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## Broadband acoustic measurement of an agar-based tissue mimicking material - a longitudinal study

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Abstract: Commercially available ultrasound quality assurance test

phantoms rely upon the long-term acoustic stability of tissue-mimicking-materials (TMMs). The measurement of the acoustic properties can be technically challenging and it is important to ensure its stability. The standard technique is to film-wrap samples of TMM and to measure the acoustic properties in a water bath. In this study, a modified technique is proposed whereby the samples of TMM are measured in a preserving fluid that is intended to maintain their characteristics. The acoustic properties were evaluated using a broadband pulse-echo substitution technique over the frequency range of 4.5 - 50 MHz at 0, 6 and 12 months using both techniques. For both techniques, the measured mean values for the speed of sound and the attenuation were very similar and within the IEC recommended value. However, the results obtained using the proposed modified technique demonstrated

greater stability over the 1-year period when compared  
with the results acquired using the standard technique.

Suggested Reviewers: Deirdre M King

Senior Medical Physicist, Radiology, Hermitage Medical Clinic  
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She has good knowledge regarding quality assurance phantoms and tissue  
mimicking material. She could provide a different point of view for this  
study.

Mark P Brewin

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He published a paper that it is relevant to this study.

Srinath Rajagopal

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He published a paper that it is relevant to this study.

Opposed Reviewers:

# Adela Rabell-Montiel

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**27<sup>th</sup> of February, 2016**

Dr. Holland  
Editor-in-Chief  
Ultrasound in Medicine & Biology  
University of Cincinnati, Cardiovascular Research Center  
Cincinnati, OH, USA

**Dear Dr. Holland:**

Please find attached my revised manuscript with the title changed to 'Broadband acoustic measurement of an agar-based tissue mimicking material – a longitudinal study.' for consideration for publication in Ultrasound in Medicine and Biology.

I declared that the 'Broadband acoustic measurement of an agar-based tissue mimicking material – a longitudinal study.' have not been and will not be submitted elsewhere for publication.

I will advise the following for potential reviewers for the manuscript:

Dr. Mark Brewin

Dr. Srinath Rajagopal

Dr. Deirdre M King

**Sincerely,**

**RABELL-MONTIEL Adela**

*The authors are grateful to the Reviewers for their thorough review and constructive feedback on our manuscript.*

*The organisation of this response document will be as follows: answers to the questions raised by the Reviewers, will be in italic font. Modifications to the paper will be added in the same format.*

Editor in Chief: You have not made any modifications to the manuscript, nor have you convinced me that none are necessary.

1. Please add discussion of the difference in measured mean attenuation over time as a function of frequency, according to the reviewer's observations.

-OUR RESPONSE: Based on the original reviewer comment: "In addition, the change in attenuation with time appears only to be analyzed very qualitatively. It is suggested that authors report the average change in attenuation over 12 months, the frequency band which changes the least (and what that frequency is), and the frequency band which changes the most (and what that frequency is); this should be reported for both phantom preservation methods. The correlation coefficient and p-value should also be reported".

-OUR RESPONSE: We have added at in Page 17 Line 332-340. *"attenuation measurements obtained from the FTMMs compared to the UTMMs. At both the lower (4.5 – 9MHz) and the higher (40 – 50MHz) frequency ranges, the data displayed is obtained from a single transducer. Nevertheless, the attenuation versus frequency for the FTMMs would suggest, that with increasing frequency there is an increasing difference in measured mean attenuation values between the 6 month data and 0 and 12 month data. The maximum difference, 7 dB cm<sup>-1</sup>, occurring over the frequency range from 30 – 42MHz and a minimum difference at 15 – 19MHz. For the UTMMs, a maximum variation of 2 dBcm<sup>-1</sup> was observed across the time points, at a frequency of 47MHz and a minimum variation from 37 to 47MHz"*.

2. Please modify the title of your paper according to the reviewers' request. Simply changing the reference medium is not sufficient to highlight a "modified technique" in the manuscript. Modified reference fluid, yes. Modified technique, no.

-OUR RESPONSE: The title has been modified. *"Broadband acoustic measurement of an agar-based tissue mimicking material- a longitudinal study"*.

3. Incorporate your response to the reviewer's third comment regarding the lack of an effect of pressure output on attenuation measurement in the manuscript (not just in the response to the reviewer's comments). I suggest augmenting the discussion.

-OUR RESPONSE: We agreed that these points need to be added into the manuscript.

-We have added at Page 17 Line 344-349. *"These effects are unlikely to be due to the difference in non-linear effects between water and TMM preserving fluid as it has previously been shown (Sun, 2012) that even in water, at these output powers, the second harmonic component of the ultrasound beam is at least 30dB smaller in magnitude than the first harmonic (fundamental). Since non-linear effects are easier to generate in water than in the TMM preserving fluid, it is unlikely that nonlinearities are significantly greater than the experimental errors identified."*

1 **Broadband acoustic measurement of an agar-based tissue mimicking**  
2 **material – a longitudinal study.**

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14 ABSTRACT

15           Commercially available ultrasound quality assurance test phantoms rely upon the long-term  
16 acoustic stability of tissue-mimicking-materials (TMMs). The measurement of the acoustic properties  
17 can be technically challenging and it is important to ensure its stability. The standard technique is to  
18 film-wrap samples of TMM and to measure the acoustic properties in a water bath. In this study, a  
19 modified technique is proposed whereby the samples of TMM are measured in a preserving fluid  
20 that is intended to maintain their characteristics. The acoustic properties were evaluated using a  
21 broadband pulse-echo substitution technique over the frequency range of 4.5 – 50 MHz at 0, 6 and  
22 12 months using both techniques. For both techniques, the measured mean values for the speed of  
23 sound and the attenuation were very similar and within the IEC recommended value. However, the  
24 results obtained using the proposed modified technique demonstrated greater stability over the 1-  
25 year period when compared with the results acquired using the standard technique.

26

27 *Key words:* ultrasound, high frequency, tissue mimicking material, speed of sound, attenuation  
28 coefficient, long-term.

## 29 INTRODUCTION

30           Commercially available quality assurance (QA) test phantoms are widely used to test the  
31 performance of clinical ultrasound scanners. These phantoms, are manufactured from tissue-  
32 mimicking-material (TMM) which is designed to closely match the acoustical properties of the speed  
33 of sound (SoS) and the attenuation coefficient soft tissue. The aim of these phantoms is to provide a  
34 reproducible method to assess the performance of ultrasound scanners. However, these phantoms  
35 are intended for use with clinical ultrasound scanners at frequencies up to 20 MHz. To the best of  
36 our knowledge, there are no commercially available test phantoms to assess the performance of  
37 ultrasound scanners employing ultrasound frequencies above 20 MHz.

