Novel Hepatitis C Virus External Quality Control That Is Stable at Ambient Temperature And Suitable For Use In Low-Resource Settings

P. Zhelev [1], S. Rivers [1], A. Alagic [1], T.Gerbaba[1], B. Aquino[1], R. Patel [1], M. Luscher [1], K. Hughes [1]

[1]*Microbix Biosystems – Mississauga, ON, Canada*

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INTRODUCTION

Notwithstanding the WHO's initiative to eliminate viral hepatitis by 2030, there are currently 58 million individuals infected with Hepatitis C Virus (HCV)[1]. 73% of infected individuals live in low- and middle-income countries (LMIC) where conventional diagnostics are inaccessible due to high costs, lack of refrigeration, equipment, and trained personnel[2]. The emergence of low complexity Point of Care Tests (POCT) is critical for improving HCV surveillance, diagnostic accessibility, and treatment turnaround time. In conjunction with POCT use, there is a need for External Quality Controls (EQC) that monitor the accuracy of test results.

Microbix strived to develop reproducible HCV EQC swab formulations that are stable at ambient temperature and suitable for use in LMIC. Since this format deviates from standard HCV diagnostic workflows, we sought to establish a simple yet reproducible workflow that can be followed by laboratories worldwide.

MATERIALS & METHODS

1 Sample Formulation
- Contrived specimen is formulated with protected synthetic HCV whole genome nucleic acid and human cells desiccated on a Copan FLOQSwab®

2 Quantification
- ddPCR

3 Established a Suitable Handling Procedure for LMIC
- Elute the swab by roll pressing against the vial tube wall, incubation, vigorous shaking and stand to let the bubbles disappear.

4 Cepheid Xpert® HCV Viral Load Fingerstick Assay
- Sample performance was evaluated with various elution buffers (PrimeStore® MTM, eNAT®, Microbix Proprietary Buffer, TE buffer)

RESULTS

1. Sample Quantification

Table 1: Sample Quantification Data

Sample	Cat. No.	Method	Results
HCV Positive Swab	VP-S-101-M1 PROCEEDFLOQ	ddPCR	4,680 copies/swab

2. Evaluating Suitable Handling Procedure for LMIC

Table 2: Detailed Sample Performance with Preferred Sample Elution Medium (*eNAT*®) by Manual Elution Procedure

Method	Elution	Ct	Log	HCV Quantification
Manual Elution Procedure for LMIC	eNAT®, 2mL	35.9	2.61	403 IU/mL
		37.1	2.35	223 IU/mL
		36.3	2.43	268 IU/mL
		36.5	2.49	306 IU/mL
		36.2	2.39	245 IU/mL
AVERAGE		**36.4**	**2.45**	**289 IU/mL**
STANDARD DEVIATION		**0.45**	**0.10**	**70.7 IU/mL**

3. Comparing Elution Buffers

Table 3: Sample Performance with Cepheid Xpert® HCV Viral Load Fingerstick Assay

Method	Elution	Ct		Log		HCV (IU/mL)	
		Avg.	SD	Avg.	SD	Avg.	SD
Manual Elution Procedure for LMIC	eNAT®, 2mL	36.4	0.45	2.45	0.10	289	70.7
	PrimeStore® MTM, 3mL	35.3	0.48	2.77	0.09	604	152.2
	Microbix PT Buffer, 3mL	37.9	1.10	2.54	0.29	391	200
	TE Buffer, 3mL	39.6	-	Invalid		Invalid	

CONCLUSION

1. Microbix successfully developed a whole-workflow HCV positive swab sample that is stable at ambient temperature (2-30°C). This prospective EQC, when handled using the manual elution procedure, is suitable for use in LMIC or remote areas where access to equipment, refrigeration, and skilled personnel are limited.

2. Swab elution buffer is a key variable that can impact EQC result reproducibility, with eNAT® and PrimeStore® MTM being the most favourable elution buffers when using the manual elution procedure. Our findings demonstrate that a universal workflow should be followed to standardize results across testing sites in LMIC as deviations in elution buffers and handling procedures can influence EQC outcomes.

3. EQC design and characterization should rely on methods with absolute and universal quantification (i.e. ddPCR). Reasons include but are not limited to:
   - WHO standards are finite and only accessible to select groups. Most laboratories use intermediate standards that have been benchmarked to a WHO standard; thereby incorporating an undefined level of variability across testing groups.
   - WHO standards are derived from patient pools, which are comprised of interfering agents that will vary across standards. Deviations amongst patient pools add a level of uncertainty for long term repeatability in the context of assay EQC.
   - Conversely, protected synthetic standards with absolute quantification are stable, reproducible, available at commercial scale, and have minimal interfering agents. ddPCR is a reliable quantification methodology for EQC that will be used to benchmark the performance of quantified assays.

4. A desiccated FLOQSwab® is one of the few EQC design formats that meets the temperature stability requirements for LMIC and is compatible with the preferred POCT methodology used in these geographies.

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CONTACT INFORMATION

Pavel Zhelev, Director, Product Management
Email: pavel.zhelev@microbix.com

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

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