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Variability in clinically measured wideband acoustic immittance over time in young and old adults

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Variability in Clinically Measured Wideband Acoustic Immittance
Over Time in Young and Old Adults

Allison G. McGrath

A dissertation submitted to the Graduate Faculty of

JAMES MADISON UNIVERSITY

In

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Abstract

Wideband acoustic immittance (WAI) measures of the middle ear have the potential to increase our ability to detect changes in the middle ear transfer function not seen using traditional tympanometry. In order to use this new tool diagnostically we must first understand its normal clinical variability. The present study aimed to investigate the variability that occurs when wideband acoustic immittance (WAI) is measured clinically within subjects as a function of subject age, as a function of time, and as a function of pressure. A total of thirty-six ears from eighteen subjects were studied (n=18 young adults ears, n=18 older adult ears). Subjects were included in the study if they had negative history of middle ear disease, normal tympanogram at 226 Hz (peak pressure ± 50 daPA), and an air-bone gap less than 10 dB. Subjects were tested on two days separated by at least a week using a commercial acoustic-immittance system (Interacoustics Titan©). Otoscopy was completed at the beginning of each session to ensure proper probe fit. Following otoscopy, wideband absorbance measurements with a hermetic seal (WBT) were obtained by recording the acoustic response to a click stimulus as a function of frequency and pressure. The wideband clicks were presented at a rate of 21.5/sec and an intensity of 100 dB peSPL (~65 dB nHL) and WAI was measured at 107 frequency data points from 226 to 8,000 Hz. WBT was first measured in the subject's right and left ears and then the probe was reinserted and a second measure was made using the same protocol. Wideband absorbance measures as a function of ambient pressure (WBA) were obtained without pressure next. A hermetically sealed ear canal was not necessary in order to successfully record a WBA measurement from a subject. A measurement was made in the subject's right and left ears, and then the probe was

reinserted for a second, identical condition. Both WBT and WBA measures were repeated on a second study session at least one week after the first study session.

Wideband absorbance measures for both age groups, under ambient pressure (WBA) were slightly more variable between days than wideband absorbance measure under tympanic peak pressure and with a hermetic seal (WBT). Variability was also seen between tests on the same day after probe reinsertion for both WBA and WBT measurements; however this variability was much smaller than the between-day measurements. Variability remained small in both age groups with slightly greater variability seen in younger adults under both WBT and WBA conditions. Regardless of these factors, essentially all average WAI responses during both test sessions (with the exception of the 6,000 Hz one-third octave interval), fell within the 95% confidence intervals provided by the Titan clinical system. Our results suggest that clinical measures of WAI are most stable when measured at tympanometric peak, and that age may play a role in the amount of variability in WAI over time. Variability over time is small and should not alter clinical decision-making.

List of Abbreviations:

WAI: Wideband Acoustic Immittance

WBA: Wideband Absorbance under Ambient Pressure

WBT: Wideband Absorbance under Tympanometric Peak Pressure

1.0 Introduction

Wideband acoustic immittance, a relatively new middle-ear function analysis technique, provides a noninvasive diagnostic test to detect conductive hearing loss and middle ear pathology (Keefe and Levi, 1996; Keefe et al., 1993, Shahnaz et al., 2009, Stinson et al., 1982; Vander Werff, Prieve, and Georgantas, 2007; Voss and Allen, 1994). Wideband immittance measures have been shown to be more sensitive to middle ear status than previous measurements using single or multiple probe-tone frequency tympanometry due to the use of wideband signals (e.g., acoustic clicks or broadband tone chirps). The outcome represents middle ear responses across a broad frequency range (up to 8,000 Hz) (Prieve, Feeney, Stenfelt, & Shahnaz, 2013).

Wideband acoustic immittance measurements have been studied at ambient pressure and tympanometric peak pressure. Ambient pressure measures have been made in normal-hearing adults (Feeney and Sanford, 2004; Keefe et al., 1993; Shahnaz and Bork, 2006; Voss and Allen, 1994), in children and infants (Keefe et al., 1993; Vander Werff et al., 2007), as well as in ears with middle ear dysfunction. Studies have shown that in the presence of middle-ear dysfunction, wideband absorbance is systematically shifted across frequencies when measured at ambient pressure (Feeney et al., 2003; Piskorski et al., 1998). There have been limited studies on wideband acoustic immittance under tympanometric peak pressure, however in general studies have shown equal or better potential to detect conductive hearing losses and middle ear pathologies when compared to the same measures at ambient pressure (Keefe et al., 2012; Simmons and Keefe, 2003).

Wideband acoustic immittance measures have the potential to improve newborn hearing screening outcomes, improve the detection and diagnosis of middle ear pathologies clinically, and improve the prediction of conductive hearing loss (Beers, Shahnaz, Westerberg, & Kozak, 2010; Ellison et al., 2012; Feeney et al., 2003; Hunter, Feeney, Lapsley Miller, Jeng, & Bohning, 2010; Margolis, Saly, and Keefe, 1999; Sanford et al., 2009; Shahnaz, Longridge, & Bell, 2009b). Also, wideband acoustic immittance is nearly independent of probe-insertion depth; consequently the measurements are interpretable across a wider frequency range than conventional tympanography measures. (Stinson et al., 1982; Voss, Horton, Woodbury, and Sheffield, 2008). However, in order to understand the diagnostic significance of these measures, we must first understand the variability that might occur normally in a clinical setting.

Within-subject variability in wideband absorbance measurements stems from numerous factors. Poor probe fit and the presence of air leaks have been the primary sources affecting within-subject variability (Keefe et al., 2000; Vander Werff et al., 2007). Vander Werff et al. (2007) examined differences in wideband acoustic immittance in adult and infant subjects to demonstrate test-retest measures within subjects on the same day with probe reinsertion. In that study, adult subjects had more consistent test-retest findings than infants. Findings by Hunter, Bagger-Sjoberg, and Lundberg (2008) also suggested “substantial reliability of repeat measures within the same test session.” While studies have shown greater within subject variability in infants than in adults, little is known about within subject variability in the elderly. Physiological and anatomical changes occur in the human middle ear and associated structures as adults age (Holte and Leonore, 1996). Feeney and Sanford (2004) showed older adults displayed an increase in

absorbance from 800 to 2,000 Hz and then a decrease near 4,000 Hz when measuring wideband absorbance. The authors proposed that the middle-ear stiffness decrease with age is most likely responsible for these findings. Changes in middle ear stiffness may affect the variability within older adult subjects, especially when compared to younger subjects (Feeney and Sanford, 2004; Feeney et al., 2014; Shahnaz, Feeney, and Schairer, 2013). However, the exact effect of the aging process in the middle ear on intra-subject variability has yet to be definitively determined.

Other factors that may influence inter-subject variability in wideband acoustic immittance includes ear canal volume, ear, sex, and ethnicity (Feeney et al., 2014; Shahnaz & Bork, 2006; Shahnaz et al., 2013; Voss et al., 2008). However, literature is mixed on if ethnicity influences variability significantly enough to be clinically relevant. Normative data have been published for infants and adults for both ambient and tympanometric peak pressure wideband immittance (Hunter et al., 2010; Lui et al., 2008; Margolis et al., 1999). Shahnaz et al. (2013) recommend using age-specific normative data when measuring wideband immittance for clinical use.

The recent availability of a clinical tool for the measurement of wideband acoustic immittance makes it important to discover whether these issues affecting variability under laboratory conditions are similar in clinical use. Clinically, it is important to know whether inter- or intra-subject variability in WAI will affect the ability to make accurate diagnoses of middle ear pathology. The primary objectives of this study were to analyze the variability that occurs when wideband immittance is measured clinically within subjects: (1) as a function of time (between days versus within day); (2) as a function of ambient pressure versus tympanometric peak pressure; and (3) as a

function of age (young adults versus older adults). Further we seek to determine whether clinical variability between subjects is generally within published normative ranges. The hypotheses derived based on these objectives can be summarized as follows:

Variability within Subjects:

- i. Wideband absorbance will be more variable over time (between days) than within the same day;
- ii. Wideband absorbance will be more variable when measured at ambient pressure than when measured at tympanometric peak pressure;
- iii. There will be a significant difference in the variability of wideband absorbance measures as function of age

Variability between Subjects

- iv. Average wideband immittance measured over time and with probe reinsertion will fall within the 95% confidence interval provided by the Titan clinical system (Lui et al., 2008) regardless of age.

2.0 Literature Review

2.1 Overview

The human auditory system is dependent on the mechanical transmission of sound through the anatomical components of the outer and middle ear. These components include the external ear canal, the tympanic membrane and the middle ear. During sound transmission to the inner ear, the frequency and amplitude of the signal is changed through the resonance, absorptive and reflective characteristics of these structures. Prior to wideband acoustic immittance (WAI), tympanometry was the main clinical tool audiologists used to quantify these mechanical transmission characteristics.

Tympanometry generally consisted of assessment of middle-ear acoustic transfer function at a single frequency and by sweeping pressure (Keefe et al., 1993; Keefe, Ling, & Bulen, 1992; Margolis, Saly, & Keefe, 1999; Voss & Allen, 1994). Contrary to tympanometry, the relatively new middle-ear analysis technique, WAI, proposes the ability to assess middle-ear function across a much wider frequency range (Lily & Margolis, 2013; Margolis et al., 1999) and without the necessity of external sweeping pressure (Keefe et al., 1993).

2.2 Historical Perspectives of Wideband Immittance

One of the first pioneer studies on acoustic immittance in human ears was Georg von Békésy's study in 1932. Békésy measured acoustic immittance in human ears at static ear-canal air pressure. His experiment demonstrated that the effects of air pressure on hearing were due to middle-ear transmission losses and not related to changes in cochlear sensitivity. Békésy's experiment led to the development of the measurement of

acoustic immittance in the ear canal as a function of ear-canal air pressure, which is now clinically known as tympanometry (Békésy, 1932 as cited in Margolis, et al., 1999).

Besides Békésy, many other researchers and studies examined middle-ear function based on changes in air pressure (Metz, 1946; Pohlman & Kranz, 1923; Politzer, 1869; Toynbee, 1865; van Dishoeck, 1937, all as cited in Margolis et al., 1999), however it wasn't until Békésy's findings were clinically applied, that the measurement began to be used diagnostically. Terkildsen and colleagues developed the first device to measure acoustic immittance clinically, based upon Békésy's findings (Terkildsen and Nielsen, 1960 as cited in Margolis et al., 1999). This device resulted in the clinical use of tympanometry at single probe frequency of 226 Hz, as it is still widely used today (Margolis et al., 1999).

The availability of a clinical device resulted in studies with the purpose of associating tympanometric patterns with specific middle ear conditions to be used for clinical purposes. Lindén (1969) as cited in Margolis et al., 1999 was one of the original researchers to assess tympanometric patterns for diagnostic purposes. However, Jerger's research in 1970, expanded on Lindén's original findings and supported three specific patterns originally described by Lindén, which came to be known as normal type A pattern, flat type B pattern, and significant negative pressure type C pattern (Lily & Margolis, 2013).

In 1972, Grason-Stadler, Co. introduced a new clinical tympanometric instrument that had two probe tones available: 226 Hz and 678 Hz. Two major advantages developed from introducing the 678 Hz probe tone. The first was that the probe-frequency was closer to the first middle ear resonance peak of the adult ear, which allowed for additional

typanometric patterns to be used for clinical assessment of middle ear function. These patterns were consistent with ossicular chain disruption (type D) and tympanic membrane hypermobility (type E). The second advantage to including the 678 Hz tone was it could be used to measure valid tympanograms in infants under the age of six months. Prior to this, it was found that the 226 Hz probe tone did not reliably or accurately assess the middle ear function of infants under the age of six months (Lily & Margolis, 2013).

The next development in tympanometry was multifrequency tympanometry and its ability to detect middle-ear pathologies. This new tool developed from the clinical use of more than two probe frequencies. Colletti and colleagues first studied multifrequency tympanometry in subjects in 1976. Multifrequency tympanometry was further developed for clinical use by additional researchers and released as a clinically available instrument in 1985 by Virtual Corporation (Colletti, 1997; Funasaka et al., 1984; Lily, 1984, all as cited in Lily & Margolis, 2013). Multifrequency tympanometry was found to be more sensitive than single frequency tympanometry for detecting middle-ear pathologies (Ibraheem, 2014; Lily & Margolis, 2013; Margolis et al., 1999). By using multiple frequency probe-tones, multifrequency tympanometry was considered better than single-frequency tympanometry at detecting high-impedance pathologies of the middle ear, as well as more sensitive in identifying normal and pathological middle ear conditions in infants (Ibraheem, 2014; Shahnaz & Polka, 1997).

