



LEIDS UNIVERSITAIR MEDISCH CENTRUM

# *Challenges and New Developments in Diagnosing Gastro-enteritis using Multiplex PCR*

*Eric C.J. Claas*



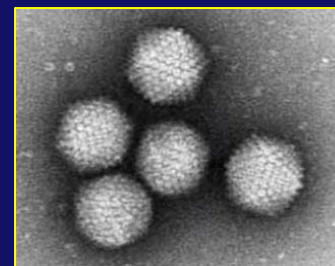
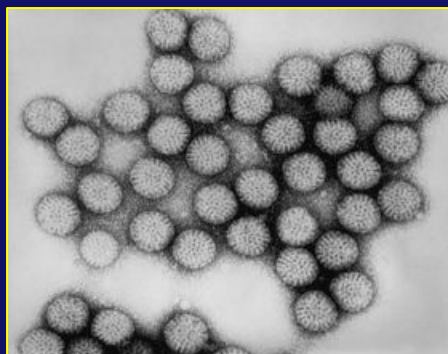
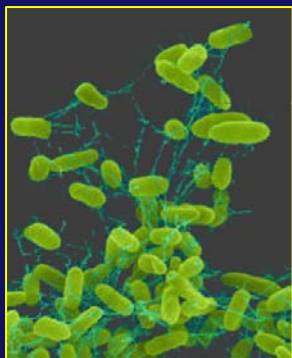
- Results as presented here will not be achieved in your lab for the simple reason that Luminex has been adapted the assay following these data.

## *Infectious gastroenteritis: diarrhea*



- Significant mortality worldwide
- In the EU: over 200 million cases per year.

*Main diagnostic challenge: the etiology of gastro-enteritis*



## *Clinical diagnosis of GE*

- Symptoms and appearance
  - bloody, watery, mucoid stool
- Duration of complaints
- Community or hospital acquired
- Relation to food or travel
- Use of antibiotics
- Underlying disease

*Laboratory based methods for correct diagnosis*

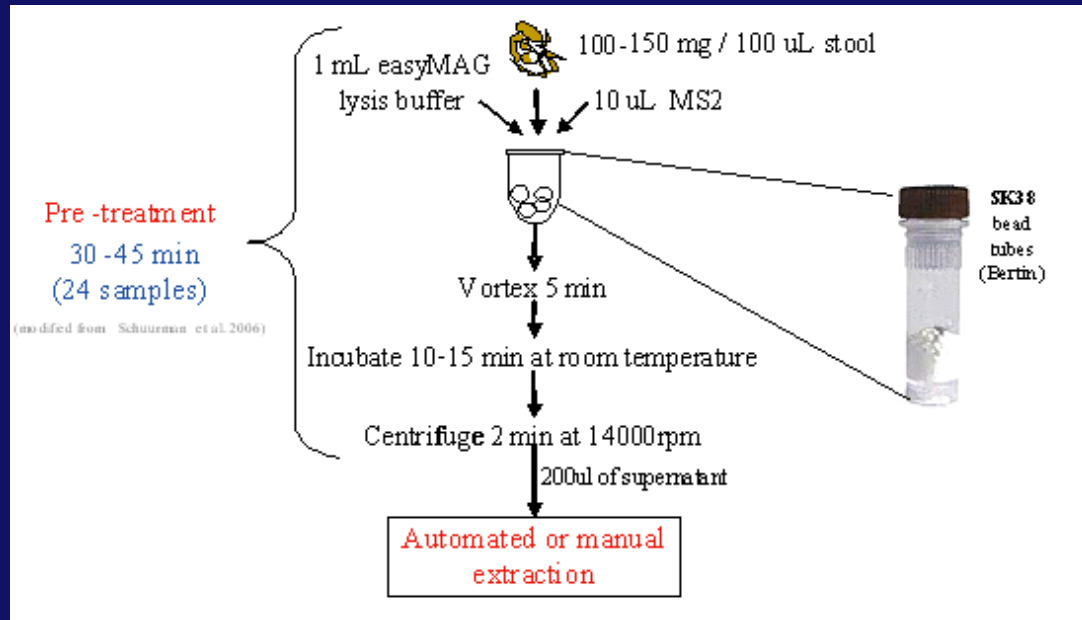
## *Diagnosis of infectious GE at the Leiden UMC*

