applied sciences

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A Novel Earwax Self-Sampling Device: A Feasibility Study

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Featured Application: We introduce a self-sampling earwax device for the analysis of chronic levels of different substances relevant to chronic diseases.

Abstract: (1) Background: Earwax might provide the long-term concentration of substances that are altered in chronic diseases. Standardised earwax extraction has to be exclusively performed by clinicians. We investigated the safety, reliability, and tolerance of a novel self-sampling earwax device in comparison with a clinical method; (2) Methods: We compared the reliability between both methods in a longitudinal study. We first cleaned both ears at baseline in 37 controls. Secondly, we obtained a sample a month after by extracting earwax from the right ear with a novel self-sampling device, and from the left ear by using the clinical method. Reliability of both methods was measured by coefficients of variation; (3) Results: The weight of the baseline samples was not significantly different between ear sides. The reliability of the two methods was not significantly different. The self-extraction method removed eight times more earwax than the clinical method. The new method proved to be well tolerated; (4) Conclusions: The novel device was as reliable as the clinical method in sampling earwax. In view of its practicality, safety, tolerability and efficiency, the new method may have clinical applications at a reduced cost.

Keywords: self-sampling earwax device; clinical method; cotton swabs

Citation: Herane-Vives, A.; Sandoval, R.; Ortega, L.; Espinoza, S.; Cleare, A.; Hayes, A.; Ortuzar, E.; Valdenegro, T.; Aguilo, B.; Benöhr, J.; et al. A Novel Earwax Self-Sampling Device: A Feasibility Study. *Appl. Sci.* **2021**, *11*, x. https://doi.org/10.3390/xxxxx

Academic Editors: Lapo Governi and Junseop Lee

Received: 12 February 2021
Accepted: 12 April 2021
Published: 16 April 2021

1. Introduction

There is a great need to develop new technologies to improve detection of common conditions, such as diabetes and depression. Earwax offers the possibility of extracting putative naturally occurring endogenous substances which could be detected for analysis. Several substances have been measured in earwax [1], which could be considered potential biomarkers for improving the detection of chronic diseases [2], like glycaemic abnormalities typical of diabetes. In this condition, currently the gold standard, raised levels of glycated haemoglobin (HbA_{1c}) are imprecise in relation to chronic average of

glucose levels [3]. We recently found that earwax glucose offers almost 60% improved detection than HbA_{1c} in measuring chronic glucose levels [4].

To date, no medical device has been designed for sampling earwax for analysis of endogenous substances. Earwax extraction is performed by clinicians in cases of external ear pathologies, in symptomatic impacted earwax [5], and to allow an unimpaired view of the tympanic membrane [6]. The irrigation method of extraction, using the Reiner-Alexander syringe is one of the most common clinical extraction methods because it is safe and effective [5]. This is a costly procedure, impractical for routine use. Due to its bacteriostatic properties [7,8], similarly to other types of wax originating from the animal kingdom, such as honeycombs [9], human wax can easily be shipped to a lab for analysis by post without any specific requirements typical of many other biological samples such as blood, which are required to comply with strict regulations with regard to safety and quality control. Nonetheless, a comprehensive systematic review showed that, although some earwaxolytic solutions (containing mineral oils) may provide some success at removing earwax, no self-cleaning device, mechanic or electric, is as effective as the clinical extraction methods [10] and commercial availability is limited.

Self-cleaning cotton swabs or "buds" popularly used to cleanse external ears and the auditive canal [11] can cause irritation, external ear diseases, and impacted earwax associated with canal bleeding [12,13]. Furthermore, by stimulating sensitive fibres surrounding the external auditive canal, cotton buds elicit various pleasurable visceral sensations, which might be associated with their continued use and potential abuse [14,15]. The amount of earwax extracted by these devices is also variable, as the method is not standardised. Hence, cotton buds would be better not be used for analytic applications of earwax.

That is why we firstly designed a pre-clinical pilot study to test the safety of using a novel self-sampling plastic earwax device inside the ear, and the wax cleaning effect of different materials for removing artificial wax from a piece of animal skin. We used porcine skin because it has striking similarities to the human skin [16]. The results confirmed that the use of this novel device, given its incorporated break (Arrow 5 in Figures 1a to 1d) may be safe for using inside the ear and showed that an organic cellulose sponge that was impregnated with a specific concentration of a mineral oil solution (50%) [10] of Magnesium Chloride ($MgCl_2$) may constitute the most effective tip for the novel device. While this sponge revealed the best abrasive and absorptive wax performance, the incorporated mineral oil solution confirmed its previously found earwaxolytic property [7] and reassured us that its appliance was going to be innocuous for the external auditory canal. It has previously shown prophylactic features for the skin [17]. A detailed diagram of the device can be found in Figures 1 and a 3D view of it can be seen in Figure 2.

In this feasibility study we tested an inexpensive self-sampling new standardised device for obtaining earwax for analysis in comparison with a clinical method, based on the Reiner-Alexander syringe in healthy volunteers. We assessed reliability in wax sampling and safety of use of the new method. We also took into consideration users' experience. We predicted that: (1) The self-sampling external ear device would be as reliable as the most commonly used clinical method at obtaining earwax from healthy ears, and (2) The use of the novel self-sampling earwax device would be considered safer than other self-cleaning methods, such as cotton swabs, and as comfortable as the clinical method.

2. Materials and Methods

Healthy participants were recruited from staff and student volunteers of the Universidad Católica del Norte (UCN) in Coquimbo, Chile and from its catchment area. We used public and internal advertisements to recruit participants. Participants were not compensated for taking part in the research. All participants were assessed by the same clinical researcher (S.E). The sample consisted of 37 healthy participants; 20 were female, the mean age was 29.9 years, and the mean Body Mass Index (BMI) was 25.6 kg/m^2.

