Anaerobic Microbiology and NEQAS

A Participant's Experience

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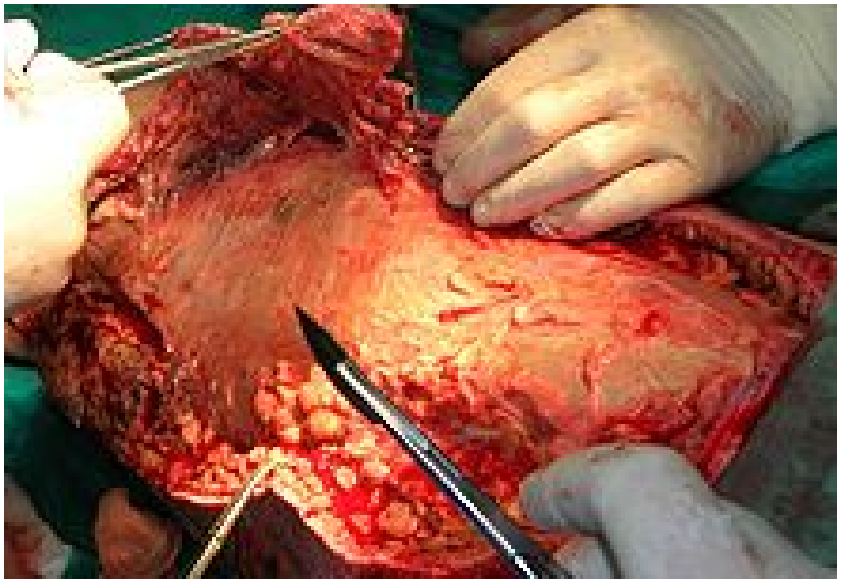
Necrotising Fasciitis

March 2010

54 year old female
Presented with necrotising fasciitis



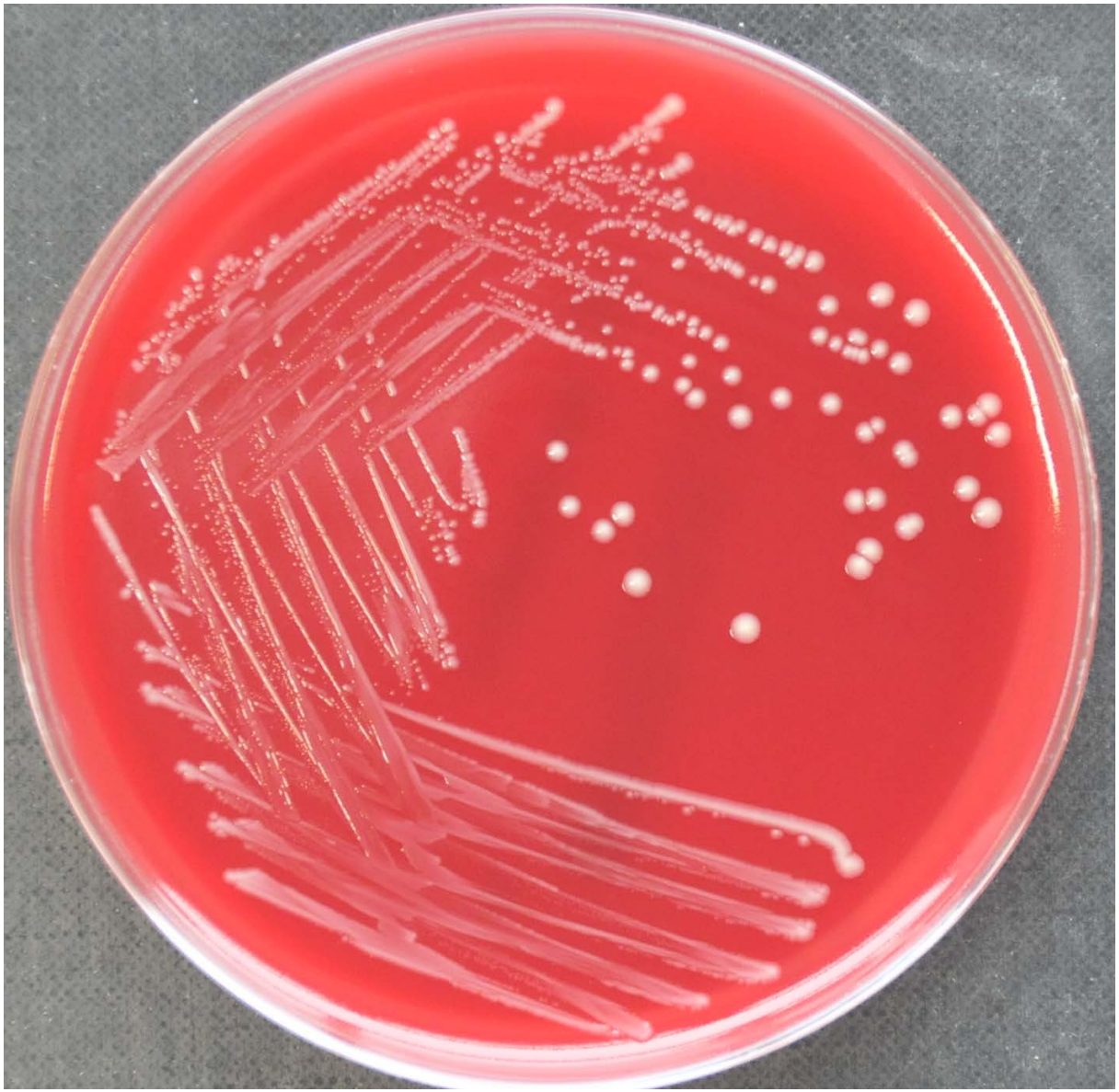
http://commons.wikimedia.org/wiki/Main_Page

