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Urine Webinar Q&A

1. Why do we check the GC content in DNA and RNA?

We don't check GC content. Silica based technologies coupled with phenol-chloroform have a bias towards binding nucleic acid fragments with higher G-C base content. This is because historically, silica columns were developed for DNA isolation. While attempts to modify this technology have been made towards the extraction of microRNA, it has been shown to not be sensitive enough. This inefficiency using silica was demonstrated in a publication that incorrectly mentioned a miRNA being down regulated, where in fact the researchers later discovered it had not been. This was due to the miRNA's low GC content, which the silica-based technology was not able capture. Norgen tested our Silicon Carbide technology to see if we have the same drawback, by testing miRNA ranging from low to high GC content. Our Silicon Carbide technology demonstrated no bias towards G-C content and isolated all sizes with equal consistency.

SiC having this capability is important for capturing the true total nucleic acid profiles, providing more reliable data, particularly within the field of novel biomarker discoveries.

2. Please Provide Webinar on ARMS PCR Designing.

Thank you, we will take this into consideration. Once our current webinar series ends, we will consider this into our schedule.

3. Do we expect 18s and 28S ribosomal peak in cfRNA isolated from urine supernatant which does not contain cellular RNA? RIN is right parameter to judge the quality of cell free RNA?

28s and 18s bands should not be expected in the cell-free fraction of urine as they are indicators of cellular RNA. In a cell-free sample, any cells present are removed during the sample preparation stage. If these bands are present within the bioanalyzer, this is a sign of cellular degradation and contamination, indicating that cells have lysed during the pre-analytical preparation phase. Since RIN values are calculated with the Bioanalyzer using the ratio of 18s and 28s, and since cell-free urine should not contain any cells, the RIN value is therefore not a proper indicator of urine sample quality. Despite the low RIN value, many urine samples are shown to perform well in downstream amplification studies

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4. **May I concentrate urine samples using SpeedVac?**

We do not recommend the use of SpeedVac for concentration or filtration of urine. The filtration has a molecular weight cut off, and the cell-free urine contains RNA and DNA bound to proteins which come in various sizes and can cause discrimination and loss in yield. To achieve a lower elution volume, the RNA Clean-Up and Concentration Micro-Elute Kit can be combined to further concentrate small amounts of RNA into 8 uL.

The purified RNA is of the highest integrity, and can be used in a number of downstream applications.

5. **Is there a specific protocol for children? since their sample is usually smaller than those mentioned, and the method is different from adults.**

No specific protocol for children, as urine is the same regardless. The optimal time to collect urine is in the morning, first void, mid stream. This is due to the fact that during the night, urine becomes concentrated and the nucleic acids will be in highest concentration during this time. If sample volume is a concern, Norgen offers a range of formats which can accommodate specific volumes. For example, the mini format offers a versatile urine input ranging from 250 µL to 2 mL.

6. **How much is the minimum amount of urine required for adults and kids?**

There is no difference between adult and children urine samples. Depending on your research, a different volume will be required. There is no limit; the higher the volume, the higher the amount of nucleic acids to be isolated. We offer urine isolation kits (mini, midi and maxi) with a versatile volume range.

7. **What are the advantages of urine biomarkers compared to plasma biomarkers for diagnosing diseases at the early stages?**

Urine is the filtrate of blood, meaning the contents will be qualitatively similar. The main advantage of urine is the ease of collection and the process being non-invasive. Also, the urine contains less proteins than plasm (up to 1000-fold less) and also does not contain molecules such as red blood cells that can disrupt downstream analysis.

8. **Any updates about Urine Biomarkers in the battle against cancers since they show promise for prostate cancer?**

There has been a lot of work done on prostate cancer and urine biomarkers. Please reach out to support@norgenbiotek.com for certified publications.

9. **We currently spin out cells from our urine, then store aliquot at -80 degrees. From your seminar, you suggest that this method is sub-optimal. You mentioned that precipitates can bind bioanalytes (nucleic acids) upon thawing the urine. Is there a way of avoiding this problem?**

Based on information that was presented, urine contains the protein uromodulin. When exposed to cold temperatures, uromodulin will precipitate and create a mesh which traps valuable biomarkers such as nucleic acids. If the precipitates are removed either via filtration or centrifugation, the risk is a lower nucleic acid yield. To avoid this, using a preservative where urine can be stabilized at room temperature is the primary recommendation; as it negates the use of freezing, precipitation and improves nucleic acid yield by preserving sample integrity. If the urine is already frozen, some of these nucleic acids can be re-solubilized by incubating the urine for 5 minutes at 37C; this will slightly relax the protein and free some of those trapped molecules. This will not release 100% of the trapped molecules, but it does assist with the overall yield.

10. **We have discussed the best time to collect urine. You suggest 1st urine in the morning. While this urine may be quite concentrated, might it be more degraded due to sitting in the bladder for extended times?**

Urine as a sample type is naturally highly fragmented and degraded. Any cells that are present in the urine are exfoliated dead cells and thus degraded. Urine DNA and RNA is more fragmented in size than when in the blood circulation, so the time of collection has no effect on the size of the fragment, but does affect the concentration of analytes. Generally speaking, nucleic acid content within urine samples will become more dilute throughout the day with daily voids and fluid intake. Therefore the first void collection offers the best time to obtain a highly concentrated sample. A concern with this collection time is that it will contain a high number of bacteria that has been accumulating in the bladder, which is why it is recommended to collect mid-stream to expel the initial bacterial content.

