

Video Article

Taste Exam: A Brief and Validated Test

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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.

Video Link

The video component of this article can be found at <https://www.jove.com/video/56705/>

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 smell¹, 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 function⁶ or the willingness of a patient to take medication⁷. 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 psychology⁸, a field with which those in medicine may be relatively unfamiliar. Moreover, renewed interest in taste has ushered in standardized taste methods⁹ that build on earlier methods¹⁰, 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 shape¹¹. Investigators have published several useful methods to quantify FP and we direct readers to these sources for measurement protocols^{12,13,14,15,16}. Foliate papillae, shaped like the pages of a book (folia), are located exclusively on the lateral posterior tongue surface¹¹. 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 twins¹⁷. Papillary density also differs among people, and although rare, at least one genetic disease (familial dysautonomia) results in a congenital absence of taste papillae^{18,19,20}. 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 behavior²¹. 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 receptors²⁴. These bitter receptors can respond to multiple bitter compounds, and a single bitter compound often stimulates more than one receptor²⁵. Despite the recent expansion of knowledge about the molecular basis of taste, novel pathways²⁶ and new discoveries beyond the traditional five taste qualities (e.g., calcium²⁷ or fatty acid²⁸ 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^{21,29,30,31}. These extraoral sites include the brain, thyroid, upper and lower respiratory tract, and the gastrointestinal tract, among many others^{21,29,30,31}. 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 response³², 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 Fox³³; later this compound was identified as an agonist of the T2R38 receptor³⁴. 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 sensitivity³⁵. 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 quinine³⁶. Likewise, variation in *TAS2R31* affects the perceived bitterness of one of the high-potency sweeteners^{37,38,39}.

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. Instruct the subject to open the mouth.
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. 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.
 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.
 3. Extend the examination anteriorly by lifting the upper and lower lips to visualize the surfaces of the labial mucosal and anterior gingiva.
 4. Finally, visualize the hard palate and retromolar trigone.
 5. Note lesions, abrasions, and masses or signs of inflammation.
 6. Again, ask the subject to open the mouth and extend the tongue.
 7. Use a light source to visualize the dorsal surface of the tongue.
 8. Identify the presence or absence of FP, e.g., a smooth tongue surface
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/>.

1. Tastant preparation

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.
 1. Denatonium benzoate (bitter): prepare a stock solution of 4.99×10^{-3} M denatonium benzoate by dissolving 2.228 g of denatonium benzoate in 1 L of water. Add 180 μ L 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 μ M.
 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 μ M.
 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 μ M.
 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.
 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. 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.
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.
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.
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)
 - PTC (red)
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.

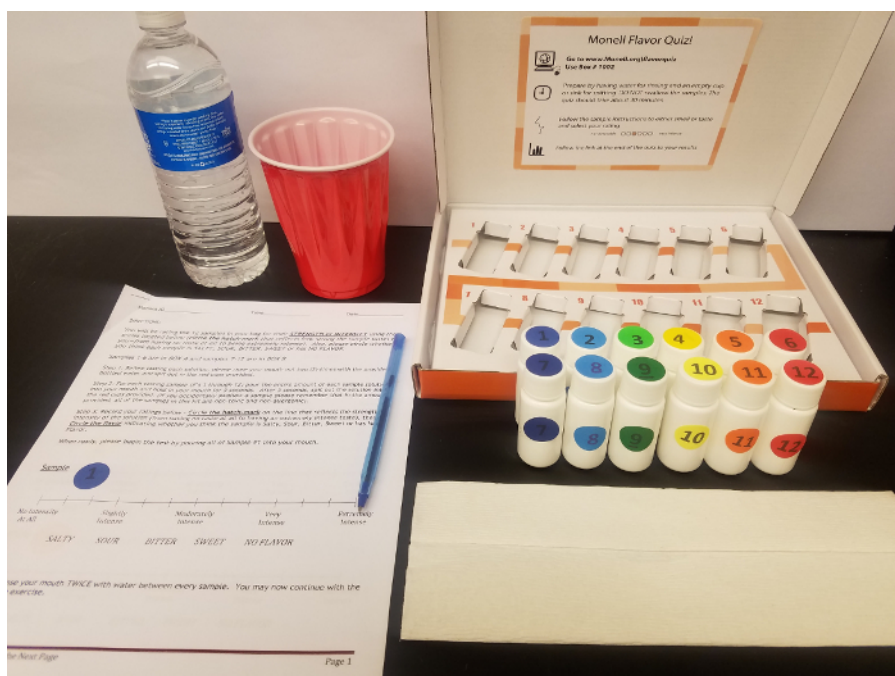


Figure 1: Taste kit. Subjects use the kit to rate taste intensity and quality of various color-coded tastants. Box A contains samples 1–6, box B contains 7–12. [Please click here to view a larger version of this figure.](#)