Infection started in the groin, ascending over the front of the abdomen

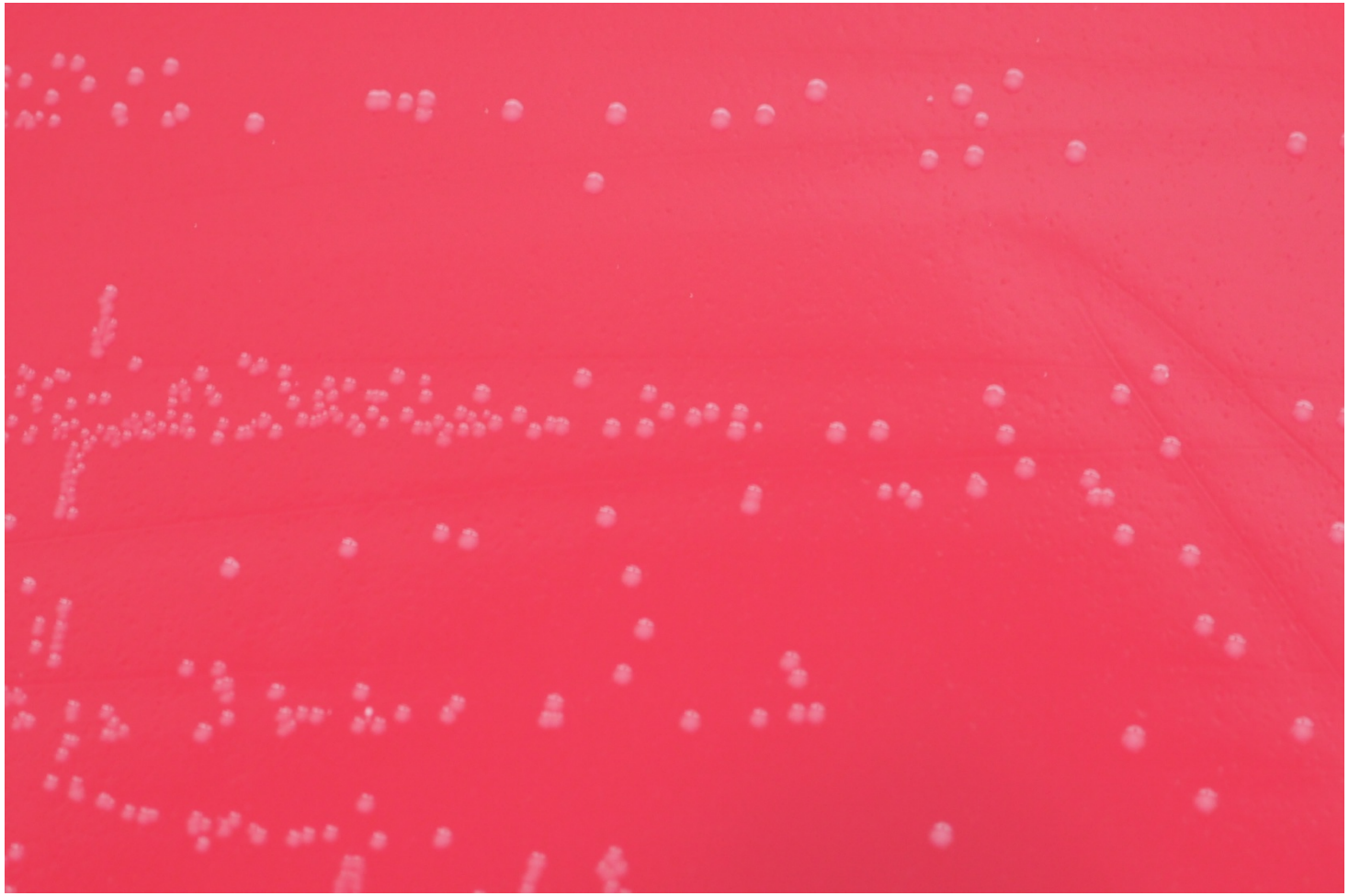
Debridement (anterior abdomen) resulted in extensive loss of skin, subcutaneous tissue and muscle

