Version of Record: https://www.sciencedirect.com/science/article/pii/S0166093422001641
Manuscript_af4aed3e63e666bacbaaaca65a0019de

Effect of the Environment on Home-Based Self-Sampling Kits for Anal Cancer Screening

Jenna Nitkowski [a, *], Anna Giuliano [b], Tim Ridolfi [c], Elizabeth Chiao [d], Maria Fernandez [e], Vanessa Schick [f], Michael D. Swartz [g], Jennifer S. Smith [h], Ellen A. Schneider [i], Bridgett Brzezinski [a], and Alan G. Nyitray [a, c]

[a] Center for AIDS Intervention Research, Medical College of Wisconsin, Milwaukee, Wisconsin, USA
[b] Center for Immunization and Infection Research in Cancer, Moffitt Cancer Center and Research Institute, Tampa, Florida, USA
[c] Clinical Cancer Center, Medical College of Wisconsin, Milwaukee, Wisconsin, USA
[d] MD Anderson Cancer Center, The University of Texas, Houston, Texas, USA
[e] Department of Health Promotion and Behavioral Sciences, The University of Texas Health Science Center at Houston School of Public Health, Houston, Texas, USA
[f] Department of Management, Policy and Community Health, The University of Texas Health Science Center at Houston School of Public Health, Houston, Texas, USA
[g] Department of Biostatistics and Data Science, The University of Texas Health Science Center at Houston School of Public Health, Houston, Texas, USA
[h] Gillings School of Global Public Health, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA
[i] Medical College of Wisconsin, Milwaukee, Wisconsin, USA

[*]Corresponding author. jnitkowski@mcw.edu. 2071 N. Summit Ave., Milwaukee, WI 53202, USA.

Abstract

Background: Anal cancer incidence has increased in Western countries in recent decades and currently there are no consensus screening guidelines. Home-based self-sampling kits might facilitate screening for anal precancer/cancer but could require travel through postal mail where they may experience extreme temperatures or long transport times.

Objective: To determine the effect of the environment on specimen adequacy for HPV genotyping of a mailed home-based self-sampling anal cancer screening kit.

Study design: The Prevent Anal Cancer (PAC) Study in Milwaukee, Wisconsin recruited men who have sex with men (MSM) and transgender persons 25 years of age and older. Participants were randomized to receive a mailed self-sampling kit or attend a clinic for screening. Kits were insulated with foam and included a device to record temperature every twenty minutes. Samples were returned via mail and underwent HPV genotyping using the SPF_{10}-$LiPA_{25}$ assay which also detected human RNase P to determine specimen adequacy by qPCR. For the first 93 kits, logistic regression assessed associations between specimen inadequacy and temperature, freeze-thaw cycle, presence of fecal matter, and number of days in an uncontrolled environment.

Results: Most specimens (92.5%) were adequate for HPV genotyping. Specimen inadequacy was not associated with temperature, freeze-thaw cycle, or transit time. Fecal matter was present more often in inadequate (71.4%) compared to adequate specimens (16.3%) (p=.004).

Conclusions: These real-world data from mailed home-based anal self-sampling kits found that environmental conditions did not affect specimen adequacy. While over 90% of specimens were adequate, presence of fecal matter predicted specimen inadequacy.

Keywords**:** self-sampling; anal cancer; human papillomavirus (HPV); temperature; specimen adequacy; MSM

Background

Anal cancer incidence rates in Western countries have steadily increased in the last three decades [1,2] and are disproportionately higher among HIV-negative and HIV-positive men who have sex with men (MSM) [3]. Squamous cell carcinoma of the anus is almost always caused by oncogenic human papillomavirus (HPV) infection [4,5]. Currently there are no consensus screening guidelines for anal cancer, although these are expected in the near future given the recent completion of a large, randomized clinical trial showing that treatment of precancerous lesions in the anal canal can reduce anal cancer incidence [6]. Guidelines are likely to reflect a cervical cancer screening model where molecular or cytological biomarkers are used to identify persons in need of follow up for detection of precancerous lesions. A number of biomarkers, including HPV DNA, are being studied to support follow up and detection of precancerous lesions in the anal canal.

