Molecular diagnosis of enteric pathogens: utility of a syndromic approach to infectious intestinal disease

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Declarations

- GPP materials and equipment (Luminex)
- Speakers fee (Luminex)

A perfect storm

- Rising demand on services
- 7 day working
- Budget constraint

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- Improving patient care

The future…..

- Low hanging fruit
  - “Salami slicing”
  - Budget silos

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- Low hanging fruit
  - “Salami slicing”
  - Budget silos
- vs
- New ways of working
  - Smarter
  - Faster
  - More efficient
  - Collaboration

NHS Tayside

- NHST Medical Microbiology Molecular Development & Implementation Group (MOLDI)
  - Aims: rationalise, simplify and improve testing across medical microbiology, through the use of molecular techniques. **Improve patient outcome**

Why faeces?

- Disease burden
  - 5000–9000 patients are admitted to a hospital with diarrhoea PA

- Infection control
  - up to 90% of cases non-infectious
  - 21% infection control time spent dealing with ?infectious diarrhoea [1]

- Patient care
  - Anecdotal evidence of unnecessary testing/interventions whilst awaiting microbiology test results

(1) HTA 18530

Why faeces?

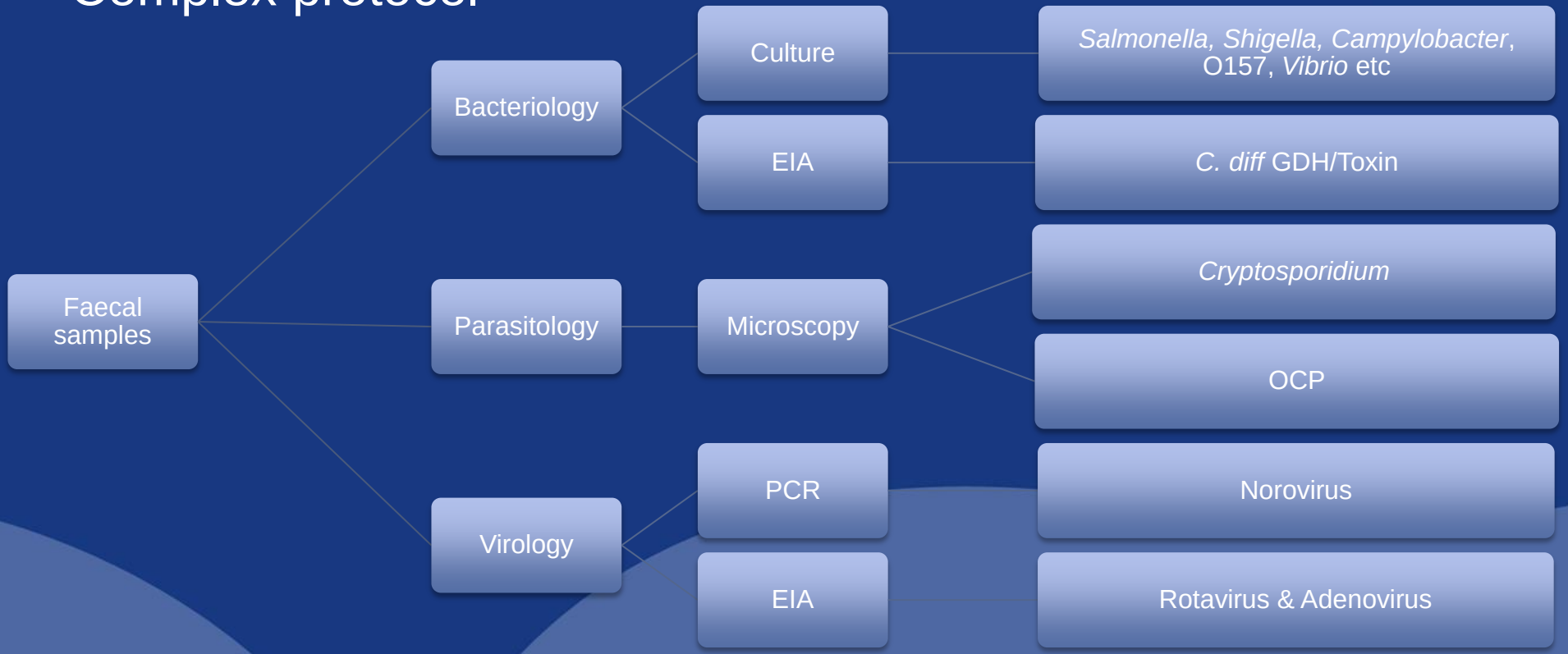
- Standard detection of GI pathogens relies on:

  - Microscopy
  - Culture
  - EIA
  - (+/- viral PCR)

Sensitivity?

