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Trends in bacterial pathogen identification-molecular or not-that is the question!

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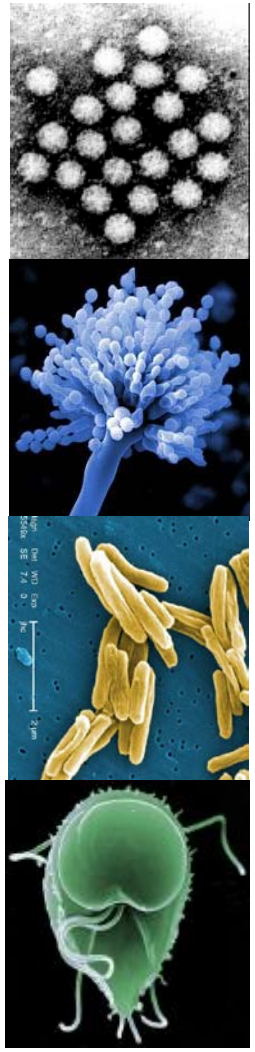
Schemes overview



44 SCHEMES

Identification
- Biochemistry
- Ag (IF / agglutination)
- Growth characteristics
- Molecular

Virology
- 23 schemes

Typing / subtyping
- Biochemistry
- Ag
- Molecular

Mycology
- 3 schemes

Quantification
- Microscopic
- Molecular

Bacteriology
- 12 schemes

Susceptibility
- phenotype
- genotype

Serology
- EIA
- Agglutination
- Line assays
- IF

Parasitology
- 6 schemes

UK NEQAS molecular schemes Overview

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- 13 molecular schemes
- 4 quantitative
- 1 quantitative and qualitative
- 8 qualitative
- Out of the 8 qualitative schemes four targets bacteria

✓ Molecular detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*

✓ Molecular detection and resistance testing of Mycobacteria

✓ MRSA screening

✓ *Clostridium difficile*

Chlamydia trachomatis (CT) and Neisseria gonorrhoeae (NG)

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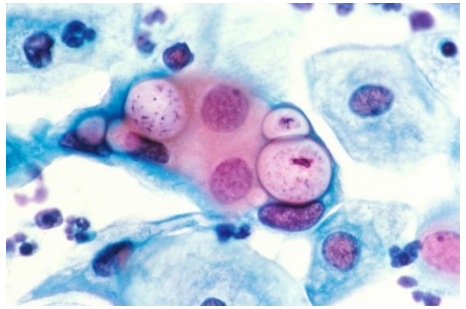
- Among the most prevalent bacterial pathogens of sexually transmitted infections (STIs)
- Estimated case numbers for CT 131 million new/year for NG 78 million new/year (http://www.who.int/mediacentre/factsheets/fs110/en/)
- CT includes three human biovars

1. Serovars A-C cause trachoma
2. Serovars D-K cause urethritis, pelvic inflammatory disease (PID) ectopic pregnancy, neonatal pneumonia and neonatal conjunctivitis
3. Serovars L1, L2 and L3 cause lymphogranuloma venerum

Diagnosis of *Chlamydia trachomatis*

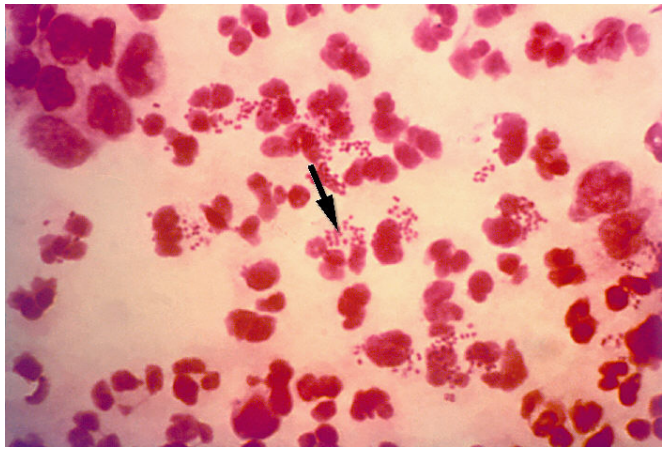
- Infection mainly asymptomatic
- Reticulate body (RB): metabolically active
- Elementary body (EB): infectious form
- Cryptic plasmid (plasmid-free isolates have been described)
- Cell culture (McCoy cell line) was for a long time the reference standard –too complex and time consuming
- Direct fluorescence antibody (DFA) test --issues with cross-reactivity
- Antigen ELISA
- Molecular tests (inhibitors: haemoglobin, low-pH cervical mucosa, b-chorionic gonadotropin, urine crystals and urine nitrites)

