Contaminated fingers: a potential cause of *Chlamydia trachomatis* positive urine specimens

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Context

Ultimately, this is intertwined with the socio-political environment of "The Intervention"

Context: why?

Approach to Menzies from Northern Territory Government, Sexual Assault Referral Centre (SARC)

- Clinical examination of possible abuse victims
- Advise child protection and law enforcement authorities

SARC's question:

- If UGT *Chlamydia trachomatis* detection in children, no "disclosure":
- **What advice to police and child protection service providers??**

- "the presence of an STI in a preadolescent is most likely the result of sexual abuse and formal assessment should always be initiated"
- **What does "most likely" mean, numerically?**

Matters because:

- **Error mode 1: a child may remain in danger**
- **Error mode 2: groundless investigation and stigmatisation of individual/family/community**.
- **Perhaps most importantly: with this fraught issue, numerical transparency based on evidence is a virtue in itself.**

Context: how?

If "sexual abuse" is inferred from presence of STI….

- STI test is a diagnostic test for sexual contact
- Positive diagnostic test in absence of sexual contact: **False positive**

"Conceivable mechanisms" of false positivity tested experimentally to determine frequency.

- **"Conceivable mechanisms" defined by front-line clinicians, not Menzies researchers**
- **Our "Mythbusters" approach**

Outputs: confidence limits on false positive frequencies

Ultimate aim: PPV of a positive STI test in a young child for sexual contact

The first (we think) C. trachomatis self contamination-based experiment

The "myth" for this study:

"Contamination of urine specimens during collection process by transfer of STI material from carer's STI agent contaminated fingers to the inside of a urine collection jar"

plausible or busted?:

To address this:

Members of the research team deliberately contaminated their un-gloved fingers with live *C. trachomatis.*

Study design

Dilution series of *C. trachomatis* suspension (largely EBs) in urine surrogate solution..

↓

Each dilution:
- Dip three un-gloved fingers into EB dilution
- Contact fresh specimen of urine surrogate

↓

Analyse original suspensions and "finger contaminated" specimens using qPCR-based diagnostic system

↓

Ratio of *C. trachomatis* loads: carry-over

Experimental details

Variables

- Three *C. trachomatis* isolates
  - *OmpA* genotypes B, E, F
  - Grown in McCoy cell, EBs purified
- Two contamination methods (dip and pour)
- Three participants
  - Members of research team
- **Diluent:** artificial urine surrogate
- **Fingers dried with paper towel** between contact with *Chlamydia* dilution and test specimen

- **Nine page SOP…**

Experimental details (Cont.)

- **Timing**
  - Experimentation on three days, all two weeks apart
  - Each day: 1 X *C. trachomatis* strain, 3 X participants, 2 X transfer methods.
  - One transfer method (dip or pour) per hand

- **Decontamination and controls**
  - Hand wash before experimentation
  - Dip or pour after this wash and before finger contamination
  - Hand wash after experimentation
  - Post-wash "dipping" specimens

