Appendix 15D

Wisconsin Cataract Grading System
AREDS Lens Opacity Grading Protocol

1.0 OVERVIEW

The AREDS lens opacity grading protocol is an adaptation of the Wisconsin System for Classification of Cataracts from Photographs.

A single nonstereoscopic photograph taken with a modified Topcon slit lamp camera according to a specified protocol (Section 8.3) is used to grade nuclear sclerosis and nuclear color. Degree of nuclear sclerosis is graded by comparing the photograph with a series of seven standard photographs. Both the interval into which the photograph being assessed falls and its approximate position within that interval are estimated. The principal characteristics evaluated are the optical density of the nucleus and the clarity of its normal landmarks (Sections 15D-2.2 and 15D-2.3). Nuclear color is graded on a four-step scale by comparing the color of the specular reflection near the posterior surface of the lens in the photograph being assessed with the comparable feature in a series of three standard photographs (Section 15D-2.4).

Two nonstereoscopic photographs taken with a modified Neitz retro-illumination camera according to a specified protocol (Section 8.3) are used to estimate the extent of cortical and posterior subcapsular lens opacities and to record the presence of other opacities. A grid superimposed on the photographs divides the dilated pupil into 17 subfields, so that the area of each occupied by opacity can be estimated (Sections 15D-3 and 15D-4). The area within a 5 mm diameter circle (the "central zone", comprising 9 of the subfields) is considered of primary importance, since it is expected that pupillary dilation to at least this degree will be achieved at most visits in most AREDS participants, thus facilitating analyses of change between baseline and follow-up visits.

A stereoscopic pair of photographs of the red reflex taken with the Zeiss fundus camera according to a specified protocol (Section 8.2.3) is used to assist grading of the Neitz photographs and to recognize opacities not visible in them. This stereo pair (albeit taken through a dilated pupil) is also used to grade degree of iris pigmentation (iris color) on a four-step scale, by comparison with three standard photographs (Section 15D-5).

The results of the grading are recorded on the AREDS Lens Grading Form, (Exhibit 15D-1). The form includes a comments section for recording unusual, presumably non-age-related lens opacities. The grading grid is illustrated in Exhibit 15D-2. The standard photographs are on file at the UW Fundus Photograph Reading Center, 610 Walnut St., Madison, Wisconsin 53705.

2.0 SLIT LAMP PHOTOGRAPHS

2.1 Camera features and artifacts

The Topcon Model SL-6E photo slit lamp camera has been modified so that the angle between the illumination beam and the observation system, the beam width and intensity, and the magnification remain the same for all cameras for all subjects. Fixation targets have been added (viewed with the eye being photographed) so that the path of the slit beam through the lens is the same for right and left eyes (the beam is to the examiner's left of the observation system, for both right and left eyes, so that the posterior surface of the lens is always to the observer's right). A detailed protocol is specified, including focusing of the camera at the center of the lens nucleus. A single (nonstereoscopic) slit lamp photograph of the lens is mounted in a plastic sheet along with the red reflex and retro-illumination photographs for that eye. The right and left eyes are mounted and graded separately.

2.2 Slit lamp appearance of the lens and definitions

As the slit lamp beam transverses the normal adult lens, differences in refractive indices result in alternating brighter and darker bands of varying widths (See SL Standard Photograph l). Because the depth of focus in the photographs is fairly shallow, these bands become less well defined the farther they are from the center of the lens, the ideal focal point of the camera. In SL Standard 1 a wide dark band can be seen running vertically through the center of the lens. This is termed the *central dark interval* or the *sulcus* of the nucleus (and corresponds to the *embryonal nucleus*). Bordering the sulcus anteriorly and posteriorly are two broad, short, bright bands. The surfaces of these bands facing the sulcus tend to be flat or only slightly curved, while the surfaces facing the lens capsule are more steeply curved. In SL Standard 1 the posterior of these two bright bands is rather steeply curved both posteriorly and anteriorly, i.e., is bean-shaped. This is a common appearance and has led to the use of the descriptive term *lentils* for these bright bands, which are part of the fetal nucleus. The anterior and posterior Y-sutures can sometimes be seen in the anterior and posterior lentils. They are not clearly visible in SL Standard 1, but part of the posterior suture can be seen in SL Standard 3.

There are differences in interpretation regarding the remaining bands between the lentils and the lens capsule. According to Berliner[2] the bright bands immediately external to the lentils, visible as narrow, tall, relucent bands with dark bands of equal or slightly narrower width adjacent to them, represent the anterior and posterior surfaces of the lens at birth, the "fetal nucleus". In the Oxford System, these bands are described as the anterior and posterior limits of "the nucleus"[1]. In this grading system these bands will be referred to as the anterior and posterior *nuclear surface bands*. That part of the lens between (and including) these bands is considered the *nucleus* and is the only part of the lens evaluated in the slit lamp photographs. The term *nuclear landmarks* will be used to refer to all the parts of the nucleus described above, i.e., the sulcus, the lentils, the nuclear surface bands, and the dark bands between the lentils and the nuclear surface bands.