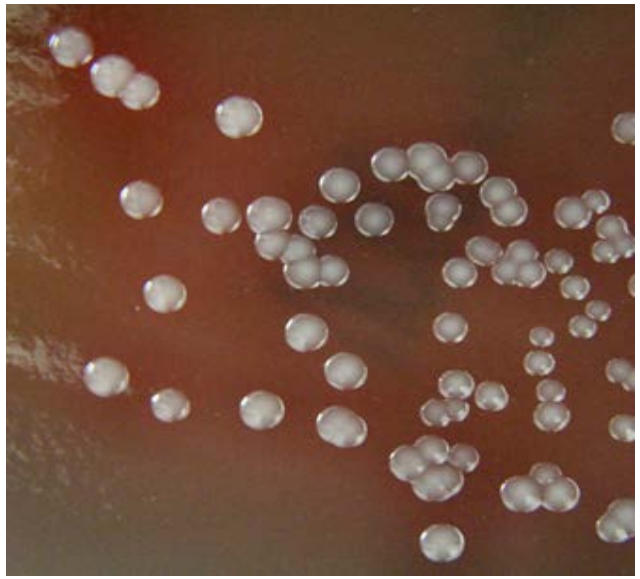


Human pap smear showing chlamydia in the vacuoles at 500x and stained with H&E. Image credit: Wikimedia Commons

Diagnosis of *Neisseria gonorrhoeae*

- Symptomatic infection (urethritis)
- Gram-stained specimen (Gram-negative diplococci)
- Culturing (fastidious organism, grow on chocolate agar with CO_2 or on Thayer-Martin agar, confirmation required)
- Molecular tests





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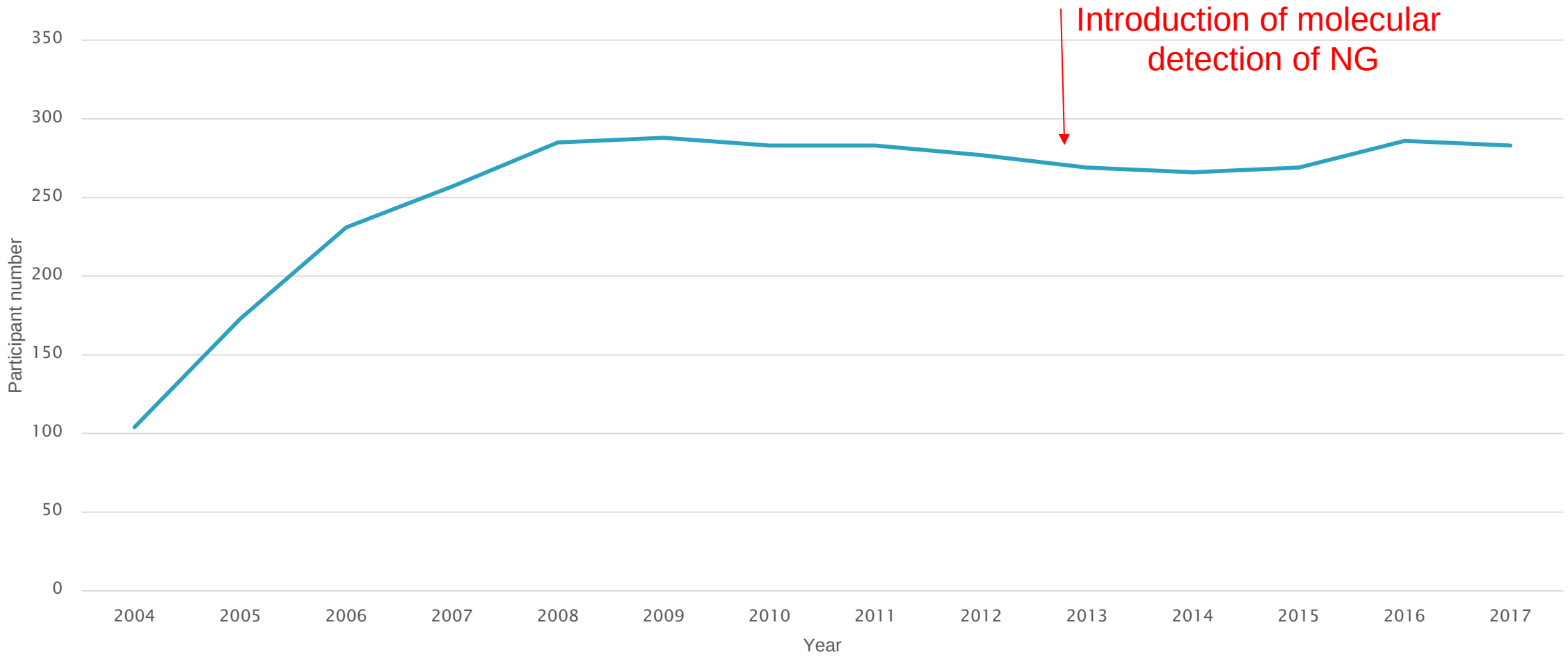
Changes in participant number over 13 years



