Journal of Visualized Experiments

Taste exam: A brief and validated test

--Manuscript Draft--

Article Type:	Methods Article - JoVE Produced Video
Manuscript Number:	JoVE56705R1
Full Title:	Taste exam: A brief and validated test
Keywords:	Taste; flavor; bitter; Genetics; genotype; tongue; sensory; bitterness; sweetness; biomarker
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Additional Information:	
Question	**Response**
If this article needs to be "in-press" by a certain date, please indicate the date below and explain in your cover letter.	

TITLE:
Taste exam: A Brief and Validated Test

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KEYWORDS:
psychophysics, human, tongue, taste cells, *TAS2R38*, phenylthiocarbamide, psychophysical taste testing

SHORT ABSTRACT:
This protocol measures human taste responses and includes a brief anatomical assessment, a short taste test, and a validation method using the subject's reported sensation and taste receptor genotype.

LONG ABSTRACT:
The emerging importance of taste in medicine and biomedical research, and new knowledge about its genetic underpinnings, has motivated us to supplement classic taste-testing methods in two ways. First, we explain how to do a brief assessment of the mouth, including the tongue, to ensure that taste papillae are present and to note evidence of relevant disease. Second, we draw on genetics to validate taste test data by comparing reports of perceived bitterness intensity and inborn receptor genotypes. Discordance between objective measures of genotype and subjective reports of taste experience can identify data collection errors, distracted subjects or those who have not understood or followed instructions. Our expectation is that fast and valid taste tests may persuade researchers and clinicians to assess taste regularly, making taste testing as common as testing for hearing and vision. Finally, because many tissues of the body express taste receptors, taste responses may provide a proxy for tissue sensitivity elsewhere in the body and, thereby, serve as a rapid, point-of-care test to guide diagnosis and a research tool to evaluate taste receptor protein function.

INTRODUCTION:
Measures of human taste perception can be both part of medical care and a target of biomedical research, yet taste has received scant attention compared with hearing and vision (**Table 1**). From the medical perspective, when clinicians evaluate patients complaining of taste loss, in most cases the actual loss is of smell[1], which has led to dismissal of taste loss as an uncommon and often invalid presenting complaint. Taste distortions (dysgeusia) are more common and frequently arise from the secondary effects of medications or peripheral nerve injury[2,3], but neither form has an effective treatment (other than stopping the medication). Clinicians have also ignored taste loss because it has hitherto had little diagnostic or prognostic value on its own. However, although the measurement of taste has been a backwater, it may now be entering mainstream medicine with the revival of a historical appreciation that taste may be a diagnostic or prognostic tool[4,5]. For instance, bitterness perception can predict immune function[6] or the willingness of a patient to take medication[7]. Nonetheless, biomedical researchers have largely neglected taste. This inattention may, in part, reflect the fact that early progress in understanding this sensory system has its roots in experimental psychology[8], a field with which those in medicine may be relatively unfamiliar. Moreover, renewed interest in taste has ushered in standardized taste methods[9] that build on earlier methods[10], which while

comprehensive are lengthy and inappropriate for clinical settings. Finally, confidence in taste measures can be weak because subjects report on their own experience and validation of their observations has hitherto been lacking. Our hope is that a simple measure that investigators or clinicians can easily administer will gain in popularity with both constituents. Here we describe a simple taste exam protocol that has three parts: an assessment of the oral cavity, the taste test, and a validation step using inborn genotype. First, we provide biological context for these procedures, which merge simple practices in medicine, sensory measures from experimental psychology and validation of responses using genotype and genetics.

Taste perception starts in the mouth, so an effective taste exam needs to include a brief clinical assessment for obvious oral diseases, redness, swelling and other discoloration. The oral cavity contains seven subsites: the tongue, gingiva, floor of mouth, buccal mucosa, labial mucosa, hard palate, and the retromolar trigone. Past studies of human taste focused on healthy participants or those with well-defined diseases, but as taste testing becomes routine in medical exams, it is important to record the condition of the oral cavity as part of the procedure.

The tongue itself is a muscular structure encased in mucosa; dotting its dorsal surface are papillae, the small raised structures that give the tongue its unique texture and contain taste receptor cells. We classify papillae by their shape: fungiform, filiform, foliate, and circumvallate. Fungiform papillae (FP) are located anterolaterally on the tongue and are round, with a mushroom shape[11]. Investigators have published several useful methods to quantify FP and we direct readers to these sources for measurement protocols[12-16]. Foliate papillae, shaped like the pages of a book (folia), are located exclusively on the lateral posterior tongue surface[11]. Circumvallate papillae, found in the sulcus terminalis of the tongue base, are large dome-shaped structures surrounded by mucosal walls (Latin *circum,* "surround," + *vallum*, "wall")[11]. The most numerous papillae, the filiform, are long and thin and do not contain taste receptors.

People differ in tongue anatomy. While the sources of this anatomic variation are unknown, it is determined in part by inborn genetic variation, with investigators reporting 31% concordance of tongue anatomy among dizygotic twins and 60% concordance among monozygotic twins[17]. Papillary density also differs among people, and although rare, at least one genetic disease (familial dysautonomia) results in a congenital absence of taste papillae[18-20]. Thus, before performing psychophysical testing, it is helpful to confirm the presence of FP as part of the brief assessment and note the relative size and color of the tongue and evidence of oral disease.

The taste papillae contain the sensory cells that when stimulated initiate taste sensation. Humans are capable of sensing at least five classes of taste: salty, sour, bitter, sweet, and umami. While salty, sweet, and umami tastes signal the presence of valuable food sources containing sodium chloride, glucose, and amino acids, respectively, bitterness and sourness signal the presence of potential toxins and acids from the bacterial decomposition of food, respectively, and induce aversive behavior[21]. Salty and sour tastes are transduced through the activation of ion channels found in some types of taste cells, though the understanding of salt transduction is evolving and it may require type I cells as well[22,23]. Bitter, sweet, and umami

arise from activation of G-protein-coupled receptors on type II taste cells, each attuned to a particular taste. Heterodimers of subunits of three particular receptors transduce sweet and umami while bitter compounds activate a group of 25 different bitter receptors[24]. These bitter receptors can respond to multiple bitter compounds, and a single bitter compound often stimulates more than one receptor[25]. Despite the recent expansion of knowledge about the molecular basis of taste, novel pathways[26] and new discoveries beyond the traditional five taste qualities (*e.g.*, calcium[27] or fatty acid[28] perception) may lie ahead.

