



**Calculation of the Cut-off value (C.O.) =  $N_c \times 2.1$**   
 ( $N_c$  = the mean absorbance value for three negative controls). If  $N_c$  is < 0.05, take it as 0.05.

**Quality control (assay validation):** The test results are valid if the Quality Control criteria are fulfilled. It is recommended that each laboratory must establish appropriate quality control system with quality control material similar to or identical with the patient specimen being analyzed.

- The A value of the Blank well, which contains only Chromogen and Stop solution, is < 0.080 at 450nm.
- The A values of the Positive control must be  $\geq 0.800$  at 450/600-650nm or at 450nm after blanking.
- The A values of the Negative control must be  $\leq 0.100$  at 450/600-650nm or at 450nm after blanking.

If one of the Negative control A values does not meet the Quality Control criteria, it should be discarded, and the mean value should be calculated by using the remaining two values. If more than one Negative control A values do not meet the Quality Control Range specifications, the test is invalid and must be repeated.

**Example:**

**1. Quality Control**  
 Blank well A value:  $A1 = 0.025$  at 450nm (Note: blanking is required only when reading with single filter at 450nm)

**Well No.:**                    **B1**    **C1**    **D1**  
 Negative control A values after blanking:            0.012    0.010    0.011

**Well No.:**                    **E1**    **F1**  
 Positive control A values after blanking:            2.363    2.436

All control values are within the stated quality control range

**2. Calculation of  $N_c$ :** =  $(0.012+0.010+0.011) / 3 = 0.011$ .  $N_c$  is < 0.05 so the value of 0.05 is used in the next step.

**3. Calculation of the Cut-off:** (C.O.) =  $0.05 \times 2.1 = 0.105$

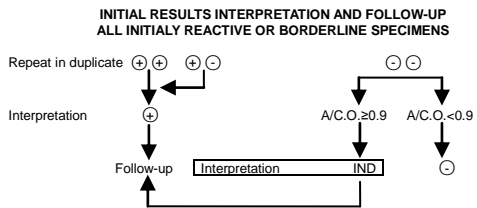
**INTERPRETATIONS OF THE RESULTS**

**Negative Results** (A / C.O. < 1): Specimens giving A value less than the Cut-off value are negative for this assay, which indicates that no SARS-CoV-2 IgM antibodies have been detected with WANTAI SARS-CoV-2 IgM ELISA.

**Positive Results** (A / C.O.  $\geq 1$ ): Specimens giving A value equal to or greater than the Cut-off value are considered initially reactive, which indicates that SARS-CoV-2 IgM antibodies have probably been detected with WANTAI SARS-CoV-2 IgM ELISA. Retesting in duplicate of any initially reactive specimen is recommended. Repeatedly reactive specimens could be considered positive for SARS-CoV-2 IgM antibodies therefore there are serological indications for current coronavirus disease COVID-19.

**Borderline** (A / C.O. = 0.9-1.1): Specimens with A value to Cut-off ratio between 0.9 and 1.1 are considered borderline and retesting of these specimens in duplicate is required to confirm the initial results.

**Follow-up, confirmation and supplementary testing of any positive specimen with other analytical system (e.g. PCR) is required. Clinical diagnosis should not be established based on a single test result. It should integrate clinical and other laboratory data and findings.**



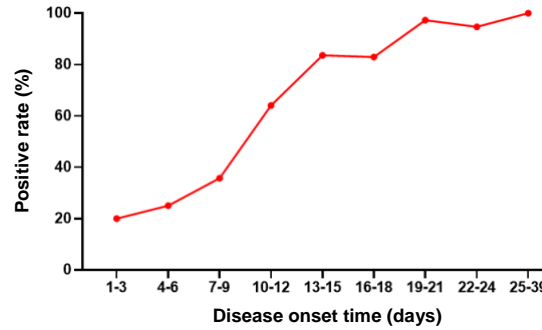
IND = non interpretable

- If, after retesting of the initially reactive specimens, both wells are negative results (A/C.O.<0.9), these specimens should be considered as non-repeatable positive (or false positive) and recorded as negative. As with many very sensitive ELISA assays, false positive results can occur due to the several reasons, most of which are connected with, but not limited to, inadequate washing step. For more information regarding Wantai ELISA Troubleshooting, please refer to Wantai's "ELISAs and Troubleshooting Guide".
- If after retesting in duplicate, one or both wells are positive results, the final result from this ELISA test should be recorded as repeatedly reactive. Repeatedly reactive specimens could be considered positive for SARS-CoV-2 IgM antibodies and therefore the patient is probably infected with SARS-CoV-2.
- After retesting in duplicate, specimens with values close to the Cut-off value should be interpreted with caution and considered as "borderline" zone specimen, or uninterpretable for the time of testing.

