

## British Columbia Farmed Fish Auditing and Surveillance Program

### Case Definitions:

#### Lesion Description, Significance, and Criteria for Assigning Severity Scores

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**Introduction:** The British Columbia Ministry of Agriculture had an active Fish Health Auditing and Surveillance Program in which staff inspected farm sites and sampled specimens volunteered by the farm companies for health evaluation. Samples were collected for bacteriology, molecular diagnostics, and histopathology. The histopathology component of the program involves microscopic examination of tissue sections prepared from formalin-fixed liver, heart, trunk kidney, head kidney, spleen, intestinal ceca and exocrine pancreas (added during the third quarter of 2006) and brain (added during the first quarter of 2007). Other organs were included if they had gross lesions (gill and mouth were most common). This document describes the most common lesions in these fish, including these criteria for assigning semiquantitative severity scores (i.e., case definitions): none (0), mild (1), moderate (2), or severe/abundant (3). Lesion and severity scores for each fish are recorded in the spreadsheet <CohenFHAS-Histopathology.xlsx>. In addition, for each fish two summary diagnoses are included:

- 1) Most significant lesion - the lesion thought most likely to impair fish health, even if it does not seem to be of sufficient severity to have killed the fish; and,
- 2) Cause of death - the lesions of sufficient severity to have either caused death directly (e.g., severe hemorrhage in the brain) or be the morphologic evidence of the cause of death (e.g., eosinophilic granules in cells lining vessels in the kidney are evidence that the fish died from systemic disease, even if the specific cause is included on the sections). If a cause of death is listed, it always includes the most significant lesion.

This document is split into chapters by organ. The first page of each chapter has a table that lists lesion abbreviations and good examples or "type specimens" of each lesion severity score (some scores are not included because good examples were never identified). The remainder of each chapter describes the lesion, lesion significance, and criteria for assigning each severity score. Examples of causative organisms are based on common causes of the lesion in farmed salmon in British Columbia.

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Chapter 1. Quality control for all organs on the slide.

Summary of type specimens				
Abbreviation	None score = 0	Mild score = 1	Moderate score = 2	Severe score = 3
ART	kidney: 6-1023-7	2006-1017-4 heart:6-3001-4	2007-2543-2A	none
PFD	2006-1014-1	2006-1033-2	2006-1026-6	none
AHT	2006-1014-1	2006-1019-5	2006-1013-4, - 1585-3	none

- ART = Artifact. Tissue changes that are not inherent in the tissue sampled. Sources of artifact included handling at necropsy, processing, sectioning, and staining. Artifact is scored on the basis that it impedes interpretation of tissue morphology. Examples of artifact include splits, folds, bubbles, or knife marks in tissues. Artifact resulting from postfixation dehydration is scored under PFD (see #2 below), and acid hematin deposits are scored under AHT (see #3 below).

score = 0; sections have no tissue alterations that would impede analysis or photography of any part of the sections.

score = 1; tissue alterations are present, but some areas could still be photographed without artifact, and analysis for lesions is unaffected. This is the most common score for paraffin-embedded sections.

score = 2; tissue alteration prevented analysis for lesions in some areas and photography would be unacceptable anywhere.

score = 3; tissue alterations are too extensive for histopathologic analysis.
- PFD = postfixation dehydration. This is a specific type of artifact. The margins of affected organs have evidence of dehydration after fixation (e.g., nuclei stain dull blue; erythrocyte cytoplasm stains yellow instead of red; cytoplasm of other cells stains pale blue or not at all). The most common cause is removal of tissues from liquid for more than a few minutes (e.g., during shipment or trimming). Other possible causes include fixation in formalin that is too concentrated (e.g., 100% formalin instead of 10% formalin), or transfer to ethanol that is too concentrated (e.g., >70% ethanol) before processing to paraffin, or immersion in hypertonic saltwater formalin.

score = 0; no postfixation dehydration.

score = 1; postfixation dehydration limited to total sectional area less than 500  $\mu$ m in diameter.

score = 2; total sectional area of postfixation dehydration >500  $\mu$ m, but no 10 $\times$  objective lens field is completely affected by PFD.

score = 3; total sectional area of postfixation dehydration fills at least one 10× objective lens field.

3. AHT = Acid hematin. This is a specific type of artifact. Acid hematin is a granular brown pigment that accumulates when tissues are not fixed in neutral buffered fixative and when tissues become acidic during fixation. Acid hematin is birefringent under polarized light; the primary differential, melanin granules, are not birefringent under polarized light. Acid hematin granules are most common where erythrocytes accumulate (e.g., anywhere in the spleen, and around congested blood vessels in the liver). In cases of decreased blood flow, acid hematin can be a "useful artifact" (i.e., consistent with lactic acid as a result of decreased perfusion).
- score = 0; no acid hematin deposits.
- score = 1; acid hematin deposits are limited to total sectional area <500  $\mu\text{m}$  in diameter.
- score = 2; total sectional area of acid hematin deposits >500  $\mu\text{m}$ , but deposits do not prevent analysis of tissues for lesions.
- score = 3; extensive acid hematin deposits impair analysis of tissues for lesions.



Chapter 2. Type specimens for liver scores. Abbreviations are explained after the table.

LIVER (Summary of type specimens)				
Abbreviation	None score = 0	Mild score = 1	Moderate score = 2	Severe score = 3
LAtly	2006-1018-3 2008-3507-3A	2006-4169-7	2006-1021-1	none
VAC	2006-1014-1 2008-3507-3A	2006-1014-3 2006-3643-4	2006-4171-2 2007-1752-2A (gly)	2007-1679-6A 2007-1752-3A (gly) 2008-3171-4A (not gly)
LIP	2006-1014-1	2006-1023-7	2004-3480-4	2008-2307-7A
BPH	2006-1014-1	2006-1018-3	2007-4184-6A	2004-3673-1, 2010-0292-1A 2006-1017-2 2007-1680-1A (subtle)
LFN	2006-1014-1	2004-3481-7	2005-0303-4	2007-1751-3A
BPC	2006-1014-1 2008-3507-3A	2006-4270-3	2006-1026-4	none
CPL	2006-1014-1	2006-1030-1	2007-3987-5A (mild Atly)	none
PMP	2006-1014-1	2006-1030-1	2007-3986-3A (mild Atly)	none
FPL	2006-1014-1	2007-1070-8A	none	none
PVL	2006-1014-1	2006-2181-3	none	none
SSC	2006-1014-1	2006-1017-1	2007-2543-9A (diffuse)	2006-1594-4 2009-1089-7A 2008-0512-1A 2009-0673-3A
AIB	2006-1014-1	2006-1017-1	2006-3004-1	2007-0781-4A
SSF	2006-1014-1	2006-3002-5 (LAtly = 1)	2006-1594-2	2007-0781-4A
MEG	2006-1014-1	2008-2981-2A	none	2006-3214-5
SCN	2006-1014-1	2008-0393-2A	2006-1791-1	2006-1791-2
HHD	2006-1014-1	2008-2307-2A 2009-0673-7A	2007-1053-6A	2006-3212-6 2007-1398-3A
LGR	2006-1014-1	2006-2181-3	2006-1014-5	2006-1009-2

				Liver Scores
LRS	2006-1014-1	none	2007-0195-2A	2006-1593-2
LPS	2006-1014-1	2006-4369-3	2006-4275-10	2007-3987-2A
LLS	2006-1014-1	none	none	none

#### *Quality Control/Quality Assurance*

LA<sub>ty</sub> = Autolysis. Changes in membrane integrity begin immediately after death, and are sometimes aided by leakage of bile onto cells. The margin of the liver adjacent to the gallbladder often has severe lysis, even when tissues further from gallbladder have mild or moderate autolysis; in such cases, the tissues away from the gallbladder are used to assign the LA<sub>ty</sub> score. Autolytic changes are enhanced by handling; therefore, foci with handling artifact are not used for determination of the LA<sub>ty</sub> score. In farmed salmon, autolyzed hepatocytes have hyperchromatic (dark) nuclei and pale cytoplasm; autolyzed hepatocytes tend to lose cell-to-cell contact (detach) and "round up." By comparison, in the primary differential, single cell hepatocellular necrosis, affected hepatocytes also have hyperchromatic (pyknotic) nuclei, but the cytoplasm of necrotic hepatocytes is hypereosinophilic.

Significance: Autolysis decreases the sensitivity and specificity of histopathology.

score = 0; no membrane changes, erythrocytes stain intensely, hepatocyte nuclei are not hyperchromatic.

score = 1; < 1/3 of hepatocyte nuclei are hyperchromatic.

score = 2; > 1/3 but < 2/3 of hepatocyte nuclei are hyperchromatic.

score = 3; > 2/3 of hepatocyte nuclei are hyperchromatic.

#### *Lesions*

1. VAC = vacuoles in hepatocyte cytoplasm. Hepatocellular cytoplasmic vacuoles vary from round (possible lipid) to angular (possible glycogen). These types of vacuoles were rare before 2007 and might be related to the significant increase in the proportion of plant-based components in commercial feeds that has occurred since 2006. Sometimes, hepatocellular vacuoles contain faint eosinophilic granules. Individual hepatocellular vacuoles are scored only in one category: use lipid (LIP) or hydropic degeneration (SCN) when vacuoles clearly fit into these categories. [Note: the VAC score was added to the list of scored lesions in July 2008; scores on earlier submission were added beginning in December 2008.]

Significance: The vacuoles might be normal for fish on high plant-based feeds. Their effect on growth and feed conversion is unknown. The eosinophilic granules might be lysosomes filled with cellular debris (i.e., the result of toxin exposure).

score = 0; vacuoles are absent from most hepatocytes.

score = 1; vacuoles are smaller than or about equal to nuclear diameter.

score = 2; vacuoles are larger than nuclei, but < 2× nuclear diameter.

score = 3; vacuoles are > 2× nuclear diameter.

2. LIP = lipidosis. A change/lesion in hepatocytes; lipid appears as clear, round, well-demarcated, cytoplasmic vacuoles (= lipid vacuoles). Pathologic change is more likely

when the vacuoles are significantly larger than nuclei. When stained with PAS, none of the vacuoles stain (this pattern rules out glycogen in the vacuoles, which would stain red with PAS). Abundant hepatocellular lipid is probably normal for black cod.

Significance: In some species, nearly all hepatocytes have uniform vacuoles that are about the size of nuclei, or smaller; in these cases, hepatocellular lipid probably is a normal form of energy storage. In salmon, hepatocellular fatty change (lipidosis) often occurs when fish are not feeding; it also occurs in cases of inadequate nutrition.

score = 0; hepatocytes have no lipid vacuoles.

score = 1; < 5% of hepatocytes in the section have lipid vacuoles larger than nuclei, or <50% have lipid vacuoles that are smaller than nuclei.

score = 2; 5-20% of hepatocytes in the section have lipid vacuoles larger than nuclei, or 50-100% of hepatocytes have vacuoles that are smaller than nuclei.

score = 3; >20% of hepatocytes in the section have lipid vacuoles that are larger than nuclei.

2. BPH = biliary preductular cell hyperplasia. Biliary preductular cells line the cavity that connects the short interhepatocellular canaliculi with the larger bile ductules. Normally, the cells are spaced so that cross sections of hepatic tubules have no more than 1 nucleus. Significance: Hyperplasia of biliary preductular epithelial cells is evidence of exposure to toxins. The toxins could be produced inside the fish (e.g., bacterial toxins) or come from outside the fish (e.g., from the water or the feed).  
score = 0; no biliary preductular cell hyperplasia;  
score = 1; >1 but < 2/3 of tubular cross sections per 40× objective lens field contain >1 preductular epithelial cell nucleus;  
score = 2; > 2/3 of tubular cross sections per 40× objective lens field contain >1 preductular epithelial cell nucleus;  
score = 3; > 2/3 of tubular cross sections per 40× objective lens field contain >1 preductular epithelial cell nucleus, and hyperplastic cells are piling up on each other.
3. LFN = focal/multifocal necrosis. A lesion primarily of hepatocytes, affected cells have hypereosinophilic coagulated cytoplasm, and pyknotic, karyorrhectic, or karyolytic nuclei. Necrosis associated with *Renibacterium salmoninarum* or *Piscirickettsia salmonis* infection is not scored here; instead, it is scored with the specific *Renibacterium salmoninarum* or *Piscirickettsia salmonis* categories below. Significance: Hepatic necrosis can be caused by inadequate vascular perfusion (e.g., as occurs with harmful algal blooms) or direct cytotoxicity from viral or bacterial infections (e.g., viral hemorrhagic septicemia virus, *Piscirickettsia salmonis*).  
score = 0; no necrotic cells in the section..  
score = 1; total area of necrosis is < 1 40× objective lens field.  
score = 2; total area of necrosis is > 1 40× but < 1 10× objective lens field.  
score = 3; total area of necrosis is >1 10× objective lens field.
4. BPC = basophilic cytoplasm (hepatocytes). Cytoplasmic staining of hepatocytes varies based on the activity of the liver, but cytoplasm is usually eosinophilic. Basophilic

cytoplasm in hepatocytes is an indication of active protein synthesis (the rough endoplasmic reticulum stains basophilic).

Significance: Basophilic hepatocellular cytoplasm is normal in mature females producing protein for deposition in their eggs. In juvenile salmon it might be related to increased protein needed as part an inflammatory response.

score = 0; hepatocytes have eosinophilic cytoplasm or abundant glycogen vacuoles.

score = 1; about 1/3 of hepatocyte cytoplasm is basophilic.

score = 2; about 2/3 of hepatocyte cytoplasm is basophilic.

score = 3; nearly all of hepatocyte cytoplasm is basophilic.

5. CPL = cholangitis/pericholangial leukocytes (lymphocytes, plasma cells, and macrophages): a lesion of the bile ductules and the surrounding connective tissue (adventitia).