2.3 Clinical Availability of Wideband Absorbance

Wideband acoustic immittance (WAI) is a relatively new approach used to evaluate middle ear dysfunction by analyzing the outer and middle ear's ability to transfer sound across a broad range of frequencies and without the necessity of external

pressure (Keefe et al., 1993). WAI measurements are typically described as energy absorbance or energy reflectance. Energy absorbance is represented as a percentage equal to the ratio of acoustic energy absorbed by the middle ear and ear canal to the acoustic energy of a stimulus presented in the ear canal and directed towards the tympanic membrane (Lui et al., 2008). Conversely, energy reflectance is the inverse ratio of energy absorbance, or the acoustic energy reflected to the total acoustic energy presented. Thus, energy reflectance equals 100% minus the percent absorbance. (Keefe & Feeney, 2009). The term “wideband acoustic immittance” is an encompassing term to describe wideband measures including both energy absorbance and energy reflectance (Feeney et al., 2013). The current study will use the term wideband acoustic immittance (WAI) to encompass both energy absorbance and energy reflectance. However, the terminology used when discussing individual studies will reflect the measurements used in that specific study.

WAI instruments are clinically available and can measure absorbance at ambient pressure and tympanometric peak pressure. Keefe et al. (1992) described a wideband reflectance system that obtained measurements at ambient pressure (without external changes to ear canal pressure). Energy absorbance and energy reflectance measurements at ambient pressure have been studied in normal-hearing adults, children and infants, and newborns (Feeney & Sanford, 2004; Keefe et al., 1993; Keefe et al., 2000; Vander Werff et al., 2007; Voss and Allen, 1994). Other studies have suggested that WAI measurements at tympanometric peak pressure offer more information about the status of the middle-ear system than the same measurements at ambient pressure (Margolis et al., 1999; Sanford & Feeney, 2008). As defined by Lui et al., 2008, tympanometric peak pressure is the ear canal pressure at which acoustic energy transmission is most efficient,

and the greatest percentage of energy absorbance occurs. However, one of the major advantages of WAI is the fact it can be measured at either ambient pressure or tympanometric pressure. This is beneficial for patients where it is more difficult to maintain a seal, or for patients, such as infants, who have more compliant ear canal walls. Another advantage to measuring WAI is that it is not as sensitive to probe location in the external auditory canal as traditional tympanometric measures, which have been shown to be greatly influenced by probe insertion depth. (Allen, Jeng, & Levitt, 2005; Margolis et al., 1999; Shanks & Lily, 1981). WAI measures also have an advantage over multifrequency tympanometry. WAI is able to obtain frequency-specific information more rapidly, and these measures cover a broader range of frequencies (Vander Werff et al., 2007). Wideband immittance measures are also capable of predicting the degree of conductive hearing loss (Keefe & Simmons, 2003; Piskorski et al., 1999).

Two currently (2015) available clinical wideband immittance systems include the Interacoustics Titan and the Mimosa RMS Acoustics system. The Mimosa Acoustics RMS system, a laptop based system, measures the overall chirp sound-pressure level in the ear canal in dBA (Mimosa Acoustics, 2002; Shahnaz & Bork, 2006). The Mimosa Acoustics RMS system is capable of measuring wideband reflectance at ambient pressure from 250 to 8,000 Hz, and reports the findings in terms of energy reflectance (Shaw, 2009). The Interacoustic Titan, also a laptop based system, on the other hand reports results in terms of energy absorbance. The Titan can also perform measurements at ambient pressure and tympanometric peak pressure from 250 to 8,000 Hz. Although these clinical instruments are available, the normative database is still being developed (Feeney et al., 2014).

2.4 Inter-subject Variability:

It is crucial to understand the factors that lead to variability within wideband immittance measurements as normative data is collected for clinical use (Voss et al., 2013). The overall goal when analyzing variability is to improve any test's sensitivity and specificity, and optimize that test for clinical use (Shahnaz et al., 2014). Several studies have looked at both the variability that occurs between subjects (inter-subject) and the variability that occurs within the same subject over time or under different conditions (intra-subject variability).

Numerous studies have found inter-subject variability in adults to be greater in the mid to high frequencies than in the low frequencies when analyzing wideband reflectance and greater overall when compared to intra-subject variability (Feeney et al., 2003; Feeney and Sanford, 2004; Keefe and Simmons, 2003; Voss and Allen, 1994; Voss et al., 2008; Werner, Levi, & Keefe, 2010). Voss and Allen (1994) were one of the first researchers to analyze variability in wideband immittance measurements between several subjects. They proposed that inter-subject variability was due to cochlear and middle ear impedance differences. However, it was found that within subjects, results were repeatable from test to test.

Voss et al. (2008) identified sources of inter-subject variability in energy reflectance measurements in temporal bones of adult cadavers. They found that probe location within the ear canal during measurement had only a minimal effect on variability between subjects. The smallest changes were noted for frequencies up to 5,000 Hz, while larger changes were observed in the 5,000 to 6,000 Hz range. Overall, Voss and colleagues concluded that absorbance was relatively stable and not dependent on

measurement location in the ear canal for much of the length of the ear canal. They noted as the probe was moved further from the tympanic membrane and within the cartilaginous portion of the ear canal, some ears showed greater increases in absorbance, due to losses along the length of the ear canal to the tympanic membrane.

Results from the same study (Voss et al., 2008) showed volume of the middle ear space significantly contributed to variability in energy reflectance across subjects. They found that increasing the middle ear space volume led to increased absorbance below 2,000 Hz. However, frequencies above 2,000 Hz displayed more variable findings as middle ear cavity volume was increased. It was concluded that middle ear volume is likely to be a cause of inter-subject variability in wideband absorbance measures.

A recent study by Feeney and colleagues also found sex, age, abnormal 226 Hz tympanometry as well as ear to be factors in wideband reflectance inter-subject variability (Feeney et al., 2014). An analysis of ear “laterality” was demonstrated at frequencies below 4,000 Hz), with the left ear showing greater reflectance values than the right ear across all subjects. These results were only significant at the 2,000 Hz and 2,500 Hz one-third octave bands. However at frequencies 4,000 Hz and greater, the right ear demonstrated greater reflectance values. This comparison was only significant at 6,300 Hz one-third octave band. Baseline reflectance measures in Feeney et al. (2014) also showed slight sex differences. Females were shown to have slightly higher mean energy reflectance differences than the male subjects at frequencies below 2,500 Hz and at 8,000 Hz. Differences were only significant at 8,000 Hz.

A review by Shahnaz, Feeney, and Schairer in 2013 analyzed the effect of ethnicity, aging, and instrumentation on normative data. Previous studies have shown

differences between Caucasian and Chinese WAI measurements, however it was suggested that this was due to differences in body size among the groups (Wan & Wang, 2002; Shahnaz & Bork, 2006; Shahnaz & Davis, 2006). Similarly, wideband immittance patterns have been found to vary by age, but this was also likely attributed to differences in body size (Shahnaz & Bork, 2006). The study by Shahnaz & Bork (2006) found that Caucasian children had significantly higher energy reflectance values at low frequencies (315 to 1,250 Hz), than Caucasian adults. However, the differences in body size equate to differences in middle ear volume, and a corresponding resonant frequency of the middle ear system. A larger middle ear space would equate to a lower resonant frequency which would allow the middle ear system to transfer low-frequency sounds better, consistent with the observed differences in reflectance values between children and adult groups (Shahnaz et al., 2013). The reverse is true for children who have smaller body sizes and middle ear volumes; they will have a higher middle ear frequency and a more suitable system to transfer high-frequency acoustic sounds.

It is unknown whether the aging process in adulthood effects the sound-transmission properties of the human middle ear. The tympanic membrane and middle ear structures have been shown to undergo structural changes at older adult ages (Ruah et al., 1991). These changes could potentially affect WAI patterns (Shahnaz et al., 2013). Feeney & Sanford (2004) found that older adults had lower reflectance values than younger adults at mid-frequency range and higher reflectance at higher frequencies than the young group. This was interpreted to suggest that the wideband reflectance pattern for the young adult group was more stiffness dominated than that of the old group (Feeney et al., 2014).

Several studies have also been done comparing variability in measurements obtained at ambient pressure and at pressure corresponding to the peak pressure (similar to tympanometric peak). Kenny, 2011 as cited in Shahnaz et al. (2013), demonstrated that absorbance obtained in Caucasian adults at ambient pressure was significantly lower at frequencies 250 to 2,500 Hz, and higher at 4,000 to 5,000 Hz than measurements at tympanometric peak. Similar findings were reported in Chinese subjects, but significant values were only measured in the frequency range of 250 to 2,500 Hz. Noticeable differences were found in the ninety percent confidence intervals as well. The ninety percent range for tympanometric peak pressure was significantly higher in the low to mid frequency range than that of the ambient pressure measurements. Lui et al. (2008) had similar findings. They reported lower absorbance estimates at low frequencies and higher absorbance estimates at high frequencies when measuring at ambient pressure. Clinically, this supports the use of separate sets of normative data for each measurement condition.

Feeney et al. (2014) suggested that using wideband immittance measurements under tympanometric peak pressure would improve the sensitivity and specificity when identifying normal middle ears from middle ears with abnormalities (negative middle ear pressure, pathologies, etc.). This would allow subjects or patients with an abnormal middle ear systems to have their measurements compared to normative data. This is possible because the abnormal middle ears would have the pathologic middle ear pressure compensated for during measurement (Lui et al., 2008).

Normative data has been reported for wideband immittance measures for clinical implications for both wideband immittance measures at ambient pressure conditions and at tympanometric peak pressure conditions (Lui et al., 2008). The general consensus is

that for normal hearing adults with normal middle ear function there is low absorbance in the low frequencies increasing to a maxima around 1,000 to 3,000 Hz, with lower absorbance at frequencies greater than the high-frequency maxima (Feeney and Sanford, 2004; Keefe et al., 1993; Margolis et al., 1999; Shahnaz and Bork, 2006; Voss and Allen, 1994; Werner et al., 2010). System-based normative data (as shown in Figure 1) has also been analyzed to see if test sensitivity and specificity improved with unique normative data for each clinical system. It was found that applying system-specific norms is not clinically significant and test sensitivity or specificity would not improve (Shahnaz et al., 2014).

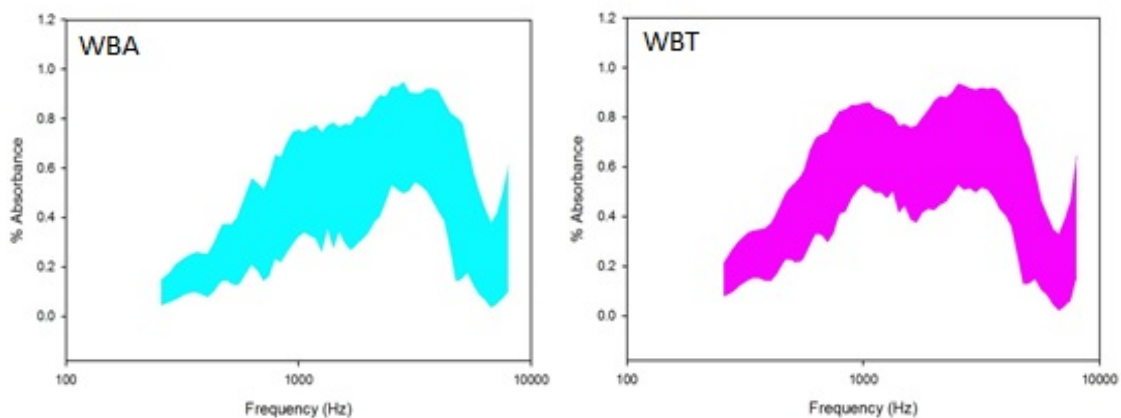


Figure 1: Redrawn from normative 5th and 95th percent confidence intervals provided by the Interacoustics Titan. Left: WBA normative data; Right: WBT normative data

2.5 Intra-subject Variability:

Voss et al. (2008) found that probe location within the ear canal during measurement had only a minimal effect on intra-subject variability between measurements. The smallest changes were noted for frequencies up to 5,000 Hz, and larger changes were observed in the 5,000 to 6,000 Hz range. Overall, Voss and colleagues concluded the WAI absorbance was relatively stable and not dependent on

measurement location in the ear canal for much of the length of the ear canal. However, a more recent study by Abur, Horton and Voss (2014) showed intra-subject standard deviations were smallest when the probe insertion depth was deepest, suggesting clinical measurements will be less variable when a deep probe insertion is made in the patient's ear canal (Abur, Horton, & Voss, 2014).

Voss et al. (2008) noted as the probe was moved further from the tympanic membrane and within the cartilaginous portion of the ear canal, some ears showed greater increases in absorbance, due to losses along the length of the ear canal to the tympanic membrane. Although the findings from the earlier study were not clinically significant, it has been agreed upon that errors in intra-subject variability among measurements will occur if a high-quality acoustic seal between the insert's tip and the ear canal is not maintained throughout measurement (Voss et al., 2013). If a deeper insertion is used clinically, it would likely lead to improved acoustic seals and air leaks and less acoustic loss during acoustic transmission along the ear canal, together resulting in less intra-subject variability.

