JOURNAL OF ONCOLOGY PHARMACY PRACTICE

Original article

J Oncol Pharm Practice
1–6

DOI: 10.1177/10781552251350633
journals.sagepub.com/home/opp
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Validation of a customized combined chemical and microbiological test for qualification of aseptic preparation processes

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Abstract

Introduction: To meet legal requirements, an initial and ongoing training program of production staff must be implemented. As part of their initial and periodic qualification to carry out aseptic preparations, a media fill test (MFT) simulating aseptic processes as closely as possible must be carried out. Moreover, and especially when the handled products are toxic, it is also crucial to carry out an analysis of the chemical contamination generated during the aseptic process. For this reason, the Pharmacy Production Department wanted to implement a method combining both the media fill test and the chemical contamination test (MFT/CCT), by coupling a culture medium with a fluorescent tracer. To gain independence and to reduce costs, the department wished to manufacture its own customized bags and vials of culture medium, subsequently referred to as the "MFT kit", needed to carry out aseptic filling tests.

Methods: The medium chosen for developing the kit was Tryptone-soy broth (TSB) while two sterile options were available for the fluorescence tracer: quinine hydrochloride and fluorescein. A list of all manipulations handled and to be submitted to the MFT/CCT was created. After aseptic compounding, the kits were quarantined for 14 days in an incubator (22.5 °C ± 2.5 °C) in order to check that the sterility was maintained.

Results: Fluorescence was visually checked for both fluorescent tracers solutions after addition of TSB and no change in fluorescence ($\lambda = 366$ nm) could be detected. Fertility tests were also carried out and the results showed that the CCT quinine solution inhibited bacterial growth and therefore gave a non-compliant result. The CCT quinine solution was subsequently withdrawn from further development of the MFT/CCT protocol. The fertility test with CCT fluorescein solution was fully compliant. The MFT/CCT protocol developed was submitted to 20 pharmacy technicians in the production department. No post-incubation turbidity was observed after handling. Post-handling UV revelation revealed an average of 2.2 [1.0–4.0] areas with traces per technician. This result reflects the non-compliance of 30% of the technicians who had to repeat the training. The average number of areas with traces among those who successfully passed the test was 1.6.

Discussion/Conclusion: The development of this combined MFT/CCT protocol is promising, and opens up excellent prospects for the training and (re-)qualification of operators involved in aseptic handling. The cost of producing in-house "MFT kits" is much lower than the cost of commercial kits, and also makes it possible to involve teams in the implementation of MFT/CCT. A stability study on the shelf life of the kits will be conducted, with the aim of manufacturing them on a larger scale and making them available at cost price for other hospitals.

Keywords

Combined MFT/CCT, fluorescent tracers, microbiological contamination, chemical contamination, operators' validation, sterile compounding

Date received: 5 February 2025; revised: 2 June 2025; accepted: 2 June 2025

Introduction

Preparation of injectable anti-cancer drugs is considered to be a high-risk activity for both the patient and the operator. The centralization of preparations in hospital pharmacies, particularly in controlled-atmosphere areas, has improved control of the quality, asepsis and chemical risks associated with these preparations. Increasingly stringent regulatory

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standards specific to this type of activity have emerged over the last few decades, in particularly through the publication of good practices for the preparation of medical products in healthcare establishments and PIC/S.[1–4]

Purposes of these guidelines are to promote safety by ensuring sterility and accuracy of compounded sterile preparations in healthcare establishments. Failure of pharmaceutical personnel to adhere to quality standards and best practice recommendations introduces the greatest risk of contamination.

Watson and colleagues reviewed pharmaceutical compounding in US and screened 2155 published reports of compounding errors with 63 errors identified which harmed 1155 patients. When broken down by type, contamination (microbiological or toxic) and errors in concentration were the most frequent reported deviations.[5,6]Therefore appropriate training of personnel is of high importance. In Belgium on September 30, 2020, a Royal Decree[7] was published on subject hospital pharmacy, adopting PIC/S 010-4 guidelines as the reference standard for compounding.[2] In these guidelines, particular emphasis is placed on the initial and ongoing training and qualification of operators carrying out the preparations.

