To the Editor

This is an excellent report dealing with significant technical matters. In this work the Authors describe the main functions of microRNAs (miRNAs) and their detection methods in biological materials including serum, plasma, urine, normal human samples and neoplastic tissues. Moreover, the paper underline the relevance of bright field in situ hybridization (ISH) methods in revealing the cells of origin of specific miRNAs. The Authors also highlight the automated ISH protocols for miRNA detection in formalin-fixed, paraffin-embedded tissues (FFPE) that allow retrospective studies. No fault whatsoever with the methods, data analysis, or conclusions were found and is fundamentally suitable to further explore the biological role of miRNAs. In addition, detection of miRNA levels in serum, plasma and tissue extracts using qRT-PCR oligonucleotide microarray, or miRNA-sequencing have been reported. However, these techniques are not able to determine the cellular origin of miRNAs. For a precise analysis of the topographical expression of miRNAs in tissues and therefore an in-depth understanding of miRNA function in development, diseases, and tumors it is crucial to employ in situ hybridization (ISH). For ISH is mandatory to optimize tissue morphology and preservation for a better detection of its localization. Published ISH protocols were developed using frozen tissues, in which the morphology is not as detailed as in formalin fixed, paraffin embedded (FFPE) tissues. Furthermore, we want to underline in this comment the benefits and pitfalls of Tissue Micro-Arrays (TMAs) technology applied to miRNA detection.

TMAs provide a fast workflow for the evaluation of biomarkers in a series of patients, in a unique experimental approach. The possibility of using small amounts of tissue blocks for building a specific TMA led to biomaterial saving, favouring multiple studies on the blocks. The combination of TMAs and clinically annotated samples represents an elegant and cost-effective approach to study panels of expression under identical experimental conditions and to develop prognostic or predictive models of patient outcomes.

Despite these considerable advantages, TMA technology is not without limitations. Pitfalls in TMA studies are mainly related to technical fields and the specific interpretative competencies of clinical personnel.

Primarily TMA sections felt all effects of not good tissue pre-analytic preservation observed in the whole section. Thus validated antibodies and standardized techniques could not provide expected results, above all when donor tissues originated from different institutions. Moreover, as whole histological section, TMA sections could be altered by oxidative effects responsible of loss quality of immunostaining. Preservation of antigenicity could be obtained storing paraffin coat slides in a nitrogen desiccator.

Tumour heterogeneity can have a significant impact on the interpretation of biomarkers, so is required the use of more spots for each one case. Generally the inclusion of two cores per case provides immunostained percentage of cells comparable to conventional tissue section.

Finally, many statistical analyses are currently used to evaluate the association of the tested biomarkers with other patients’ clinic-pathological parameters.

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data and survival. The choice of the critical cut-off for novel biomarkers remains often arbitrary, but the use of sophisticated biostatistical algorithms could solve the definition of the optimal cut-off 2.

TMA technology can be used for high-throughput marker trial in order to save tissue, time and effort and to review many markers on numerous cases to select the markers with the most promising results to use them on the full sections for more differentiated results. TMA should allow an efficient and rapid analysis of large tissue numbers, whereby the representativity of the core for the corresponding full-section slide is crucial for this technique.

One major problem of taking samples of full-section tumour blocks is the heterogeneity of marker expression of the tumour cells, which is predicted to show a unique expression pattern for each marker. The studies of comparison and validation of TMA both in the field of stem cell markers that the immune system have shown that the TMA technology is not a good tool for the study of cellular populations poorly represented within the tumor. In conclusion, TMA technology could be recommended to be used with markers of a high expression and a more homogeneous staining pattern. Therefore, for markers expressed in few cells which show a focal and heterogeneous staining pattern, the full section slides is currently the superior method.

Based on these purpose, the clinician and the lab manager may join together to evaluate advantages and limitation, in terms of costs and applicability, of the most appropriate methods to detect miRNA.

CONFLICT OF INTERESTS:
The Authors declare that they have no conflict of interests.

REFERENCES