TÜBERKÜLOZ TANISINDA SORUNLAR YENİLİKÇİ ÇÖZÜMLER

Prof. Dr. Tanıl Kocagöz

ACIBADEM
MEHMET ALİ AYDINLAR
ÜNİVERSİTESİ

Tüberküloz

- Dünya nüfusunun 1/3'ü (2 milyardan fazla) taşıyıcı
- Her yıl 10 milyon kadar aktif yeni tüberküloz hastası ortaya çıkıyor ve 1,6 milyon kişi (günde 5000 kişi) tüberkülozdan ölüyor. Bunların 1/3'üne hiç tanı konmadığı tahmin ediliyor.
- Tüberküloz gelişmekte olan ülkelerde önlenebilir ölümlerin %25'ini oluşturmakta
- ÇİD olgularda hızlı bir artış yaşanıyor

?

- BCG’nin koruyuculuğu düşük
- Halen tüberkülozdan toplumu korumanın en etkili yolu balgamında tüberküloz basili bulunan kişileri erken saptamak ve başkalarına bulaştırmadan etkili şekilde tedavi etmektir.

Klinik bulgu ve belirtiler

- Halsizlik, yorgunluk
- Zayıflama
- Uzun süren ateş, gece terlemesi
- Uzun süren (2 haftadan fazla) öksürük, balgam çıkarma (ilerlemiş olgularda kanlı balgam)
- Akciğer dışı organların tutulumunda, bunlara ait bulgular (menenjit, artrit, organ işlev bozuklukları)

2 HAFTADIR ÖKSÜRÜYOR MUSUNUZ ?

Tüberküloz olabilir misiniz?

Tüberküloz tedavi edilebilir.

- Halk arasında verem diye bilinir.
- Akciğerlerde başlar ve tüm vücuda yayılabilir.
- İnsandan insana hava-yoluyla bulaşır.
- Tüm tedavi masrafları devlet tarafından karşılanır.
- Erken teşhis tüberkülozun yayılmasını engeller.

BALGAM ÖRNEĞİ VERMENİZ TANI İÇİN YETERLİ

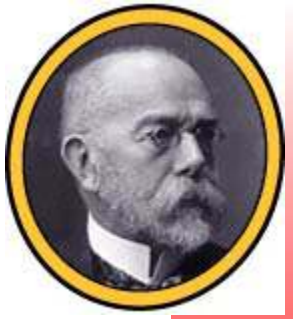
Sizin ve çevrenizdekilerin sağlığı için...

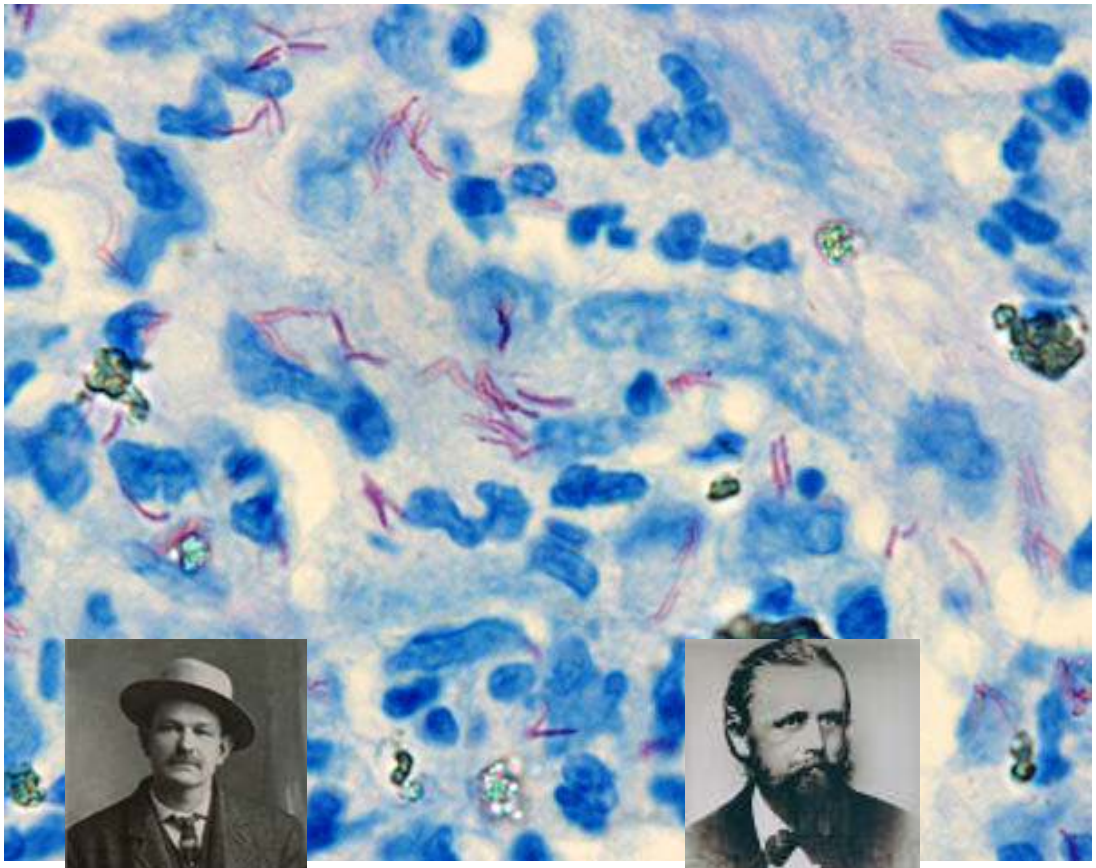
ACIBADEM ÜNİVERSİTESİ

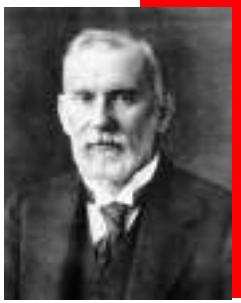
İLETİŞİM: 0534 685 91 32
0539 960 79 57
0505 317 12 12

Elämän tähden

M. tuberculosis - Robert Koch -1882





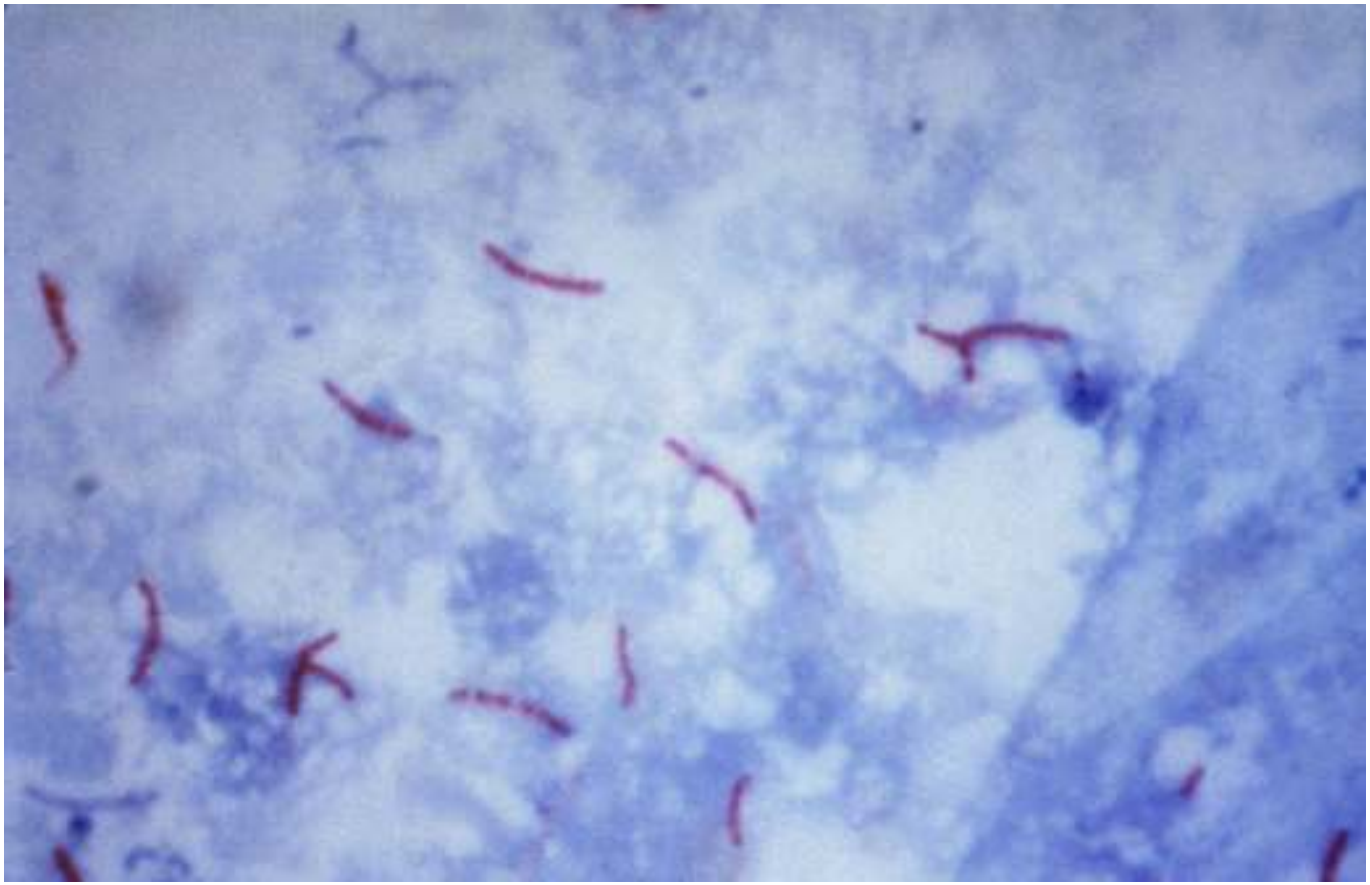


Erlich

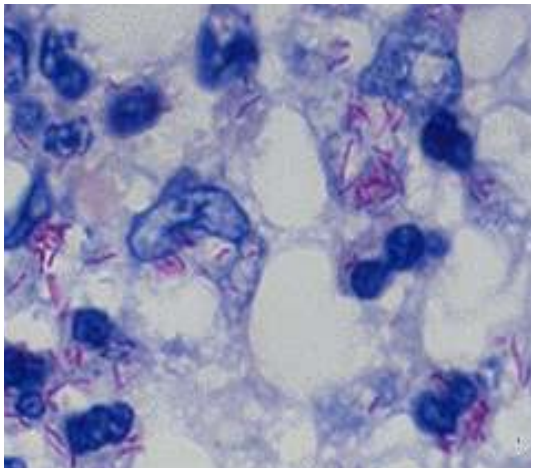
Ziehl

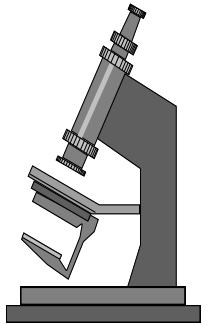
Neelsen

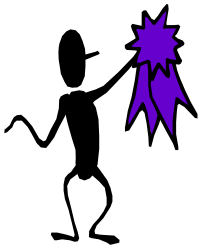
- Asit-alkol karışımı mikobakteriler dışındaki tüm hücrelerin boyasını çıkartır. Mikobakteriler kırmızı kalır. Boyası çıkmış organizmalar daha sonra metilen mavisi ile maviye boyanır.