To meet these legal requirements, an initial and ongoing training program is being developed within the pharmacy's production department. As part of their initial and periodic qualification to carry out aseptic preparations, a media fill test (MFT) simulating aseptic processes as closely as possible must be carried out by staff.[2,8] The MFT is used to assess the microbiological risk associated with preparation. The principle is to replace the actual solutions by a culture medium, and to mimic the various production operations under real-world operating conditions.[9,10] Moreover, and especially when the products handled are toxic, it is also crucial to carry out an analysis of the chemical contamination generated in the working environment during the aseptic process.[11] For this reason, the Pharmacy Production Department wanted to implement a method combining both the media fill test requested by PIC/S (010.4–5.4 (4)) and a chemical contamination test (CCT), by coupling a culture medium with a fluorescent molecule adapted to the particular domain of chemotherapy compounding from *Fersing* et al. for the qualification of radiopharmacy operators.[12]

To gain independence and to reduce costs, the department wished to produce its own customized bags and vials of culture medium, subsequently referred to as the "MFT kit", required to carry out aseptic filling tests. In order to combine MFT with UV visible CCT, a fluorescence-emitting molecule is required. An added value in this approach is to find tracers available on the market in ready-to-use sterile pharmaceutical forms and detectable at wavelengths commonly found in microbiology and research laboratories, i.e., 366 nm. A review of the literature relating to CCT was performed to identify candidate molecules enabling both quinine and fluorescein to be considered for the combined approach. The literature describes the quinine CCT approach, which requires the addition of citric acid for fluorescence visualization (pH < 4.0). Optimum fluorescence is described for a solution containing a final concentration of 5.0 mg/ml quinine HCl and 2.5 mg/ml citric acid (= quinine CCT solution).[11] For the combined MFT/CCT approach with fluorescein, the optimum final concentration described for UV-detection is 0.1 mg/ml (0.01%) sodium fluorescein.[12] The advantage of the quinine CCT solution is that it is clear and lightly colored, so contamination cannot be visualized by the naked eye during the various manipulations in the TSB, unlike the sodium fluorescein solution, which is yellow-orange in color and can be directly visualized by the operator if a contamination occurs. On the other hand, fluorescein has an intrinsic green-yellow fluorescence (λ=366 nm), whereas to obtain the blue-green fluorescence (λ=366 nm) of quinine, acidification of the solution is requested.[13,14] Both molecules are therefore perfectly suitable as fluorescent tracers for CCT. Before combining the CCT and the MFT we had to check that the fertility of the TSB was not affected by addition of the tracer solutions and that the fluorescence of the tracer was maintained after addition to the culture medium. It was also necessary to define the contents (bags and vials) of the custom-made kits required for the simulation activity.

Materials & methods

MFT/CCT "KIT" compounding

The culture medium used for the MFT kits is the one recommended by the Eur.Ph. for fertility testing (chapter 2.6.1.). This is a tryptone-soy broth (TSB), allowing the growth of aerobic bacteria as well as yeasts, fungi and facultative anaerobic bacteria.[15] These tests were performed with TSB in 1000 ml bottles [Tritium Microbiology, Netherlands]. The fluorescence tracers chosen were ampoules of sterile quinine hydrochloride solution 300 mg/ml [Sterop, Belgium] and Faure fluorescein 10% (500 mg/5 ml) [SERB, France]. The UV lamp used for detection emits at 366 nm [VWR, Belgium].

The quinine HCl solution was acidified using a sterile citric acid solution at a concentration of 25 mg/ml, produced in-house from anhydrous citric acid [Magis Pharma, Belgium] and water for injection [Baxter, Belgium], sterilized by 0.2 µm filtration through a Mediakap 2 0.2 µm W/O filling bell filter [Repligen, USA]. Sterilized 50 ml white (type 1) glass vials [Pontos, Belgium] were filled with this solution and fitted with a 20 mm butyl stopper [Derco, Belgium] and a tear-off aluminum cap [Pontos, Belgium]. Prior to release, the product underwent sterility testing in accordance with local practices.

Aseptic production of MFT kits (bags and vials) was carried out in a biosafety cabinet type IIb [Berner International GmbH, Germany] starting from a TSB

Table 1. MFT/CCT kit composition.