2.1. Study Design

The study included two interviews that were conducted one month apart. A baseline (day = 1) and a follow-up (day = 30) visit. During the baseline assessment, participants had a comprehensive clinical interview to rule out the presence of any medical illness, including any ear pathologies or abnormality, such as a narrow ear canal. During the baseline assessment, a range of demographic, clinical and environmental factors were systematically assessed. Once participants were included in the study, both external ears were cleaned using the Reiner-Alexander syringe. This syringe is commonly used by general practice doctors for removing impacted earwax. Participants were instructed to avoid using "cotton buds" or the use of any other external ear self-cleaning method during the follow-up period. This allowed us to collect a standardised amount of secreted earwax 30 days after the baseline visit (the follow-up assessment). It has previously been found that 3–8 mg of earwax represents four weeks of earwax production in healthy ears [18]. Furthermore, since it has been shown that the amount of earwax does not differ between ear sides [19], at least in term of lipids, which constitutes the largest earwax weight fraction [20], we were then capable of designing a prospective case-control, rather than a prospective cross-sectional research study. Thus, at the follow-up assessment, left earwax samples were obtained using the clinical method (controls) and the right using the self-sampling earwax device (cases). A standardised satisfaction survey was administrated for evaluating participants' self-sampling external ear device user experience after the follow-up visit. This instrument was designed using attitude scale construction techniques for summated *(Likert)* rating scales of 5 points [21]. Some categorical and continuous variables, such as cotton swab frequency of use were also recorded in the follow-up survey. We excluded Asian people and people intellectual disabilities, because differences in the type of earwax and production of it [21; 22].

2.2. Study Population

All participants were recruited during a southern hemisphere winter (between 6 July and 3 August 2018). It has previously been found that different seasons vary the triglyceride composition of earwax [19]. We excluded people of Asian ethnicity and people with intellectual disabilities, due to their differences regarding earwax composition and quantity, respectively [10,11]. Participants were free from medical illness, either chronic, current or during the previous month. Participants also reported no history of any ear pathologies, such as impacted earwax, perforated eardrum, currently or over the previous month.

2.3. Earwax Sample Collection

The clinical research assistant (S.E) was trained in the use of the Reiner-Alexander syringe by an Ear-Nose-Throat (ENT) specialist doctor in May 2018. Before cleaning both ears, the external auditory canal was examined using an otoscope to rule out the presence of any external ear pathology, such as impacted earwax or a perforated eardrum or ear canal shape variation, such a narrow one, which may disregard the use of the novel self-sampling device. The Reiner-Alexander syringe slowly injects 50 cc of warm tap water at 37 °C inside the external ear canal. The process of syringing creates a sensation of mild pressure in the ear as the warm water from the syringe flushes the wax out. The expelled water and the obtained earwax secretion were collected in a kidney basin. The otoscopic status was checked again after the use of this clinical method of earwax extraction. The earwax solution was dried using the displacement method of N_2 at 25 °C. We did not repeat this clinical procedure. During the follow-up visit, earwax samples from the left ear were again taken using the clinical method, whereas participants self-collected earwax samples from their right ears using the self-sampling device, according to the manufacturer instructions (www.trears.com). Each tip of the self-sampling device was weighed before its use, using a highly precise digital analytical balance. It was not needed

to dry the earwax samples obtained by the self-sampling device since it uses a dry method of extraction which bypassed that stage before analysis. The four labelled earwax samples were stored at 4 °C until they were weighed, using again the same digital analytical balance.

2.4. Data Analysis

Results were reported according to the STROBE guidelines for observational studies. The data were checked for normality using the *Shapiro–Wilk* test statistical and graphical methods, including histograms. All samples were non-normally distributed (all $p < 0.05$). Therefore, we used an adapted version of the Coefficients of Variation (CVs) for assessing the reliability of both earwax sampling methods. This is because, while there are plenty for mean comparisons, there are not well documented non-parametric methods for comparing CVs in the common statistical practice. Therefore, CV comparisons were performed through the Vector of Sample's Squared Relative Dispersion (VSSRDs) (see Appendix A). Nonetheless, an equalness between the VSSRD mean of each sample is equivalent to equalness with its respective CV.

Thus, *Wilcoxon* matched-pairs signed-ranks test was used for comparing the weight and CV between both baselines, both follow-up and both samples of the same ear side. We used Generalised Linear Models (GLMs) with a *gamma* distribution and a *log link* function to assess the association between the obtained amount of earwax by the self-sampling external ear device and biological variables. The GLM is a flexible generalization of ordinary linear regression that allows for response variables that have error distribution models other than a normal distribution [23]. The level of significance was set at $p \leq 0.05$ (two-tailed).

3. Results

The sample included mainly mixed-race people. Participants had normal weight, BMI and waist circumference on average (Table 1). The visual exploration of the external ear canal using an otoscope did not reveal the presence of any external ear pathology, such as impacted earwax, perforation nor the presence of ear canal shape abnormalities. No biological variable was associated with variations in the volume of collected earwax using the self-sampling earwax device (all $p > 0.05$) (Table 2). Overall, most participants considered the use of the self-sampling earwax device very comfortable, effective and safe (Table 3). They also felt that it was generally safer, more effective and as comfortable as the use of "cotton buds" (Table 3).

Table 1. Socio-demographic and Anthropometric Variables.

Socio-Demographic Variables		Result
N: Female (%)		20, (54)
Age (Years), Mean (SD)		29.9, (1.4)
Ethnicity	**Mixed race,** N (%)	36, (96)
	White N (%)	1, (4)
Alcohol	**Yes** $^{\delta}$, N (%)	10, (27)
	Units $^{\varphi}$, Mean, (SD)	1.3, (0.5)
Tobacco (yes), N (%)		9, (24)
Contraceptive pill (yes), N (%)		9, (53)

Medical or psychiatric illness, N(%)				0, (0)
Anthropometric Variables	**Q1**	**Median**	**Mean, (SD)**	**Q3**
Weight (Kg) Mean,(SD)	62	72	72.5, (2.5)	78
BMI (Kg/m^2), Mean (SD)	23.3	24.9	25.6, (0.6)	26.7
Waist circumference (cm), Mean (SD)	77	86	85.9, (2.4)	95

$^{\delta}$: at least one unit last week; &: any medication, including psychotropic and steroidal medication; $^{\varphi}$: One alcohol unit is measured as 10 mL or 8 g of pure alcohol. This equals one 25 mL single measure of whisky (Alcohol by Volume [ABV] 40%), or a third of a pint of beer (ABV 5–6%) or half a standard (175 mL) glass of red wine [ABV 12%]. BMI: Body Mass Index.

Table 2. Generalised Linear Models between the among of extracted earwax using the self-sampling external ear device and some biological variables.