There are at least two surprising aspects of the taste families of receptors: genes that code for these receptors can differ markedly in DNA sequence and hence function among people, and many tissues of the body express these genes[21,29-31]. These extraoral sites include the brain, thyroid, upper and lower respiratory tract, and the gastrointestinal tract, among many others[21,29-31]. While the taste receptors at these locations do not participate in taste perception in the traditional sense, they likely sense the local chemical environment[29,32]. For example, the ciliated epithelium of the upper respiratory tract expresses the bitter receptor T2R38 (Bitter Taste Receptor 38), which responds to chemical compounds produced by bacteria and influences the innate immune response[32], such as increasing mucociliary clearance and levels of anti-microbial peptides and nitric oxide. This finding has medical implications for chronic rhinosinusitis, a disease of chronic bacterial infection and inflammation of the upper respiratory tract and paranasal sinuses.

Of particular relevance to the taste exam we describe here is that the T2R38 bitter taste receptor, encoded by the *TAS2R38* gene, exhibits genetic variability and therefore variable taste sensitivity. Perceptual differences for the bitter compound phenylthiocarbamide (PTC) were first described by the chemist Arthur Fox[33]; later this compound was identified as an agonist of the T2R38 receptor[34]. Individual differences arise from the DNA sequence of the *TAS2R38* gene, which has three single-nucleotide polymorphisms, each yielding amino acid substitutions (A49P, A262V, and I296V; A=Alanine, P=Proline, V=Valine, I=Isoleucine). Two common haplotypes result, PAV and AVI, with PAV/PAV individuals being highly sensitive to PTC ("tasters"), AVI/AVI individuals being relatively insensitive ("non-tasters"), and heterozygous AVI/PAV individuals being more variable in their sensitivity[35]. There are more examples of genetic variation affecting bitter perception, *e.g.*, taste receptor T2R19, encoded by the *TAS2R19* gene, similarly exhibits genetic variability and differing taste sensitivity to the bitter compound quinine[36]. Likewise, variation in *TAS2R31* affects the perceived bitterness of one of the high-potency sweeteners[37-39].

Here we describe a rapid method to characterize a patient's sense of taste that draws on high-yield protocols in clinical medicine, experimental psychology and genetics.

PROTOCOL:

The University of Pennsylvania Institutional Review board approved this protocol. We excluded subjects if they were under 18 years of age or were pregnant.

1. Oral Cavity Evaluation: Disease Assessment and Papilla Identification

1.1. Instruct the subject to open the mouth.

1.2. Using a light source such as a penlight or headlamp, illuminate the oral cavity and examine the seven subsites of the area (tongue, floor of mouth, buccal mucosa, labial mucosa, gingiva, hard palate, and retromolar trigone).

1.2.1. Visualize the dorsal surface of the tongue. Instruct the subject to lift up the tongue, and examine the ventral tongue surface and floor of mouth, making sure to extend the examination posteriorly to the molars.

1.2.2. Using a tongue depressor, lateralize the subject's cheek to visualize the buccal mucosa, as well as the lateral gingiva bilaterally surrounding the upper and lower teeth.

1.2.3. Extend the examination anteriorly by lifting the upper and lower lips to visualize the surfaces of the labial mucosal and anterior gingiva.

1.2.4. Finally, visualize the hard palate and retromolar trigone.

1.2.5. Note lesions, abrasions, and masses or signs of inflammation.

1.2.6. Again, ask the subject to open the mouth and extend the tongue.

1.2.7. Use a light source to visualize the dorsal surface of the tongue.

1.2.8. Identify the presence or absence of FP, *e.g.*, a smooth tongue surface

1.3. Note the results of the oral cavity examination before proceeding with taste testing. If investigators conduct this taste test in a medical context, unexpected findings should prompt further work-up.

2. Psychophysical Taste Testing

Note: Resources and descriptions for the psychophysical taste testing that follow are also available from the following web page: https://osf.io/hn87s/.

2.1. Tastant preparation

2.1.1. Prepare solutions as directed below. Make each solution using a volumetric flask to ensure precision of concentrations to ± 0.0002 M. Dissolve samples using ultrapure water. Tailor the choice of compounds to the research goals. The compounds included here are meant as one example.

2.1.1.1. Denatonium benzoate (bitter): prepare a stock solution of 4.99 x 10^{-3} M denatonium benzoate by dissolving 2.228 g of denatonium benzoate in 1 L of water. Add 180 µL of this stock solution to a 500 mL volumetric flask. Add water to bring the volume to 500 mL, producing a solution with a final concentration of 1.8 µM.

2.1.1.2. PTC (bitter): Place 0.0135 g of PTC in a 500 mL volumetric flask. Add water to bring the volume to 500 mL. PTC is difficult to dissolve, so place a stir bar in the flask and heat the solution to 70 °C on a hot plate. Use the stir bar to mix the solution until all solute has dissolved (~15 min). This produces a solution with a final concentration of 180 µM.

2.1.1.3. Quinine (bitter): Place 0.011 g of quinine HCl dihydrate in a 500 mL volumetric flask. Add water to bring the volume to 500 mL, producing a solution with a final concentration of 56 µM.

2.1.1.4. Sodium chloride (salty): Place 7.5 g of sodium chloride in a 500 mL volumetric flask. Add water to bring the volume to 500 mL, producing a solution with a final concentration of 0.25 M.

2.1.1.5. Sucrose (sweet): Place 60 g of sucrose in a 500 mL volumetric flask. Add water to bring the volume to 500 mL, producing a solution with a final concentration of 0.35 M.

2.1.2. Store taste solutions at 4 °C. Some commonly used taste compounds are light-sensitive, and investigators should wrap them in foil or other materials to reduce their exposure to light.

2.1.3. To identify common errors in solution preparation, fill one tasting cup with the old solution and one with the new solution. Taste each solution to verify they are identical in strength.

