocol implemented a 1:1:1:1 randomization to the investigation treatments Zmapp, Mab114, Remdesivir and Regeneron. Additional secondary endpoints include Survival at Day 58 and to evaluate the presence of Ebola viral RNA in the semen of male survivors at discharge or Day 28 (whichever comes first) and Day 58.

The PALM protocol was designed as a multi-outbreak, multi-country protocol with the aim of implementation in additional countries should the outbreak spread.

During the protocol implementation, various discussions were held with regard to data collection strategies. Strategies included either collection of clinical research data at the Ebola Treatment Centers via paper Case Report Forms (CRFs; which then included an off-site double data entry process into the central database); OR directly entering clinical research data into a database deployed on tablets (with a data-sync to the central database). The study team decided that paper CRFs would be the most risk-adverse option due to the following reasons:

- Internet connectivity challenges and downtimes at various Ebola Treatment Centers would disallow continued data collection.
- The outbreak was continuously moving within the country and new Ebola Treatment Centers needed to be added in response in a very timely manner.
- Technical inexperience of study team members in the field with regard to technology devices such as tablets; Team members felt more familiar with paper CRFs.
- Considerations regarding what is called "The Hot Zone." It was going to be difficult to support tablets (for data capture) in a "Hot Zone." It is important to remember what goes into a "Hot Zone" cannot come out.



Study team development of the first batch of Ebola Participants CRFs during a very late night preparing for first participant to be enrolled into PALM

The Data Management process consists of clinical research data collection on paper CRFs at the various Ebola Treatment Centers. The paper CRFs are then scanned and emailed to an online CRF management system developed by the University of Minnesota (UMN). A double data entry process then follows, with the 1st data entry happening in the DRC, followed by a 2nd entry that happens in the USA. Reconciliation of 1st and 2nd data entry happens ongoingly to ensure that data entry mistakes are minimized. Additionally, data queries are generated within the clinical database and released on the paper format to the Ebola Treatment Centers. In response to the data queries, the CRFs are updated/corrected, then rescanned and again emailed to the online CRF management system.

Important considerations needed to be instituted at the various Ebola Treatment Centers considering that data were being collected inside the Hot Zone. One such strategy was the deployment of the CUBE (the Biosecure Emergency Care Unit for Outbreaks) at the Beni site. The CUBE was developed in the aftermath of the West African Ebola Epidemic, drawing from the lessons learned during ALIMA's (The Alliance for International Medical Ac-

tion) response in Guinea in 2014. The CUBE is designed to improve communication between patients, medical teams, and families.

The CUBE offers the added advantage of needing fewer medical personnel. It reduces the risk of exposure and contamination and requires fewer medical materials. Medical personnel benefit from being able to work comfortably, protected from contamination without needing to wear personal protective equipment. It is also essential that clinical research data can be collected outside of the Hot Zone since study team members do not need always to enter the CUBE.

In the event the outbreak spreads, the CUBE has been designed to be easily transportable and can be decontaminated, disassembled and reinstalled in other areas if needed.

As with most clinical trials, sometimes unexpected events happen; however, none of the study team members realized how volatile the situation was until the attacks happened on the Ebola Treatment Centers at the Beni and Katwa sites on the evenings of 25 and 27 Feb 2019.

Katwa attack on 25 Feb 2019

Burned CRFs during the attack

Although many CRFs were burned during the attacks, fortunately very little clinical research data was lost since most of the CRFs were already submitted into the online CRF system and available electronically (while the paper copies were burned). It was very fortunate that no primary outcome data of the study was lost due to these two attacks.

The PALM protocol has currently enrolled 676 participants at the 4 Ebola Treatment Centers (Beni, Butembo, Katwa, and Mangina) and will likely complete enrollment of 725 EVD participants by mid-Aug 2019. It is planned that rapid analysis of the study data will occur very soon after the primary study endpoint of Day 28. Really important clinical decisions will then be made with regards to the further deployment of the investigational drugs within the context of the current Ebola outbreak in the DRC, as well as any future Ebola outbreaks.

Biography

Michael Duvenhage has 19 years' experience in Clinical Data Management and is currently employed by Leidos Biomedical Research, Inc. and functions as the Clinical Trials Data Operations Manager within the Division of Clinical Research at the National Institute of Allergy and Infectious Diseases. In his role, he is supporting data management operations for emerging infectious diseases and other infectious diseases within Africa, Asia, and the USA.

