INTERPRETATION INFORMATION SHEET

Testing for COVID-19 Convalescent Plasma Qualification

FDA Guidance issued in April 2020 (https://www.fda.gov/media/136798/download) provides recommendations for convalescent plasma collected from individuals who have recovered from COVID-19 during the public health emergency. Creative Testing Solutions has the ability to provide in-process testing to meet current regulations associated with distribution of COVID-19 convalescent plasma.

Below is a summary of the guidance document requirements:
- Prior diagnosis of COVID-19 documented by a laboratory test
- Complete resolution of symptoms at least 28 days prior to donation
  OR
  Complete resolution of symptoms at least 14 days prior to donation AND negative results by either from one or more nasopharyngeal swab specimens or by a molecular diagnostic test from blood.
- Female donors negative for HLA antibodies or male donors
- Defined SARS-CoV-2 neutralizing antibody titers of at least 1:160. A titer of 1:80 may be considered acceptable if an alternative matched unit is not available

Creative Testing Solutions is implementing the following tests for SARS-CoV-2, each of which may be required prior to qualification of a donation as acceptable for COVID-19 convalescent plasma (CCP). FDA-licensed establishments may submit requests for applicable testing directly to CTS.

Ortho Clinical Diagnostics VITROS® Ortho Anti-SARS-CoV-2 Total

The Ortho VITROS® Anti-SARS-CoV-2 Total test qualitatively detects antibody (including IgG and IgM) to SARS-CoV-2 S protein. This test may be used to detect the immune response to SARS-CoV-2 infection and may be used in conjunction with other measures to qualify a donation as acceptable for CCP. It is not intended for use as a routine donor screening test and results may not be used for donor management or donor counseling.

An immunometric technique is used which involves a two stage reaction.
- In the first stage antibodies to SARS-CoV-2 present in the sample bind with SARS-CoV-2 antigen coated on the test well. Unbound sample is removed by washing. In the second stage horseradish peroxidase (HRP)-labeled recombinant SARS-CoV-2 antigen is added in the conjugate reagent. The conjugate binds specifically to any anti-SARS-CoV-2 captured on the test well in the first stage. Unbound conjugate is removed by the subsequent wash step.
- The bound HRP conjugate is measured by a luminescent reaction. A reagent containing luminogenic substrates and an electron transfer agent is added to the test wells. The HRP in the bound conjugate catalyzes the oxidation of the luminol derivative, producing
light. The electron transfer agent (a substituted acetanilide) increases the level of light produced and prolongs its emission. The light signals are read by the system. The amount of HRP conjugate bound is indicative of the amount of SARS-CoV-2 antibody present.

CTS will report results of the Ortho VITROS® Anti-SARS-CoV-2 Total test as Nonreactive or Reactive.
- Samples with reactivity at or above the defined Signal/Cutoff (S/CO) threshold required for qualification of COVID-19 convalescent plasma will be sent to an alternate laboratory for SARS-CoV-2 Reporter Viral Particle Neutralization (RVPN) if applicable.
- No further testing is performed if reactivity is below the defined S/CO threshold.

**Vitalant Research Institute (VRI) SARS-CoV-2 Reporter Viral Particle Neutralization (RVPN)**

VRI RVPN testing is performed when the qualitative SARS-CoV-2 antibody screening test is reactive at or above the defined S/CO threshold to help determine the specificity and relative neutralizing capability of the antibody. The RVPs represent a safe and rapid way of quantitatively measuring neutralization, by using SARS-CoV-2 Spike glycoprotein pseudotyped onto a rhabdovirus reporter virus. The assay is performed by mixing serial dilutions of serum with constant concentrations of reporter viruses, in equal volumes. The mixture is then added to cell culture monolayers to assess antibody presence. Inhibited reporter virus growth in the cell culture monolayers is indicative of the presence of neutralizing antibody in serum. The SARS-CoV-2 RVPN detects the presence of neutralizing antibody directed against the virus but there is still a potential that cross-reactivity with other coronavirus species may occur.

