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Review Article

Good Medical Entomology Laboratories Practices (GMELP) in India: A Concept Note

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A B S T R A C T

Vectors of malaria, filaria, dengue, chikungunya, zika, japanese encephalitis, and kala-azar play an important role in the disease transmission in different eco-settings with variable climatic conditions. Ticks, mites, and fleas also pose a threat to new emerging and re-emerging vector-borne diseases, i.e. Kyasanur Forest Disease (KFD), Crimean Congo Hemorrhagic Fever (CCHF), scrub typhus, and other rickettsioses. Now, the time has come that field oriented entomological work has to shift from open, field based towards closed medical entomological laboratories for undertaking molecular research and pathogen/ virus detection among vector species handling them by minimising human risks. It is imperative to note that there is a strong need for a standard protocol for effective medical entomological laboratory practices while handling the pathogen carrying vector species under laboratory conditions. This may help to prevent the transmission of pathogens/ viruses in case of accidental release of vectors carrying pathogens/ viruses from the entomology laboratories. Such protocols would always help the scientists to minimise risks working in closed conditions. Though, there are guidelines/ procedures available for developing medical entomology laboratory, having facilities for insect rearing, its handling and equipment, however, no specific published protocol or guidelines exist presently in the Indian context. In the present manuscript, the need for a standard protocol for arthropod containment levels (ACLs 1- 4) along with the appropriate bio-safety levels based on the risk potential of pathogen carried by the vector species has been discussed for its application at the ground by the respective health authorities/ institutions.

The presence of Standard Operating Procedures (SoPs) and guidelines on Good Medical Entomology Laboratory Practices (GMELP) would help the professionals working in a medical entomology laboratory to minimise risks. There is a need to develop and follow Good Medical Entomology Laboratory Practices (GMELP) for handling the vectors (Arthropods) carrying the pathogens/ viruses at the national/ state/ district level as well as by the research institutes, medical colleges, and universities. The present concept note shall help to provide a guiding principle to develop standard operating procedure (SoP)/ Guidelines for GMELP.

Keywords: GMELP, ACL, BSL, SoP, Pathogen, Vector, GLP, NABL

Introduction

In India, presently six vector-borne diseases namely malaria, filaria, dengue, chikungunya, Japanese Encephalitis, and kala-azar attribute to a major public health concern and have influenced the life of a common man related to high mortality and morbidity.

There are two basic components of vector surveillance, i.e. field-based and laboratory-based. Vector collections are done to study taxonomy and bionomics in different eco-types, while laboratory-based studies are done to undertake molecular studies including pathogen/ virus detection among vector populations. All medical entomology laboratories engaged in the testing of biological samples carrying pathogen/ viruses including arthropods of public health importance should be well-structured keeping in view the bio-safety aspects of the technical workforce engaged in the labs.

A bio-safety level (BSL) is a set of standard operating procedures (SoPs) highlighting the need for biocontainment principles, which may be essentially required to isolate the pathogen/ agent in an enclosed laboratory condition. On the other hand, arthropod containment levels (ACL) can also be classified as a set of principles on human risk assessment to adhere to in order to prevent contact of vector species infected with the pathogen/ virus with the lab personnel in the enclosed environment of a medical laboratory.

The American Committee of Medical Entomology (ACME), affiliated with the American Society of Tropical Medicine and Hygiene (ASTMH), developed a set of arthropod containment guidelines that are in line with the Centre for Disease Control and Prevention (CDC) Biosafety in Microbiology and Biomedical Laboratories (BMBL).[1,2] The Arthropod Containment Guidelines (ACG) by ACME divided the arthropod containment level into 4 levels based on risk assessment.[3]

The Arthropod Containment Guidelines were published by the American Committee of Medical Entomology, a sub-committee of the American Society of Tropical Medicine and Hygiene in the years 2004 and 2008. The guidelines provide a reference for research laboratories to assess the risk and establish protocols for the safe handling of arthropod vectors of human and animal disease agents. The guidelines deal with the arthropod handling practices, safety equipment, and facilities constituting Arthropod Containment Levels 1-4 (ACL-1 to 4).

Though, ICMR Guidelines for Good Clinical Laboratory Practices (GCLP) of 2008 have been revised and published during 2021,[4] and are very well described and have information with regard to infrastructure, training, equipment, reagents, sample handling and processing, biosafety levels, ethical considerations, quality management and data management, however, it is lacking in the area of medical entomology laboratories, where studies of the vectors carrying/ infected with pathogens/ viruses involving the same human exposures and risks are dealt with. Good Laboratory Practices (GLP) are a set of principles that define a quality system concerned with the organisational process and the conditions under which laboratory studies are planned, performed, monitored, recorded, archived, and reported. It is intended to promote quality test data.[5,6]

There is a need to develop and follow Good Medical Entomology Laboratory Practices (GMELP) for handling the vectors (arthropods) carrying the pathogens/ viruses at the national/ state/ district level as well as by the research institutes, medical colleges, and universities. As of now, no such approved national guidelines or protocol exist for medical entomology laboratories handling the vectors carrying pathogens or viruses of concern to human health risks. Such SoPs can be the guiding principle for standard design, procedure, quality, performance, monitoring, recording, analysing, and reporting of vector incrimination and virus antigen detection.

