IJPDTM

"organo ufficiale della"

SOCIETA ITALIANA DI MEDICINA DIAGNOSTICA E TERAPEUTICA

VOLUME 2 - NUMERO 2
2019

SIMEDET.EU

PODCAST

Italian Journal of Prevention, Diagnostic and Therapeutic Medicine (IJPDTM)

Italian Journal of Prevention, Diagnostic and Therapeutic Medicine
IJPDTM *Vol2. N°2. 2019.*

SAFECAPSULE: INNOVATION IN ANATOMIC PATHOLOGY FOR CHEMICAL & CLINICAL RISK MANAGEMENT IN HEALTHCARE FACILITIES

Martina Verri[1], Anna Crescenzi[1], Carmelo Lupo[2], Roberto Virgili[1]

ABSTRACT

Formaldehyde, as main fixative of choice in medical services, was reclassified as carcinogenic and mutagenic by European Directive N. 895/2014.

Since no valid alternatives are available for the fixation of biological samples intended for microscopic examination, the industry has proposed a series of pre-filled devices with formalin release at closure, which have however shown different logistic and use problems.

For effective formalin management according to current safety standards, a new device with the CE-IVD mark: "SafeCapsule" has been developed by the Campus Bio-Medico University of Rome and the Diapath company. It consists of a container with a blue screw cap pre-filled with buffer solution and a red safety capsule containing concentrated fixative.

The aim of the study was to evaluate the qualities and safety of SafeCapsule during work practice in an Anatomic Pathology Service. For this purpose, a satisfaction questionnaire and a series of laboratory tests were used.

For operators of medical services, SafeCapsule is ergonomic, very handy, safe and easy to use.

All lab tests have shown: intact cell morphology and cell features staining; accessibility of nuclear, cytoplasmic and membrane antigens; unaltered probe's specificity on DNA sites investigated (as ALK e ROS1 rearrangements, HER2 amplification) in FISH tests; good results in molecular investigations (EGFR mutations).

In conclusion SafeCapsule enables:

- the elimination of chemical and clinical risk into the work place;
- the overcoming of technical failure of conventional devices;
- the great fixation of biological samples for right histological examinations.

[1]UOC Anatomia Patologica, Policlinico Universitario Campus Bio-medico, Roma,

[2]DIAPATH S.p.A, Martinengo (BG)

Keywords: Formaldehyde, Chemical/Clinical Risk, SafeCapsule, Pathology

This article was published on June 24, 2019, at SIMEDET.EU . doi.org/10.30459/2019-10

Italian Journal of Prevention,
Diagnostic and Therapeutic Medicine.

ABSTRACT

Formaldehyde, as main fixative of choice in medical services, was reclassified as cancerous and mutagen by European Directive N. 895/2014.

Suitable substitutes to formaldehyde do not exist at the time, so industry has proposed a set of closed-circuit safety devices pre-filled with formalin.
These devices have shown a lot of technical failure, so the University Campus Bio-medico in collaboration with Diapath company have developed "SafeCapsule", in order to promote an effective management of formalin use in compliance with safety measures and directives.

SafeCapsule (patent pending) is composed by a blue screwcap container pre-filled with buffer solution and a safety red capsule pre-filled with concentrate fixative solution.

The aim of the work is to evaluate all intrinsic SafeCapsule features into the normal work routine. For operators of medical services, SafeCapsule is ergonomic, very handy, safe and easy to use. All lab tests have shown:

- intact cell morphology and cell features staining;
- accessibility of nuclear, cytoplasmic and membrane antigens;
- unaltered probe's specificity on DNA sites investigated (as ALK e ROS1 rearrangements, HER2 amplification) in FISH tests;
- good results in molecular investigations (EGFR mutations).

In conclusion SafeCapsule enables the great fixation of biological samples for right histological examinations; the elimination of chemical and clinical risk into the work place and the overcoming of technical failure of conventional devices.

INTRODUCTION

Formaldehyde, a colorless gas with a pungent and irritating odor, is produced and marketed as a water solution with the name of formalin. It is the main fixative of choice because of the following features: easily accessible, low-cost, it does not change (if kept at normal temperature and protected from direct sunlight), allows the preservation of cell morphology and tissue architecture, guarantees standardized protocols for the histochemical, immunohistochemical and molecular investigation. With EU Regulation No. 895/2014, formaldehyde has been reclassified as carcinogenic (category 1B) and mutagenic (category 2) [(1)].