Significance: Lymphocytic inflammation around bile ductules is evidence of chronic immune stimulation. This type of inflammation can result from bacteria ascending from the intestine to the liver through the biliary system.

score = 0; <3 leukocytes in the region of every bile ductule in the section.

score = 1; 3 to many leukocytes infiltrate or surround at least one bile ductule in the section, but leukocytes do not extend into the surrounding parenchyma.

score = 2; leukocytes extend into the surrounding parenchyma.

score = 3; none are severe.

6. PMP = Pigments in the liver are usually within macrophages and vary from yellow-brown to yellow-green. Pigments include lipofuscin, hemosiderin, or both. Melanin pigment occurs normally in some salmonids; melanin pigments are not scored here. Significance: Accumulation of lipofuscin in the liver is a nonspecific change that can result from a variety of insults, including rancid feed, low levels of antioxidants in the feed, chronic infections, and exposure to organic contaminants. In pen-reared salmon, hepatic lipofuscin accumulation is a common feature of netpen liver disease (microcystin-LR). Conditions that lead to moderate to abundant hepatic lipofuscin have been associated with decreased growth and survival in several studies. Hemosiderin accumulation in the liver is evidence of increased turnover of red blood cells.  
score = 0; < 1 focus of pigment in most 20× objective lens fields.  
score = 1; > 1 but <2 foci of pigment in most 20× objective lens fields.  
score = 2; ≥2 foci of pigment in most 20× objective lens fields.  
score = 3; none are severe.

7. FPL = focal/multifocal parenchymal leukocytes. Leukocyte aggregates are usually less than 500 µm in diameter and composed mostly of lymphocytes. Significance: these represent foci of chronic immune stimulation (e.g., bacterial infection).  
score = 0; no focal parenchymal leukocytes.  
score = 1; <1 focus of parenchymal leukocytes per 10× objective lens field.  
score = 2; 1-2 foci of parenchymal leukocytes per 10× objective lens field.  
score = 3; none are severe

8. PVL = perivascular leukocytes (eosinophilic granular cells, lymphocytes, and plasma cells). Leukocytes infiltrate the connective tissue (adventitia) around blood vessels. Leukocytes within the tunica intima and tunica media are NOT included in this category. Significance: these probably are foci of chronic immune stimulation (e.g., bacterial infection) that are derived from vasculature.  
 score = 0; <3 leukocytes in the adventitia of any vessel in the section.  
 score = 1; 3 to many leukocytes in the adventitia of at least one vessel in the section, but leukocytes do not extend into the surrounding parenchyma or the muscular tunics of the vessel.  
 score = 2; perivascular leukocytes extend into the surrounding parenchyma, and more than one vessel is involved.  
 score = 3; none are severe.
  
9. SSC = Sinusoidal congestion is the accumulation of greater than normal numbers of erythrocytes in hepatic capillaries/sinusoids. Congested capillaries often have acid hematin deposits.  
 Significance: Sinusoidal congestion probably is a nonspecific result of sinusoidal damage or systemic vasodilators. In BC Atlantic salmon, sinusoidal congestion is an uncommon feature of infection with viral hemorrhagic septicemia virus (VHSV) and *Listonella anguillarum*. Sinusoidal congestion is one of the classic lesions associated with infectious salmon anemia virus (ISAV) infection, but ISAV has never been identified in British Columbia. Sinusoidal congestion has also been described in wild fish (dab) surveyed in the North Atlantic (source: <http://www.cefas.co.uk/publications/aquatic/aemr41.pdf>), but the cause was not determined. I have seen sinusoidal congestion in farmed rainbow trout fed rancid feed with high mycotoxin concentrations (unpublished data). Acid hematin accumulates when tissues are acidic during fixation; therefore, acid hematin deposits in congested foci, but nowhere else in the section, are evidence that the congested foci were acidic. This might have occurred before death as a result of lactic acid accumulation in a region of decreased vascular perfusion.  
 score = 0; hepatic capillaries contain very few erythrocyte.  
 score = 1; erythrocytes in hepatic capillaries are sometimes piled up to 3 cells thick, or foci of sinusoidal erythrocytes are 500 - 1000 µm in diameter.  
 score = 2; erythrocytes in hepatic capillaries are sometimes piled 3 - 6 cells thick, or foci of sinusoidal erythrocytes are 1 - 3 mm in diameter.  
 score = 3; erythrocytes in hepatic capillaries are sometimes piled > 6 cells thick, or piles 3 - 6 cells thick are > 3 mm in diameter.
  
10. AIB = amphophilic/eosinophilic inclusion bodies (in hepatocytes). Golden to amphophilic or eosinophilic cytoplasmic inclusions in hepatocytes are sometimes large, up twice the size of hepatocyte nuclei. The amphophilic form usually occurs in the presence of sinusoidal congestion. The eosinophilic form is associated with large well-demarcated but not-round cytoplasmic vacuoles.  
 Significance: The inclusions might be remnants of ingested erythrocytes; this type of inclusion has not been described with any salmon virus.

- score = 0; hepatocytes have no amphophilic inclusion bodies  
 score = 1; hepatocytes have >0 but <2 amphophilic/eosinophilic inclusion bodies per 40× objective-lens field.  
 score = 2; hepatocytes have >2 but <5 amphophilic/eosinophilic inclusion bodies per 40× objective-lens field.  
 score = 3; hepatocytes have >5 amphophilic/eosinophilic inclusion bodies per 40× objective-lens field.
11. SSF = sinusoidal fibrin. Fibrin deposits are brightly eosinophilic and acellular. Fibrin associated with *Renibacterium salmoninarum* or *Piscirickettsia salmonis* infection is not scored here; instead, it is scored with the specific *Renibacterium salmoninarum* or *Piscirickettsia salmonis* categories below.  
 Significance: Multifocal fibrin deposits in the liver are evidence of endothelial damage, probably from exposure to toxins. The toxins could be of bacterial origin or inflammatory cell origin; toxins in the water or feed are less likely. I have also seen sinusoidal fibrin in fish that are PCR positive for VHSV. Inclusion of fibroblasts in the fibrin is evidence of remodelling and chronicity; lack of fibroblasts is consistent with formation just before death (this is the most common pattern).  
 score = 0; hepatic sinusoids have no fibrin deposits.  
 score = 1; total cross sectional area of fibrin deposits fills <1 40× objective-lens field.  
 score = 2; total cross sectional area of fibrin deposits fills >1 40× objective-lens field, but <1 10× objective-lens field.  
 score = 3; total cross sectional area of fibrin deposits fills >1 10× objective-lens field.
12. MEG = hepatocellular karyomegaly and megalocytosis. Karyomegaly is the most prominent feature of hepatocellular megalocytosis. Hepatocyte nuclei are considered enlarged if they are >2.5× the diameter of normal nuclei.  
 Significance: Hepatocellular karyomegaly/megalocytosis results from sublethal injury to hepatocytes, and affected cells may be able to survive for several months. Hepatic megalocytosis can result from exposure to several types of toxins, including aflatoxins, pyrrolizidine alkaloids, complex chemical mixtures from marine sediment extracts, and the algal toxin microcystin-LR. In BC salmon, megalocytosis is one of the main diagnostic criteria for "net pen liver disease."  
 score = 0; <3 hepatocyte nuclei are >2.5× the diameter of any other hepatocyte nuclei.  
 score = 1; > 3 and <20 hepatocyte nuclei are >2.5× the diameter of any other hepatocyte nuclei.  
 score = 2; karyomegalic hepatocytes are in > 50% of the 40× objective lens fields but are always <10% of the hepatocytes in any 40× objective lens field.  
 score = 3; karyomegalic hepatocytes are in > 50% of the 40× objective lens fields and often involve >10% of the hepatocytes in a 40× objective lens field.
13. SCN = hepatocellular single cell necrosis/apoptosis. Cell death can be normal (apoptosis) or abnormal (single cell necrosis). Acute cell injury is sometimes followed by single cell necrosis, affected hepatocytes have pyknotic nuclei and condensed cytoplasm that often stains more deeply eosinophilic than normal cells. Because of cytoplasmic collapse, individual necrotic cells are sometimes surrounded by a clear ring



or halo. The section might have only remnants of nuclear material (karyorrhectic debris). SCN must be differentiated from artifact. Rough handling can result in hepatocytes with dark-staining cytoplasm, and autolysis results in hyperchromatic nuclei, but only necrosis results in hypereosinophilic cytoplasm.

Significance: Single cell necrosis is evidence of cellular damage. Causes of hepatocellular single cell necrosis have not been well defined in fish. Possible differentials include exposure to toxins (endogenous or exogenous), viral infection (VHSV), or re-oxygenation after a period of hypoxia. Apoptosis occurs with remodelling of the liver in rapidly growing fish that suddenly go off feed about 24 hours before death. Apoptosis is the normal way in which hepatocyte numbers are decreased (i.e., the hepatocytes are not needed when growing fish stop feeding because few to no nutrients are being absorbed into the blood and entering the liver for processing). Single cell necrosis is a common feature of "net pen liver disease."

score = 0; <3 necrotic/apoptotic hepatocytes in the section.

score = 1; <1 necrotic/apoptotic hepatocytes per 40× objective lens field.

score = 2; 1 to 2 necrotic/apoptotic hepatocytes per 40× objective lens field, or 20 to 50 necrotic/apoptotic hepatocytes per section.

score = 3; >2 necrotic/apoptotic hepatocytes per 40× objective lens field.

14. HHD = hepatocellular hydropic degeneration. Acute hepatocyte injury is sometimes followed by hydropic degeneration; cytoplasm of affected hepatocytes is expanded by fine to large foamy vacuoles. Sometimes the vacuoles coalesce and are larger than normal hepatocytes; nuclei of affected cells often have normal morphology.  
Significance: Hepatocellular hydropic degeneration is evidence of cellular damage. Causes of hepatocellular hydropic degeneration necrosis have not been well defined in fish. Possible differentials include exposure to toxins (endogenous or exogenous), or re-oxygenation after a period of hypoxia. It is not associated with VHSV infection.  
score = 0; <3 degenerative hepatocytes in the section.  
score = 1; <1 degenerative hepatocytes per 40× objective lens field.  
score = 2; 1 to 2 degenerative hepatocytes per 40× objective lens field, or 20 to 50 degenerative cells per section.  
score = 3; >2 degenerative hepatocytes per 40× objective lens field.
15. LGR = granulomatous hepatitis. Granulomatous hepatitis spans the range from diffuse granulomatous inflammation to focal granulomas. Granulomas often surround radiating globular material (it can be yellow, green, or pale red, in any combination of these colours); this material is consistent with vaccine material. Lesions with *Renibacterium salmoninarum* or *Piscirickettsia salmonis* are not scored here (instead, use LRS and LPS). When present, radiating globular material in LGR multinucleate giant cells (MNGs) is a key feature differentiating LGR from the primary differential, LRS (LRS occasionally contains MNGs, but LRS granulomas do not contain radiating globular material). In Pacific salmon, a major differential is LLS; do a Twort's stain to look for Gram positive spores.  
Significance: This form of granulomatous hepatitis might be a reaction to a vaccine; differentials include chronic bacterial disease (e.g., *Yersinia ruckeri* or *Renibacterium salmoninarum* infection).

score = 0; the liver has no granulomatous inflammation.

score = 1; total cross sectional area of granulomatous inflammation fills <1 40× objective-lens field.

score = 2; total cross sectional area of granulomatous inflammation fills >1 40× objective-lens field, but <1 10× objective-lens field.

score = 3; total cross sectional area of granulomatous hepatitis fills >1 10× objective-lens field.

16. LRS = *Renibacterium salmoninarum*. *Renibacterium salmoninarum*, the cause of bacterial kidney disease, causes a wide range of lesions in the liver, from acute necrosis with fibrin exudation, to chronic granulomatous inflammation with fibrosis. Numbers of organisms in affected livers varies from almost none to very abundant. Twort's Gram stain works well for highlighting the short Gram-positive rods; however, because the lesions alone are fairly diagnostic, a Gram stain is not routinely done with auditing cases. Infection in the liver is usually associated with infection in other organs (those lesions are scored separately in the chapter for each organ). Unless a Gram stain is done, lesions suggestive of LRS usually are scored in the LGR category (i.e., the LRS prevalence probably underestimates the true prevalence of LRS). Lack of radiating globular material in multinucleate giant cells of LRS is the key feature differentiating granulomatous inflammation of LRS from LGR (radiating globular material is a prominent feature of multinucleate giant cells of LGR).

Significance: *Renibacterium salmoninarum* is a significant cause of mortality, particularly with Pacific salmon.

score = 0; no *R. salmoninarum*-associated lesions in the section.

score = 1; total cross sectional area of *R. salmoninarum*-associated lesions fills <1 40× objective-lens field.

score = 2; total cross sectional area of *R. salmoninarum*-associated lesions fills >1 40× objective-lens field, but <1 10× objective-lens field.

score = 3; total cross sectional area of *R. salmoninarum*-associated lesions fills >1 10× objective-lens field.

17. LPS = *Piscirickettsia salmonis*. *Piscirickettsia salmonis* is a Gram-negative coccoid organism (primitive bacterium) that is the cause of Salmonid Rickettsial Septicemia. Fish with *P. salmonis* commonly present with multiple pale foci in the liver. Organisms are most common in macrophages, where they are about 1 µm in diameter. They can be positively identified using an immunohistochemical stain optimized at the Animal Health Centre; however, morphologic features on H&E are usually sufficient for diagnosis. Microscopically, *P. salmonis* infection is associated with foci of vasculitis, coagulative necrosis, fibrinous peritonitis, and variable numbers of macrophages with cytoplasmic organisms. *P. salmonis* is most easy to identify when a cluster of uniform-diameter organisms occur in an otherwise clear vacuole in macrophages. Differentials include (1) melanin granules, which are brown and smaller; and (2) karyorrhectic debris, which usually have variable diameter and stain more basophilic on H&E. Sensitivity of histopathology and PCR for diagnosis of *P. salmonis* is about the same. Significance: *Piscirickettsia salmonis* is associated with sporadic mortality in pen-reared salmon in BC.



score = 0; no *P. salmonis*-associated lesions in the section.

score = 1; total cross sectional area of *P. salmonis*-associated lesions fills <1 40× objective-lens field, or reaction limited to infected macrophages that can be disseminated throughout the liver.

score = 2; total cross sectional area of *P. salmonis*-associated lesions fills >1 40× objective-lens field, but <1 10× objective-lens field.

score = 3; total cross sectional area of *P. salmonis*-associated lesions fills >1 10× objective-lens field.

