Research Article
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Chemical-physical investigations of nine types of nasopharyngeal swabs for the PCR analyses

Abstract

During the SARS-Cov-2 pandemic, a test of samples collected through naso/oropharyngeal swab was set up; for this aim new swabs were developed. Materials and methods: The laboratory New Nanodiagnostics srl (Modena - Italy) analyzed nine different types of swabs used to collect human organic material for PCR diagnostic test for the SARS-CoV-2 infection. The swabs were observed under Optical Microscope and analyzed by Field Emission Gun Environmental Scanning Electron Microscope (FEGE-SEM) coupled with an Energy Dispersive System (EDS), to verify their morphology and chemical composition. Three different morphologies and nanostructures, along with their chemical composition were identified. Surprisingly enough, the presence of identified chemical contaminants like Titanium-Silicon-Aluminum or Silicon- Aluminium-Chromium-Manganese does not be understandable nor is it explained in the data sheet. Some fibers also present a nanostructured coating of Silicon-Zirconium. This might be of concern, as their presence could invalidate the accuracy of the PCR testing. In addition to that, the biocompatibility of the medical devices is discussed since a particular tendency of the glassy fibers to break has been verified. The presence of broken fibers in the nasal and oropharyngeal mucosa can cause irreversible damages. Finally, after their use, the swabs must be incinerated and their fumes contribute to the increase of the environmental pollution.

Keywords: swab, covid-19, fiber, contamination, coating

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Introduction

During the SARS-Cov-2 pandemic, a test of samples collected through naso/oropharyngeal swab was set up. The test employed reverse transcriptase reaction of viral RNA into complementary DNA (cDNA), and subsequent Polymerase Chain Reaction (PCR) of a given part of cDNA, using specific primers and a suitable cycling protocol. For the first time, this test was used as a main diagnostic tool to claim someone with a positive SARS-CoV-2 virus test as a case, although no symptoms are presented. Even the developer of the system, the Nobel prize winner Dr. Kary Mullis, advised against its use as a sole diagnostic tool for a virus (HIV).[1,2] The system is indispensable in molecular biology research, but when used as the sole criterion in infection diagnostics, disregarding the importance of clinical symptoms, or even causality principles in epidemiology,[3] it is subjected to strong critics. The PCR protocol suggested by its inventor, indicated number of amplification cycles for the viral signal, was very different from what has been applied worldwide. Moreover, these numbers varied from state to state, as if there was no consensus to what the right number of cycles is. In Italy, the value was 40-50, but it depends on the particular PCR protocol validated by its manufacturer. Anyone who had worked with PCR in molecular biology laboratory knows that sequence-of- interest amplification above 30 cycles yields unreliable results, and that increasing the cycles can give results indicating virtually any piece of nucleic acid. It is obvious that the higher the value of amplification, the higher the probability of falsely confirming any, even viral, sequences. Nevertheless, screening for SARS-Cov-2 infection and applying following measures require a suitable sample-collecting tool.[4–8] The WHO declared in 2020 that there are false positive PCR tests, but also false negative results are possible. So, "any positive result (SARS-CoV-2 detected) or negative results (SARS-CoV-2 not detected)" must be "in combination with specimen type, clinical observations, patient history, and epidemiological information" in order to identify clearly thedisease.[9,10] Aim of the present study is to investigate the morphology and chemical compositions of the swabs currently used in the worldwide market.

Materials and methods

The swabs

Nine different types of swabs were used for morphology and chemical composition study analysis (Figure 1). According to the manufacturers' label, the swabs were sterilized in ethylene oxide atmosphere (labelled as EO) or treated with gamma rays at 2.5 Megarad (labelled as R). Note that normally packs of enveloped swabs are exposed to ethylene gas in a suitable room. Then, they are removed and put in an air- pressurized room to eliminate the gas for at least 24h. After that, they can be used in a safe way (Table 1).

Table 1 List of the analysed swabs

Name	N° swabs analysed	Manufacturer	Name	Production country	Sterilization method
A	1	BioComma Limited	Disposable Swab	China	R
B	1	LP Italiana	Swab	Italy	R
C** / E	1	Copan italia	FLOQSwab	Italy	EO
D	2	Copan italia	eSwab	Italy	R
F	1	Jiangsu Chang Feng Medical	Disposable Swab	China	EO
G	2	Labweare manufacturing	CitoSwab	China	R

Table 1 Continued...