Variables	β	*p*-Value	CI
Age	0.6	0.77	−3.9; 5.3
Sex	53.8	0.13	−15.1; 122.8
Alcohol (unit) $^{\Omega}$	−2.2	0.70	−13.5; 9.1
Tobacco	−26.0	0.49	−101.1; 49.0
BMI	5.9	0.34	−6.3, 18.2
Waist circumference	0.8	0.53	−1.7; 3.3
Anti-conceptive pill	65.5	0.14	−21.6; 150.7

$^{\Omega}$: One alcohol unit is measured as 10 mL or 8 g of pure alcohol. This equals one 25 mL single measure of whisky (alcohol by volume (ABV) 40%), or a third of a pint of beer (ABV 5–6%) or half a standard (175 mL) glass of red wine (ABV 12%).

Table 3. The Self-Sampling External Ear Device Satisfaction Survey.

N	Questions:		Results:					
		Meaning	**1st Quartile**		**Median**	**Mean (s.d)**	**3rd Quartile**	
1	*How would describe your experience using the self-sampling external ear device?*	1 = Very uncomfortable, 5 = Very comfortable	4		4	4.0 (1.0)	5	
2	*How effective was the self-sampling external ear device for cleaning your external ears?*	1 = Very ineffective, 5 = Very effective	4		4	4.2 (0.9)	5	
3	*How safe do you consider the use of the self-sampling external ear device inside your ear?*	1 = Very unsafe, 5 = Very safe	4		4	4.3 (0.6)	5	
4	*Do you know of "cotton buds"?*	n, (%)	**Yes**			**No**		
			28, (100)			0, (0)		
5	*How often do you use "cotton buds"?*	n, (%)	**Every day**	**Almost every day**	**Sometimes**	**Seldom**	**Nearly never**	**Never**
			2, (7.1)	8, (28.6)	8, (28.6)	1, (3.6)	3, (10.7)	6, (21.4)
		Meaning	**1st Quartile**		**Median**		**Mean (s.d)**	**3rd Quartile**
6	*Do you think that the use of the self-sampling external*	1 = Extremely disagree,	2		3		3.1,	4.5

	ear device was more comfortable than "cotton buds"?	5 = Extremely agree			(1.4)	
7	*Do you think that the use of the self-sampling external ear device for cleaning your ears was more effective than "cotton buds"?*	1 = Extremely disagree, 5 = Extremely agree	3	4	3.9, (1.2)	5
8	*Do you think that the use of the self-sampling external ear device was safer than "cotton buds"*	1 = Extremely disagree, 5 = Extremely agree	4	4	4.2, (0.9)	5

The amount of obtained earwax at baseline sampling did not differ between left and right ears (both $p > 0.05$), although there was a clear tendency for a relatively increased left baseline sample weight in comparison to the right one ($p = 0.08$). At follow up, the self-sampling external ear device collected eight times more earwax than the Reiner-Alexander syringe ($p < 0.01$) (Table 4). Both ear canals significantly increased their earwax production after the baseline external ear cleaning procedure (both $p < 0.05$). The CV results were: Right-Baseline: 51%, Left-Baseline: 59%, Right-Follow-up: 71% and Left-Follow-up: 59%. CVs comparisons between sampling methods were not significant (all $p > 0.05$); this result was reinforced by the similitude between samples, in terms of the relative deviation of each participant's earwax weight (Figure 3a–3b). No side-effects were reported by extraction method at any assessment.

Table 4. Earwax Extraction Method Comparisons.

Ear Side	**Left**				**Right**				
Extraction procedure	**Clinical Method ω** (mg)				**Clinical Method ω** (mg)				**P-value Ψ**
Visit	Q1	Median	Mean (s.d)	Q3	Q1	Median	Mean (s.d)	Q3	
Baseline (day = 0)	6.22	8.54	10.70, (6.36)	15.69	5.06	7.92	9.23, (4.73)	13.67	0.08
Extraction procedure	**Clinical Method ω** (mg)				**Self-extraction method ϕ** (mg)				***P*-value Ψ**
Visit	Q1	Median	Mean (s.d)	Q3	Q1	Median	Mean (s.d)	Q3	
Follow-up (day = 30)	12.0	17.3	19.1 (11.3)	19.6	79.8	124.7	155.8 (110.7)	200.3	<0.01 *

ω: Using the Reiner-Alexander syringe; ϕ: Using the self-sampling external ear device; Ψ: *P*-value was obtained using Wilcoxon matched-pairs signed-ranks test; *: *p*-value significant < 0.05.

Fig. 1a is a left side view of a first embodiment of the earwax self-sampling device showing the cross section of the tip including the sponge.

Fig. 1b is a perspective view of the tip of the medical device of the earwax self-cleaning device.

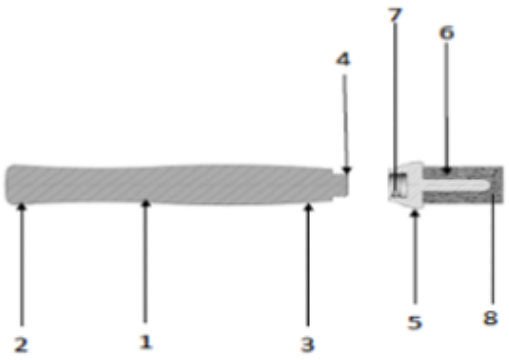
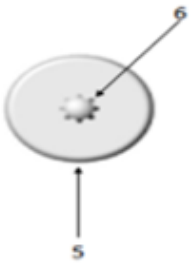


Fig. 1c is an upper view of the tip of the earwax self-sampling device.

Fig. 1d is a left side view of a second embodiment of the earwax self-sampling device showing the cross section of the tip including the sponge.



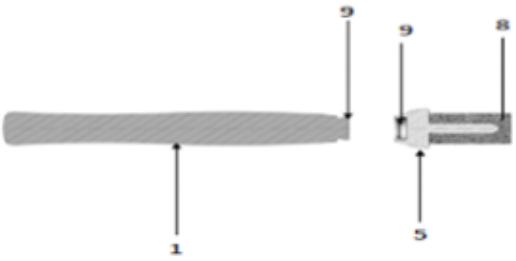


Figure 1. A detailed diagram of the earwax self-sampling device.

