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Human Immunoglobulin G ELISA kit
Catalog # F160
Validation Summary
Report dated April 23, 2000

The data summarized below was generated by *Cygnus Technologies* to establish the performance parameters and validity of this kit to measure Total Human Immunoglobulin G (hIgG). This data is intended to supplement and not replace user generated validation data. The data is representative of what a laboratory can expect to achieve when following the kit insert recommended protocols. Significant differences in these performance parameters may be indicative of problems with reagents, laboratory equipment, or technique and should be investigated before reporting results.

It is recommended that a user validation study include at least the following experiments to validate this kit for use with their product: (1) Each user should perform intra and inter assay precision experiments to establish their procedural proficiency. (2) Each user should perform recovery experiments using their test sample matrices and diluents. Such a study can be performed by adding known amounts of the 50ng/mL standard provided with this kit to the final product or diluent which are to be evaluated. Ideally these test sample matrices should be devoid of any hIgG or have very low levels (< 0.5ng/mL) determined prior to adding the 50ng/mL standard. Such an experiment will establish the degree of sample or diluent matrix interference in the recovery of hIgG. (3) Laboratories should also perform dilutional recovery experiments on their actual samples. This experiment assumes that at least some of the test samples will have significant levels of hIgG. Such samples will be serially diluted by some appropriate diluent previously shown to give acceptable recovery. When diluted, samples should give essentially the same value at each dilution when multiplied by the appropriate dilution factor. This experiment establishes the condition of antibody excess for accurate quantitation and determines that typical process samples do not have hIgG in the “Hook Region” of the concentration response curve.

Materials: Kit Lot 13030, 3040, 11040.

Methods: The protocol as defined in the kit insert was used in this validation.

Data References: Raw data for these experiments are recorded in Notebooks #1-hIgG pages 1-23.

Precision: Precision is defined as the percent coefficient of variation (%CV). This is calculated by dividing the standard deviation by the mean value for a number of replicate determinations of two different control samples in the low and high concentration range of the assay. The design goal specifications are given in the last column of each experiment. While actual precision may vary from laboratory to laboratory and technician to technician it is recommended that all operators achieve precision below these design goals before reporting results.

| Intra-assay | | | | Inter-assay | | | |
|-------------|------------|-----|---------------------------|-------------|------------|-----|---------------------------|
| # of tests | Mean ng/mL | %CV | Design Goal Specification | # of assays | Mean ng/mL | %CV | Design Goal Specification |
| 20 | 1.5 | 4.7 | <10% | 5 | 1.5 | 6.2 | <12% |
| 20 | 5.08 | 6.0 | <8% | 5 | 5.08 | 5.7 | <10% |
| 20 | 13.05 | 5.4 | <8% | 5 | 13.05 | 5.7 | <10% |

Sensitivity: The hIgG concentration corresponding to a signal 2 standard deviations above the mean of the zero standard is defined as the limit of detection (LOD). This was determined from 10 replicates of the zero standard. The mean signal of the zero standard plus 2 SD yielded a LOD of <0.1ng/mL. The limit of quantitation (LOQ) is defined as the lowest concentration for which the CV is <20%. This is determined by performing a precision profile for the assay at several low concentration points and then interpolating that concentration which corresponds to a 20% CV. The LOQ was ~0.2ng/mL.

Specificity: In sandwich ELISA, cross reactivity can manifest itself either as a false increase in hIgG (positive cross reactivity) or as a false decrease in hIgG (negative cross reactivity) when hIgG present in the sample competes with the cross reactant for the kit antibodies. Animal IgG fractions at ~2mg/mL and/or undiluted sera from the various animal species shown below were tested for positive cross reactivity by assaying the sample as an unknown. Negative cross reactivity was evaluated by spiking 25ng/mL of hIgG into each of the potential cross reactants and dividing the recovered value by 25ng/mL. None of the materials below showed either type of cross reactivity except for mouse IgG, which gave a percent cross reactivity of 0.001%. The antibodies used in this kit have been affinity purified to minimize cross reactivity but it is recommended that each user test their particular sample matrix material for cross reactivity in a similar experiment.

| Animal Species | % Cross Reactivity |
|-----------------------|---------------------------|
| Cat | Not detectable |
| Chicken | Not detectable |
| Cow | Not detectable |
| Dog | Not detectable |
| Goat | Not detectable |
| Guinea pig | Not detectable |
| Hamster | Not detectable |
| Horse | Not detectable |
| Mouse | ~0.001% |
| Pig | Not detectable |
| Rabbit | Not detectable |
| Rat | Not detectable |
| Sheep | Not detectable |

Because the antibodies used in this kit were generated and affinity purified against hIgG heavy and light chains there is some cross reactivity to other human immunoglobulin classes such as hIgA, and hIgM.

Recovery/Interference Studies: Various buffer matrices have been evaluated by adding known amounts of the hIgG preparation used to make the standards in this kit. Because this assay is designed to minimize matrix interference, most of these buffers yielded acceptable recovery defined as between 80-120%. In general, extremes in pH (<5.0 and >8.5) as well as some detergents like SDS and Tween can cause under-recovery. Very high concentrations of certain proteins can also interfere in accurate detection of hIgG. Each user should validate that their sample matrices yield accurate recovery by performing a similar experiment. For example, this experiment can be performed by diluting one part of the 50ng/mL standard provided with this kit into 4 parts of the sample matrix in question. Recovery should be on the order of 8 to 12 ng/mL hIgG.

Hook Capacity: Very high concentrations hIgG were evaluated for the hook effect. At concentrations exceeding 50ng/mL the apparent concentration of hIgG may read less than the 50ng/mL standard. The hook capacity, defined as that concentration which will give an absorbance reading less than the 50 ng/mL standard was ~30 µg/mL.