	Presentation	Labelled volume	Content	Unit/ kit	Name
Culture media	Bag	100 ml	104 ml TSB	1	MFT(1)
	Vial	45 ml	45 ml TSB	2	MFT(2)
		18 ml	18 ml TSB	1	MFT(3)
Tracer	Vial	30 ml	30 ml UV tracer	1	CCT

solution using a pump and a fluid transfer tube set Baxa Repeater® [Baxter, Belgium]. The bags used were Freeflex NaCl 0.9% 100 ml [Fresenius, Germany] which were previously emptied of their contents. Sterilized 50 ml and 20 ml white (type 1) glass vials [Pontos, Belgium] were filled with respectively 45 ml and 18 ml of this solution and fitted with a 20 mm butyl stopper [Derco, Belgium] and a tear-off aluminum cap [Pontos, Belgium]. Each element of the kit was quarantined for 14 days in an incubator [Evermed, Italy] at a temperature of 22.5 °C (± 2.5 °C). At the end of the quarantine period, a visual check for turbidity was carried out in order to assess the preservation of fertility. Kits were considered compliant and released in the absence of turbidity.[15]

The elastomeric diffusers used in our development were the Folfusor® 120 ml 2 days [Baxter, Belgium] and the eye drop vials were screw-in bottle with specific tips [Pontos, Belgium]. Syringes were 3 pieces 3 ml syringes [Becton Dickinson, Belgium]. Empty 100 ml bags made of EVA Easyflex® [Macopharma, France] were used for this development. Kits were assembled individually for each operator and were composed of 1 bag and 4 vials (1 MFT(1), 2 MFT(2), 1 MFT(3), 1 CCT(1)). Kit composition is available in Table 1.

Kit's uv & fertility testing

To ensure that the UV absorbance of the fluorescent tracer in the TSB medium is not inhibited, a UV absorbance test at 366 nm is performed on TSB alone and on the UV tracer diluted 1:10 in TSB (final concentration).

Fertility tests were carried out in accordance with Chapter 2.6.1. of the European Pharmacopeia "Sterility - Growth promotion test of aerobes, anaerobes and fungi". This test provides evidence of the ability of media to support microbial growth from an inoculum ≤ 100 CFU, after incubation at 35°C for less than 3 days for bacteria and less than 5 days for fungi, and attests to the persistence of fertility at the end of the processes applied.[15]

The method used for fertility testing is direct inoculation. After dilution of the UV tracer to 1:10 in TSB an inoculum of a small number of viable micro-organisms (not more than 100 CFU) is added to the medium. The micro-organisms used are: *Staphylococcus aureus* (ATCC 29213), *Pseudomonas aeruginosa* (ATCC 27853), *Bacillus subtilis* (ATCC 6633), *Candida albicans* (ATCC 10231) [Oxoid, Thermoscientific]. Inoculation is followed by an incubation period of less than 3 days for bacteria and less than 5 days for fungi at 35°C.

A positive control is also performed using TSB medium without a UV tracer. After incubation, if visible microbial growth is observed, comparable to that of the positive control, it indicates that the product has no antimicrobial activity or that such activity has been effectively neutralized. However, if growth is reduced or absent, it means that the UV tracer exhibits antimicrobial activity that has not been completely eliminated under the test conditions.

MFT/CCT training activity

The activity was carried out in a positive-pressure sterile isolator to mimic non-toxic sterile preparations and in a negative-pressure sterile isolator to mimic cytotoxic sterile preparations [JCE Biotechnology, France].

All 20 operators working in the department took part in training activities, either as part of their initial or continuing training.

Manipulations routinely carried out by cytotoxic production staff were listed. The production/preparation mainly consists of bags, syringes, elastomeric diffusors and eye-drop vials. The list of manipulations to be submitted to the MFT/CCT is representative of the preparations routinely carried out in the department.

Solutions were aseptically removed or transferred from bag and vials to obtain final products defined according to the defined protocol, simulating as closely as possible a working session.

1. **Aseptic transfer of a solution into a 100 ml bag:**
   - 10.0 ml of culture media were withdrawn from the bag *MFT(1)* and injected in an empty bag *(S1) ;*
   - 10.0 ml of tracer were added in the *MFT(1)* bag. The final bag containing the media and the tracer, named *S2*.
2. **Aseptic transfer of a solution into a 10 ml eye drop bottle:**
   - 9.0 ml of *S1* were withdrawn and transferred in the sterile eye drop vial ;
   - 1.0 ml of tracer was added in the eye drop vial.
3. **Aseptic packaging of a solution in syringes with stopper :**
   - 5.0 ml of tracer were added in the vial containing 45 ml of TSB, *MFT(2),* to obtain a final volume of 50 ml *(S3) ;*
     - 3 syringes were prepared using the vial *S3*:
       - 7.2 ml,
       - 8.3 ml,
       - 9.5 ml.

