Silver Impregnation Stain for
Leptospira and Flagella

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Leptospires are difficult to stain with the usual bacterial stains; many different stains have been
proposed, most of which are designed for staining of tissue sections. Silver stains that are available
for smear preparations yield variable, unreliable results. Staining of bacterial flagella is often
avoided because of difficulties involved. There exists a need for a simple technique which does
not greatly alter the morphology of the leptosporal cell, or which will reliably stain flagella.

The silver impregnation technique described here is a modification of a technique described by
D. S. Kim of the Veterinary Research Institute, Pusan, Korea (unpublished data). The modified
technique is rapid, employs only two reagents which are easily applied, and requires no special
fixation or heating. Alteration of the morphology of leptospires and flagella is minimal. The spiral
structure of the spirochetes can often be seen in a relatively normal state.

Reagent A is composed of 100 ml of distilled water containing 5 g of tannic acid, 1.5 g of ferric
chloride, 2.0 ml of 15% formalin, and 1.0 ml of 1% sodium hydroxide.

Reagent B, ammoniated silver nitrate solution, is prepared by use of 100 ml of 2% silver nitrate.
About 10 ml of this volume is removed and saved; to the remaining 90 ml, ammonium hy-
droxide is added dropwise until the heavy precipitate that is formed is dissolved. From the 10
ml previously removed, 2% silver nitrate is added dropwise until a slight clouding appears
and persists. At this point, the pH is adjusted to 10.0 with the ammonium hydroxide and silver
nitrate. Reagent B is relatively unstable, and must be used within 4 hr of preparation. Read-
justment of the pH to 10.0 after it changes has not produced satisfactory results.

Smears are prepared in a routine fashion except that extreme caution must be employed in
handling bacterial suspensions for flagella stain. The slides are alcohol-cleaned and a loopful
of distilled water is placed on each. A loopful of culture or suspension is placed just touching the
distilled water, so that the two diffuse together to achieve a gradation of dilution of the medium.

Slides are then allowed to air-dry. Fixation by heat is unnecessary.

For the preparation of bacteria to produce and maintain maximal numbers of flagella, incubation
at 20°C, harvesting organisms during the logarithmic or early stationary phase, preparation of
a faintly cloudy suspension in distilled water, and extreme care in handling, all as described by

Blood films from animals with leptospirosis are prepared in the fashion routine for hematologi-
al examination and are not fixed prior to the addition of reagent A. It appears, however, that
leptospires are more easily found in very thin smears, and thick areas should be avoided. The
cells will appear thicker, and spirals are not usually visible due to the presence of serum on the
cells. (Fig. 2).

Tissue impression smears have been made by adding a small drop of water to the slide and
making the touch preparation through this water. The preparation is air-dried.

The smears are covered by reagent A for 2 to 4

mill; they are then rinsed in distilled water.

Distilled water and varying concentrations of ammonium hydroxide and ethyl alcohol have
been tried, both as rinses and as 10-min washes, with no apparent difference. After the water
rinse, reagent B (pH 10.0) is added for about 30

sec. The smears are immediately washed with

distilled water, air-dried, and examined under oil

immersion.

Leptospires are stained dark-brown to black on

a light-to-golden background. Other structures,

for example, erythrocytes or other bacteria,

stain dark, but the characteristic shape and

hooked ends of the leptospires should be sufficient

for differentiation from other cells. Also, spirals

can often be observed under high-power magnifi-

cation. The flagella of such organisms as Proteus

vulgaris and Vibrio fetus (Fig. 3 and 4) are readily

stained if care has been taken to prevent their

mechanical removal in the preparation of the

smear.

This stain seems to be dependable and is easily
reproduced. The reagents are composed of readily available chemicals. The time involved in staining is minimal, and the smears often show striking cellular detail. No problem with precipitates has been encountered when the pH of reagent B is correct. The technique would seem to have application in both clinical and research laboratories.

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