To date, a fixative that is a valid alternative to formaldehyde is not available. Therefore, it was necessary to update the Risk Assessment Document according to the indications of Title IX of Legislative Decree 81/08 and to implement technical-organizational and procedural measures according to the guidelines issued by the Ministry of Health (LG Ministry of Health CSS May 2015: Traceability, collection, transport, storage and conservation of cells and tissues for diagnostic investigations of Anatomic Pathology) [(2)] and the document of recommendations drawn up by the Scientific Society of Anatomic Pathology (Notes relating to the use of formalin, reclassified " carcinoegnic "- Guidelines SIAPEC-IAP Italian Division February 2016) [(3)].

To eliminate or minimize the risk of exposure to formalin and its vapors in a free environment, the industry has proposed several solutions of "empty" containers with formalin release at closure. In daily practice these containers have shown numerous technical problems, such as:

1. Increase in size compared to conventional containers with consequent logistic and storage problems in the warehouse;

2. Instability of the container due to the weight concentrated in the upper part of the container;
3. Absence of liquid for rinsing forceps/needle and release of the biological sample at the time of collection;
4. Impossibility of combining multiple samples in a single container after formalin release;
5. Drying of the biological sample in case of failure to release formalin;
6. Difficulty in visualizing the sample in containers containing oily gels.

To overcome the multiple problems of the devices on the market and to allow an effective formalin management according to the new safety standards, a new device has been proposed for the collection, storage and/or transport of biological samples.This device, called "SafeCapsule", is the subject of a patent by the Università Campus Bio-Medico di Roma and the Diapath company. After obtaining the CE-IVD mark for use as a medical device, it entered the market in October 2018.

It is characterized by 2 distinct but matchable containers:
1. **Container A**, which contains a quantity of non-toxic buffered solution to house the biological samples, usable in a free environment in the absence of a chemical hood;
2. **Container B**, which contains a concentrated fixative solution toxic/harmful to humans.

The fixative is obtained "in situ" by mixing the two solutions after the biological samples taken by the patient have been placed in the buffer solution. This mixing is achieved by manually screwing the container 2 onto the container 1.

A device of this type offers surprising advantages compared to conventional containers, overcoming the problems they have and guaranteeing total absence of contact between the operator and toxic and harmful substances and their vapors.

FIGURE 1. SAFECAPSULE DEVICE

OBJECTIVES

In agreement with SSPP of the Campus Bio-Medico, a sampling of SafeCapsule was provided to allow, in primis, an assessment by the operators about the quality and functionality of the device during work practice.
In secundis, the morphological conservation and biochemical and molecular properties of the samples (taken from different organs and apparatuses) were evaluated following the fixation in these devices.

METHODS

The "SafeCapsule" device was introduced into the routine of surgical ambulatories and laboratories of the Anatomic Pathology Department of the Policlinico Universitario Campus Biomedico and was validated by filling in a dedicated evaluation sheet distributed to the experts. Following the collection of the questionnaires, a database was developed, from which it was possible to extract relevant data on the qualities and functionalities most appreciated by the operators of the sector.

From the point of view of environmental safety, the

Anatomic Pathology Laboratory is equipped with a safety sensor for detecting formalin vapors. At the time of delivery to the laboratory the containers were placed on the acceptance table near the sensor to ensure their containment of the vapors.

After acceptance, the biological samples were processed as routine and the ability of the SafeCapsule device to ensure adequate storage and fixation of the biological sample was evaluated.
To allow a correct histological diagnosis and to avoid changes in the structural characteristics of the tissue under examination, complete and perfect fixation of the biological sample is a basic condition [(4)]. For this reason, the microscopic evaluation of tissue morphology and architecture was conducted through laboratory tests such as Hematoxylin-Eosin staining and special histochemical staining such as Giemsa, PAS, Perls, Masson Trichrome (DIAPATH Staining Kit).

