

3° CONGRESSO NEWMICRO
...The need for speed: il
laboratorio di Microbiologia e le
Urgenze Infettive



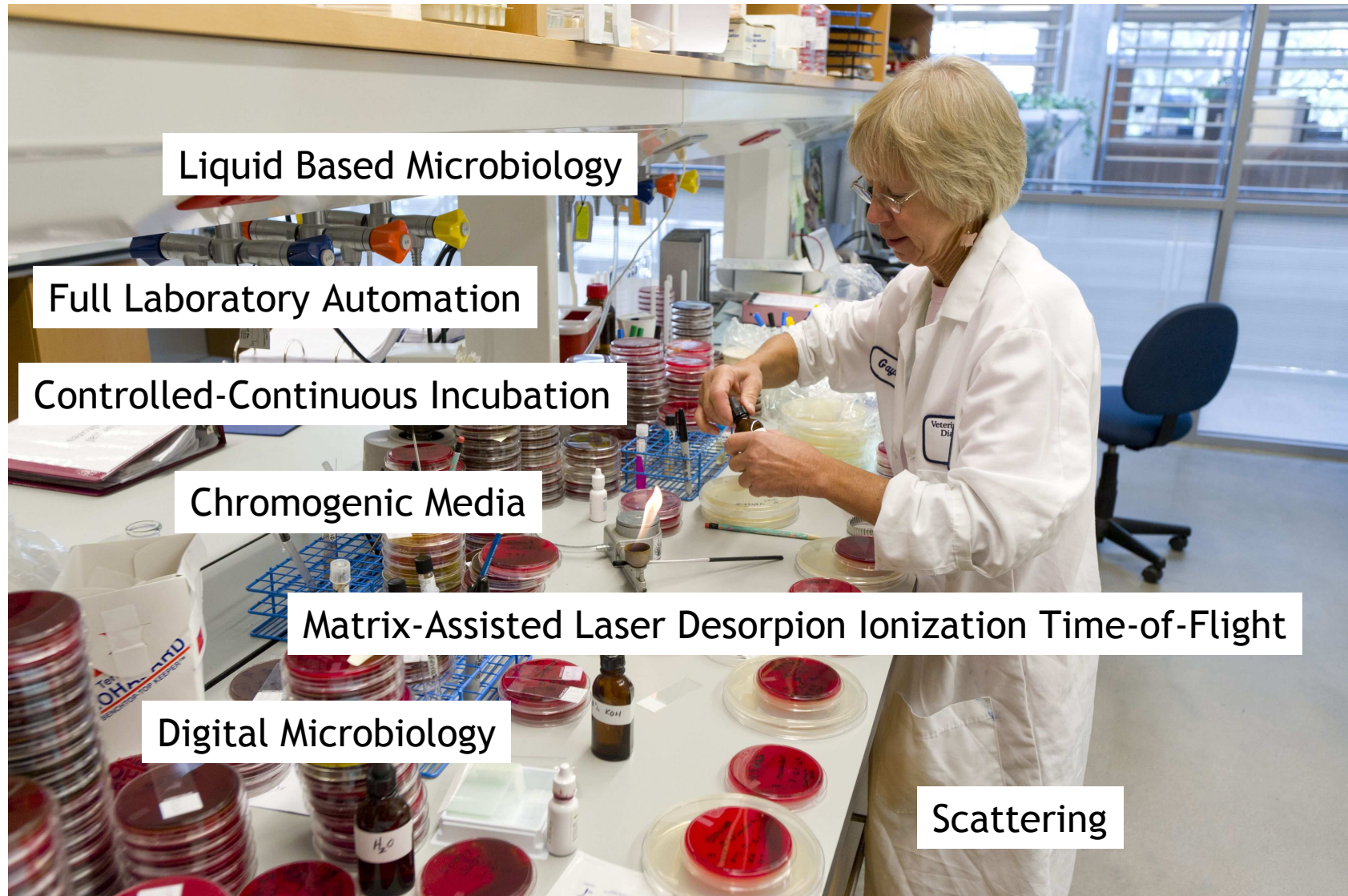
Coltura batterica su piastra, una nuova primavera

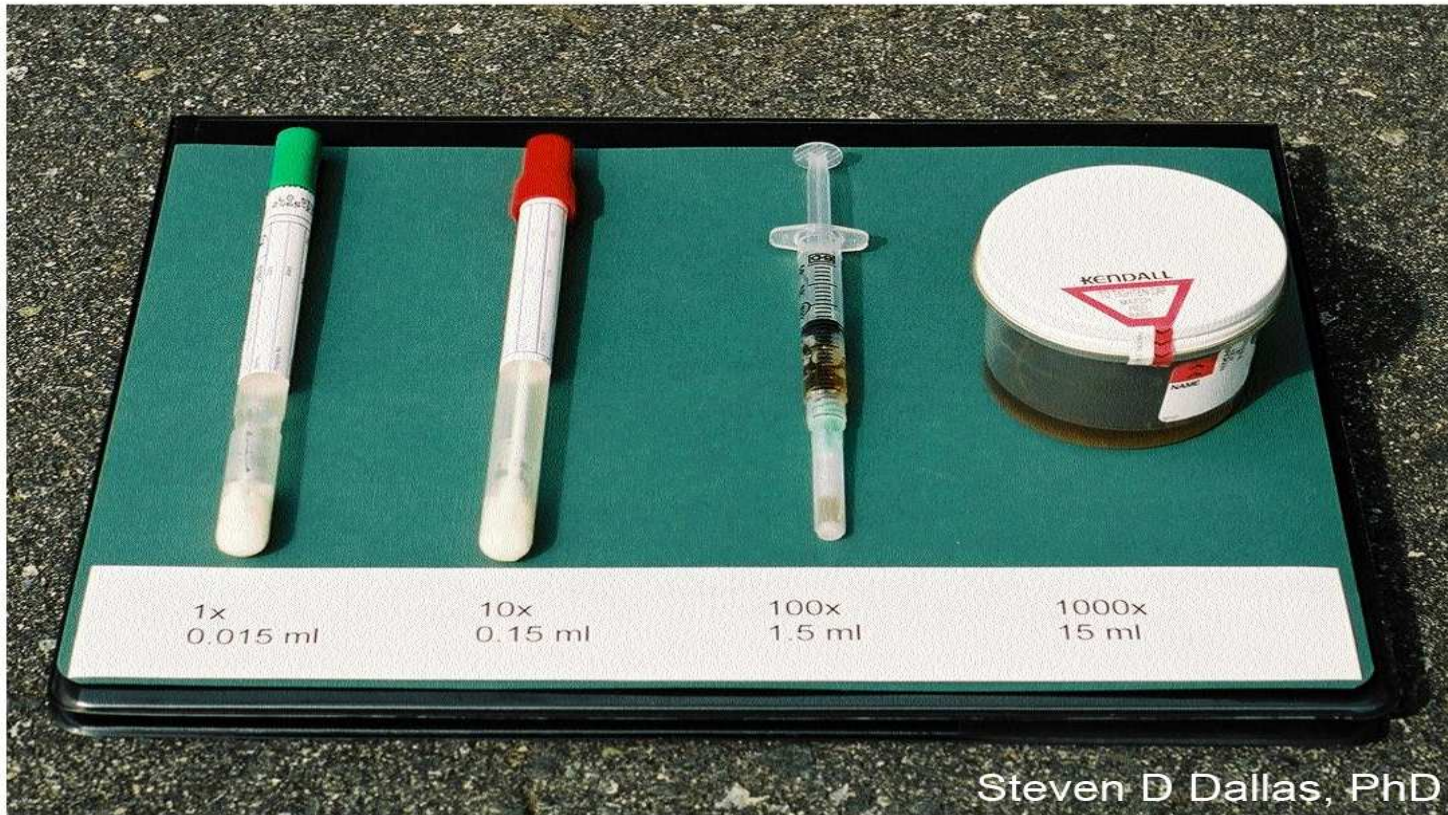
Giovanni P. Gesu
S.C. Microbiologia e Virologia
Ospedale Niguarda Ca' Granda
Milano



Padenghe sul Garda, 21 marzo 2013

“Classic” Bacteriology

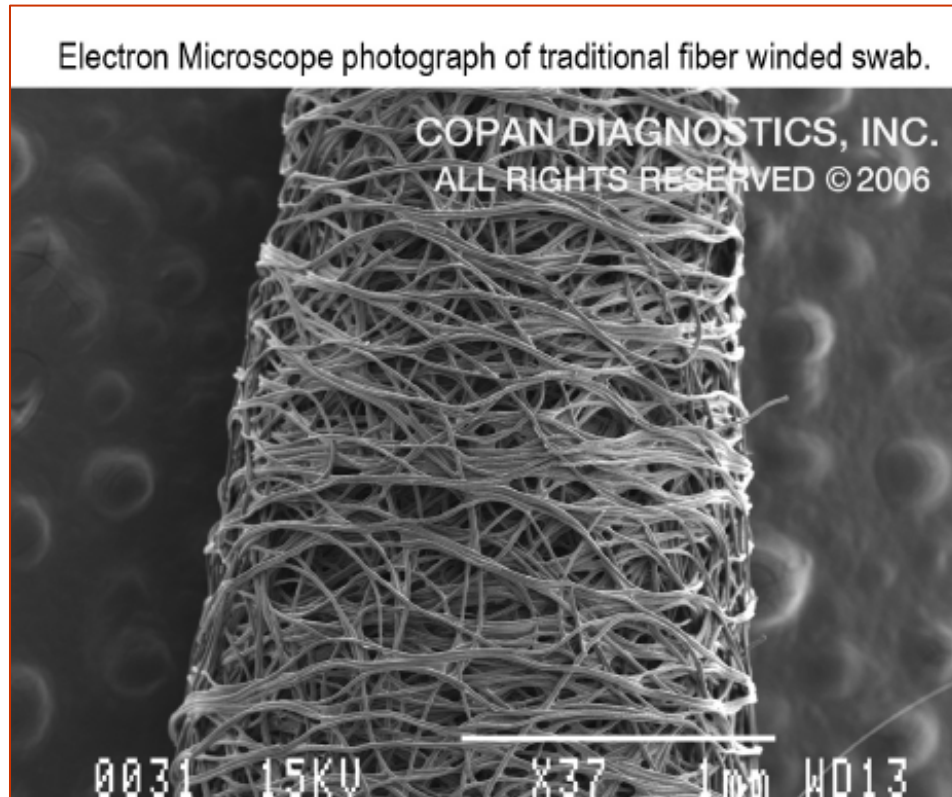




Steven D Dallas, PhD

Struttura dei tamponi

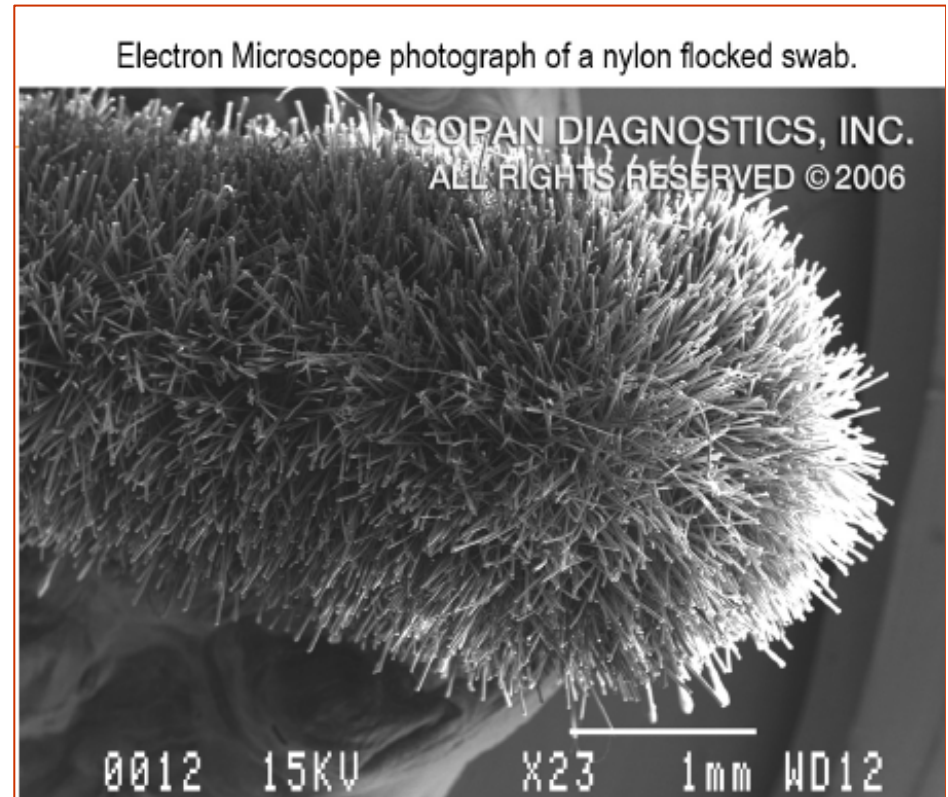
Tampone Standard



Matrice di fibra caotica e irregolare

Fino a 2 km di microfibra

Tampone Floccato



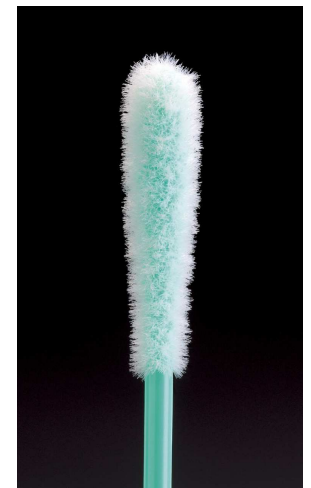
Fibra regolare e incollata in modo
perpendicolare