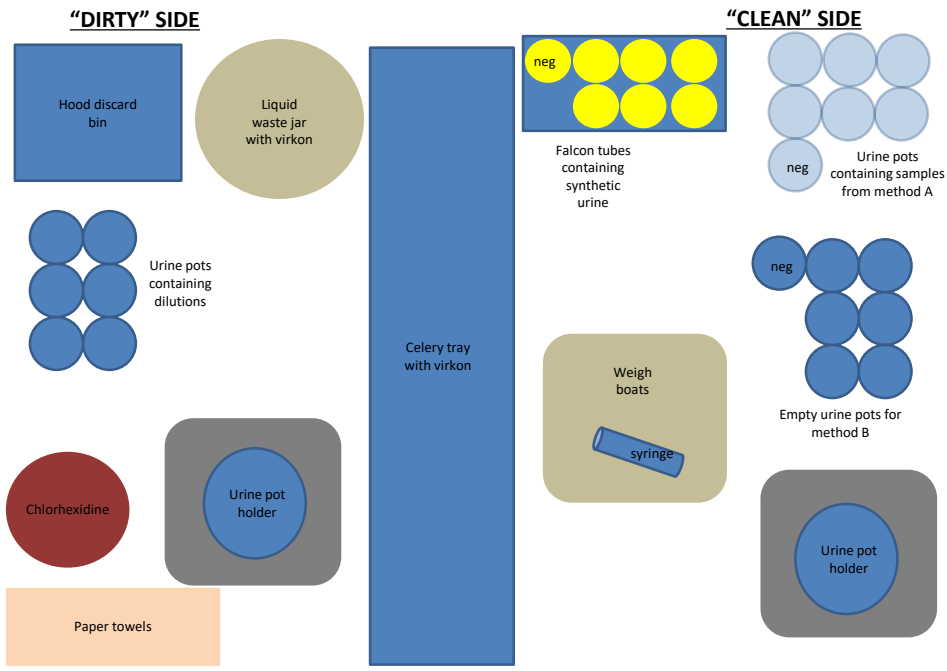
- **QPCR:** Roche COBAS 4800CT/NG

- **Data analysis**
  - **Cq values excluded:** ≥40, and when C. trachomatis DNA not detected in more concentrated equivalents

Urine surrogate

Di Martino *et al* 2003. Can J. Microbiol. 49:443-449

Method B – Hood Set Up



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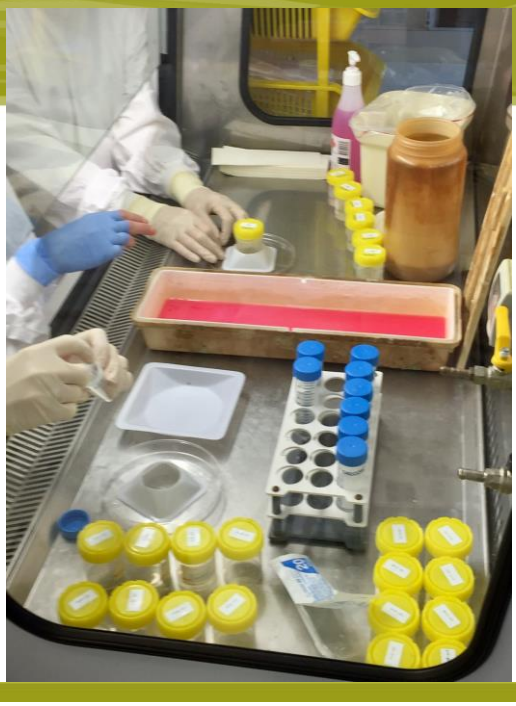

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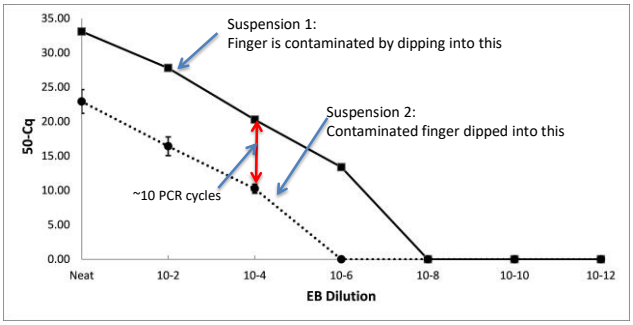
All results looked very like this



Where *C. trachomatis* was detectable in contaminated specimen..:

- *C. trachomatis* dilution vs corresponding contaminated specimen: **~10 PCR cycle difference**
- ~2^{10}=1024-fold difference in *C. trachomatis* load
- ~10 µl of EB suspension transferred.

