The Effects of Collection Procedures on Telomere Length Measurement in Population-based Surveys

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THE TELOMERE’S STRUCTURE AND BIOLOGY IS MUCH MORE COMPLEX THAN COMMONLY PORTRAYED
Telomere length at age represents a balance between cell division and telomerase activity. Other factors may disturb this balance or damage telomeric DNA, resulting in shortening or lengthening of the telomere. It is this ‘disturbance’ that makes the telomere length an interesting biomarker.
BACKGROUND

• Studies show associations between shorter TL and age-related diseases such as: cardiovascular disease (e.g., stroke, ASHD), cancer, and diabetes as well as osteoporosis, cognitive function, dementia, depression, and autoimmune diseases.

• TL is also measured cross-sectionally and longitudinally to characterize positive or negative effects of social environment, nutritional or behavioral factors.

• Measuring telomere length (TL) accurately has become increasingly important as interest grows in TL as a biomarker of aging and stress – which differences are real and which are measurement error?
SIGNIFICANCE

• Many past TL studies collected samples in a lab, but in the extension to field studies—with longer delays between collection and DNA extraction—some fundamental issues were left untested.

• This project examines one possibly strong confounder of measured TL—time stored at room temperature prior to DNA extraction and storage at -80°C to -196°C.

• Does storage time affect TL outcomes?
PROTOCOL

• Saliva collected in lab using from 30 volunteers (18-65 years, avg. 35 y; 13 F) over 3 days
• Subject spits into a 50 ml conical tube
• Saliva divided into 6 DNA Oragene saliva collection kits and mixed with Oragene solution
• DNA isolated from 1 kit (time 0) and stored at -80°C
• The remaining aliquots stored at room temperature for 1, 2, 6 or 12 months after which time DNA isolated and frozen.
ANALYSIS

• At 1 year, all samples analyzed simultaneously using quantitative PCR (Mitchell et al., 2014; O'Callaghan 2011) in the Notterman Lab at Princeton
• DNA quantified by picogreen (measures dsDNA)
• Telomere length from all samples measured in triplicate by qPCR at 12 months (c.v. assay < 0.1).
• Significance tested by ANOVA, Tukey.
World Spitathon Champs
This ancient Indian sport needs to get its due recognition

India might have the strongest lungs in the world but because of the emphasis that they make their lungs undergo by living in a high altitude the sport collapses. But as generations of spitting competition builds the lungs become stronger. The present generation is still trying to learn from the old spitters and their techniques. The modern generation is not in a position to learn from the old spitters as they are not interested in the sport.

One of the reasons why the sport is not very popular in the rural areas is that it is not a sport that is played in the schools. The sport is more popular in the urban areas where the schools have sports facilities. The sport is not very popular in the rural areas where the schools do not have sports facilities.

In the city the sport is more popular in the sports clubs. The sport is more popular in the clubs as they have facilities to train the spitters. The sport is not very popular in the rural areas as the schools do not have facilities to train the spitters.

Spitting is a skill that needs to be learned. The spitters have to learn how to spit accurately and how to spit far. The modern generation is not in a position to learn from the old spitters as they are not interested in the sport.

If you are interested in the sport you can look for the old spitters who are still active. They can teach you the techniques of spitting.

Harshad Oak

Guest Column

The Maharashtra Herald, Saturday, January 26, 2008
dsDNA Yield is dramatically reduced between 6 and 12 months of storage

*p<1*10^{-6}
Measured TL decreases between 6 and 12 months of storage

* p < 10^{-3}
DNA distribution at 12 months in stable sample
DNA distribution at 12 months in an unstable sample
DNA Degradation over 12 months of Saliva Storage at RT
Subject 16

DNA Degradation over 12 months of Saliva Storage at RT
Density plot—TL stable until 6-12 months
Percent change in TL vs DNA Yield: at ~50% loss of yield measured TL decreases sharply.
Picogreen (dsDNA) vs Nanodrop (all nts) measurement of DNA Yield

\[ y = -32.496 + 4.2925x \quad R = 0.78621 \]
RECOMMENDATIONS

• Saliva sample storage duration at room temperature should be minimized, even when using a stabilizing kit. Our lab processes samples within 2 weeks to a month.

• Determine DNA yield following extraction, and reject samples with substantially less DNA than expected (~18 μg in this experiment; ~50% of original yield).

• Extracted samples stored in several aliquots at -80°C. Do not freeze, thaw, re-freeze samples.

• Preprocessing stability of blood and blood spot samples should be prospectively evaluated.

• Long term goal: possible mechanisms for adjusting already collected material if time at room-temperature is known.

• Further research on improved room temperature stabilizing solutions.
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