SPECIMEN COLLECTION CONSIDERATIONS FOR SARS-COV-2 PCR TESTING WHEN SHIPPING ON DRY ICE

SARS-CoV-2 samples' quality has to be preserved during shipment to avoid interferences during the PCR amplification. Bacteria contamination or a drop in pH can affect the quality of the sample and the efficiency of the testing.

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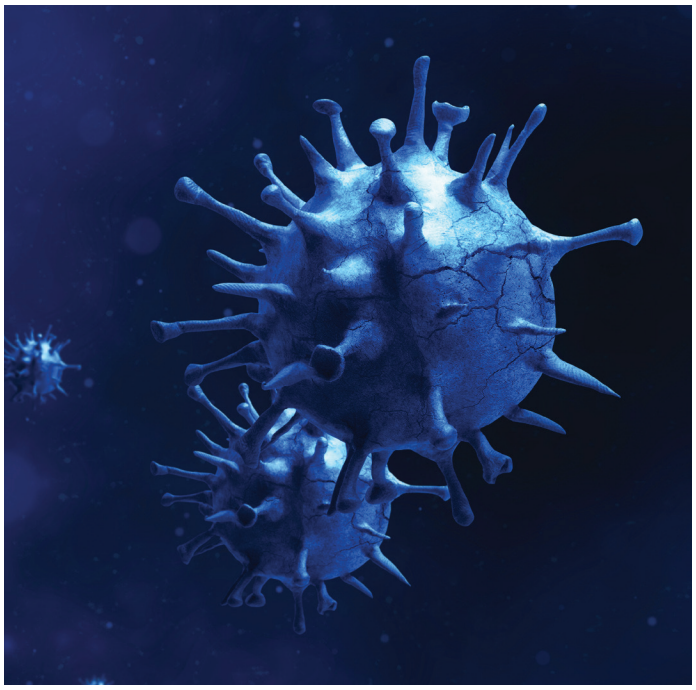
INTRODUCTION

For initial diagnostic testing for SARS-CoV-2 by polymerase chain reaction (PCR), the Centers for Disease Control and prevention (CDC) recommends collecting an upper respiratory specimen. This can be either a nasopharyngeal (NP) or an oropharyngeal (OP) swab collected by a trained healthcare provider[1]. Upper respiratory specimen are generally collected using sterile flocked synthetic fiber swabs with plastic or wire shafts and placed into viral transport media (VTM) or universal transport media (UTM).

Medpace's central laboratories performs SARS-CoV-2 testing on multiple specimens, including NP and OP swabs collected in VTM or UTM. SARS-CoV-2 samples are collected around the globe and shipped frozen on dry ice to Medpace's central laboratories in Cincinnati (Ohio-USA) and Leuven (Belgium).

Bacterial growth in the transport media must be prevented, as this can compromise the sample integrity before analysis. The majority of UTM or VTM tubes contain a pH indicator dye, generally phenol red,



which exhibits a gradual transition from yellow to red over a pH range of 6.2 to 8.2. This enables simple visual monitoring of bacterial contamination and stability of the Covid sample.

It is recommended to store SARS-CoV-2 specimens at 2-8°C for up to 72 hours after collection. Samples should be stored at -70°C or below if specimens are to be tested more than 72 hours after collection[1]. When transit from the local collection site to the specialized central laboratory is expected to take more than 72 hours, samples are usually frozen and shipped on dry ice. SARS-CoV-2 samples are usually placed into Category B UN3373 certified specimen transport bags prior to shipment, to ensure safety, sample isolation and preservation. The specimen transport bags contain an adhesive tape which, if properly closed, ensures the tube isolation and protection in case of leak.

Maintaining the red color of the UTM is indicative that sample quality is not compromised during shipping, while a change in color is indicative of a sample contamination with bacteria. In addition, a drop in pH can affect the quality of the sample and the efficiency of the PCR amplification. Given the importance of the pre-analytic phase in SARS-CoV-2 PCR analysis and to better understand the root cause of the UTM color change when samples are shipped on dry ice, a series of experiments were designed to mimic different shipping conditions.

UTM tubes were stored for 48 hours on dry ice in a shipping box. After 48 hours, samples were thawed with closed caps under a biological safety cabinet. Our results suggest that the fact that UTM tubes do not have an O-Ring, can allow CO_2 released from sublimating dry ice to infiltrate the tubes. The CO_2 is confined to the tube headspace when the samples are frozen and the change in pH only occurs when the solution is thawed.

