

# MALDI-TOF - vad är nytt?

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# Klinisk Mikrobiologi

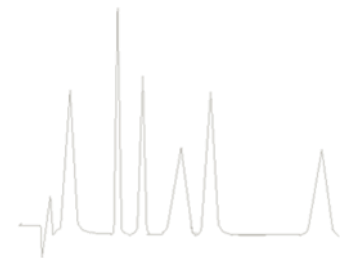
## Växjö-Karlskrona

- Bruker Microflex
- Karlskrona 2010
- Växjö 2011



# MALDI-TOF

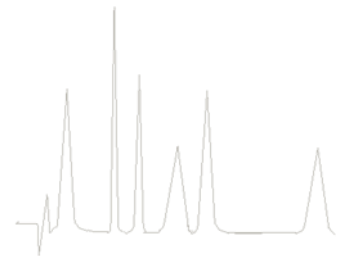
- Artbestämning
- Subtypning
- Resistens



Artbestämning

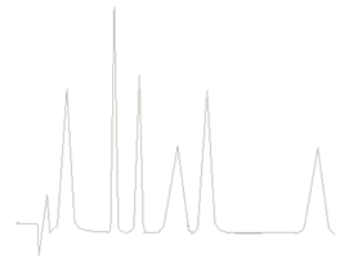
# Artbestämning

- ID (från platta- direkt transfer/extraktion)
- Direkt ID – blododlingar
- Direkt ID – urinodlingar
- Direkt ID – buljonger/andra vätskor



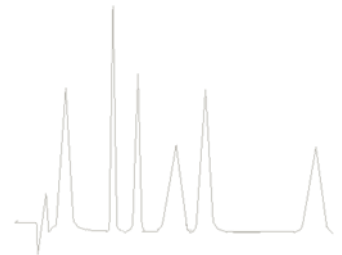
# Direkt ID -blododlingar

- Olika metoder –vilken är bäst?
- Infört i Växjö 1/6
- In-house metod (Martiny D et al Eur J Microbiol Infect Dis, 2012)
- 79.5% kunde prelsvaras direkt
- Exempel på fynd (utöver koliformer, *S aureus*, KNS):  
*Listeria monocytogenes*, *Candida tropicalis*, *Bacteroides thetaotaomicron*, *Veillonella atypica*, *Streptococcus pyogenes*
- Lägre cut-off ( $>1.6$ ): 87%



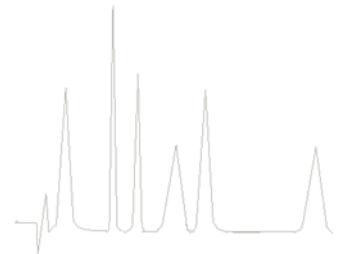
# Direkt ID -urinodlingar

- **Metoder** (Ferreira JCM 2011, Wang JMM 2013)
- **Direkt ID Urinodlingar** (Olafsson J, Poster ECCMID 2013)- endast enterobacteriaceae 148/238



# Direkt ID -buljonger

- *Salmonella* sp i selektiv buljong – 100/108  
(K Sparbier et al Eur J Clin Microbiol Infect Dis (2012))
- GBS? Utvärdering Karlskrona
- VRE/MRSA-buljong?
- Annat? Likvor, ledvätska, CAPD-vätska...

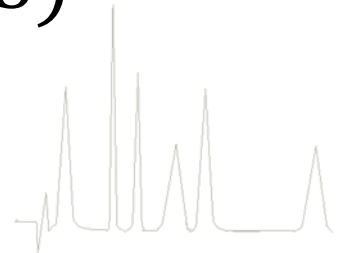




# Subtyping

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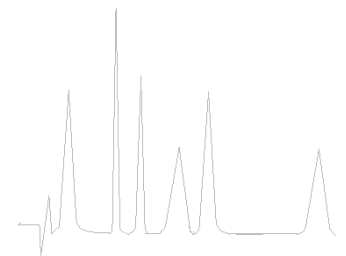
- Strept oralis/mitis vs Strept pneumoniae (Ikryanikkova et al CMI 2012)
- Shigella vs E coli (Khot et al JCM 2013)
- GBS ST-1/ST-17 (Lartigue et al JMM 2011)
- Prop acnes Phylotypes I,II,III (Nagy et al, Anaerobe 2013)
- MRSA (spa?, CC?) (Josten et al JCM 2013)
- VRE (Griffin et al JCM 2012) -utbrott



