Wisconsin State Laboratory of Hygiene

UNIVERSITY OF WISCONSIN–MADISON

PCR Contamination and Molecular Best Practices

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Polymerase Chain Reaction (PCR)

- A molecular technique used to detect and amplify (i.e. make multiple copies) of a specific DNA target.

PCR Terminology

Target – gene sequence (5 – 20 nucleotides) detected and amplified.

Primer – short DNA fragments (5 – 20 nucleotides) with a complementary sequence to the target region. (Amplification)

Probe – short DNA fragments (5 – 20 nucleotides) linked to a reporter molecule that bind downstream of the target. (Detection)

Polymerase – thermostable DNA replication enzyme (Taq).

Nucleotides (dNTPs) – building blocks (A,T,C,G) for replicating DNA.

Master mix – suspension containing all reaction components.

Amplicon – One copy of replicated (amplified) DNA target.

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PCR Reaction

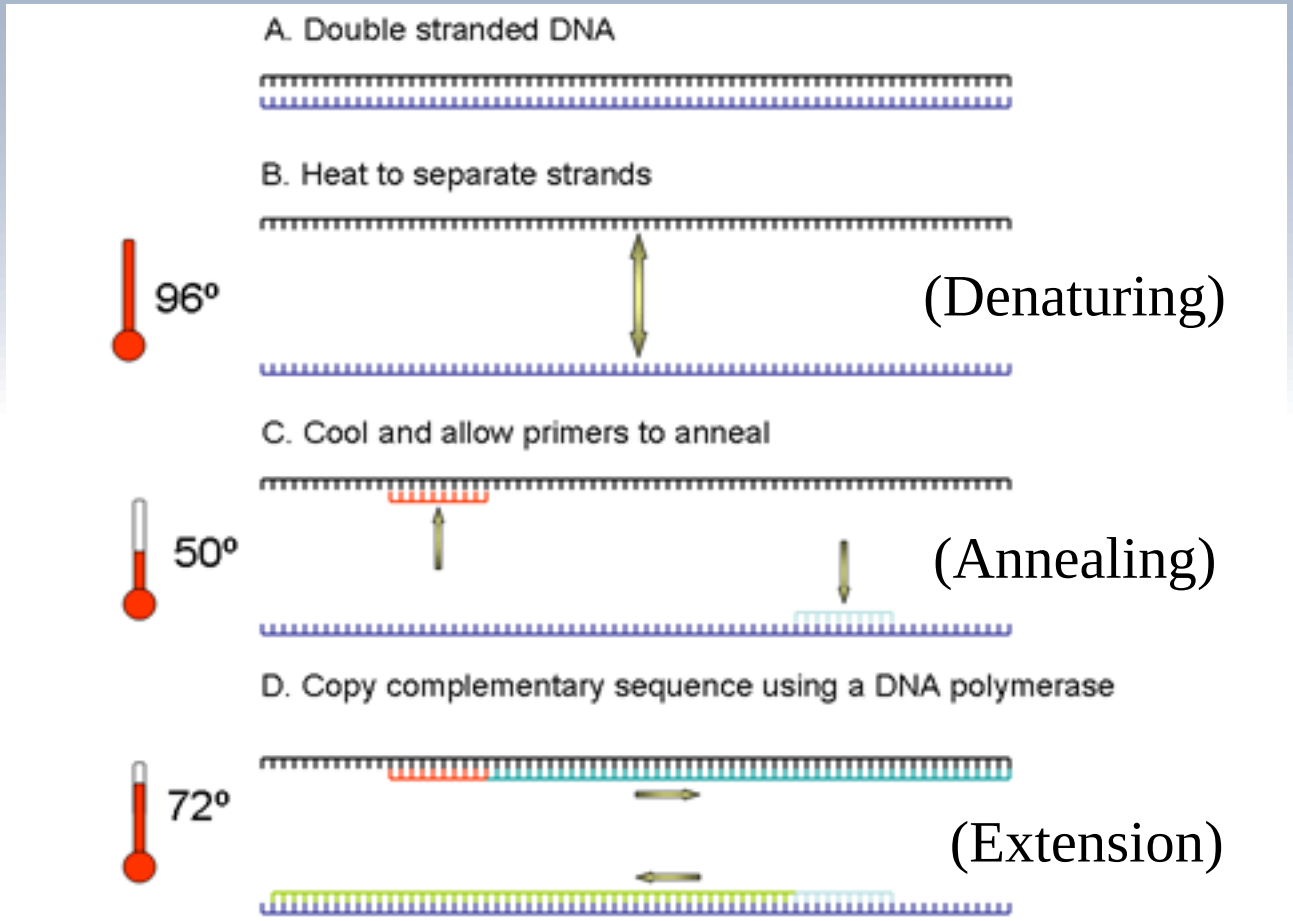
Steps (4)