INA-RESPOND Newsletter

LEADERS TAKE THE LEAD… FOLLOWERS HAVE THE COURAGE

By: Aly Diana

I just joined a leadership workshop, and from my self-reflection, I concluded that all of us is a leader to some extends. Therefore, we need to cherish our roles as a leader. One thing that the cartoon is right though is that there will be no leader if there is no follower. And to be the best leader, although there is some guidance for it, by the end of the day it's most likely based on the perceptions of the followers. Also, situations where we are in, make a difference. Overall, leadership depends not only on the leader but also depends on the followers and the circumstances.

Different followers and different situations need different leadership styles. However, being great leaders is not only about leadership style or type but also understanding who our followers are and what conditions they are facing. Nevertheless, there is another significant step to take before trying to understand others - we must learn to know better about ourselves. Self-awareness is essential, and please remember that how we see ourselves is also a matter of perception. We usually compare ourselves with the values and the standard that we believe. But is it true? Is it correct?

We hear a lot of theories, and I am sure that we have heard or read things written here somewhere else before this. However, no matter how much we read/learn, practicing is the primary key. Nothing will change if we don't practice what we learn. Let's start with the basic to increase our self-awareness: we can make a daily journal, learn from experiences, face new/unusual experiences, pay attention to reflections from others (remember that not everything negative said to our face is mere critiques), and make self-assessment inventories by asking ourselves – have I improved from yesterday? Whatever happens, please try to be grateful for the day, to embrace our achievement, and to plan things that we can develop the next day. Being a happy and confident person seems to be a significant trait in making ourselves a good leader.

Another way to help us understand ourselves is by using the Myers Briggs Type Indicator (MBTI). MBTI may help us to identify how consistently we prefer one tendency over others, help us to understand human behavior, and then learn more about other preferences too. The main postulates of the theory are: 1) people have inborn behavioral tendencies and preferences; 2) each type and individual has unique gifts – there is no right or wrong type, no better or worse combination of types in work or relationships; 3) each person is unique; and 4) the purpose of knowing about personality type is to help us understand ourselves and to enhance our relationship through appreciation of individual differences. Knowing others help us to be less defensive when we are involved in a disagreement. Others have different preferences and different ways of dealing with things. By understanding this, life will feel much more manageable.

OH, I'VE ALREADY FOUND A NATURAL BORN LEADER. NOW I'M LOOKING FOR SOMEONE TO FOLLOW BLINDLY.

COMIC CORNER

Extra note: Most of us is a leader in some sorts, but we are also a follower in some ways. So, for all followers in the world, there are some tips to deal with our leaders: find our leaders' "weakness," his/her favorite way communication, and his/her ideal time to receive inputs. There will be some trials and errors, but if we keep trying and then do some more self-reflection, we will find an excellent way to get what we expect from our leaders. If change doesn't happen, it's most likely because we don't want change to happen.

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INA-RESPOND Newsletter

EXERCISING FOR A BETTER SLEEP

By: dr. Monica Surjanto

Introduction

Exercise can be a vital contributor to your sleep health. Overall, people tend to sleep better when their lifestyle includes some physical activity and exercise. For the last decade, exercise has been recommended as a significant factor for improving health in the general population, elderly and in many groups with chronic diseases such as obesity, type 2 diabetes, cardiovascular diseases, depression, and even cancer. Exercise has also been found beneficial for reducing weight, preventing pain, improving mood, and also enhancing the quality of sleep in patients with insomnia. Having sufficient sleep has been recommended as insufficient sleep has been identified as an associated risk factor for major health concerns such as obesity, type 2 diabetes, cardiovascular diseases, depression, and accidents. Sleeping duration for 7-8 hours is associated with lower risks of morbidity and mortality.[1] For every age groups, there is the sleep recommendation as we can see below. [2]

The effects of exercise on sleep can be affected by many factors, such as individual characteristics and exercise protocol. Personal characteristics include sex, age, fitness level, type of sleeper and Body Mass Index (BMI), whereas exercise protocol includes acute or regular, aerobic or anaerobic, and different characteristics such as intensity, duration, environment (indoor or outdoor, hot or cold environment) and the time of day.[1]

