1. Objective:
This document describes the standard procedure for administering the Trier Social Stress Test (TSST). It is adapted from the procedure described in: http://www.macses.ucsf.edu/Research/Allostatic/notebook/challenge.html
This SOP assumes the only biological measure obtained is salivary cortisol. Additional measures can be added but are not discussed in this SOP.

II. TSST Set-up:
a) Participants
i. Experimenter 1 (E1): E1 is responsible for guiding the subject from one room to another and debriefing the subject.
ii. Confederates (C): Two confederates are used. Neither should have had contact with S prior to the TSST. The confederates may be of any gender.
   1. Confederate 1 (C1): C1 will be the only person to speak to S during the TSST.
   2. Confederate 2 (C2): C2 is the only person to take notes during the procedure.
b) Materials
i. Lab coats (for each confederate)
ii. Questionnaires
iii. Scripted Material: Script for introduction to the TSST, instructions for the mathematics task, and debriefing script. Copies of the Speaking Task and Debriefing scripted material can be found in the Appendix A and B of this SOP. Evaluators should be familiar with this script well in advance of task.
   i. Two timers with an audible ticking and alarm. ii. Video camera
   iii. Cassette recorder (to play instructions)
   iv. Two clipboards and notepads (for each C)
   v. Salivettes for salivary cortisol samples.
   vi. No microphone stand or TV monitor (for subject to observe themselves) is to be used. c) Environment
i. Preparatory Room:
   1. This room should have a comfortable chair or sofa, and bland reading material for the subject.
   2. The subject is placed in this room before and after the TSST.
   3. Paper and pencil/pen with a writing area (clipboard or small table) are available for writing.
ii. Testing Room:
   1. Testing Room should be a plain room containing a desk with two chairs behind it.
   2. The Testing Room is the room in which the instructions are given and the speech/math task is conducted.
   3. The video camera is set on a tripod behind the Cs. III. If only one room is available, the room should have all the available equipment listed in the two rooms above, including two chairs in front of the desk (for the subject and E1) and two chairs behind the desk (for the Cs).
d) Set-up
i. Preparatory Room: Salivettes for salivary samples are labeled. No TV is allowed while the subject is in this
room. Light reading material is available prior to basal measures.
ii. Testing room:
1. Cs wear white lab costs and are seated behind desk.
2. Clipboards with notepad and pencil/pen for each C.
3. Video camera is focused on subject. The record light should be on and visible to the subject.
4. Timer is easily visible to subject and the ticking is audible.
Subject can see the time left on the timer.
5. C1 will have scripts to read.
6. Cassette recorder is on desk.

III. Initial Procedures Prior to Speaking Task
a) Subject should arrive between 1:00 and 6:00 PM (during a period of relative quiescent of the HPA axis). All times should be similar (within 60 minutes) within a study.
b) Upon arrival the subject is greeted by E1.
c) Smoking: Nicotine dependent subjects should have a cigarette two hours prior to TSST. Alternately, subjects may be placed on an appropriate dose of nicotine patch.
d) Caffeine: no caffeinated beverages should be consumed within two hours of the TSST.
e) Diet: The subject should not eat within three hours of testing. Alternatively, a calorie-controlled meal is served and eaten at this time upon arrival.
f) Pre-study measures are obtained, including breathalyzer and urine drug screen.
g) If plasma measures are to be obtained, the intravenous catheter or heplock is inserted at this time. The subject should be at rest for 60 minutes following insertion of the catheter/heplock.
h) If other equipment is to be used (i.e. galvanic skin response, vagal tone), these monitors are to be attached to the subject at this time. i) The subject is then escorted to Preparatory Room to relax.

IV. Basal Measures
a) Following 10 minutes of Preparatory (60 minutes if an intravenous catheter/heplock is inserted), testing procedures are begun.
b) The following questionnaires are administered: VAS “How stressed are you right now?”
State-Trait Anxiety Inventory (STAI), State Version
c) Other site-specific questionnaires are administered after the VAS.
d) Salivary cortisol and plasma measures are obtained following completion of the Questionnaires. Basal hormone measures are obtained every fifteen minutes. At least two, and optimally three, basal measures are obtained. Salivary cortisol measures are obtained at fifteen minute intervals.

V. Instructions for Speaking Task
a) Immediately following completion of basal measures, the subject is escorted by E1 to the Testing Room. E1 will knock on the Testing Room door and wait until E1 says to “come in.” The subject should be standing in front of the desk, the two Cs, and the video camera.
b) The Cs acknowledge the arrival of the subject with a brief nod of their head. The Cs remain expressionless during the encounter and maintain eye contact with the subject throughout. Each C has a notepad on a clipboard in front of them.
c) The E1 turns on the cassette recorder and the instructions for the TSST are played. The instructions are on Appendix A.
d) After the instructions are read, E1 leads the subject back into Preparatory Room. If the participant asks E1 any questions regarding the task, E1 responds “Do whatever you think is best” or “I do not know any other details.”

