Handy Tips
that might be useful when developing, editing, and executing technological programs for FS-9-25, FS-12-25, FS-16-25 automated slide stainers

V1.2 English – 05.08.2020

IVD

Dubna 2020 r.

The latest supplementary documents for the devices FS-9-25, FS-12-25, and FS-16-25 are available on the website www.fastainer.com, section http://fastainer.com/resources.

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Revision history

Revision	Effective date	Change	Written by
1.2	04.08.2020	First revision manual	Bezrukov A.V.

Handy Tips
that might be useful when developing, editing, and executing technological programs for FS-9-25, FS-12-25, FS-16-25 automated slide stainers

List of terms and abbreviations:

AFB – acid-fast bacillus is a type of bacteria that causes tuberculosis and certain other infections.

IM - Automated Slide Stainer FS-12-25 Instruction Manual V1.5 English

SFE – STAINER FIRMWARE EDITOR - specialized PC software for TP developing and firmware editing.

Stainer – any of FS-9-25, FS-12-25, FS-16-25 automated slide stainers.

TO – technological operation.

TP – technological program.

1 Preliminary comments

1.1 When developing technological programs (TP), do not fill in the template of TP manually, but use a computer: this allows more convenient storage, editing, and comparison of TP options.

1.2 It Is most suitable and productive to develop new TP using a specialized computer program «Stainer Firmware Editor» (SFE). SFE allows you to develop new TP automatically and select the optimal interval for launching racks in operation when processing 2-3 racks in parallel. It will be possible to get access to SFE after registration on the website FASTAINER.COM soon. If you do not have such a program, you can use the template for recording in *.rtf format by downloading it from the site FASTAINER.COM.

1.3 When developing a TP, it is advisable to choose a configuration (placement of reagents at stations) that minimizes the path of the racks with slides movements.

1.4 **FORBIDDEN** TO OPERATE THE STAINER WITHOUT PRIOR READING THE INSTRUCTION MANUAL. First of all, be sure to read and comply with chapter 2 "Safety" carefully (IM).

2 Selection of optimal processing regimes

2.1 Agitation of racks in troughs and dipping mode of TO might give intensification of process and substantially shorten the time of TP.

2.2 Agitation should not be used in reagents that are prone to precipitation – in particular for the Giemsa working solution.

2.3 It is necessary to balance the agitation period and the time of the technological operation. A short agitation period of 1-2 seconds is appropriate only for short TO. In

some cases, it is advisable to use the dipping mode instead. This is particularly true for rapid rinsing in tap water.

2.4 After processing preparation in the fixative (by Leishman or May-Grunwald or Wright), in order to avoid drying of the glass and the appearance of a blue background on slides, it is advisable to transfer the rack as quickly as possible to the Giemsa working solution (Leishman, Wright), or rinse in water. The draining delay after TO in fixative (Leishman, May-Grunwald) is usually must be no more than 5 seconds. It is not advisable to use a blotter after a fixative.

2.5 When staining cytological preparations (Papanicolaou stain technique) and histological preparations, it is necessary to keep the preparations moist until they are enclosed in the balm, in order to prevent the appearance of artifacts associated with drying. For this purpose should be used XYLENE-E reagent in the last TO. After processing at the XYLENE-E station, the slides with preparations remain in the trough until the user unloads the processed rack.

2.6 There is no sense in double (repeated) TO on blotter. Repeated blotting removes a too small amount of the reagent.

3 Fixation

3.1 **Fixation of hematological preparations.** Here it is a useful quotation from one of the best hematology books (Bain**)**: *«Dry the films in the air, then fix by immersing in a jar of methanol for 5–10 min. For bone marrow films, allow a longer time to ensure thorough drying and then leave it for 15–20 min in the methanol. Films should be fixed as soon as possible after they without spoiling the staining of the blood cells. It is important to prevent any contact with water before fixation is complete. Methyl alcohol (methanol) is the fixative of choice, although ethyl alcohol ('absolute alcohol') can also be used. To prevent the alcohol from becoming contaminated with absorbed water, it must be stored in a bottle with a tightly-fitting stopper and not left exposed to the atmosphere, especially in humid climates …. As little as 1% water may affect the appearance of the films, and a greater water content causes gross changes»*

Before fixing hematological preparations in dye fixative, especially in a humid climate, it may be advisable to pre-dry the preparations in the drying station.

The probability of blue background arising on the sides is less for Leishman fixative, in comparison with a May-Grunwald fixative (because of lower concentration of dye).

When working in a humid climate, it is advisable to take measures to prevent the degradation of methanol-based fixatives due to the absorption of water vapor.

3.2 **Fixation of cytological preparations.** *Fixation does not require more than a few minutes, but a minimum of 15 minutes is advisable for proper adherence of the smear to the slide.* (Papanicolaou).