In SL Standard 1, three additional bright bands are visible: (1) a broad one at the posterior surface of the lens, (2) a narrower one at the anterior surface of the lens, and (3) another relatively narrow one between the anterior surface of the lens and the anterior nuclear surface band. The third of these bands is considered by Berliner to represent the anterior surface of the "adolescent" or "adult" nucleus; in the Oxford System it is considered to be part of the lens cortex. In SL Standard

1 the reflection of the slit beam from this band and from the posterior lens surface is particularly bright ("specular reflection"). The anterior chamber lies between the surface of the anterior capsule and the wide steeply curved band of the cornea. The downward pointing arrow that appears to be within the anterior chamber is a reflex from a mirror in the slit illumination system and ideally should fall midway between the anterior surface of the lens and the posterior surface of the cornea, as it does here.

2.3 Grading nuclear sclerosis

2.3.1 Characteristics graded

In grading the severity of nuclear sclerosis two factors are considered: (1) the optical density (perceived as brightness or relucency, sometimes described as "opalescence") of the nuclear landmarks, especially the sulcus, and (2) the definition of these structures (contrast between the bright and dark bands). Optical density is given greater weight, in part because it is less influenced by suboptimal focus than is definition of nuclear landmarks. In the early stages of nuclear sclerosis, increased optical density is noticeable only in the normally dark bands, particularly the sulcus (see SL Standards 2, 3, and 4), but in advanced stages the density of all bands becomes greater (see SL Standards 5, 6 and 7). With increasing nuclear sclerosis, the definition of nuclear landmarks decreases, and finally disappears.

When the focus of the photograph being graded is too anterior, posterior landmarks will be blurred, and vice versa. In judging the definition of landmarks in such cases, primary emphasis should be placed on the part of the lens that is in better focus. The optical density of the sulcus also may appear to increase as the plane of focus moves farther from it, but it is difficult for the grader to make any allowance for this, and no such attempt should be made. "Cannot grade" may be assigned in extreme cases of incorrect focus (see Sections 8.3.4.1 and 15D-2.6). When analyses of change between two visits are carried out, the plane of focus grades from the Quality Gradings (see 8.3.4.1) of these visits will be compared and eyes with disparities flagged for special consideration (such as exclusion, assignment to a special category, or editing by an algorithm to be developed, or by a direct side-by-side comparison).

2.3.2 The grading scale

The grader compares the photograph being graded with seven standard photographs, which show progressively increasing severity of nuclear sclerosis (SL Standards 1-7). The grader first determines the interval between adjacent standards into which the photograph being graded falls and then estimates its position in the interval to the nearest tenth. For example, if the lens being graded is considered to have nuclear sclerosis only slightly more severe than that in SL Standard 2, the grade 2.1 is assigned; if half way between SL Standards 2 and 3, the grade 2.5 is assigned. Photographs with less optical density and greater definition of landmarks than Standard 1 (virtually a normal lens) are assigned the grade 0.9; those with nuclear sclerosis exceeding that of SL Standard 7 are assigned the grade 7.1.

2.3.3 Description of slit lamp standard photographs

To be comprehensive, this description of standard photographs includes a description of the Y-sutures. However, their status is ignored in judging definition of the lentils. Not only is visibility of the sutures highly vulnerable to changes in the plane of focus, but they may in fact become more distinct as the relucency of the lentils increases (i.e., with the onset of mild sclerosis).

In **SL Standard 1** the nuclear landmarks are easily identified. The sulcus is well defined and appears dark throughout the vertical extent of the nucleus. (The small circular red dot on the anterior edge of the sulcus is a reflection of the fixation target.) The density of the posterior lentil is greater than that of the anterior, and its edges are more clearly defined. The Y-sutures are not visible in this photograph, except perhaps for a faint line centrally in the posterior lentil. The anterior and posterior nuclear surface bands and the narrow dark bands bordering them are easily discerned. As one would expect because of their distance from the point of focus, the remaining bright bands are out of focus.

In **SL Standard 2** the sulcus is denser (or more relucent, thus less black) and is less well defined, particularly superiorly and inferiorly. Although the anterior and posterior nuclear surface bands are still well defined, the dark bands between them and the lentils are denser.

In **SL Standard 3** the density of all the dark bands has increased, leading to a decrease in distinctness of the landmarks. The density of the sulcus has increased to the point that its definition has been lost, except for its central one-third. The anterior nuclear surface band cannot be distinguished at all, except perhaps centrally. The posterior nuclear surface band can only be distinguished centrally. The anterior lentil has become denser. Part of the posterior Y-suture is easily identified in the posterior lentil.

In **SL Standard 4** there appears to have been a further increase in the density of the dark bands. Only a suggestion of the sulcus can be detected. Towards the upper and lower ends of the sulcus segments of what appears to be the equator of the fetal nucleus (or a zone just beneath its surface) are visible as steeply curved white lines. Only a small part of the anterior lentil is visible. The posterior nuclear surface band cannot be seen at all and the anterior one is very faint.