VI. Preparation for Speaking Task
a) Upon arrival in the Preparatory Room, E1 sets the timer for ten minutes. In the Preparatory Room, E1 will give subject a notepad to make notes for their speech. The subject will be given 10 minutes to prepare. Subject is told that the notepad is to help him/her prepare for their speech, but
they will not be able to take their notes into Testing Room with them.

b) E1 leaves the room for ten minutes.
c) After the alarm goes off, E1 returns to the Preparatory Room and a cortisol sample is obtained.

VII. Speaking and Math Task
a) After the cortisol sample has been obtained (Section V), E1 will escort the subject from the Preparatory Room into the Testing Room. E1 will knock on the Testing Room door and wait until E1 says to “come in.” E1 should step outside the room and close the door. E1 should remain outside Testing Room until S has completed the TSST.
b) Cs are to remain neutral in expression throughout the speaking and math task and to maintain eye contact with subject throughout the tasks. c) Only C1 is the only C to speak during the tasks.
d) Speaking Task
i. The timer is set for five minutes and C1 tells the subject “Please begin.”

ii. C2 should take notes appropriately every one minute, as if noting the subject’s performance. The comments should be brief so that C2s eyes are not taken off the participant for more than a glance.

i. If the subject pauses for 20 seconds, the C1 will prompt the subject with “You still have some time. Please continue.”

iv. If the subject asks the Cs a question, C1 should make neutral comments, such as “Do whatever you think is best,” “Say whatever comes to your mind,” or “Be as creative as you like.”
v. When the alarm sounds, C1 says “Please stop, your time is up.”
e) Math Task
i. C1 then tells the subject “Now we would like you to subtract number 13 from 6233, and keep subtracting 13 from the remainder until we tell you to stop. You should do the subtraction as fast and as accurately as possible.”

ii. Whenever the subject makes an error, the subject needs to restart at 6233. C1 instructs the subject “That's incorrect. Please start again from the beginning.” If the subject has forgotten the starting number, C1 provides the number (6233) again.

iii. At the end of 5 minutes , C1 instructs the subject ”Please stop, your time is up. You can go back to your room now.”
iv. If the subject asks questions as to how he/she did, C1 responds “I am not allowed to tell you that. Someone will give you that information later.”
f) Adverse Response
If at any time the subject appears to be having an adverse reaction, i.e. begins to cry or seems overly agitated, C1 should ask the subject “Are you okay?” “Do you want to stop?” or “Are you okay to continue?” If the subject indicates that they wish to stop, C1 should stop the study immediately and notify the person in charge of the stress test that the participant has had an adverse reaction and needs to be debriefed.

VIII. Follow-up Measures
a) E1 accompanies the subject back to the Preparatory Room. b) A salivary cortisol salivette is obtained (at 0 min post-task). c) VAS and other questionnaires are obtained.
d) Subsequent salivary cortisol measures are obtained at 10, 20, 30, 45, 60, and 90 minutes post-task.
i. Cortisol measures is obtained for at least 90 minutes post-task.
ii. During the first half-hour, cortisol is obtained at least every ten minutes.
e) VAS is obtained immediately after each cortisol sample.
IX. Debriefing
If subject is not to participate in another TSST, E1 debriefs the subject. See Appendix B for the Debriefing Script.

Appendix A: TSST Speaking Script

“These two trained interviewers are here to assess how outgoing, gregarious, and comfortable you are in situations in which you must project yourself as an expert. This is a type of personality test for a trait called extraversion. You will be given a hypothetical situation in which you will be applying for your ideal job. In this hypothetical situation, you are applying for your ideal job. You have dreamed about working in this job for as many years as you can remember. You have just seen an advertisement for this perfect job and decided to apply. After submitting your application, you have been invited for an interview. The job pays a very large salary. You are competing against a lot of other candidates, and the final selection will be made based on your ability to convince the interviewers of how your experiences, abilities, and education make you a better candidate than the others. You will try to convince this panel of interviewers that you are the best candidate for the position. In addition, you will be asked to perform a mental math test, which will give us additional information about your working memory capacity.”

“You will have 10 minutes to prepare a detailed speech. After the preparation time has elapsed, you will return and deliver your speech to these interviewers. Your speech should explain why you should get the job.”

“Remember, you should try to perform better that all of the other participants. These examiners are specially trained to monitor and rate your speech for its believability and convincingness, and they will compare your performance to that of the others who perform this task. Also, you will be videotaped during the task so that the examiners can go over the videotape carefully and rate the contents of your speech as well as your nonverbal behavior. Now let us go back to your room so that you can prepare for your job interview in the given 5 minutes.”

Appendix B: Debriefing Script

“You were not actually being evaluated or scored. You were not actually being recorded. Your performance is not compared to other participants. We are measuring a naturally occurring stress hormone on the body called cortisol. We wanted to see what happens to this hormone in your body under stress, that’s why we have been collecting samples from you. We are sorry that we didn’t tell you the truth about everything, but if we had, the situation wouldn’t be stressful. You did a good job. Thank you for participating. Do you feel okay to go home/leave?”