

AD A049293
AU NO.
DDC FILE COPY

AD

12

14
TRANSLATION NO.: MUL-0537

6
TITLE: An Accelerated Method for Staining Tularemia Bacteria

10
AUTHOR(S): Kudelina, R. I. / Kudelina

11
REFERENCE: Tr. Inst. of Laboratornoye Delo 4:250, 1974

(USSR) n4 p.54 1974.

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SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) An accelerated method for staining Tularemia bacteria		5. TYPE OF REPORT & PERIOD COVERED Translation
7. AUTHOR(s) Kudelina, R. I.		6. PERFORMING ORG. REPORT NUMBER MIL 0537
9. PERFORMING ORGANIZATION NAME AND ADDRESS Lab Delo 4:250, 1974		8. CONTRACT OR GRANT NUMBER(s)
11. CONTROLLING OFFICE NAME AND ADDRESS USAMRIID Library, Ft. Detrick, Md.		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		12. REPORT DATE 18 January 1978
		13. NUMBER OF PAGES 3
		15. SECURITY CLASS. (of this report) Unclassified
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE
16. DISTRIBUTION STATEMENT (of this Report) Approved for public release; distribution unlimited		
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)		
18. SUPPLEMENTARY NOTES		
19. KEY WORDS (Continue on reverse side if necessary and identify by block number) Bacteria Tularemia Staining		
20. ABSTRACT (Continue on reverse side if necessary and identify by block number)		

ACCESSION for	White Section	☒
	Buff Section	☐
	DDC	☐
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JUSTIFICATION		
BY		
DISTRIBUTION/AVAILABILITY CODES		
Dist.	AVAIL. and/or SPECIAL	
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UDC576.851.45.086.15

AN ACCELERATED METHOD FOR STAINING TULAREMIA BACTERIA

/Article by R.I. Kudelina, Tularemia Laboratory/ Institute of Epidemiology and Microbiology imeni N.F. Gamaleya/ USSR Academy of Medical Sciences/ Moscow, Laboratornoye Delo, No 4, 1974, p 250, /

At the present, time the standard procedure for staining tularemia bacteria in animal tissue is the staining of the smear-impression according to Romanovskiy-Gimze. During the cultivation of tularemia strains in the organism of chick embryos we came up against the fact that this stain makes it difficult to differentiate tularemia bacteria in the smears of vitelline sacs. The duration of the staining procedure, from several hours to days and the need to fix the smear also create difficulties in its application.

In a search for a more optimal method, we decided on the method of accelerate staining using the Gimze solution, which is employed in staining the blood of farm animals /1/. This method has not been previously employed to stain the tularemia microbe, and literature available to us did not provide references thereto.

The advantage of the method over others is found in the rapidity of the staining procedure (15 min). Moreover, preliminary fixing is not required since the staining and fixing of the smear are carried out simultaneously.

The accelerated staining of tularemia bacteria calls for a mixing of equal parts of the Romanovskiy-Gimze stain and methyl alcohol or chemically pure acetone. This mixture enclosed in vials with ground stoppers may be kept for several months.

In order to use single smears we use Petri dishes with a closed cover, and for a staining of a large number of smears we use ice trays (without the screen) contained in each freezer.

The staining technology is as follows: a fresh (not less than 24 hour old) air dried and unfixed smear is covered with 20 drops of the mixture of the Romanovskiy-Gimze stain and methyl alcohol¹. after 1 minute we add to the stain 10 ml of alkalized (pH7.2) distilled water. Carefully mixe the mixture with water. After 10-15 minutes the water and the mixture are combined, the smear is washed with running water, dried and examined under a microscope.

Using this method of staining the tularemia bacteria, we have a dark-violet color, and is clearly differentiated from the surrounding light-lilac cellular background.

This method described by us was tested during the staining of the smears-stains (over 2,000) from vitelline sacs of chick embryos and organs of white rats, guinea pigs infected with tularemia microbes. In addition we obtained good results allowing us to recommend this method for practical use.

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1. Niktin, V.N. Atlas of blood cells of farm and laboratory animals Moscow, 1949, p. 8.

Submitted 15 December 1972