Performance characteristics of a CT positive (serovar K) clinical specimen

Dilution factor	Theoretical Bacterial load in copies/mL	Measured Bacterial load in copies/mL (in-house real-time PCR)	Consensus
30	1,600,000	1,549,644	99.30%
50	1,000,000	1,312,148	98.50%
96	520,833	36,236	100.00%
139	359,712	19,761	99.20%
250	200,000	23,800	98.80%
1080	46,296	not tested	97.70%
1250	40,000	7,031	99.60%
2500	20,000	14,500	96.90%
3600	13,888	21,588	94.30%
7200	**6,944**	**6,000**	**87.40%**

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Performance characteristics of a cultured CT positive (serovar J) specimen

Dilution factor	Theoretical Bacterial load in copies/mL	Measured Bacterial load in copies/mL (in-house real time PCR)	Consensus
200	2,800,000	Not tested	99.20%
1400	400,000	114,871	98.80%
3600	155,556	155,861	99.60%
5000	112,000	26,226	98.80%
15000	37,333	12,803	100%
18000	31,111	39,910	98.90%
72000	7,778	10,657	96.90%
80000	**7,000**	**7,079**	**88.70%**

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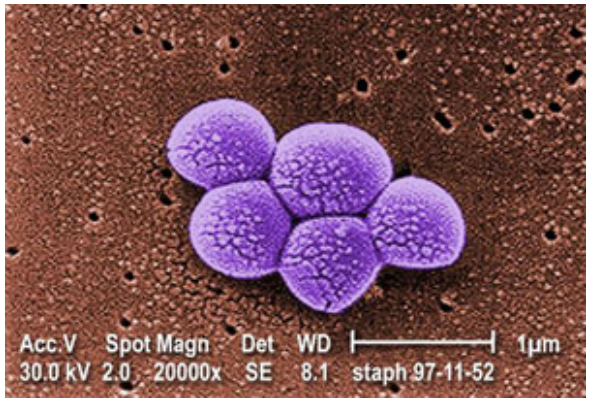
Performance characteristics of NG positive cultured specimen

Dilution factor	Consensus
5000	98.10%
10000	97.80%
25000	96.40%
50000	97.90%

0.13mMcF was the lowest concentration of NG positive cultured specimen that was distributed with 98.4% of participants reporting the correct result

Methicillin-resistant Staphylococcus aureus (MRSA)



- Gram-positive bacteria
- Causing mainly healthare-associated infections
- Distinctions:

1. HA-MRSA –healthcare associated or hospital acquired
2. CA-MRSA –community acquired
3. LA-MRSA –livestock associated

- Culturing (screening with chromogenic media) and then confirming (cefoxitin disk screen test, latex agglutination test for PBP2a or a plate containing oxacillin in Mueller-Hinton agar supplemented with NaCl)
- Molecular tests to detect mecA gene however novel resistance mechanism occurs due to mecC gene

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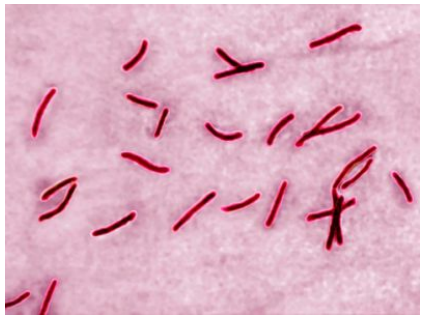
MRSA screening

- Introduced in 2009
  - 180 participants
    - 180 culture based
    - 31 molecular assays
- 8 years later
  - 315 participants
    - 315 culture based (chromogenic agar)
    - 166 molecular assays

	Distribution (Year)	
Assay	**2474 (May 2009)**	**4087 (July 2017)**
Culture	180	315
Amplex: easyplex	-	1
BD: GeneOhm	14	5
BD: MAX	-	2
BD: MAX MRSA	-	4
Cepheid: Xpert	-	71
Cepheid: Xpert NxG	-	12
GenomEra: Diagnose	-	2
PCR: Multiplex	1	4
PCR: Single target	1	3
Real-Time Multiplex	2	8
Real-Time Single target	1	4
Hain	3	-
Roche: LightCycler	-	6
Other	2	44
Σ	**180**	**315**
Culture based total	**180 (100%)**	**315 (100%)**
Molecular total	**31 (14.6%)**	**166 (52.7%)**



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Tuberculosis –key facts (WHO data)

- One of the top 10 causes of death worldwide
- Six countries account for 60% of the total (India, Indonesia, China, Nigeria, Pakistan and South Africa)
- In 2015, an estimated 1 million children became infected with MTB and 170 000 children died of TB
- TB is a leading killer of HIV-positive people
- Globally in 2015, an estimated 480 000 people developed multidrug-resistant TB (MDR TB)
- Ending the TB endemic by 2030 is among the health targets of the newly adopted Sustainable Development goals

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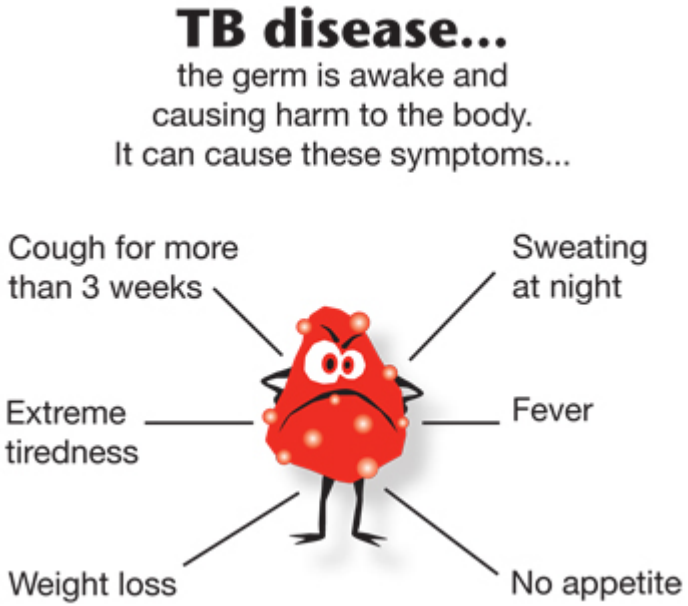
Tuberculosis: symptoms and diagnosis

- Cough, chest pain, weakness, weight loss, fever and night sweats

Diagnosis:

- ✓ Chest X-ray
- ✓ Sputum smear microscopy (acid-fast bacilli)
- ✓ Culture
- ✓ Interferon gamma release assay (IGRA)
- ✓ MGIT (Mycobacteria Growth Indicator Tube)
- ✓ Molecular tests