Why faeces?

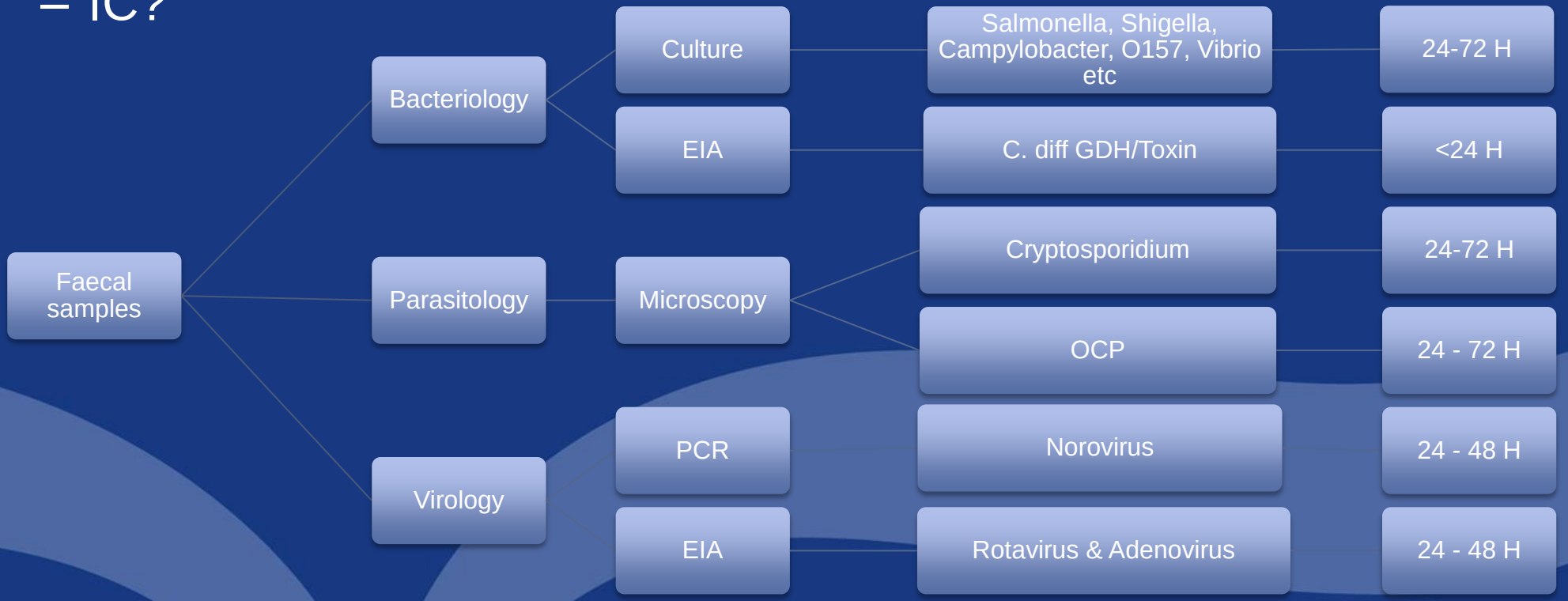
- Complex protocol



- Multiple technologies
- Little coordination
- Large numbers of well trained staff doing