18. LLS = *Loma salmonae*. *Loma salmonae* is a microsporidian parasite that forms discreet clusters (xenomas) of spores in the circulatory system. Most xenomas are 50 to 100 µm in diameter. Spores, about 5.5 × 3 µm, have a single polar capsule that is weakly Gram positive and birefringent under polarized light. Reaction to the xenomas varies from none to severe necrotizing vasculitis. Reaction to spores outside of xenomas is granulomatous.  
Significance: Infection with *Loma salmonae* is often associated with inflammation and tissue destruction, but these lesions often do not surround the xenomas. It may be that the xenomas stimulate the release of inflammatory mediators that cause most of the damage in the host.  
score = 0; no *Loma salmonae*.  
score = 1; <7 *Loma salmonae* in the section, and no significant inflammation.  
score = 2; <7 *Loma salmonae* in the section, with significant inflammation, or >7 xenomas in the section with no associated inflammation.  
score = 3; > 7 *Loma salmonae* in the section, with severe associated inflammation.

Chapter 3. Type specimens for kidney scores. Abbreviations are explained after the table.  
Lesions in head kidney and trunk kidney are combined into this chapter.

KIDNEY (Summary of type specimens)				
Abbreviation	None score = 0	Mild Score = 1	Moderate score = 2	Severe score = 3
KAtly	2007-4655-2A	2007-3233-2A	2006-1019-1	
ISH	2007-4655-2A	2005-3191-1	2006-3643-1	2006-3219-1
TEP	2007-4655-2A	2006-4662-4	2005-0629-3 2007-4519-7A	
GRP	2007-4655-2A	2006-2182-1, 3002-5	2006-4369-5	2008-0631-1A
TDI	2007-4655-2A	2007-2820-4A	2005-3586-2	2007-1561-4A
MIN	2007-4655-2A	2006-3643-1	2009-3071-2A	2005-0839-4
HEM	2007-4655-2A	2006-1033-1 2009-0344-3A	2007-0260-4	
EGC	2007-4655-2A	2007-1191-4A	2005-1245-2	2007-3732-4A 2007-4658-2A
IPC	2007-4655-2A	2006-1585-3		
IFB	2007-4655-2A			2009-4680-2A
RTN	2007-4655-2A	2006-2182-1	2006-1019-5	2006-2351-2 2007-0260-4
ICN	2007-4655-2A		2006-4267-14	
IEA	2007-4655-2A		2010-0628-2A(atrophy)	
MGN	A2006-1022-3 P2008-1504-3A	2006-3643-1	2004-3947-3	
KGR	2007-4655-2A	2006-4496-9	2006-1009-2 2006-2349-3	2006-1014-5
KRS	2007-4655-2A	2006-4369-4A	2007-1679-2A	2006-1593-2
KPS	2007-4655-2A	2006-4369-3		2006-4275-10

*Quality Control/Quality Assurance*

Part of kidney examined: scored as yes (Y) or not present (NP) on the slide.

HKN = head kidney; the head kidney is not always sampled for histopathology. However, even when a full section of head kidney is not available, the sample of trunk kidney often contains a region of atubular hematopoietic cells that should have the same range of lesions as the head kidney. If the contiguous sectional area of atubular hematopoietic cells covers an area equivalent to 2 10× objective lens fields (i.e., about 2 × 4 mm), then the head kidney is considered to be present for analysis.

TKN = trunk kidney; this is most commonly sampled for histopathology; it includes a mixture of tubules and interstitial hematopoietic cells.

KAtly = Autolysis. Changes in membrane integrity begin immediately after death. In farmed salmon, autolysis is usually most obvious in the proximal tubules (i.e., proximal tubules will have obvious autolytic changes while adjacent distal tubule morphology will still be fairly normal). Therefore, scores for autolysis are based on the morphology of the most affected tubules. Autolyzed tubular epithelial cells usually have contracted nuclei, and cytoplasm staining remains the same or becomes pale (i.e., autolysis decreases the intensity of cytoplasmic staining). By comparison, in the primary differential, renal tubular necrosis, affected tubular epithelial cells have condensed nuclei (pyknosis), karyorrhexis, or karyolysis, and the cytoplasm of necrotic epithelial cells is hypereosinophilic. Autolysis decreases the crisp definition of the apical brush border of renal tubular epithelial cells, and autolyzed cells tend to pull away from the basement membrane.

Significance: Autolysis decreases the sensitivity and specificity of histopathology.

score = 0; brush border is prominent, tubular epithelial cell nuclei are intact, and cytoplasmic staining is intense.

score = 1; some nuclei are condensed and hyperchromatic, brush border is intact, but not prominent; some tubular epithelial cells have pulled away from the basement membrane.

score = 2; sections of up to 50% of the tubules are composed entirely of cells with condensed nuclei and pale eosinophilic cytoplasm; brush border of affected tubules is not discernable.

score = 3; >50% of nuclei of tubular epithelial cells are hyperchromatic.

*Lesions*

1. ISH = interstitial (hematopoietic) cell hyperplasia. The renal interstitium normally contains basophilic hematopoietic cells; hyperplasia is diagnosed when the numbers of these cells increases. The relative volume of hematopoietic cells varies depending on where in the kidney the sample is taken; therefore, the scoring system comes with the assumption that each kidney is sampled from approximately the same part of each fish. Significance: Interstitial cell hyperplasia results from increased demand for erythrocytes or white blood cells somewhere in the body. In Chinook salmon, this lesion is often

associated with the clinical diagnosis of "Marine anemia"; differentials include infection with *Nucleospora salmonis* and viral erythrocytic necrosis virus (VENV).

score = 0; cross-sectional area of hematopoietic cells is about equal to area of tubules and glomeruli.

score = 1; cross-sectional area of hematopoietic cells is > area of tubules but < 2× area of tubules and glomeruli.

score = 2; cross-sectional area of hematopoietic cells is >2× area of tubules and glomeruli.

score = 3; none are severe.

2. TEP = renal tubular epithelial protein droplets. The cytoplasm of renal tubular epithelium contains protein droplets that are homogeneous, eosinophilic, and range from about 3 to 12 µm in diameter. Droplets are most common in proximal tubules. Nuclei of affected cells are NOT undergoing degeneration.

Significance: Renal tubular epithelial protein droplets are normal in some species, or they may be an indication of glomerular disease (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson).

score = 0; no renal tubular epithelial cells contain cytoplasmic protein droplets.

score = 1; fewer than 10% of renal tubular epithelial cells contain protein droplets.

score = 2; more than 10% of renal tubular epithelial cells contain protein droplets.

score = 3; none are severe.

3. GRP = golden renal pigment. Foci of golden pigment (probably lipofuscin) can occur in several tissues or spaces in the kidney: tubular epithelial cells (intracellular), tubular lumens, urinary spaces, and interstitial macrophages. Pigmented foci vary from 5 to 30 µm in diameter. Intracellular pigment in tubular epithelial cells is sometimes associated with eccentric pyknotic nuclei.

Significance: Accumulation of lipofuscin is a nonspecific change that can result from a variety of insults, including rancid feed, low levels of antioxidants in the feed, chronic infections, and exposure to organic contaminants. When tubular epithelial cells are involved, variation in size of nuclei and cytoplasm is evidence of cellular degeneration and regeneration, and it is consistent with persistent damage to the tubules.

score = 0; the kidney contains ≤1 foci of golden pigment per 5 40× objective lens field.

score = 1; foci of golden pigment >1 per 5 40× objective lens field, but <2 per 40× objective lens field.

score = 2; > 2 foci of golden pigment per 40× objective lens field, but <10% of volume of affected fields is pigmented.

score = 3; > 2 foci of golden pigment per 40× objective lens field, and >10% of volume of affected fields is pigmented.

4. TDI = tubular dilation (of lumen). A renal tubule is considered dilated when luminal diameter is more than 2× the thickness of the tubular epithelium. Dilation associated with MIN is not scored here (see MIN below).

Significance: Dilation of renal components is evidence that flow of urine is abnormal. The most probable cause is some type of flow blockage.

score = 0; tubules are not dilated.

score = 1; <50% of the tubules are dilated.

score = 2; >50% of the tubules are dilated.

score = 3; >50% of the tubules are dilated, and at least one tubule dilated is >500  $\mu\text{m}$  in diameter.

5. MIN = mineralization. Basophilic calcium salts sometimes precipitate and accumulate in tubules and ducts of the trunk kidney. In more severe cases, the ducts expand and sometimes rupture; granulomatous inflammation is commonly associated with ruptured ducts.

Significance: Renal mineralization is common in cultured fish species; when severe, the condition is termed nephrocalcinosis. The lesion is not considered fatal, although feed conversion may be adversely affected. The pathogenesis is not fully understood, but renal mineralization has been experimentally reproduced through high carbon dioxide levels, magnesium deficiency, selenium toxicity, and a diet low in minerals (source, "Systemic Pathology of Fish", Second edition, 2006, edited by H. Ferguson). Clinically, renal mineralization is most commonly associated with high carbon dioxide levels.

score = 0; tubules are not dilated.

score = 1; <5 mineralized tubules per 10 $\times$  objective lens field, and minimal fibrosis.

score = 2; >5 but <10 mineralized tubules per 10 $\times$  objective lens field with no fibrosis, or <5 mineralized tubules per 10 $\times$  field but moderate associated fibrosis.

score = 3; >10 mineralized tubules per 10 $\times$  objective lens field with no fibrosis, or total sectional area of fibrosis greater than 2 mm in diameter.

6. HEM = interstitial hemorrhage/congestion. Because the kidney has abundant thin-walled vessels, and tremendous capacity for congestion, it is often difficult to differential renal congestion from renal hemorrhage. Erythrocytes within tubular lumens are clear evidence of hemorrhage. Hemorrhage sometimes is more obvious in the head kidney. Significance: In British Columbia, renal congestion and hemorrhage is associated with VHSV and bacterial infections. Renal congestion and hemorrhage is one of the classic signs of infectious salmon anemia (ISA), but ISAV has never been isolated from fish in BC.

score = 0; erythrocytes are in <10% of the cross sectional area and are contained within blood vessels.

score = 1; erythrocytes are in >10% but <20% of the cross sectional area, or erythrocytes outside of blood vessels affect an area < a 40 $\times$  objective lens field.

score = 2; erythrocytes are in >20% but <50% of the cross sectional area, or erythrocytes outside of blood vessels affect a total area > a 40 $\times$  objective lens field, but < a 10 $\times$  objective lens field.

score = 3; erythrocytes are in >50% of the cross sectional area, or erythrocytes outside of blood vessels affect a total area > a 10 $\times$  objective lens field.

7. EGC = eosinophilic granular cells in the interstitium. Cells with eosinophilic granules are sometimes common lining the thin-walled vessels of the kidney of Atlantic salmon. Granules often have pale staining, and their margins are usually poorly defined. These cells are either eosinophilic granular cells or endothelial cells that contain eosinophilic granules. EGC does not occur in Pacific salmon.

Significance: These cells probably are part of a specific type of inflammatory response that can be induced by more than one cause. I have seen these granules associated with cases of *Piscirickettsia salmonis* (e.g., type specimen for a severe case in the brain, 2007-1061-6B) and cerebral *Renibacterium salmoninarum* (2007-3732-5A), and these cells have been associated with experimental infection of rainbow trout with *Listonella anguillarum* (Lamas et al. 1991).

score = 0; the interstitium has <6 cells with eosinophilic granules per 40× objective lens field.

score = 1; the interstitium has >6 but < 40 cells with eosinophilic granules per 40× objective lens field.

score = 2; the interstitium has >40 cells with eosinophilic granules per 40× objective lens field.

score = 3; the interstitium has >40 cells with eosinophilic granules per 40× objective lens field, and the cells are more than 1 cell thick along the vessel walls.

Lamas, J., Bruno, D.W., Santos, Y., Anadon, R., and A.E. Ellis. 1991. Eosinophilic granular cell response to intraperitoneal injection with *Vibrio anguillarum* and its extracellular products in rainbow trout, *Oncorhynchus mykiss*. *Fish Shellfish Immunol.* 1(3):187-194.

8. IPC = intratubular (luminal) protein casts. Protein casts within tubules tend to be more eosinophilic than the cytoplasm of renal tubular epithelial cells. This lesion has two differentials. First, small amounts of pale eosinophilic material in tubules are considered normal and are not scored as a lesion. Second, necrotic cells within the lumen are scored as part of renal tubular necrosis (RTN) in the next category.

Significance: Renal tubular protein casts result from glomerular or tubular dysfunction; either excess protein leaks through glomeruli or tubules are unable to reabsorb protein.

score = 0; no intratubular (luminal) protein casts

score = 1; <10% of tubules have intratubular (luminal) protein casts.

score = 2; >10% but <50% of tubules have intratubular (luminal) protein casts.

score = 3; >50% of tubules have intratubular (luminal) protein casts.

9. IFB = interstitial fibrin. Fibrin deposits are brightly eosinophilic and acellular. Significance: Multifocal fibrin deposits in the kidney are evidence of endothelial damage, probably from exposure to toxins. The toxins could be of bacterial origin or inflammatory cell origin; toxins in the water or feed are less likely.
 

score = 0; interstitial tissues have no fibrin deposits.

score = 1; total cross sectional area of fibrin deposits fills <1 40× objective-lens field.

score = 2; total cross sectional area of fibrin deposits fills >1 40× objective-lens field, but <1 10× objective-lens field.

score = 3; total cross sectional area of fibrin deposits fills >1 10× objective-lens field.