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Tuberculosis

- Introduced in 2007
  - 53 participants
    - 10 direct only
    - 5 post culture only
    - 38 both
- 10 years later
  - 137 participants
    - 61 direct only
    - 6 post culture only
    - 70 both

Assay	Distribution (Year)			
	2181 (May 2007) Direct	2181 (May 2007) Indirect- post culture	4077 (June 2017) Direct	4077 (June 2017) Indirect – post culture
AccuPower	-	-	1	-
Beckman: GenomeLab	-	-	1	-
BD MAX	-	-	1	-
BD MGIT	-	-	1	-
BD ProbeTec	9	2	1	2
Cepheid: GeneXpert	-	-	98	13
GeneProof	-	-	1	-
Gen-Probe: MTD	9	1	2	-
Gen-Probe: Accuprobe	-	8	-	11
HAIN	1	13	9	30
Illumina: WGS	-	-	-	1
Infopia	-	-	1	-
Innogenetics: InnoLiPa	1	3	-	1
LG: AdvanSure	-	-	1	-
Nanogen AD	-	-	3	1
Pyrosequencing/sequencing	1	-	-	2
Qiagen: Artus	8	1	2	-
Roche: Amplicor	2	-	-	-
Roche: Cobas Amplicor	6	3	-	-
Roche: Cobas TaqMan	-	-	6	-
PCR: Single target	2	1	1	2
Real-Time Single target	5	1	2	1
Real-Time Multiplex	-	-	-	2
Sacace	-	-	1	-
Seegene	-	-	3	-
Unspecified	9	10	2	9

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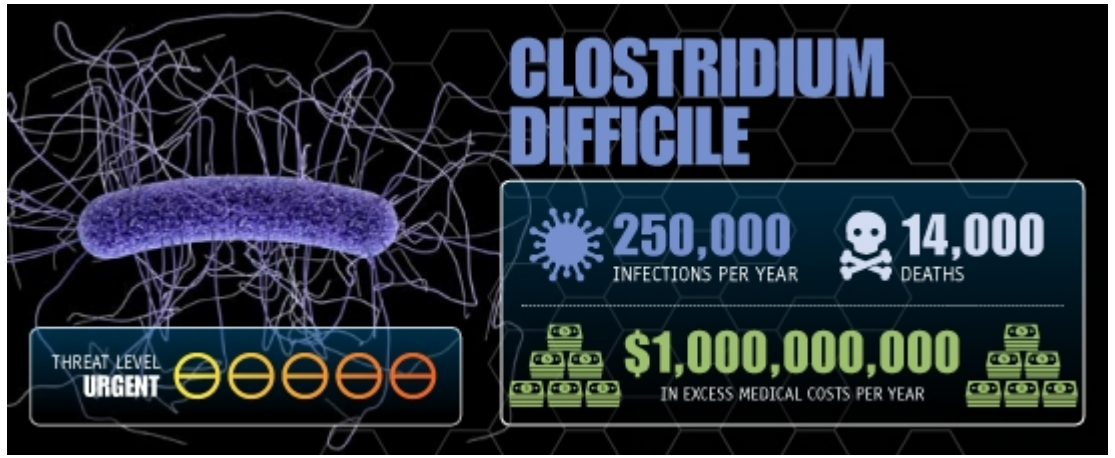
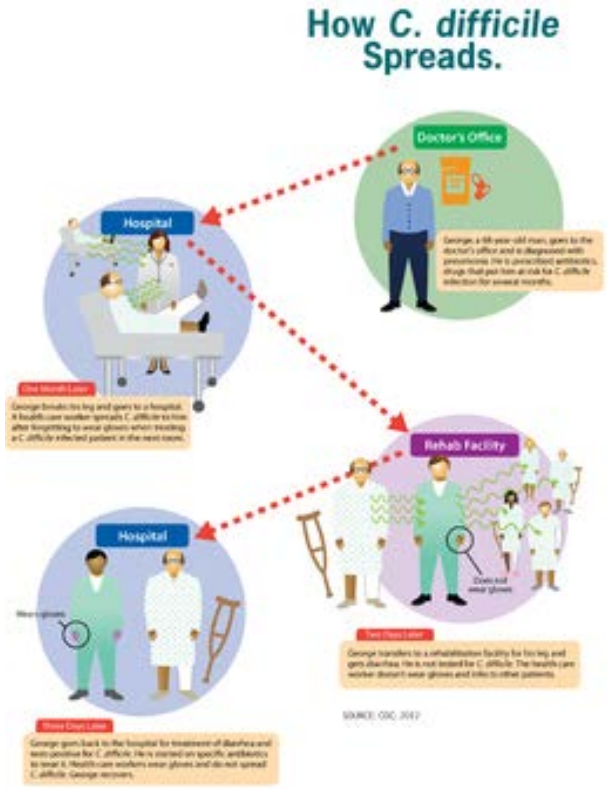
Clostridium difficile

