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DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

TEST REPORT NUMBER 712 OF TEST STATION 53

/Following is a translation of a German-language document issued by Test Station 53 of the Armed Forces of the Federal Republic of Germany (Bundeswehr), 3042 Munster-Lager, Germany, telephone Munster-Lager 2831/583, dated 14 June 1963./

Testing Assignment

Subject: US Sampling Kit, Biological Agent, Equipment Set E 25 R 2

Reference: Testing Assignment PT 357 - PT III 1/01/427 Z, dated 28 June 1962

Reporting: Doctor of Medicine and Veterinarian von Sprockhoff

Test Report Number 712 of Test Station 53 - V [illegible]/63
(1st Intermediate Report)

In the tests conducted thus far, the American prepared solid culture medium has proved to be a poor medium for growing and testing for bacteria. These negative results are very likely due to the age of the material tested (packing date July 1959), however. A definite statement as to its suitability or lack of suitability will therefore be possible only after testing material of recent manufacture.

The US vacuum pump, used in conjunction with the Impinger effect, is well suited for the taking of air samples. The detection of aerified bacteria (Serratia marcescens) could be carried out satisfactorily with it.

/s/ Dr Waitz
/t/ Dr Waitz

/s/ Sprockhoff
/t/ Dr. v. Sprockhoff

/s/ Kramer
/t/ Kramer

Bundeswehr Testing Station 53 3042 Munster-Lager, 14 June 1963
Doc Log No V1 - 72 - 22 - 66 - 65 Tel: Munster-Lager 2831/583

Test Report Number 712 of Test Station 53 - V 15/63
(1st Intermediate Report)

Subject: US Sampling Kit, Biological Agent, Equipment Set E 25 R 2

Reference: Testing Assignment PT 357 - PT III 1/01/427 Z, dated 28 June 1962

Reporting: Doctor of Medicine and Veterinarian von Sprockhoff

A) Equipment Tested:

US sampling kit for biological warfare agents, Equipment Set E 25 R 2.

B) Purpose of Test:

To determine whether this equipment is suitable for taking samples of biological warfare agents. Testing should yield a comparison with the equipment produced by the firm of Bartels & Rieger.

C) Conduct of Test:

The testing included:

1. Attempts to cultivate various microorganisms in the prepared solid culture medium of the Equipment Set E 25 R 2.

2. Examination of experimentally produced germ aerosols with the US vacuum pump.

The following strains of bacteria were used in the accomplishment of Part 1: Staphylococcus aureus "P 6538" from the American type culture collection (ATCC), Escherichia coli of the serological group O 26 : B 6, Salmonella gallinarum "Münster," Brucella melitensis "Erbach," Pasteurella multocida "Münster," Listeria monocytogenes of the serological group I and Bacillus cereus var. mycoides "9634" of the ATCC. They were used partly as 18-20 hour bouillon cultures, partly as inclined agar suspensions. 0.1 ml of these was placed on the carton disks, which were put into plastic dishes and soaked with the American liquid culture medium shortly before use.

Parallel to this, a growth control was conducted by means of innoculation of normal agar media. Growing time was from 1-3 days at 37°C (Tests 1 and 2).

Further, some of the test germs described above were placed in sterilized water and filtered through the original US membrane filter of the equipment set E 25 R 2, using the American vacuum pump. As a means of control, these germs were filtered through the "K 5" membrane filter, of German manufacture, in the bacteria detection instrument Coli 5 of the firm Membranfiltergesellschaft (Membrane Filter Corporation) of Göttingen, Germany. The breeding of the bacteria then took place again at 37°C on the US nutrient carton disks or on freshly prepared agar (Test 3).

Serratia marcescens served as the test germ for the aerosol tests in accordance with C) 2. First pre-tests were conducted to determine which conditions would best produce a clear formation of the red pigment which is characteristic of this bacterium. The optimum conditions to produce this prodigiosin were found to be attained within 24-48 hours while breeding on Sabouraud-agar at pH 5.6, under the influence of sunlight and at room temperature (about 22°C). These conditions were maintained during all tests.

The breeding of Serratia marcescens (also called Bacterium prodigiosum) for the purpose of obtaining a concentrated germ solution was done in Difco-bouillon nutrient with subsequent centrifugation at 3000 revolutions per minute and placing the sediment in 8-10 ml bouillon. In some tests, several milliliters of inclined agar solution of Serratia marcescens were also added.

The aerosol was produced with the Piccolo aerosol device of the firm C. Heyer of Bad Ems, Germany. The particles produced with the fine adjustment of the jet, according to the literature of the manufacturer, had a diameter of 0.5 - 2 μ. Due to the lack of an aerosol chamber, all tests had to be conducted in an air shaft (digestorium) of the laboratory which enclosed a spaced of about 1.4 m^3 and which was made air tight with the use of foam rubber and Tesafilm. The ventilation facility was not in operation during the test. A lab worker, wearing CBR protective clothing and mask, entered this shaft and performed the air filtration both during and after the fogging.

