Visium CytAssist Spatial Gene Expression for FFPE: A Tissue Slide Shipping and Extended Storage Case Study

Introduction

The Visium CytAssist Spatial Gene Expression for FFPE workflow is designed to analyze mRNA in fixed tissue sections after processing via the CytAssist instrument. The assay is compatible with archived slides as well as freshly placed samples on glass slides, which are processed with Hematoxylin and Eosin (H&E) or Immunofluorescence (IF) staining. Staining allows users to identify and select an area of interest for analysis.

Slides with tissue sections will be referred to as tissue slides throughout this document. In some cases, tissue slides may require shipping. This Technical Note examines the effect of shipping slides with formalin fixed & paraffin embedded (FFPE) tissue sections by measuring RNA quality, assay sensitivity, and provides suggested shipping conditions.

In this document, archived slides are defined as stored glass slides with FFPE sections, stained or unstained, with or without coverslips. This Technical Note assesses the changes in RNA quality and assay sensitivity in archived slides stored over a period of 90 days and provides suggestions for extended storage conditions.

Contents

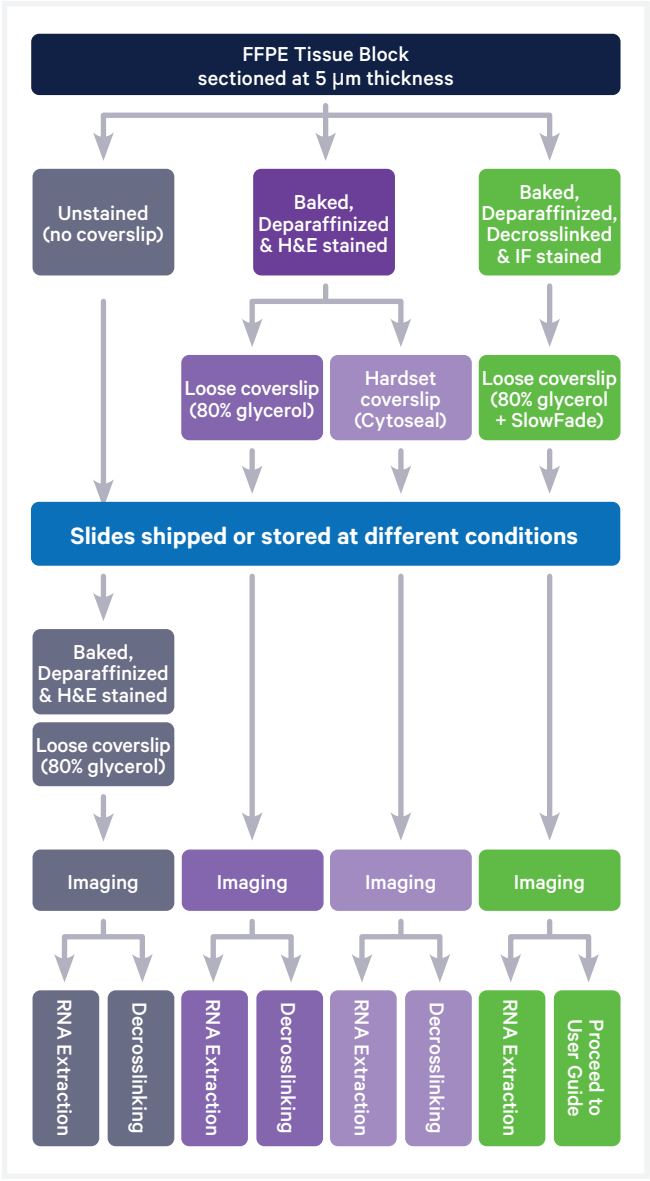

Figure 1. Sample preparation experimental setup. Before variation in storage or shipping conditions, FFPE tissue blocks are sectioned onto SuperFrost Plus slides and processed as described in Demonstrated Protocol CG000518. For any tested condition, serial sections were used to assess RNA quality (n=1) and assay sensitivity (n=2).

Methods

Sectioning & Staining

A human breast cancer FFPE block previously demonstrated to have low variability in RNA quality across the thickness of the block was selected for analysis. However, some minor variability in quality across the block is expected.

5 µm sections were generated and placed on VWR SuperFrost Plus slides (PN 48311-703) according to the CytAssist Tissue Preparation Guide (Document CG000518). Sections were H&E stained (Document CG000520), IF stained (Document CG000519), or were left unstained.

Stained tissue slides were mounted with either of the following methods:

- Loose mounting: 85% glycerol (Acros Organics, PN 27255000) or SlowFade Diamond (Thermo Fisher Scientific, PN S36967) in glycerol as described in the relevant Demonstrated Protocol
- Hardset mounting: Cytoseal XYL (Thermo Fisher Scientific, PN 8312-4)

For glycerol based loose mounting, coverslips were held in place by applying coverslip sealant (Biotium, PN 23005) on all four coverslip corners. To prevent mounting media leaking during storage, slides were stored flat in boxes. Unstained tissue slides were not coverslipped during shipping or storage.

Storage & Shipping

Sections were stored or shipped as described in subsequent chapters.

RNA Quality

RNA quality was assessed from a single section for each stated storage and shipping condition. To extract RNA, coverslips were removed (if applicable) at stated time points by removing the sealant (if applicable) from the corners of the coverslip with a blade, then according to the following Demonstrated Protocols:

- Loose coverslip removal of H&E stained tissue slides - Document CG000520
- Loose coverslip removal of IF stained tissue slides - Document CG000519
- Hardset coverslip removal - Document CG000518

Unstained sections were deparaffinized in xylene and rehydrated in an ethanol gradient. Sections were scraped into tube strips and processed with Qiagen RNeasy FFPE Kit (PN 73504) according to the RNA extraction protocol listed in the Visium CytAssist Spatial Gene Expression for FFPE – Tissue Preparation Guide (Document CG000518). Extracted RNA was stored at -80°C. RNA quality was assessed by calculating the DV200 score using a Tapestation High Sensitivity kit (Agilent, PN 5067-5579).

Assay Sensitivity

Assay sensitivity was assessed from two sections that were serial to the section used to assess RNA quality for each stated storage and shipping condition. To measure assay sensitivity, H&E and IF stained tissue slides were imaged and coverslips were removed at stated time points. Coverslip removal and subsequent destaining or decrosslinking were performed using the documentation above. Slides were then processed as described in the Visium CytAssist Spatial Gene Expression Reagent Kits User Guide (CG000495). Assay sensitivity was determined by analyzing the mean UMI counts and mean genes detected at 20,000 read pairs per tissue-covered spot. This sequencing depth differs from the minimum recommended sequencing depth of 25,000 read pairs per tissue-covered spot.

Effect of Shipping on Tissue Slides

To examine the effect of shipping on tissue slides, the following conditions were compared:

- No shipping: tissue slides were generated and stored for 14 days at 4°C or at room temperature, with or without a desiccator.
- Shipped: tissue slides were placed in sealed bags, with or without desiccant, and with or without ice packs to match the storage conditions of tissue slides that were not shipped. Sealed bags were boxed and shipped via vehicle for 24 h with vehicle temperatures fluctuating between 14–30°C.

At day 14, shipped tissue slides were analyzed and compared to tissue slides that were not shipped, with equivalent storage conditions. For comparison, tissue slides that were not stored or shipped for any length of time were analyzed for RNA quality and assay sensitivity. The exact experimental designs for each staining condition are shown in Figures 2, 5, and 8.

The human breast cancer FFPE block used to generate tissue slides was expected to give ~100 sections without any change in morphological features and minimal variations in RNA quality arising from variable fixation at different tissue depths.