- 2.0 ml of tracer were added in the vial containing 18 ml of TSB, *MFT(3),* to obtain a final volume of 20 ml *(S4) ;*
  - 3 syringes were prepared using the vial *S4*:
    - 1.5 ml,
    - 2.2 ml,
    - 2.9 ml.

4. **Aseptic transfer of a solution into a 120 ml elastomeric diffuser.**
   - 5.0 ml of tracer were added in a *MFT(2)* vial to obtain a volume of 50 ml *(S3').*
   - 45.0 ml of bag *S2*, 20.0 ml of *S3* and 50.0 ml of *S3'* were injected in the diffusor.

Prior to filling, the diffuser was connected to an empty bag to allow the solution to flow and to enable post-incubation visual turbidity control.

Fluorescent tracer spot detection using a 366 nm UV lamp after the production session is performed by the operator at the following points in the work area: field, gloves, needle caps, vials, compresses and bags under supervision of the responsible pharmacist. The preparations are then collected and identified for each operator and placed in incubation for 14 days according to in-house procedures [7 days (20°C–25°C) followed by 7 days (30°C–35°C)], with a visual check for the absence of post-incubation turbidity. MFT/CCT is considered compliant if there are ≤ 2 spotted areas and there is no post-incubation turbidity in the media handled.

Results

Fluorescence was visually checked for each fluorescent tracer solution: quinine solution, fluorescein solution, quinine solution + TSB media and fluorescein solution + TSB media. No change in fluorescence could be detected after addition of TSB. As expected quinine solutions exhibited a light blue color while fluorescein solutions were deep yellow green when exposed to UV lamp ($\lambda = 366$ nm).

Fertility tests were carried out in accordance with Chapter 2.6.1. of the European Pharmacopeia "Sterility - Growth promotion test of aerobes, anaerobes and fungi". Results showed that the CCT quinine solution inhibited bacterial growth and therefore gave a non-compliant result. The CCT quinine solution was subsequently withdrawn from further development of the MFT/CCT protocol. The fertility test with CCT fluorescein solution was fully compliant. Growth promotion test results are summarized in Table 2.

Forty kits were produced in the pharmacy department by the operators. After incubation and quarantine for 14 days, all the kits were in conformity and could be released for use in methodological validation tests, followed by operator training by MFT/CCT.

The MFT/CCT protocol developed was submitted to 20 pharmacy technicians in the production department. The average technician year of service is 6.6 [0.3–33.4] years.

No post-incubation turbidity was observed in the prepared media.

Post-handling UV revelation revealed an average of 2.2 [1.0–4.0] areas with traces per technician. This result reflects the non-compliance of 30% of the technicians who had to repeat the test. The average number of areas with traces among those who passed the test was 1.6 (limit : ≤ 2). The distribution of the areas spotted by the technicians during MFT/CCT is available in Figure 1.

Discussion

This development of a combined MFT/CCT protocol is promising, and opens up excellent prospects for the training and (re-)qualification of operators involved in aseptic handling.

Table 2. Growth promotion tests results.

Micro-organisms	Medium	Bacterial growth after inoculation with 10 CFU, visible to the eye
Staphylococcus aureus	TSB	Positive at 24 h
(ATCC 29213)	TSB + fluorescein 0,01%	Positive at 24 h
	TSB + quinine HCl acidified 5 mg/ml	**Negative at 72 h**
Pseudomonas aeruginosa	TSB	Positive at 24 h
(ATCC 27853)	TSB + fluorescein 0,01%	Positive at 24 h
	TSB + quinine HCl acidified 5 mg/ml	**Negative at 72 h**
Bacillus subtilis	TSB	Positive at 24 h
(ATCC 6633)	TSB + fluorescein 0,01%	Positive at 24 h
	TSB + quinine HCl acidified 5 mg/ml	**Negative at 72 h**
Candida albicans (ATCC 10231)	TSB	Positive at 24 h
	TSB + fluorescein 0,01%	Positive at 24 h
	TSB + quinine HCl acidified 5 mg/ml	Positive at 24 h
Aspergillus niger	TSB	Positive at 24 h
(QCE NEQAS 1517)	TSB + fluorescein 0,01%	Positive at 24 h
	TSB + quinine HCl acidified 5 mg/ml	Positive at 24 h

