قسم : طب الحيوان وأمراض الدواجن . كلية الطب البيطرى - جامعة أسيوط .
رئيس القسم . أ . د . / سيد عبد الرحيم العمروسى .

بعض الدراسات عن تشخيص اللاهوائيات باستعمال الاختبارات المتعددة والمجهزة

طه العلاوى ، بترويتش سها

- تم دراسه الخصائص الكيميائية لخمسة عترات من ميكروب التيتانوس باستخدام اختبارات متعدده ومجهزه (Api 20 A) .
- وجد أن ٤٨ ساعة حضانة على درجة ٣٧ م هى مناسبة لنمو اللاهوائيات.
- يعتبر التشخيص بواسطه هذه الاختبارات الكيميائيه المتعددة وسابعه التجهيز سريع وبسيط فى استعماله واقتصادى فى تشخيص اللاهوائيات.

Dept. of Medicine & Infectious Diseases,
Faculty of Vet. Med. Assiut University,
Head of Dept. Prof. Dr. S. El-Amrousi.

SOME STUDIES ON THE LABORATORY DIAGNOSIS OF ANAEROBES BY USING MULTISET KITS*

(With One Table and One Figure)

By

T. EL-ALLAWY and P. SAHA**

(Received at 29/10/1980)

SUMMARY

Study on biochemical properties of Cl.tetani were made with Api 20 A system. 48 hrs. incubation was found to be sufficient and reasonable for the routine use of kits in anaerobic bacteriology.

Lombard Dowell medium is necessary only when solid media is used. The Lombard Dowell medium could be inoculated with fluid cultures too, even this medium can be successfully substituted with fluid medium like thioglycollat medium. Api 20 A multikit system was found to be rapid, simple and economic method for the identification of anaerobes in routine diagnosis.

INTRODUCTION

In recent years many trials were carried out toward developing simple, practical and rapid technique in the field of diagnosis of clinical anaerobic bacteria.

Several commercial micromethod multitests were described for diagnosing enterobacteriaceae by using Api 20 system and Analytab products, Inc. SMITH et al. (1972) and WASHINGTON et al. (1971).

STARR et al. (1973) discussed the need of simple method for identifying anaerobic bacteria isolated from clinical specimens, they compared anaerobic system Api 20 and Anatylab products and found that H2S and indole were over 90% aggreement between the two systems.

The investigations made with Api 20 system by DOWELL,(1972) and SMITH et al. (1972) still need some questions to be answered. These include incubation, utilization of Lombard Dowel medium to make this system more applicable in the field of anaerobic bacteria. There fore the present study was designed to investigate the following points:

1- The time of incubation of Api 20 strips in the anaerobic glove box.
2- Studying the biochemical properties of several strains of Cl.tetani in order to find the accurate diagnostic biochemical properties.
3- Comparative study between two media used for identification of strips using fluid thioglycollate media cultures.
4- Comparing inoculating sources of Lombard Dowell media from fluid or solid media containing strain colonies of Cl.tetani.

MATERIALS AND METHODS

a- Strains: Five strains of Cl.tetani:

NCTC No.	Symbolic Names Used
1- NCTC 540	CT2
2- NCTC 5410	FT4
3- NCTC 9569	CT6
4- NCTC 9568	CT7
5- NCTC 9575	CT9

These strains were obtained from the national collection of the type cultures (NCTC), Central Health Laboratory, London.

---

* This work was done in the Institute of Animal Hygiene and Animal Medicine, Hohenheim University, Director Prof. D. Strauch.

** Co-worker at the Institute of Animal Hygiene and Animal Medicine Hohenheim Univ.,Federal Republic of Germany.

b- Multitest Kits Api 20 A System:

Api 20 A strips for anearobic bacteria were used,each strip contained 20 biochemical tests,these are: Indole, urease, glucose, mannite, lactose, sucrose, maltose, salicin, xylose, arabinose, gelatin, esculin (H2S), glycine, cellobiose, melzeitose, raffinose, sorbitol, rhaminose and trehalose.

The test was carried out according to Api 20 A instruction manual (Api Labor system GmbH Bismarking 24-6200 Wiesbaden). In the microtube esculin we read out results of (H2S) production instead of asculin.

c- Fluid Media:

Thioglycollate media USP (BBL, 11260).

d- Solid Media:

Blood agar base No: 2 (oxid CH271) with 5% sheep blood. The tubes of fluid media (10 ml/tube) were heated up to 80°C in water bath just before use for purpose of reduction, the tubes were then inoculated after it was cooled to room temperatue.

Agar plates and tubes were already prepared 3 days prior to investigations. All media and biochemical strips were inoculated in anaerobic glove box (National Heinicke Company, USA Model 3650) at 37°C. For each strain 12 strips were used, 6 strips were incubated for 48 hrs and another 6 strips for 72 hrs.

Fluid media were inoculated with the used strains for the preparation of the inoculum for the solid cultures. The inoculated culture media were incubated in anaerobic glove box for 24 hrs.

