ANNEX II: Atlas of histopathological lesions of Isolated Chicken Eyes
(from Triskelion, Zeist, The Netherlands)

1. Introduction
In the Isolated Chicken Eye test (ICE) the eyes (cornea) of spring chickens acquired from the slaughter house are exposed to test chemicals according to standardized protocols. At the end of the test, the eyes are collected and processed and the cornea is evaluated for histopathological changes by light microscopy. The goal of this atlas is to present photomicrographs of chicken corneas, untreated as well as treated, and to show a variety of possible histopathological changes.

2. Semi-quantitative microscopic evaluation of the cornea
Eyes were fixed in phosphate buffered formalin, trimmed, embedded in paraffin wax, sectioned at 5 µm and stained with Periodic Acid Schiff (unless indicated otherwise). The grading of the changes observed are based on the criteria given in Table 1 below. This set of criteria proposes a semi-quantitative evaluation which is as objective as possible and enables comparison of effects caused by different test chemicals. Using this system, an experienced observer should be able to detect subtle changes and discriminate treatment-related changes from artefacts.
Table 1. Semi-quantitative scoring system used for Isolated Chicken Eyes that were fixed, trimmed, embedded in paraffin wax, sectioned and stained. Photomicrographs of epithelial erosion, epithelial vacuolation, epithelial necrosis, stromal effects and endothelial necrosis are shown in section 4.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Observation</th>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Epithelium:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>erosion</td>
<td>Very slight</td>
<td>½</td>
<td>Few single cells up to the entire single superficial layer</td>
</tr>
<tr>
<td></td>
<td>Slight</td>
<td>1</td>
<td>Up to 3 layers are gone</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>2</td>
<td>Up to 50 % of the epithelial layer is gone*</td>
</tr>
<tr>
<td></td>
<td>Severe</td>
<td>3</td>
<td>Epithelial layer is gone up to the basement membrane</td>
</tr>
<tr>
<td><strong>Epithelium:</strong> vacuolation</td>
<td>Very slight</td>
<td>½</td>
<td>Few scattered cells</td>
</tr>
<tr>
<td></td>
<td>Slight</td>
<td>1</td>
<td>Groups of vacuolated cells or single string of cells with small vacuoles</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>2</td>
<td>Up to 50% of the epithelium consists of vacuolated cells</td>
</tr>
<tr>
<td></td>
<td>Severe</td>
<td>3</td>
<td>50 – 100% of the epithelium consists of vacuolated cells</td>
</tr>
<tr>
<td><em>Separately scored for the top, mid, and lower parts of the epithelium</em>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Epithelium:</strong> necrosis ***</td>
<td>Normal</td>
<td>-</td>
<td>&lt; 10 necrotic cells</td>
</tr>
<tr>
<td></td>
<td>Very slight</td>
<td>½</td>
<td>10 – 20 necrotic cells</td>
</tr>
<tr>
<td></td>
<td>Slight</td>
<td>1</td>
<td>20 – 40 necrotic cells</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>2</td>
<td>Many necrotic cells but &lt; 50% of the epithelial layer*</td>
</tr>
<tr>
<td></td>
<td>Severe</td>
<td>3</td>
<td>50 – 100% of the epithelial layer is necrotic.</td>
</tr>
<tr>
<td><strong>Stroma:</strong> pyknotic nuclei†, ††, †††</td>
<td>Normal</td>
<td>-</td>
<td>&lt; 5 pyknotic nuclei</td>
</tr>
<tr>
<td><em>In top or bottom region</em></td>
<td>Slight</td>
<td>1</td>
<td>5 – 10 pyknotic nuclei</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>2</td>
<td>&gt; 10 pyknotic nuclei</td>
</tr>
<tr>
<td><strong>Stromal disorder of fibres</strong>†††</td>
<td>Present</td>
<td>P</td>
<td>Irregular appearance of the fibres.</td>
</tr>
<tr>
<td><strong>Endothelium:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>necrosis</td>
<td>Present</td>
<td>P</td>
<td>The endothelium consists of only one layer, so a grade is not relevant</td>
</tr>
</tbody>
</table>

*Over the entire cornea except in case of test chemicals (e.g. some solid chemicals) causing localized effects. In this case the evaluation should be based on the localized effects at the site(s) of exposure.