For samples that were not shipped with ice packs, temperatures fluctuated between 14–30°C in the shipping vehicle, which is described in the following sections as ambient temperature. The 14 day time point was selected as it provided a sufficient time interval to identify an area of interest and to ship tissue slides.

Tissue Slide Shipping Results - IF Stained Tissue Slides

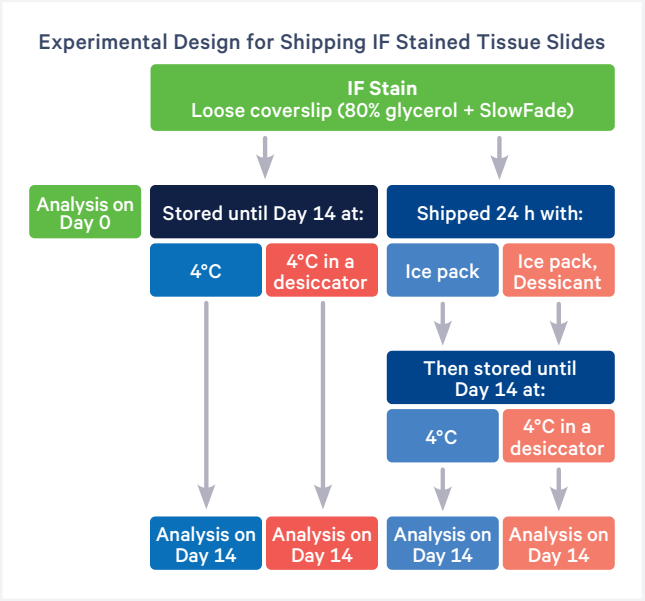

Figure 2. Experimental design for shipping IF stained tissue slides. Slides were divided into three groups: analysis on day 0 (slides were immediately processed), shipped, or storage without shipping. Slides stored without shipping were stored for 14 days at 4°C with or without a desiccator. Shipped slides were shipped for 24 h with ice packs, with or without desiccant, then stored until day 14. These groups were compared to the slides from day 0. Analysis was performed on serial sections to assess RNA quality by measuring DV200 scores (n=1), and assay sensitivity (n=2) by analyzing the mean UMI counts and mean genes detected at 20,000 read pairs per tissue-covered spot.

Shipping with Ice Packs Does Not Impact RNA Quality of IF Stained Tissue Slides Compared to Not Shipped Controls

IF stained tissue slides were shipped for 24 h with ice packs, with or without desiccant (Figure 2). Upon return, tissue slides were stored until day 14 at 4°C with or without a desiccator. Changes in RNA quality and assay sensitivity were estimated by analyzing the shipped or stored slides and comparing to tissue slides with freshly placed sections from day 0.

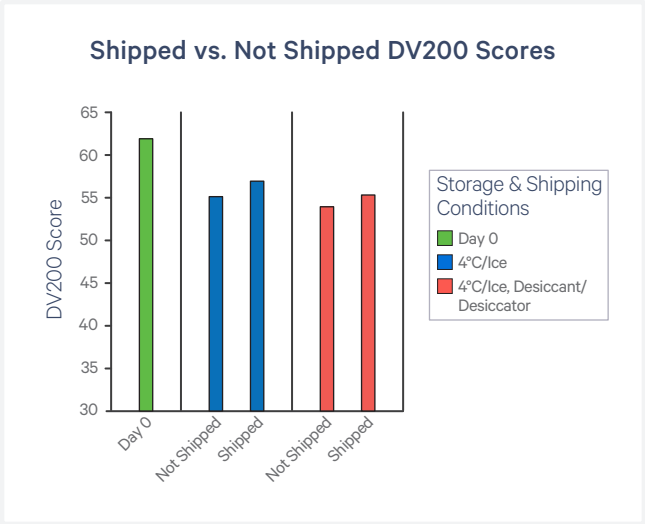

Figure 3. Shipping does not impact RNA quality in IF stained tissue slides. On Day 0, human breast cancer FFPE sections were placed on SuperFrost Plus slides, stained and stored or shipped according to conditions described in Figure 2. At day 14, RNA was extracted from the sections and DV200 score was determined. One section was analyzed for each condition. Day 0 shows the RNA quality of the freshly placed section on a SuperFrost Plus slide from the same tissue block.

IF stained tissue slides stored for 14 days showed a ~10% decrease in DV200 scores, but similar DV200 scores were observed between shipped and not shipped tissue slides as shown in Figure 3. These data suggest that shipping with ice packs, with or without desiccant, did not impact the RNA quality of IF stained sections.

Shipping with Ice Packs Does Not Significantly Impact Assay Sensitivity of IF Stained Tissue Slides Compared to Not Shipped Controls

Compared to day 0 tissue slides, slides shipped at 4°C without desiccant showed similar number of mean genes detected as well as mean UMI counts per tissue-covered spot (Figure 4). Two users handled the shipped and not shipped tissue slides, which likely lead to consistently better performance of shipped sections vs. sections that were not shipped.

Slides shipped with ice packs with desiccant showed an average ~5% decrease in the mean genes detected compared to the tissue slides shipped with ice packs without desiccant (Figure 4). However, this decrease was not significant, likely due to the small sample size. Despite this limitation, the data trends observed still suggest shipping IF stained tissue slides with ice packs and without desiccant.

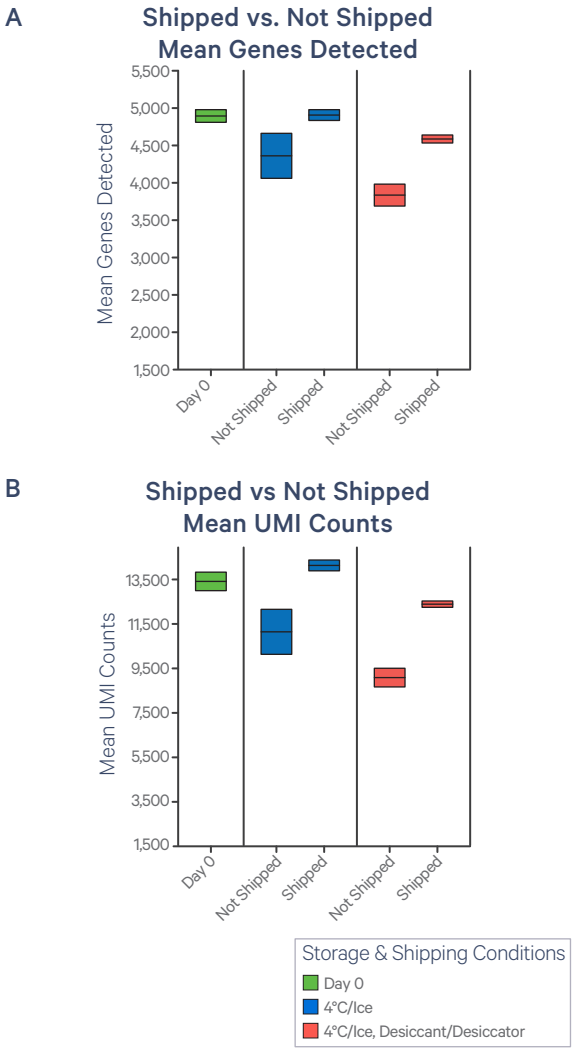

Figure 4. Shipping does not significantly impact assay sensitivity in IF stained tissue slides. **A**. Mean genes detected at 20,000 read pairs per tissue-covered spot. **B**. Mean UMI counts at 20,000 read pairs per tissue-covered spot. Two sections were analyzed for each condition.