3.3 **Fixation of microbiological preparations.** For microbiological preparations, especially preparations for the diagnosis of AFB, recommended (5) thermal (heat) fixation: «*According to this method, slides with smears are laid out on tin or enamel trays and placed in a drying cabinet, where they are first dried at 37 degrees C. Then the temperature is raised to 105 degrees. C and, after 10 minutes, the cabinet is turned off».* It must be taken into account that heat fixation might change the tinctorial properties of the preparation.

Fixation in the flame (Allen), as well as chemical fixation in alcohol (Chedore), does not guarantee the disinfection of AFB preparations.

3.4 Caution should be managed when using pure methanol for fixation due to its high toxicity and the risk of confusing methanol with ethanol. When using methanol, be sure to connect the machine to the ventilation, or install it in the fume hood.

4 Configuration

4.1 (1.3) When developing a TP, it is advisable to choose a configuration (placement of reagents at stations) that minimizes the path of the racks with slides movements.

4.2 It is advisable to choose such a configuration (such placement of troughs with reagents) to avoid carrying the rack over the fixatives, alcohols, xylene, xylene substitute after TO in tap water or aqueous solution.

4.3 Methanol-based fixatives should be placed further away from the tap water trough and the drying station.

4.4 If there is a need in high capacity staining, It will be useful to stain 2 or 3 racks simultaneously. Such an opportunity is managed by optimization of rack operation launching interval. You can define the optimal launching interval with SFE.

Simultaneous staining of 2 or 3 racks is only possible if the agitation period is set to more than 5 seconds.

The TP with simultaneous staining of 2 or 3 racks will run without errors for each rack only if the interval is selected so that there is no need to move two or more racks simultaneously and use the same bath for two racks at the same time.

4.5 If the technique involves a long-term TO (such as in auramine-rhodamine AFB staining), and there is a need for high capacity staining, it is advisable to use

configurations with two or three troughs with the reagent that is used for long-term operation (auramine-rhodamine solution). This can significantly increase productivity.

4.6 Each time you change the program (or enter a new program), the compliance of the configuration of the Stainer and the reagents locations should be checked.

5 Precautionary measures

5.1 Use the troughs according to the reagent used:

TV-25-PP – a polypropylene trough **(color: either white or natural half-transparent)** for RV-25 racks; **recommended when handling aqueous solutions; spirits; and acetone**.

TV-25-POM – a polyoxymethylene trough **(color: grey)** for RV-25 racks; **recommended when handling xylene; substitutes of xylene**; aqueous solutions; and solution with pH≥7. (IM)

5.2 Fill the bath with reagents outside the working chamber of the machine..

Recommended volume of liquid in troughs depending on the number of glasses in RV-25 rack loaded.:

when there are 25 slides loaded into the rack – 210 ± 10 ml.

when there are 12 slides loaded into the rack – 220 ± 10 ml.

when there are 5 slides loaded into the rack – 230 ± 10 ml. []

5.3 It is recommended to use separate racks for each staining technique or to thoroughly clean the racks of dye residues before using another staining technique.

5.4 Slides with preparations should be loaded only in dry racks.

5.5 When processing is complete, unload the racks and switch off the Stainer.

If a break in operation is not long (several hours), it is acceptable to cover the troughs with lids and to close the lid of the Stainer.

If a break in operation is long, turn over the water supply tap, pour off the reagents, wash the troughs. If necessary, clean the working chamber of the Stainer. Close the lid of the Stainer.

FORBIDDEN TO LEAVE BOTH CLOSED AND OPEN TROUGHS WITH CHEMICALLY ACTIVE, HIGHLY FLAMMABLE, COMBUSTIBLE AND TOXIC REAGENTS (ACID SOLUTIONS, IODINE SOLUTIONS, SPIRITS, XYLENE, ACETONE, METHANOL-BASED FIXATIVES, ETC.) IN THE WORKING CHAMBER OF THE STAINER AFTER OPERATION. (IM).

5.6 The critical thing we have to remember is that TP must be user friendly. It must be convenient for lab personnel. Better user friendly but not so productive TP, then so productive, but inconvenient in daily lab work.

6 References with quotations

1. Allen BW Survival of tubercle bacilli in heat-fixed sputum smears J Clin Pathol 1981; 34:719-722

SUMMARY Tubercle bacilli, which survived heat fixation, were detected with a slide culture technique, which allowed the entire smear to be examined. Both conventional flame fixation and the use of a controlled hot-plate failed to render tuberculous sputum smears safe for further handling. Smears which were stained with the phenol-auramine method, failed to yield growth on culture. If the delay between preparation and staining is unavoidable, it is recommended that smears are given additional treatment to prevent the survival of tubercle bacilli.