As with cervical cancer screening, home-based options for anal cancer screening might facilitate screening for anal precancers. Self-sampling allows a person to collect a sample themselves and mail it to a laboratory facility for processing and analysis. Home-based self-sampling can be a convenient, private way to screen for anal cancer while alleviating barriers to in-person anogenital screening such as stigma or embarrassment [7,8]. Previous research has demonstrated that MSM find anal self-sampling highly acceptable and are willing to self-administer a test at home [9,10].

However, home-based self-sampling kits could require transport through the postal mail. Thus, kits may be subjected to uncontrolled conditions such as extreme temperatures during different seasons or long transport times on their way to laboratories. Kits may also experience freeze-thaw cycles [11], such as being exposed to 0°C (freezing) to 20°C (room temperature) during transit which can impact specimens. While limited research has been conducted on the

effect of time and temperature on self-samples for cervical cancer screening [12], no studies have evaluated how environmental factors may affect the adequacy of anal exfoliated cell specimens.

Objectives

We aimed to assess the effect of environmental conditions, like temperature, on specimen adequacy of mailed home-based anal self-sampling swabs.

Study design

Data for this study come from the Prevent Anal Cancer (PAC) Study which is recruiting MSM and transgender persons from 2020 to 2022 in Milwaukee, Wisconsin, USA to participate in an anal cancer screening study. The PAC Study randomizes eligible participants to either a home- or clinic-based arm. We used data here from the home-based arm, since those participants received a mailed anal self-sampling kit (PAC Pack) through the postal mail at baseline and 12 months later. Kits contained a flocked swab (COPAN Italia SPA, Brescia, Italy), a vial containing 2 mL of standard transport media (Qiagen, Germantown, MD, USA) labeled with a unique participant number and kit number, self-sampling instructions written at a sixth-grade reading level, and a biohazard bag. Kits also contained return instructions and packaging for postal mail return. Each kit was packaged in foam insulation and included a temperature monitoring device (LogTag Recorders, Auckland, New Zealand) which captured and recorded the temperature of the kit every twenty minutes. Research staff started the temperature recording device when they sent out the mailed kit and stopped it after the completed kit was picked up from the laboratory. Participants were asked to record the date that they collected the swab in their returned kit.

After completing the self-collection, participants mailed their completed kit to the Medical College of Wisconsin (MCW) Tissue Bank laboratory where the specimen was processed and aliquoted into cryovials and stored in -80.0°C until shipping. The average

processing time was 4.7 days. Laboratory staff noted any presence of visible fecal matter and/or other kit-related details and notified research staff when completed kits were received. Research staff then picked up the temperature recorders and downloaded the data onto study computers. Swabs were overnighted on dry ice to Moffitt Cancer Center and Research Institute for DNA extraction, HPV genotyping, and assessment of specimen adequacy. Anal self-collected samples were HPV genotyped using the SPF_{10}-$LiPA_{25}$ assay which detected human RNase P to determine specimen adequacy by qPCR. Human RNase P and L1 HPV both have an amplicon size of 65 bp. As of June 17, 2022, complete temperature and genotyping data were available for 93 returned kits.

Measures

Temperature data from each kit were compiled into a dataset containing temperature and time variables. Exposure variables consisted of temperature (the lowest, the highest, and the range of temperatures experienced by each kit), time (the number of days in an uncontrolled environment), presence of a freeze-thaw cycle (yes/no), and presence of fecal matter (yes/no). The number of days in an uncontrolled environment was measured by calculating the number of days between when the kit was mailed to a participant to when the completed kit was received by the MCW Tissue Bank. These dates were entered into REDCap [13] by study staff. Freeze-thaw cycle was a binary variable (yes/no) that measured whether a kit temperature changed from 0°C (freezing) to 20°C (room temperature). The outcome variable of specimen inadequacy was a binary variable (1=inadequate, 0=adequate).