Mikroskop







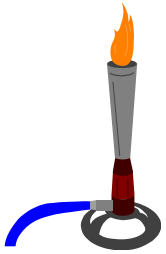
- Hızlı tanı sağlar
- Maliyeti düşük.

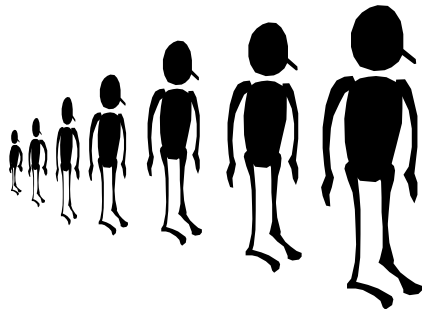
Sorunlar

- Deneyimli tekniker gerektirmekte
- Yalancı negatif veya yalancı pozitif sonuçlar elde edilebilir
- Duyarlılığı düşük
- Atipik mikobakterilerin, *M.tuberculosis*' ten ayrımını yapamaz.
- İlaç duyarlılığı belirlenemez

Kültür

- Tüberkülozun kesin tanısı bakteriyolojik inceleme ile konur.
- Altın standart bakterinin kültürde üretilmesidir.
- *M. tuberculosis* yavaş çoğaldığı için Löwenstein Jensen (LJ) besiyerinde kültür uzun sürer.
- Teksif (dekontaminasyon konsantrasyon) yapılması mutlaka gereklidir.

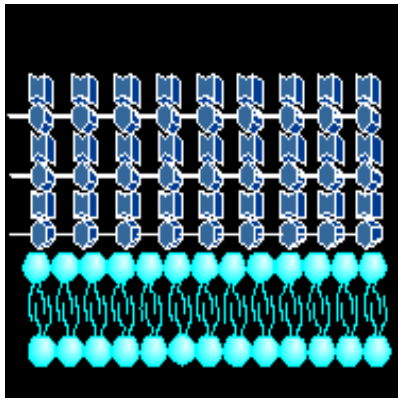




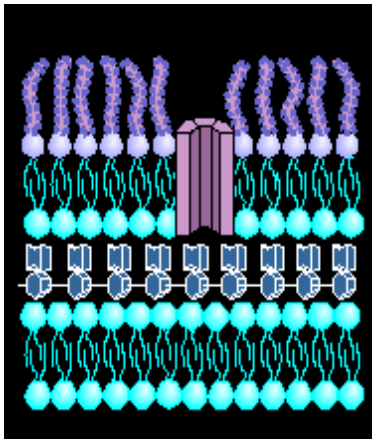
Clever Tube

Mikobakteri hücre çeperi

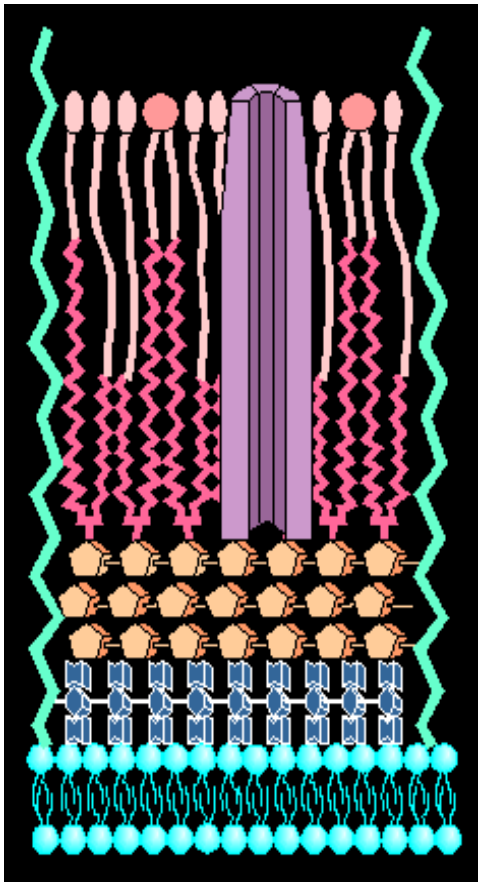
Mikolik asitler çok uzun zincirli yağ asitleridir.



Gram +



Gram -



Mikobakteri

Dekontaminasyon Konsantrasyon

- Balgamda çok sayıda hızlı üreyen bakteri bulunur. M. tuberculosis çok yavaş ürediği için balgam doğrudan besiyerine ekilirse hızlı üreyen bakteriler besiyerini kaplayarak mikobakterilerin görülmesini engeller. Bunu önlemek için bu bakterileri öldüren, mikobakterilere zarar vermeyen kimyasallar ile örnek dekontamine edilir.
- Dekontaminasyon konsantrasyon için önerilen ve en yaygın kullanılan yöntem NaOH-NALC (N-asetil-L-cysteine) yöntemidir.

Dekontaminasyon ve Konsantrasyon

Petroff Yöntemi: 1915

- Balgam 1N NaOH ile dekontamine edilir
- pH nötralizasyonu 1N HCl ile yapılır
- pH indikatörü olarak brom timol mavisi kullanılır.
- Çözeltileri hazırlaması kolay
- pH ayarlaması zor
- Santrifüj gerektiriyor

Kubica Yöntemi: 1963

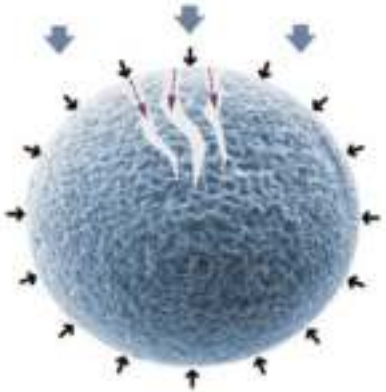
- Decontaminasyon için NaOH-NALC kullanılır
- Nötralizasyon için fosfat tamponu kullanılır
- pH ayarlaması daha iyi ama deneyim ve dikkat gerektiriyor
- Çözeltilerin hazırlanması zor, yarı ömrü kısa
- Kontaminasyon olasılığı yüksek

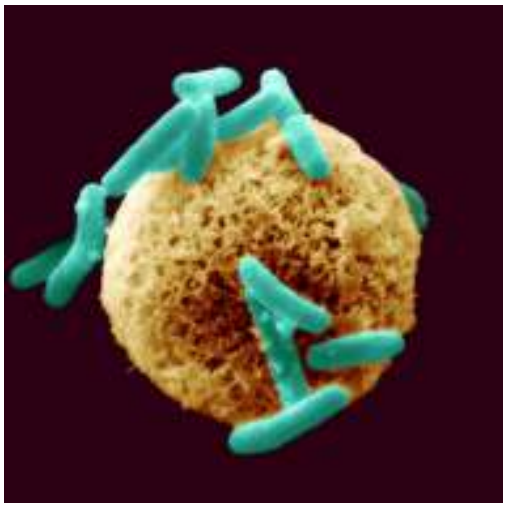
MYCOPROSAFE (2001)

- Kubica yöntemini kolay uygulamak için hazırlanmış kit
- Her hasta için ayrı steril çözeltiler içeriyor
- Kontaminasyon oranını çok düşürüyor
- Çözelti hazırlama zahmetini ortadan kaldırıyor
- Her örnek için taze çözelti hazırlanmasını sağlıyor

Santrifüj gereksinimini ortadan kaldıran dekontaminasyon ve konsantrasyon yöntemi

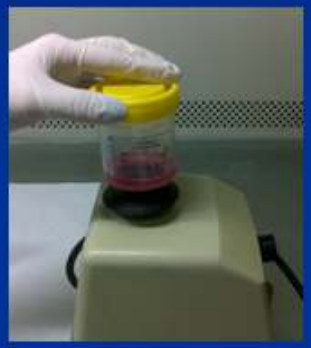
- Emici boncuklarla çalışır.
- İşlem süresini 45'den 23 dakikaya indirir.





DECOMICS İLE ÖRNEK İŞLEME

Örnek kaba konur



Karıştırılır

10 dakika beklenir

Boncuk paketi açılır

Emici boncuklar eklenir

Karıştırılır
5 dakika beklenir

Nötralizasyon
sıvısı eklenir

3 dakika sonra hazır
olan örnekten ekim yapılır

Hızlı Kültür Sistemleri

- BACTEC (Becton Dickinson, USA)
- MGIT (Becton Dickinson, USA)
- Bac-T Alert (Biomerieux, France)
- Versatrek (Thermoscientific, USA)
- TK Culture System (TiBO)



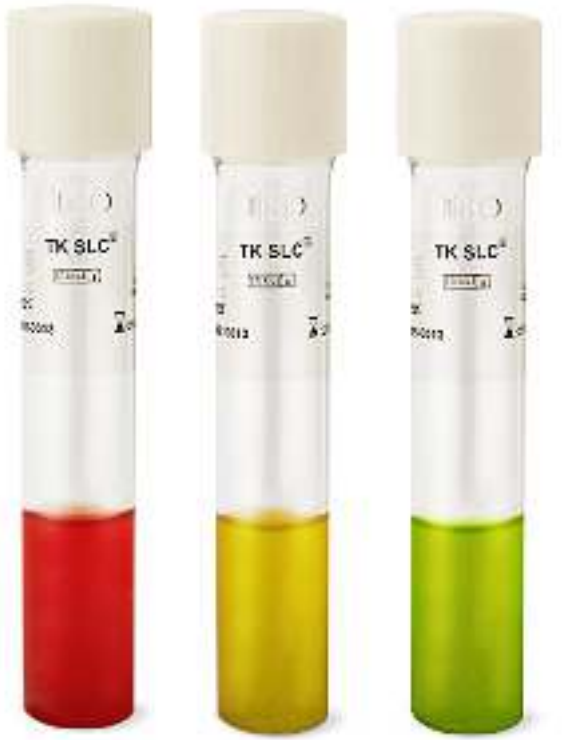







TK MEDIUM

- Hızlı kültür sonucu, kontaminasyonu ayırt etme
- TK-SLC diğer bakteri türleri ve mantarların üremesini engelleyen ilaç içeren seçici TK-Besiyeri
- Hızlı ilaç duyarlılığı belirleme
- Hızlı *M. tuberculosis* ve atipik mikobakteri ayrımı