Average Sleep Needs by Age	
Age	Hours Needed
Newborn to 3 months old	14 - 17 hrs
4 to 11 months old	12 - 15 hrs
1 to 2 years old	11 - 14 hrs
3 to 5 years old	10 - 13 hrs
6 to 13 years old	9 - 11 hrs
14 to 17 years old	8 - 10 hrs
Young adults (18 to 25 years old)	7 - 9 hrs
Adults (26 to 64 years old)	7 - 9 hrs
Older adults (65+ years)	7 - 8 hrs
Source:National Sleep Foundation	

The Stages of Sleep

The stages of sleep may be divided into two phases: Rapid eye movement REM) sleep and non-rapid eye movement (NREM) sleep. According to the American Association of Sleep Medicine (AASM), the manual scoring of NREM is divided into N1, N2, N3 in the basis of EEG waves. REM sleep has two phases – Tonic and Phasic.[3]

Effect of Exercise on Sleep

According to the American Sleep Disorder Association (ASDA), exercise is one of the non-pharmacological intervention used to promote sleep.[3] In 2003, National Sleep Foundation reported that compared to those who reported exercising <1 time per week, those who exercised more frequently had fewer complaints on almost every index of disturbed sleep, including fair or poor sleep, symptoms of insomnia, difficulty falling or staying asleep, waking up feeling unrefreshed, day time sleepiness, or any reported sleep problem.[4]

The possible mechanisms effects of exercise on sleep are:[3]

Impact on Mental Health

The result of exercise on mental health is closely related to depression and anxiety. The number of awakenings in the night is one of the important indicators of anxiety which is

effectively decreased by exercises. Many studies have been published about the positive effect of acute exercises in decreasing trait anxiety.

Effect on Thermoregulatory Mechanism

Temperature regulation is an important aspect of homeostasis. Modulation in core body temperature affect the sleep parameters, which can be brought about actively (by exercises) or passively (by a warm bath, thermo suit, effects blankets, etc.)

Effects on Circadian Rhythm

Circadian rhythm is the 24-hour interval clock which responds to endogenous factors like core body temperature and exogenous factors like the light. A study reported that daily exercise of moderate-intensity had differential effects on circadian melatonin rhythm, rectal temperature during nocturnal sleep, sleep stages, and heart rate variability depending on the time of day the exercise is performed. The interpretation of these results suggests that the timing of exercise is vital for sleep quality. The authors concluded that exercise earlier in the day might improve the quality of nocturnal sleep because exercise stimulates the sympathetic nervous system.

The effect of exercise on improving Sleep Onset Latency (SOL) and decreasing Wake After Sleep Onset (WASO) was found positive when the exercise took place 4-8 hour before bedtime, and negative when the exercise was performed more than 8 hours or less than 4 hours before sleep. However, The National Sleep Foundation has amended its sleep recommendations for good sleepers to encourage exercise without any caveat as to the time of day as long as this is not at the expense of sleep duration. Furthermore, once the working day is over, the evening is a moment when exercise can be incorporated into the daily routine as a leisure activity.

Studies about Exercise and Sleep

Many studies support the use of exercise to improve sleep quantity and quality. One study demonstrated that 12 weeks of exercise training, three times per week (30 min of continuous aerobic exercise with 60-80% VO2 peak and 20 min of resistance training) increased sleep duration and variables of sleep quality in adolescents. These investigators found exercise training to decrease NREM stage N1 (very light sleep) while increasing REM sleep, sleep continuity, and sleep efficiency when using polysomnography.[5]

A review by Lang et al. found that participants who engaged in high levels of physical activity were more likely to experience better sleep quality. Harp evaluated the chronic effects of exercise on sleep in young adults who participated in a 15 -week aerobic exercise intervention and completed sleep quality questionnaires, at the start and end of the study. Harp found that age, gender, and body composition are significantly related to sleep quality. Importantly, participants classified as overweight or obese were found to experience more inferior sleep quality than those of leaner body composition. However, the author did not find 15 weeks of exercise to change sleeping patterns for the majority of the participants. Instead, those who were considered overweight/obese before the study showed improved sleep quality, likely the result of a decrease in body fat percentage from participating in regular exercise.[1]

Exercise and sleep apnea

5 to 10% of adults are affected by sleep apnea. Most of them are males and those who are overweight or obese. Epidemiologic studies have shown an association of exercise with reduced symptoms and diagnoses of sleep apnea. It seems that combining both exercise and weight loss with CPAP (Continuous positive airway pressure) may provide the most effective treatment for many patients with obstructive sleep apnea. From a clinical point of view, two recent studies have shown that exercise may improve the severity of sleep apnea by up to 50% of the AHI (Apnea-Hypopnea Index), independently of the effect of weight loss.

