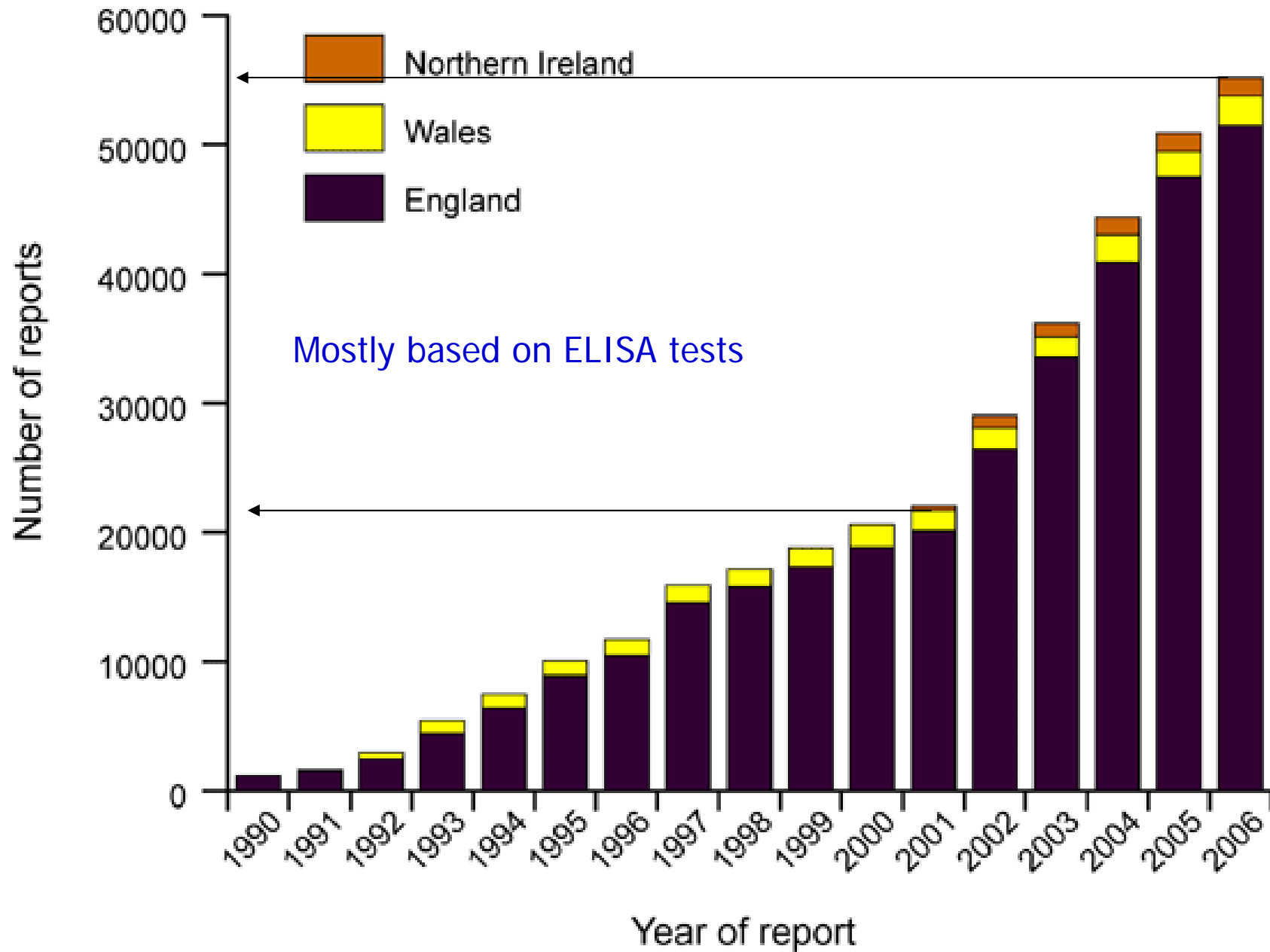


The laboratory diagnosis of
Clostridium difficile infection:
What's the real deal?