2. Bain Barbara J., Bates Imelda, Laffan Mike A Dacie and Lewis Practical Haematology, p.53 E-Book Elsevier Health Sciences 11 aug. 2016 r. 600 p. ISBN: 9780702069253

Fixing blood films To preserve the morphology of the cells, films must be fixed as described on page 53. This must be done without delay, and the films should never be left unfixed for more than a few hours. If films are sent to the laboratory by post, it is essential that, when possible, they are thoroughly dried and fixed before dispatch. P.51 Dry the films in the air, then fix by immersing in a jar of methanol for 5–10 min. For bone marrow films, allow a longer time to ensure thorough drying and then leave it for 15–20 min in the methanol. Films should be fixed as soon as possible after they without spoiling the staining of the blood cells. It is important to prevent any contact with water before fixation is complete. Methyl alcohol (methanol) is the fixative of choice, although ethyl alcohol ('absolute alcohol') can also be used. To prevent the alcohol from becoming contaminated with absorbed water, it must be stored in a bottle with a tightly-fitting stopper and not left exposed to the atmosphere, especially in humid climates (see Chapter 26). As little as 1% water may affect the appearance of the films, and a greater water content causes gross changes (Fig. 4-2). Methylated spirits must not be used because it contains water. P.53

3. Chedore P. et all Method for Inactivating and Fixing Unstained Smear Preparations of *Mycobacterium tuberculosis* for Improved Laboratory Safety J Clin Microbiol. 2002 Nov; 40(11): 4077–4080.doi: 10.1128/JCM.40.11.4077-4080.2002 PMCID: PMC139704 PMID: 12409378

ABSTRACT
The inactivation of smears that contain Mycobacterium tuberculosis for microscopy before removal of the material from a biosafety cabinet is an important safety factor in preventing the potential transmission of tuberculosis to laboratory workers. The fixing and inactivating properties of heat flaming, 70% ethanol, and 1, 3, and 5% phenol in ethanol for smears containing M. tuberculosis were investigated. Heat flaming failed to inactivate the smear material, whereas 5% phenol in ethanol successfully fixed and inactivated all smears containing M. tuberculosis, both from concentrated sputum samples and from culture material.

4. Papanicolaou G. ATLAS OF Exfoliative Cytology, Commonwealth Fund by Harvard University Press, Cambridge, Mass.1963. p.5
Fixation does not require more than a few minutes, but a minimum of 15 minutes is advisable for proper adherence of the smear to the slide.

5. ПРИКАЗ МИНЗДРАВА РФ ОТ 21.03.2003 N 109 "О СОВЕРШЕНСТВОВАНИИ ПРОТИВОТУБЕРКУЛЕЗНЫХ МЕРОПРИЯТИЙ В РОССИЙСКОЙ ФЕДЕРАЦИИ"

9.4. Фиксация мазков
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В плане охраны труда оптимальным является метод, предложенный A. Hain. Этот метод фиксации мазков используется как при окраске по Ziehl-Neelsen, так и с люминесцентными красителями. Согласно этому методу, предметные стекла с мазками раскладывают на жестяные или эмалированные подносы и помещают в сушильный шкаф, где сначала высушивают при 37 град. С. Затем температуру повышают до 105 град. С и, спустя 10 минут, шкаф выключают. При таком методе достигается надежное прикрепление материала к стеклу и гибель микобактерий, как находящихся в материале мазка, так и случайно попавших на поднос. Фиксирующая температура не должна превышать 105 град. С, чтобы не изменить тинкториальные свойства микобактерий.*

THE ORDER OF MINISTRY OF HEALTHCARE OF THE RUSSIAN FEDERATION DATED 21.03.2003 N 109 "ON IMPROVING TB CONTROL IN THE RUSSIAN FEDERATION"

9.4. Fixation of smears
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In terms of labor protection, the method proposed by A. Hain is optimal. This method of fixing smears is used both for staining by Ziehl-Neelsen, and with fluorescent dyes. According to this method, slides with smears are laid out on tin or enamel trays and placed in a drying cabinet, where they are first dried at 37 degrees C. Then the temperature is raised to 105 degrees. C and, after 10 minutes, the cabinet is turned off. With this method, a reliable attachment of the material to the glass is achieved and the death of mycobacteria, both located in the smear material, and accidentally fallen on the tray. The fixing temperature should not exceed 105 deg. C, so as not to change the tinctorial properties of mycobacteria.*

* Highly likely, this means the method proposed in the article:
HAIN E Zur Hitzefixation mikroskopisher Präparate, insbezondere in der Tuberkulose-Diagnostik. [Heat fixation of microscopical preparations especially in diagnosis of tuberculosis] Zentralblatt fur Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene. Zweite Naturwissenschaftliche Abt.: Allgemeine, Landwirtschaftliche und Technische Mikrobiologie, 01 Mar 1953, 159(4):313-316 Language: ger. PMID: 13079168
It is a pity, but we still do not have the full text of this article.

6. Automated Slide Stainer FS-12-25 Instruction Manual (IM) V1.8 English – 05.08.2020