# COVID-19

## Serologic Immunoassay solutions

**DRG International, Inc.**
*Springfield, NJ, USA*
•Global Headquarters
•Opening of new facility in 2012
•Launch of R&D program in 2012
•Administrative and Operations base of global distribution and business


**DRG Instruments, GmbH**
*Marburg, Germany*
•Main DRG production facility, currently capable of producing >160 ELISAS
•Acquisition of NOVUM Diagnostics in 2003 added 70 Infectious Disease ELISAS to production portfolio
•Seat of DRG:Hybrid-XL project development

# Company History

- DRG International, Inc. was founded in 1970 as a multinational specialty clinical diagnostics and medical equipment manufacturer and distributor.
- 1970 – Sales began with Radioimmunoassays
- 1973 – DRG Instruments, GmbH founded in Munich
- 1990 – DRG Medtek founded in Warsaw, Poland
- 1991 – DRG Biomed founded in St. Petersburg, Russia
- 1993 – DRG Techsystems founded in Moscow, Russia
- 2000 – DRG Group receives ISO Certification in Marburg
- 2014 – Launch of DRG:Hybrid-XL®
- 2017 – DRG International launches Ecommerce platform
- 2017 – Dr Geacintov, CEO and Founder passes away.
- 2018 DRG International: ISO 13485 Certification
- 2019 – MDSAP Certification *(Medical Device Single Audit Program)*