TK MEDİUM MİKOBAKTERİ KONTAMİNASYON

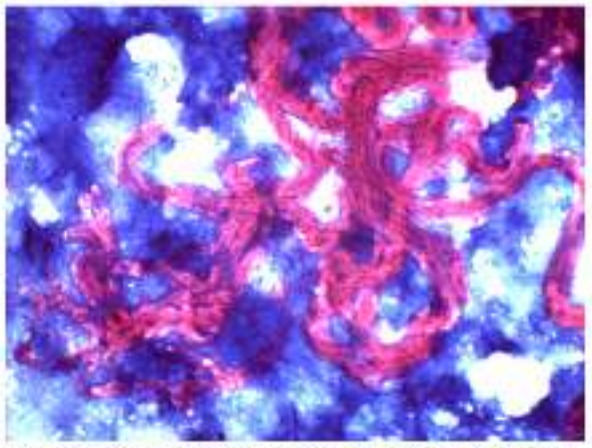
TK MEDIUM

- Kullanıma hazır: OADC ve seçici antimikrobiyal eklenmesini gerektirmiyor.
- Besiyerinin rengi kırmızıdan sarıya değişerek üremeyi gösteriyor. Üreme 7-15 günde saptanıyor.
- Mikobakteri dışında bir tür üreyecek olursa renk kırmızıdan yeşile dönerek kontaminasyonun ayırt edilmesini sağlıyor.

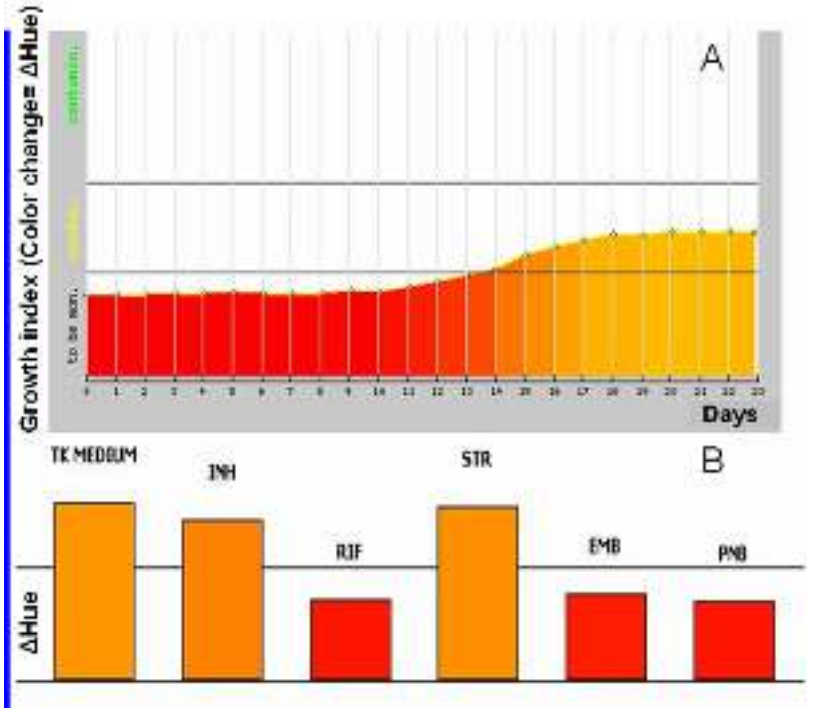
Mikobakteri

TK Medium

Kontaminasyon

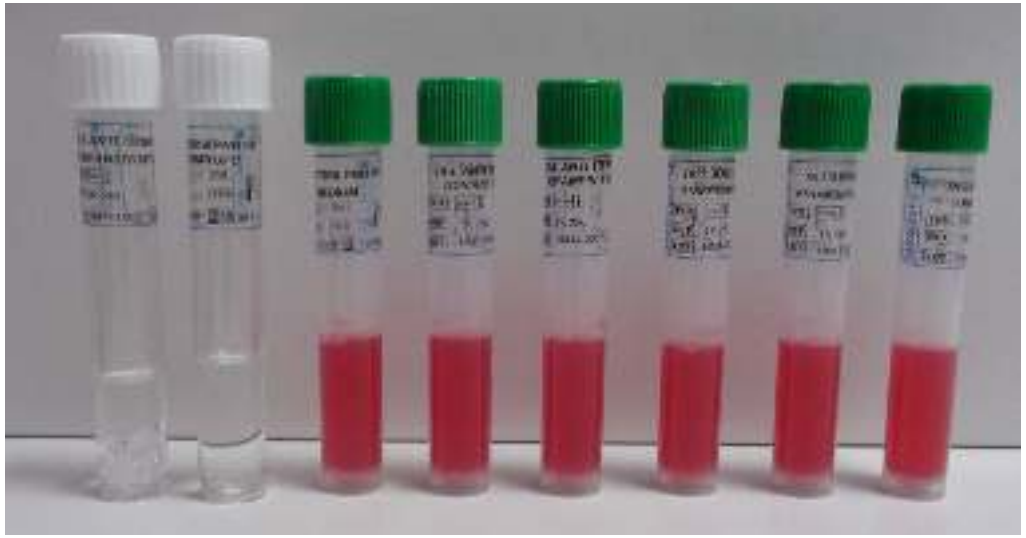


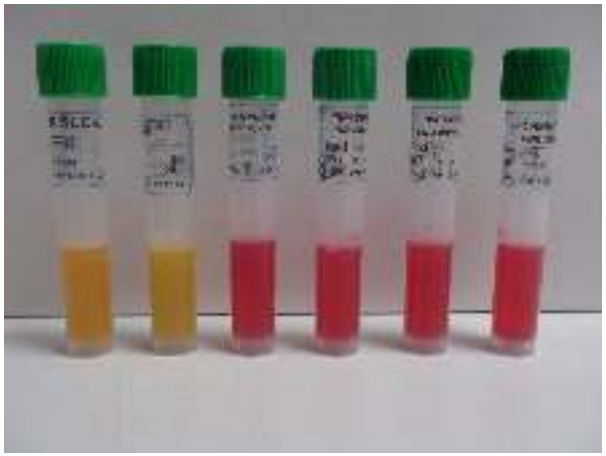
Mycolor TK



- TK Medium için bilgisayarlı otomatik inkübatör ve okuyucu
- Her örnek için üreme eğrileri çiziyor
- Uzman sistemi ile M. tuberculosis, atipik mikobakteri, kontaminant organizma ayrımı yapabiliyor
- Otomatik ilaç duyarlılığı belirleme ve tiplendirme yapabiliyor

Kontrol ilaçsız tüp ve antitüberküloz ilaç içeren tüplerdeki üremeler karşılaştırılarak ilaç duyarlılık testi yapılır.

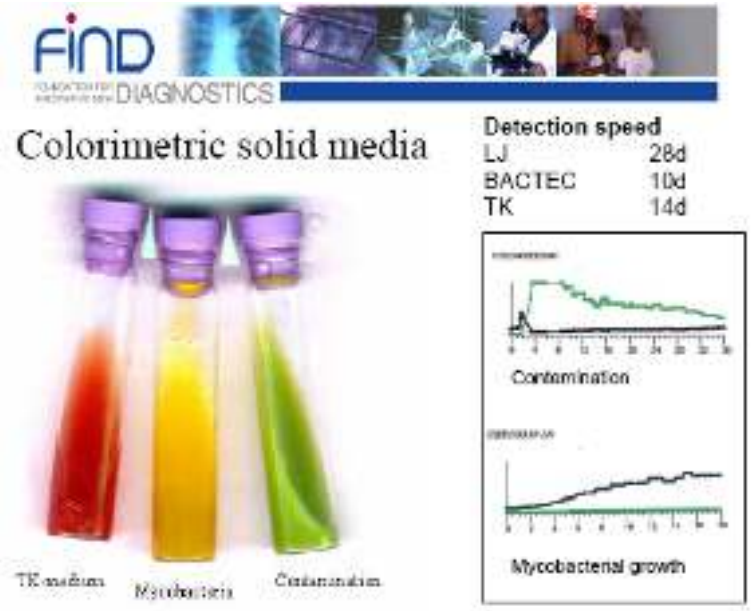




FOUNDATION FOR INNOVATIVE NEW DIAGNOSTICS
FIND: 71, av.Louis Casaï, P.O.Box 93, 1216 Cointrin/Geneva, Switzerland, Tel +41(22)710 0590

Press Release July 2004

FIND to invest in visual TB test from SALUBRIS that cuts culture time in half



SALUBRIS

Development, Distribution and Licensing Agreement for TB rapid culture media

- Cuts TB detection time in half (vs. standard LJ method)
- Color differentiation between positives, contamination, and negatives
- Inexpensive

EUROWARDS
TÜRKİYE

Sayın **Tanıl Kocagöz,**
İnovatif Biyoteknoloji Organizasyonu Tic. Ltd. Şti.,

2005 Yılında Türkiye genelinde düzenlenen
Eurowards Girişimcilik Yarışması'nda
TK Hızlı Tüberküloz Kültür Sistemi Projesi ile
Başlangıç Kategorisi'nde **Birinciliğe** layık görülerek
bu sertifikayı almaya hak kazanmıştır.