10. ICN = interstitial cell necrosis. Necrotic hematopoietic cells of the renal interstitium have some form of nuclear degeneration (pyknosis, karyorrhexis, or karyolysis); because of the relatively small volume of cytoplasm in hematopoietic cells, necrotic cells might have only small amounts of coagulated hypereosinophilic cytoplasm.

Significance: Large numbers of necrotic interstitial cells is the hallmark lesion of Infectious Hematopoietic Necrosis Virus (IHNV). Milder cases might also be a result of infection with bacteria or other viruses.

score = 0; no interstitial cells are necrotic.

score = 1; <3 necrotic interstitial cells per 40× objective-lens field.

score = 2; 3 - 20 necrotic interstitial cells per 40× objective-lens field.

score = 3; >20 necrotic interstitial cells per 40× objective-lens field.

11. IEA = interstitial edema and/or interstitial cell atrophy. Edema is diagnosed when the space between renal tubules is increased without an increase in the number of hematopoietic cells. Interstitial cell atrophy is diagnosed when there is a decrease in the number of hematopoietic cells. They are scored together because they often occur together, and without morphometry the diagnoses cannot be reliably distinguished from each other. The primary differential is postmortem autolysis, in which a space is formed when the base of the tubular epithelial cells pulls away from the tubular basement membrane.

Significance: Interstitial edema is evidence of dysfunctional circulation, and interstitial cell atrophy is evidence of decreased immune function; both are often a result of a viral infection (e.g., VHSV).

score = 0; cross-sectional area of interstitium is about equal to area of tubules and glomeruli, and most of the interstitial space is filled with hematopoietic cells.

score = 1; cross-sectional area of the interstitium is > area of tubules and glomeruli but < 2× area of tubules and glomeruli, and/or total numbers of hematopoietic cells is less than normal.

score = 2; cross-sectional area of the interstitium is > 2× the area of tubules and glomeruli, and/or total numbers of hematopoietic cells is less than half of normal.

score = 3; cross-sectional area of the interstitium is > 3× the area of tubules and glomeruli, and/or hematopoietic cells are absent between most tubules.

12. RTN = renal tubular necrosis. Necrotic tubules contain cells with pyknotic to karyorrhectic nuclei, and condensed hypereosinophilic cytoplasm. Necrotic cells often slough and are in the lumen. Tubules lined by attenuated cytoplasm are common in subacute to chronic cases. Chronic cases are sometimes surrounded by fibrosis. Necrosis associated with *Renibacterium salmoninarum* or *Piscirickettsia salmonis* infection is not scored here; instead, it is scored with the specific *Renibacterium salmoninarum* or *Piscirickettsia salmonis* categories below.

Significance: Renal tubular epithelial necrosis results from acute damage to renal epithelial cells; damage is reversible if the basement membrane is spared. Causes in fish include viral hemorrhagic septicemia virus (VHSV) and exposure to toxins (e.g., bacterial toxins, or aminoglycoside antibiotics such as gentamicin).

score = 0; sections have no renal tubular necrosis.

score = 1; total sectional area affected by renal tubular necrosis is <40× objective lens field.

score = 2; total sectional area affected by renal tubular necrosis is > 40× but <10× objective lens field.

- score = 3; total sectional area affected by renal tubular necrosis is  $>10\times$  objective lens field.
13. MGN = Membranous glomerulonephritis. Affected glomeruli have glomerular basement membranes thickened by homogeneous eosinophilic material  
Significance: Membranous glomerulonephritis is fairly common in older salmonids, particularly Chinook salmon; it is often associated with infections in other parts of the fish, but a link to immune complex deposition has not been demonstrated. Membranous glomerulonephritis has been associated with cardiomyopathy syndrome (in Atlantic salmon), nephrocalcinosis, and infections with a number of bacteria and parasitic species (e.g., *Loma salmonae*).  
score = 0; sections have no membranous glomerulonephritis.  
score = 1; thickness of eosinophilic deposits is less than the thickness of glomerular nuclei  
score = 2; thickness of eosinophilic deposits is greater than the thickness of glomerular nuclei.  
score = 3; thickness of eosinophilic deposits is greater than  $2\times$  the thickness of glomerular nuclei.
14. KGR = granulomatous interstitial nephritis. Granulomatous interstitial nephritis spans the range from diffuse granulomatous inflammation to focal granulomas. Granulomas often surround radiating globular material (it can be yellow, green, or pale red, in any combination of these colours); this material is consistent with vaccine material. Mild cases of granulomatous inflammation that might be *Renibacterium salmoninarum* or *Piscirickettsia salmonis* are scored here (if organisms are confirmed, use KRS and KPS). When present, radiating globular material in KGR multinucleate giant cells (MNGs) is a key feature differentiating KGR from the primary differential, KRS (KRS occasionally contains MNGs, but KRS granulomas do not contain radiating globular material). In Pacific salmon, a major differential is *Loma salmonae* (below, KLS); do a Twort's stain to look for Gram positive spores.  
Significance: When associated with radiating globular material, granulomatous nephritis might be a reaction to a vaccine; differentials include chronic bacterial disease (e.g., *Yersinia ruckeri* or *Renibacterium salmoninarum* infection).  
score = 0; the kidney has no granulomatous inflammation.  
score = 1; total cross sectional area of granulomatous inflammation fills  $<1/40\times$  objective-lens field.  
score = 2; total cross sectional area of granulomatous inflammation fills  $>1/40\times$  objective-lens field, but  $<1/10\times$  objective-lens field.  
score = 3; total cross sectional area of granulomatous inflammation fills  $>1/10\times$  objective-lens field.
15. KRS = *Renibacterium salmoninarum*. *Renibacterium salmoninarum*, the cause of bacterial kidney disease, causes a wide range of lesions in the kidney, from acute necrosis with fibrin exudation, to chronic granulomatous inflammation with fibrosis. Numbers of organisms in affected kidneys varies from almost none to very abundant. Twort's Gram stain works well for highlighting the short Gram-positive rods; however, because the



lesions alone are fairly diagnostic, a Gram stain is not routinely done with Auditing cases. Some cases have a subtle but diffuse population of macrophages that would be diagnosed as interstitial hyperplasia unless the presence of organisms is revealed with a Gram stain (often, these cases have moderate numbers of organisms). Infection in the kidney is usually associated with infection in other organs (those lesions are scored separately in the chapter for each organ). Unless a Gram stain is done, lesions suggestive of KRS usually are scored in the KGR category (i.e., the KRS prevalence probably underestimates the true prevalence of KRS) or the ISH category. Lack of radiating globular material in multinucleate giant cells of KRS is the key feature differentiating granulomatous inflammation of KRS from KGR (radiating globular material is a prominent feature of multinucleate giant cells of KGR).

Significance: *Renibacterium salmoninarum* is a significant cause of mortality, particularly with Pacific salmon.

score = 0; no *R. salmoninarum*-associated lesions in the section.

score = 1; total cross sectional area of *R. salmoninarum*-associated lesions fills <1 40× objective-lens field.

score = 2; total cross sectional area of *R. salmoninarum*-associated lesions fills >1 40× but <1 10× objective-lens field, or, diffuse organisms are associated with minimal inflammation (this type requires a Gram stain for diagnosis; e.g., 2007-1679-2A).

score = 3; total cross sectional area of *R. salmoninarum*-associated lesions fills >1 10× objective-lens field.

16. **KPS = *Piscirickettsia salmonis*.** *Piscirickettsia salmonis* is a Gram-negative coccoid organism (primitive bacterium) that causes Salmonid Rickettsial Septicemia. Fish with *P. salmonis* commonly present with multiple pale foci in the liver, but many cases also have organisms in the kidney. Microscopically, *P. salmonis* infection is usually associated with vasculitis, and variable numbers of macrophages with cytoplasmic organisms (each about 1 µm in diameter). *P. salmonis* is most easy to identify when a cluster of uniform-diameter organisms occur in an otherwise clear macrophage vacuole. Differentials include (1) melanin granules, which are monomorphic, brown-black, and smaller, and (2) karyorrhectic debris, which is polymorphic, often larger, and usually stains more basophilic on H&E. Positive identification is best done using an immunohistochemical stain optimized at the Animal Health Centre. Without using the immunohistochemical stain, it is likely that mild cases in the kidney will be missed. Significance: *Piscirickettsia salmonis* is associated with sporadic mortality in pen-reared salmon in BC.

score = 0; no *P. salmonis*-associated lesions in the section.

score = 1; total cross sectional area of *P. salmonis*-associated lesions fills <1 40× objective-lens field, or reaction limited to infected macrophages that can be disseminated throughout the parenchyma.

score = 2; total cross sectional area of *P. salmonis*-associated lesions fills >1 40× but <1 10× objective-lens field.

score = 3; total cross sectional area of *P. salmonis*-associated lesions fills >1 10× objective-lens field.

17. KLS = *Loma salmonae*. *Loma salmonae* is a microsporidian parasite that forms discreet clusters (xenomas) of spores in the circulatory system. Most xenomas are 50 to 100  $\mu\text{m}$  in diameter. Spores, about  $5.5 \times 3 \mu\text{m}$ , have a single polar capsule that is weakly Gram positive and birefringent under polarized light. Reaction to the xenomas varies from none to severe necrotizing vasculitis. Reaction to spores outside of xenomas is granulomatous.
- Significance: Infection with *Loma salmonae* is often associated with inflammation and tissue destruction, but these lesions often do not surround the xenomas. It may be that the xenomas stimulate the release of inflammatory mediators that cause most of the damage in the host.
- score = 0; no *Loma salmonae*.
- score = 1;  $<7$  *Loma salmonae* in the section, and no significant inflammation.
- score = 2;  $<7$  *Loma salmonae* in the section, with significant inflammation, or  $>7$  xenomas in the section with no associated inflammation.
- score = 3;  $> 7$  *Loma salmonae* in the section, with severe associated inflammation.

Chapter 4. Type specimens for heart scores. Abbreviations are explained after the table.

HEART (Summary of type specimens)				
Abbreviation	None score = 0	Mild score = 1	Moderate score = 2	Severe score = 3
HAtly	2007-1752-3A	2006-4267-14	none	none
MKM	2007-1752-3A	2004-3670-3	2008-1511-4A	none
EPL	2007-1752-3A	2009-3711-1A	2007-2043-2A	2007-1561-3A
EPH	2007-1752-3A	2006-4496-7	2007-3732-2A	none
ENL	2007-1752-3A	2007-3984-7A	none	2009-4023-4A
ENE	2007-1752-3A	2006-4267-14	2007-3158-3A	none
ENH	2007-1752-3A	2009-4384-6A	none	2007-3907-9A 2006-4368-2
ECH	2007-1752-3A	2007-0260-12	2006-3001-2	2009-0344-1A
HTH	2007-1752-3A	2006-2349-4	2007-1060-2A	none 2006-1009-2 2006-2167-3 (epicarditis)
HGR	2007-1752-3A	2006-4268-6	2006-1014-5	2007-1398-7A
HRS	2007-1752-3A	2006-1794-2	2006-1593-2	none
HPS	2007-1752-3A	2006-4369-11	2006-4275-10	none
HLS	2007-1752-3A	2006-1031-6 (free spores only)	none	none

*Quality Control/Quality Assurance*

HAtly = Autolysis. Although changes in membrane integrity begin immediately after death, the heart is the last organ to show significant autolysis. In farmed salmon, autolyzed myocytes have pale cytoplasm.

Significance: Autolysis decreases the sensitivity and specificity of histopathology.

score = 0; no membrane changes, erythrocytes stain intensely, myocyte cytoplasm stains intensely.

score = 1; myocyte cytoplasm stains less intensely.

score = 2; myocyte cytoplasm stains less intensely, and some nuclei are fragmented.

score = 3; myocyte cytoplasm stains poorly; nuclei are mostly lysed.

*Lesions*

1. MKM = myocardial karyomegaly. Nuclei of cardiac muscle cells are considered enlarged when they are at least 2× as large as normal myocardial cells. Enlarged nuclei are most common in cells at the junction of the internal spongy and peripheral compact layers of the heart. Enlarge nuclei are often associated with enlarged myocardiocytes; however, because cell margins are less distinct than nuclear margins, only nuclear margins are scored.  
Significance: Myocardial karyomegaly probably is an indication of multiple copies of DNA within the affected nuclei; this could represent a developmental anomaly or a response to toxicant exposure. Karyomegaly in other cell types has been associated with exposure to algal toxins (e.g., hepatocytes exposed to microcystin LR in netpen liver disease).  
score = 0; ≤2 myocardial cells in the section have enlarged nuclei.  
score = 1; >2 myocardial cells in the section have enlarged nuclei, but <3 enlarged nuclei per 40× objective lens field.  
score = 2; >2 myocardial cells in the section have nuclei that are > 4× larger than normal, or the section has 3 - 5 enlarged nuclei per 40× objective lens field.  
score = 3; >2 myocardial cells in the section have nuclei that are > 6× larger than normal, or the section has >5 enlarged nuclei per 40× objective lens field.
2. EPL = epicarditis, lymphohistiocytic. Inflammation in the epicardium is mostly composed of a mixture of small lymphocytes and larger cells with more open nuclei; these are either large lymphocytes or histiocytes; pure lymphocytic inflammation is rare, but is included in this category, as are plasma cells. Small perivascular foci of inflammatory cells in the peripheral compact layer of the heart are also included with the EPL score.  
Significance: Epicarditis is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine.  
score = 0; the epicardium contains ≤6 inflammatory cells per 40× objective lens field.  
score = 1; epicarditis limited to a few foci, with total sectional area affected <500 μm in diameter.  
score = 2; total sectional area of epicarditis is >500 μm but <2 mm in diameter.  
score = 3; total sectional area of epicarditis is >2 mm in diameter.
3. EPH = epicarditis, histiocytic. Inflammation in the epicardium is mostly composed of histiocytes (macrophages), but the macrophages are not organized into granulomatous inflammation. Small perivascular foci of histiocytes in the peripheral compact layer of the heart are also included with the EPH score. When histiocytic epicarditis is associated with a mixture of fibrin and neutrophils, is usually is a result of infection with *Renibacterium salmoninarum* and not scored here; instead, it is scored under HRS below.  
Significance: Epicarditis is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine.  
score = 0; the epicardium contains ≤6 inflammatory cells per 40× objective lens field.  
score = 1; epicarditis limited to a few foci, affecting a total sectional area <500 μm in diameter.  
score = 2; total sectional area of epicarditis is >500 μm but <2 mm in diameter.

score = 3; total sectional area of epicarditis is >2 mm in diameter.