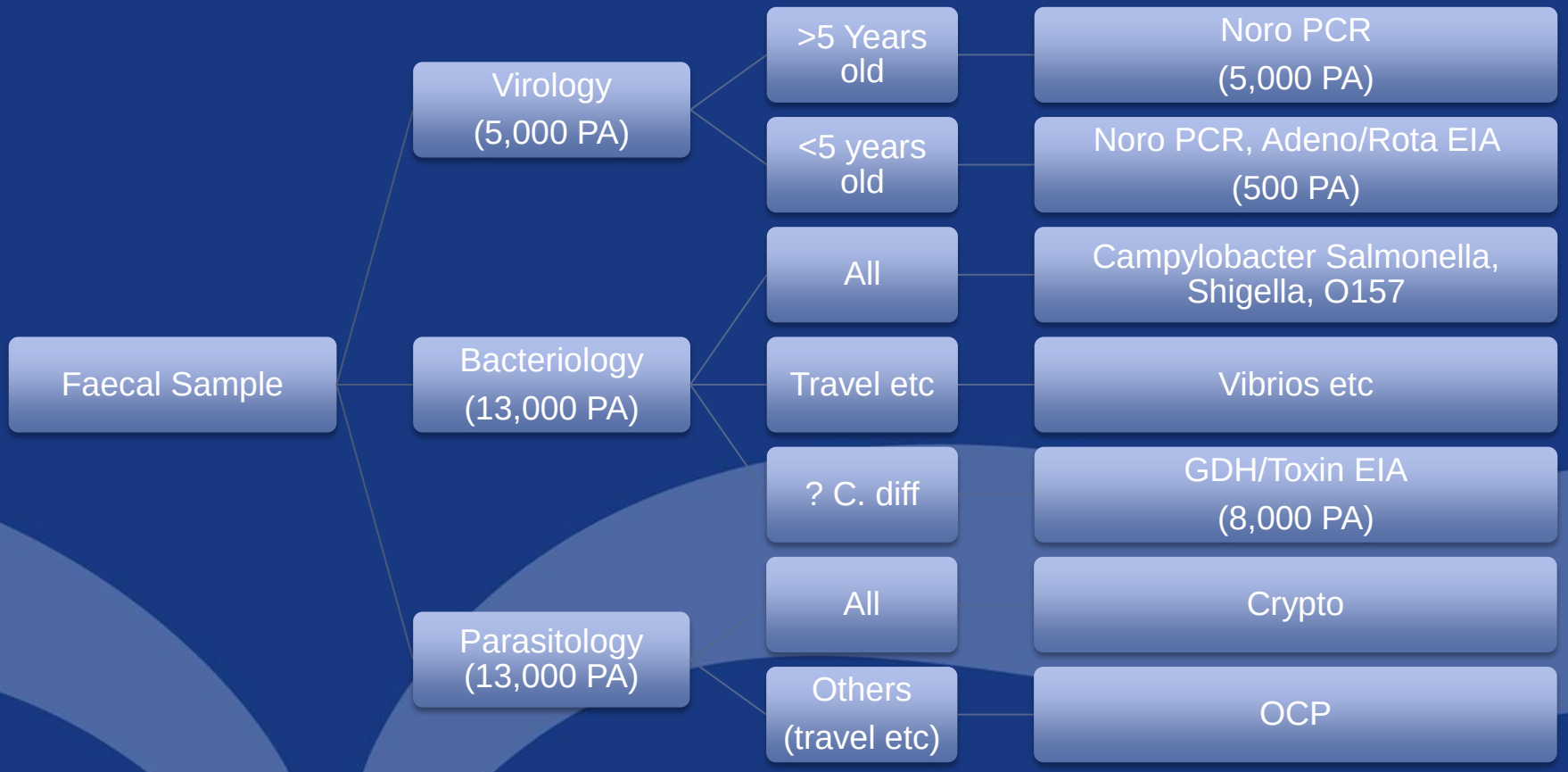
Why faeces?

- Time consuming
  - Patient care
  - IC?



Why faeces?

- Are we getting & testing the right samples?



Why GPP

- All patients tested for all pathogens
- Simplified protocol
  - One department
  - Less staff
- Faster
- More sensitive

Luminex GPP study

- Objectives
  - “Determine whether systematic syndromic testing of faecal samples with multiplex PCR increases diagnostic yield in patients with diarrhoea compared with conventional methods using a clinician initiated, selective testing, strategy”
  - “Analyse the cost, healthcare and infection control implications of introducing such technology”

Method: overview

- Single centre study
- Retrospective stored samples
- Prospective
  - Ninewells Hospital & Medical School, Dundee (NW)
    - In-patient and community samples
      - Routine methods vs Luminex GPP
        - » Sensitivity, specificity, PPV, NPV
    - In-patient only
      - Healthcare economics, IC, patient management

Methods: Microbiology

- Clinician request
- All samples:

  - *Campylobacter spp*
  - *E. coli O157*
  - *Salmonella spp*
  - *Shigella spp*

Methods: Microbiology

- Additional tests
- Clinician request and clinical history
  - C. difficile
    - All patients >5 (unformed)
  - Vibrio
    - Travel history & clinician request
  - Yersinia
    - Clinical details & clinician request
  - O157/STEC referral
    - patients with bloody diarrhoea, HUS & failed to yield another pathogen

Methods: Parasitology

- Clinician request
  - Cryptosporidium
    - All samples
      → Modified ZN stain