Another analysis procedure, conducted on the samples for which the SafeCapsule device was used, is the immunohistochemistry test. This was performed on paraffin sections of the samples processed to evaluate the expression of specific cellular proteins, their preservation and the absence of fixation artifacts that could prevent their accessibility to the antigen by the specific antibody used during the reaction. Immunohistochemistry tests were performed on an automated Dako Autostainer OMNIS instrument, evaluating the following antigens:

- Nuclear: Estrogen or ER receptor, Progesterone or PGR receptor, Ki67, p63, GATA3;
- Cytoplasmic: high and low molecular weight cytokeratins or CK, Racemasi, Hepar1;
- Membrane: Epithelial Membrane Antigen or EMA, CD3, Her2neu.

On breast and lung tissue samples, a FISH (Fluorescence In Situ Hybridization) analysis was also performed to detect and localize the presence or absence of rearrangements of the ALK and ROS1 genes (FISH Probe Kit), useful for the detection of lung cancer [(5)], and amplification of the Her2neu gene (PathVision Probe Kit), essential for the detection of breast cancer [(6)].

Finally, molecular investigations were carried out, first of all evaluating the purity and quality of the extracted DNA (QIAmp DNA FFPE TISSUE QIAGEN) from the samples fixed in SafeCapsule and in secundis the search for the main mutations of the EGFR gene using the PCR technique (Easy EGFR Diatech Pharmacogenetics Kit).

RESULTS

The questionnaires distributed were collected and the data reported in histograms:

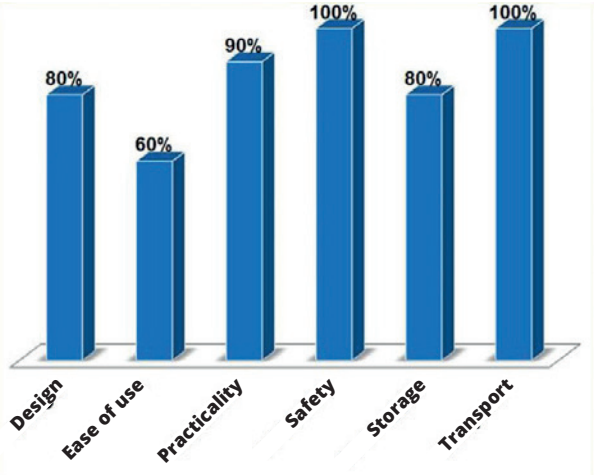

FIG.2 HISTOGRAM OF THE DATA COLLECTED ON THE SAFECAPSULE DEVICE

Following the evaluation of the questionnaires, the data obtained showed that the SafeCapsule device:

- it is ergonomic, practical and easy to use during working practice;
- showed 100% integrity and perfect seal, without the occurrence of formalin spills during transport and/or loss of biological material;
- Its use is 100% safe without dispersing its vapors;
- It showed a high level of satisfaction from health service operators.

Environmental monitoring has not registered the presence of formaldehyde gas in the air.

At the microscopic evaluation, in all the cases examined, the cellular morphology was of high quality with preservation of the staining properties of the cellular components (basophilia and acidophilia) and with high resolution in the display of the diagnostic relief characters.

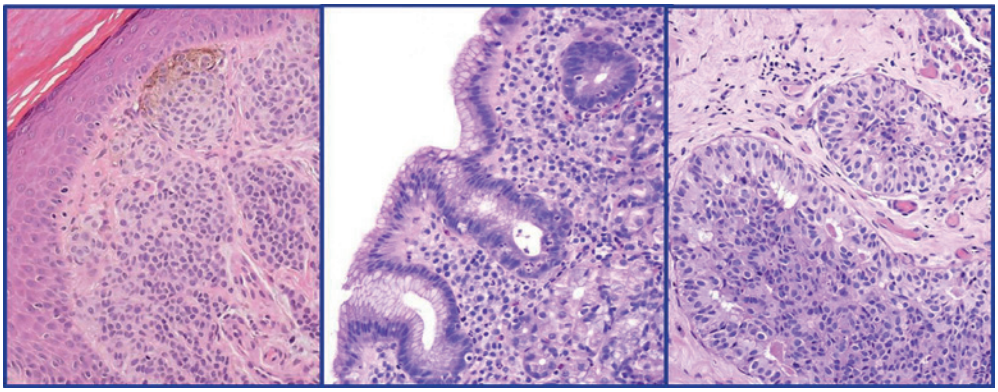

FIG.3 EE STAINING ON SKIN, GASTRIC BIOPSY AND LIVER BIOPSY

Even the histochemical stains set up to highlight specific cellular constituents were perfectly executable and consistent in results, showing excellent maintenance of staining properties.