Tissue Slide Shipping Results - H&E Stained Tissue Slides

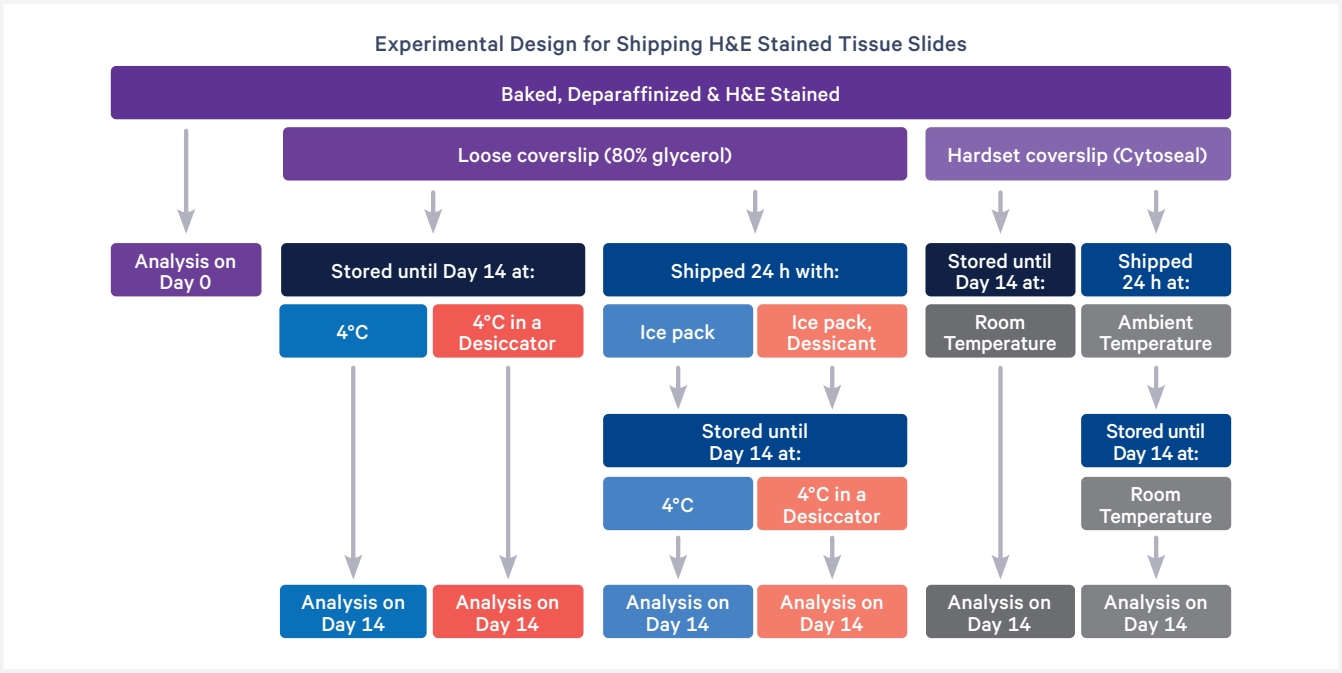

Figure 5. Experimental design for shipping H&E stained tissue slides. Slides were divided into three groups: analysis on day 0 (slides were immediately processed), shipped, or storage without shipping. Slides stored without shipping were stored for 14 days at 4°C with or without a desiccator, or at room temperature. Shipped slides were shipped for 24 h with ice packs with or without desiccant, or at ambient temperature, then stored until day 14. These groups were compared to the slides from day 0. All slides shipped with ice packs or stored at 4°C received loose coverslips, while slides shipped at ambient temperature or stored at room temperature received hardset coverslips. Analysis was performed on serial sections to assess RNA quality (n=1) by measuring DV200 scores, and assay sensitivity (n=2) by analyzing the mean UMI counts and mean genes detected at 20,000 read pairs per tissue-covered spot.

Shipping at Ambient Temperature Does Not Impact RNA Quality of H&E Stained Tissue Slides Compared to Not Shipped Controls

H&E stained tissue slides were shipped for 24 h with ice packs, with or without desiccant or at ambient temperature (Figure 5). Upon return, tissue slides were stored until day 14 at 4°C with or without a desiccator or at ambient temperature. Changes in RNA quality and assay sensitivity were estimated by comparing tissue slides with freshly placed sections from day 0.

H&E stained tissue slides across the tested storage conditions for 14 days show a 15-20% decrease in DV200 score. However, no difference in DV200 scores was observed between shipped and not shipped tissue slides as shown in Figure 6, suggesting that shipping did not further impact RNA quality.

Shipping at Ambient Temperature Does Not Impact Assay Sensitivity of H&E Stained Tissue Slides Compared to Not Shipped Controls

H&E stained tissue slides shipped at ambient temperature or with ice packs (with or without desiccant) show similar number mean genes detected as well as mean UMI counts compared to their not shipped controls. This suggests that H&E stained tissue slides can safely tolerate 24 hours of shipping.

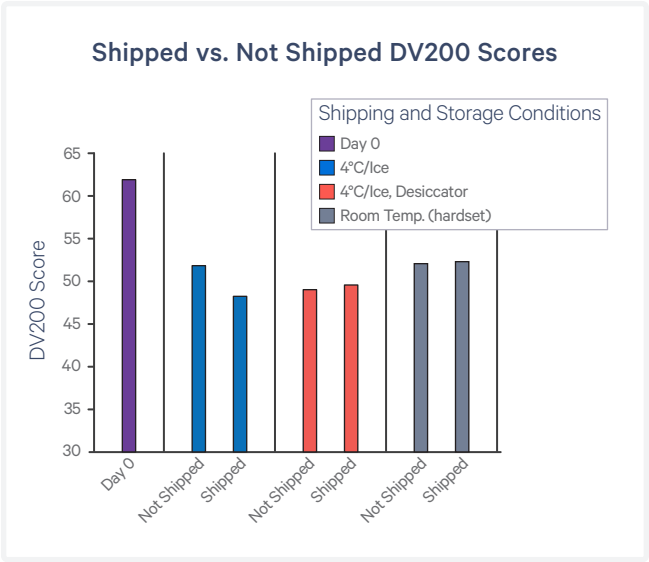

Figure 6. Shipping does not impact RNA quality in H&E stained tissue slides. On day 0, human breast cancer FFPE sections were placed on SuperFrost Plus slides, stained and stored or shipped according to conditions described in Figure 5. At 14 days, RNA was extracted from the sections and DV200 score was determined. One section was analyzed for each condition. Day 0 shows the RNA quality of the freshly placed section on a SuperFrost Plus slide from the same tissue block. H&E stained tissue slides stored at 4°C and at 4°C with a desiccator were loosely mounted with glycerol, while tissue slides stored at room temperature were mounted with hardset mounting media.

