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Optimal Serum Cotinine Levels for Distinguishing Cigarette Smokers and Nonsmokers Within Different Racial/Ethnic Groups in the United States Between 1999 and 2004

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Cotinine, a metabolite of nicotine, is widely used to distinguish smokers from nonsmokers in epidemiologic studies and smoking-cessation clinical trials. As the magnitude of secondhand smoke exposure declines because of proportionally fewer smokers and more clean-indoor-air regulations, the optimal cotinine cutpoint with which to distinguish smokers from nonsmokers is expected to change. The authors analyzed data on 3,078 smokers and 13,078 nonsmokers from the National Health and Nutrition Examination Survey for 1999–2004. Optimal serum cotinine concentrations for discriminating smokers from nonsmokers were determined using receiver operator characteristic curve analysis. Optimal cotinine cutpoints were 3.08 ng/mL (sensitivity = 96.3%, specificity = 97.4%) and 2.99 ng/mL (sensitivity = 86.5%, specificity = 93.1%) for adults and adolescents, respectively. Among adults, optimal cutpoints differed by race/ethnicity: They were 5.92 ng/mL, 4.85 ng/mL, and 0.84 ng/mL for non-Hispanic blacks, non-Hispanic whites, and Mexican Americans, respectively. Among adolescents, cutpoints were 2.77 ng/mL, 2.95 ng/mL, and 1.18 ng/mL for non-Hispanic blacks, non-Hispanic whites, and Mexican Americans, respectively. Use of the currently accepted cutpoint of 14 ng/mL overestimates the number of nonsmokers in comparison with the proposed new overall cutpoint of 3 ng/mL or the race/ethnicity-specific cutpoints of 1–6 ng/mL.

African Americans; continental population groups; cotinine; Mexican Americans; smoking; tobacco; tobacco smoke pollution

Abbreviations: MEC, mobile examination center; NHANES, National Health and Nutrition Examination Survey; ROC, receiver operating characteristic.

Cotinine, the major proximate metabolite of nicotine, has been widely used as a biomarker of exposure to tobacco and both active and secondhand tobacco smoke (1). While cotinine has been the main biomarker used to distinguish tobacco users from people who do not use tobacco (2), cotinine reflects the extent of exposure, not how the exposure was derived. There is an overlap in cotinine values between less intense or less frequent active smokers and persons heavily exposed to secondhand smoke. Biochemical validation of smoking status is commonly used to improve the accuracy of self-reported status in studies of smoking exposure and smoking-related health risks and in studies of cessation interventions. The overlap in exposure to tobacco smoke between less intense active smokers and persons with heavy secondhand smoke exposure necessitates selection of a cutpoint for defining active smokers as separate from nonsmokers exposed to secondhand smoke.

The optimal cutpoint with which to distinguish smokers from nonsmokers varies according to the distribution of cotinine levels among exclusive cigarette smokers (excluding the use of nicotine from other sources) and the distribution of cotinine levels among nonsmokers exposed to secondhand smoke. The most widely cited cutpoint for discriminating smokers from nonsmokers—14 ng/mL—was

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determined by Jarvis et al. (3) on the basis of cotinine samples taken from persons visiting outpatient clinics in the United Kingdom in the early 1980s. There were few smoke-free indoor air regulations at that time in the United Kingdom, and exposure to secondhand smoke was considerable. A high level of secondhand smoke exposure would be expected to result in a higher cutpoint. Using 1988–1991 data, Pirkle et al. (4) reported serum cotinine levels in a nationally representative US sample as part of the National Health and Nutrition Examination Survey (NHANES). Cotinine levels of nonsmokers who reported no secondhand smoke exposure were quite low, with a geometric mean of 0.15 ng/mL. Nonsmokers who reported secondhand smoke exposure at home and/or at work had geometric mean values ranging from 0.32 ng/mL to 0.93 ng/mL. Likewise, Venn and Britton (5), using NHANES data from 1988–1994, found a median cotinine level of 0.205 ng/mL in nonsmokers. Using NHANES data from 1988–2002, Pirkle et al. (6) compared cotinine levels in nonsmokers and found a decrease in the geometric mean value from 0.25 ng/mL to 0.05 ng/mL. Low levels of cotinine found in nonsmokers in these and other recent studies suggest that the optimal cutpoint for distinguishing smokers from nonsmokers in the United States and other countries with effective smoke-free ordinances might be much lower than that determined in the United Kingdom in the 1980s (2, 3).

In addition, racial/ethnic differences in cigarette smoking and in the rate of metabolism of nicotine and cotinine have been well described (7–9). At the same daily level of cigarette smoking, higher serum cotinine concentrations are observed in blacks than in whites (10, 11). This is a result of slower metabolism of cotinine and greater intake of nicotine per cigarette in blacks. Such racial/ethnic differences in smoking behavior and nicotine metabolism could also affect the optimal cutpoint for distinguishing smokers from nonsmokers. In this paper, we present results from an analysis of serum cotinine data from the NHANES surveys conducted between 1999 and 2004, the most recent data available. We aimed to determine an optimal cutpoint that would reflect the current extent of secondhand smoke exposure in the United States and the influence of racial/ethnic differences on that cutpoint.

MATERIALS AND METHODS

The NHANES is an ongoing survey conducted by the National Center for Health Statistics that is designed to examine a nationally representative sample of the US civilian, noninstitutionalized population aged 2 months or older. It is based upon a complex, stratified, multistage, probability cluster sample design (see the NHANES website for more information (http://www.cdc.gov/nchs/nhanes.htm)). The protocols include a home interview followed by a physical examination and additional interviews administered in a specially equipped mobile examination center (MEC), where blood samples are drawn, from which serum cotinine concentrations are determined. The MEC represents a private and nonjudgmental environment, and subjects reply to questions directly on a computer screen rather than respond to an interviewer. This method of data collection is thought to be especially effective in eliciting truthful answers from young people.

Subject selection

We included NHANES participants aged 12 years or older from 1999–2000, 2001–2002, and 2003–2004 who completed a tobacco-use questionnaire in the MEC and who were classified, on the basis of self-described race/ethnicity, as non-Hispanic white, non-Hispanic black, or Mexican-American, who had a serum cotinine measurement, and who reported not using a significant source of nicotine other than cigarettes in the previous 5 days. We excluded persons with conditions that might have interfered with nicotine metabolism, including pregnant women and persons with a history of kidney disease, a serum creatinine concentration greater than 2 mg/dL, or use of anticonvulsant drugs, disulfiram, or rifampin.