The effect of consecutive WAI trials on intra-subject variability has also been studied. Werner and colleagues (2010) analyzed test-retest differences in energy reflectance in adult subjects. Their results showed test retest differences were smaller in the mid-frequency range than at the high frequencies when test sessions were separated by approximately two weeks. These findings replicated similar findings by Vander Werff et al. (2007) from a comparable subject pool.

A recent study by Rosowski et al. (2012) made four reflectance measurements each one-week apart on seven subjects. They found that repeated measurements had

similar standard deviations to the population standard deviation. Also, when there was a week of time between each measurement, larger variations were shown than the repeated measures during the same session, as had been previously reported by Vander Werff et al. (2007). Test-retest absolute differences in reflectance tended to be generally small and fall within the ninety percent confidence interval values. In both studies, any unique features of an individual's wideband immittance pattern were found to repeat on retest patterns (Rosowski et al., 2012; Werner et al., 2010).

Burdiek and Sun (2014) also showed a small, but significant effect of consecutive trials on intra-subject variability. They found that wideband immittance changes in a frequency specific manner over trials for both ambient and tympanometric peak pressure measurements. In their study, absorbance increased in low frequencies (below 1,500 Hz) and decreased in specific high frequency regions (around 2,000 Hz and 5,000 to 6,000 Hz). Changes in absorbance from trial to trial were compared in sequence, and were generally found to gradually increase over trials, with the largest change typically occurred at the second trial.

In order to fully understand changes that may occur within pathological patients, we must first understand the changes that might occur normally in a clinical setting. Literature is still accumulating on the effect of age, pressure, and time on variability in wideband immittance measures. Studies under clinical conditions using clinically available instrumentation are needed to add to the foundational research that has already been done. Understanding how such factors contribute to both inter and intra-subject variability clinically can result in a more specific and sensitive test for clinical use. The purpose of the present study was to assess sources of inter-subject and intra-subject

variability in wideband absorbance under a variety of test conditions. Repeated measures on the same day as well as over two test sessions at ambient pressure and at tympanometric peak pressure were analyzed in younger and older adults with normal middle ear function in hopes to better understand the diagnostic significance of these measures.

3.0 Methods

3.1 Subjects

Measurements were made in a total of 36 ears from 18 subjects. Subjects were separated into two groups by age and ears were measured and treated independently. The first group of younger adults consisted of 18 ears from 9 younger adults (1 male and 8 females), ages 22-25 years (mean = 23 ± 1.24 years). The second group consisted of 18 ears from 9 older adults (2 males and 7 females), ages 51-65 years (mean = 60 ± 4.36 years).

The James Madison University (JMU) Institutional Review Board (IRB) approved the study, and each subject was provided written consent. Participants were recruited through word of mouth, bulk email to JMU faculty and students, and IRB approved flyers and advertisements. Each subject was given a unique code at the time of the study in order to keep the patient's identity anonymous while data was being recorded, analyzed, and stored. Participation required two testing sessions separated by at least one week.

Prior to participating in the study, each subject completed a history that screened for exclusionary criterion. Subjects were excluded from the study if they had a positive history of middle ear pathology or if they had an air-bone gap greater than 10 dB at any frequency. Each of the subjects' ears were examined before both study sessions to assure normal appearing tympanic membrane (TM) via otoscopy. Subjects were included in the study if they had a normal 226 Hz tympanogram bilaterally on both session days. A normal 226 Hz tympanogram for the purpose of this study was defined as middle ear pressure ± 50 daPa and peak compliance between 0.3 and 2.0 ml. Pure-tone

audiometric thresholds were measured from 250 to 8,000 Hz for each subject using a Grason-Stadler model GSI-61 clinical audiometer calibrated according to American National Standards Institute criteria (re: S3.6.1989). EARtone ER-3A insert earphones or Telephonics TDH-50P headphones were used to obtain air-conduction thresholds on each subject. Bone-conduction thresholds were obtained as well using a Radioear B-71 clinical bone oscillator. Hearing sensitivity was not an exclusionary criterion for the purposes of this study, but was measured for informational purposes only.

3.2 Instrumentation

All testing was conducted in a double-walled, sound-treated booth (IAC model 107498) at the James Madison University Speech-Language-Hearing Applied Laboratory. Otoscopy was performed using a Welch Allyn MacroView manual otoscope. A commercial acoustic-immittance system (Interacoustics Titan©), was used to perform wideband absorbance measurements and tympanometry during both test sessions for all 36 ears. The Titan© system was calibrated before each test session by measuring ear volume for 2.00 cc (tolerance = ± 0.1 cc) and 5.00 cc (tolerance = ± 0.25 cc) cavities as recommended by the manufacturer. Wideband absorbance measurements were made using the Interacoustics Titan© IMP440 module. Measurements were made with a hermetic seal and pressure sweep from positive 200 daPa to negative 400 daPa at 200 daPa/sec as well as at ambient pressure without the necessity for a hermetic seal. The Titan IOW probe assembly consists of a loudspeaker, a microphone and pressure pump. The probe delivers a wideband click stimulus at 100 dB peSPL at a rate of 21.5 Hz per second and records absorbance as a function of frequency (226 to 8,000 Hz; 1/12 octave step size) and sweeps pressure from positive 200 to negative 400 daPa. A PC based

laptop using Interacoustics Otoaccess© software was used to create original research protocols and store all participants' data from each session. Wideband absorbance was made using two unique protocols, one measured at tympanometric peak pressure (wideband absorbance tympanometry or WBT) and the other measured at ambient barometric pressure (wideband absorbance at ambient pressure or WBA). WBT measurements were made first in all subjects in order to determine if the tympanometric pressure and compliance were within normal limits. The WBT protocol would automatically begin to make measurements once a probe seal was detected by the Titan system©. Determination of an acoustic leak was assessed through an estimation made by the Titan system©. If a leak was detected, the experimenter was prompted by a yellow light on the IOW probe assembly to check the probe fit and run the protocol again. WBA measurements were made second and would automatically begin once the probe was placed in the ear canal.

3.3 Procedures

Four wideband absorbance measures were made in each ear during each test session. Otoscopic examination was completed prior to beginning wideband absorbance measurements on both session days to rule out ear canal and eardrum abnormalities. For purposes of this study, right and left ears were randomly assigned as the first test ear. The first wideband absorbance measurement made on each subject was the WBT protocol measurement (wideband admittance as a function of tympanometric peak pressure (TPP)). This measurement required a hermetic seal between the probe and the ear canal. Once a hermetic seal was detected by the Titan probe system, the measurement would automatically be taken. The WBT protocol automatically measured a multifrequency

tympanogram prior to the absorbance measurement. The data set was displayed as a three dimensional tympanogram and a 226 Hz tympanogram was extracted through the Titan software in order to ensure clinically normal middle-ear function (± 50 daPa; .02 to 03 ml compliance). Tympanometric peak pressure (TPP), defined as the ear canal pressure at which maximal absorbance occurred, was also obtained during the WBT protocol. Once the WBT protocol measurement was completed, the probe was removed from the ear and the WBT measurement was repeated in the opposite ear. This procedure was repeated a second time for both the initial test ear and the opposite test ear, so that a total of two separate WBT measurements in each ear were made in each ear during each test session.

After WBT measurements were made twice in each ear, wideband absorbance under ambient pressure (WBA protocol) measurements were obtained. The same initial test ear was used for WBA measurements as WBT measurements. The WBA protocol measurements did not require a hermetic seal, rather it only required a proper fit (no acoustic leak) and no occlusion of the probe. If these conditions were satisfied, the WBA measurement would automatically start and an absorbance measurement would be obtained. WBA measurements were obtained using the same stimulus as used in the WBT protocol. Once the WBA measurement was completed in the initial test ear, the probe was removed and inserted to the opposite ear for WBA measurement. After completion of WBA measurement in the second ear, the procedure was repeated in the initial ear and the opposite ear a second time.

The procedures described above for WBT protocol measurements and WBA protocol measurements were repeated on the second test day as well. The second test day was scheduled at least seven days from the first test day. In total, four WBT

measurements and four WBA measurements were made on day one of testing, and four WBT measurements and four WBA measurements were made on day two of testing for each participant.

Pure-tone audiometry was performed for each subject during the first session only. There was no audiometric threshold criterion that the subjects had to meet in order to qualify for the study. Participants were only required to have no air-bone gaps larger than 10 dB at any frequency. Audiometry was measured for information purposes only. Two different testers performed all of the protocols over the course of the study.

3.4 Data Analysis

Sixteen one-third octave band measures (226 to 8,000 Hz) of percent absorbance in WBA and WBT were downloaded from the Interacoustics Titan using the research protocol. This protocol provided numerical values for absorbance within each frequency band. The usual clinical protocol only provides a visual graphic representation of the data. Three differences in wideband absorbance measurements were calculated for each frequency band: first a difference as a function of time (day 2 time 1 minus day 1 time 1), second a difference as a function of probe reinsertion on day 1 (day 1 time 2 minus day 1 time 1), third a difference as a function of probe reinsertion on day 2 (day 2 time 2 minus day 2 time 1). These difference scores were analyzed as a function of age (young adults and older adults), and as a function of tympanometric peak pressure (WBT protocol and WBA protocol).

The variance for each difference was computed across all frequencies and an F-test determined whether one variance was significantly different than another. The purpose was to find out if there was more variability in within day measures (probe

reinsertion), between day measures (over time), as well as any differences in variability between WBT measures and WBA measures (at tympanometric peak pressure or at ambient pressure). These same variability measures were used to determine whether subject age influenced variability. An alpha level of 0.05 was used to test significance.

A learning effect analysis was also performed to assess if the two testers' experience over the course of the study affected variability. The maximum difference in wideband absorbance across frequencies was determined for each subject on each day of the study for both the WBA and WBT protocols for both testers. Linear regression of these measures of maximum differences over dates from the beginning to end of the study for both WBT and WBA quantified any significant change over time, as the testers gained experience. Linear regression analysis was calculated using all tests pooled as well as for the tests separated by tester.

4.0 Results

4.1 Learning Effect Analysis

An analysis of learning effect was performed to ensure any variability found in the results were not a bias of either examiners' experience over the course of the study. Results were analyzed as a function of condition and as a function of examiner. The learning effect analysis was performed for both the WBT condition and WBA condition ($n=72$; 36 ears tested twice). Subjects were divided into two groups: those tested earlier in the study (February to March, 2014; $n=50$) and those tested later in the study (September to October, 2014; $n=22$). Results were designated as early or later in the study regardless of whether they were a first or second test day of the same subject. The data was also split by age for further analysis. The maximum difference in wideband absorbance across frequencies was determined for each subject on each day of the study for both conditions, WBA and WBT. Results, as displayed in Figure 2, showed tester experience did not decrease variability for the WBA condition or for the WBT condition.

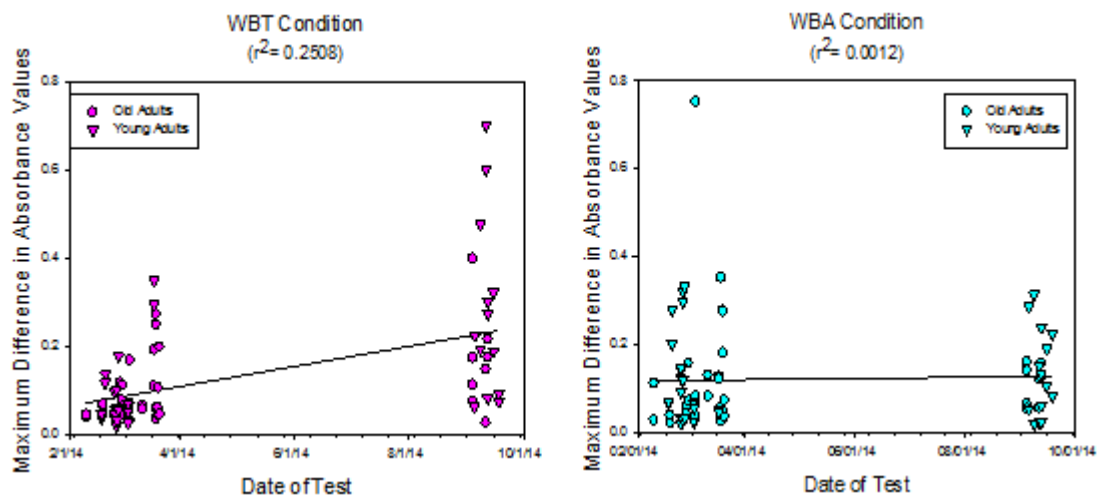


Figure 2: Maximum absolute differences over dates from the beginning to end of the study shown for WBT condition (left) and WBA condition (right), as well as for older adults (circles) and younger adults (triangles). Maximum differences in wideband absorbance did not decrease over time.