Lombard Dowell media were inoculated with Iml of 24 hrs. fluid culture. When the solid media were used as a source of inoculation, 6 strips were employed for this purpose. For direct inoculations with fluid media (thioglycollate) 24 hrs. cultures were used.

RESULTS

The results of biochemical tests for Cl.tetani strains after 48 hrs. and 72 hrs. incubation could be presented in the table. From the obtained results, it is obvious that strains used in the present study were in 100% negative for indole, urease; glucose, mannite, salicin, xylose, arabinose, cellobiose, mannose, malezitose, raffinose, sorbitol, rhaminose, trehalose, and catalase while it was in 100% positive in case of gelatin and asculin (H2S).

In case of lactose,the percentage of negative for CT7 was 83.3% after 48 hrs. incubation and also it was 83.3% for CT9 after 48 hrs. and 72 hrs. incubation while it was 100% negative after 48 hrs. and 72 hrs. incubation for the other strains.

For maltose, it was found that CT4 showed 83.3% negative after 72 hrs. incubation while for other strains it was 100% negative after 48 hrs. and 72 hrs. incubation.

Concerning glycine, the biochemical tests showed negative results that ranged from 50%-100%. In this respect it is difficult to have as particular biochemical behaviour with glycine.

Time Of Incubation:

The effect of incubation time on bicohemical reaction for all strains used was the same except for CT2 for glycine, CT4 for maltose and CT7 for lactose where variations were recorded. In case of CT4, the decomposition of maltose was less after 72 hrs. incubation than that of 48 hrs.

For lactose decomposition in case of CT7, it was 83.3% negative and 100% negative after 48 hrs. and 72 hrs. incubation respectively. In case of glycine, biochemical results were different either after 48 hrs. or 72 hrs. of incubation for CT2 only.

In this study the fluid medium was used with strain colonies of CT2, CT4 and CT6 (6 strips for each) with and without passing through Lombard Dowell medium inoculated strips which were incubated 48 hrs. as we experienced.

The results of indole, urease, glucose, mannite, lactose, sucrose, maltose, salicin, xylose, arabinose, glycine, cellobiose, mannose, malzetose, raffinose, sorbitol, rhaminose, trehalose and catalase were 100% negative while in case of gelatin and asculin (H2S) were 100% positive. These results are the same with both media used with different strains with 48 hrs. incubation.

In case of using strains obtained from solid media to Lombard Dowell media according to directions of Api 20 A system, we used CTI strains. The same results as those with fluid media except gelatin and asculin (H2S) were positive in 82% were obtained.

DISCUSSION

The purpose of this study was to investigate the effecinecy of Api 20 A system for diagnosing biochemical properties of Cl.tetani with regard to the suitable time of incubation and comparing different media used for inoculation.

In general, it was found that Api 20 A system was a reliable simple, practical besides it does not need much time to reach a reliable diagnosis. This is in aggreement with STARR et al. (1973).

Concerning the biochemical properties of Cl.tetani strains (5 strains), the results were 100% negative for all biochemical tests except gelatin and asculin (H2S) were 100% positive and maltose results were 83.3% negative after 72 hrs. incubation for CT6 while the other strains showed 100% negative after 48 hrs.and 72 hrs. incubation. On the other hand, glycine showed frequent changes ranged from 50 - 100% negative. For lactose 83.3% negative for CT7 after 48 hrs. and CT9 after 48 hrs. and 72 hrs. while it was 100% negative with other strains.

To sum up, we could state that only gelatin will be hydrolysed and (H2S) will be produced through Cl.tetani strains, but they do not play any role in the acid formation with carbohydrates. However the changes in case of glycine were irrigular and that is why it is of no particular value to identify this bacteria at least. In this respect the available literature concerning the biochemical properties of Cl.tetani with Api 20 A system lacks too much to be compared with the obtained results.

The effect of incubationn time showed that there was no such difference between 48 hrs.and 72 hrs. incubation with different strains. However, if we compare our results with others (STEPHAN, 1955 and LOTHER et al. 1974) concerning biochemical properties of Cl.tetani and aggreement could be reached.

The investigations given by the manifactures of Api 20 A system recommened 24 hrs. incubation. Our results, however, showed that this time of incubation was not sufficient to reach the desired reaction and the recommened time could reach 48 hrs. or 72 hrs. incubation with very insignificant differences between both that can be overlocked and the 48 hrs. are quite sufficient.

Reconstitution of the strains were performed in the thioglycollate media either inoculated directly to the strip or passed through Lombared Dowell media.

From the observations during the course of this study, it was found that the use of heavy inoculum for the inoculation of agar plates is recommended toget quick regular results. In addition to this, the given media should be shaked vigrously for the purpose of the proper distribution of the germs before agar inoculation. We believe that these tests are necessary for definite identification of anaerobic bacteria and such a system may be of a great help in the field of clinical bacteriology. Moreover, it is relatively more economic, rapid, simple and practical.

REFERENCES

Dowell,V.R. (1972): Comparison of techniques for isolation and identification of anerobic bacteria. Amer. J. Clin, Nutr. 25: 1335-1345.