Of 15,332 adults aged 20 years or older, 735 (4.8%) did not answer the tobacco-use questionnaire, 2,130 (13.9%) had no serum cotinine measurement, and 515 (3.4%) reported using a source of nicotine in the previous 5 days other than cigarettes (i.e., cigars, pipes, smokeless tobacco, or a nicotine patch, nicotine gum, or any other product containing nicotine). We excluded persons who consumed nicotine from sources other than cigarettes because of differences in how those products are used in comparison with cigarettes and because they represent only a small proportion of the population of all tobacco users. Additionally, 1,165 (7.6%) of the 15,332 eligible adults described themselves as being of a race/ethnicity other than non-Hispanic white, non-Hispanic black, or Mexican-American; all were excluded from the analyses. Non-Hispanic blacks and Mexican Americans were oversampled in NHANES in 1999–2004. Mexican Americans were oversampled rather than all Hispanics because they represent the majority of Hispanics in the United States (70%). Data for other racial/ethnic groups were analyzed separately because the numbers of subjects in these groups were too low for adequate statistical precision.

Similarly, of 15,794 young people aged less than 20 years, 8,589 (54.4%) were children under age 12 and were excluded. Of the remaining 7,205 adolescents aged 12–19 years, 156 (2.2%) did not answer the adolescent version of the MEC tobacco-use questionnaire, 899 (12.5 %) had no serum cotinine measurement, 262 (3.6%) reported intake of nicotine from other sources, and 498 (6.9%) were of a race/ethnicity other than the 3 primary groups.

A total of 16,156 (out of 31,126) NHANES subjects simultaneously met all eligibility requirements (3,078 exclusive cigarette smokers with no other sources of nicotine and 13,078 non-cigarette-smokers with no other sources of nicotine).

The MEC tobacco questionnaire asked participants, ''During the past 5 days (including today), on how many days did you smoke cigarettes?'' A smoker was defined as a person who reported having smoked 1 or more cigarettes during the previous 5 days; everyone else was considered a nonsmoker. In NHANES 1999–2000, the laboratory limit

Table 1. Demographic Characteristics of Study-Eligible[a] non-Hispanic Whites, non-Hispanic Blacks, and Mexican Americans (Overall) by Age Group, Smoking Status, and Gender, National Health and Nutrition Examination Survey, 1999–2004

	Adolescents (Subjects Aged 12–19 Years)				Adults (Subjects Aged ≥20 Years)			
	Nonsmokers		Smokers		Nonsmokers		Smokers	
	Estimate[b]	95% CI	Estimate[b]	95% CI	Estimate[b]	95% CI	Estimate[b]	95% CI
Males								
No. of subjects	2,339		249		3,512		1,377	
Prevalence, %	90		9		70		30	
Age, years	15.0	14.9, 15.1	17.4	17.1, 17.8	48.1	47.3, 48.8	40.1	39.3, 41.0
No. of cigarettes/day			8.5	7.1, 9.9			15.8	15.0, 16.7
Body weight, kg	67.3	65.9, 68.6	76.9	74.7, 79.0	89.2	88.3, 90.1	83.4	82.4, 84.4
Educational level, %								
Less than high school	88	85, 90	67	59, 75	16	14, 17	27	24, 30
High school diploma/GED	7	6, 9	26	18, 35	22	20, 24	33	29, 37
More than high school	5	4, 7	7	4, 13	62	59, 64	40	36, 44
Females								
No. of subjects	2,284		266		4,049		963	
Prevalence, %	84		16		77		23	
Age, years	15.1	14.9, 15.3	17.1	16.8, 17.3	49.9	49.2, 50.7	41.8	40.7, 43.0
No. of cigarettes/day			7.1	6.0, 8.1			14.0	13.0, 14.9
Body weight, kg	60.6	59.6, 61.6	65.3	62.9, 67.7	75.1	74.1, 76.1	73.5	72.2, 74.8
Educational level, %								
Less than high school	87	84, 89	75	67, 81	17	15, 18	25	22, 28
High school diploma/GED	6	5, 8	18	12, 27	24	23, 26	34	31, 37
More than high school	7	5, 10	7	5, 10	59	56, 62	41	37, 45

Abbreviations: CI, confidence interval; GED, General Education Diploma.

[a] Eligibility criteria: 1) includes nonsmokers and persons who smoked exclusively cigarettes within the last 5 days; 2) excludes persons with tobacco or nicotine intake from other sources; 3) includes persons with available mobile examination center data and nonmissing serum cotinine values; 4) excludes women who were pregnant at the time of the examination; 5) excludes persons with a history of kidney disease; 6) excludes persons with serum creatinine concentrations greater than 2 mg/dL; and 7) excludes users of anticonvulsants, disulfiram, or rifampin.

[b] Mean or percentage, as appropriate.

of detection for cotinine was 0.050 ng/mL; however, the laboratory methods improved over time, and the limit of detection was lowered to 0.015 ng/mL for NHANES 2001–2002 and NHANES 2003–2004. For consistency in using NHANES data from 1999–2004, we employed a limit of detection of 0.050 ng/mL for all subjects. Nonsmokers with cotinine concentrations below 0.050 ng/mL were considered either to have not recently been exposed to secondhand smoke or to have been exposed at such low levels that the exposure could not be detected. For values below the limit of detection (LOD), we used the equation $\mathrm{LOD}/\sqrt{2}$ (0.035 ng/mL), which follows the NHANES coding for 1999–2004 data. Tables 1 and 2 show the subjects' demographic characteristics according to age group (12–19 years and $\geq$20 years), smoking status, gender, and (Table 2) race/ethnicity.

Analytical chemistry

Serum cotinine concentrations were measured by means of a high-performance liquid chromatography/atmospheric-pressure ionization tandem mass spectrometry method that has been described previously (7, 12, 13).

Statistical analysis

We used receiver operating characteristic (ROC) curve analysis to identify optimal rule cutpoints that maximize the total probability of correct classification, using dichotomous self-reported cigarette smoking status as the classifier. ROC analysis is a graphical and quantitative technique (14) which, for a given continuous criterion variable X, can determine an optimal cutpoint c for a classified decision. Here, the criterion variable X is serum cotinine, the classified condition is self-reported smoking status, sensitivity is the percentage of smokers exceeding the cutpoint, and specificity represents the percentage of nonsmokers below the cutpoint.

Because there is no formula for determining the standard error of the optimal cutpoint itself, we used a bootstrap approach with over 2,000 resampling simulations, to build confidence intervals for optimizing c and its corresponding optimal sensitivity and specificity for various demographic subgroups (15). We took the survey weights into account, and we validated our customized SAS program for equally weighted settings against commercially available software for ROC analysis (MedCalc Software, Mariakerke,

Belgium). Although the bootstrap means may not necessarily converge to the onetime point estimates or be the best choice of bootstrap statistic in this situation, the corresponding confidence intervals should give us an estimate of the variability that might be expected in the onetime estimate of c.

