Validated Inactivation Methods
for SARS-CoV-2 and COVID-19 positive samples

Internally validated methods for listed samples:
[CLICK ON LISTING TITLE TO GO DIRECTLY TO THAT ENTRY]

- Whole blood
- Serum/plasma
- Urine
- Saliva
- Cell culture monolayers
- Tissue samples
- Oropharyngeal (OP)/Nasopharyngeal (NP) swabs (in Viral Transport Medium (VTM))
- Endotracheal Aspirate
- Other Reagents
- CDC-approved RNA Extraction Buffers

**Whole blood**

*Heat inactivation* at 56 °C • incubate for 1 hour •
(validated by Dr. Sara Cherry)

**Serum/plasma**

*Heat inactivation* at 56 °C • incubate for 1 hour •
(validated by Dr. Sara Cherry)

*Paraformaldehyde* (4% final concentration) • incubate at room temperature • 15 min •

*Trizol Reagent* (900μL Trizol per 100μL sample) • incubate at room temperature • 10 min •

*Trizol LS Reagent* (3 parts Trizol LS reagent / 1 part sample) • incubate at room temperature • for 10 min •

*Triton X-100* (4% final concentration) • incubate at room temperature • 5 min •
(validated by Dr. Sara Cherry)

**Ineffective inactivation**

*Triton X-100* (1% final concentration) • incubation at room temp or 37 °C • 15 min •
(validated by Dr. Susan Weiss’ lab)

**Inactivation being evaluated**

*UV inactivation*
(testing by Dr. Sara Cherry)
Urine

**Heat inactivation** at 56 °C • incubate for 1 hour •
(validated by Dr. Sara Cherry)

Saliva

**Heat inactivation** at 95 °C • incubate for 5 min •
(validated by Dr. Sara Cherry)

Cell monolayers

**Glutaraldehyde solution** (2.5% final concentration) • incubate at room temperature • 15 min •

**Paraformaldehyde solution** (4% final concentration) • incubate at room temperature • 30 min •
(validated for MERS coronavirus)

**Trizol Reagent** with 300-400 μL Trizol per 1x10⁵ - 1x10⁷ cells • incubate at room temperature • 10 minutes • sample volume must not exceed 10% of the volume of Trizol Reagent used for lysis

Inactivation being evaluated

**Qiagen RNease Mini Plus: RLT-plus lysis buffer**

**NP-40 lysis buffer** (containing 1% NP-40, 2mM EDTA, 10% glycerol, 150mM NaCl, 50mM Tris pH 8.0, and Roche protease inhibitors)

Tissue Samples

**Trizol Reagent**, 1 mL per 50-100 mg tissue for homogenization in closed system; sample volume must not exceed 10% of the volume of Trizol Reagent used for lysis

Oropharyngeal (OP)/Nasopharyngeal (NP) swabs in Viral Transport Medium (VTM)

**Heat inactivation** at 56 °C • incubate for 1 hour •
(validated by Dr. Sara Cherry)
## Endotracheal Aspirate

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Preparation Note</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trizol Reagent</strong> (900μL Trizol per 100μL sample)</td>
<td>incubate at room temperature • 10 min •</td>
</tr>
<tr>
<td><strong>Trizol LS Reagent</strong> (3 parts Trizol LS reagent and 1 part sample)</td>
<td>incubate at room temperature • 10 min •</td>
</tr>
</tbody>
</table>

## Other Reagents

- **COPAN eNAT medium**  
  *(validated by Dr. Susan Weiss’ lab)*

**Inactivation being evaluated**

- Qiagen CD1 solution  
  *(testing by Dr. Susan Weiss’ lab)*
Documented methods: CDC-approved RNA extraction buffers

(FDA Emergency Use Authorization: https://www.fda.gov/media/134922/download)
Must be used following product manual and manufacturer’s recommendations.

<table>
<thead>
<tr>
<th>Instrument/Manufacturer</th>
<th>Extraction Kit</th>
<th>Catalog No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>QIAGEN</td>
<td>$^2$QIAmp DSP Viral RNA Mini Kit</td>
<td>50 extractions (61904)</td>
</tr>
<tr>
<td></td>
<td>$^2$QIAmp Viral RNA Mini Kit</td>
<td>50 extractions (52904)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>250 extractions (52906)</td>
</tr>
<tr>
<td>QIAGEN EZ1 Advanced XL</td>
<td>$^2$EZ1 DSP Virus Kit</td>
<td>48 extractions (62724)</td>
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<td></td>
<td>Buffer AVL (19073 or 19089)</td>
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<tr>
<td></td>
<td></td>
<td>EZ1 Advanced XL DSP Virus Card (9018703)</td>
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<td></td>
<td>$^2$EZ1 Virus Mini Kit v2.0</td>
<td>48 extractions (955134)</td>
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<td>Buffer AVL (19073 or 19089)</td>
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<td>EZ1 Advanced XL Virus Card v2.0 (9018708)</td>
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<td>Roche MagNA Pure 24</td>
<td>$^2$MagNA Pure 24 Total NA Isolation Kit</td>
<td>96 extractions (07 658 066 001)</td>
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<td>External Lysis Buffer (06 374 913 001, 12 239 469 103, 03 246 779 001 or 03 246 752 001)</td>
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<td>Roche MagNA Pure 96</td>
<td>$^2$DNA and Viral NA Small Volume Kit</td>
<td>576 extractions (06 543 588 001)</td>
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<td></td>
<td>External Lysis Buffer (06 374 913 001, 12 239 469 103, 03 246 779 001 or 03 246 752 001)</td>
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<tr>
<td>$^3$Roche MagNA Pure LC</td>
<td>$^2$Total Nucleic Acid Kit</td>
<td>192 extractions (03 038 505 001)</td>
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<tr>
<td>$^3$Roche MagNA Pure Compact</td>
<td>$^2$Nucleic Acid Isolation Kit I</td>
<td>32 extractions (03 730 964 001)</td>
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<tr>
<td>$^3$QIAGEN QI/Acube</td>
<td>$^2$QIAmp DSP Viral RNA Mini Kit</td>
<td>50 extractions (61904)</td>
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$^1$Equivalence and performance of these extraction platforms for extraction of viral RNA were demonstrated with the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel (K190302). Performance characteristics of these extraction platforms with 2019-nCoV (SARS-CoV-2) have not been demonstrated.

$^2$CDC has confirmed that the external lysis buffer used with this extraction method is effective for inactivation of SARS-CoV-2.

$^3$CDC has compared the concentration of inactivating agent in the lysis buffer used with this extraction method and has determined the concentration to be within the range of concentrations found effective in inactivation of SARS-CoV-2.