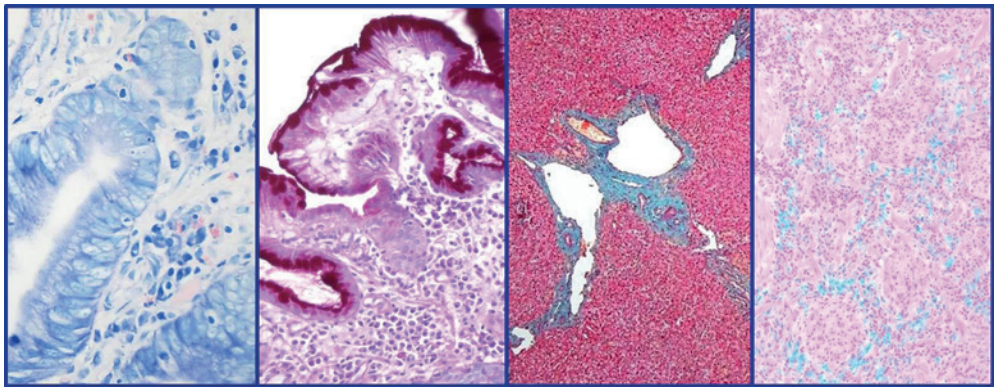

FIG.4 SPECIAL HISTOCHEMICAL STAINS: GIEMSA. PAS, MASSON TRICHROME AND PERLS

Immunohistochemical tests showed perfect preservation of the antigen and its accessibility and high signal cleanliness without the presence of a non-specific staining background.

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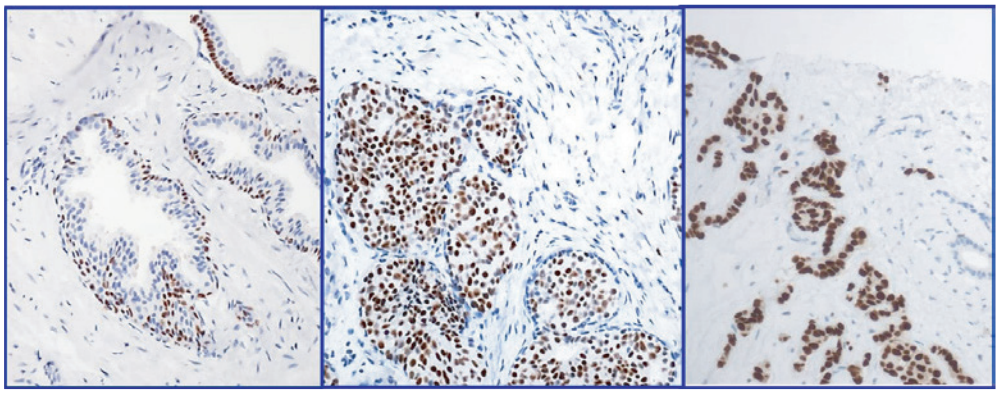

FIG.5 IMMUNOHISTOCHEMICAL REACTIONS FOR NUCLEAR ANTIGENS (P63, ER, GATA3)

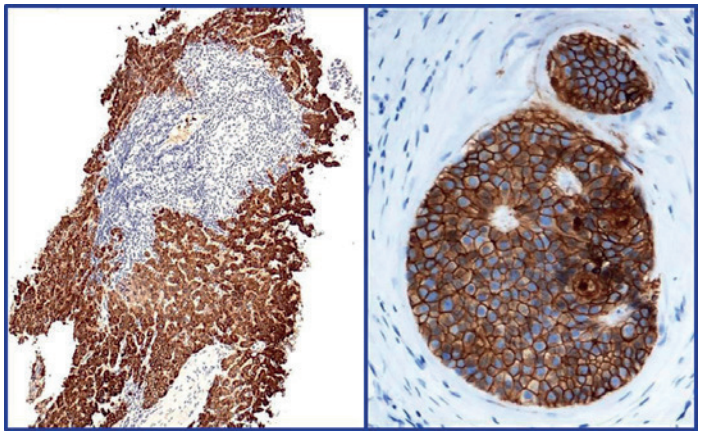

FIG.6 IMMUNOHISTOCHEMICAL REACTIONS FOR THE CYTOPLASMIC ANTIGEN, HEPAR1, AND MEMBRANE, HER2NEU

The FISH survey was found to be executable with excellent conservation of the specificity of the probe's specificity.