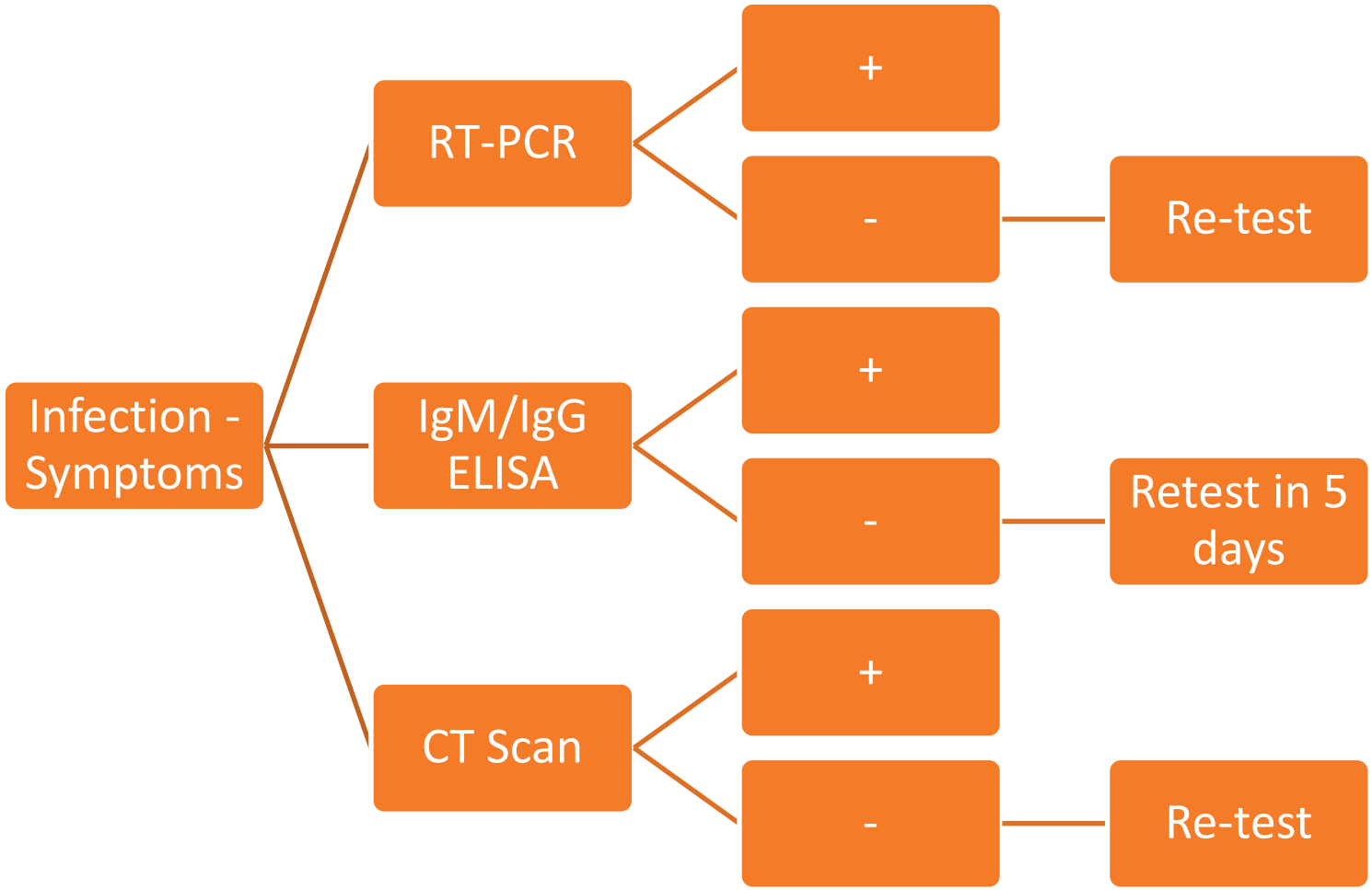
# COVID-19

- The pandemic COVID-19 is a new disease caused by a new coronavirus named SARS-CoV-2 that has not previously been seen in humans.
- SARS-CoV-2 is composed of a single positive RNA strand, which is the target of the widely used **rRT-PCR** (Real time Reverse transcription polymerase chain reaction) tests, as well as several proteins including the spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins.
- It has been suggested that the spike protein has sufficient affinity to the angiotensin converting enzyme 2 (ACE2), providing the entry point for the viruses.
  - ACE2 is mainly expressed in vascular endothelial cells, the renal tubular epithelium, and in Leydig cells in the testes. It is also expressed in the lung, kidney, and gastrointestinal tract, tissues shown to harbor SARS-CoV.
  - Patients with higher level of ACE2 due to the use of certain drugs (for example, ACE inhibitors) to treat underlying diseases (such as cardiac diseases, hypertension, or diabetes), are at higher risk for severe COVID-19 symptoms.
- Human-to-human transmission is primarily thought to occur among close contacts via respiratory droplets generated by sneezing and coughing.
- Reported illnesses have ranged from very mild (including some with no reported symptoms) to severe, including illness resulting in death. Older people and people of all ages with severe underlying health conditions, such as cardiac diseases, hypertension, or diabetes, are at higher risk of developing serious COVID-19 illness.


CDC

World Health Organization

# COVID-19 Clinical Diagnosis Algorithm



# Laboratory Examinations

Laboratory Examination for suspected patients include the following:

1. General Examination
2. Etiological and serological examination
    I. Novel Coronavirus nucleic acids can be detected in nasopharyngeal swabs, sputum and other lower respiratory tract secretions, blood and feces by using rRT-PCR. Detection of lower respiratory tract speciments (sputum or airway extracts) are more accurate. Submit specimens for inspection as soon as possible after collection
    ***II. Serological examination:***
        ***a. Most of the novel coronavirus-specific IgM antibodies begin to show positive after 3-5 days of onset.***
        ***b. The recovery phase of IgG antibody titers is 4 times or more higher than that of the acute phase.***
3. Chest Imaging

**National Health Commission of the People's Republic of China**
New Coronavirus Pneumonia Diagnosis and Treatment Program (Trial Version 7)

# Diagnostic Criteria for Confirmed Cases

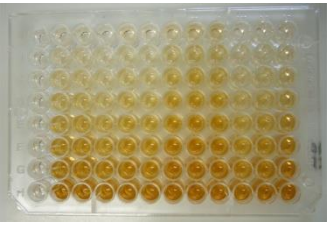
Suspected cases with one of the following etiology and serology evidence:

1.rRT-PCR detection of the novel Coronavirus nucleic acid is positive

2.Viral gene sequencing, highly homologous to known novel coronaviruses

***3.Serum new coronavirus-specific IgM and IgG antibodies were positive; serum new coronavirus-specific IgG antibodies turned positive from negative or the positive IgG value turned 4 times or higher in the recover phase than acute phase.”***

**National Health Commission of the People's Republic of China**
New Coronavirus Pneumonia Diagnosis and Treatment Program (Trial Version 7)