Meno di 6 m di fibra

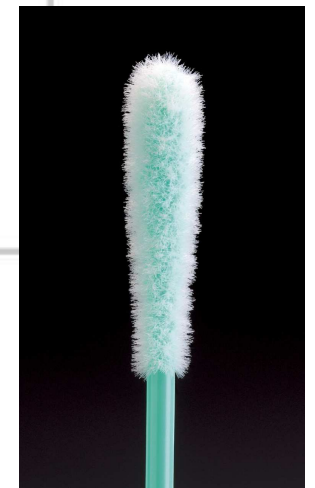
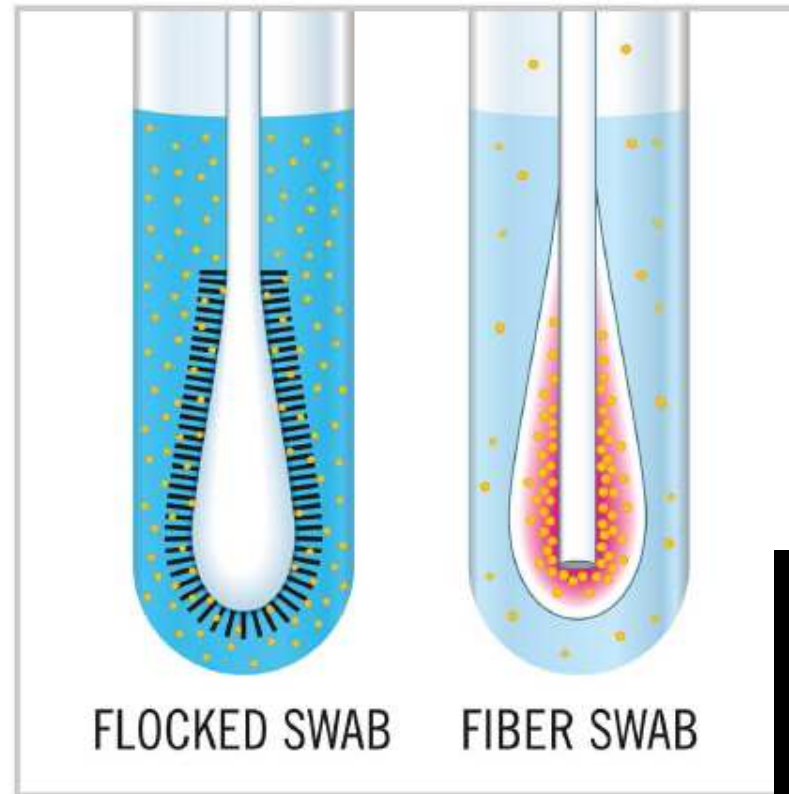
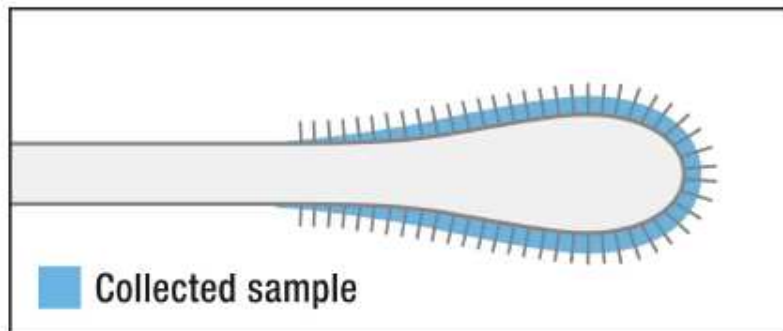
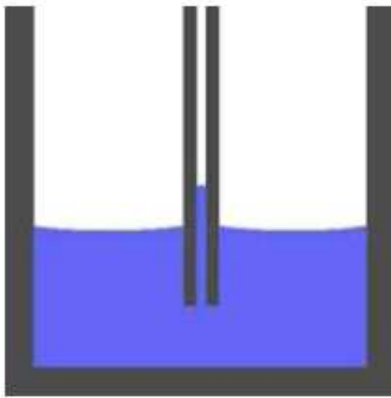
Prelievo → Assorbimento



Fiber	Average absorption*
Cotton	50 μL
Polyester	143 μL
Rayon	149 μL
Nylon Flocked	158 μL



Idrofilicità versus capillarità



Struttura dei tamponi



Liquid-Based Microbiology

- Trasferimento di un campione dal mezzo di prelievo (tampone) al terreno di trasporto (liquido) che diviene a sua volta il campione stesso.
 - Il campione biologico viene prelevato per capillarità (fenomeno indipendente dal tipo di fibra utilizzata)
 - Il campione viene poi rilasciato in fase liquida non appena immerso nel terreno di trasporto
 - Il liquido di trasporto diventa il campione





Urine

Feci



Tamponi



12 x 80 mm

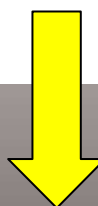
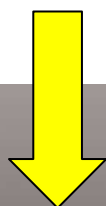
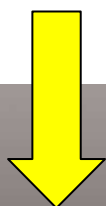
**Unico formato del
contenitore**

Codice Colore per le Provette

Colturali (qualsiasi)

Feci

Urina



Coagulazione

Ematologia
VES

Biochimica
Sierologia

Tutte le provette trasportano i campioni in fase liquida



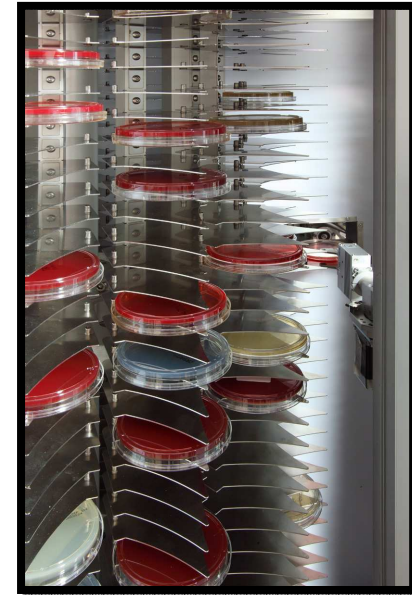
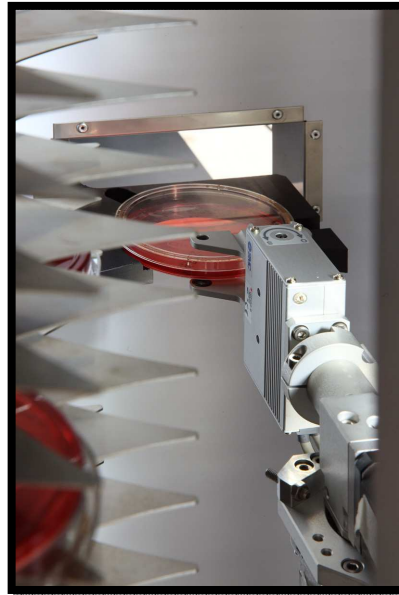
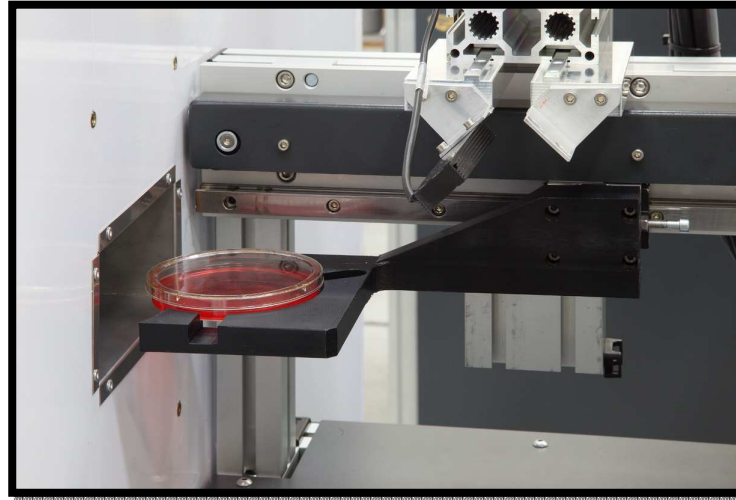
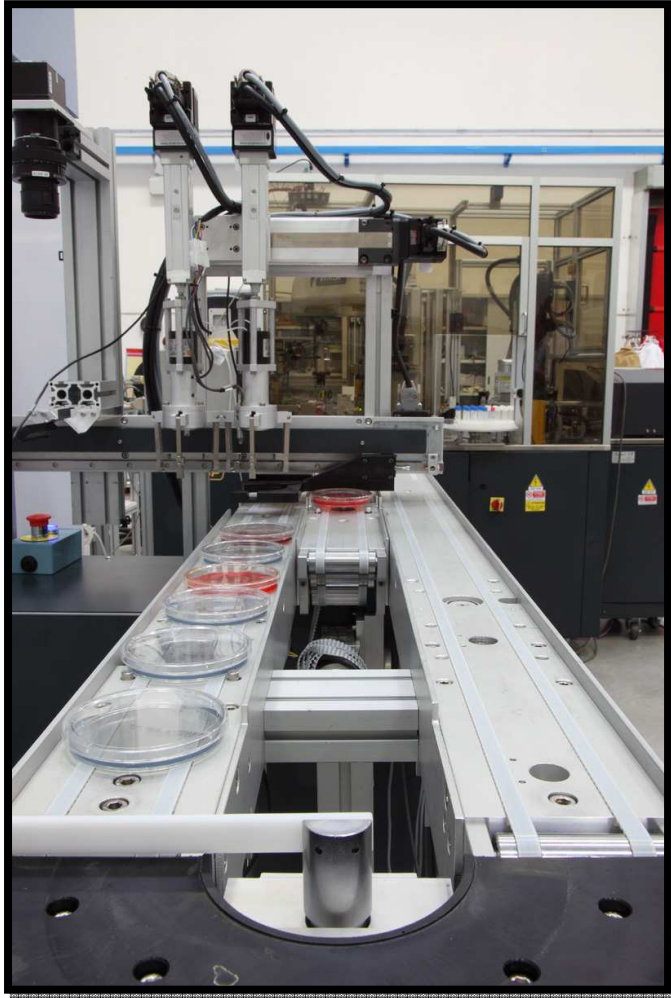
Microbiologia in fase liquida