Gökhan Gürşen
Eurowards Türkiye
Genel Müdür

Frédéric Béguin
Eurowards Avrupa
Başkan

World Health Organization

Diagnostics for tuberculosis

UNITAID

2014

TUBERCULOSIS

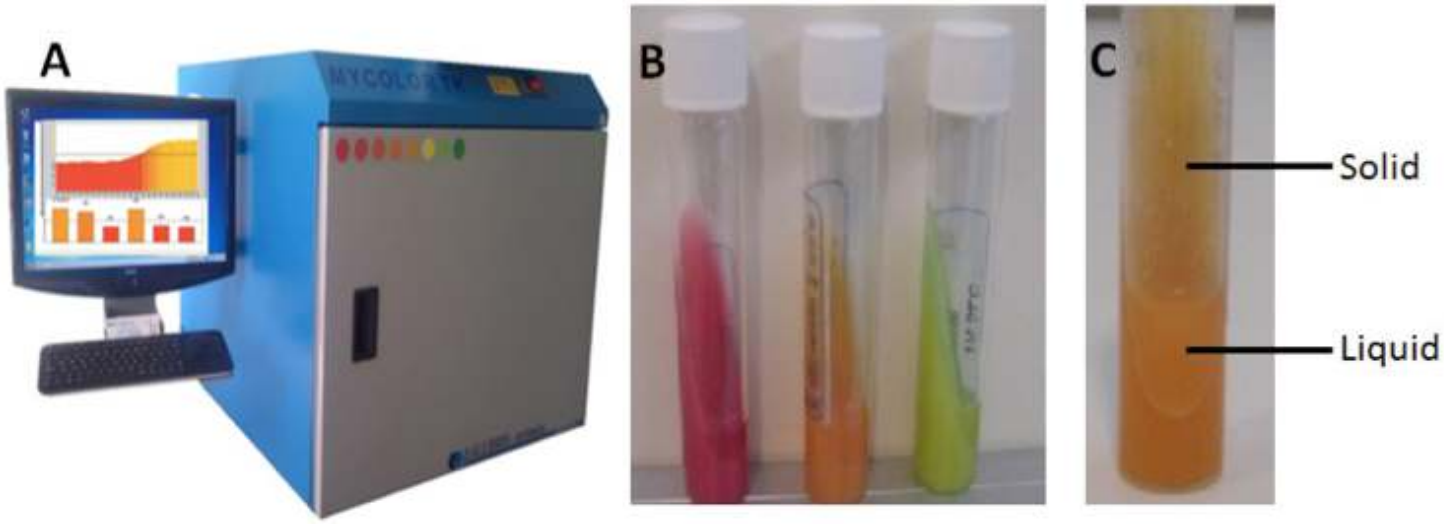
Diagnostics Technology and Market Landscape

3RD EDITION

World Health Organization

Salubris Inc. offers a series of products that also perform with automated TB culture (Figure 14). They manufacture: TK MEDIUM® SLC; a biphasic, culture medium that can be incubated and automatically monitored in the MYCOLOR TK® instrument (Figure 14); and TK MEDIUM SLC-L, a liquid formulation.

Figure 14. A: MYCOLOR TK automated incubator; B: TK colorimetric media with changes from red to orange (mycobacterial positive culture) red to green (contaminated culture); C: biphasic format of TK media with both solid and liquid media



Source: Images reproduced with permission from Salubris Inc.

TK MEDIUM® is an egg-based solid medium similar to L-J.[92] Unlike the liquid media used in other automated platforms, the TK media do not require any additives, creating a simplified workflow and reducing risk of contamination.[92] The media contain dyes that react upon the growth of microorganisms (Figure 14).[93] Upon mycobacterial growth, the original red colour turns orange and then yellow. The colour change is indicated before colonies are visible on the agar, improving the time to detection. Contamination by fungi or Gram-negative bacteria produces a green pigment; some Gram-negative bacteria can produce orange/yellow. The TK SLC medium is biphasic having both solid and liquid media (Figure 14). The TK

assessment between this system and the other liquid systems has not been performed, but recently a new liquid medium, TK SLC Liquid®, was compared to MGIT.[94] Overall, the performance of both media was similar, but MGIT™ had a faster median time to result, 7.7 days as compared to 15.1. Contamination was much more prevalent in the MGIT™ tubes (13.7%) compared to TK SLC Liquid (1.3%), which may be due to the preparation steps prior to inoculation of MGIT.

Çiftci and Karakeçe *BMC Infectious Diseases* 2014, **14**:130
http://www.biomedcentral.com/1471-2334/14/130

BMC
Infectious Diseases

RESEARCH ARTICLE **Open Access**

Comparative evaluation of TK SLC-L, a rapid liquid mycobacterial culture medium, with the MGIT system

İhsan Hakkı Çiftci* and Engin Karakeçe

UNITAID

2015

TUBERCULOSIS

Diagnostics Technology and Market Landscape

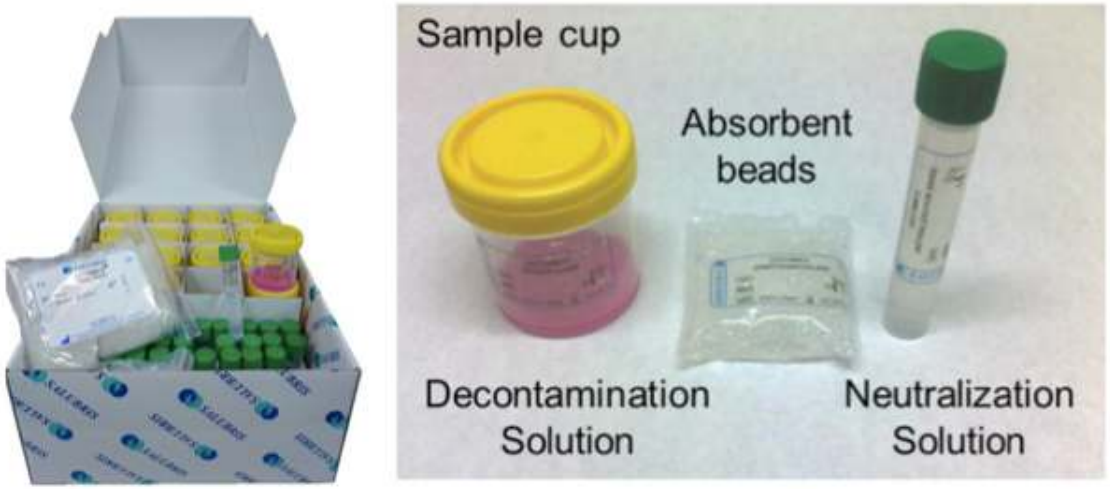
4TH EDITION

World Health Organization

4.2.4. Culture-based tools for the diagnosis of TB and DST

There is little to report in terms of new product information from developers regarding culture-based diagnosis of TB. Salubris Inc. (USA) no longer supplies the biphasic TK MEDIUM® SLC for use in its MYCOLOR TK® automated culture system. The company now offers the TK MEDIUM® SLC-L, a liquid media that is now housed in a plastic tube to limit risks of mishandling. A consistent issue with the conventional decontamination method of sodium hydroxide and N-acetyl cysteine (NaOH-NALC) is the risk of incomplete decontamination or overexposure to reagents killing the MTB cells. The NaOH-NALC method also requires centrifugation that can limit processivity rate and incorrect use of this can result in infection risks via aerolisation. The Decomics® kit (Figure 9) uses absorbent beads and reagents to liquefy, decontaminate and neutralize samples in less than 25 minutes without requiring a centrifuge or NaOH-NALC.

Figure 9. Decomics® kit from Salubris Inc.: individual components including sample cup, decontamination solution, beads and neutralization solution



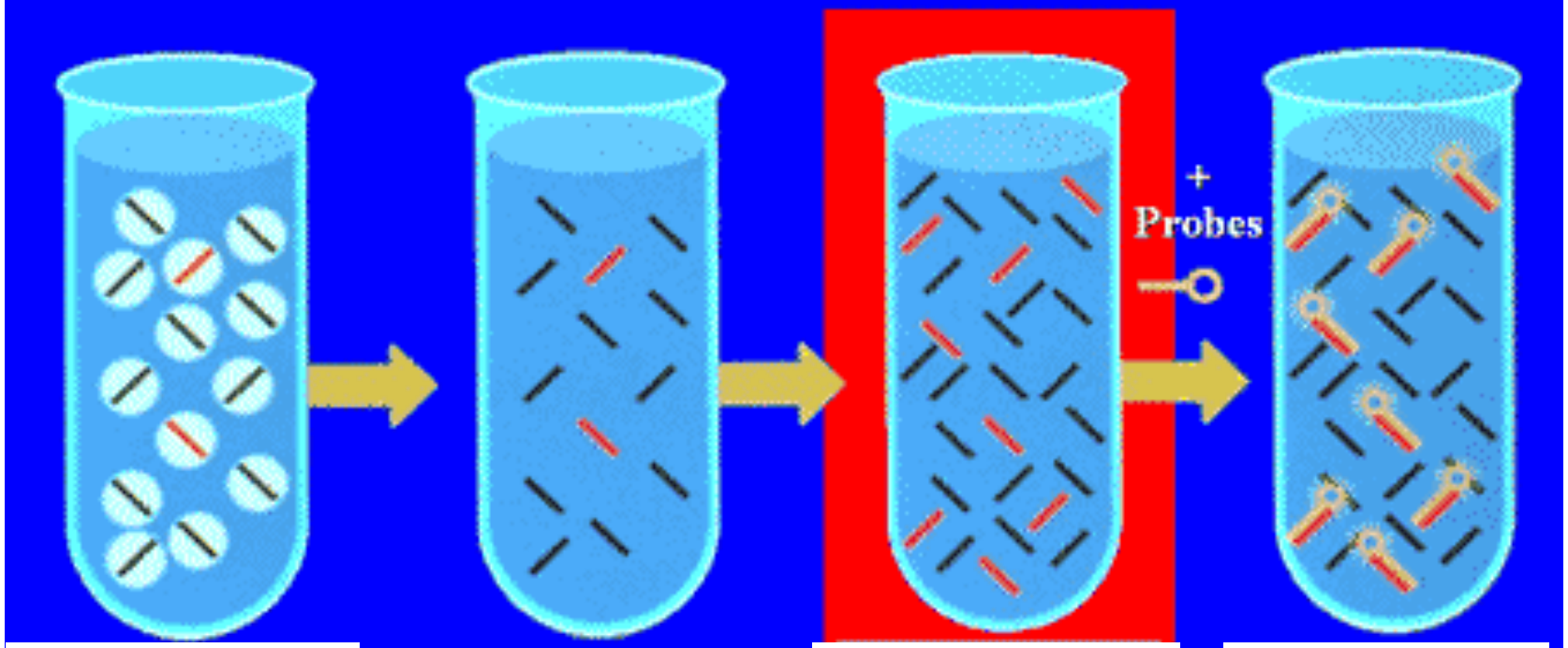
Source: Image reproduced with permission from Salubris Inc.