The Isolates

The Isolates



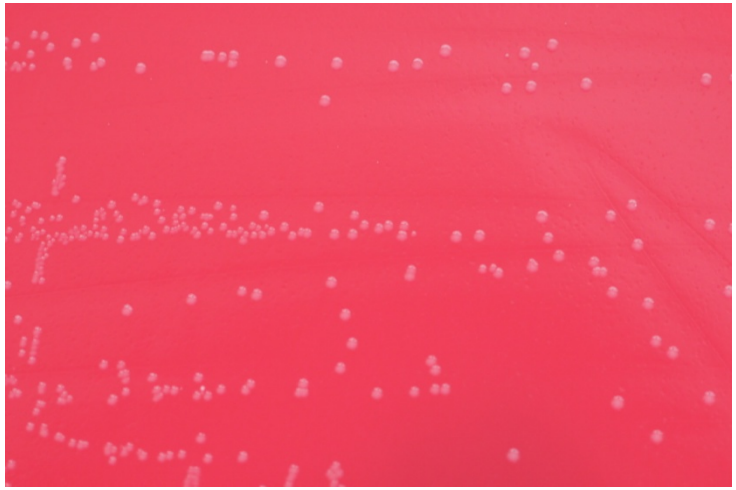
The Isolates



The Isolates

Bacteroides fragilis

Peptostreptococcus anaerobius



Actinomyces turicensis

What is NEQAS to a Microbiologist

- Allows an assessment of standards
- A teaching tool
- A catalyst for change

Reporting of Anaerobes: Pre August 2009

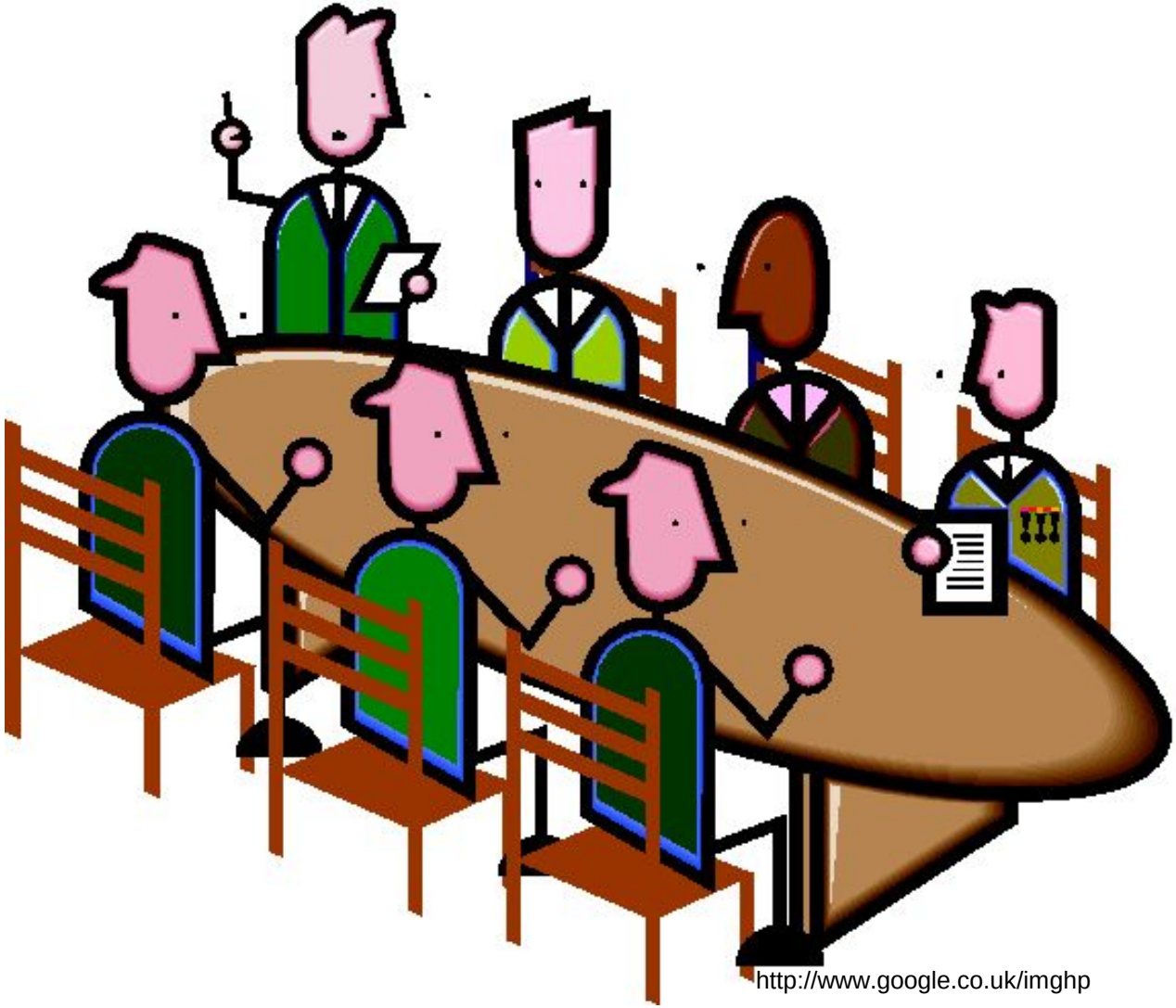
Mixed Non-Sporing Anaerobes