2.1.4. Include water as a control solution, presented in the first position to verify subjects understand the testing procedure. Present subjects with each tastant and the control tastant twice, taking care to avoid presenting the same tastant consecutively. For example, to test five unique tastants, feature twelve samples in the questionnaire (see 2.2): the five tastants and water, each presented twice. Aliquot 5 mL of water into individual glass scintillation vials. Label the vial caps with a dark blue sticker bearing the number 1.

2.1.5. Repeat this process for each tastant. Label vial caps with a circular sticker according to the order of presentation and some color code, example detailed below; match the vial labels with the labels on the taste questionnaire (see 2.2).
Water (dark blue)
Quinine (light blue)
NaCl (green)
PTC (yellow)
Sucrose (orange)
Denatonium benzoate (red)

NaCl (dark blue)
Denatonium benzoate (light blue)
Water (green)
Quinine (yellow)
Sucrose (orange)
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2.1.6. Package samples by placing them into two boxes, samples 1–6 and 7–12 in boxes labeled “box A” and “box B,” respectively (**Figure 1**); other packaging strategies are possible.

[Place **Figure** 1 here]

2.2. *Taste questionnaire*

2.2.1. Prepare the taste questionnaire using a category scale for rating taste intensity and a forced choice for identifying the taste quality of each tastant (**Figure 2**).

2.2.2. Place circular labels of the appropriate color and number next to the appropriate sample in the taste questionnaire (see 2.1.6).

*[Place **Figure** 2 here]*

2.3. *Taste Test Administration*

2.3.1. Provide subjects with box A, box B, a bottle of water, empty cup, pen, and pen-and-paper taste questionnaire containing entries for 12 samples. Use the same brand of bottled water throughout the duration of any given study. As an alternative to a paper questionnaire, port the material into online survey software and administered via tablet or desktop/Laptop. A sample template is available at https://osf.io/hn87s/.

2.3.2. Instruct subjects that they will be asked to rate both the intensity and quality (*e.g.*, salty, sour, bitter, sweet, or no flavor) of each tastant. Also, inform subjects they may not experience all qualities.

2.3.3. Explain the testing procedure, as follows: Rinse your mouth twice with water and spit it out in the cup provided. Pour all of sample 1 into your mouth and hold it there for 5 seconds before spitting the solution into the cup. Do not gargle or swallow the solution. Circle one of the 13 vertical lines corresponding with the sample’s intensity, on a scale of 0 to 12, from “no intensity at all” to “extremely intense” and choose a single quality to describe the taste. Afterward, rinse your mouth with water twice before proceeding to the next sample.

2.3.4. Observe the subject tasting and rating sample 1 (water). Should the rating deviate from “no intensity at all” and “no flavor,” reiterate the questionnaire instructions before allowing the test to proceed.

2.3.5. Review the finished questionnaire for completeness.

2.3.6. Score intensity ratings on a scale of 0 to 12 from the vertical lines that the subjects circled. Average the two intensity ratings for each tastant; this value will be used for analysis.

3. Genotype

3.1. Collect saliva from each subject using a saliva DNA collection kit.

3.2. Purify genomic DNA from the sample by following manufacturer instructions.

3.3. Determine *TAS2R38* genotype using SNP genotyping assays (*rs713598, rs1726866*, *rs10246939*).[40]

3.4. Determine *TAS2R19* genotype using SNP genotyping assays (*rs10772420*).[36]

4. Genotype-Phenotype Validation

4.1. Review the available pooled control data from over 800 subjects genotyped for *TAS2R38* (rs713598, rs1726866, rs10246939) and *TAS2R19* (rs10772420) at the following web page: https://carayata.shinyapps.io/TasteBoxplots/.

4.2. Based on a subject's *TAS2R38* genotype, compare his or her psychophysical taste response for the bitter compound PTC with the norms for individuals of the same genotype. Responses should match; however, in rare cases, *TAS2R38* genotype does not perfectly predict PTC sensory results.[35]

4.3. Should responses show significant divergence, compare the subject's taste response for quinine with the norms for individuals of the same *TAS2R19* genotype. Responses for quinine intensity and genotype should match.[36] Should all taste results fail to correspond with genotype, it is possible that the subject (a) does not understand the task (b) is providing spurious ratings or malingering or (c) there has been an data collection error on the part of the investigator.

4.4. Identify data point outliers, and perhaps exclude them from analysis (**Figure 3**). Correspondence of sensory results with objective genotyping validates the reliability of the psychophysical testing procedure.

REPRESENTATIVE RESULTS:

Results from the taste test have been pooled for all subjects evaluated (n = 840) and are presented after segregation by genotype. The full data set is accessible at https://carayata.shinyapps.io/TasteBoxplots/ and can be reviewed for each tastant assessed and for *TAS2R38* and *TAS2R19* genotypes. Results confirm the existence of perceptual taste

differences for PTC among subjects grouped by *TAS2R38* receptor genotype (**Figure 3**). Ratings of PTC intensity are significantly different across *TAS2R38* genotypes (AVI/AVI, 0.86; AVI/PAV, 6.95; PAV/PAV, 8.18; one-way ANOVA, $\boldsymbol{p} < 0.0001$). Results of quinine intensity are also significantly different across *TAS2R19* genotype (A:A, 3.77; A:G, 3.08; G:G, 2.26; one-way ANOVA, $\boldsymbol{p} < 0.0001$).

Figure 1: Taste kit. Subjects use the kit to rate taste intensity and quality of various color-coded tastants. Box A contains samples 1-6, box B contains 7-12.

Figure 2: Taste questionnaire entry, comprising a category scale for intensity rating and forced choice response for tastant quality. The taste questionnaire will include one entry for each of the color-coded tastants tested.

Figure 3: PTC taste questionnaire results by *TAS2R38* genotype. The taste questionnaire can be used to segregate individuals by *TAS2R38* genotype based on PTC bitterness intensity ratings on a category scale (*p < 0.0001). Here we see a few outliers, data points located outside the fences ("whiskers") of this boxplot (*e.g.*, outside 1.5 times the interquartile range above the upper quartile and bellow the lower quartile)

Table 1. Records identified using 'Taste' versus 'Hearing' and 'Vision' as keywords

DISCUSSION:

The significance of this method is that it uses a multidisciplinary approach with features from medicine (the oral exam), experimental psychology (the taste test) and genetics (a validation step). Taste information is likely to develop as a diagnostic and prognostic tool because taste provides a window into the function of proteins elsewhere in the body. From an experimental psychology viewpoint, the addition of a simple exam can identify subjects who are not appropriate for the study of normative taste function. From a genetics point of view, these procedures provide a simple way to study easily reproducible genotype-phenotype relationships.

