# Development of a rapid DNA extraction protocol for qPCR detection of foodborne pathogens

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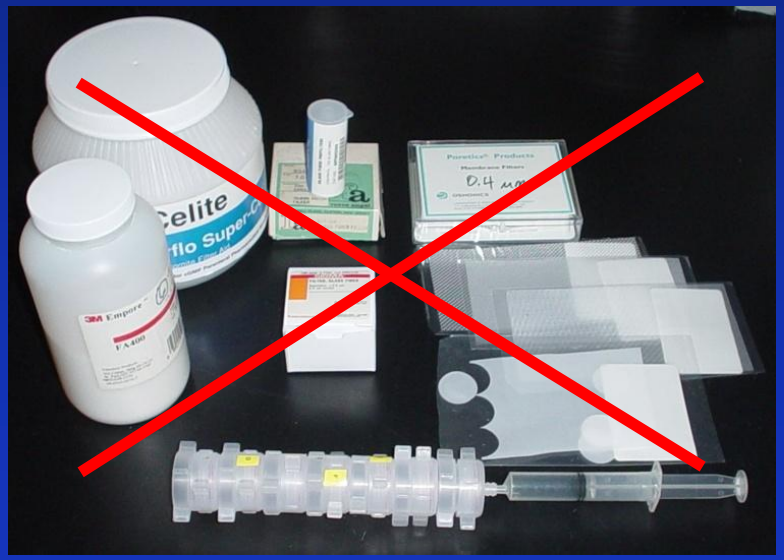
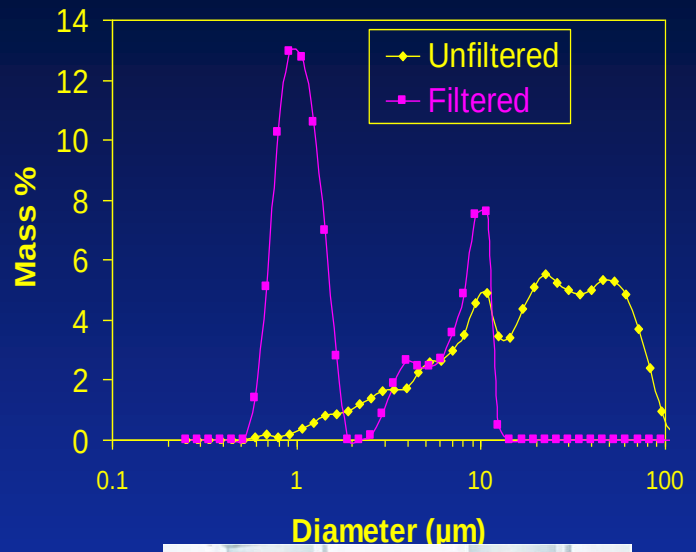
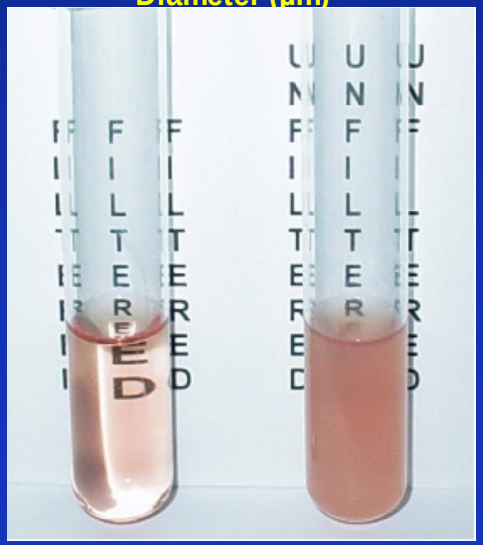
# High Speed Filtration - Goals

- Alternative to enrichment
- Handle ~ 10% solids (stomached hamburger)
- 10,000x concentration of 100 - 1000 ml
- 1 hour or less
- Cost < $10
- Disposable sterile module
- Conventional filters don't work
  - clog
  - slow
  - high retention of bacteria
  - high cost