Sensitivity analysis. Participants were asked to record the date they collected their sample on a label inside the kit. A total of 11 kits out of the 93 returned kits used in this analysis (11.8%) did not have a collection date recorded. An alternative measure of the number of days in an uncontrolled environment was constructed using this participant-reported swab collection date.

This alternative number of days variable was calculated as the number of days between the participant-reported swab collection date and the date the completed kit was received. Data from each kit were compiled into a dataset containing temperature and time data starting at the collection date (instead of the date the kit was mailed to a participant) to when the completed kit was received by the MCW Tissue Bank. Sensitivity analyses were then conducted using this dataset.

Statistical methods

Chi-square tests assessed the associations between the categorical exposures (presence of a freeze-thaw cycle and presence of fecal matter) and specimen inadequacy. Fisher's exact test was used due to small cell sizes. T-tests assessed the associations between the means of the continuous variables (lowest temperature, highest temperature, temperature range, and number of days in an uncontrolled environment) and specimen inadequacy. Specifically, Welch's t-test was used due to unequal variance in the outcome variable. Univariate logistic regression analyses were conducted to examine associations between exposure variables and specimen inadequacy. Multivariable logistic regression analyses examined the associations between each temperature variable (the lowest, the highest, the range) and specimen inadequacy adjusted for the number of days in an uncontrolled environment, since number of days could be considered a potential confounder. These steps were also repeated using the alternate number of days variable. Firth's penalized likelihood estimation was used for all univariate and multivariable logistic regression analyses to account for unequal variances in the outcome variable. All statistical analyses were conducted in IBM SPSS Statistics 28.0 [14] and Stata/SE 17.0 [15].

Results

Between January 2020 and June 2022, a total of 208 participants enrolled in the PAC Self-Swab Study. Study activities were paused between March 14, 2020 and November 2, 2020

due to the COVID-19 pandemic. As of June 2022, a total of 104 participants were randomized to the home-based arm and sent a baseline PAC pack; 93 returned a kit and 11 did not return a kit. Complete temperature and adequacy data were available for 83 of the baseline PAC packs and 10 of the 12-month PAC packs, resulting in a sample of 93 kits returned between January 2020 and April 2022 (n=93). Kits were shipped during summer (n=27, 29.0%), autumn (n=17, 18.3%), winter (n=23, 24.7%), and spring (n=26, 28.0%).

A total of 92.5% (n=86) of anal swabs self-collected in the home were adequate for HPV genotyping and 7.5% (n=7) were inadequate (Table 1). Kits experienced an average of 13.1 days in an uncontrolled environment, with a range of 4.0 to 105.0 days. The average temperature a kit experienced ranged from 9.5°C to 25.9°C (mean=20.0°C). Kits were subjected to low temperatures ranging from -16.0°C to 21.8°C, with an average lowest temperature of 8.5°C. Highest temperatures ranged from 22.0°C to 46.3°C, with an average highest temperature of 27.7°C. Boxplots illustrating the lowest and highest temperatures experienced by kits grouped by specimen adequacy are shown in Figure 1. On average, kits experienced a temperature range of 19.3 degrees during their journey (min=3.8; max=40.2). A total of 20.4% (n=19) of kits experienced a freeze-thaw cycle. One fifth (20.4%) of specimens (n=19) had fecal matter.

There were no significant differences in time or temperature between adequate and inadequate specimens (Table 1). Although these differences were not precise, inadequate specimens were subjected to a greater range of temperatures and number of days in an uncontrolled environment compared to adequate specimens. A larger percentage of inadequate specimens also experienced a freeze-thaw cycle (28.6%) compared to adequate specimens (19.8%), although differences were imprecise (p=.63). The presence of fecal matter was positively associated with specimen inadequacy. A large majority of inadequate specimens (71.4%) had visible fecal matter compared to 16.3% of adequate specimens (p=.004).