11. **What can we do if we already collected urine as part of trial and have not used Norgen's preservative? Can we salvage these samples?**

This all depends on what your downstream application is and your target analyte. Freezing urine samples is a common practice and we have recommendations for retaining some of the lost content due to cell lysing or precipitation removal. If you froze the urine sample as is, after thawing you may expect some loss of molecules (DNA, RNA, etc). If you are interested in working with cellular nucleic acids, cell lysing during the freeze storage will have degraded the integrity of the sample. However, to recover some of your losses, it is essential that the cold-induced precipitates are not filtered or removed. Instead after sample thawing, a quick incubation at 37 degrees for five minutes can help to recover some of these losses.

12. **Most metabolites in the urine are polar, using silica, which is also polar, I want to know if the interaction won't be too long?**

Norgen has not done any studies using silica for purification. With silicon carbide, the technology is highly optimized for interactions and bonding.

13. **Do you recommend to preserve cell free fraction of urine to urine preservative tube? if yes, then what are the benefits of storing cell free fraction over whole urine in Norgen's urine preservative tube**

Once your cell free urine sample is prepared after spin down/debris/cell removal, yes we recommend placing it in our preservative tube. For the benefits of storing cell free versus whole urine, it depends on the project you are working with. Some research requires whole urine and some require cell free urine samples

14. **Is there a method or technique to determine whether the cf-nucleic acid material is from human as opposed to bacterial source (particularly in urine samples)**

For cell free nucleic acid, yes. By amplifying the 16s from the cf DNA captured, this can be used to distinguish between human and bacterial sources. Metagenomic high-throughput DNA sequencing offers an unbiased approach for the detection of pathogens in clinical samples.

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15. **I am using Norgen Urine preservation solution for my urine samples. How long can I keep the urine with preservation solution at room temperature?**

Norgen's Urine Preservative can preserve RNA, DNA, exosomes and proteins for 2+ years at room temperature.

16. **How much volume of urine can be collected using NORGEN urine preservative tube?**

We offer different versions of our preservatives. Our dry urine preservative is available in 5mL, 15mL, 50mL tubes and a 120mL cup. Also, we offer a 2ml ampoule with the preservative in liquid form, compatible with urine sample volumes of 5-50mL.

17. **Are these test kits available on the market?**

Yes, please see norgenbiotek.com for all of our products.

18. **What I was looking for is a list of biomarkers for stress, depression, anxiety etc. to show before and after interventions or treatments. What can be measured in urine ? For example we use cortisol to measure stress before and after in Saliva**

Norgen has not performed research for these biomarkers, but a lot of information can be obtained from urine, including investigations on epigenetics, mutations, proteins etc. Cortisol levels can also be measured in an individual's urine - many of the markers you may be used to analyzing in saliva could also be found in urine. We recommend referring to recent literature on urine biomarkers for the most up-to-date information on specific analytes of interest.

19. **What causes the occasional orange precipitate pre-lysis buffer suspension? (Following slurry addition + centrifugation @ 2500 rpm for 2 minutes) ***regarding Urine Exosome kit**

There should not be any orange precipitate through your protocol, please reach out to support@norgenbiotek.com if you have this issue.

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20. **Which is more suited method for normalization in urine samples? bradford assay for total protein concentration or creatinine normalization?**

Normalizing by volume and using a common housekeeping gene is recommended for normalization such as 5s ribosomal RNA or miR-21. Bradford assay is used for measuring protein concentration. This can't be used for assessing different gene expression.

21. **Prior to analysis, what centrifugation protocol should be followed with regards to spin speed and duration?**

Prior to cf-DNA and cf-RNA purification, first spin at 1500-2000 rpm for 10 minutes to separate human cells and debris, second spin at max speed for 10-15 minutes to pellet bacteria. This supernatant is now a cell free urine sample. These steps are carefully described in our kit protocols.

22. **Can you store Preserve tubes of samples at any temperature?**

With Norgen's preservative tubes, room temperature (18-25 C) is recommended for 2+ years.

23. **How stable are miRNA's and cfDNA in the urine? Are you worried about DNase and RNase degradation?**

miRNAs and cfDNA are not found freely floating in urine, they are bound to respective proteins/histones and are short fragments. Thus, they are protected from DNAse and RNAse degradation. Once proteins denature, this is when DNAse and RNAse can lyse the nucleic acids further, some publications claim within a few hours/days without proper preservation.

24. **After isolation of miRNAs. what downstream kit and application do you recommend for identification of miRNAs in a sample (ex profiling a sample)**

To profile miRNA in sample, microarray, nanostring technology or Next Generation Sequencing is recommended. For example, with these methods, gene expression for cancer biomarkers can be studied.

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25. **Mainly interested in proteins included in urine samples. Do you recommend any depletion method for the high abundance proteins?**

We currently do not have a kit for depleting highly abundant protein from urine, however, we did some work on this and we can discuss what can be done for depleting the highly abundant proteins. Please reach out to us at support@norgenbiotek.com

26. **How soon should the sample be added to the kit?**

The sample should be added immediately to the preservation tube/cup. If immediate is not possible, urine remains stable for up to 3 hours at room temperature.

27. **Why should the sample be cell-free?**

This depends on your project. If you wish to study any cell free nucleic acids, without any contamination from genomic/other nucleic acids, cell-free urine is required. Sample must be “clean” so no additional information from other cell components change your results.

28. **if someone having his/her urine blood why they have in urine found blood which disease cause and why they happen ? If you answer me that was great for me again thank you**

While we appreciate your inquiry, this is a question more suited for a medical professional as blood in urine can have many causes. Please contact a medical professional for further information.