

Introduction

Advancements in nucleic acid testing have enabled point-of-care testing solutions to provide clinicians with greatly improved time to diagnosis and patient care. However, complex technologies and regulatory requirements demand close monitoring of assay performance to accurately identify shifts, trends, and random errors to ensure accurate patient reporting. To support these requirements, MMQCI has developed a synthetic, easy-to-use, external control that simultaneously monitors the accurate identification of 15 respiratory pathogens (11 viral, 4 bacterial targets) detected with the BIOFIRE® SPOTFIRE® Respiratory (R) Panel.

Materials and Methods

The multiplex control contains genome segments of all pathogens detected by the SPOTFIRE® R Panel. Sequences were designed *in silico*, ligated into MMQCI vectors, and transformed to create stable frozen clone stocks. *In vitro* RNA transcripts were generated, quantified, and formulated in a proprietary matrix to stabilize and carry the RNA through the entire test process. Validation studies were conducted to demonstrate precision across multiple reagents, operators and sites. Failure-mode, shipping and stability studies were conducted to confirm robustness and reliability. Three unique lots of the SPOTFIRE® RSP Positive and Negative controls were manufactured to include variability in key components and tested across 6 different sites, incorporating multiple reagent lots, operators and instruments. A total of 361 runs were performed across the positive and negative controls using the SPOTFIRE® R Panel.