FDA recommends neutralizing antibody titration for CCP qualification. The current Ortho VITROS assay has been compared to the VRI research use only RVPN assay demonstrating that the recommended titer of at least 1:160 may be achieved with samples demonstrating reactivity at or above a defined S/CO threshold.

CTS will automatically submit Ortho VITROS antibody positive samples with reactivity at or above the defined S/CO threshold to Vitalant Research Institute (VRI) for SARS-CoV-2 RVPN. CTS will report the titer results provided by VRI as follows:

- Negative, titer <1:40
- Positive, 1:40
- Positive, 1:160
- Positive, 1:640
- Positive, 1:2560
- Positive, 1:10240
- Positive, >1:10240

**Grifols Procleix® SARS-CoV-2 Research Use only (RUO) Assay**

The Grifols Procleix® SARS-CoV-2 RUO assay is a qualitative in vitro nucleic acid assay system for the detection of SARS-CoV-2 RNA in individual plasma and serum specimens.
The assay is intended solely for detection of SARS-CoV-2 nucleic acid and may be used in conjunction with other measures to qualify a donation as acceptable for CCP. This test will only be performed if the Ortho VITROS antibody test is reactive at or above the defined S/CO threshold required for qualification of COVID-19 convalescent plasma. It is currently performed as a research use only test and is not intended for use as a routine donor screening test and results may not be used for donor management or donor counseling.

The assay involves three main steps which take place in a single tube: sample preparation, SARS-CoV-2 target amplification by Transcription-Mediated Amplification (TMA), and detection of the amplification products (amplicons) by the Hybridization Protection Assay (HPA). TMA uses two enzymes, one enzyme to generate a DNA copy of the target RNA sequence (if present in the sample) and a second enzyme to produce multiple copies of the RNA amplicon from the DNA copy template. Detection is achieved using probes with chemiluminescent labels complementary to the amplicon which specifically hybridize to the amplicons which are then measured in a luminometer and reports as Relative Light Units (RLU). Internal controls are included within each sample tube to verify amplification and detection processes have successfully occurred. Calibrators are used to determine the assay cutoff and assess run validity.

CTS will report results of the Procleix® SARS-CoV-2 RUO NAT as Nonreactive, Reactive or Invalid.

- A specimen is considered nonreactive if the analyte signal is less than the analyte cutoff value and the internal control (IC) signal is greater than or equal to the IC cutoff.
- A specimen is considered reactive if the analyte signal is greater than or equal to the analyte cutoff value and the IC signal is less than or equal to 750,000 RLU.
- A specimen is invalid if the analyte signal is less than the analyte cutoff and the IC signal is less than the IC cutoff. A specimen is also invalid if the IC value is greater than 740,000 RLU.

IMPORTANT:

- The Ortho Clinical Diagnostics VITROS® Anti-SARS-CoV-2 Total test has been granted an FDA Emergency Use Authorization (EUA)
- The Grifols Procleix® SARS-CoV-2 RUO Assay and VRI SARS-CoV-2 RVPN tests have not been reviewed by the FDA.
- Blood centers requesting a SARS-CoV-2 Research Use Only (RUO) NAT for qualification of COVID-19 convalescent plasma must include a statement regarding research use authorization in applicable donor information prior to donation.
- Negative results do not rule out SARS-CoV-2 infection, particularly in those who have been in contact with the virus.
- Results from these tests should not be used as the sole basis to exclude or include SARS-CoV-2 infection or to inform infection status.
- Positive results may be due to past or present infection with non-SARS-CoV-2 coronavirus strains, such as coronavirus HKU1, NL63, OC43, or 229E.
- These tests are NOT intended for the screening of donated blood and results may not be used for donor management or donor counseling.

References:

Revision History

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<thead>
<tr>
<th>Revision</th>
<th>Implemented</th>
<th>Reason</th>
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<tbody>
<tr>
<td>Initial Release</td>
<td>04/20/20</td>
<td>Implementation of testing for COVID-19 convalescent plasma qualification</td>
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