INA RESPOND
INDONESIA RESEARCH PARTNERSHIP ON INFECTIOUS DISEASE

INA-RESPOND Secretariat. Badan Litbangkes, Kemenkes RI, Building 4, Level 5,
Jl. Percetakan Negara No. 29, Jakarta, 10560. Phone: +62 21 42879189.
Email: INA.Secretariat@s-3.com. Website: www.ina-respond.net

NEWSLETTER

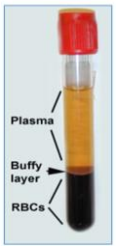
Issue #19
April 2015

IN THIS ISSUE

POTENTIAL RESEARCH WITH PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMC)

by Ungke Antonjaya

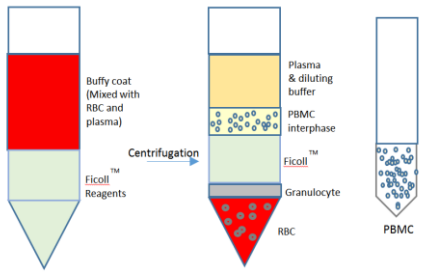
Currently, INA RESPOND is conducting two clinical studies, AFIRE and SEPSIS, at its hospital sites. Both studies collect buffy coat, plasma and/or serum as the main biological sample for laboratory testing as well as other types of samples based on the diseases symptoms. Why buffy coat and what can we do with it?



Picture 1 Separation of Buffy Coat from Whole Blood

Buffy coat is a blood fraction that sits in the middle ring when an anti-coagulated blood tube goes through centrifugation; the upper part is the plasma, and the bottom part is the packed red blood cells (see picture 1.) Here the buffy coat looks reddish because centrifugation cannot completely separate it from plasma and red blood cells. The buffy coat fraction consists of all various types of leucocyte (PBMC and Granulocytes) and platelets.

The PBMC is like an aircraft's black box that stores all package of information about host immune response after experiencing an infection attack. With the currently available advance technologies, PBMC is becoming precious biological sample for research when the sample has clinical information or epidemiological data linked to it. This is exactly what we are doing in all INA-RESPOND studies.



Picture 2 Separation of PBMC with Ficoli Reagent

PBMC is isolated from other leucocytes and platelets through widely-used gradient separator reagents using centrifugation like Histopaque or Ficolpaque™ (see picture 2.) PBMC contains lymphocyte as the majority (70-90%), monocyte, and dendritic cells.

Lymphocyte can be further sub fractionated by its three components: T cells (T helper, T cytotoxic and T suppressor and T regulator) as the major components, B cells (known as source of humoral immune response), and NK cells.

Each type of cells plays typical role by producing specific proteins as 'weapon' for fighting an infection, such as production of cytokines, chemokines, or inflammation/ anti-inflammation signaling factors and antibodies. They can also respond by multiply themselves rapidly. All those cell types in the PBMC respond to an infection like an orchestra.

[continue to page 4]

Every year, the World Health Organization selects a priority area of global public health concern as the theme for World Health Day, which falls on 7 April, the birthday of the Organization. The theme for World Health Day 2015 is Food Safety, a theme of high relevance to all people on the planet, and multiple stakeholders, including government, civil society, the private sector, and intergovernmental agencies. World Health Day 2015 is an opportunity to alert governments, manufacturers, retailers and the public to the importance of food safety—and the part each can play in ensuring that the food on peoples' plates is safe to eat.

insight

Last year we had Site Profile section in our newsletter, and we received interesting feedback from our readers. To follow up, this year we decided to focus our interest to the Steering Committee member at sites. We have conducted interviews with the network's SC member at sites, so you can get to know them much better. Check them out! ☺