Figure 1. Design Strategy for SPOTFIRE RSP Control

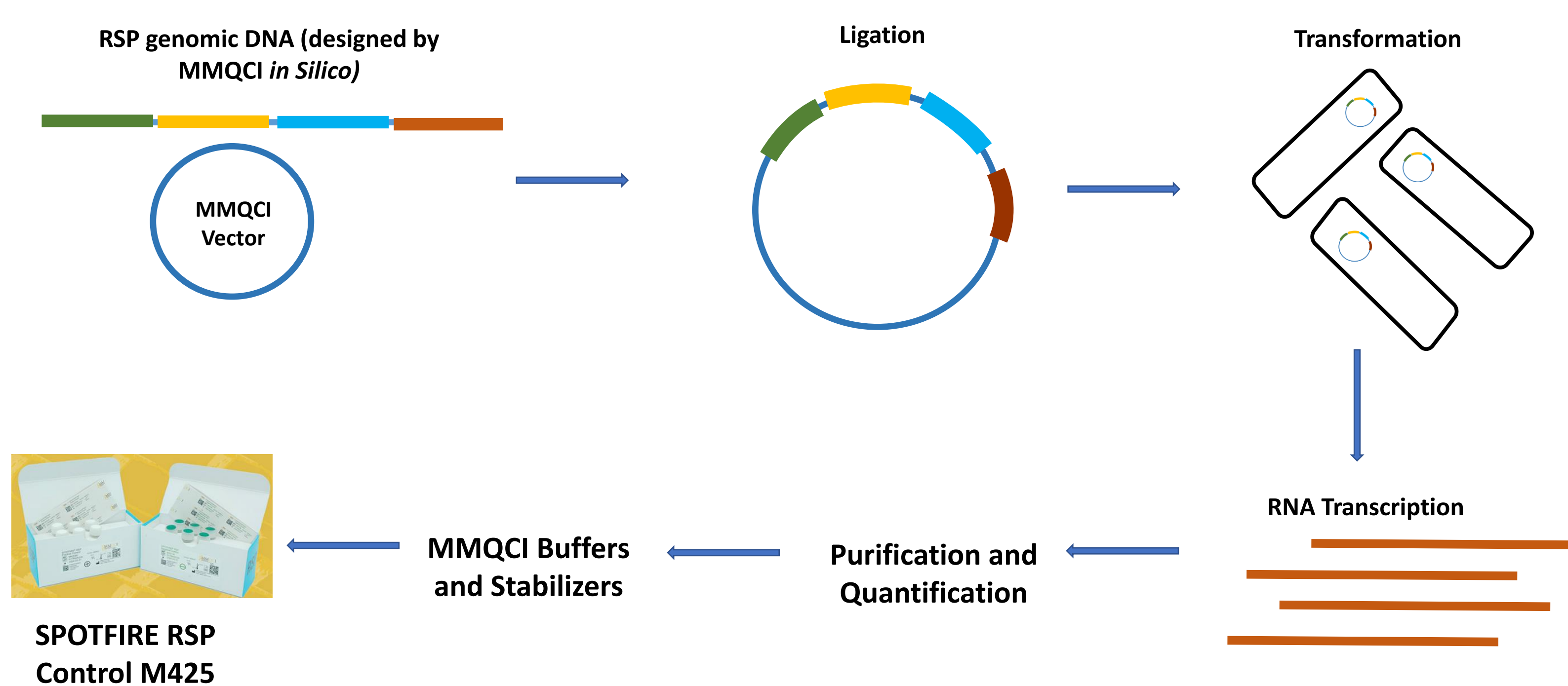


Table 1. SPOTFIRE RSP Analytes

| Table 1: SPOTFIRE RSP Analytes | | |
|---|--------------------------------|--|
| Viruses | | |
| • Adenovirus | • Human Rhinovirus/Enterovirus | • Influenza B Virus |
| • Coronavirus 229E, HKU1, OC43, NL63 ¹ | • Influenza A Virus | • Parainfluenza Virus serotypes 1-4 ² |
| • Coronavirus SARS-CoV-2 | • Influenza A Virus A/H1-2009 | • Respiratory Syncytial Virus |
| • Human Metapneumovirus | • Influenza A Virus A/H3 | |
| Bacteria | | |
| • <i>Bordetella parapertussis</i> | • <i>Chlamydia pneumoniae</i> | |
| • <i>Bordetella pertussis</i> | • <i>Mycoplasma pneumoniae</i> | |

1. Reported as seasonal Coronavirus (undifferentiated).
2. Reported as Parainfluenza Virus (undifferentiated).

Results

Validation of SPOTFIRE RSP Positive and Negative Control

Table 2. Summary Table of Clinical and MMQCI Internal Data

Of the 361 total runs across the 6 sites, 351 were valid and used for analysis. A total of 171/172 positive controls resulted in correct calls (99.3%), and all 179 negative controls were called correctly (100%).

| Sites | Total Tests | Invalid * | Correct Positive Control Result | Incorrect Positive Control Result | Percent Correct Positive Control | Correct Negative Control Result | Incorrect Negative Control Result | Percent Correct Negative Control | Total Percent Correct |
|-------|-------------|-----------|---------------------------------|-----------------------------------|----------------------------------|---------------------------------|-----------------------------------|----------------------------------|-----------------------|
| 6 | 361 | 10 | 171 | 1 | 99.3% | 179 | 0 | 100% | 99.7% |

*The Invalid samples were not included in the Percent Correct analysis.

Table 3. Internal Site Testing Results

Internal reproducibility was performed at MMQCI, by testing 3 lots over 21 different days across 3 SPOTFIRE® R Panel pouch lots, incorporating 4 operators on 1 BIOFIRE® SPOTFIRE® System (n=66 positive control samples, n=63 negative control samples). All positive controls gave correct results except for 3 which gave invalid results. Repeat tests gave correct results on the first retest. All negative controls gave correct results. The overall correct result rate at MMQCI was 100% (3 invalid results were not included in percent correct.)

| Control | Control Lot # | No. of Tests | Invalid* | Correct Results | Incorrect Results | Percent Correct |
|-------------------------------|---------------|--------------|----------|-----------------|-------------------|-----------------|
| SPOTFIRE RSP Positive Control | A02NOV20A | 23 | 2 | 21 | 0 | 100% |
| SPOTFIRE RSP Positive Control | B06NOV20A | 21 | 0 | 21 | 0 | 100% |
| SPOTFIRE RSP Positive Control | C10NOV20A | 22 | 1 | 21 | 0 | 100% |
| SPOTFIRE RSP Negative Control | F09JUL19P | 21 | 0 | 21 | 0 | 100% |
| SPOTFIRE RSP Negative Control | D11MAR20H | 21 | 0 | 21 | 0 | 100% |
| SPOTFIRE RSP Negative Control | H05MAY20M | 21 | 0 | 21 | 0 | 100% |
| TOTAL | | 129 | 3 | 126 | 0 | 100% |

*Invalid results were not included in percent correct.