A greater degree of separation between the distributions of cotinine among smokers and nonsmokers will result in a higher area under the ROC curve. Paradoxically, this can result in a wider confidence interval for the cutpoint because of the flatness of the objective function. Therefore, there may be widely varying choices in determining a cutpoint within the bounds of its corresponding confidence interval, without much loss due to overall misclassification. For the form of our rule, increasing the cutpoint will decrease sensitivity and increase specificity for identifying tobacco users. If one were deemed more important than the other for a given application, the cutpoint could be adjusted accordingly within the cutpoint confidence interval, with performance bounds indicated through the bootstrap confidence intervals for the sensitivity and specificity. For comparison purposes, we also calculated the onetime sensitivity and specificity for use of the fixed cutpoint 14 ng/mL, which is currently recommended on the basis of the work of Jarvis et al. (2, 3).

All data manipulations were carried out using SAS, version 12 (SAS Institute, Inc., Cary, North Carolina); demographic statistics and histograms were prepared with SAS-callable SUDAAN, version 9 (RTI International, Research Triangle Park, North Carolina). PROC SURVEYSELECT was used to generate the weighted bootstrap samples; it showed reasonable numeric stability with 2,010 replicates.

Misclassification of self-reported smoking status based on serum cotinine concentration using different cotinine cutpoints was computed as 1 minus specificity for self-reported smokers and 1 minus sensitivity for self-reported nonsmokers.

RESULTS

Tables 3 and 4 show geometric mean values for serum cotinine concentration according to smoking status, gender, and race/ethnicity in adults and adolescents, respectively. Among adult smokers, cotinine levels were similar in males and females; they were highest in non-Hispanic blacks and lowest in Mexican Americans. Among adult nonsmokers, cotinine levels were highest in non-Hispanic blacks and similar in non-Hispanic whites and Mexican Americans. The same racial differences were seen in adolescent smokers, though cotinine levels overall were much lower in adolescents than in adults.

Figures 1 and 2 show the distributions of serum cotinine levels and ROC characteristics in adults and adolescents, respectively, overall and by gender and race/ethnicity. There were a few persons who reported being nonsmokers but had cotinine values spread over the range of the values for smokers. While we cannot exclude the possibility of very heavy secondhand smoke exposure for these individuals, it is highly likely that many of them were in fact active smokers who misrepresented their smoking status in their self-reports. The distributions of cotinine values for adolescent self-reported smokers and for Mexican-American adult smokers contained many persons with very low cotinine levels that were in the middle range of those found for nonsmokers. This distribution probably reflects the much higher prevalence of nondaily smoking among adolescents and Mexican Americans.

The optimal serum cotinine cutpoint for discriminating adult smokers from adult nonsmokers was 3.08 ng/mL, with sensitivity of 96.3% and specificity of 97.4% (Table 5). Table 5 shows the corresponding sensitivity and specificity for the currently recommended value of 14 ng/mL to be 92.4% and 98.5%, respectively. Lowering the cutpoint from 14 ng/mL to 3.08 ng/mL gives the optimal combination of sensitivity and specificity, which in this case is only a small improvement of 2.8% in total correct classification. Table 5 also shows that the optimal cutpoint for females (4.47 ng/mL) exceeds that for males (1.78 ng/mL) and that non-Hispanic blacks have the highest discriminating cutpoint (5.92 ng/mL) and Mexican Americans the lowest (0.84 ng/mL).

In all groups, improvement in total correct classification among adults was realized by lowering the previously recommended cutpoint of 14 ng/mL to the values indicated in Table 5. Table 6 shows similar tabulations for adolescents aged 12–19 years. For both adults and adolescents, the greatest improvement in total correct classification obtained by using the ROC cutpoint occurred within the Mexican-American population.

Bootstrap mean estimates of the ROC cutpoint are provided in Web Tables 1 and 2, which are posted on the *Journal*'s website (http://aje.oxfordjournals.org/). Web Tables 1 and 2 provide a rough estimate of the sampling variability of the cutpoints through the corresponding 95% confidence intervals. This allows us to make some inferences regarding differences in cutpoints across groups. We recommend the estimates in Tables 5 and 6.

As Table 6 shows, among all adolescents, the optimal serum cotinine cutpoint was similar to that of adults, at 2.99 ng/mL. The optimal cotinine cutpoint was higher in males than in females, but there was considerable variability in these subgroup estimates. Optimal cutpoints were higher in non-Hispanic whites and blacks and lower in Mexican Americans (2.95 ng/mL, 2.77 ng/mL, and 1.18 ng/mL, respectively).

Table 7 presents data on misclassification of self-reported smoking status based on serum cotinine concentration, using both the optimal ROC value and the fixed 14-ng/mL cutpoint. Among adult self-reported nonsmokers, the misclassification rate was higher for the optimal cotinine cutpoint than for the previously recommended cutpoint. Among adult self-reported smokers, misclassification was considerably lower using the optimal cutpoint as compared with the previous standard. This was particularly true for Mexican-American smokers, for whom the previous standard cutpoint resulted in 28%–33% misclassification.

Misclassification rates were generally higher in adolescents than in adults. The pattern of differences observed when comparing use of the optimal standard cutpoints with use of the previous standard cutpoints was similar in adolescents to that noted above in adults.

Table 2. Demographic Characteristics of Study-Eligible[a] Subjects by Age Group, Smoking Status, Race/Ethnicity, and Gender, National Health and Nutrition Examination Survey, 1999–2004