Name	N° swabs analysed	Manufacturer	Name	Production country	Sterilization method
H	1	Zhejiang Gongdong Medical Technology	Gongdong	China	EO
I	1	Taizhou Sun Trine Biotecnology	Swab	China	*
***	2	Miraclean Technology	Man Tacc	China	EO
***	3	Noble Bio	Swab	China	R

*The company did not declare the method of sterilization. **The sample C was cut longitudinally in order to see the core. *** The samples were not photographed since they are similar to G.

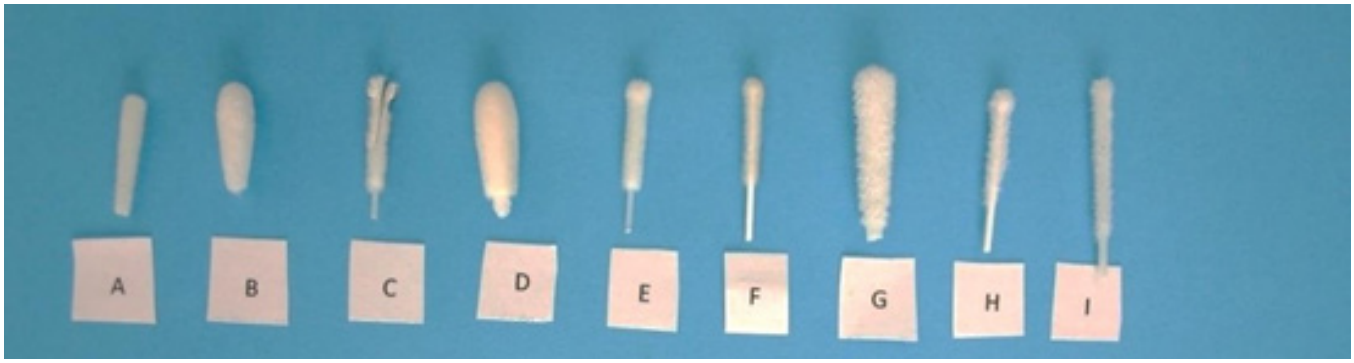

Figure 1 Different types of analyzed swabs present on the international market.

Microscopy

First, while still inside the plastic envelope (original packaging), the swabs were observed under Optical Microscope (OM, Stereo Olympus, Japan), thus preserving their state of sterile conditions. Then, the swabs were observed under a stereomicroscope Olympus (SZ61, Japan), to verify their macroscopic structure. The part that entraps the biological sample was cut and deposited on an Aluminium support made of an adhesive Carbon disc. Then, for more detailed structural and chemical investigations, the specimens were introduced in the chamber of the Field Emission Gun Environmental Scanning Electron Microscope (FEGESEM, Quanta 650, Thermofisher, USA) equipped with an X- ray microprobe of an Energy Dispersive System (Thermofisher, USA). The FEGESEM setup has a very high sensitivity for details, being able to reach a 20nm scanning resolution. It allows identification of nanostructures as well as determination of their chemical composition. The only drawback with this method is the possible bias in the identification of Carbon-Oxygen-based products. For instance, the plastic materials are composed essentially of Carbon and Oxygen only, so, the system does not distinguish for example, among polyethylene, polycarbonate and polypropylene. Similar cases occur with vegetal products, as they are composed primarily of Carbon and Oxygen. On the other hand, it is possible to distinguish between polysulfone (PSU) and polyvinyl chloride (PVC), thanks to the presence of Sulphur and Chlorine, respectively.

Results

The observations under OM revealed that inside the envelope there were already broken swab fibers. The envelope was open, the fibers extracted and deposited on a stub. In general, the swabs were made of a plastic core with white nylon fibers, suitable for the collection of biological specimens. In addition to that, there were black fibers, clearly not part of the swab-head collection fibers, most likely a contaminant present in an almost systematic way. Figure 2 shows two examples of the black fibers entrapped among the white fibers.