Table 7. Shipping Study Testing Results

MMQCI SPOTFIRE RSP Positive lots A02NOV20A and B06NOV20A were placed on dry ice in MMQCI's standard shipping box, packaged according to MMQCI's standard protocol for 6 days at ambient temperature. To simulate a shipping delay, samples were tested at 2, 4 and 6 days at ambient temperature, incorporating a total of three freeze/thaw events. All SPOTFIRE RSP Positive controls were stable with no significant difference from pre-shipment conditions even after exposure to shipping delays up to 6 days at room temperature incorporating three freeze/thaw events.

| SPOTFIRE RSP Analytes | Standard Shipping Study | | | Shipping Delay Study | | | | | |
|-----------------------------------|-------------------------|-------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|
| | Pre-shipment | Standard Shipping | Shipping Delay 2 days | Shipping Delay 2 days | Shipping Delay 4 days | Shipping Delay 4 days | Shipping Delay 6 days | Shipping Delay 6 days | Shipping Delay 6 days |
| Bacteria | | | | | | | | | |
| <i>Bordetella parapertussis</i> | Detected | Detected | Detected | Detected | Detected | Detected | Detected | Detected | Detected |
| <i>Bordetella pertussis</i> | Detected | Detected | Detected | Detected | Detected | Detected | Detected | Detected | Detected |
| <i>Chlamydia pneumoniae</i> | Detected | Detected | Detected | Detected | Detected | Detected | Detected | Detected | Detected |
| <i>Mycoplasma pneumoniae</i> | Detected | Detected | Detected | Detected | Detected | Detected | Detected | Detected | Detected |
| Viruses | | | | | | | | | |
| Adenovirus | Detected | Detected | Detected | Detected | Detected | Detected | Detected | Detected | Detected |
| Coronavirus SARS-CoV-2 (COVID-19) | Detected | Detected | Detected | Detected | Detected | Detected | Detected | Detected | Detected |
| Coronavirus (Seasonal) | Detected | Detected | Detected | Detected | Detected | Detected | Detected | Detected | Detected |
| Human Metapneumovirus | Detected | Detected | Detected | Detected | Detected | Detected | Detected | Detected | Detected |
| Human Rhinovirus/Enterovirus | Detected | Detected | Detected | Detected | Detected | Detected | Detected | Detected | Detected |
| Influenza A Virus | Detected | Detected | Detected | Detected | Detected | Detected | Detected | Detected | Detected |
| Influenza A Virus A/ H1-2009 | Detected | Detected | Detected | Detected | Detected | Detected | Detected | Detected | Detected |
| Influenza A Virus A/ H3 | Detected | Detected | Detected | Detected | Detected | Detected | Detected | Detected | Detected |
| Influenza B Virus | Detected | Detected | Detected | Detected | Detected | Detected | Detected | Detected | Detected |
| Parainfluenza Virus | Detected | Detected | Detected | Detected | Detected | Detected | Detected | Detected | Detected |
| Respiratory Syncytial Virus | Detected | Detected | Detected | Detected | Detected | Detected | Detected | Detected | Detected |

Table 4. External Clinical Site Testing Results

Testing performed across 5 clinical sites included 3 lots of SPOTFIRE RSP Positive Control and 4 lots of SPOTFIRE RSP Negative Control using a total of 25 SPOTFIRE® R Panel pouch lots, incorporating multiple operators and instruments for a total of 232 runs. Of the 232 controls tested, there were 7 with Invalid results which were repeated for a total of 225 controls with valid results. Of the 7 Invalid results, 4 were due to failed internal pouch controls and 3 were due to instrument errors. Correct results were obtained on the first test of 224 controls. One Positive control gave initial false negative results. All produced the correct results upon a single retest for an overall correct result rate of 99.6%.