	Adolescents (Subjects Aged 12–19 Years)				Adults (Subjects Aged ≥20 Years)			
	Nonsmokers		Smokers		Nonsmokers		Smokers	
	Estimate[b]	95% CI	Estimate[b]	95% CI	Estimate[b]	95% CI	Estimate[b]	95% CI
Non-Hispanic Whites								
Males								
No. of subjects	625		70		1,995		681	
Prevalence, %	90		10		71		29	
Age, years	15.0	14.8, 15.2	17.5	17.0, 17.9	49.6	48.8, 50.5	40.3	39.3, 41.4
No. of cigarettes/day			10.1	8.3, 12.0			17.9	17.0, 18.9
Body weight, kg	67.1	65.4, 68.9	77.5	74.8, 80.3	89.9	88.8, 91.0	84.0	82.8, 85.2
Educational level, %								
Less than high school	86	83, 89	64	53, 73	10	9, 12	20	16, 24
High school diploma/GED	8	6, 11	29	19, 41	22	20, 25	36	31, 41
More than high school	6	4, 9	7	3, 16	67	64, 71	44	39, 49
Females								
No. of subjects	585		135		2,225		580	
Prevalence, %	81		19		77		23	
Age, years	15.1	14.8, 15.3	17.0	16.7, 17.3	51.5	50.6, 52.3	42.1	40.7, 43.4
No. of cigarettes/day			7.5	6.4, 8.7			15.0	14.0, 16.0
Body weight, kg	59.2	57.9, 60.6	65.2	62.4, 68.0	74.0	73.0, 75.1	72.2	70.7, 73.8
Educational level, %								
Less than high school	86	81, 89	74	65, 82	12	10, 14	22	19, 25
High school diploma/GED	6	4, 9	19	12, 28	25	23, 28	35	32, 39
More than high school	8	6, 11	7	5, 10	63	60, 67	43	38, 48
Non-Hispanic Blacks								
Males								
No. of subjects	819		75		594		343	
Prevalence, %	92		8		62		38	
Age, years	15.0	14.9, 15.2	17.3	16.8, 17.7	42.8	41.4, 44.3	42.6	41.4, 43.8
No. of cigarettes/day			4.6	3.6, 5.6			11.0	10.2, 11.9
Body weight, kg	68.3	66.5, 70.1	78.4	73.4, 83.5	90.7	88.4, 93.0	82.8	79.9, 85.7
Educational level, %								
Less than high school	92	90, 94	81	69, 89	30	25, 35	42	36, 48
High school diploma/GED	5	4, 6	13	7, 23	23	19, 27	24	20, 29
More than high school	3	2, 5	7	2, 17	48	41, 54	34	29, 39

Table continues

DISCUSSION

This analysis of data from a large, nationally representative group of US smokers and nonsmokers indicated that the optimal overall cutpoint for minimizing the rate of misclassification of self-reported smoking status is a serum cotinine concentration of 3 ng/mL. This cutpoint had a high degree of sensitivity and specificity for adults, giving it excellent discriminative ability. The cutpoint was similar, though sensitivity was substantially lower, in adolescents. This was most likely due to a higher prevalence of occasional smoking and potentially more underreporting of smoking among adolescents.

Our analysis assumed that the self-reported information was correct (i.e., the ''gold standard''). As was discussed above in Materials and Methods, the MEC data collection setup was designed to minimize the likelihood of misreporting. Nonetheless, there was some evidence of misrepresentation of smoking status, particularly among persons defining themselves as nonsmokers who had cotinine values that were in the upper range of those reported for smokers. However, this was relatively uncommon. It would be inaccurate to assume that the cutoffs recommended from our analyses can be used to definitively identify persons who are misrepresenting their smoking status by self-report. Most

Table 2. Continued

	Adolescents (Subjects Aged 12–19 Years)				Adults (Subjects Aged ≥20 Years)			
	Nonsmokers		Smokers		Nonsmokers		Smokers	
	Estimate[b]	95% CI	Estimate[b]	95% CI	Estimate[b]	95% CI	Estimate[b]	95% CI
Females								
No. of subjects	779		56		778		227	
Prevalence, %	93		7		76		24	
Age, years	15.1	14.9, 15.2	17.3	16.9, 17.7	45.6	44.4, 46.8	42.0	40.5, 43.4
No. of cigarettes/day			5.2	4.0, 6.3			9.5	8.3, 10.7
Body weight, kg	66.0	64.6, 67.3	68.3	62.2, 74.5	84.8	83.3, 86.2	82.9	80.2, 85.5
Educational level, %								
Less than high school	90	87, 92	83	69, 91	28	24, 32	35	27, 44
High school diploma/GED	6	4, 7	14	7, 27	22	19, 26	32	25, 40
More than high school	5	3, 7	3	1, 11	50	46, 54	33	26, 41
Mexican Americans								
Males								
No. of subjects	895		104		923		353	
Prevalence, %	90		10		68		32	
Age, years	15.1	14.9, 15.2	17.4	17.1, 17.8	39.1	37.5, 40.6	35.4	34.0, 36.8
No. of cigarettes/day			4.1	3.2, 5.0			6.1	5.1, 7.1
Body weight, kg	66.7	65.3, 68.1	72.0	68.6, 75.4	81.4	79.8, 82.9	79.5	77.2, 81.9
Educational level, %								
Less than high school	91	89, 93	71	62, 79	51	46, 55	63	55, 70
High school diploma/GED	6	4, 7	22	15, 30	23	19, 27	19	15, 25
More than high school	3	2, 6	7	3, 16	26	22, 31	18	13, 25
Females								
No. of subjects	920		75		1,046		156	
Prevalence, %	91		9		84		16	
Age, years	15.2	14.9, 15.5	17.4	17.1, 17.6	40.1	38.3, 41.9	37.2	34.8, 39.6
No. of cigarettes/day			2.9	2.3, 3.6			7.5	6.1, 8.9
Body weight, kg	60.4	59.3, 61.4	63.5	59.3, 67.6	72.0	70.3, 73.7	72.7	69.4, 76.0
Educational level, %								
Less than high school	89	85, 91	67	58, 75	51	47, 56	51	42, 60
High school diploma/GED	7	5, 10	23	16, 31	19	16, 22	24	16, 35
More than high school	4	3, 7	10	5, 19	30	26, 35	24	17, 34

Abbreviations: CI, confidence interval; GED, General Education Diploma.

[a] Eligibility criteria: 1) includes nonsmokers and persons who smoked exclusively cigarettes within the last 5 days; 2) excludes persons with tobacco or nicotine intake from other sources; 3) includes persons with available mobile examination center data and nonmissing serum cotinine values; 4) excludes women who were pregnant at the time of the examination; 5) excludes persons with a history of kidney disease; 6) excludes persons with serum creatinine concentrations greater than 2 mg/dL; and 7) excludes users of anticonvulsants, disulfiram, or rifampin.

[b] Mean or percentage, as appropriate.

misclassification is based on the overlap in actual exposures to tobacco smoke among less intense smokers and heavily exposed nonsmokers rather than misrepresentation of smoking status by self-report. Determination of the optimal cutpoint depends on the frequency of the misclassification rather than the cotinine level. Thus, inclusion of a small number of subjects who have misreported their smoking status will not affect the cutpoint very much.