Resistens

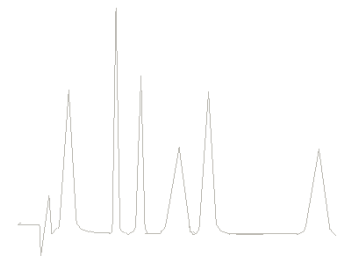
# Detektion av resistens

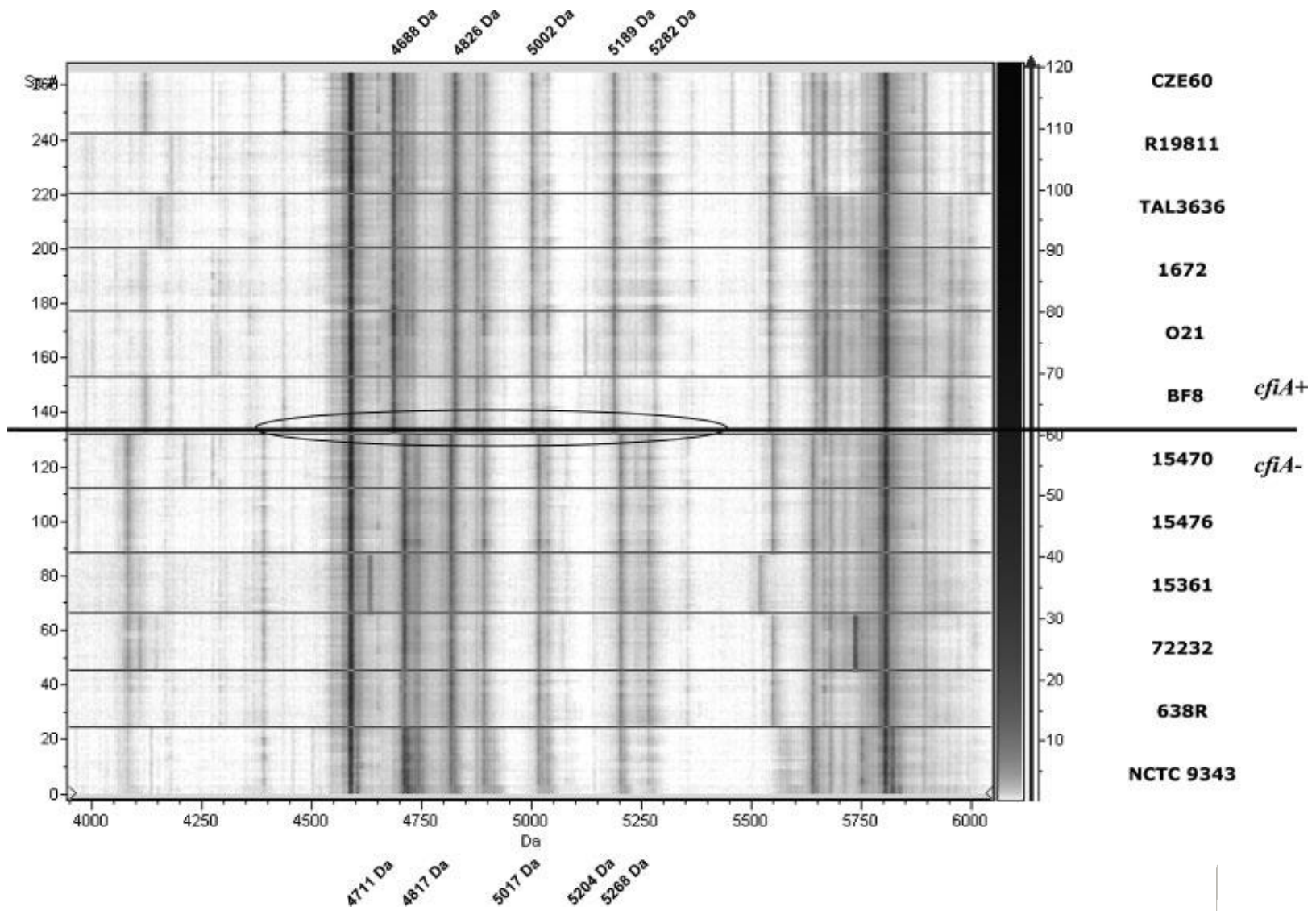
- Via specifikt topp-mönster associerat till resistens (subtypning) alt via specifika toppar bevisat kopplat till resistens
- Beta-laktamas assay (betalaktamantibiotika)
- Isotop-inmärkta aminosyror