The earwax is obtained by means of the extraction device of the novel device which comprises: a handle [1] having a first end [2] and a second end [3], said second end [3] having coupling means which in a preferred embodiment of the self-sampling device may comprise a thread [4];a detachable head [tip] comprising a base [5] and a longitudinally extending elongated sponge holder [6] directly depending from an upper portion of the base, wherein the lower portion of the base has a housing including an internal threaded pattern [7] for receiving the thread [4] of the handle [1], and wherein the sponge holder [6] has a star-shaped cross-section; an elongated sponge [8] having a centrally located longitudinal housing [not shown] having a star-shaped cross-section for receiving the sponge holder [6] of the base [5]. The handle [1] and the base [5] may include any suitable coupling means for coupling the handle, such as a snap joint [9]. The sponge [8] may be made preferable of cellulose and is glued to the sponge holder [6] using a non-allergenic glue. As previously described, the sponge holder [6] has a star-shaped cross-section, which improves the earwax extraction while rubbing the sponge [8] inside the ear, however, its cross-section may have any suitable shape. The base [5] is wider than the handle [1] and acts as a safety brake which hinders to introduce the tip inside the ear canal. The handle [1] is characterised by having a rotationally symmetric form, which allows the user to rub the sponge inside the ear by rotating it inside the outer ear canal. The sponge [8] is packaged and sealed in wet condition to keep it soft. The used moistener is magnesium chloride [MgCL], which acts as an antimicrobial agent to prevent microorganism growth during storage shelf-life. The magnesium chloride not only

prevents the sponge from growing microorganism but it also supports the extraction of earwax. The earwax is obtained by inserting the tip with the sponge [8] in the ear and rotating the sponge [8] inside the ear canal for around 30 to 60 seconds.

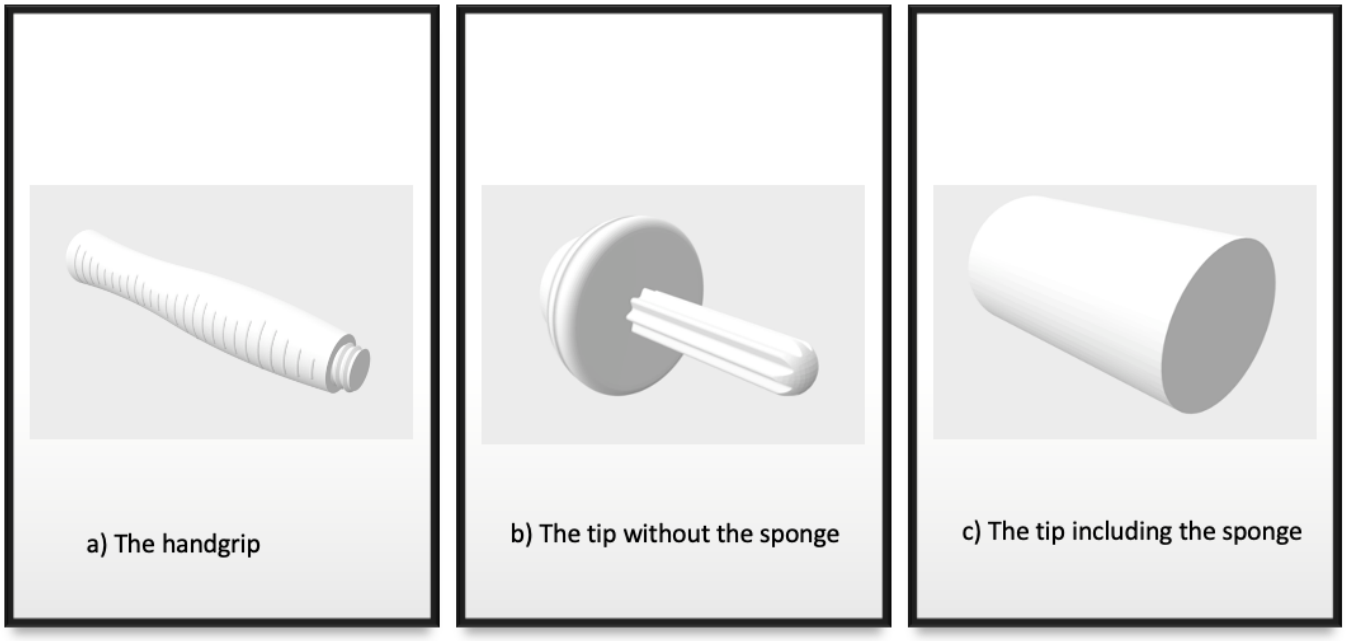


Figure 2. Three dimensional drawings of the device structure.