1. Denaturation – converts dsDNA to ssDNA using heat (95° C).
2. Annealing –primers bind to their target sequence (50-60°C).
3. Extension – replication of DNA by DNA polymerase (72° C).
4. Detection – measurement of fluorescent signal.

PCR Cycle – one round of denaturing, annealing, extension, and detection (Thermocycling).

Amplification – logarithmic increase in copies of DNA target that occurs with the completion of each PCR cycle.

PCR Reaction



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PCR Kinetics

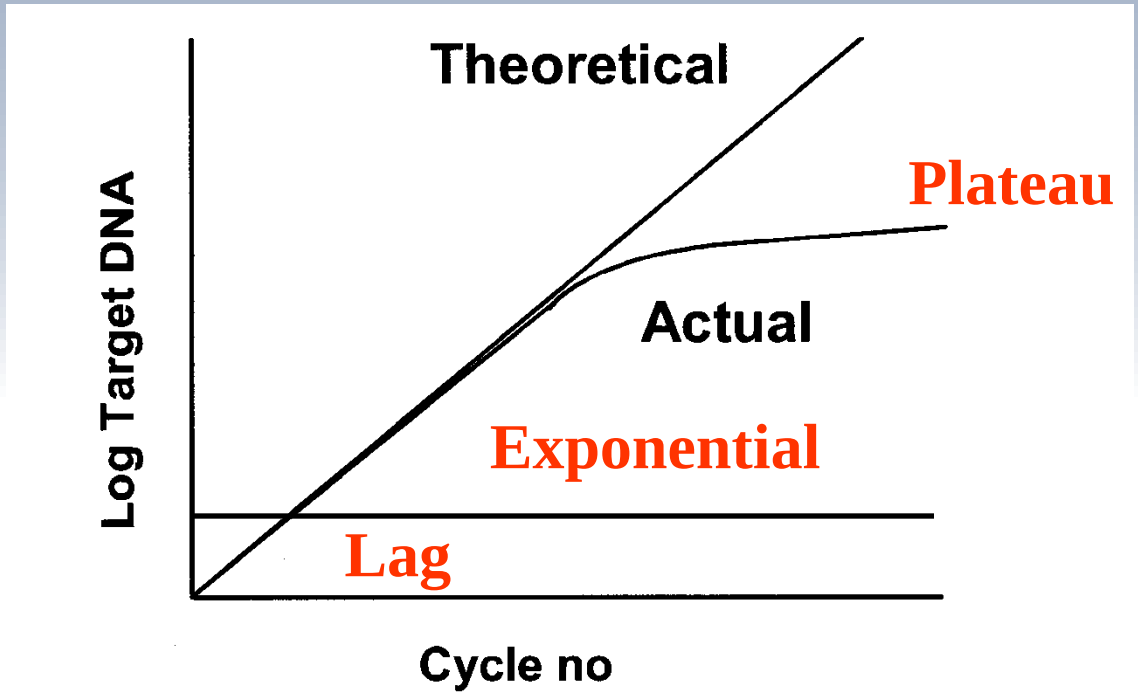

Figure 1.1. Kinetics of PCR amplification. Theoretically, the amount of product doubles during each cycle of the PCR reaction. In practice, the fraction of template replicated during each reaction cycle is less than 100%.