A recent evaluation of the TK MEDIUM® SLC-L and Decomics® beads was performed in India with 500 specimens with comparison to NaOH-NALC processing and L-J culture. The authors noted an improved performance of MTB diagnosis from the liquid media versus L-J (130 MTB vs 110 MTB) and with an earlier median time to detection (12 days vs 30). Rates of contaminated culture were reduced from 7% observed with the NaOH-NALC method to only 2% with Decomics®. This kit also reduced the median time for specimen processing to approximately 23 minutes as compared to NaOH-NALC, which took 45 minutes. The Decomics® product is CE-IVD marked and US FDA (510k) cleared. Current pricing of this product is unknown.

Moleküler Tanı

- Duyarlılığı yüksek, yalancı pozitif sonuç verebilir
- İyi standardize edilmezse yüksek oranda yalancı negatif sonuç elde edilebilir
- Canlı ve ölü bakterileri birbirinden ayırt edemez
- Sonuçlar diğer tanı yöntemlerinden elde edilenler ile birlikte değerlendirilmelidir
- Pahalı, karmaşık cihazlar ve özel eğitimli çalışanlar gerektirmektedir
- Hızlı tür saptama ve ilaç duyarlılığı belirlemede çok başarılıdır.

Nükleik Asit Çoğaltma ile Enfeksiyon Etkenlerinin Saptanması



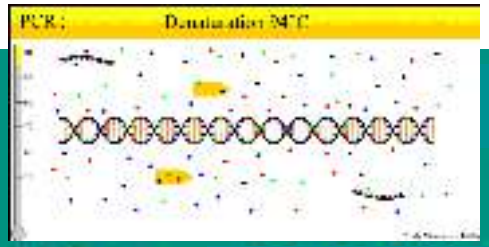
Örnekteki hücreler

DNA eldesi

DNA çoğaltma

Ürün saptama

Polimeraz Zincirleme Tepkimesi



(Polymerase Chain Reaction –PCR-)

