Wideband Measurements in Newborns: Relationship to Otoscopic Findings

Jacob Pitaro MD

Department of Experimental Surgery
McGill University, Montreal

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Abstract

Introduction: Current newborn hearing screening include testing with otoacoustic emission and automated auditory brainstem response. Unfortunately, both tests are affected by the presence of material in the ear canal and middle ear such as vernix, meconium and amniotic fluid. The primary objective of this study was to perform wideband measurements and otoscopy on newborns in order to determine whether occlusion of the ear canal affects the wideband measurements. A secondary objective was to compare the wideband measurements obtained after birth to those taken at 14 to 28 days later. A third objective was to obtain additional wideband normative data in newborns.

Materials and Methods: Newborns from a well-baby nursery were enrolled. Wideband measurements under both ambient and pressurized conditions and otoscopy were done immediately after the hearing screening and between 14 and 28 days later. Occlusion of the ear canal as seen on otoscopy was described on a scale of 0 to 100% in increments of 10.

Results: A total of 156 babies were enrolled. On the first measurement, a statistically significant difference in reflectance was found between 0-70% and 80-100% occlusion groups and between 6 groups of frequencies between 250 Hz and 8 kHz. There was no significant difference in reflectance between the right and the left ears. A comparison of reflectance under pressurised conditions between the first and second measurements has shown a higher reflectance at the negative pressure region during the first few days of life.
Conclusion: Significant increase in reflectance occurs when 70% to 80% of the ear-canal diameter is occluded. A trend of higher reflectance appears to be present when the canal is pressurized to negative values. A comparison of reflectance between the present study and previous studies is given.
Résumé

Introduction: Le dépistage néonatal de la surdité actuel inclut l’évaluation d’émissions oto-acoustiques automatisées et la réponse évoquée auditive du tronc cérébral. Malheureusement, les résultats de ces tests peuvent être affectés par la présence de matériaux transitoires dans le conduit auditif externe et l'oreille moyenne comme par exemple le vernix, le méconium et le liquide amniotique. L’objectif primaire de cette étude était d'effectuer des mesures de réflectance à large bande et un examen otoscopique sur les nouveau-nés afin de déterminer si l'occlusion du conduit auditif externe affecte les mesures à large bande. Un objectif secondaire était de comparer les mesures après la naissance à celles obtenues 14 à 28 jours plus tard. Le troisième objectif était d'obtenir des données normatives supplémentaires.

Matériels et méthodes: De nouveau-nés d’une pouponnière de bébés en santé ont été inscrits. Les mesures à large bande, effectuées dans des conditions ambiantes et sous pression, et l’examen otoscopique ont été exécutés immédiatement après le dépistage de la surdité et entre 14 et 28 jours plus tard. L’occlusion du conduit auditif externe, vu par examen otoscopique, a été décrite par une échelle de 0 à 100% d’obstruction, par étapes de 10%.

Résultats: Un total de 156 bébés ont été inscrits. Lors de la première mesure, une différence statistiquement significative a été observée entre les groupes 0-70% et 80-100% d’obstruction du conduit, et également entre les 6 groupes de fréquences auditives comprises entre 250 Hz et 8 kHz. Il n’y avait aucune différence significative entre l’oreille droite et la gauche. Une comparaison des résultats dans
des conditions sous pression, entre la première et la deuxième mesure, a démontré un coefficient de réflectance supérieur à la zone de pression négative pendant les premiers jours de vie.

**Conclusion:** Une augmentation significative de la réflectance se produit lorsque le conduit auditif externe est obstrué de 70% à 80%. Une tendance de réflectance plus élevée semble être présente lorsque le canal est sous pression, à des valeurs négatives. Une comparaison des résultats de cette étude et d’études antérieures est discutée.
I dedicate this thesis to my family.

Without their love and support I could not accomplish this work.
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AABR: automated auditory brainstem response
ABR: auditory brainstem response
DPOAE: distortion product otoacoustic emission
OAE: otoacoustic emission
TM: tympanic membrane
Chapter 1: Introduction

1.1 Background

Since its endorsement by the Joint Committee on Infant Hearing, universal newborn hearing screening has become a standard procedure in many countries around the world, with a primary goal of detection and intervention for infants with hearing loss (Hunter et al., 2010). For example, “approximately 95% of newborn infants in the United States were screened for hearing loss before hospital discharge” (Joint Committee on Infant Hearing, 2007). It has been shown that early identification and intervention for children with hearing loss results in better speech and language outcomes and so an effort is made to identify and treat this population as early as possible (e.g. Yoshinaga-Itano, 2003; Pimperton & Kennedy, 2012).

The two main types of hearing loss are conductive and sensorineural. Conductive hearing loss involves the outer ear and/or the middle ear while sensorineural hearing loss involves the inner ear, auditory nerve and/or higher centres. The prevalence of congenital hearing loss has been shown to be about 1 to 3 per 1000 newborns (e.g. Barsky-Firkser & Sun, 1997; Mehl & Thomson, 1998; Fortnum et al., 2001).

Current neonatal screening protocols include the use of otoacoustic emission (OAE) testing and automated auditory brainstem response (AABR) testing. OAE are sounds produced by the cochlear outer hair cells spontaneously or in response to sound stimuli and can be detected in the ear canal using a microphone (e.g. Kemp, 2002). The auditory brainstem response (ABR) is an
evoked potential appearing as a series of waves following acoustic stimulation and representing electrical activity in the auditory nerve and brainstem (e.g. Jewett & Williston, 1971). The automated auditory brainstem response (AABR) technology includes “pass” or “refer” criteria. The automation procedure makes the screening objective and eliminates the need for a professional to interpret the results while keeping it accurate and cost effective (van Straaten, 1999). Although both technologies have been shown to be highly effective in detecting hearing loss in universal newborn hearing screening (e.g. Norton et al., 2000; Thompson et al., 2001), a major problem is that both tests are affected by obstruction of the newborn’s ear canal and/or middle ear by materials such as vernix, meconium and amniotic fluid (e.g. McLellan & Webb, 1961; Balkany et al., 1978; Doyle et al., 2000). Such transient conditions can result in false positive results on newborn hearing screening even though the cochlea and auditory nerve are normal (e.g. Hunter et al., 2010). For example, Doyle et al. (2000) examined the relationship between ear-canal and middle-ear factors and hearing screening with ABR and OAE. Two hundred babies aged 5 to 48 hours underwent otoscopic examination that showed occluding vernix in 112 (28%) of the ears. Of these 112 ears, only 12.5% passed OAE and 78.5% passed ABR before cleaning. After cleaning, the pass rate improved to 51% for OAE and 96% for ABR.

False-positive rates are defined as the “the proportion of normally hearing children who are referred for diagnostic testing” (Patel & Feldman, 2011). For example, Mehl and Thomson (1998) found a cumulative false-positive rate of 6% when all newborn infants were screened with OAE, AABR or ABR while
Clemens and Davis (2001) found a false positive rate of 0.8% in healthy term newborns screened with AABR.

False positives on screening tests may cause parental anxiety, a negative attitude toward the baby, prolongation of diagnostic time, increased costs to the system and decreased confidence in Universal Newborn Hearing Screening program (e.g. Magnuson & Hergils, 1999; Poulakis et al., 2003; Kennedy, 1999; Kemper & Downs, 2000). In addition, it is important to identify the type of hearing loss because intervention differs between conductive and sensorineural hearing loss (Shahnaz, 2008). For all of these reasons it is recommended that an improved and more reliable technology be developed for evaluating middle-ear function in order to differentiate specific types of hearing loss at the time of newborn hearing screening (Joint Committee on Infant Hearing, 2007; Hunter et al., 2010).

Tympanometry is a standard, quick clinical test that is used to assess middle-ear admittance and can help to distinguish between conductive hearing loss and sensorineural hearing loss (Further information about admittance is given in Section 3.4). Tympanometry is performed by introducing a probe tone into the sealed ear canal while changing the air pressure in the canal and measuring the sound pressure that is developed within the canal (ASHA, 1988).

Tympanometry using a 226-Hz probe tone is a widely used clinical tool that provides an effective measure of middle-ear function in adults and older children (Jerger, 1970). However, since the newborn middle ear undergoes developmental changes during the first few months of life, conventional 226-Hz tympanometry produces different tympanograms in this age group and is not
recommended in infants under 7 months of age (e.g. Paradise et al., 1976; Meyer et al., 1997).

It has been shown that by using a higher probe-tone frequency more consistent tympanograms are produced that can predict middle-ear status in newborns more accurately during the first few months of life (Kei et al., 2003; Alaerts et al., 2007). It is currently recommended to use 1-kHz probe-tone tympanometry in infants up to 6 month of age (Joint Committee on Infant Hearing, 2007). However, even when using a high probe-tone frequency, variable results exist in newborns.