38           A variety of TMM materials are currently produced both commercially and within  
39 laboratories. These include: agar-based TMMs (Teirlinck et al., 1998), condensed milk TMMs  
40 (Madsen et al., 1998), gelatine TMMs (Culjat et al., 2010), konjac-carrageenan TMMs (Kenwright et  
41 al., 2014; Meagher et al., 2007), urethane rubber TMMs (Culjat et al., 2010), Poly (vinyl alcohol)  
42 Cryo-gel (PVA-C) TMMs (Cournane et al., 2010; Culjat et al., 2010; King et al., 2011) and Zerdine™  
43 TMMs (CIRS, Inc. Norfolk, VA). The International Electrotechnical Commission (IEC) agar-based TMM  
44 has become widely used and popular for clinical and preclinical test objects (Brewin et al., 2008;  
45 Browne et al., 2003; Cannon et al., 2011; Culjat et al., 2010; Inglis et al., 2006; Moran et al., 2011;  
46 Rajagopal et al., 2014; Sun et al., 2012; Yang et al., 2013). The acoustical properties of this agar-  
47 based TMM are designed to comply with the ultrasound acoustical parameters provided by the IEC  
48 (IEC, 2001) with the recommended SoS and attenuation over the frequency range 2 – 10 MHz being  
49  $1540 \pm 15 \text{ ms}^{-1}$  and  $0.5 \pm 0.05 \text{ dB cm}^{-1}$  respectively.

50           Moderately high-frequency ultrasound scanners (up to 20 MHz) have been manufactured for  
51 many years and have been utilised clinically in the assessment of skin (Machet et al., 2009), vascular  
52 structures (Rhee, 2007) and retinal imaging. In recent years, reliable high (20 – 50 MHz) and very  
53 high (>40 MHz) ultrasound scanners have become mainstream technology for the imaging of



54 superficial structures in clinical imaging and for preclinical imaging applications due to  
55 improvements in transducer engineering and software technology (Banchhor et al., 2016; Moran et  
56 al., 2011; Schmitt et al., 2010; Sundholm et al., 2015; Xu et al., 2012).

57 With the increase of high-frequency ultrasound imaging applications, there is a need to  
58 develop and to acoustically characterise TMMs suitable for high-frequency ultrasound QA and  
59 training phantoms. It has been shown that above 10 MHz the TMMs in the commercial test  
60 phantoms, do not have optimum acoustic properties as the attenuation starts to exhibit a nonlinear  
61 response with increasing frequency (Browne et al., 2003), whereas the IEC guidelines for TMM  
62 properties recommend a linear relationship between attenuation and frequency up to 10 MHz.

63 The agar-based TMM developed under the IEC guidelines and used in this study, has  
64 previously been found to have a non-linear response when acoustically investigated at frequencies  
65 up to 23 MHz by Brewin et al., (2008), in our own lab and in the National Physical Laboratory at  
66 frequencies up to 47 MHz and 60 MHz respectively (Sun et al., 2012 and Rajagopal et al., 2014). In  
67 these studies, the use of test cells or TMM samples wrapped with film material (Saran Wrap® or  
68 Mylar®) was employed to preserve the samples during acoustic characterisation when degassed,  
69 deionised water was used as the reference medium. Moreover, thin slices of TMM ranging in  
70 thickness from 2.5 – 30mm were used, enabling higher ultrasound frequencies to propagate through  
71 the TMM slices (Brewin et al., 2008; Rajagopal et al., 2014; Sun et al., 2012). The encasing of the  
72 TMM in film is important as, without the film, the TMM will degrade rapidly. This degradation is due  
73 to leaching of the glycerol from the TMM into the water reference medium, thus altering the  
74 acoustic properties of the TMM (Brewin et al., 2008). A reference water test cell, also encapsulated  
75 in Saran Wrap® or Mylar® film, was used in the reference measurement in order to account for the  
76 effect of the film on measurements (Rajagopal et al., 2014; Sun et al., 2012). However, the  
77 production of both the TMM slices wrapped in film and water test cells is time-consuming and  
78 technically challenging, especially for thin TMM samples. Therefore, the aim of this study was to

79 compare this well-established technique for the measurement and preservation of an IEC agar-TMM  
80 to a technique where TMM is characterised and preserved in a preserving fluid. Furthermore, this  
81 method was evaluated over a 1-year period, to determine the longitudinal stability of the acoustic  
82 properties.

## 83 MATERIALS AND METHODS

### 84 *Acoustical measurements*

85 Data was captured using two different acoustical systems, described briefly here and  
86 elsewhere (Sun et al., 2012). Firstly, a Vevo 770® preclinical ultrasound scanner (Visualsonics Inc.,  
87 Toronto, Canada) was used at the University of Edinburgh and secondly, a Scanning Acoustic  
88 Macroscope (SAM) system developed in-house at the Dublin Institute of Technology (Cannon et al.,  
89 2011). The SAM system was used to provide additional acoustic data and to extend the lower limit of  
90 the bandwidth of the measurements to 4.5 MHz.

### 91 *Manufacture of UTMMs and FTMMs samples.*

92 A batch of the IEC agar-based TMM was manufactured following a widely used standard  
93 recipe and method (Brewin et al., 2008; Browne et al., 2003; Cannon et al., 2001; Ramnarine et al.,  
94 2001; Teirlinck et al., 1998). This mixture was poured at 42°C onto a pre-warmed metal plate. The  
95 plate was pre-warmed to ensure that the mixture spread uniformly. The TMM mixture was then left  
96 to cool to room temperature. From this batch of TMM, 22 cylindrical slices of TMM of diameter  
97 5.5cm were cut using a thin-walled plastic tube. Due to the short focal lengths associated with high  
98 frequency transducers (Table 1), the thickness of the TMM slices was constrained to less than  
99 3.2mm and ranged in value from 1.8mm – 3.2 mm.

100 After being cut, eleven of these cylindrical TMM samples were left uncovered, and placed in  
101 a sealed container with TMM preserving fluid. This TMM preserving fluid was manufactured in-  
102 house (Brewin et al., 2008; Cannon et al., 2011; Inglis et al., 2006). These samples will be referred to  
103 as unwrapped-TMM (UTMM) (Figure 1a).

104 The remaining eleven TMM samples were used to manufacture the samples which were  
105 subsequently covered with clear film in the following manner. Initially, a layer (0.015mm thick) of  
106 Saran Wrap® film (SC Johnson Inc., Racine, USA) was stretched over an embroidery ring, of 10cm

107 diameter. A fast-hardening epoxy (Araldite Rapid; Huntsman Advanced Materials, Basel, Switzerland)  
108 was then applied to one side of a PVC ring (2mm thick, 5.8mm outer diameter) and the stretched  
109 Saran Wrap<sup>®</sup> was lowered onto the PVC ring. This was left to set overnight. The TMM was then  
110 manufactured as described above. After setting and cutting, the eleven samples were placed into  
111 the PVC rings. Five drops of TMM preserving fluid were added to the surface of the TMM to ensure  
112 good acoustic coupling between the film and the TMM, then a second layer of Saran Wrap<sup>®</sup> was  
113 glued to the other side of the ring, similar to that described above, such that the TMM slices were  
114 “sandwiched” between the two films and thereby, film-wrapped-TMM (FTMMs). These final film-  
115 wrapped samples were left to set overnight. Finally, epoxy was used to seal the edges of the film-  
116 ring-film to ensure the FTMMs did not leak. This was re-enforced with insulating tape to ensure that  
117 the film would not peel off during the 1-year period of investigation (Figure 1b). These FTMMs were  
118 preserved in a box with tissue paper moistened with TMM preserving fluid to create a humid TMM  
119 preserving fluid saturated environment. In a similar manner, a water test cell was manufactured  
120 whereby the TMM was replaced by degassed deionized water in the manufacturing process.

