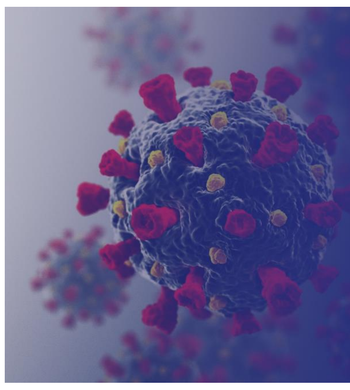

SIMULATED SWAB SPECIMENS FOR WHOLE WORKFLOW QUALITY CONTROL OF SARS-COV-2 MOLECULAR DIAGNOSTIC TESTING

Pavel Zhelev[1], Anu Rebbapragada[1], Sydney Rivers[1], Dang Hoang Pham[2], Janet Wong[2], Hossam Abdelrahman[2], Reshmi Thulasi Devi[2], Amer Alagic[1], Shane Niyamuddin[1], Mark Luscher[1], Ken Hughes[1]

[1]Microbix Biosystems Inc. – Mississauga, ON, Canada
[2]FH Health - Toronto, ON, Canada

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INTRODUCTION

Pre-analytical variables are key determinants of test result accuracy; the diagnostic value chain begins at specimen collection and conditions prior to testing can profoundly impact final results. Pre-analytical perturbations are the leading cause of errors in molecular diagnostic laboratories, accounting for 46%-68% of all errors[1]. The design of QC material often does not adequately assess the impact of the pre-analytical process on downstream test result accuracy. Most laboratories prepare "contrived" samples spiked with liquid quality controls to test the analytical process (equipment, reagents, operators) but overlook verification of the pre-analytical process (specimen collection, impact of pre-test storage time and temperature on specimen integrity). Diagnostic laboratories would benefit from using whole workflow external quality controls to simulate the entire journey experienced by actual swab-based patient specimens, identify risks & errors in testing workflow, fulfill accreditation requirements for Molecular Individual Quality Control Plan (IQCP) and ultimately, ensure accuracy and confidence in the entire diagnostic value chain.

OBJECTIVES

This study leverages Microbix's control formulations desiccated on Copan FLOQSwab® to simulate SARS-CoV-2 positive and negative swab specimens i.e. Whole Workflow External Quality Control (WWEQC) indicators for end-to-end testing. The influence of pre-test temperature & duration (during transit or storage), and collection medium were evaluated by testing both simulated and clinical respiratory swab specimens.

MATERIALS

1) Simulated Respiratory Swab specimens on a Copan FLOQSwab®
Positive: SARS-CoV-2 whole genome cDNA and human fibroblast cells
Negative: Human fibroblast cells to meet specimen adequacy/cellularity requirements (RNAseP or beta-globin molecular detection).

Figure 1. REDx FLOQ® SARS-CoV2 Swab Positive Control, P/N# RED-S-19-01

2) Viral Transport Medium: Microbix DxTM™ Viral Transport Media; 3ml per tube
3) Molecular Transport Medium with inactivation: Bioer Sample Preservation Fluid (w/ guanidine isothiocyanate); 2ml per tube
4) Whole Workflow External Quality Controls (WWEQC): prepared in either Microbix DxTM™ Viral Transport Medium (VTM) or Bioer molecular transport medium by immersing the respective positive or negative swab into the collection tube as follows:
- "Strong" positive: single swab into 1 tube of medium, Ct values 25-30
- "Weak" positive: 1:100 dilution of "strong" positive in medium, Ct values >35

5) Clinical specimens in respective transport medium:
- Strong positive: Viral target Ct values <20
- Weak positive: Viral target Ct values >30

6) FDA and Health Canada approved SARS CoV2 RT-PCR Assays:
- Osang GeneFinder™ COVID-19 Plus RealAmp Kit: N, RdRP, E, Human RNAseP
- ThermoFisher TaqPath ™ COVID-19 Combo Kit: N, S, ORF1ab, MS2 spike-in process control added during extraction

7) Testing System: KingFisher Extraction & QuantStudio5 Real-Time thermocycler
All specimens were assayed in triplicate following the standard operating protocols for diagnostic testing at the ISO 15189 accredited FH Health Laboratory:
- 200µl of specimen extracted on KingFisher with MagMAX ™ Viral/Pathogen Nucleic Acid Isolation kit and recovered as 50µl of eluate.
- Osang GeneFinder™: 5µl eluate in 20µl total reaction volume; LoD 2 copies
- ThermoFisher TaqPath™ : 10µl eluate in 25µl total reaction volume; LoD 4 copies