Kalıp DNA

95°C zincirlerin ayrılması

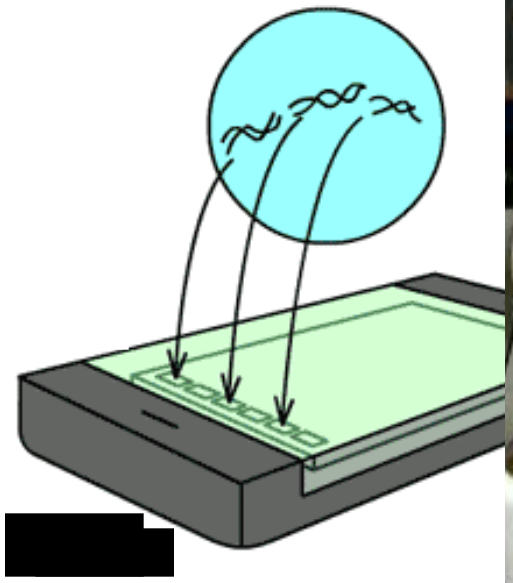
50-70°C

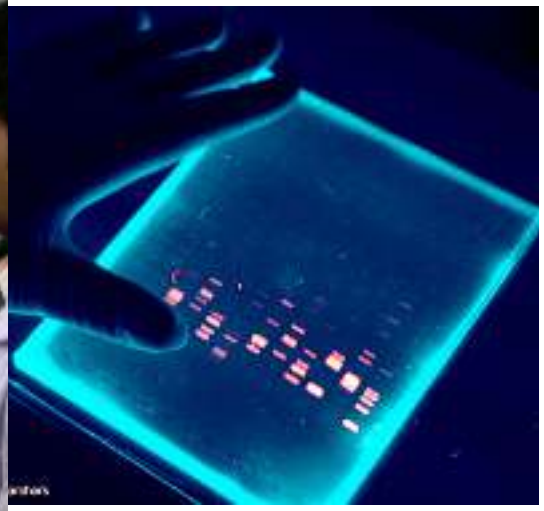
primerlerin birleşmesi

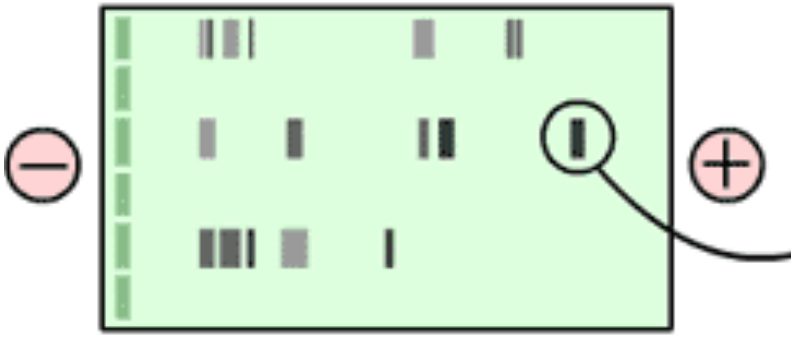
72°C DNA yapımı

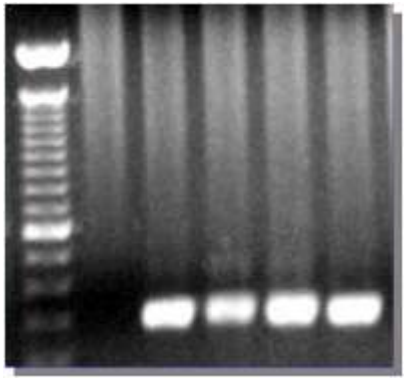
Ürünler

Elektroforez





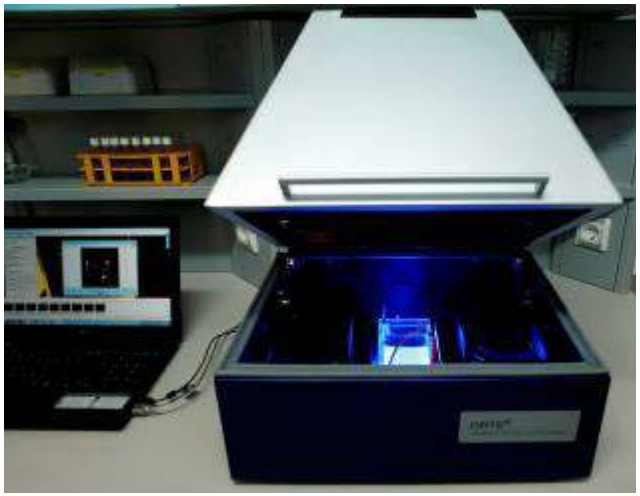


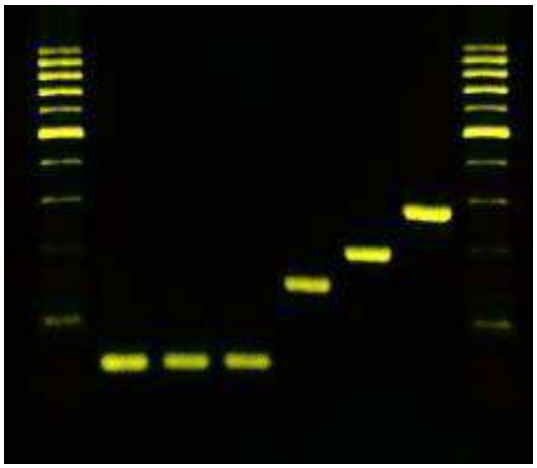


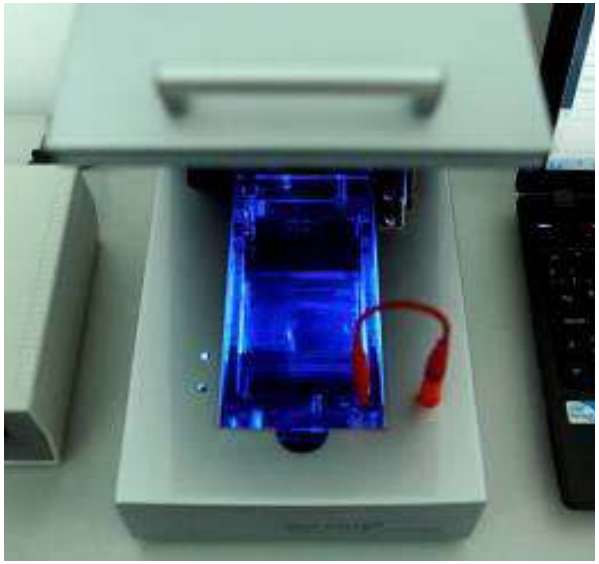
İzlenebilir Elektroforez ORTE

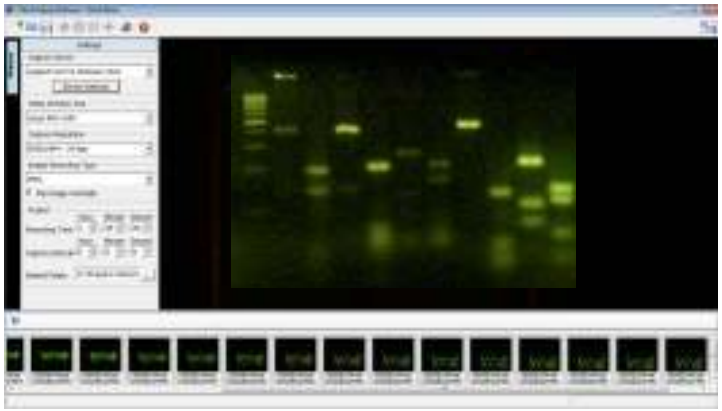
Observable Real Time Electrophoresis

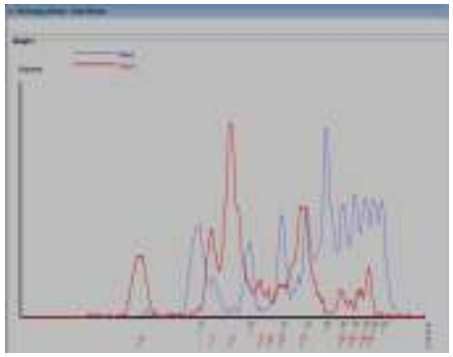
Observable Real Time Electrophoresis



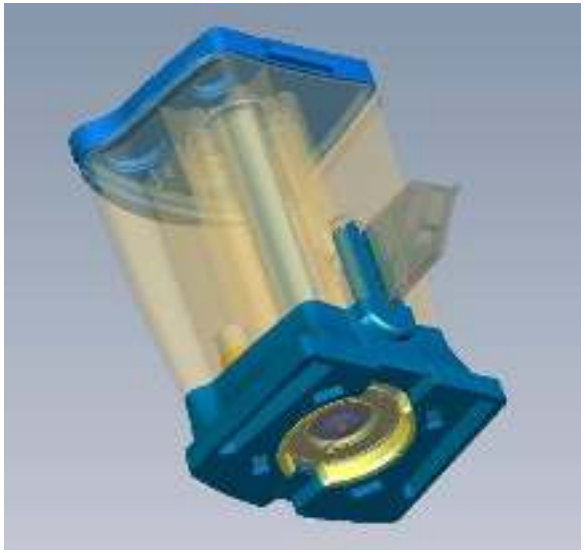








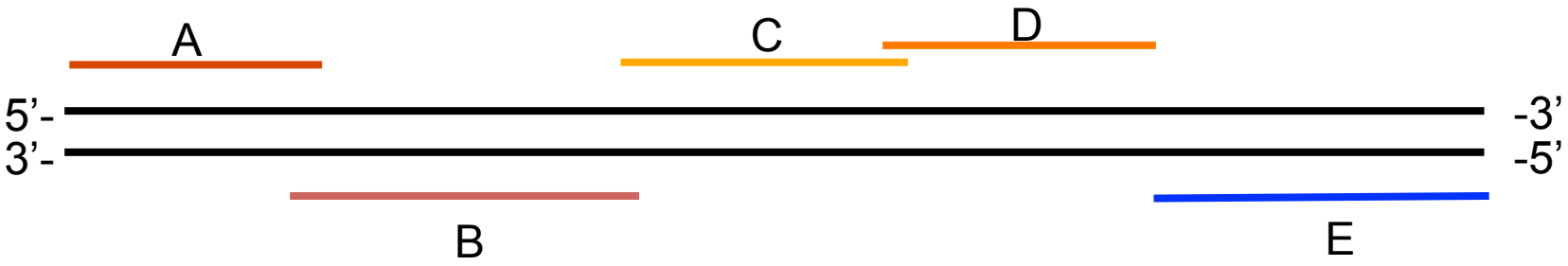
Gene Xpert

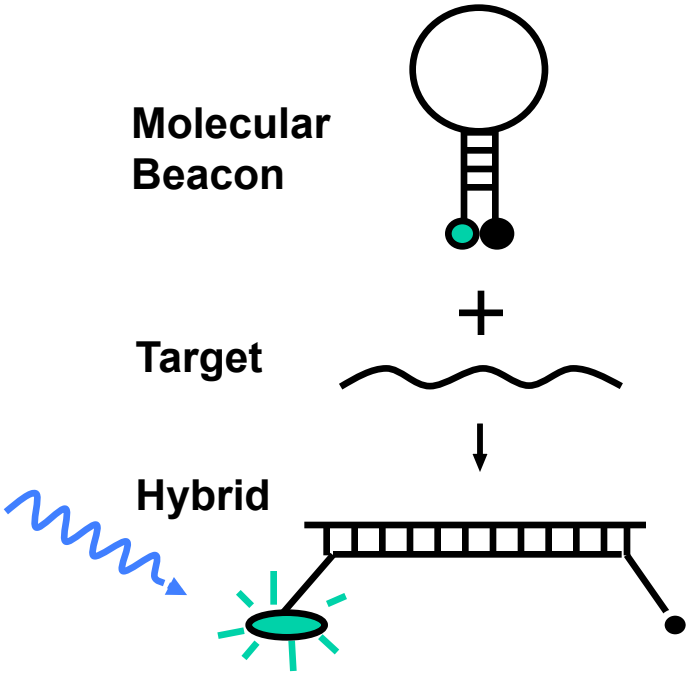


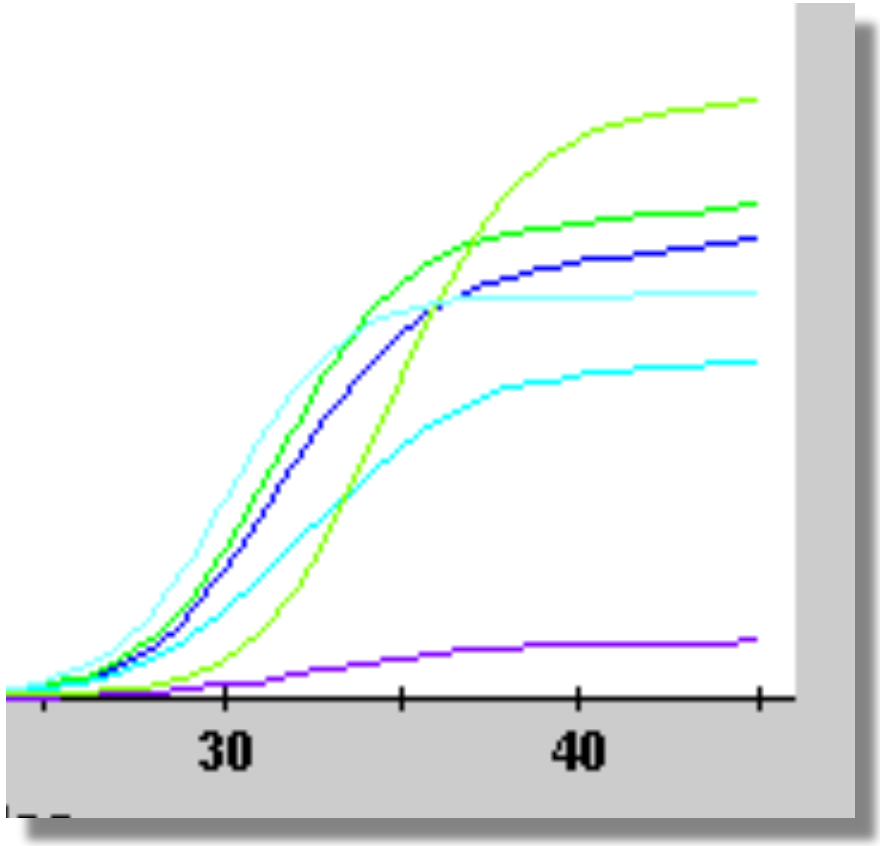
- *M. tuberculosis* ve Rifampisin direnci

Test Hazırlama

Sürüntü çubuğu elüsyon sıvısına konup tutacak kısmı kırılır
Kapak kapatılıp vorteksle çalkalanır.
Kartuşun örnek kısmına dökülür.
Aygıta konup test işlemi başlatılır.

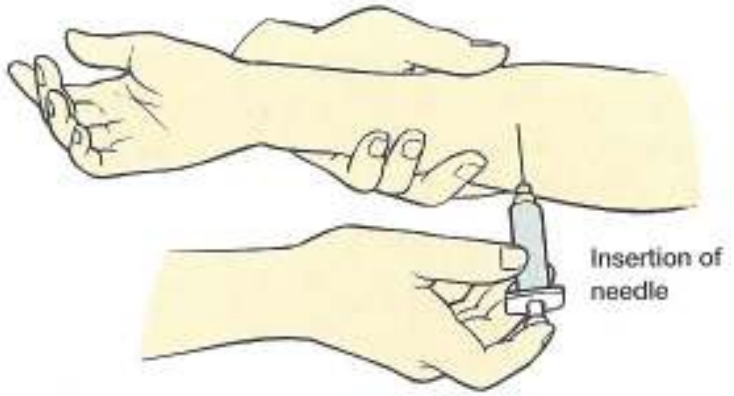

A
C
D
5'-
3'-
-3'
-5'
B
E

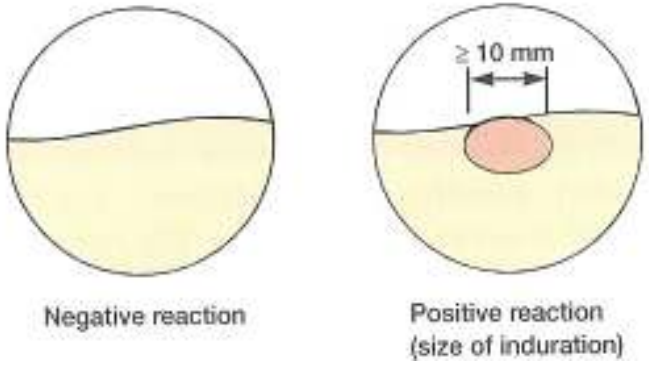

Molecular
Beacon
Target
Hybrid


30
40

Tüberkülin Deri Testi

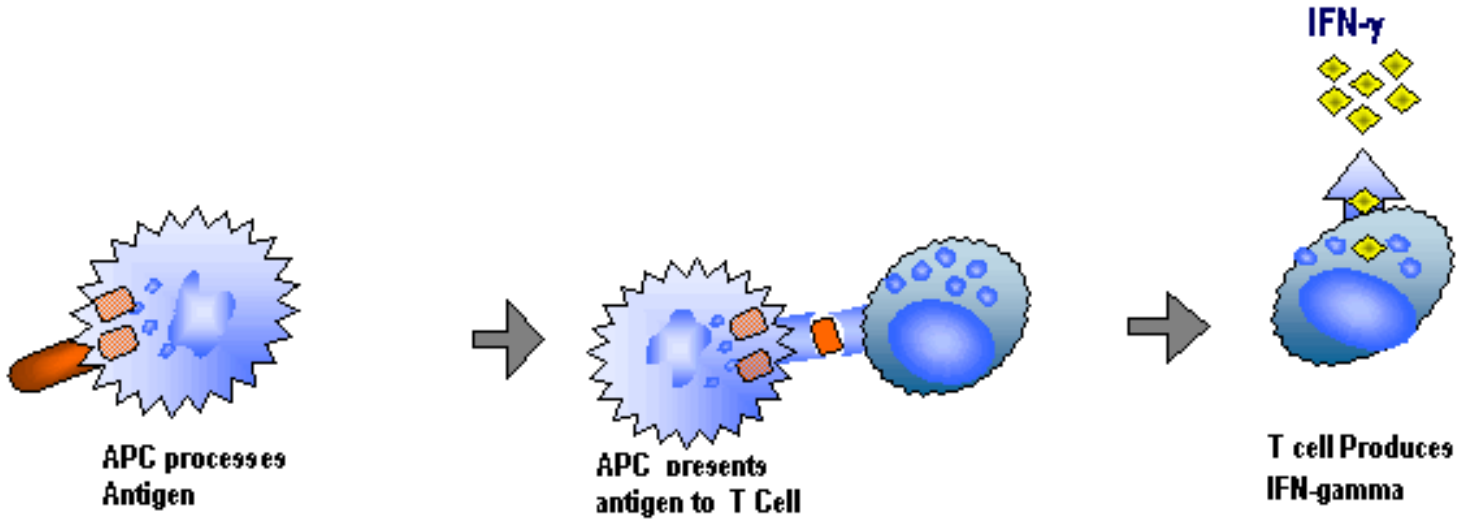
PPD (Purified Protein Derivatives)





Interferon Gamma Salınım Deneyi

Tüberküloz antijenleri ile uyarılan T-lenfositlerden gama interferon yapımı ELISA ile belirleniyor



Tüberküloza özgü antijenlerle deri testi?