Logistic regression analyses were conducted between each of the exposure variables and specimen inadequacy. In the univariate analyses, none of the temperature or time variables were associated with specimen inadequacy. In multivariable analyses adjusting for the number of days in an uncontrolled environment, temperature and time did not appear to be associated with specimen inadequacy, including lowest temperature (aOR=0.96, 95% CI 0.88 – 1.04, *p*=.27), highest temperature (aOR=0.98, 95% CI 0.83 – 1.16, *p*=.85), and temperature range (aOR=1.05, 95% CI 0.96 – 1.16, *p*=.27).

With analyses using participant-reported collection date, the average number of days in an uncontrolled environment was reduced to 3.5 days (min=0, max=11). In multivariable logistic regression analyses adjusted for this alternative variable, point estimates of variables remained consistent with the primary analysis except for presence of freeze-thaw cycle which increased in magnitude along with a much wider confidence interval (see Appendix Table 1A).

Discussion

To our knowledge, this is the first study to use real-world time and temperature data from mailed home-based self-sampling kits for detecting anal precancers. Home-based options for anal cancer screening may require transport through the postal mail, so research on the environmental conditions that kits experience during their journey can help inform future implementation. This research demonstrated that despite transit during all four seasons, specimen inadequacy was not significantly associated with any of the temperature or time conditions.

Presence of fecal matter on the swab was the only exposure in this study that was associated with specimen inadequacy, although the low overall number of inadequate specimens hinders interpretation. Previous research found that anal canal specimens yield higher proportions of inadequate specimens compared to other anatomical sites such as the penis, potentially due to more PCR inhibitors in anal samples [16,17]. Study participants were asked to not do any extra

bathing before using the swab because extra washing may remove exfoliated cells and increase the potential for inadequate specimens. It is also important to note that not all specimens with fecal matter were inadequate, since 73.7% (n=14) of 19 specimens with fecal matter were adequate. Given the relatively small sample size of our study, the potential effect of fecal matter on home-based anal self-sampling adequacy needs further study.

There are limitations to note. While the sampling and laboratory methods resulted in over 90% adequacy in these home-based self-collected swabs, the few remaining inadequate specimens (n=7) limited our power and ability to detect exposures associated with inadequacy. We used the LiPA assay, but it is possible an alternative assay might be used in a screening program which could result in swabs with different levels of adequacy. Second, while our primary definition of days in an uncontrolled environment included verified dates and no missing values, the alternative definition of this variable (participant-reported date of swabbing) may appropriately limit this exposure to days when the swab carried anal canal exfoliated cells and thus was subject to DNA degradation. However, about 12% of participant-reported swab collection dates were missing and those recorded may be subject to recall bias. For example, the range for this alternative variable was 0 to 11 days, with zero days indicating the swab was used, mailed, and then received at the laboratory on the same day which seems unlikely. It is possible that participants wrote down the day they mailed the swab, rather than the date they collected it. Third, while Wisconsin experiences a wide range of temperatures, substantially hotter or colder climates could impact adequacy which we could not detect in this study. Finally, while adequacy was high for both self-sampled and clinician-sampled specimens, this does not necessarily also mean that the genotypes detected in self-sampled vs clinician-sampled specimens are equally accurate. Our study design did not allow for this type of comparison.

In terms of study strengths, this research provides a strong contribution to the literature on home-based anal self-sampling. The PAC Study is the first research to use data from actual mailed home-based self-sampling kits to determine whether environmental conditions affect anal specimen adequacy. Most studies examining the effect of time and temperature subject specimens to specific temperature and time thresholds in a laboratory. A major strength of our study is that it uses data from kits that experienced the U.S. postal mail, thus mirroring real-world conditions kits may undergo if this method is implemented. The temperature recorders allowed us to collect detailed, precise "real-world" temperature data every 20 minutes. We utilized an experienced HPV genotyping laboratory to assess the outcome of specimen adequacy. Kits were also subjected to spring, summer, fall, and winter in Milwaukee, Wisconsin where temperatures can vary greatly by season. For example, the average low temperature in January in Milwaukee is around -9°C and the average high temperature in July is 27°C [18]. These conditions subjected specimens to a large range of temperatures as well as a wide range of the number of days in an uncontrolled environment. In spite of these exposures, 92.5% of specimens were adequate. This research provides evidence that participants can self-collect adequate anal specimens in their own home and that uncontrolled conditions such as time and temperature may have limited effect on the adequacy of these specimens. In contrast, the presence of fecal matter appeared to result in higher specimen inadequacy which requires confirmation in future at-home anal HPV self-sampling studies.