**Top, mid and lower parts represent equal one third parts of the epithelial layer each. If the top layer is gone, the mid layer will not become the ‘new’ top layer, but is still the mid layer (see figure 1).

***Includes also detached cells.

† Necrotic cells are counted across the entire length of the cornea (there is no need for a specific fixed length to report cell counts because the entire length of the cornea is consistent on each slide as there is almost no variation at all in the size of the chicken eyes used and in the size of the samples evaluated microscopically). The scoring system uses absolute cell counts from ‘normal’ to ‘slight’, versus a percentage for ‘moderate’ and ‘severe’. This is due to the way the evaluation is performed by the examiner: necrotic cells are seen as individual items. If there are more, they are usually scattered. Therefore the examiner counts them to get an impression of the amount of necrosis. This is in contrast to epithelial erosion, which the first thing the examiner notices is that part of the epithelium is missing, so it makes sense to use an estimated percentage-loss.

†† The ICE test includes precise measurement of the thickness of the cornea at evaluation with the slit lamp microscope. Therefore, swelling of the stroma is not separately scored during the subsequent histopathological evaluation.

†††The stromal effects that are scored consist of (1) pyknotic nuclei, which originate from the scoring system used by Maurer (2001) based on his observations in corneas of rabbits after in vivo exposure (described as keratocyte loss/necrosis), and of (2) disorder of fibres. Regarding (1), the presence of pyknotic nuclei is observed only occasionally and the development of pyknotic nuclei is proposed to be dependent on the depth of injury and/or the inflammation process of the cornea (in vivo). Furthermore, due to the elongated form of the stromal fibroblasts, normal nuclei could be misleadingly considered as pyknotic nuclei depending on the section orientation of cells. Regarding (2), the observation and scoring of disorder of fibres may be difficult because the stromal fibres already show a “natural” disorder. The processing of the cornea for microscopy can also contribute to an artificial disorder of stromal fibres. In both cases (pyknotic nuclei and disorder of fibres), these observations coincide with severe corneal effects already observed by the slit-lamp microscope observations, and with effects observed in the mid and/or lower epithelial layer.
Additional terms observations

Wrinkling Epithelial layer is wrinkled but the basement membrane is not.
Undulating Epithelial layer including the basement membrane is wrinkled.
Detachment Epithelial layer is (partly) detached from the basement membrane.

The terms are descriptive. Their relevance is difficult to assess, but these findings never occur in controls and are definitely treatment related.

General

Unless otherwise indicated, lesions are often diffuse. In ‘diffuse’ lesions the central part of the cornea is usually more affected than the peripheral part. This may be due to the fact that the test chemical, which is applied on the centre of the cornea, dilutes when it flows to the peripheral parts of the cornea. In contrast, lesions can be called focal or multifocal if they are actually confined to certain spots. This may be observed when the test chemical is a powder.

The corneal parts directly adjacent to the limbus should be ignored when scoring.

In the case of ‘diffuse’ lesions caused e.g. by liquid test chemical, when scoring the histopathological change ‘vacuolation’ the whole picture must be taken into account. For example: the epithelial layer shows complete vacuolation of the mid part at one or a few spots. Although at those spots 100% of the mid layer is vacuolated the criterion ‘groups of vacuolated cells’ applies, hence: score 1 (slight) for mid layer. In contrast, in case of solid or viscous test chemicals that cause local effects, scoring should be conducted based on these localized effects.