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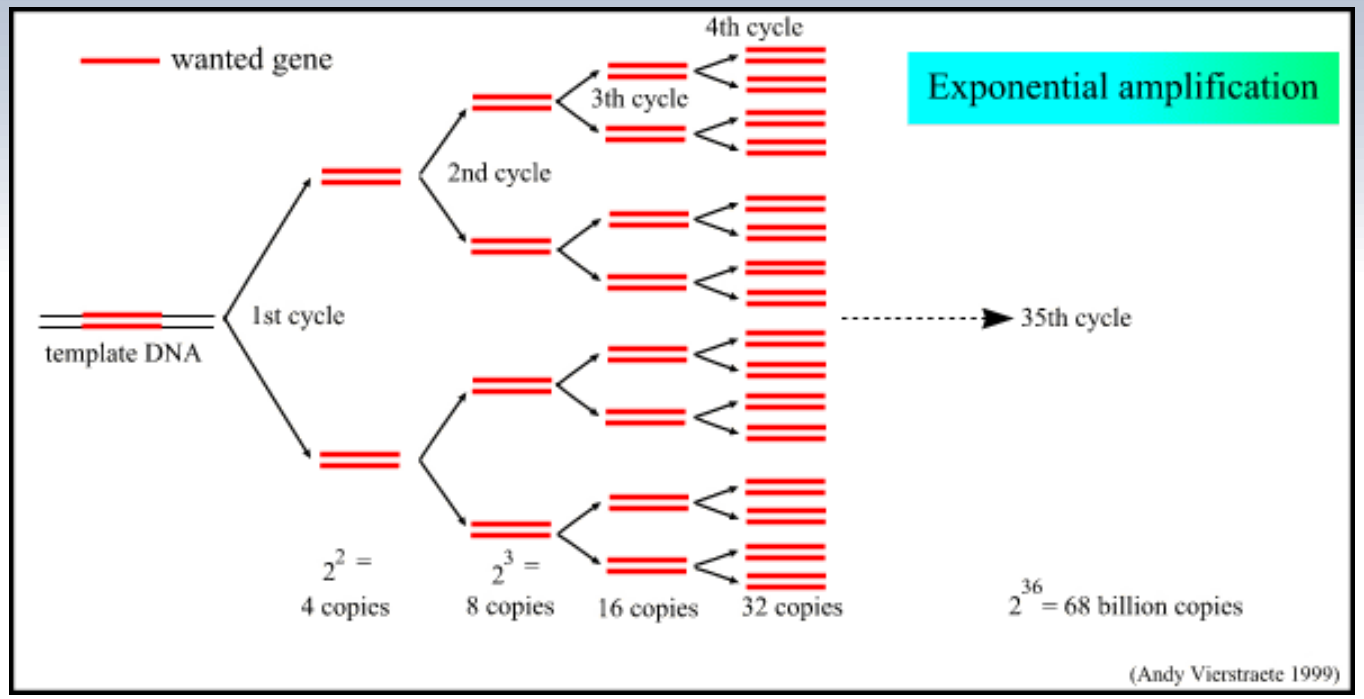
Amplification



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Sensitivity (Limit of detection)

- Usually measured in number of targets (copies) detectable per reaction; also CFUs, $TCID_{50}$
- The most sensitive assays can detect 1-10 copies of template, most assays range between10-100 copies.
- Greatest strength / Greatest weakness

Sources of DNA contamination

- Environmental – insufficient containment or separation
- Unprocessed patient or research samples
- Previously amplified material

Open PCR Systems

Conventional PCR

-Requires post-amplification processing (agarose gel)