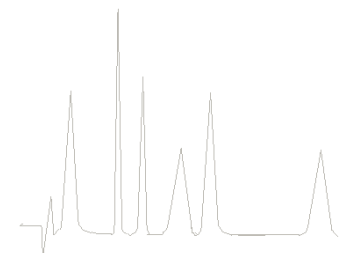
# Via specifika topp-mönster som associeras till resistens (subtypning)

- Ökad förekomst av resistens hos *B fragilis*
  - 10% av *B fragilis* från blododlingar (Danmark) (Justesen Int J Antimicrob Agents, 2013)
- metallo-betalaktamas (MBL)
- *cfiA* - Division I (neg) resp II (pos)



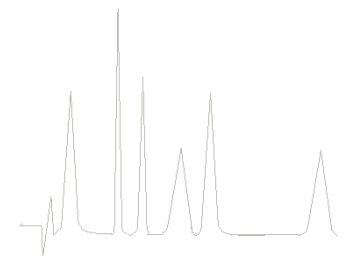


Nagy et al JMM 2011



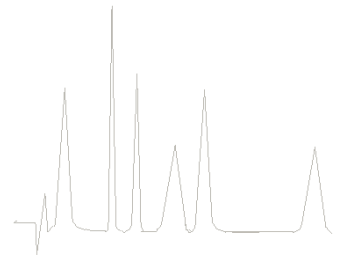
| Analyte Name                          | Organism (best match) | Score Value           | Organism (second best match)       | Score Value           |
|---------------------------------------|-----------------------|-----------------------|------------------------------------|-----------------------|
| <a href="#">16997</a><br>( ++ ) ( A ) | cfiA pos              | <a href="#">2.251</a> | <b>not reliable identification</b> | <a href="#">1.308</a> |
| <a href="#">16997</a><br>( ++ ) ( A ) | cfiA pos              | <a href="#">2.272</a> | <b>not reliable identification</b> | <a href="#">1.56</a>  |
| <a href="#">1776</a><br>( ++ ) ( A )  | cfiA pos              | <a href="#">2.271</a> | <b>not reliable identification</b> | <a href="#">1.358</a> |
| <a href="#">1776</a><br>( +++ ) ( A ) | cfiA pos              | <a href="#">2.305</a> | <b>not reliable identification</b> | <a href="#">1.458</a> |
| <a href="#">2685</a><br>( ++ ) ( A )  | cfiA pos              | <a href="#">2.19</a>  | <b>not reliable identification</b> | <a href="#">1.46</a>  |

Resultatfil från egna MSP:s för uppdelning av *B fragilis* cfiA pos/neg



# Via specifika topp-mönster som associeras till resistens (subtypning)

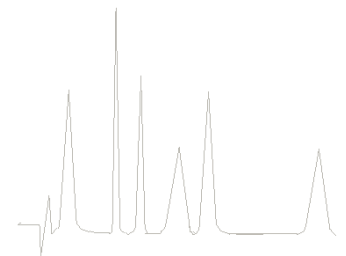
- Subtypning  $\uparrow$
- Optimalt i ID range
- Stor potential
- Utveckling av mjukvara





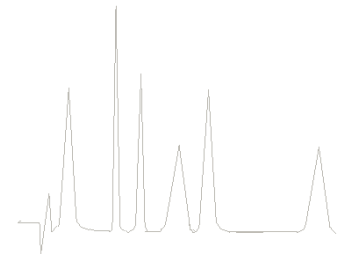
# Via specifika toppar bevisat kopplat till resistens