MATERIAL AND METHOD

- UTM tubes (Mantacc, Cat# MBT-010, lot 2020030058, expiration date 10 Mar 2022)
- Bemis Parafilm (Fisher Scientific, Cat#11762644, PM-992)
- Dry ice
- Category B UN3373 certified specimen transport bags

UTM tubes were divided into several groups and different conditions to mimic different shipping scenarios:

- Group 1: 10 UTM tubes with perfectly closed caps but with unclosed safety bags (the adhesive tape was not glued on the bag).
- Group 2: 10 UTM tubes with caps not properly closed but safety bags properly closed.
- Group 3: 10 UTM tubes with both caps and safety bags not properly closed.
- Group 4: 10 UTM tubes with parafilm sealed caps but not properly closed safety bags.
- Group 5: 10 UTM tubes with properly closed caps and properly closed safety bags.
- Group 6: 10 UTM tubes with parafilm sealed and properly closed cap and properly closed safety bags.

RESULTS

In group 1, 7/10 UTM tubes turned yellow after 48 hours in dry ice (*Figure 1*). The caps of all tubes were perfectly closed but with the adhesive tape was not properly glued on the safety bag, leading to an unproperly closed safety bags.

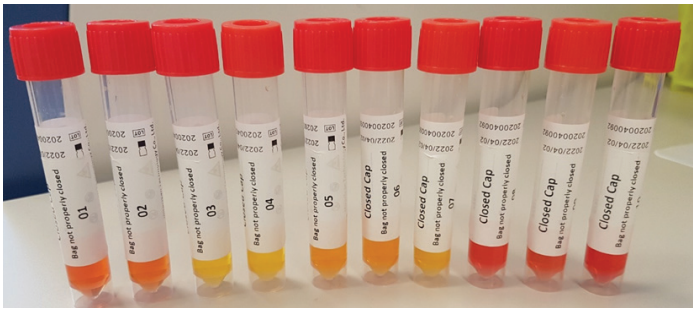


Figure 1: UTM tubes with perfectly closed cap but with unclosed safety bags. 7 out of 10 UTM tubes turned yellow after 48 hours in dry ice.

In group 2, 4/10 UTM tubes turned yellow after 48 hours in dry ice (*Figure 2*). The tube caps were not properly closed but the adhesive tape was properly glued on the safety bag.

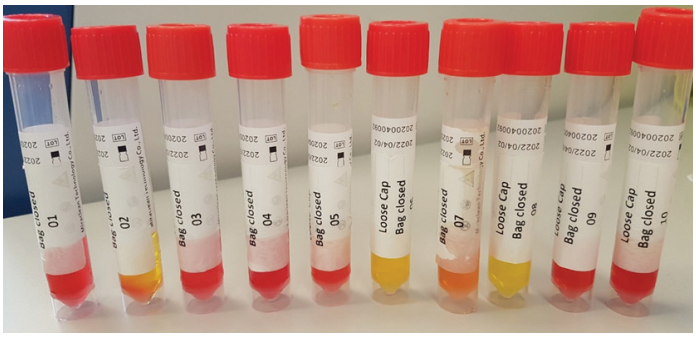


Figure 2: UTM tubes with perfectly unclosed caps but with closed safety bags. 4 out of 10 UTM tubes turned yellow after 48 hours in dry ice.

In group 3, all UTM tubes turned yellow after 48 hours in dry ice (*Figure 3*). In this group, tube caps were not properly closed and the adhesive tape was not properly glued on the safety bag.

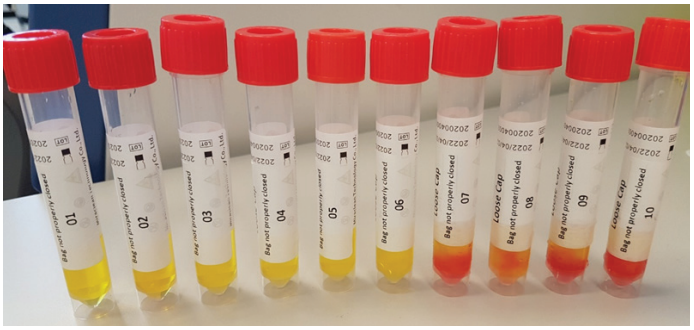


Figure 3: UTM tubes with both unclosed caps and safety bags. All UTM tubes turned yellow after 48 hours in dry ice.

In group 4, all 10 UTM tube caps were sealed with parafilm, but safety bags were not properly closed. All 10 UTM tubes turned yellow after 48 hours in dry ice (*Figure 4*).

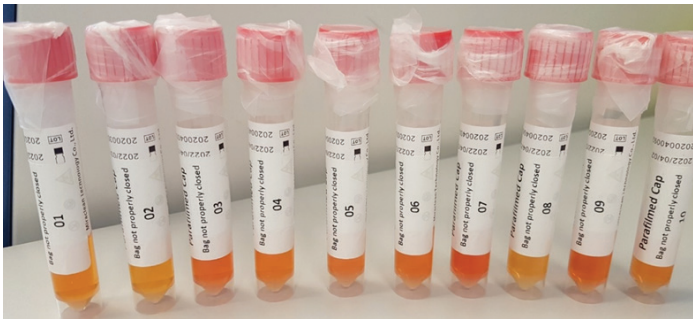


Figure 4: UTM tubes with closed and parafilm sealed caps but unclosed safety bags. All 10 UTM tubes turned yellow after 48 hours in dry ice.