Funding

This clinical trial was supported by the National Cancer Institute of the National Institutes of Health (grant number R01CA215403 to AGN) and Clinical and Translational Science Institute grant support (2UL1TR001436). COPAN Italia SPA donated swabs. These funding entities had no involvement in the design, collection, analysis, or interpretation of data, writing of this report, or decision to submit this research for publication.

Competing interests

None.

Ethical approval

Study activities were approved by the Medical College of Wisconsin Human Protections Committee (protocol number PRO00032999).

Acknowledgements

We would like to thank the participants and the PAC Study Team (Bridgett Brzezinski, Cameron Liebert, Esmeralda Lezama-Ruiz). The authors acknowledge the contributions of Dave Wenten, Andrew Petroll, Brian Hilgeman, Sarah Lundeen and Leslie Cockerham. Thanks also to Mary Rau, Bradley Sirak, April Johnsen, Winsome Panton, Jonathan Weimer, Kartikey Acharya, Sol Aldrete, Sharon O'Dwyer, Christine Hogan, Janaki Shah, Kathryn Hoffman, Nicole Gerboth, Kathryn Kerhin, Anne Lakatos, Sally Anderson, Adrienne Parnon, Stacie Ciesielski and Mary Kay Schuknecht. Thank you to the Biorepository and Tissue Analytics (BTA) department at the Medical College of Wisconsin Cancer Center and to COPAN Italia SPA for donating swabs.

References

[1] Chaturvedi, A. K. (2010). Beyond cervical cancer: Burden of other HPV-related cancers among men and women. *Journal of Adolescent Health*, *46*(4), S20-S26.

[2] Deshmukh, A. A., Suk, R., Shiels, M. S., Sonawane, K., Nyitray, A. G., Liu, Y., ... & Sigel, K. (2020). Recent trends in squamous cell carcinoma of the anus incidence and mortality in the United States, 2001–2015. *JNCI: Journal of the National Cancer Institute*, *112*(8), 829-838.

[3] Machalek, D. A., Poynten, M., Jin, F., Fairley, C. K., Farnsworth, A., Garland, S. M., ... & Grulich, A. E. (2012). Anal human papillomavirus infection and associated neoplastic lesions in men who have sex with men: a systematic review and meta-analysis. *The Lancet Oncology*, *13*(5), 487-500.

[4] Clifford, G. M., Georges, D., Shiels, M. S., Engels, E. A., Albuquerque, A., Poynten, I. M., ... & Stier, E. A. (2021). A meta-analysis of anal cancer incidence by risk group: Toward a unified anal cancer risk scale. *International Journal of Cancer*, *148*(1), 38-47.

[5] de Martel, C., Georges, D., Bray, F., Ferlay, J., & Clifford, G. M. (2020). Global burden of cancer attributable to infections in 2018: a worldwide incidence analysis. *The Lancet Global Health*, *8*(2), e180-e190.

[6] Fernandez, E. (2021, October 7). *Treating anal cancer precursor lesions reduces cancer risk for people with HIV: Groundbreaking national clinical trial halted due to therapy's high success rates* [Press release]. Retrieved from https://anchorstudy.org/sites/default/files/newsletters/anchor_press_release_07oct2021.pdf

[7] Foster, S., Carvallo, M., Wenske, M., & Lee, J. (2021). Damaged masculinity: how honor endorsement can influence prostate cancer screening decision-making and prostate cancer mortality rates. *Personality and Social Psychology Bulletin*, 1-13.

