

## **HBT ENDOCAB TEST KIT**

The EndoCab test kit has been developed by the Scottish National Blood Transfusion Service (SNBTS) in Edinburgh<sup>(1)</sup>. The assay determines endotoxin core antibodies (EndoCab) in human plasma or serum in patients or healthy individuals. Several studies show a consistent drop in postoperative levels of circulating anti endotoxin core antibodies from the preoperative value. This drop has been interpreted as consumption of antibodies to endotoxin by systemic release of endotoxin<sup>(2)</sup>. A hypothesis is that if the patients' pre-operative EndoCab level is low, even moderately low, patients may not be able to cope with the efflux of endotoxin, which may have mild to severe clinical consequences. The assay is of interest for various experimental conditions ranging from in vitro LPS neutralization by plasma components to in vivo studies on kinetics of antibodies to endotoxin in health and diseases.

The EndoCab standard median-units IgG, IgM and IgA (MU) are arbitrary and are based on medians of ranges for 1000 healthy adults in a particular locality. It has not been established whether normal EndoCab values vary by region, culture or race. Users should establish appropriate local statistical controls for their studies. It is advised that studies of any patient group should always be controlled by studies of appropriately selected contrasting clinical groups and/or healthy individuals recruited locally.

### **LITERATURE**

1. Barclay GR, Endogenous endotoxin-core antibody (EndoCab) as a marker of endotoxin exposure and a prognostic indicator: a review. *Prog Clin Biol Res* 392:263-272
2. Allan E et al., Anti-bacteroides lipopolysaccharide IgG levels in healthy adults and sepsis patients. *Immunol Med Microbiol* 115-12
3. Barclay GR, Antibodies to endotoxin in health and disease. *Rev Med Microbiol* 1:133-142

### **PRINCIPLE OF THE TEST**

The Hbt EndoCab test kit is a solid-phase enzyme-linked immunosorbent assay. Samples and standards are incubated in microtiter wells coated with equimolar amounts of endotoxin rough-lipopolysaccharides from four Gram-negative bacterial species, each comprised of a complete inner core, but lacking complete outer core or O-specific polysaccharide chain. These endotoxins, complexed with polymyxin B, constitute the solid phase antigen. During this incubation anti endotoxin core antibodies are captured by the solid bound endotoxins. Unbound material present in the sample is removed by washing. Next anti-human Ig(G or M or A) tracer antibody is added to the wells. If EndoCab antibodies were present in the sample, the tracer antibodies will bind to the captured EndoCab antibodies. The excess tracer is removed by washing and substrate, tetramethylbenzidine (TMB) is added to the wells. Colour develops proportionally to the amount of anti endotoxin core antibodies present in the sample. The enzyme reaction is stopped by the addition of citric acid and the absorption at 450 nm is measured with a spectrophotometer. A standard curve is obtained by plotting the absorptions versus the corresponding concentrations of the known standards. The human EndoCab antibody concentration of samples with unknown concentrations, which are run concurrently with the standards, can be determined from the standard curve.

### **SPECIAL FEATURES OF THE KIT**

- Ready-to-use (i.e. pre-coated microwells).
- High reproducibility
- High sensitivity. The minimum concentration which can be measured is 0.125 GMU/ml of IgG EndoCab antibodies, 0.055 MMU/ml of IgM EndoCab antibodies and 0.156 AMU/ml of IgA EndoCab antibodies.
- Large measurable concentration range.
- Standard curve of IgG from 0.125-8 GMU/ml. Standard curve of IgM from 0.055-3.5 MMU/ml. Standard curve of IgA from 0.156-10 AMU/ml.
- Efficient format. One plate with each twelve 8-well strips allow free choice of batch size for the assay.
- Simple, rapid procedure. Three pipetting steps are required to complete the assay. Working time 2½ hours.

### **AVAILABILITY**

The Hbt EndoCab test kit is available in a kit for 1 x 96 determinations.

**PRODUCT NUMBER: HK504**

Hbt Endocab test kit

For research purposes only.

Caution: Not for use in humans.