- E.coli, m/z peak of 29 kDa=betalaktamas (AmpR) (Camara Anal Bioanal Chem 2007)
- F.nucleatum m/z peak of 29 kDa =betalaktamas (AmpR) (Al-Haroni Oral Microbiol. Immunol. 2008)
- OmpK36 porin loss in K pneumoniae (Cai JCM 2012)



# Resistensbestämning

- Via specifikt topp-mönster associerat till resistens (subtypning) alt via specifika toppar bevisat kopplat till resistens
- Beta-laktamas assay (betalaktamantibiotika)
- Isotop-inmärkta aminosyror



# Beta-laktamas assay

- Betalaktamantibiotika
- Övergripande artikel: Sparbier et al JCM 2012
- Enkel metod

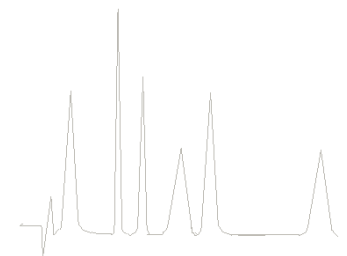
Ertapenem Mol wt [M]: **475.5 Da**

Intakt ertapenem

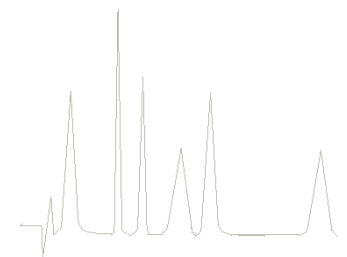
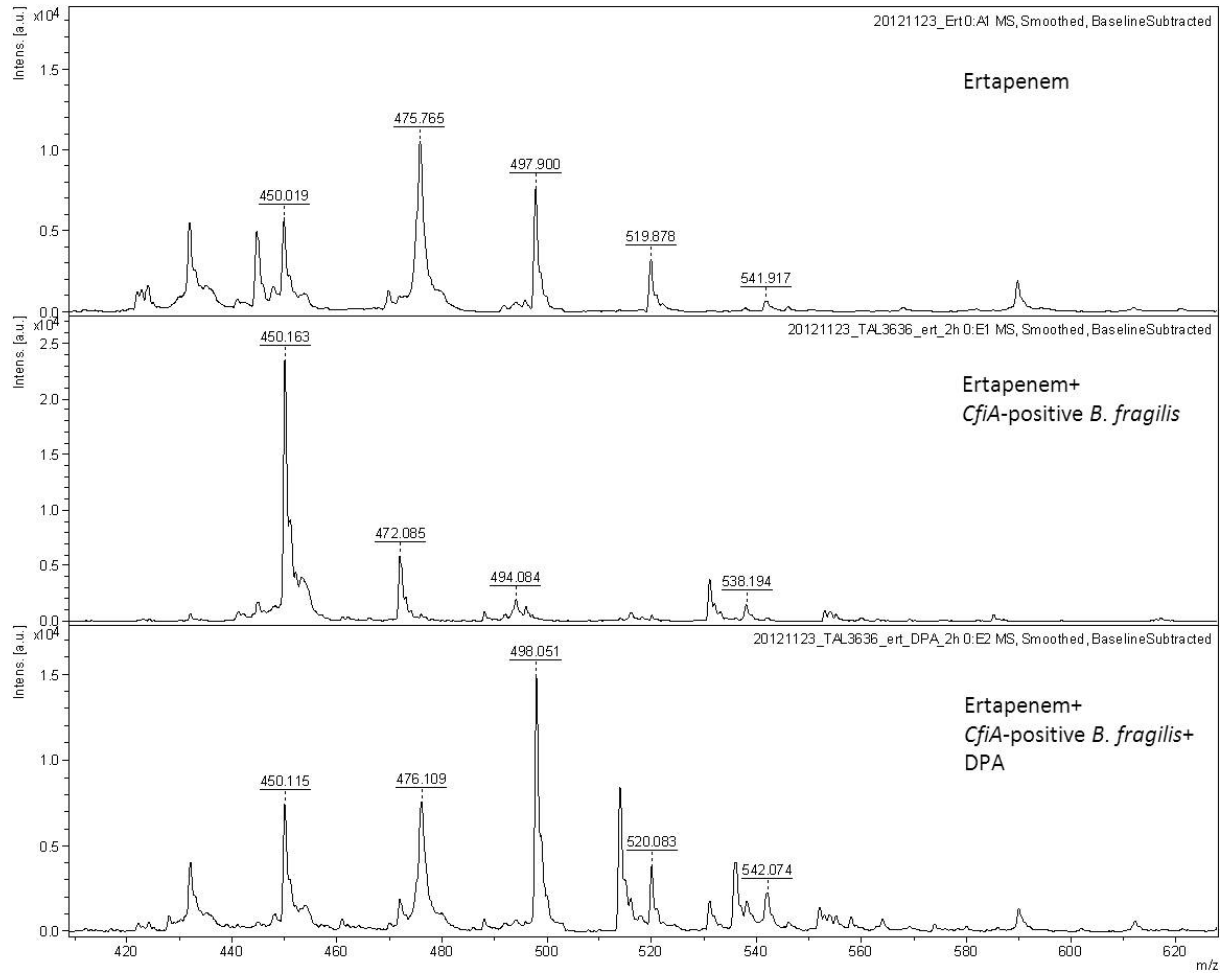
| $[M+H]^+$ | $[M+Na]^+$ | $[M+2Na]^+$ | $[M+3Na]^+$ |
|-----------|------------|-------------|-------------|
| 476.5     | 498.5      | 520.5       | 542.5       |