What exercise to recommend for sleep-disorder patients?

Exercise needs to be progressive in patients suffering from sleep disorders, starting at low intensity and preceded by a physical/cardiovascular checkup. Growing epidemiological evidence indicates that short duration sleepers and those with sleep disorders are at higher risk of sudden cardiac death, coronary heart disease, myocardial infarction, angina, stroke, or diabetes. Moreover, exercise must be progressive and initially moderate to improve endothelial function and decrease the risk of death. Compared to resistance exercise, aerobic exercise appears to be more beneficial for improving endothelial function.

Conclusion

Exercise is a positive behavioral modification tool for all age groups to bring about an improvement in sleep quality. Following practical recommendations, exercise training could be prescribed as a non-pharmacological treatment of sleep disorders.

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INA-RESPOND Newsletter

NGS FOR DIAGNOSIS OF INFECTION

By: Ungke Anton Jaya

In the last decade, molecular testing has been the chosen method to identify pathogen causality of infection due to highly sensitive, specific, and faster turnaround time compared to classical culture or serology assay. The molecular assay also has the capability to diagnose an organism that difficult to grow in routine culture media due to being fastidious or early administration of broad-spectrum of antimicrobial drugs. The molecular assay is based on the detection of the genetic material of the pathogen. The powerful of PCR to amplify from a single copy of the target genome to billion copies just by conducting 40 cycles of amplification. PCR is highly specific to detect down to species level or even strain level of the pathogen. The complexity with PCR occurs when a disease shows non-specific symptoms, which creates a lot of possibilities of suspected pathogens to detect and require many PCR assays. Unfortunately, the specimen is often limited as well as the time as doctors need to start the patient's treatment immediately. A similar situation also applies in an outbreak when the health authorities need to identify the pathogen causing the epidemic to start the mitigation action. As an example, a recent study at INA-RESPOND has required more than 40 PCR assays to detect various pathogens of infection in around 1,400 acute febrile cases requiring hospitalization to get about 68% of infection diagnosed.

What if we have a molecular tool that can detect all kinds of genetic materials present in the sample, including virus, bacteria, or fungal target, so we can tell which pathogen is responsible? Would it help? That approach is called metagenomics, which is interpreted as analyzing a mixture of microbial genomes without separating the genomes or culturing the organisms.

NGS was born

In 2005 The first commercial Next Generation Sequencing (NGS) platform was initially released by 454 Life Sciences with the GS20 sequencer followed by other instrument brands, Ion Torrent PGM, and Illumina and PacBio. The NGS, also known as second-generation sequencing, use sequencing by synthesis platform. This offers more robust, faster, and accurate sequencing capacity over the preceded sequencing by Sanger method for whole-genome sequencing (WGS) or metagenomics analysis NGS (mNGS).

The general protocol for NGS application starts with extraction of genomic material from a sample including genomic RNA, DNA, plasmid, or mitochondrial DNA. Converting to cDNA is often required to sequence the RNA target. All DNA will be enzymatically/physically spliced into thousands to billions smaller fragment typically 200-400 bases. The fragmented DNA is called "library" and work as template injected into the flow cell/chip. Considering the high cost per run, the library will have to meet several quality control parameters, i.e., high quantity, high purity, and the expected length of the DNA fragment template for successful run. The NGS instruments will sequence each fragment independently and simultaneously from forward and reverse direction. Therefore, NGS is called a high throughput or massively parallel sequencing method. The sequence output of each DNA fragment is called a read that determines the capacity of various NGS instruments to produce sequence data in a single run. For example, the MiSeq-Illumina can produce 1 million to 25 million reads per run depend on the library preparation kit. This is just halfway since the data output from the instruments will need a series of bioinformatics software to get meaningful information, a process called bioinformatics pipeline. Data from NGS run is typically a large file, for example, one sequencing run using MiSeq - a widely used NGS instrument from Illumina, can produce 0.3 to 24-gigabyte hard disk size containing 15-25 million sequence reads. Large data requires large infrastructure data storage, and the analysis requires powerful computational to run many steps of bioinformatics analysis (Figure 1).