The learning effect analysis was also performed for each of the two testers of the study. Figure 3 shows the learning effect analysis for tester 1 on the left (n=80 ears; 40 ears total, each tested on two separate days for two conditions, WBT and WBA) and on the right for tester 2 (n= 64 ears; 32 ears total, each tested on two separate days for two conditions, WBT and WBA). Subjects were divided into two groups as in the first analysis: those tested earlier in the study (tester 1 n=26; tester 2 n=24) and those tested later in the study (tester 1 n=14; tester 2 n=8). Results, as displayed in Figure 3, show that tester experience did not decrease variability for the WBA condition or for the WBT condition.

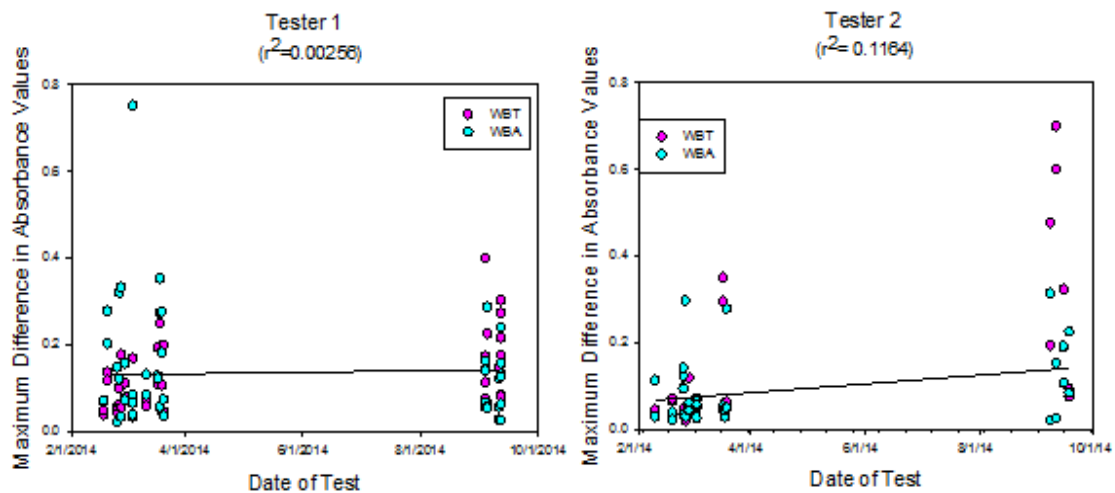


Figure 3: Learning effect of maximum differences over time from the beginning to end of the study shown for tester 1 (left) and tester 2 (right). WBT condition (pink circles), WBA condition (aqua circles). Maximum difference did not decrease as a function of tester experience.

4.2 Between Day Variability

The difference in absorbance between days (day 2 time 1 minus day 1 time 1) for the WBT and WBA conditions is shown in Figure 4 (top row) for all subjects regardless of age. Each subject's difference in absorbance is represented as a function of one-third octave band frequency. Wideband absorbance variability between days was small, but

significantly greater at ambient pressure (WBA), regardless of age ($F_{575, 575} = 1.495$, $p < 0.0001$). Previous studies suggested that there were differences in variability as a function of test ear (Feeney et al., 2014). We found more variability over time in right ear measurements ($F_{287, 287} = 1.41$, $p < 0.05$) for the WBT condition but no significant difference for left and right ears in the WBA condition ($F_{287, 287} = 1.15$, $p > 0.05$).

4.3 Within Day Variability (Probe Reinsertion)

We also calculated the difference in WBA and WBT absorbance between tests on the same day (variability as a function of probe reinsertion; see bottom row Figure 4). Wideband absorbance variability as a function of probe reinsertion was small regardless of condition (WBA variance day 1 = 0.0078, day 2 = 0.0038; WBT variance day 1 = 0.0132, day 2 = 0.0052) and was not significantly different on day 1 or day 2 regardless of condition (WBT: $F_{575, 575} = 0.3942$, $p > 0.05$; WBA: $F_{575, 575} = 0.4969$, $p > 0.05$). However, there was significantly more variability as a function of probe reinsertion in the WBT condition ($F_{1151, 1151} = 1.5945$, $p < 0.0001$).

4.4 Between Days Versus Within Day Variability

An analysis was also completed to compare between day and within day differences for both the WBA and WBT condition (Figure 4 top row versus bottom row). An F ratio was calculated for each absorbance condition using the two variances (between day and within day). Variability in wideband absorbance was significantly greater between days than within the same day for both the WBT and WBA conditions (WBT: $F_{1151, 575} = 1.1422$, $p < 0.05$; WBA: $F_{1151, 575} = 2.7222$, $p < 0.001$).

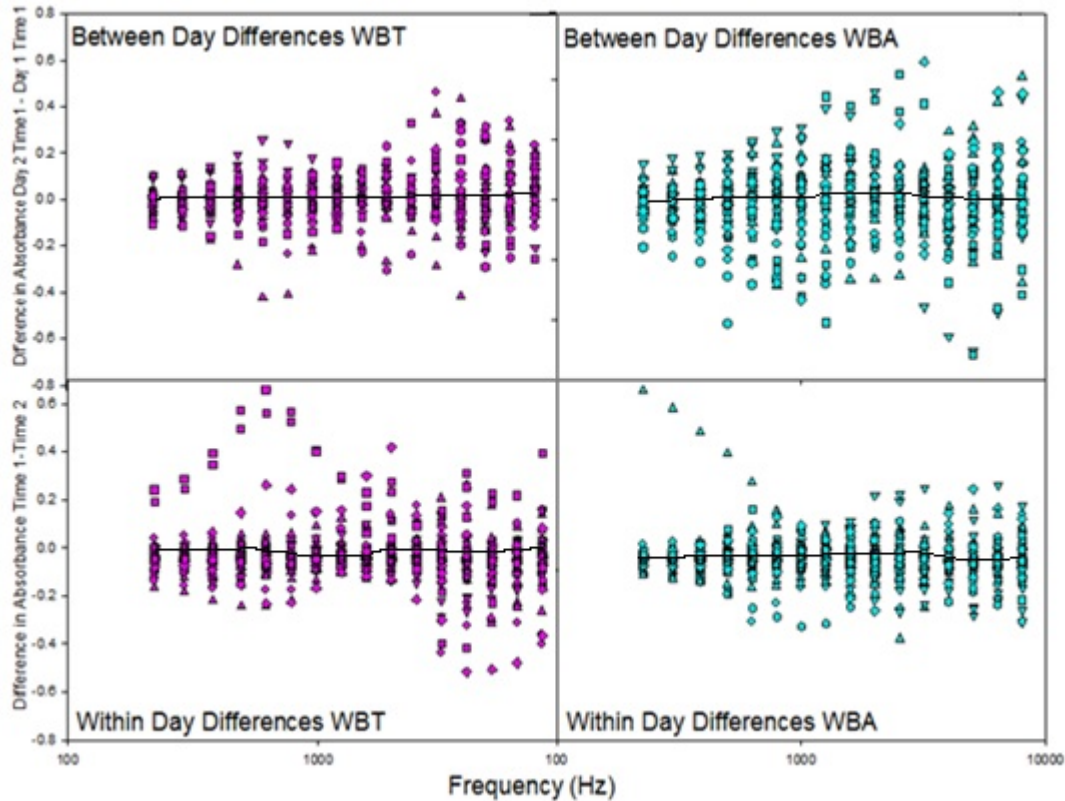


Figure 4: (Top Row- Between Day) Difference between day 2 time 1 and day 1 time 1 absorbance measurements for WBT and WBA conditions for all subjects regardless of age.

(Bottom Row- Within Day) Difference between time 1 and time 2 absorbance measurements for WBT and WBA conditions on day 1 and day 2 for all subjects regardless of age.

4.5 Variability as a Function of Age

4.5a Between Day Variability as a Function of Age

Wideband absorbance values were analyzed as a function of age: young adults (22-25 years of age; n=18 ears) and older adults (51-65 years of age; n=18 ears). Between day differences (day 1 time 1 minus day 2 time 1) were calculated for both age groups for WBT and WBA conditions. Variance values were measured for each analysis and then compared based on absorbance condition (younger adult WBT differences compared to older adult WBT differences; younger adult WBA differences compared to older adult

WBA differences) using an F-test. Results, as shown in Figure 5, indicated wideband absorbance variability between days was significantly greater in the young adults regardless of absorbance condition (WBA or WBT condition) (WBT: $F_{287, 287} = 1.4153$, $p < 0.05$; WBA: $F_{287, 287} = 1.6244$, $p < 0.001$).

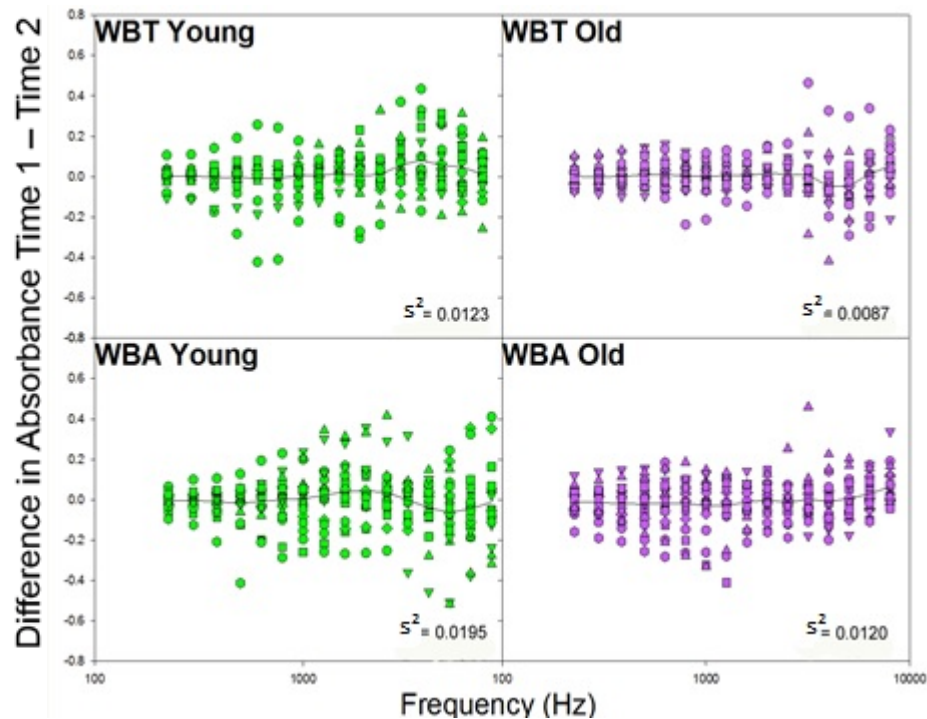


Figure 5: Difference between day 1 time 1 and day 2 time 1 absorbance measurements for WBT and WBA conditions for younger and older adults. The solid line shows mean difference in absorbance as a function of frequency in the WBT and WBA conditions.

4.5b Within Day Variability as a Function of Age

Within day differences were analyzed for younger versus older adults as well.

Within day differences (time 1 minus time 2) were calculated for both younger and older adult groups for the WBT and WBA conditions for both sessions for each subject.

Variance values were measured for each analysis and then compared based on absorbance condition using an F-test. Results overall generally showed wideband absorbance variability was greater in the younger adults for both absorbance conditions.

However, only the WBT condition reached statistical significance (WBT: $F_{575, 575} = 3.879$, $p < 0.001$; WBA: $F_{575, 575} = 1.051869321$, $p = 0.2723$). Within day differences for both age groups for the WBT and WBA condition are shown in Figure 6.

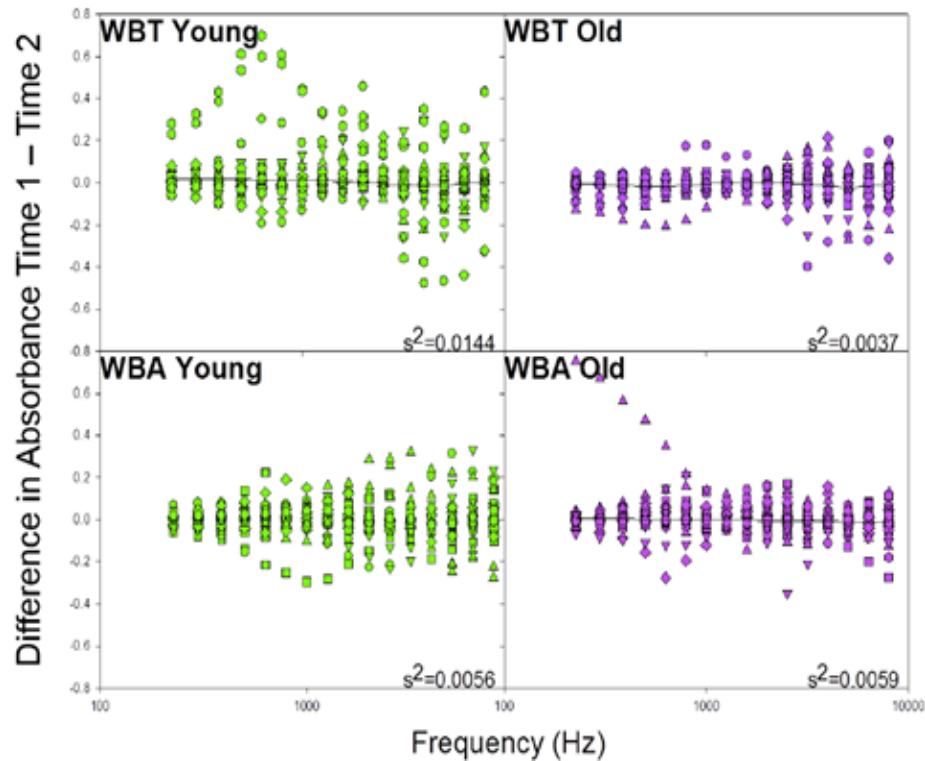


Figure 6: Difference between time 1 and time 2 absorbance measurements for WBT and WBA conditions on day 1 and day 2 for younger and older adults. The solid line shows mean difference in absorbance as a function of frequency in the WBT and WBA conditions.