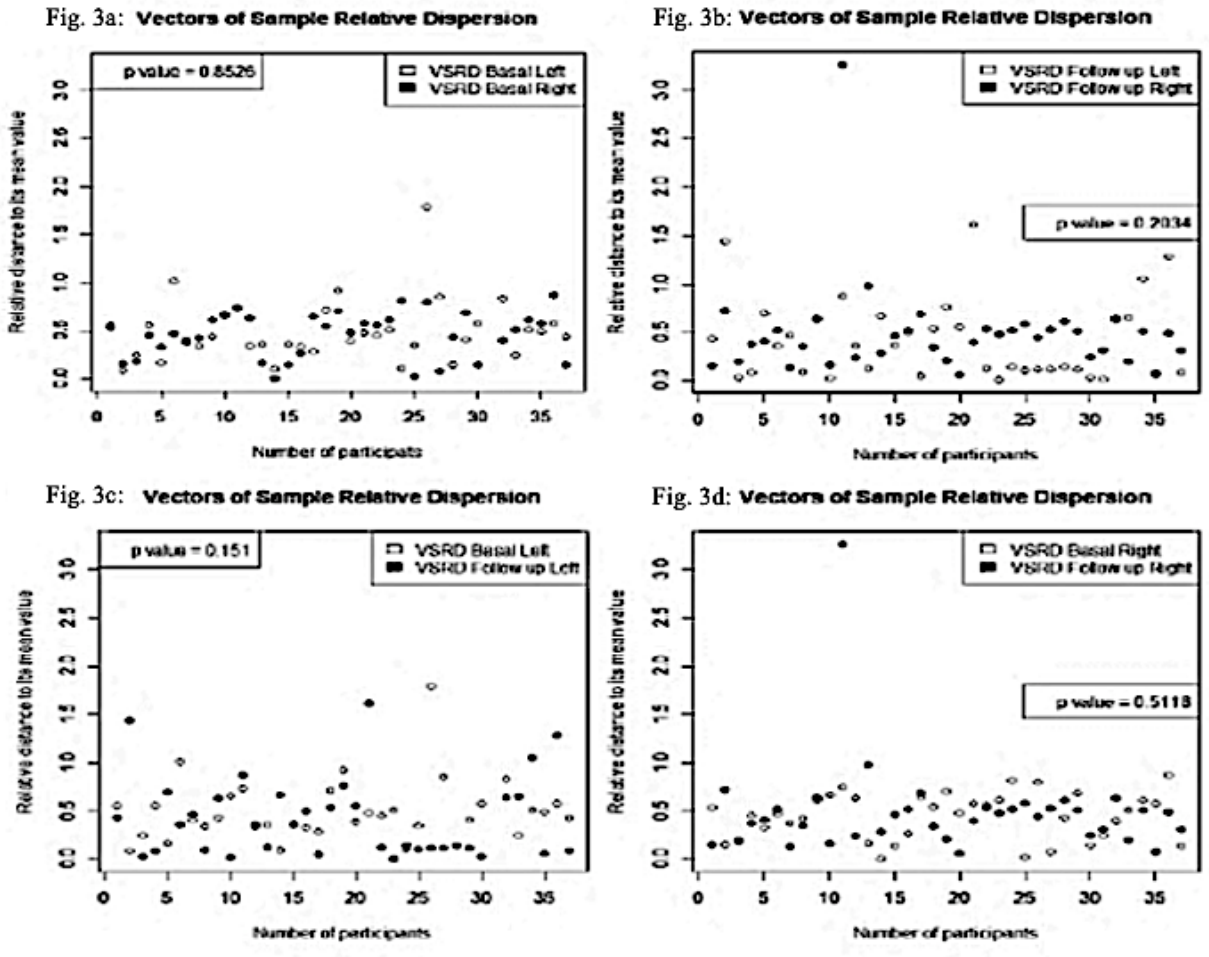


Figure 3. Vectors of Sample Relative Dispersion [VSRS], rather than the Vectors of Sample's Squared Relative Dispersion [VSSRS] were graphed for a better conception of data dispersion. P-values were obtained comparing the Coefficient of Dispersions of each sample**.**

4. Discussion

We found that the self-sampling earwax device obtained more earwax than one commonly used clinical syringing method. Our results showed that the amount of obtained earwax was greater at follow up in both ear sides after the baseline extraction. Regardless of the sampling method used, and the amount of earwax, the obtained volume in relation to the mean, varied little among participants. Most participants considered that the self-sampling earwax device to be comfortable, effective and safe. They also reported that its use felt safer, more effective and as comfortable as the use of "cotton buds".

It is noticeable that irrespective of the sampling method used, the amount of extracted earwax increased significantly during the follow-up period. This might indicate that the ceruminous glands of the external ear canal increased their earwax production, possibly as a compensatory mechanism, due to the removal of earwax that the baseline cleaning procedure caused. However, this difference may also be explained by our study protocol. We instructed participants to avoid cleaning their external ears during the follow-up period to obtain a standardised amount of earwax that could represent the retrospective month of earwax production. This possibility was reinforced after observing that before of the study period, 36% of our participants were "heavy" cotton swab users, and another 29,6% least sporadically used them (Table 3). It is also possible that the baseline cleaning loosened some earwax, entailing that the follow up clinical procedure was then more efficient at sampling earwax.

The syringing method obtained more than a double amount of earwax (4.7 ± 2.8 mg/week) than the most effective cleaning method used by Cipriani et al. (1986) when they used an unspecified mechanical method, syringing one mixed solution of alcohol/ether 3:1 *v*/*v* (2.02 ± 0.22 mg/week) in healthy participants. It is quite likely than our considerably larger sample may have explained this result. We replicated the finding of no difference in earwax volume between different ear sides, as also found by Cipriani et al. (1990).

The self-sampling earwax device obtained greater than eight times the amount of secretion than the syringe. It is not completely clear why this novel device showed this considerable improvement in earwax sampling in comparison to the clinical method. This might be explained by the design of the syringe intended to extract impacted earwax. Thus, the clinical method removal effect appears to be related to the volume, rather than the weight of earwax, since both follow-up samples increased, rather than decreased their mass. The clinical method removal effect, influenced by the pressure exerted by warm water, may not be appropriate for sampling thin layers of oily secretion from healthy ears, irrespective of their weight. The most recently secreted earwax may likely be tightly adhered to the external auditory canal epidermis, due to its oily, rather than waxy features. By contrast, we previously conducted a preclinical pilot study that showed that the tip material of this novel device was effective at removing artificial wax from a piece of animal skin. These results made us predict that the self-sampling earwax device may be useful for this feasibility study. In addition to this, we incorporated into the tip of the device a mineral oil solution that has shown some effect at cleaning earwax [10]. The cumulative effect of the tip material (an absorptive and abrasive sponge) and the oily solution may explain its superior effectiveness at earwax sampling in healthy ears. One potential limitation of this study is that while the clinical method requires to dry the earwax samples before analysis, this step was not needed when the novel device was used. Thus, it might be possible that some amount of earwax was dissolved in the water syringed by the clinical method. Even though no water content is found in the earwax secretion [24], Saxby et al. (2013) showed that distilled water solutions have some properties for dissolving earwax [25]. Oil solubility—the main earwax component (50%)

[24]—in water is larger when distilled water is used than the solubility when saltwater solution with 44 g L^{-1} of NaCl is used [26]. This is explained by the reduced density of distilled water in comparison to salt water. However, the tap water injected by the clinical method of this study contained high concentrations of many salts. Apart from NaCl, drinking water has large concentrations of Na_2SO_4, $CaSO_4$, KCl and $CaCl_2$ in Chile [27]. Furthermore, oil solubility in water is observed when water is warmed at 25 °C or above, conversely to 4 °C that we stored our samples. These results make it extremely unlikely that the vast earwax weight difference between both methods had been explained by some potential earwax solubility in the water injected by the medical device. More importantly, the amount of earwax is not an important variable when the main aim of this novel device is to constitute a reliable method for self-sampling earwax from healthy ears, rather than an effective device for extracting impacted earwax from diseased ears. Other variables need to be considered for assessing whether or not this novel device meets this requirement.