[8] Ferrante, J. M., Shaw, E. K., & Scott, J. G. (2011). Factors influencing men's decisions regarding prostate cancer screening: a qualitative study. *Journal of Community Health*, *36*(5), 839-844.

[9] Botes, L. P., Jin, F., Bourne, C., Pett, S., Marriott, D., Carr, A., ... & Hillman, R. J. (2011). Participants' perspectives of self-collected anal cytological swabs. *Sexual Health*, *8*(2), 257-258.

[10] Thompson, J. (2014). *Acceptability of Anal Cancer Screening Tests Among Gay and Bisexual Men*. [Master's thesis, University of North Carolina at Chapel Hill]. UNC University Libraries Carolina Digital Repository.

[11] Catomeris, P., Baxter, N. N., Boss, S. C., Paszat, L. F., Rabeneck, L., Randell, E., ... & Tinmouth, J. (2018). Effect of temperature and time on fecal hemoglobin stability in 5 fecal immunochemical test methods and one guaiac method. *Archives of Pathology & Laboratory Medicine*, *142*(1), 75-82.

[12] Ejegod, D. M., Pedersen, H., Alzua, G. P., Pedersen, C., & Bonde, J. (2018). Time and temperature dependent analytical stability of dry-collected Evalyn HPV self-sampling brush for cervical cancer screening. *Papillomavirus Research*, *5*, 192-200.

[13] Harris, P. A., Taylor, R., Thielke, R., Payne, J., Gonzalez, N., & Conde, J. G. (2009). Research electronic data capture (REDCap)—a metadata-driven methodology and workflow process for providing translational research informatics support. *Journal of Biomedical Informatics*, *42*(2), 377-381.

[14] IBM Corp. Released 2021. IBM SPSS Statistics for Windows, Version 28.0. Armonk, NY: IBM Corp.
[15] StataCorp. 2021. *Stata Statistical Software: Release 17*. College Station, TX: StataCorp LLC.
[16] Fife, K. H., Coplan, P. M., Jansen, K. U., DiCELLO, A. C., Brown, D. R., Rojas, C., & Su, L. (2003). Poor sensitivity of polymerase chain reaction assays of genital skin swabs and urine to detect HPV 6 and 11 DNA in men. *Sexually Transmitted Diseases*, *30*(3), 246-248.
[17] Flores, R., Abalos, A. T., Nielson, C. M., Abrahamsen, M., Harris, R. B., & Giuliano, A. R. (2008). Reliability of sample collection and laboratory testing for HPV detection in men. *Journal of Virological Methods*, *149*(1), 136-143.
[18] U.S. Climate Data. (2022). Climate Milwaukee-Wisconsin. Retrieved from https://www.usclimatedata.com/climate/milwaukee/wisconsin/united-states/uswi0455

Table 1. Conditions by kit and specimen adequacy in the Prevent Anal Cancer (PAC) Study, Milwaukee, Wisconsin, January 2020 – April 2022 (n=93).