Clostridium species

Bacteroides fragilis

NEQAS RESULTS?

Identification and Reporting: The Decision



http://www.google.co.uk/imghp

Practical Anaerobic Microbiology Course, Cardiff

Run By:

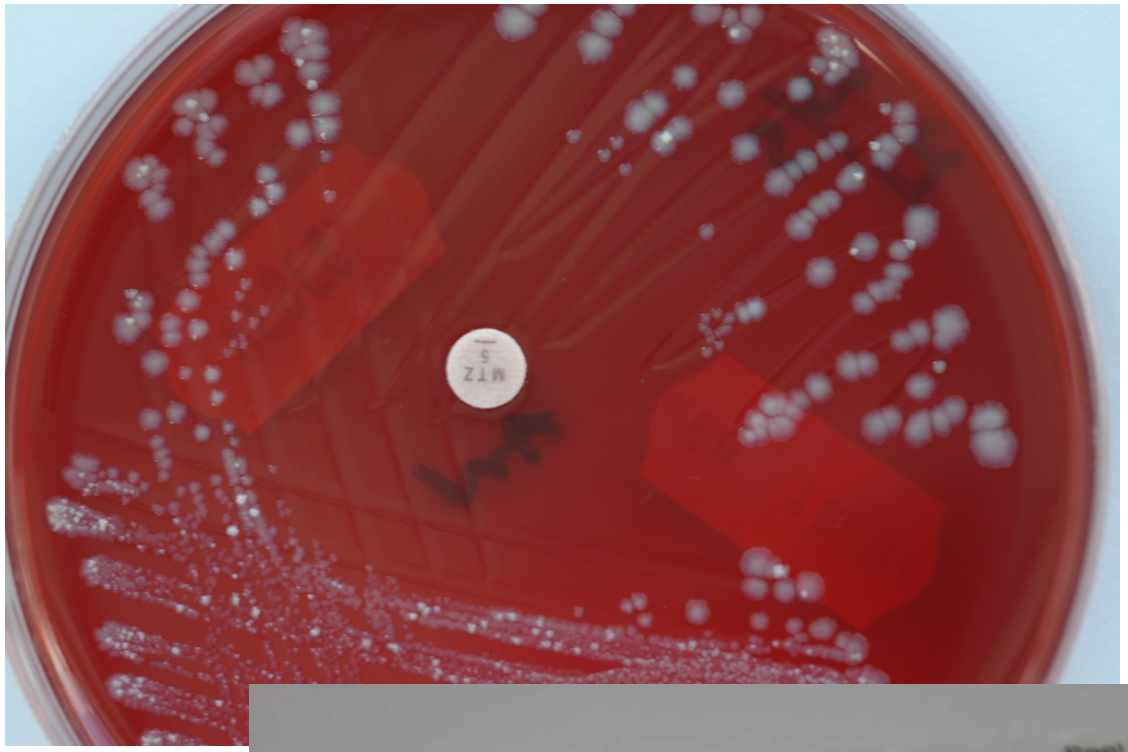
Prof. Mike Wren, UCH, London
Dr. John Brazier, Anaerobic Reference Laboratory, Cardiff
Dr. Val Hall, Anaerobic Reference Laboratory, Cardiff