- Aerosolize amplicons

Contaminate workspace and downstream samples

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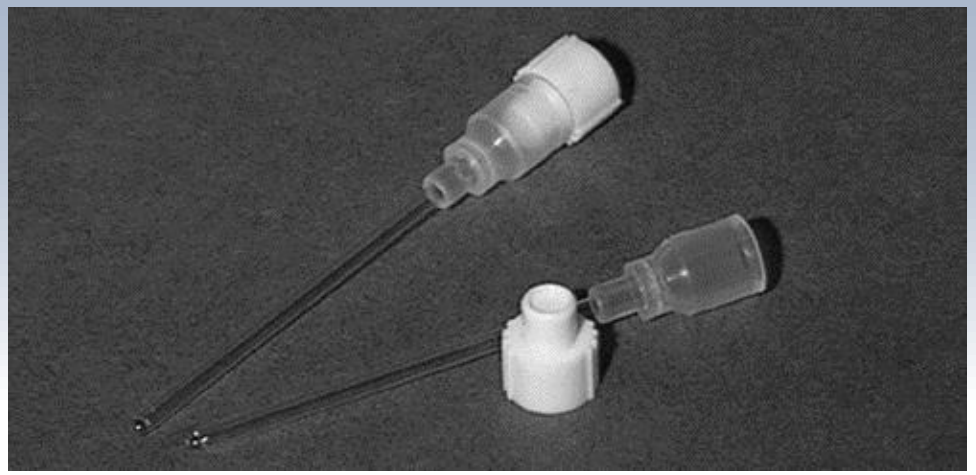
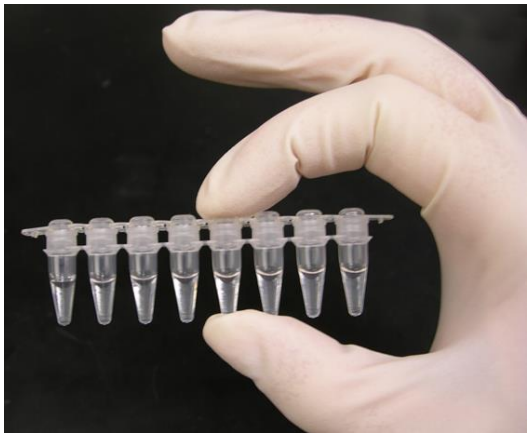
Closed PCR Systems

- Examples
  - Real-time PCR
  - Sample-to-answer platforms (ARIES®, GeneXpert)

- Advantage
  - Amplicon containment

Closed PCR Systems





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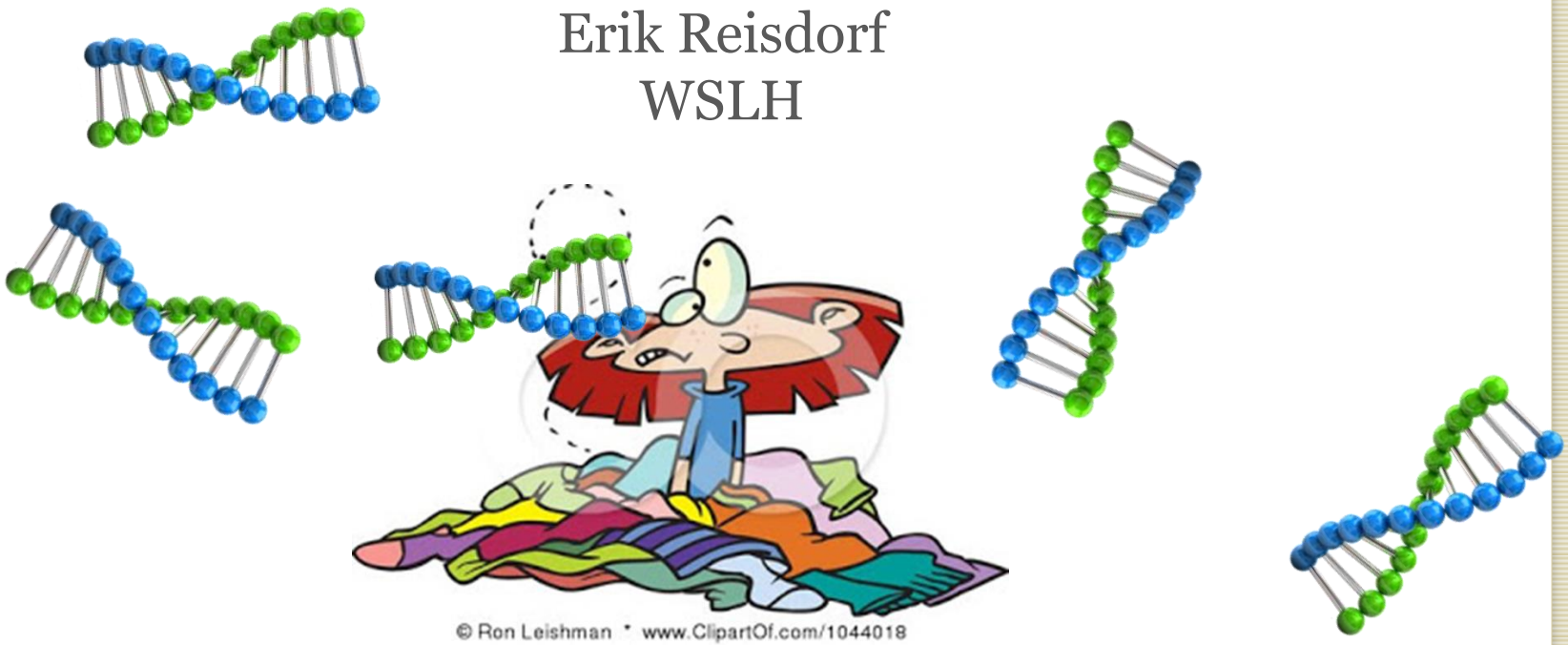
Recognizing Contamination

