Preface

These guidelines are devoted to disseminate molecular methods that can be used to analyze DNA, RNA, and proteins in formalin-fixed and paraffin-embedded (FFPE) tissues. We have called them guidelines because this is the first method book that is entirely dedicated to archive tissues. They are addressed to pathologists, biologists, and biotechnologists who do research in FFPE tissues. The protocols presented in these guidelines derive from the experience of the laboratories participating in the European project "Archive tissues: improving molecular medicine research and clinical practice" – IMPACTS (www.impactsnetwork.eu). The 20 participants, from 11 European countries, are some of the most experienced groups in molecular analysis of fixed and paraffin-embedded human tissues. Among them are some of the groups that developed this type of molecular analysis for the first time.

These guidelines are mostly dedicated to the homemade protocols that were developed, tested, and even validated by multicentric analyses performed by the IMPACTS groups. Experience in basic molecular methods is necessary to develop molecular pathology activities in human tissues. This allows research without too many technical limitations. In this way the researcher is also capable to better evaluate commercial kits and validate them. Also commercial kits are reported in some protocols, especially when the authors have consolidated habits in kit utilization or when advanced techniques are used without being directly developed by the participants. Thus the reported commercial reagents and kits reflect in the same way the experience of the IMPACTS laboratories. However, in our experience standardization of molecular methods by commercial kits is not sufficient to guarantee reproducibility; good laboratory practice is absolutely necessary to obtain reliable results. Sometimes a slightly different "lab jargon" is maintained in the different chapters; this reflects the authors' consuetude but it does not compromise its clarity. Basic footnotes are repeated in many chapters to make it easy to access the single protocol.

We recognize that semi- or fully automated instruments for nucleic acids extraction from FFPE could be very useful in establishing standardization and method reproducibility. In the nearest future they will represent an important task for any molecular pathology laboratory devoted to routine diagnostic molecular analyses in FFPE. However, we did not mention them in these guidelines, because we did not have the chance to compare the performances of the different commercial options.

These guidelines are divided into different parts related to tissue processing and macromolecule preservation, molecular analysis of DNA, RNA, and proteomics, and a short chapter about internal quality control. Small chapters about the new developing technologies are also included, these are often confined to the major research centres, but, as past experience has showed us, they sometimes diffuse very quickly.

Also a chapter dedicated to forensic methods has been added. Since nucleic acid degradation is a common problem with FFPE tissues, useful suggestions can be obtained also from this issue together with specimen identification through DNA analysis.

Most of the methods here reported are more or less similar to those used in fresh cells and tissues, but the modifications made in the protocols are necessary to obtain a positive result in human FFPE tissues.

In the intention of the authors all the methods and protocols are described for a laboratory direct use, and the addition of explanatory notes should help even less experienced researchers, but a basic experience of laboratory research methods is required. If during the reading and practical use of the protocols errors or unclear and insufficient explanations are found, please contact directly the IMPACTS Group by e-mail (secretary@impactsnetwork.eu).

Bioethical norms are of pivotal importance in the use of human tissues for research, but we avoided ethical considerations because of the lack of uniformity in the directives on clinical residual tissue research in European countries. The only general suggestion is to contact the local ethical committees.

I would like to thank very much all the contributors of the IMPACTS group that collaborated not only in the preparation of the chapters but also in the multicentric project of methods validation, and took part in the extensive discussions held within the IMPACTS meetings.

I would like to thank Serena Bonin, who has worked with me for the past fifteen years, for her continuous interest and effort in developing molecular methods in archive tissues.

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This book could have not been edited without the continuous effort of Valentina Melita, Danae Pracella, and Renzo Barbazza who dedicated a lot of time to reading, correcting, and improving the comprehension and the presentation of the protocols.

Trieste, January 2011 Giorgio Stanta

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