Concerning this, the earwax weight variability (relative distance to the mean) was similar between the clinical and self-extraction method (Inter-Reliability) ($p = 0.20$) (Figure 3b). This result indicates that the novel device is indeed a consistent method for sampling earwax, since the comparator or reference device, also showed to be consistent in sampling earwax from healthy ears, when the VRSDs from both baseline samples that were obtained using the same clinical method (Intra-Reliability) were compared between them ($p = 0.85$) (Figure 3a). It might be also possible that the novel device is even more reliable than the clinical device at sampling larger volume of this oily secretion since the p-value observed when both follow-up VRSDs were compared between them ($p = 0.20$) (Figure 1b) was much smaller than the p-value obtained from the comparison between both baseline VRSDs ($p = 0.85$) (Figure 3a). This might be explained because the clinical method did not uniformly perform at sampling large volume of earwax that do not constitute a pathologic condition, such as impacted earwax. The p-value obtained was much smaller when the clinical device was also used for sampling a greater volume of earwax ($p = 0.15$) (Figure 3c) than the p-value obtained when the novel device was used for obtaining a larger volume of this specimen ($p = 0.51$) (Figure 3d). These results might imply that the novel device could also perform well at sampling earwax specimens with less weight, considering its less data dispersion. Nonetheless, all these p-values are still not significant. Therefore, future larger studies should investigate the novel device reliability at sampling earwax from both ear sides, as part of a baseline visit.

An effective earwax self-sampling device may also allow the measurement of a variety of substances, such as glucose levels over different periods. A reliable and effective earwax self-sampling device is therefore needed because current self-sampling products or devices have shown no or minimal effect at removing earwax. Although we did not carry out a cost-analysis, the self-sampling device might be very likely more economical to potentially extract endogenous potential biological markers, than sophisticated technologies. For example the Realtime Continuous Glucose Monitoring (RT-CGM) device currently used to measure chronic glucose levels. Moreover, it could be utilised by patients themselves since it was well-tolerated by our participants. Patients could use it in their own home, sending the samples by post to a specialised clinic/lab. Lessons learned from the current COVID-19 pandemic suggests that in situations when patients may not be able to attend health facilities, self-sampling methods may be a practical solution if devices were enabled to detect putative biomarkers to confirm diagnoses, especially in case of an increased prevalence of common disorders (e.g., Arora et al., 2020)[28]. Importantly, earwax holds similar properties to the beeswax hence is a bacteriostatic agent [7,9] immune to the most common strains of the epidermal flora [8,29]. The self-sampling external ear device is safer than cotton buds because its design cannot harm the eardrum [30] and participants reported no side-effects. Furthermore, the earwaxolytic solution into the tip device has successfully used for treating dermatitis [31], the most commonly associated side-effect by the chronic use of "cotton buds" [12]. Depression may be another

common disorder which might potentially benefit from the use of earwax, as a potential medium to extract putative biomarkers. A depressive disorder is characterised by chronic cortisol level alteration [32]. Although hair cortisol extraction is a reliable method for measuring that level, it has significant shortcomings [33] and cortisol extraction from earwax might be a viable alternative, as we have recently suggested [34].

There are other clinical earwax extraction methods, apart from the Reiner-Alexander syringe. In the United States, for instance, the majority of the clinicians prefer to extract earwax under microscopic vision, rather than the syringing method we used as a comparator, as this may be more precise [6]. We considered the use of Reiner-Alexander syringe because there is no clear consensus about what constitutes a gold standard earwax removal method, and no previous earwax device was designed for sampling earwax. Pothier, Hall and Gillett, (2006) found that endoscopic removal, when compared to microscopic extraction, was less uncomfortable and less painful for patients, as well as easier to perform by non-specialists, a common occurrence outside the United States [35–37]. However, to our knowledge, no study has ever compared the use of the Reiner-Alexander syringe with either endoscopic or microscopic dewaxing. Both procedures, in comparison to the syringing method that we used are expensive and may be unavailable. Therefore, we used the Reiner-Alexander syringe as a reference test which is largely available "under reasonable conditions" an essential characteristic for a gold standard test as suggested by Versi (1992) [38]. In terms of reliability, the clinical method chosen by us showed to be reliable at sampling a small volume of earwax from healthy ears, and the novel method was more effective than the clinical method. Thus, the use of the Reiner-Alexander syringe as a comparison method appears a rational decision based on safety, effectiveness and availability [5].

Although we always checked the otoscopic status after cleaning ears, we never repeated the irrigation using the Reiner-Alexander syringe. Therefore, it might be possible that, if that procedure had been repeated, more earwax would have been extracted. Nonetheless, the evidence for repeating earwax irrigation, if needed, at least using electronic devices is low to moderate [39]. Hence, although speculative, it appears legitimate to think that the Reiner-Alexander may not differ from the earwax irrigation method. Another potential limitation of our study is the fact that the use of common equipment such as earphones or earplugs, may affect the efficiency of the new method by removing earwax to a small extent. Future studies may investigate this potential earwax removal effect. More work could also test the self-sampling earwax device reliability amongst Asian and in people with intellectual disabilities. It has been found that these populations produce a different type and amount of earwax [19,22].

A further limitation is that sampling bias might have occurred in the absence of randomisation. Hence, this work is the first step towards a controlled study which could specifically test this extraction method. A single-blind, randomized controlled trial in comparison with a sham self-sampling earwax intervention could test the effectiveness of this device in relation to specific characteristics of the human ear including for example the impact of the device tip on physiological variations of the diameter of ear canals.

5. Conclusions

This novel device may be more reliable, economical and effective than the most common clinical method for sampling earwax from healthy ears. This earwax device may also prove to be safer and as comfortable as "cotton buds". Future studies could compare the reliability of this device with other self-cleaning earwax methods in controlled studies and the potential use of it to extract putative biomarkers to improve and/or corroborate the diagnosis and/or response to treatment in the context of various illnesses such as diabetes and major depression.

6. Patents

The earwax self-sampling device is patented (PCT/IB2018/060470).

Author Contributions: Conceptualization, A.H.V.; methodology, A.H.V. and E.O.; software, A.H.V., T.V. and B.A.; validation, A.H.V., J.B.; review and editing, A.H.V., A.H.; analysis, A.H.V., R.S.; supervision, A.H.V., R.S., A.C., D.A.; project administration, R.S., L.O., S.E.; funding acquisition, A.H.V., J.B. All authors have read and agreed to the published version of the manuscript.