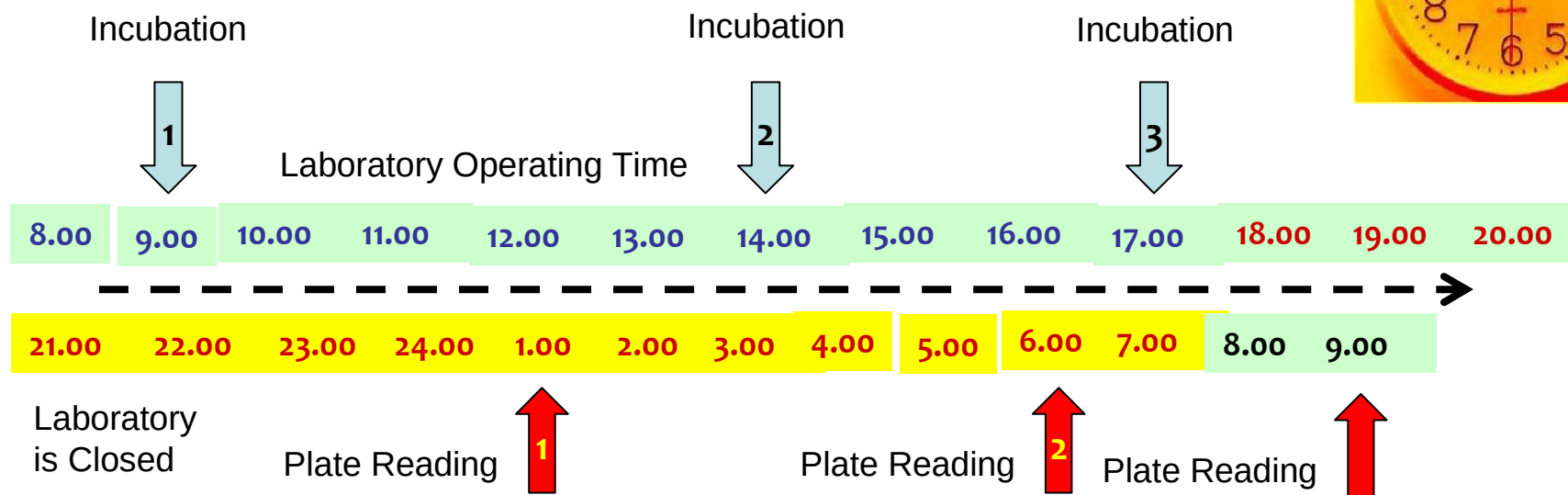
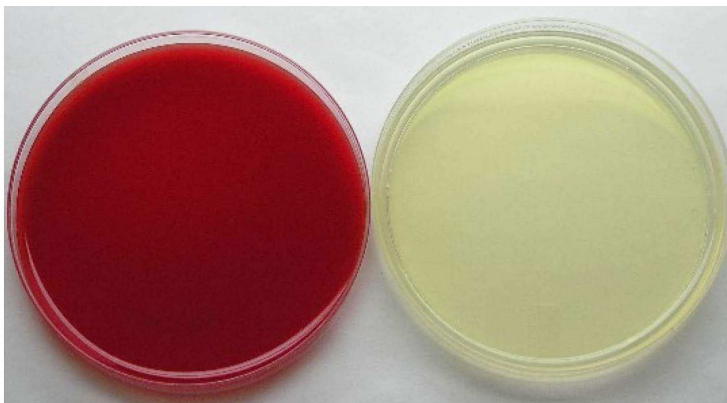
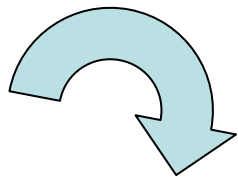
Punti di forza

- Prelievo di volumi adeguati
- Rilascio massimale del volume raccolto
- Facilità di trasporto e stoccaggio
 - volumi di ingombro minimi
- Possibilità di effettuare analisi/metodiche differenti
 - microscopia, coltura, antigeni, test molecolari...
- AUTOMAZIONE...



Robotic Incubation

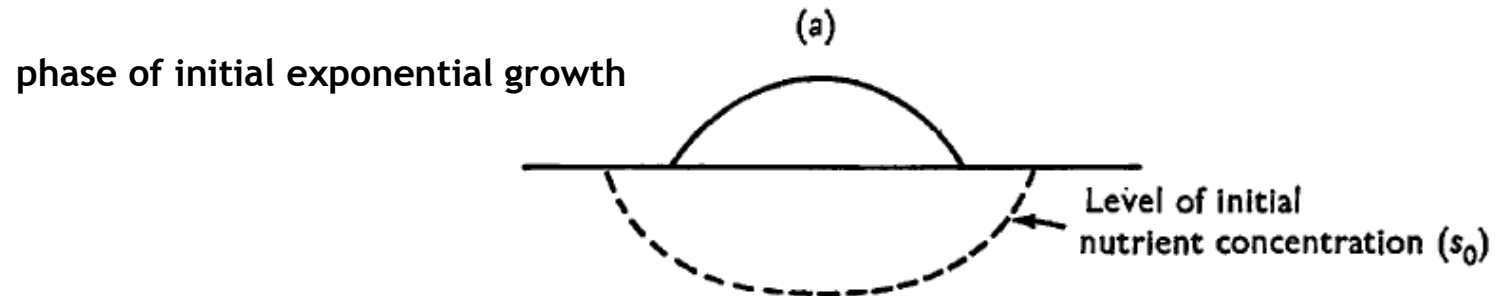




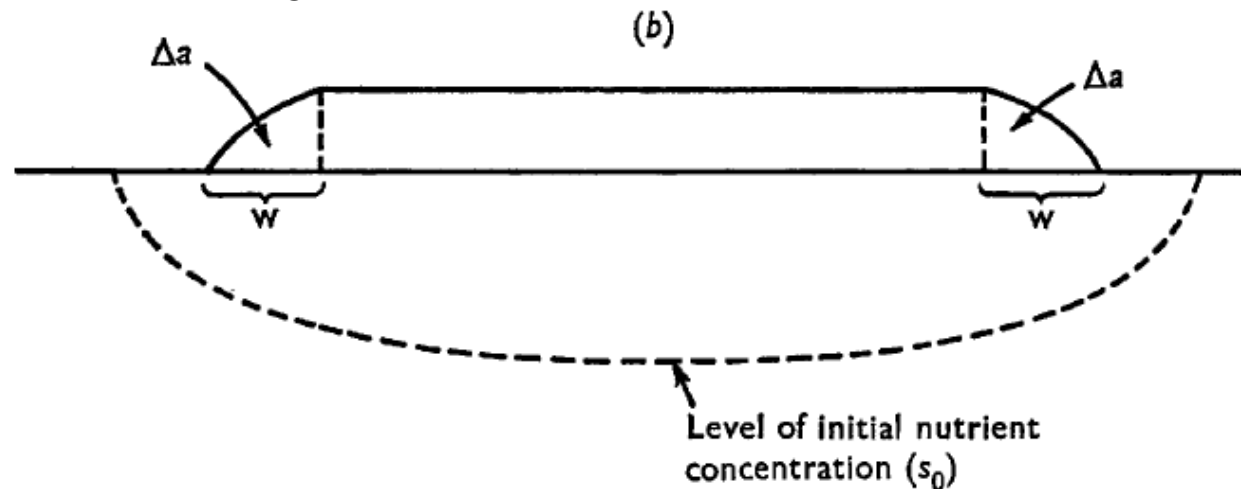
A Kinetic Study of the Mode of Growth of Surface Colonies of Bacteria and Fungi

By S. J. PIRT

Department of Microbiology, Queen Elizabeth College, University of London, Campden Hill, London, W.8



phase of constant radial growth rate



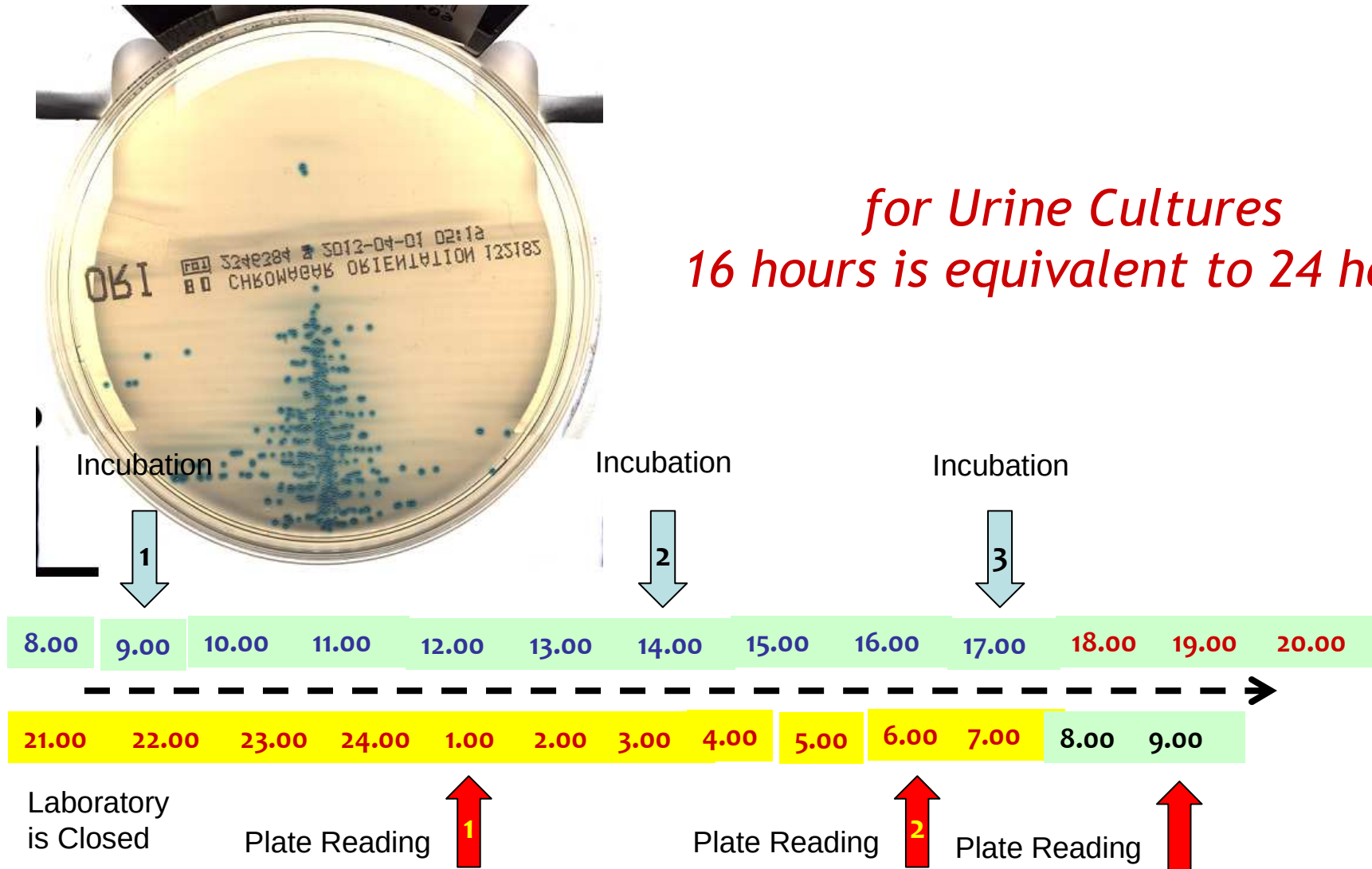
Variables Influencing Colony Development

- initial nutrient concentration depth of agar
- maximum specific growth rate
- oxygen partial pressure
- humidity of gas phase
- temperature