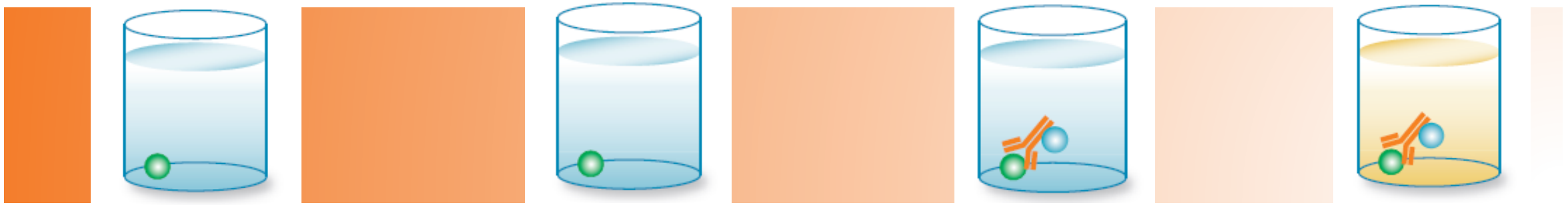
# Advantages of ELISA vs. rRT-PCR

| | ELISA | rRT-PCR |
|---|---|---|
| Purpose of Test | Is/ was there an immune response to virions? | Does virions exist in the samples? |
| Accessibility | Widely Available | Limited |
| Samples | Serum | Lower respiratory tract speciment (sputum or airway extracts), although nasopharyngeal swabs can be used |
| Level of Difficulty | Easy | Can be difficult |
| Safety | Safe | Riskier to handle samples with live virus |
| Sample Stablility | Very Good | RNA very prone to degradation |
| Results | Very reliable when both IgM and IgG are tested | High rate of false negative |
| Quantitation | Possible | Not Possible |
| Therapeutic Application | Possible | Not Possible |

The ELISA tests are used in conjunction with other tests such as rRT-PCR for diagnosis and monitoring treatment. ***They complement each other***!

# Enzyme Linked Immunoassay (ELISA)

*A well-established plate-based assay technique designed for detecting and quantifying substances such as peptides, proteins, antibodies and hormones.*



### Coating

Antigen or (capture antibody) is adsorbed onto wells in ELISA plate

### Blocking

A buffer containing unrelated protein is used to block free sites in the wells

### Detection

Enzyme conjugated detection antibody binds antigen

### Readout

Substrate is catalyzed by enzyme to generate colored readout

# EIA-6146/EIA-6146R : Novel Coronavirus COVID-19 IgG ELISA Kit

IgG is the most abundantly found immunoglobulin to be produced in response to an antigen and will be maintained in the body after initial exposure for long term response *

| | |
|---|---|
| Principle | Indirect Method |
| Sample Type | Serum |
| Sample Volume | 10 µL |
| Assay Incubation | 80 minutes, RT |
| Total Wash Steps | 2 |
| Limit of Detection | 5U/mL |
| Repeatability | CV < 15% |
| Reproducibility | CV < 20% |

- Utilizes Indirect ELISA method.
- An immune complex of “COVID-19 recombinant antigen – human anti-COVID-19 IgG antibody (in the serum sample)- HRP labeled anti-human IgG tracer antibody" is formed if there is anti-coronavirus IgG antibody present in the tested materials.
- Can easily be converted to quantitative measurement if IgG antibody-based treatments for COVID-19 are desired.
- EIA-6146 is for regions outside the USA, EIA-6146R (RUO) is for the USA

# Novel Coronavirus COVID-19 IgG Clinical Testing in China

| | Confirmed Positive | Confirmed Negative |
|---|---|---|
| IgG Test Positive | 30 | 0 |
| IgG Test Negative | 0 | 54 |
| IgG Test Borderline | 0 | 0 |

**Sensitivity = 100%**

**Specificity = 100%**

**PPV* = 100%**

**NPV** = 100%**

*** : Positive Predictive Value**

****: Negative Predictive Value**

- Serum samples from two cohorts of patients were tested using the IgG ELISA kit in China.
- The first cohort consisted of serum samples from normal healthy patients collected prior to the COVID-19 outbreak [December 3, 2019] (n = 54)
- The second consisted of serum samples from RT-PCR confirmed positive patients after two weeks of the onset of the disease (n = 30).