Admittance measured during tympanometry includes the admittance of the ear-canal air volume and that of the middle ear. However in clinical practice most of the interest is in the admittance of the middle ear. By pressurizing the canal during tympanometry, the ear-canal admittance can be measured which is than subtracted from the total measured admittance and the result is the middle-ear admittance of interest. However, this process may be problematic in newborns where the canal can extend or collapse during pressurization. In addition, the probe location within the canal can cause substantial effect on the results.

Energy reflectance is defined as the ratio between the energy reflected back to the probe and the incident energy delivered by the probe. Assuming that the energy absorbed in the canal is negligible (a better approximation for adults than for infants), then the energy reflectance equals the ratio of the energy reflected from the middle ear to the incident energy, uninfluenced by the location of the probe within the canal (Voss et al., 2008; Sanford et al., 2009).
Wideband measurements for both energy reflectance and admittance are performed over a wide range of frequencies at the same time (Keefe et al., 2000). This is in contrast to conventional pure-tone tympanometry in which admittance is measured at a single frequency at a time. Wideband measurements have the advantage of being a very fast test (seconds), much faster than measurements with pure tones at multiple frequencies. Both reflectance and admittance can be measured under either pressurized (tympanometric) or non-pressurized (ambient) conditions.

Wideband measurements may be able to serve as a complementary tool in newborn hearing screening. However, wideband measurements have not been described in relation to otoscopic findings and it is unknown how the results are affected by occlusion of the ear canal and middle ear by different amounts of materials occurring immediately after birth. Furthermore, additional measurements are needed to establish normative data. The first objective of this research project was to determine whether a correlation exists between wideband measurements and different levels of occlusion of the ear canal and middle ear. A second objective was to compare the wideband tympanometric measurements obtained immediately after birth to measurements obtained 14 to 28 days later in order to identify any trends in the measurements. Finally, the current measurements will provide an additional normative database of wideband measurements in newborns.
1.2 Outline

Chapter 2 contains an overview of the relevant anatomy of the human ear. Chapter 3 presents the basic principles of newborn hearing screening, including standard and research techniques. In Chapter 4 the materials and methods of the current research project are presented followed by the results in Chapter 5. In Chapter 6 a discussion of the results is presented. Finally, in Chapter 7 the conclusion is given together with future directions.
Chapter 2: Anatomy of the Ear

2.1 Overview

The auditory system is divided into peripheral and central sections. The peripheral section includes the outer (external) ear, the middle ear, the inner (internal) ear and the cochlear nerve while the central portion includes the brainstem and the brain with their nuclei, fibres, tracts and commissures (e.g. Katz et al., 2002, pp. 9-15). This research deals with the outer and middle ear and therefore the relevant anatomy of these parts of the auditory system will be described (Figure 1). This review is based largely on Standring et al. (2008, Chap. 36) as well as other references that are explicitly cited.

The outer ear comprises the pinna (auricle) and the ear canal and it is separated from the middle ear by the tympanic membrane (TM). The middle-ear cavity contains the three ossicles; the stapedius and tensor tympani muscles; some ligaments; and the chorda tympani nerve. The inner ear comprises the bony labyrinth that contains the cochlea, the vestibule and the semicircular canals. The cochlea is part of the auditory system while the vestibule and semicircular canals belong to the vestibular system. The cochlea is shaped like a snail’s shell and in it is the organ of Corti with its sensory hair cells (three rows of outer hair cells and one row of inner hair cells). The primary role of the middle ear is that of an impedance-matching transformer between the low-impedance air in the outer ear and the high-impedance liquid in the cochlea. This transformer is often said to involve three mechanisms: the area ratio between the TM and the stapes footplate,
the lever ratio of the malleus and incus, and the TM curvature. These three mechanisms cannot, however, be clearly separated (Funnell, 1996).

Following exposure to sound, the TM vibrates and in turn moves the osicular chain. Next, the footplate of the stapes moves in a piston-like fashion in the oval window, where the sound energy is transferred to the fluid in the cochlea. The wave created within the fluid displaces the basilar membrane on which the sensory hair cells rest. This eventually causes cilia on top of the hair cells to bend, leading to the generation of action potentials in the auditory nerve. The electrical signal created is transmitted through the auditory nerve to the brainstem and from there to the auditory cortex.

**Figure 1.** Anatomy of the ear (Adapted from Drake et al., 2010)
2.2 Pinna

The pinna (auricle) is the lateral part of the outer ear. It is supported by a frame of elastic cartilage covered by skin. The cartilage has different eminences and depressions creating unique anatomical structures including the helix, antihelix, tragus, antitragus, auricular tubercle (Darwin’s tubercle) and concha. The lobule lacks cartilage at the bottom and is made of fibrous tissue and fat. The pinna continues to grow throughout life (Heathcote, 1995). The growth was found to be faster during childhood and adolescence than in the elderly, with much of the pinna’s length reached by 4 to 5 years of age (Sforza et al., 2009). The pinna helps to distinguish whether a sound arrives from the back or front of the head, as seen during gradual occlusion of the pinna (Gardner & Gardner, 1973).

2.3 External Ear Canal

The external ear canal is the medial part of the outer ear (Figure 2). It extends from the concha to the TM. In the adult, the lateral one-third of the ear canal is cartilaginous, while the medial two-thirds are bony. Superiorly the cartilage is open but this gap is closed by a dense fibrous tissue so a closed tube is formed (Donaldson & Anson, 1992). The TM is positioned obliquely, so the floor and the anterior wall of the canal are longer than the roof and the posterior wall. The skin over the cartilaginous part has thick subcutaneous tissue containing hair follicles, sebaceous and cerumen-producing glands, while the skin over the bony part has very few glands. Differences exist between the newborn ear canal and that of the adult. Otoscopic examinations have shown that under 24 hours of age, most of the canals are
obstructed to a certain degree by vernix caseosa and the ear-canal walls are close to each other. In addition, the anatomic transition between the skin and the TM is not well defined, in contrast to the adult ear where the TM is in a more vertical position so the angle formed between the TM and the ear canal is better identified (Balkany et al., 1978). Some have described the newborn ear canal as curved (e.g. McLellan & Webb, 1957, 1961) while others describe it as almost straight (e.g. Crelin, 1973, p. 18). The adult ear canal is not straight and is often described as S-shaped. The newborn canal wall comprises mainly elastic cartilage and soft tissue, whereas the adult ear contains more bone. The ear canal is not ossified at birth except for the tympanic ring, located at the medial end of the canal and has 4 ossification centers at nine weeks of gestation (Glasscock & Gulya, 2003, pp. 4-
During the first year of life, the tympanic ring grows in a lateral direction. The ear canal completes its ossification by the second year of life but the ear canal reaches adult size only at 9 years of age (Bluestone et al., 1996, p. 114). The length of the newborn ear canal from the outer surface of the tragus to the pars tensa of the TM, was found to range between 23 to 25 mm (McLellan & Webb, 1957). Keefe et al. (1993) estimated the length of the ear canal at one month of age and found it to be 14 mm. The variability between reported results is probably due to different measurement techniques. The ear-canal diameter at one month was found to be approximately 4.4 mm (Keefe et al., 1993). In the adult, the length of the ear canal is estimated to be 23 mm and the ear-canal diameter is approximately 10.4 mm (Keefe et al., 1993). Both the pinna and the ear canal have acoustical resonance properties between 2 to 7 kHz that increase the sound pressure at the TM (Shaw & Teranishi, 1968).

### 2.4 Tympanic Membrane

The TM is an oval, thin and semitransparent structure that separates the ear canal from the middle ear (Figure 3). Most of the TM circumference is surrounded by a fibrocartilagenous ring attached in the tympanic sulcus, a groove located in the tympanic ring at the medial end of the ear canal. Superiorly, the tympanic ring is not complete, forming the notch of rivinus. The manubrium of the malleus is attached to the TM on its medial aspect. The tip of the attachment corresponds to the umbo of the TM.
The angle between the superior canal wall and the TM is usually about 140 degrees but this angle can vary (Donaldson & Anson, 1992, pp. 147-148). In a study of 20 adult TMs, Lim (1970) found that the longest diameter of the TM was from 9 to 10.2 mm while the shortest diameter varied between 8.5 to 9 mm.

The TM is divided into two main parts, the pars tensa and the pars flaccida. The pars tensa comprises the largest area of the TM while the pars flaccida is the smaller area found above the short process of the malleus. Both parts contain three distinct layers: outer epidermal, middle lamina propria and inner mucosal layer. In the pars tensa the lamina propria is formed by a subepidermal connective tissue layer, outer radial and inner circular fibres and a submucosal connective tissue layer. Between the radial and circular fibres, there are few parabolic fibres (e.g. Lim, 1970). At birth, the TM has completed its full
growth (Crelin, 1973, p. 19), however, it was shown that the TM’s thickness changes in some areas from birth to old age. Ruah et al. (1991) examined 46 TMs from patients aged two days to 91 years and found that the pars flaccida thickness decreases throughout life but mainly during the first year of life and particularly during the first month. In the pars tensa, it was shown that only the posterosuperior quadrant and the umbo areas of the TM decreased in thickness from birth to old age.