# Leukocyte Removal Filter


Capacity >100 ml10% ground beef slurry

> 10 ml/min flow rate

< 5% Retention of *E. coli.* O157:H7

# PCR Detection Problems

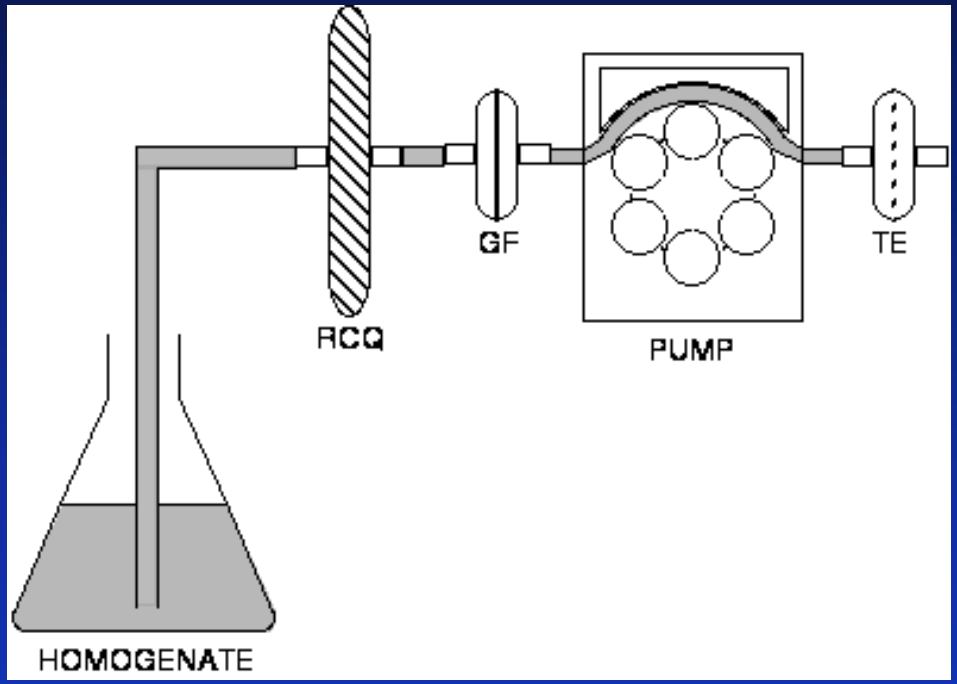
- In theory detects 1 cfu
- In practice detects ~1000 cfu
- Fast methods  discard >90% of DNA to avoid inhibition
- More elaborate methods are slow and exhibit poor recovery for very low DNA levels
- Recovery from gram positive << gram negative

# PCR inhibition by direct lysis reagents

| Reagent | qPCR copies | PicoGreen copies |
|---|---|---|
| MicroLysis Plus | 0.2 | 996,000 |
| Yeast Protein Extraction Reag | 0 | 872,000 |
| Bacterial Protein Extraction Reag | 4 | 617,000 |
| HotShot | 71 | 702,000 |
| 10x Modified HotShot + Tween | 891 | 429,000 |
| 10x HotShot +Tween | 122,059 | 281,000 |
| UltraPrepMan | 7 | 276,000 |
| 10x Modified HotShot + CTAB | 0 | 212,000 |