| Site | Total Tests | Invalid | Correct Positive Control Result | Incorrect Positive Control Result | Percent Correct Positive Control | Correct Negative Control Result | Incorrect Negative Control Result | Percent Correct Negative Control | Total Percent Correct |
|------------------|-------------|-----------|---------------------------------|-----------------------------------|----------------------------------|---------------------------------|-----------------------------------|----------------------------------|-----------------------|
| 1 | 26 | 0 | 13 | 0 | 100% | 13 | 0 | 100% | 100% |
| 2 | 33 | 1 | 13 | 0 | 100% | 19 | 0 | 100% | 100% |
| 3 | 120 | 4 | 53 | 1 | 98.1% | 62 | 0 | 100% | 99.1% |
| 4 | 44 | 1 | 21 | 0 | 100% | 22 | 0 | 100% | 100% |
| 5 | 9 | 1 | 8 | 0 | 100% | NA | NA | NA | 100% |
| All Sites | 232 | 7* | 108 | 1 | 99.1% | 116 | 0 | 100% | 99.6% |

*Invalid results were not included in percent correct.

Table 5. Summary of Within-run Precision at MMQCI

Within-run precision (repeatability) was demonstrated by 1 operator testing 2 lots each of SPOTFIRE RSP Positive and SPOTFIRE RSP Negative, with 1 lot of SPOTFIRE® R Panel pouches on 1 SPOTFIRE® System, all within one day at MMQCI.

| Control | Control Lot # | Number of Tests | Correct Results |
|-------------------------------|---------------|-----------------|-----------------|
| SPOTFIRE RSP Positive Control | B06NOV20A | 6 | 6/6 |
| SPOTFIRE RSP Positive Control | C10NOV20A | 6 | 6/6 |
| SPOTFIRE RSP Negative Control | F09JUL19P | 6 | 6/6 |
| SPOTFIRE RSP Negative Control | H05MAY20M | 6 | 6/6 |

Table 6. Failure Mode of MMQCI Positive control and Simulated clinical Sample (Viral Transport Media) spiked with inactivated RSV.

Failure modes tested: (1) mimic poor hydration, ¼ volume of hydration buffer (n=2 for each sample), (2) mimic operator error, pouch inserted incorrectly on loading dock (n=2 for each sample), (3) mimic operator error, fail to mix the sample and sample buffer in the sample injection vial prior to loading into pouch (n=2 for each sample). All runs performed under failure mode analysis for criteria (1) and (2) were reported as invalids for both Positive Control and simulated clinical samples. All runs performed under failure mode analysis for criteria (3) were reported as detected calls, however upon analysis of raw data a delay in amplification for both the Positive Control and simulated clinical samples was observed.

| Tested Correctly | | Inadequate Hydration | | Pouch inserted Incorrectly | | Fail to Mix | |
|-------------------------------|---------------------------|-------------------------------|---------------------------|-------------------------------|---------------------------|-------------------------------|---------------------------|
| SPOTFIRE RSP Positive Control | Simulated clinical Sample | SPOTFIRE RSP Positive Control | Simulated clinical Sample | SPOTFIRE RSP Positive Control | Simulated clinical Sample | SPOTFIRE RSP Positive Control | Simulated clinical Sample |
| Detected | Detected | Invalid | Invalid | Invalid | Invalid | Detected | Detected |

Conclusion

Validation studies demonstrated reproducibility, overall precision and robustness of SPOTFIRE RSP Positive and Negative Controls. The SPOTFIRE RSP Positive and Negative Controls provide a stable, easy to use solution for training, verification and routine monitoring of the performance of the SPOTFIRE® R Panel.

Acknowledgements

- Reagents for this study were kindly provided by bioMérieux, Salt Lake City, Utah.

