FLUXERGY

Fluxergy Analyzer
Trial Evaluation

See suggested testing protocols for organizations evaluating the performance of the Fluxergy Test Kit CoVID-19 RT-PCR test. Fluxergy strongly recommends use of suggested control materials for optimal performance.

A suggested protocol is listed for the following:

- Limit of Detection (LoD) – Analytical Sensitivity
- Clinical Evaluation (Pos and Neg Agreement)

1. Suggested Materials

The following table describes recommended control materials and clinical samples for quality control testing as well as validating performance of the Fluxergy Test Kit COVID-19 RT-PCR Test.

Table 1: Recommended input materials for quality controls

Sample/ Control	Recommended Sample Input	Notes
Positive Control	ZeptoMetrix – NATtrol™ SARS- CoV-2 (recombinant) Stock 1mL Catalog #: 0831042	• Chemically modified, non-infectious and refrigerator stable qualitative control
	If copies/mL is desired, use Qnostics SARS CoV-2 Medium Q 01 SCV2MQC01-B	• Add directly without nucleic acid extraction (recommended Fluxergy workflow)
Negative Control	UTM or VTM	• Have validated BD UVT™, Copan UTM™, Puritan UniTranz-RT™ Transport Systems (UTM)
LOD – Analytical Sensitivity	ZeptoMetrix -SARS-CoV-2 (Isolate: USA-WA1/2020) Culture Fluid (Heat Inactivated) 0.5 mL Catalog # 0810587CFHI	• Tests both lysis and amplification of whole virus • Dilute in UTM or equivalent transport medium • NOTE: Titer by $TCID_{50}$ assay can be obtained from CoA titer
Clinical Samples	Nasopharyngeal swab samples (NPS) in 3mL of VTM or UTM	• Critical to use 3 mL UTM/UVT due to direct PCR • Have validated BD UVT™, Copan UTM™, Puritan UniTranz-RT™ • 3-D printed or cotton swabs has not been validated

2. LoD and Analytical Sensitivity

Fluxergy Sample Dilution and Tentative LoD Determination

- The heat inactivated virus (0810587CFHI-0.5 mL) was serially diluted in pooled negative nasopharyngeal swab matrix. Nasopharyngeal swab matrix was collected with FLOQSwabs™ in Puritan UniTranz-RT 3 ml Universal Transport Media (SKU#: UT-300) and qualified as negative by a reference RT-PCR before using in spiking studies.
- The viral stock can simply be diluted in an acceptable UTM or VTM.
- To generate samples for LoD/Analytical sensitivity testing, the ZeptoMetrix control stock was added to the UTM or NPS in UTM starting with a 1:10 dilution (0.1 mL Zepto to 0.9 mL UTM). For instance, we continued to dilute the stock material down to 1:1,000,000 to reach LoD. A total of n=3 was tested for each sample dilution, a total of 6 groups.

To determine a tentative LoD, Fluxergy performed the following experiments listed in Table 2.

Table 2: LoD testing

Tentative LoD of SARS-CoV-2 Culture Fluid (Heat Inactivated virus, ZeptoMetrix Catalog # 0810587CFHI-0.5mL) in pooled negative NPS matrix		
Dilution	Concentration ($TCID_{50}$/mL)	n
1:1,000	355	3
1:10,000	35.5	3
1:100,000	3.55	3
1:200,000	1.78	3
1:1,000,000	**0.**35	3
1:2,000,000	0.18	3

* The LoD was found to be 0.3 $TCID_{50}$/mL in an internal performance evaluation. Determination of the LoD will be depend on LOT, source, and type of positive control used.

Confirming the LOD

Confirmation of LoD can be assessed with 20 replicates. The LoD using the Zeptomatrix Catalog # 0810587CFHI was found to be 0.3 $TCID_{50}$/mL. The limit of detection as defined as the lowest concentration at which target is detected at least 95% of the time. The LoD of the Fluxergy Test Kit CoVID-19 was determined in an internal performance evaluation to be 0.3 $TCID_{50}$/mL.

Table 3: Confirmation of LoD testing

Confirmatory LoD of of SARS-CoV-2 Culture Fluid (Heat Inactivated virus, ZeptoMetrix Catalog # 0810587CFHI-0.5mL) in pooled negative NPS matrix	
Concentration	Replicates
0.3 $TCID_{50}$/mL	10

3. Clinical Evaluation Sensitivity and Specificity

To ensure performance in a clinical setting, the Fluxergy CoVID RT-PCR test can be challenged with clinical samples. If using contrived samples, use a positive control with a concentration above LoD (i.e. 2x–5x). See Table 4 for suggested number of tests for assessing clinical performance of Fluxergy assay. See Table 1 for suggested positive and negative sample inputs.

In a performance evaluation, the Fluxergy Analyzer's clinical performance were determined to 93.54 % (Positive agreement) and 95.08 % (Negative agreement). The clinical evaluation consisted of 31 positive samples (including 10 contrived) and 61 negative samples (including 36 contrived). .

Table 4: Clinical Evaluation

Group	Reference Result	n
Positive	Positive by Reference RT-PCR	20
Negative	Negative by Reference RT-PCR	20

Additional Considerations

- It is recommended that all NPS be diluted in an accepted 3mL UTM or VTM. Because the test is a direct PCR, sample dilution into correct volume of transport media is important.
- All samples or matrix used for clinical evaluation must be characterized by an authorized Molecular assay. The user must acknowledge the LoD of the comparator assay and Fluxergy assay when selecting samples for clinical evaluation..
- Improperly collected, transported, or handled samples risk the potential for false positive, false negative or erroneous results. The detection of viral nucleic acid is dependent upon proper sample collection, handling, transportation, storage and preparation.
- Multiple freeze thaws can affect stability of viral RNA. The exogenous internal control will not indicate whether nucleic acid has been lost to inadequate collection, transport and storage of samples.

Contact us

Fluxergy
13766 Alton Pkwy
Irvine, CA 92618

Email: customersupport@fluxergy.com
Phone: 949-305-4201
http: // fluxergy.com / SARS-CoV-2