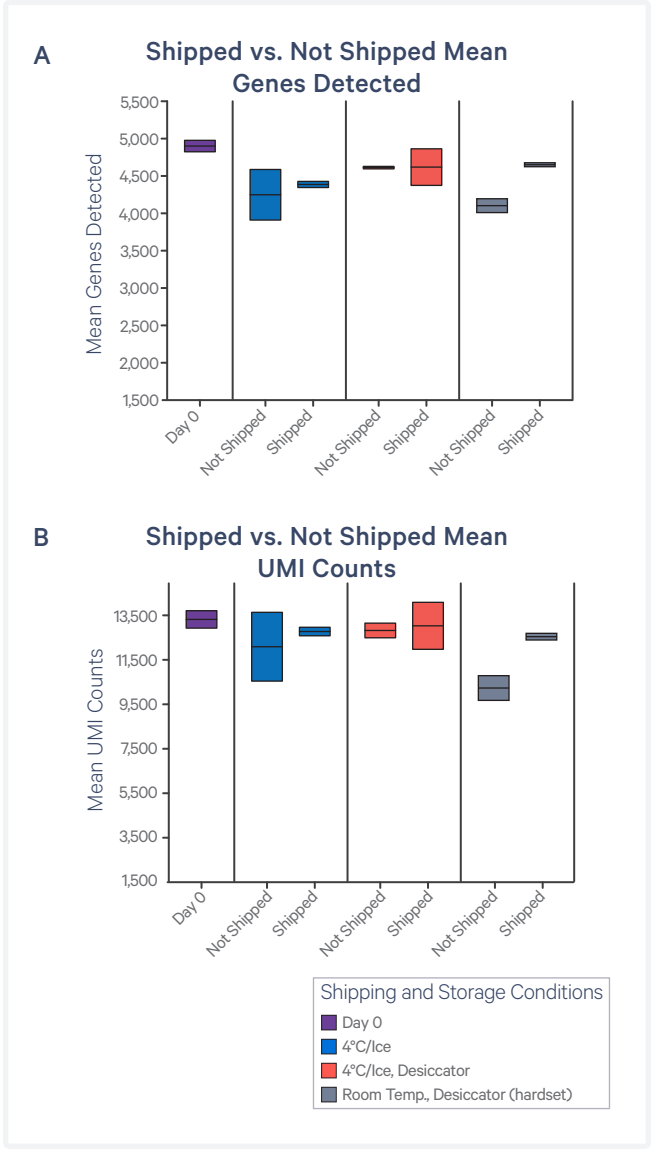

Figure 7. Shipping does not impact assay sensitivity in H&E stained tissue slides. **A**. Mean genes detected at 20,000 read pairs per tissue-covered spot. **B**. Mean UMI counts at 20,000 read pairs per tissue-covered spot. H&E stained tissue slides stored at 4°C and at 4°C with a desiccator were loosely mounted with glycerol, while tissue slides stored at room temperature were mounted with hardset mounting media. Two sections were analyzed for each condition.

Tissue Slide Shipping Results - Unstained Tissue Slides

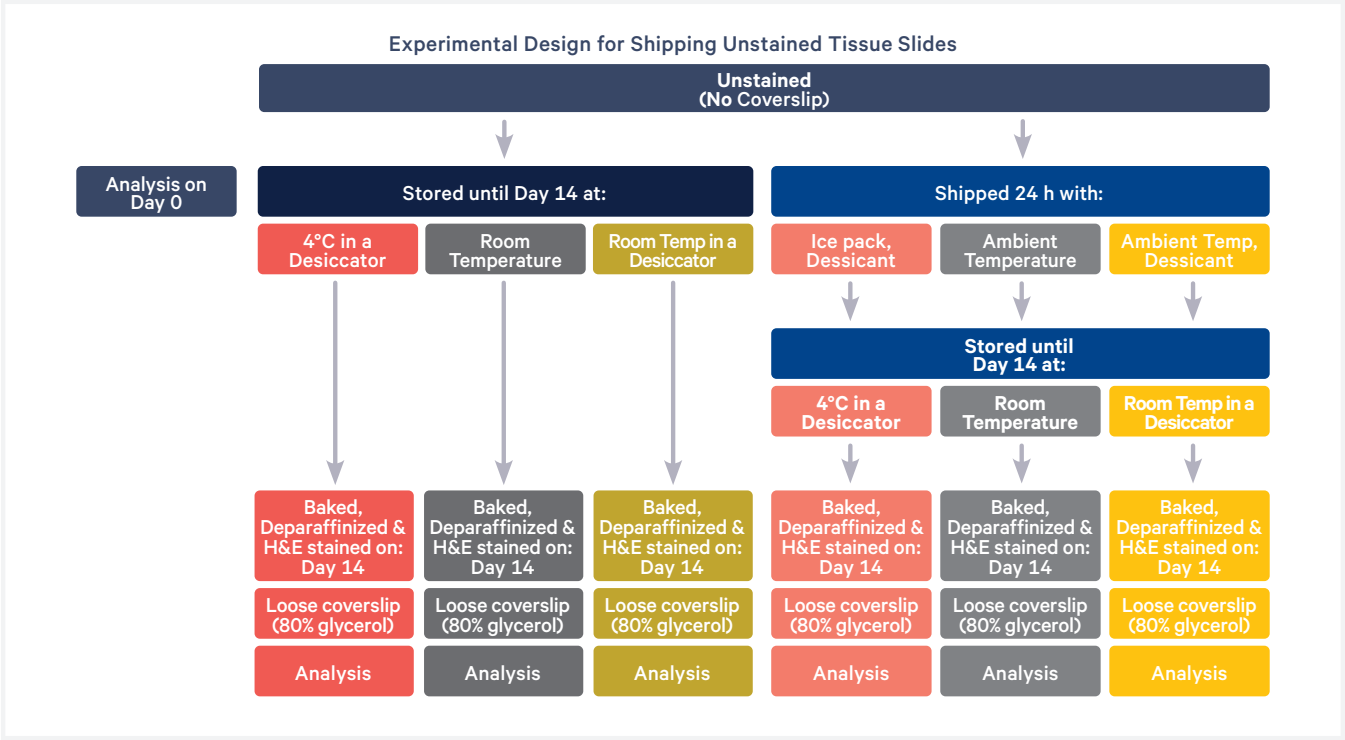

Figure 8. Experimental design for unstained tissue slides. Slides were divided into three groups: analysis on day 0 (slides were immediately processed), shipped, or storage without shipping. Slides stored without shipping were stored for 14 days at 4°C in a desiccator, or at room temperature with or without a desiccator. Shipped slides were shipped for 24 h with ice packs with desiccant, or at ambient temperature, then stored until day 14. These groups were compared to the slides from day 0. Analysis was performed on serial sections to assess RNA quality (n=1) by measuring DV200 scores, and assay sensitivity (n=2) by analyzing the mean UMI counts and mean genes detected at 20,000 read pairs per tissue-covered spot.

Shipping Does Not Adversely Impact RNA Quality of Unstained Tissue Slides Compared to Not Shipped Controls

Unstained tissue slides were shipped for 24 h with ice packs and desiccant or at ambient temperature with or without desiccant (Figure 8). Upon return, tissue slides were stored until day 14 at 4°C in a desiccator or at room temperature, with or without a desiccator. Changes in RNA quality and assay sensitivity were estimated by comparing tissue slides with freshly placed sections from day 0.

Over a period of 14 days, unstained sections showed a ~30% loss in DV200 score compared to sections analyzed at day 0. However, shipping did not further adversely affect DV200 scores. Shipped tissue slides had better DV200 scores than tissue slides that were not shipped (Figure 9). Since a single section was analyzed per condition, the statistical significance of this change cannot be predicted. Despite this, the consistent trend across conditions suggests that shipping did not further impact RNA quality.

Shipping at Ambient Temperature without Desiccant Negatively Impacts Assay Sensitivity of Unstained Tissue Slides Compared to Not Shipped Controls

Though similar RNA quality between shipped and not shipped samples was observed, a 44% decrease in mean genes detected and 66% decrease in mean UMI counts was observed in tissue slides shipped at ambient temperature without desiccant (Fig 10 A, 10B). The decrease in assay sensitivity at ambient

temperature is based on one replicate, suggesting that shipping at ambient temperature can negatively impact assay sensitivity in an unpredictable manner. Tissue slides shipped at other tested conditions did not show a difference in mean genes detected or mean UMI counts compared to not shipped controls. Based on these data, shipping samples at ambient temperature without desiccant should be avoided. Ignoring the outlier section shipped at room temperature, unstained sections on average showed ~5% loss in mean genes detected compared to sections analyzed on day 0 (Figure 10A), showing that large changes in DV200 scores (~30%) resulted in only small (~5%) changes in the assay sensitivity.