**PERFORMANCE CHARACTERISTICS**

**Sensitivity and specificity:** clinical validation study of Wantai SARS-CoV-2 IgM ELISA was conducted in 2020 in Shenzhen, China. 310 specimens from confirmed COVID-19 patients and 333 specimens from healthy individuals were tested. The kit demonstrated sensitivity of 86.8% (269/310) and specificity of 100% (333/333).

Samples were collected from COVID-19 confirmed cases with clinical symptoms, laboratory abnormalities or pulmonary imaging manifestations. No tests have been performed on specimens from latent infections or patients in the incubation period. It was observed that the detection rate of the kit was closely related to the time of disease onset, the kit showed higher positive detection rate in specimens from patients with delayed onset. Therefore, the interpretation of the test results should consider the specimen's collection time.



**LIMITATIONS**

1. Positive results must be confirmed with another available method and interpreted in conjunction with the patient clinical information.
2. Antibodies may be undetectable during the early stage of the disease and in some immunosuppressed individuals. Therefore, negative results obtained with SARS-CoV-2 IgM ELISA are only indication that the specimen does not contain detectable level of IgM antibodies and any negative result should not be considered as conclusive evidence that the individual is not infected with SARS-CoV-2.
3. If, after retesting of the initially reactive specimens, the assay results are negative, these specimens should be considered as non-repeatable (false positive) and interpreted as negative. As with many very sensitive ELISA assays, false positive results can occur due to the several reasons, most of which are related but not limited to inadequate washing step. For more information regarding Wantai ELISA Troubleshooting, please refer to Wantai's "ELISAs and Troubleshooting Guide", or contact Wantai technical support for further assistance.
4. The most common assay mistakes are: using kits beyond the expiry date, bad washing procedures, contaminated reagents, incorrect assay procedure steps, insufficient aspiration during washing, failure to add specimens or reagents, improper operation with the laboratory equipment, timing errors, the use of highly hemolyzed specimens or specimens containing fibrin, incompletely clotted serum specimens.
5. The prevalence of the marker will affect the assay's predictive values.
6. This kit is intended ONLY for testing of individual serum or plasma specimens. Do not use it for testing of cadaver specimens, saliva, urine or other body fluids, or pooled (mixed) blood.
7. This kit is a qualitative assay and the results cannot be used to measure antibodies concentrations.

**SUMMARY OF THE MAJOR COMPONENTS OF THE KIT:**

Use this summary only as a reference and always follow the comprehensive method sheet when performing the assay. Note: the components of individual kits are not lot- interchangeable.

1. Microwell plate	Code 5	one
2. Negative Control	Code 8	1x0.5ml
3. Positive Control	Code 7	1x0.5ml
4. HRP-Conjugate	Code 6	1x12ml
5. Specimen Diluent	Code 9	1x12ml
6. Wash Buffer	Code 1	1x50ml
7. Chromogen Solution A	Code 2	1x6ml
8. Chromogen Solution B	Code 3	1x6ml
9. Stop Solution	Code 4	1x6ml

**SUMMARY OF THE ASSAY PROCEDURE:**

Use this summary only as a reference and always follow the detailed method sheet when performing the assay.

Add Specimen Diluent	100µl
Add Specimen	10µl
Incubate	30 minutes
Wash	5 times
Add HPR-Conjugate	100µl
Incubate	30 minutes
Wash	5 times
Coloring	50µl A + 50µl B
Incubate	15 minutes
Stop the reaction	50µl stop solution
Read the absorbance	450nm or 450/600-650nm

**EXAMPLE SCHEME OF CONTROLS / SPECIMENS DISPENSING:**

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	S3										
B	Neg.	...										
C	Neg.	...										
D	Neg.	...										
E	Pos.											
F	Pos.											
G	S1											
H	S2											

**SYMBOLS:**



In Vitro Diagnostic Medical Device



+2°C~+8°C Storage Conditions



Use By



Batch



Content Sufficient For <n> Tests



Instructions For Use



Catalog Number



Manufacturer



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