Table 1. Pre-Analytical Conditions and Storage Durations Prior to RT-PCR Assay

Conditions	Storage Durations Prior to RT-PCR Assay			
	d0	d1	d3	d5
Room Temperature (20ºC-25ºC)	√	√	√	√
Refrigerated (4ºC-8ºC)	√	√	√	√
Freeze-Thaw from -20ºC	√	√	√	√
Warm Temperature (35ºC-40ºC)	√	√	√	√

Reference:
1. A Review of Medical Errors in Laboratory Diagnostics and Where We Are Today. Julie A. Hammerling, *Lab Medicine*, **43**(2): 41-44.

RESULTS

Average Ct values for representative viral target genes (N gene, S gene) and Human RNaseP assayed in simulated and clinical specimens (prepared in VTM and molecular medium) across a set of pre-analytical conditions.

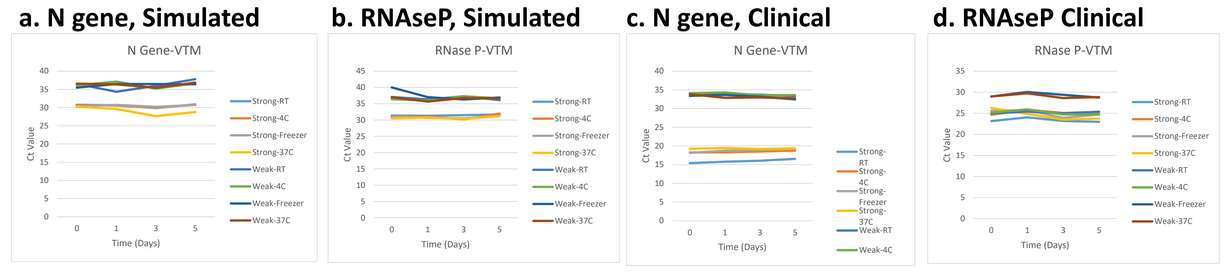

Figure 2. Osang RT-PCR, Simulated & Clinical Specimens in Microbix DxTM™ VTM

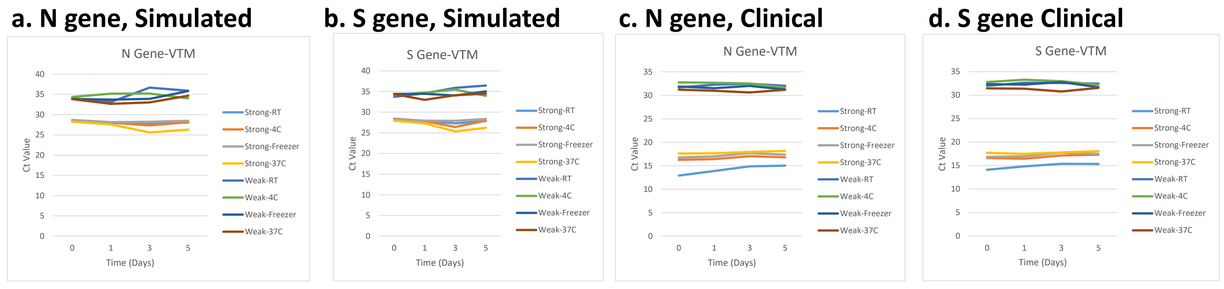

Figure 3. ThermoFisher RT-PCR, Simulated & Clinical Specimens in DxTM™ VTM

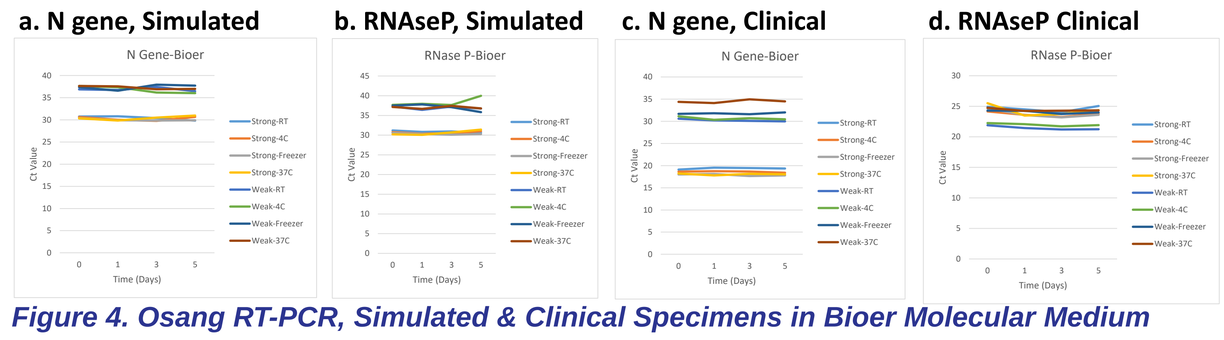

Figure 4. Osang RT-PCR, Simulated & Clinical Specimens in Bioer Molecular Medium

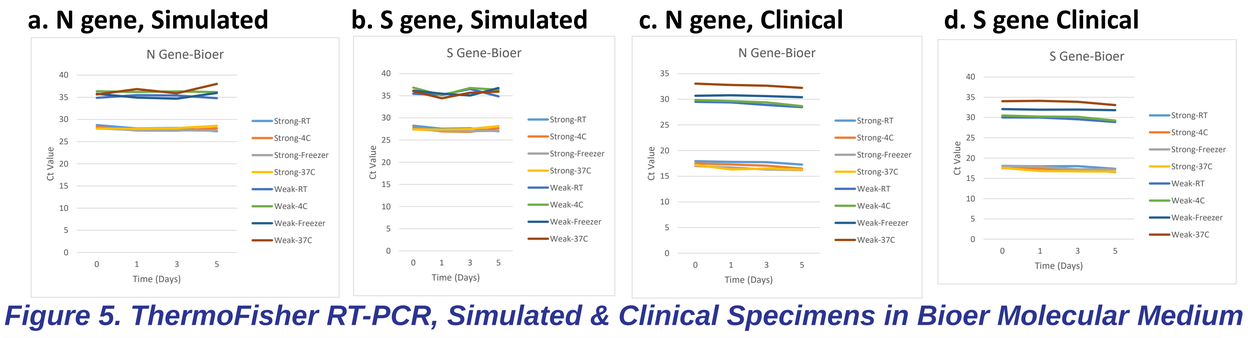

Figure 5. ThermoFisher RT-PCR, Simulated & Clinical Specimens in Bioer Molecular Medium

RESULTS SUMMARY

1) Weak Positive specimens (both simulated and clinical) exhibited the greatest change in Ct values across every condition tested (up to 3 Ct values).
2) As expected, specimens tested after freeze-thaw and prolonged storage at warm temperature exhibited the greatest change in Ct values.
3) Increases in RT-PCR assay Ct values were attributed to changes in specimen integrity and nucleic acid recovery which compromised molecular test performance.