Measuring human taste has several critical features that are intangible but important, including helping the subject feel at ease and oriented to the task, and especially in medical settings, keeping the procedure short, so the attention of the subject does not waver. It is also important to intervene if subjects appear uncomfortable, and trouble-shoot their concerns, such as regarding the nature of the testing stimuli. Subjects are often reassured to learn that most of the testing stimuli are in foods, *e.g.*, salt and sugar. This procedure, while simple, has significant limitations. While the oral exam is routine for investigators with medical training, those with experimental psychology or genetics training are likely to be less facile at recognizing oral disease. Another limitation is the rating scale that while easily understood by subjects with no prior training, may obscure differences between individuals, or groups of individuals[41]. Finally, the guidance on how to treat subjects with mismatches between genotype and phenotype in the statistical processing of the data is not yet codified into simple rules, *e.g.*, dropping subjects who fail to meet certain criterion.

Looking ahead to future applications, taste exams may become routine parts of medicine like vision and hearing tests, which would increase our understanding of how taste relates to human disease and well-being and will allow us to refine this simple test.

ACKNOWLEDGMENTS:

Awards from the National Institutes of Health supported this research (R01DC013588 to NAC, R21DC013886 to NAC and DRR, and NIDCD Administrative Research Supplement to Promote Emergence of Clinician-Scientists in Chemosensory Research to JED). We collected genotype data from equipment purchased in part with NIH funds from OD018125.

DISCLOSURES:

NAC and DRR are co-inventors on a patent under review (Therapy and Diagnostics for Respiratory Infection 61/697,652, WO2013112865).

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41 Bartoshuk, L. M. *et al.* Labeled scales (*e.g.*, category, Likert, VAS) and invalid across-group comparisons: what we have learned from genetic variation in taste. *Food Quality Prefererences.* **14** (2), 125-138, doi:https://doi.org/10.1016/S0950-3293(02)00077-0 (2003).

Figure 1

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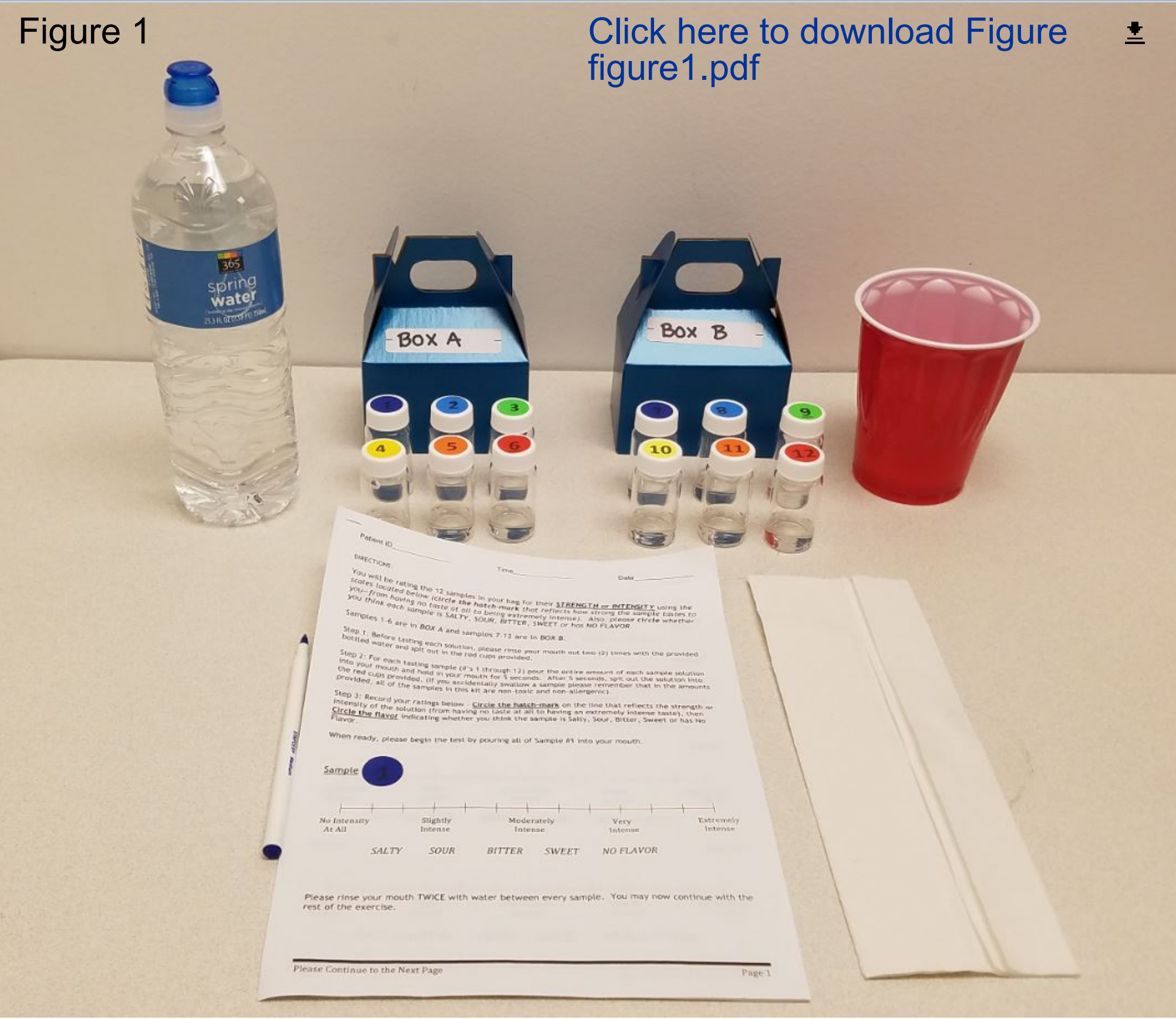