Hydrolyserat ertapenem

| $[M_{hydr.}+H]^+$ | $[M_{hydr.}+Na]^+$ | $[M_{hydr.}+2Na]^+$ | $[M_{hydr./decarb.}+H]^+$ |
|-------------------|--------------------|---------------------|---------------------------|
| 494.5             | 516.5              | 538.5               | 450.5                     |

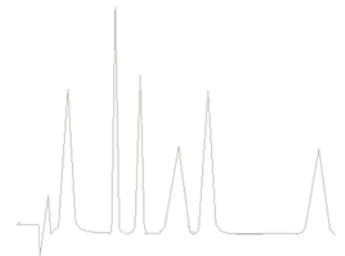


# Beta-laktamas assay



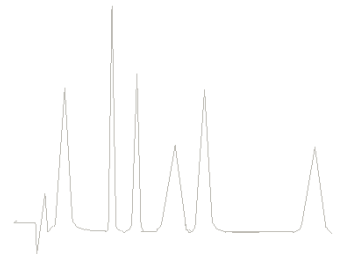
# Testat i Växjö...

- KPC+MBL (VIM+NDM-1) *K pneumoniae* -15' resp  
2h  
MBL (VIM) *Ps aeruginosa* -2h  
OXA *Acinetobacter baumannii* -24h  
OXA-48 *E coli*, *K pneumoniae* -24h  
Ertapenem, APBA, DPA  
(*Johansson et al, Manuscript*)
- MBL *B fragilis*  
Ertapenem, DPA  
(*Johansson et al, Manuscript*)
- TEM *Neisseria gonorrhoeae*  
Ampicillin  
(*Unemo et al, Manuscript*)



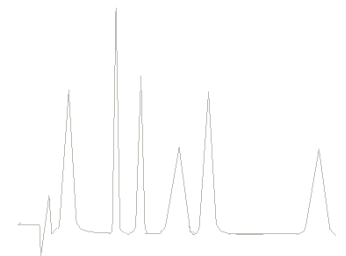
# Beta-laktamas assay

- Mycket enkelt, men manuellt arbete
- Visuellt ganska lätt att tolka
- Mjukvara som tolkar
- Lätt att modifiera ex tid, Ab/art