2.5 Ossicles

The middle ear contains three ossicles: the malleus, incus and stapes (Figures 1 and 4). The most lateral and the largest ossicle is the malleus. The manubrium of the malleus is firmly attached to the TM. The radial fibers of the TM are attached to the manubrium, and medially it is covered by the mucous membrane of the middle ear (Donaldson & Anson, 1992, p. 152). The malleus articulates with the middle ossicle, the incus, via the incudomallear joint, and the incus articulates with the medial ossicle, the stapes, through the incudostapedial joint. The stapes footplate is attached around the margin of the oval window by the annular ligament. The ossicles have ligamentous attachments to the walls of the middle-ear cavity and there are two muscles attached to the ossicles. The tensor tympani muscle inserts in the neck of the malleus It draws the manubrium medially and so tightens the TM (Donaldson & Anson, 1992, p. 156). The stapedius muscle inserts on the posterior surface of the neck and posterior crus of the stapes. It draws the anterior base of the stapes laterally (Donaldson & Anson, 1992, p. 156). It has been shown that ossicular development continues in both size and weight after
birth. Olszewski (1990) examined ossicles from fetuses and adults up to 40 years of age and found that the weight of the malleus, incus and stapes increased by about 22%, 26% and 12% respectively. In addition, Yokoyama et al. (1999) demonstrated that in newborns both malleus and incus contain bone marrow that gradually disappears by the age of 25 months.

**Figure 4:** The human ossicular chain (Adapted from Sobotta et al., 2008)
2.6 Middle-Ear Cavity and Eustachian Tube

The middle-ear cavity is an air space in the temporal bone lined by mucous membrane (Figure 1). Posteriorly the middle ear communicates with the mastoid antrum and the mastoid air cells. Anteriorly, the middle ear communicates with the nasopharynx through the Eustachian tube. The Eustachian tube is usually closed but can open during swallowing, yawning and valsalva manoeuver. The Eustachian tube is responsible for pressure equalization between the two ends of the tube and for mucociliary clearance. In children the angle between the tube and the horizontal plane is about 10 degrees while in adults it changes to 30 to 40 degrees (Proctor, 1967). The length of the tube also changes, from approximately 21 mm at 3 month to approximately 34 mm at 17 years of age (Ishijima et al., 2000).

Ikui et al. (2000) measured the volume of the middle-ear cavity in six infant temporal bones and eight adult bones. Under the age of one year the mean volume was 451±68 mm$^3$ while in adults over 18 years, the middle-ear cavity volume was 640±69 mm$^3$, about 1.5 larger than that of infants.
Chapter 3: Newborn Hearing Screening

3.1 Basic Principles

Newborn hearing screening was recommended initially only for babies who had a risk factor for hearing loss (Table 1) (Joint Committee on Infant Hearing, 1982).

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1. **Family history of hereditary childhood sensorineural hearing loss**
2. **In utero infection** (e.g. cytomegalovirus, rubella, syphilis, herpes and toxoplasmosis)
3. **Craniofacial anomalies, including pinna and ear canal**
4. **Birth weight < 1500 gr**
5. **Hyperbilirubinemia requiring exchange transfusion**
6. **Ototoxic medications** (e.g. aminoglycosides)
7. **Bacterial meningitis**
8. **Apgar scores of 0 to 4 at 1 minute or 0 to 6 at 5 minutes**
9. **Mechanical ventilation lasting 5 days or longer**
10. **Stigmata or other findings associated with a syndrome known to include hearing loss**

**Table 1.** Risk factors for hearing loss

(Joint Committee on Infant Hearing, 1995)

However, later on it was found that screening babies with risk factors identified only 50% of those with hearing loss and the other 50% who do not have risk factors, are diagnosed at a later stage often after 18 months of age (Mauk et al., 1991; Katz et al., 2002, p. 469). As a result, in 1994 the Joint Committee on Infant Hearing endorsed the goal of screening all newborns for hearing loss before the age of three months and when indicated, treat before six months of age (Joint Committee on Infant Hearing, 1995). Screening protocols may differ between
centres. Some departments use a one stage protocol with either OAE or AABR and rescreen if necessary with the same technology before discharge. Others will conduct a two stage protocol in which the baby is screened first with OAE and when referred, is rescreened with AABR before discharge (Joint Committee on Infant Hearing, 2007). It has been shown that the rate of false positives on universal newborn hearing can be decreased with the two stage program (e.g. Mason & Herrmann, 1998; Clemens & Davis, 2001). Current screening protocols in the well-infant nursery include a hearing screening after birth with either OAE or AABR. If the result on the first attempt is “refer” than a second attempt should be done preferably before discharge from the hospital; and if the test could not be completed or missed then it should be done before the age of one month (Joint Committee on Infant Hearing, 2007). In our centre, the first test is done with OAE preferably after the first 24 hours of life. If the result on the first test is “refer” then a second attempt is done with AABR before hospital discharge. If the result is “refer” on the second test then an AABR is repeated in two weeks.

### 3.2 Otoacoustic Emission

OAE are sounds that are produced in the cochlea, transmitted through the middle ear into the ear canal and detected using a sensitive microphone in the canal (Katz et al., 2002, p. 440). It is believed that OAE originate from the outer hair cells. This theory is supported by the fact that even in the absence or abnormal auditory nerve activity OAE can still be recorded. An example of such a situation is auditory neuropathy (Vlastarakos et al., 2008). In addition, it has been shown that when outer hair cells are destroyed by ototoxic medication, OAE
levels are reduced (Brown et al., 1989). OAE measurements are used to detect hearing loss. It has been shown that OAE are missing when sensorineural hearing loss exceeds 40 dB hearing level at 1 kHz and 45 dB mean hearing level at 500 Hz, 1, 2 and 4 kHz (Collet et al., 1989).

The two main types of OAE are spontaneous and evoked. Spontaneous OAE occur in the absence of a stimulus while evoked OAE are measured as a response to acoustic stimuli. Spontaneous OAE occur in approximately 50% of the normal hearing population (Katz et al., 2002, p. 443). It is unknown whether the prevalence of spontaneous OAE is different between newborns, older children and adults. Kok et al. (1993) measured spontaneous OAE on newborns aged 1 to 10 days and found the prevalence to be 77.8% which was higher when compared to data from older children and adults. On the contrary, Burns et al. (1992) did not find a significant difference between the prevalence in newborns compared to adults. The latter study also found that in neonates the majority of spontaneous OAE were recorded between 2.5 and 5 kHz while in adults they were recorded mainly between 1 and 2 kHz. Since they are found only in 50% of individual, spontaneous OAEs are not useful clinically (Katz et al., 2002, p. 443).

There are two types of evoked OAE, transient-evoked and distortion product OAE. Transient-evoked OAE are recorded following a transient or a brief stimulus given as a click or tone burst (Katz et al., 2002, p. 443). Collet et al. (1993) have shown that in the first day of life, click-evoked OAE were present in 33% of the subjects, rose to about 70% in second day of life and reached 100% at the fifth day of life. The same study showed a higher intensity of OAE in neonates aged up to 25 days compared to adults between 18 and 37 years.
Distortion product OAE are produced when the cochlea is stimulated by 2 different frequencies and the resulted output is in a different frequency than the original stimulus (Katz et al., 2002, p. 447). The two stimulus frequencies are denoted by f1 and f2 whereas f1<f2. As with transient-evoked OAE, it has been shown that distortion product OAE levels in infants are higher than those in older children and adults (Prieve et al., 1997).

Both transient-evoked and distortion product OAE have been used in newborn hearing screening. The test performance of transient-evoked OAE, distortion product OAE and ABR as tools for identification of neonatal hearing impairment was compared using visual reinforcement audiometry at 8 to 12 months corrected age. Test performance was similar in all three tests when used to detect hearing loss between 2 to 4 kHz. However, when 1 kHz was added to the test, ABR performed better (Norton et al., 2000).

