Contamination with Nucleic Acid in Molecular Settings: Detection, Removal, Monitoring and Prevention

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Agenda

- Contamination with NA in molecular settings and impact on laboratory results
- Potential source of contamination, detection and cleaning/removing
- Monitoring, removing and prevention of contamination (available publications)
- Results from lab survey on contamination, detection, cleaning and prevention
- Lesson learned, summary, discussion and
- recommendations

Contamination with NA in molecular settings

- NA molecular techniques have become important diagnostic tools in clinical laboratories
- These techniques are powerful and have exquisite sensitivity and specificity
- A typical PCR reaction can generate 10^{12} molecules of amplified DNA in a 0.1mL reaction
- High sensitivity of these techniques, makes them vulnerable to contamination

Contamination

- Contamination is an introduction of unwanted NA in test (PCR) reaction
- Contamination is a problem that may make scientist to move to a new location
- Contamination by unwanted NA leads to false positive results
- Implications on diagnosis and patient treatment
- Negative impact in laboratory performance
- Financial negative impact

False Positive Results

- False positive results can be caused by
  1. general contaminants
  2. sample contaminants
- Those caused by general contaminants will generally affect every sample in the run
- Those caused by a sample contaminant, only affect a limited number of samples in the run

Source of Contamination

- The products of the amplification reaction or amplicons
- Positive QC, mainly consists of plasmid or patient DNA
- Specimen extracted DNA
- Reagents, contaminated water, enzymes (taq polymerase), primers
- Biohazard waste, disposables, benches, plastic supply, clothing, and equipments

Contamination Detection

- There are no publications on techniques or methods on how to detect contamination
- Good assay QC, (NTD, or other negative controls)
- Test reagents (master mix, primers) before used as unknown
- Detecting aerosol DNA by using NTD
- Monitoring assay positive rates
- Environmental studies (wipe test)