Some manufacturers give details on the production method, whereas others give insuffi- cient details for a medical device of Class 2B, according to ISO/EN 10993. The swab called FLOQSwab®(COPAN Diagnostics Inc. (Carlsbad, CA)) is a "flocked swab that consists of a molded plastic applicator stick with a variable size and shape tip. The tip of the applicator is formed with short Nylon® fibers that are arranged in a perpendicular fashion" as a "porcupine-like" structure. The analyses show that there is a coating of a patented material, which we show to be Silicate-Zirconia-Titanium. Such coating makes the fiber harder, so, more efficient in scratching the internal nose/throat mucosa and detach more biological material. The FEGESEM observations showed 3 main structures of the swabs Figure 3 able to catch and entrap biological material: a) like a porcupine, b) like a bundle, c) like a sponge. The following images Figure 4 show swab surface details, while EDS spectra show the chemical composition of the wires / bundle / sponge. The core of the fibers in Figure 3 & 4 are polymeric, probably Nylon 6.6. Many flocked swabs present similar structures - a hard core from which longitudinal fibers depart. The bundle swab is made of cotton fibers. The swab in Figure 3c has a polymeric foam structure. Many swabs present similarities: "ManTac" swab (China) is a sort of a porcupine with hard fibers stemming from the core of the device, which is similar to Biocomma swab (Shenzhen, China). The distribution of the fibers is either random or geometrically arranged. At higher magnification, the fibers appear to have a coating that is not homogenously distributed.

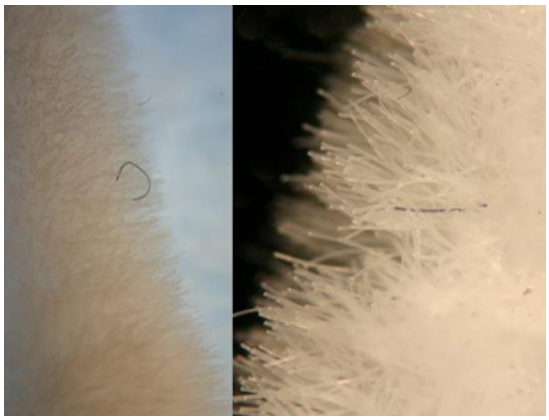

Figure 2 The OM macro photos show black wires entrapped in the white structure of the swabs.

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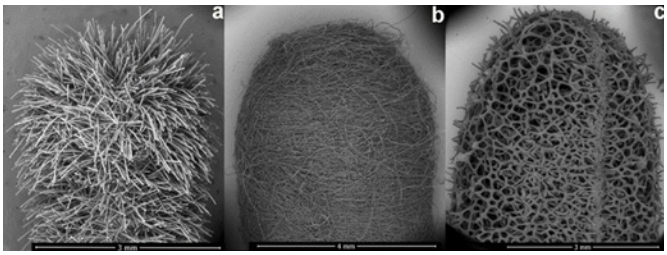

Figure 3 The images show three different morphologies: a) porcupine-like (Mantac, Zhejiang Gongdong Medical Technology); b) bundle-like (LP Italiana); c) porous surface (Jiangsu Chang Feng Medical).

Figure 4 Superficial structure at higher magnification of the swabs showed in Figure 3, respectively.

In Figure 5, the EDS chemical spectra of the FLOQSwab (a, b) show that the fibers have probably a Silicon-Zirconia coating. The "Taizhou Sun Trine" swab (c, d) shows a coating made of Silicon-Aluminium-Titanium-Zirconium- Sulphur. The "Jiangsu Chang Feng" medical swab is coated with Aluminium-Chromium-Iron-Titanium (e, f). The analysis revealed that all fibers have a high content of Carbon and Oxygen, pointing towards the possibility that the fibers core is a polymeric material, for example Nylon 6,6, with a very low hardness. Most likely, the fibers were either coated to increase their resistance and be able to detach the first layer of the nasopharyngeal mucosa, or the manufacturer made a mixture of polymer and ceramic compounds. In addition, the swab's structures reveal a dust contamination likely to origin during a production process which was not performed in a clean room. In Figure 6, we see particulate matter that contaminates only the Citoswab. The contaminants contain Titanium, Iron. Chromium, Calcium, Aluminium and Silicon. Even though it is impossible to determine the origin of this pollution, manufacturing process in a dirty environment might be one of the possible scenarios.