Funding: This study was funded by private funds provided by A.H.V. and J.B.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board (or Ethics Committee) of Universidad Católica del Norte (UCN), Coquimbo, Chile approved Decree number 75 year 2017. The research and written informed consent were obtained from all participants.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. Participants were not compensated for taking part in the research.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to ongoing research projects.

Acknowledgments: The views expressed are those of the author[s] and not necessarily those of University College London, Institute of Psychiatry, Psychology and Neuroscience in King's College London (IoPPN) of King's College London, Universidad Católica del Norte or the United Arab Emirates University. We thank all participants of this study.

Conflicts of Interest: No author has received funding from any funding agency for designing or running this study. The funders (A.H.V. and J.B.) had no role in running the study and collecting the data. D.A. has received travel grants from Jansenn-Cilag and Servier and sponsorship from Lundbeck. AJC has in the last three years received honoraria for speaking from Lundbeck, honoraria for consulting from Allergan, Janssen, Lundbeck and Livanova, and research grant support from Lundbeck.

Abbreviations

HbA_{1c}	Glycated Haemoglobin
BMI	Body Mass Index
CV	Coefficients of Variation
VSSRDs	Vector of Sample's Squared Relative Dispersion

Appendix A

Let $X = (X_1, \dots, X_n)$ be a sample of a random variable and

Let $\overline{X} \coloneqq \frac{1}{n}(\sum_{i=1}^{n} X_i)$, $S_X \coloneqq \sqrt{\sum_{i=1}^{n}(X_i - \overline{X})^2/(n-1)}$ and

$\widehat{CV}_X \coloneqq \frac{S_X}{\overline{X}}$ be the unbiased estimators of mean and standard deviation, and the estimator of coefficient of variation of the variable, respectively.

We define $D_X \coloneqq \left(\frac{n(X_1-\overline{X})^2}{(n-1)\overline{X}^2}, \dots, \frac{n(X_1-\overline{X})^2}{(n-1)\overline{X}^2}\right)$ to be the Vector of Sample's Squared Relative Dispersion (VSSRD) and notice that if $\overline{D}_X$ is the mean of the VSSRD, then $\widehat{CV}_X^2 = \overline{D}_X$.

Therefore, the coefficient of variation is nonnegative measure, if $X = (X_1, \dots, X_n)$ and $Y = (Y_1, \dots, Y_m)$ are samples of two random variables, then $\widehat{CV}_X = \widehat{CV}_Y$, only if $\overline{D}_X = \overline{D}_Y$. Then, it is possible to use tests for significant difference between means, of D_X and D_Y-, to test significant difference between coefficients of variation, of X and Y-.

References

1. Prokop-Prigge, K.A.; Thaler, E.; Wysocki, C.J.; Preti, G. Identification of volatile organic compounds in human cerumen. *J. Chromatogr. B* **2014**, *953-954*, 48–52, doi:10.1016/j.jchromb.2014.01.043.
2. Shokry, E.; De Oliveira, A.E.; Avelino, M.A.G.; De Deus, M.M.; Filho, N.R.A. Earwax: A neglected body secretion or a step ahead in clinical diagnosis? A pilot study. *J. Proteom.* **2017**, *159*, 92–101, doi:10.1016/j.jprot.2017.03.005.
3. Dagogo-Jack, S. Pitfalls in the use of HbA1c as a diagnostic test: The ethnic conundrum. *Nat. Rev. Endocrinol.* **2010**, *6*, 589–593, doi:10.1038/nrendo.2010.126.

