INTENDED USE

The **Human anti- Meningococcus Group ACWY IgG** ELISA Kit is an immunoassay suitable for detecting and quantifying IgG antibody activity specific for the Group A, C, W135 & Y polysaccharides of *N. meningitidis* in serum or plasma of vaccinated, immunized, and/or infected hosts. This immunoassay is suitable for:

- Determining **immune status** of humans
- Assessing efficacy of **vaccines**, including dosage, adjuvantcy, route of immunization and timing
- Qualifying and standardizing vaccine batches & protocols

GENERAL INFORMATION

Meningococcal disease is an infection caused by the bacterium *Neisseria meningitidis* (also termed meningococcus) including meningitis, meningococcemia, and septicemia. *N. meningitidis* has 13 clinically significant serogroups classified according to the antigenic structure of their polysaccharide capsule. Six **serogroups: A, B, C, Y, W135 and X** are responsible for virtually all cases of the disease in humans. There are currently several vaccines to prevent meningococcal disease, all quadrivalent in nature, targeting serogroups A, C, W-135, and Y: two conjugated vaccines (MCV-4), Menactra (Polysaccharides conjugated to Diphtheria Toxoid) and Menveo (Conjugated to CRM197); one polysaccharide vaccine (MPSV-4), Menomune, produced by Sanofi Pasteur; Mencevax (GlaxoSmithKline, CRM197 conjugate) and NmVac4-A/C/Y/W-135 (JNI conjugated to Diphtheria Toxoid). Conjugated vaccines provide enhanced duration of protection and increased immunity. The antibodies are produced against both the carbohydrate part and the toxoid. Diphtheria Toxoid when conjugated to bacteria polysaccharides acts as carrier protein and adjuvant.

PRINCIPLE OF THE TEST

The Human Anti- **Meningococcus Group ACWY IgG** ELISA kit is based on the binding of IgG in samples to the antigen immobilized on the microwells. Bound antibody is detected by anti-human IgG-HRP (horseradish peroxidase) enzyme. After a washing step, chromogenic substrate (TMB) is added and color (blue) is developed by the enzymatic reaction of HRP on the substrate which is directly proportional to the amount of IgG present in the sample. Stopping Solution is added to terminate the reaction and convert blue to yellow color; absorbance at 450nm is then measured using an ELISA reader. The presence of **Anti-Meningococcus Group ACWY IgG** antibody in samples is determined relative to Calibrators.

PRODUCT SPECIFICATIONS

Specificity
A mixture of purified polysaccharide of groups ACWY, of equal concentration, is used to coat the microwells (no toxin or toxoid). Therefore, only antibodies to ACWY are detected in the test. The anti-human IgG HRP conjugate reacts specifically with human IgG class antibodies; IgM, IgA ,and IgE antibody would not be measured above background signals. ADI has other ELISA kits to detect group specific individual A, C, W, or Y-specific antibodies.

Assay Sensitivity

The ACWY antigen coating level, HRP conjugate concentration, and Low NSB Sample Diluent are optimized to differentiate anti-ACWY IgG from background (non-antibody) signal with human serum or plasma samples diluted 1:100 or more.

KIT CONTENTS

The microtiter well plate and all other reagents, if unopened, are stable at 2-8°C until the expiration date printed on the box label. Stabilities of the working solutions are indicated under Reagent Preparation.

To Be Reconstituted: Store as indicated.

Component	Preparation Instructions
Wash Solution Concentrate (100x) Cat. No. WB-100, 10ml	Dilute the entire volume 10ml + 990ml with distilled or deionized water into a clean stock bottle. Label as **Working Wash Solution** and store at 4°C for long term and ambient temp. for short term.
Sample Diluent Concentrate (20x) Cat. No. SD-20T, 10ml	Dilute the entire volume, 10ml + 190ml, with distilled or deionized water into a clean stock bottle. Label as **Working Sample Diluent** and store at 2-8°C until the kit lot expires or is used up.
Anti-Human IgG-HRP Conjugate Concentrate (100x) Part: H-HuG.2a11, 0.15ml	Peroxidase conjugated anti-human IgG in buffer with detergents and antimicrobial as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of **Working Sample Diluent** is sufficient for 1 8-well strip. Use within the working day and discard. Return 100X to 2-8°C storage.