In group 5, none of the 10 UTM tubes turned yellow. For all tubes, caps were properly tightened, and the adhesive tape was properly glued on the safety bags.

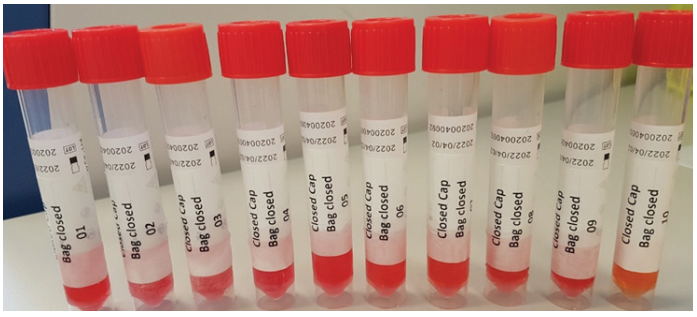


Figure 5: UTM tubes with both closed caps and safety bags. None of the 10 UTM tubes turned yellow.

In the last group, none of the 10 UTM tubes turned yellow. 10/10 tubes had properly closed caps, sealed with parafilm and put in safety bags with properly glued adhesive tape. No pH change was observed when the tubes were frozen (*Figure 6*) or after thaw (*Figure 7*).

Before Thaw

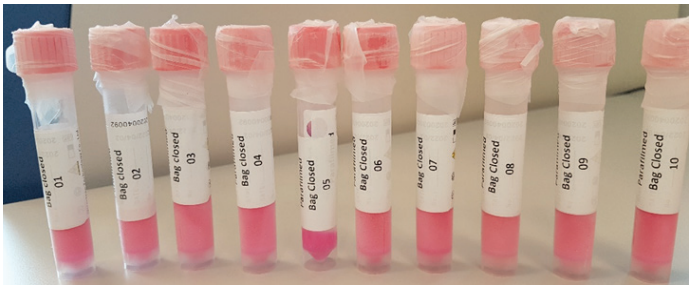


Figure 6: UTM tubes with closed caps and parafilm seal, and closed safety bags immediately after being placed in dry ice for 48 hours. None of the 10 UTM tubes turned yellow.

After Thaw

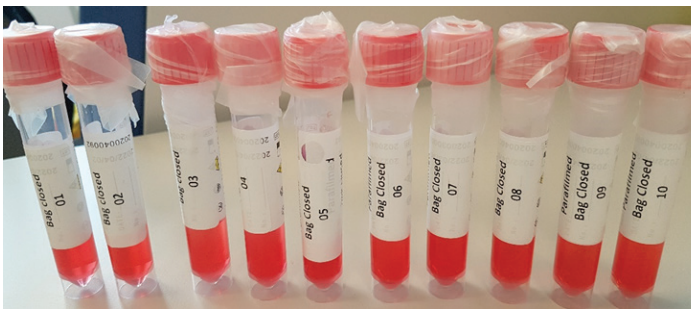


Figure 7: UTM tubes with closed caps and parafilm seal, and closed safety bags after thaw. None of the 10 UTM tubes turned yellow.

DISCUSSION

Dry ice is the solid form of carbon dioxide (CO_2), a molecule consisting of a single carbon atom bonded to two oxygen atoms, and it is commonly used as a cooling agent to ship reagents and specimens. Dry ice sublimates (transition from solid to gas state) at temperatures below −56.4 °C and CO_2 changes from a solid to a gas state with no intervening liquid form, and it is released in the atmosphere[2].

The yellow color indicative of lower pH, can be detrimental to both viral RNA template through depurination which will result in more partial products and decreased overall yield as well as on the activity of polymerase used for PCR amplification.

Before thawing, all samples were at pH of 7.4 as indicated by no change in color of the UTM color. To explain the color change after thawing, we have identified two possibilities: i) Tube lid is not tightly closed, or ii) shipment bag adhesive tape was not properly sealed. Through an internal investigation, having properly closed caps and parafilm-sealed tubes, combined with tubes being enveloped into safety bag with properly glued adhesive tape, is the best option to prevent CO_2 access to tubes and, consequently, a pH drop in the tubes which will interfere with the quality of the sample and the result of the PCR test.

At Medpace's central laboratories, we strive to deliver the best-quality results, therefore, samples with a yellow UTM are not accepted for analysis. However, we provide detailed instructions to our collaborators to prevent this scenario.

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REFERENCES

1.https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html

2. “The sublimation temperature of carbon dioxide”, C R Barber 1966 Br. J. Appl. Phys. 17 391

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