Figure 2

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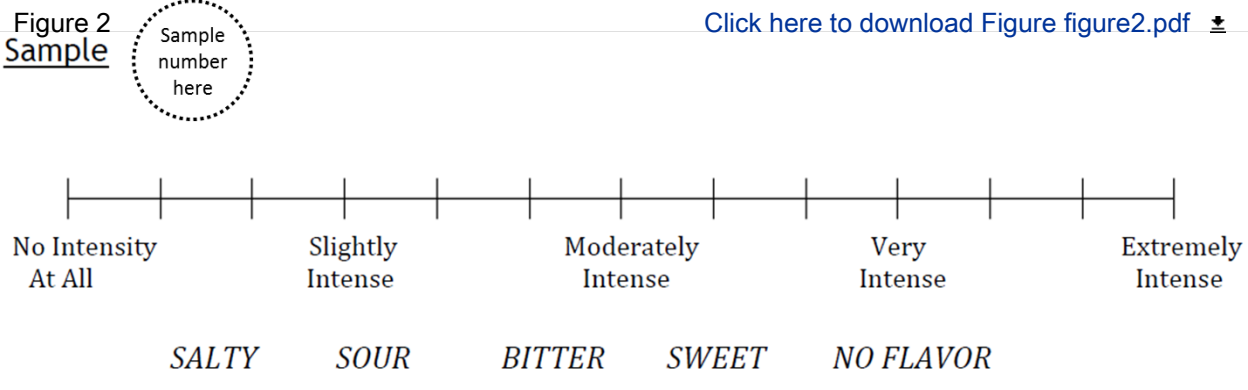

Figure 3

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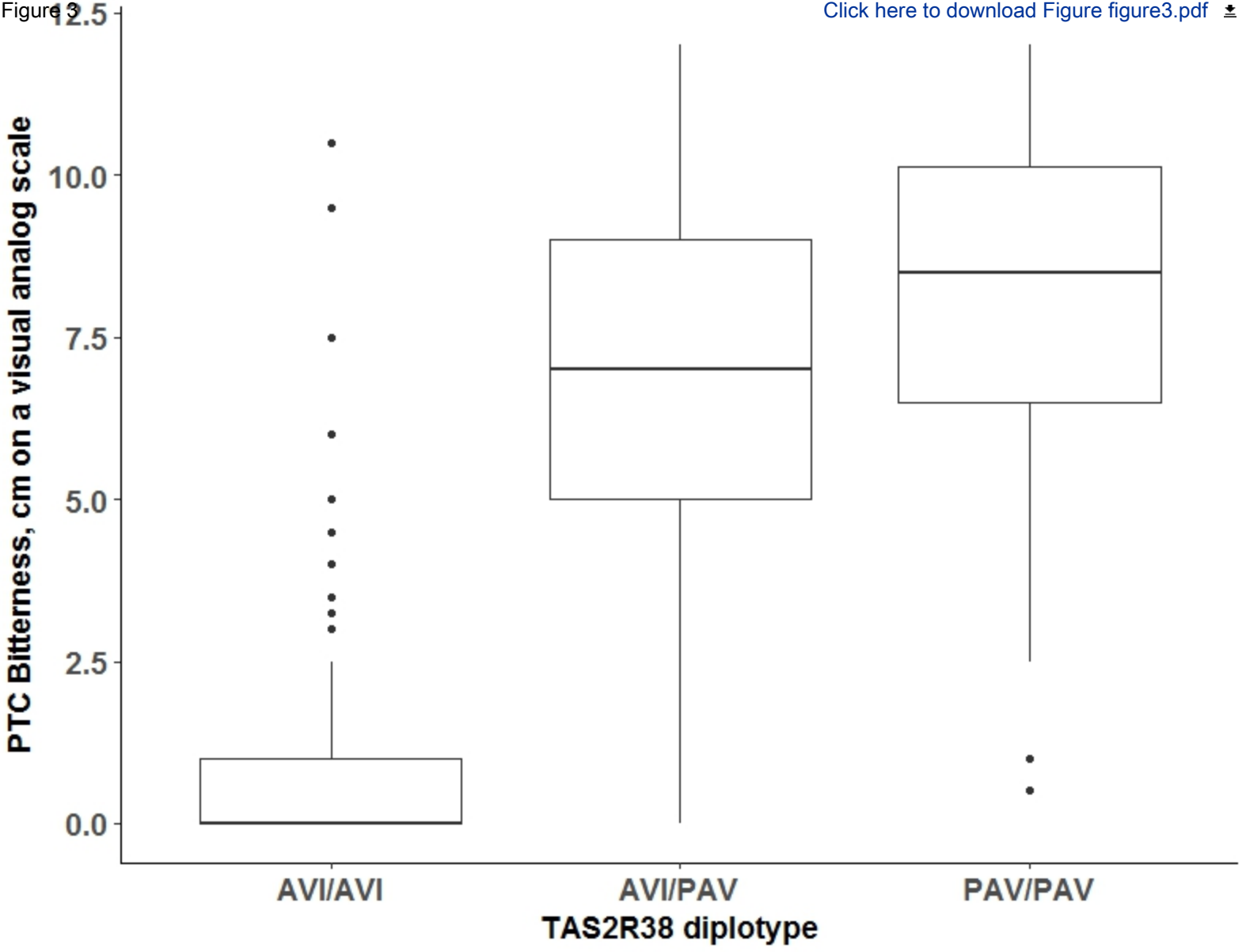

Table

Metric	Taste	Hearing	Vision	Hearing/T	Vision/Taste
PubMed	36,302	123,101	171,522	3.39	4.72
NIH Repor	6,144	23,873	54,858	3.89	8.93

We conducted a text search of *PubMed* and *NIH Reporter* using a particular sense as a key wo
https://www.ncbi.nlm.nih.gov/pubmed/
https://projectreporter.nih.gov/reporter.cfm

rd. *PubMed* is an online database of published experimental results and *NIH Reporter* is an onl

ine database that lists research projects funded through the National Institutes of Health. Value

es in the 'Hearing/Taste' column show a ratio, *i.e.* , there are over three times as many publicati

ons referencing hearing compared with taste. We accessed the URLs below on January 31, 2018

3 at 10 am EST.