3.3 Auditory Brainstem Response

ABR is represented by a series of waves occurring within the first 15 milliseconds following a sound stimulus. The ABR waves represent synchronous electrical activity in the auditory nerve and brainstem. The stimulus can be in the form of a broadband click which stimulates a whole region of the cochlea or a tone burst that stimulates a limited region. The waves are recorded from electrodes placed on the scalp (Katz et al., 2002, p. 274). There are five primary waves each arising from one or more neural generator sites beginning at the auditory nerve and ending in the brainstem. Wave five is the most robust due to high traveling wave velocity in the high frequency region of the cochlea and
therefore is used to evaluate hearing thresholds (Jewett & Williston, 1971). As mentioned previously, ABR is better than OAE in detecting hearing loss in newborns with regard to the frequency range. The reason is that ABR measures electrophysiological activity from the auditory nerve to the brainstem while OAE is a measure of the outer hair cells activity (Norton et al., 2000). Other important advantages of ABR include its stability when the examinee is in resting state, the ease of recording and the high correlation with abnormal findings and hearing loss (Katz et al., 2002, p. 471). As mentioned in the introduction, AABR has been used successfully in newborn hearing screening. A comparison between OAE and AABR is presented in table 2.

<table>
<thead>
<tr>
<th>Advantages</th>
<th>OAE</th>
<th>AABR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple testing technique (relatively minimal training needed to perform); Cheaper than AABR screening; Fast</td>
<td>Superior evaluation of the auditory system (vs. assessment of outer hair cells alone as in OAE)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Disadvantages</th>
<th>OAE</th>
<th>AABR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Limited assessment of the auditory system; Impacted by middle ear fluid or vernix or wax in the ear canal; Optimal to perform in a quiet environment</td>
<td>Requires more operator knowledge than OAE testing; Requires sleeping or quiet infant; Optimal to perform in a quiet environment; Potential for electrical and noise artifacts; Requires longer time than OAE screening; Typically more costly than OAE screening</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2.** Comparison between OAE and AABR (Adapted from Choo & Meinzen-Derr, 2010).
3.4 Tympanometry

Tympanometry is a test of middle-ear function. It is done by measuring the immitance in the ear canal while the ear-canal air pressure is changing. Immitance is a term that includes both admittance and impedance. Admittance expresses how easy a sound flows through a system whereas impedance is the opposition to sound flow through an acoustic system. Admittance and impedance are reciprocals. However, most tympanometry instruments use admittance to present the results (Katz et al., 2002, p. 161). Tympanometry is an integral part of the clinical audiologic evaluation. It provides useful information regarding the middle-ear status such as the presence of middle ear effusion, patency of pressure-equalizing tubes and perforated TM.

Figure 5. Tympanometer diagram (Adapted from Bess & Humes, 2009)

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Figure 5 illustrates a tympanometer. A probe with a soft tip is inserted into the ear canal to ensure a proper canal seal. The probe consists of three parts: a loudspeaker, a microphone and a pump. A probe tone which is an acoustic signal at a specific frequency is introduced while the air pressure in the canal changes. The sound pressure reflected from the TM and detected at the microphone is continually measured and used as a reference to maintain a constant probe tone in the canal. The measured sound pressure level at the microphone is used to calculate the admittance (Katz et al., 2002, pp. 167-168). The resulted admittance depends on the frequency at which it is measured and the physical dimensions and physical properties of the system in which it is measured. In the normal ear, when admittance is measured at low frequencies such as 226 Hz it is primarily characterised by the stiffness of the TM, round window membrane, ossicular ligaments, middle ear muscles and air within the outer ear and middle ear. At higher frequencies such as 1 kHz, the admittance is more mass dominated since the relative contribution of each anatomic structure changes (Katz et al., 2002, pp. 162-164).

The admittance has a real component which is the conductance ($G$) and an imaginary component which is the susceptance ($B$). From these two parameters which act as two vectors opposing each other, the admittance can be derived (Katz et al., 2002, pp. 162-165). Admittance measured at the probe tip includes the admittance between the probe tip and the TM and the middle ear. A better estimation of the middle-ear admittance would be to have it measured just lateral to the TM. However, this is not feasible technically and so the admittance of the volume between the probe tip and the TM is subtracted from the overall
admittance. The result is termed compensated admittance and it represents the middle-ear admittance (Katz et al., 2002, p. 169).

The equivalent ear-canal volume is an estimate of the volume between the probe tip and the TM. It is calculated from the admittance since a given equivalent volume has a known admittance (Katz et al., 2002, pp. 180-181). The equivalent volume can give a clue on the reason for a flat tympanogram whether it is due to a perforation, middle-ear effusion or patent pressure equalizing tube. Normative data for equivalent volume have been published for different age groups. In preschool-age group of children with a mean age of 4.7 years the mean equivalent volume was 0.74 cm$^3$ whereas in a group of adults with a mean age of 30.5 years the mean equivalent volume was 1.05 cm$^3$. Interestingly the equivalent volume was significantly larger for male ears in both subject groups (Margolis & Heller, 1987). Keefe et al. (2000) measured the equivalent volume in the frequency range of 0.250 to 1 kHz on a group of newborns. The median equivalent volume varied between 0.1 cm$^3$ and slightly below 0 cm$^3$ at high frequencies.

The tympanogram is a plot of the measured admittance as a function of ear-canal pressure change from positive to negative. The pressure sweep changes the TM stiffness until a peak is reached. The peak represents the point at which maximum energy enters the middle ear (Katz et al., 2002, p. 176). There are five types of tympanograms. The different types are shown in figure 6 and an explanation is given in table 3.
Figure 6. Types of tympanograms (Adapted from Goodman et al., 2007).

X and Y axes represent pressure and admittance respectively.

<table>
<thead>
<tr>
<th>Type</th>
<th>Clinical presentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Normal middle-ear</td>
</tr>
<tr>
<td>As</td>
<td>Ossicular fixation</td>
</tr>
<tr>
<td>Ad</td>
<td>Ossicular disarticulation, TM scarring, Tympanosclerosis, post-stapedectomy</td>
</tr>
<tr>
<td>B</td>
<td>Middle-ear effusion, TM perforation, cerumen occlusion, probe against canal</td>
</tr>
<tr>
<td>C</td>
<td>Negative middle-ear pressure</td>
</tr>
</tbody>
</table>

Table 3. Types of tympanograms (Katz et al., 2002, p. 177)
Tympanometry in newborns has been shown to result in different traces from the adult. The exact mechanism or the difference is unknown; however, it has been proposed that since the ear canal of the newborn contains mainly soft tissue it is mass controlled in the lower frequencies and so it responds differently to low frequency tympanometry than the adult ear. In addition, it has been shown that by pressurizing the newborn ear canal, the latter may distend up to 70% of its volume and can affect the tympanogram shape (Holte et al., 1990).

3.4.1 226-Hz Tympanometry in Newborns

Paradise et al. (1976) performed otoscopy and 220-Hz tympanometry on a group of children aged 10 days to five years and 11 months. A subgroup of the children also underwent myringotomy. Otoscopic and myringotomy findings were then correlated with tympanometry findings. Results from this study have shown that in children under seven months of age, at least half of the ears that were suspected or proven to have middle-ear effusion had normal tympanograms. In another study, Himelfarb et al. (1979) performed tympanometry with 220-Hz probe tone on 63 infants aged 8 to 96 hours. Otoscopy was also performed and demonstrated vernix caseosa in all ears. The TM however, appeared normal although not as translucent as the normal adult TM. Even though otoscopic findings demonstrated normal TM, doubled-peaked tympanograms were present in more than one third of ears. Meyer et al. (1997) performed repeated 226-Hz tympanometry on an infant aged two weeks to six and a half months. During the first four weeks of life, admittance measurements showed notched tympanograms while thereafter tympanograms became single peaked. Interestingly, when
measurements were done using 1-kHz probe tone, results did not show notching before 148 days of age.

3.4.2 1-kHz Tympanometry in Newborns

It is thought that 1-kHz tympanometry is more effective in diagnosing neonatal middle-ear status in comparison to the 226-Hz tympanometry. Some studies reported normative data for 1-kHz tympanometry in infants younger than seven months of age, however it is not exactly clear what characterizes normal or abnormal 1-kHz tympanograms in this age group. For example Baldwin (2006) performed 226-Hz, 678-Hz and 1000-Hz tympanometry on 211 babies aged 2 to 21 weeks. All babies were tested with OAE and ABR. Babies were divided into two groups. One group had normal hearing while the second group had conductive hearing loss. Tympanometry were classified as “normal” or “abnormal” according to trace patterns. It was found that the 226-Hz tympanometry could not differentiate between the normal-hearing group and the hearing-loss group while the 1-kHz tympanometry demonstrated the best differentiation between the two groups. Unfortunately some of the tracings could not be classified. In another study, Swanepoel et al. (2007) measured OAE and 1-kHz tympanometry in 278 healthy neonatal ears aged 1 to 28 days. When compared to the OAE results, the sensitivity and specificity for middle-ear pathology of 1-kHz tympanometry were 57% and 95% respectively. In addition, admittance values were found to increase with increasing age. Hence the need of age specific norms for 1-kHz tympanometry in neonates.
3.5 Wideband Reflectance

When sound energy reaches the ear, it passes through the ear canal, the TM and the middle ear. Some of the sound energy is absorbed at these locations and some of it is reflected back out of the ear canal (Shahnaz & Bork, 2006). The ratio between the reflected pressure wave and the incident wave at a probe tip located within the ear canal is described quantitatively as reflectance $R$ where $0 \leq |R| \leq 1$. The reflected energy can be also described quantitatively as energy reflectance that equals $|R|^2$. When energy reflectance equals 1 all the energy is reflected back and when it equals 0 no energy is reflected (Voss et al., 2008).