# EIA-6147/ EIA-6147R: Novel Coronavirus COVID-19 IgM ELISA Kit

IgM is the first immunoglobulin to be produced in response to an antigen and will be primarily detectable during the early onset of the disease

| Principle | Capture Method |
|---|---|
| Sample Type | Serum |
| Sample Volume | 20 µL |
| Assay Incubation | 80 minutes, 37 °C |
| Total Wash Steps | 2 |
| Limit of Detection | 5IU/mL |
| Repeatability | CV < 15% |
| Reproducibility | CV < 20% |

- Utilizes a Sandwitch ELISA method.
- An immune complex of “Anti-hIgM antibody - human COVID-19 IgM antibody (in the serum sample) - HRP labeled COVID-19 antigen" is formed if there is novel coronavirus IgM antibody present in the tested materials.
- National Health Commission of the People's Republic of China states that IgM antibodies begin to show positive after 3-5 days of onset of COVID-19.
- EIA-6147 is for regions outside the USA, EIA-6147R (RUO) is for the USA

# Novel Coronavirus COVID-19 IgM Clinical Testing in China

| | Confirmed Positive | Confirmed Negative |
|---|---|---|
| IgM Test Positive | 9 | 0 |
| IgM Test Negative | 10 | 54 |
| IgM Test Borderline | 1 | 0 |

**Sensitivity = 45.0%**
**Specificity = 100 %**
**PPV = 100 %**
**NPV = 83.1%**

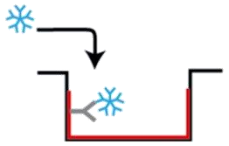
- Serum samples from two cohorts of patients were tested using the IgG ELISA kit in China.
- The first cohort consisted of serum samples from normal healthy patients collected prior to the COVID-19 outbreak [December 3, 2019] (n = 54)
- The second consisted of serum samples from RT-PCR confirmed positive patients after two weeks of the onset of the disease (n = 20).

- IgM is the first immunoglobulin to be produced in response to SARS-CoV-2 and will be primarily detectable during the early onset of the disease. It can be detected after 3-5 days of onset of COVID-19.
- Serum samples showing here were from patients after *two weeks* of the onset of the disease, at which point antibody class switching (from IgM to IgG) has already occurred, resulting in low level of IgM and high level of IgG.
- Therefore, low sensitivity of the IgM tests can be attributed to the sample collection date after the onset of the disease.

# Assay Protocols

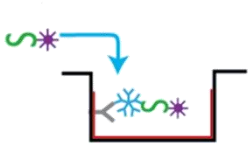
## EIA-6146 SARS-CoV-2 IgG ELISA Kit

Add **100 µL** of controls and 1:100 diluted samples into the designated microwells coated with COVID-19 peptide antigen.



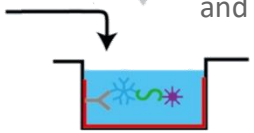
Incubate for **30** minutes and wash

Add **100 µL** of HRP-labeled COVID-19 Tracer Antibody



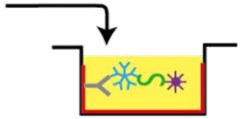
Incubate for **30** minutes and wash

Add **100 µL** of substrate



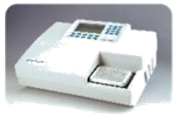
Incubate for **20** minutes

Add **100 µL** of stop solution



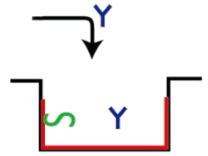
Read within **10** minutes

Read absorbance at **450 nm** with a microplate reader



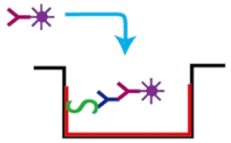
## EIA-6147 SARS-CoV-2 IgM ELISA Kit

Add **100 L** of controls and 10**µL** samples plus 100 **µL diluent** into the designated microwells coated with Anti-hIgM antibody.