- **Bacteria** (*25% of feces is bacteria*)
  - Culture on selective media: ID, antibiogram
  - Real-time PCR
  
- **Parasites**
  - Microscopy (TFT)
  - Multiplex real-time PCR (since 2006)
    - Verweij et al. J.Clin.Microbiol.2004
  
- **Viruses**
  - Antigen detection assays
  - Multiplex real-time PCR (since 2007)
    - Van Maarseveen et al. J. Clin.Virol.2010



- Luminex MD xTAG® Gastro-intestinal Pathogen Panel:
  - single tube amplification and detection of 15 bacteria, parasites and viruses
- Multicenter study:
  1. Mount Sinai Hospital, Canada
  2. St Louis Children' Hospital, USA
  3. Edinburgh Royal Infirmary, Scotland
  4. Leiden University Medical Center, Netherlands

# Luminex MD xTAG® Gastro-intestinal Pathogen Panel: Universal NA isolation

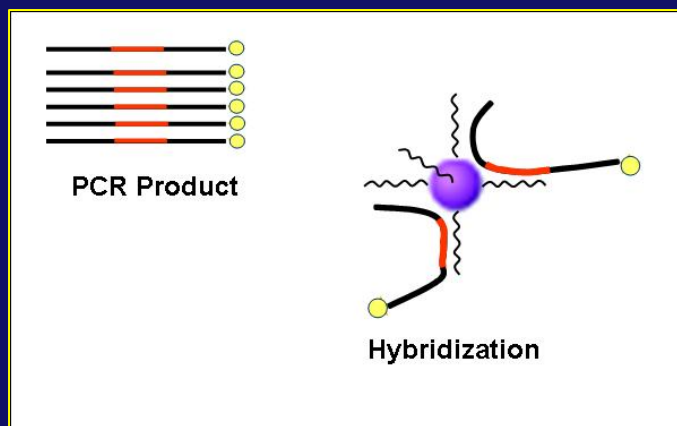
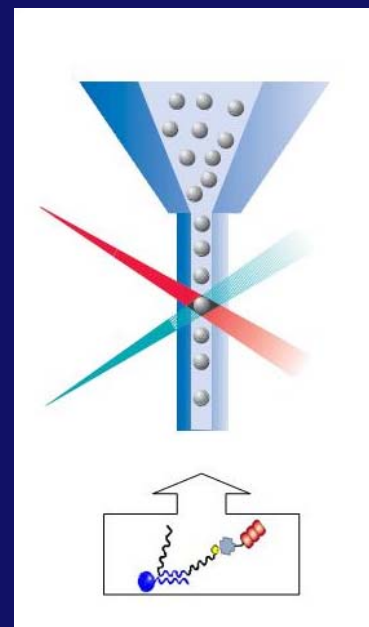
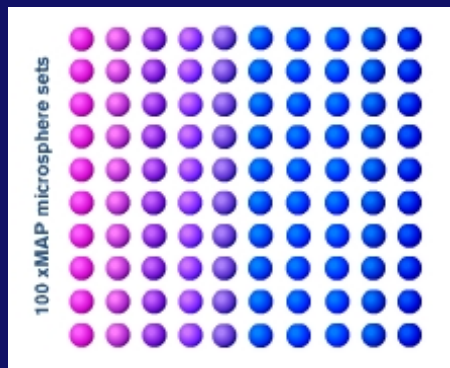


- Easy Mag (BioMerieux), Qiasymphony or columns (Qiagen)
- MagNApure (Roche): pretreatment step in MP lysis buffer
- Result: purified Nucleic Acid with MS2 RNA internal control