4.6 Variability Falling Within Normative Percentiles

All of the data collected in the study, regardless of condition or test session, was compared against the normative data provided by Lui et al., 2008 used in the Interacoustics Titan clinical system. Figure 7 shows the 5th and 95th percentile intervals for the WBT and WBA conditions, respectively shaded pink and blue (redrawn from Titan system norms) with all subject average data from day 1 and day 2 (time 1).

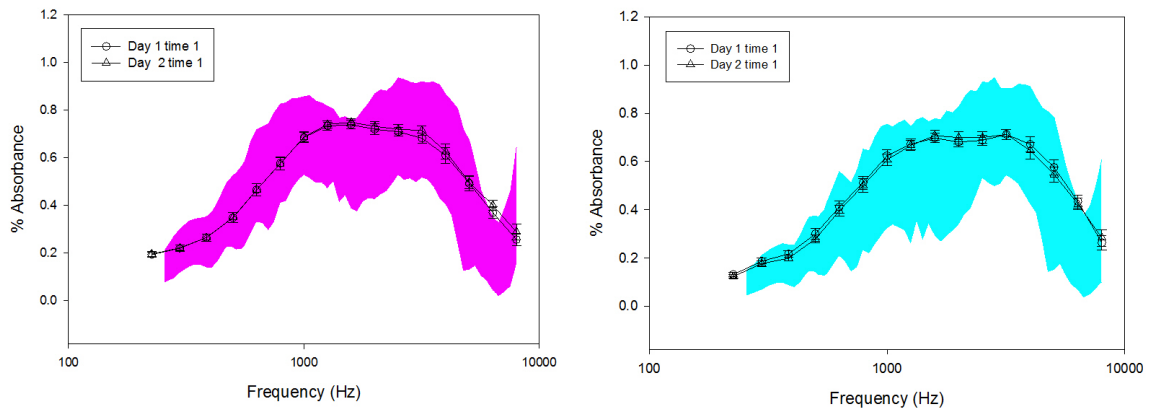


Figure 7: Subject average data with standard error bars for Day 1 Time 1 and Day 2 Time 1 for the WBT (left) and WBA (right) conditions; Pink and blue shading represents the 5th and 95th percent confidence intervals (Lui et al., 2008) as provided by the Interacoustics Titan

With the exception of WAI at the 6,000 Hz one-third octave interval, all average WAI responses measured either on day 1 or day 2 fall within the 95% confidence intervals provided by the Titan system. Figure 8 shows inter-subject variability for each age group. While there does appear to be some differences in average WAI as a function of age, average WAI (with the exception of 1,500 and 6,000 Hz one-third octave intervals) fell within the 95% confidence intervals.

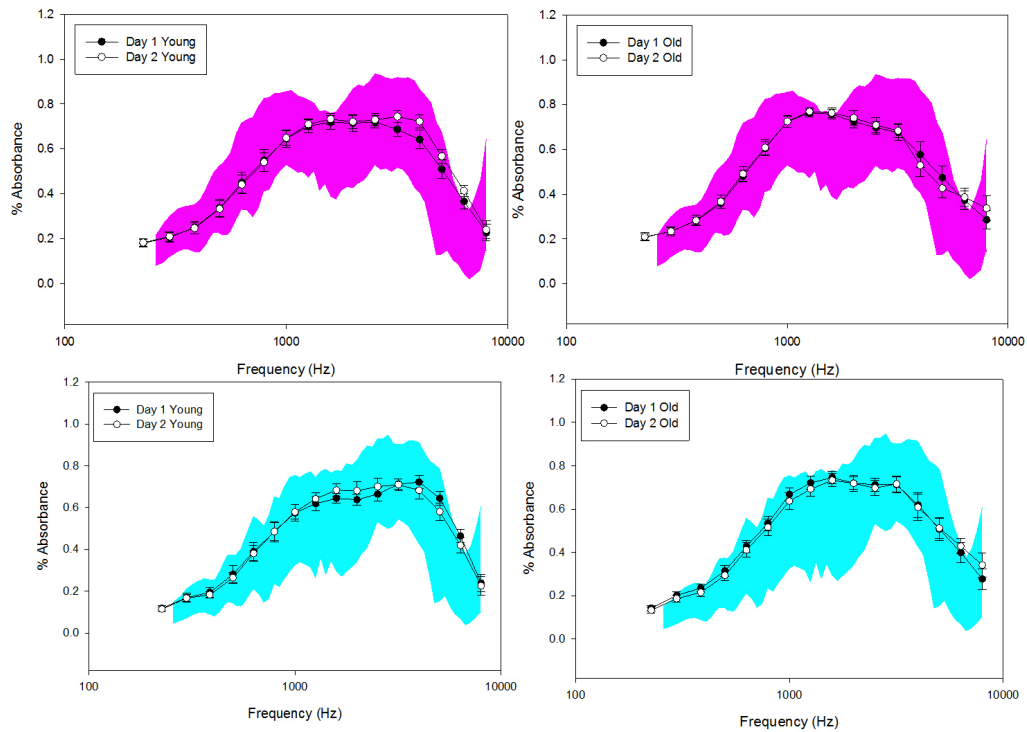


Figure 8: Inter-subject variability for young adult group (left) and older adult group (right) with standard error bars for day 1 and day 2 for the WBT (top) and WBA (bottom) conditions; Pink and blue shading represents the 5th and 95th percent confidence intervals (Lui et al., 2008) as provided by the Interacoustics Titan; WBT absorbance values for both groups fell within normative data 76% of the time, while WBA absorbance values for both groups were within normative data 77% of the time

Figure 9 provides a more detailed examination of inter-subject variability by showing all ears, all conditions (n=144) WAI tracings against the normative data. The majority of individual tracings fall within the 95% confidence intervals for these norms, however there are several instances, particularly for the WBA condition where individual wideband immittance fall outside these norms. In general, the individuals whose WAI fell outside the norms showed greater absorbance across all measured frequencies, particularly for the lowest and highest frequencies.

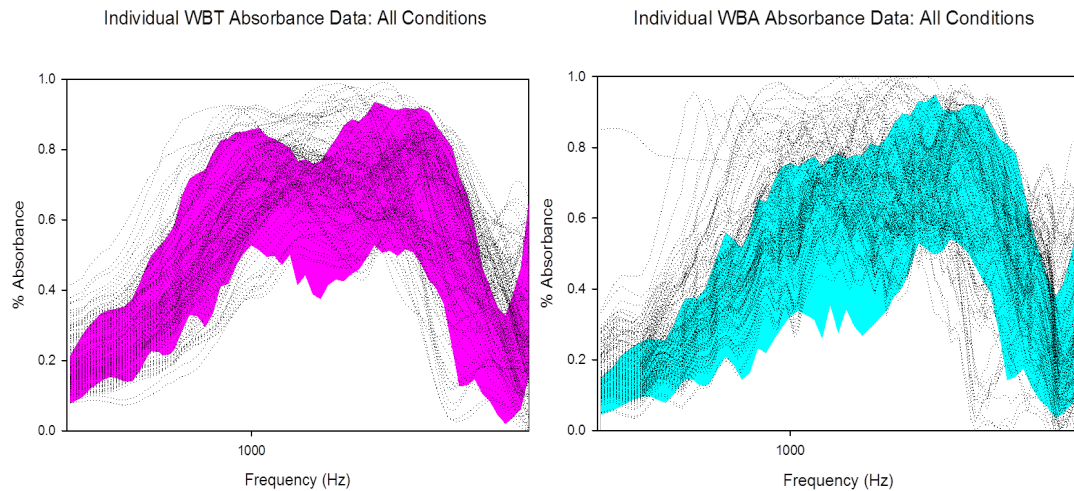


Figure 9: All ears, all conditions (n=144, WBT and WBA conditions) absorbance data; Pink and blue shading represents the 5th and 95th percent confidence intervals (Lui et al., 2008) as provided by the Interacoustics Titan; WBT absorbance values for both groups fell within normative data 76% of the time, while WBA absorbance values for both groups were within normative data 77% of the time

5.0 Discussion

5.1 Intra-subject Variability

5.1a Test-Retest Findings as a Function of Condition

One of the primary purposes of the present study was to determine if test-retest measurements in wideband immittance would be more variable between two tests on the same day with probe reinsertion, or between two test sessions on separate days. We expected test-retest variability to be greater between test sessions on separate days regardless of the WAI condition and age. Previous studies on WAI over multiple sessions found that measurements on separate days show substantially larger variability than repeated measurements with probe reinsertion in the same session (Vander Werff et al., 2007). Hunter et al. (2008) analyzed test–retest measurements of power reflectance where consecutive measurements were made within the same test session with probe reinsertion for each measurement. The intraclass correlation coefficient at various frequencies presented evidence of “substantial reliability of repeat measures within the same test session” (Hunter et al., 2008). However, other studies have shown that probe reinsertion presents a higher risk for air-leaks, which in turn, has been shown to increase variability (Voss et al., 2013). When all of our subjects’ data are analyzed together, both the WBA and WBT conditions are more variable between days than within the same day. These results repeat similar findings by Vander Werff et al. (2007) and Rosowski et al. (2012).

The greater likelihood of air-leaks with probe placement during WBA measurements led us to hypothesize that the WBA measurements would be more variable than the WBT measurements for both between day and within day comparisons.

We found this to be true for the between day measurement, but within day measurements revealed unexpected greater variability in the WBT condition. A possible explanation of this finding is the potential effect of outlying data. When individual subject data was examined, two outlying WAI tracings were noted in the WBT condition. The outlying data belonged to the same subject on day 1 test 2 for both the right and left ear. When these data points are removed from the statistical analysis, there is no significant difference between variability in within day measures for the WBT or WBA conditions ($F_{1151, 1120}=1.01, p > 0.05$). This would suggest that even though the probe is being reinserted, the changes noted from test 1 to test 2 do not make a significant difference in absorbance for either WAI condition. Clinically, this is useful to know when basing a diagnosis of middle ear dysfunction or pathology on WAI patterns. It is also interesting to note that even though the outlier subject had abnormal WAI tracings with normal middle ear status as defined by the current study's criteria, there was not a high difference in absorbance from time 1 to time 2 tracings for this subject. This reinforces a statement made by Rosowski et al. (2012) and Warner et al. (2010) that unique characteristics of an individuals' WAI tracings were found to repeat on retest tracings.

In the current study's protocol, the probe was reinserted just after the WBT condition. Because the probe had just been previously inserted with a sufficient seal to measure WAI with pressure, it is possible that the probe reinsertion may not have resulted in a large change in position. This could have resulted in minimal changes in the amount of air-leak between test 1 and test 2 in the same session. This would have had a greater influence on variability for within day measurements as opposed to between day measurements and could be one reason we found WBA more variable only in the

between day situation. As previously mentioned, controlling or monitoring the amount of air leak during measurements could shed more light on this finding.

One possibility for monitoring the influence of air-leak on variability would be to explore the variability that occurs when using foam tips for non-pressurized conditions. Vander Werff et al. (2007) found test-retest differences in power reflectance in infants were smaller for foam tips than rubber tips. Foam tips have the ability to expand to the shape of the ear canal, reducing the amount of acoustic leak during testing. Clinically, air-leak effects on absorbance were the least for mid frequencies (1,000 to 5,000 Hz) (Vander Werff et al., 2007). This is advantageous for clinical implications of absorbance because this frequency range may have the greatest potential for diagnosing middle-ear pathology (Hunter et al., 2013; Keefe et al., 1993; Sanford & Feeney, 2008).