In **SL Standard 5** there has been a further increase in density of both the dark and bright bands. A very faint shadow centrally marks the sulcus; the normally dark bands separating the nuclear surface bands from the lentils have increased in density to equal the increased density of the remaining nucleus. Only the dark bands external to the nuclear surface bands are well defined, and these are not included in the assessment of nuclear opacity.

In **SL Standard 6** there has been a further increase in density of both dark and bright bands. The sulcus and lentils cannot be distinguished and the density of the area assessed is greater than that in SL Standard 5.

In **SL Standard 7**, nuclear landmarks are indistinguishable due to increased density, which in the area assessed is even greater than that in SL Standard 6.

SL Standard 8 is used only in grading lens color (see below). Its grade for nuclear sclerosis would be near the lower end of the 4.0-4.9 range, because the optical density of the nucleus is a little greater than that of SL Standard 4 (although nuclear landmarks are better defined).

2.4 Grading lens color

2.4.1 Characteristics graded

In most adults, the gray-blue nucleus typical of youth begins to yellow with increasing age, although there is great variation in the age at which this begins. Yellowing may not develop at the same rate in both eyes of an individual and in rare cases may appear in only one eye[2]. The color may continue to deepen and intensify, turning from pale yellow to gold to orange and finally to brown ("brunescent"). In rare cases the nucleus may appear nearly black in color. The greatest intensity of the color change is seen in the specular reflection from the central zone of the posterior surface of the lens. It is at this point that the grader should assess the color, comparing it with the same area in the standard photographs.

2.4.2 The grading scale and descriptions of standard photographs

Slit lamp Standard Photographs 2, 4, and 8 are used as standards for grading lens color. The grader compares the photographs to the standards both with and without the Donaldson stereo viewer. Sometimes subtle changes in color can be perceived more easily without the stereo viewer. The standards for lens color and their use in grading are described below.

In **SL Standard 2** slight yellowing can be seen at the posterior surface of the lens and extending forward into the posterior part of the nucleus. This is best appreciated by comparison with SL Standard 1, in which there is no yellowing. If color in the photograph being graded is less yellow than that in SL Standard 2, the grade is 1; for yellowing $\geq$ SL Standard 2 but $<$ SL Standard 4 the grade is 2.

In **SL Standard 4** a broad, bright, pale yellow reflex can be seen at the posterior pole of the lens. If yellowing in the photograph being graded equals or exceeds that in SL Standard 4 (but $<$ SL Standard 8), the grade is 3. SL Standard 3 provides an example of yellowing very nearly the same as that in SL Standard 4, but with less specular reflection. For example in SL Standard 5 color is slightly more yellow than in SL Standard 4 and would receive the grade 3.

In **SL Standard 8** the reflex from the posterior pole of the lens has a deeper yellow color than that in SL Standards 3 or 4. If color in the photograph being graded is as yellow as or more yellow than that in SL Standard 8, the grade is 4. To illustrate, in SL Standard 6 color is substantially more yellow than in SL Standard 8 and would receive the grade 4.

If lens color cannot be graded the grade is 8.

2.5 Cortical flecks

Small, discrete white flecks or dots are often visible in the peripheral cortex of the lens in the slit lamp photograph. Although such opacities occur commonly with some systemic (metabolic) diseases, they are also often found in healthy individuals. The relationship of cortical flecks to other age-related changes is unclear. These small snowflake-like opacities may be found scattered throughout the lens periphery, but are most often seen, in photographs taken according to the AREDS protocol, at the superior and inferior poles of the cortex. Their presence or absence is recorded for the lens as a whole.

2.6 Gradability of slit-lamp photographs (use of "cannot grade")

Three factors determine gradability of slit-lamp photographs: focus, placement of the slit beam, and area of the lens visible (determined by pupil size and position of the eyelids). The depth of focus of the slit lamp camera is shallow (1 - 2 mm), but broad enough so that the entire thickness of the nucleus can be in satisfactory focus simultaneously when the plane of focus is in the sulcus. If the plane of focus is at or anterior to the anterior lens capsule in the optic axis, <u>or</u> at or posterior to the posterior capsule in the optic axis, photo quality is graded inadequate (see 8.3.4.1). However, if such photographs are presented to the grader, a grade is assigned if possible.

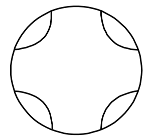
If the slit beam is displaced laterally so that an imaginary vertical line through the sulcus does not fall between vertical lines that would cross the pupil margin from 10:30 to 7:30 and from 1:30 to 4:30, too little of the nucleus will be visible to allow grading. Similarly, if a small pupil or drooping upper lid allows less than one-half of the vertical extent of the nucleus to be seen, cannot grade is assigned.