Numerical results

Table 1 Ranges of ΔCq values and inferred volumes of EB dilutions transferred

Category (n)	Mean ΔCq (SD)	ΔCq range	Mean µL transferred (inferred from±2xΔCq SD range)(inferred from observed ΔCq range)	
All (49)	10.0 (1.6)	6.7–13.3	9.8 (1.1–89.7)(1.0–96.2)	
Participant 1 (17)	8.8 (0.9)	6.7–9.8	22.4 (6.4—78.1)(11.2–96.2)	p<0.0001*
Participant 2 (16)	10.5 (1.2)	8.6–13.0	7.1 (1.4–32.6)(1.2–25.8)	Participant 1 vs participant 2: p<0.01†
Participant 3 (16)	10.9 (1.6)	7.7–13.3	5.2 (0.6–48.1)(1.0–48.1)	Participant 1 vs participant 3: p<0.01†
				Participant 2 vs participant 3: not significant†
Dipping (23)	10.6 (1.7)	7.4–13.3	6.4 (0.6–68.0)(1.0–59.2)	p=0.015‡
Pouring (26)	9.5 (1.3)	6.7–12.4	13.8 (2.3–83.7)(1.0–96.2)	
E_Aus56 (17)	9.7 (1.6)	6.7–12.2	12.0 (1.3–110.5)(2.1–96.2)	Not significant; p=0.41*
F_Aus51 (15)	10.0 (1.6)	7.4–13.3	9.8 (1.1–89.8)(1.0–59.2)	
B_Aus45 (17)	10.4 (1.5)	8.4–13.0	7.4 (0.9–59.2)(1.2–29.6)	
Undiluted (18)	9.4 (1.7)	6.7–12	14.8 (1.4–156.3)(2.4–96.2)	p=0.004*
10^{-2} diluted (18)	11.0 (1.5)	9.0–13.3	4.8 (0.6–39.1)(1.0–19.5)	10^{0} vs 10^{-2}: p<0.01†
10^{-4} diluted (13)	9.5 (1.0)	7.7–11.1	13.8 (3.5–55.2)(4.6–48.1)	10^{0} vs 10^{-4}: not significant†
				10^{2} vs 10^{-4}: p<0.05†

The 49 data points were collated according to different criteria to reveal possible correlates. This encompasses data only from specimens that yielded a positive C. trachomatis test with a Cq <40.
*One way analysis of variance.
†Tukey HSD.
‡t-test (two tailed).

Statistically significant differences between:

- Participants
- Method
- Dilutions

BUT

- Magnitudes small
- We cannot see any translational implications of these differences

Numerical results (continued)

Effect of post-experimental hand-washing on *C. trachomatis* DNA load on fingers

Hand washing:

- Wash with Avagard 9230-D (hand and body wash)
- Rinse with water
- Rinse with Avagard 9250 P (0.5% chlorhexidine gluconate in 70% ethanol)

Reduction was ~1000X but quite variable

Inferred range: 85-28526X

Post decontamination specimen	ΔCq	Reduction *in C. trachomatis* DNA
1	10.3	1261
2	11.1	2195
3	14.8	28526
4	9.2	588
5	8.2	294
6	7.7	208
7	9.2	588
8	10.4	1351
9	6.4	85
10	8.3	315
11	11.4	2702
12	10.7	1664
13	13.3	10086
14	9.5	724
15	12.6	6208
16	10.8	1783
17	11.4	2702
Mean (SD)	10.3 (2.1)	1261

Implications

1. **Does urine ever have enough *C. trachomatis* DNA to underpin this mechanism of specimen contamination?**

- The highest Cq (lowest DNA load) for a positive test: Cq = ~40
- ΔCq from our experiments = ~10
- Any urine with enough DNA to provide a Cq of ≤40-10=30 could underpin this contamination mechanism
- Snapshot of 30 actual *C. trachomatis* positive urine specimens from collaborators:
  - 6/30=20% gave Cq≤30

Conclusion: A significant minority of actual *C. trachomatis* positive urine specimens could underpin this mechanism of contamination.

Implications

2. **Do clinical guidelines foreshadow this mode of specimen contamination?**

- **No!**
- **Universally:**
  - Guidelines for mid-stream urine collection (e.g. for UTI test) **DO** incorporate precautions against specimen contamination
  - Guidelines for first stream urine collection (for STI testing) **DO NOT** incorporate guidelines against specimen contamination
    - **Presumably mind-set that specimen is already contaminated**
  - **Possibility of exogenous contamination of first stream urine is not considered**

- **Conclusions**
  - **Recommend extending the simple anti-contamination precautions for mid-stream urine collection to first stream urine collection .**
  - **Perhaps urine specimens from children for STI testing should not be collected by carer alone.**

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