Another factor to consider in the findings from the current study is a phenomenon called “tympanometric preconditioning of the ear drum,” as coined by Gaihede (1996). Burdick and Sun (2014) discussed tympanometric preconditioning of the ear drum and how it may relate to changes in wideband absorbance measurements. Burdick and Sun proposed tympanometric preconditioning can be attributed to a change in the stiffness component of the eardrum after repeatedly varying the pressure in the ear canal. The findings from their study showed that consecutive wideband absorbance with variation in ear canal pressure resulted in an increase in low frequency (below 1,500 Hz) absorbance and a decrease in high frequency (at 2,000 Hz and 5,000 to 6,000 Hz) absorbance. The changes observed were small but significant. Larger absorbance changes were noted in ambient conditions that were performed after a conventional tympanogram. These findings may have been a factor in the variability noted in our study. The research

protocol used called for the WBT condition to be performed first in order to validate the middle ear status of each subject during each session. A direction for future studies could be to observe the implications of tympanometric preconditioning on clinical measurements of wideband absorbance in light of factors similar to the ones analyzed in the current study.

Feeney et al. (2014) also reported that the left ear demonstrated greater reflectance values than the right ear when measured at ambient pressure. Findings were only significant at 2,000 and 2,500 Hz one-third octave bands. Interestingly, the right ear showed greater reflectance values at one-third octave bands 4,000 Hz and greater. Our study looked at ear-specific variability as a function of test condition as well and found more variability between days in right ear measurements for the WBT condition. However, no significant difference was found for left and right ears in the WBA condition. These differences in findings may be related to the differences in the length of time between each test session and the number of test sessions in each study. In Feeney's study, wideband energy reflectance measurements were obtained at baseline and annually for up to four additional tests. In our study, each subject was only tested on two days, which were separate by at least seven days and at most 198 days.

Previous studies have showed WAI changes in a frequency specific manner over trials for both ambient and tympanometric peak pressure measurements. Burdick and Sun (2014) found between trials, absorbance increased in low frequencies (below 1,500 Hz) and decreased in specific high frequency regions (around 2,000 Hz and 5,000 to 6,000 Hz). When observing the current study's Figure 7 and Figure 8, there does not appear to be a consistent frequency effect across all the subjects, nor does there appear to be a large

change or difference between absorbance values on the two session days, as suggested in Burdick and Sun (2014).

5.1b Test-Retest Findings as a Function of Age

We were also interested in variability by age in the current study. Anatomical and physiological changes of the tympanic membrane have been studied and an increase in stiffness with age has been shown. Previous studies have shown wideband immittance patterns vary by age (Feeney and Sanford, 2004; Gaihede and Koefoed-Nielsen, 2000). Feeney and Sanford (2004) found significant age effects in wideband absorbance under ambient conditions. Their results showed that older adults displayed a decrease in reflectance from 800 to 2,000 Hz followed by an increase around 4,000 Hz. These findings were proposed to be the consequence of a decrease in middle-ear stiffness with age (Feeney and Sanford, 2004).

In the current study, younger and older adults' wideband acoustic immittance measurements were compared within the same day and between two separate days. Both between day and within day measurements were more variable in the younger adult group. The WBT condition was significantly more variable in younger adults for both within day and between day measures. The WBA condition only reached significance for between day measures in the younger adults. The question arose again regarding the effect of the outlying data, as seen in the variance analysis for all subjects. However, when the outlier subject was removed from the data set, WBT continues to be significantly more variable in younger adults ($F_{575,544} = 1.93, p < 0.05$).

It has been shown that ear canal volume continues to increase into the ninth decade of life (Hunter and Shahnaz, 2013). Keefe et al. (1993) and Sanford and Feeney

(2008) proposed absolute admittance differences found between adults and infants were due to differences in ear-canal volume. Studies on factors influencing variability in WAI (Voss et al., 2008) also suggest that ear canal volume is likely to be a cause of inter-subject variability in wideband absorbance measures. While there have been differing conclusions regarding the exact increase or decrease in ear canal volume with age (Feeney and Sanford, 2004; Wiley et al., 1996; Wiley et al., 2005), if ear canal volumes were more homogenous among the older adult group than the younger adult group in the current study, the absorbance test-retest differences would likely be less variable in the older adult group as demonstrated in the findings. Future research could analyze if ear canal consistency among an age group contributes to the variability seen in test-retest measures in absorbance.

5.2 Inter-subject Variability for Clinical Use

The present study demonstrated ambient and tympanometric WAI measures in 36 ears tested twice per test session on two separate days. In Figure 9, WAI tracings for all ears, all conditions (n=144) are plotted against normative data provided by Lui et al. (2008). The normative data used for comparison was selected because the Interacoustic Titan, the clinical tool utilized in the present study, used this set of data to determine clinically “normal” WAI measurements versus measurements indicative of a pathological condition. The majority of individual tracings from the present study fall within the 95% confidence intervals, however there are several instances, particularly for the WBA condition, where individual patterns fall outside these norms. When the individual subject data was examined separately, the subjects whose WAI fell outside the normative range exhibited greater absorbance across several frequencies, particularly for the lowest and

highest frequencies. However, the findings from the present study are comparable to the findings by Rosowski et al. (2012). Their repeated measurements over the course of four sessions had similar standard deviations to the population standard deviation. Similarly to the present results, test-retest absolute differences in reflectance tended to have small confidence intervals (0.2 to 0.3) and remain consistent with the normative population data's confidence interval values.

Groon et al. (2015) suggested that changes in absorbance could be a result of air-leaks with probe placement. Their findings showed changes in absorbance were largest for lower frequencies, but could also be observed at frequencies up to 10,000 Hz. However, the extent and direction of the effects on absorbance were unpredictable above 1,000 Hz. These findings are similar to the tracings seen in Figure 9, where WAI measures exhibited greater absorbance specifically in the low and high frequencies.

One of the disadvantages of ambient WAI measurements is that they can be obtained without assessing the extent of the acoustic seal during measurements (WBA condition in the current study) (Groon et al., 2015). The Titan clinical system provides an LED light notification on the probe system that estimates if a proper fit is obtained during these non-pressurized measurements (WBA condition in the current study). Yet, this notification does not provide information if an accurate acoustic seal, free of air-leaks, is obtained during such measurements. Therefore, the protocol used in our study did not measure or control the amount of air-leak between measurements and this certainly could have influenced the individual variability we saw under ambient conditions.

Figure 7 from the present study displays WAI tracings by pressure condition and further depicts variability against normative data. With the exception of WAI at the 6,000

Hz one-third octave interval, all average responses measured either on day 1 or day 2, WBA and WBT conditions, fell within the 95% confidence intervals of the normative data. While there do appear to be some differences in average WAI as a function of age, as depicted in Figure 8, all average WAI (with the exception of 1,500 and 6,000Hz one-third octave intervals) fell within the normal confidence intervals. Clinically speaking, despite the individual differences in WAI tracings (Figure 9), it appears, on average, both young and old adults fall within normative ranges for both conditions (Figure 7 and 8). One of the primary objectives of the present study was to see whether inter-subject variability of clinically measured WAI (using the Titan system) would result in average wideband absorbance values that fell within the published 95% confidence interval provided by Titan. Our results suggest that, on average, and regardless of age, the WAI measured clinically does fall within those normative values. Importantly, even when individual WAI responses fell outside the 95% confidence intervals, the resultant WAI response was most similar to the “lose probe fit” example provided by the Titan template and did not resemble common pathological conditions such as negative middle ear pressure or middle ear fluid (Figure 10).

Examples of WAI in pathological conditions

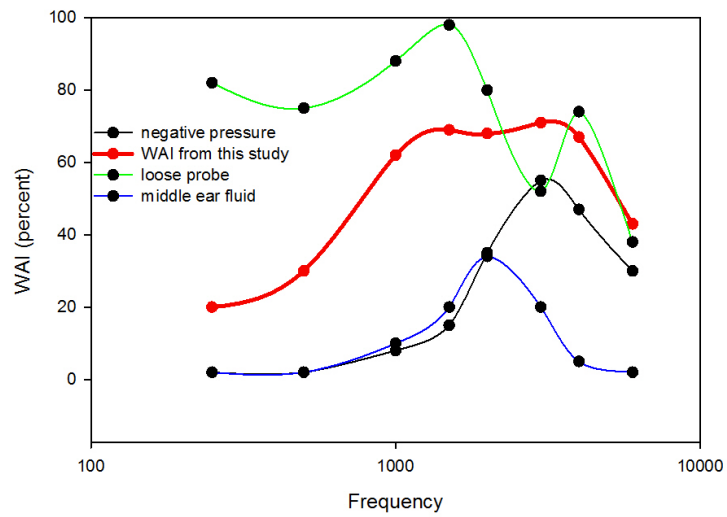


Figure 10: Examples of WAI patterns in typical middle ear pathology (redrawn from Titan manual; negative middle ear pressure and middle ear fluid) as well as artifactual WAI response with loose probe fit (green line). Average WAI from the present study is shown in red.

I. Figure 2: Learning Effect WBT Data

TESTER	SUBJECT	TEST DATE	MAXIMUM DIFFERENCE IN ABS	
			Tester 1	Tester 2
Tester 2	4CO1RDIFF	2/10/14		0.038
Tester 2	4CO1LDIFF	2/10/14		0.044
Tester 1	ADY1RDIFF	2/18/14	0.03625	
Tester 1	ADY1LDIFF	2/18/14	0.046625	
Tester 2	4CO2RDIFF	2/19/14		0.062875
Tester 2	4CO2LDIFF	2/19/14		0.068
Tester 1	10CY1RDIFF	2/20/14	0.1355	
Tester 1	10CY1LDIF	2/20/14	0.116375	
Tester 2	QCY1RDIFF	2/25/14		0.03575
Tester 2	QCY1LDIFF	2/25/14		0.034
Tester 2	7CY1RDIFF	2/25/14		0.044
Tester 2	7CY1LDIFF	2/25/14		0.095125
Tester 1	ADY2RDIFF	2/25/14	0.041625	
Tester 1	ADY2LDIFF	2/25/14	0.056875	
Tester 2	QHY1RDIFF	2/26/14		0.017625
Tester 2	QHY1LDIFF	2/26/14		0.03025
Tester 1	9DY1LDIFF	2/26/14	0.099125	
Tester 1	9DY1RDIFF	2/26/14	0.06	
Tester 1	10CY2RDIFF	2/27/14	0.176	
Tester 1	10CY2LDIFF	2/27/14	0.053666667	
Tester 2	2CO1RDIFF	2/28/14		0.046125
Tester 2	3DO1RDIFF	2/28/14		0.0415
Tester 2	2CO1LDIF	2/28/14		0.116875
Tester 2	3DO1LDIFF	2/28/14		0.060375
Tester 1	8DO1RDIFF	3/1/14	0.076875	
Tester 1	8DO1LDIFF	3/1/14	0.1102	
Tester 2	QCY2RDIFF	3/4/14		0.047375
Tester 2	QHY2RDIFF	3/4/14		0.069625
Tester 2	QCY2LDIFF	3/4/14		0.052375
Tester 2	QHY2LDIFF	3/4/14		0.02625
Tester 1	3CO1RDIFF	3/5/14	0.168125	
Tester 1	6SO1RDIFF	3/5/14	0.070375	
Tester 1	3CO1LDIFF	3/5/14	0.06875	
Tester 1	6SO1LDIFF	3/5/14	0.0315	
Tester 1	KSO1RDIFF	3/12/14	0.06375	
Tester 1	KSO1LDIFF	3/12/14	0.0575	
Tester 1	8DO2RDIFF	3/18/14	0.10825	
Tester 1	8DO2LDIFF	3/18/14	0.191875	
Tester 2	7CY2RDIFF	3/18/14		0.294
Tester 2	7CY2LDIFF	3/18/14		0.348875
Tester 2	3DO2RDIFF	3/19/14		0.0345
Tester 1	3CO2RDIFF	3/19/14	0.247875	
Tester 2	3DO2LDIFF	3/19/14		0.058875
Tester 1	3CO2LDIFF	3/19/14	0.271625	
Tester 2	2CO2RDIFF	3/20/14		0.055625
Tester 1	6SO2RDIFF	3/20/14	0.0435	
Tester 2	2CO2LDIFF	3/20/14		0.061375
Tester 1	6SO2LDIFF	3/20/14	0.105	
Tester 1	KSO2RDIFF	3/21/14	0.198375	
Tester 1	KSO2LDIFF	3/21/14	0.04425	
Tester 1	8CO1RDIFF	9/4/14	0.398375	
Tester 1	8CO1LDIFF	9/4/14	0.074125	
Tester 1	JCO1RDIFF	9/4/14	0.172375	
Tester 1	JCO1LDIFF	9/4/14	0.111875	
Tester 1	6DY1RDIFF	9/5/14	0.22425	
Tester 1	6DY1LDIFF	9/5/14	0.062375	
Tester 2	9CY1LDIFF	9/8/14		0.192333333
Tester 2	9CY1RDIFF	9/8/14		0.475875
Tester 2	7SY1LDIFF	9/11/14		0.699333333
Tester 2	7SY1RDIFF	9/11/14		0.599166667
Tester 1	JCO2LDIFF	9/11/14	0.148	
Tester 1	JCO2RDIFF	9/11/14	0.026	
Tester 1	6DY2RDIFF	9/12/14	0.272875	
Tester 1	6DY2LDIFF	9/12/14	0.082125	
Tester 1	8CO2RDIFF	9/12/14	0.174125	
Tester 1	8CO2LDIFF	9/12/14	0.214625	
Tester 1	9DY2RDIFF	9/12/14	0.0805	
Tester 1	9DY2LDIFF	9/12/14	0.300875	
Tester 2	9CY2LDIFF	9/15/14		0.187875
Tester 2	9CY2RDIFF	9/15/14		0.32175
Tester 2	7SY2LDIFF	9/18/14		0.09175
Tester 2	7SY2RDIFF	9/18/14		0.073625