- Symptoms: watery diarrhoea, fever, nausea and abdominal pain
- Contributes up to 20% of cases of antibiotic associated diarrhoea
- Complications: pseudomembranous colitis, toxic megacolon, perforation of the colon and sepsis





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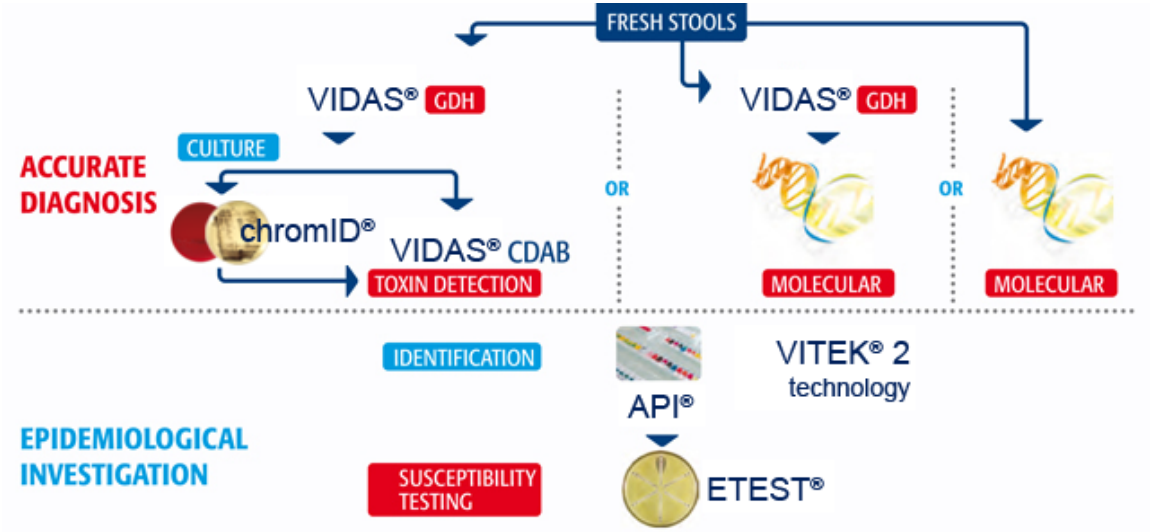
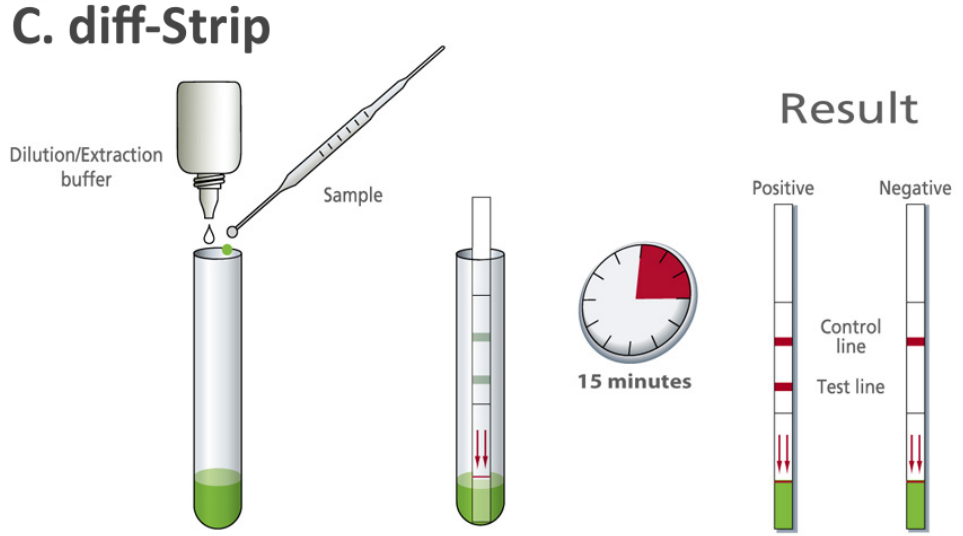
Diagnosis of *Clostridium difficile*