121 *Experimental set-up of Vevo 770<sup>®</sup> preclinical ultrasound scanner.*

122 In this study, the radio frequency (RF) data was collected and analysed from 11 FTMMs and  
123 11 UTMMs. To measure the acoustic properties, the FTMMs were submerged in a tank filled with  
124 degassed, deionised water as the reference medium, while for the UTMM measurements, the tank  
125 was filled with TMM preserving fluid. For both measurements, a Polymethylpentene (TPX, Boedeker  
126 Plastics, Texas, USA reflector) of 2.5cm diameter and 5mm thickness was located beneath the  
127 samples at the focal position of each transducer as illustrated in Figure 2.

128 Measurements were made using 4 transducers (Table 1) at 10% output power, using a high-  
129 frequency ultrasound scanner Vevo 770<sup>®</sup>. This power output was considered sufficient signal  
130 magnitude to obtain good signal-to-noise data without the generation of significant nonlinear  
131 effects (Sun et al., 2012). The regions of interest (ROI) were located at the upper surface of the TPX

132 reflector with and without the sample in place and from the lower and upper surfaces of each  
133 sample. For each measurement the RF data was collected from 10 scan-lines within these pre-  
134 selected ROIs at 4 different positions on the FTMMs or UTMMs. The data was analysed off-line using  
135 a MatLab script (MatLab R2013a MathWorks, Inc). The calculated angular separation between the RF  
136 acquisition lines is approximately  $0.15^\circ$ , so the lines were considered parallel and perpendicular to  
137 the TPX reflector. For the FTMMs, identical acoustic measurements were also taken through the  
138 water test cell to take into account potential reflections from the Saran Wrap® interfaces and to  
139 obtain absolute values of attenuation (Cannon et al., 2011; Sun et al., 2012).

140 The performance of both FTMMs and UTMMs were assessed over a period of one year at  
141 approximately 0, 6 and 12 month time points.

142 The 3dB bandwidth of each transducer (Table 1) was measured from the frequency spectra  
143 taken from the TPX reflector in a degassed, deionized water tank without the sample in place, where  
144 the reflector was placed at the focal length of the transducer.

145 *Analysis of speed of sound, thickness and attenuation data of FTMMs and UTMMs samples.*

146 The analysis of the FTMMs was performed based on a broadband pulse-echo substitution  
147 technique (AIUM, 2014). The pulse-echo return times from the front and rear surfaces of the FTMMs  
148 and from the front surface of the TPX reflector were used to determine the thickness and SoS of the  
149 samples. The magnitude of these pulse-echoes were used to calculate the attenuation. In addition,  
150 the echoes from the TPX reflector with the water test cell was acquired. This data was then used to  
151 calculate the SoS, thickness and attenuation of the FTMMs samples in a manner similar to that in  
152 Sun et al., (2012).

153 For the SoS and thickness of the UTMMs, the measurement technique and analysis was  
154 carried out in a similar manner to the FTMMs. However, since the UTMMs were not wrapped in  
155 Saran Wrap®, no water test cell was required and the reference measurements from the TPX were

156 taken through the bath of TMM preserving fluid. The absence of Saran Wrap® meant that the  
 157 magnitude of the echoes from the boundaries of the TMM was reduced. This, necessitated manual  
 158 selection of the position of the boundaries from the raw RF signals. This was performed by selection  
 159 of the largest pulse-echo at each interface of the UTMMs. The criterion for this was that the peak  
 160 selected was at least 100% greater than the magnitude of the previous peak within 2 μs time-  
 161 window. This was carried out for each of the 10 lines of the raw RF signal inside the ROI of the  
 162 UTMMs, enabling the thickness of the sample to be determined at each of these positions. In  
 163 addition, the thickness values of the UTMMs were measured manually in five different positions on  
 164 each UTMM with a digital calliper (DURATOOL, TM Taiwan, 0 – 150mm). Each sample was placed  
 165 between 2 plastic plates of known thickness in order to avoid excessive compression of the UTMMs  
 166 (Brewin et al., 2008; Rajagopal et al., 2014). This was performed at the initial time-point before the  
 167 acoustic measurements commenced.

168 The SoS in the UTMMs was calculated using Equation (1).

$$SoS_{UTMM} = \left( 1 + \frac{T_R - T_{TMMR}}{T_{TMMLW} - T_{TMMUp}} \right) SoS_{TMMfluid} \quad (1)$$

169 Where  $T_R$  is the time from the transducer to the TPX in the tank with no sample in the acoustical  
 170 path,  $T_{TMMR}$  is the time from the transducer to the TPX reflector through the sample,  $T_{TMMLW}$  and  
 171  $T_{TMMUp}$  are the time from the transducer to the lower and upper surface respectively of the sample  
 172 and  $SoS_{TMMfluid}$  is the SoS of the TMM preserving fluid in which the samples were immersed.

173 Calculation of the attenuation of the UTMMs was carried out in a similar manner to the  
 174 FTMMs, but without the use of the water test cell, to compensate for the interfacial attenuation  
 175 loss.

176 *Acoustical properties of TMM preserving fluid and degassed, deionized water.*

177 The acoustic properties of the TMM preserving fluid were measured by the National Physical  
178 Laboratory (Teddington, UK). The SoS was found to be  $1538.15 \pm 0.22 \text{ ms}^{-1}$  at  $19.3 \pm 0.1^\circ\text{C}$  and a 2<sup>nd</sup>  
179 degree polynomial function was fitted to the attenuation data ( $\alpha$  [dB cm<sup>-1</sup>]) as a function of  
180 frequency ( $f$  (MHz)): as  $\alpha_{TMMfluid} = 0.00309f^2 - 0.004996f$  ( $R^2=0.99$ ) over the frequency range  
181 of 1 – 60 MHz.

182 The acoustic properties of the degassed deionized water have previously been measured  
183 and found to have an attenuation proportional to  $f^2$  over the range of 7.5 – 67.5 MHz (Duck, 1990;  
184 Pinkerton, 1949). Furthermore, the SoS of degassed deionized water varies with temperature  
185 (Bilaniuk et al., 1992; Del Grosso et al., 1972). In this study all measurements were undertaken at  
186  $22.2 \pm 0.5^\circ\text{C}$  with a SoS of  $1488.88 \text{ ms}^{-1}$ .

187 *Experimental set-up and data analysis using the SAM system (Dublin Institute of Technology).*

188 The SAM system uses broadband transducers which work as both transmitter and receiver  
189 (Olympus NDT Inc., Waltham, MA, USA). The experimental setup was similar to that used with the  
190 Vevo 770<sup>®</sup> (Figure 2). Three different transducers were used (Table 1) where the 3dB bandwidth was  
191 measured in a similar manner to that of the transducers of the Vevo 770<sup>®</sup>.

192 A pulser-receiver (Model 5052PR; Panametrics, Waltham, MA, USA) was used to transmit  
193 and receive the pulses. The received reflected pulse was digitised and captured using a data  
194 acquisition card (PCI-5144: National Instruments, Austin, TX, USA) with the data acquisition  
195 controlled by a LabView (National Instruments Corporation, TX, USA) program.