  - Parasites cysts & ova
    - Clinician request
    - Clinical history (e.g. prolonged diarrhoea, foreign travel)
      → Wet prep of concentrate

Methods: Virology

- Clinician request
  - Norovirus
    - All samples >5 YO (<5YO if Rotavirus & Adenovirus negative)

    → Norovirus GTI/II PCR

  - Rotavirus & Adenovirus testing
    - Specific request
    - Clinical details (<5 YO)

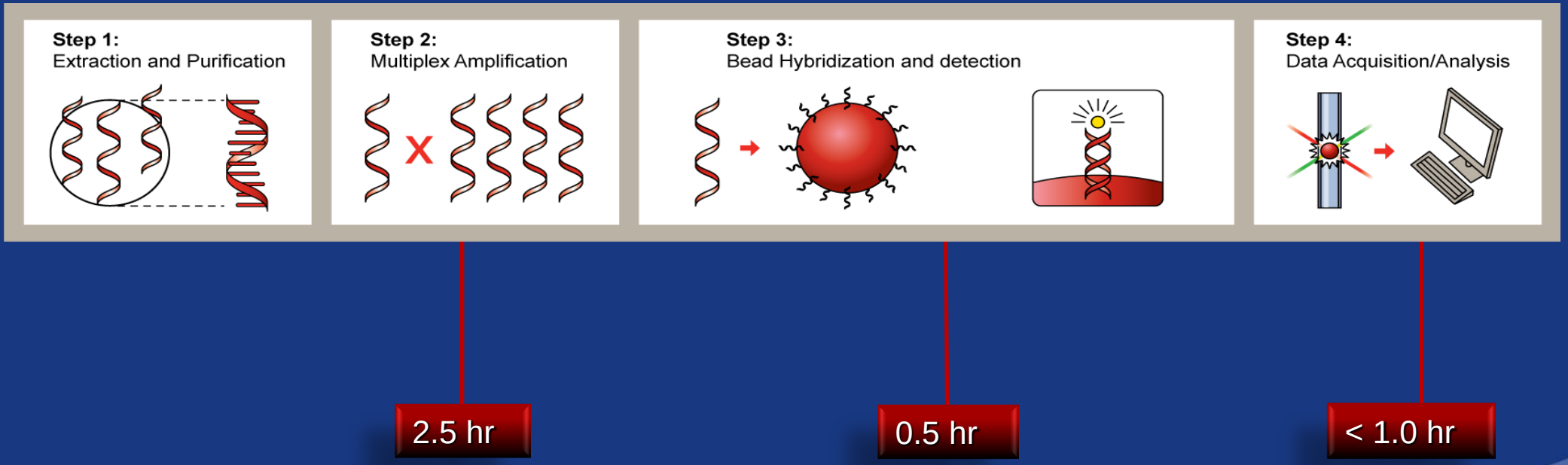
    →Lateral flow EIA

Methods: xTAG GPP

- Single sample
- All targets

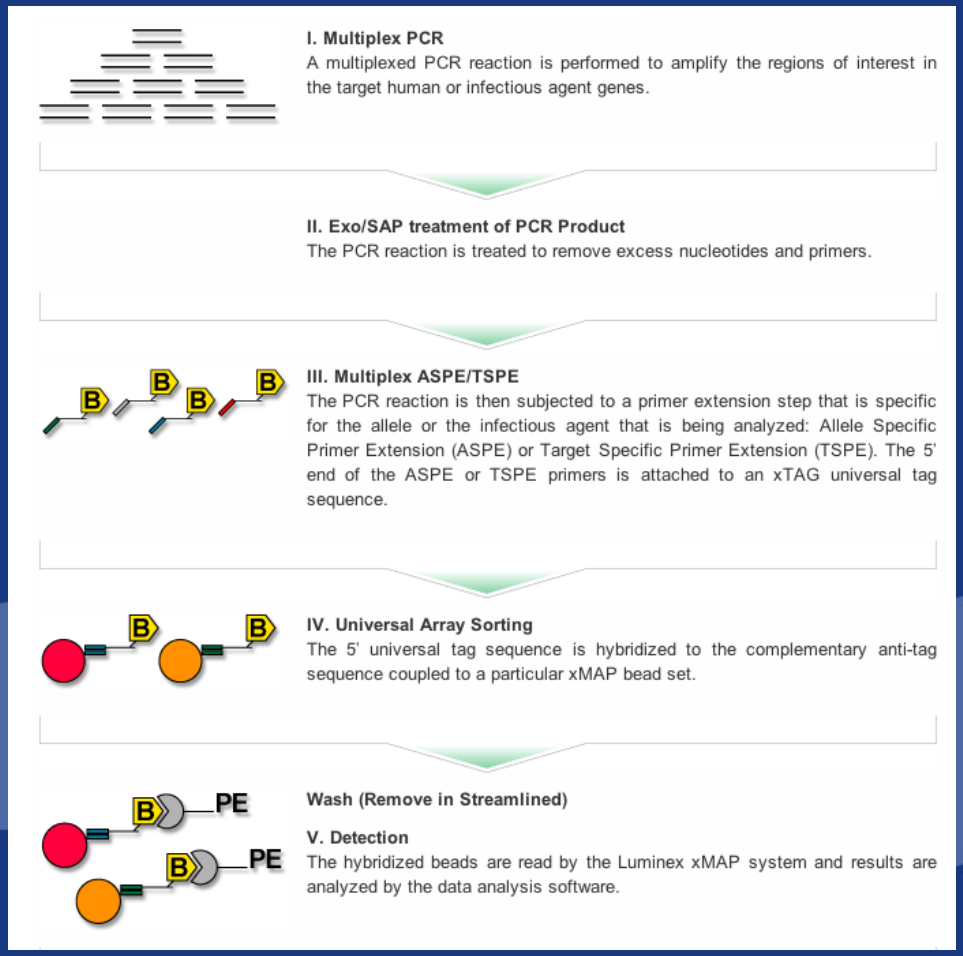
	Pathogen
Bacterial gastroenteritis	Campylobacter
	C. diff toxin A/B
	E. coli O157