Differences in cutpoints among subsets based on race/ethnicity and gender were observed. Given the lack of precision in the cutpoint estimates by combinations of race/ethnicity and gender, we recommend that the overall cutpoint be used for persons of both genders. However, given the known racial/ethnic differences in smoking behavior, serum cotinine levels, and nicotine metabolism (7, 9, 11, 16), we recommend the use of race/ethnicity-specific cutpoints. For adults, we recommend cutpoints of 5 ng/mL for non-Hispanic whites, 6 ng/mL for non-Hispanic blacks, and 1 ng/mL for Mexican Americans; for adolescents, we recommend 3 ng/mL for non-Hispanic whites and blacks and

Table 3. Geometric Mean Values for Serum Cotinine Concentration Among Study-Eligible Adults Aged 20 Years or Older, Overall and by Smoking Status, Gender, and Race/Ethnicity, National Health and Nutrition Examination Survey, 1999–2004

	Total			1999–2000			2001–2002			2003–2004		
	Cotinine Value	95% CI	Sample Size	Cotinine Value	95% CI	Sample Size	Cotinine Value	95% CI	Sample Size	Cotinine Value	95% CI	Sample Size
						Smokers						
All subjects	122.37	112.04, 132.69	2,527	120.12	103.95, 136.28	759	121.56	104.93, 138.19	895	125.20	102.50, 147.90	873
Gender												
Male	122.08	110.79, 133.37	1,492	118.77	98.81, 138.72	448	121.75	104.42, 139.09	530	125.52	101.32, 149.72	514
Female	122.72	108.52, 136.91	1,035	121.88	102.77, 140.99	311	121.33	97.72, 144.94	365	124.82	92.96, 156.67	359
Race/ethnicity												
Non-Hispanic white	143.44	130.35, 156.54	1,261	145.64	126.71, 164.58	340	136.55	117.04, 156.05	451	148.45	118.05, 178.86	470
Non-Hispanic black	181.65	162.28, 201.03	570	190.39	164.12, 216.65	169	195.63	145.12, 246.14	205	161.55	133.71, 189.39	196
Mexican-American	32.10	23.57, 40.64	509	34.55	15.56, 53.55	179	35.75	24.26, 47.24	171	27.69	11.79, 43.59	159
						Nonsmokers						
All subjects	0.08	0.08, 0.09	8,123	0.09	0.08, 0.10	2,508	0.07	0.07, 0.08	2,847	0.08	0.07, 0.10	2,768
Gender												
Male	0.09	0.08, 0.10	3,758	0.10	0.08, 0.12	1,149	0.08	0.07, 0.10	1,327	0.10	0.08, 0.12	1,282
Female	0.07	0.07, 0.08	4,365	0.08	0.07, 0.09	1,359	0.07	0.06, 0.07	1,520	0.07	0.06, 0.09	1,486
Race/ethnicity												
Non-Hispanic white	0.08	0.07, 0.08	4,220	0.09	0.07, 0.10	1,158	0.07	0.06, 0.07	1,551	0.08	0.06, 0.09	1,511
Non-Hispanic black	0.16	0.13, 0.19	1,372	0.16	0.11, 0.20	425	0.16	0.12, 0.21	459	0.16	0.10, 0.22	488
Mexican-American	0.07	0.06, 0.08	1,969	0.07	0.06, 0.08	726	0.09	0.05, 0.12	649	0.07	0.05, 0.08	594

Abbreviation: CI, confidence interval.

Table 4. Geometric Mean Values for Serum Cotinine Concentration Among Study-Eligible Adolescents Aged 12–19 Years, Overall and by Smoking Status, Gender, and Race/Ethnicity, National Health and Nutrition Examination Survey, 1999–2004

	Total			1999–2000			2001–2002			2003–2004		
	Cotinine Value	95% CI	Sample Size	Cotinine Value	95% CI	Sample Size	Cotinine Value	95% CI	Sample Size	Cotinine Value	95% CI	Sample Size
						Smokers						
All subjects	40.61	30.15, 51.07	551	45.27	28.44, 62.10	187	38.82	23.01, 54.63	192	38.14	14.00, 62.28	172
Gender												
Male	54.41	34.08, 74.73	267	63.32	10.87, 115.77	96	52.88	26.64, 79.11	98	47.32	7.55, 87.08	73
Female	33.42	22.80, 44.05	284	36.74	24.49, 48.98	91	29.33	8.34, 50.32	94	34.32	11.65, 56.99	99
Race/ethnicity												
Non-Hispanic white	48.92	34.21, 63.62	205	54.30	32.10, 76.50	65	44.61	17.98, 71.24	76	48.53	12.78, 84.27	64
Non-Hispanic black	56.25	38.77, 73.73	131	41.84	18.41, 65.27	43	82.67	1.96, 163.37	40	52.97	24.84, 81.10	48
Mexican-American	13.70	7.68, 19.72	179	16.61	5.23, 27.99	68	16.42	3.48, 29.36	59	9.62	0.42, 18.83	52
						Nonsmokers						
All subjects	0.15	0.13, 0.18	4,955	0.19	0.14, 0.24	1,624	0.12	0.09, 0.16	1,709	0.17	0.13, 0.21	1,622
Gender												
Male	0.17	0.14, 0.20	2,506	0.23	0.14, 0.31	811	0.13	0.10, 0.16	842	0.18	0.13, 0.23	853
Female	0.14	0.11, 0.16	2,449	0.15	0.12, 0.19	813	0.12	0.08, 0.16	867	0.15	0.11, 0.20	769
Race/ethnicity												
Non-Hispanic white	0.15	0.12, 0.18	1,210	0.21	0.13, 0.29	292	0.11	0.08, 0.14	498	0.17	0.11, 0.22	420
Non-Hispanic black	0.29	0.25, 0.33	1,598	0.33	0.27, 0.39	453	0.29	0.22, 0.35	525	0.27	0.19, 0.36	620
Mexican-American	0.09	0.08, 0.10	1,815	0.09	0.07, 0.11	764	0.09	0.07, 0.11	552	0.08	0.06, 0.10	499

Abbreviation: CI, confidence interval.