Table of Materials/Equipment

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Name of Material/ Equipment	Company	Catalog Number	Comments/Description
Disposable diagnostic penlight	Primacare	DL-9223	
UltraLite Pro headlight	Integra LifeSciences	AX2100BIF	
Millipore Q-Gard 2 water purification system	EMB Millipore	QGARD00D2	
Denatonium benzoate	Sigma Aldrich	D5765	
Phenylthiocarbamide	Sigma Aldrich	P7629	
Quinine hydrochloride dihydrate	Sigma Aldrich	Q1125	
Sodium Chloride	Sigma Aldrich	S1679	
Sucrose	Sigma Aldrich	S0389	
Glass scintillation vials	Thomas Scientific	1230L59	Same as Wheaton catalog no. 986580
Oragene Discover OGR-500 DNA collection kit	DNA Genotek	OGR-500	
prepIT L2P Protocol reagents	DNA Genotek	PT-L2P-5	
rs713598 TaqMan SNP genotyping assay	ThermoFisher Scientific	C___8876467_10	
rs1726866 TaqMan SNP genotyping assay	ThermoFisher Scientific	C___9506827_10	
rs10246939 TaqMan SNP genotyping assay	ThermoFisher Scientific	C___9506826_10	
rs10772420 TaqMan SNP genotyping assay	ThermoFisher Scientific	C___1317426_10	

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Author(s): Douglas, J.E., Mansfield, C.J., Arayata, C.J., Cowart, B.J., Blasetti, M.T., Cohen, N.A., and Reed, D.R.

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Thursday, January 25, 2018

To: Editorial Board, *Journal of Visualized Experiments*

From: Danielle Renee Reed, Ph.D.

Re: Revision: "Taste exam: A brief, validated, and high-yield test for clinically pertinent chemical perception"

To the concerned member(s) of the Editorial Board,

We are submitting our revised manuscript titled "*Taste exam: A brief, validated, and high-yield test for clinically pertinent chemical perception*" for consideration for the *Journal of Visualized Experiments*. Below we respond to the comments from the editor and reviewers.

We numbered the material in a specific way to ensure that the origin of all comments was clear ('E' for editor and 'R' for reviewer) and distinguished the comment from our answers (comment, 'C'; answer, 'A'). Multipart comments were unpacked and renumbered to ensure we addressed each point. Our answers are italic font, comments in regular font. As an example, the first comment of the second reviewer is 2R.1C and our answer to this comment is 2R.1A.

As instructed, we state that the authors have (a) conferred, (b) approved the final manuscript, and (c) will assume full responsibility for its written contents.

We further state that neither the manuscript nor any parts of its content are currently under consideration or published in another journal.

We appreciate your further consideration of our submission. Please feel free to contact me at (267) 519-4915 or *reed@monell.org* if you have any questions.

Sincerely,

Danielle Renee. Reed, Ph.D.
Associate Director
Monell Chemical Senses Center

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E1A. *To the best of our ability, we have corrected grammatical and spelling errors.*
E2C. Please abbreviate all journal titles.
E2A. *We have abbreviated all journal titles. Journal editors provided no guidance regarding the abbreviation convention, so we followed the journal abbreviations used by PubMed.*
E3C. Please define all abbreviations before use.
E3A. *We have defined all abbreviations prior to first use, although we assume that DNA is on the list of commonly used abbreviations that require no definition.*
E4C. Please use focused images of uniform size/resolution (at least 300 dpi).
E4A. *We will ensure that all images uploaded will be at least 300 dpi.*
E5C. Please revise the table of the essential supplies, reagents, and equipment. The table should include the name, company, and catalog number of all relevant materials in separate columns in an xls/xlsx file.
E5A. *We now provide the relevant table in the requested format.*
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E6A. *We ensured that the manuscript has no commercial language including trademark or registered symbols.*
E7C. Please include an ethics statement before the numbered protocol steps, indicating that the protocol follows the guidelines of your institution's human research ethics committee.
E7A. *We added the following ethics statement: "The University of Pennsylvania Institutional Review Board approved this protocol."*
E8C. Please specify inclusion/exclusion criteria for subjects.
E8A. *We added the inclusion and exclusion criteria as follows: "We excluded subjects if they were under 18 years of age or were pregnant."*
E9C. Please provide citations for protocol steps 3 and 4.
E9A. *We have added reference (a) to step 3 and references (b) and (c) to step 4, below.*

a) *Guo, S. W. & Reed, D. R. The genetics of phenylthiocarbamide perception. Ann of Hum Biol.* ***28*** *(2), 111-142, doi:10.1080/03014460151056310, (2001).*
b) *Reed, D. R. et al. The perception of quinine taste intensity is associated with common genetic variants in a bitter receptor cluster on chromosome 12. Hum Mol Genet.* ***19*** *(21), 4278-4285 (2010).*
c) *Bufe, B. et al. The molecular basis of individual differences in phenylthiocarbamide and propylthiouracil bitterness perception. Curr Biol.* ***15*** *(4), 322-327, doi:http://dx.doi.org/10.1016/j.cub.2005.01.047, (2005).*

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E10C. Please highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol. The highlighted steps should form a cohesive narrative with a logical flow from one highlighted step to the next. Remember that non-highlighted Protocol steps will remain in the manuscript, and therefore will still be available to the reader. Please ensure that the highlighted steps form a cohesive narrative with a logical flow from one highlighted step to the next. Please highlight complete sentences (not parts of sentences). Please ensure that the highlighted part of the step includes at least one action that is written in imperative tense. Steps 1 and 2 are most amenable to filming.
E10A. *We have highlighted approximately 2.5 pages with the essential steps of the protocol most appropriate for video recording.*
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E12C. As we are a methods journal, please revise the Discussion to explicitly cover the following in detail in 3-6 paragraphs with citations: 'critical steps within the protocol', 'any modifications and troubleshooting of the technique', 'any limitations of the technique', 'significance with respect to existing methods', and 'any future applications of the technique'.
E12A. *We have included these requested points in the discussion section (page 8).*