	STX 1 & 2
	E. coli LT/ET
	Salmonella
	Shigella
	V. cholera
Viral gastroenteritis	Adenovirus
	Norovirus 1&2
	Rotavirus
Parasite gastroenteritis	Cryptosporidium
	E. histolytica
	Giardia lamblia

Methods: xTAG GPP



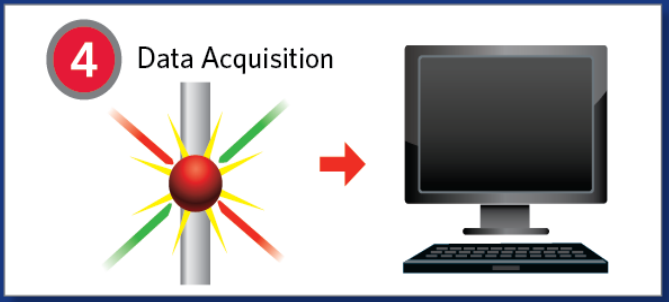
Methods: xTAG GPP

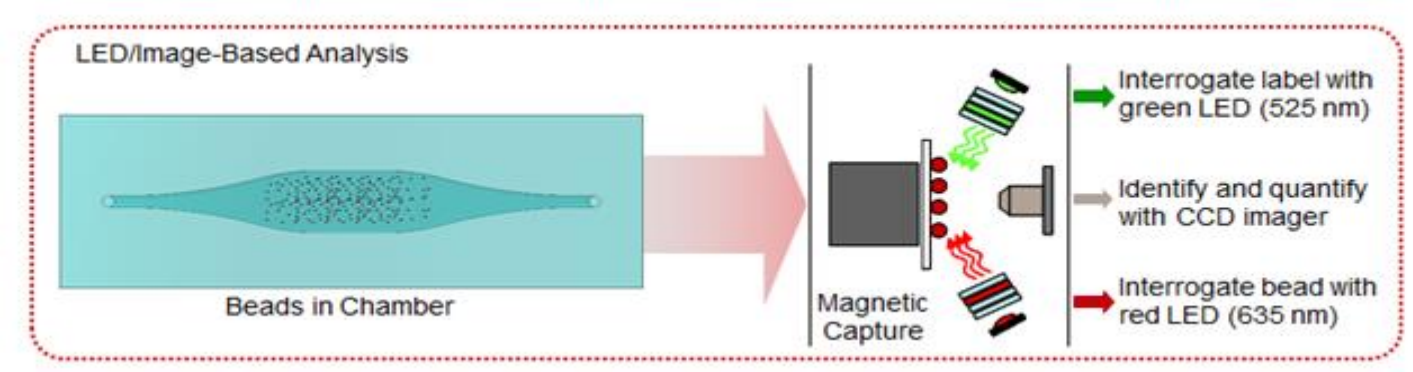
- Single sample
- All targets
- EasyMag extraction
- PCR
- Hybridisation
- Detection
- TAT c. 5H



xTAG® GPP Data Acquisition

NHS Tayside





Methods: discrepant results

- Discrepant testing
  - 2nd molecular test
    - FTD +/- reference lab
  - Study definition result validity assigned to each result:

Assignment	Abbreviation	Definition
True positive	TP	≥ 2/3 methods positive
False positive	FP	1/3 methods positive
True negative	TN	≥ 2/3 methods negative
False negative	FN	1/3 methods negative

Results: prospective testing

- 594 faeces samples tested by conventional methods & GPP
- Request type
  - Bacteriology requests = 442
  - Virology requests = 152
- Request location
  - In patient 59%
  - Out patient 41%
- Age
  - Average = 45.4 years (min’ 4 days, max’ 98 years)

Results: prospective

– Selective conventional testing

Standard Microscopy & Culture	Supplementary Culture	Supplementary Microscopy	Virology	GPP
98%	4%	13%	51%	100%

Results: prospective

- Inhibition rate
  - 4.7% initial testing
  - 0.3% following dilution

- 23 samples excluded as no confirmatory test available
  - Descriptive statistics available for 571 samples

Results: Virology

	Adenovirus		Norovirus		Rotavirus	
	Conventional	Luminex	Conventional	Luminex	Conventional	Luminex
True positive	2	4	10	13	3	6
False positive	0	3	0	2	0	0
True negative	567	564	558	556	565	565
False negative	2	0	3	0	3	0
Sensitivity	50.0%	100.0%	76.9%	100.0%	50.0%	100.0%
Spesificity	100.0%	99.5%	100.0%	99.6%	100.0%	100.0%
PPV	100.0%	57.1%	100.0%	86.7%	100.0%	100.0%
NPV	99.6%	100.0%	99.5%	100.0%	99.5%	100.0%

Results: Bacteriology

	Campylobacter		C. difficile	
	Conventional	Luminex	Conventional	Luminex
True positive	33	37	20	24
False positive	1	1	2	3
True negative	532	532	545	544
False negative	5	1	4	0
Sensitivity	86.8%	97.4%	83.3%	100.0%
Spesificity	99.8%	99.8%	99.6%	99.5%
PPV	97.1%	97.4%	90.9%	88.9%
NPV	99.1%	99.8%	99.3%	100.0%

Results: Bacteriology

	Salmonella		Shigella	
	Conventional	Luminex	Conventional	Luminex
True positive	7	8	2	3
False positive	0	2	0	2
True negative	563	561	568	566
False negative	1	0	1	0
Sensitivity	87.5%	100.0%	66.7%	100.0%
Spesificity	100.0%	99.6%	100.0%	99.6%
PPV	100.0%	80.0%	100.0%	60.0%
NPV	99.8%	100.0%	99.8%	100.0%

Results: Bacteriology

	O157		STX		E. Coli ET/LT	
	Conventional	Luminex	Conventional	Luminex	Conventional	Luminex
True positive	1	3	1	4		
False positive	0	0	0	0		
True negative	570	568	567	567		
False negative	2	0	3	0	4 x positive by GPP Negative by Culture Awaiting confirmatory testing	
Sensitivity	33.0%	100.0%	25%	100.0%		
Spesificity	100.0%	100%	100.0%	100%		
PPV	100.0%	100%	100.0%	100%		
NPV	99.6%	100.0%	99.5%	99.8%		