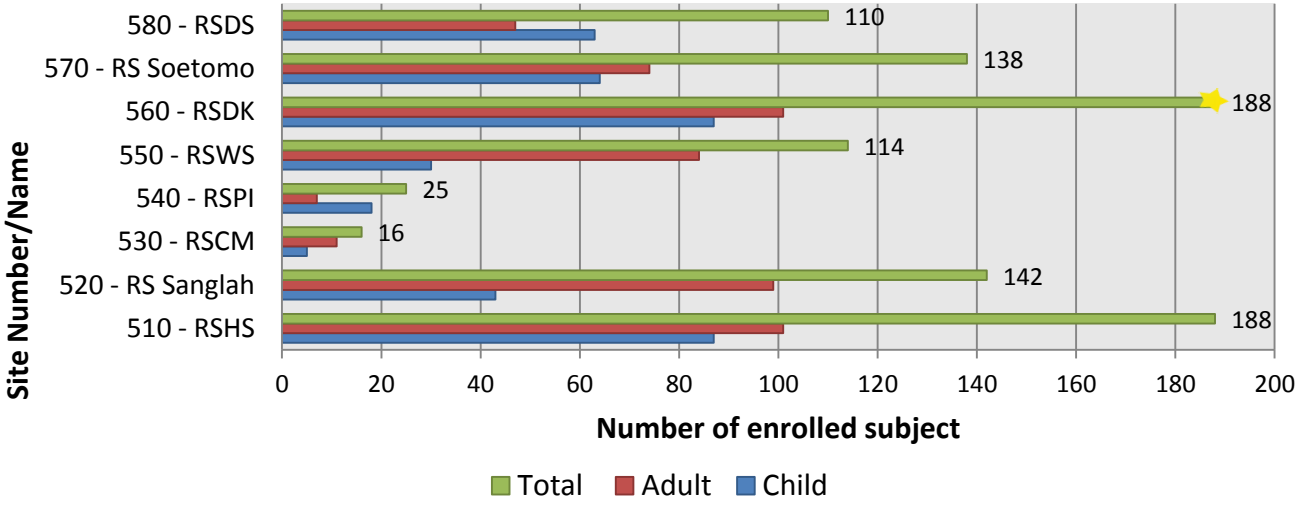
Studies' Progress and Updates

by dr. Anandika Pawitri,
dr. Nurhayati,
dr. Retna Mustika.

SPV 2, RS. Sardjito, Yogyakarta

AFIRE STUDY

Up to March 29, 3,081 patients had been screened. 921 subjects had been enrolled (524 adults and 397 children). Description of screening and enrollment progress can be seen in the chart below:



Detailed screening and enrollment progress is available in portal folder: Studies\INA101\Screening progress.pdf or go to the following link: https://ina-respond.s-3.com/EdmFile/getfile/797233

*510– RSUP dr Hasan Sadikin, Bandung
520 – RSUP Sanglah, Denpasar
530 – RSUPN dr Cipto Mangunkusumo, Jakarta
540 – RSPI Prof Dr Sulianti Saroso, Jakarta
550 – RSUP dr Wahidin, Makassar
560 – RSUP dr Kariadi, Semarang
570 – RSUD dr Soetomo, Surabaya
580 – RSUP dr Sardjito, Yogyakarta

For further information on this study,go to http://www.ina-respond.net/afire-study/

SEPSIS

To date RS Wahidin Sudirohusodo, Makassar, the first site activated for Sepsis study, has screened 15 patients and enrolled 2 adult subjects. The patient cannot be included in the study mainly because they don't meet the inclusion criteria. As for the exclusion criteria, 50 % of the screened patients have been hospitalized more than 72 hours in other hospitals. CRA visited RS Sardjito, Yogyakarta on Site Initiation Visit (March 31 – April1). All essential documents are mostly complete, and we are hoping that the site will be ready for enrollment in 2 weeks. Jakarta site, RSCM, is preparing itself by recruiting new RA and submitting protocol to local IRB.

ReDEFINe

Under this study, INA-RESPOND is involved in the study initiation visit, study monitoring, and DSMB. The site started screening in December 2014, and as of March 7, 2015 a total of 33 patients were screened, from which 17 subjects were enrolled. As for SAEs, up to now we have had 5 cases, from which 3 have been reported.

The DSMB members had a closed-session meeting at the Secretariat on March 11 and they are planning for another meeting on March 26. The upcoming meeting with include discussion with the study investigators and sponsor. The 2nd Site Monitoring Visit (SMV) is scheduled for April 15-17.

Birthdays and Celebrations!

ApRiL

- 4 April – **Ms. Hofiya Djauhari**, Msi. (INA101 Lab Technician at site 510)
- 6 April – **dr. Heni Kismayawati** (National Institute of Health Research and Development/ *Badan Litbangkes*)
- 8 April – **Mr. Ungke Antonjaya** (INA-RESPOND Secretariat)
- 21 April – **Mr. Budhi Kusnadi** (INA-RESPOND Secretariat)
- 22 April – **dr. M.M.D.E.A.H. Hapsari**, Sp.A(K) (INA101 Co-PI at site 560)
- 25 April – **dr. Nugroho Hari Susanto** (INA-RESPOND Secretariat)
- 27 April – **Prof. dr. Emiliana Tjitra** (National Institute of Health Research and Development / *Badan Litbangkes*)

Congratulations to our last month's quiz winner, **dr. Yan Mardian** (INA101 Research Assistant at site 580.) For those who have not yet won, keep on following our newsletter for more fun quizzes, and send your answers before the submission date passes.