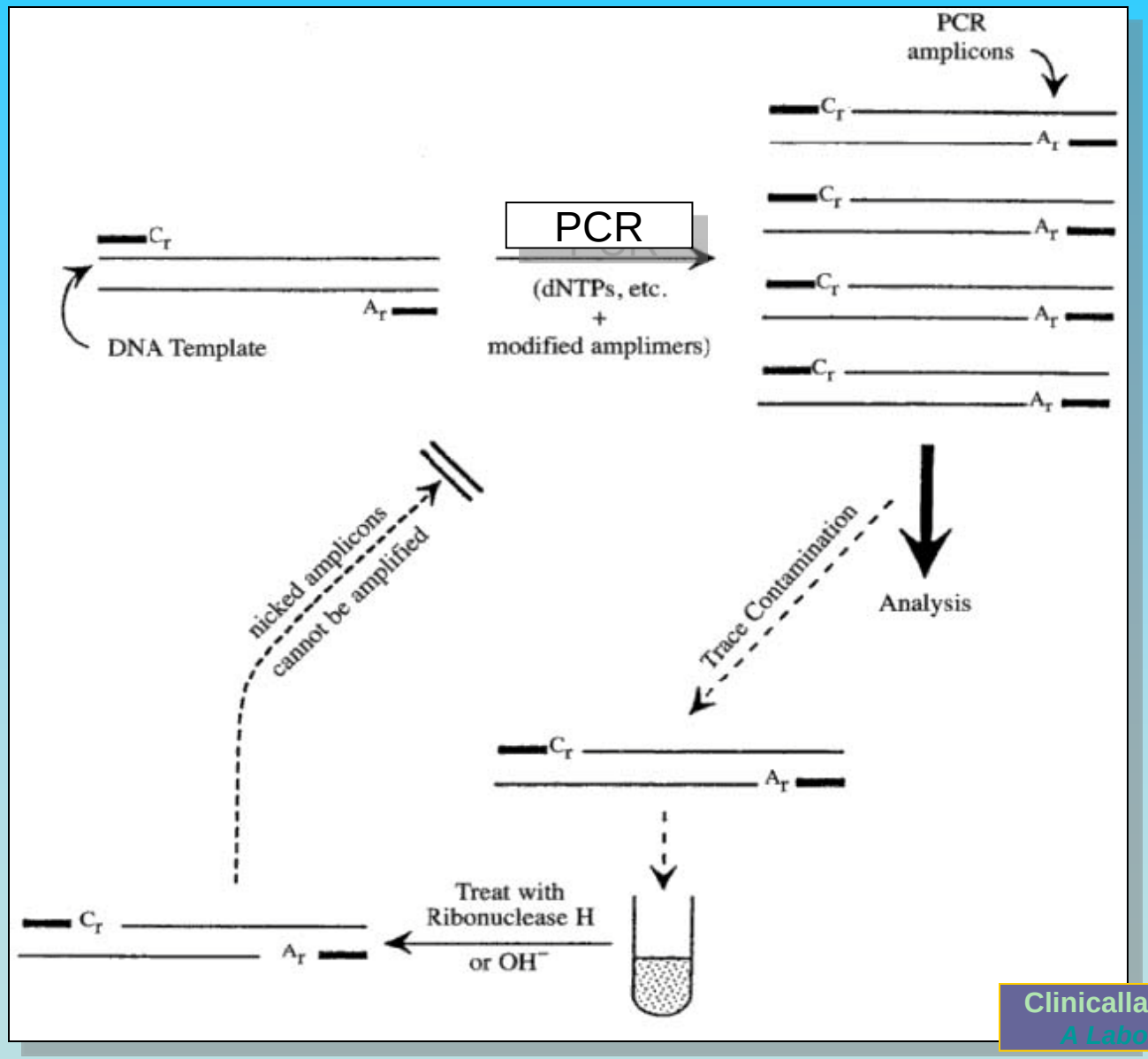
Cleaning/Removing Contamination

- Cleaning agents that are suitable for and dedicated to decontaminating NA contamination
- NA removing agents (License to