1. Evaluation of No Detect Control (NDC) Contamination

Please consult NDC_Check_1691478213.csv to see the initial evaluation of the NDC negative controls tested in this experiment. In this file, assays are flagged for which the NDC samples have tested positive, after being thresholded against the assay-specific NTC mean.

If any of the NDC samples show a positive result for any assay, then that assay should be evaluated for contamination with nucleases likely at the sample mastermix preparation step in the experimental workflow. However, other sources for NDC contamination may exist.

Please be advised to check the output files as well.

2. Evaluation of Combined Positive Control (CPC) Validity

Please consult CPC_Check_1691478213.csv to see the initial evaluation of the CPC positive controls tested in this experiment. In this file, assays are flagged for which the CPC samples have tested negative, after being thresholded against the assay-specific NTC mean.

If any of the CPC samples show a negative result for any assay excluding the 'no-crRNA' negative control assay, then that assay should be considered invalid for this experiment.

Please be advised to check the output files as well.

3. Evaluation of Human Samples for the Internal Control (RNaseP)

Warning: First verify that your experiment included a RNaseP assay. If yes, proceed to the following RNaseP analysis.

Please consult RNaseP_Check_1691478213.csv to see which samples are negative for the RNaseP assay(s). In this file, the samples that appear negative for the RNaseP assays have been flagged after thresholding against the NTC. The negative controls (NTC and NDC) are expected to be negative for the RNaseP assay and should be listed here (if you have included them in this experiment). All other samples should be evaluated for being negative for the RNaseP assay.

Possible reasons for a sample testing negative for the RNaseP assay:

(A) If the sample is negative for all assays (including RNaseP), then the most plausible hypothesis is that the viral extraction protocol used in this experiment needs to be examined. For optimal results, the extraction must be compatible with the Standard Operating Procedure (SOP) advised by the CARMEN team in the Sabeti Lab.

** Note: If the sample is negative for RNaseP and ALL other crRNA assays tested in this experiment, the sample should be rendered invalid.

(B) If the sample is negative for RNaseP BUT positive for any other viral crRNA assay (excluding RNaseP or no-crRNA), then the most plausible hypothesis is that the sample’s viral titer may be too high compared to its RNaseP titer. This, thereby, renders the system possibly unable to detect RNaseP, leading to the sample testing negative for RNaseP.

** Note: If the sample is negative for RNaseP but positive for any other viral crRNA assay (excluding RNaseP or no-crRNA) tested in this experiment, the sample can still be included in the final results.

(C) The source sample may have insufficient material, leading to a negative RNaseP signal and an invalid sample result.

Please be advised to check the output files as well.

4. Evaluation of No Target Control (NTC) Contamination

Please consult NTC_Contamination_Check_1691478213.csv to see which NTC samples may be potentially contaminated.

This file contains a list of samples that have a raw fluorescence signal above 0.5 a.u. These samples are being flagged for having a higher than normal signal for an NTC sample. The range for typical raw fluorescence signal for an NTC sample is between 0.1 and 0.5 a.u.

Please be advised to check the output files to further evaluate potential NTC contamination.

5. Evaluation of Potential Co-Infected Samples

Please consult Codetection_Check_1691478213.csv to see which samples may be potentially co-infected.

A preliminary evaluation for co-infection of a given sample against all tested assays has been completed:

(A) If you have included Combined Positive Controls (CPCs) in this experiment, as recommended, these positive controls should be identified and listed among the flagged samples. CPCs are expected to show a “co-detection” with ALL of the assays being tested in this experiment.

(B) Samples are not flagged as “co-detected” based on positivity with RNaseP and a second assay. For a sample to be flagged during this Co-detection Check, it must test positive for at least two assays, excluding RNaseP.

(C) All other flagged samples should be further evaluated for potential co-infection.

Please be advised to check the output files to further evaluate potential co-infection.