# Single one step multiplex cDNA and PCR amplification

Organism	Target
<b>Adenovirus</b>	Adenovirus 40 and 41
<b>Rotavirus</b>	Group A
<b>Norovirus</b>	Group I and II (differentiation possible)
<b><i>Clostridium</i></b>	<i>C. difficile</i> and toxin A and B (differentiation possible)
<b><i>Salmonella spp.</i></b>	<i>S. enteritidis</i> , <i>S. typhi</i> , <i>S. typhimurium</i> , <i>S. paratyphi</i> and <i>S. cholerasuis</i>
<b><i>Shigella spp.</i></b>	<i>S. dysenteriae</i> , <i>S. sonnei</i> , <i>S. boydii</i> and <i>S. flexneri</i>
<b><i>Campylobacter</i></b>	<i>C. jejuni</i> , <i>C. lari</i> , <i>C. coli</i> , and maybe <i>C. doylei</i> and <i>C. upsaliensis</i>
<b><i>E. coli</i> O157</b>	<i>E. coli</i> O157 specific gene and <i>stx1</i> , <i>stx2</i> (differentiation possible)
<b><i>E. coli</i> enterotoxic</b>	Heat stable (ST) and labile (LT) enterotoxins (differentiation)
<b><i>Yersinia</i></b>	<i>Y. enterocolitica</i>
<b><i>Vibrio</i></b>	<i>V. cholerae</i>
<b><i>Giardia</i></b>	All relevant species
<b><i>Cryptosporidium</i></b>	All relevant species
<b><i>Entamoeba</i></b>	<i>E. histolytica</i>

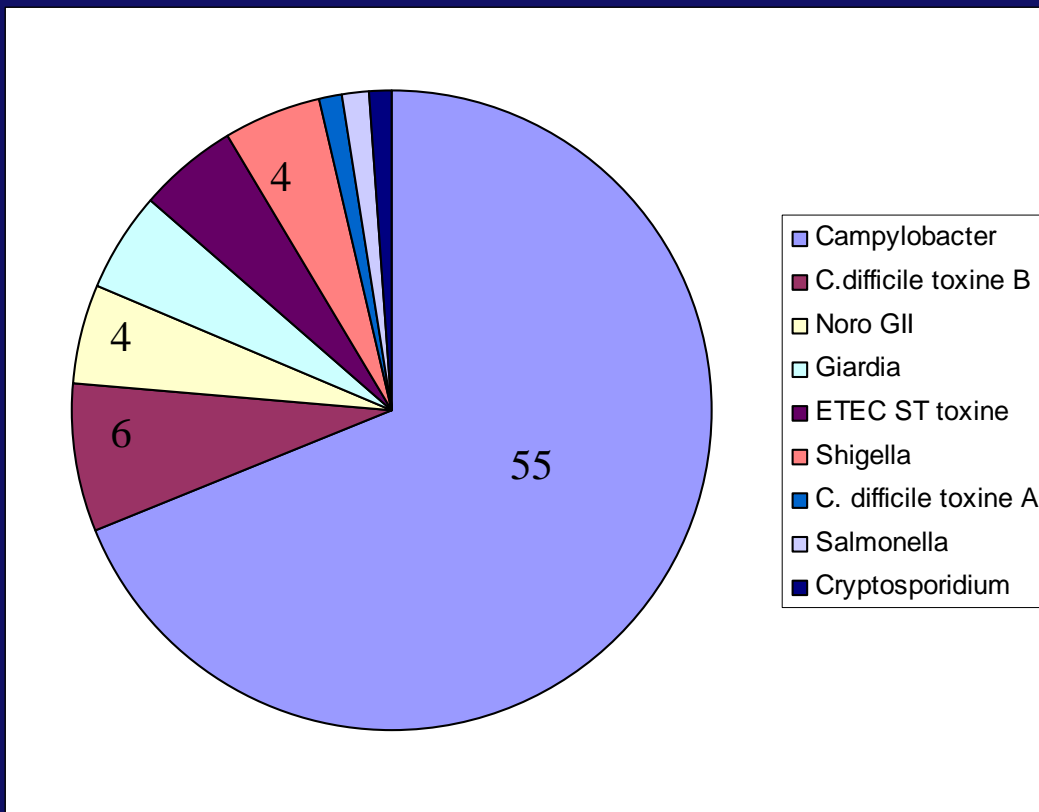
# Detection using Luminex beads



- 300 fecal samples
  - 200 consecutive samples (dd bacteria, virus and parasite) from June to August 2010
  - 100 selected samples
    - 50 known positives from LUMC
    - 50 samples from a general hospital (25 culture positive and 25 negative)
- compare xTAG® -GPP results to conventional diagnosis
  - Bacterial culture and multiplex real-time PCR