3.0 NEITZ RETRO-ILLUMINATION PHOTOGRAPHS

3.1 Camera features and artifacts

Because the illuminating and viewing axes of the Neitz camera coincide, the image it records is provided by light reflected from the fundus. This image is reddish-orange because of pigment in the retinal pigment epithelium and choroid, and because of blood in the choroidal, and to a lesser extent, the retinal vessels. Lens opacities (other than nuclear sclerosis) are visible as dark spots because they partially or completely block the light reflected from the fundus behind them (retro-illumination). Two nonstereoscopic color photographs are taken with the Neitz CT-R cataract camera through the dilated pupil, one focused on the iris (corresponding to the anterior cortex of the lens) and one focused 3-5mm more posteriorly (at or near the posterior capsule).

To reduce the bright reflection from the cornea of the Neitz camera's flash, polarizing filters are placed (at opposite axes) in front of the light source and in front of the film. In the Neitz photograph there is a characteristic unevenness in color in the form of a darker orange cross, similar in shape to the central part of a celtic cross, as illustrated below.



In the four peripheral areas not covered by the orange cross, the fundus reflex is lighter and more yellow in color. This nonuniformity is normal for the system and should not be confused with lens abnormality.

Frequently in the anterior Neitz photograph a pale white rectangular reflection from a mirror in the illuminating pathway can be seen centrally (with a longer axis vertical). A similar but fainter, out-of-focus image can be seen in many posteriorly focused Neitz photographs. These images should be ignored when grading.

3.2 Appearance of lens opacities in Neitz retro-illumination photographs

Lens fibers grow in concentric layers and continue to develop throughout life. Each new layer grows beneath the capsule and around the older fibers internal to it pushing these fibers inward. For this reason opacities of the lens tend to be location-specific, depending on the stage of lens development at which they occur. For example , a common type of congenital cataract is confined to a zone within the nucleus ("zonular cataract").

In retro-illumination, the normal lens appears uniformly transparent. Discrete lens opacities are visible as dark interruptions of this transparency, even when their appearance in direct illumination is subtle. The principal types of age-related lens opacities assessed in the Neitz photographs are *cortical* and *posterior subcapsular*. Typical cortical opacities are wedge-shaped and oriented radially. Another, less common, appearance of cortical opacities is a collection of fine granular dots ("stippling"). Posterior subcapsular opacities are located just beneath the posterior lens capsule. Typically they are centered at or near the posterior pole of the lens and extend for varying distances towards the lens equator. The areas involved by cortical and posterior subcapsular opacities are estimated separately for each of the 9 subdivisions of the central zone of the grading grid (within the 5 mm diameter circle). In addition, the number of cortical "vacuoles" in each of these subdivisions is counted up to a limit of 10 (excluding any that form part of a cortical spoke or a posterior subcapsular opacity and are therefore included in those estimates). Vacuoles have the appearance of small, round or oval, clear cyst-like spaces (see Training Photograph 9). The presence or absence of anterior cortical opacities that appear white from directly reflected light rather than black from blocking of light reflected from the fundus (white anterior cortical opacities, or WACOS) is also recorded for each of these subdivisions. The 8 outer subdivisions of the grid are graded for cortical lens opacities (see 15D-3.3.2).

In nonstereoscopic retro-illumination photographs, artifacts can mimic lens opacities because they also block light reflected from the fundus. Common problems are debris on the cornea (such as mascara, eyelashes, or strands of mucus) and irregularities of the cornea following examination procedures (such as applanation tonometry or use of a diagnostic contact lens). A broad tear meniscus may form above the lower lid margin, appearing as a narrow blurred area in the Neitz photograph; care should be taken not to confuse this with an abnormality in the lens cortex. Many of these problems can be detected by careful scrutiny of the Zeiss stereo red reflex photograph.

3.3 Grading Neitz photographs

3.3.1 Measurement of pupil diameter

The diameter of the pupil is measured from the anterior Neitz photograph using a magnifier with a built-in millimeter scale (Bausch and Lomb, 7X). Both the horizontal diameter from 9:00 to 3:00 and the vertical diameter from 12:00 to 6:00 are measured on the film and recorded to the nearest tenth of a millimeter (these may not always be the widest and narrowest dimensions). If the pupil is particularly distorted the grader measures the horizontal and vertical meridians as specified and notes the distortion under the Comments section. Because the Neitz camera has a magnification of 2X, the film measurements will be twice the actual pupil size, that is, in an eye with a 5mm pupil the measurement on film will be 10mm.

3.3.2 Lens grading grid

In order to allow the grader to specify the location and extent of lens opacity more precisely, a grid is used to divide the Neitz photograph into subfields. The grid is formed by three concentric circles: a central circle with radius 2mm; an inner circle with radius 5mm and an outer circle with radius of 8mm. The outer circle is used only to facilitate placement of the grid, <u>not</u> to define the limits of the outer subfields, which are defined by the pupillary margin.