196 The SAM system displayed the RF data in real time during the measurements. Ten lines of  
197 data were acquired from each of four different positions on the reflector with and without the  
198 sample (FTMMs or UTMMs) in place.

199           The calculation of the SoS and the attenuation coefficient was also based on the broadband  
200 pulse-echo substitution technique. However, here the thickness of the sample was inputted into the  
201 calculation of SoS and attenuation coefficient. The thickness value input for each FTMMs and  
202 UTMMs was the mean of the 10 different measurements at each of 8 locations calculated using the  
203 4 transducers of the Vevo 770<sup>®</sup> ultrasound scanner.

204           At all-time points, measurements taken with the Vevo 770<sup>®</sup> were performed before  
205 measurements with the SAM system.

206 *Unpreserved samples, batch to batch variation, and repeatability of the UTMMs.*

207           The acoustic properties of two uncovered TMM samples (UTMM) were measured in an  
208 identical manner to the UTMMs described previously using the RMV704 transducer (centre  
209 frequency 40 MHz, Table 1). Measurements were undertaken initially and then approximately once  
210 every 24 hours over a 96 hour period. However, in-between measurements, the samples were left  
211 exposed to the air. These samples will be referred to unpreserved samples.

212           An indication of TMM batch-to-batch variability was assessed by measuring the acoustical  
213 properties of 6 UTMMs manufactured from a different batch of TMM. These samples will be  
214 referred to as UTMM2. These samples had a mean thickness of  $2.01 \pm 0.05$  mm as measured using  
215 the Vevo 770<sup>®</sup> scanner. The acoustical properties were measured with the Vevo 770<sup>®</sup> and with the  
216 SAM system at the 6 and at 12 month time points.

217           Data analysis was performed in an identical manner to that with the 11 UTMMs described  
218 above.

219           To assess the repeatability of the measurement system, the acoustic properties of the 11  
220 UTMMs were measured with one transducer RMV710 with the Vevo 770<sup>®</sup> at 5 different times over 1  
221 month period. The reference medium was TMM preserving fluid. The temperature for these  
222 measurements was  $22.0 \pm 0.4^{\circ}\text{C}$ .



223 RESULTS

224 The mean thickness of the FTMMs and UTMMs calculated using the RF ultrasound signals  
225 from the Vevo 770<sup>®</sup> over all the time points showed a maximum variation of 0.25mm and 0.08mm  
226 for the FTMMs and UTMMs respectively. The thicknesses, measured by the digital calliper, from 11  
227 UTMMs showed a maximum variation of 0.03mm.

228 Table 2 shows the mean SoS of FTMMs and UTMMs at each time point. It can be seen that  
229 the SoS of the FTMMs exhibited larger variability than the SoS of the UTMMs. Using a Student's t-  
230 test it was shown that the mean SoS value of FTMMs and UTMMs samples were not statistically  
231 different ( $p>0.5$ ) at 0 time point, but displayed a significant difference ( $p<0.05$ ) at the remainder of  
232 the time points. The results after 1 year showed that the SoS of the FTMMs decreased  $22.1 \text{ ms}^{-1}$   
233 when compared with the first measurement at 0 months, whereas the SoS of the UTMMs samples  
234 decreased  $4.1 \text{ ms}^{-1}$  over the same 12 month period. The SoS of UTMM2 samples calculated over a 6  
235 month time period showed a decrease from  $1558.1 \pm 5.3 \text{ ms}^{-1}$  to  $1544.8 \pm 3.3 \text{ ms}^{-1}$ , with a difference  
236 of  $13.3 \text{ ms}^{-1}$ .

237 Table 3 shows the mean SoS averaged all time-points for each of the measurement systems.  
238 It was found that the SoS measurements using the SAM system showed smaller variability than the  
239 SoS measurements using the Vevo 770<sup>®</sup> for the FTMMs and UTMMs. The mean values over all the  
240 samples over all time points, using both measurement systems, were found to be  $1538.2 \pm 14.5 \text{ ms}^{-1}$   
241 for the FTMMs and  $1544.0 \pm 3.5 \text{ ms}^{-1}$  for the UTMMs (Table 4). The mean SoS for the UTMM2 was  
242 found to be  $1551.4 \pm 6.2 \text{ ms}^{-1}$ . Table 4 also shows the SoS results in comparison with those values  
243 published for IEC agar TMM (Brewin et al., 2008; Browne et al., 2003; IEC, 2001; Rajagopal et al.,  
244 2014; Sun et al., 2012). The mean SoS of FTMMs and UTMMs are within the values specified by the  
245 IEC (IEC, 2001).

246 Figure 3 and Figure 4 show the attenuation as a function of frequency for the FTMMs and  
247 the UTMMs respectively at 0, 6 and at 12 month time points measured using the seven different  
248 transducers. It can be seen that the variation in mean attenuation values for the UTMMs is small in  
249 comparison to the variation observed in mean attenuation values measured from the FTMMs.

250 Figure 5 and Figure 6 shows the mean attenuation data over the frequency range 4.5 – 50  
251 MHz, averaged over all 11 FTMMs and over all 11 UTMMs and time points, respectively.

252 The batch to batch variation of TMM was assessed by the UTMM2s ( $2.01 \pm 0.05$  mm  
253 thickness). These samples were measured with both the Vevo 770<sup>®</sup> and the SAM system (at 6 and 12  
254 month time points). The mean SoS of UTMM2s was found to be 0.44% higher ( $7.4 \text{ ms}^{-1}$ ) than the  
255 mean SoS of the 11 UTMMs. For the attenuation, the UTMM2s were found to have a maximum  
256 difference of  $\pm 1 \text{ dB cm}^{-1}$  in mean attenuation across the frequency range shown in Figure 7.

257 For the unpreserved samples, after 96 hours of exposure to air, the samples were visibly  
258 dehydrated. The mean thickness of the two samples had decreased by 1.22mm and the diameter  
259 was decreased by 1.5cm. Additionally, the SoS was shown to increase by  $140 \text{ ms}^{-1}$  for sample 1 and  
260  $180 \text{ ms}^{-1}$  for sample 2 over the total period of time. The attenuation was found to increase by  
261 approximately  $10 \text{ dBcm}^{-1}$  per day.

262 In the assessment of repeatability, the mean SoS over the five measurements taken from the  
263 11 UTMMs was calculated to be  $1543.0 \text{ ms}^{-1}$  with a range in SoS of  $\pm 11.0 \text{ ms}^{-1}$ . The mean SoS was  
264 found to be smaller by  $1 \text{ ms}^{-1}$  when compared to the mean SoS calculated using all the transducers  
265 at all time points (Table 4) of the UTMMs. The variation in attenuation as a function of frequency  
266 was  $\pm 1 \text{ dBcm}^{-1}$  over the frequency range of the Vevo770<sup>®</sup> RMV710B probe.

267 Polynomial functions were calculated for the attenuation as a function of frequency at each  
268 time point for the FTMMs and for the UTMMs. The best fit polynomial function was determined over  
269 all the attenuation versus frequency data in the range 4.5 – 50 MHz as a combination of Vevo 770<sup>®</sup>

270 and SAM system data over all time points (Figure 8). The polynomial fit found for FTMM was  
271  $0.4649f + 0.007363f^2$  ( $R^2=0.80$ ) and  $0.4897f + 0.008366f^2$  ( $R^2=0.99$ ) for UTMM. The goodness  
272 of fit ( $R^2$ ) of the three polynomial fits at each of the three time points for the FTMMs ranged  
273 between 0.78 – 0.92 whereas for the UTMMs this value ranged between of 0.96-0.99. In addition, in  
274 Figure 8, for comparison, the attenuation data of the IEC agar TMM from studies already published is  
275 included.

