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Observations on the number of saliva cotinine positives over a nine-year period

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In the past, many insurance companies would take customers at their word when completing application forms. With the rising cost of healthcare, these companies now increasingly insist on independent checks to identify fraudulent claims.[1,2] Companies are especially interested in using laboratory tests to identify controllable activities such as smoking, which in turn helps them to set insurance premiums correctly. Smokers may have to pay up to twice as much for some types of insurance, especially life assurance, because they face a higher risk of ill health and premature death. Passive smoking (usually seen in people who live with smokers) also has an impact on health.[3,4]

When nicotine from tobacco smoke is taken into the lungs and enters the bloodstream, it is metabolised in the liver and converted to cotinine by enzymes such as cytochrome P450 2A6, then eventually excreted in the urine as trans-3'-hydroxycotinine.[5,6] Cotinine diffuses easily from the blood into saliva, and salivary and blood levels have been shown to correlate.[7,8] Cotinine in saliva has a longer half-life than nicotine (greater than 10 hours), and is a specific and sensitive marker for determining exposure to tobacco and nicotine both in smokers and passive smokers.[9]

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The levels of cotinine considered significant have changed over the years. Previous cut-off levels were 10 ng/mL: anything greater was considered as positive and consistent with smoking, and anything less was considered negative.[10] In more recent years, this cut-off has been changed to 13 ng/mL and now includes an equivocal range of 7–13 ng/mL, to try to discriminate between active smokers, passive smokers and non-smokers.[11–13]

Up to March 2011, Quest Diagnostics provided services for a number of insurance companies to test potential life insurance clients for smoking by measuring salivary cotinine levels as part of the application criteria. All samples were self-collected by insurance applicants using the Omin-SAL collection device. The collected samples were processed and assayed using a standard saliva cotinine assay (Cozart oral fluid microplate enzyme immunoassay [EIA]).

Over a nine-year period, 39,651 saliva samples were assayed for cotinine. Some of the samples either did not have the optimal amount of fluid in the specimen tube (2.1 mL) or lacked fluid completely. Of the samples received, 104 (0.3%) had to be discarded because of the absence of buffer in the collection tube.

Of the samples tested, the majority (93.6%) were reported as negative (<7 ng/mL); only 2433 (6.1%) samples were repeatedly positive (>13 ng/mL) (Table 1). There were also 80 samples that fell into the equivocal range (7–13 ng/mL).

From the 1970s onwards, smoking prevalence fell rapidly until the mid-1990s. Since then, the rate has continued to fall slowly, and in 2007 around a fifth (22%) of men (aged 16 and over) were reported as cigarette smokers. The rate remained stable between 2007 and 2009, but fell to 21% in 2010.[14,15] In 2007, smoke-free legislation was implemented in England, making virtually all enclosed public places and workplaces smoke-free.[16]

Over a nine-year period (2003–2011), the laboratory tested nearly 40,000 self-taken saliva samples for cotinine level. Some of these had to be discarded as they were unsuitable for testing due to lack of buffer; usually the container arrived with no buffer and only a dried, slightly blue collector strip. The assumption was that the buffer had leaked out during transport or the patient had discarded it, not realising its importance. Of the remaining samples, the majority were shown to be negative for cotinine, and only 6% were positive.

What was interesting was that, from 2003 to 2008, the positivity rate remained fairly constant (average: 6.3%), but in 2009 and 2010 this figure fell to 4.7%. Although the number of data points is small, a Fisher two-tailed test gave a P value of 0.0043, indicating a significant reduction in positivity rate. Further analysis of the number of positives for 2011 onwards is needed to see if this represented a true fall or simply a reflection of the reducing number of samples tested. The drop in the percentage of positive samples does seem to be in line with the drop in smoking among the general population since the introduction of smoke-free legislation in 2007. However, as the laboratory did not have access to the smoking habits of the people who provided the samples, it would be difficult to draw a solid conclusion.

What about the 80 samples (0.2%) that were in the 7–13 ng/mL range? Studies have shown that passive smokers, usually people who live with a smoker, will often

Table 1. Saliva cotinine results, 2003–2011.

	Negative <7 ng/mL	Positive >13 ng/mL	Equivocal 7–13 ng/mL	TNP	Total	%
2003	5426	374	11	10	5800	6.45
2004	5696	409	4	9	6105	6.70
2005	5533	332	11	13	5865	5.66
2006	5550	349	14	13	5899	5.92
2007	6914	510	15	20	7459	6.84
2008	3495	231	12	12	3750	6.16
2009	3410	175	9	10	3604	4.86
2010	1068	53	4	16	1141	4.65
2011	26	1	0	1	28	
Total	37,118	2434	80	104	39,651	6.14
%	93.6	6.1	0.2	0.3		

P=0.0043; CI 95%: 0.6901–2.37766; df: 6.

fall into this range. These samples may also come from smokers who have abstained or are trying to quit and their bodies could be eliminating the cotinine. It would have been an interesting follow-on experiment to retest these samples in three to six months to see if they still gave equivocal results or had become negative.

It should be stressed that this is only a snapshot of the results. Nothing is known about the insurance applicants, including their ethnicity, the number of cigarettes smoked, when the sample was taken, and whether or not they are non-smokers living with a smoker. All of these factors have been shown to influence cotinine levels and should be taken into consideration before any conclusions can be drawn.[17–20] The assay simply detected and quantified cotinine levels in the saliva sample that was presented at the time. Further information about the subject would be required to ascertain if the person was a smoker, non-smoker or passive smoker. □

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