Standard operating procedure to prepare agar phantoms

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Abstract. Agar phantoms are widely used as soft tissue mimics and some preparation techniques are described in the literature. There are also standards that describe the recipe of a soft tissue mimicking material (TMM). However some details of manufacture process are not clearly defined. The standardization of the phantom's preparation can produce a metrological impact on the results of the acoustic properties measured. In this direction, this paper presents a standard operating procedure (SOP) to prepare the agar TMM described on the IEC 60601-2-37.

1. Introduction

Ultrasonic phantoms are probe bodies used to mimic the ultrasound properties of biologic tissues such as acoustic impedance, ultrasound propagation velocity, and attenuation coefficient, allowing the study of its interactions with the ultrasound [1]. In general, the use of these tissues mimicking material (TMM) is intended to many applications, such as the calibrating ultrasound imaging diagnostic and therapeutic equipment, evaluating heating generated by ultrasonic transducers, among others.

The advantage of tissue phantoms is that tissue models can be constructed with defined acoustic properties, dimensions and internal features, thereby simplifying and standardizing the imaging environment. Phantoms are available commercially, mimicking many tissues organs and organ systems. Commercial phantoms costs hundreds to thousands of dollars and are often preferred for training and calibration of ultrasound systems [2].

It is worth pointing out agar phantoms are widely used as soft tissue mimics and some preparation techniques described in the literature [2][3].

The standards IEC 60601-2-5 [4] and IEC 60601-2-37 [5] present a recipe of agar TMM to evaluate safety on ultrasound therapeutic and diagnostic equipment, respectively. Although described in standards, some details of manufacture procedure are not clear, or some aspects that could influence the homogeneity of the TMM and some details on its manufacture process are not well defined, which lead each one to perform different procedures.


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2. Materials and Methods

2.1. Phantom

Many materials have been studied looking for the optimum acoustical properties for the manufacture of phantoms such as gelatin, agar, polyacrylamide Zerdine®, urethane rubbers epoxy, among others [1]. This paper presents the agar TMM recipe.

The broad use of agar phantom is a result of their well characterized performance, the ease of fabrication and the flexibility that the process provides, allowing the incorporation of additional components to achieve a range of acoustic properties [2].

The accurate reporting of acoustic properties is highly dependent on preparation, and handling of TMM and the inherent dependence of acoustic properties, especially attenuation and backscatter. According to the standards [4][5] the acoustic properties of the TMM are listed in Table 1.

Table 1. Acoustic properties.

Material	**Velocity (m/s)**	**Density (kg/m^3)**	**Attenuation Coefficiente (dB/cm MHz)**	**Acoustic Impedance (10^8 kg/m^2 s)**
TMM	1540	1050	0.5	1.6

2.2. Materials

The list of materials used to prepare the phantom is described as follows:

- Beaker of 1000 ml;
- Beaker of 25 ml;
- Glass rod;
- Erlenmeyer of 2000 ml;
- Mechanical Stirrer;
- Foil.

2.3. Components

The mixture is made from the materials provided in Table 2 [4][5]. The second column provides a percentage weight of each pure component. It should be calculated the weight of the component according to the necessary amount of water. Considering 660 g of water, the weights of components are shown in the third column of Table 2.

Table 2. Components.

Component	**Weight (%)**	**Weight (g)**
Glycerol	11.21	89.19
Deionized Water	82.95	660
Benzallkonium chloride	0.47	3.74
Silicon Carbide (Sic(-400 mesh)	0.53	4.22
Aluminium Oxide (Al2O3 (0,3µm))	0.88	7
Aluminium Oxide (Al2O3 (3µm))	0.94	7.48
Agar	3.02	24.03
Sum	100	795.66

2.4. Stirring Technique

There are three possible stirring methods: manual, mechanical or magnetic. None of them was mentioned in the standards [4][5]. The literature still does not present conclusive works about that. However, Culjat and colleagues show the difference among these methods [2]. In this paper, the method chosen was the mechanical stirring because of the large volume of mixture.

2.5. Standard operating procedure

The standard operating procedure to prepare agar phantoms was divided in seven steps, described as following.

1º) Initially, all the components must be weighed and the deionized water, to add into the mix, heated up to 90 °C (Figures 1 and 2). A calibrated balance shall be used. Thereafter, the components must be mixed together in the following sequence:

Add Al_2O_3, SiC, glycerin and benzalkonium chloride in a container and mix them using a glass rod until a homogeneous appearance is obtained (looks like a viscous paste light gray). Then, add the agar and the water homogenizing the mixture.