276 DISCUSSION

277 This aim of this study was to develop a robust and easy-to-use technique for the  
278 characterisation and preservation of the IEC agar TMM and to compare the acoustic properties  
279 obtained using this modified technique with the standard technique over a period of one year.

280 The thickness used in the calculation of SoS from the SAM system was a mean thickness  
281 measured by the Vevo 770<sup>®</sup> ultrasound scanner at eight different locations on the UTMMs (10 lines  
282 at each position). Although this mean thickness was used, the SD of the SoS values from the SAM  
283 system were smaller than the SD variation calculated using the Vevo 770<sup>®</sup> (Table 4) which would  
284 suggest that the use of this mean thickness in the SAM system measurements did not contribute  
285 significantly to the experimental error.

286 The acoustic properties of the UTMMs were measured in TMM preserving fluid whose  
287 acoustical properties were assessed by NPL at  $19.3 \pm 0.1^\circ\text{C}$ , whereas the UTMMs in this study were  
288 measured at  $22.2 \pm 0.5^\circ\text{C}$ . The TMM preserving fluid is composed of the same fluid as used in the  
289 TMM manufacture process and Brewin et al (2008) has previously shown a TMM SoS temperature  
290 dependence of  $2.1 \text{ ms}^{-1} \text{ }^\circ\text{C}^{-1}$ . Consequently, there is likely to be a maximum variation of  $6 \text{ ms}^{-1}$  in the  
291 SoS of the TMM preserving fluid which could be attributable to the temperature change. Such a  
292 change would result in a potential error of less than  $7 \text{ ms}^{-1}$  in the measured SoS of the UTMMs.  
293 Nevertheless, the SoS values of the UTMM were found to be in good agreement with Rajagopal et  
294 al., (2014); Sun et al., (2012) and Brewin et al., (2008). Furthermore, the SoS of the UTMMs was  
295 found to decrease by  $4.1 \text{ ms}^{-1}$  over a 12 month period compared with FTMMs which showered a  
296 decrease in the mean SoS of  $22.1 \text{ ms}^{-1}$  over the same period of time. Additionally the standard  
297 deviation of the mean SoS values for FTMM samples was larger than that for UTMMs at all time-  
298 points. This increased variation in SoS values for the FTMMs in comparison to UTMMs may be  
299 attributable to a number of reasons. Firstly, although a visual inspection was performed on each of  
300 the FTMMs before each acoustical measurement and no evidence of leakage was observed,

301 nevertheless, in several of the samples, the epoxy securing the film to the rings showed signs of  
302 ageing and the Saran Wrap® film appeared to become less taut over the 1 year period. This could  
303 have potentially increased the permeability of the film allowing the glycerol from the TMM to leach  
304 into the water medium resulting in a decrease in the measured SoS properties of the FTMM.  
305 However, although a decrease in SoS in FTMMs was measured between 0 and 12 months, this did  
306 not decrease continuously over the 1-year period which would suggest that the measured variation  
307 is not likely to be attributable to glycerol leakage. Secondly, for the FTMMs, the position of the  
308 water-film interfaces was selected using Matlab code based on the identification of the position of  
309 the maximum rectified RF signal and it was assumed that this signal also marked the TMM interface.  
310 Although this is a reasonable assumption, if any of the FTMM samples were subject to shrinkage (by  
311 drying out) over the 1-year period, this would represent a potential source of error.

312           The SoS results of both FTMMs and UTMMs in this study were compared with previously  
313 published work (Table 4). It can be seen that the UTMM mean SoS values are in good agreement  
314 with those published, whereas the mean SoS of FTMM were found to be  $5.8 \text{ ms}^{-1}$  smaller when  
315 compared with Rajagopal et al., 2014 and up to  $9.6 \text{ ms}^{-1}$  smaller when compared with Sun et al.,  
316 2012. In Rajagopal et al., (2014) the manufacture of FTMM was achieved by sandwiching the TMM  
317 slice between 2 sheets of Mylar® ( $\sim 12\mu\text{m}$  thickness) affixed into Perspex frames, whereas in Sun et  
318 al., (2012) the manufacture process of the FTMMs was similar to the method used in this study  
319 (referred to as TMM test cells in that study). However, in Rajagopal even though the acoustic  
320 measurement was completed relatively quickly (within seconds) the edges of the TMM were not  
321 covered, which is likely to have led to some undefined glycerol leakage and potentially affect the  
322 measured acoustical properties. Furthermore, in Brewin et al., (2008) the acoustical properties of 2  
323 different batches of TMM were measured over a 3 year period with a thickness range from 3mm to  
324 12.7mm. In Brewin, the first batch consisted of TMM samples which were not protected by a film  
325 and were measured in double degassed, deionized water. As a result of glycerol leaching from the  
326 samples in this batch, the SoS was found to decrease by  $2.1 \text{ ms}^{-1} \text{ }^\circ\text{C}^{-1}$ . The second batch consisted of

327 TMM samples protected by Saran Wrap® and were also measured in water. Using this method  
328 thinner samples (3mm) displayed the largest SoS variation of  $13.4 \text{ ms}^{-1}$ . This value is comparable to  
329 the SD found in this study for the FTMMs (Table 6) but considerably higher than that measured for  
330 the UTMMs.

331 Figure 3 and Figure 4 show the attenuation of FTMMs and UTMMs respectively, at 0, 6 and  
332 12 month time points. It can be observed, that there is a much larger variation in the attenuation  
333 measurements obtained from the FTMMs compared to the UTMMs. At both the lower (4.5 – 9MHz)  
334 and the higher (40 – 50MHz) frequency ranges, the data displayed is obtained from a single  
335 transducer. Nevertheless, the attenuation versus frequency for the FTMMs would suggest, that with  
336 increasing frequency there is an increasing difference in measured mean attenuation values between  
337 the 6 month data and 0 and 12 month data. The maximum difference,  $7 \text{ dB cm}^{-1}$ , occurring over the  
338 frequency range from 30 – 42MHz and a minimum difference at 15 – 19MHz. For the UTMMs, a  
339 maximum variation of  $2 \text{ dBcm}^{-1}$  was observed across the time points, at a frequency of 47MHz and a  
340 minimum variation from 37 to 47MHz.

341 Moreover, the difference in mean attenuation values between the UTMMs and FTMMs  
342 would suggest that, despite compensation for the effects of the Saran Wrap®, some additional  
343 acoustic effects are introduced which are not fully compensated using the Saran-wrapped reference  
344 water test-cell. These effects are unlikely to be due to the difference in non- linear effects between  
345 water and TMM preserving fluid as it has previously been shown (Sun, 2012) that even in water, at  
346 these output powers, the second harmonic component of the ultrasound beam is at least 30dB  
347 smaller in magnitude than the first harmonic (fundamental). Since non-linear effects are easier to  
348 generate in water than in the TMM preserving fluid, it is unlikely that nonlinearities are significantly  
349 greater than the experimental errors identified.