Energy absorbance is the ratio of acoustic energy that is absorbed by the middle ear and the ear canal to the acoustic energy of an incident sound presented in the ear canal and directed towards the TM. Through conservation of energy, energy absorbance and energy reflectance are related by energy absorbance=1-energy reflectance. Energy absorbance also varies between 0 and 1. Advantages of using energy absorbance include a single-peaked function as in conventional admittance tympanometry and a maxima that occur around frequencies at which the middle ear is most efficient in absorbing sound energy (Liu et al., 2008; Sanford et al., 2009). Most studies report the reflectance. In the present study we report on energy reflectance and for convenience, reflectance means energy reflectance.

An important advantage of using reflectance in comparison to impedance measurements is that reflectance does not depend on the location of the probe in the ear canal considering that loss of energy in the ear canal is minimal (Voss & Allen, 1994; Voss et al., 2008). For example, Voss et al. (2008) measured
reflectance in 9 cadaveric ears with the probe at different locations in the canal and found that the effect of probe location was small in most ears.

Keefe et al. conducted the first study of wideband reflectance in neonates. This study included babies from 3 populations: neonates in neonatal intensive care units, neonates in well-baby nurseries and neonates with one or more risk factors for hearing loss. Reflectance averaged across all frequencies and was defined as high if was higher than 0.6. The highest percentage of ears was found to have higher than 0.6 reflectance during the first 24 hours of life. This finding may be explained by low absorption of energy immediately after birth by transient materials in the ear canal and middle ear (Keefe et al., 2000). Hunter et al. (2010) compared reflectance measurement performance to 1-kHz tympanometry for prediction of OAE results. Measurements from 324 healthy full-term neonates 3 to 102 hours old showed that reflectance in the range of 2 kHz had greater discriminability of OAE status compared to 1-kHz tympanometry. It was also found that reflectance decreased during the first 4 days of life with improved middle-ear function. Sanford et al. (2009) reported on reflectance data from healthy, full term newborns that had passed OAE screening. The results were compared to those from a group that had not passed the OAE screening and to results of 1-kHz admittance tympanometry. The findings demonstrated that wideband reflectance measures were more accurate than 1-kHz admittance tympanometry in classifying OAE results during hearing screening. Aithal et al. (2013) reported on normative wideband reflectance data in 66 ears of healthy neonates with a mean age of 46 hours and normal middle-ear function. The results showed two maxima of 0.59 and 0.52 reflectance at 0.5 and 4 kHz, respectively.
and two minima of 0.21 and 0.24 reflectance at 1.5 and 6 kHz, respectively. Keefe et al. (1993) performed reflectance measurement on normal subjects including one month olds and adults. The results have shown that at all ages, the energy reflectance are highest below approximately 1 kHz and above approximately 4 kHz while the lowest reflectance is between 1 and 4 kHz.
Chapter 4: Materials and Methods

4.1 Subjects

Infants enrolled in the study were recruited from the well-baby nursery at the Royal Victoria Hospital, Montreal, Canada. Parents were informed of the study and were given a flyer describing the research before the standard newborn hearing screening took place. After reading the flyer, the project was presented to the parents and their questions were answered. If the parents agreed to participate, an institutional review board approved consent form was used to obtain parent’s permission. All parents willing to participate had their newborn included; unless a medical issue before or during the test, prevented participation or completion of the measurement. Babies whose parents agreed to participate during their hospital stay but could not return for the second measurement were also included in the study. In addition, parents were asked to fill out a questionnaire regarding race and ethnicity origin based on the Canadian Census 2011 [http://www12.statcan.gc.ca/NHS-ENM/ref/Questionnaires/2011NHS-ENM-eng.cfm#Q17].

The study was approved by the institutional review board of McGill University Health Centre.
4.2 Instruments

4.2.1 Otoscopy

Ear examination was performed using a diagnostic manual otoscope (Welch Allyn Inc.). A scale of eleven grades was used to document how much of the ear canal was occluded by debris. Clear canal with no debris was denoted by 0% whereas complete occlusion was denoted by 100%. The TM status were included in the description if were clearly seen. In two cases, otoscopy was not performed due to a very narrow canal where the otoscope tip could not fit in.

4.2.2 Wideband Measurement System

The wideband system (WBTym 3.2, Interacoustics Inc.) was used to perform measurements under ambient and tympanometric pressures (Figure 7). The system is a Windows-based computer consisting of a sound card (CardDeluxe), a modified acoustic immitance instrument with a pressure pump and a controller that is connected via cables to the sound card (Interacoustics, AT235 device modified by the manufacturers). The device includes a Titan probe assembly with a loudspeaker and a microphone and channel to change the pressure in the canal. The probe is supplied from the immitance instrument. Plastic ear tips (Interacoustics Inc.) are placed over the probe tip for both calibration and testing to provide a hermetic seal. The system performs measurements under ambient and tympanometric pressures.
Figure 7: Wideband measurement system.
4.2.2.1 Calibration

Calibration was performed daily before a new set of data was obtained. The calibration determines changes in the performance of the probe if occurred since the last calibration. The procedure uses two sets of short and long rigid-walled tubes. One set has a large diameter of 0.794 cm for use in testing adults. The set used in this study has a diameter of 0.476 cm and is therefore better suited for testing infant-sized ear canals. One end of the tube is closed and the probe is inserted into the opposite side while performing the calibration. The display informs the examiner whether the results met the current standards for successful calibration. Unsuccessful calibration was repeated until a valid calibration was achieved.

4.2.2.2 Ambient Measurements

Ambient measurements are obtained by recording the acoustic response to clicks. The clicks are rapid stimuli in the range of 0.250 to 8 kHz containing 60 frequency data points. For newborns up to three month of age, responses for 16 clicks are acquired and analyzed according to the last calibration available. The number of clicks is lower compared to that for adults with a goal to reduce the testing time. During measurement, the system is able to detect a leaky insertion. If the latter occurs, a warning appears on the screen with a recommendation to reinsert the probe and repeat the measurement. The response is recorded by the probe microphone.
4.2.2.3 Wideband Tympanometry

As in ambient measurements, the wideband tympanometry measures responses over 60 data points between 0.250 to 8 kHz however, as a function of air pressure changes. The measurements were done with a positive to negative pressure sweeps at a rate of 100 daPa/sec. The maximum and minimum pressure points are +200 daPa and -300 daPa respectively. While the pressure changes within the canal, clicks are presented and the response is recorded by the probe microphone. Recording is possible only in a sealed canal.

4.3 Procedure

4.3.1 Timing

The first set of wideband measurements and otoscopy took place following the standard hearing screening procedure. If the baby had a “refer” result on OAE, the research measurements were done after the AABR. A second set of measurements including otoscopy was done 14 to 28 days following the initial screening depending on the parent’s convenience. A phone call reminder was given few days before a possible second measurement. All second visits except one, took place at the otolaryngology outpatient clinic of the Montreal Children’s Hospital.

4.3.2 Wideband Measurements and Otoscopy

The wideband signal is introduced through the probe into the subject’s sealed ear canal. The physical procedure is identical to that for standard clinical tympanometry. Measurements were obtained under both ambient and pressurized conditions. The ambient measurements were done before pressurizing conditions
and the otoscopy was done after the wideband measurements have been completed. Testing was done while the baby in the crib or mother’s arms. The more conveniently positioned ear was tested first. Whenever possible, an attempt was done to test both ears. Data was collected and saved in the computer and later on was transferred into a spreadsheet using MATLAB software for further analysis (MATLAB R2012b, The MathWorks Inc.).