- Increase in positivity rate for low prevalence pathogens
- Increase in low level (high Ct) positives
- Unusual patterns of out-of-season positives
- Results that do not fit clinical diagnosis
- Swabs from sites within the testing environment test positive

PCR Contamination & Laboratory Best Practices

Dean Jobe
Gunderson Lutheran

Erik Reisdorf
WSLH



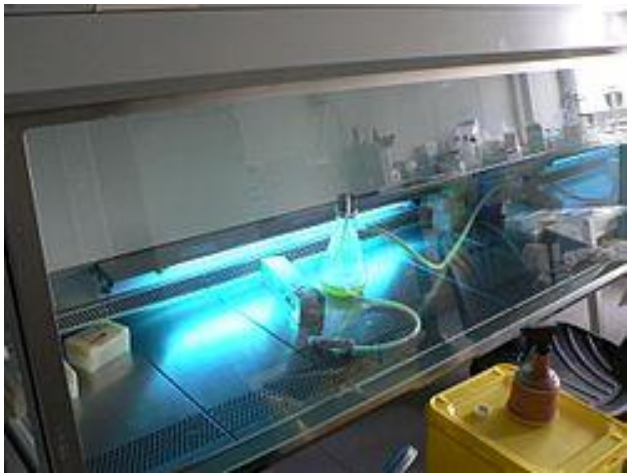
Cleaning-up Contamination in the Lab

Chemical Disinfection

- Bleach (~5%) solution
  - Corrosive
  - Instrumentation
- HCL (1.0N)
  - Depurination of nucleic acid
  - Stable, long contact time
  - Effectiveness
- Commercial Products
  - Effectiveness??

Image source: VWR.com

Cleaning-up Contamination in the Lab



Ultraviolet Light (UV)

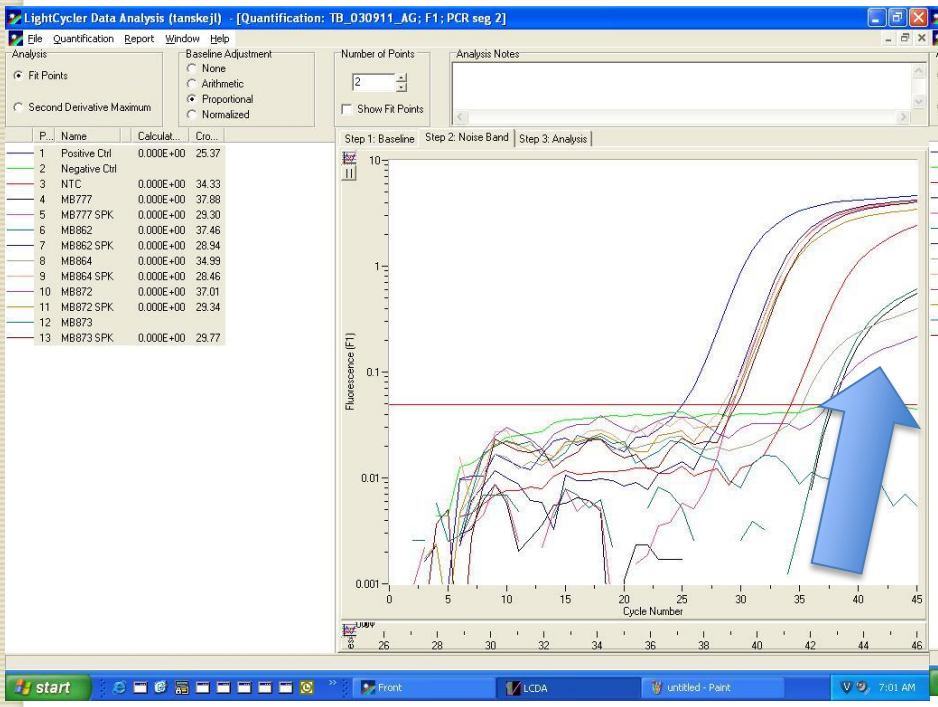
- Common in BSC's
  - 10-15 minutes
  - Automated extraction platforms.
  - Not practical for most lab surfaces.
  - Prolonged exposure degrades plastic.
  - Decreased effectiveness over time.

