

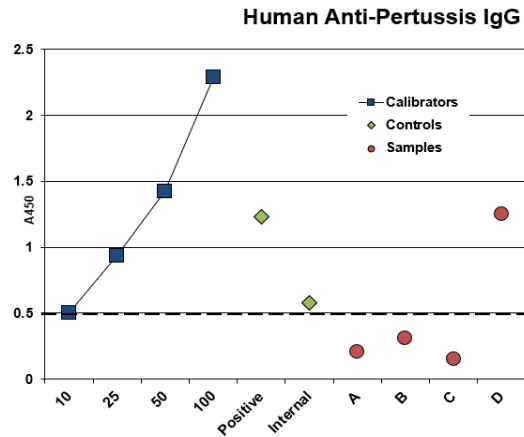


## INTERPRETATION OF RESULTS

### A. Antibody Activity Threshold Index

Compare Samples to **10 U/ml Calibrator** or **Internal Control**  
= **Positive/Negative Cut-off.**

#### Example:



#### Results

The **sensitivity** of the assay to detect anti-Pertussis Antigens IgG, from either natural infection or vaccination, is controlled so that the **10 U/ml Calibrator** represents a threshold OD for most true positives in human serum diluted to 1:200 or greater. Visual inspection of the data in the above graph shows the following:

**Calibrators** – dilution curve of antiserum from pertussis antigen immunization shows the OD range of the assay; high value indicates optimal sensitivity of the assay.

**10 U/ml:** a 'Cut-off' line has been drawn to indicate a threshold distinguishing between **Positive/Negative**. This is not a clear-cut threshold, rather a low OD area that could represent either low positives or high background negatives.

**Positive Control** – serum showing reactivity to pertussis antigens; the value range is on the label. This Control may be used to gauge precision and to normalize between-assay variation.

**Internal Control** – a true positive from an immune human that represents the investigator's experience in distinguishing low positive from negative samples (not in kit). This should be run in each assay to supplement the 10 U/ml Calibrator for Positive/Negative discrimination purposes.

**Samples A,B,C,D** – 3 samples (1:200) (A, B, C) are **negative**: below the threshold; 1 sample (D) is **positive**: clearly above the threshold.

The 10 U/ml Calibrator can be used to calculate a **Threshold Index** that numerically discriminates Positive/Negative:

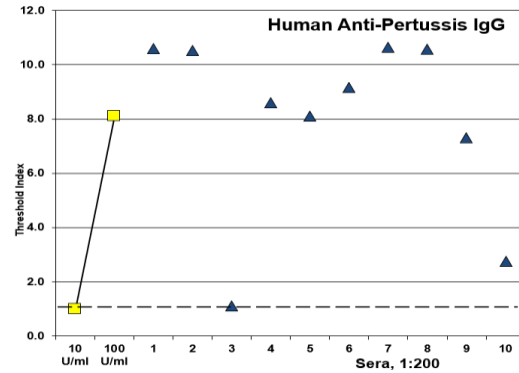
- ❖ Divide each Sample net OD by the 10 U/ml Calibrator net OD. Values above 1.0 are a measure of **Positive** Antibody Activity; below 1.0 are **Negative** for antibody.

## INTERPRETATION OF RESULTS (cont)

### Example:

#### Human Serum/Plasma IgG

A panel of human serum/plasma of unknown history was tested for anti-pertussis IgG (1:200 dilution in Low NSB Sample Diluent). **Threshold Index** was calculated using the **10 U/ml Cal.**



#### Results

##### Anti-Pertussis Antigens IgG:

Nine (9) NHS/P were distinctly positive (above 1.0 TI); one serum (3) was borderline. When a significant portion of the positives are **>4** Index, it may be more useful to run dilution curves to calculate titers (see next page).

#### Notes:

- Positives** may be due to prior encounter with the bacteria or from pertussis immunization.
- When the **Positive Index** is **above 5.0**, using a dilution curve to calculate titer is a more accurate quantitation method (see Method C).
- The **sensitivity** of the assay may be adjusted by changing the sample dilutions: a) increase dilution (e.g., 1:300) to lower the signals of borderline positives to negative; b) decrease dilution (e.g., 1:100) to convert borderline samples to positive. With the latter, the values of negatives may increase, so an alternative threshold should be considered using known negatives to develop a **Positive Index** (see below) or use an **Internal Control** (Page 5).