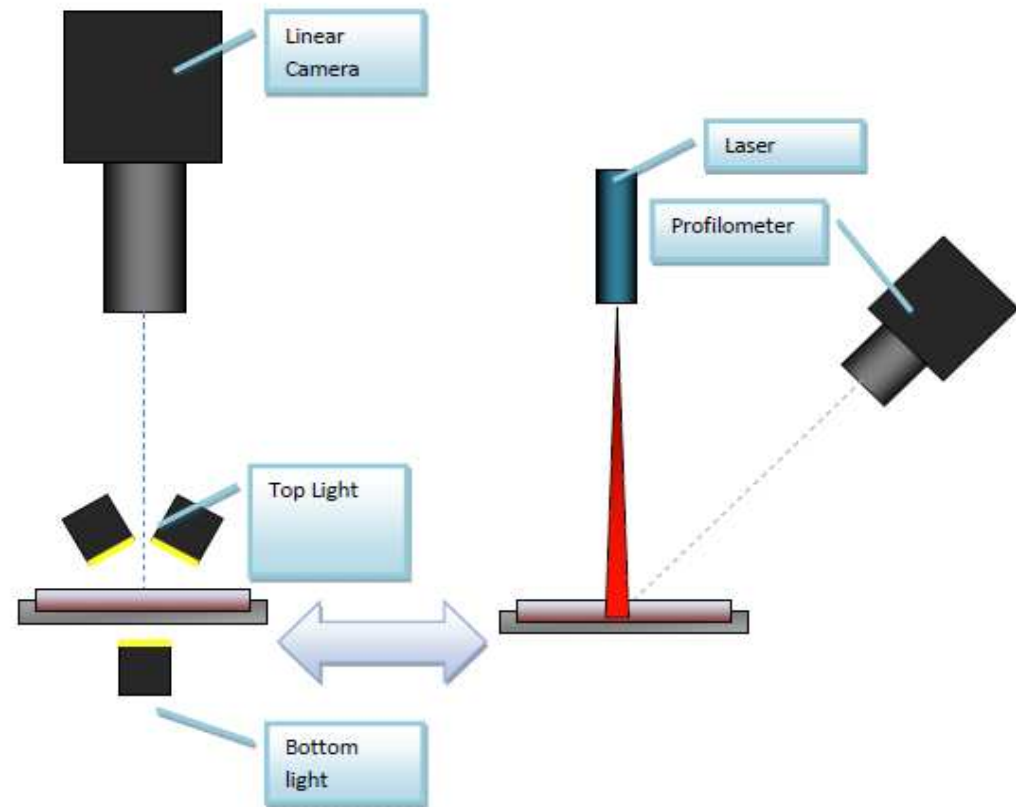
Effect of Temperature on initial colony radial growth rate and on the specific growth rate of three bacteria

Temp.	Maximum specific growth rate (α_m , hr ⁻¹)	Initial colony radial growth rate (K_r , μ /hr)	$K_r/\sqrt{\alpha_m}$	
<i>Escherichia coli</i> (glucose, 1.1 g./l.)				
37°	0.70	20.0	24.9	
30°	0.445	20.4	31.1	
25°	0.282	18.0	33.9	
<i>Klebsiella aerogenes</i> (glucose, 1.0 g./l.)				
37°	0.49	23.3	33.3	
30°	0.72	26.2	30.9	
25°	0.48	28.3	40.8	
<i>Streptococcus faecalis</i> (glucose, 1.0 g./l.)				
37°	1.36	22.8	19.5	
31°	1.05	21.7	20.6	
25°	0.65	17.8	22.1	

Robotic Incubation @ Stable Temperature and Colony Growth Rate



Highly Efficient Image Analysis



3 Images per plate

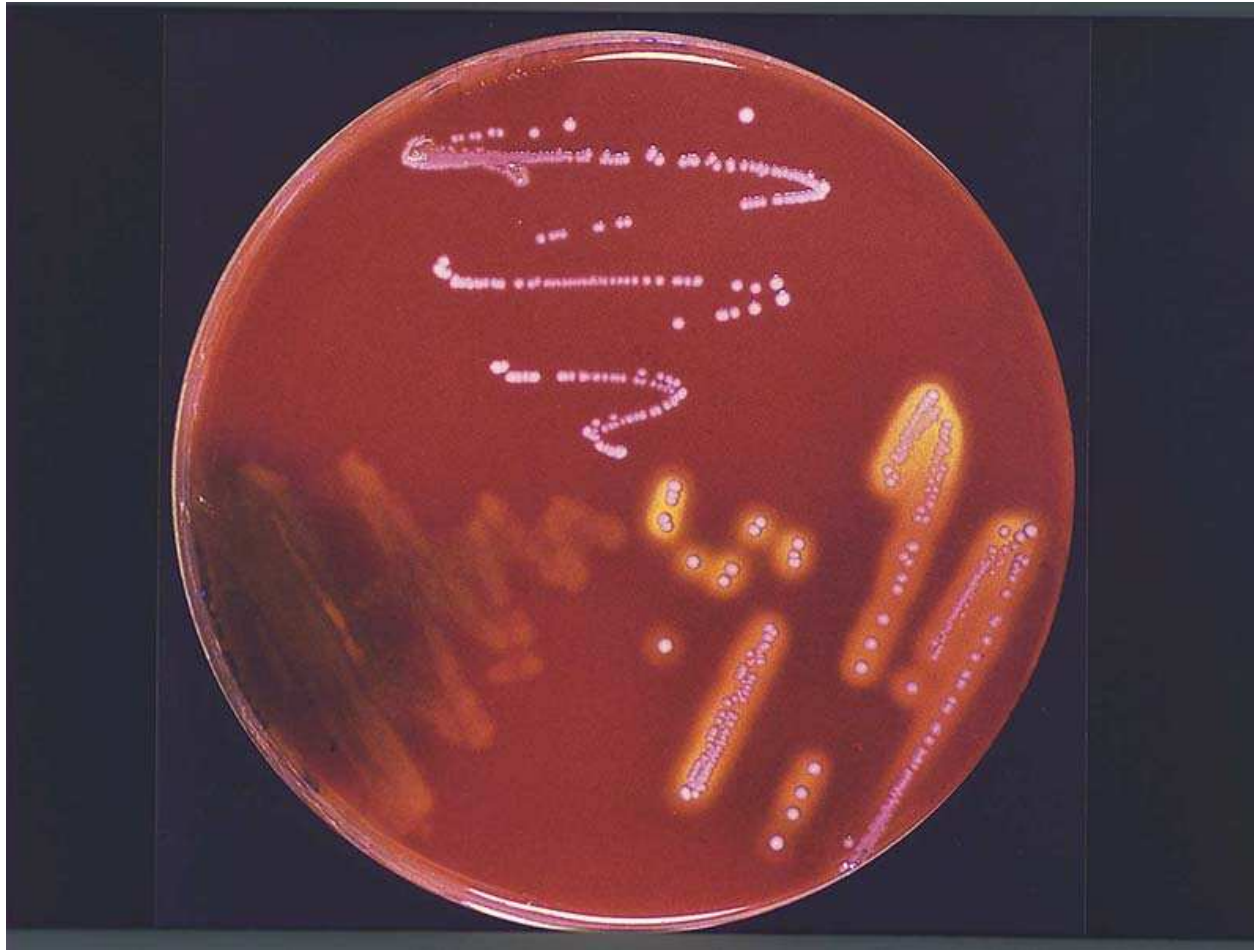
- Top
- Bottom
- Angle

Early identification of bacterial growth

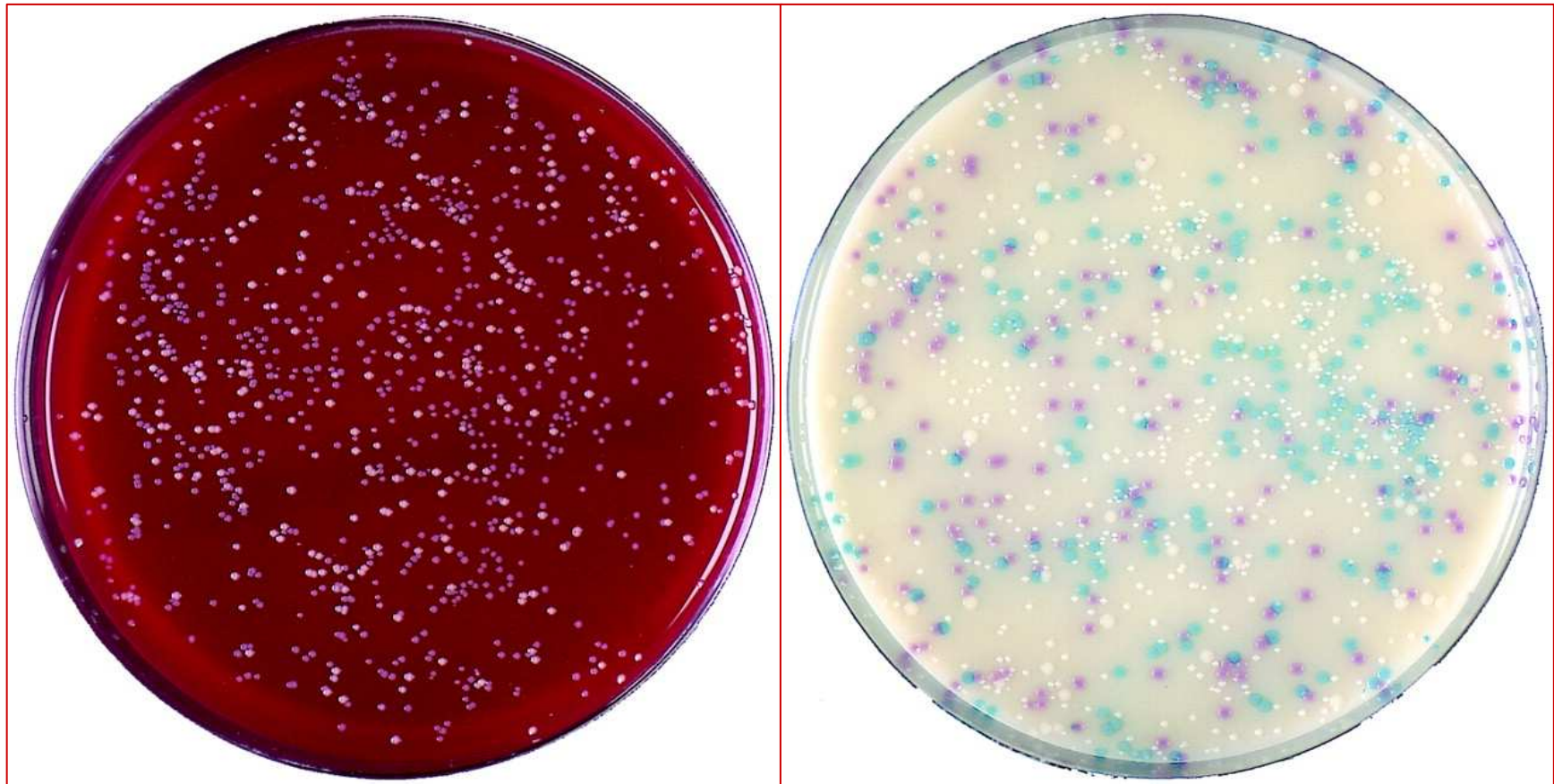
Automatic detection of NO GROWTH (**85% of all plates**)