2. Taste questionnaire

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

Sample Sample number here

No Intensity At All	Slightly Intense	Moderately Intense	Very Intense	Extremely Intense
SALTY SOUR BITTER SWEET NO FLAVOR				

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. [Please click here to view a larger version of this figure.](#)

3. Taste Test Administration

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/hn87sf>.
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.
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.
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.
5. Review the finished questionnaire for completeness.
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

1. Collect saliva from each subject using a saliva DNA collection kit.
2. Purify genomic DNA from the sample by following manufacturer instructions.
3. Determine *TAS2R38* genotype using SNP genotyping assays (*rs713598*, *rs1726866*, *rs10246939*)⁴⁰.
4. Determine *TAS2R19* genotype using SNP genotyping assays (*rs10772420*)³⁶.

4. Genotype-phenotype Validation

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/>.
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 results³⁵.
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 match³⁶. 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. 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, $p < 0.0001$). 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, $p < 0.0001$).

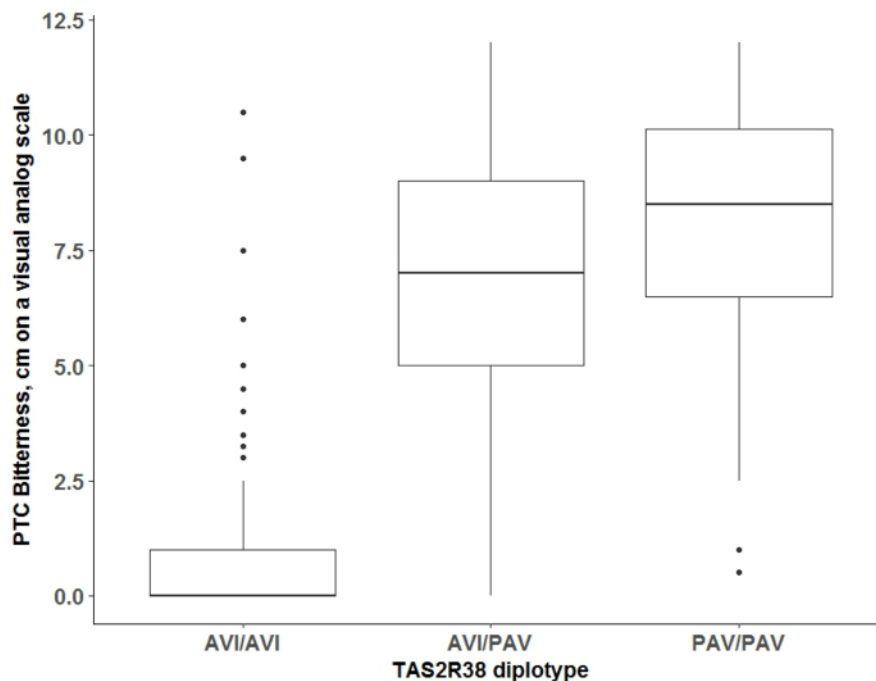


Figure 3: PTC taste questionnaire results by *TAS2R38* genotype. The taste questionnaire can be used to segregate individuals by *TAS2R38* genotype based on PTC bitterness intensity ratings on a category scale ($*p < 0.0001$). Here we see a few outliers, data points located outside the fences ("whiskers")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

Metric	Taste	Hearing	Vision	Hearing/Taste	Vision/Taste
PubMed	36,302	123,101	171,522	3.39	4.72
NIH Reporter	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 word. *PubMed* is an online database of published experimental results and *NIH Reporter* is an online database that lists research projects funded through the National Institutes of Health. Values in the 'Hearing/Taste' column show a ratio, *i.e.*, there are over three times as many publications referencing hearing compared with taste. We accessed the URLs below on January 31, 2018 at 10 am EST.

https://www.ncbi.nlm.nih.gov/pubmed/	
https://projectreporter.nih.gov/reporter.cfm	

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 individuals⁴¹. 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.

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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