The NGS platform has increased the speed and accuracy of the sequencing. To sequence of the first human genome was around 3 billion base pairs, had taken more than ten years and cost billions of dollars with Sanger sequencing. Today with NGS instruments, a researcher can do it in a week and costs less than two thousand US dollars. The capability of NGS to sequence all DNA in the sample give researcher around the world a euphoria to sequence sample of interest to know what organisms are in the sample, from dust in air conditioner to dust from aerospace, microbiome in our human gut or in water sewage, and microbe in deep seawater or in Antarctic's glacier. Very interesting.

NGS for infectious diseases

Today, the NGS application can be done using various choices of sequencing instruments that differ in the output of size, accuracy (error rate), and capability to sequence long sequence. Cost per reaction is in parallel with the quality output.

In infectious diseases research, NGS has been widely successfully applied using DNA from a positive bacterial culture of virus culture. Whole Genome Sequencing (WGS) application with NGS using bacteria isolate can be used to identify antibiotic resistance gene that can guide doctors for patient treatment, track down hospital infection control, surveillance programs, and community outbreak investigations. For examples, NGS was used to track origins of a series of nosocomial carbapenem-resistant Klebsiella pneumoniae infections or the origins of the 2010 Haitian Vibrio cholerae epidemic

Using the virus isolates or specimen with high viral load, NGS was successfully used to get the data of whole genome virus even when there is more than one virus variant in the sample, for example, the minor variant of antiviral resistance in HIV. NGS has the sensitivity to detect low variant as low as 5% of the viral population while with Sanger method, the sensitivity is 15-20%. This can be applied to other genotyping viruses with drug resistance mutation like hepatitis and Influenza. A challenge when using NGS in assessing minor variants is distinguishing true mutations from artifacts generated during PCR amplification, library preparation, or sequencing where many researchers are still struggling to find solution by optimizing the assay or through the bioinformatics stage.

Diagnosis of infection using NGS from direct clinical sample

With the speed, accuracy, and robustness of NGS, sequencing of pathogen DNA directly from clinical samples is really in high expectation as it offers the possibilities of rapid diagnosis.

Unfortunately, until now, the diagnosis from the clinical samples is still proof-of-concept studies due to the major challenge to make the NGS assay powerful enough to pick the tiny amount of bacterial or viral genome among the abundance of dominating human genome. With genome pathogen of interest that may represent 0.01% of the total DNA, it is like finding a needle in-haystack situation.

Current major approaches are to enrich the input material for target sequences (viral or bacterial, fungal) and, or depletion of non-target DNA (human, other bacteria. This involved a combination of many approaches. Clinical samples can be pre-treated to purify viral particle before genomic extraction by filtration, ultracentrifugation, density gradient centrifugation or selectively lyse human lymphocytes using Saponin or chemical agent. DNA/RNA extraction can be followed by pre-treatment of the sample with nucleases to remove host nucleic acids or use of ligation of adaptors PCR amplification (i.e., 16s RNA bacteria metagenomics). Newer methods have used hybridization approaches to capture viral nucleic acids with oligonucleotide probe as baits that are designed based on current knowledge of the bacterial or viral sequence (BacCap Seq/ VirCapSeq kit) (Wei Gu 2019). At the bioinformatics stage, computational tools have been developed for subtracting host sequences from the initial read pool containing mixed human and microbial sequences. Although second-generation NGS has been successfully used to identify the pathogens in direct clinical specimen in many studies, the high cost for a single run (can hold many samples per run), days of analysis in the machine and lengthy bioinformatics analysis has put the NGS platform as a drawback in practicality for diagnosis of infection in hospitals. By the end of 2018, three laboratories in the US had offered NGS service for diagnostic. The clinical microbiology laboratory at the University of California San Francisco provides mNGS assay on cerebrospinal fluid for encephalitis and meningitis, ID byDNA Inc. provides similar mNGS testing on bronchioalveolar lavage fluid for lower respiratory tract infections, and Karius Inc. targets microbial cell-free DNA from plasma. (Greninger 2018)

The future of portable NGS instruments

In 2014, The Oxford Nanopore Technologies (Oxford, United Kingdom) released MinION, GridION, and PromethION, recognized as the third generation of sequencing using nanophore platforms. The Nanophore offers several advantages over the second generation NGS-sequencing by synthesis platform. The Nanopore sequencer is small size, portable and low-cost sequencers. The most exciting features of MinION sequencer is that it can provide a long read data, real-time, and on-site analysis of any genetic material which should be useful, especially for clinical applications. However, the nanopore approach currently required more DNA mixture (around 1ng), produce more sequencing errors, and has lower throughput sequence data. This makes the existing NGS protocol required optimization for MinION application.