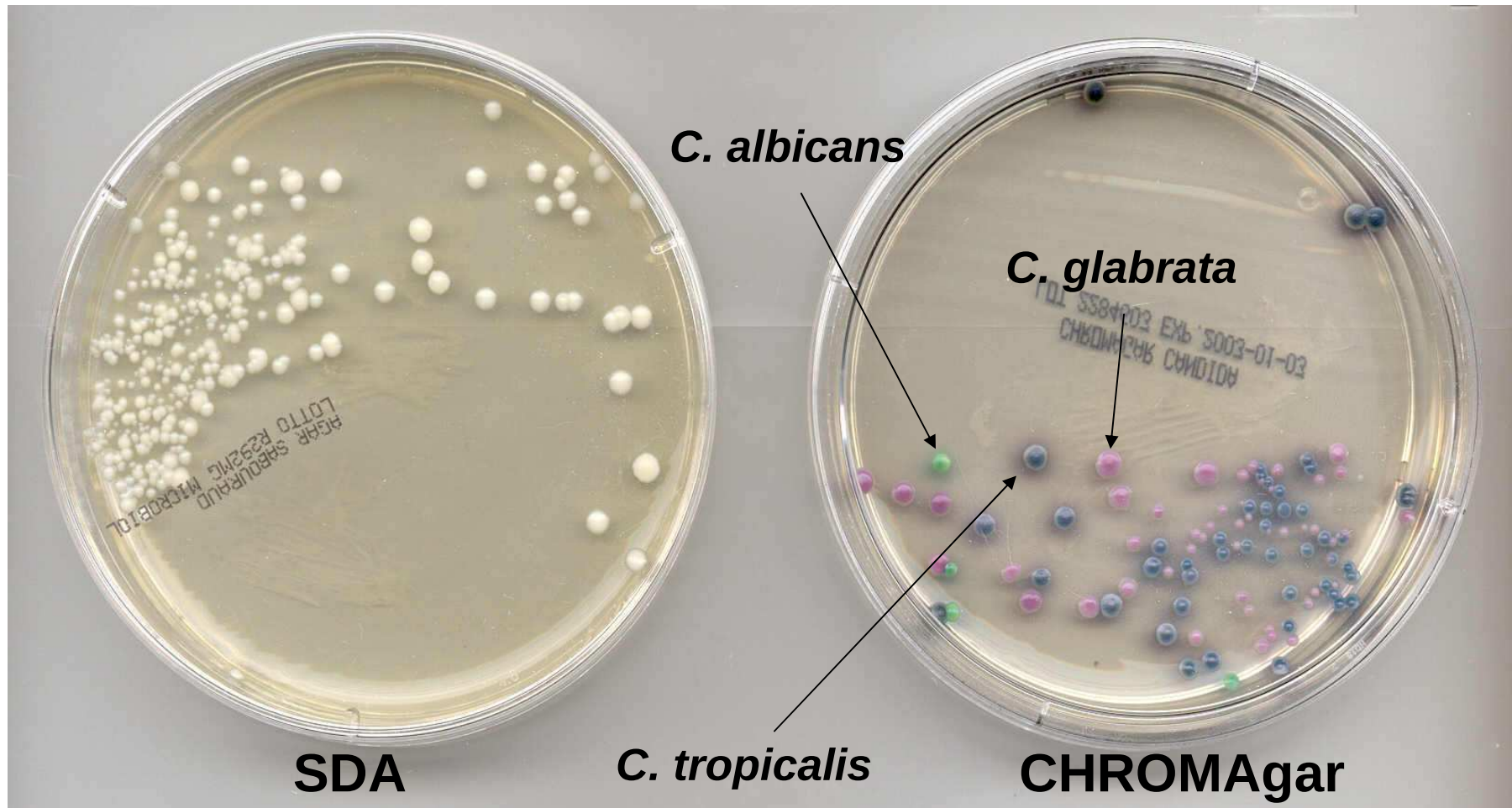
Emolisi

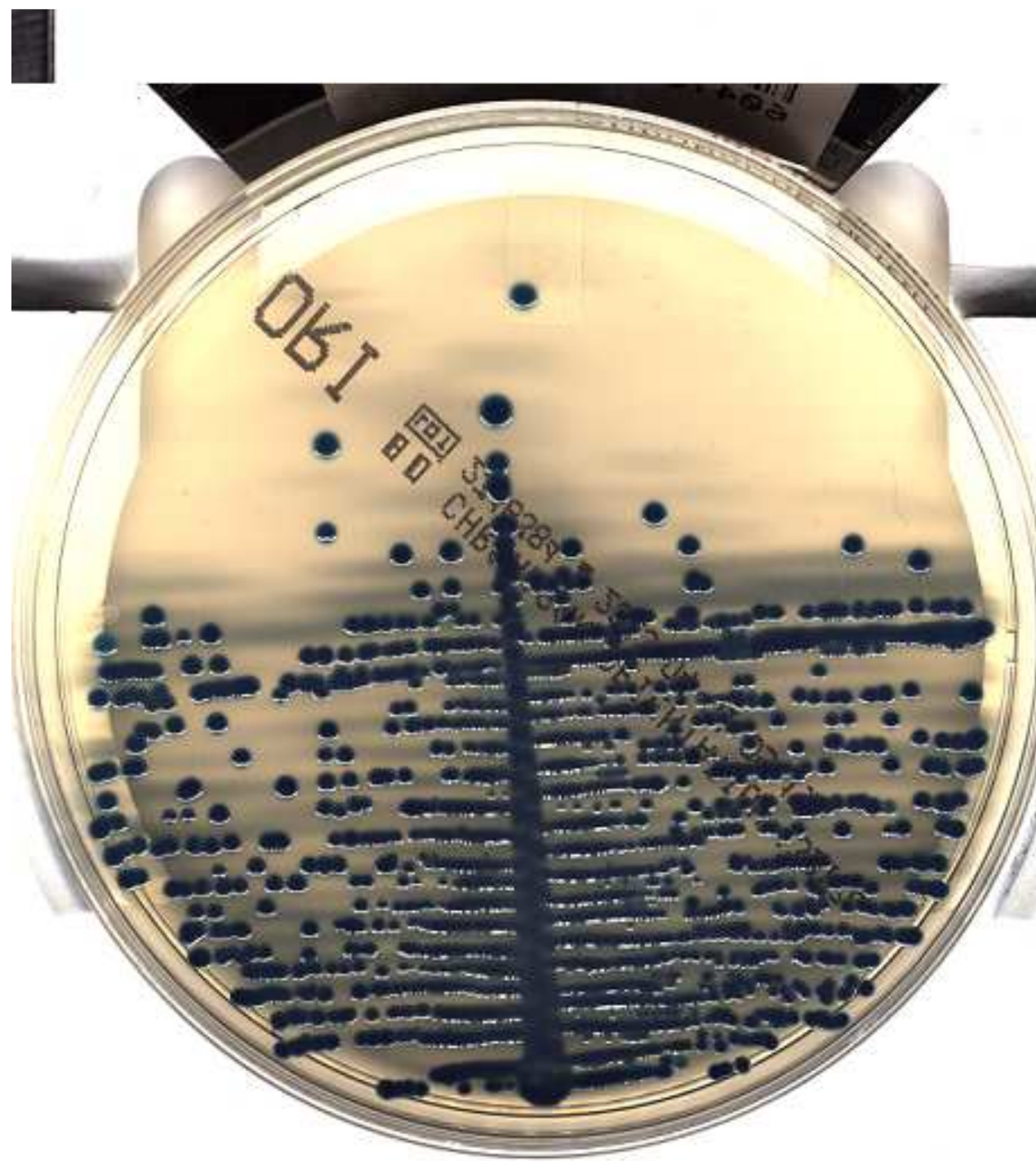


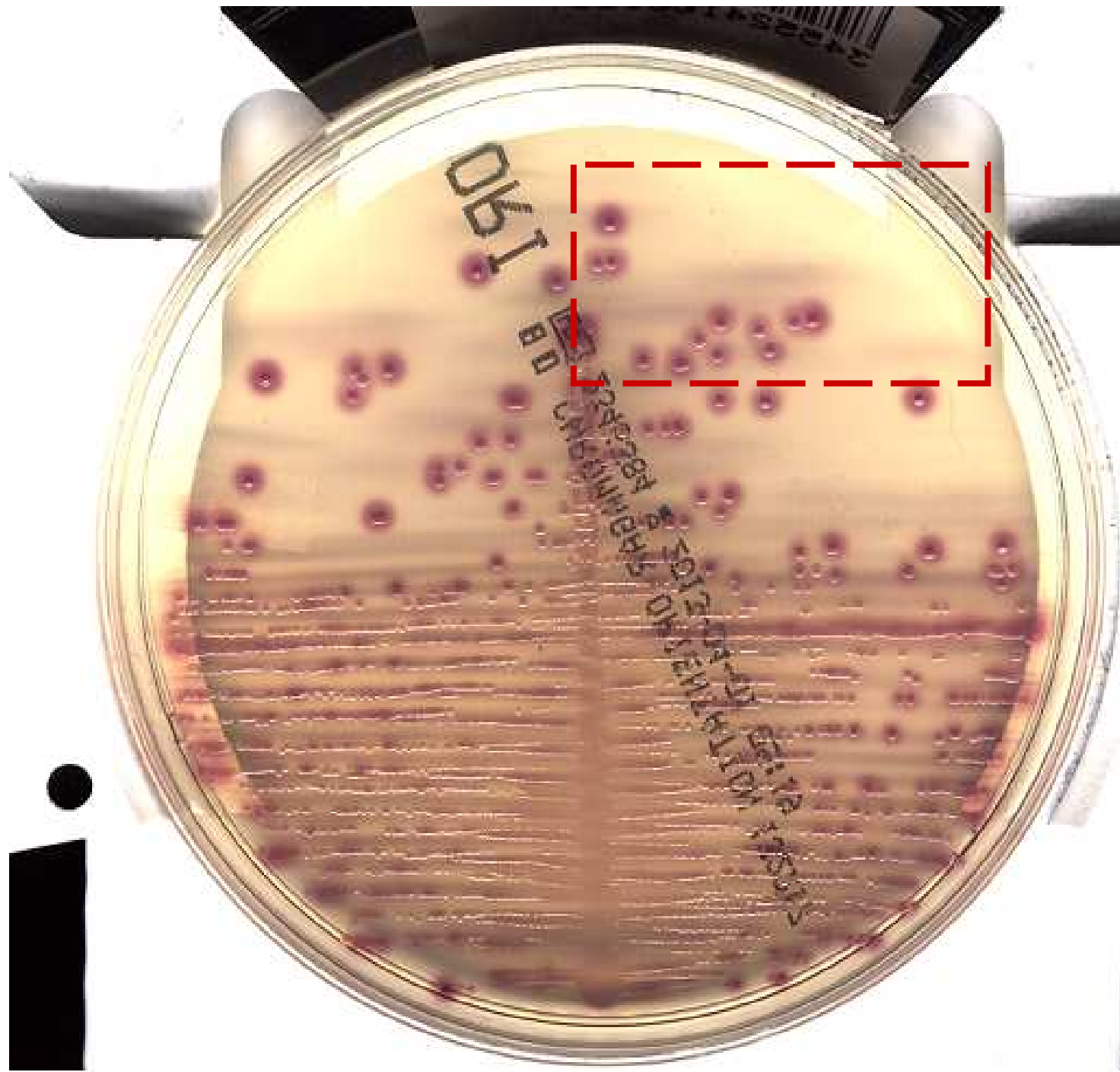
Agar Sangue vs. Chromagar

