Laboratoire de sciences judiciaires et de médecine légale Québec
100 ANS D'EXPERTISE

Do plastic leachables interfere with forensic DNA control solutions?

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Green Mountain DNA Conference,
August 2016

Plastic labware leachables

- 2007 - Reports of leaching bisphenol A and antimony into food and beverages from polycarbonate and polyethylene containers
- 2008 - 1st scientific communication concerning the presence of leachables in plastic labware used in enzymatic studies:

  G. R. MacDonald et all, Bioactive contaminants leach from disposable laboratory plasticware, Science 2008 [1]

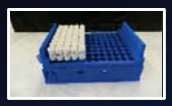
- 2008 – 2012 - Several publications concerning a broad range of biological assays affected by plastic leachables (ex: enzymatic, receptor binding and photometric) [1, 2, 3, 4, 5, 6]

LSJML Biology/DNA - Quantification

- Quantifiler Duo DNA Quantification Kit, Applied Biosystems ®
- 7500 Real-Time PCR System, Applied Biosystems ®
- Positive control : NIST
- Negative control
- Automated procedure: Freedom EVO, Tecan ®

LSJML Biology/DNA - Quantification

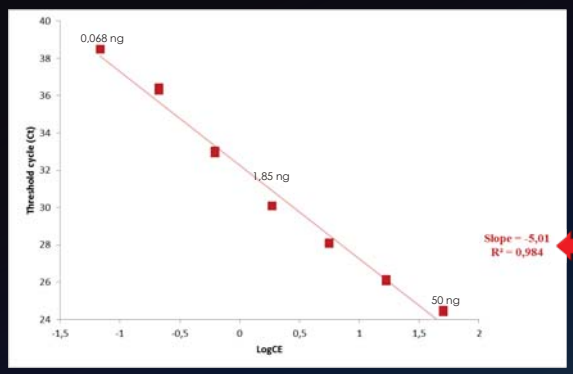
- Standard curve with 7 points: 50, 16.7, 5.56, 1.85, 0.62, 0.21 and 0.068 ng
- Quantifiler Duo standards dilutions stored in our regular DNA storage tubes ("M")



- Standard curve with expected slope between -3.0 and - 3.6 and $R^2 \geq 0.98$

Abnormal quantification DUO standard curves

November 1st 2011

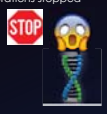


Troubleshooting timeline

Nov 1st 2011
Abnormal standard curves detected

Immediate troubleshooting:
- Quantifiler Duo Standard dilutions
- Quantifiler Duo Kit lot
- 7500 Real-Time PCR System verification (parameters ok)
- Freezing vs refrigerating Quantifiler Duo standard dilutions
- Manual vs robot

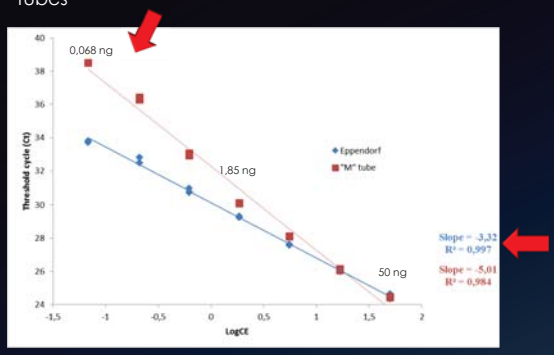
2nd week Nov 2011
Biology / DNA operations stopped



DUO Standard dilutions storage tubes ?

Abnormal quantification DUO standard curves

Fresh Quantifiler Duo standards dilutions were prepared and stored in regular Eppendorf ® tubes



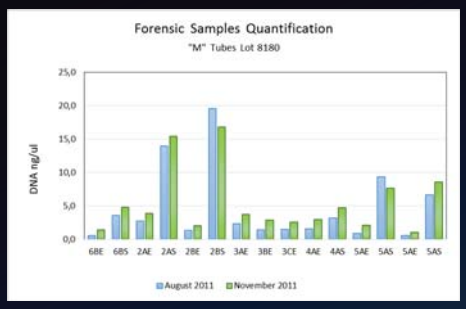
The culprit

- Normal quantification curves were obtained
- The culprit seemed to be found

"M" tubes lot number 8180

Effect on stored forensic samples

- 15 casework samples, stored in the problematic lot tubes were re-quantified 3 months after