Cleaning-up Contamination in the Lab

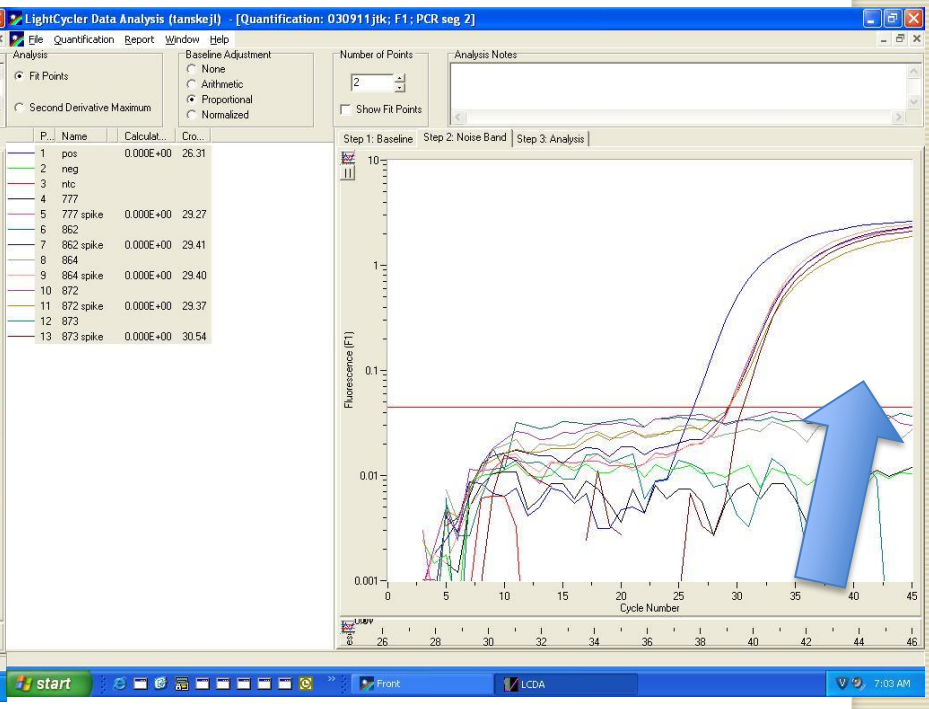
Reaction Chemistries

- Uracil DNA Glycosylase (UNG)
  - Works by destroying dUTP incorporated into the DNA amplicon.
  - Native DNA does not contain uracil so the sample (template) is not degraded by this procedure
  - Effective for low level contami
  - Must be heat-labile
  - $$$
  - Check with you manufacturer!