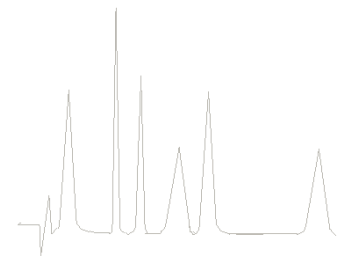
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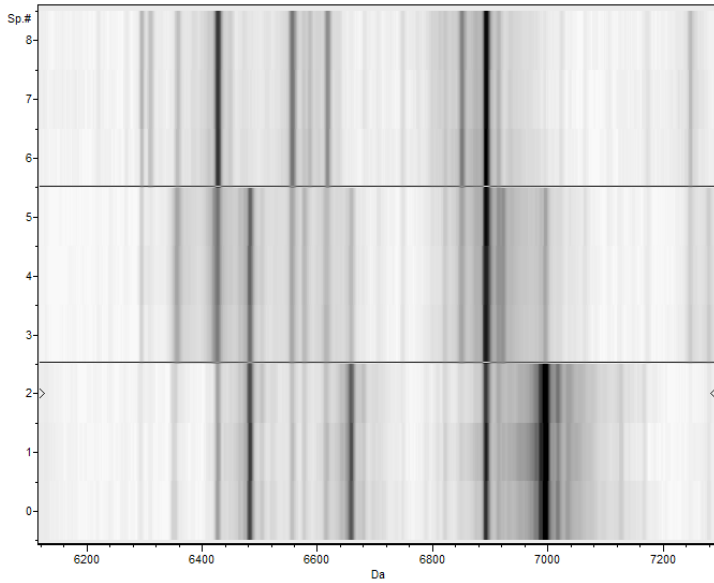


# Isotop-inmärkning

- Sparbier et al JCM 2013
  1. Bakterier i vanlig buljong
  2. Bakterier i buljong utan vissa aminosyror +isotop-inmärkta aminosyror +Ab
  3. Bakterier i buljong utan vissa aminosyror +isotop-inmärkta aminosyror





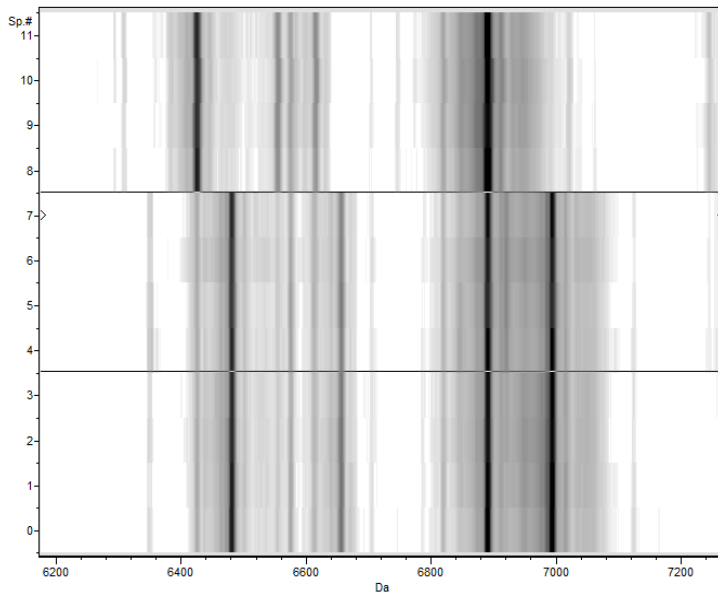


Normal

Heavy+Ab

Heavy

**Känslig stam!**

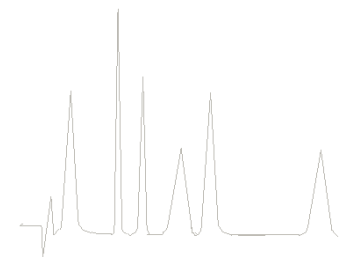


Normal

Heavy+Ab

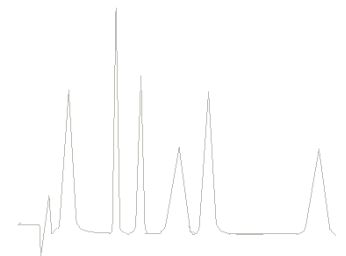
Heavy

**Resistent stam!**



# Isotop-inmärkning

- Svårtolkat?
- Automatiserad tolkning...
- Applikation- när?
- Automation –lång väg kvar...



# Vad kommer vi se framöver?

- Publikationer -subtypning
- Mjukvarulösningar för subtypning ("om MT svarar E. coli, undersök om Shigella")
- Multicenterstudier –stort behov! "En gång är ingen gång..."

Tack!