Each time, 6-7 ml of bacteria concentrate were aerified. The relative humidity varied from 45-50% at the start of the test and from 65-88% at the end of the test. The temperature in the chamber was 22-24°C at the start and 27-28°C at the end of the test.

The US vacuum pump was used for the air filtrations. According to tests made at the CBR Defense Station at Sonthofen (reference: letter from the CBR Defense Station, dated 30 May 1962, to the Troop Headquarters Cologne, Inspector of Engineers, Section Troop CBR Techniques), the American pump has a capacity of 184 ml per revolution, or a sure capacity of about 10 ml per 55 (54.5) revolutions. One second was selected for the time duration of one revolution. The stated 184 ml of air per revolution are caused, by the design of the instrument, to flow through very small slots into a testing container made of plastic. Prior to use, a suitable liquid (a physiological salt solution or bouillon) is placed in the container with its meniscus reaching just to the slots. The so-called Impinger effect is thus attained, i.e., the airborne germs are transferred from the dry to the liquid phase.

As the initial liquid for the aerosol germ, m/90 phosphate-buffered physiological table salt solution was used. 20 ml were placed in each container. The plastic containers, as shown by tests at the CBR Defense Station at Sonthofen and at Testing Station 53, are not heat resistant. They were therefore sterilized in a Sterivit apparatus with an ethyleneoxide-carbonic acid mixture.

Prior to the subsequent processing of this 20 ml of liquid which had been seeded with germs via the Impinger effect, the liquid was filtered in portions (two of 5 ml, one of 10 ml) through the K 5 membrane filter of German manufacture in the Coli 5 bacteria detection instrument. The purpose of dividing the 20 ml into three portions was to diffuse the possibly high germ content over a larger membrane filter surface so as to enable a positive count of bacteria colonies.

The utilization of the US membrane filter and the American filter provision was omitted because the tests reported here were intended mainly to determine the performance of the vacuum pump in conjunction with the Impinger effect and also because the filter material was available only in limited supply. Later tests will be concerned with this question. Following the filtration of the three liquid samples, the membrane filters were placed on the above-mentioned Sabouraud-agar and brooded at room temperature (about 22°C) under the influence of light. The final reading of the results -- counting the red bacteria colonies which occurred -- took place on the second or third day.

D) Test Results:

1. Attempts to cultivate various microorganisms in the prepared solid culture medium of the Equipment Set E 25 R 2.

The results of Tests 1 and 2 are shown in Table 1. Staphylococci, Coli-bacteria and Cereus-bacilli showed extensive growth after one day, as in the control on agar. For Pasteurella, Brucella and Listeria, the detection was delayed until the second day; furthermore, the bacteria growth could only be recognized here under magnification. Certain identification of germ growth was only possible after removing a portion of the culture growth from the surface of the culture medium by means of a wire loop and smearing it upon slides, staining (via the Gram method) and observation under a microscope. The cultivation of Salmonella gallinarum on the US nutrient carton disks failed in both tests.

Test 3 (Table 2) resulted in only a very weak growth and a lack of germ multiplication with the use of the American prepared solid culture medium and the corresponding membrane filter. On the other hand, identification of germs on agar with and without the membrane filter of German manufacture was possible with ease.

2. Examination of experimentally produced germ aerosols with the US vacuum pump.

The results of this test are shown in Table 3.

Tests 1, 4-6 and, in part, 2 yielded certain identification of the test germ. The membrane filters were often completely covered with bacterial growth; in many cases, the colonies could also be counted.

In Test 2, the germ determination succeeded without difficulty through sample number 7. From number 8 on, only isolated colonies of prodigiosus bacteria could be found, however. This is very apparently the result of the time when the samples were taken -- in this case, after the aerification.

The initially heavy growth, but later very limited growth, of Serratia marcescens in Test 3 is probably due to certain conditions of the test environment which are not known at this time, possibly varying flow relationships in the shaft. Exact data could only be obtained with a special aerosol chamber. Construction of such a chamber is planned.

Table 1. Results of Tests 1 and 2 on the cultivation of various species of bacteria on the US nutrient carton and agar.