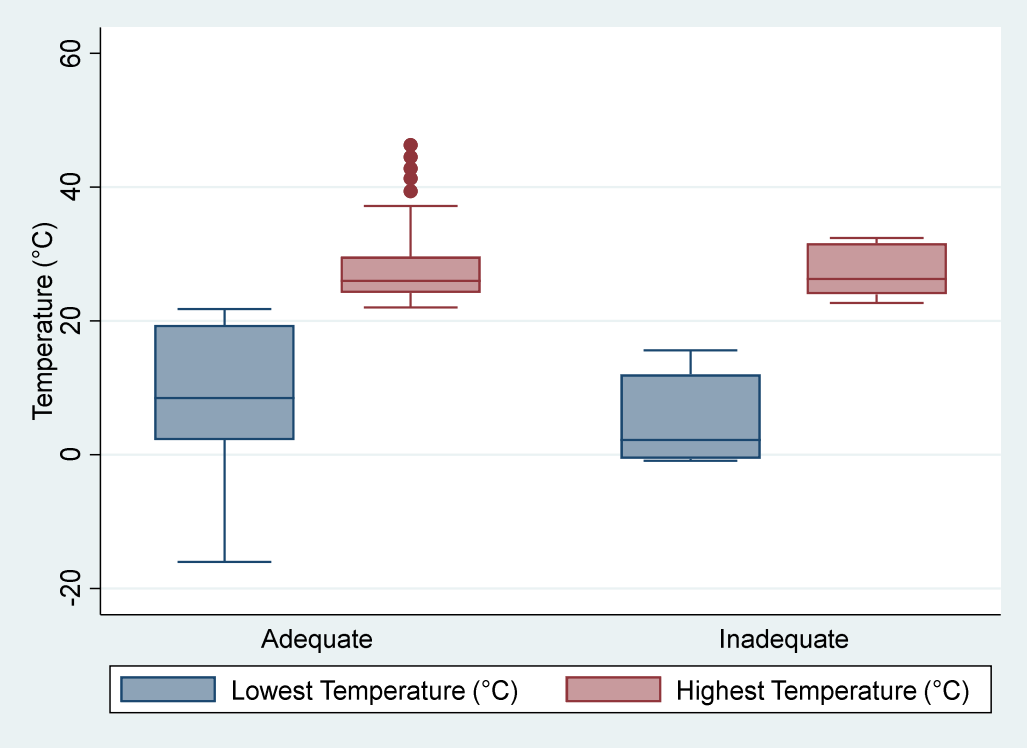
	Total (n=93) Mean (SD)	**Adequate** (n=86) Mean (SD)	**Inadequate** (n=7) Mean (SD)	*p*-value[1]
Specimen adequacy, n (%)				
Adequate	86 (92.5)	--	--	--
Inadequate	7 (7.5)	--	--	--
Days in uncontrolled environment	13.1 (13.8)	12.9 (13.6)	16.0 (16.5)	.64
Temperature (°C)[2]				
Lowest	8.5 (9.3)	8.8 (9.5)	4.7 (6.4)	.17
Highest	27.7 (5.0)	27.7 (5.1)	27.5 (3.8)	.90
Range	19.3 (8.0)	19.0 (8.2)	22.8 (4.5)	.08
Freeze-thaw cycle, n (%)[3]				
Yes	19 (20.4)	17 (19.8)	2 (28.6)	.63
No	74 (79.6)	69 (80.2)	5 (71.4)	
Presence of fecal matter, n (%)				
Yes	19 (20.4)	14 (16.3)	5 (71.4)	.00
No	74 (79.6)	72 (83.7)	2 (28.6)	

[1] Welch's t-test was used for days in an uncontrolled environment and temperature (lowest, highest, range). Fisher's exact test was used for freeze-thaw cycle and fecal matter variables.

[2] Lowest/highest temperature measured the lowest/highest temperature a kit experienced. Temperature range represented the difference between the highest and lowest temperatures a kit experienced.

[3] Freeze-thaw cycle measured whether a kit temperature changed from 0°C (freezing) to 20°C (room temperature).

Figure 1. Boxplot of lowest and highest temperatures experienced by kits grouped by specimen adequacy in the Prevent Anal Cancer (PAC) Study, Milwaukee, Wisconsin, January 2020 – April 2022 (n=93).



Appendix

Table 1A. Logistic regression sensitivity analyses of exposures and specimen inadequacy in the PAC Study Jan 2020-April 2022 (n=82).

	OR (95% CI)	aOR (95% CI)[1]
Temperature (°C)		
Lowest	.89 (.79 – 1.00)	.89 (.79 – 1.00)
Highest	.86 (.57 – 1.30)	.88 (.60 – 1.28)
Range	1.11 (.97 – 1.26)	1.12 (.99 – 1.26)
Days in uncontrolled environment	.87 (.53 – 1.44)	--
Freeze-thaw cycle (yes)	6.71 (1.12 – 40.18)	7.05 (1.16 – 42.83)
Presence of fecal matter (yes)	15.72 (2.25 – 109.87)	16.16 (2.20 – 118.75)

[1]Adjusted for number of days in uncontrolled environment.