350 Figure 5 and Figure 6 show the mean attenuation of the 11 FTMMs and the 11 UTMMs  
351 across the 7 different transducers and measured 3 times during the time period of 1 year. The

352 FTMMs (Figure 5) showed larger variability ( $\sim 15\text{dB cm}^{-1}$ ) across samples and transducers. This may  
353 be due to inadequate acoustical correction for the interface layers when using the reference water  
354 test cell, leading to an increased uncertainty in the attenuation measurements in addition to the  
355 factors previously described. The UTMMs (Figure 6) showed good consistency and little variability in  
356 the attenuation over the frequency range of 4.5 – 50 MHz.

357           Polynomial fits from FTMM and UTMMs were in good agreement with previous studies in  
358 the frequency range of 17 – 23 MHz (Brewin et al., 2008), 10 – 47 MHz (Sun et al., 2012) and 1 – 60  
359 MHz (Rajagopal et al., 2014). The polynomial fits were also in good agreement at lower frequencies  
360 4.5 to 10 MHz as reported by IEC (IEC, 2001) and in other studies (Brewin et al., 2008; Inglis et al.,  
361 2006) as can be seen in Figure 8. The attenuation from FTMMs and UTMMs does not increase  
362 linearly with frequency as shown by the quadratic terms of the polynomial fit. This quadratic term  
363 was found to be 0.0073 for FTMMs and 0.0083 for UTMMs which is in good agreement with 0.0076  
364 reported by Sun et al., 2012 and with 0.0081 reported by Rajagopal et al., 2014.

365           Finally the unpreserved samples displayed significant visual degradation and changes in SoS  
366 and attenuation, over the 96 hours. These results are consistent with those of Brewin et al., (2008)  
367 who also reported shrinking and hardening of TMM samples which were not preserved.

368 CONCLUSIONS

369 In this study, two different measurement techniques were evaluated for assessing the  
370 temporal stability of the acoustic properties of the IEC agar TMM over the frequency range 4.5 – 50  
371 MHz. In the first technique thin slices were wrapped and stored in Saran Wrap® and measured in  
372 degassed, deionised water. In the second technique, thin slices of TMM were preserved and  
373 measured in TMM preserving fluid. Measurements were undertaken, over the period of 1 year. The  
374 measured SoS of an IEC agar TMM calculated by the Vevo 770® and SAM system was found to be  
375  $1538.2 \pm 14.5 \text{ ms}^{-1}$  for the FTMMs and  $1544.0 \pm 3.5 \text{ ms}^{-1}$  for the UTMM. For FTMMs the SoS results  
376 were less than  $10 \text{ ms}^{-1}$  lower when compared with those published. The acoustic properties of  
377 UTMMs (SoS and attenuation values) were found to be in good agreement with results in earlier  
378 studies by Brewin et al., (2008) over the range of 17 – 23 MHz, Sun et al., (2012) over the range of 10  
379 – 47 MHz and Rajagopal et al., (2014) over the range of 1 – 60 MHz. Nevertheless, the results for  
380 both FTMMs and UTMMs were consistent at low frequencies (Browne et al., 2003; Inglis et al., 2006)  
381 and within the range provided by the IEC (IEC, 2001). However, the attenuation coefficient was  
382 shown to be nonlinear as a function of frequency. The attenuation was found to increase as  
383  $0.4649f + 0.007363f^2$  for FTMMs and as  $0.4897f + 0.008366f^2$  for UTMMs with increasing  
384 frequency. This second degree polynomial fit was derived based on the data generated in this study  
385 using two different measurement systems and was shown to be able to estimate the attenuation of  
386 this IEC agar TMM in the frequency range of 4.5 – 50 MHz. The quadratic term was also found to be  
387 in good agreement with previous studies.

388 Finally, this study has demonstrated that using unwrapped TMM slices (UTMM), maintained  
389 and measured in TMM preserving fluid, results in approximately 4 times smaller SD values for the  
390 SOS and up to 5 times smaller variation for the attenuation values when compared with the  
391 common method of film-wrapped TMM samples (FTMM) measured in degassed, deionised water.  
392 This suggests that, despite compensation within the calculation of the attenuation effects of the



393 Saran Wrap<sup>®</sup>, additional acoustic effects are introduced which are not fully compensated using the  
394 standard technique (FTMMs). Moreover, this study has also brought into question, the validity and  
395 subsequent stability of encasing gel TMM QA phantoms in a sealed film-dry environment.

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399 characterisation of the TMM preserving fluid. This study was funded by a CONACyT (Becas al  
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487

488 LIST OF FIGURES

489 Figure 1. a) Non-wrapped TMM slices (UTMMs), arrow indicates the identification mark on the  
490 sample and b) wrapped TMM slices (FTMMs).

491 Figure 2. Experimental set-up using a high frequency ultrasound scanner Vevo 770<sup>®</sup> used at the  
492 University of Edinburgh and a SAM system used at the Dublin Institute of Technology.

493 Figure 3. Measured attenuation with the Vevo 770<sup>®</sup> and SAM system of 11 FTMM at 0, 6 and 12  
494 month time points as a function of frequency. Each line represents one of the seven different  
495 transducers used at each time point [RMV704 (18 – 40 MHz), RMV707B (12 – 32 MHz),  
496 RMV710B (12 – 28 MHz), RMV711 (25 – 50 MHz), V320 (4.5 – 9 MHz), V317 (14 – 25 MHz),  
497 V390 (20 – 40 MHz)].

498 Figure 4. Measured attenuation with the Vevo 770<sup>®</sup> and SAM system of 11 UTMM at 0, 6 and 12  
499 month time points as a function of frequency. Each line represents one of the seven different  
500 transducers used at each time point [RMV704 (18 – 40 MHz), RMV707B (12 – 32 MHz),  
501 RMV710B (12 – 28 MHz), RMV711 (25 – 50 MHz), V320 (4.5 – 9 MHz), V317 (14 – 25 MHz),  
502 V390 (20 – 40 MHz)].

503 Figure 5. Attenuation data as a function of frequency averaged over all time points (data set: 11  
504 FTMM measured in degassed deionized water by the Vevo 770<sup>®</sup> and SAM system in the  
505 frequency range of 4.5 – 50 MHz).

506 Figure 6. Attenuation data as a function of frequency averaged over all time points (data set: 11  
507 UTMM preserved and measured in TMM preserving fluid by the Vevo 770<sup>®</sup> and SAM system in  
508 the frequency range of 4.5 – 50 MHz).

509 Figure 7. Attenuation data as a function of frequency averaged over all time points from the 6  
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511 770<sup>®</sup> and SAM system at 6 and 12 month time points in the frequency range of 4.5-50 MHz).

512 Figure 8. Polynomial curve-fit of all the attenuation data as a function of frequency and the  
513 attenuation (compensated for the attenuation in water) of TMM in 2 – 10 MHz (IEC, 2001), 17  
514 – 23 MHz (Brewin et al., 2008), 2.25 – 15 MHz (Browne et al., 2003), 6 – 15 MHz (Inglis et al.,  
515 2006), 10 – 47 MHz (Sun et al., 2012) and 1 – 60 MHz (Rajagopal et al., 2014). Also the  
516 attenuation as a function of frequency for the TMM preserving fluid and degassed deionised  
517 water.