For scoring erosion the approach is slightly different: if only part of the epithelial layer is no longer present, up to the basement membrane, this clearly shows that the test chemical is able to damage the entire epithelium in that way, so the score 3 (severe) is justified. This would also be the case for focal lesions produced by powders.

Histopathological changes should only be scored when they are actually present in the slide. Any assumption should not be scored. For example: when the top layer is completely gone one may assume that necrosis of the cells of the top layer (i.e. the top one third part of the epithelial layer) may have been the cause of the erosion. However, only the erosion should be scored. If necrotic cells are detached/eroded from the epithelial layer, but still present in the slide, they should be counted. Sometimes a combination of changes is present, for example there is severe erosion but part of the epithelium is still present and shows necrosis. Then both changes should be scored.

Occasionally, part of the epithelial layer is detached from the basement membrane. This should be mentioned as a ‘note’.
**Vacuolation effects**

Vacuolation is a degenerative change. The vacuoles may represent accumulations of water, lipids or (parts of) damaged cellular organelles. The cause may be a pathological metabolic change of the cell or damage of the cellular membrane resulting in the cell losing the ability to maintain homeostasis. Either way, the vacuolation is an intracellular process. Vacuoles are spherical and usually empty spaces of variable size, sometimes causing considerable enlargement of the cell (‘ballooning’). Very fine vacuolation causes a foamy appearance of the cytoplasm.

Sometimes a small space around the nuclei (like a halo) can be observed. This represents a shrinking artefact and should not be mistaken for vacuolation. Histological processing of the tissue may result in displacement of the nucleus, leaving an open space in the cell (ghost cells). This is also to be considered an artefact.

A degenerating cell may recover unless a point of no return is reached and then the cell dies. If the cell membranes of adjacent cells containing large vacuoles disintegrate, the large vacuoles merge. The epithelial layer above then loses connection with the below layer. At that point this change should be scored as ‘erosion’.

When evaluating vacuolation, the part of the epithelial layer in which the effects are observed should be indicated: top (outer part of the epithelial layer), mid or low (closest to Bowman’s membrane). As shown in Figure 1, the top, mid and lower parts represent equal one third parts of the epithelial layer each. If the top layer is gone, the mid layer will not become the ‘new’ top layer, but is still the mid layer. In contrast to the scores for vacuolation (for three different layers), the scores for erosion and necrosis should be applied to the entire cornea.

![Figure 1: Schematic representation of the top, mid and lower layers of the ICE epithelium](image)

**Artefacts**

Treatment of the cornea may result in damage of intercellular junctions known as desmosomes. This may show in the slide as regular intercellular ‘cracks’ (expanded intercellular spaces). It is unclear whether the histotechnical procedures may contribute to the visibility of these ‘cracks’. This phenomenon may sometimes resemble vacuolation at first sight. However, it should not be scored as vacuolation, because it does not represent an intracellular degenerative process as described above. When such effects are observed they are always accompanied by other histopathological changes.
scored within the prediction model, so they do not need to be taken into account in the scoring of the histopathological effects.

Indeed, when the microscopic slides are evaluated, the examiner must be aware of the possibility that artefacts may cause confounding morphological changes. Commonly encountered artefacts should be recognised as such and should not be confused with treatment-related pathological changes (pictures of various artefacts are presented in section 4.8). Some examples include:

- Variation in staining intensity. This may occur due to slight differences between batches of the staining chemicals or the staining procedures applied (see also notes in section 4.3).
- ‘Saw teeth appearance’. The top layer of the epithelium shows a regular pattern resembling the appearance of saw teeth. This might be mistaken for very slight erosion, but is, in fact, the result of the cutting procedure which occasionally results in this phenomenon.
- Complete detachment of the endothelium. This is occasionally observed. The endothelium as such looks fine, however, it has apparently detached from the cornea and is present at an unusual location, for example double folded and adjacent to the lens. This can never be the effect of a test compound, but should be recognised as a histotechnical artefact.
- ‘Cracks’ or folds in the tissue may occur during the histological procedure as described above.
- Abrupt absence of part of the epithelium
- Shrinking artefact resulting in a clear halo around the nuclei.
- Ghost cells resulting from nuclear displacement.