Results: Parasitology

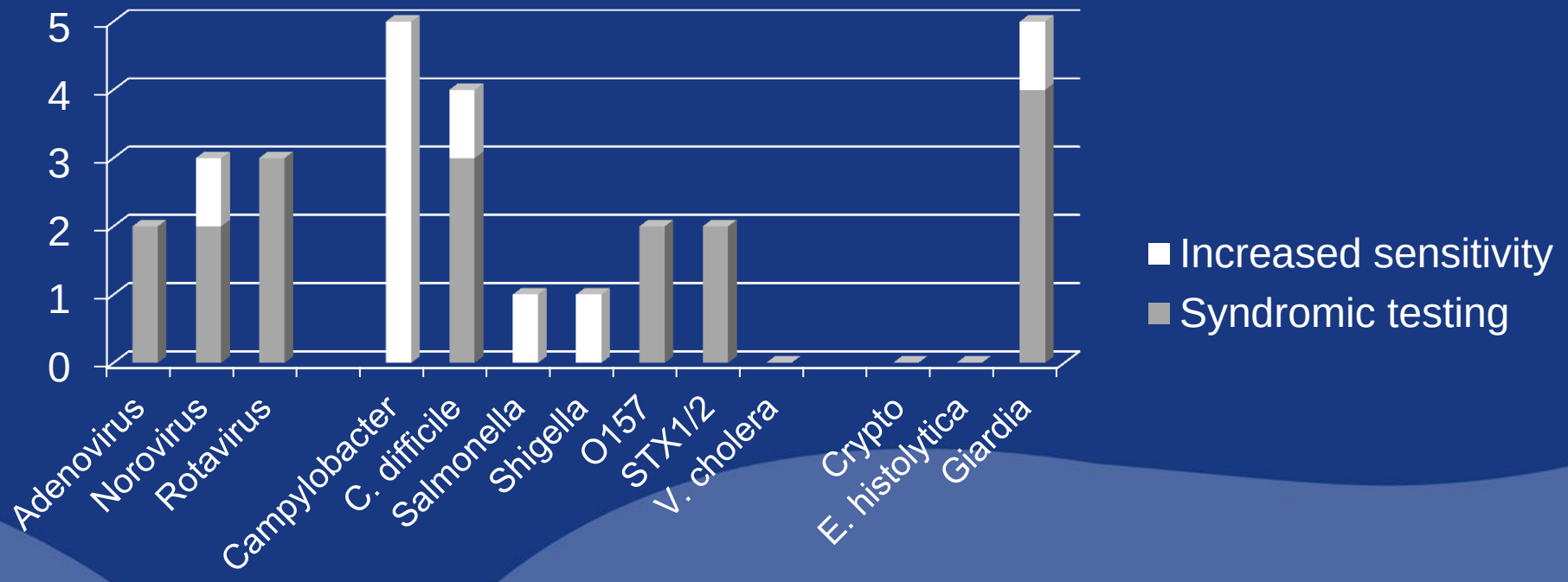
	Cryptosporidium		E. Histolytica		Giardia	
	Conventional	Luminex	Conventional	Luminex	Conventional	Luminex
True positive	1	1	0	0	0	5
False positive	0	1	0	1	0	0
True negative	570	569	571	570	566	566
False negative	0	0	0	0	5	0
Sensitivity	100.0%	100.0%	-	-	0.0%	100.0%
Spesificity	100.0%	99.8%	-	-	100.0%	100.0%
PPV	100.0%	50.0%	-	-	-	100.0%
NPV	100.0%	100.0%	-	-	-	100.0%

Results: summary

	Conventional	Luminex GPP
Total True Positives	80	108
Total False Positives	3	15
Total True Negatives	7885	7870
Total False Negatives	26	1
Sensitivity	73.4%	99.1%
Spesificity	100.0%	99.8%
PPV	96.4%	87.8%
NPV	99.6%	100.0%
Yield	14.0%	18.4%

- 28 additional true positive pathogens identified by GPP
- 18 false positives by GPP
- High NPV = screening method
  - ?Confirmation of positives

Results: additional positives



- 28 additional positives:
  - 64% (n=18) due to syndromic testing
  - 36% (n=10) due to increased sensitivity

Summary

- Luminex GPP
  - High NPV → screening assay +/- confirmation
  - Increased diagnostic yield
    - Syndromic testing & increased sensitivity
  - Staffing
    - 1/1.5 BMS Luminex GPP
    - 3 BMS conventional methods
  - Expensive compared with conventional methods
  - Potential for
    - Improved patient management
    - savings elsewhere within the hospital
      - » SILOS