1R.1C. – none
1R.1A. – no response required

Introduction to Reviewer 2. Four themes emerged from the comments raised below and addressing them in this introduction allows us then to respond fully to the remaining concerns. The four themes are intended audience, the goal of this manuscript, clarifying the clinical example used and the practical value of genotyping. We address each theme below. [Theme 1# Audience]: *This method paper has at least three intended audiences: (a) researchers conducting taste testing who are interested in methods validated by genetic testing, (b) clinicians who are interested in taste testing as a research and eventually a diagnostic tool and (c) trainees, especially sensory scientists, who might benefit from an explicit method with a genetic validation step. We offer these methods not only to clinicians but also broadly, to basic science researchers, many of whom have little or no training in psychophysics.* ***[Theme 2# Goal]:*** *We now more clearly state the main message of this methods approach, which is to direct attention to three points that can improve the quality of taste data collected in a research or clinical setting. Building on the Boolean logic example provided by the reviewer: if there are papillae, and there is no evidence of oral disease, and intensity ratings match genotypes, it is 'true' that taste intensity ratings are more likely to be valid.* ***[Theme 3# Clinical Example]****: Although our current interest is in chronic*

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rhinosinusitis (CRS), our goal is to ensure that taste testing becomes standard in many areas of medicine, because almost every tissue studied to date expresses one or more bitter or sweet taste receptors. We provide but one example with CRS. We explain both the broad applications of this method and the use of CRS as a test case more clearly in the introduction. ***[Theme 4# Use and Value of Genotyping]:*** *Sensory ratings by their nature are subjective because people report on their personal experience. Evaluating genotype provides a way of matching the reported experience to an objective measurement. If there is no agreement between genotype and reported sensation for an individual subject, it may be that the subject is inattentive or otherwise not reporting accurately. The use of genotype gives sensory psychophysics a new tool to evaluate data quality. However, it is up to individual investigators to use this tool in the evaluation of their own data. For instance, the match between genotype and sensory ratings can identify errors in data collection, distracted subjects or people who did not understand the scales.*

2R.1C. The short abstract is clear and helpful, it lists that there are three things assessed and the importance. The long abstract is more muddled. It jumps from an introduction to the method (seemingly incomplete, listing only 2 things assessed, then jumps into an incoherent explanation of the genotyping, and then back to background-like statements again. Tighten it up and remove the rambling prose and it would be good to go.

2R.1A. *We have given the long-abstract a logic overhaul; it has been revised by both the senior authors and a science editor who helped polish the final version.*

2R.2C. The entire paragraph about tongue anatomy (127-136) seems a little odd. The afterthought of the clinical assessment of "evidence of oral disease, such as lesions or lumps, or other clinically remarkable features" seems more important to me than just "do they have papillae?" The section on oral cavity evaluation needs to be more detailed about what to look for and why. e.g. the method explains to look at the 7 subsites, but not what you should be looking for in any of these sites and why each site is important and what it means if you do find something noteworthy in any given site… beyond the absence or presence of papillae.

2R.2A*. Recognizing that the section on oral pathology was thin in comparison to other sections, we have added more description and an explicit checklist of what to look for when assessing oral disease. Many published studies with human taste data make no mention of any type of oral exam so we emphasize this simple but overlooked step.*

2R.3C. The psychophysical taste test includes a seemingly random assortment of taste tests with a vague reference that assessors could use any combination or other taste tests as necessary. It is not clear to me why these specific tastes tests were selected (3 bitter, 1 salty, 1 sweet), with the exception of PTC which ties back to the original rationale about CRS and has a genetic component that is included in the methodology presenting (thus strengthening the case for testing that taste ligand). Quinine it seems is being used as a second opinion (section 4.3)? The rationale for the tests included could be better explained and again, why this measurement matters. What does one do with this data by itself and now in conjunction with the oral assessment?

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2R.3A. *We provide a rational in the section for some of the taste compounds but we emphasize that other stimuli could be included to tailor the method to particular research questions.*

2R.4C. There is a decent amount of space dedicated to papillae density, but it looks like the protocol is literally "see if they have them". Is it more important to look for presence vs absence of papillae than density? No rationale is given for density; however the authors do explain why it is important to look for presence or absence. The latter appears more important, in which case density is not necessary for the oral assessment in this method. Other minor note related to papillae: the density methods listed are all for fungiform papillae, and not for foliate or circumvallate. If density is in fact necessary, authors need to point to methods to assess density for all types of papillae or explain why FP is good enough a measure.

2R.4A. *The goal of this manuscript is in part to advocate for simple steps all investigators can use to increase the quality of their human taste data. One such example is to ensure that each subject has fungiform papillae. These papillae are on the tip of the tongue and easy to see in contrast to foliate or circumvallate, which are harder to visualize because of their location. Evaluating fungiform papillae density contributes more information, and we direct investigators to other protocols for this procedure.*

2R.5C. The taste questionnaire entry form should have the forced choice (quality) listed first, then the VAS should follow (it currently is shown with the VAS first). Also it should be clear that you are asking for the intensity of the primary taste detected, not the overall intensity of the sample. It is unclear if you give patients the chance to list secondary tastes and intensities for a given sample, or if they are only being asked to rate the primary attribute.

2R.5A*. We appreciate these suggestions, but participants see the computerized instruction to 'circle only one quality' on the same 'page' that they rate intensity. They are also instructed to rate overall intensity, not the intensity of individual attributes.*

2R.6C. Better job needs to be done explaining what is the most important measure and why, in case resources are not unlimited and the steps in the proposed method need to be prioritized. What if an MD doesn't have access to genotyping, what alternative method could be proposed in that case?

2R.6A*. We have no alternative to genotyping. However, genotyping is now a routine part of protocols in both clinical research and medicine, and our goal is to move the field forward by describing the value of genotyping in the evaluation of self-report sensory data.*

2R.7C. I guess I wonder what the point of the phenotyping is, especially when the authors go on to say the PAV/AVI diplotype confounds everything.

2R.7A. It is unclear how data from heterozygous people "confounds everything". Ratings of one bitter compound are tied to the diplotype of one bitter receptor gene, and data from people with a heterozygous diplotype fit a well-described genetic model that predicts a wide range of responses compared to people with either homozygous diplotype.

2R.8C. Or is it simply that taste loss may have occurred? This was not adequately highlighted for me.

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2R.8A. One of the uses of the genotyping information could be to identify taste loss, for instance, if someone with the taste diplotype rating the bitter ligand like water. However, this is not the intended use of the information, because in the case of taste loss, participants would rate other compounds as being like water too. This is one rationale for including compounds that elicit taste qualities other than the one of primary interest in any given research protocol.

2R.9C. What is the point of figure 1? Perhaps add labels to the different subsites for identification purposes, and add other images of things at each subsite to look for/abnormalities as indicated in 1.2 and 1.3.