Ready For Use: Store as indicated on labels.

Component	Part	Amt	Contents
Meningo-coccal ACWY Coated Strip Plate	600-ACWY	8-well strips (12)	Coated with purified Group A,C,W135,Y polysaccharides and post-coated with stabilizers.
Anti-Meningococcal Calibrators			
1 U/ml 2.5 U/ml 5 U/ml 10 U/ml	6008AXSrB 6008AXSrC 6008AXSrD 6008AXSrE	0.65 ml 0.65 ml 0.65 ml 0.65 ml	Four (4) vials, each containing anti-meningococcal antibody; in buffer with antimicrobial as stabilizers.
Anti-Menin-gococcal Positive Control	600-8AX-SrPC	0.65 ml	Antiserum with meningococcal reactivity; [value range on label]
Low NSB Sample Diluent Reduces non-specific binding	TBTm	30 ml	Buffer with protein, detergents and antimicrobial as stabilizers. Use as is for sample dilution. See **Assay Design**, page 3. **Not** for HRP Conjugate dilution.
TMB Substrate	80091	12 ml	Chromogenic substrate for HRP containing TMB and peroxide.
Stop Solution	80101	12 ml	Dilute sulfuric acid.

Materials Required But Not Provided:

- Pipettors and pipettes that deliver 100ul and 1-10ml.
- Disposable glass or plastic 5-15ml tubes
- Stock bottle to store diluted Wash Solution; 0.2 to 1L.
- Distilled or deionized water to dilute reagent concentrates.
- ELISA reader at 450 nm and ELISA plate washer

ASSAY DESIGN AND SET-UP

Sample Collection and Handling
Serum and other biological fluids may be used as samples with proper dilution to avoid solution matrix interference. For **serum**, collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature. For other samples, clarify the sample by centrifugation and/or filtration prior to dilution in Sample Diluent. If samples will not be assayed immediately, store refrigerated for up to a few weeks or frozen for long-term storage.

Caution: Human serum and other bodily fluids may contain infectious material. Always wear gloves when handling human samples, including the standards and controls (which have been tested non-reactive for HbsAg and Anti-HIV), and dispose of these samples and containers as biohazard waste.

Antibody Stability & Dilution
Initial dilution of serum into **Working Sample Diluent** (WSD) is recommended to stabilize antibody activity. This enhances reproducible sampling, and stabilizes the antibody activity for years, stored refrigerated or frozen. Further dilution into **Low NSB Sample Diluent** (LNSD), which provides the lowest assay background, should be at least 10 times the initial dilution and performed the same day as the assay.
Example:
Initial (1:5): **10**ul serum + **40**ul WSD [or 0.1ml + 0.4ml]
Further (1:50): **10**ul initial (1:5) + **90**ul LNSD (1:50)

Assay Design
Review Interpretation of Results and Limits of the Assay (p. 5-7) before proceeding:

- Select the proper sample dilutions accounting for expected potency of positives and minimizing non-specific binding (NSB) and other matrix effects; for example, net signal for non-immune samples should be lower than the **1 U/ml Calibrator**. This is usually 1:100 or greater dilution for human serum/plasma with normal levels of IgG and IgM.
- Run the **Anti-Meningococcal Positive Control**; value range is on the label.
- Run a Sample Diluent **Blank**. This signal is an indicator of proper assay performance, especially of washing efficacy, and is used for net OD calculations, if required. Blank OD should be <0.3.
- Run a set of **Calibrators**, which validate that the assay was performed to specifications: **10 U/ml** should give a high signal (>1.5 OD); **1 U/ml** should give a low signal which can be used to discriminate at the Positive/Negative threshold (see Interpretation of Results, p. 5).