Equally spaced radial lines (meridians) at 10:30, 12:00, 1:30, 3:00, 4:30, 6:00, 7:30, and 9:00 o'clock divide the zones between the central and inner circles and between the inner circle and the pupillary margin into eight subfields each. The grid therefore has 17 subfields: the central subfield; eight equal inner subfields; and eight outer subfields, the areas of which vary with pupil size. On the grading form the subfields are designated as follows:

Central,	
1A (10:30-12:00),	1B (12:00-1:30),
2A (1:30-3:00),	2B (3:00-4:30),
3A (4:30-6:00),	3B (6:00-7:30),
4A (7:30-9:00),	4B (9:00-10:30).

Thus for right eyes inner and outer subfields 2A and 2B are nasal, while for left eyes inner and outer subfields 4A and 4B are nasal. A diagram of the grid is provided as Exhibit 15D-2. The central and inner subfields are referred to as the "central zone".

Short perpendicular ticks are spaced at 1mm intervals along each meridian to facilitate placement of the grid. The grid is affixed to the front of the anterior Neitz transparency so that the central circle is equidistant from the pupillary margins vertically and horizontally. Occasionally the eyelid may obscure a portion of the pupil superiorly, making placement of the grid more difficult. Despite possible interference from the lid, the best determination of the center of the pupil should be made and the grid placed accordingly.

With the grid placed on the anterior photograph, both the anterior and posterior Neitz photographs are mounted side-by-side in the plastic sheet so that they can be viewed simultaneously with the stereoscopic viewer. This allows the grader to combine opacities seen in the anterior cortex with those seen in the posterior cortex, resulting in a single grade for each type of cortical opacity. To determine whether a posterior lesion falls within a particular subfield without attaching an additional grid to the posterior photograph, both the anterior and posterior photographs are viewed simultaneously with the stereo viewer as a pair, thus allowing the grid to be visually superimposed over the posterior photograph. Another technique is to close one eye and then the other in rapid succession, so that the immediate memory of the position of the grid on the anterior photograph helps determine the location of an opacity seen in the posterior photograph.

For both cortical and PSC opacities, each of the 9 subfields comprising the central zone (those within the 5 mm diameter circle) will be graded for presence and extent of opacities. For cortical opacities alone, each of the outer subfields will also be graded. Although opacities in the outer subfields have little or no effect on visual function, cortical cataract typically begins here and almost 50% of lens area visible with a 7 mm pupil lies outside the central zone. Thus it seems undesirable to exclude this area totally from consideration.

However, variations in degree of pupillary dilation from visit to visit, and the tendency for dilation to decrease with increasing age, suggest that for most analyses assessing change in extent of lens opacities between visits only the central zone should be considered. Pupillary dilation of at least 5 mm should be attainable at nearly all visits for nearly all AREDS participants.

3.3.3 Grading rules

When determining the area of each subfield involved by definite cortical or posterior subcapsular opacities, the grader records the percentage to the nearest whole number. However, when the total percentage of the subfield involved is less than one percent (for example when one or two small isolated dots are present), the grader by convention records the percentage as 1%.

When it cannot be determined with ≥90% certainty that the lesion being graded is indeed that lesion, but the grader is ≥50% although <90% confident of the identity of the lesion, the grade is "questionable" and is recorded in the appropriate box for that lesion as "Q". If the grader is <50% confident, the grade is "absent," code 0. If a subfield cannot be graded the grade is recorded as "CG" (see 15D-3.6).

3.4 Grading cortical lens opacities

Cortical opacities vary in shape, size, location in the pupil, and depth within the anterior and/or posterior cortex of the lens. The lesions graded are cortical opacities (spoking or stippling), vacuoles, and WACOS.

3.4.1 Cortical spokes and cortical stippling

Cortical spokes are linear or wedge-shaped radially oriented opacities that partially or completely block light reflected from the fundus. Their appearance varies from dense black solid opacity to diffuse collections of dots with intervening clear areas. They usually originate near the peripheral edge of the lens (the equator) and extend toward the center of the pupil. They are frequently broader at the base, tapering as they extend centrally. Spokes are more often seen in the anterior cortex, although they often appear in both the anterior and posterior cortex and occasionally only in the posterior cortex. Occasionally strings of vacuoles are aligned in radial spoke-like formations, (see Training Photograph 14); these, too, are graded as cortical spokes (not vacuoles, see 15D-3.4.2).

Cortical opacities may also appear as collections of uneven granular dots that do not form spokes; these are referred to as stippling. Typically, in zones of stippling much of the lens between the dots is clear. Therefore, in estimating area involved by stippling the grader mentally sweeps the opacities together and estimates the area they would cover if contiguous. An example of stippling can be seen in Training Photograph 12 in the anterior image at 12:00.

When grading cortical opacities, the grader mentally combines the anterior and posterior images, then estimates and records the percentage of area in the composite image covered by opacities (spokes and stippling combined) in each subfield. Care should be taken to confirm that the opacities seen in the posterior photograph are not merely out-of-focus images of the same opacities seen anteriorly, so as not to over-estimate the area involved.