Effectiveness of UNG



Without UNG



With UNG

Strategies to Minimize Risk

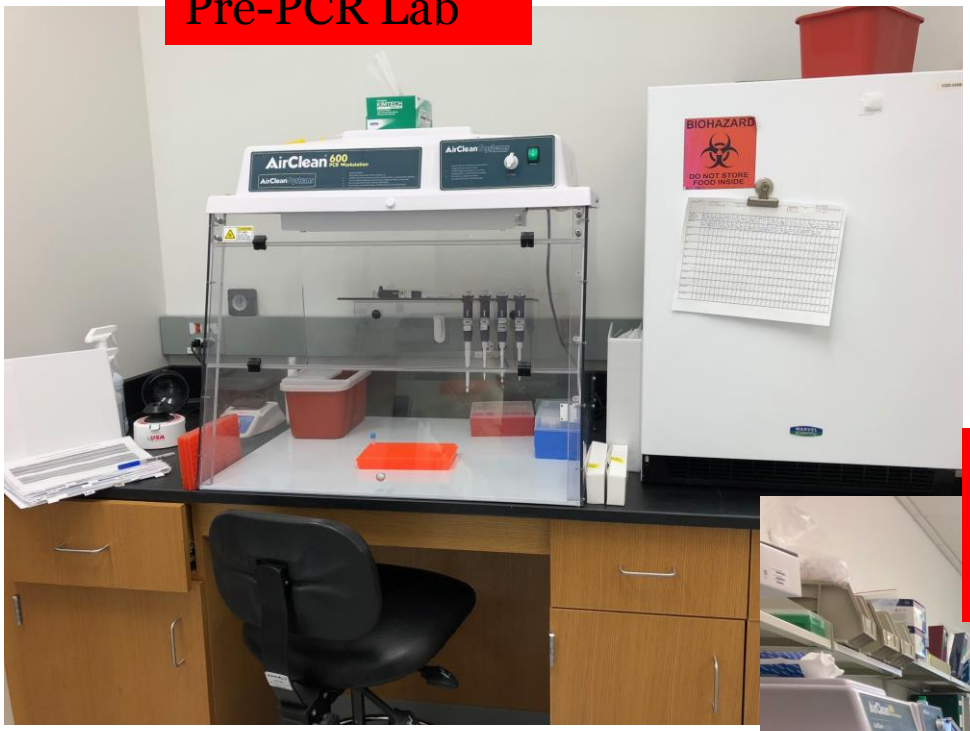
Lab Design & Workflow

- Pre-PCR Lab Area
  - Preparing & storage of PCR reagents.
  - Mini "Clean Room"
  - Dedicated refrigerator/*freezer*
  - Dedicated PPE, pipetters and supplies.
- Specimen Processing Area
  - Handling nucleic acid & control material
- PCR Lab Area
  - "Dirty lab", High risk area
  - Amplicons
  - Special procedures considered

Unidirectional Workflow

Image source: http://www.medicalexpo.com/prod/biofire-diagnostics/product-93999-817662.html

Pre-PCR Lab



Specimen Processing Lab

Strategies to Minimize Risk

Dedicated Equipment, Reagents & Consumables

- Refrigerator/freezer
- PCR workstations
- Pipettes with aerosol resistant tips (ART)
- PPE
- Practice color coding
- Do NOT borrow without decontaminating first!

Image source: www.aircleansystems.com

Strategies to Minimize Risk

Molecular Training Program

- Molecular lab orientation program.
- Knowledgeable about amplicon risk.
- Importance of good lab practice.
- Pipetting practice.
- Competency assessments.
- Incident management.

Molecular Best Practices

- Process one specimen at a time.
- Frequent glove changes.
- Robust lab cleaning program.
- Monitor positivity rates.
- Use low level positive controls.
- Frequent negative controls.
- Avoid testing if ill.
- Bleach (~5%) is most effective for labs performing molecular testing.

Molecular Best Practices

- Use non-frost free freezers.
- Aliquot reagents, avoid repeated freeze/thaw cycles.
- Strategy to dispose of used cartridges.
- Molecular spill clean-up procedures & follow-up monitoring.
- Avoid loading cartridges in areas where cultures are performed.
- Store your “clean” reagents separate from controls.
- Single use procedure pad

Molecular Best Practices

Recognize potential contamination

- Look for unusual patterns.
- Out of season positives.
- Monitor positivity rates.
- Review specimens that are close to the cut-off.

Periodic environmental wipe testing:

- Wet polyester swab
- Wipe lab areas
- Place in 0.5ml water & vortex
- Test

Where are we know??

CLIA waived Molecular Testing

- First waiver granted 2015.
- Transforming where molecular testing is performed.

Alere Receives FDA CLIA Waiver for Alere™ i RSV Rapid Molecular Test
First molecular test to detect RSV infection in 13 minutes or less now widely available in a broad range of healthcare settings

Image source: molecular.roche.com

Examples of Lab Contamination

JCM
Journals.ASM.org

Aerosolized Vaccine as an Unexpected Source of False-Positive *Bordetella pertussis* PCR Results

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When 13 of 13 nasal wash specimens from a single pediatrician's office tested positive for low quantities of *Bordetella pertussis* DNA, we suspected prelaboratory contamination. Investigation revealed that Pentacel and Adacel vaccines contain high copy numbers of *B. pertussis* DNA, which can be aerosolized, causing false-positive *B. pertussis* PCR results.