4.4 Data Analysis

Results on newborn hearing screening were documented for each baby as well as demographic data including pregnancy and birth history, birth type, gestational age, birth weight, head circumference, body length, dysmorphic features and family history of hearing loss. Maternal history was documented as well. Descriptive statistics were calculated and are presented in the results section. Screening results on both OAE and AABR were presented as pass or refer. Equivalent volumes as a function of frequency in ambient pressure were plotted for each ear to explore any trends in shapes. Wideband absorbance measurements were transformed into reflectance and were analyzed in this form. The reflectance 10th, 25th, 50th, 75th and 90th percentiles were plotted for all ears to be compared with results from previous studies. The 50th percentile of each occlusion group was than plotted as well to identify trends and grouping of the reflectance results in relation to occlusion grade of the ear canal. The tympanometry three dimensional graphic presentation of the absorbance as a function of pressure and frequency are given and were used to compare and identify any trends in reflectance between birth and at 14 to 28 days of later.
Statistical analysis with linear mixed model was used to examine whether a difference exists in reflectance between right and left ears, occlusion groups and frequencies. The statistical software package, SPSS 18, was used to analyze the results.
5.1 Subjects

A total of 156 babies were enrolled in the study. There were 80 females and 76 males with a mean age at hearing screening of 25 hours (range, 12-54 hours). The first measurements were done on 292 ears immediately after the hearing screening. The second measurements were done on 26 babies at 14 to 28 days after the first measurement and included a total of 49 ears. Results on newborn hearing screening were documented for each baby as well as demographic data including birth type, gestational age, birth weight, head circumference, body length, dysmorphic features if presented and family history of hearing loss. Table 4 summarizes demographic characteristics as were documented after birth. During the first few days of life, most of the ear canals were found to contain some amount of material and in most cases, it was difficult to identify and evaluate the status of the TM. During the second measurements, most of the ear canals were found to contain no debris and most TM’s were normal. Middle-ear effusion was identified in four ears. Measurements were not performed if the baby was crying or moving and when the canal was too narrow to insert the measuring probe. The results of the ethnic origin questionnaire are presented in table 5.
<table>
<thead>
<tr>
<th>Babies</th>
<th>156</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gestational age (wks)</strong></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>35-42</td>
</tr>
<tr>
<td>Mean</td>
<td>39</td>
</tr>
<tr>
<td>SD</td>
<td>10 days</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>80</td>
</tr>
<tr>
<td>Males</td>
<td>76</td>
</tr>
<tr>
<td><strong>Birth type</strong></td>
<td></td>
</tr>
<tr>
<td>Vaginal</td>
<td>108</td>
</tr>
<tr>
<td>Cesarean Section</td>
<td>48</td>
</tr>
<tr>
<td><strong>Ears</strong></td>
<td></td>
</tr>
<tr>
<td>Right ear</td>
<td>146</td>
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<tr>
<td>Left ear</td>
<td>146</td>
</tr>
<tr>
<td><strong>Age at screening (hrs)</strong></td>
<td></td>
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<tr>
<td>Range</td>
<td>12-54</td>
</tr>
<tr>
<td>Mean</td>
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<tr>
<td>SD</td>
<td>8</td>
</tr>
<tr>
<td>Median</td>
<td>24</td>
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<tr>
<td><strong>DPOAE</strong></td>
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<td>Pass</td>
<td>155</td>
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<tr>
<td>Refer</td>
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<tr>
<td><strong>Ear canal occlusion (%)</strong></td>
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</tr>
<tr>
<td>RE Mean</td>
<td>50</td>
</tr>
<tr>
<td>RE SD</td>
<td>30</td>
</tr>
<tr>
<td>LE Mean</td>
<td>50</td>
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<tr>
<td>LE SD</td>
<td>30</td>
</tr>
<tr>
<td><strong>Birth weight (gr)</strong></td>
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</tr>
<tr>
<td>Mean</td>
<td>3406</td>
</tr>
<tr>
<td>SD</td>
<td>472</td>
</tr>
<tr>
<td><strong>Head circumference (cm)</strong></td>
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<td>Mean</td>
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</tr>
<tr>
<td>SD</td>
<td>2</td>
</tr>
<tr>
<td><strong>Body length (cm)</strong></td>
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</tr>
<tr>
<td>Mean</td>
<td>51</td>
</tr>
<tr>
<td>SD</td>
<td>3</td>
</tr>
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Table 4. Demographic data.
<table>
<thead>
<tr>
<th>Origin</th>
<th>Mother</th>
<th>Father</th>
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</thead>
<tbody>
<tr>
<td>First Nations (North American Indian)</td>
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<tr>
<td>Metis</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Inuk (Inuit)</td>
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<td>2</td>
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<tr>
<td>Latin American</td>
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<td>8</td>
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<tr>
<td>White</td>
<td>79</td>
<td>75</td>
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<td>Black</td>
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<tr>
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</tr>
<tr>
<td>Filipino</td>
<td>2</td>
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<tr>
<td>South Asian (e.g. East Indian, Pakistani, Sri Lankan, etc.)</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>Southeast Asian (e.g. Vietnamese, Cambodian, Malaysian, Laotian, etc.)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>West Asian (e.g. Iranian, Afghan, etc.)</td>
<td>3</td>
<td>3</td>
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<tr>
<td>Arab</td>
<td>30</td>
<td>31</td>
</tr>
<tr>
<td>Korean</td>
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<td>1</td>
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<tr>
<td>Japanese</td>
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<td>0</td>
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<tr>
<td>Other:</td>
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<td>0</td>
</tr>
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</table>

Table 5. Ethnic origin as reported by the parents.
5.2 Exclusion Procedure

In one month old babies the equivalent volume may be slightly negative at low frequencies (Keefe et al., 2000). However, large negative volumes are not expected to occur since they may represent a probe leak. A procedure was employed to exclude cases of large negative equivalent volume. As was described by Keefe et al. (2000), cases with equivalent volume value below \(-1.15 \text{ cm}^3\) were excluded from the study. By using this procedure, 3 cases were excluded from the first set of measurements and 3 cases were excluded from the second set of measurements. Figure 8 shows examples of large equivalent volumes that were excluded from analysis due to large negative volumes.

Figure 8. Examples of excluded cases due to larger negative volumes.
5.3 Equivalent Volume

In order to explore the equivalent volume results, the 10th, 25th, 50th, 75th and 90th percentiles were plotted as a function of frequencies. As is seen in Figure 9, the 50th percentile of the equivalent volume is between approximately 0.4 cm$^3$ at low frequencies and about 0.1 cm$^3$ at high frequencies. The 90th percentile shows the largest difference from the median equivalent volume values at the low and the very high frequencies. Similar results were shown by the 10th percentile. In general, between approximately 1.5 and 6 kHz the difference between the percentile groups was the smallest.

**Figure 9.** 10th, 25th, 50th, 75th and 90th percentiles of the equivalent volume from both ears.
Next we wanted to find whether the equivalent volumes follow a specific pattern. By plotting individual cases of equivalent volumes it was possible to identify two common patterns. As seen in Figure 10, the first pattern shows an up-shooting to different levels of volumes occurring at the very low frequencies. Next, values descend and then ascend to a plateau between approximately 400 and 790 Hz. The values then descend to below 0.2 cm$^3$ at around 1 kHz and remain at this level along the middle and high frequencies.

![Figure 10. First pattern of equivalent volume](image-url)
A second pattern of equivalent volume is shown in Figure 11. As it can be seen, the values at low frequencies are still high however, the up shooting that was found in the previous pattern is not seen here, instead there is a gradual descend to a value below 0.2 cm³ from approximately 600 Hz. From approximately 5 kHz some of the measurements show a trend of increase in volume and around 7 kHz, some of the values decrease again.

**Figure 11**: Second pattern of equivalent volume.
5.4 Ear-Canal Occlusion

In order to explore whether there is a correlation between the ear-canal occlusion and the reflectance results, the 50th percentile of each occlusion group was plotted for the right and left ear. As is seen in Figures 12 and 13, the largest separation between the occlusion groups occurs between the 0 to 70% occlusion groups and the 80 to 100% occlusion groups. This separation is more pronounced below approximately 400 Hz and between 840 Hz and 6 kHz in the right ear and bellow 400 Hz and between 1.6 and 3.2 kHz in the left ear. These findings support the hypothesis that as occlusion of the ear canal increases, less energy will enter the middle ear and will be reflected back at the probe. However, as it was seen these differences occur at specific frequency regions. To better appreciate the difference between the 0-70% and 80-100% occlusion groups, each main group was plotted in a different color as seen in Figures 14 and 15.
Figure 12. Median reflectance at each level of occlusion in the right ear.

Figure 13. Median reflectance at each level of occlusion in the left ear.
**Figure 14.** Median reflectance of the 0-70% and 80-100% occlusion in the right ear.

**Figure 15.** Median reflectance of the 0-70% and 80-100% occlusion in the left ear.
Table 6 summarises reflectance values at some of the frequency data points as a function of ear-canal occlusion. The largest difference in reflectance values between the 70% and 80% occlusion groups occurs at 2-kHz data point.