**Staining of the histological slides**

The treated isolated chicken eyes are collected in a neutral aqueous phosphate buffered 4% solution of formaldehyde at termination, i.e. 4 hours after treatment, of the standard ICE test according to the OECD TG 438\(^1\). For this purpose, the eyes are first incised almost completely in half with a scalpel just behind the level of the lens and through the vitreous body, leaving a part of the posterior tissue still attached where eyes can be held (that will later be discarded) to ensure that the cornea is not damaged during manipulation by dropping on the surface. The sectioned eyes are placed in a container with approximately 20 mL of formalin. After fixation for at least 24 hours, the tissue is trimmed with scissors in such a way that a thin piece containing the entire cornea and the adjacent sclera is embedded in paraffin wax. Longitudinal serial slides (sectioned at 5 µm) are prepared from the central area of the cornea and further processed with the staining. The directions given in the manual AFIP Laboratory Methods in Histotechnology\(^2\) are followed using the Periodic Acid-Schiff (PAS) staining as described previously\(^3\).

---


Semi-quantitative microscopic evaluation of PAS stained corneas is then performed according to the criteria described in the present document.

Staining histological slides alternatively with HE (haematoxylin and eosin) also gives excellent results. However, a better visibility of the basement membrane can be obtained when PAS is used. Apart from the effect on the visibility of the basement membrane, both stainings are suitable for histopathological evaluation of all relevant endpoints in the ICE test. To illustrate the differences in appearance of both types of staining some examples are presented in section 4.9.

Peer review

The laboratory conducting the histopathological evaluation of the isolated chicken eyes should have a peer review system in place, where a proportion of the slides (e.g., 1 out of 3) are re-evaluated by another person. This enhances the quality, consistency and reproducibility of the evaluation. Both the first evaluator and the peer reviewer should have experience in evaluating isolated chicken eyes and the application of the scoring system.

3. Histopathology Criteria for Identification of test chemicals according to UN GHS

Currently only criteria for identification of UN GHS category 1 test chemicals have been developed. The International Association for Soaps, Detergents and Maintenance Products (A.I.S.E.) conducted an in vitro study from 2010 to 2012 where specific ICE histopathological effects were found to be correlated with serious eye damage classification induced by non-extreme pH detergents\(^4,5\). The study comprehended a total of 30 non-extreme pH detergents (2<pH<11.5)\(^4\) and 18 extreme pH detergents (pH \(\leq 2\) or pH \(\geq 11.5\))\(^5\). Epithelial vacuolation (mid and lower layers) and epithelial erosion (at least moderate level) were found to be the most typical histopathological effects induced by the non-extreme pH detergents classified in vitro as UN GHS Cat. 1. Use of these histopathology criteria substantially increased the sensitivity of the standard ICE prediction model for UN GHS Cat. 1 identification (from 0% to at least 75%, n=8) whilst maintaining a good concordance (73%, n=30), and an acceptable specificity (from 100% to 73%, n=22). In particular, it allowed correctly identifying 5 of 6 non-extreme pH detergents classified as UN GHS Cat. 1 based on in vivo persistence of effects i.e., having tissue effects that do not reverse 21 days after treatment and that do not lead to severity of effects that would warrant a UN GHS Cat. 1 classification\(^4\). In contrast, for extreme pH detergents, 5 of the 6 tested in vivo UN GHS Cat. 1 were classified in vivo due to severity of effects and not persistence. In this case, the A.I.S.E. histopathology criteria did not improve the sensitivity of the standard ICE test method (83%, n=6), whilst it strongly decreased specificity (from 83% to 33%, n=12), and concordance (from 83% to 50%, n=18)\(^5\).