Save The Date

The SEAICRN (The South East Asia Infectious Disease Clinical Research Network) has a collaborative partnership with INA-RESPOND network and will hold the **SEAICRN Annual Meeting** on 2-3 June 2015 in Jakarta.

This year we will have oral and poster presentation in the meeting, and we welcome any scientific studies that you did or you are doing. You don't have to be an INA-RESPOND study team member to participate in this event. The submitting/ presenting author does not need to be the primary author or the one who has made the most contribution to the paper. The presenting author is the one who intends to personally present the poster at the meeting.

Please be informed that the presenting author needs to submit the abstract on May 1, 2015 at the latest. We have prepared 8 slots for selected presenting authors to attend the SEAIRCN Annual Meeting in Jakarta (travel and accommodation covered). For more information, please contact INA-RESPOND secretariat or send email to INASiteSupport@s-3.com.

Network Steering Committee Meeting and Network's One-Day Seminar

The next NSC Meeting will be held on **29 April 2015** followed by a one-day seminar on **30 April 2015** at Hotel JS. Luwansa, Jln. HR. Rasuna Said Kav. C-22, Jakarta Pusat, 12940, Indonesia.

FOR MORE INFORMATION

Please contact Mr. Dedy Hidayat or Ms. Yayu Nuzulurrahmah at +62 21 42879189 ext. 102 or 112 during office hours (08.00 – 16.00)

It is very interesting that against each pathogen, T cells and B cells respond uniquely, and this unique response pattern is the most important piece of information to understand the disease progress. Understanding the disease progress better can help us find better therapy or protection for that particular infection. Referring to picture 2, PBMC represents the human immune response, as the response's producer, while the plasma contains the actual products of the released immune response (i.e. cytokine, chemokine, antibodies, etc.).

Typical study with PBMC as the target resource is determining variation in immune response within infected or non-infected population, severe or mild symptoms, and persistent or recovered population. The information collected from those study could be developed to characterize specific immune response pattern for each disease, to find a biomarker for diagnostic, or to find new drugs. More extensive research, like pathogen epitope mapping to know which epitope at the pathogen particle that leucocyte cells recognize and respond to, is vital for developing therapeutic agent or vaccine.

Several interesting findings from typical studies employing PBMCs are:

a. A study has successfully showed that PBMC is a useful source to identify early onset biomarkers for TB progressions or non-progressions. The progressors group had lower proportion of CD_4+ T cells, NK cells, and B cells and lower gene expressions of Bcl2 but higher CCR7 gene expressions relative to non-progressors group. (Shuterland etal. 2011. PLOSOne 6(9).
b. PBMC from TB active participants and healthy household contact (HHC), a group positive with tuberculin test, was challenged with 30 kda antigen of Mycobacterium tuberculosis. After it was challenged, cytokine profile and mRNA gene expression were characterized. The result confirms that the TB active group produced stronger humoral and lower cellular immune response. Study has shown that particular 30 kda antigen triggers protective immune response in HHC participants, which could potentially be developed as purified antigen for better vaccine candidate. (Torres et.al. Infection and immunity 1998. 66:1).
c. For certain viruses like Dengue, Chikungunya, and HIV, specific lymphoid cells (monocyte and dendritic) and macrophages are targets for infections and become sites for their multiplication. PBMC is becoming an important object to study those viruses for diagnostic purposes. For example, a study detected positive strand virus RNA in CD_4+ cells, which is significantly higher in DHF patients compared to DF in secondary dengue cases (Srikiatkachorn 2012). It could lead to development of biomarker for diagnostic and disease severity.
d. A study has successfully described distinct immune response between two populations that experienced chronic or recovered inflammation caused by Chikungunya virus infections. (Hoarau et al 2015). The study was conducted using participants' PBMC from each population where immunophenotyping and gene expression profiling was measured. The distinct immune response profile is definitely important for disease therapy protection.

Although for most of the diseases a lot have been done to characterize immune response using PBMC as the source of information, there are still many questions that remain unanswered and need further studies. A good example is the infection of H_5N_1 in Indonesia. Among 26 identified clusters, hypotheses were raised that one of the risk factors was genetic factor since the majority of the secondary cases was first-degree relatives (sons/ daughters). However, the question remains which gene is responsible. Undoubtfully, PBMC is a vital source of sample to answer the question.