TOTALS	
Tester 1	40
Tester 2	32
First Half of Study	
Tester 1	26
Tester 2	22
Second Half of Study	
Tester 1	14
Tester 2	8

J. Figure 2: Learning Effect WBA Data

TESTER	SUBJECT	TEST DATE	MAXIMUM DIFFERENCE IN ABS	
			Tester 1	Tester 2
Tester 2	4CO1RDIFF	2/10/14		0.111625
Tester 2	4CO1LDIFF	2/10/14		0.02775
Tester 1	ADY1RDIFF	2/18/14	0.067	
Tester 1	ADY1LDIFF	2/18/14	0.070625	
Tester 2	4CO2RDIFF	2/19/14		0.03825
Tester 2	4CO2LDIFF	2/19/14		0.02125
Tester 1	10CY1RDIFF	2/20/14	0.27675	
Tester 1	10CY1LDIFF	2/20/14	0.201	
Tester 2	QCY1RDIFF	2/25/14		0.09225
Tester 2	QCY1LDIFF	2/25/14		0.121625
Tester 2	7CY1RDIFF	2/25/14		0.031
Tester 2	7CY1LDIFF	2/25/14		0.1405
Tester 1	ADY2RDIFF	2/25/14	0.019625	
Tester 1	ADY2LDIFF	2/25/14	0.145875	
Tester 2	QHY1RDIFF	2/26/14		0.027375
Tester 2	QHY1LDIFF	2/26/14		0.295625
Tester 1	9DY1LDIFF	2/26/14	0.1195	
Tester 1	9DY1RDIFF	2/26/14	0.31825	
Tester 1	10CY2RDIFF	2/27/14	0.331625	
Tester 1	10CY2LDIFF	2/27/14	0.031875	
Tester 2	2CO1RDIFF	2/28/14		0.056125
Tester 2	3DO1RDIFF	2/28/14		0.053
Tester 2	2CO1LDIF	2/28/14		0.058375
Tester 2	3DO1LDIFF	2/28/14		0.042375
Tester 1	8DO1RDIFF	3/1/14	0.072	
Tester 1	8DO1LDIFF	3/1/14	0.156	
Tester 2	QCY2RDIFF	3/4/14		0.066125
Tester 2	QHY2RDIFF	3/4/14		0.023125
Tester 2	QCY2LDIFF	3/4/14		0.055375
Tester 2	QHY2LDIFF	3/4/14		0.02575
Tester 1	3CO1RDIFF	3/5/14	0.751	
Tester 1	6SO1RDIFF	3/5/14	0.081875	
Tester 1	3CO1LDIFF	3/5/14	0.036	
Tester 1	6SO1LDIFF	3/5/14	0.06425	
Tester 1	KSO1RDIFF	3/12/14	0.129875	
Tester 1	KSO1LDIFF	3/12/14	0.081625	
Tester 1	8DO2RDIFF	3/18/14	0.1275	
Tester 1	8DO2LDIFF	3/18/14	0.121833333	
Tester 2	7CY2RDIFF	3/18/14		0.0525
Tester 2	7CY2LDIFF	3/18/14		0.044375
Tester 2	3DO2RDIFF	3/19/14		0.026375
Tester 1	3CO2RDIFF	3/19/14	0.351125	
Tester 2	3DO2LDIFF	3/19/14		0.043
Tester 1	3CO2LDIFF	3/19/14	0.0555	
Tester 2	2CO2RDIFF	3/20/14		0.277333333
Tester 1	6SO2RDIFF	3/20/14	0.180375	
Tester 2	2CO2LDIFF	3/20/14		0.049
Tester 1	6SO2LDIFF	3/20/14	0.274375	
Tester 1	KSO2RDIFF	3/21/14	0.07225	
Tester 1	KSO2LDIFF	3/21/14	0.034625	
Tester 1	8CO1RDIFF	9/4/14	0.1605	
Tester 1	8CO1LDIFF	9/4/14	0.064375	
Tester 1	JCO1RDIFF	9/4/14	0.053625	
Tester 1	JCO1LDIFF	9/4/14	0.13975	
Tester 1	6DY1RDIFF	9/5/14	0.286	
Tester 1	6DY1LDIFF	9/5/14	0.053	
Tester 2	9CY1LDIFF	9/8/14		0.019125
Tester 2	9CY1RDIFF	9/8/14		0.313
Tester 2	7SY1LDIFF	9/11/14		0.024
Tester 2	7SY1RDIFF	9/11/14		0.1512
Tester 1	JCO2LDIFF	9/11/14	0.12025	
Tester 1	JCO2RDIFF	9/11/14	0.054625	
Tester 1	6DY2RDIFF	9/12/14	0.1315	
Tester 1	6DY2LDIFF	9/12/14	0.238625	
Tester 1	8CO2RDIFF	9/12/14	0.124375	
Tester 1	8CO2LDIFF	9/12/14	0.15575	
Tester 1	9DY2RDIFF	9/12/14	0.024125	
Tester 1	9DY2LDIFF	9/12/14	0.0615	
Tester 2	9CY2LDIFF	9/15/14		0.105625
Tester 2	9CY2RDIFF	9/15/14		0.190625
Tester 2	7SY2LDIFF	9/18/14		0.083166667
Tester 2	7SY2RDIFF	9/18/14		0.224166667

TOTALS	
Tester 1	40
Tester 2	32
First Half of Study	
Tester 1	26
Tester 2	22
Second Half of Study	
Tester 1	14
Tester 2	8

L. Between Day WBT Data, All Subjects

Frequency (Hz) on x-axis by Subject Code on y-axis

	226	297.3	385.55	500	629.96	793.7	1000	1259.9	1587.4	2000	2519.8	3174.8	4000	5039.7	6349.6	8000
2COR	-0.031	-0.029	-0.035	-0.051	-0.052	-0.025	-0.015	-0.015	-0.019	-0.012	-0.019	-0.009	-0.002	-0.012	-0.054	-0.051
3DOR	0.03	0.0297	0.037	0.0376	0.0278	0.012	0.0099	0.0116	0.0061	-0.004	-0.018	-0.042	-0.061	-0.087	0.0131	0.1313
8DOL	0.091	0.093	0.1215	0.1452	0.1607	0.1218	0.0354	0.0524	0.0641	0.0199	-0.016	-0.004	-0.093	-0.095	0.012	0.1445
3COR	-0.038	-0.044	-0.049	-0.032	-0.006	0.0659	0.0827	0.0753	0.0689	0.1318	0.001	-0.286	-0.417	-0.216	-0.082	-0.04
6SOR	-0.063	-0.061	-0.059	-0.051	-0.055	-0.05	-0.04	-0.019	-0.002	0.0279	0.0448	0.0374	-0.019	-0.133	-0.198	0.0508
KSOR	0.032	0.034	0.0435	0.0558	0.054	0.0478	0.0425	0.0428	0.0119	-0.036	-0.077	-0.062	-0.049	0.0001	0.0821	0.1599
2COL	-0.035	-0.037	-0.041	-0.049	-0.057	-0.066	-0.07	-0.068	-0.053	-0.04	-0.059	-0.076	-0.053	-0.049	0.1079	0.0306
3DOL	0.016	0.01	0.012	0.0124	0.0285	0.0406	0.0333	0.0266	0.0121	-0.015	-0.042	-0.07	-0.095	-0.044	0.0683	0.1348
8DOL	0.102	0.1103	0.1303	0.133	0.0962	0.0474	0.0689	0.0668	0.0592	0.0106	-0.02	-0.083	-0.119	-0.093	0.07	0.2059
3COL	-0.075	-0.085	-0.101	-0.096	-0.09	-0.065	-0.016	-0.011	-0.011	0.0364	0.0965	0.114	-0.134	-0.226	-0.115	-0.21
6SOL	0.039	0.046	0.0663	0.1016	0.1367	0.094	0.0918	0.0928	0.0722	0.1524	0.0819	-0.001	-0.198	-0.291	-0.25	0.0885
KSOL	-0.015	-0.018	-0.017	-0.01	-0.026	-0.027	-0.02	0.0006	-0.006	0.0036	-0.011	-0.006	-0.013	-0.023	0.0027	0.0016
QCVR	0.033	0.027	0.0205	0.012	-0.007	-0.043	-0.051	0.0035	0.1224	0.085	0.0678	0.0829	0.0349	0.0019	-0.032	0.0085
QHVR	-0.01	-0.016	-0.025	-0.023	-0.008	0.0104	0.029	0.0399	0.0426	0.0519	0.0643	0.0701	0.0998	0.0356	-0.042	-0.118
ADVR	0.003	0.003	0.0052	0.0068	0.013	0.0204	0.0221	0.0096	0.0095	0.0259	0.0291	0.0192	0.0094	-0.034	-0.083	-0.041
10CYR	0.029	0.0333	0.0428	0.0534	0.0583	0.0505	0.0378	0.0163	0.0016	-0.083	-0.142	-0.163	-0.102	0.0458	0.3121	0.1913
QCYL	0.033	0.0343	0.0343	0.0324	0.0155	0.0008	-0.03	-0.033	0.0311	0.039	-0.005	-0.018	-0.021	-0.015	-0.05	0.0401
QHVL	0.014	0.0137	0.0155	0.0194	0.02	0.0248	0.0306	0.0358	0.0295	0.0304	0.0254	0.0229	0.035	-0.038	-0.078	-0.02
ADVL	0.009	0.0087	0.016	0.0244	0.032	0.0529	0.0594	0.0559	0.049	0.0343	0.0223	0.0096	0.0081	-0.064	-0.125	-0.077
10CYL	0	0.0027	0.001	0.006	0.0083	0.0096	0.0071	-0.005	-0.023	-0.044	-0.064	-0.089	-0.063	0.0653	0.2059	0.0895
6DYR	0.038	0.038	0.047	0.0638	0.0597	0.0456	0.048	0.0505	0.0178	-0.002	-0.032	-0.024	0.0912	0.0158	0.0338	0.0541
6DYL	-0.018	0.006	0.0283	0.024	0.0145	0.0008	0.0311	0.0596	0.0706	0.0889	0.0753	-0.027	0.0131	-0.085	-0.062	-0.015
7SYL	-0.021	-0.011	-0.029	-0.081	-0.12	-0.102	-0.098	-0.086	-0.226	-0.304	-0.237	0.1623	0.3299	0.2579	0.2056	0.0291
7SYR	0.006	-0.003	-0.052	-0.045	0.0382	0.0109	0.0124	-0.049	-1E-04	0.0069	-0.014	0.0088	0.0365	0.134	0.0761	0.0786
8COR	0.031	0.0223	0.0043	-0.019	-0.106	-0.238	-0.213	-0.124	-0.146	-0.082	0.0638	0.4638	0.3256	0.2956	0.3374	0.2305
8COL	-0.041	-0.025	-0.014	0.015	0.0615	0.1011	0.1173	0.13	0.1111	0.1464	0.1631	0.0329	-0.074	-0.14	-0.045	-0.084
9CYL	0.106	0.1093	0.141	0.191	0.257	0.2416	0.1769	0.0844	0.0715	-0.017	-0.018	-0.025	-0.17	-0.041	0.0203	0.1191
9CYR	-0.081	-0.106	-0.175	-0.284	-0.423	-0.411	-0.222	-0.106	-0.203	-0.271	0.035	0.37	0.434	0.2604	0.2353	0.0739
9DYR	-0.107	-0.112	-0.157	-0.15	-0.185	-0.149	-0.142	-0.124	-0.075	-0.036	-0.045	0.1784	0.1724	0.102	0.1575	-0.044
9DYL	-0.031	-0.025	-0.043	-0.011	-0.022	0.0374	0.1045	0.1589	0.1291	0.1629	0.3273	0.196	0.0034	-0.193	-0.177	-0.259
JCOL	0	-0.019	-0.038	0.0094	0.0283	0.0384	0.0246	-0.055	-0.024	0.051	0.0439	-0.058	0.0834	0.0391	-0.012	0.0494
JCOR	-0.019	-0.045	-0.032	-0.056	0.007	0.0092	-0.071	-0.007	-9E-04	-0.021	0.0071	0.2151	0.1125	0.1229	0.1038	0.0315
4COR	0.005	0.0063	0.0045	-0.009	-0.018	-0.021	-0.02	-0.009	-6E-04	-0.016	-0.026	-0.023	-0.013	0.0525	0.0978	0.0061
4COL	-0.009	-0.011	-0.014	-0.015	-0.021	-0.027	-0.034	-0.041	-0.017	-0.017	-0.016	-0.012	0.0067	0.0549	0.1123	0.0438
7CYR	0.025	0.0367	0.0555	0.0776	0.0797	0.0604	0.0265	0.0226	0.1143	0.231	0.0884	0.1279	0.2448	0.2725	0.1013	-0.001
7CYL	0.013	0.018	0.0215	0.0176	0.0108	0.0096	0.0275	0.0164	0.0861	0.1036	-0.009	0.1349	0.2988	0.314	0.1475	0.1148
MEAN	0.0017	0.001	-9E-04	0.0015	0.0004	-0.002	0.0021	0.0083	0.0104	0.0123	0.0102	0.0324	0.0179	0.0053	0.0305	0.0318