3.4.2 Vacuoles

Vacuoles appear as small round or oval, clear, cyst-like spaces with sharply defined borders. With retro-illumination some or all of the borders of a vacuole usually appear dark. Vacuoles may be found at any level in the cortex. Isolated vacuoles are counted up to a maximum of ten in each of the 9 subfields of the central zone. If vacuoles appear as part of a cortical spoke or if their configuration is spoke-like, they are considered as spoking and are not tallied in the vacuole count. Similarly, if vacuoles appear to be part of a posterior subcapsular opacity (see 15D-3.5), they are considered PSC and not included in the vacuole count.

3.4.3 White anterior (and/or posterior) cortical opacities (WACOS)

In the Neitz photographs cortical opacities sometimes are seen as white or yellow-white spots of variable size and shape with hazy, ill-defined borders. WACOS are located mainly in the anterior cortex, but may be seen in both the anterior and posterior Neitz photographs. WACOS do not appear to be flat but rather to have some volume. These opacities vary greatly in number and are usually located centrally rather than peripherally. WACOS are graded in each of the 9 subfields of the central zone as being absent (code 0), questionably present (code 1), present (code 2), or cannot grade (code 8).

Often WACOS appear as pale *gray* opacities in the red reflex photographs. Because of the color difference, there is a risk that the grader may fail to identify them as WACOS and erroneously indicate the presence of an opacity in the red reflex photograph not present in the Neitz photograph (item 300 on the form). Careful comparison of the location of the appearances in the two photographs is necessary to avoid this error.

3.5 Grading of posterior subcapsular (PSC) opacities

Posterior subcapsular (PSC) lens opacities are a less frequent but visually important finding in the older population. They are usually located in the central part of the pupil, and are often accompanied by cortical opacities. PSC opacities develop in what appears to be a single layer immediately anterior to the posterior lens capsule, and thus can be in sharp focus only in the posterior Neitz photograph. Because of the camera's shallow depth-of-field, if the focus is not directly on the PSC opacities they may be somewhat out-of-focus even in the posterior photograph, but will still be sharper than in the anterior photograph. PSC opacities may vary from a darkly opaque network to a thin brown or gray barely discernible haze. These opacities are usually lacy in configuration, often with discrete round or oval "bubbles"or vacuoles within them. Less frequently PSC opacities may appear granular. Any vacuole touching or part of the PSC network is graded as PSC opacity and not included in the vacuole count. Usually PSC opacities have irregular edges, are asymmetrical, and are limited to the central and inner subfields. The Zeiss stereoscopic red reflex photograph may be helpful in determining whether a central opacity that is visible but out-of-focus in the posterior Neitz photograph is in fact a PSC opacity.

The grader evaluates the area covered by PSC opacities by positioning the grid on the posterior Neitz photograph and estimating the percentage of involvement in each of the 9 subfields of the central zone. Because PSC opacities are usually fairly compact, extent of opacity is estimated without any attempt to subtract clear spaces even if a PSC opacity is lacy with small open areas. If PSC is so large or eccentrically located that it extends beyond the central zone, the grader notes this fact in the comments section.

Care should be taken not to confuse a Mittendorf dot, a remnant of the fetal hyaloid vascular system sometimes seen on the posterior capsule, with PSC opacities (Section 15D-4.1).

3.6 Gradability of Neitz photographs (use of "cannot grade")

3.6.1 Anterior Neitz photograph

To grade for cortical opacities, vacuoles, and WACOS, the anterior Neitz photograph must be present and gradable; that is, the anterior photograph must be in reasonably good focus and at least two-thirds of the subfield being graded must be visible and free from major artifacts. Thus, if actual pupillary diameter were less than 4mm (defined by the first tick posterior to the middle circle on each meridian, corresponding to 4mm radius on the grid or 2mm radius of the pupil), all inner subfields would be assigned cannot grade. When grading each of the outer subfields the procedure followed is similar to that used for grading the inner subfields, except that the total area of each subfield used as the denominator in applying the two-thirds rule is variable rather than fixed. However, if a portion of one of the outer subfields is obscured by an eyelid, it is easy to estimate the size of the portion obscured, and this is considered part of the ungradable portion in applying the two-thirds rule.

3.6.2 Posterior Neitz photograph

To grade for posterior subcapsular cataract, the posterior Neitz photograph must be present and at least the central subfield must be gradable. The distance between the anterior and posterior surfaces of the lens varies from subject to subject, and increases with age. The protocol takes this variability into consideration by allowing the posterior photograph to be taken from 3 to 5mm posterior to the anterior photograph. If the distance recorded by the photographer between the anterior and posterior photograph differs substantially from this range (< 2.5mm or > 6.0mm) and no PSC opacities are visible, "cannot grade" is assigned. However, if PSC appears to be present, though out of focus, the grader should attempt to grade it if at all possible, without regard to the distance recorded.