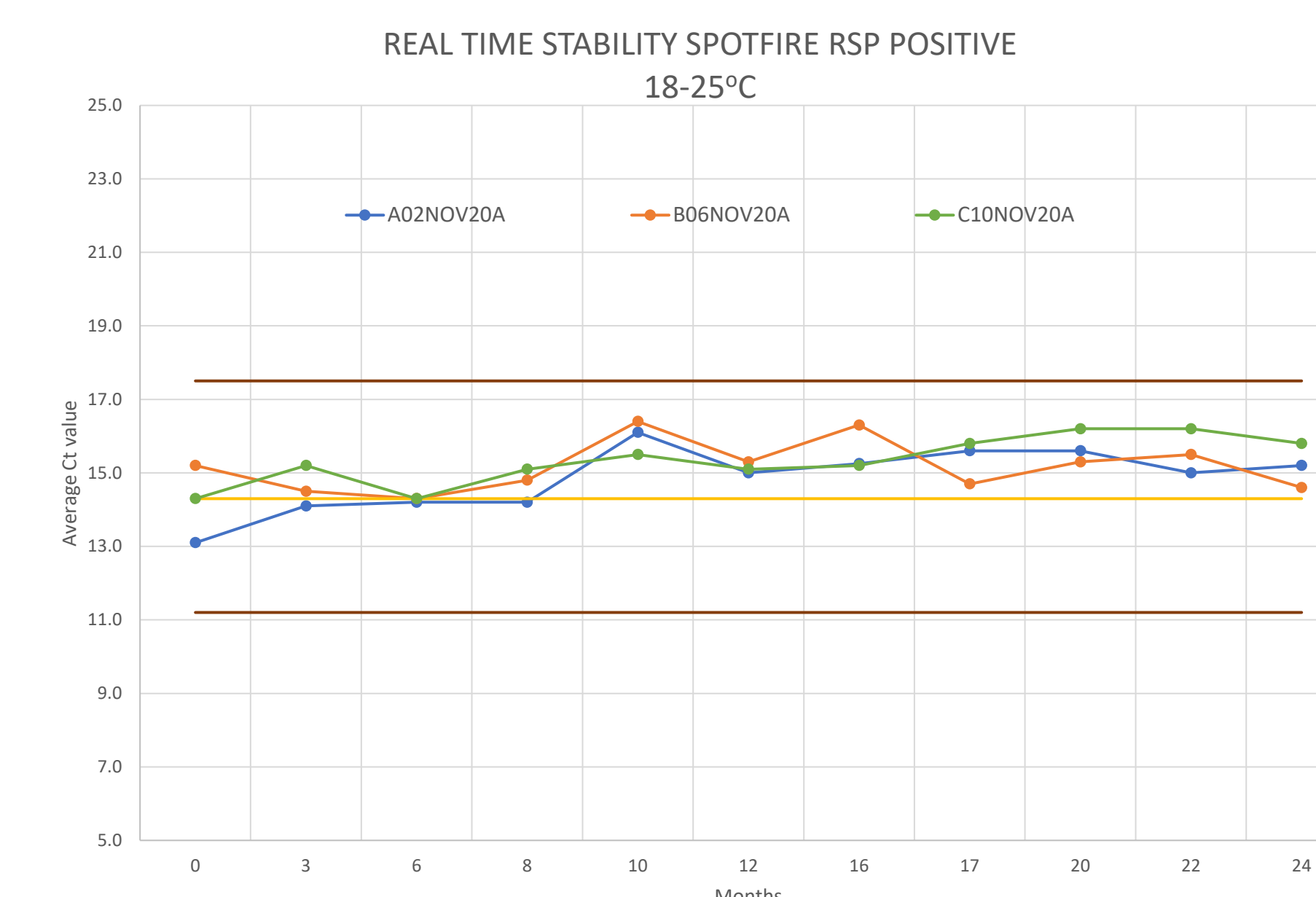


Figure 2: Three lots of SPOTFIRE RSP Positive controls stored at 18-25°C were tested over 24 months using MMQCI in-house qPCR assay. Linear regression was performed for all three lots and no significant trend or shift was observed. Testing is ongoing.