Course Aims:

- To improve standards of anaerobic microbiology in clinical laboratories
- To promote an understanding and awareness of anaerobic bacteria in clinical material

Changes

- Media



- Identification Methods

Media

- Fastidious Anaerobe Agar (FAA) to replace Columbia Blood Agar

Columbia Blood Agar 48hrs

Fastidious Anaerobe Agar 48hrs

Bacteroides fragilis

Media

- FAA supplemented with Nalidixic Acid and Tween

- Good for –
  - o Anaerobic Gram negative rods
  - o Gram positive anaerobic cocci
  - o Non sporing Gram positive bacilli

- Not so good for –
  - o Clostridia

Media

- FAA supplemented with Neomycin

- Good for –
  - o Clostridia
  - o Most anaerobic Gram negative rods

- Not so good for –
  - o Fusobacteria
  - o Gram positive anaerobic cocci
  - o Non sporing Gram positive bacilli

Identification Methods

- Changed from reliance solely on commercial kit ...
(Remel RapID ana II system)

To

- Simple tests (plus commercial kit, if required)
    - o Colonial Appearance
    - o Cellular Morphology
    - o Smell
    - o Mast Ring
    - o UV Fluorescence (365nm)
    - o Spot Indole
    - o Lethicinase / Lipase production
    - o Bile Tolerance

Standard Operating Procedure

6.13 Examination and Interpretation.

				Mast ring							
Gram Negative Rods	**MTZ S/R**	**Bile Tolerance**	**UV Fluorescence**	**E**	**RP**	**CO**	**PG**	**K**	**VA**	**Spot Indole**	**Lethicinase / Lipase**
Bacteroides	S	Tolerant	None	/	/	/	R	R	R	Neg	No
Porphyromonas	S	Intolerant	Red	/	/	/	S	R	S	POS	No
Prevotella	S	Intolerant	Red / None[1]	/	/	/	S[2]	R	R	Neg[3]	No
Fusobacterium necrophorum	S	Intolerant	Green/Yellow	/	/	/	S	S	R	POS	Lipase
Fusobacterium nucleatum	S	Intolerant	Green/Yellow	/	/	/	S	S	R	POS	No
Other Fusobacteria	S	Variable	Green/Yellow	/	/	/	S	S	R	Variable	No

/ Results of these not helpful in identification
[1] Those that form pigment tend to fluoresce
[2] Majority of species sensitive
[3] Majority negative

The Results of Change

- More anaerobes grown
- It is easier to see anaerobic growth on new media
- More confidence in identification
- Increased confidence in BMS staff
- Targeted treatment
- More *Actinomyces* ...

More *Actinomyces* ...