Plate Set-up
Bring all reagents to room temperature (18-30°C) equilibration (at least 30 minutes).

- Determine the number of wells for the assay run. Duplicates are recommended, including 8 Calibrator wells and 2 wells for each sample control to be assayed.
- Remove the appropriate number of microwell strips from the pouch and return unused strips to the pouch. Reseal the pouch and store refrigerated.
- Add 200-300ul Working Wash Solution to each well and let stand for about 1-5 minutes. Aspirate or dump the liquid and pat dry on a paper towel before sample addition.

Assay Procedure

ALL STEPS ARE PERFORMED AT ROOM TEMPERATURE. After each reagent addition, gently tap the plate to mix the well contents prior to beginning incubation.

1. 1st Incubation [100ul – 60 min; 4 washes]

- Add 100ul of calibrators, samples, and controls each to pre-determined wells.
- Tap the plate gently to mix reagents and incubate for 60 minutes.
- Wash wells 4 times and pat dry on fresh paper towels. As an alternative, an automatic plate washer may be used. Improper washes may lead to falsely elevated signals and poor reproducibility.

2. 2nd Incubation [100ul – 30 min; 5 washes]

- Add 100ul of diluted Anti-Human IgG HRP to each well.
- Incubate for 30 minutes.
- Wash wells 5 times as in step 1.

3. Substrate Incubation [100ul – 15 min]

- Add 100ul TMB Substrate to each well. The liquid in the wells will begin to turn blue.
- Incubate for 15 minutes in the dark, e.g., place in a drawer or closet.

Note: If your microplate reader does not register optical density (OD) above 2.0, incubate for less time, or read OD at 405-410 nm (results are valid).

4. Stop Step [Stop: 100ul]

- Add 100ul of Stop Solution to each well.
- Tap gently to mix. The enzyme reaction will stop; liquid in the wells will turn yellow.

5. Absorbance Reading

- Use any commercially available microplate reader capable of reading at 450nm wavelength. Use a program suitable for obtaining OD readings and data calculations if available.
- Read absorbance of the entire plate at 450nm using a single wavelength within 30 minutes after Stop Solution addition. If available, program to subtract OD at 630nm to normalize well background.

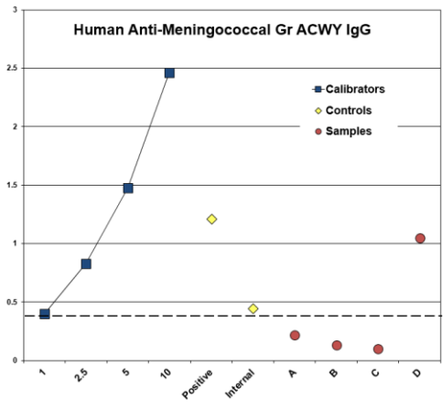
INTERPRETATION OF RESULTS

A. Antibody Activity Threshold Index

Compare Samples to **1 U/ml Calibrator** or **Internal Control**

= Positive/Negative Cut-off.

Example:



Results

The **sensitivity** of the assay to detect anti-ACWY IgG, from either natural infection or vaccination, is controlled so that the **1 U/ml Calibrator** represents a threshold OD for most true positives in human serum diluted to 1:100 or greater. Visual inspection of the data in the above graph shows the following:

Calibrators – dilution curve of anti-ACWY antiserum, derived from ACWY vaccination, shows the OD range of the assay; high value indicates optimal sensitivity of the assay.
1 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 – antiserum with reactivity to Meningococcal Group A; value range is on the label. This Control can be used to assess reproducibility and/or normalize between-assay variation.

Internal Control – a true positive from a normal individual that represents the lab's experience in distinguishing low positive from negative samples. This should be run in each assay to supplement the 1 U/ml Calibrator for Positive/Negative discrimination purposes.

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

The 1 U/ml Calibrator can be used to calculate a **Threshold Index** that numerically discriminates Positive/Negative:

- Divide each Sample net OD by the 1 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)

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:

1. Calculate the net OD mean + 2 SD of the Control/Non-immune samples = **Positive Index**.
2. 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.