Mike Wren

Clinical Microbiology

UCH



Nosocomial acquisition and carriage of *C difficile*

No Patients tested on admission (culture)	428
No Patients positive on admission	29(7%)
No Patients acquiring CD after admission	83(19.4%)
No Patients remaining asymptomatic	52(12.1%)
No Patients developing CD infection	31 (7.2%)

No health care attendants having CD spores on hands 59%

Patients room environment contaminated with CD spores

49% in symptomatic patients
29% in asymptomatic patients

No patients with *C difficile* positive on discharge 68(15.9%)

? Risk from asymptomatic patients with *C difficile* colonisation

Asymptomatic patients tested	69
Patients carrying tox+ strains	35
Epidemic strains	13

Skin colonisation (chest / abdomen)	61%
Environmental contamination	59%

Predictive of asymptomatic carriage

Previous history of CDAD
Previous antibiotic therapy

Riggs et al. Clin Infec Dis 2007 45: 992-998

Current problems in the diagnosis of CDI

1. Faecal cytotoxin said to be the “gold standard” BUT
2. ELISA tests are often used as stand alone tests
3. Difficult to differentiate mild disease from the carrier state
4. Difficult to predict which patients will develop severe disease
5. Delayed faecal ELISA positivity occurs in some patients with acute and severe disease (**examples to follow**)
6. In hospitalised patients the number of “carriers” may far exceed truly diseased patients (63% vs 37%). These “carriers” may have large amounts of toxin in their faeces
7. Need to re-assess the laboratory approach to help diagnose diseased patients and to “predict” those who have or will develop serious disease

Faecal cytotoxin said to be Gold Standard

BUT

Differences in cell lines used
No declared optimal faecal dilution
No standard interpretation of cytotoxicity

Miss up to 30% of CDAD cases
In PMC patients Faecal Cytotoxin only detected 27/56 cases with PMC
Of the 29 negative faeces 9 were culture positive with toxigenic isolate

Johal et al, Gut, 2004

The commercial tests

ELISA:

Sensitivity of ELISA is approx 75-80% overall

They miss samples that are culture pos with a tox+ isolate

Delayed positivity in some patients

Tox A only ELISA misses those strains that are A-B+

GDH (Glutamate DeHydrogenase):

Common antigen in all CD strains

Does NOT distinguish Tox- from Tox+ strains

GDH offers high negative predictive value (99.2% Fenner et al; 100.0% Wren)
(mouse monoclonal Ab against the CD GDH)

Lactoferrin (not specific for CD)

Indicator of intestinal inflammation (key role in how quickly the disease progresses to colitis)

High levels in patients with severe/advanced CDAD compared to mild or no disease

Toxin assays: Merits and shortcomings

Test for toxin	Advantages	Disadvantages
Tissue Culture	“Gold Standard”? Most Sens & Spec	24-48Hr Relatively expensive technically exacting
EIA	Standard in most labs Commercial kit form Good specificity/ cheap 2-4Hr	Only ~75% sensitive
Culture	Sensitive/specific	48-72Hr
Culture + toxin on cols Common Ag / Toxin EIA	Sensitive & specific	48-72Hr 4Hr?

What test and when?