Kaynakları kısıtlı yörelerde tüberküloz tanısı

Tanzanya'da gezici TB saptama

Uganda'da güneş enerjisi ile çalışan sistem

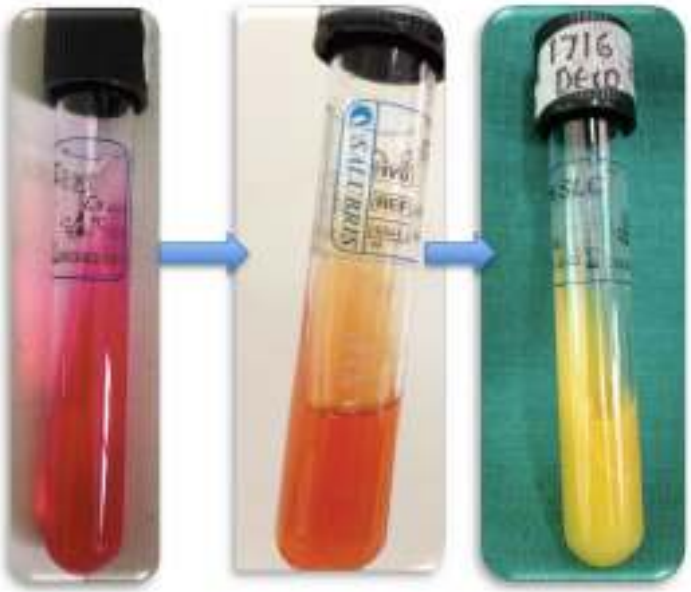
Güney Afrika'da Infinity-48 sistemi kuruldu

Kaynakları kısıtlı ortamlarda tüberküloz tanısı

DECOMICS

TK SLC

TK ANTI TB PNB KIT



Color Change to Yellow (MYCOBACTERIAL GROWTH)

1	2	3	4	5	6
No Growth		Possible Growth or Contamination	Definite Growth*		

Color change due to mycobacterial growth (to yellow) is usually observed between 3 days and 3 weeks depending on the type and amount of mycobacteria present in the inoculated sample.

Color Change to Green (CONTAMINATION)

1	2	3	7	8
No Growth		Possible Growth or Contamination	Definite Contamination*	

Rapid Mycobacterial Culture in Resource Limited Settings by Decomics and TK SLC Medium

Shivya Jamwal
MMIMSR, Department of Microbiology, Mullana, Ambala, India

- 500 balgam örneği Decomics ve Kubica yöntemleri ile işlenip LJ ve TK SLC besiyerlerine ekildi
- Decomics ve TK SLC ile 130, Kubica ve LJ ile 110 M. tuberculosis izole edildi
- TK SLC ile Mtb üretilen örneklerin %77'sinde mikroskop ile aside dirençli basil görüldü.
- Ekilen tüm örneklerde TK SLC'de kontaminasyon %2, LJ'de %7 oranındaydı.
- Mtb üreme süresi ortalama TK SLC'de 12 tün, LJ'de 30 gün idi.

Rapid mycobacterial culture in resource-limited settings by Decomics and TK-SLC medium

Dr Shivya Thakur, Tanil Kocagöz, Dr. Varsha A Singh, Dr. Tanya Sharma, Dr kanishtha Sharma, Dr. Bhawna Sharma, Dr Payal Dutta, Dr. Harman multani, Dr. Rumana ifarooq , Dr. Gomty mahajan, Dr. shaveta dhiman

Introduction

Tuberculosis remains the single infectious disease causing the highest mortality in humans, scavenging both developing as well as developed countries. In India, TB kills two people every three minutes - nearly 1,000 every day.

Gold Standard method used for years for the diagnosis of pulmonary tuberculosis is the traditional **culture** of *M. tuberculosis* on conventional, internationally accepted solid media like Löwenstein-Jensen. Present study used culture as the confirmatory tool and assessed the efficiency of a new ready to use, rapid, biphasic culture medium **"TK-SLC medium® "** in rapid detection and isolation of *Mycobacterium tuberculosis* in comparison to the conventional LJ Medium.

Decontamination is the crucial preliminary step before any culture media is inoculated. All current decontamination and concentration methods, like Petroff and Kubica (NaOH-NALC), require centrifugation and also a lot of time and effort. These limit their application to laboratories with elaborate capabilities. Present study used **"Decomics®"** kit based on absorbent beads, which does not require centrifugation and is easy to use and swift.

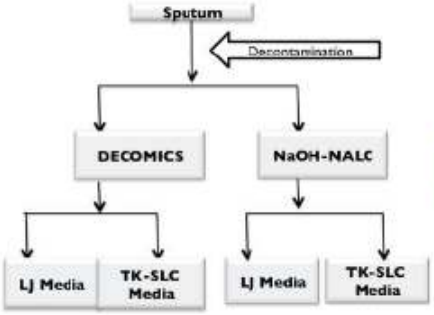


Figure 1: Summary of the application

Figure 2: Decontamination concentration by Decomics

Material and Methods

A prospective study was done in **MMIMSR, Ambala, India** on clinically suspected cases of pulmonary tuberculosis. 500 sputum samples of at least 2ml, were split into two equal parts and processed by NaOH-NALC method and Decomics (figure 2).

Decomics consists of three components:

1- Sample cup containing decontamination solution with pH indicator;

2- Absorbent beads;

3- Neutralization solution

- Absorbent beads, which have pores smaller than bacteria, absorb most of the solutions and leave 2-3ml concentrated sample behind (figure 2).
- Processed samples, were inoculated to Löwenstein Jensen (LJ) and TK SLC (rapid mycobacterial culture medium). Both LJ tubes and TK-SLC tubes were followed visually on weekly and daily basis respectively.
- Color changes in TK-SLC tubes were interpreted according to color chart provided

Table 1: Rate of smear positivity before and after Decontamination by NaOH-NALC and Decomics

Total n=500	Direct ZN Smear	After Decontamination	
		NaOH-NALC	Decomics
Positivity	90(18%)	100 (20%)	106 (21.2%)

Figure 3: Color change in TK Media by mycobacterial growth and contamination

TK Medium that enables early detection of mycobacterial growth by changing its color .TK Medium includes egg and additional nutrients like glutamic acid and iron. To lower the rate of contamination, TK Medium is supplemented with selective antimicrobials, polymyxin B, piperacillin, amphotericin B, nalidixic acid, and trimethoprim, to produce ready to use **TK- SLC Medium®.**

Mycobacterial growth causes its original red color to turn yellow. The color change occurs even before the colonies have grown sufficiently to become visible on the solid part of the medium. TK-SLC Medium® has the advantage of differentiating mycobacterial growth from the growth of most common contaminants like fungi and Gram-negative bacilli because the growth of those contaminants causes the medium to change to green instead of yellow (figure 3).

Results

- Among the 500 clinically suspected cases of pulmonary tuberculosis, *M. tuberculosis* was isolated from 130 (26%) specimens.
- After decontamination and concentration there was an increase in the total **ZN** smear positivity from **18%** to **20%** and **21.2%** with NaOH-NALC and Decomics respectively (table 1).
- There was a significant increase of 2.4% and 3.9% positivity in ZN smear negative samples after decontamination with NaOH-NALC and **Decomics®** respectively.
- Decomics® Kit required less time i.e. around 20-25 minutes as compared to NaOH-NALC method, which takes around 40-45 minutes.
- Among 500 specimens, *M. tuberculosis* was isolated from 130 sputum samples by TK-SLC Medium® and 110 by LJ which referred to 84% of the samples positive by TK-SLC. (All samples positive by LJ were also positive by TK-SLC).
- Average time to detection (TTD) was 12 days by TK-SLC Medium® and 30 days by LJ medium.
- In culture after **Decomics®** in **TK-SLC Medium®**, the minimum TTD in 3+ grade specimens was 5 days and maximum 17 days for scanty samples.
- Rate of Contamination was **7%** and **2%** on LJ and TK-SLC Medium® respectively, among all 500 specimens.

n=500	L J Medium	TK-SLC Medium
Mycobacterial isolates	110	130
Average time to detection in days	30	12
Contamination Rate (%)	35 (7%)	10 (2%)

Table 2: Mycobacterial growth and contamination rates in LJ and TK-SLC Media

Conclusion

"Decomics ®" was a better decontamination and concentration method in enabling isolation of mycobacteria, than classical and widely used NaOH-NALC method. Elimination of need for centrifugation, decreased processing time by half and higher isolation rate of mycobacteria were important advantages of Decomics.

TK-SLC medium® was superior to the conventional LJ medium in being rapid, easy to use and interpret, significantly low time-to-growth detection, and lower contamination rate. It is indeed a boon for resource limited settings for effective and early diagnosis and treatment of tuberculosis.

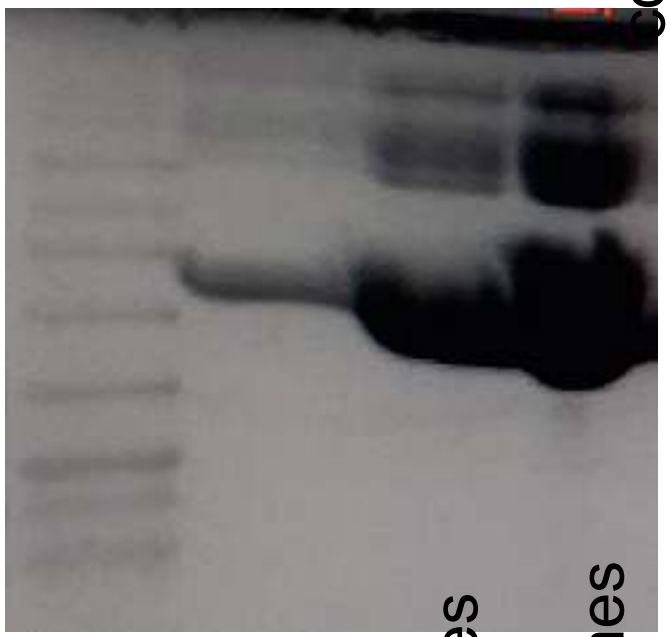
Balgam yoksa tüberküloz tanısı nasıl konur?