A study using pooled plasma specimen of 5 septic patients has been applied on MinIOn. Without any enrichment, the DNA was run for 1 to 8 hours in MinIOn resulting in 99 % of reads is still host genome but the 1 % to staphylococcus and streptococcus group. This result also comparable to the more accurate NGS Illumina platform. (Stevens P et.al 2017)

Using 16S rDNA amplification followed by NGS MniNION sequencing has correctly detected 20 bacterial communities in a mock sample with data reads obtained only 5 minutes after the sequence data obtained. The result was consistent with the results of those obtained from 4 hours

of Ion PGM sequencing. The same protocol identified major bacterial pathogen in a clinical sample using a clinical sample extracted from the empyema patient's pleural effusion. The results are comparable to conventional 16S rDNA sequencing results using an IonPGM sequencer. (Mitsuhasi et al. 2017). The commercial kit to perform bacterial 16sRNA amplification for mNGS has been available from several manufacturers.

MinION correctly identified pathogens without culture in ten heavily infected (107 cfu/mL) clinical urines from patients. The protocol also correctly identified 51 out of 55 acquired resistance genes detected in parallel by Illumina sequencing. The protocol still needs improvement to reach sensitivity since bacteriuria is determined as 105 CFU/ml) (Schimdt 2017).

Using blood-derived specimen, a protocol using by MinION nanopore sequencing has successfully detected chikungunya virus (CHIKV), Ebola virus (EBOV), and hepatitis C virus (HCV) from four human clinical samples. The specimen has been previously confirmed by specific real-time for each virus with genomic titers in specimen ranging from 107–108 copies per milliliter in two EBOV patients with acute hemorrhagic fever and an asymptomatic blood donor with CHIKV. Virus was detected within 4 to 10 min of data acquisition. Although the identification of correct viral strain is permitted within 6-hour turnaround time of the test the drawbacks are high error rate (10–30 %,) and low throughput 100.000 reads/flow cells that may challenge the application for mNGS application Figure 1 (Greningger et al. 2015).

Bioinformatics analysis

One data output from NGS (reads) are generated from the instruments, and the file needs to go through a series bioinformatics application to generate meaningful information. The bioinformatics part has more advance than the assay protocol. Output sequence is mapped to human genome and subtracted for example using minimap2. The unmatched reads are then mapped to the existing pathogen genome database similar to BLAST analysis. Some popular application for mNGS are SURPI (sequence-based ultrarapid pathogen identification), Kraken, Taxonomer and GenomeSync database http://genomesync.org/) for 16s RNA bacteria, while for MinION Nanopipe or Onecodex.com for metagenomics analysis which user can upload FASTQ file and get the highly matched pathogen sequence in the database.

Metagenomic NGS for pathogen identification has some drawbacks. Due to the sensitivity of mNGS, the sequence data is prone to sequence from contaminating microbes that may be present in reagents, the environment, or normal human flora. Strict workflow quality control procedures are needed to maintain a sterile and nucleic acid–free as possible such as the use of negative controls and periodic swipe tests to check for cross-contamination or false-positive results.

Data Interpretation

Typical mNGS's result after bioinformatics analysis will show that our sample contains genome that matches many pathogens at species or higher level of taxonomic classification. In general, the more abundant sequence read that matches similar organisms (sequencing depth) or the longer size of genome fragment (sequencing coverage) that matches a reference sequence of an organism, the more confident the results are. Newly identified pathogen from the NGS sequence can be reconfirmed by PCR.

In Indonesia, MiSeq Illumina instruments and also IonTorrent PGM including all the required reagents are available from the local vendor. The users at many research institutions mostly run for whole-genome sequencing approach for pathogen characterization, genetics inherited disease or cancer while mNGS work for infectious disease is still rare. Just recently, a local vendor for MinION is available in Indonesia that will open the possibility for more application in research and diagnostic tools. The bioinformatics analysis should not be a big obstacle since cloud computing is available through open -source software or collaboration with other institution worldwide.