---


These data indicate that there are specific applicability domains for the use of the ICE histopathology for detergents that are likely based on the mode of action of the tested detergents. Indeed, the decision criteria developed by A.I.S.E. (described below) were found to be applicable to non-extreme pH detergents but not to extreme pH detergents. In order to expand the applicability of the ICE histopathology decision criteria to other chemistries it would be necessary to generate appropriate and relevant data to demonstrate such applicability.

3.1. Histopathology Criteria for Identification of Non-Extreme pH Detergents as UN GHS Cat. 1

Based on the study described above A.I.S.E. developed decision criteria that are to be used in addition to the standard validated ICE prediction model as described in OECD TG 438 (see table 2). The A.I.S.E. histopathology decision criteria shown in Table 2 were found most suitable to identify UN GHS Cat. 1 detergent and cleaning products having non-extreme pH (2<pH<11.5) that are classified in vivo mainly based on persistence of effects, and could be used in addition to the standard validated ICE prediction model as described in OECD TG 438. Furthermore, the between-laboratory reproducibility of the below criteria is currently under evaluation.

Table 2: Histopathology decision criteria recommended to be used in addition to the standard validated ICE test method (OECD TG 438) for the identification of UN GHS Cat. 1 non-extreme pH detergents* (2<pH<11.5)

<table>
<thead>
<tr>
<th>Tissue layer</th>
<th>Effects triggering eye serious damage (GHS Cat 1) identification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelium</td>
<td>- erosion ≥ moderate (score 2) in at least 2 out of 3 eyes</td>
</tr>
<tr>
<td></td>
<td>- and/or, any vacuolation (≥ very slight, score ½) observed in the mid and/or lower parts in at least 2 out of 3 eyes</td>
</tr>
<tr>
<td></td>
<td>- or, if erosion ≥ moderate (score 2) in 1 out of 3 eyes + vacuolation ≥ very slight in mid and/or lower part (score ½) is observed in at least another eye out of the 3 eyes</td>
</tr>
<tr>
<td></td>
<td>- and/or, necrosis ≥ moderate (score 2) observed in at least 2 out of 3 eyes</td>
</tr>
</tbody>
</table>

* Detergents here are defined as a mixture (excluding dilutions of single surfactant) containing one or more surfactants at a final concentration of > 3%, intended for washing and cleaning processes. Detergents may be in any form (liquid, powder, paste, bar, cake, moulded piece, shape, etc.) and marketed for or used in household, or institutional or industrial purposes.

In addition, in case stromal pyknotic nuclei scores ≥ slight (score 1) in at least 2 out of 3 eyes are observed; or any endothelium effects are observed in at least 2 out of 3 eyes, such effects should be noted as observations to give indication on the severity of effects. Such effects are however not integral part of the decision criteria due to the fact that according to depth of injury principle (Maurer et al. 2002), effects on stroma and endothelium shall occur only if effects in the mid and/or lower epithelial layer are already observed.
4 Atlas

The slides were scanned with 3DHistech Midi scan and pictures were prepared from the scans with Pannoramic Viewer software, except Fig 4.4.8, 4.4.9 and 4.6.2, that were made using a Zeiss AxioCam ICc 1 digital camera mounted on a microscope. Every effort was made to present pictures of optimal quality. However, it was not always possible to retrieve pictures with the presence of a representative lesion without artefacts, perfectly stained and with all parts of the photomicrograph in focus.

Multiple pictures for similar observations were included to show a certain bandwidth of biological variation for each observation, of which one should be aware. Furthermore, slight variations in the histotechnical process (the technician involved, the temperature, thickness of the slide, etc…) can also cause variation in morphology.

The collection of slides with representative morphology will continue to increase in the future. A regular update of the atlas will be made with new observations added and, if better quality pictures become available, pictures of lesser quality will be replaced.

