INTRODUCTION

In recent years there have been advances in cell culture technology, immunochemical procedures, and the use of peripheral nervous and non-nervous tissues for rabies diagnosis. Since the advent of monoclonal antibodies directed against various components of the rabies virion and their use to distinguish different rabies and/or rabies-like strains, it has become increasingly apparent that many of the biological curios noted in the past are probably valid expressions of different strains of rabies. It is no longer correct to say that all rabies virus isolates behave in a similar manner or that the characteristics of street strains can be inferred from the study of fixed strains. This is becoming evident in diagnostic situations. The laboratory worker must always be on guard for the unusual or altered reaction. We hope to address some of these aberrations in this review.

The principal routine diagnostic procedures in most industrialized countries consist of immunofluorescent staining of brain tissue and inoculation of suspect tissue into mice or cell cultures. However, for a variety of climatic or socio-economic reasons, different procedures may be used in different laboratories. Not all diagnostic laboratories are equipped with fluorescence microscopes, cryostats or CO₂ incubators or have access to laboratory animals. In preparing Table 1, we have attempted to present a choice of techniques suitable for different situations. The listing in Table 1 is not necessarily complete but hopefully contains the publications in which the particular techniques are more fully described.

We have relied heavily upon experience gained by personnel of the Animal Diseases Research Institute, Nepean (ADRI). During the twenty
Table 1. Selected References Containing Techniques for Rabies Diagnosis

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Fresh</th>
<th>Fixed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain (CNS)</td>
<td>FAT*: 1-4, 6, 93, 147, 148</td>
<td>FAT (formalin): 24, 29, 31, 32, 34, 36, 37</td>
</tr>
<tr>
<td></td>
<td>FAT (frozen sections): 71, 106, 113, 131</td>
<td>FAT (acetone): 149, 150</td>
</tr>
<tr>
<td></td>
<td>TC: 57, 58, 63, 65-68, 70</td>
<td>EM: 57, 71, 94, 98, 106, 113, 115</td>
</tr>
<tr>
<td></td>
<td>HIST: 40, 138, 148, 152</td>
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<tr>
<td></td>
<td>ELR: 90, 99</td>
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<tr>
<td></td>
<td>EM: 112, 153</td>
<td></td>
</tr>
<tr>
<td>Salivary gland</td>
<td>FAT (frozen section): 106, 131, 154</td>
<td>EM: 106</td>
</tr>
<tr>
<td>Saliva</td>
<td>TC: 56, 117</td>
<td></td>
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<tr>
<td></td>
<td>MIT: 48, 56, 121, 125</td>
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</tr>
<tr>
<td>Cornea</td>
<td>FAT: 130, 132, 138, 155</td>
<td></td>
</tr>
<tr>
<td>Skin</td>
<td>FAT: 138-141, 143</td>
<td></td>
</tr>
</tbody>
</table>

* FAT = fluorescent antibody staining; MIT = mouse inoculation; TC = cell culture; HIST = histological staining; ELR = enzyme-linked reactions; EM = electron microscopy.

In a period between 1966 and 1986, some 150,000 brain or nervous tissue specimens have been submitted for rabies diagnosis and the number of annual submissions is now approaching 13,000. With a staff of 5 devoted almost exclusively to routine rabies diagnosis, the procedures and techniques used must be both reliable and adaptable to the examination of large numbers of specimens on a daily basis. Although several techniques have been described in the literature and are acceptable as diagnostic procedures, some are impractical in laboratories burdened by large numbers of submissions. We have included some of our procedures which we have found to produce satisfactory results (See Appendix).