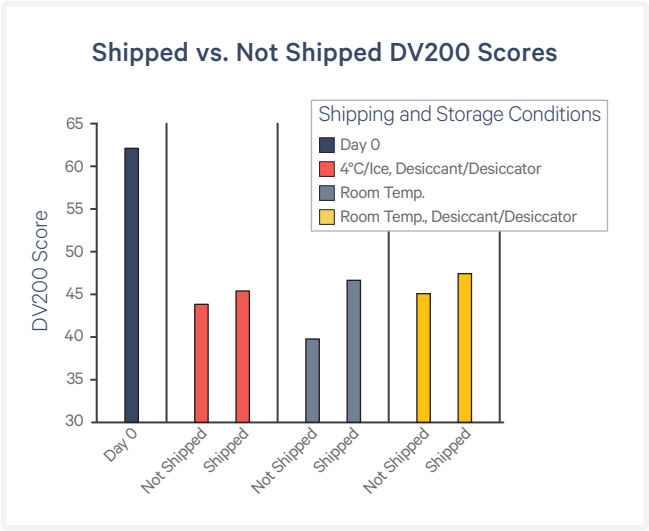

Figure 9. Shipping does not adversely impact RNA quality in unstained tissue slides. On day 0, human breast cancer FFPE sections were placed on SuperFrost Plus slides, stained and stored or shipped according to conditions described in Figure 8. At 14 days, RNA was extracted from the sections and DV200 score was determined. One section was analyzed for each condition. Day 0 shows the RNA quality of the freshly placed section on a SuperFrost Plus slide from the same tissue block.

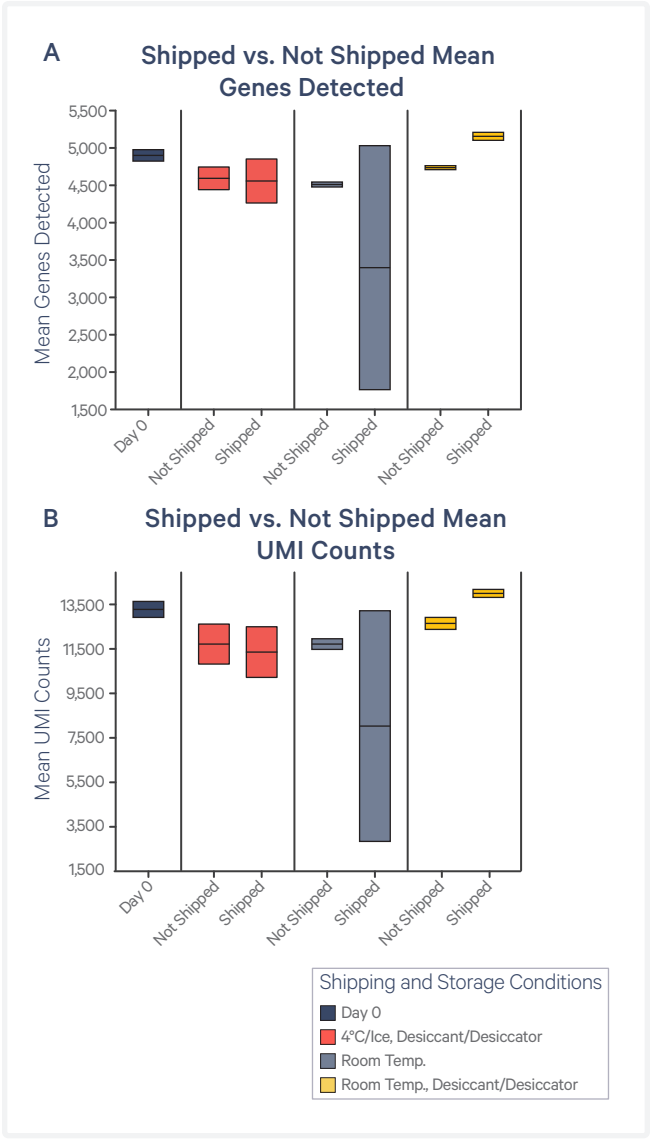

Figure 10. Shipping at ambient temperature without desiccant negatively impacts assay sensitivity in unstained tissue slides **A**. Mean genes detected at 20,000 read pairs per tissue-covered spot. **B**. Mean UMI counts at 20,000 read pairs per tissue-covered spot. Two sections were analyzed for each condition.

Effect of Storage on Tissue Slides

To examine the effect of storage over time on tissue slides, tissue slides were separated into four different storage conditions:

1. 4°C
2. 4°C in a desiccator
3. Room temperature
4. Room temperature in a desiccator

Tissue slides were analyzed at Day 0 (no additional storage), Day 14, Day 28, and Day 90. For a given time point and storage condition, serial sections were tested for RNA quality (n=1) and sequenced for assay performance (n=2).

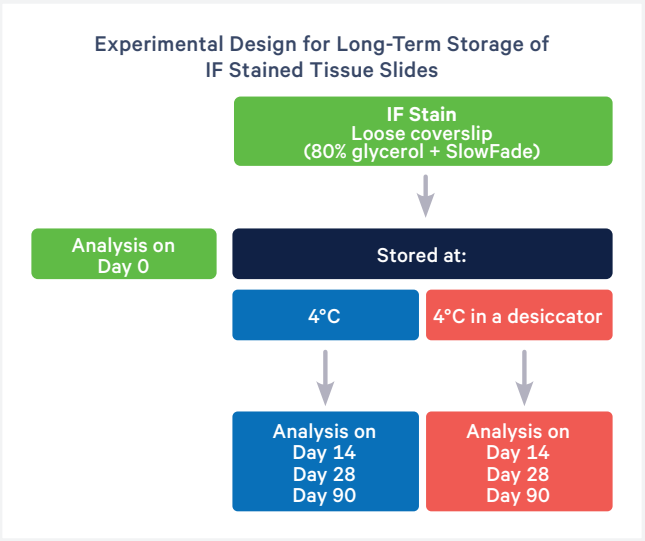

Figure 11. Experimental design for stored IF stained tissue slides. Slides were divided into three groups: analysis on day 0 (slides were immediately processed), stored at 4°C with a desiccator, stored at 4°C without a desiccator. Slides were stored for 14, 28, or 90 days and compared to slides from day 0. Analysis was performed on serial sections to assess RNA quality (n=1) by measuring DV200 scores, and assay sensitivity (n=2) by analyzing the mean UMI counts and mean genes detected at 20,000 read pairs per tissue-covered spot.

Storage Results - IF Stained Tissue Slides

Storing IF Stained Tissue Slides at 4°C in a Desiccator Results in Better Maintenance of RNA Quality

IF stained tissue slides were stored at 4°C with or without a desiccator and analyzed on day 14, 28 and 90 (Figure 11). Changes in RNA quality and assay sensitivity were estimated by comparing tissue slides with freshly placed sections from day 0.

As shown in Figure 12, IF stained tissue slides stored at 4°C at different time points on average showed a ~13% decrease in DV200 score compared to day 0 tissue sections. Similarly, IF stained tissue slides stored at 4°C in a desiccator a showed ~6% decrease in DV200 score. The small sample size is insufficient to reach significance, but the data trends towards better maintenance of RNA quality of IF stained tissue slides stored at 4°C in a desiccator compared to 4°C without a desiccator.