Lother,H. and Friedrich,B. (1974): Klinische Mikrobiologie. George Thieme Verlog, Sturttgart.

Starr,S., F.Thompson., V.Dowell,and A.Balows. (1973): Micromethod system for identification of anerobic bacteria. App. Microbiol. 25: 713-717.

Stephan,W. (1955): Mikrobiologische und Serologische Diagnostik. 2- Auflage. (1955). Gustav Fischer Verlog, Stuttgart.

Smith,P., K.Rhoden. and A.Balows. (1972): Api system, multitube micromethod for identification of enterobacteriaceae. Appl. Microbiol. 24: 44ç-452.

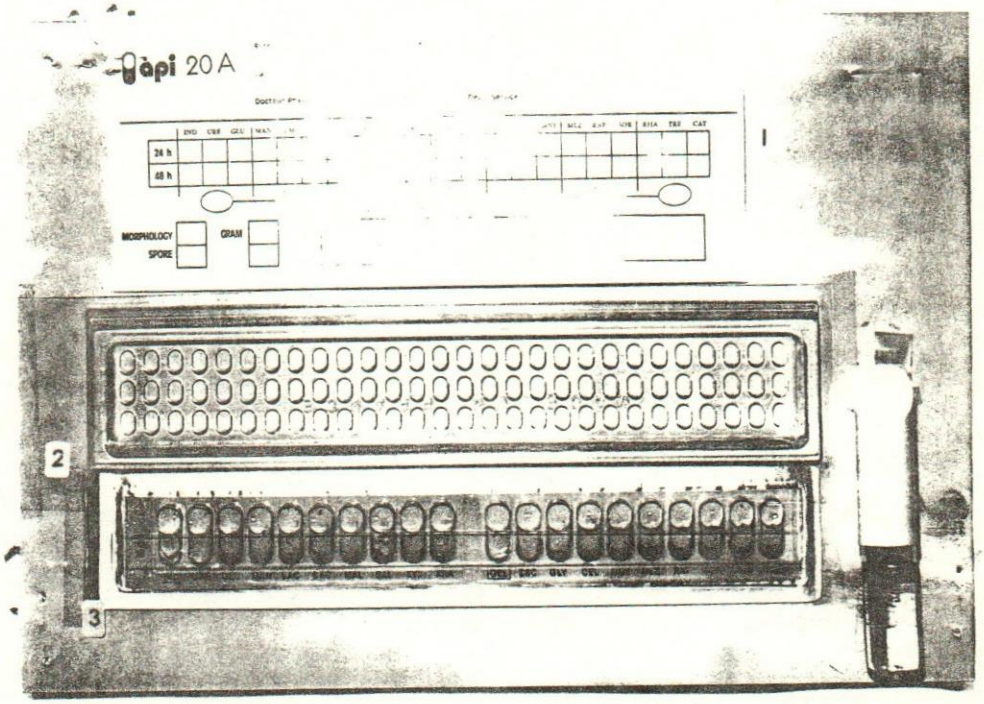
Washington,J., P.Yu. and W.Martin. (1971): Evaluation of accuracy of multitest micrmethod system for identification of enterobacteriaceae. Appl. Microbiol. 22: 267-269.

TABLE (1)

Results of biochemical tests for Cl.tetani strains after 48 hrs. and 72 hrs. incubation.

Strains	CT2		CT4		CT6		CT7		CT9	
Biochemical tests	48	72	48	72	48	72	48	72	48	72
Indole	100-	100-	100-	100-	100-	100-	100-	100-	100-	100-
Urease	"	"	"	"	"	"	"	"	"	"
Gluoose	"	"	"	"	"	"	"	"	"	"
Mannite	"	"	"	"	"	"	"	"	"	"
Lactose	"	"	"	"	"	"	83.3-	"	83.3-	83.3-
Succrose	"	"	"	"	"	"	100-	"	100-	100-
Maltose	"	"	"	83.3-	"	"	100-	"	"	"
Salicin	"	"	"	100-	"	"	"	"	"	"
Xylose	"	"	"	"	"	"	"	"	"	"
Arabinose	"	"	"	"	"	"	"	"	"	"
Gelatin	100+	100+	100+	100+	100+	100+	100+	100+	100+	100+
Asculin (H2S)	"	"	"	"	"	"	"	"	"	"
Glycine	50-	83.3-	100-	100-	66.6-	66.6-	66.6-	66.6	83.3-	83.3-
Cellobiose	100-	100-	100-	100-	100-	100-	100-	100-	100-	100-
Mannose	"	"	"	"	"	"	"	"	"	"
Malezitose	"	"	"	"	"	"	"	"	"	"
Raffinose	"	"	"	"	"	"	"	"	"	"
Sorbit	"	"	"	"	"	"	"	"	"	"
Rhaminose	"	"	"	"	"	"	"	"	"	"
Trehalose	"	"	"	"	"	"	"	"	"	"
Catalase	"	"	"	"	"	"	"	"	"	"

48,72 time of incubation. (-&+) positive and negative in 100%.



Multitest kits Api 20 A system