Example:

Sample	Assay Net OD		Calculated Antibody Activity	
	Control	Exptl	Control	Exptl
1	0.325	2.281 **C**	0.75	**5.29**
2	0.272	1.581 **C**	0.63	**3.67**
3	0.133	0.998 **C**	0.31	**2.32**
4	0.194	0.453 **C**	0.45	**1.05**
5	0.289	0.767 **E**	0.67	**1.78**
6	0.319	0.982 **E**	0.74	**2.28**
7	0.332	0.401 **I**	0.77	0.93
8	0.291	0.351 **E**	0.68	0.81
9	0.402	0.325 **E**	0.93	0.75
10	0.253	0.16 **E**	0.59	0.37
Mean	0.281			
SD	0.075			
Mean +2 SD	**0.431**	= **Positive Index**		

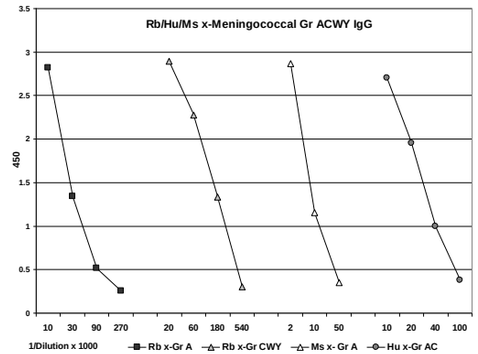
Results

Experimental Samples are represented as follows:

C – Calibrator
I – Internal Control; lab's threshold positive serum
E – Experimental sample

ASSAY PERFORMANCE

Antibody Titer & Specificity
Dilutions of antibodies with specificity for the different Group antigens were run in the assay to demonstrate the efficacy of the multiple antigen (ACWY) coated plate. Titers were calculated as inverse of the dilution that produced a **1.0 OD** in the assay.



Results

Rb x-Gr A: rabbit immunized with the Group A segment of the Menveo vaccine by Sanofi Pasteur. Titer: **47.5 k**

Rb x-Gr CWY: rabbit immunized with the Group CWY segment of the Menveo vaccine by Sanofi Pasteur. Titer: **255 k**

Ms x-Gr A: mouse monoclonal antibody specific to Group A antigen; a candidate WHO standard. Titer: **13.6 k**

Hu x-Gr A/C: a human serum pool reactive with Groups A and C provided by the CDC as a Reference Pool. Titer: **40 k**

Quality Control
The calibrators and controls must perform as stated in the manual. High blanks or Negative controls (A450 >0.5) is usually due to inefficient plate washing or too much sample or high amount of antibody-HRP conjugate. Please read the manual carefully before using the kit.

PRECAUTIONS AND SAFETY INSTRUCTIONS

Calibrators and controls may contain diluted human serum, although tested –ve for HIV/HCV etc, but all precautions must be considered and samples treated as potentially infectious.

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.

Instruction Manual No. M-600-880-XHG

Human Anti-Meningococcal Group ACWY IgG ELISA Kit

Cat. #600-880-XHG, 96 Tests

For the Quantitation of IgG Antibodies to Group A,C,W,Y Oligosaccharides of *Neisseria meningitides* in Human Serum/Plasma

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

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ELISA Kit Components	Amount	Part #
Meningococcal ACWY Coated Strip Plate	8-well strips (12)	600-ACWY
Anti-Meningococcal Positive Control	0.65 ml	600-8AX-SrPC
Anti-Meningococcal Calibrator 1 U/ml	0.65 ml	600-8AX-SrB
Anti-Meningococcal Calibrator 2.5 U/ml	0.65 ml	600-8AX-SrC
Anti-Meningococcal Calibrator 5 U/ml	0.65 ml	600-8AX-SrD
Anti-Meningococcal Calibrator 10 U/ml	0.65 ml	600-8AX-SrE
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-600-880-XHG