2R.9A. We provided Figure 1 to show the tongue, one of the seven subsites we recommend examining as part of a thorough oral cavity evaluation. We now include descriptive labels to assist the reader in identifying structures relevant to the oral exam.

2R.10C. Is figure 4 a previously published result or new data? It contradicts many reports that show no statistical difference between heterozygotes and dominant (PAV) homozygotes.

2R.10A. *The data reported here are new but are consistent with published results. Whether TAS2R38 heterozygotes differ statistically from homozygotes often depends on the number of participants studied. For studies with smaller numbers of subjects, there are sometime no statistical differences between heterozygotes and people with the homozygous taste (PAV/PAV) diplotype owing to reduced statistical power, whereas there almost alwaysare for larger numbers of participants. See Figure 2 in doi:10.1097/01.ALC.0000145789.55183.D4 for a published example of these heterozygous vs taster homozygous differences.*

2R.11C. PTC elicits retronasal olfaction, therefore propylthiouracil is a better ligand choice for this reason

2R11A. We selected PTC over propylthiouracil because it is a higher affinity ligand for the T2R38 receptor (see doi:10.1016/j.cub.2005.01.047).

2R.12C. In all cases nose clips or instructing the patient to plug their nose would be valuable to eliminate olfactory cues and allow them to be mindful only of taste detection.

2R.12A. The odor associated with PTC might affect threshold measurement, but comparison of ratings during pilot testing confirmed the odor contributes little to the overall perception of the stimulus at the concentration used here.

2R.13.C. Step 2.2.3 is a non-scientific measurement for quality control that would lead to flavor drift over time, decreasing the validity of this method. Might there be a quantitative method you could propose?

2R13.A. Tasting newly prepared solutions is a common sense procedure to avoid serious errors such as using a different chemical during solution preparation (sucrose versus salt). We include this point because investigators can overlook this simple step. This is just a final step in quality control, after following the standard procedures for the preparation of solutions of given concentrations.

2R.14C. The genotype section should explain the relationship between the genes proposed for testing and the taste tests that were administered, e.g. TAS2R38 PTC/PROP… TAS2R19 quinine? It is not fully explained in the manuscript, and wouldn't necessarily make sense to someone who wasn't in genetics/sensory science (i.e. if this is written for MDs it is not a fair assumption that they would know the reason why these genes are selected).

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2R.14A. In this revised version, we clarify the connection between inborn genetic variants of the bitter receptor gene TAS2R19 and quinine taste perception.
2R.15C. Why are there two boxes, are the patients to pause in between samples 6 and 7. Are they just holders for the samples? In which case are they necessary? Better to let administrators prepare and store/hold samples in whatever manner works for them, and list the boxes as an example?
2R.15A. We use this two-box configuration because these small boxes fit easily into the limited storage space of our clinic. We now indicate in this revision that this configuration is one of many possible configurations.
2R.16C. There is a reference that there is one particular disease that causes an absence of FP (lines 130-131) but the authors don't talk about what that disease is. It is brought up again in the discussion so might be worth explaining a bit more. I recommend the authors call it out by name and speak a little more about it.
2R.17A. We now provide more information about familial dysautonomia and provide more context about this disorder.
2R.18C. Line 89 add "loss" after "taste".
2R.18A.We corrected this typo (omitted word) as advised.

Reviewer #3 Comments
Introduction to Reviewer 3. The overview comment is that we have studied the material covered in the six references highlighted by Reviewer 3 and have incorporated it into the manuscript. We address the remaining points below.

3R.1C. Previous articles on the clinical assessment of taste have been published (e.g., Bartoshuk, Gent, Catalano, & Goodspeed, 1983; Mueller et al., 2003), but are not cited; these articles should be mentioned, though it is obvious that the proposed exam does not simply replicate previous publications
3R.1A. These papers are now referenced and our work is now better placed in historical context for the reader.

3R.2C. Firstly, the oral cavity evaluation as described ignores the lateral surfaces of the tongue. In doing so, the foliate papillae are not examined. Although fungiform papillae are perhaps best associated with genetic diversity, the foliate papillae are nevertheless important in taste perception and contribute differentially from other papillae to the perception of a taste stimulus (Collings, 1974; Ishida, Ugawa, Ueda, Murakami, & Shimada, 2002). Thus, any examination of the tongue for conditions that could affect taste function should include an assessment of all visible taste papillae - not just the fungiform papillae.
2R.2A. While logical to evaluate the presence of the foliate and circumvallate papillae as well as the more easily spotted fungiform papillae, this is harder to do in practice. Our pilot testing indicates that visualizing the rear of the tongue can make subjects gag and that foliate papillae can be hard to

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see depending on individual tongue anatomy. While we appreciate the suggestion, here we prioritize speed and subject comfort over comprehensiveness.

3R.3C. Next, the psychophysical taste testing as described suffers from a lack of sensitivity to potential disturbances in taste. The whole-mouth testing that is described is less sensitive than regional testing to the effects of aging and other conditions that may affect gustation (Matsuda & Doty, 1995; Bartoshuk, 1989). In addition, the scaling used to assess the tastants may obscure differences between individuals, or groups of individuals (e.g., Bartoshuk, Duffy, Fast, Green, Prutkin, & Snyder, 2003).

3R.3A. *The value of regional testing is appreciated but we have weighed the value of the knowledge gained against the time constraints of most clinical testing, and we will pursue the whole-mouth approach, mindful of the limitations of the whole-mouth approach. As a remediation measure, we now direct interested researchers to the NIH Toolbox regional taste protocols. We are also aware that the scale selected has limitations, but it requires little training time, unlike the labeled magnitude scale. However, drawing on this comment from the reviewer, we now point out the limitation of the scale we have selected to the reader.*

3R.4C. Lastly (and this is a small thing, but important), Figure 1 simply shows the dorsal portion of the tongue. Of the seven subsites that are mentioned for oral assessment, this particular part is likely the most recognizable - and thus, the least in need of illustration. Perhaps a different figure would better assist your readers/viewers in completing the evaluation.

3R.4A. As an improvement, we are illustrating all seven regions of the tongue as part of the filmed portion of this article, which we agree were not adequately illustrated by our former Figure 1.

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