DNA extracted from ~$2x10^6$ cfu *L. monocytogenes*

# Original Filtration Method
# 3 stage filtration/capture

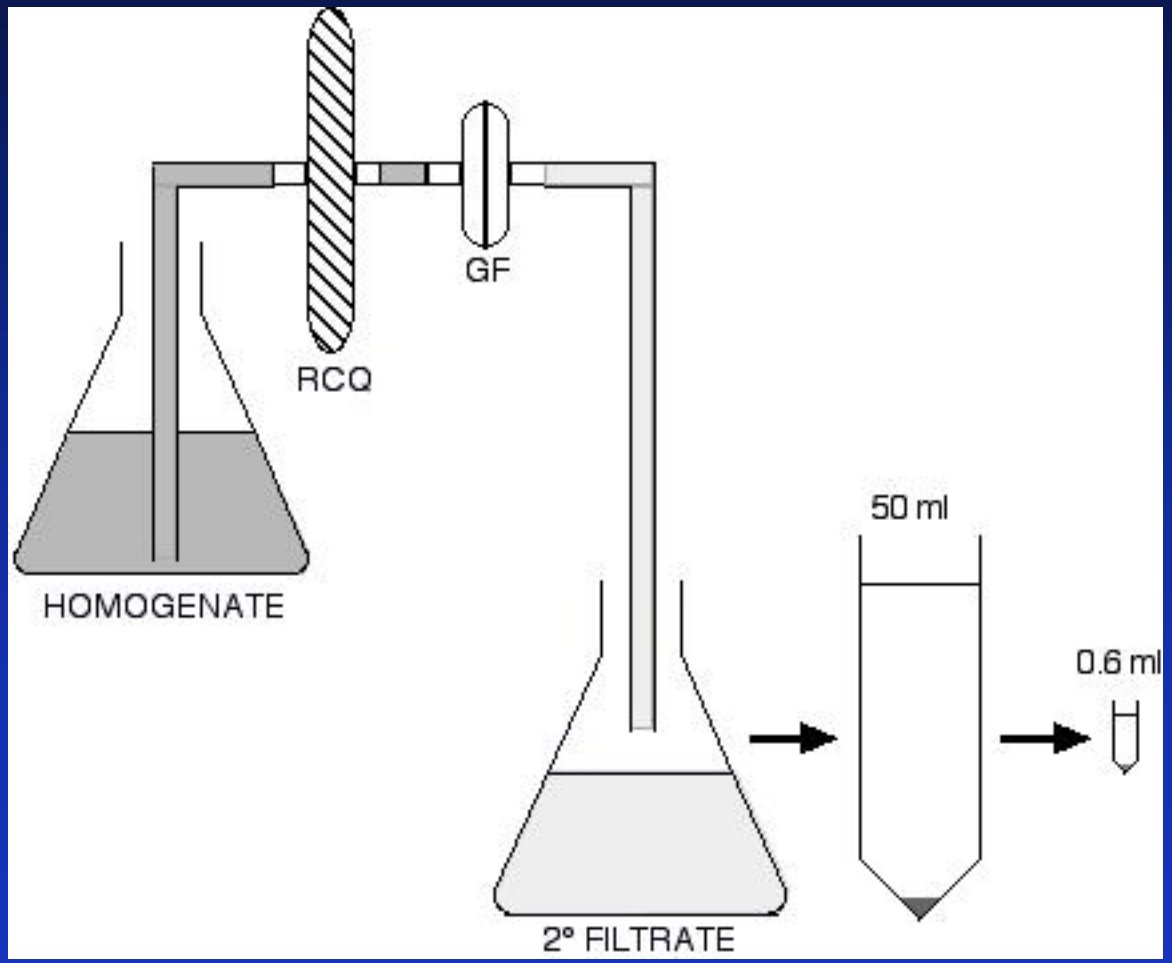



RCQ pre-filter
Glass fiber pre-filter
Track-etched capture filter
10 ml/min max flow
(limited by TE filter)

Need to elute cells
Clogs with high levels of background flora

# Modified Filtration Method

## 2 stage filtration + centrifugation




RCQ pre-filter
Glass fiber pre-filter
Gravity flow (~1 m)
50-100 ml/min

2 centrifugations

30 min total

# Sample Prepration

- Stomach 170 g ground beef + 360 ml isotonic dextrose
- Filter through RCQ and  glass fiber filter
- Aliqout into ten 50 ml tubes
- Add  3 x 0 cfu, 3 x 13 cfu, 3 x 130 to tubes 1-9
- Add 1300  cfu to tube 10
- Centrifuge 8 min at 4200 x g, remove supernate
- Resuspend pellet, transfer to 0.6 ml tube
- Centrifuge 3 min at 21000 x g, remove supernate

# Direct DNA Extraction and PCR

- Add 6 µl reagent to cell pellet in 600 µl tube
- Heat 10 min at 65° C
- Add 3 µl 2x neutralizer
- Transfer 8 µl to PCR tube
- Add 2 µl primers and 10 µl Master Mix
- Run qPCR assay (2 hour)

# *E. coli* O157:H7 in ground beef

| Sample | | cfu/rxn | cfu/g | $C_T$ | $C_T$ | $C_T$ | Copies |
|---|---|---|---|---|---|---|---|
| Blank 3° | filtrate | 0 | 0 | U | U | U | 0 |
| Spiked 3° | filtrate | 13 | 1.5 | 37.2 | 35.6 | 36.0 | 10 |
| Spiked 3° | filtrate | 130 | 15 | 32.8 | 32.5 | 32.1 | 104 |
| Spiked 3° | filtrate | 1300 | 150 | 28.9 | - | - | 990 |
| Bacteria standard | | 1.3 | - | 38.2 | 38.9 | - | 3 |
| Bacteria standard | | 13 | - | 36.5 | 35.7 | - | 11 |
| Bacteria standard | | 65 | - | 32.7 | 34.1 | - | 64 |
| Water | | - | - | U | U | U | 0 |

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