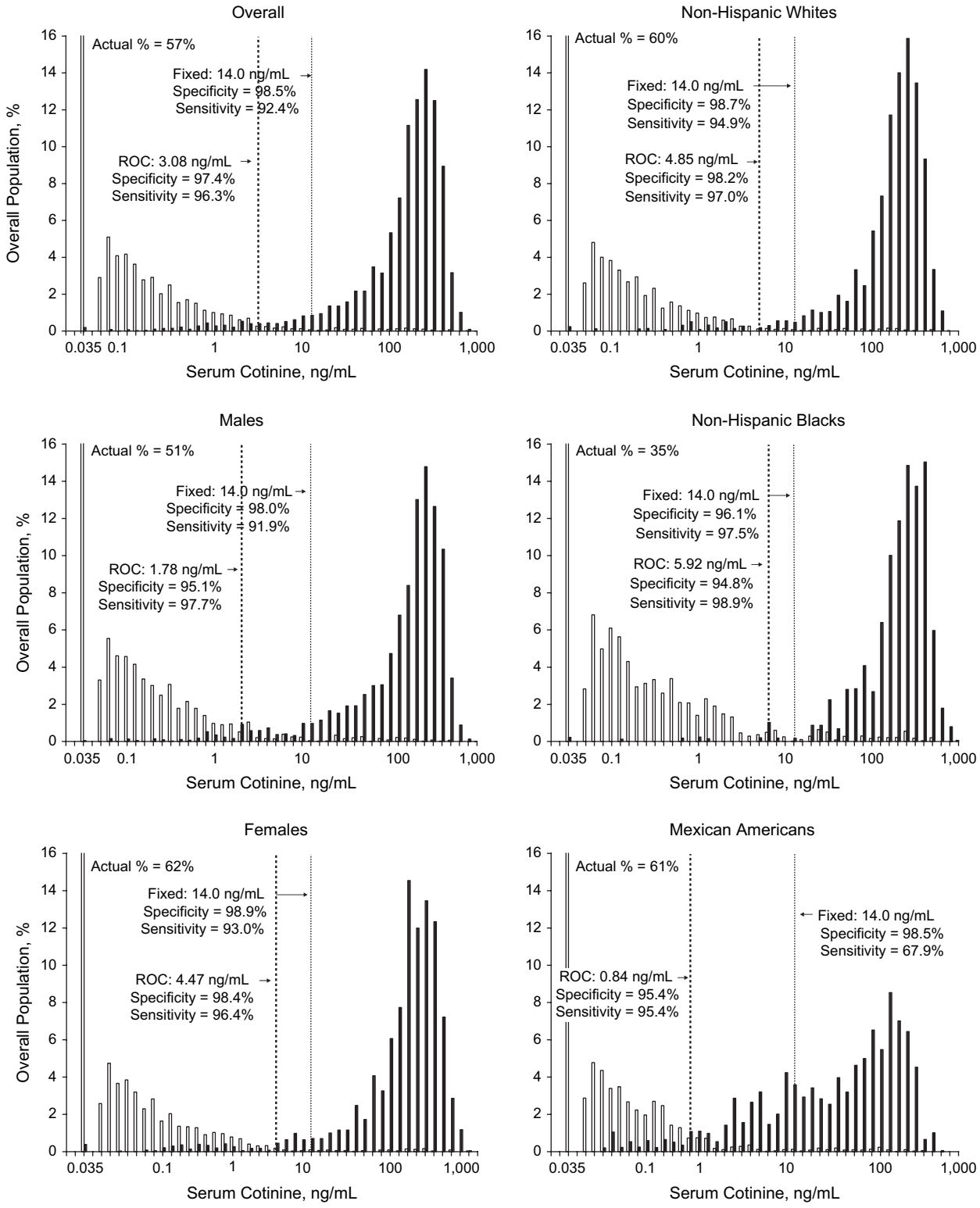

Figure 1. Distribution of serum cotinine concentrations and selected receiver operating characteristic (ROC) and other operating characteristics of cutpoint rules among adult smokers and nonsmokers aged 20–85 years, overall and by gender and race/ethnicity, National Health and Nutrition Examination Survey, 1999–2004. The solid and open bars represent the self-reported distributions of smokers and nonsmokers, respectively; a heavy dotted line is drawn at the optimal ROC cutpoint; and a lighter dotted line is drawn at the common fixed cutpoint of 14 ng/mL. "Overall Population, %" on the *y*-axis refers to the percentage of all smokers or nonsmokers in the respective groups. "ROC" is the optimal cutoff point derived using the ROC curve, which is the representation of the tradeoffs between sensitivity and specificity. All percentages of nonsmokers with serum cotinine levels of 0.035 ng/mL or less are cropped at 16%.