As a conclusion, although metagenomics NGS (mNGS) application for diagnostic of infection is still at proof of concept stage, but the speed of development is high-speed worldwide. For Indonesia, it would be essential and exciting research to use copyright since the tropical region is hypothetically highly suspected for various endemic pathogens that have not been well discovered.

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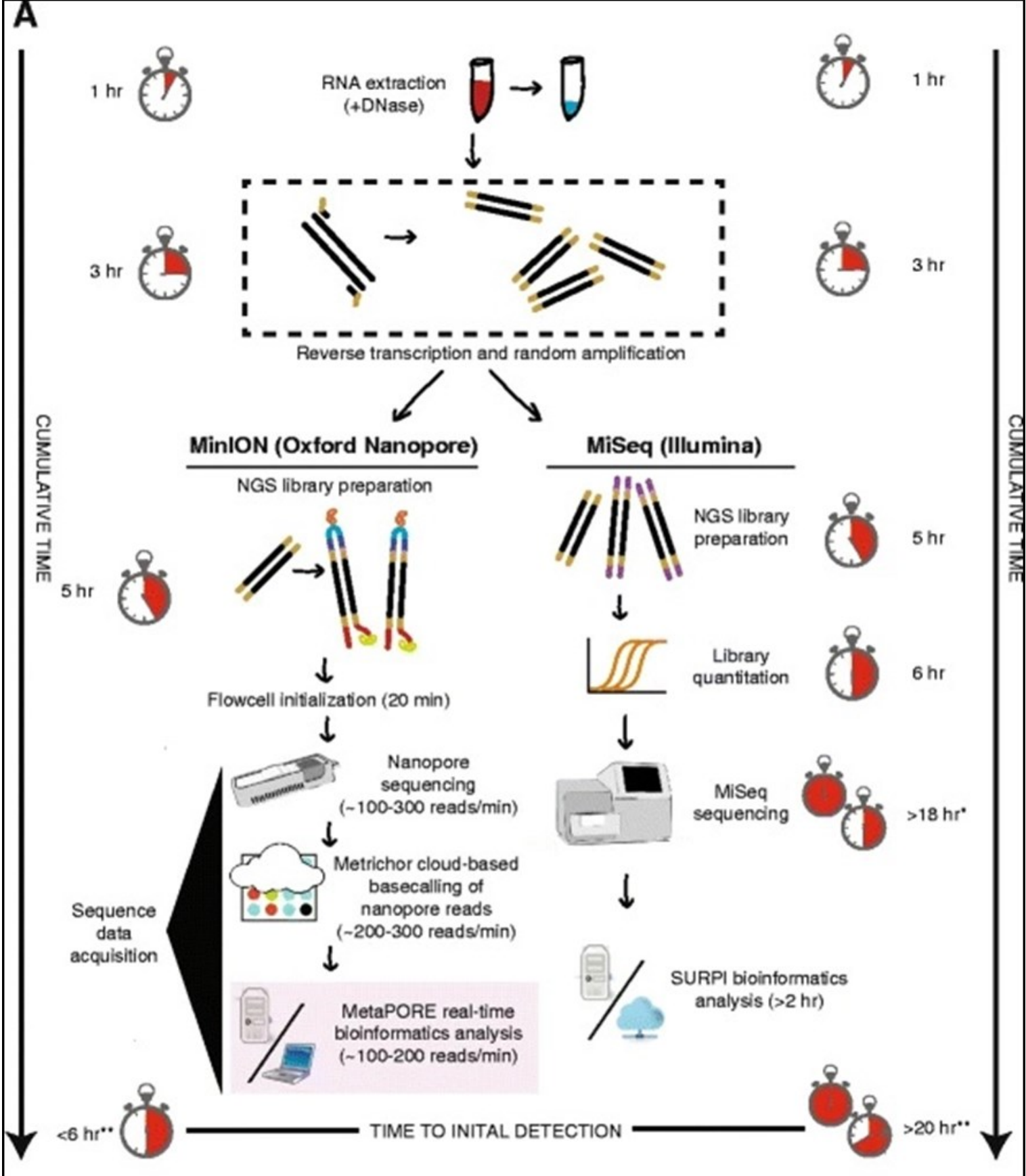

Figure 1. Example of protocol for virus metagenomics sequencing using MinION and Illumina and the MetaPORE bioinformatics pipeline. An Overall workflow. B. Steps in the MetaPORE real-time analysis pipeline. (copyright by Greninger et al. 2015)

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