3.7 Opacities absent in the Neitz photographs but present in the Zeiss red reflex photograph

Occasionally opacities not apparent in the Neitz photographs can be identified in the Zeiss red reflex stereo photograph. One explanation is that the very shallow depth of field of the Neitz camera (approximately 1mm) does not allow opacities outside this range to be seen while the greater depth of field of the Zeiss camera captures them. Another reason is that since some stereoscopic effect is present in Zeiss fundus reflex photographs (even though this effect is less than in fundus photographs or in external stereo photographs taken with slit lamp, Donaldson, or other cameras), the grader can determine that opacities are within the portion of the lens under consideration. In contrast, the location of out-of-focus opacities in the Neitz photograph (if they can be seen at all) may not be determinable. The presence of such opacities is recorded in item 300 of the form for the lens as a whole (absent; questionable; definite, but not PSC opacity; definite PSC opacity, with or without other opacities; or cannot grade). Care should be taken not to record any gray opacities seen in the fundus reflex photograph, and identifiable as white cortical opacities in the Neitz photographs, as opacities <u>not</u> present in the Neitz photograph (see WACOS, Section 15D-3.4.3). In addition, because of the optics involved, a slight shift in gaze may change the perceived position of an opacity seen in the fundus reflex photograph from the position observed in the Neitz photograph, thus giving the erroneous impression of the presence of two opacities instead of one.

4.0 OTHER OPACITIES (OBSERVED IN ANY TYPE OF PHOTOGRAPH)

Other opacities are assessed for the lens as a whole.

4.1 Mittendorf dot

Infrequently, a Mittendorf dot, a remnant of the fetal hyaloid vascular system, can be seen attached to the surface of the posterior lens capsule. It is usually located slightly nasal to the center of the lens (see Training Photograph 36 LE) and appears as a small, round or oval, dense black dot approximately 125-350 µm in diameter. It should not be confused with PSC opacities. When a Mittendorf dot is present, the grader checks the appropriate box.

4.2 Pseudoexfoliation of the lens capsule

Occasionally pseudoexfoliation of the lens capsule, a deposit on the anterior lens capsule that has the appearance of a curling or scrolling back of a thin transparent membrane, can be seen in a circular zone inside the edge of the dilated pupil. The everting free ends of the tissue sometimes may appear to curl toward the lens equator in strips of varying widths. Pseudoexfoliation is often very subtle and easily can be missed or mistaken for cortical opacities. The origin of pseudoexfoliation is unknown, but it is sometimes associated with glaucoma. When pseudoexfoliation is present, the grader checks the appropriate box.

4.3 Miscellaneous opacities

Presence of opacities other than those described above is indicated by checking the "other" box and describing the opacities in the Comments section. There are many types of non-age-related lens opacities, which may be seen in any layer of the lens. These include polar cataracts (white or gray circular or oval opacities within the fetal nucleus which may or may not extend beyond it, as in Training Photograph 41); stellate cataracts associated with the Y-sutures, traumatic cataracts (one type of which appears as a central rosette formation in Training Photograph 33), and cataracts of unknown origin (such as the "barbed-wire" opacity seen posteriorly in Training Photograph 39). Vitreous opacities may also confound grading. For example, in Training Photograph 38 a scattered granular pattern can be seen in the posterior photograph; these opacities are asteroid bodies in the anterior vitreous.

5.0 GRADING IRIS PIGMENTATION (COLOR) IN ZEISS STEREO RED REFLEX PHOTOGRAPHS

5.1 Introduction

The iris is a thin membrane separating the anterior and posterior chambers. Peripherally the iris is continuous with the ciliary body; anteriorly, in the undilated state, the pupillary margins rest on the surface of the lens. The iris consists of a layer of mesothelium anteriorly, a stroma made up mostly of blood vessels centrally and two layers of pigment epithelium posteriorly. Located within the stroma are melanin-containing pigment cells which vary greatly in number. The color of the iris depends mainly on the presence and number of these cells. When light passes through the translucent stroma unimpeded by stromal pigment cells, the rays of shorter wavelength (blue) are reflected back preferentially, resulting is a blue-appearing iris.(2) This phenomenon is analogous to the sky appearing blue due to scattering (diffraction) of light as it passes through atmospheric haze. As the melanin-containing pigment cells in the stroma increase in number, the iris color changes from blue to various shades and combinations of blue, green, yellow and brown.

In the lightly-pigmented iris the radial, thick-walled, opaque iris blood vessels are well defined, appearing as slightly curving white lines that run from the periphery of the iris to within about 1mm of the pupillary margin, where they end by joining a circular vessel at the "collarette". Between the collarette and the pupil margin, the color of the iris often appears darker, in part because the stroma is thinner, allowing the dark color of the pigment epithelium to be seen more clearly, and in part because of the black fringe of pigment epithelium that is directly visible at the pupil margin (see Iris Standard Photograph 1, with undilated pupil). The zone of iris between the collarette and the pupil margin is called the pupillary zone to distinguish it from the remainder of the iris, called the ciliary zone. When the pupil is dilated the pupillary zone is pulled partially under the ciliary zone, so that it appears narrower and is often in partial shadow (see Iris Standard Photograph 1, with dilated pupil). When grading iris pigmentation the pupillary zone is excluded from consideration. Also excluded are iris crypts and iris freckles (see below). The characteristic assessed is the degree of *browness* of the iris stroma, on the blue-green-yellow-light brown-dark brown continuum.