IF Stained Tissue Slides Across All Conditions Show Similar Decreases in Assay Sensitivity

Figure 13 illustrates that across all three time points, IF stained sections stored at 4°C with or without a desiccator show a ~10% decrease in mean genes detected and ~15% decrease in the mean UMI counts per tissue-covered spot. The Day 14, 4°C in a desiccator storage condition did not follow the expected trend in either assay sensitivity or RNA quality and should be considered an outlier.

These data suggest that storage of IF tissue slides at 4°C with or without a desiccator lead to a slight loss in mean genes detected. Although tissue slides stored at 4°C with or without a desiccator show a similar impact on assay sensitivity, better RNA quality is observed at 4°C in a desiccator. Based on these combined results, 10x Genomics suggests storing IF stained, loose coverslipped tissue slides at 4°C in a desiccator.

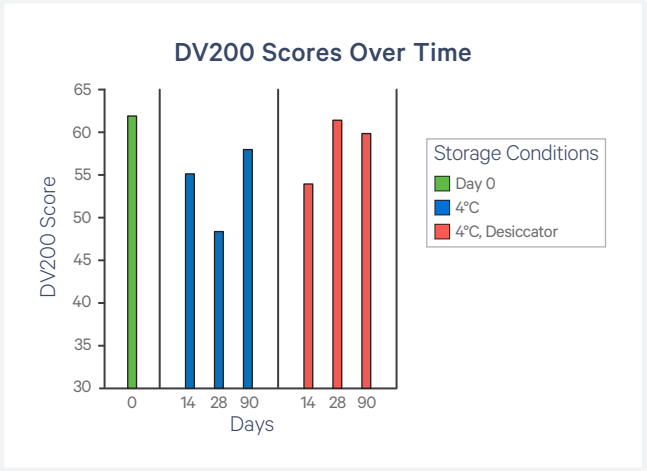

Figure 12. Storing IF stained tissue slides at 4°C in a desiccator results in better maintainence of RNA quality. On day 0, human breast cancer FFPE sections were placed on SuperFrost Plus Slides, stained and stored according to conditions described in Figure 11. One section was analyzed for each condition.

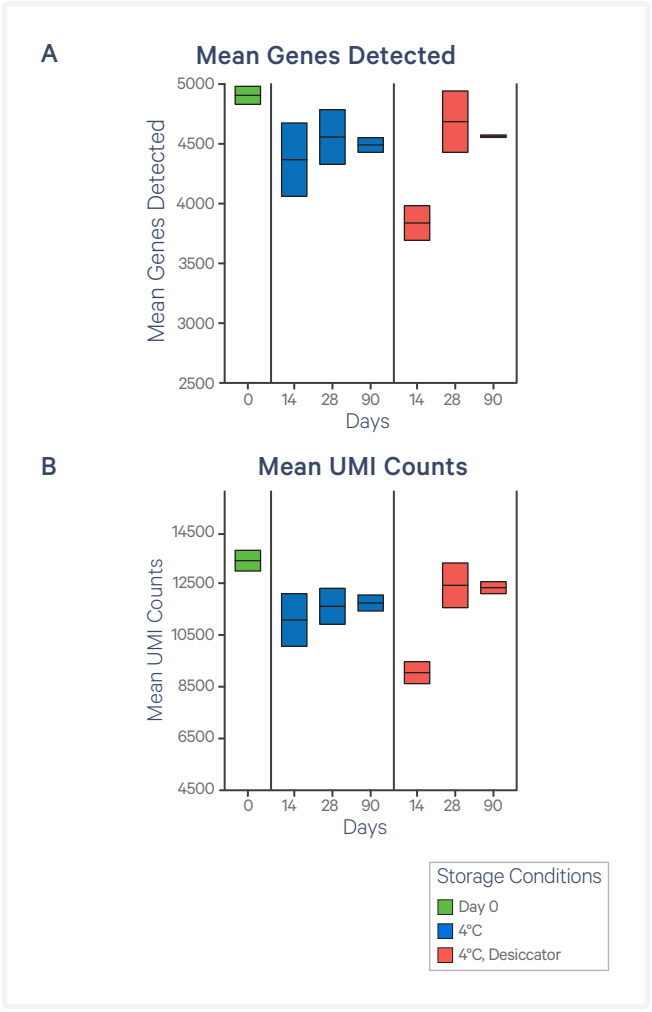

Figure 13. IF stained sections stored at 4°C with or without a desiccator show similar decreases in assay sensitivity. **A**. Mean genes detected at 20,000 read pairs per tissue-covered spot. **B**. Mean UMI counts at 20,000 read pairs per tissue-covered spot. Two sections were analyzed for each condition. Day 14 sections stored at 4°C in a desiccator were considered outliers.

Storage Results - H&E Stained Tissue Slides

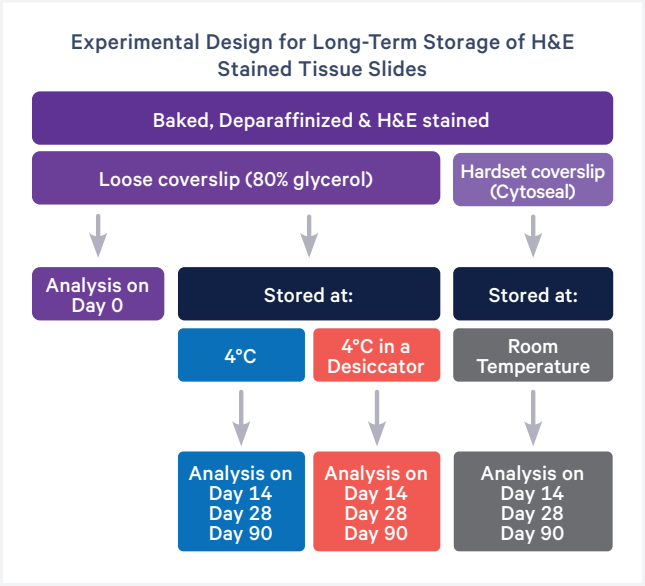

Figure 14. Experimental design for stored H&E stained tissue slides. Slides were divided into four groups: analysis on day 0 (slides were immediately processed), stored at 4°C with a desiccator, stored at 4°C without a desiccator, or stored at room temperature. Slides were stored for 14, 28, or 90 days and compared to slides from day 0. All slides stored at 4°C received loose coverslips, while slides stored at room temperature received hardset coverslips. Analysis was performed on serial sections to assess RNA quality by measuring DV200 scores (n=1), and assay sensitivity (n=2) by analyzing the mean UMI counts and mean genes detected at 20,000 read pairs per tissue-covered spot.

Storing H&E Stained Tissue Slides at 4°C without a Desiccator Results in Better Maintenance of RNA Quality

H&E stained tissue slides were stored at 4°C with or without a desiccator and at room temperature. Slides stored at 4°C with or without a desiccator were loosely mounted with glycerol, while slides stored at room temperature were mounted with hardset mounting media (Figure 14). Tissue slides stored for 90 days at 4°C show a 17% decrease in DV200 score, while the tissue slides stored at 4°C in a desiccator show a 26% loss in DV200 score compared to the sections analyzed at day 0 (Figure 15). The data trends suggest that RNA quality was preserved better at 4°C without a desiccator when day 14, 28 and 90 were analyzed together (p value=0.08, Students t-test).

Hardset coverslipped sections stored for 90 days at room temperature showed a ~30% decrease in DV200 score over time.