<table>
<thead>
<tr>
<th>Frequency (kHz)</th>
<th>0.25</th>
<th>0.5</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Occlusion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RE 0%</td>
<td>0.522</td>
<td>0.571</td>
<td>0.460</td>
<td>0.308</td>
<td>0.627</td>
<td>0.286</td>
</tr>
<tr>
<td>RE 10%</td>
<td>0.461</td>
<td>0.567</td>
<td>0.481</td>
<td>0.266</td>
<td>0.574</td>
<td>0.354</td>
</tr>
<tr>
<td>RE 20%</td>
<td>0.491</td>
<td>0.592</td>
<td>0.335</td>
<td>0.398</td>
<td>0.641</td>
<td>0.128</td>
</tr>
<tr>
<td>RE 30%</td>
<td>0.419</td>
<td>0.549</td>
<td>0.415</td>
<td>0.320</td>
<td>0.627</td>
<td>0.171</td>
</tr>
<tr>
<td>RE 40%</td>
<td>0.395</td>
<td>0.521</td>
<td>0.351</td>
<td>0.400</td>
<td>0.541</td>
<td>0.439</td>
</tr>
<tr>
<td>RE 50%</td>
<td>0.729</td>
<td>0.556</td>
<td>0.531</td>
<td>0.452</td>
<td>0.655</td>
<td>0.340</td>
</tr>
<tr>
<td>RE 60%</td>
<td>0.666</td>
<td>0.584</td>
<td>0.493</td>
<td>0.522</td>
<td>0.663</td>
<td>0.340</td>
</tr>
<tr>
<td>RE 70%</td>
<td>0.714</td>
<td>0.602</td>
<td>0.497</td>
<td>0.481</td>
<td>0.652</td>
<td>0.421</td>
</tr>
<tr>
<td>RE 80%</td>
<td>0.799</td>
<td>0.571</td>
<td>0.614</td>
<td>0.779</td>
<td>0.776</td>
<td>0.551</td>
</tr>
<tr>
<td>RE 90%</td>
<td>0.795</td>
<td>0.632</td>
<td>0.656</td>
<td>0.801</td>
<td>0.782</td>
<td>0.582</td>
</tr>
<tr>
<td>RE 100%</td>
<td>0.912</td>
<td>0.678</td>
<td>0.662</td>
<td>0.817</td>
<td>0.805</td>
<td>0.669</td>
</tr>
<tr>
<td>LE 0%</td>
<td>0.518</td>
<td>0.621</td>
<td>0.471</td>
<td>0.426</td>
<td>0.598</td>
<td>0.218</td>
</tr>
<tr>
<td>LE 10%</td>
<td>0.587</td>
<td>0.603</td>
<td>0.454</td>
<td>0.345</td>
<td>0.601</td>
<td>0.161</td>
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<tr>
<td>LE 20%</td>
<td>0.437</td>
<td>0.580</td>
<td>0.431</td>
<td>0.363</td>
<td>0.627</td>
<td>0.174</td>
</tr>
<tr>
<td>LE 30%</td>
<td>0.515</td>
<td>0.555</td>
<td>0.332</td>
<td>0.357</td>
<td>0.621</td>
<td>0.162</td>
</tr>
<tr>
<td>LE 40%</td>
<td>0.520</td>
<td>0.571</td>
<td>0.432</td>
<td>0.335</td>
<td>0.523</td>
<td>0.254</td>
</tr>
<tr>
<td>LE 50%</td>
<td>0.534</td>
<td>0.561</td>
<td>0.424</td>
<td>0.435</td>
<td>0.659</td>
<td>0.434</td>
</tr>
<tr>
<td>LE 60%</td>
<td>0.693</td>
<td>0.584</td>
<td>0.526</td>
<td>0.455</td>
<td>0.657</td>
<td>0.283</td>
</tr>
<tr>
<td>LE 70%</td>
<td>0.702</td>
<td>0.652</td>
<td>0.609</td>
<td>0.550</td>
<td>0.713</td>
<td>0.542</td>
</tr>
<tr>
<td>LE 80%</td>
<td>0.907</td>
<td>0.717</td>
<td>0.596</td>
<td>0.776</td>
<td>0.696</td>
<td>0.459</td>
</tr>
<tr>
<td>LE 90%</td>
<td>0.807</td>
<td>0.653</td>
<td>0.620</td>
<td>0.694</td>
<td>0.800</td>
<td>0.619</td>
</tr>
<tr>
<td>LE 100%</td>
<td>0.888</td>
<td>0.681</td>
<td>0.573</td>
<td>0.723</td>
<td>0.714</td>
<td>0.564</td>
</tr>
</tbody>
</table>

Table 6. Median reflectance as a function of ear-canal occlusion

(RE-right ear, LE-left ear).
5.5 Mean Reflectance

To explore the pattern of the reflectance measurements, the 10th, 25th, 50th, 75th and 90th percentiles plots were produced. As is seen in Figure 16, a common reflectance pattern seen in newborns can be identified in the 50th percentile plot. This pattern includes a high reflectance below the region of 1 kHz, low reflectance between approximately 1 and 2.5 kHz and then an increase at the high frequencies to a maximum around 4 kHz from where at the higher frequencies a decrease occurs.

Figure 16. Reflectance percentiles from right and left ears together.
5.6 Normality Plots

To explore whether a normal distribution of the reflectance results exists, histograms were plotted for 6 different frequency data points. These points can be seen in Figure 16 in red as the cut points. We chose these points since they represent regions where the percentiles plots are close or far from each other. Figures 17, 20 and 23 show histograms for the 250 Hz, 1.3 kHz and 4.5 kHz data points respectively. As it can be seen, the 250 Hz and 1.3 kHz show bimodal distribution while the 4.5 kHz shows an even distribution. We assumed that this bimodal distribution occurs between the 0-70% and the 80-100% groups since a more pronounced separation between these groups occurs at 250 Hz and 1.3 kHz as is seen in figures 14 and 15. Plots were then produced for the two main occlusion groups separately at 250 Hz and 1.3 kHz data points. The resulted plots are seen in Figures 18, 19, 21 and 22. As it can be seen, when the 0-70% and 80-100% groups are plotted separately, the bimodal distribution does not occur. The conclusion was that the bimodal distribution was a result of larger difference in reflectance between the 2 main groups at 250 Hz and 1.3 kHz while at 4.5 kHz, the difference is smaller. These finding supported the hypothesis that the reflectance increases as the occlusion increase. We could also conclude that there is probably a critical point of occlusion level that causes a much larger increase in reflectance compared to a lower level of occlusion.
Figure 17. Reflectance distribution of all occlusion groups at 250 Hz

Figure 18. Reflectance distribution in 0-70% occlusion at 250 Hz

Figure 19. Reflectance distribution in 80-100% occlusion at 250 Hz
**Figure 20.** Reflectance distribution of all occlusion groups at 1.3 kHz

**Figure 21.** Reflectance distribution in 0-70% occlusion at 1.3 kHz

**Figure 22.** Reflectance distribution in 80-100% occlusion at 1.3 kHz
Figure 23: Reflectance distribution of all occlusion groups at 4.5 kHz
5.7 Linear Mixed Model Analysis

A linear mixed model was applied on the data to examine whether a difference in reflectance exists between the right and left ears, main occlusion groups and frequencies. The 60 frequency data points were divided into groups of 10 so a total of 6 groups of frequencies were analyzed. The latter step was done in order to increase the number of results per frequency group. Since it was found from the graphic presentation (Figures 14 and 15) that the most prominent difference in reflectance appears to be between the 70% and 80% occlusion, the 11 occlusion levels were divided into 2 main groups: 0-70% and 80-100% and a comparison was done between these 2 groups. The reflectance was the dependent variable nested within each ear separately.

The analysis showed a significant difference in reflectance between the six frequency groups in both the right and the left ear. In addition, a significant difference was found between the 2 main occlusion groups in both ears. There was no significant difference in reflectance between the right and the left ears.

5.8 Wideband Absorbance Tympanometry

The wideband system provides 3 dimensional plots of the absorbance as a function of air pressure and frequency. There were 23 plots available for comparison between the first and the second measurements. A graphic comparison revealed that in 20 of the tympanograms, the absorbance was lower in the region of negative pressure during the first measurement as compared to the second measurement. Examples of such findings are given in Figures 24 and 25.
**Figure 24:** Example of absorbance tympanometry from first (left) and second (right) measurement.

**Figure 25.** Another example of absorbance tympanometry from first (left) and second (right) measurement.
Following these findings, a plot was produced representing the reflectance at -300 daPa which is the most negative pressure point in the tympanometry. Figure 26 shows the comparison between the two time-point measurements. The red lines represent the first measurements while the black lines represent the second group of measurements. A tendency towards separation between the groups can be seen however, some overlap exists. Higher reflectance is seen in most cases of the first measurement group.