#### B. Positive Index

Experimental sample values may be expressed relative to the values of Control or Non-immune samples, by calculation of a **Positive Index**. One typical method is as follows:

- Calculate the net OD mean + 2 SD of the Control/Non-immune samples = **Positive Index**.
- Divide each sample net OD by the Positive Index. Values above 1.0 are a measure of **Positive** Antibody Activity; below 1.0 are **Negative** for antibody.

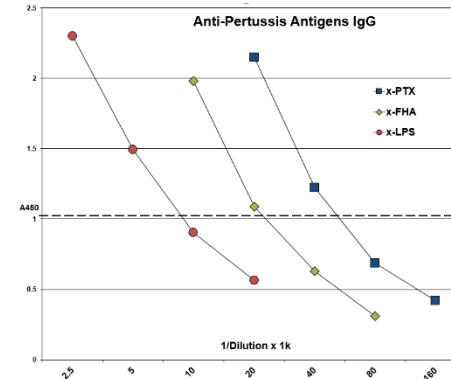
A sample value would be **Positive** if significantly above the value of the pre-immune serum sample or a suitably determined non-immune panel or pool of samples, tested at the same sample dilution.

This calculation also **quantifies** the positive Antibody Activity level, assigning a higher value to samples with higher Antibody Activity, and vice versa.

## INTERPRETATION OF RESULTS (cont)

### C. Antibody Titer

The most accurate method for comparing antibody potencies is by calculation of a titer, using an OD reading in the linear range of dilution curves of each antibody as **Index**. In the example below, **IgG** titers were calculated as inverse of the dilution that produced a **1.0 OD** in the assay.



#### Results

Antisera specific to each of the coated pertussis antigens showed moderate to high titers.

**Mouse Anti-PTX:** Titer: **55.8 k**

**Rabbit Anti-FHA:** Titer: **21.2 k**

**Human Anti-LPS:** Titer: **7.6 k**

#### Calibrator Curve Quantitation

To quantitate antibody activity from a calibrator curve (such as provided with the kit), the dilution curve of the samples must be parallel to the calibrator curve to avoid different values being obtained from different regions of the curve. In cases of non-parallelism, antibody activity is best expressed as a titer relative to the titer of a reference positive, as shown above.

#### Assay Sensitivity

The pertussis antigen coating level, HRP conjugate concentration and Low NSB Sample Diluent are optimized to differentiate anti-pertussis antigen IgG from background (non-antibody) signal with human serum samples diluted 1:200.

## PRECAUTIONS AND SAFETY INSTRUCTIONS

Calibrators, Sample Diluent, and Antibody HRP contain bromonitrodioxane (BND: 0.05%, w/v). Stop Solution contains dilute sulfuric acid. Follow good laboratory practices and avoid ingestion or contact of any reagent with skin, eyes, or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water. MSDS for TMB, sulfuric acid, and BND can be requested or obtained from the ADI website.

# Human Anti-B. Pertussis Antigens IgG ELISA Kit

Cat. No. 960-110-PHG, 96 tests

For Quantitation of Anti-B. Pertussis Toxin (PTX), Filamentous Hemagglutinin (FHA), & Lipopolysaccharide (LPS) IgG in Serum, Plasma, or other Biological Fluids

For research use only, not for diagnostic or therapeutic use.



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ELISA Kit Components	Amount	Part #
Pertussis Antigens Coated Strip Plate	8-well strips (12)	960-101
Anti-Pertussis Positive Control	0.65 ml	960-122PC
Anti-Pertussis Calibrator 10 U/ml	0.65 ml	960-122B
Anti-Pertussis Calibrator 25 U/ml	0.65 ml	960-122C
Anti-Pertussis Calibrator 50 U/ml	0.65 ml	960-122D
Anti-Pertussis Calibrator 100 U/ml	0.65 ml	960-122E
Anti-Human IgG HRP Conjugate (100X)	0.15 ml	H-HuG.2a11
Sample Diluent (20X)	10 ml	SD20T
Low NSB Sample Diluent	30 ml	TBTm
Wash Solution Concentrate (100X)	10 ml	WB-100
TMB Substrate	12 ml	80091
Stop Solution	12 ml	80101
Product Manual	1 ea	M-960-110-PHG