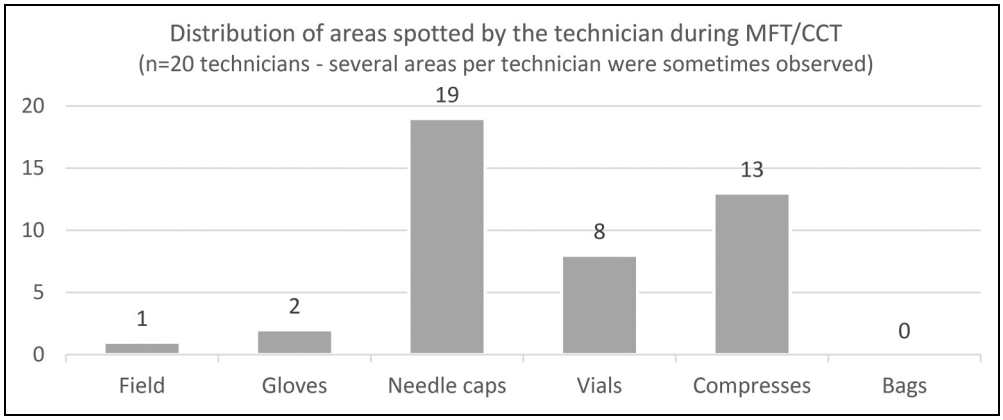

Figure 1. Distribution of areas spotted by technicians during MFT/CCT (n = 20 technicians).

This method development also revealed the inhibition of TSB fertility by acid quinine solution. The use of fluorescein as a fluorescence tracer was therefore preferred. As expected, chemical contamination is more visible where chemotherapy is more susceptible to appear due to aseptic transfer of a drug : septum, needle caps and gazes used for decontamination. These results are in line with those previously obtained internally in unpublished data. The results are also relatively consistent with the existing literature.[11,16]

The prospects for this method development include the search for a UV fluorescence tracer invisible to the naked eye. In this context, optical brighteners used in the bleaching industry could be studied. Those products are non-toxic and exhibit an intense fluorescence whilst being colorless. In terms of future developments, a semi-quantitative approach is considered for spots detection in future sessions, based on a risk analysis of the criticality of contamination, and inspired by the approach described by Sadeghipour et al.[6] as follows: low (0–5 spots) - medium (6–10 spots) - high (>10 spots).

It is also important to consider the lack of sensitivity of the MFT approach. Despite the fact that the method is well known, it has not been quantified to a significant extent in the literature. The risk of contamination of a sterile preparation carried out according to the rules of the art in a cleanroom is less than 2.2 per million preparations. This means that a colossal number of MFTs would have to be carried out to identify possible contamination. It is therefore important to take this into account when validating results. That said, the MFT remains a useful tool for training and (re-)qualifying personnel, as it also enables us to observe operator actions.[17,18]

The cost of producing in-house "MFT kits" is much lower than that of commercial kits, and also makes it possible to involve teams in the implementation of MFT/CCT. A stability study on the shelf life of the kits will soon be conducted, with the aim of producing them on a larger scale and making them available at cost price for other hospitals.

Compounding is more relevant than ever. Appreciating that the need for compounding is unlikely to decrease in the near future, we can only re-emphasize the critical nature of our recommendations for the federal and state governments to fully fund the oversight of outsourcing facilities, for healthcare practices to refuse medications compounded without strict adherence to GMP-like regulations, for pharmacy schools to expand compounding training and certification, and for physicians to think critically about the risks of prescribing medications that are not commercially produced.[5]

Conclusion

Our combined MFT/CCT approach enables both production operations and operators to be microbiologically and chemically qualified in a single step, making the requirements of regulatory texts more accessible to all hospital pharmacists involved in aseptic preparations.

Abbreviations

PIC/S	Pharmaceutical Inspection Convention and Pharmaceutical Inspection Co-operation Scheme
MFT	Media fill test
CCT	Chemical contamination test
UV	Ultraviolet
TSB	Tryptone-soy broth
λ	Wavelengths

Author contributions

Marie Gils: Conceptualization, Methodology, Investigation, Writing – Original Draft, Project administration. **Elisa Gava:** Investigation, Project administration.
Charlotte Hanze: Conceptualization, Methodology.
Isabelle Roland: Conceptualization, Supervision.

Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

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