Isolate	2005 - 2006	2007 - 2008	2009 - 2010	2011
Total Actinomyces	1	2	10	7
Actinomyces spp.	1	2	2	1
A. europaeus	-	-	1	1
A. gerencseriae	-	-	2	1
A. meyeri	-	-	1	-
A. naeslundii	-	-	1	-
A. odontolyticus	-	-	1	-
A. turicensis	-	-	1	1
Scardovia spp.	-	-	1	-
B. dentium	-	-	-	2
V. cambriense	-	-	-	1

Actinomyces gerencseriae

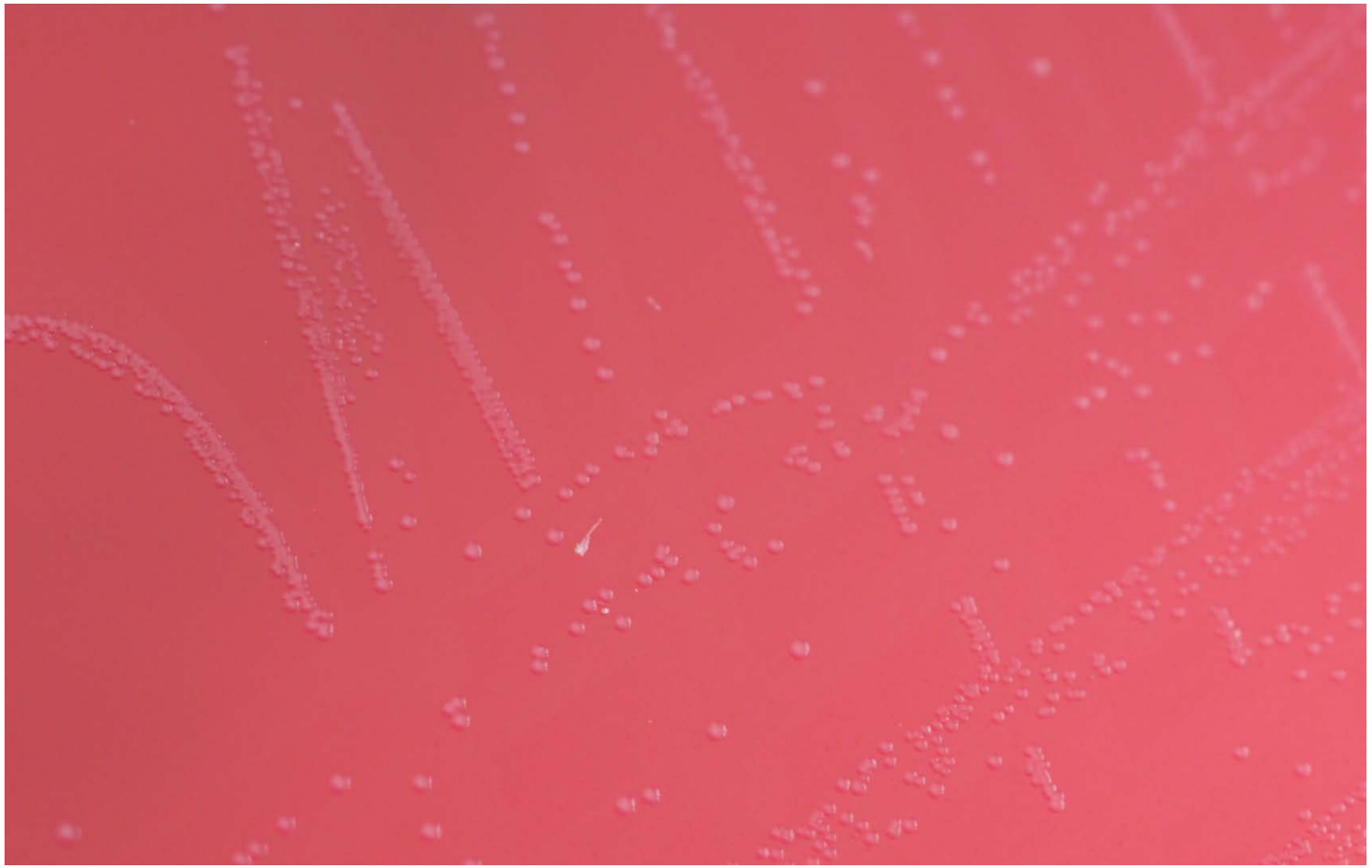
Subphrenic abscess (4 Days incubation)

Actinomyces naeslundii

Hip abscess following THR (48 hours incubation)

Actinomyces turicensis

Necrotising fasciitis (72 hours incubation)



In Conclusion

- Involvement in NEQAS has had a direct impact on anaerobic culture and identification techniques
- More anaerobes are being grown
- Identification has improved
- Increased confidence in staff
- Improved quality of treatment