Storing H&E Stained Tissue Slides at 4°C with or without a Desiccator Show Similar Decreases in Assay Sensitivity

Sections stored at 4°C with or without a desiccator showed a similar decrease in mean genes detected (~10%, average of 3 time points) when compared to day 0 tissue sections (Figure 16). One of the replicates at day 90 stored at 4°C with desiccator showed a 30% reduction in sensitivity compared to the day 0 sections, suggesting that storage of H&E slides in 4°C in a desiccator can have adverse impact on RNA quality and assay sensitivity when compared to storage at 4°C without a desiccator. The hardset coverslipped sections show a trend towards greater loss in mean genes detected (~15%, average of 3 time points) compared to day 0 tissue sections, but the change was statistically insignificant (Figure 16). Based on these data, 10x Genomics suggests storing H&E stained tissue slides at 4°C without a desiccator for up to 90 days.

In this study, H&E stained tissue slides stored at 4°C without any mounting media were not tested.

Stained Tissue Slides Conclusions

On average, all storage conditions for IF stained sections over the analyzed timepoints show a 9% decrease in DV200 score (Figure 12), while H&E stained sections with glycerol based mounting media show 23% decrease in DV200 score over time (Figure 15). Despite the variable decrease in RNA quality, both H&E and IF stained sections over the period of 90 days showed a similar loss (~10%) in mean genes detected (Figures 13, 16). This suggests that the assay is robust despite variable RNA quality, and that sections with ~25% lower DV200 scores perform equally as well as sections with higher DV200 scores.

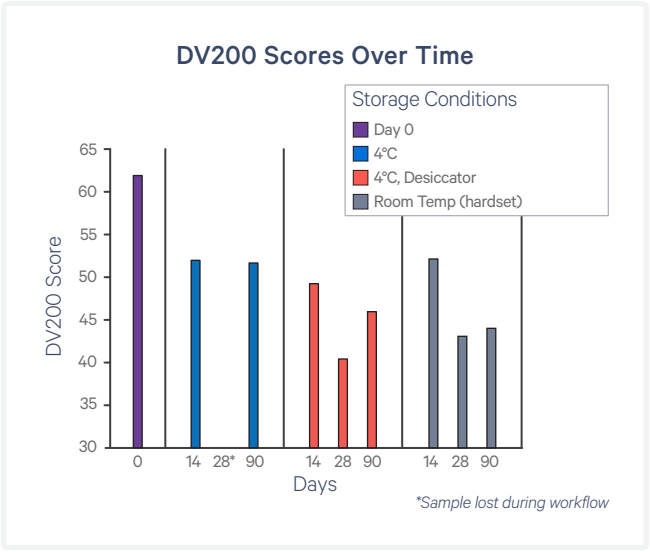

Figure 15. Glycerol mounting H&E stained tissue slides better preserves RNA quality compared to hardset mounting. On Day 0, human breast cancer FFPE sections were placed on SuperFrost Plus Slides, stained and stored according to conditions described in Figure 14. One section was analyzed for each condition.

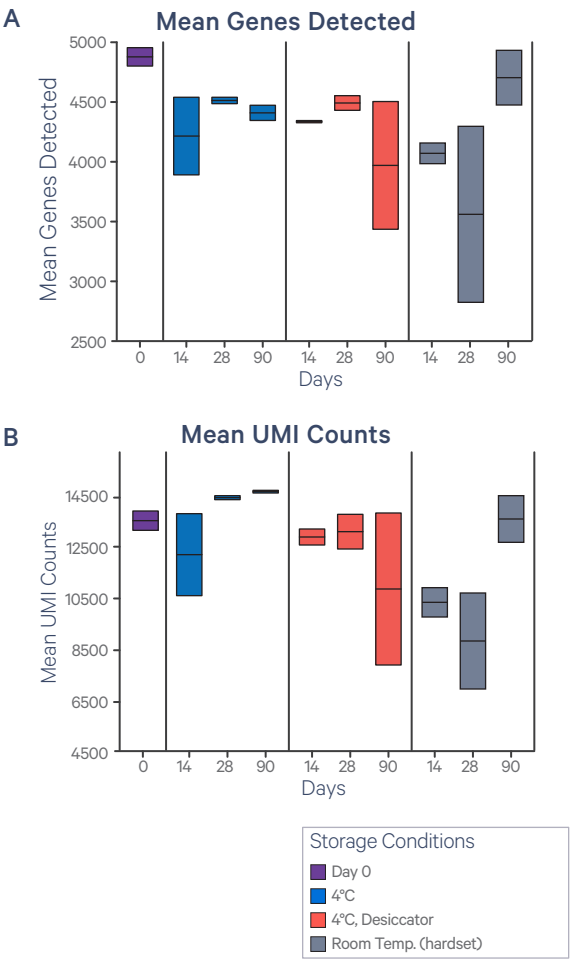

Figure 16. H&E stained tissue slides stored at 4°C with or without a desiccator showed a similar decrease in assay sensitivity **A**. Mean genes detected at 20,000 read pairs per tissue-covered spot. **B**. Mean UMI counts at 20,000 read pairs per tissue-covered spot. Two sections were analyzed for each condition.

Storage Results - Unstained Tissue Slides

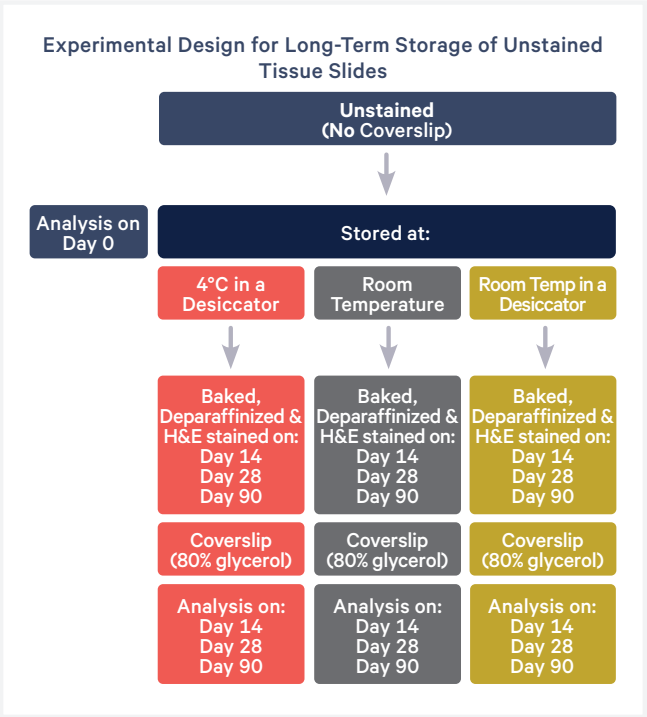

Figure 17. Experimental design for stored unstained tissue slides. Slides were divided into four groups: analysis on day 0 (slides were immediately processed), stored at 4°C with a desiccator, stored at room tempreature without a desiccator, or stored at room temperature with a desiccator. Slides were stored for 14, 28, or 90 days and compared to slides from day 0. Analysis was performed on serial sections to assess RNA quality by measuring DV200 scores (n=1), and assay sensitivity (n=2) by analyzing the mean UMI counts and mean genes detected at 20,000 read pairs per tissue-covered spot.

Unstained Sections Retain Similar RNA Quality Across Tested Storage Conditions

Unstained tissue slides were stored as described in Figure 17. Sections stored unstained at room temperature, with or without a desiccator, showed a 7-30% decrease in DV200 score across the time points compared to sections analyzed at day 0, which suggests a loss in RNA quality (Figure 18).