- Akciğer tüberkülozu erken dönem
- Çocuk hastalar
- Akciğer dışı tüberküloz olguları

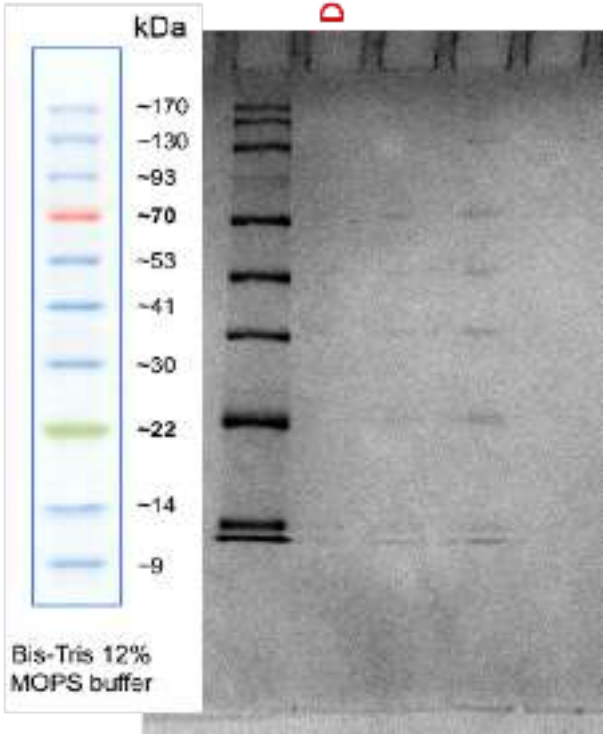
MyMagiCon

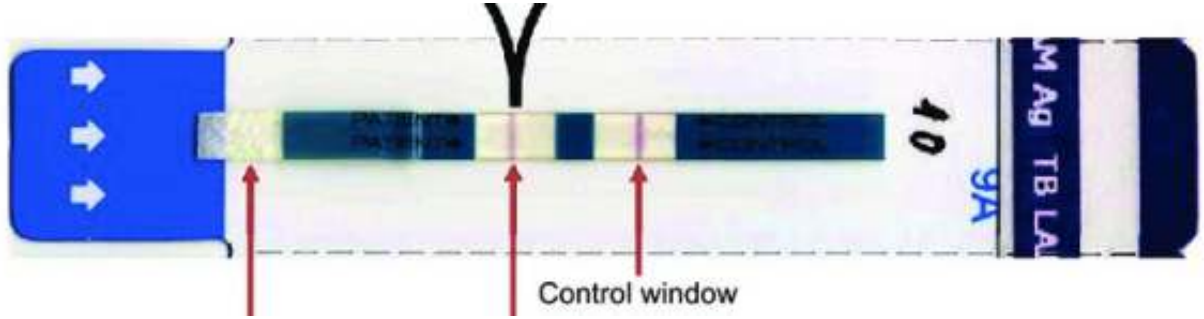
INCUBATION Center

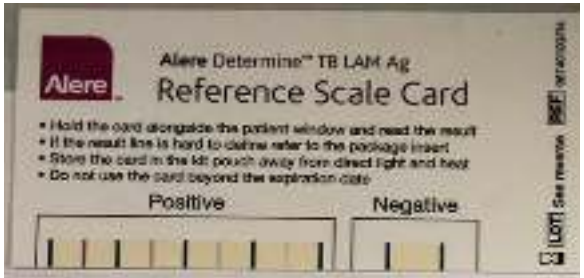
Molecule Concentrator − Gigabiomol -



Urinary Tb LAM test - MyMagiCon







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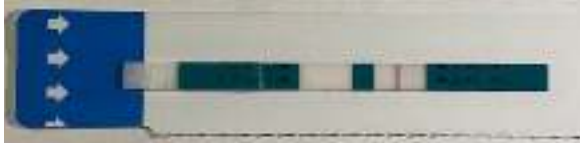

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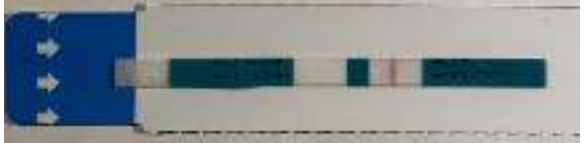

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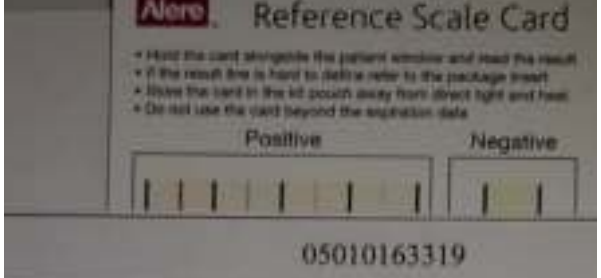

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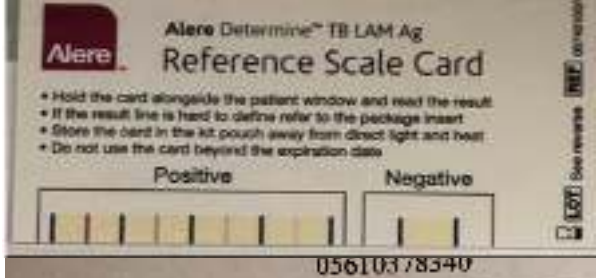

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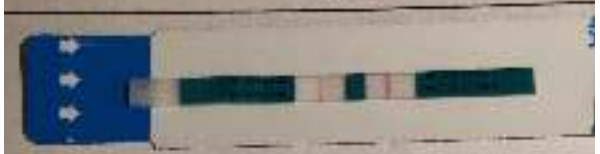

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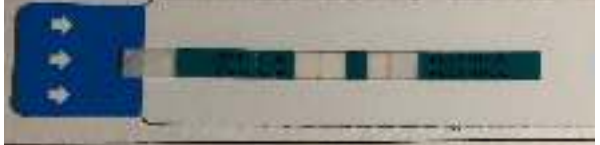

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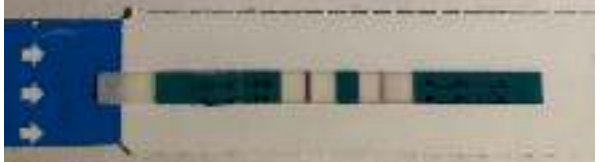
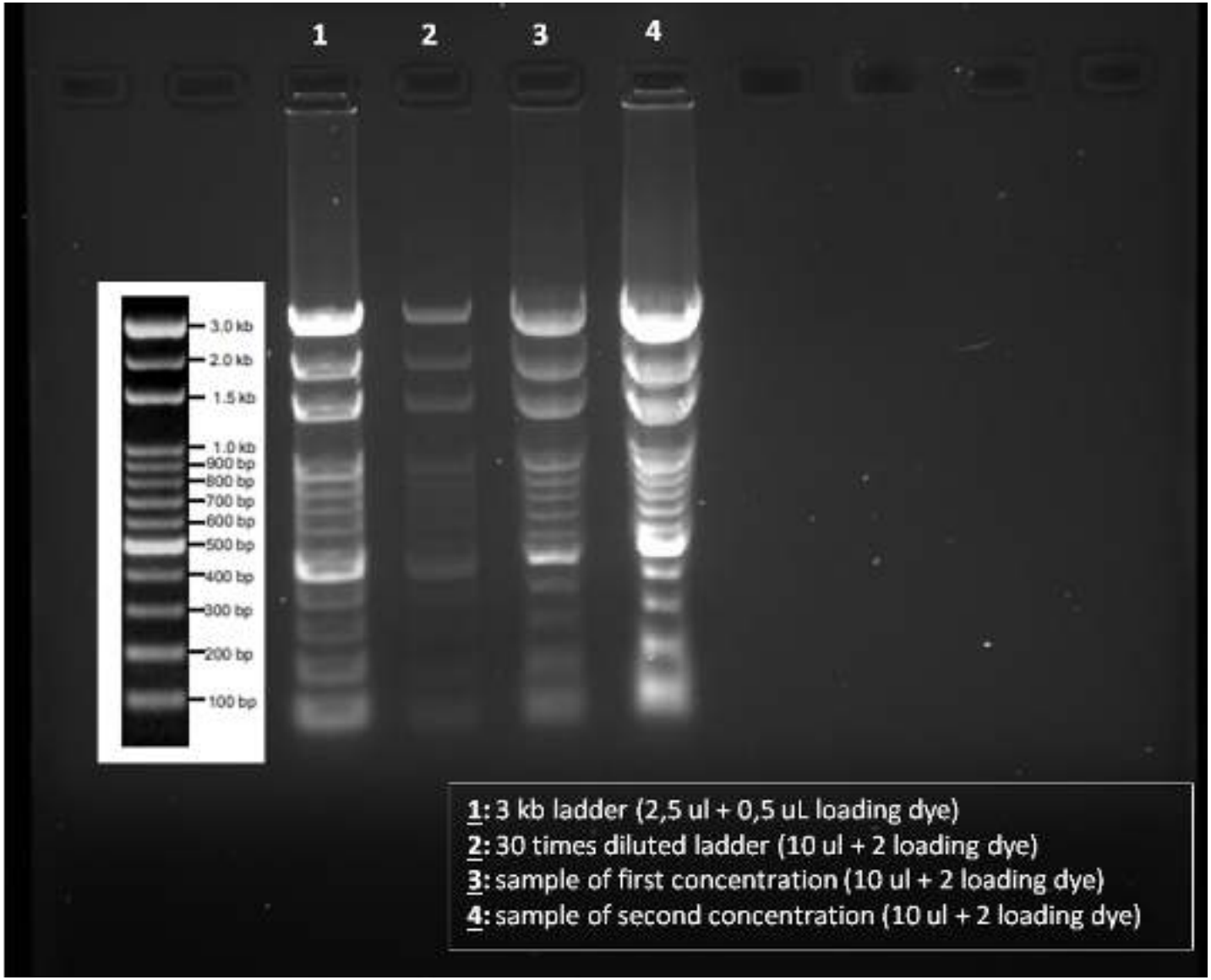


Concentration of DNA molecules by MyMagiCon



İDRAR ÖRNEĞİ VERMENİZ TANI İÇİN YETERLİ

TEŞEKKÜRLER

Teşekkürler