4. ENL = endocarditis, lymphohistiocytic. Inflammation of the endocardial lining includes a mixture of small lymphocytes and larger cells with more open nuclei; these are either large lymphocytes or histiocytes; inflammation composed entirely of small lymphocytes is rare. Plasma cells are included in this category.  
Significance: Lymphoplasmacytic inflammation in the heart is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine. In wild salmonids, lymphocytic inflammation affecting more than 1 or 2 foci is usually associated with presporogonic stages of a myxosporean (*Sphaerospora* sp.).  
score = 0; the endocardium contains  $\leq 6$  inflammatory cells per 40 $\times$  objective lens field.  
score = 1; endocarditis limited to a few foci, affecting a total sectional area <500  $\mu\text{m}$  in diameter.  
score = 2; total sectional area of endocarditis is >500  $\mu\text{m}$  but <2 mm in diameter.  
score = 3; total sectional area of endocarditis is >2 mm in diameter.
5. ENE = endocarditis, eosinophilic. This type of inflammation involves the accumulation of cells with eosinophilic granules in the endocardium--often most prominent in the bulbus arteriosus. Eosinophilic granules often have pale staining and poorly defined margins. These cells are either eosinophilic granular cells or endothelial cells that contain eosinophilic granules. ENE seems to be unique to Atlantic salmon; I have not seen this lesion in Pacific salmon.  
Significance: These cells probably are part of a specific type of inflammatory response that can be induced by more than one cause. I have seen these granules associated with a case of *Piscirickettsia salmonis*, and these cells have been associated with experimental infection of rainbow trout with *Listonella anguillarum* (see EGC in the kidney for more details).  
score = 0; the endocardium contains  $\leq 6$  inflammatory cells per 40 $\times$  objective lens field.  
score = 1; endocarditis limited to a few foci, affecting a total sectional area <500  $\mu\text{m}$  in diameter.  
score = 2; total sectional area of endocarditis is >500  $\mu\text{m}$  but <2 mm in diameter.  
score = 3; total sectional area of endocarditis is >2 mm in diameter.
6. ENH = endocarditis, histiocytic. Inflammation in the endocardium is mostly composed of histiocytes (macrophages), but the macrophages are not organized into granulomatous inflammation, and some cases also have plump (hypertrophied) endothelial cells. Histiocytes are rarely more than 2 cell-layers thick.  
Significance: Histiocytic inflammation in the heart is evidence of chronic immune stimulation; differentials include a low grade bacterial infection and reaction to a vaccine.  
score = 0; the endocardium contains  $\leq 6$  inflammatory cells per 40 $\times$  objective lens field.  
score = 1; endocarditis limited to a few foci, affecting a total sectional area <500  $\mu\text{m}$  in diameter.  
score = 2; total sectional area of endocarditis is >500  $\mu\text{m}$  but <2 mm in diameter.  
score = 3; total sectional area of endocarditis is >2 mm in diameter.

7. ECH = endothelial cell hypertrophy. This form of endocarditis is composed mostly of hypertrophic endothelial cells. Although ECH sometimes includes small numbers of inflammatory cells, endocarditis dominated by inflammatory cells is not scored here, in favor of ENL, ENE, or ENH.  
Significance: Endothelial cell hypertrophy is evidence of systemic immune stimulation: probably resulting from inflammatory cell mediators released into the circulation.  
Examples include *Yersinia ruckeri* infection (e.g., 2006-4495-3) and *Piscirickettsia salmonis* (where it is scored only as part of SPS, not here).  
score = 0; no endothelial cell hypertrophy.  
score = 1; endothelial cell hypertrophy limited to a few foci; total affected sectional area <500  $\mu\text{m}$  in diameter.  
score = 2; endothelial cell hypertrophy may be diffuse, but <2/3 of cells affected.  
score = 3; endothelial cell hypertrophy is diffuse, and >2/3 of cells affected..
  
8. HTH = thrombosis. Thrombi are stationary blood clots attached to the endocardium. They can include a wide range of ratios between fibrin and thrombocytes. Thrombi are more organized than their primary differential, postmortem clots. Thrombi tend to stain more intensely eosinophilic than plasma (thrombi have a greater protein content than plasma). Thrombi associated with *Renibacterium salmoninarum* or *Piscirickettsia salmonis* infection is not scored here; instead, it is scored with the specific *Renibacterium salmoninarum* or *Piscirickettsia salmonis* categories below.  
Significance: Thrombosis in the heart is evidence of increased coagulability. This can result from endothelial damage related to virus, bacterial, or parasitic infection.  
score = 0; no thrombi in the section, but can include a single thrombus <60  $\mu\text{m}$  in diameter.  
score = 1; at least one thrombus  $\geq 60$   $\mu\text{m}$  in diameter, but total affected sectional area <500  $\mu\text{m}$  in diameter; or, >10 thrombi per section.  
score = 2; total affected sectional area of thrombi >500  $\mu\text{m}$  but <2 mm in diameter.  
score = 3; total affected sectional area of thrombi >2 mm in diameter.
  
9. HGR = granulomatous carditis. Granulomatous carditis spans the range from diffuse granulomatous inflammation to focal granulomas; it can include epicarditis, myocarditis, and endocarditis. Granulomas sometimes surround radiating globular material (it can be yellow, green, or pale red, in any combination of these colours); this material is consistent with vaccine material. Thrombi that seem to be part of HGR are scored here and NOT separately in the HTH category. Lesions with *Renibacterium salmoninarum* or *Piscirickettsia salmonis* are NOT scored here (instead, use HRS and HPS). When present, radiating globular material in HGR multinucleate giant cells (MNGs) is a key feature differentiating HGR from the primary differential, HRS (HRS occasionally contains MNGs, but HRS granulomas do not contain radiating globular material). In Pacific salmon, a major differential is HLS; do a Twort's stain to look for Gram positive spores.  
Significance: This form of granulomatous carditis might be a reaction to a vaccine; differentials include chronic bacterial disease (e.g., *Yersinia ruckeri* or *Renibacterium salmoninarum* infection).  
score = 0; the heart has no granulomatous inflammation.

score = 1; total cross sectional area of granulomatous inflammation fills <1 40× objective-lens field.

score = 2; total cross sectional area of granulomatous inflammation fills >1 40× objective-lens field, but <1 10× objective-lens field.

score = 3; total cross sectional area of granulomatous inflammation fills >1 10× objective-lens field.

10. **HRS = *Renibacterium salmoninarum*.** *Renibacterium salmoninarum*, the cause of bacterial kidney disease, causes a wide range of lesions in the heart, from acute necrosis with fibrin exudation, to chronic granulomatous inflammation with fibrosis; other cases are primarily epicarditis composed of a mixture of macrophages, neutrophils, and fibrin. Numbers of organisms in affected hearts varies from almost none to very abundant. Twort's Gram stain works well for highlighting the short Gram-positive rods, but a Gram stain is not routinely done with Auditing cases. Therefore, some mild cases of *Renibacterium salmoninarum* might be scored under the general categories epicarditis, endocarditis, or HGR. Any definitive prevalence study would require a Gram stain of tissues from every fish (particularly Pacific salmon). Infection in the heart is usually associated with infection in other organs (those lesions are scored separately in the chapter for each organ). Lack of radiating globular material in multinucleate giant cells of HRS is the key feature differentiating granulomatous inflammation of HRS from HGR (radiating globular material is a prominent feature of multinucleate giant cells of HGR). Significance: *Renibacterium salmoninarum* is a significant cause of mortality, particularly with Pacific salmon.

score = 0; no *R. salmoninarum*-associated lesions in the section.

score = 1; total cross sectional area of *R. salmoninarum*-associated lesions fills <1 40× objective-lens field.

score = 2; total cross sectional area of *R. salmoninarum*-associated lesions fills >1 40× but <1 10× objective-lens field.

score = 3; total cross sectional area of *R. salmoninarum*-associated lesions fills >1 10× objective-lens field.

11. **HPS = *Piscirickettsia salmonis*.** *Piscirickettsia salmonis* is a Gram-negative coccoid organism (primitive bacterium) that causes Salmonid Rickettsial Septicemia. Fish with *P. salmonis* commonly present with multiple pale foci in the liver, but many cases also have organisms in the spleen. Microscopically, *P. salmonis* infection is usually associated with vasculitis, and variable numbers of macrophages with cytoplasmic organisms (each about 1 µm in diameter). *P. salmonis* is most easy to identify when a cluster of uniform-diameter organisms occur is an otherwise clear macrophage vacuole. Differentials include (1) melanin granules, which are monomorphic, brown-black, and smaller, and (2) karyorrhectic debris, which is polymorphic, often larger, and usually stains more basophilic on H&E. Some cases have fibrinous endocarditis. Positive identification is best done using an immunohistochemical stain optimized at the Animal Health Centre. Without using the immunohistochemical stain, it is likely that mild cases in the heart will be missed. Significance: *Piscirickettsia salmonis* is associated with sporadic mortality in pen-reared salmon in BC.

score = 0; no *P. salmonis*-associated lesions in the section.

score = 1; total cross sectional area of *P. salmonis*-associated lesions fills <1 40× objective-lens field, or reaction limited to infected macrophages that can be disseminated throughout the parenchyma.

score = 2; total cross sectional area of *P. salmonis*-associated lesions fills >1 40× but <1 10× objective-lens field.

score = 3; total cross sectional area of *P. salmonis*-associated lesions fills >1 10× objective-lens field.

12. HLS = *Loma salmonae*. *Loma salmonae* is a microsporidian parasite that forms discreet clusters (xenomas) of spores in the circulatory system. Most xenomas are 50 to 100 µm in diameter. Spores, about 5.5 × 3 µm, have a single polar capsule that is weakly Gram positive and birefringent under polarized light. Reaction to the xenomas varies from none to severe necrotizing vasculitis. Reaction to spores outside of xenomas is granulomatous.  
Significance: Infection with *Loma salmonae* is often associated with inflammation and tissue destruction, but these lesions often do not surround the xenomas. It may be that the xenomas stimulate the release of inflammatory mediators that cause most of the damage in the host.  
score = 0; no *Loma salmonae*.  
score = 1; <7 *Loma salmonae* in the section, and no significant inflammation.  
score = 2; <7 *Loma salmonae* in the section, with significant inflammation, or >7 xenomas in the section with no associated inflammation.  
score = 3; > 7 *Loma salmonae* in the section, with severe associated inflammation.



Chapter 5. Type specimens for spleen scores. Abbreviations are explained after the table.

SPLEEN (Summary of type specimens)				
Abbreviation	None Score = 0	Mild score = 1	Moderate score = 2	Severe score = 3
SAtly	2006-4171-4	2006-1019-1	2008-4633-1A	2010-1057-1A
PER	2006-4171-4	2004-3480-4	2004-3480-1	2007-1053-6A
LKR	2006-4171-4	2008-3691-1A	2007-3987-3A	2006-1031-5
PGP	2006-4171-4	2005-1786-1	p2006-1011-1 a2008-1120-2A	none
PFB	2006-4171-4	2006-4496-2		2006-1031-5 (Atly = 1)
EHP	2006-4171-4	2007-1678-8A	2005-4044-9, 2009-4781-8A	2005-4045-1
SGR	2006-4171-4	2007-3156-3A	2006-4368-6	2006-1014-5
SRS	2006-4171-4	2006-4369-4A	2007-1679-2A	2006-1593-2
SPS	2006-4171-4	2006-4369-3		2006-4275-10
SLS	2006-4171-4		2007-3987-1A	none

#### *Quality Control/Quality Assurance*

SAtly = Autolysis. Changes in membrane integrity begin immediately after death. In farmed salmon, autolyzed cells in the spleen have fragmented nuclei and pale cytoplasm. By comparison, in the primary differential, splenic necrosis, affected cells in the spleen have nuclear pyknosis or karyorrhexis, and the cytoplasm of necrotic spleen cells is hypereosinophilic.

Significance: Autolysis decreases the sensitivity and specificity of histopathology.

score = 0; no membrane changes, spleen cells, including erythrocytes, stain intensely.

score = 1; cytoplasm of spleen cells stains less intensely.

score = 2; spleen cell cytoplasm stains poorly; many nuclei are fragmented.

score = 3; individual cells are no longer discernable; most nuclei are fragmented and many are completely lysed.

#### *Lesions*

1. PER = peritonitis. Inflammation on the surface of the spleen is composed of variable amounts of granulomatous inflammation, lymphoplasmacytic infiltrates, and fine fibrocellular fronds. In some cases, the inflammation includes intralesional vacuoles 50 - 200 µm in diameter. Sometimes peritonitis includes fibrin. Peritonitis sometimes extends into surrounding exocrine pancreas and mesenteric adipose tissue (scored separately under IPR).

Significance: Splenic peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated (Mutoloki et al. 2004). Vacuoles probably represent lipophilic vaccine material that was removed during tissue processing (alcohol

and xylene remove lipid from tissues before staining). Peritonitis can also result from a bacterial infection (e.g., *Yersinia ruckeri* or *Aeromonas salmonicida*).

score = 0; no peritonitis.

score = 1; peritonitis covers <2 mm of the surface of the spleen, or the inflammation is <300  $\mu$ m thick.

score = 2; peritonitis covers 2 - 5 mm of the surface of the spleen, or the inflammation is 300 - 500  $\mu$ m thick.

score = 3; peritonitis covers >5 mm of the surface of the spleen, or the inflammation is >500  $\mu$ m thick.