Next steps

- IC analysis
- Cost/benefit analysis

Thank you

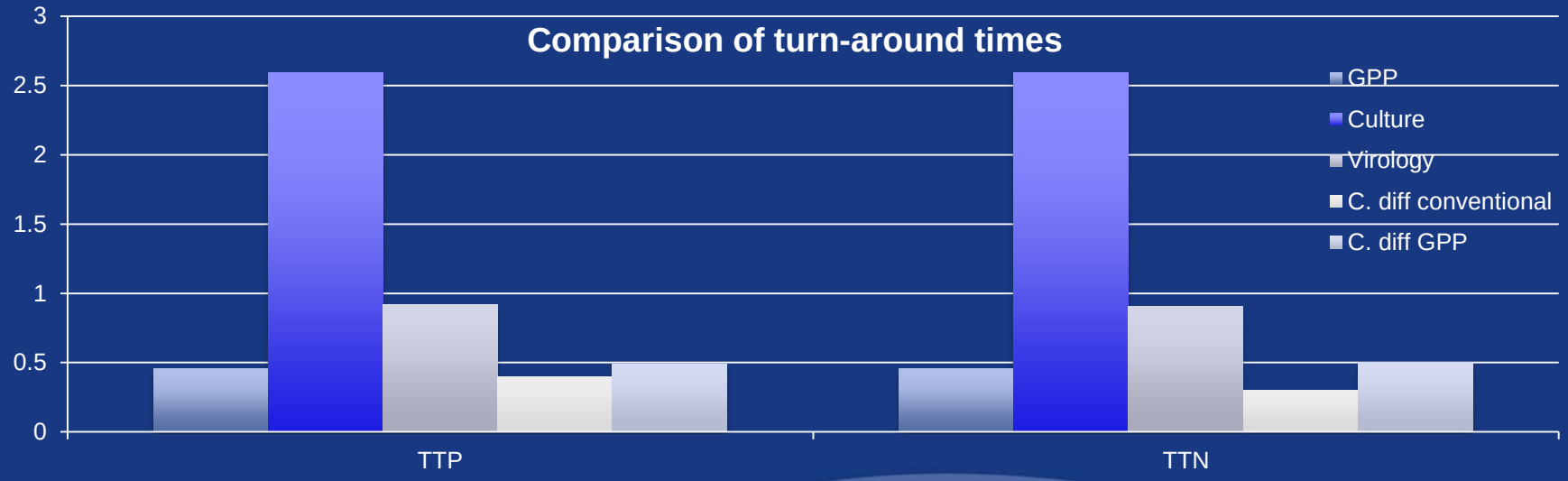
BMS staff, Ninewells Hospital
Clinical staff, Ninewells Hospital
Luminex

QUESTIONS?

Results: Turn-around time & repeat testing

	TTP	TTN
Luminex GPP	8.54	8.54
Culture & microscopy	62:14	62:14
Virology	22:03	21:40
C. diff conventional	09:32	9.32
C. diff luminex	11:42	11:59

Results: Turn-around time & repeat testing



Repeat samples previous/next 7 days	Percentage
0	58.6
1	22.1
2	11.6
3	6.6
4	1.1

21 % repeat samples received before culture results available

Results: clinical impact

- When comparing the TAT of the GPP assay to conventional methods (In-patients),
  - Potential to
    - Avoid 1 x endoscopy
      - » Campylobacter positive by GPP & culture
    - Amend 2 x cases antibiotic therapy earlier

- Infection control and cost analysis in progress

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Results: assay sensitivity vs syndromic testing

	Additional cases	Case	Improved sensitivity	Syndromic testing	Details
Giardia	5	1	No	Yes	No tested
		2	No	Yes	No tested
		3	No	Yes	No tested
		4	No	Yes	No tested
		5	Yes	No	Missed by microscopy

Results: assay sensitivity vs syndromic testing

	Additional cases	Case	Improved sensitivity	Syndromic testing	Details
Adenovirus	2	1	No	Yes	No virology sample
		2	No	Yes	No virology sample
Norovirus	3	1	Yes	No	Not detected
		2	No	Yes	No virology sample
		3	No	Yes	No virology sample
Rotavirus	3	1	No	Yes	No virology sample
		2	No	Yes	No virology sample
		3	No	Yes	Age >5

Results: assay sensitivity vs syndromic testing

	Additional cases	Case	Improved sensitivity	Syndromic testing	Details
Campylobacter	5	1-5	Yes	No	Culture negative
C. difficile	4	1	No	Yes	Not tested - formed
		2	No	Yes	Not tested - formed
		3	No	Yes	Insufficient
		4	Yes	No	EIA negative
Salmonella	1	1	Yes	No	Culture negative
Shigella	1	1	Yes	No	Culture negative
E. coli ET/LT	4	1-4	No	Yes	No tested by culture

Results: assay sensitivity vs syndromic testing

	Additional cases	Case	Improved sensitivity	Syndromic testing	Details
E. coli O157(STX – ve)	2	1	Yes	Yes	Not sent to ref' lab
		2	Yes	Yes	Not sent to ref' lab
STX 1/2 (non-O157)	3	1	Yes	Yes	Not sent to ref' lab
		2	Yes	Yes	Not sent to ref' lab
		3	Yes	Yes	Not sent to ref' lab