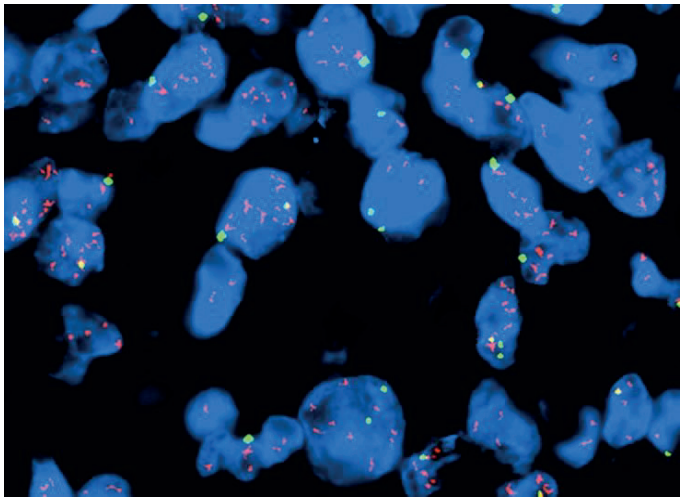

FIG.7 FISH FOR AMPLIFICATION OF THE HER2NEU GENE

Furthermore, the DNA extracted from fixed biological samples showed a good concentration and A260/280 ratio and subsequent mutational analyzes confirmed that the samples fixed with SafeCapsule are perfectly usable for molecular investigations.

DISCUSSION

Safety of the workplace is a current issue of great importance and the health activity often exposes workers to potentially harmful substances, just think of the preparation of chemotherapeutic drugs or, as in the case of histological examinations, the use of aldehyde fixatives.

In working areas where formaldehyde is used routinely, the generally applied solution is the use of chemical hoods for handling samples.

However, these devices cannot be implemented, due to space and costs, in clinics and in endoscopic or surgical rooms, where the samples are collected and placed in fixative. It was therefore necessary to study pre-loaded post-closure release containers, which avoided the environmental dispersion of formalin vapors.

Many devices have entered the market in recent years, most of them based on containers with an empty chamber in which to introduce the sample and a chamber with formalin that is released after closure to allow fixation.

Although this solution is generally effective in terms of environmental protection, its use has been complex both for logistical reasons (doubling of the overall dimensions) and for practical difficulties such as the difficult release of the sample in the empty chamber and the impossibility of inserting a second sample after the release of the fixative.

Last but not least, it must be considered that in the case of failure to release the fixative due to defect or forgetfulness, the sample is irreparably damaged by drying.

The SafeCapsule patent project has overcome all these complexities by separating a non-harmful liquid part (not requiring pictograms) and buffered, from a concentrated toxic part that is added only after completion of the patient sampling procedures.

After the screwing of the upper capsule, the mixing of the two substances is immediate and has been studied so that the production of the "in situ" fixative is complete and fast. The studies we conducted on morphology, preservation of antigenic properties and maintenance of the requirements for molecular investigations and FISH showed that the formalin solution, reconstituted when the SafeCapsule is closed, has all the qualities required for modern histological diagnosis and is absolutely comparable to formalin solutions already complete at the time of use.

This datum assumes particular importance in consideration of the numerous molecular pathology exams that are currently required on formalin-fixed and paraffin-embedded material [7]. Of particular importance was the satisfaction of the operators expressed by completing the questionnaire, about the practicality of use and ease of transport. The most appreciated aspect was safety, confirmed by an environmental assessment through the use of a specific sensor, calibrated to international standards.

CONCLUSIONS

In conclusion, we can say that:

- The SafeCapsule device has shown a high level of satisfaction by health service operators for Transport, Practicality and Safety. It allows the isolation of harmful and hazardous components in a free environment, eliminating the chemical and clinical risk;

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- SafeCapsule guarantees the overcoming of logistic and utilization criticalities linked to conventional devices, thanks to its quality and functionality;
- SafeCapsule allows optimal storage and fixation of the biological sample, which are the basic conditions for a correct histological diagnosis.

In working areas where formaldehyde is used routinely, the generally applied solution is the use of chemical hoods for handling samples.

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