kill, DNAZap™, DNA remover, DNA-exit plus DNA-free™
- 10% solution of sodium hypochloride
- UV irradiation

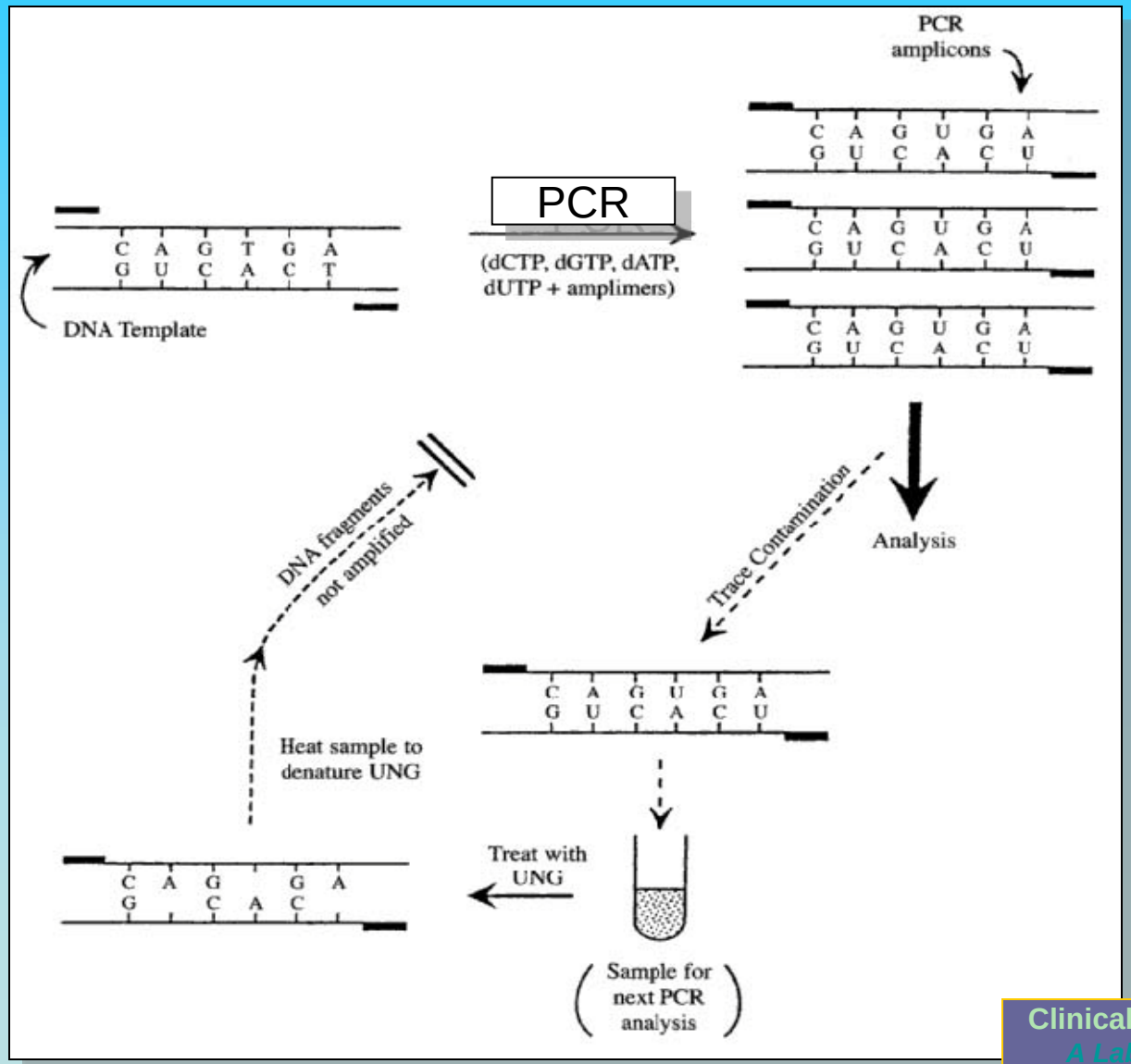
Recommended Protocols for Prevention and Controlling