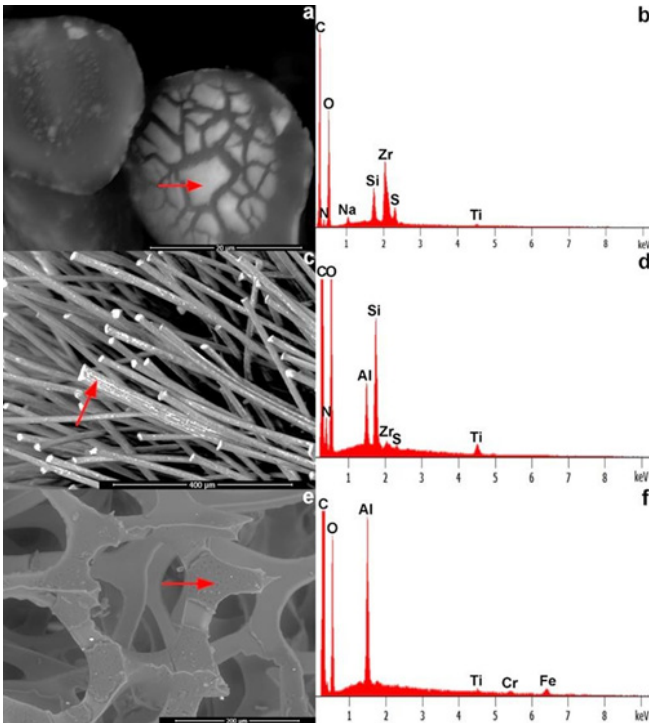

Figure 5 Shows the surface of the swab at three different magnifications.

The microphotographs show the swab morphologies at different magnifications, with the EDS spectra related to their chemical composition. (a, b) The FLOQSwab presents a non-homogenous fiber-coating, similar to the Taizhou Sun Trine swab (c, d). The Jiangsu Chang Feng swab (e, f) is a foam composed of Carbon, Oxygen, Aluminium, Titanium, Chromium and Iron

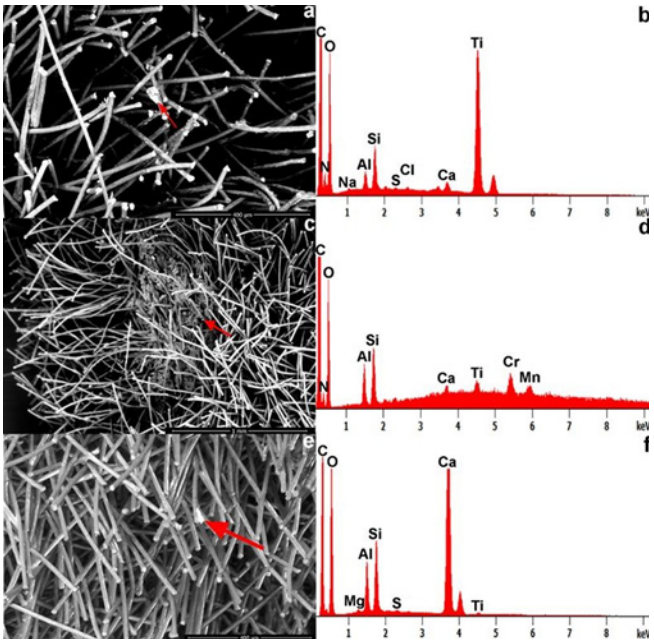

Figure 6 The microphotographs show different debris with their composition contaminating the Cito Swabs fiber.