GDH could be used as a sensitive “screening” test

Positives then go forward for **A/B ELISA**

ELISA and culture

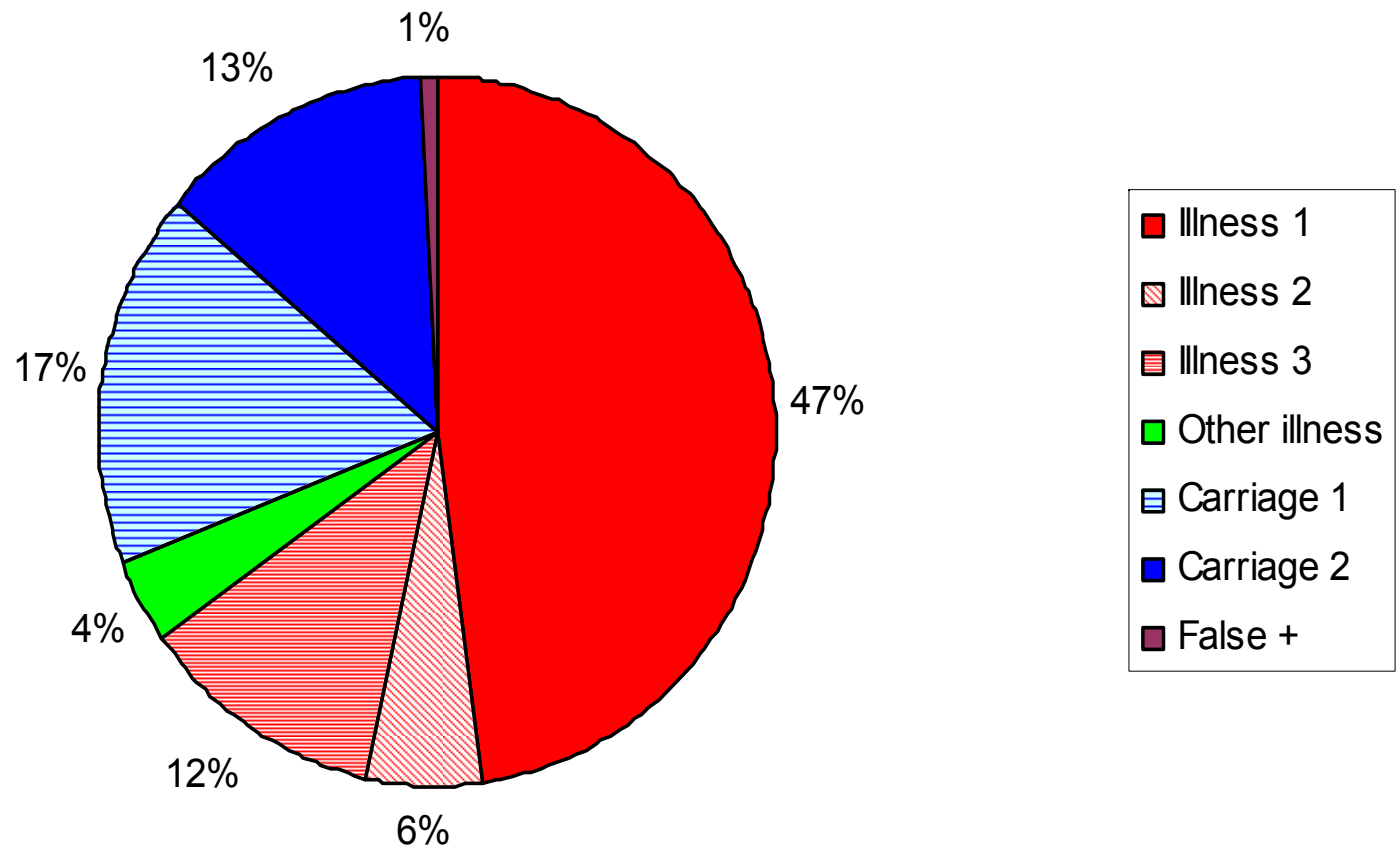
A/B ELISA and parallel culture; ELISA +ves look at clinical data

ELISA – culture pos with a tox+ strain look at clinical data

Accurate testing becoming more important especially when the role of CD is explored in other intestinal diseases

(eg. IBD, Hirschsprung’s, Chron’s, UC,etc.)

Role of a marker for intestinal inflammation?