- Use of modified primers that use ribonucleotides
- Use of UNG with dUTP
- Iso-psoralen and long wavelength UV photoactivation
- Sterilizing the PCR-mixture directly before amplification starts (UV-Induced thymine dimers)
- Good laboratory QA/QC
- Good monitoring program (environmental study)

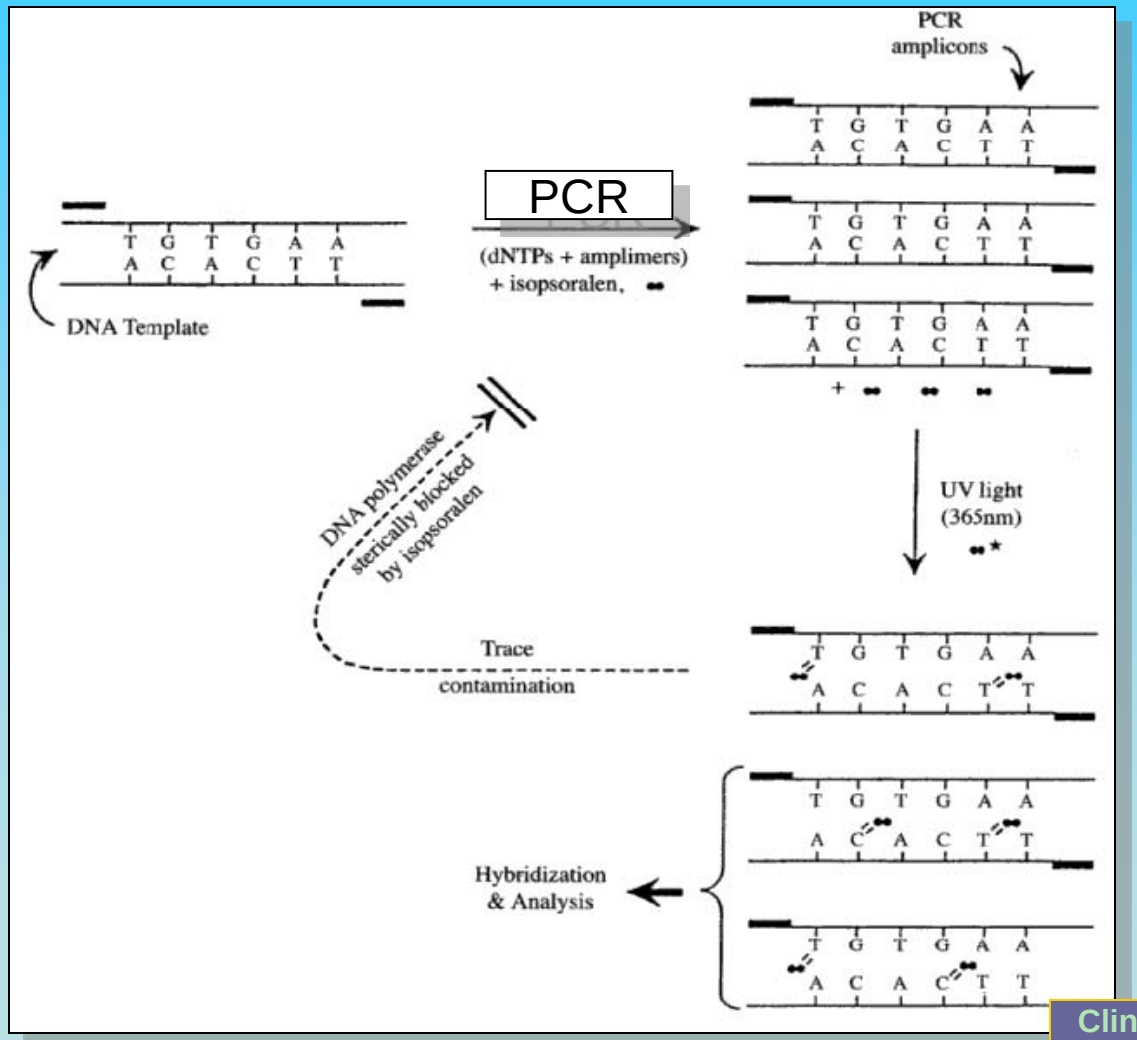
Modified Primers



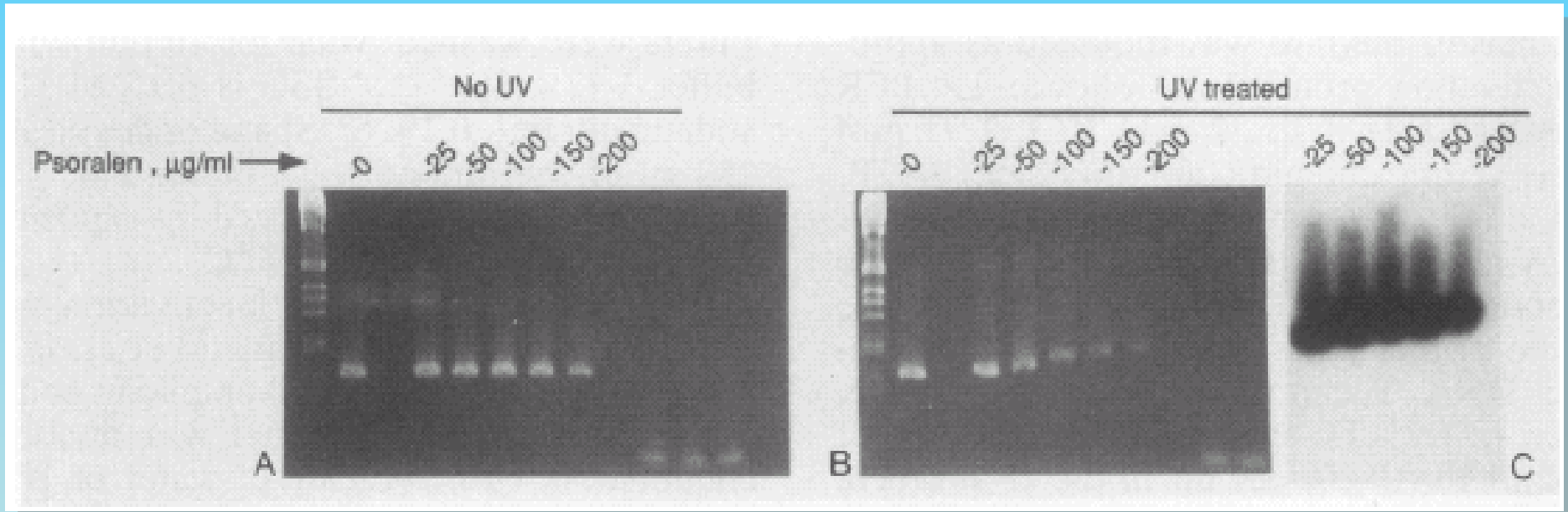
Use of UNG



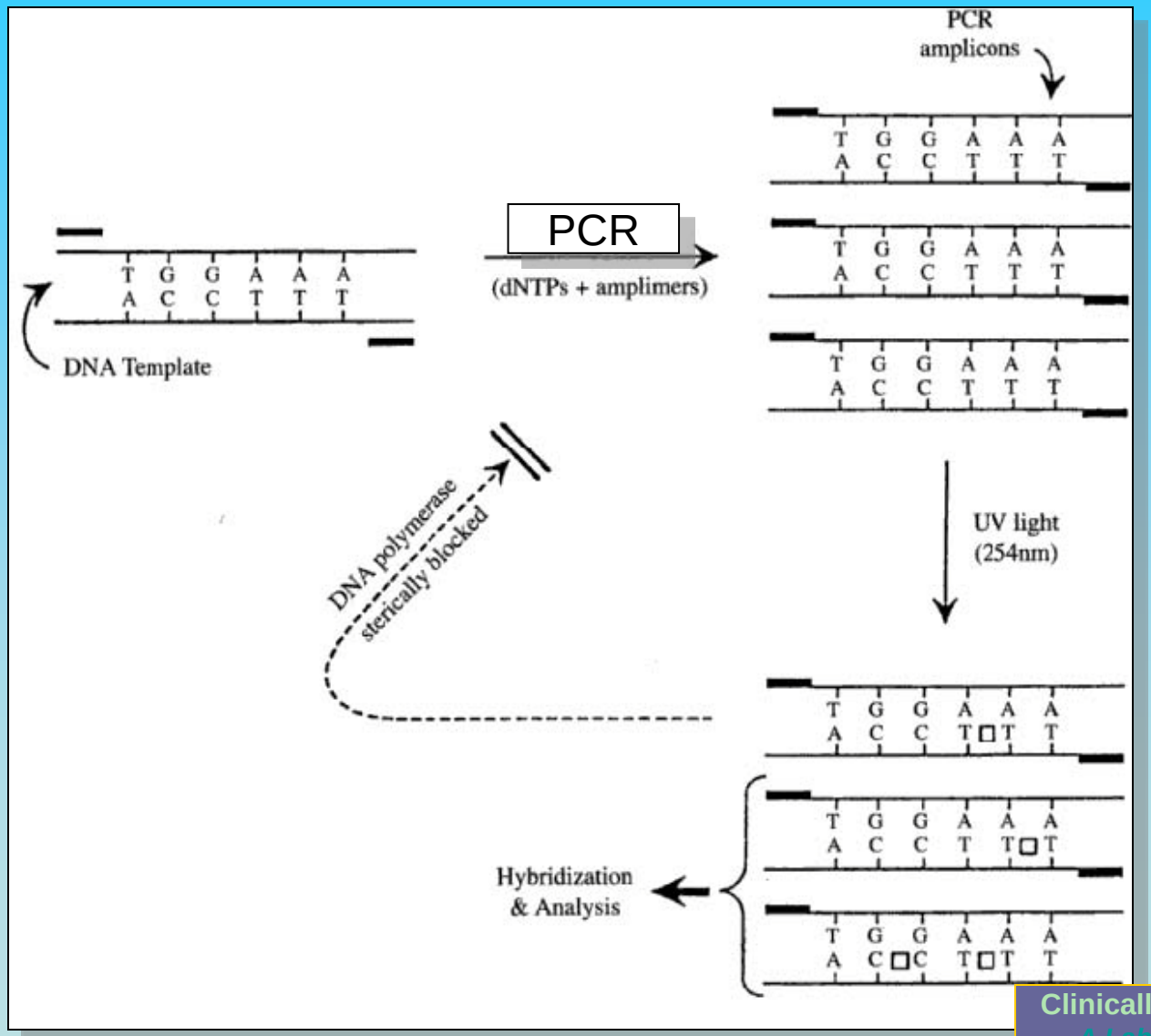
Iso-psoralen UV photoactivation