- Culture
- ELISA/POCT (antigen and toxin detection)
- Cell cytotoxicity assay(toxin detection)
- Molecular tests





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Clostridium difficile

- Introduced in 2009
  - 167 participants
    - 3 rapid assays
    - 2 EIA assays
    - 1 semi/automated assay
- 8 years later
  - 401 participants
    - 17 rapid assays
    - 7 EIA assays
    - 17 semi/automated/ molecular assays

	Distribution (Year)	
Assay	**2467 (May 2009)**	**4042 (March 2017)**
Beta Diag: Rapid Card	-	1
BIOHIT: GDH		1
C. difficile QuikChek (GDH)	-	18
CerTest GDH	-	1
CoproStrip	-	1
Corisbio: Clostridium K-SeT	-	3
Coris Strip	-	1
EuroClone: C.difficile GDH	-	1
Meridian: ImmunoCard	-	19
Meridian: ImmunoCard A+B	33	21
Proflow C. difficile GDH	-	17
Proflow C. difficile Tox A-B	-	5
QuikChek Complete	-	112
Techlab: C. diff Tox A/B QuikChek	17	30
Trinity : Unigold	-	1
Ridaquick	-	9
Remel: Xpect C. diff Tox A/B	1	1
Rapid total	**51**	**242**

Clostridium difficile

Assay	Distribution (Year) 2467 (May 2009)	4042 (March 2017)
CoproELISA GDH	-	1
Premier GDH	-	23
Premier Tox A+B	55	11
Prolisa C.difficile GDH	-	4
Ridascreen	-	6
Techlab: C. diff Chek	-	57
Techlab: C. diff Tox A/B II	27	37
EIA total	**82**	**139**

Assay	Distribution (Year) 2467 (May 2009)	4042 (March 2017)
Cell culture	12	3
Culture	15	50
Microgen : LA	-	3
Oxoid: C. diff Tox A	2	-
AmpliVue C. difficile	-	4
BD: GeneOhm	-	1
BD: MAX	-	13
BioMerieux: Vidas*	33	37
Cepheid: Xpert	-	110
Great Basin: C.difficile	-	1
Liaison C.difficile A&B	-	15
Liaison C.difficile GDH	-	12
Luminex xTAG	-	1
MALDI-TOF	-	4
Meridian: illumigene	-	25
PCR: Single target	-	1
Qiagen: Artus	-	1
Real-Time Multiplex	-	2
Real-Time Single target	-	7
Ridagene	-	1
Serosep: EntericBio	-	13
Unspecified	24	23
Culture based total	**29**	**56**
Semi/automated/ molecular total	**33**	**248**

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Summary : when is Molecular ID the best?

Molecular method

Advantages

- ✓ provides a rapid ID answer compared to culture (if detected directly from the clinical sample)
- ✓ Reduces TAT if potential pathogen identified from culture compared to conventional methods (e.g biochemical)
- ✓ Excellent for epidemiological data collection (to source outbreaks)

Limitations

- X cannot provide a battery of AST (only for those encoded by genes)
- X method unsuitable for monitoring the effectiveness of therapy
- X more expensive then conventional
- X requires highly skilled staff for performing and evaluating test results
- X interpretation of test result can be complicated

Thank you for listening!

Acknowledgements:
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