Figure 1. Component being weighed.



Figure 2. Heating of water.

2º) The mixture should be maintained in a water bath under continuous mechanical stirring. The water bath temperature can vary in the range of 90-100 °C. In the procedure described herein, the water bath temperature was around 97 °C. It is noteworthy that the water bath should be preheated while weighing the components of the mixture for 30 minutes with thermal bath at 120 °C. The rotational speed varies with the volume of the mixture in question. Initially, the mechanical stirring was set at 200 rpm. It is important that no vortex shall occur to avoid the bubbles formation in the mixture. As the viscosity of the mixture increases, the rotational speed must increase. During the phantom

preparation, the mechanical stirring was increased in steps of 100 rpm, until it reaches 1000 rpm, according to the viscosity increasing.
3º) Heat the mixture for one hour in the temperature range of between 80-90 °C. In the present procedure, the mixture temperature was around 83 °C. To minimize evaporation to get out of the flask, it is desirable that the mixture container remains covered during the entire process. An Erlenmeyer was used due to its smaller nozzle diameter, in order to reduce evaporation.
4º) Therefore, the mixture should be cooled to 47 °C, under continuous mechanical stirring at 1000 rpm. Again, it is desirable that the mixture remains covered during this process in order to prevent evaporation.
5º) Dump the substance into a mold (Figure 3) and let it cool down. To avoid water evaporation, the mold is kept covered. After 24h the phantom can be demolded, cut (Figure 4), and it is ready to be stored.



Figure 3. The mixture being poured into the mold.

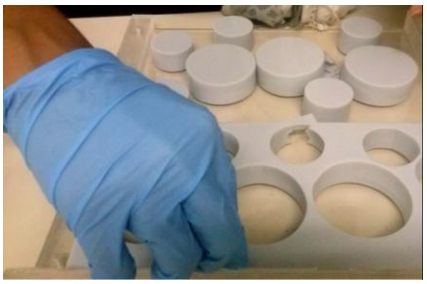


Figure 4. The phantoms being cut.

6º) The material should be stored in a closed container under normal laboratory conditions (18°C – 25 °C). While stored, keep the material in a water/glycerol mixture to prevent it from drying out and to avoid air contact. This mixture contains 88.1% (weight) demineralized water and 11.9% (weight) glycerol (purity > 99%).

The shelf life of the material is at least one year, if it is preserved without air contact. The addition of a 0.5% (weight) solution of benzalkonium chlorid acts as an antifungal agent extending the life of the phantom. Produced samples with shelf lives over 2 years were found [4][5].

Two independent batches of phantoms where produced in different days, both applying the procedure described in this paper. The speed of sound of two phantoms, each one from one batch, is presented in Table 3. While its expanded uncertainties (U) were calculated based on the method described in [7] and are presented in the same table. The values of speed of sound were measured at Labus using the procedure described in [8]. One can observe that, based on the normalized error (En), the values of speed of sound cannot be considered statistically different. This result suggests that, using the proposed procedure, it will possible to produce phantoms with similar ultrasonic properties.

Table 3. The values of speed of sound measured from phantoms obtained using the proposed standard operating procedure, but cooked in two different days.

Phantom	**Speed of sound (m/s)**	**U (m/s)**
Day 1	1551.8	5.5
Day 2	1550.3	5.3
E_n	0.2	

3. Discussions and conclusions

The lack of details in the standardizing document of agar phantom preparation could affect negatively on its ultrasonic properties. The aim of this paper is ultimately provide a step-by-step procedure to develop an agar phantom, according to the recipe disclosed at international standards. Furthermore, a metrologically based comparison between two phantoms produced in two dissimilar batches was performed. Ultrasonic quantity "speed of sound" was the comparison parameter. Results depicted from the statistical lead to the conclusion that the procedure to produce phantoms can be considered good enough, regarding its repeatability.

A worldwide international comparison is under agreement among many National Metrology Institutes and some ultrasonic devices manufactures. Agar phantoms will be the circulated among the participants, and the ultrasonic parameters "speed of sound" and "attenuation coefficient" will be the quantities to be assessed. The goal of such work is to stablish both a standardized agar phantom detailed procedure for manufacturing, as well as mutually accepted measurement procedure and uncertainties. The manufacturing procedure is the first step, and the work described within this paper will be used for such activity.

4. References

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