4) The pattern of Ct value changes in simulated specimens resembled what was observed with clinical specimens, indicating that these are valuable surrogates to assess impact of pre-analytical variables on downstream testing.
5) Specimen storage in Microbix DxTM™ provided stability (for up to 5 days) at levels comparable to molecular transport medium, with the added benefit of not containing toxic chemicals (guanidine isothiocyanate) which pose a health hazard.

CONCLUSIONS

1) SARS-CoV-2 positive and negative swab WWEQC simulate clinical specimens, serving as surrogates to examine risk in the entire diagnostic testing workflow.
2) Simulated specimens are particularly beneficial to high volume labs that receive specimens from a wider catchment area with lengthy transportation and higher potential for pre-analytical perturbations.
3) This study provides a model for proper design and utility of whole workflow external quality controls to assess risks in the entire molecular diagnostic testing pathway and fulfill CAP accreditation requirements for Molecular IQCP.

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CONTACT US

Pavel Zhelev: Director of Product Management (QAPs)
Email: pavel.zhelev@microbix.com
Dr. Anu Rebbapragada D(ABMM) FCCM: Director, Senior Advisor for Diagnostic Strategy & Services
Email: anu.rebbapragada@microbix.com

Microbix Biosystems Inc.
+1-800-794-6694
+1-905-361-8910
www.microbix.com

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