MALARIA VACCINE INITIATIVE

FACT SHEET

Growth Inhibition Assay (GIA) and Standard Membrane Feeding Assay (SMFA) Reference Center

The Project: Functional antibody testing for SSM-TBV and blood stage vaccine candidates

GIA Services

As part of a comprehensive effort to properly evaluate the potential of new malaria vaccine candidates, the PATH Malaria Vaccine Initiative (MVI) continues its support for a range of laboratory tools, including those that employ the growth inhibition assay (GIA) at the Laboratory of Malaria and Vector Research (LMVR), US National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health.

Established in 2004 with MVI support, the purpose of the GIA Reference Center has evolved from support for a primarily research assay to support for an assay that is used more widely as a decision-making tool in the assessment of candidate vaccines that target the malaria parasite when at its most destructive—when it enters human red blood cells.

The GIA Reference Center measures and assesses the ability of blood-stage vaccines to induce an antibody response—one that inhibits the ability of malaria parasites to infect and replicate in human red blood cells. MVI has used GIA as a potency assay to compare the immunogenicity of various blood-stage vaccine candidates.

SMFA Services

In 2009, LMVR added the Standard Membrane Feeding Assay (SMFA) to its repertoire of immunological assays to meet the increasing need for impartial immunological evaluation of TBV candidates. This assay measures the ability of vaccine-induced antibodies to block transmission of the malaria parasite to mosquitoes feeding through a membrane on a blood meal from an immunized individual. Performance of this assay is dependent on a well established insectary that supplies mosquitoes to the Center and a number of other labs. The Center has recently qualified their SMFA in reference to ICH Q2 (R1) guidelines on assay validation with regard to linearity, precision, range, and specificity.

The Potential: More Reliable Assays to Evaluate New Vaccine Candidates

Experience shows that properly evaluating the potential of new vaccine candidates requires reliable and sensitive laboratory tools. MVI is therefore eager to direct significant resources toward developing and refining assays. As such, several areas of focus for these assays have been identified for assay improvement, including the handling of human samples, the development of reference reagents, and assay variability which is especially high in the SMFA.

Human samples: The quality of serum is a major concern. Purification of the immune globulin IgG from serum can eliminate some of the variability introduced by the use of serum. Also, difficulties arise in attempting to purify IgG from small volumes of serum such as that available from children and infants. The lab has optimized the GIA to accommodate low volumes of serum and continues to identify quality criteria that serum, plasma, or IgG samples need to meet for use in both GIA and SMFA.

Reference reagents: Standard reference reagents for controls when measuring human samples are needed. An independent critique on the development of this assay argued that a bank of serum samples should be collected from protected individuals living in endemic areas and that these samples should be tested individually in functional assays before pooling. This standard reagent could be stored and made available to the malaria community either as whole serum or as purified IgG. While this effort is still ongoing, the Center has produced and continues to make available reference AMA1 IgG and monoclonal antibodies for conduct of the GIA, and has made large quantities of the transmission-blocking monoclonal antibodies 4B7 directed to the sexual stage protein Pfs25 which has been characterized for use as a SMFA standard, as well as 3E12 directed to the sexual stage protein Pfs48/45.

Parasites: Experts have pointed to parasite culture conditions and the percentage of gametocytemia or infected red blood cells at the start of the assay as important sources of variation that should be agreed upon by the community. The consensus is to use a selected set of strains and a single source of parasite for each strain, at least as a start to identify causes for the variability.

Proficiency testing: Proficiency testing can help to relate results obtained in different laboratories performing assays using different methods. Our approach is to run a proficiency panel in which different labs perform assayss using common reagents, standards, and coded samples. These exercises help identify sources of variation that can then be harmonized if the community deems appropriate. Additionally MVI seeks consensus regarding the ways in which data are reported and which reagents are used in the assays.

Parasite detection read out: A number of different assays are used for detection of parasites, whether infected mosquito midguts in the SMFA or infected red blood cells from an infected mammal in the GIA. The choice of a method should allow for some degree of automation and treatment of large numbers of samples. MVI plans to establish criteria for selection of optimal parasite detection.