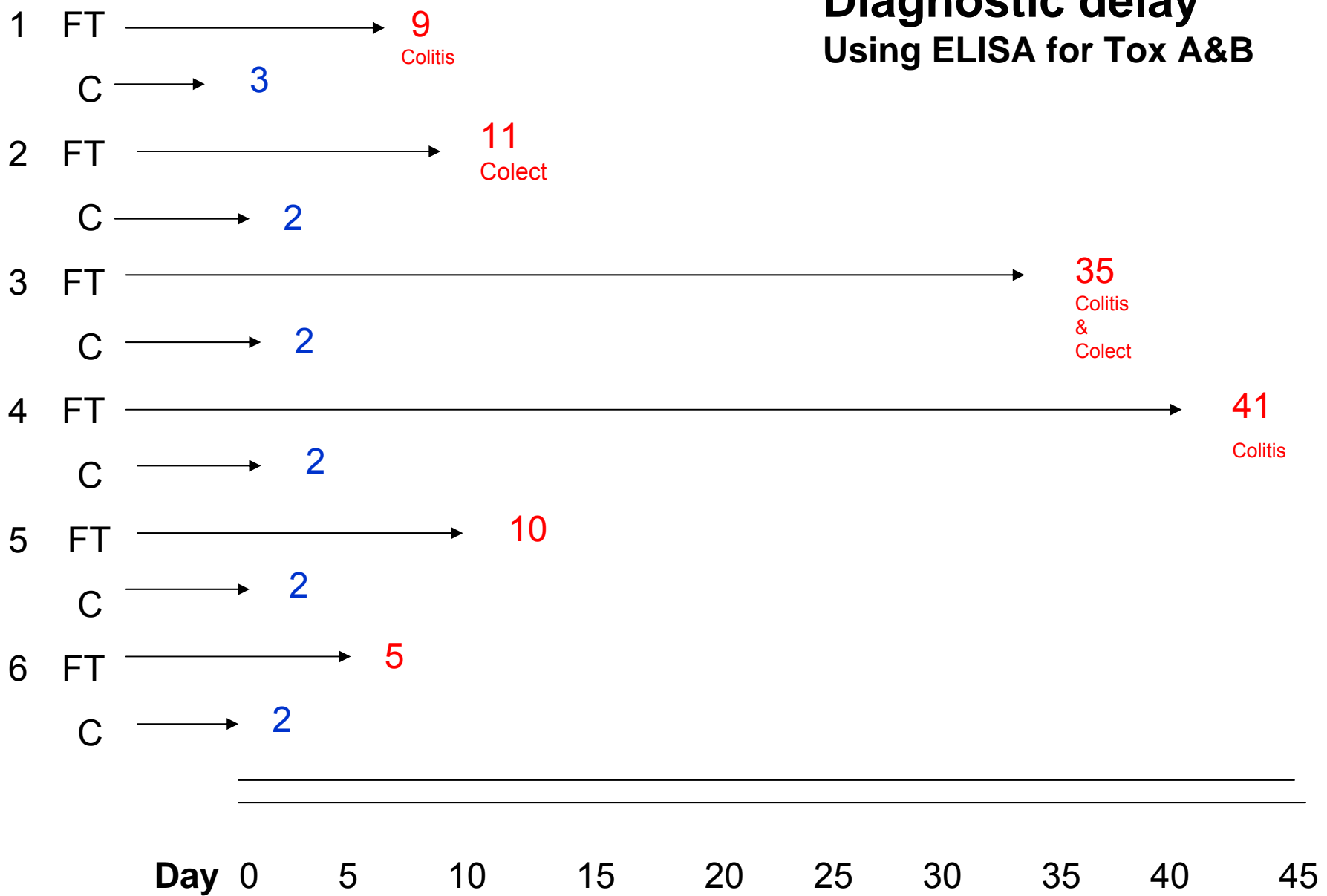
Correlation of Faecal Lactoferrin with Peripheral WBC Count*