4. Herane-Vives, A.; Espinoza, S.; Sandoval, R.; Ortega, L.; Alameda, L.; Young, A.H.; Arnone, D.; Hayes, A.; Benöhr, J. A Novel Earwax Method to Measure Acute and Chronic Glucose Levels. *Diagnostics* **2020**, *10*, 1069, doi:10.3390/diagnostics10121069.
5. Sevy, J.O.; Singh, A. *Cerumen Impaction*; StatPearls Publishing: Treasure Island, FL, USA, 2019.
6. Pothier, D.D.; Hall, C.; Gillett, S. A comparison of endoscopic and microscopic removal of wax: A randomised clinical trial. *Clin. Otolaryngol.* **2006**, *31*, 375–380, doi:10.1111/j.1749-4486.2006.01288.x.
7. Ghanem, N. The Antimicrobial Activity of Some Honey Bee Products and some Saudi Folkloric Plant Extracts. *J. King Abdulaziz Univ.* **2011**, *23*, 47–62.
8. Stoeckelhuber, M.; Matthias, C.; Andratschke, M.; Koechler, C.; Herzmann, S.; Sulz, A.; Welsch, U. Human ceruminous gland: Ultrastructure and histochemical analysis of antimicrobial and cytoskeletal components. *Anat. Rec. Part A Discov. Mol. Cell. Evol. Biol.* 2006, *288*, 877–884, doi:10.1002/ar.a.20356.
9. Fratini, F.; Cilia, G.; Turchi, B.; Felicioli, A. Beeswax: A minireview of its antimicrobial activity and its application in medicine. *Asian Pac. J. Trop. Med.* **2016**, doi:10.1016/j.apjtm.2016.07.003.
10. Clegg, A.J.; Loveman, E.; Gospodarevskaya, E.; Harris, P.; Bird, A.; Bryant, J.; Scott, D.A.; Davidson, P.; Little, P.; Coppin, R. The safety and effectiveness of different methods of earwax removal: A systematic review and economic evaluation. *Health Technol. Assess.* **2010**, *14*, 1–192, doi:10.3310/hta14280.
11. Khan, N.B.; Thaver, S.; Govender, S.M. Self-ear cleaning practices and the associated risk of ear injuries and ear-related symptoms in a group of university students. *J. Public Health Afr.* **2017**, *8*, 555, doi:10.4081/jphia.2017.555.
12. Ahmed, S.; Zaheer, S.A.I.; Shabbir, S.M.A.; Rao, S.; Islam, T.; Ahmed, B. Association of Dermatological Conditions of External Ear with the Use of Cotton Buds. *J. Enam. Med. Coll.* **2014**, *4*, 174–176, doi:10.3329/jemc.v4i3.20956.
13. Nussinovitch, M.; Rimon, A.; Volovitz, B.; Raveh, E.; Prais, D.; Amir, J. Cotton-tip applicators as a leading cause of otitis externa. *Int. J. Pediatr. Otorhinolaryngol.* **2004**, *68*, 433–435, doi:10.1016/j.ijporl.2003.11.014.
14. Pata, Y.S.; Ozturk, C.; Akbas, Y.; Gorur, K.; Unal, M.; Ozcan, C. Has cerumen a protective role in recurrent external otitis? *Am. J. Otolaryngol.* **2003**, *24*, 209–212, doi:10.1016/s0196-0709(03)00034-6.
15. Mochizuki, H.; Tanaka, S.; Morita, T.; Wasaka, T.; Sadato, N.; Kakigi, R. The cerebral representation of scratching-induced pleasantness. *J. Neurophysiol.* **2014**, *111*, 488–498, doi:10.1152/jn.00374.2013.
16. Summerfield, A.; Meurens, F.; Ricklin, M.E. The immunology of the porcine skin and its value as a model for human skin. *Mol. Immunol.* **2015**, *66*, 14–21, doi:10.1016/j.molimm.2014.10.023.
17. Denda, M.; Katagiri, C.; Hirao, T.; Maruyama, N.; Takahashi, M. Some magnesium salts and a mixture of magnesium and calcium salts accelerate skin barrier recovery. *Arch. Dermatol. Res.* **1999**, *291*, 560–563, doi:10.1007/s004030050454.
18. Cipriani C, Taborelli G, Cardo PP, et al. A technique for measuring the rate of cerumen production. *Laryngoscope* **1986**;96:204–5. doi:10.1288/00005537-198602000-00015
19. Bortz JT, Wertz PW, Downing DT. Composition of cerumen lipids. *J Am Acad Dermatol* 1990;**23**:845–9. doi:10.1016/0190-9622(90)70301-W
20. Spector, P.E. Measurement of human service staff satisfaction: Development of the Job Satisfaction Survey. *Am. J. Community Psychol.* **1985**, *13*, 693.
21. Cipriani, C.; Taborelli, G.; Gaddia, G. Production rate and composition of cerumen: Influence of sex and season. *Larygoscopegoscope* **1990**, *100*, 275.
22. Crandell, C.C.; Roeser, R.J. Incidence of excessive/impacted cerumen in individuals with mental retardation: A longitudinal investigation. *Am. J. Ment. Retard.* **1993**, *97*, 568.
23. Nelder, J.; Baker, R. Generalized linear models. *Encycl. Stat. Sci.* **1972**, doi:10.1002/0471667196.ess0866.pub2.
24. Chiang, S.P.; Lowry, O.H.; Senturia, B.H. Micro-chemical studies on normal cerumen. I. The lipid and protein content of normal cerumen as affected by age and sex. *Laryngoscope* **1955**, *65*, 927–934.
25. Saxby, C.; Williams, R.; Hickey, S. Finding the most effective cerumenolytic. *J. Laryngol. Otol.* **2013**, *127*, 1067.
26. Hamam, S.E.M.; Hamoda, M.F.; Shaban, H.I.; Kilani, A.S. Crude oil dissolution in saline water. *Water. Air. Soil Pollut.* **1988**, *37*, 55.
27. Herrera, V.; Carrasco, C.; Araneda, P.; Sandoval, J.M. Chemical quality of urban and rural drinking water, in Tarapaca, northern arid area of Chile. *J. Chil. Chem. Soc.* **2019**, *64*, 4421.
28. Arora T, Grey I, Östlundh L, *et al.* The prevalence of psychological consequences of COVID-19: A systematic review and meta-analysis of observational studies. *J Health Psychol* 2020;:135910532096663. doi:10.1177/1359105320966639
29. Lum, C.L.; Jeyanthi, S.; Prepageran, N.; Vadivelu, J.; Raman, R. Antibacterial and antifungal properties of human cerumen. *J. Laryngol. Otol.* **2009**, *123*, 375.
30. Hexa Reports. *Global Cotton Bud Market Research Report*; 2017. http://www.hexareports.com/report/global-baby-cotton-budsindustry/details
31. Zhai, H.; Willard, P.; Maibach, H.I. Putative skin-protective formulations in preventing and/or inhibiting experimentally-produced irritant and allergic contact dermatitis. *Contact Dermatitis* **1999**, *41*, 190.
32. Herane-Vives, A.; de Angel, V.; Papadopoulos, A.; Wise, T.; Chua, K.-C.; Strawbridge, R.; Castillo, D.; Arnone, D.; Young, A.H.; Cleare, A.J. Short-term and long-term measures of cortisol in saliva and hair in atypical and non-atypical depression. *Acta Psychiatr. Scand.* **2018**, doi:10.1111/acps.12852.

33. Fischer, S.; Duncko, R.; Papadopoulos, A. Sociodemographic, lifestyle, and psychosocial determinants of hair cortisol–Evidence from a south London community sample. *Psychoneuroendocrinology* 2016. doi: 10.1016/j.psyneuen.2016.11.011. Epub 2016 Nov 17.
34. Herane-Vives, A.; Ortega, L.; Sandoval, R.; Young, A.H.; Cleare, A.; Espinoza, S.; Hayes, A.; Benöhr, J. Measuring Earwax Cortisol Concentration Using a Non-Stressful Sampling Method. *Heliyon* **2020**, *6*, e05124, doi:10.1016/j.heliyon.2020.e05124.
35. Sharp, J.F.; Wilson, J.A.; Ross, L.; Barr-Hamilton, R.M. Ear wax removal: A survey of current practice. *BMJ* **1990**, *301*, 1251.
36. Lamberts (Hendrik), H. *In Het Huis van de Huisarts*: *Verslag van Het Transitieproject*; Meditekst , Lelystad: 1991.
37. Memel, D.; Langley, C.; Watkins, C.; Laue, B.; Birchall, M.; Bachmann, M. Effectiveness of ear syringing in general practice: A randomised controlled trial and patients' experiences. *Br. J. Gen. Pract.* **2002**, *52*, 906.
38. Versi, E. "Gold standard" is an appropriate term. *BMJ* **1992**, *305*, 187.
39. Ftouh, S.; Harrop-Griffiths, K.; Harker, M.; Munro, K.J.; Leverton, T. Hearing loss in adults, assessment and management: Summary of NICE guidance. *BMJ* **2018**, *361*, doi:10.1136/bmj.k2219.