Despite of its importance, conducting research with PBMCs is very challenging. Several standard methods for assay, which require certain instruments, need to be established. A basic method for PBMC isolation from whole blood or further isolation of subset cells population from

PBMC i.e CD4+Tcells, monocyte and B cells is needed. These cells are source of host genetic information to study genetic factors and/or to characterize immune response. The study is conducted by stimulating targeted PMBCs cells population using specific pathogen. This requires lymphoid cells that are still 'alive' (viable). Good quality of sample, cold storage, and careful work are critical. Output of immune response from the stimulated PBMcs will be measured through immunophenotyping (cytokine, chemokine, etc) or gene expression profiling from mRNA. Methods such as flowcytometry, ELISPOT, RT-PCR/qRT-PCR, and ELISA are standard assays for studying lymphoid cells functions. These assays may sound complicated. Fortunately, now researchers are facilitated with wide options of commercial kits and instruments to perform the assays. Kits are available for each stage of assays, from cells isolation, cells stimulation, to parameters measurements.

Potential study with PBMC samples collected from AFIRE and Sepsis study (and soon Tuberculosis study) should start with the identification of groups with distinct clinical characteristics to be further explored. For example, identification of patients with known pathogen but with unusual clinical symptoms, identification of groups with severe versus non-severe conditions of an infection, or group with chronic versus recovered outcome. The research will be feasible to be done when several factors such as informative clinical data, good cold chain storage that guarantees the viability condition of PBMCs and plasma, and comprehensive laboratory testing are available. Although it is still a long way to get there, the findings will give fundamental contributions in fighting the pathogens.

Source:

1. Boyum, A. Isolation of mononuclear cells and granulocytes from human blood. Scand. J. Clin. Lab. Invest. 21, Suppl 97 (Paper IV), 77-89, 1968.
2. Boyum A. Isolation of lymphocytes, granulocytes and macrophages. Scand J Immunol. (Suppl 5):9–15, 1976.
3. Gooding et.al. 2002. Cytokine profiles of patients infected with Mycobacterium ulcerans and unaffected household contacts. Infection and Immunity. 1998; 70(10):5562-5567.
4. Torres M, etal 1998. Cytokine Profiles for Peripheral Blood Lymphocytes from Patients with Active Pulmonary Tuberculosis and Healthy Household Contacts in Response to the 30-Kilodalton Antigen of Mycobacterium tuberculosis. Infection and Immunity. 1998; 66(1):176-180.
5. (Shuterland etal. 2011. PLOSOne 6(9).
6. Srikiatkhachorn A, etal. 2012. Dengue Viral RNA Levels in Peripheral Blood Mononuclear Cells Are Associated with Disease Severity and Preexisting Dengue Immune Status. PLoS ONE 7(12): e51335. doi:10.1371/journal.pone.0051335
7. Hoarau J et.al. 2010. Persistent chronic inflammation and infection by chikungunya arthritogenic alphavirus in spite of robust host immune response. J Immunol 2010; 194:5914-5927.

PROFILE: INSIGHT TO THE NETWORK'S STEERING COMMITTEE MEMBER AT SITES

by RA from site 530 dr. Caleb Leonardo Halim, dr. Suratno Lulut Ratnoglik,
RA from site 570 dr. Mochammad Helmi Aziz, dr. Akbar Fahmi

Prof. Pratiwi Sudarmono
Johnson Space Center, USA

JAKARTA – You know their names, you have seen them roaming across the halls of your institutions, and you have probably met them in our network meetings, but do you really know them? We have interviewed our Steering Committee (SC) members and bring you a brief report about it. Starting from this issue, our newsletter will feature two of our ten SC members, so we have the chance to get to know them much better, understand some of the challenges faced at sites by the research team members, and learn what their hopes for the network are.

JAKARTA - Professor Pratiwi P. Sudarmono is one of the INA RESPOND's Steering Committee (SC) members. She earned her medical degree in 1976 from the Faculty of Medicine, *Universitas Indonesia*, Jakarta. She then received her PhD research course in Molecular Biology from Osaka University, Japan. After returning from Japan, she went to Johnson Space Center, USA where she underwent rigorous training and earned the Payload Specialist Astronaut certificate in 1985. Finally, in 1992 she attained recognition as a Clinical Microbiology Specialist. Currently, she is the Vice Dean of Faculty of Medicine, *Universitas Indonesia (FKUI)* and an active lecturer of the Department of Microbiology, *FKUI*. In February 2008 she was appointed as Honorary Professor of Microbiological Science in Faculty of Medicine, *Universitas Indonesia*.

