

## Appendix 3. Measuring the immune response using keyhole limpet hemocyanin (KLH)

### Measuring immune response

Comprehensive evaluation of the immune system calls for analysis of cellular and humoral immunity. Evaluation of cellular immunity relies on measurement of T-cell numbers and subtypes with the use of special stains and flow cytometry. Delayed hypersensitivity testing assesses *in vivo* T-cell function. Humoral immunity is quantified through measurement of immunoglobulin levels or immunoglobulin subtypes. Determination of antibody levels to particular antigens can be used to estimate an individual's ability to mount a humoral immune response. For instance, specific antibodies should be present in vaccinated individuals. The ability to mount a humoral response may also be evaluated by deliberately injecting the patient with an agent such as keyhole limpet hemocyanin (KLH) and then measuring levels of natural antibodies binding to KLH antigen.

### What is KLH?

Keyhole limpet hemocyanin or KLH is a copper-containing carrier protein isolated from the Giant Keyhole Limpet (*Megathura crenulata*), a marine mollusk. KLH possesses many immunostimulatory properties, including the ability to increase antigenic immune responses *in vivo* by interacting with T cells, monocytes, macrophages, and polymorphonuclear leukocytes.

In addition to immune competence testing, KLH is widely used as an immunotherapeutic agent. KLH may serve as a carrier for vaccines and antigens, and in human medicine, a major clinical use of KLH is the treatment of bladder carcinoma. KLH may also be used as part of adjunctive therapy for other carcinomas, particularly adenocarcinomas.

### Use of KLH in birds

Measurement of antibody response to KLH antigen has been well documented in the chicken. Since one would not expect domestic fowl to encounter KLH in nature, antibody response to this antigen reflects the ability of the humeral immune system to respond, particularly cytotoxic T-cells. Interestingly, variations in antibody response to KLH have been documented among different types of chickens. Broilers primarily demonstrate a high IgM antibody response whereas layers have a high IgY response. Chicken IgY is the functional and structural equivalent to mammalian IgG. This major circulating antibody is passed from the hen to the embryo via the egg yolk.