- S. Mutoloki, S. Alexandersen, and Ø. Evensen. 2004. Sequential study of antigen persistence and concomitant inflammatory reactions relative to side-effects and growth of Atlantic salmon (*Salmo salar* L.) following intraperitoneal injection with oil-adjuvanted vaccines. *Fish & Shellfish Immunology* 16(5):633-644.

2. LKR = leukocytic karyorrhexis. Karyorrhexis is the phase of nuclear degeneration in which the chromatin disintegrates into roughly spherical granules that are smaller than nuclei. The main differential for LKR is postmortem autolysis. Autolyzed nuclei within the spleen tend to lyse but not clump, whereas karyorrhexis involves clumping into distinctive small hyperchromatic basophilic clumps.

Significance: Karyorrhexis in the spleen is evidence of increased cell turnover, possibly as part on an active inflammatory response.

score = 0; the spleen contains  $\leq 2$  karyorrhectic cells per 40 $\times$  objective lens field.

score = 1; 2 - 4 karyorrhectic cells per 40 $\times$  objective lens field.

score = 2; 5 - 10 karyorrhectic cells per 40 $\times$  objective lens field.

score = 3; >10 karyorrhectic cells per 40 $\times$  objective lens field.

3. PGP = parenchymal golden pigment (lipofuscin). Pale yellow-green to yellow-brown pigment in the spleen accumulates primarily in macrophages, but this pigment sometimes distends melanomacrophages. Accumulations of golden pigment are usually >10  $\mu$ m in diameter. Differentials include (1) melanin granules of melanomacrophages, which are dark brown, monomorphic, and < 1  $\mu$ m diameter; and (2) acid hematin deposits, which are granular, 1 - 4  $\mu$ m in diameter, and birefringent under polarized light. Mild accumulations of golden pigment are common in per-reared Chinook salmon in British Columbia.

Significance: The golden pigment in the spleen most likely is lipofuscin. Accumulation of lipofuscin is a nonspecific change that can result from a variety of insults, including rancid feed, low levels of antioxidants in the feed, chronic infections, and exposure to organic contaminants. Small amounts of splenic lipofuscin are fairly common in pen-reared Chinook salmon. In pen-reared salmon, hepatic lipofuscin accumulation is a common feature of netpen liver disease (microcystin-LR). Conditions that lead to moderate to abundant lipofuscin have been associated with decreased growth and survival in several studies.

score = 0; the spleen contains  $\leq 1$  foci of golden pigment per 5 40 $\times$  objective lens field.

score = 1; foci of golden pigment >1 per 5 40 $\times$  objective lens field, but <2 per 40 $\times$  objective lens field.

score = 2; > 2 foci of golden pigment per 40× objective lens field, but <10% of volume is pigmented.

score = 3; > 2 foci of golden pigment per 40× objective lens field, and >10% of volume is pigmented.

4. PFB = parenchymal fibrin. Fibrin is homogeneous, eosinophilic to hypereosinophilic, and acellular. Fibrin deposits are usually a few microns thick and they extend many microns along vascular channels and cellular margins. Fibrin accumulates within capillaries and in the interstitium.  
Significance: Fibrin deposits are evidence of endothelial damage, probably from exposure to toxins. The toxins could be of bacterial origin or inflammatory cell origin. I have seen this response in salmon that are PCR positive for VHSV. Lack of remodelling of the fibrin is consistent with these deposits forming just before death.  
score = 0; no fibrin deposits.  
score = 1; fibrin deposits limited to total sectional area <500 µm in diameter.  
score = 2; total sectional area of fibrin deposits 500 µm to 2 mm in diameter.  
score = 3; total sectional area of fibrin deposits >2 mm in diameter.
5. EHP = endothelial cell hypertrophy. This form of splenitis is composed mostly of hypertrophic endothelial cells that often contain eosinophilic granules. Alternatively, this lesion might be a result of eosinophilic granular cells accumulating on the endothelial cells of the spleen.  
Significance: Endothelial cell hypertrophy is evidence of systemic immune stimulation: probably resulting from inflammatory cell mediators released into the circulation.  
score = 0; no endothelial cell hypertrophy.  
score = 1; endothelial cell hypertrophy limited to a few foci (>6 but < 40 cells with eosinophilic granules per 40× objective lens field).  
score = 2; endothelial cell hypertrophy involves most of the spleen (>40 cells with eosinophilic granules per 40× objective lens field).  
score = 3; >40 cells with eosinophilic granules per 40× objective lens field, and the cells are more than 1 cell thick along the vessel walls.
6. SGR = granulomatous splenitis. Inflammation is composed of variable amounts of organized macrophages, which usually include multinucleate giant cells. Multinucleate giant cells sometimes surround globular green-yellow material. Inflammation may also include variable numbers of fibroblasts, lymphocytes, and plasma cells. Lesions with *Renibacterium salmoninarum* are scored under SRS and those with *Piscirickettsia salmonis* are scored under SPS. When present, radiating globular material in SGR multinucleate giant cells (MNGs) is a key feature differentiating SGR from the primary differential, SRS (SRS occasionally contains MNGs, but SRS granulomas do not contain radiating globular material). In Pacific salmon, a major differential is GLS; do a Twort's stain to look for Gram positive spores.  
Significance: Granulomatous splenitis is evidence of chronic immune stimulation. This most likely is a vaccine reaction (i.e., the globular green-yellow material); differentials include chronic bacterial disease (e.g., *Yersinia ruckeri* or *Renibacterium salmoninarum* infection).

score = 0; no granulomatous inflammation.

score = 1; granulomatous inflammation limited to total sectional area <500  $\mu$ m in diameter.

score = 2; total sectional area of granulomatous inflammation 500  $\mu$ m to 2 mm in diameter.

score = 3; total sectional area of granulomatous inflammation >2 mm in diameter.

7. SRS = *Renibacterium salmoninarum*. *Renibacterium salmoninarum*, the cause of bacterial kidney disease, causes a wide range of lesions in the spleen, from acute necrosis with fibrin exudation, to chronic granulomatous inflammation with fibrosis. Numbers of organisms in affected spleens varies from almost none to very abundant. Twort's Gram stain works well for highlighting the short Gram-positive rods; however, because the lesions alone are often diagnostic, a Gram stain is not routinely done with Auditing cases. Infection in the spleen is usually associated with infection in other organs (those lesions are scored separately in the chapter for each organ). Unless a Gram stain is done, lesions suggestive of SRS usually are scored in the SGR category (i.e., the SRS prevalence probably underestimates the true prevalence of SRS) or as normal. Indeed, many cases in the spleen have a subtle but diffuse population of macrophages that resembles normal splenic parenchyma; the presence of organisms is revealed only with a Gram stain (often, these cases have moderate numbers of organisms). Lack of radiating globular material in multinucleate giant cells of SRS is the key feature differentiating granulomatous inflammation of SRS from SGR (radiating globular material is a prominent feature of multinucleate giant cells of SGR).

Significance: *Renibacterium salmoninarum* is a significant cause of mortality, particularly with Pacific salmon.

score = 0; no *R. salmoninarum*-associated lesions in the section.

score = 1; total cross sectional area of *R. salmoninarum*-associated lesions fills <1 40 $\times$  objective-lens field.

score = 2; total cross sectional area of *R. salmoninarum*-associated lesions fills >1 40 $\times$  but <1 10 $\times$  objective-lens field, or diffuse organisms are associated with minimal inflammation (this type requires a Gram stain for diagnosis; e.g., 2007-1679-2A).

score = 3; total cross sectional area of *R. salmoninarum*-associated lesions fills >1 10 $\times$  objective-lens field.

8. SPS = *Piscirickettsia salmonis*. *Piscirickettsia salmonis* is a Gram-negative coccoid organism (primitive bacterium) that causes Salmonid Rickettsial Septicemia. Fish with *P. salmonis* commonly present with multiple pale foci in the liver, but many cases also have organisms in the spleen. Microscopically, *P. salmonis* infection is usually associated with vasculitis, peritonitis (sometimes fibrinous), and variable numbers of macrophages with cytoplasmic organisms (each about 1  $\mu$ m in diameter). *P. salmonis* is most easy to identify when a cluster of uniform-diameter organisms occur in an otherwise clear macrophage vacuole. Differentials include (1) melanin granules, which are monomorphic, brown-black, and smaller, and (2) karyorrhectic debris, which is polymorphic, often larger, and usually stains more basophilic on H&E. Positive identification is best done using an immunohistochemical stain optimized at the Animal

Health Centre. Without using the immunohistochemical stain, it is likely that mild cases in the spleen will be missed.

Significance: *Piscirickettsia salmonis* is associated with sporadic mortality in pen-reared salmon in BC.

score = 0; no *P. salmonis*-associated lesions in the section.

score = 1; total cross sectional area of *P. salmonis*-associated lesions fills <1 40× objective-lens field, or reaction limited to infected macrophages that can be disseminated throughout the parenchyma.

score = 2; total cross sectional area of *P. salmonis*-associated lesions fills >1 40× but <1 10× objective-lens field.

score = 3; total cross sectional area of *P. salmonis*-associated lesions fills >1 10× objective-lens field.

9. SLS = *Loma salmonae*. *Loma salmonae* is a microsporidian parasite that forms discreet clusters (xenomas) of spores in the circulatory system. Most xenomas are 50 to 100 µm in diameter. Spores, about  $5.5 \times 3$  µm, have a single polar capsule that is weakly Gram positive and birefringent under polarized light. Reaction to the xenomas varies from none to severe necrotizing vasculitis. Reaction to spores outside of xenomas is granulomatous.

Significance: Infection with *Loma salmonae* is often associated with inflammation and tissue destruction, but these lesions often do not surround the xenomas. It may be that the xenomas stimulate the release of inflammatory mediators that cause most of the damage in the host.

score = 0; no *Loma salmonae*.

score = 1; <7 *Loma salmonae* in the section, and no significant inflammation.

score = 2; <7 *Loma salmonae* in the section, with significant inflammation, or >7 xenomas in the section with no associated inflammation.

score = 3; > 7 *Loma salmonae* in the section, with severe associated inflammation.

Chapter 6. Type specimens for scores in the intestine, exocrine pancreas, and mesenteric adipose tissue. Abbreviations are explained after the table.

Intestine/Exocrine Pancreas (Summary of type specimens)				
Lesion Abbreviation	None Score = 0	Mild score = 1	Moderate score = 2	Severe score = 3
IAtly	2006-3212-6	2006-3213-1	2006-3003-3	2006-4658-8
IPR	2006-4273-7	2006-3212-6	2006-3667-2	2007-0260-4
EPN	2006-3212-6			
MCC	2006-3212-6		2010-1057-4B 2007-2101-5B	
FEN	2006-3212-6	2007-4519-7B	2009-1466-5B	2009-4674-2B
IGR	2006-3212-6		2007-1061-5B	
IRS	2006-3212-6	2007-1070-3B	2007-1398-7B	2008-0392-5B 2009-1871-2B

Scores for intestine include all segments of intestine, including intestinal (pyloric) ceca. Mesenteric adipose tissue and exocrine pancreas are scored here, regardless of whether they are adjacent to the spleen or adjacent to the intestinal ceca.

#### *Quality Control/Quality Assurance*

IAtly = Autolysis. Changes in membrane integrity begin immediately after death, and the intestine is one of the first organs to autolyze. Digestive enzymes continue to function after the fish dies, resulting in autodigestion before tissues are fixed by the formalin. In farmed salmon, intestinal autolysis most commonly involves lysis of the tips of villi; the crypts are usually the last to autolyze. Nuclear and cellular detail is lost, and staining intensity of autolyzed cells tends to decrease.

Significance: Autolysis rapidly decreases the sensitivity and specificity of histopathology.

score = 0; no membrane changes, erythrocytes stain intensely, the tips of intestinal villi are intact.

score = 1; < 1/3 of intestinal villi are autolyzed.

score = 2; > 1/3 but < full length of intestinal villi are autolyzed.

score = 3; full length of intestinal villi are autolyzed.

#### *Lesions*

1. IPR = peritonitis. Inflammation on the surface of the intestine is composed of variable amounts of granulomatous inflammation, lymphoplasmacytic infiltrates, and fine fibrocellular fronds. In some cases, the inflammation includes intralesional vacuoles 50 - 200 µm in diameter. Peritonitis sometimes extends into the surrounding spleen (scored separately under PER).

Significance: Intestinal peritonitis is consistent with a reaction to foreign material; it is common in fish that have been vaccinated. Vacuoles probably represent lipophilic vaccine material that was removed during tissue processing (alcohol and xylene remove

lipid from tissues before staining). Peritonitis can also result from a bacterial infection (e.g., *Yersinia ruckeri* or *Aeromonas salmonicida*).

score = 0; no peritonitis.

score = 1; peritonitis covers <2 mm of the surface of the intestine, or the inflammation is <300 µm thick.

score = 2; peritonitis covers 2 - 5 mm of the surface of the intestine, or the inflammation is 300 - 500 µm thick.

score = 3; peritonitis covers >5 mm of the surface of the intestine, or the inflammation is >500 µm thick.

2. EPN = exocrine pancreas necrosis. General features of necrotic exocrine pancreatic cells are similar to hepatocellular necrosis. Affected cells have hypereosinophilic coagulated cytoplasm, and pyknotic, karyorrhectic, or karyolytic nuclei. The primary differential is autolysis: cytoplasmic staining intensity of autolyzed cells decreases, whereas staining intensity of necrotic cells increases. Some slides that lack intestine have exocrine pancreas near the spleen; these foci are scored here.

Significance: Necrosis of pancreatic acinar cells is a hallmark of infection with Infectious Pancreatic Necrosis virus (IPNV, Birnaviridae) and salmon pancreas disease (SPDV, salmonid alphavirus, Togaviridae). These diseases have not been identified in pen reared salmon in BC.

score = 0; no necrotic cells in the section.

score = 1; total area of necrosis is < 1 40× objective lens field.

score = 2; total area of necrosis is > 1 40× but < 1 10× objective lens field.

score = 3; total area of necrosis is >1 10× objective lens field.