## *Results 200 prospectively collected samples*

- 62 out of 200 samples (31%) were positive
- 80 pathogens were detected



## *Results 200 prospective samples*

### Mixed detection of pathogens

#### Campylobacter: (12/200)

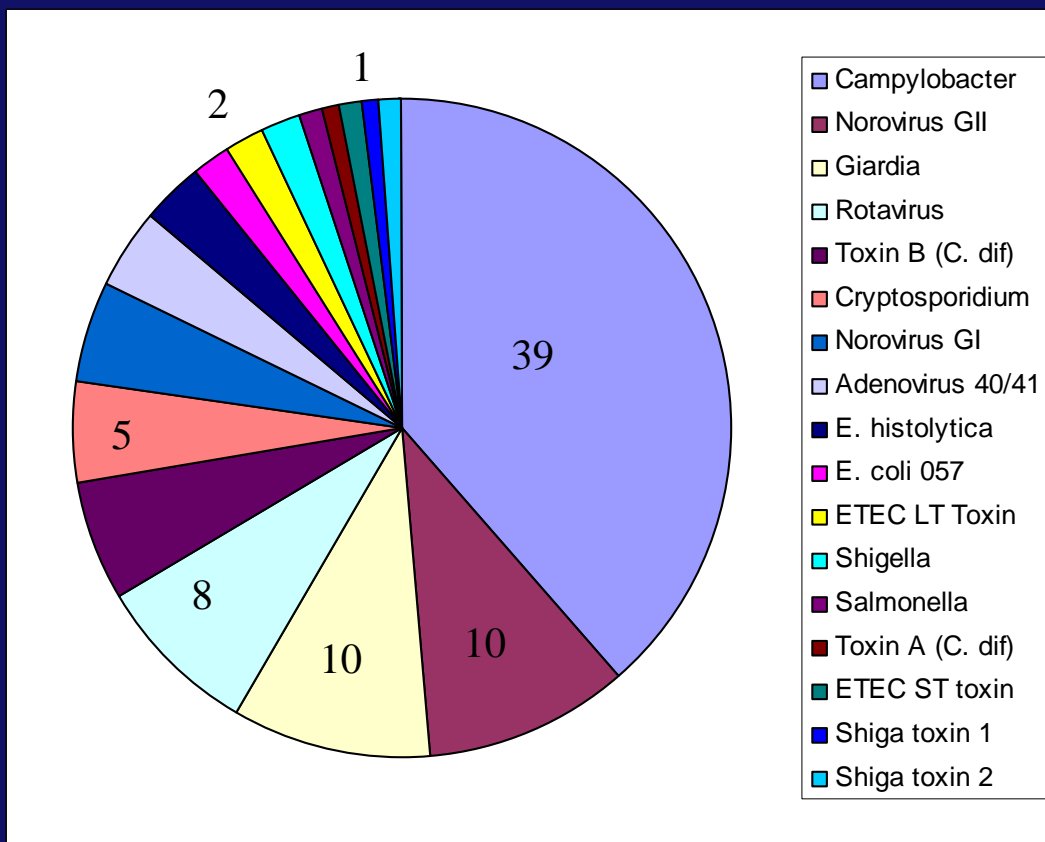
- Norovirus GII (1/200)
- C. dif toxin B (1/200)
- C. dif toxin B + ETEC ST toxin + Norovirus GII (1/200)
- C. dif toxin B + ETEC ST toxin (1/200)
- Salmonella (1/200)
- Shigella (3/200)
- Cryptosporidium (1/200)
- Giardia (3/200)

#### Other dual detections

- C. dif toxin A + B (1/200)
- C. dif toxin B + ETEC ST toxin (1/200)
- ETEC ST toxin + Shigella (1/200)

# Results selected samples

- 74 out of 100 samples (74%) were positive
- 101 pathogens were detected



## *Compare to LUMC diagnostic results*

- Bacteria
  - Culture
  
- Parasites
  - Feces in 2% PVPP,
  - o/n incubation in lysis plus protease
  - MagNApure LC isolation, Bact III kit (Roche)
  
- Viruses
  - Feces in 2% PVPP
  - MagNApure LC isolation, HP kit (Roche)

## *Confirmation xTAG results: bacteria*

- Culture positive results were confirmed with xTAG

### However:

- Two Salmonella positive (xTAG) results were “viral diarrhea requests”: no culture performed.
- 3/6 luminex Shigella positive (xTAG) samples were “parasite PCR requests”, 3/6 were subjected to culture with 2 positive and 1 negative result.