Unstained Sections Retain Similar Assay Sensitivity Across Tested Storage Conditions

Observed decreases in RNA quality translated to 0–8% decrease in mean genes detected, and 0–14% decrease in the number of mean UMI counts compared to sections analyzed at day 0 (Figure 19).

These data support earlier observations that a decrease in DV200 score does not translate into a significant decrease in assay sensitivity at the same magnitude.

All tested storage conditions show similar changes in RNA quality and assay sensitivity (except an outlier at day 90). Based on replicate variability (day 28) and the trend towards lower mean genes detected (day 90) for sections stored at room temperature in a desiccator, 10x Genomics suggests storing unstained tissue sections at 4°C in a desiccator.

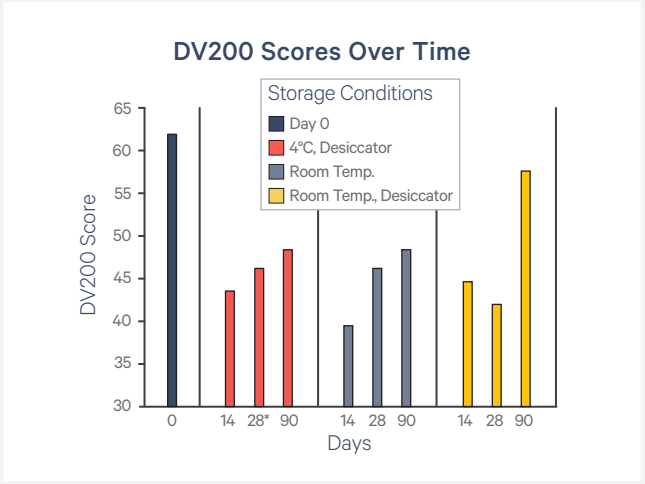

Figure 18. Unstained sections show similar trends in DV200 score across tested storage conditions. On Day 0, human breast cancer FFPE sections were placed on SuperFrost Plus Slides, stained and stored according to conditions described in Figure 17. One section was analyzed for each condition.

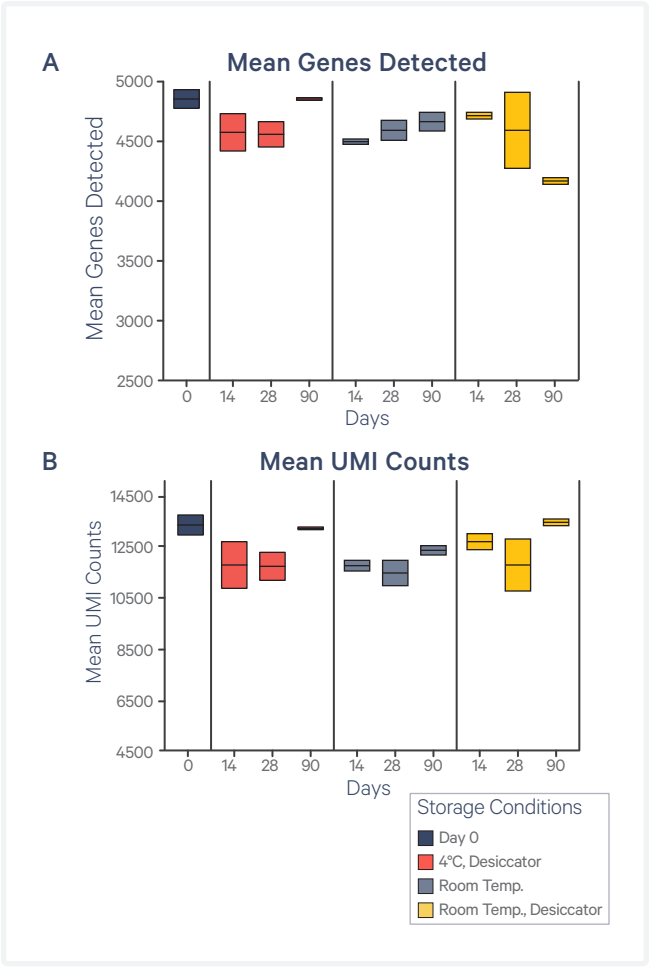

Figure 19. Unstained sections retain similar assay sensitivity across tested storage conditions. **A.** Mean genes detected at 20,000 read pairs per tissue-covered spot. **B.** Mean UMI counts at 20,000 read pairs per tissue-covered spot. Two sections were analyzed for each condition.

Discussion

This Technical Note examines the impact of storage and shipping conditions for stained (H&E and IF) and unstained tissue slides. It is important to note that though the trends observed in this Technical Note are likely to extend to other tissue blocks, the experiments were performed on a single block with a single tissue type. Additionally, only human tissues were analyzed in this study. The conclusions within this Technical Note may not extend to mouse tissues, as 10x Genomics mouse probe sets differ in gene coverage (~1 probe pair per gene) vs the human probe set v2 (~3 probe pairs per gene).

For shipped tissue slides, this study shows that the process of shipping for 24 h does not further impact RNA quality compared to controls that were not shipped across all staining and shipping conditions. For stained tissue slides, shipping also did not impact assay sensitivity. However, the data in this study shows that unstained tissue slides shipped at ambient temperature without desiccant did result in a significant decrease in assay sensitivity. Based on these data, suggested shipping conditions are presented in Table 1.

Tissue Slide	Suggested Shipping Conditions (24 h)
IF Stained, SlowFade + Glycerol Mount	Ice packs without desiccant
H&E Stained, Glycerol Mount	Ice packs without desiccant
H&E Stained, Hardset Mount	Ambient temperature
Unstained	Ice packs with desiccant

Table 1. Suggested shipping conditions for prepared tissue slides. Suggested conditions were based on data derived from a single tissue block with a single human tissue type.

For stored tissue samples, this study shows that RNA quality decreases in all staining and storage conditions over time. However, a linear trend in the loss of RNA quality with time in storage was not observed. Unstained and H&E stained tissue slides both showed similar drops in RNA quality over time. However, RNA quality in IF stained tissue slides was impacted the least. These slides likely maintained RNA quality due to the presence of RNase inhibitors in the IF staining workflow, which prevent RNA degradation from RNases present in tissue sections. Interestingly, even a ~25% drop in DV200 score did not impact assay sensitivity in this study. However, the DV200 scores of samples studied in this Technical Note are still above the 30% threshold recommended by 10x Genomics. 10x Genomics recommends acquiring a DV200 score immediately before running the Visium CytAssist Spatial Gene Expression assay.

This study examined associations between variability in RNA quality with variability in assay sensitivity. Based on the data presented, RNA quality deteriorates with time but does not impact

assay sensitivity. One explanation for this is that the DV200 score measures the percentage of total RNA that is ≥ 200 nucleotides, while successful gene calling theoretically requires ~50 nucleotides of intact RNA complimentary to the probe sequence. Thus, samples with degraded RNA may still perform well with the assay provided that RNA fragments are longer than 50 nucleotides.

This Technical Note analyzed sections stored over a period of 90 days. Based on the data observed in this Technical Note, suggested extended storage conditions for up to 90 days are provided in Table 2. Slides stored for longer periods of time may not show similar data trends.

Tissue Slide	Suggested Extended Storage Conditions (up to 90 days)
IF Stained, SlowFade + Glycerol Mount	4°C with a desiccator
H&E Stained, Glycerol Mount	4°C without a desiccator
H&E Stained, Hardset Mount	Room temperature
Unstained	4°C in a desiccator

Table 2. Suggested extended storage conditions for tissue slides. Suggested conditions were based on data derived from a single tissue block with a single human tissue type.

Document Revision Summary

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