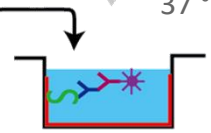
Incubate for **30** minutes at 37 °C and wash

Add **100 µL** of HRP-labeled COVID-19 Antigen



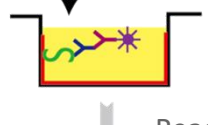
Incubate for **30** minutes at 37 °C and wash

Add **100 µL** of substrate



Incubate fat RT or **20** minutes

Add **100 µL** of stop solution



Read within **10** minutes

Read absorbance at **450 nm** with a microplate reader

# Interpretation of IgG Assay Results

**Defined assay cut-off to minimize inter-assay and inter-lab OD differences.**

1. Calculate the average value of the absorbance of the negative control (xNC).
2. Calculate the cutoffs using the following formulas:
   - Positive cutoff = 1.1 X (xNC + 0.18)
   - Negative cutoff = 0.9 x (xNC + 0.18)
3. Determine the interpretation of the sample by comparing the OD to the following table:

| Interpretation | Interval | Results |
|---|---|---|
| Negative | Measured value ≤ negative cutoff | The sample does not contain the new coronavirus (COVID-19) IgG-related antibody |
| Positive | Measured value ≥ positive cutoff | The sample contains novel coronavirus (COVID-19) IgG-associated antibodies. |
| Borderline | Negative Cutoff < Measured value < Positive cutoff | Retest the sample in conjunction with other clinical tests. |

# Interpretation of IgM Assay Results

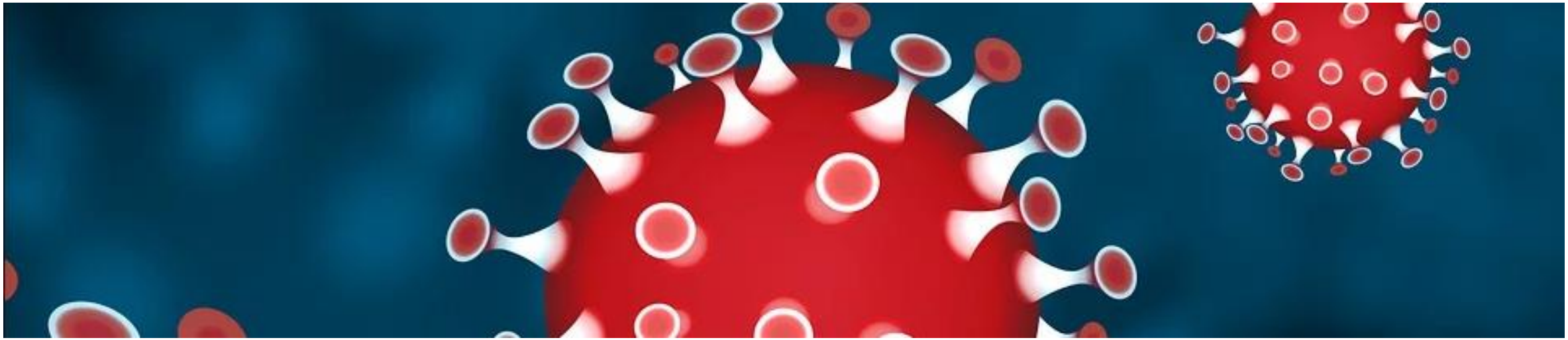
**Defined assay cut-off to minimize inter-assay and inter-lab OD differences.**

1. Calculate the average value of the absorbance of the negative control (xNC).
2. Calculate the cutoffs using the following formulas:
   - Positive cutoff = 1.1 X (xNC + 0.10)
   - Negative cutoff = 0.9 x (xNC + 0.10)
3. Determine the interpretation of the sample by comparing the OD to the following table:

| Interpretation | Interval | Results |
|---|---|---|
| Negative | Measured value ≤ negative cutoff | The sample does not contain the new coronavirus (COVID-19) IgM-related antibody |
| Positive | Measured value ≥ positive cutoff | The sample contains novel coronavirus (COVID-19) IgM-associated antibodies. |
| Borderline | Negative cutoff < Measured value < Positive cutoff | Retest the sample in conjunction with other clinical tests. |

# Benefits of ELISA Testing

- IgM and IgG tests can be combined for efficient clinical diagnosis at multiple stages.
- Established industry technology.
- Easy-to-use and cost efficient product.
- Minimal error in sample handling.
- Low risk and low incidence of cross-contamination.



# THANK YOU!