N. Between Day WBA Data, All Subjects
Frequency (Hz) on x-axis by Subject Code on y-axis

	226	297.3	385.55	500	629.96	793.7	1000	1259.9	1587.4	2000	2519.8	3174.8	4000	5039.7	6349.6	8000
2COR	-0.031	-0.0293	-0.035	-0.0512	-0.0515	-0.0246	-0.0149	-0.0145	-0.0189	-0.0116	-0.0185	-0.0086	-0.002	-0.0123	-0.0535	-0.0513
3DOR	0.03	0.0297	0.037	0.0376	0.0278	0.012	0.0099	0.0116	0.0061	-0.0035	-0.0179	-0.042	-0.0609	-0.0866	0.0131	0.1313
8DOR	0.091	0.093	0.1215	0.1452	0.1607	0.1218	0.0354	0.0524	0.0641	0.0199	-0.0164	-0.0043	-0.0933	-0.0948	0.012	0.1445
3COR	-0.038	-0.0437	-0.049	-0.032	-0.0063	0.0659	0.0827	0.0753	0.0689	0.1318	0.001	-0.286	-0.4168	-0.2158	-0.0819	-0.0403
6SOR	-0.063	-0.0613	-0.0593	-0.051	-0.0548	-0.0499	-0.0398	-0.0185	-0.002	0.0279	0.0448	0.0374	-0.0189	-0.133	-0.1984	0.0508
KSOR	0.032	0.034	0.0435	0.0558	0.054	0.0478	0.0425	0.0428	0.0119	-0.0364	-0.0766	-0.062	-0.049	0.0001	0.0821	0.1599
2COL	-0.035	-0.037	-0.0413	-0.0486	-0.0567	-0.0664	-0.0704	-0.068	-0.0529	-0.0396	-0.0589	-0.076	-0.0525	-0.0491	0.1079	0.0306
3DOL	0.016	0.01	0.012	0.0124	0.0285	0.0406	0.0333	0.0266	0.0121	-0.0154	-0.0424	-0.07	-0.0947	-0.044	0.0683	0.1348
8DOL	0.102	0.1103	0.1303	0.133	0.0962	0.0474	0.0689	0.0668	0.0592	0.0106	-0.0203	-0.0834	-0.1189	-0.0929	0.07	0.2059
3COL	-0.075	-0.0853	-0.1008	-0.0964	-0.0903	-0.0645	-0.0158	-0.0114	-0.0106	0.0364	0.0965	0.114	-0.1341	-0.2261	-0.1149	-0.2104
6SOL	0.039	0.046	0.0663	0.1016	0.1367	0.094	0.0918	0.0928	0.0722	0.1524	0.0819	-0.0014	-0.1978	-0.2908	-0.2501	0.0885
KSOL	-0.015	-0.018	-0.0165	-0.0098	-0.026	-0.0267	-0.0197	0.0006	-0.006	0.0036	-0.011	-0.0064	-0.0126	-0.0231	0.0027	0.0016
QCYR	0.033	0.027	0.0205	0.012	-0.007	-0.0434	-0.0506	0.0035	0.1224	0.085	0.0678	0.0829	0.0349	0.0019	-0.0324	0.0085
QHYP	-0.01	-0.016	-0.0245	-0.0234	-0.0077	0.0104	0.029	0.0399	0.0426	0.0519	0.0643	0.0701	0.0998	0.0356	-0.0418	-0.118
ADYP	0.003	0.003	0.0052	0.0068	0.013	0.0204	0.0221	0.0096	0.0095	0.0259	0.0291	0.0192	0.0094	-0.034	-0.083	-0.041
10CYR	0.029	0.0333	0.0428	0.0534	0.0583	0.0505	0.0378	0.0163	0.0016	-0.0825	-0.1415	-0.1631	-0.1021	0.0458	0.3121	0.1913
QCYL	0.033	0.0343	0.0343	0.0324	0.0155	0.0008	-0.0301	-0.033	0.0311	0.039	-0.0054	-0.0176	-0.0206	-0.0153	-0.0498	0.0401
QHYP	0.014	0.0137	0.0155	0.0194	0.02	0.0248	0.0306	0.0358	0.0295	0.0304	0.0254	0.0229	0.035	-0.0384	-0.0784	-0.02
ADYP	0.009	0.0087	0.016	0.0244	0.032	0.0529	0.0594	0.0559	0.049	0.0343	0.0223	0.0096	0.0081	-0.064	-0.1254	-0.0774
10CYL	0	0.0027	0.001	0.006	0.0083	0.0096	0.0071	-0.005	-0.0225	-0.0435	-0.0636	-0.0895	-0.0629	0.0653	0.2059	0.0895
6DYP	0.038	0.038	0.047	0.0638	0.0597	0.0456	0.048	0.0505	0.0178	-0.0015	-0.0316	-0.0238	0.0912	0.0158	0.0338	0.0541
6DYL	-0.018	0.006	0.0283	0.024	0.0145	0.0008	0.0311	0.0596	0.0706	0.0889	0.0753	-0.0266	0.0131	-0.0853	-0.0619	-0.0151
7SYL	-0.021	-0.0107	-0.0287	-0.0806	-0.1197	-0.1024	-0.0984	-0.0862	-0.2256	-0.304	-0.2365	0.1623	0.3299	0.2579	0.2056	0.0291
7SYR	0.006	-0.003	-0.0515	-0.0452	0.0382	0.0109	0.0124	-0.0495	-0.0001	0.0069	-0.0143	0.0088	0.0365	0.134	0.0761	0.0786
8COR	0.031	0.0223	0.0043	-0.0192	-0.1058	-0.2378	-0.2134	-0.1236	-0.146	-0.082	0.0638	0.4638	0.3256	0.2956	0.3374	0.2305
8COL	-0.041	-0.025	-0.0143	0.015	0.0615	0.1011	0.1173	0.13	0.1111	0.1464	0.1631	0.0329	-0.0739	-0.1401	-0.0448	-0.0843
9CYL	0.106	0.1093	0.141	0.191	0.257	0.2416	0.1769	0.0844	0.0715	-0.0165	-0.0176	-0.025	-0.1696	-0.0412	0.0203	0.1191
9CYR	-0.081	-0.1057	-0.1748	-0.2836	-0.4228	-0.4113	-0.222	-0.1063	-0.2031	-0.2708	0.035	0.37	0.434	0.2604	0.2353	0.0739
9DYP	-0.107	-0.1117	-0.1565	-0.1504	-0.1848	-0.1493	-0.1415	-0.1243	-0.0754	-0.0359	-0.0454	0.1784	0.1724	0.102	0.1575	-0.044
9DYL	-0.031	-0.0247	-0.043	-0.0114	-0.0223	0.0374	0.1045	0.1589	0.1291	0.1629	0.3273	0.196	0.0034	-0.1934	-0.177	-0.2589
JCOL	0	-0.0187	-0.0383	0.0094	0.0283	0.0384	0.0246	-0.0545	-0.0244	0.051	0.0439	-0.0582	0.0834	0.0391	-0.012	0.0494
JCOR	-0.019	-0.0453	-0.0323	-0.0562	0.007	0.0092	-0.0713	-0.0071	-0.0009	-0.0209	0.0071	0.2151	0.1125	0.1229	0.1038	0.0315
4COR	0.005	0.0063	0.0045	-0.0086	-0.0178	-0.0214	-0.0204	-0.0087	-0.0006	-0.0156	-0.0259	-0.0234	-0.0133	0.0525	0.0978	0.0061
4COL	-0.009	-0.0113	-0.0143	-0.0154	-0.0212	-0.0271	-0.0339	-0.0411	-0.0175	-0.0173	-0.016	-0.0116	0.0067	0.0549	0.1123	0.0438
7CYR	0.025	0.0367	0.0555	0.0776	0.0797	0.0604	0.0265	0.0226	0.1143	0.231	0.0884	0.1279	0.2448	0.2725	0.1013	-0.0014
7CYL	0.013	0.018	0.0215	0.0176	0.0108	0.0096	0.0275	0.0164	0.0861	0.1036	-0.0086	0.1349	0.2988	0.314	0.1475	0.1148
Mean	0.0017	0.001	-0.0009	0.0015	0.0004	-0.002	0.0021	0.0083	0.0104	0.0123	0.0102	0.0324	0.0179	0.0053	0.0305	0.0318

O. Percentage of Subject Data for WBT and WBA Within Normative Data
Normative Data Confidence Interval Percentages on x-axis,
Frequency (Hz) on x-axis

WBA Day 1						WBA Day 2						
	5%conf	95%conf	count 5%	count 95%	count total	5%conf	95%conf	count 5%	count 95%	count total	% within CI	
297.3	0.2102	0.0672	10	0	36	0.2102	0.0672	11	1	36		
385.55	0.255	0.0863	10	0	36	0.255	0.0863	9	1	36		
500	0.3741	0.1415	9	3	36	0.3741	0.1415	7	2	36		
629.96	0.5581	0.2072	6	2	36	0.5581	0.2072	6	2	36		
793.7	0.6516	0.2314	5	0	36	0.6516	0.2314	5	1	36		
1000	0.7544	0.3218	6	0	36	0.7544	0.3218	5	0	36		
1259.92	0.7448	0.2588	12	0	36	0.7448	0.2588	9	0	36		
1587.4	0.778	0.2932	8	0	36	0.778	0.2932	8	0	36		
2000	0.8262	0.3366	4	0	36	0.8262	0.3366	7	0	36		
2519.84	0.9292	0.5294	2	3	36	0.9292	0.5294	3	3	36		
3174.8	0.9018	0.5422	2	1	36	0.9018	0.5422	2	3	36		
4000	0.9112	0.4231	1	3	36	0.9112	0.4231	2	6	36		
5039.68	0.7839	0.1492	2	1	36	0.7839	0.1492	2	0	36		
6349.6	0.4336	0.0661	21	1	36	0.4336	0.0661	20	0	36		
8000	0.6082	0.0989	1	7	36	0.6082	0.0989	3	6	36		
sum			99	21	540			99	25	540	-0.774074	

WBT Day 1						WBT Day 2						
	5%conf	95%conf	count 5%	count 95%	count total	5%conf	95%conf	count 5%	count 95%	count total	% within CI	
297.3	0.2993	0.1165	5	1	36	0.2993	0.1165	8	3	36		
385.55	0.3521	0.139	6	0	36	0.3521	0.139	8	3	36		
500	0.5248	0.2231	3	5	36	0.5248	0.2231	4	6	36		
629.96	0.7164	0.3288	3	5	36	0.7164	0.3288	2	7	36		
793.7	0.8244	0.4088	3	4	36	0.8244	0.4088	2	6	36		
1000	0.8558	0.5269	4	3	36	0.8558	0.5269	3	3	36		
1259.92	0.8166	0.4706	8	1	36	0.8166	0.4706	8	0	36		
1587.4	0.7583	0.3868	17	0	36	0.7583	0.3868	16	0	36		
2000	0.8669	0.4246	5	0	36	0.8669	0.4246	5	0	36		
2519.84	0.9348	0.5262	0	1	36	0.9348	0.5262	2	2	36		
3174.8	0.9177	0.5149	2	4	36	0.9177	0.5149	1	1	36		
4000	0.8654	0.4038	1	6	36	0.8654	0.4038	1	5	36		
5039.68	0.6727	0.1285	5	2	36	0.6727	0.1285	5	0	36		
6349.6	0.3491	0.0437	19	0	36	0.3491	0.0437	23	0	36		
8000	0.6454	0.1527	0	10	36	0.6454	0.1527	2	11	36		
			81	42	540			90	47	540	-0.759259	

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