The GMELP aims to ensure timely and accurate processing of entomological samples enabling early and accurate detection leading to desired outcomes. Presently, there are no standard protocols for establishing good medical entomology laboratory practices in India.

All laboratory-based studies involving vector carrying pathogens need to be undertaken as per the laid down basic and general ethical principles. The present concept note for Good Medical Entomology Laboratory Practices (GMELP) aims at establishing minimum criteria, which should be followed by medical entomology laboratories involved in handling vector studies including vector incrimination and virus detections in arthropods.

In India, National Accreditation Board for Testing and Calibration Laboratories (NABL) has been providing accreditation services to medical laboratories based on International Standard (ISO 15189: 2012) which specifies requirements for competence and quality that are particular to medical laboratories.[7] However, Medical Entomology Laboratories in the country have not been specifically covered yet under this provision.

Background

The concept of arthropod containment was thought of in 1915, when the Chagas disease vector, Rhodnius prolixus, is believed to have accidentally escaped from a research laboratory in El Salvador.[8] It subsequently established itself throughout Central America where, because of its close association with humans, it remains the most important vector of Chagas disease throughout Central America and northern South America. In 1930, the highly efficient African

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malaria vector, Anopheles gambiae, was detected in Natal, Brazil,[9] thought to be accidentally introduced by ships from Africa.

The invasion and expansion of Aedes aegypti (the yellow fever mosquito) into the New World, was presumably via ships from Africa that were part of the European colonisation of the western hemisphere and the slave trade.[10] In 1985, the Asian tiger mosquito, Aedes albopictus, was detected in Houston, Texas.[11,12] Its introduction was traced to the importation of tires from Asia. From the point of introduction, it expanded its range to the north and east and became an important pest mosquito in much of its new habitat.[13] The introduction of the West Nile virus (WNV1) into North America in 1999 may have elevated Ae. albopictus from a pest to a public health threat.[14]

In 1980, a set of guidelines were published by the sub-committee on Arboviral Laboratory Safety (SALS1).[15] In 1984, the US Department of Health and Human Services expanded the format developed in the SALS report to include all microbial infectious agents (DHHS 1999). Biosafety in Microbiological and Biomedical Laboratories (BMBL) emphasises containment in the context of "laboratory practice and technique, safety equipment, and facility design". In 1996, equipment, procedures, and facilities were recommended to provide safe containment of small, zoophilic arthropods that are infected with biosafety level (BSL1)-3 pathogens.[16] Again, other institutions, facilities, and practices successfully applied for several decades of research on mosquitoes infected with BSL-3 arboviruses at Colorado State University.[17] The American Committee of Medical Entomology (ACME), a group affiliated with the American Society of Tropical Medicine and Hygiene (ASTMH), adopted a resolution to develop arthropod containment guidelines consistent with the format of and augmenting the BMBL.

Scope

All Medical Entomology laboratories, where vectors (arthropods) carrying pathogens/ viruses are processed, may be tested under the following disciplines:

- Vector Incrimination
- Vector Rearing/ Insectary
- Molecular Biology
- Antigen Virus Detection through ELISA
- Typing of Viruses through RT-PCR
- Genetics/ Genotyping

Levels of Medical Entomology Laboratory

From the Indian perspective, the laboratory services are an integral part of the 3-tier public health system at the primary, secondary, and tertiary levels. Besides these, there may be Reference Laboratories, Research Laboratories and Specific Disease Reference Laboratories to provide services for complex and special tests. Medical laboratories for clinical diagnosis have been classified as per NABL.[7] However, Levels of Medical Entomology Laboratory are needed to be planned at the national, state, zonal/ district level for public health programmes dealing with vector-borne diseases.[18] A national reference laboratory may be provisioned at the Centre level managed by the Ministry of Health & FW, Govt of India. Services at this level are to be highly specialised and the techniques used are often complex and automated, including research and specialised tests.[19] The national reference laboratory can also provide training for technical personnel at central laboratories and conduct external quality control evaluations.

Risk Assessment for Arthropod Vectors

''Risk'' can be stated as the probability that harm, injury, or disease will occur among medical entomologists/ technicians due to the accidental escape of a pathogen-infected vector. The risk factors may include the efficiency of the vector to transmit the disease, preference in feeding habits on humans, and kinds of pathogen/ virus involved. However, handling the vector species with the pathogen/ viruses can be easily classified based on the risk involved to the human life while handling them in laboratory conditions. Therefore, in the present document, an attempt has been made to discuss whether the ACLs can be directly correlated with the appropriate BSL of the agents with which they are naturally or experimentally infected or may transmit in the event of accidental release.