4.1 General

Figure 4.1.1 Spring chicken as eye-donor.
Fig. 4.1.2 Cross section of the chicken eye.
(Adapted from: http://www.class.cvm.uiuc.edu/eurell/eye14.htm).

Fig. 4.1.3 After trimming, only the front part of the eye is further processed.
4.2 Control cornea

Fig. 4.2.1 Control cornea (treated with physiological saline).

Fig. 4.2.2 Control cornea. A normal epithelium consists of 6-8 layers of epithelial cells. Basement membrane and Bowman’s membrane between the epithelium and stroma.
4.3 Epithelium: erosion

Fig. 4.3.1 Very slight erosion (score ½): few single cells up to the entire single superficial layer.

Fig. 4.3.2 Very slight erosion (score ½): few single cells up to the entire single superficial layer.
Fig. 4.3.3. Very slight erosion (score ½): few single cells up to the entire single superficial layer.

Fig. 4.3.4 Slight erosion (score 1): up to three layers are gone.
Fig. 4.3.5 Moderate erosion (score 2): up to 50% of the epithelial layer is gone.

Fig. 4.3.6 Moderate erosion (score 2): up to 50% of the epithelial layer is gone.
Fig. 4.3.7 Moderate erosion (score 2): up to 50% of the epithelial layer is gone.

Note: Figures 4.3.5 to 4.3.7 show that subtle variations in morphology may occur due to, for example, the result of the staining, the visibility of individual cells, the quality of the slide, etc. The conclusion, however, is that up to 50% of the epithelial layer is gone; hence the diagnosis ‘moderate erosion’ (score 2).

Fig. 4.3.8 Severe erosion (score 3): epithelial layer is gone up to the basement membrane. Most of the epithelium is gone. Bowman’s membrane is still present.
Fig. 4.3.9 Severe erosion (score 3): epithelial layer is gone up to the basement membrane. Basement membrane and Bowman’s membrane are gone.

Note: Figures 4.3.8 to 4.3.10 show that subtle variations in morphology may occur due to, for example, the result of the staining, the visibility of individual cells, the quality of the slide, etc. The conclusion, however, is that the epithelial layer is gone up to the basement membrane; hence the diagnosis ‘severe erosion’ (score 3).
4.4 Epithelium: vacuolation

Fig. 4.4.1 Control cornea.

Fig. 4.4.2 Very slight vacuolation (score ½), low: few scattered cells (arrows). Very slight erosion (score ½): few single cells up to the entire single superficial layer.
Fig. 4.4.3 Very slight vacuolation (score ½), low: few scattered cells (arrows). Very slight erosion (score ½): few single cells up to the entire single superficial layer.

Fig. 4.4.4 Slight vacuolation (score 1) in top layer: groups of vacuolated cells or single string of cells with small vacuoles.
Fig. 4.4.5 Slight vacuolation (score 1) in mid- and low layer: groups of vacuolated cells or single string of cells with small vacuoles. Slight erosion (score 1): up to three layers are gone.

Fig. 4.4.6 Slight vacuolation (score 1) in mid- and low layers (arrow): groups of vacuolated cells or single string of cells with small vacuoles. Moderate erosion (score 2): up to 50% of the epithelial layer is gone.
Addendum to Draft Revised GD 160 v. 21 Dec. 2016

Fig. 4.4.7 Moderate vacuolation (score 2) in top layer: Up to 50% of the epithelium consists of vacuolated cells. Slight erosion (score 1): up to three layers are gone.

Fig. 4.4.8 Moderate vacuolation (score 2) in top layer: Up to 50% of the epithelium consists of vacuolated cells. Wrinkling of epithelium (partly detached from the basement membrane). Pyknotic nuclei in stroma. Haematoxylin and eosin staining.
Addendum to Draft Revised GD 160 v. 21 Dec. 2016

Fig. 4.4.9 Higher magnification of figure 4.4.8.