Iso-psoralen UV photoactivation



UV Decontamination



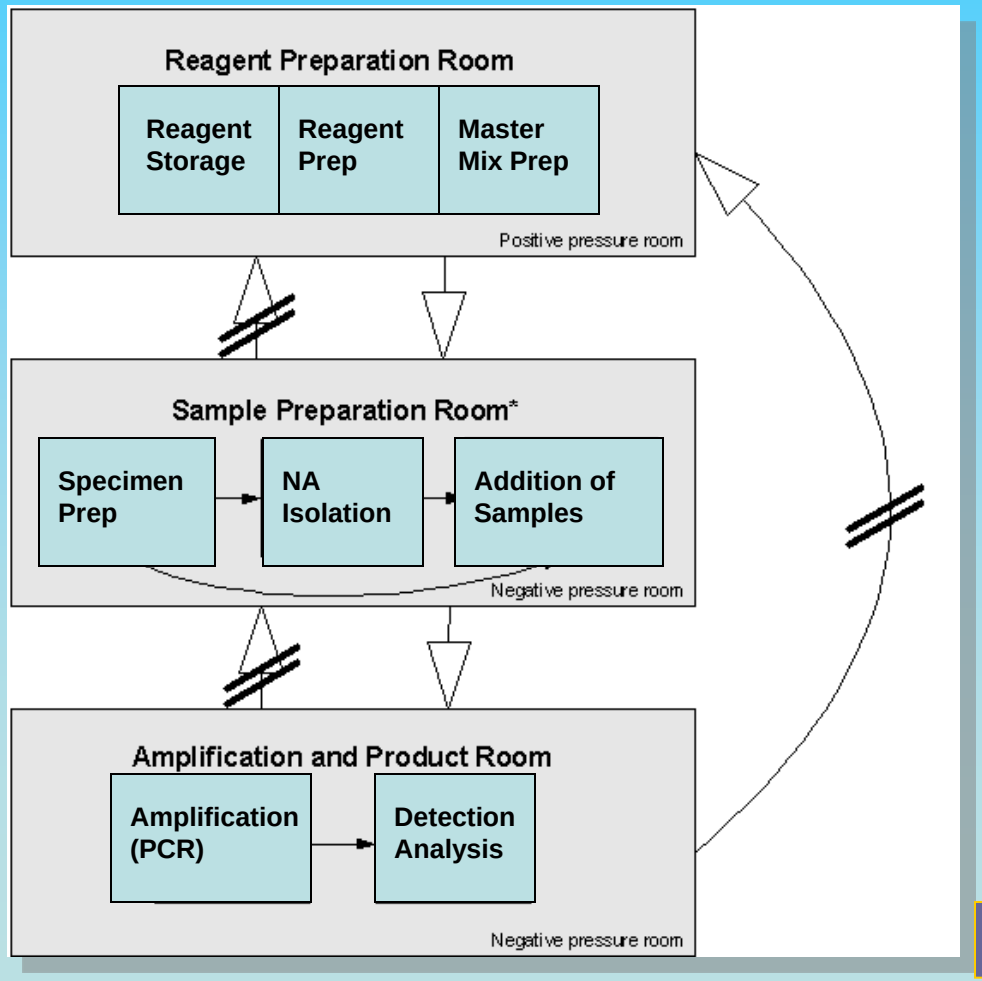
Sterilization Products

Table 1. Comparison of amplification products sterilization techniques to control PCR carryover contamination.