3. MCC = mesenteric capillary congestion and hemorrhage. Distension of capillaries in the mesenteric adipose tissue is often part of the inflammatory response to many infectious diseases; hemorrhage sometimes occurs in severe cases.

Significance: In British Columbia, congestion and hemorrhage is most commonly associated with VHSV and bacterial infections.

score = 0; no capillary congestion or hemorrhage; most sections of mesenteric adipose tissue have <12 RBC-containing capillaries per 40× objective-lens field.

score = 1; most sections of mesenteric adipose tissue have >12 but <24 RBC-containing capillaries per 40× objective-lens field; or, erythrocytes outside of blood vessels affect an area < a 40× objective lens field.

score = 2; most sections of mesenteric adipose tissue have >24 RBC-containing capillaries per 40× objective-lens field, but erythrocytes are rarely stacked more than 2 cells thick; or, erythrocytes outside of blood vessels affect a total area > a 40× objective lens field, but < a 10× objective lens field.

score = 3; most sections of mesenteric adipose tissue have >24 RBC-containing capillaries per 40× objective-lens field, and erythrocytes are often stacked more than 2 cells thick; or, erythrocytes outside of blood vessels affect a total area > a 10× objective lens field.

4. FEN = fibrinocellular enteritis. The lumen of affected intestinal ceca contains plugs of fibrin and various numbers of inflammatory cells or bacteria; some cases have few or no

inflammatory cells or bacteria. The fibrin is usually more eosinophilic than the surrounding absorptive epithelium. Fibrin tends to cover the surface of the villi and can accumulate in larger volumes in the central lumen.

Significance: Fibrinous plugs in the intestinal lumen are evidence of enteritis. The inflammation might be local or systemic, with bacterial or viral infection the most likely differentials. The lesion is considered classic [but not pathognomonic] for infectious pancreatic necrosis (IPNV).

score = 0; no fibrinocellular enteritis.

score = 1; thickness of fibrin plugs between villi and in the central lumen is less than the width of adjacent villi; if only one plug present, it can be up to 3× villus width.

score = 2; thickness of fibrin plugs in the central lumen is 1 - 2× the width of adjacent villi.

score = 3; thickness of fibrin plugs in the central lumen is > 2× the width of adjacent villi.

5. IGR = granulomatous enteritis. Inflammation is composed of variable amounts of organized macrophages, which usually include multinucleate giant cells. Multinucleate giant cells sometimes surround globular green-yellow-brown material. Inflammation may also include variable numbers of fibroblasts, lymphocytes, and plasma cells. Lesions with *Renibacterium salmoninarum* are scored under IRS. When present, radiating globular material in IGR multinucleate giant cells (MNGs) is a key feature differentiating IGR from the primary differential, IRS (IRS occasionally contains MNGs, but IRS granulomas do not contain radiating globular material).

Significance: Granulomatous enteritis is evidence of chronic immune stimulation. This most likely is a vaccine reaction (i.e., the globular green-yellow-brown material); differentials include chronic bacterial disease (e.g., *Yersinia ruckeri* or *Renibacterium salmoninarum* infection).

score = 0; no granulomatous inflammation.

score = 1; granulomatous inflammation limited to total sectional area <500 µm in diameter.

score = 2; total sectional area of granulomatous inflammation 500 µm to 2 mm in diameter.

score = 3; total sectional area of granulomatous inflammation >2 mm in diameter.

6. IRS = *Renibacterium salmoninarum*. *Renibacterium salmoninarum*, the cause of bacterial kidney disease, causes a wide range of lesions in the intestine, from acute necrosis with fibrin exudation, to chronic granulomatous inflammation with fibrosis. Numbers of organisms in affected intestines varies from almost none to very abundant. Twort's Gram stain works well for highlighting the short Gram-positive rods; however, because the lesions alone are fairly diagnostic, a Gram stain is not routinely done with Auditing cases. Infection in the intestine is usually associated with infection in other organs (those lesions are scored separately in the chapter for each organ). Unless a Gram stain is done, lesions suggestive of IRS usually are scored in the IGR category (i.e., the reported IRS prevalence probably underestimates the true prevalence of IRS). Lack of radiating globular material in multinucleate giant cells of IRS is the key feature differentiating granulomatous inflammation of IRS from IGR (radiating globular material is a prominent feature of multinucleate giant cells of IGR).



Significance: *Renibacterium salmoninarum* is a significant cause of mortality, particularly with Pacific salmon.

score = 0; no *R. salmoninarum*-associated lesions in the section.

score = 1; total cross sectional area of *R. salmoninarum*-associated lesions fills <1 40× objective-lens field.

score = 2; total cross sectional area of *R. salmoninarum*-associated lesions fills >1 40× but <1 10× objective-lens field.

score = 3; total cross sectional area of *R. salmoninarum*-associated lesions fills >1 10× objective-lens field.

Chapter 7. Type specimens for Brain scores. Abbreviations are explained after the table.

BRAIN (Summary of type specimens)				
Lesion Abbreviation	None Score = 0	Mild score = 1	Moderate score = 2	Severe score = 3
BAtly	2007-0195-3B	2007-0261-4A	2007-2728-2B	
BHM	2007-0261-4A	2007-0261-5B		2007-0261-1A
BCC	2007-0261-4A			2007-1680-2B
MEN	2007-0261-4A	2007-0546-4B	2009-5112-4B 2007-1678-1 2007-0545-2B	2008-2314-4B
MSC	2007-0261-4A	2007-781-6B	2007-4656-5B	2007-0545-3B
MXA	2007-0261-4A		2007-0375-10B	
PMX	2007-0261-4A		2007-4431-1B	2010-4660-1A
BGR	2007-0261-4A	2007-0261-5B	2007-0613-1B	
BRS	2007-0261-4A	2008-4314-3B	2007-0375-6B	2007-0613-3B
BPS	2007-0261-4A		2007-3907-4B	2007-1061-6B
BLS	2007-0261-4A		2008-0629-1B	

#### Quality Control/Quality Assurance

BAtly = Autolysis. Changes in membrane integrity begin immediately after death

Significance: Autolysis rapidly decreases the sensitivity and specificity of histopathology.

score = 0; no membrane changes, Nissl substance in neuron cell bodies is discrete; erythrocytes stain intensely.

score = 1; margins of neuron cell bodies have small vacuoles (<3 µm in diameter); erythrocytes are pale.

score = 2; margins of neuron cell bodies have larger vacuoles (>3 µm in diameter); Nissl substance is clumped, neuron cell bodies begin to collapse (outlines are irregular) or expand (pale, with clumps of Nissl substance that resemble a *Loma salmonae* xenoma).

score = 3; .

#### Lesions

1. BHM = brain hemorrhage. Erythrocytes outside of vessels are evidence of hemorrhage. Vessels distended by erythrocytes, but still intact, are scored under BCC below. Significance: Microhemorrhage is evidence of increased fragility of vessel walls, often as a result of circulating toxins; differentials include viral and bacterial infections. Massive hemorrhage is more likely a result of trauma; the primary differential for severe hemorrhage is a "bonk on the head" used to kill the fish before sampling the tissues. score = 0; no hemorrhage.

- score = 1; hemorrhage limited to total sectional area  $<500\ \mu\text{m}$  in diameter.  
 score = 2; total sectional area of hemorrhage  $500\ \mu\text{m}$  to 2 mm in diameter, or hemorrhage extends  $>0.5\ \text{mm}$  and  $<2\ \text{mm}$  along a single line.  
 score = 3; total sectional area of hemorrhage  $>2\ \text{mm}$  in diameter, or hemorrhage extends  $>2\ \text{mm}$  along a single line.
2. BCC = brain capillary (vascular) congestion. The neuropil contains a rich network of capillaries, but in any given section, the majority of capillaries normally contain no erythrocytes. By comparison, when cerebral capillaries are congested, a greater proportion of capillaries in the section will contain erythrocytes. Congestion of meningeal vessels (some of which are larger than capillaries) are also scored in this category. Erythrocytes that leak outside of vessel walls are scored under BHM above. Significance: congestion of brain capillaries is evidence of circulating vasodilators; differentials include viral, bacterial (e.g., filamentous bacteria of "mouth rot"), and parasitic infections. Focal capillary congestion might be a result of vascular occlusion (e.g., a thrombus). BCC is representative of lethal change when severe; when moderate, BCC may be listed as a cause of death in combination with another lesion.  
 score = 0; no capillary congestion; most sections of grey matter have  $<12$  RBC-containing capillaries per  $40\times$  objective-lens field.  
 score = 1; most sections of grey matter have  $>12$  but  $<24$  RBC-containing capillaries per  $40\times$  objective-lens field.  
 score = 2; most sections of grey matter have  $>24$  RBC-containing capillaries per  $40\times$  objective-lens field, but erythrocytes are rarely stacked more than 2 cells thick.  
 score = 3; most sections of grey matter have  $>24$  RBC-containing capillaries per  $40\times$  objective-lens field, and erythrocytes are often stacked more than 2 cells thick.
3. MEN = meningitis or encephalitis. Any mixture of inflammation of the brain and meninges that is not associated with a scored organism diagnosis is included in this category; inflammatory cells could include macrophages, lymphocytes, plasma cells, and neutrophils. Vasculitis and fibrin exudation are also included. By comparison, pure granulomatous inflammation is scored only under BGR (below). Significance: meningitis and encephalitis are evidence of immune stimulation; differentials include viruses, bacteria, or parasites (e.g. in Pacific salmon, cases of *Loma salmonae* in which the organisms are not seen in the section examined).  
 score = 1; meningitis and encephalitis limited to total sectional area  $<500\ \mu\text{m}$  in diameter.  
 score = 2; total sectional area of meningitis and encephalitis  $500\ \mu\text{m}$  to 2 mm in diameter.  
 score = 3; total sectional area of meningitis and encephalitis  $>2\ \text{mm}$  in diameter.
4. MSC = *Microsporidium cerebralis*. *Microsporidium cerebralis* is a microsporidian that affects pen-reared salmon. Clusters of spores sometimes occur within large neuron cell bodies. More severe infections are associated with inflammation composed mostly of dissemination macrophages, with scattered lymphocytes. Significance: *Microsporidium cerebralis* is associated with sporadic mortality in pen-reared salmon in BC.  
 score = 0; no *Microsporidium cerebralis* in the section.

- score = 1; *Microsporidium cerebralis*-associated lesions limited to total sectional area <500  $\mu\text{m}$  in diameter.
- score = 2; total sectional area of *Microsporidium cerebralis*-associated limited to total sectional area 500  $\mu\text{m}$  to 2 mm in diameter.
- score = 3; total sectional area of *Microsporidium cerebralis*-associated lesions >2 mm in diameter.
5. **MXA - *Myxobolus arcticus*.** *Myxobolus arcticus* is a myxosporean common in the brains of Pacific salmon. Clusters of spores are mostly limited to the brain stem. The spores seem to mostly be space-occupying lesions: inflammation is rare.  
Significance: *Myxobolus arcticus* is mostly an incidental finding in Pacific salmon.
- score = 0; no *Myxobolus arcticus* in the section.
- score = 1; *Myxobolus arcticus* -associated lesions limited to total sectional area <500  $\mu\text{m}$  in diameter.
- score = 2; total sectional area of *Myxobolus arcticus* -associated limited to total sectional area 500  $\mu\text{m}$  to 2 mm in diameter.
- score = 3; total sectional area of *Myxobolus arcticus* -associated lesions >2 mm in diameter.
6. **PMX - presporogonic myxosporean.** An unidentified organism, tentatively classified as a presporogonic myxosporean, is sporadically associated with meningitis in Atlantic salmon. Organisms are most common in macrophages and ependymal cells, but at least one case has some organisms that seem to be in nerve processes. Organisms vary from  $2.5 \times 3 \mu\text{m}$  to  $3 \times 4 \mu\text{m}$ ; they seem to be intracytoplasmic and unicellular, but some are either bi- or tri-nucleate or have deeply lobed nuclei. Some seem to be surrounded by host nuclei, but I think this is just a function of the thick sections. Most of the parasites occur within macrophages, including melanophages, but others seem to be within neurons (e.g., case 2007-3158-4B). Inflammation is common, varying from neutrophilic, to lymphocytic and granulomatous.  
Significance: Significant inflammation associated with parasite is evidence that it is the cause of death in most affected fish. It does not occur in Pacific salmon.
- score = 0; no presporogonic myxosporea in the section.
- score = 1; lesions associated with the presporogonic myxosporean are < 100  $\mu\text{m}$  thick, or total volume of inflammation is <500  $\mu\text{m}$  in diameter.
- score = 2; lesions associated with the presporogonic myxosporean are 100 - 300  $\mu\text{m}$  thick, or total volume of inflammation is 500  $\mu\text{m}$  to 2 mm in diameter.
- score = 3; meningitis associated with the presporogonic myxosporea is >300  $\mu\text{m}$  thick, or total volume of inflammation is > 2 mm in diameter.
7. **BGR = granulomatous meningoencephalitis.** Inflammation is composed of variable amounts of organized macrophages, which might include multinucleate giant cells. Multinucleate giant cells sometimes surround globular green-yellow material. Inflammation may also include variable numbers of fibroblasts, lymphocytes, and plasma cells. Lesions with *Renibacterium salmoninarum* are scored under BRS and those with *Piscirickettsia salmonis* are scored under BPS. When present, radiating globular material in BGR multinucleate giant cells (MNGs) is a key feature differentiating BGR from the

primary differential, BRS (BRS occasionally contains MNGs, but BRS granulomas do not contain radiating globular material). In Pacific salmon, a major differential is BLS; do a Twort's stain to look for Gram positive spores.