**Figure 26.** Comparison of reflectance results between first and second measurements at minus 300 daPa pressure point (Red-first measurements, Black-second measurements).
Interestingly, below 1 kHz a clearer and a more consistent separation exists between the groups which is in contrast to what one would expect to find with conventional low-frequency tympanometry. Both groups show some kind of pattern under 1 kHz. The first group shows a more consistent pattern above 1 kHz. The patterns occurring in the first measurement are surprising since after birth the canal may collapse more easily at negative pressures and so it would be expected to find less consistent results.
Chapter 6: Discussion

The present study has shown that occlusion of the newborn ear canal by transient materials affects the wideband reflectance measurements. In addition, the study has provided further baseline data of wideband reflectance from a large population of newborn babies. Previous studies have reported reflectance values in newborns. However, this is the first study that has correlated otoscopic findings to reflectance measurements. Statistical analysis has shown that there is a significant difference in reflectance between low and high levels of occlusion. The critical point of the occlusion level appears to be where the ear-canal is occluded by 70 to 80% of its space. In the present study it was found that the largest difference in reflectance between neighbouring occlusion groups occurred between the 70 and 80% occlusion groups at some frequency regions. Figure 27 shows a comparison of the median reflectance in newborns between the present study and previous studies. As can be seen, the reflectance pattern in the present study follows that of previous studies. The current study agrees with previous findings that have shown a high reflectance below approximately 1 kHz and around 4 kHz, and a low reflectance between 1 and 4 kHz and at frequencies higher than 4 kHz. However, the reflectance values in the present study are higher in comparison to previous studies. There may be different explanations for these differences. The study by Aithal et al. (2013) included only ears with normal middle-ear function as was determined by a battery of tests. This inclusion can explain a lower reflectance than in the present study since normal middle ear
implies lower reflectance. The plot taken from the study by Hunter et al. (2010) represents babies who passed the hearing screening only with OAE. The present study included reflectance measurements that were taken after birth from babies that passed the hearing screening either on the first attempt with OAE or on the second attempt with AABR and so, if middle-ear function was abnormal in a subgroup of the babies, this could be an explanation for higher reflectance in the present study. However, since in the present study all babies except one have passed the hearing screening, the current study may better represent a database for the majority of newborns who normally do not have a test for middle-ear function at birth. Another factor to be considered is the age of participants. In the study by

Figure 27. Comparison of median reflectance between different studies
(Modified from Aithal et al., 2013)
Aithal et al. (2013) the mean age of the babies was 46 hours. Keefe et al. (2000) included babies from intensive care unit. Hearing screening for babies in the intensive care unit is often delayed and so the test might have been performed when the ear canal and middle ear were already cleared from materials. As a result, inclusion of this population may reduce the overall reflectance values. In the study by Hunter et al. (2010), the mean age of the babies was 29 hours. In the present study, the mean age was 25 hours. It has been shown that reflectance decreases with increasing newborn age and so, the findings of higher reflectance in the present study could be explained by the age differences (Keefe et al., 2000; Hunter et al., 2010).

The present study included healthy babies. Risk factors included family history of hearing loss however not in first degree relatives. Aithal et al. (2013) included healthy babies and did not mention any risk factors. In the study by Hunter et al. (2010) “risk factors were rare, because all babies were considered well babies”. In contrast, the subject population in the study by Keefe et al. (2000) included a subgroup of babies with risk factors for hearing loss such as cleft palate, dysmorphic features, ventilator support and positive family history as well as babies from intensive care unit who as a group has the risk for low birth weight and ventilatory support. The latter study has shown that in patients with cleft lip or palate, the reflectance was higher in all frequencies compared to the group without risk factors. Interestingly, as it can be seen in Figure 27, the mean reflectance was lower compared to other studies even though a mixed population was included.
Another difference between the studies includes the usage of different instruments, calibration methods and ear tips. Aithal et al. (2013) used the Reflwin system produced by Interacoustics, the same instrument that was used in the present study. Hunter et al. (2010) has used the HeardID R4 system (Mimosa Acoustics, Inc.). Keefe et al. (2000) has used a custom made system. The different calibration procedures may as well contribute to the different results.

Hunter et al. (2010) reported on results between 2 test sites. They found a difference that occurred under 1 kHz. Their assumption was that the difference could have been explained by different techniques of probe insertion, depth in the canal and seal confirmation. This difference in using the probe may also contribute to differences in reflectance between the studies. It is worth mentioning that although ambient measurements are not dependent on the probe depth, the position of the probe in relation to the axis of the canal may influence the measurements. For example if the probe faces the canal wall the results may be different if it would face the TM.

In the present study, no significant difference in reflectance was found between the right and left ears. This finding agrees with those of Aithal et al. (2013) and Hunter et al. (2010). The latter study also examined whether there was a difference in reflectance between the ears separately for each group that had passed or referred on the screening. No significant difference was found between the ears in each of the group. In contrast to the latter studies, Keefe et al. (2000) found that below 1414 Hz, the left ear had a higher reflectance than the right ear while above 1414 Hz, they found a lower reflectance in the left ear compared to the right ear.
Hunter et al. (2010) have shown that at 2 kHz, reflectance best discriminate between the babies who passed or referred on OAE. Interestingly, from the present study it seems that the largest difference in reflectance occurring between the two main groups was around the region of 2 kHz. This finding may point to the fact that in the region of 2 kHz the middle ear is more sensitive to a disturbance in sound transmission.

Even though the newborn ear canal is narrower, it was possible to perform otoscopy in most of the babies. Most ear canals were found to contain some amount of material and it was difficult to differentiate between the canal wall and the TM. However, when the TM was identified, the more horizontal position of the newborn TM could be seen. These findings are not surprising since earlier studies have documented similar otoscopic results (McLellan & Webb, 1957; Balkany et al., 1978).

The present study has demonstrated a significant difference between the 6 frequency regions. Aithal et al. (2013) that used the same instrument as in the present study have found significant difference between individual frequencies however, when specific frequency regions were compared, the reflectance was not significantly different between 250 to 800 Hz and 3 to 4 kHz, and between 1 to 2.5 kHz and 6 to 8 kHz. However, these regions are different from the 6 regions analyzed in the present study.

The tympanometric reflectance data has shown a tendency towards a higher reflectance at the negative pressure region during the first measurement compared to a lower reflectance in the second measurement. Although a lower reflectance is expected to be found in the first days after birth, it is possible that
during this time, when negative pressure is introduced to the ear canal, it may cause collapse of the canal walls and increase the reflectance values. However, there were no earlier studies that provided a similar data for comparison. This question should be investigated on a larger group of newborns.

In some of the ambient measurements the absorbance values were negative. Sanford et al. (2009) reported on a similar finding however during tympanometric measurements. They considered negative absorbance as a measurement error and attributed it to maturational changes of the ear canal which may cause closure of the canal during negative pressurization. All negative absorbance in their study were set to zero. In the present we used reflectance and so when negative absorbance has occurred, reflectance was set to 1. It is unknown however whether these changes affect the overall results.

Finally, in the current study we used 4 types of rubber probe tips that have been supplied by the company (Interacoustics Inc.). Two of the probe tips had a tube shape while the other two had a cone shaped. The question of how the probe tip type affects the measurements was addressed in earlier studies. Merchant et al. (2010) have used rubber and foam tips during their methodological development. They reported that the rubber tip had a tendency to fall out and that it was difficult to achieve a proper seal. Eventually they used the foam tips during their study. Vander Werff et al. (2007) also reported that the rubber tip was easily slipping out of the canal and that it was difficult to get a tight seal which resulted in a high degree of variability when the probe was reinserted. In the latter study they also compared reflectance measurements between the rubber tip and the foam tip. When test-retest was performed, the differences between repeated tests using the
rubber tip were greater than with the foam tips. It is unknown whether different tip shapes had influenced the measurements in the present study since no test-retest was performed on the same individual with different tip shapes.
Chapter 7: Conclusions and Future Directions

The present study was the first to describe correlation between otoscopic findings and ambient wideband reflectance. It has been shown how occlusion of the ear canal by transient materials occurring after birth affects the reflectance. Wideband tympanometry has shown a tendency of higher reflectance in the negative pressure region after birth. However the latter finding is limited by the small number of subjects during the second measurement. Since wideband measurements are currently being studied as a possible future complementary tool in newborn hearing screening, the current study provides additional normative database in the newborn population who passed the hearing screening. It has been shown that variability exists between the results of various studies in regard to reflectance results due to the different possible factors. Future studies should investigate how probe position and different probe-tip shapes affect the measurements. In addition, it is unknown how different physiological factors such as respiratory rate, heart rate and sucking can affect the measurements. The number of participants in the second measurement should be increased to further investigate the differences in wideband tympanometry between the first and second measurement. A comparison of wideband measurements to a single-probe tone tympanometry should be made in order to explore the accuracy of these tests in diagnosing the middle-ear function in newborns. The use of a video-otoscope could further enhance diagnosis and documentation of the ear canal and middle ear status and enable inter-observer agreement. Finally additional wideband
measurements will increase the normative database in the newborn population and will allow further comparisons with other studies.
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