## *Comparison results for Campylobacter*

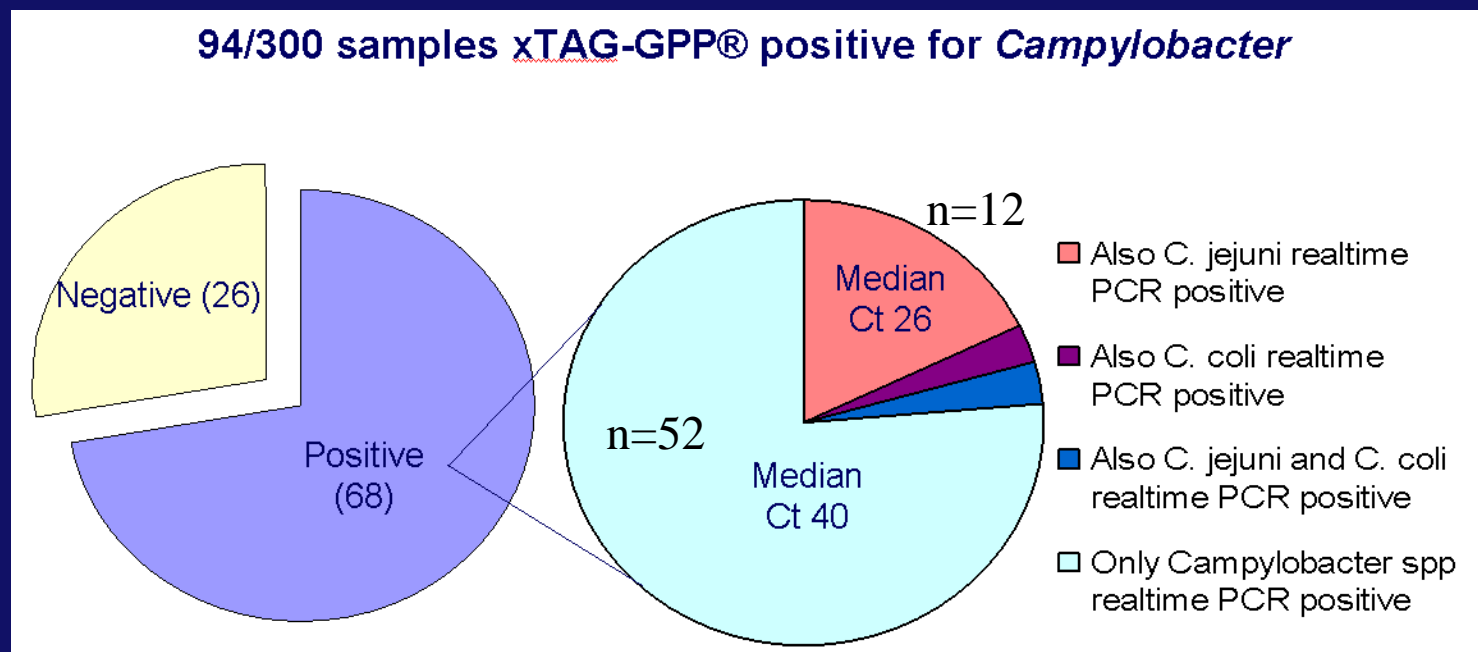
94 out of 300 samples (31%) xTAG positive for Campylobacter

Campylobacter	xTAG pos	xTAG neg
Culture positive	10	0
No culture requested	43	197
Culture negative	26	68
No information on culture	15	35

## Confirmation Luminex results: *Campylobacter*

Realtime PCRs targeting:

- *Campylobacter* spp (16s rRNA gene)
- *Campylobacter jejuni* (mapA gene)
- *Campylobacter coli* (ceuE gene)



