LABIAL SALIVARY GLAND BIOPSY
Standard Operating Procedures for Diagnosis and Focus Scoring
(Performed at the SICCA Coordinating Center)

Specimen handling:

1. Labial salivary gland biopsy specimens will be delivered to Oral Pathology (S-512) by DD along with a SICCA *Baseline Labial Salivary Gland Biopsy Report Form* for each specimen. She will complete the participant ID number and items #1 and #2 on the forms. If no stained sections are present, but unstained sections are, one of the latter should be taken to the OP lab to have it stained. If a block is present but there are no stained or unstained sections, the block should be taken to the OP lab to have two H&E-stained sections cut. If neither a block nor sections are available, inquiry should be made to the SICCA Group which accessioned that participant.

2. The oral pathologist on service (RJ, JG, or DC) the day the specimens arrive will examine them and complete the report forms. After completing the examinations and reports, the slides and completed forms are placed in their slide folder in the bottom tray, next to the dictation microscope and TD is notified of their presence.

3. Later that day, or as soon as possible, the specimens will be reexamined by TD. If there is disagreement in the two examinations, the two examiners will meet and resolve the difference by consensus.

4. The Results forms and accompanying slides are then given to Peggy Rasmussen for transmission of the forms to CIDEA by DataFax and conveyance of the slides to DD.

Histopathological examination and diagnosis:

1. First, complete items #3 and #4 on the Results Form.

2. In item #5 on the form, indicate the specimen's histopathological diagnosis, in the categories of: Focal lymphocytic sialadenitis (FLS),* Focal sclerosing lymphocytic sialadenitis (FSLS),* Within normal limits, Non-specific chronic sialadenitis, Sclerosing chronic sialadenitis, or Granulomatous inflammation.

3. If the diagnosis is <u>not</u> FLS or FSLS, sign and date the form.

4. Indicate the presence or absence of germinal centers, epimyoepithelial islands or ductal hyperplasia, and/or fatty replacement in #12 through #14, respectively.

Focus score measurement:

1. The goal is to count lymphocytic aggregates that have 50 or more cells, which surround or are closely associated with a duct or blood vessel, and acini adjacent to the foci appear within normal limits.* This measure does not distinguish between small and large foci.

2. If a case exhibits both FLS <u>and</u> non-specific chronic or sclerosing sialadenitis and FLS is the

most prominent feature in the specimen, proceed to focus score measurement. In such a case, the lymphocytic focus counting should be done where ever they occur, but all glands should be included in the area measurement.

3. First, count the number of lymphocytic foci in the specimen and enter that value in #6. In glands exhibiting confluent foci (*i.e.* focal lymphocytic infiltrates that have become too large or numerous to be counted separately), count the lobules of a gland so affected, instead of foci.

4. Next, count the total number of eyepiece-graticule squares (through the 10x10 mm eyepiece graticule) that are necessary to cover all the glands exhibiting FLS, using the 4x objective lens. The area counted is everything within the gland outline and excludes interglandular connective tissue, if present. Enter that value in #7. (**NB**: Prior calibration with a stage micrometer has determined that, on the multi-head Olympus microscope in room S-516, 12.53 squares, at that magnification, are equal to an area of one (1) mm^2 without magnification.)

5. Divide the number of squares, corresponding to the area of gland (#7), by 12.53 and enter that value, which is the glandular area of the specimen in mm^2, into #8. If the specimen area is <4 mm^2, indicate no in #9, do not complete the focus score, and go to item #12.

6. Divide the number of foci (#6) by the glandular area of the specimen (#8) and multiply the result by 4. This calculates the number of lymphocytic foci per 4 mm^2 (*i.e*. the focus score). Enter that value in #10.

7. In #11, indicate the number of glands in the specimen that are composed of confluent lymphocytic foci (*i.e*. each lobule of a gland exhibits extensive lymphocytic infiltration, in which individual foci cannot be counted).

Interpreting focus scores:

1. Performing a focus score offers a semi-quantitative assessment, or approximation, of each specimen's severity of FLS. However, focus scoring provides a low degree of precision, mainly because it is intrinsically difficult to standardize counting lymphocytic foci in most specimens. In general, the larger the specimen, the more accurate the focus score, and conversely, the smaller the specimen, the less accurate the focus score.

2. Imprecision in area measurement comes from having to visually fit the irregular contours of a gland's section into the regular squares of the eyepiece grid and deciding which tissue to include in the area count.

3. Imprecision in focus counting occurs in two general categories. 1) In specimens that have extensive lymphocytic infiltration, it may be unclear whether to count a large focus as one, or as several smaller ones (we usually depend on its relationship with a duct to make such a decision). 2) In specimens with many small infiltrates, the difficulty comes from applying the 50 lymphocyte threshold (easier said than done on an actual microscopic section) and from making decisions about whether an infiltrate is dense enough to constitute a focus.

4. In the focus score, there is usually one number after the decimal point, but that is not a significant figure and should be used only for rounding. Also note that if step sections of a LSG biopsy specimen were to be counted, there would be small variance in the area measurements, number of foci, and focus scores from one section to another. A focus score should be considered a categorical, not parametric, statistic.

5. At the outset of examining SICCA LSG specimens, a calibration exercise was developed and conducted for the faculty members in UCSF oral pathology who would be examining the specimens. It used 50 existing cases that included a range of diagnoses and severity. In the day-to-day examination of SICCA specimens, one of three UCSF oral pathologists (RJ, JG, or DC) provides the first assessment of each SICCA specimen and TD provides a second assessment. If there are any significant differences between the two assessments, the specimen is reexamined by both observers to develop a consensus. In addition to quality control, this process also serves to provide ongoing calibration of this procedure.

*Focal lymphocytic sialadenitis (FLS) is defined as the presence of one or more dense aggregates of 50 or more lymphocytes (usually several hundred or more), generally in perivascular or periductal locations. These foci contain no more than a minority proportion of plasma cells and are located adjacent to normal-appearing acini in gland lobes or lobules that do not show generalized duct dilation or fibrosis. This diagnosis is assigned when these foci are the only inflammation present in a specimen, or the most prominent feature. Focal/sclerosing lymphocytic sialadenitis (FSLS) is defined as above, in addition to intralobular fibrosis within one or more glands in the specimen.

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