Table 1. Characteristics of the Vevo 770<sup>®</sup> and SAM system transducers. The central frequency and focal length are parameters provided by the manufacturer from Vevo 770<sup>®</sup> (VisualSonics, Inc., Toronto, Canada) and SAM system (Olympus Panametrics NDT), the 3dB bandwidth from measurements and the peak pressure from (Sun, 2012).

Transducer model and measurement system		Central Frequency (MHz)	Focal Length (mm)	Measured 3dB bandwidth (MHz)	Peak negative pressure (MPa)
RMV 704	Vevo 770 <sup>®</sup>	40	6	18 – 40	0.52
RMV 707B		30	12.7	12 – 32	1.05
RMV 710B		25	15	12 – 28	1.06
RMV 711		55	6	25 – 50	0.23
V320	SAM system	7.5	95	4.5 – 9	0.05
V317		20	65	14 – 25	0.021
V390		50	12	20 – 40	0.022

Table 2. The mean and SD of SoS ( $\text{ms}^{-1}$ ) measured with the Vevo 770<sup>®</sup> and SAM system at each time point for the FTMMs and UTMMs samples.

SoS $\pm$ SD ( $\text{ms}^{-1}$ )	0 months	6 months	12 months
<b>FTMM</b>	1547.4 $\pm$ 19.2	1547.2 $\pm$ 21.5	1525.5 $\pm$ 16.5
<b>UTMM</b>	1545.9 $\pm$ 10.4	1544.2 $\pm$ 11.0	1541.8 $\pm$ 1.6

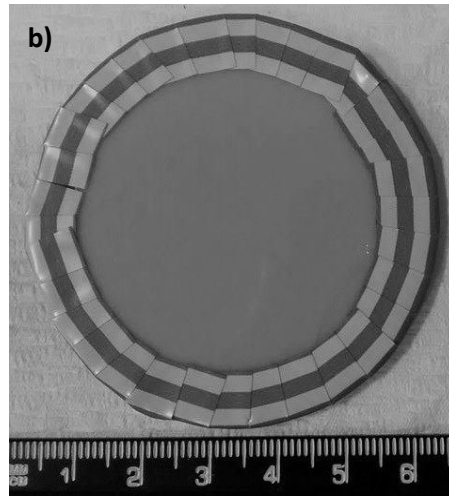
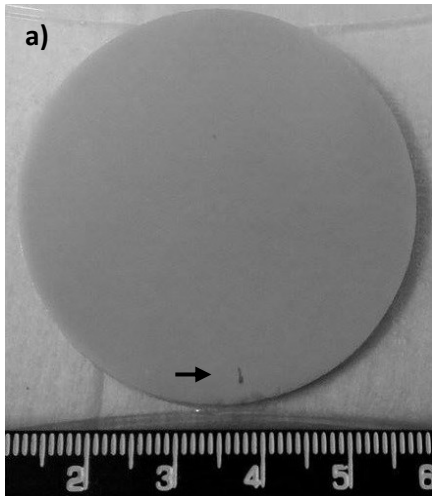
Table 3. The mean and SD of SoS ( $\text{ms}^{-1}$ ) over all time points of 11 FTMM and 11 UTMM measured by the four transducers of the Vevo 770<sup>®</sup> and by the three transducers of the SAM system.

<b>SoS <math>\pm</math> SD (<math>\text{ms}^{-1}</math>)</b>	<b>Vevo 770<sup>®</sup></b>	<b>SAM system</b>
<b>FTMM</b>	1539.6 $\pm$ 17.1	1536.3 $\pm$ 10.3
<b>UTMM</b>	1542.9 $\pm$ 3.6	1545.3 $\pm$ 3.0

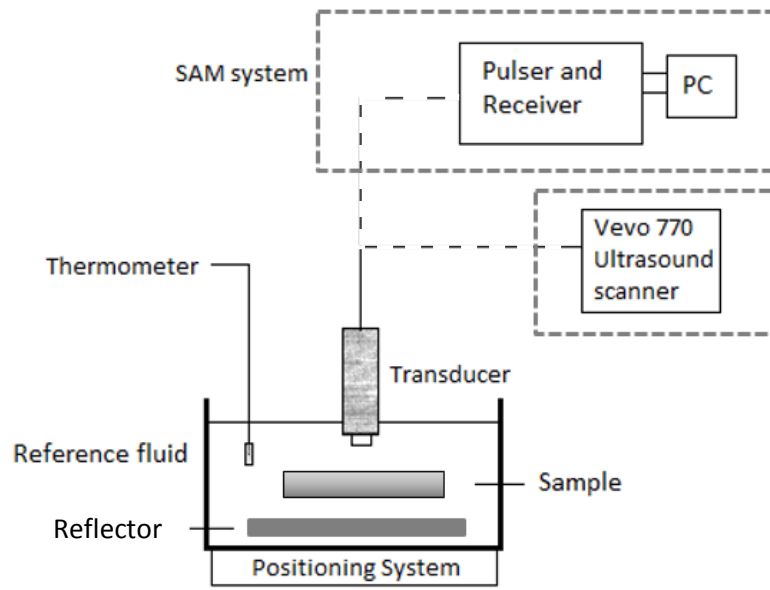
Table 4. Values of SoS ( $ms^{-1} \pm SD$ ) measured in this study using 2 different methods, compared with the published data.

Sources	Type of samples (covered with film or uncovered)	Mean SoS $\pm$ SD ( $ms^{-1}$ )	Frequency range (MHz)
IEC, 2001		1540 $\pm$ 15	2 – 10
Browne et al., 2003	TMM uncovered measured in degassed water	1546.5 $\pm$ 3	2.25 – 15
Brewin et al., 2008	TMM uncovered measured in degassed water	1537 $\pm$ 2.6	17 – 23
	TMM covered	1540.9 $\pm$ 8.7	
Sun et al., 2012	TMM covered	1547.8 $\pm$ 3.7	10 – 47
Rajagopal et al., 2014	TMM covered	1544 $\pm$ 3.1	1 – 60
This study	TMM covered (FTMM)	1538.2 $\pm$ 14.5	4.5 – 50
	TMM uncovered (UTMM) measured in TMM fluid	1544.0 $\pm$ 3.5	