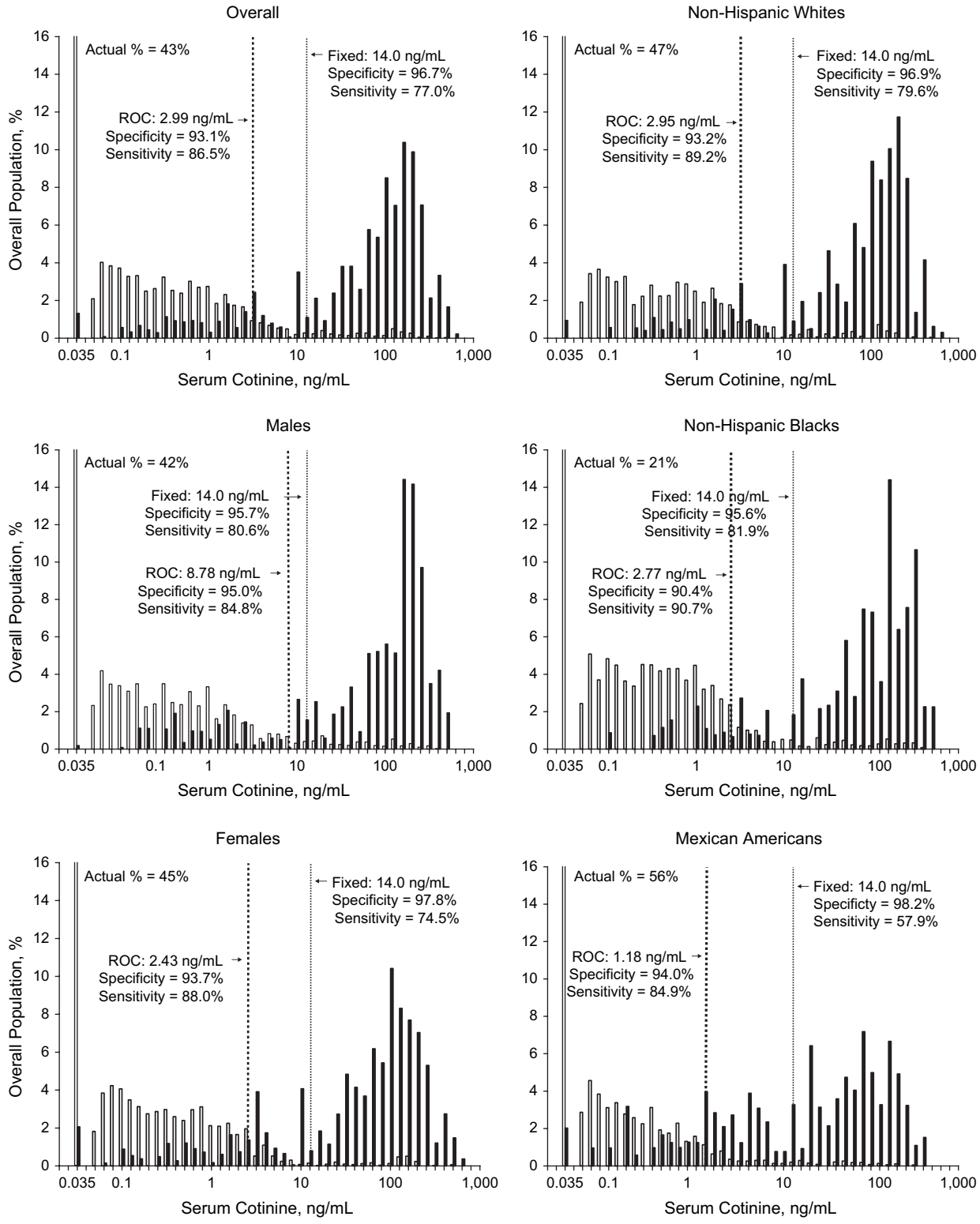

Figure 2. Distribution of serum cotinine concentrations and selected receiver operating characteristic (ROC) and other operating characteristics of cutpoint rules among adolescent smokers and nonsmokers aged 12–19 years, overall and by gender and race/ethnicity, National Health and Nutrition Examination Survey, 1999–2004. Plot characteristics are the same as those for Figure 1. "Overall Population, %" on the *y*-axis refers to the percentage of all smokers or nonsmokers in the respective groups. All percentages of nonsmokers with serum cotinine levels of 0.035 ng/mL or less are cropped at 16%.

Table 5. Receiver Operating Characteristic Optimal Cutpoints and Operating Characteristics, Including Those for the Commonly Used Cutpoint of 14.0 ng/mL, for Cotinine Classification of Smokers Versus Nonsmokers in Adults, Overall and by Gender and Race/Ethnicity, National Health and Nutrition Examination Survey, 1999–2004

	No. of Smokers	ROC Optimal Cutpoint					Fixed Cutpoint of 14 ng/mL[a]	
		Cutpoint, ng/mL	Sensitivity, %	Specificity, %	AUROC	95% CI[b]	Sensitivity, %	Specificity, %
All subjects	2,527	3.08	96.3	97.4	0.991	0.989, 0.993	92.4	98.5
Gender								
Male	1,492	1.78	97.7	95.1	0.991	0.988, 0.993	91.9	98.0
Female	1,035	4.47	96.4	98.4	0.990	0.987, 0.994	93.0	98.9
Race/ethnicity								
Non-Hispanic white	1,261	4.85	97.0	98.2	0.993	0.990, 0.995	94.9	98.7
Non-Hispanic black	570	5.92	98.9	94.8	0.987	0.980, 0.993	97.5	96.1
Mexican-American	509	0.84	95.4	95.4	0.983	0.976, 0.989	67.9	98.5
Gender and race/ethnicity								
Male								
Non-Hispanic white	681	6.79	96.4	97.8	0.993	0.990, 0.996	95.1	98.3
Non-Hispanic black	343	13.3	98.1	95.4	0.985	0.978, 0.993	97.8	95.4
Mexican-American	353	0.79	96.0	93.5	0.976	0.967, 0.985	67.1	97.5
Female								
Non-Hispanic white	580	4.73	97.5	98.8	0.992	0.988, 0.996	94.4	99.1
Non-Hispanic black	227	5.92	99.0	95.8	0.988	0.979, 0.997	97.2	96.5
Mexican-American	156	0.84	93.9	97.1	0.988	0.980, 0.996	72.3	99.4

Abbreviations: AUROC, area under the ROC curve; CI, confidence interval; ROC, receiver operating characteristic.
[a] No data are presented for the AUROC, since it is not applicable.
[b] All AUROCs (dimensionless quantities) were significantly greater than the null hypothesis of true AUROC = 0.50.

Table 6. Receiver Operating Characteristic Optimal Cutpoints and Operating Characteristics, Including Those for the Commonly Used Cutpoint of 14.0 ng/mL, for Cotinine Classification of Smokers Versus Nonsmokers in Adolescents, Overall and by Gender and Race/Ethnicity, National Health and Nutrition Examination Survey, 1999–2004

	No. of Smokers	ROC Optimal Cutpoint					Fixed Cutpoint of 14 ng/mL[a]	
		Cutpoint, ng/mL	Sensitivity, %	Specificity, %	AUROC	95% CI[b]	Sensitivity, %	Specificity, %
All subjects	551	2.99	86.5	93.1	0.950	0.942, 0.958	77.0	96.7
Gender								
Male	267	8.78	84.8	95.0	0.957	0.945, 0.968	80.6	95.7
Female	284	2.43	88.0	93.7	0.948	0.937, 0.959	74.5	97.8
Race/ethnicity								
Non-Hispanic white	205	2.95	89.2	93.2	0.959	0.946, 0.973	79.6	96.9
Non-Hispanic black	131	2.77	90.7	90.4	0.953	0.933, 0.972	81.9	95.6
Mexican-American	179	1.18	84.9	94.0	0.948	0.933, 0.963	57.9	98.2
Gender and race/ethnicity								
Male								
Non-Hispanic white	70	8.78	87.0	96.1	0.966	0.949, 0.984	82.6	96.6
Non-Hispanic black	75	6.01	88.6	90.7	0.950	0.918, 0.982	84.8	92.5
Mexican-American	104	1.18	88.4	90.9	0.951	0.930, 0.972	67.5	97.0
Female								
Non-Hispanic white	135	2.95	90.4	94.0	0.956	0.936, 0.976	78.0	97.2
Non-Hispanic black	56	2.81	89.5	94.7	0.952	0.930, 0.974	78.4	98.6
Mexican-American	75	0.66	85.8	93.3	0.948	0.928, 0.967	45.7	99.5

Abbreviations: AUROC, area under the ROC curve; CI, confidence interval; ROC, receiver operating characteristic.
[a] No data are presented for the AUROC, since it is not applicable.
[b] All AUROCs (dimensionless quantities) were significantly greater than the null hypothesis of true AUROC = 0.50.

Table 7. Rates of Misclassification of Self-Reported Smoking Status Based on Serum Cotinine Concentration Using Receiver Operating Characteristic Optimal Cutpoints and a Fixed Cutpoint of 14 ng/mL Among Adults Aged 20 Years or Older, National Health and Nutrition Examination Survey, 1999–2004

	Cotinine Cutpoint, ng/mL	Misclassification, %	
		Self-Reported Nonsmokers	Self-Reported Smokers
Gender			
Male			
ROC optimal cutpoint	1.78	4.9	2.3
Fixed cutpoint	14.0	2.0	8.1
Female			
ROC optimal cutpoint	4.47	1.6	3.6
Fixed cutpoint	14.0	1.1	7.0
Race/ethnicity and gender			
Non-Hispanic white			
Male			
ROC optimal cutpoint	6.79	2.2	3.6
Fixed cutpoint	14.0	1.7	4.9
Female			
ROC optimal cutpoint	4.73	1.2	2.5
Fixed cutpoint	14.0	0.9	5.6
Non-Hispanic black			
Male			
ROC optimal cutpoint	13.30	4.6	1.9
Fixed cutpoint	14.0	4.6	2.2
Female			
ROC optimal cutpoint	5.92	4.2	1.0
Fixed cutpoint	14.0	3.5	2.8
Mexican-American			
Male			
ROC optimal cutpoint	0.79	6.5	4.0
Fixed cutpoint	14.0	2.5	32.9
Female			
ROC optimal cutpoint	0.84	2.9	6.1
Fixed cutpoint	14.0	0.6	27.7

Abbreviation: ROC, receiver operating characteristic.