In Figure 7 a & 7b, we can see that LP-Italiana cotton swabs are composed of Carbon and Oxygen as expected, while the dust granules are composed of Silicon, Carbon, Aluminium, Potassium, Oxygen, Magnesium, Titanium, Iron and Sulphur. In Fig.7 c and d, we see that the BioComma swab has a 350-micro-sized organic contaminant, where micro- and sub-microsized particles made of Carbon, Oxygen, Sulphur, Potassium, Silicon and Phosphorus are entrapped. In Figure 7e & 7f, we see many particles made of Carbon, Oxygen, Silicon and Aluminium, entrapped on the surface of Nobel Bio swab fibers. In Figure 7g & 7h, we see particles composed of Carbon, Oxygen, Magnesium, Silicon, Aluminium, Iron, Calcium, Chlorine and Potassium, adhered to the surface of Miraclean Technology swab. Figure 6 & 7 show the chemical composition of contaminants found in many swabs, proving that these medical devices are made in a dirty environment. They are indeed biologically sterile (no live bacteria are present), but their organic and inorganic chemical contamination is extremely high. It seems evident that the broken white and black fibers Figure 8, can be released during the use of the device. It is unknown whether the black wire was added, or it is simply dirt captured during the manufacturing process. In Figure 9, we see Silver nanoparticles detected at the base (neck) of a FLOQ swab. We suppose that this coating is used against bacteria, but we do not know if it also works with viruses. However, we point out that Silver is not declared in the data sheet issued by the Manufacturer.

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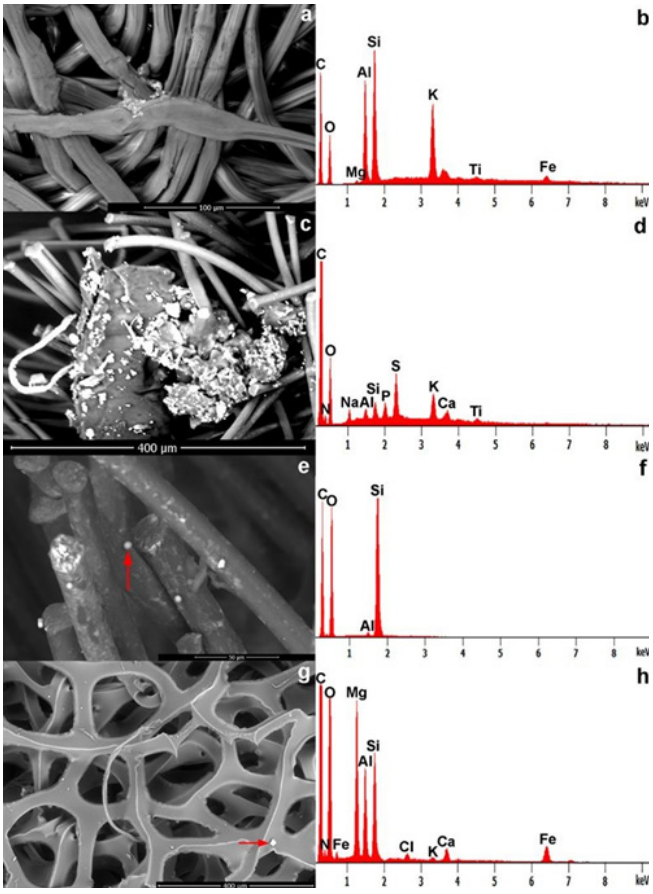

Figure 7 Micro-photographs showing foreign debris contained in different swabs.

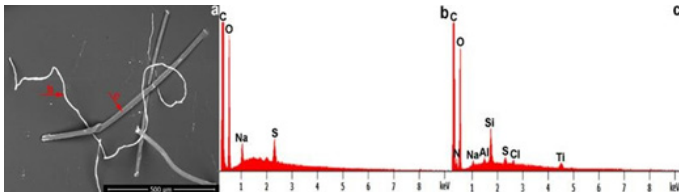

Figure 8 (a) The micro-image shows broken fibers and a black contaminating fiber collected from the swab; (b) the black fiber is "only" composed of Carbon-Oxygen-Sodium and Sulphur, (c) while the broken white fiber has a much richer chemical composition.

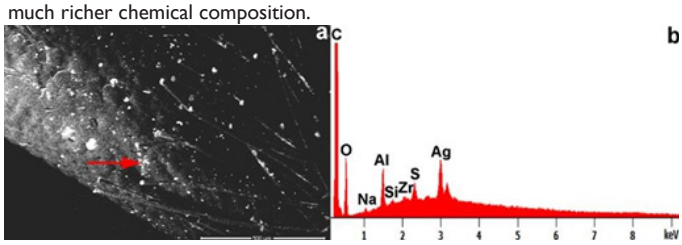

Figure 9 Image of the neck of a FLOQSwab.The small white dots (arrow) are Silver nanoparticles.