The unknown photograph to be classified, taken with the pupil dilated, is compared with a series of standard photographs, also taken with the pupil dilated. An undilated iris photograph might be preferable, but since AREDS lens and fundus photographs require dilation, obtaining the undilated photograph would necessitate an additional session (considered unjustifiably inconvenient for the participant). Undilated variants of two of the standard photographs are provided to illustrate iris anatomy.

5.2 Conditions which may confound grading

Several conditions may make it difficult to judge the density of the stromal pigmentation. A portion of the iris may be obscured (for example, when a broad arcus senilis of the cornea is present). Overall pigmentation may appear darker (blacker) because of the presence of many iris crypts, through which the dark pigment epithelium of the iris is plainly visible. Similarly, the presence of many iris freckles or nevi may give the impression of a browner iris. These conditions, described below, should be ignored when assessing iris pigmentation.

Arcus senilis, an aggregate of lipid material ringing the cornea, is seen as a white or grayish-white opaque or nearly opaque band located near the limbus and separated from it by a narrow uninvolved clear band. The opacity may involve only one or two clock hours or may encircle the cornea completely. If the opaque band is broad and completely encircles the cornea, it may make grading for iris pigmentation difficult when the pupil is dilated. The grader should select an area where the band is narrowest and grade the area between the posterior edge of the band and the collarette (or the pupil margin, if the collarette is not visible).

Iris crypts are openings or spaces in the stroma through which the dark pigment epithelium below can be seen. The blackness of the exposed pigment epithelium is not a consideration in the evaluation of iris pigmentation.

Iris freckles are isolated spots of brown or reddish brown pigment located in the superficial layers of the iris. They appear to be "thin" (as opposed to the dense or "solid" appearance of a nevus) and vary widely in shape and size. Prominent iris freckles can be seen in Iris Standard Photograph 2 (undilated pupil) at 11:30 and 5:30. At 4:30, two smaller, lighter brown freckles can be seen (almost touching one another). Near them (toward the pupil margin) is an iris crypt. All of these features can also be seen in Iris Standard Photograph 2 (pupil dilated), but less clearly. A small freckle is visible at 5:00 near the limbus in Iris Standard Photograph 3.

An **iris nevus** is a solid-appearing dark area with regular or irregular borders. Nevi are usually darker, fewer in number and appear to extend deeper into the stroma than freckles. They are thought to be composed of groups of melanophores within the stroma and are benign.

5.3 Grading iris pigmentation (color)

In **Iris Standard 1** there is little or no evidence of stromal pigment. Except for a tiny freckle at 6:00 over the collarette, no brown pigmentation can be seen in the iris. The gray-white radial blood vessels are prominent when the pupil is small (i.e., in the undilated variant of Iris Standard 1), but are less distinct when it is dilated. Because of the paucity of stromal melanophores, the dark pigment epithelium beneath the stroma can be seen easily through the iris crypts (to be ignored when evaluating the degree of pigmentation present). Photographs with iris pigmentation equal to or less than that in Iris Standard 1 are graded 1. The dilated pupil versions of the Iris Standard Photographs are to be used in grading.

In **Iris Standard 2** there is an increase in pigmentation, which results in the appearance of a very faint wash of pale brown throughout the iris and leads to a muted graying of the color. The radial blood vessels are prominent (in the undilated variant) but appear more yellow-gray in color, reflecting the increase in pigmentation. The iris crypts are not as noticeable as in Iris Standard 1. The iris freckles, described above (Section 15D-5.2) are to be ignored. Eyes with iris pigmentation greater than Standard 1, but equal to or less than Standard 2, are graded 2.

In **Iris Standard 3** there is a marked increase in pigmentation from the previous standard, giving the iris an overall appearance of a light to medium brown. Eyes with iris pigmentation greater than Standard 2, but equal to or less than Standard 3, are graded 3.

Eyes with iris pigmentation greater than Standard 3, are graded 4. Cannot grade, code 8, is assigned when iris pigmentation cannot be determined.

6.0 REFERENCES

1. Klein BEK, Magli YL, Neider MW, Klein R. Wisconsin System for Classification of Cataracts from Photographs, NTIS Accession No. PB90-138306, US Department of Commerce, National Technical Information Service, Springfield, VA 22161.

2. Berliner ML: Biomicroscopy of the eye: Slit lamp microscopy of the living eye. Vol II, New York, Harper & Bros, 1949.

3. Sparrow JM, Bron AJ, Brown NAP, Ayliffe W, Hill AR: The Oxford Clinical Cataract Classification and Grading System. *Intl Ophthalmol* 1986;9:207-225.

Exhibit 15D-1. DETAILED LENS GRADING FORM

Exhibit 15D-2. GRADING GRID