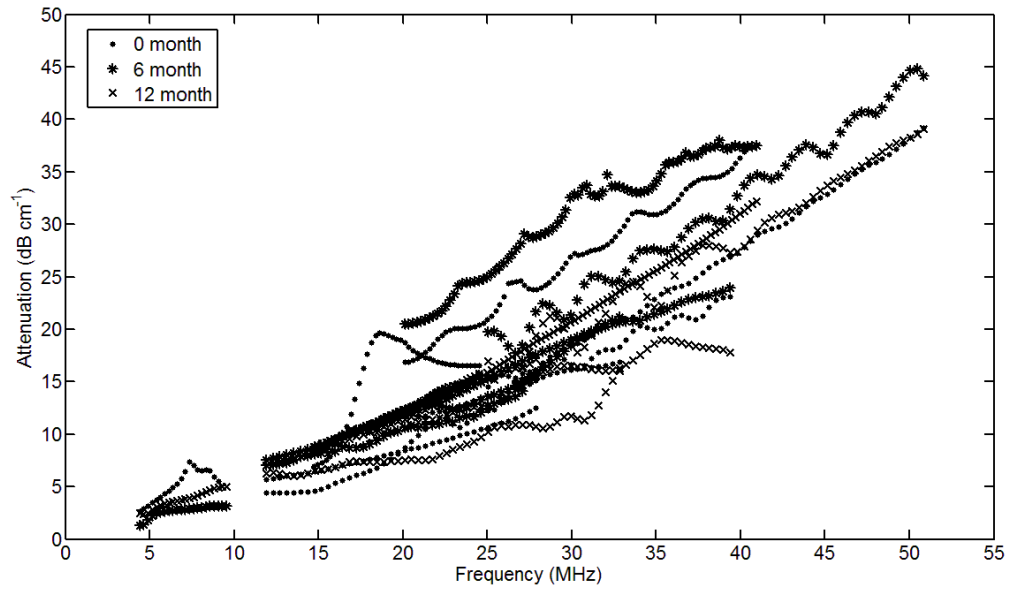
**Figure 1**  
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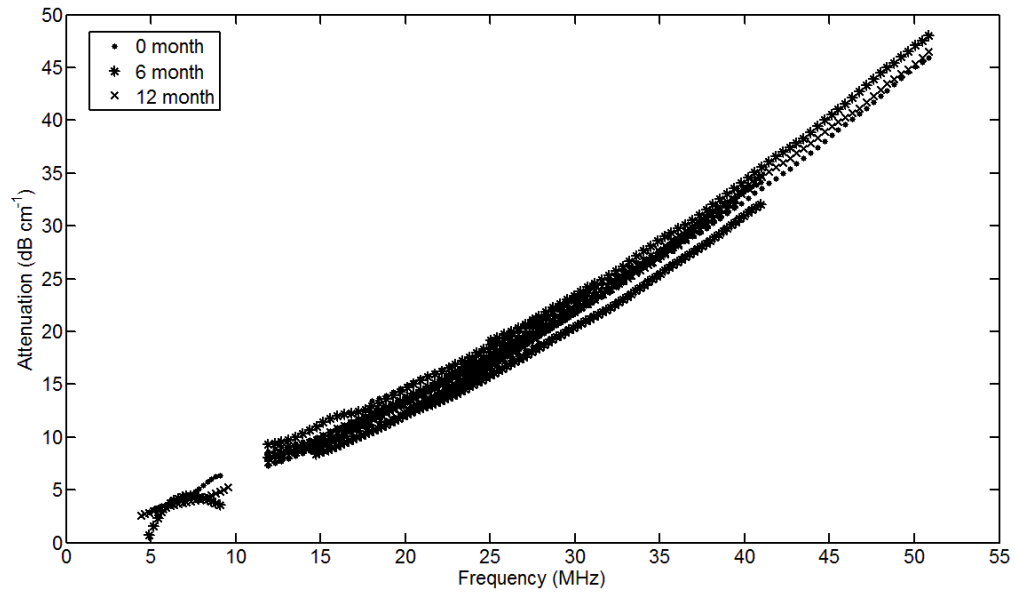
**Figure 2**  
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**Figure 3**  
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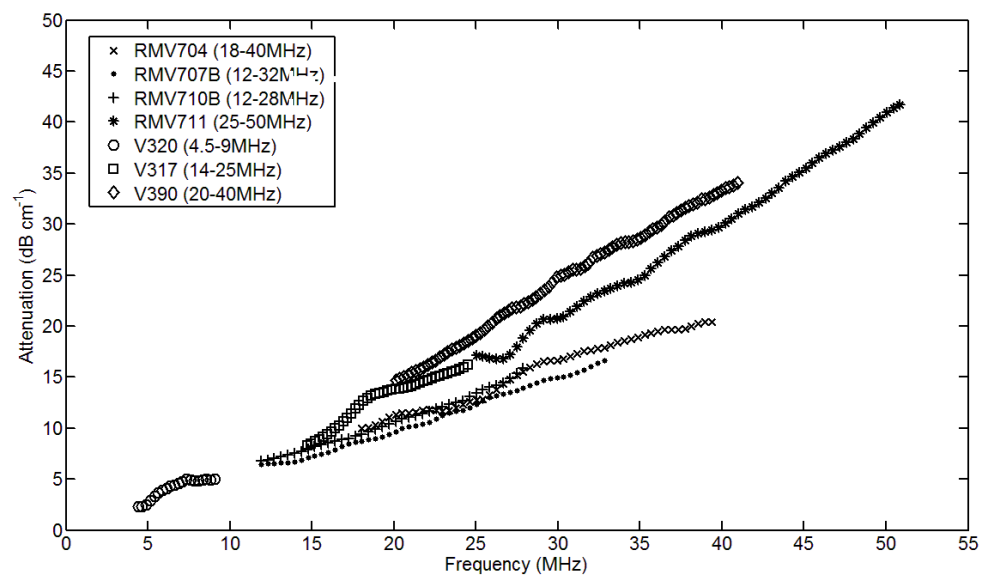


**Figure 4**  
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**Figure 5**  
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**Figure 6**  
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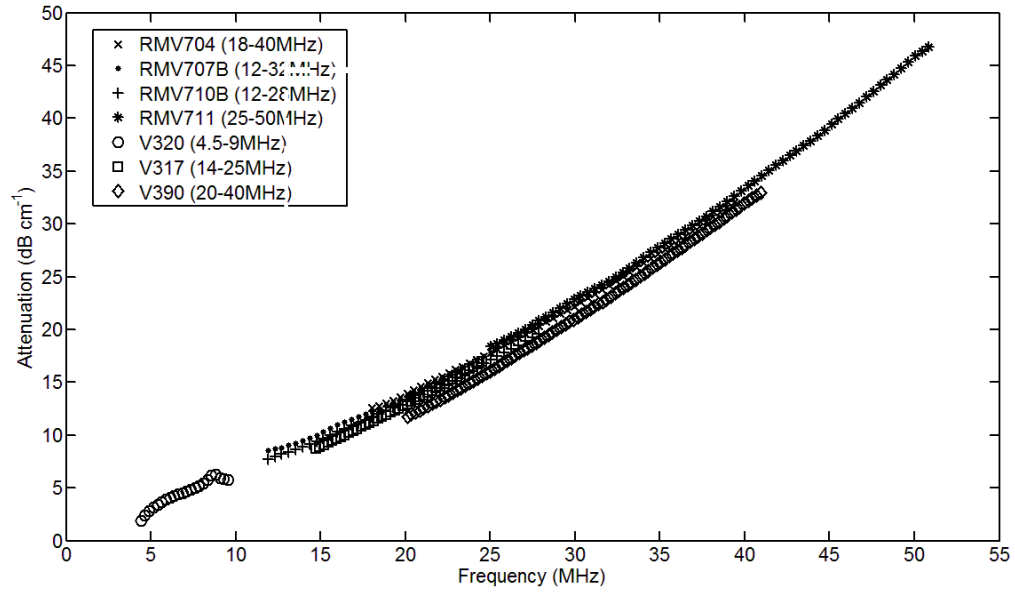


Figure 7

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