Fig. 4.4.10 Severe vacuolation (score 3) in mid layer: 50 – 100% of the epithelium consists of vacuolated cells.
4.5  Epithelium: necrosis

Fig. 4.5.1 Control cornea.

Fig. 4.5.2 Slight necrosis (score 1) of the epithelial layer. Necrotic cells are indicated by the arrows. This picture only shows part of the cornea, but along the entire length of the cornea 20 – 40 necrotic cells were observed.
Fig. 4.5.3 Moderate necrosis (score 2) of the epithelial layer. Several necrotic epithelial cells with pyknotic nucleus (arrows) are seen. This picture only shows part of the cornea, but along the entire cornea many (> 40) of these necrotic cells but less than 50% of the epithelial layer were observed.

Fig. 4.5.4 Moderate necrosis (score 2) of the epithelial layer. Most cells of the superficial epithelial layer show signs of necrosis, i.e. pyknotic nuclei and disintegration (arrows). This picture only shows part of the cornea, but along the entire cornea many (> 40) of these necrotic cells but less than 50% of the epithelial layer were observed.
Fig. 4.5.5 Focal severe necrosis (score 3) of the epithelial layer. Part of the squamous epithelium is disconnected: erosion (between arrows). Underneath a group of necrotic epithelial cells. Epithelial cells at both sides are normal. In this case the test substance was a solid. Hence, in contrast to exposure to a fluid, the lesions are present focally and the scores are applied to the affected area.

Fig. 4.5.6 Higher magnification of Fig. 4.5.2: Necrotic epithelial cells: cells are disintegrated and show pyknotic nuclei and debris (arrows).
Fig. 4.5.7 Severe necrosis (score 3) of the epithelial layer (arrows). No viable epithelial cell is left. Also necrosis of the stroma.

Fig. 4.5.8 Severe necrosis (score 3) of the epithelial layer. The entire layer consists of necrotic ghost cells.
4.6  Effects on the stroma

Fig. 4.6.1 Control cornea.

Fig. 4.6.2 Few pyknotic nuclei (arrows) in stroma (score 1: 5 – 10 pyknotic nuclei)
Fig. 4.6.3 Necrosis of stromal keratocytes in which nuclei are completely gone. Epithelial necrosis is also observed.

Fig. 4.6.4 Disorder of stromal fibres. Necrosis of stromal keratocytes and endothelial necrosis are also observed.
4.7 Effects on the endothelium

Fig. 4.7.1 Control cornea. Normal endothelium.

Fig. 4.7.2 Necrosis of endothelium in which nuclei are gone. Amorphous remnant.
4.8 Artefacts

Fig. 4.8.1 Artefact: saw tooth pattern of the squamous layer.

Fig. 4.8.2 Artefact: folds in the tissue slide (arrows).
Fig. 4.8.3 Artefact: dent in the tissue slide.

Fig. 4.8.4 Artefact: part of the epithelial layer is missing, very abrupt change from normal epithelium (left) to complete absence (right).
Fig. 4.8.5 Artefact: focal disruption of the stroma.

Fig. 4.8.6 Artefact: diagonal cutting pattern in the stroma.
Fig. 4.8.7 Artefact: detachment of endothelial layer including Descemet’s membrane.
4.9 Staining with HE or PAS

In this section photomicrographs of corneas with similar changes are presented side by side, stained with either HE (left) or PAS (right).

**Fig 4.9.1** Very slight epithelial erosion (score ½)

**Fig 4.9.2** Very slight epithelial erosion (score ½)

**Fig 4.9.3** Moderate epithelial erosion (score 2)
Fig 4.9.4 Severe epithelial erosion (score 3)

Fig 4.9.5 Slight epithelial vacuolation (score 1)

Fig 4.9.6 Slight epithelial vacuolation (score 1)
Fig 4.9.7 Very slight epithelial erosion (score ½) and slight epithelial vacuolation (score 1)

Fig 4.9.8 Slight epithelial vacuolation (score 1) and epithelial detachment