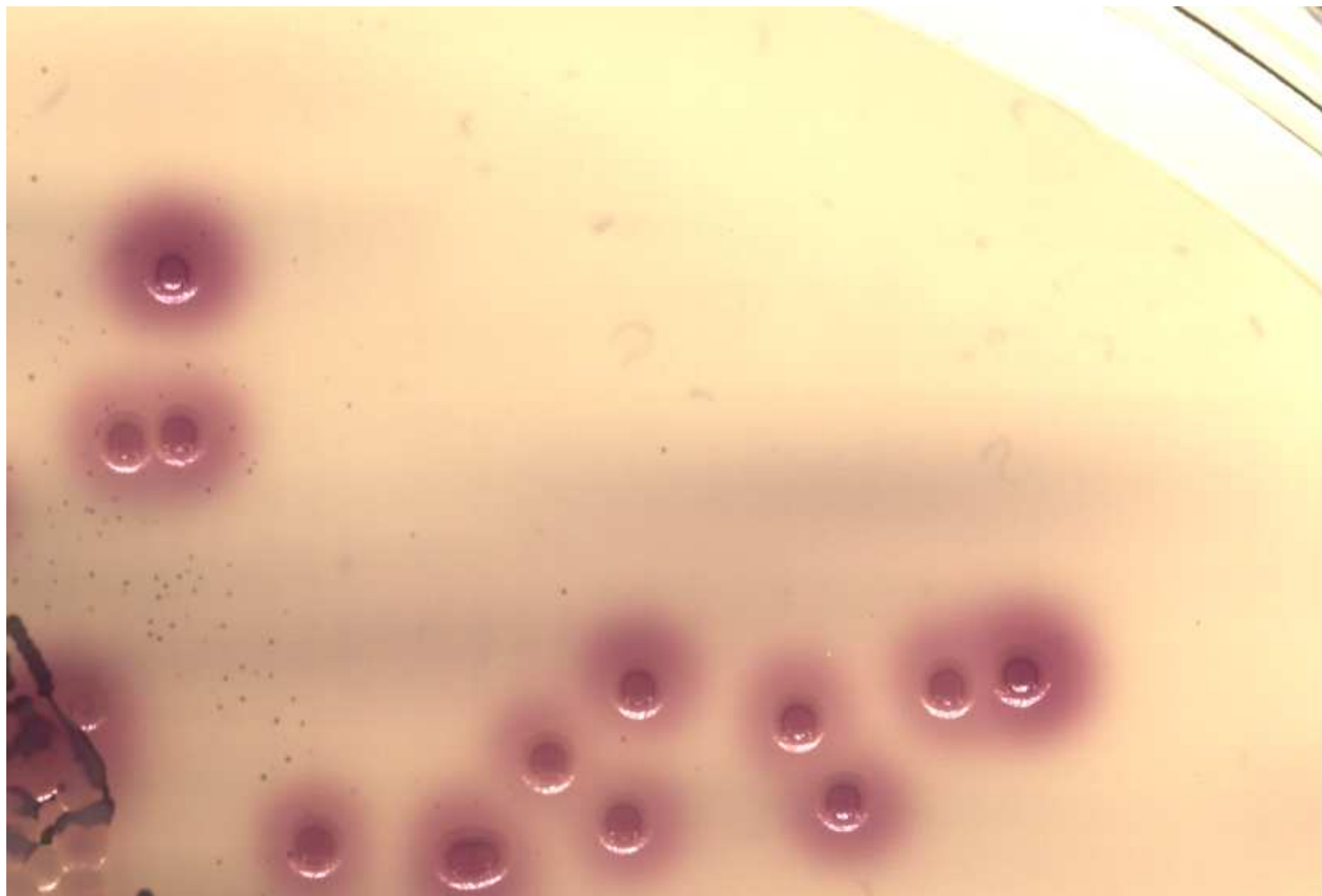


Sabouraud vs. ChromAgar

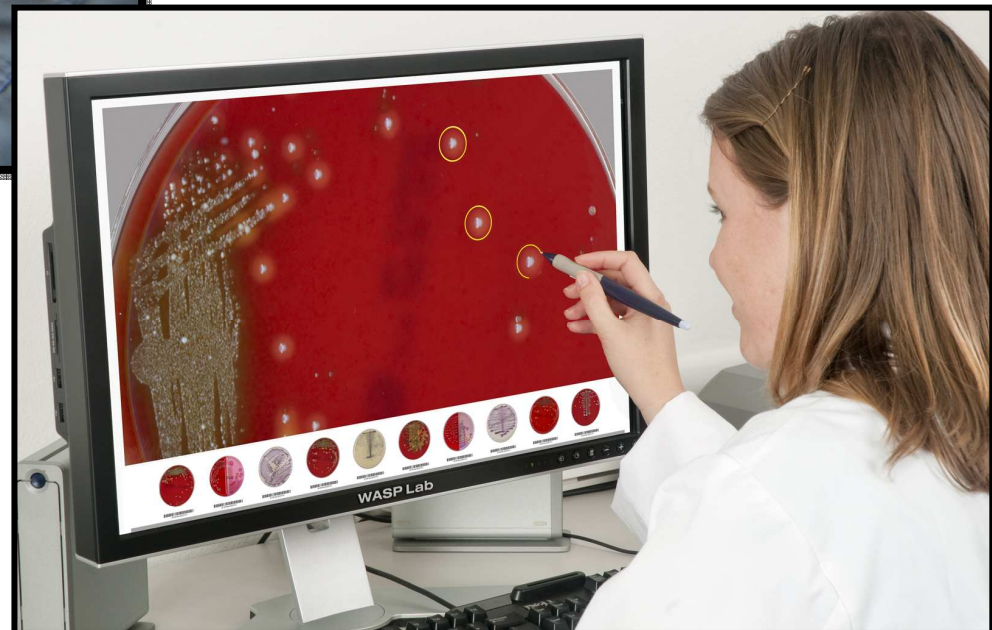


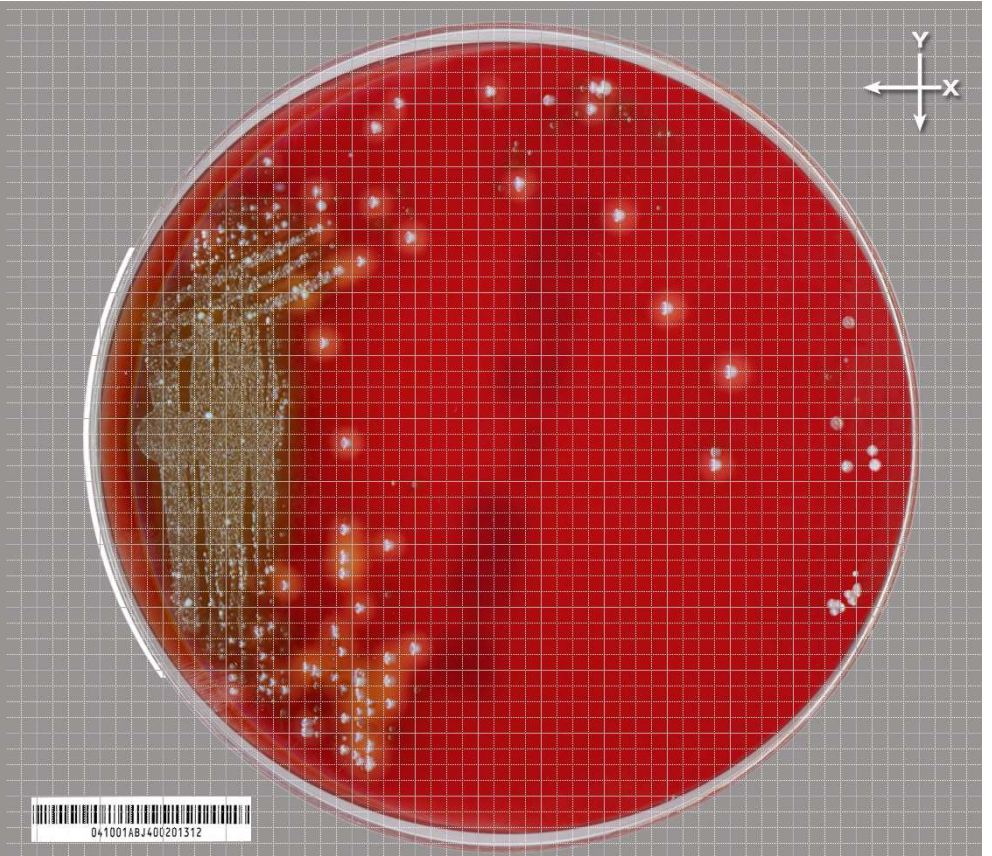
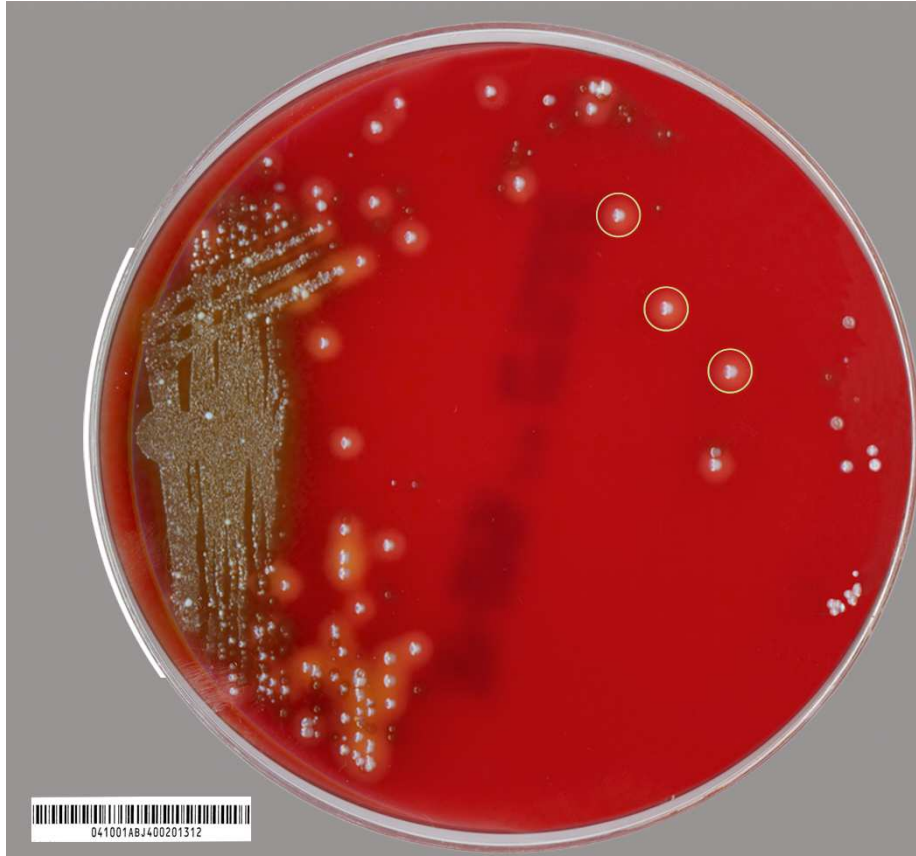