Table 8. Rates of Misclassification of Self-Reported Smoking Status Based on Serum Cotinine Concentration Using Receiver Operating Characteristic Optimal Cutpoints and a Fixed Cutpoint of 14 ng/mL Among Adolescents Aged 12–19 Years, National Health and Nutrition Examination Survey, 1999–2004

	Cotinine Cutpoint, ng/mL	Misclassification, %	
		Self-Reported Nonsmokers	Self-Reported Smokers
Gender			
Male			
ROC optimal cutpoint	8.78	5.0	15.2
Fixed cutpoint	14.0	4.3	19.4
Female			
ROC optimal cutpoint	2.43	6.3	12.0
Fixed cutpoint	14.0	2.2	25.5
Race/ethnicity and gender			
Non-Hispanic white			
Male			
ROC optimal cutpoint	8.78	3.9	13.0
Fixed cutpoint	14.0	3.4	17.4
Female			
ROC optimal cutpoint	2.95	6.0	9.6
Fixed cutpoint	14.0	2.8	22.0
Non-Hispanic black			
Male			
ROC optimal cutpoint	6.01	9.3	11.4
Fixed cutpoint	14.0	7.5	15.2
Female			
ROC optimal cutpoint	2.81	5.3	10.5
Fixed cutpoint	14.0	1.4	21.6
Mexican-American			
Male			
ROC optimal cutpoint	1.18	9.1	11.6
Fixed cutpoint	14.0	3.0	32.5
Female			
ROC optimal cutpoint	0.66	6.7	14.2
Fixed cutpoint	14.0	0.5	54.3

Abbreviation: ROC, receiver operating characteristic.

1 ng/mL for Mexican Americans. The higher cotinine cutpoint for adult non-Hispanic blacks is most likely due to slower metabolism of cotinine in comparison with the other racial/ethnic groups (7). The lower cotinine cutpoint for Mexican Americans is most likely due to lighter smoking associated with lower average cotinine levels among smokers.

Our finding was that for the overall US population, a cotinine cutpoint of 3 ng/mL was optimal. This represents a large change from the value of 14 ng/mL determined by Jarvis et al. (3) more than 20 years ago. As we noted above, the Jarvis analysis was carried out at a time when secondhand smoke exposure in the population was higher than it is today. Heavy secondhand smoke exposure can

produce serum cotinine levels greater than 10 ng/mL in nonsmokers, and a relatively high cutpoint is necessary to exclude most nonsmokers. In the United States currently, secondhand smoke exposure is generally low, and it is unusual to see serum cotinine levels greater than 1 ng/mL in nonsmokers. It is notable that in an analysis of the cotinine cutpoint based on saliva levels in a relatively small number of university students, faculty, and staff in Switzerland in 1995, Etter et al. (17) found an optimal value of 7 ng/mL.

To compare the impact of using a lower cutpoint than 14 ng/mL, we compared specificity and sensitivity for the new and old cutpoints. As expected, specificity was slightly higher with the higher cutpoint; however, sensitivity was substantially better using the lower cutpoint. Sensitivity was especially improved with the lower cutpoint for Mexican-American smokers and adolescents in general—groups that have higher proportions of occasional smokers and smokers of a few cigarettes per day. Thus, the use of the 14-ng/mL cutpoint results in many more smokers' being incorrectly classified as nonsmokers than the new cutpoint of 3 ng/mL, particularly among Mexican Americans.

The analysis presented here is relevant in countries where the prevalence of heavy secondhand smoke exposure is low. The major concern with using a lower cutpoint would be inaccuracy in groups of persons with extremely high secondhand smoke exposure, in whom there would be a greater likelihood of misclassifying a nonsmoker as a smoker.

A comment on cotinine cutpoints that use biologic fluids other than serum or plasma is warranted. Saliva and plasma cotinine concentrations are quite similar, so the optimal cutpoint in saliva would be the same as that recommended for blood (2). Urinary cotinine concentrations based on unconjugated cotinine alone are approximately 5 times those of plasma cotinine (1). Therefore, a urinary cotinine concentration of 15 ng/mL would be the appropriate cutpoint corresponding to our serum estimate. This level contrasts with urinary cutpoints proposed in various other studies ranging from 20 ng/mL to 550 ng/mL (18).

It is notable that the average serum cotinine concentration in our adult smokers was 122 ng/mL. This is considerably lower than the average of 200 ng/mL reported by O'Connor et al. (19) in their analysis of NHANES data from 1998–2002. There are 2 possible explanations for this discrepancy. First, our adult smokers included persons aged 20 years or older, while O'Connor et al. included only those aged 25 years or older. Many of our younger adult smokers were likely to still be in the process of becoming an established smoker. When we consider only smokers aged 25 years or more, we find cotinine values similar to those presented by O'Connor et al. (data not shown). A second explanation is that O'Connor et al. presented arithmetic means in their paper (19), whereas in our analysis we used geometric means, which produce lower mean values.

In conclusion, we find that in the United States at present, secondhand smoke exposure is generally low, such that the optimal serum cotinine cutpoint for distinguishing smokers from nonsmokers is considerably lower than that determined in the 1980s. Use of the revised cutpoint is important for researchers investigating the relation between smoking and health and potentially for clinicians. Verification of nonsmoking status in smoking-cessation clinical trials and by clinicians is often based on cotinine levels, and the lower cutpoint will reduce misclassification of light or occasional smokers who falsely report having stopped smoking entirely. Epidemiologic research using cotinine levels should better identify light and occasional smokers using the recommended 3-ng/mL cutpoint. However, caution should be exercised in using the new cutpoints in studying the health risks of secondhand smoke. Our recommended cutpoints are likely to misclassify nonsmokers with heavy secondhand smoke exposure, who are the persons most likely to suffer from injury due to secondhand smoke.

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Dr. Benowitz has served as a paid expert witness in litigation against tobacco companies. Drs. Bernert, Caraballo, and Holiday have no conflicts to declare.

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