Professor Pratiwi's primary interest has been clinical microbiology, especially the emerging and re-emerging infectious diseases. Over her 30 years of teaching Microbiology in FKUI, she has never stopped emphasizing to her students that infectious diseases will always be a part of their daily life as a medical doctor. Therefore, it is principal to understand the importance of clinical microbiology to assure patient's and community's surveillance through early detection and proper management, and to deliver prompt treatment and prevent further spread of the infectious disease. Furthermore, with the always budding infectious diseases, it is of highly importance to always develop research in clinical microbiology field.

As a Steering Committee member from *Universitas Indonesia*, Professor Pratiwi understands that the one of the roles of SC is to guard and to guide all INA-RESPOND programs in relation to the government's (Ministry of Health) policies, educational and research institutions' needs, as well as the researchers' development. To fulfill this role, the network's SC is comprised of researchers from various institutions such as NIHRD, universities, and hospitals. Developing international cooperation research is not easy. Therefore, the SC also helps to align the vision and mission of INA-RESPOND in Indonesia, which is to help the development of health research, transfer of technology, and capacity building. All planned programs should be discussed and decided through the SC meeting.

Prof. Pratiwi – SC Member at site 530, RS Cipto Mangunkusumo, Jakarta

According to Professor Pratiwi, so far, routine SC meetings have already been held in a good system, which include activities reporting and inputs sharing from the Ministry of Health and NIH. The difficulties we faced are more to non-academic constraint such as the agreement between Indonesia and US, which has not yet been completed. Non-technical factors like this decrease the speed of research development in Indonesia compared to that in some other countries such as Thailand and Vietnam. Therefore, a more intense dialogue and coordination meeting between the Indonesian Ministries to avoid misunderstanding about the relationship between Indonesia and US related to INA-RESPOND is needed. Diplomatic communication between the Foreign Minister and the Health Minister should clarify that the INA-respond is a mutual relationship and both countries should respect each other and open continuing communication. With this cooperation we can also initiate and develop our own programs according to our needs. For now, the benefit of the cooperation for the development of science has not been fully understood by all parties in Indonesia yet.

As a SC member, Professor Pratiwi hopes that cooperation agreement of science and technology between Indonesia and US will be completed soon. Also, she hopes that The Ministry of Health should be able to see INA-RESPOND not as a threat or obstacle but as an opportunity to develop research in many other health fields besides infectious disease. Through this network, we can learn much to develop our knowledge and capacity in research ethics, scientific writing, conducting good clinical trials, and creating or maintaining data management and reporting system. She also hopes that in the future Indonesia government will be able to contribute

actively and equally as NIH to provide research facilities for INA-RESPOND programs.

SURABAYA - Professor Suharto, born in Madiun on 2 August 1947, is one of the network SC members and a passionate Internist in infectious disease. He is currently managing the Infectious Disease Hospital (*Rumah Sakit Khusus Infeksi*) Airlangga University, Surabaya as the Vice Director of Health Care Management.

He started his career after he graduated from Faculty of Medicine, Airlangga University, Surabaya as a General Practicioner in 1973 and earned his Internal Medicine Specialist title in 1979. He received his Diploma in Tropical Medicine and Hygiene (DTM&H) and his Master of Science in Clinical Tropical Medicine (MCTM) from Bangkok School of Tropical Medicine, Mahidol University, Thailand. In 1999 he obtained his Doctor of Philosophy (PhD) from Faculty of Medicine, Airlangga University, and in 2008 he received his Master of Medical Education (MPdk) from Faculty of Medicine, *Universitas Indonesia*.

Prof. Suharto
Steering Comittee Member at Site 580
RS. Dr. Soetomo, Surabaya

Suffice to say, Professor Suharto is a man of great potential and many experiences. He has held several strategic positions such as the Vice Dean of Faculty of Medicine, Airlangga University (2002-2007) and the Chairman of Medical Education, Research, Staff Development Unit (MERSDU), Faculty of Medicine, Airlangga University (2008-2013). Professor Suharto has dedicated more than 40 years of his life to tropical and infectious diseases research.