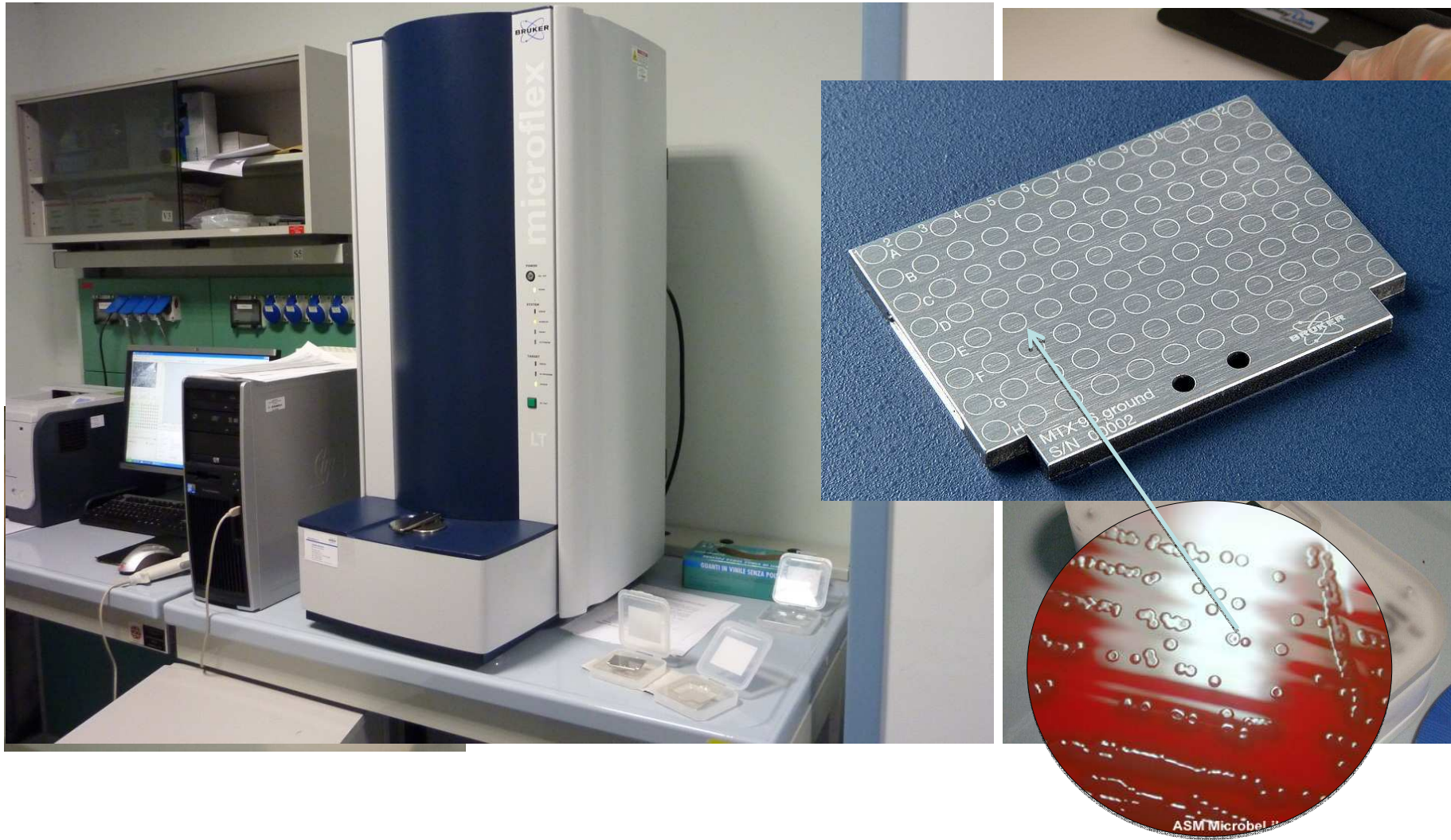


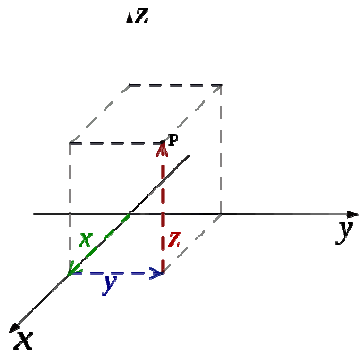
Digital Microbiology



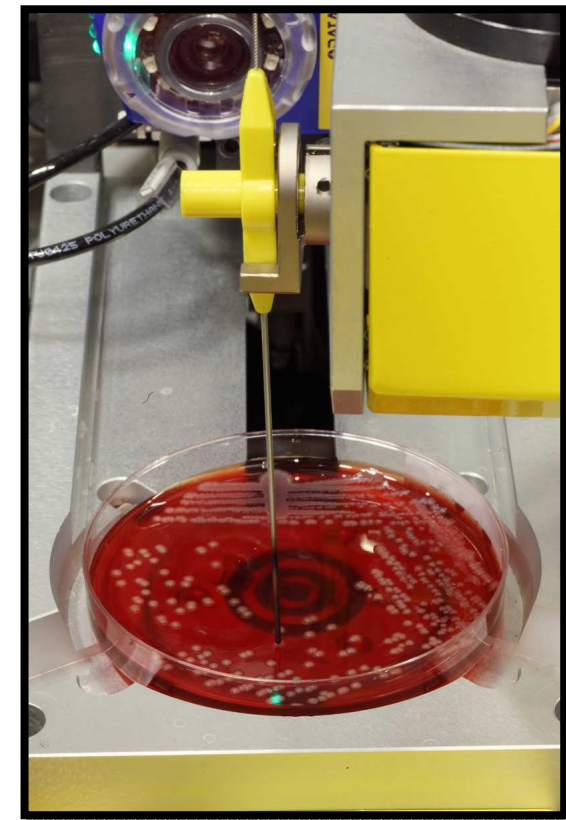
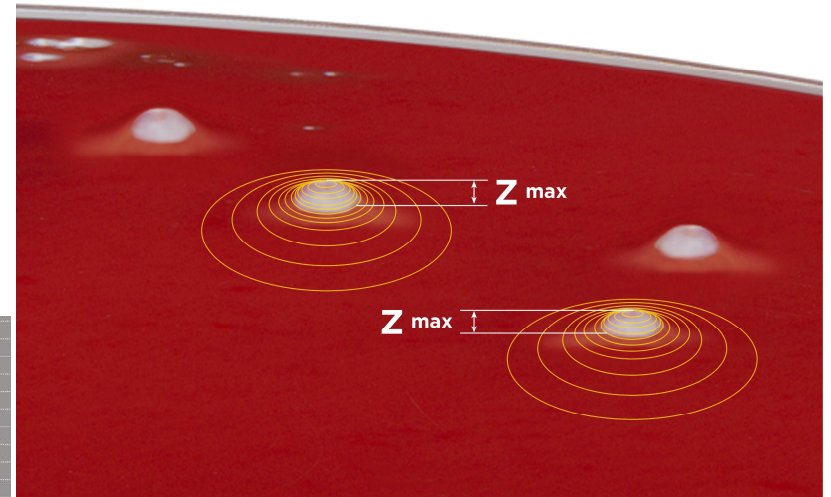
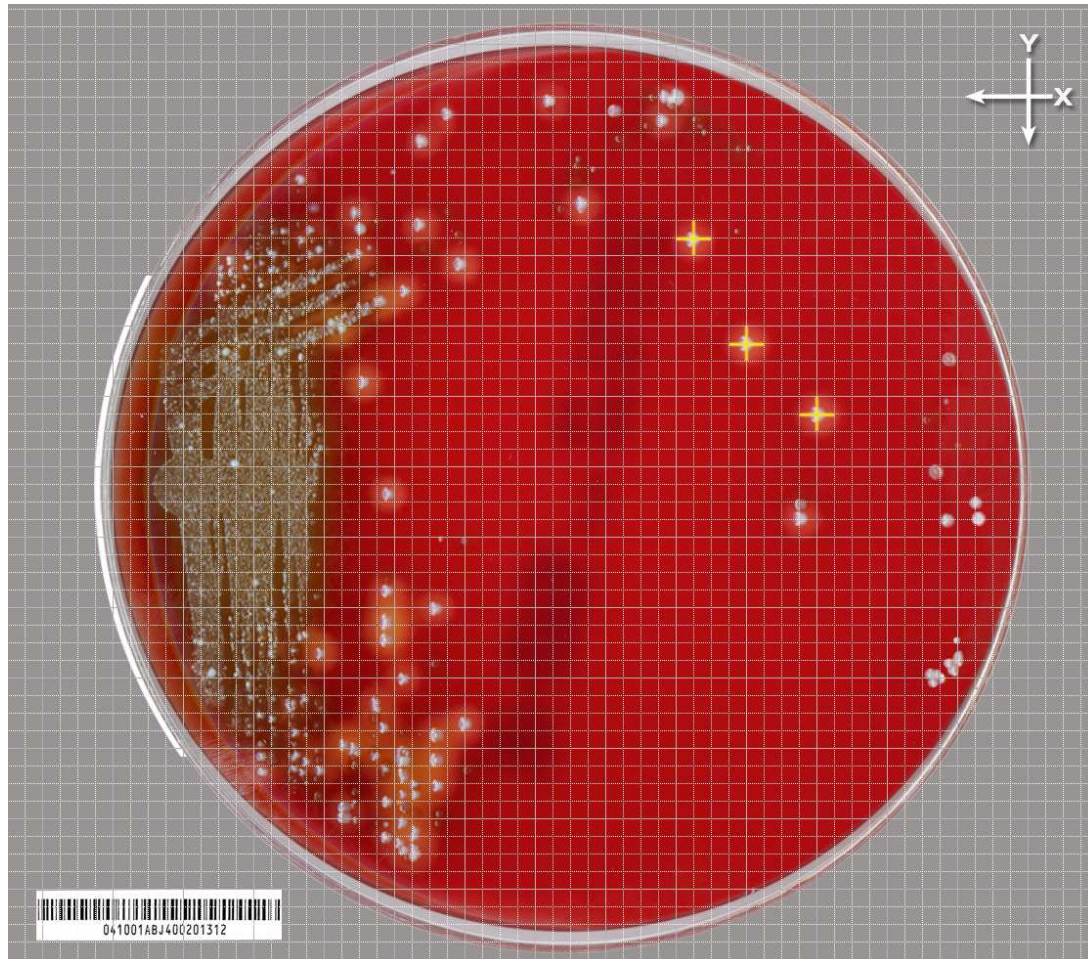


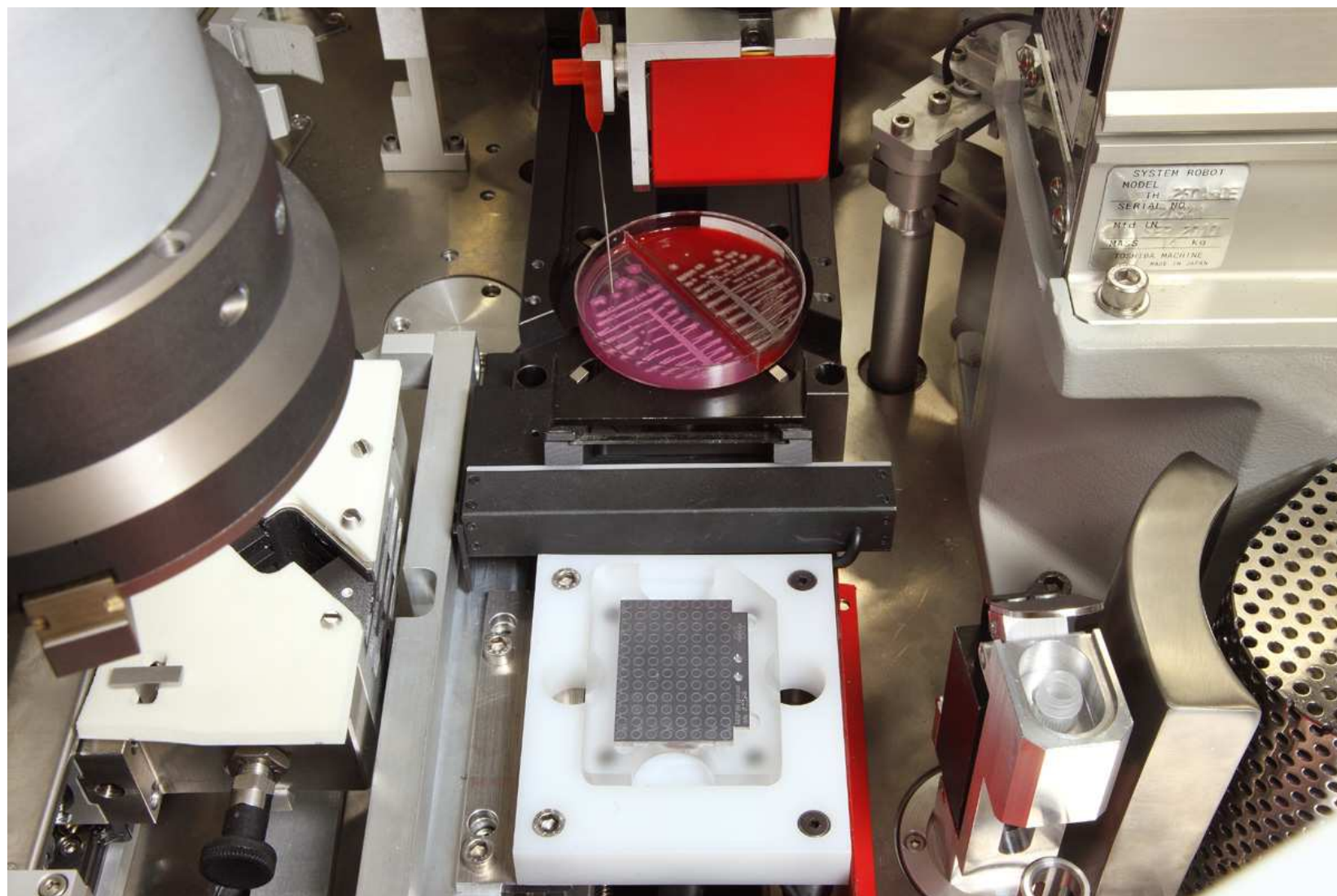
Matrix-Assisted Laser Desorption Ionization Time-of-Flight