Test Germ	Test 1						Test 2					
	US nutrient carton			agar			US nutrient carton			agar		
	1st day	2nd day	3rd day	1st day	2nd day	3rd day	1st day	2nd day	3rd day	1st day	2nd day	3rd day
Staphylococcus aureus	++			++			++			++		
Escherichia coli	++			++			++			++		
Salmonella gallinarum	-	-	-	++			-	-	-	++		
Pasteurella multocida	-	-	+	++			-	-	++	++		
Brucella melitensis	-	-	+	-	+	++	-	-	++	-	++	
Listeria monocytogenes	-	-	+	++			-	-	++	++		
Bacillus cereus	++		-	++			++			++		

Legend:

- \- = no growth
- \+ = weak growth
- ++ = heavy growth, solid cover of bacteria or numerous individual colonies

Table 2. Results of Test 3 on the cultivation of several test germs on US nutrient carton and US membrane filters and on agar and K 5 membrane filters of German origin.

Test Germ	Growth after 72 hours at 37°C on		
	US Nutrient Carton and US Membrane Filter	Agar and German Membrane Filter	Agar
Salmonella gallinarum	-	++	++
Pasteurella multocida	+	++	++
Brucella melitensis	+	++	++
Listeria monocytogenes	-	++	++

Legend: see Table 1

Table 3. Results of the tests on bacteria aerosols with the US Vacuum pump

Sample No.	Test 1		Test 2		Test 3		Test 4		Test 5		Test 6	
	Air 1	Agar Colonies	Air 1	Agar Colonies	Air 1	Agar Colonies	Air 1	Agar Colonies	Air 1	Agar Colonies	Air 1	Agar Colonies
1	10	complete coverage	10	complete coverage	0.18	183	0.18	complete coverage	0.18	ca. 1500	0.9	complete coverage
2	10	complete coverage	1.8	complete coverage	0.18	65	0.9	complete coverage	0.9	ca. 1630	3.7	complete coverage
3	20	complete coverage	0.9	complete coverage	1.8	0	0.9	ca. 4100	1.8	complete coverage	0.9	141
4	1.8	complete coverage	0.18	complete coverage	5	1	0.18	102	0.9	complete coverage	1.8	18
5	1.8	complete coverage	0.9	complete coverage	5	0	0.9	331	5	complete coverage	5	42
6	9.2	complete coverage	0.9	complete coverage	0.9	0	3.7	170	5	complete coverage	1.8	765
7	0.9	complete coverage	0.18	complete coverage	5	0	1.8	[illegible]	0.9	complete coverage	0.18	71
8	5	complete coverage	0.18	2	5	1	5	179	1.8	complete coverage	0.9	2
9	0.9	complete coverage	0.18	0	10	0	5	40	5	complete coverage	1.8	7
10	1.8	ca. 7[illegible]	0.9	3	5	0	10	14	10	complete coverage	10	234
11	5	[illegible]	5	2	40	4	20	[illegible]	20	ca. 2240	5	ca. 4
Number of germs in one ml bacteria concentrate	ca. $4 \cdot 10^8$		ca. $1 \cdot 10^8$		ca. $6.6 \cdot 10^8$		ca. $6 \cdot 10^{13}$		not possible to determine		not possible to determine	
Samples taken during the fogging	No 1-10		No 1-7		No 1		No 1-3		No 1-7		No 1-2	
Samples taken after the fogging			No [illegible]		No 2-11		No 4-11		No [illegible]-11		No 3-11	

E) Discussion:

The American prepared solid culture medium, its nutrient liquid stored in a sterile container and poured on the carton disk prior to use, proved to be of little value for detecting pathogenic bacteria in the foregoing tests. This negative result could well be due to the age of the tested nutrient substrate, however.

According to the label, the date when the nutrient was filled was July 1959. A definite statement as to the suitability or lack of suitability of the US prepared culture medium is therefore possible only after testing material of recent manufacture. A request for delivery of additional refill packets for the equipment set E 25 R 2 was made on 21/22 May 1963 at the BWB Coblenz PT III and the Federal Ministry of Defense T III.

The detection of aeriated bacteria with the aid of the US vacuum pump was successfully accomplished. Definite advantages of this pump are its capacity to draw a relatively large volume of air in a short time (five liters in ca. one minute) and its simple and easy operation.

The comparison of the results obtained in these tests with those obtained with the German sample taking instrument for biological warfare agents (Test Report Number 699 of Testing Station 53 dated 30 May 1963) will follow in a later report.

F) Summary:

The American prepared culture medium has shown itself in tests conducted up to this point as a poor medium for cultivating, and therefore also for the detection, of bacteria. This negative result is probably due to the age of the material tested (packing date July 1959). A final statement as to its suitability or unsuitability is therefore possible only after testing material of most recent manufacture.

The US vacuum pump used in conjunction with the Impinger effect is well suited for taking air samples. The identification of aerified bacteria (Serratia marcescens) was accomplished satisfactorily. ( )

/s/ Sprockhoff
/t/ (Dr. von Sprockhoff)