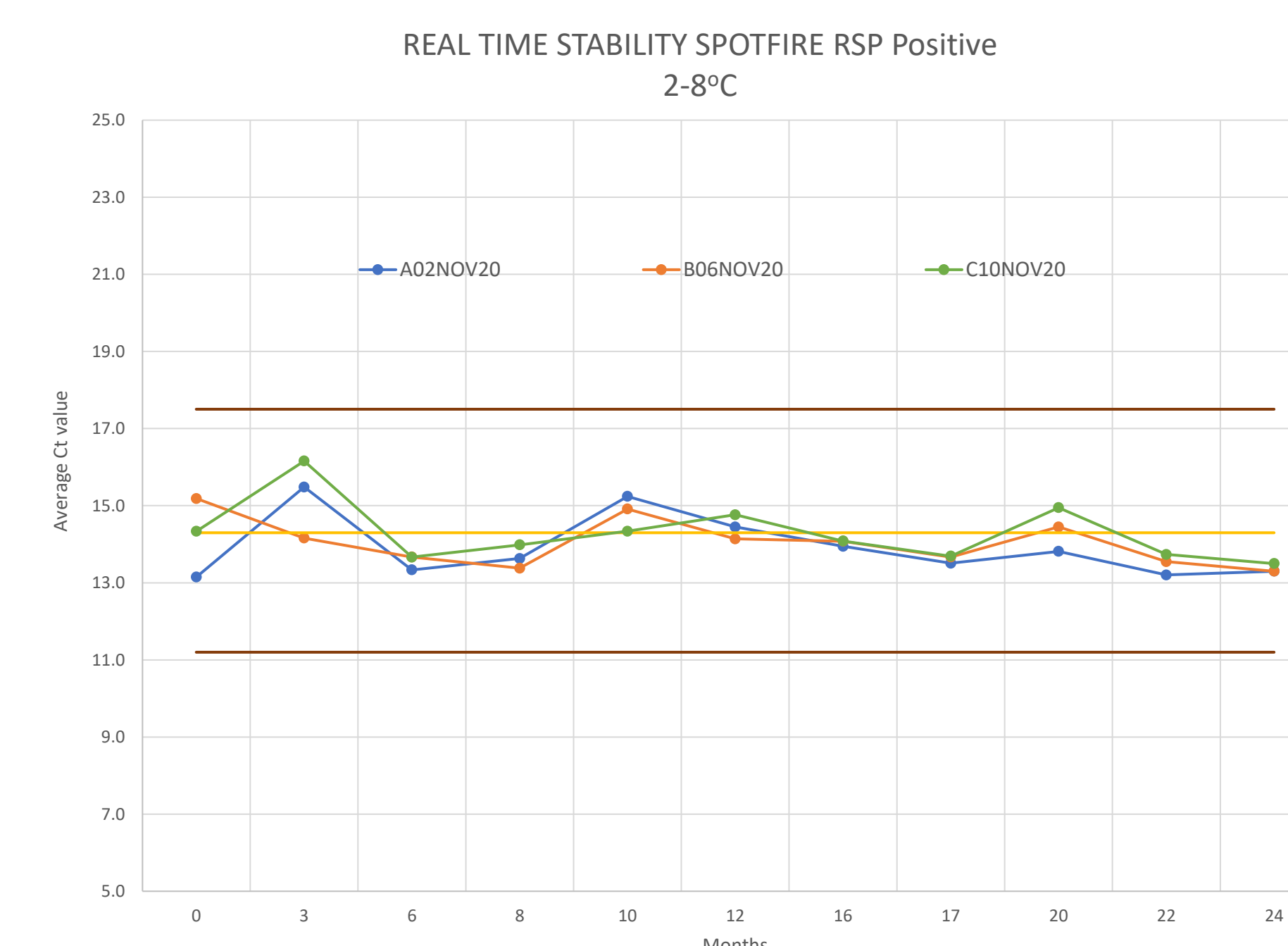


Figure 3: Three lots of SPOTFIRE RSP Positive controls stored at 2-8°C were tested over 24 months using MMQCI in-house qPCR assay. Linear regression was performed for all three lots and no significant trend or shift was observed. Testing is ongoing.