# Comparison of xTAG results to realtime PCR

## Viruses

	xTAG + PCR pos	xTAG pos	PCR pos
Norovirus G1	5 (Ct 15 – 29)	0	0
Norovirus G2	13 (Ct 18 – 39)	1	3 (Ct 32*, 36, 39)
Rotavirus	8 (Ct 15 – 28)	0	0
Adenovirus	4 (Ct 15 – 17)	0	2 (Ct 36, 38)

\* xTAG Giardia positive

## Parasites

	xTAG + PCR pos	xTAG pos	PCR pos
Giardia	13 (Ct 20 – 38)	1*	0
Cryptosporidium	6 (Ct 26 – 34)	0	3 (Ct 33, 36, 38)
E. histolytica	3 (Ct 29 – 33)	0	3 (Ct 28, 35, 36)**

\* PCR NoroGII positive, Giardia negative

\*\* xTAG extracts were PCR positive

## *Results: Inhibition in the assay*

### xTAG®-GPP

- 33 of 300 (11%) samples were inhibited
- 12/300 (4%) samples remained inhibited after testing a 1:10 dilution of the nucleic acid

### LUMC method (NA isolation and real-time PCR):

#### *Viruses (DNA + RNA):*

- 56/300 (19%) samples inhibited
- 13/300 (4%) remained inhibited after testing 1:10 dilution

#### *Parasites (DNA):*

- 3/300 (1%) samples inhibited in undiluted test
- all resolved after 1:10 dilution

## Conclusions

- Using xTAG®-GPP more pathogens are being detected in comparison to our current diagnostic procedures
  - Mainly as a result of the diagnostic procedure that is being requested
  - Poor discrimination based on clinical presentation
- Majority of xTAG®-GPP positive results are confirmed by other assays
  - Some low positive real-time PCR results not detected
  - One *E. histolytica* (Ct 28) not detected
- For *Campylobacter* many weak positive results
  - Clinical relevance weak positive *Campylobacter*s?
  - xTAG format has changed format to detect *C. jejuni*, *C. coli* and *C. lari*

## Overall results multicenter study (901 samples)

*Data generated with updated kit and provided by Luminex MD*

Target (Analyte)	Sensitivity	Specificity
<i>Salmonella</i>	84.6% (66/78)	98.8% (423/428)
<i>Shigella</i>	97.7% (43/44)	97.8% (451/461)
<i>Campylobacter</i>	97.5% (120/123)	97.7% (438/448)
<i>Clostridium difficile</i> Toxin A/B	97.7% (44/45)	94.8% (424/447)
ETEC LT/ST	N/A	97.3% (288/296)
<i>Escherichia coli</i> 0157	88.2% (15/17)	98.8% (407/412)
STEC stx1/stx2	100% (14/14)	99.0% (292/295)
<i>Yersinia enterocolitica</i>	N/A	100% (500/500)
<i>Vibrio cholerae</i>	N/A	100% (414/414)
<i>Giardia</i>	100% (22/22)	97.5% (835/856)
<i>Entamoeba histolytica</i> <sup>2</sup>	100% (6/6)	98.9% (644/651)
<i>Cryptosporidium</i>	91.7% (22/24)	99.9% (853/854)
Rotavirus A	94.7% (18/19)	99.7% (852/854)
Adenovirus 40/41	100% (9/9)	100% (235/235)
Norovirus GI/GII	93.5% (72/77)	97.0% (771/795)

- Updated xTAG®-GPP
  - Campylobacter adjusted to *C. jejuni*, *C. coli* and *C. lari* detection
  - Additional Salmonella primer set
  - No discrimination norovirus G1 and G2; ETEC ST and ETEC LT; Stx 1 and Stx2
  
- Reanalysis of discrepant results using the updated kit
  
- Validation plan for transition to generic testing.

# *Pro- and con's of xTAG-GPP*

## Advantages

Universal NA isolation for all targets

Easy analysis of results

General information on GE etiology

## Disadvantages

Post PCR processing required

No quantitative information



Hands on time?

Costs?

- Leiden University Medical Center, Dept Microbiology

*Lisette Rusman*

*Mario van Bussel*

*Els Wessels*



- St. Franciscus Hospital, Rotterdam

*Peter de Man*

- Luminex Molecular Diagnostics, Canada

*Lan Lan*

*David Himsworth*