JCM (2012) Feb. 472-474

Examples of Contamination

JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 1996, p. 1949–1951 Vol. 34, No. 8
0095-1137/96/$04.00+0

Amplification of Residual DNA Sequences in Sterile Bronchoscopes Leading to False-Positive PCR Results

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PCR has been used successfully for the direct detection of *Mycobacterium tuberculosis* in uncultured patient samples. Its potential is hindered by the risk of false-positive results as a result of either amplicon carryover or cross-contamination between patient samples. In the present study, we investigated whether residual amplifiable human or *M. tuberculosis* DNA could remain in sterile bronchoscopes and potentially be a cause of false-positive PCR results in subsequent patient samples. Sterilized bronchoscopes were flushed with sterile saline, and the collected eluate was submitted for PCR amplification of IS*6110* sequences and exon 8 of the human p53 gene. Of a total of 55 washes of sterile bronchoscopes from two institutions, 2 (3.6%) contained amplifiable *M. tuberculosis* DNA and 11 (20%) contained residual human DNA. These findings indicate that residual DNA can remain in sterilized bronchoscopes and can be a source of false-positive PCR results.

Key Points

- Consider molecular risk assessments.
- Molecular specific training program.
- Lab design and dedicated equipment.
- Set up a monitoring program with key indicators.
- Contamination will occur, plan now!!

Useful Resources

Strategies to Identify and Eliminate Contamination in the Molecular Testing Laboratory

Diagnostic tests that amplify nucleic acids (e.g. PCR) are highly sensitive procedures capable of detecting small quantities of target DNA. During amplification ≥10^9 copies (amplicons) of target DNA may be produced. Therefore, laboratories must implement stringent quality control procedures to prevent contamination of the laboratory and generation of false-positive results. The following guidelines are intended to help laboratories identify, eliminate, and minimize future risk of DNA contamination.

How to recognize contamination:

- Look for an increase in positivity rates for low prevalence organisms.
- Numerous "low level" positives (e.g. just above the cut-off values).
- Unusual patterns of "out of season" positives (e.g. RSV in the summer months).
- Results that do not fit the clinical diagnosis.
- Perform periodic environmental testing (e.g. swab testing).

What to do when you suspect amplicon contamination:

- Determine what areas, instruments, or reagents are contaminated through environmental wipe testing.
- Use fresh 5-10% bleach (shown to be the most effective means to remove amplicon contamination) to disinfect contaminated surfaces. After drying, remove bleach residue by wiping with 70% alcohol (isopropyl or ethanol).
- Dispose of affected test reagents and kits.
- Rigorously clean (at least 3 consecutive days) contaminated areas (especially handles, door knobs, light switches, etc.).
- It may take days or weeks to remove lab contamination depending upon the level of contamination present.
- Monitor positivity rates. Retest low level positive specimens.

Strategies to minimize risk:

- Store testing reagents in a designated "clean" area free from positive controls and patient specimens.
- Have dedicated lab supplies, pipettes, PPE, refrigerators/freezers for pre-PCR, sample processing and amplification areas. Color code if possible.
- Practice unidirectional workflow.
- Use aerosol resistant pipette tips.
- Daily decontamination with 5% bleach followed by ethanol wipe.
- Process one specimen at a time with frequent glove changes.
- Paper and pens can become contaminated. Do not move between areas.

Useful Resources

MM19 Establishing Molecular Testing in Clinical Laboratory Environments, 1st Edition. CLSI

Strategies for Avoiding Amplicon Contamination in the Molecular Laboratory (2018). MLO. 50:2 36-37.

Preventing PCR Amplification Carryover Contamination in a Clinical Laboratory

http://www.annclinlabsci.org/content/34/4/389.full

Clean Up Your Act! How To Clean Up PCR Contamination

https://bitesizebio.com/20773/clean-up-your-act-how-to-clean-up-pcr-contamination/

10 Ways to Minimize Contamination in a Molecular Laboratory

https://www.luminexcorp.com/eu/blog/2014/10/14/10-ways-minimize-contamination-molecular-laboratory/

Could your PCR be affected by contamination?

http://www.idtdna.com/pages/education/decoded/article/could-your-pcr-be-affected-by-contamination

Control of contamination associated with PCR and other amplification reactions

https://tools.thermofisher.com/content/sfs/brochures/D21053.pdf


THANK
YOU