Arthropods with Risk carrying Pathogen/ Viruses

- Malaria - Anopheles spp
- Filaria - Culex quinquefasciatus and Mansonia spp
- Dengue, Zika, Chikungunya, Yellow Fever - Aedes Mosquitoes
- Japanese encephalitis - Culex vishnui gpp.
- KFD - Ticks
- CCHF - Ticks
- Scrub Typhus - Mites
- Plaque - Rat Fleas
- Kala-azar - Sandfly

It would be quite better and necessary to review and take into consideration the WHO Protocol for laboratory work (based on the Risk group), which is as follows:

Classification of Infective Micro-organisms by Risk Group

This risk group classification is to be used for laboratory work only.

Risk Group 1 (no or low individual and community risk): A microorganism that is unlikely to cause human or animal disease.

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Risk Group 2 (moderate individual risk, low community risk): A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread of infection is limited.

Risk Group 3 (high individual risk, low community risk): A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available.

Risk Group 4 (high individual and community risk): A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available.

The above understanding would help the concerned medical lab professionals to take into consideration the need and requirement of arthropod containment levels (ACL 1 to 4) with regard to the risk involved while handling the vectors carrying pathogen/ viruses.

Biosafety

Medical entomology laboratories may also be designed in such a way as to comply with biosafety requirements in accordance with the risk classification of the organisms and agents being handled. An assessment should be done to ensure that the required biosafety measures are clearly identified since each laboratory has its own combination of risks. The person in charge of the laboratory and the internal biosafety committee are responsible for evaluating the risks and properly implementing recommended biosafety measures. The country's prevailing standards for risk assessment should be applied. Recommendations from the Centre for Disease Control and Prevention (CDC, 2009) and the World Health Organization (WHO, 2005) can also be followed.

Proposed Arthropod Containment Level

Containment Levels

In 2003, a sub-committee of ACME[20,21] published the recommendations for practices, equipment, and facilities that constitute arthropod containment levels (ACLs) 1 to 4. It is mentioned that personnel, who work with pathogen-infected vectors and vertebrates should simultaneously consider vertebrate animal biosafety levels, which are discussed in the BMBL.

ACL-1

ACL-1 is needed for the medical entomologists/ researchers for undertaking field surveillance activities for the vectors, i.e. malaria, filaria, houseflies, cockroaches etc. as they constitute the lowest level of human risk. These vectors that are infected even with parasites/ micro-organisms do not cause direct disease in humans and domestic animals. ACL-1 facility can be field-based or lab-based with standard insectary practices used for entomological work. However, the vectors collected from outbreaks situations, are held in cages or collection tubes that minimise/ reduce contact with vectors for chances to escape and bite laboratory personnel. Though, minimum risks are involved while handling vector arthropods, but, the cages are to be kept secure during the processing of samples under laboratory conditions. The vector species are to be properly labelled for identification (e.g., species, strain, data collected). All design and standards keeping in view the bio-safety and laboratory facilities are to be kept in place with standard insectaries. The staff working in medical entomology labs should wear gloves and white gowns/ coats when handling vertebrates for feeding adult vectors in the insectary. A high level of alert and caution should be in place, which will prevent any vector species to be accidentally released outside the insectary or medical entomology laboratory of ACL-1 standard.

ACL-2

ACL-2 may be needed for vectors of dengue, chikungunya, Zika and Japanese Encephalitis that are considered to have a moderate potential for human risk. This level pertains to and may be recommended for vectors infected or suspected

Table 1.Proposed Arthropod Containment Level

Arthropod Status of Infection	Malaria, Filaria, Kala-azar	Dengue, Zika, Chikungunya, Scrub Typhus	Yellow Fever, JE, KFD	CCHF, Plague
Lab Practices with Infection Status	Up to BSL-1	Up to BSL-2	Up to BSL-3	Up to BSL-4
Arthropod Containment Level Practices	ACL-1 standard handling practices	ACL-2, limited access, training signage, containment and disposal	ACL-3, restricted access, training, appropriate PPE, signage, containment and disposal	ACL-4, Isolation, training, appropriate PPE, signage, containment, record keeping and disposal

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of being infected with BSL-2 infectious agents (DEN 1 to 4, JE arboviruses). This is also to keep in mind that Aedes spp. are also vectors for yellow fever and therefore, one should be very careful and cautious under such conditions and a higher level of arthropod containment level may be thought of. ACL-2 may comprise most of the components recommended for ACL-1 so that the requirements for procedures, equipment, and facilities are more stringent than those for ACL-1. Facilities and procedures are to be in place as a pre-requisite for improved detection of escaped vector mosquitoes.