In our INA-RESPOND network, Professor Suharto is one of the SC members. When we asked him what his thoughts and advice for INA-RESPOND future developments, he says that INA-RESPOND has a great impact for clinical research, especially tropical infectious diseases research. There are some big gaps between the management of local clinical research and the ideal concept. International network provides us role model to implement an ideal management system on our clinical research projects, and INA-RESPOND has been proven to give this opportunity. In Surabaya, more and more foreign researchers are in touch with us, which of course helps create new international networks and strengthen the existing ones. So, it is crucial to have and maintain our good clinical practice research culture.

Seeing our condition using the S.W.O.T analysis, Our greatest strength besides the systematic and scalable project management support provided by the network is our human resource. However, we are sometimes still constrained by our unit bureaucracy. For example, the funding for the studies is often delayed so operational performance becomes low. Surely, advocacy in each institution has still to be addressed.

With the global health conditions that tends to pay more attention to infectious diseases, high-burden and high-prevalence infectious diseases such as Sepsis and Dengue Hemorrhagic Fever should take precedence. Moreover, exotic and neglected infectious diseases should also receive serious attention. Malaria and zoonotic diseases will provide great opportunity for future studies.

Last but not least, we definitely should take into account external factors and variables that could affect INA-RESPOND future development such as the MTA (Material Transfer Agreements). We need to realize that these factors pose threats, yet they could benefit us even more were we able to identify and understand them better.

CENTRAL LAB TO SUPPORT CLINICAL TRIALS

by Dona Arlinda and M. Karyana

The number of clinical trials conducted in Indonesia has risen steadily within the last decade. Indonesia as the world's fourth most populous country offers abundant pool of subjects with diverse characteristics needed for clinical trials to evaluate therapies or prevention strategies, whether healthy subjects or those attributed with certain illness, whether drug-naïve or drug-regular. Some trials, usually large or sponsor-initiated pharmaceutical ones, may involve multisite or multi country and will need various clinical or analytical laboratory assays. The testing of human samples could be done locally at the study sites or remotely at a central laboratory. Having multiple laboratories to perform assays may require extensive efforts to produce reliable and reproducible results for effective comparisons. A central laboratory is a more reasonable option to assure reliability, quality, and integrity of the work and its results.

A local laboratory is usually attached to a hospital at the study site, while a central laboratory is often (but not limited to) an independent service offered by a specialized company. The National Institute of Health Research and Development (*Badan Litbangkes*) has incorporated the concept of central laboratory to its nationwide epidemiological studies, such as the Baseline Health Research conducted in 2007, 2010, and 2013. The NIHRD central laboratory is a service provided by the Center for Biomedical and Basic Technology of Health. Samples collected from the representative subjects across Indonesian regions underwent specific handling, processing, and shipping procedures to be analyzed at the central laboratory in Jakarta. Afterwards, the central laboratory will create the necessary documentations and valid reports covering both individual and collective results, all with respect to the subject's privacy and confidentiality.

Nowadays there are many organizations or institutions eager to conduct clinical trials in Indonesia, and most of them are supported by overseas sponsors and laboratories. Having a central laboratory in Indonesia would settle most of the issues related to material transfer across countries. This is also seen as an appealing business to some specialized companies such as Prodia Laboratories, ABC Laboratories, etc., that are willing to invest and promote their own central laboratory service.

With the experiences gained from running a central laboratory for epidemiological studies, NIHRD just needs to improve certain laboratory capabilities to support clinical trials in Indonesia. The ability to comply with the regulations (GCP, GLP and GCLP) and standards is a must. In addition, a central laboratory must be able to cover at least two basic functions, i.e. clinical and analytical. Clinical laboratory is the one conducting medical screening or diagnostic tests such as hematology or biochemistry assays whereas an analytical laboratory would measure, for instance, drug or metabolite concentrations for bioequivalence or pharmacokinetics studies.

It would be best if the NIHRD could start to take the necessary steps towards establishment of a central laboratory. It would definitely take some time and effort to build the appropriate environment, so in the meantime collaboration with other laboratories or harmonization of testing procedures across laboratories can be a suitable concept to explore. Last but not least, this approach will surely require common perception from all stakeholders, including the clinical laboratory community, industry, clinicians, professional societies, IT providers, and governmental bodies.

INA-RESPOND Newsletter

Advisor : dr. M. Karyana, dr. Herman Kosasih
Chief Editor : dr. Anandika Pawitri
Art & Language : Dedy Hidayat S, S.Kom
Columnists : Dona Arlinda, dr. M. Karyana, dr. Ungke Antonjaya, dr. Caleb Leonardo Halim, dr. Suratno Lulut Ratnoglik dr. Mochammad Helmi Aziz, dr. Akbar Fahmi
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