Significance: Granulomatous meningoencephalitis is evidence of chronic immune stimulation. This most likely is a vaccine reaction (i.e., the globular green-yellow material); differentials include chronic bacterial disease (e.g., *Yersinia ruckeri* or *Renibacterium salmoninarum* infection).

score = 0; no granulomatous inflammation.

score = 1; granulomatous inflammation limited to total sectional area <500  $\mu$ m in diameter.

score = 2; total sectional area of granulomatous inflammation 500  $\mu$ m to 2 mm in diameter.

score = 3; total sectional area of granulomatous inflammation >2 mm in diameter.

8. BRS = *Renibacterium salmoninarum*. *Renibacterium salmoninarum*, the cause of bacterial kidney disease, in the brain is mostly commonly is associated with meningitis. As in other organs BRS causes a wide range of lesions in the brain: from acute necrosis with fibrin exudation, to chronic granulomatous inflammation. Numbers of organisms in affected brains varies from almost none to very abundant; abundant pale basophilic organisms are readily diagnosed on H&E without a Gram stain. When organism numbers are not abundant, Twort's Gram stain works well for highlighting the short Gram-positive rods. Gram staining is not routinely done with Auditing cases. Infection in the brain is usually associated with infection in other organs (those lesions are scored separately in the chapter for each organ). Unless a Gram stain is done or organisms are obvious on H&E, lesions suggestive of BRS are scored in the MEN or BGR categories (i.e., the BRS prevalence probably underestimates the true prevalence of BRS). Lack of radiating globular material in multinucleate giant cells of BRS is the key feature differentiating granulomatous inflammation of BRS from BGR (radiating globular material is a prominent feature of multinucleate giant cells of BGR).

Significance: *Renibacterium salmoninarum* is a significant cause of mortality, particularly with Pacific salmon.

score = 0; no *R. salmoninarum*-associated lesions in the section.

score = 1; total cross sectional area of *R. salmoninarum*-associated lesions fills <1 40 $\times$  objective-lens field.

score = 2; total cross sectional area of *R. salmoninarum*-associated lesions fills >1 40 $\times$  but <1 10 $\times$  objective-lens field.

score = 3; total cross sectional area of *R. salmoninarum*-associated lesions fills >1 10 $\times$  objective-lens field.

9. BPS = *Piscirickettsia salmonis*. *Piscirickettsia salmonis* is a Gram-negative coccoid organism (primitive bacterium) that causes Salmonid Rickettsial Septicemia. Fish with *P. salmonis* commonly present with multiple pale foci in the liver, but many cases also have organisms in the spleen. Occasionally, the brain is the site of most severe inflammatory response. Microscopically, *P. salmonis* infection is usually associated with vasculitis, peritonitis (sometimes fibrinous), and variable numbers of macrophages with cytoplasmic organisms (each about 1  $\mu$ m in diameter). *P. salmonis* is most easy to

identify when a cluster of uniform-diameter organisms occur is an otherwise clear macrophage vacuole. Differentials include (1) melanin granules, which are monomorphic, brown-black, and smaller, and (2) karyorrhectic debris, which is polymorphic, often larger, and usually stains more basophilic on H&E. Positive identification is best done using an immunohistochemical stain optimized at the Animal Health Centre. Without using the immunohistochemical stain, it is likely that mild cases in the brain will be missed.

Significance: *Piscirickettsia salmonis* is associated with sporadic mortality in pen-reared salmon in BC.

score = 0; no *P. salmonis*-associated lesions in the section.

score = 1; total cross sectional area of *P. salmonis*-associated lesions fills <1 40× objective-lens field, or reaction limited to infected macrophages that can be disseminated throughout the parenchyma.

score = 2; total cross sectional area of *P. salmonis*-associated lesions fills >1 40× but <1 10× objective-lens field.

score = 3; total cross sectional area of *P. salmonis*-associated lesions fills >1 10× objective-lens field.

10. BLS = *Loma salmonae* xenomas, spores, and associated inflammation. *Loma salmonae* is a microsporidian parasite that forms discreet clusters (xenomas) of spores in the circulatory system. Most xenomas are 50 to 100 µm in diameter. Spores, about 5.5 × 3 µm, have a single polar capsule that is weakly Gram positive and sometimes birefringent under polarized light. Reaction to the xenomas varies from none to severe necrotizing vasculitis. Vasculitis in the brain is rare, but can be associated with cerebral edema. Reaction to spores outside of xenomas is granulomatous. Significance: Infection with *Loma salmonae* is often associated with inflammation and tissue destruction. Inflammation is more commonly associated with free spores than with xenomas. A Gram stain is usually needed to localize free spores within inflammatory foci.

score = 0; no *Loma salmonae*.

score = 1; <7 *Loma salmonae* in the section, and no significant inflammation.

score = 2; <7 *Loma salmonae* in the section, with significant inflammation, or >7 xenomas in the section with no associated inflammation.

score = 3; > 7 *Loma salmonae* in the section, with severe associated inflammation.

Chapter 8. Type specimens for gill scores. Abbreviations are explained after the table.

GILL (Summary of type specimens)				
Lesion Abbreviation	None Score = 0	Mild score = 1	Moderate score = 2	Severe score = 3
GAtly	2007-3234-2C	2006-3003-4		2007-3527-2G
GLH	2006-3003-4			
GLF	2006-3003-4		2007-3525-5G	2007-3525-7G
LSE	2006-3003-4			2006-1588-2
GLT	2006-3003-4			
GGR	2006-3003-4	2010-4659-4G		
GRS	2006-3003-4	2006-1031-8 (xenomas and free spores)	2007-0375-4G 2006-1031-6 (xenomas and free spores)	2006-2048-4
GLS	2006-3003-4			2006-3663-2

Anatomy note: I use the terms gill arch, gill filament, and gill lamellae to describe the 3 basic levels of organization of the gill (I do not use the terms "primary lamella" or "secondary lamella").

#### *Quality Control/Quality Assurance*

GAtly = Autolysis. Changes in membrane integrity begin immediately after death, and the gill is the first organ to autolyze; lamellar epithelial cells are the first to autolyze. In farmed salmon, autolyzed lamellar epithelial cells slough off the supporting pillar cells, membranes stain poorly, and nuclei lyse. The primary differential for separation of lamellar epithelium is toxicant exposure. Protein-rich fluid beneath the separated epithelium is evidence of a lesion, whereas clear fluid deep to the separated epithelium cannot be differentiated from postmortem change. Reliable differentiation of transudate and postmortem change requires sampling of gills from anesthetized fish and comparison of exposed and control fish--conditions not possible with the design of our Auditing program. Staining intensity of autolyzed epithelial cells tends to decrease (i.e., autolyzed gill stains pale).

Significance: Autolysis rapidly decreases the sensitivity and specificity of histopathology.

score = 0; no membrane changes, erythrocytes stain intensely, and lamellar epithelium is intact.

score = 1; lamellar epithelium is partly separated from supporting cells, but more than half of the epithelium is intact.

score = 2; more than half of the lamellar epithelium is separated from the supporting cells, but most cells remain intact.

score = 3; more than half of the lamellar epithelium is separated from the supporting cells, and >10% of cells are detached from each other or have lost nuclear detail.



*Lesions*

1. GLH = gill lamellar hyperplasia/hypertrophy. The thin layer of cells lining the gill provides a vital permeable barrier between the water and the blood, allowing for exchange of oxygen, carbon dioxide, and ammonia. When these delicate cells are irritated, they often respond by increasing in number (hyperplasia) and size (hypertrophy). In some cases, the tips of lamellae fuse (fusion is scored under GLF below).  
Significance: Gill lamellar hyperplasia/hypertrophy decreases the efficiency of gas exchange. Common causes of GLH include physical and chemical irritants in the water, including parasites (e.g., *Paramoeba* spp.).  
score = 0; lamellar epithelium is uniformly simple and squamous.  
score = 1; foci of lamellar hyperplasia/hypertrophy total < 500  $\mu$ m of filament affected.  
score = 2; foci of lamellar hyperplasia/hypertrophy total > 500  $\mu$ m but < 5 mm of filament affected.  
score = 3; foci of lamellar hyperplasia/hypertrophy total > 5 mm of affected filament.
2. GLF = gill lamellar fusion. Fusion of adjacent lamellae can be either partial or complete. GLF is often associated with lamellar hyperplasia/hypertrophy (scored separately under GLH above).  
Significance: Lamellar fusion decreases the available surface area for gas transfer. Gill lamellar fusion may be a result of physical damage from exposure to a parasite or diatoms (e.g., *Chaetoceros* spp.). It has been associated with exposure to heavy metals and with hypoxia (thought to be from fish gasping for oxygen at the water-air interface).  
score = 0; lamellae are not fused.  
score = 1; foci of lamellar fusion total < 500  $\mu$ m of affected filament(s).  
score = 2; foci of lamellar fusion total > 500  $\mu$ m but < 5 mm of affected filament(s).  
score = 3; foci of lamellar fusion total > 5 mm of affected filament(s).
3. LSE = lamellar subepithelial edema. Separation of gill lamellar epithelium from underlying pillar cells can be a result of edema, and it also is a common postmortem artifact. In this case, accumulation of proteinaceous fluid deep to the separated epithelium supports the diagnosis of edema over artifact.  
Significance: Lamellar edema is commonly associated with exposure to toxins, including formalin and hydrogen peroxide overdose. Lamellar edema is reversible if the inciting cause is removed.  
score = 0; no subepithelial edema.  
score = 1; foci of subepithelial edema total < 500  $\mu$ m of filament affected.  
score = 2; foci of subepithelial edema total > 500  $\mu$ m but < 5 mm of filament affected.  
score = 3; foci of subepithelial edema total > 5 mm of filament affected.
4. GLT = gill lamellar telangiectasis. Telangiectasis is the permanent dilation of lamellar capillaries. It is usually associated with trauma, and affected foci are usually >40  $\mu$ m in diameter. Some dilated lamellar capillaries contained thrombi.  
score = 0; no dilated lamellar capillaries per section.  
score = 1; <20 dilated lamellar capillaries per section, and  $\leq$ 2 with thrombosis.  
score = 2; >20 dilated lamellar capillaries per section, or >2 with thrombosis.



score = 3; >20 dilated lamellar capillaries per section, with associated thrombosis, lamellar hyperplasia, and necrosis.

5. GGR = granulomatous branchitis. Inflammation is composed of variable amounts of organized macrophages, which usually include multinucleate giant cells. Multinucleate giant cells sometimes surround globular green-yellow material. Inflammation may also include variable numbers of fibroblasts, lymphocytes, and plasma cells. Lesions with *Renibacterium salmoninarum* are scored under GRS and those with *Piscirickettsia salmonis* are scored under GPS. When present, radiating globular material in GGR multinucleate giant cells (MNGs) is a key feature differentiating GGR from the primary differential, GRS (GRS occasionally contains MNGs, but GRS granulomas do not contain radiating globular material). In Pacific salmon, a major differential is GLS; do a Twort's stain to look for Gram positive spores.  
Significance: Granulomatous branchitis is evidence of chronic immune stimulation. This most likely is a vaccine reaction (i.e., the globular green-yellow material); differentials include chronic bacterial disease (e.g., *Yersinia ruckeri* or *Renibacterium salmoninarum* infection).  
score = 0; no granulomatous inflammation.  
score = 1; granulomatous inflammation limited to total sectional area <500  $\mu$ m in diameter.  
score = 2; total sectional area of granulomatous inflammation 500  $\mu$ m to 2 mm in diameter.  
score = 3; total sectional area of granulomatous inflammation >2 mm in diameter.
5. GRS = *Renibacterium salmoninarum*. *Renibacterium salmoninarum*, the cause of bacterial kidney disease, causes a wide range of lesions in the gill, from acute necrosis with fibrin exudation, to chronic granulomatous inflammation with fibrosis. Numbers of organisms in affected gills varies from almost none to very abundant. Twort's Gram stain works well for highlighting the short Gram-positive rods; however, because the lesions alone are fairly diagnostic, a Gram stain is not routinely done with Auditing cases. Infection in the gill is usually associated with infection in other organs (those lesions are scored separately in the chapter for each organ). Unless a Gram stain is done, lesions suggestive of GRS usually are scored in the GGR category (i.e., the GRS prevalence probably underestimates the true prevalence of GRS). Lack of radiating globular material in multinucleate giant cells of GRS is the key feature differentiating granulomatous inflammation of GRS from GGR (radiating globular material is a prominent feature of multinucleate giant cells of GGR).  
Significance: *Renibacterium salmoninarum* is a significant cause of mortality, particularly with Pacific salmon.  
score = 0; no *R. salmoninarum*-associated lesions in the section.  
score = 1; total cross sectional area of *R. salmoninarum*-associated lesions fills <1 40 $\times$  objective-lens field.  
score = 2; total cross sectional area of *R. salmoninarum*-associated lesions fills >1 40 $\times$  but <1 10 $\times$  objective-lens field.  
score = 3; total cross sectional area of *R. salmoninarum*-associated lesions fills >1 10 $\times$  objective-lens field.

6. GLS = *Loma salmonae* xenomas, spores, and associated inflammation. *Loma salmonae* is a microsporidian parasite that forms discreet clusters (xenomas) of spores in the circulatory system. Most xenomas are 50 to 100  $\mu\text{m}$  in diameter. Spores, about  $5.5 \times 3 \mu\text{m}$ , have a single polar capsule that is weakly Gram positive and birefringent under polarized light. Reaction to the xenomas varies from none to severe necrotizing vasculitis. Reaction to spores outside of xenomas is granulomatous. Significance: Infection with *Loma salmonae* is often associated with inflammation and tissue destruction. Inflammation is more commonly associated with free spores than with xenomas. A Gram stain is usually needed to localize free spores within inflammatory foci.
- score = 0; no *Loma salmonae*.
- score = 1;  $<7$  *Loma salmonae* in the section, and no significant inflammation.
- score = 2;  $<7$  *Loma salmonae* in the section, with significant inflammation, or  $>7$  xenomas in the section with no associated inflammation.
- score = 3;  $> 7$  *Loma salmonae* in the section, with severe associated inflammation.