All vectors (infected or uninfected) in the laboratory conditions or collected from outbreak situations are to be autoclaved or incinerated before disposal. Escape of infected vectors/ insects, if any, within the insect colony may be prevented by handling them in a glove box, biosafety cabinet or special devices that are used to prevent escape.

ACL-3

The provision of ACL-3 for medical entomology laboratory is to be recommended for handling those vector species that are carrying or may be infected with infectious pathogen/ agents approved for BSL- 3 biosafety requirements. To isolate virus/ pathogen from arthropod/ animal reservoir or from cell line culture, a BSL-3 laboratory is required in view of the risk involved with the personnel involved under a closed environment. The role of this level of facility needs strengthening for upgradation with the need to extend laboratory facilities. The laboratory implies its significance beyond the ACL-2 facility with the main focus on pathogen containment and all necessary steps be put in place for putting more restricted access to the insectary. To work in medical entomology laboratory to handle BSL-3 agents may essentially require ACL-3 level with the use of Class II biosafety cabinets. The biosafety cabinets must be fitted with a high efficiency particulate air-filtered exhaust system to protect laboratory personnel and prevent pathogen release.

An isolated area within the insectary may be identified and used for work with vector species known or suspected to be infected with a BSL-3 agent. A small, secure room that constitutes several layers of containment and where escaped arthropods can be readily detected is often best for this purpose.

A standard ACL-3 facility should focus on strict pathogen containment and decontamination protocol in place. Due care and caution are required for consideration in view of risks associated with the possible generation of an infectious pathogen. According to the ACME recommendations, only arthropods vectors requiring ACL-3 containment should be held in an ACL-3 insectary with efficient containment barriers in place. Materials used in the ACL-3 lab would require a stringent process for autoclave before they are discarded. Use of personal protection (eg., gloves, garments, and foot) is to be strictly in place. Pesticides are to be kept in hand, in case required to knockdown escaped vector species. The selection of a BSL-3 laboratory with an ACL-3 insectary may be isolated from the main building areas of the institute/ organisation. The insectary may be built up with a double-door entrance with a secure outer door (eg., key lock or card key) with no windows. An autoclave is to be made available in the insectary area. Design and operations in the ACL-3 insectary may be approved before use and annually by the Institutional Biosafety Committee (IBC).

ACL-4

ACL-4 facility is intended to design and work to handle the vector species that are suspected to be infected with the most dangerous BSL-4 infectious agents (CCHF, plague), which can cause life-threatening illnesses. As there are chances for nosocomial infection with the serious illness through aerosol transmission, i.e. simple contact/ inhalation, all necessary bio-safety protocol and containment guidelines may be included which are required for BSL-4. In addition, the personnel working in the medical entomology laboratory must ensure to shower before entering and when leaving, while working with infectious agents. The staff deployed should be wearing a positive pressure laboratory safety suit. The design of BSL-4 facilities and procedures must be followed strictly. Arthropods in an ACL-4 must be contained properly at all times, whether or not they are being manipulated. Although insects can be used experimentally in BSL-4 agent research, most vectors of agents in this category are ticks. The highly infectious and virulent nature of BSL-4 agents mandates rigorous training of staff who works in the facility, specialised equipment specifically designed for ACL-4 research, and facilities and protocols approved by the IBC.

Conclusion

Presently, limited medical entomology laboratories with BSL and ACL facilities exist in the country with regard to handling the vectors carrying pathogens/ viruses for emerging and re-emerging vector-borne diseases. An update on the important criteria to consider while designing, constructing, commissioning, and operating the facility for the establishment of the Biosafety Level-3 (BSL-3) laboratory in the Indian setting have been well explained in the year 2014 by NIV, Pune.[22] However, there is no mention of the arthropod containment levels vis-à-vis biosafety levels required as Good Medical Entomology Laboratory Practices (GMELP) while handling vector species carrying pathogens/ viruses. The Good Medical Entomology Laboratory Practices in the Indian context are quite critical and become essential for an improved understanding of

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the role of the vector in pathogen transmission and for the development of enhanced disease prevention strategies. One must understand the bio-safety requirements based on the human risks involved by the pathogen/ viruses while considering the need for the BSL 1-4 levels approach and also taking into consideration the arthropod containment levels in the medical entomology laboratory having the risk of exposure/ bite from the pathogen carrying vector species. The level of lab facilities (ACL1-4) at different tiers of the country should put into practice all the norms and procedures to minimise or eliminate the effect of any vector species or pathogens and should make all efforts to protect the laboratory personnel working in that environment. In medical entomology laboratories, the study on pathogen-infected vectors may pose an immediate risk/ threat and therefore, there is an immediate need for utmost care in the selection of BSL levels and ACL levels of medical entomology laboratories at the national/ state level.

Conflict of Interest: None

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