Discussion

Our analyses verified the morphology and chemical composition of swabs used world-wide to collect nose-throat biological samples, in the PCR process of Sars-CoV-2 viral RNA detection. On the swabs analyzed chemically interesting contamination (black wires, dust) was detected, most likely originating from the manufacturing or storage process. On the other hand, intentional swab's coating meant to harden the fibers makes them more harmful to the mucosa and, what is as important, is not declared in the data sheet. The "porcupine" swabs are made of hard fibers. There is a possibility that the pressure used during the swabbing maneuvers can break some fibers that remain in situ. When the operators are scraping the oropharyngeal mucosa, they can cause damage, like bleeding, or other tissue trauma. Some brittle fibers can be broken and left in-situ. If so, they can induce a foreign-body reaction that can damage the mucosa and hinder respiration and speech, something similar to type I (acute) and type IV (delayed) hypersensitivity. During the healing process of the mucosa, the broken fibers enter the tissue. If non-biodegradable, they are hardly or not at all eliminated, inducing inflammation and formation of granuloma and/or fibrotic tissue, even at anatomical districts distant from the collection site.[11] That is what happens as a normal body reaction towards all foreign bodies.[12] The non-biodegradability of the polymeric fibers and dirtiness can cause a severe long-term reaction.[13]

Conclusion

Considering that the swabs analyzed are medical devices, we are bound to conclude that they are not fully biocompatible, therefore they are not in line with ISO10993 standard, and should not have the CE mark.

In conclusion:

- Some swabs can be dangerous to the nasopharyngeal mucosa. The glassy fibers, hard and brittle, can scratch the mucosa and create lesions such as bleeding, that is an expression of invasive testing procedure. Their effect on the respiratory mucosa health is undeniable and known for a long time.[13]
- Repeated tests with swabs can induce chronic lesions. Of special concern is applying dirty swabs deeply inside nasal cavity, reaching and damaging the olfactory epithelium at the ethmoidal bone. Whatever delivered in this way can finally reach the brain.[14] The release of fragments of the brittle glassy fibers can induce immunological reactions, for example granuloma formation or tissue fibrosis, or can even, the nanosized structure of the coating in particular, reach the brain via the olfactory nerve.

In addition, these swabs represent a significant risk for babies' and children's health. In case tests are necessary (which is not yet scientifically demonstrated), children must be treated with smaller and softer swabs. In some schools in Germany, swabs are chewed to collect the biological sample, which is against the use indicated by the manufacturers, as this means sending poison into the gut system of a child. Any damage produced while applying such procedure is a responsibility that schools' authorities must take on. Finally, is it possible that swab's contamination can be a confounding factor during the PCR procedure and signal read- out? What about different lots of swabs having different chemical "decoration"? Knowing that most PCR tests are done with no technical replicate, if there are no clean swabs to be used as a control, how to know if the results are reliable? Unfortunately, this issue hasn't been addressed or taken into consideration from the normative organizations (WHO, EMA, etc.), though the issue can be responsible for inconsistencies and lack of validity of PCR results. Finally, the swabs used and the envelope where they are packaged show to be a great amount of waste that must be treated. At present, the only solution since plastic can hardly be degraded, the treatment is in the in incinerator plants or their disposal in landfill sites. In the former case, the burnt products concur to the increase of the CO_2, NOx, and carbonaceous pollution dispersed in the air, in the latter case, they are waste needing a long time to degrade. For the latter case, there is always the risk of the run-off due

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to the rain. This type of plastic is subjected to aging and a following cracking susceptibility. That induces its fragmentation in small debris. Since the landfill sites are never fully waterproof, the run- off can move them to the water tables and whence, via rivers, they reach the sea. A very recent paper reports how, through the ingestion of water and food (vegetables irrigated with this water or fish lived in the sea or rivers), the micro plastics can pollute man.[15]

Acknowledgments

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Conflicts of interest

The authors declare that there is no conflict of interest.

Funding

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