	>15000	4-15000	<4000
Lactoferrin +	67	9	0
Lactoferrin -	1	21	12

*Taken at the time of faecal submission

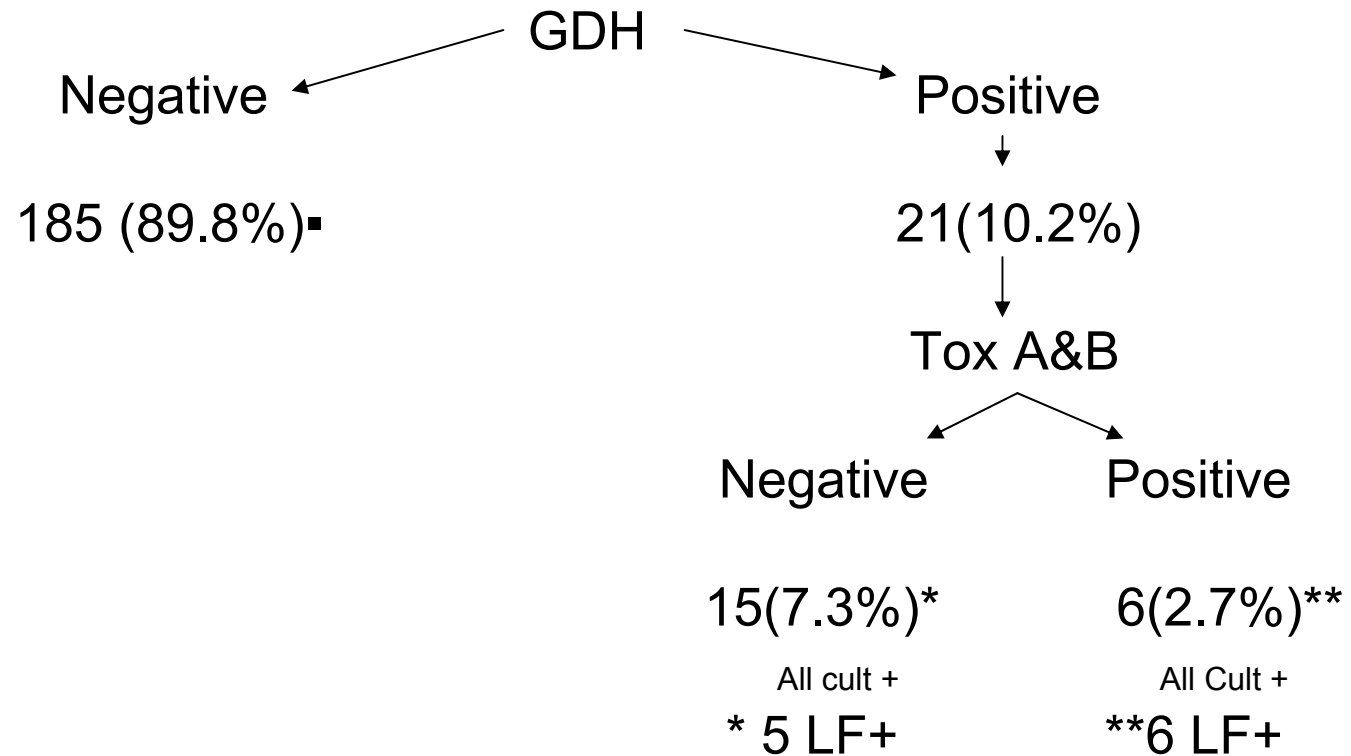
Patient

Diagnostic delay
Using ELISA for Tox A&B



The use of CD antigen (Glutamate DeHydrogenase) as a screening test

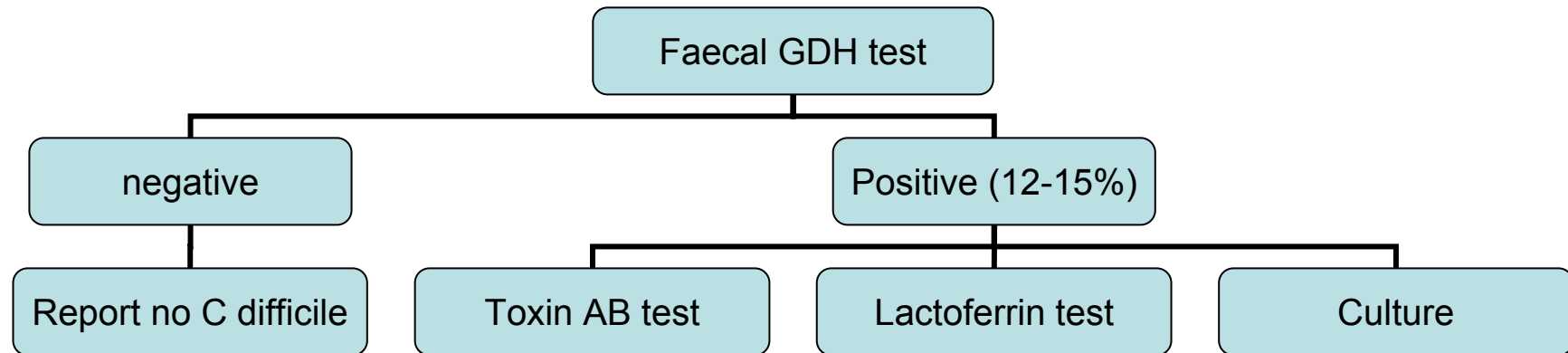
UCH patients (205)



Comparative studies of GDH assay

	% faeces negative	% faeces positive	Sensitivity	NPV
Fenner et al	87.2	12.8	93.4%	99.2%
Wren (Prelim)	89.8	10.2	94.7%	99.4%

CDI testing algorithm



Established/

severe CDI

+

+

+

Probable CDI

+

-

+

(Possible carriage)

Carriage?

-

-

+

Conclusions and future plans

ELISA's are not stand alone tests

Repeat testing may be required to establish a diagnosis of CDI

A diagnostic algorithm needs to be established for the diagnostic laboratory

GDH offers a preliminary screening test with a high NPV to rule out the presence of *C difficile*

Toxin tests may then be restricted to GDH positives

Lactoferrin may offer additional information particularly those patients who have or who are developing serious/ severe disease

Planned future

1. Aim is to look at 2000-3000 patients (one year)
2. Screen all with GDH (vs culture)
3. Test all GDH positives for toxins A&B and for Lactoferrin. Relate findings to clinical condition of the patient
4. Follow those who are GDH+ Tox- initially with weekly tests and culture
5. If the toxin test becomes positive; go back to the sequential isolates for investigation