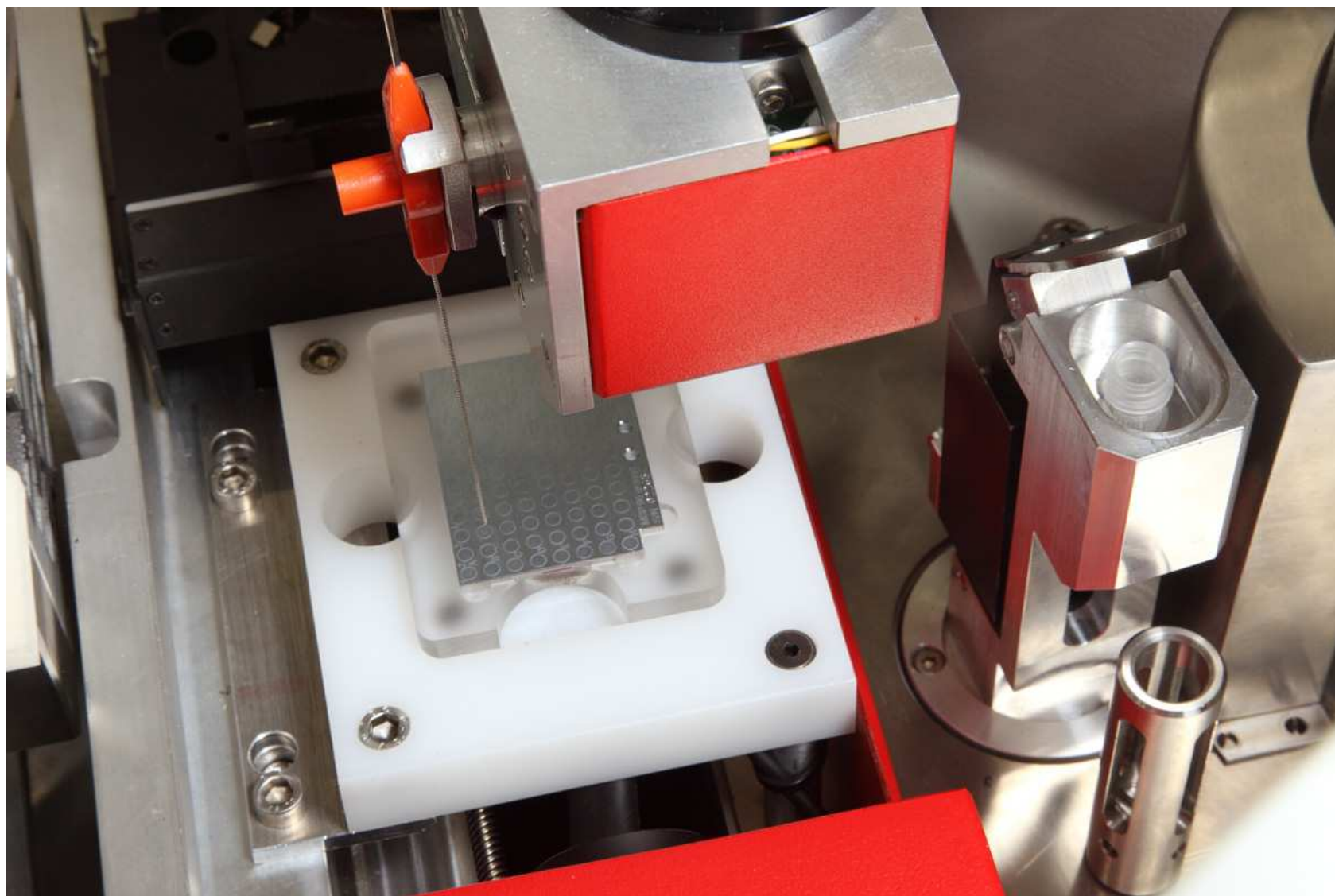




X / Y / Z coordinates



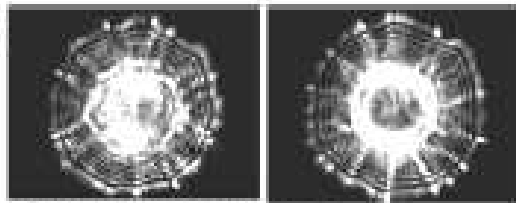




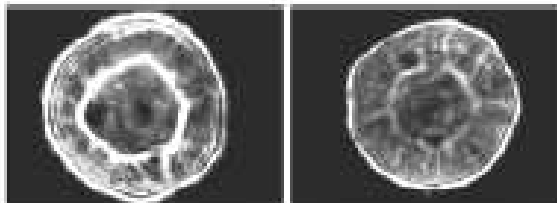
Typical *E. coli* O157:H7 colonies on selective agars



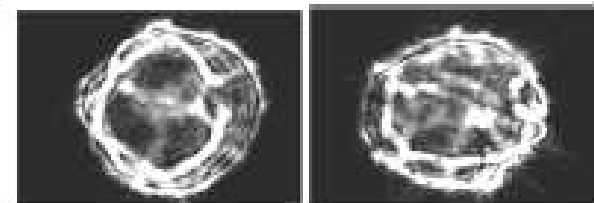
(A)



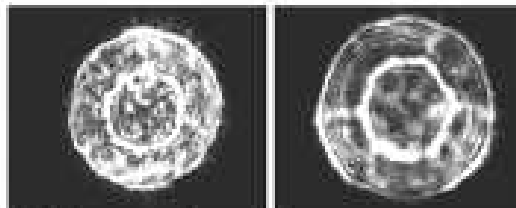
Listeria monocytogenes



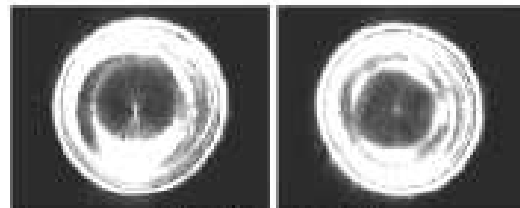
E. coli O157:H7 EDL933



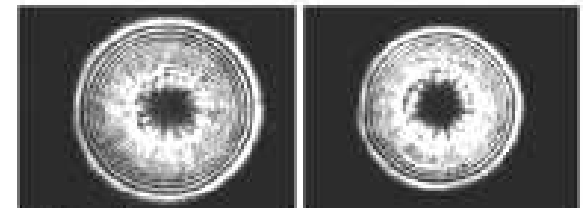
Salmonella Typhimurium
(Tennessee)



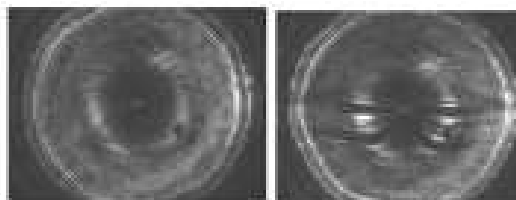
Vibrio parahaemolyticus



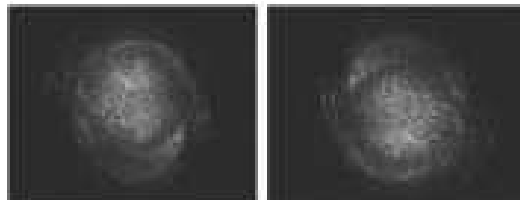
Staphylococcus
aureus 103



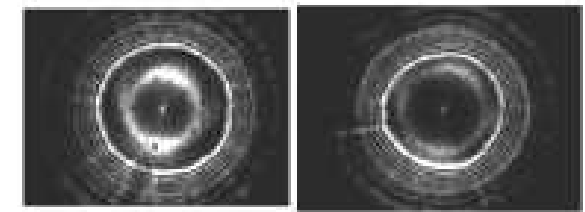
Enterobacter aerogenes



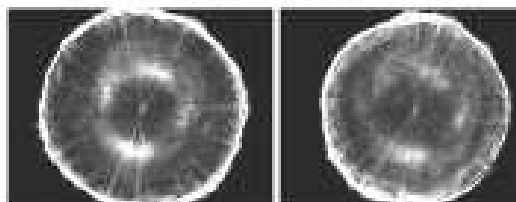
Enterococcus faecalis D516



Bacillus cereus MS1-9



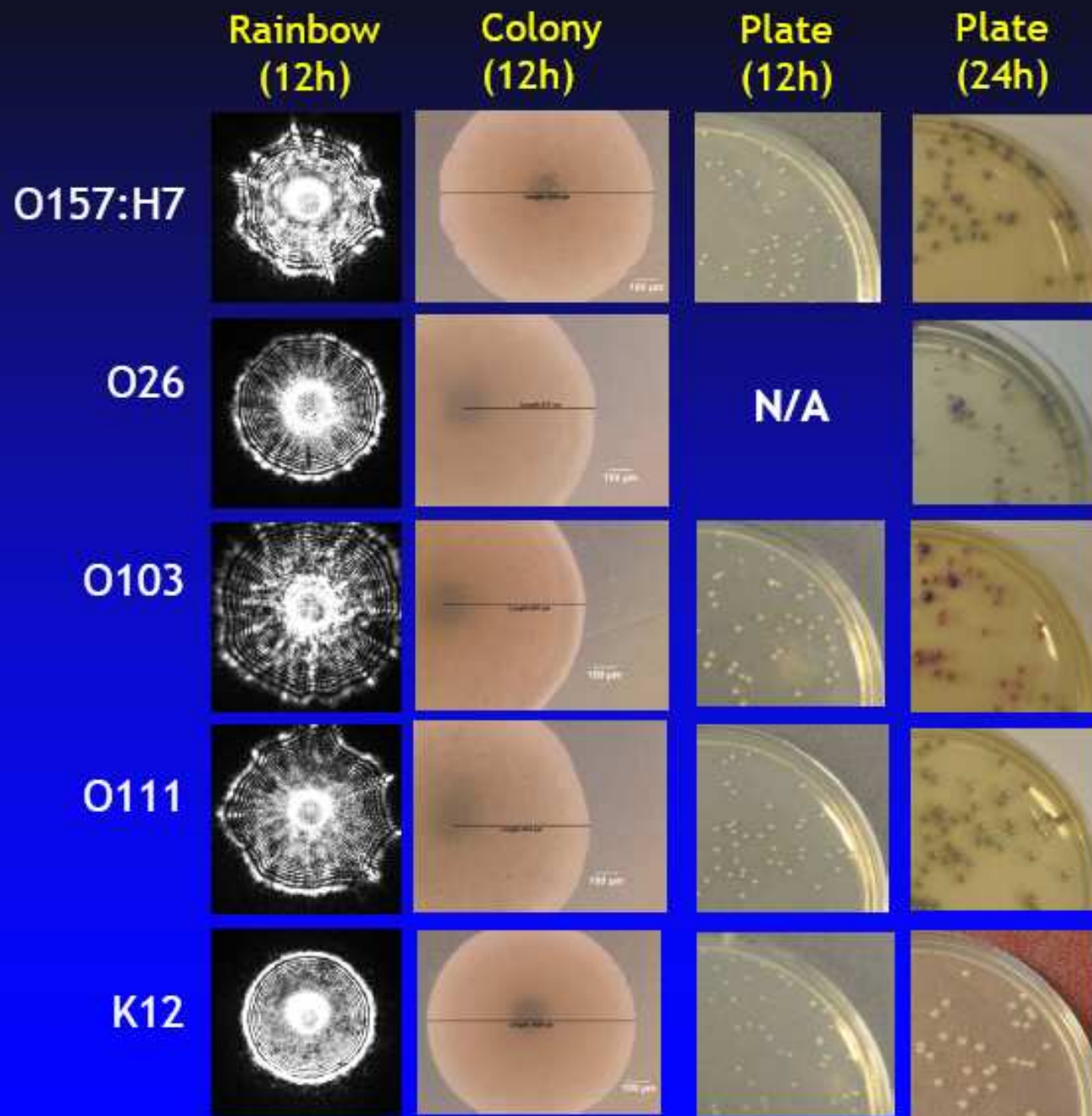
Lactococcus lactis



Aeromonas hydrophila K144

elastic light scatter (ELS) detection

courtesy of Prof. A. Bhunia



	EDL933	O111	K12	O103	O26
EDL933	98.9	0	1	0.1	0
O111	0	95.5	0	3.1	1.3
K12	0.1	0	98.6	0	1.2
O103	2.1	5.7	0	90	2.2
O26	5.6	9.7	0	3.1	81.7

Rainbow agar O157





Grazie per l'attenzione