Method	Mode of action	Advantages	Disadvantages
UV light	thymidine dimer	inexpensive, requires no change in PCR protocol	ineffective against G+C-rich and short (>300 bp) amplification products
UNG*	enzymatic hydrolysis of the aerosolized amplicons	easy to incorporate, most active against T-rich amplicons	expensive, may reduce amplification efficiency
Hydroxylamine	chemically modifies C and prevents C+G pairing	inexpensive, effective on short and G+C-rich amplicons	carcinogenic, may interfere with amplicon analysis
Isopsoralen (IP)	modifies target by cyclobutane adduct	relatively inexpensive, requires minor modification of the PCR protocol	carcinogenic, inhibitory effect on PCR not very effective for controlling G+C-rich and short amplicons, requires added equipment
Psoralen	same as IP	same as IP	may interfere with amplicon analysis
Primer hydrolysis	post PCR hydrolysis of RNA residues of the amplicons by NaOH	equally effective on G+C-rich amplicons	variable efficacy, may generate aerosol during NaOH addition

Good Laboratory QA/QC

Facility Design



Good Laboratory QA/QC

- Unidirectional workflow
- Use of equipment, reagents and disposals is room specific
- Always use high quality of reagents (aliquot in small amount)
- Clean all used areas with 10% bleach or NA removing agent before and after use
- UV Decontaminate instruments and hoods (ceilings) after use
- Use appropriate waste containers

Good Laboratory QA/QC

- Clean extensively if you suspect contamination
- Communicate to your colleagues if suspect contamination
- Clean heat/cooling blocks, trays, carousels, with DNA removing agents (or bleach) after use
- Bleach all used plastic racks
- Use positive and negative (NTD) QC in each run and through the whole assay (including extraction)
- Use PCR grade water for reagent prep
- Use RNase/DNase free pipette tips that prevent aerosol formation (filter, positive displacement tips)

Good Laboratory QA/QC

- Avoid opening of tubes
- Wipe used pipettes with DNA removing agents after use and UV for >30 minutes
- Use UNG if necessary
- Perform environmental studies
- Develop laboratory QA/QC daily, weekly and monthly check list
- Educate and train

Shared Experience from Labs

- Dr. Pritt's study
- Our study

Bobbi Pritt MD, Mayo Clinic

- Total # Labs Responded = 18
- Total # Labs Performing Environmentals = 10

Sites routinely monitored:	
Clean Room or Mastermix prep room	10
Extraction Area	10
Sample Loading Area	9
Amplification/Detection Area	10
Instrumentation	7

Actions taken if a positive is obtained:	
Nothing	0
Repeat monitoring	0
Repeat after routine cleaning	1
Repeat after extensive cleaning	7
Relocate testing/stop testing (until resolved)	3

Specifics of Environmental Monitoring

Monitoring Interval (All Labs Responded)

Weekly	1
Monthly	5
Every other Month/6 times per year	2
Quarterly/4 times per year	0
Varies with area monitored	1

Volume	Extent of Monitoring	Site monitored
10-40K	Monthly	3
<10K	monitoring 6 times per year	4
10-40K	Monthly	4
<10K	Every other month	3 to 5
<10K	Monthly	5
>100K	Weekly	>8
<10K	Quarterly monitoring	20
<10K	Monthly	Varies - 12 to 24 sites
<10K	Monthly	varies by assay
<10K	Monthly	Not S

Dr. Pritts Study Summary

- 10 of 12 labs are still performing conventional PCR
- Only 7 of 18 labs used UNG/dUPT
- 11 of 18 perform environmental studies, 10 of these 11 perform conventional PCR and 6 of these perform sequencing
- Most of these lab perform environmental studies monthly
- Most of these perform wipe test on 3-24 sites testing for all or some of tests
- Most of them take wipe test after cleaning
- If positive after testing, they would do extensive cleaning
- Of these most test pre- and post-amp rooms

Our Study

- Survey of 11 Questions and case description
  - 6 general questions
  - 5 contamination questions
  - 1 summary question
- Number of labs participating in survey = 18

GENERAL QUESTIONS

1. Please indicate the number of molecular tests your lab performs each year:
   - <10,000 = 3
   - 10,000 – 50,000 = 9
   - 50,000 – 100,000 = 3
   - >100,000 = 3

Type of Molecular Tests

2. Please indicate the type of molecular testing you perform (check all that apply):
   - End Point PCR = 10
   - Real-Time PCR = 18
   - Other Target Amplification = 9
   - Signal Amplification Method = 11
   - Other Methods (sequencing, gel) = 3

Contamination Problems

3. Have you experienced any contamination/ cross-contamination in your lab?
   - Have had contamination = 16
   - No contamination = 2

4. Do you have a QA/QC contamination protocol adapted specifically for molecular?
   - Yes = 11
   - No = 4
   - No answer = 3

Monitoring and Prevention

5. Do you use UNG or other cross-contamination preventing agents in your PCR assays?
   - Use UNG = 8
   - No UNG = 5
   - No Answer = 5

6. Do you perform environmental studies for contamination?
   - Perform environmental Study = 8
     - Monthly = 5
     - Quarterly = 1
     - As needed = 1
     - Not specified = 2
   - Don't perform environmental study = 7
   - No answer =

Labs Reporting Contamination

CONTAMINATION QUESTIONS

1. Check the number of contamination events your lab has had in the past two years:
   - No contamination = 2
   - 0-4 contaminations = 13
   - 5-9 contaminations = 3
   - 10-15 contaminations = 0
   - >15 contaminations = 1

Assay or Method Contaminated

2. Indicate the type of assay(s) that showed contamination (check all that apply):
   - End Point PCR = 5
   - Real-Time PCR = 10
   - Other Target Amp. (APTIMA) = 6
   - Other Methods (gels, sequencing) = 2

3. Check all types of contamination errors found within
& 4. the last two years:
   - Unknown = 6
   - Broken capillaries = 11
   - Operator's error = 11
   - Reagents = 3
   - Consumables = 1
   - Instrumentation = 1
   - Other (controls, infection) = 2

Contamination Cases

5. Please describe in 1 -2 sentences, the following for each contamination event you had in your lab during the past two years (feel free to add more contamination events):
   - Labs described 2 cases = 5
   - Labs described 1 case = 12
   - Labs described no cases = 1

5a. How was contamination discovered
   - Negative QC (NTD) failed = 14
   - Most of patients positive = 8
   - Environmental studies = 2

Contamination Cases

5b. What was done to eliminate contamination

- Cleaning with bleach/alcohol = 18
- Cleaning with NA remover = 4
- UV = 5

5c. How long did it take to completely clean

- < One week (hours) = 12
- < One month = 4
- > One month = 2

Contamination Cases

5d. Was testing suspended during decontamination

- Testing suspended = 13
- Testing not suspended = 4

5e. Indicator that contamination was clean

- Negative control pass = 15
- Samples tested as expected = 6
- Negative pass after wipe test = 2

Contamination Cases

5f. Any change in QA/QC protocol to prevent future contamination

- Yes, change/improvement = 7
- No, the same = 8

5g. Did you introduce UNG after contamination event

- Yes = 7
- No = 8

Contamination Cases

5h. Did you increase frequency of environmental survey

- Yes = 2
- The same = 12

5i. Other prevention that works

- Education = 18
- Training = 14

5j. Additional Comments

- Good laboratory practice (QA/QC) = 12

Our Study Summary

Of 18 reported labs:

- 16 reported contamination, 2 reported no contamination
- Only 11 had Lab QA/QC
- Only 8 used UNG in their testing
- Only 8 performed environmental studies
- Of the 16 that reported contamination:
  - 5 used endpoint PCR
  - 10 Real-time PCR
  - 6 APTIMA
  - 2 other methods (gels, sequencing)

Our Study Summary

- Of the 16 that reported contamination:
  - 11 had operator's error (broken capillaries and control contamination)
  - The major indicator was the negative control becoming positive or too many positives
  - Only 2 were discovered using environmental studies
  - All labs used bleaching and UV, some DNA removing reagents
  - 13 suspended their assay
  - 4 were longer then a week
  - Only some change their QA/QC
  - 7 introduced UNG after contamination
  - All reported personnel training and education

Lessons Learned

- Place and enforce good Lab QA/QC
- Educate and train personnel regularly (observe and check)
- Develop good laboratory habits
- Good and frequent assay QC (NTD and IC)
- Use highest quality of reagents (aliquot)
- Use UNG if you had any contamination
- Report double verification and sign off
- Monitor your positive rates
- Use closed extraction, amplification and detection systems

Acknowledge

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Questions ???????