No evidence of decrease on DNA concentration

Operations resumed

- End November 2011 operations were resumed with the following changes:
  - "M" tubes lot 8180 removed from operations
  - Quantifiler DUO standard dilutions prepared freshly in Eppendorf ® tubes
  - New "M" tubes lots tested before use
  - Use of "M" Tubes traced in our database (lot number and date of use)
  - Manufacturer contacted to enquire about recent changes on their production line

Troubleshooting timeline

1st Nov 2011
Abnormal standard curves detected

Immediate troubleshooting:
- Quantifiler Duo Standard dilutions
- Quantifiler Duo Kit lot
- 7500 Real-Time PCR System verification (parameters ok)
- Freezing vs refrigerating Quantifiler Duo Standard dilutions
- Manual vs robot

2nd week Nov 2011
Biology / DNA operations stopped

End Nov 2011
Biology / DNA Operations resumed

Storage effect on Duo standards over time

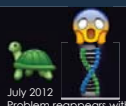
- Quantifiler DUO standards dilutions prepared and stored in "M" tubes (lot 8180)
- Standard curves established after 24h, 96h and 12 days

Storage effect on Duo standards over time

Troubleshooting timeline

1st Nov 2011
Abnormal standard curves detected

Immediate troubleshooting:
- Quantifiler Duo Standard dilutions
- Quantifiler Duo Kit lot
- 7500 Real-Time PCR System verification (parameters ok)
- Freezing vs refrigerating Quantifiler Duo Standard dilutions
- Manual vs robot



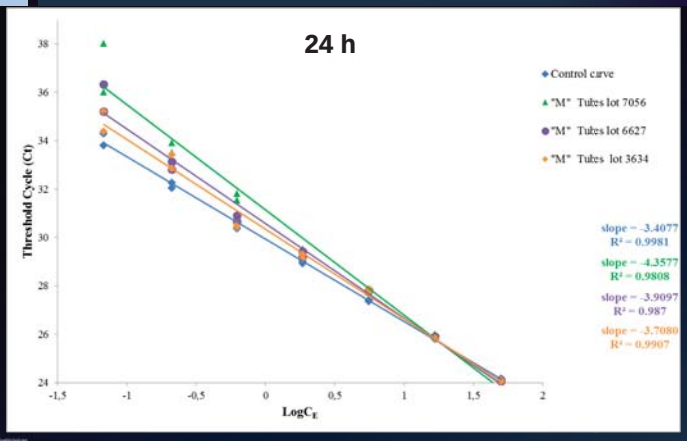
July 2012
Problem reappears with 3 "M" tube lots
Biology / DNA operations slowed down

8 months later ...

2nd week Nov 2011
Biology / DNA operations stopped

End November 2011
Biology / DNA Operations resumed:
- Minitubes lot 8180 removed from use
- New "M" tubes – tested before use
- "M" tubes lots traced in our database

Three new « M » tube lots affected

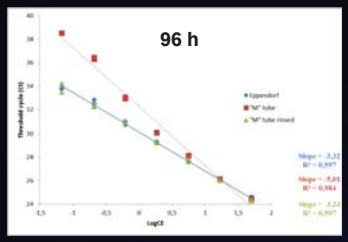


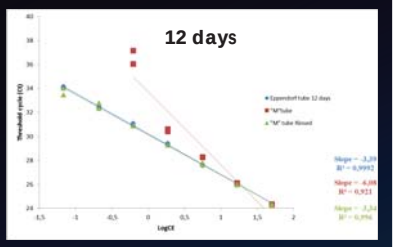
What caused the problem?

- Were ethylene oxide or other chemicals used?
- DNA adsorption to the tube walls?
- Leachable substance released from the plastic?

Further testing needed

Tube rinsing*: effect on quantification standards





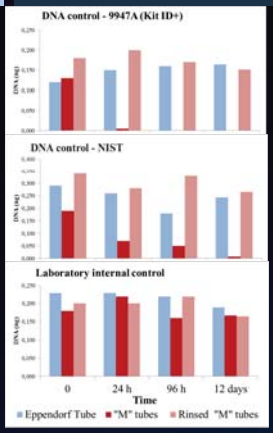
R^2 and slope restored when tubes rinsed

*Tubes were rinsed with 0,25X TE by inverting 4 times

Effect on stored control DNA

- Three DNA controls:
  - 9947A (commercial)
  - NIST (commercial)
  - Lab internal control
- Three storage tubes tested:
  - Standard eppendorf
  - "M" tube (problematic lot: 8180)
  - "M" tube (problematic lot: 8180) rinsed prior to use
- Quantified at different time points after storage:
  - 24h
  - 96h
  - 12 days

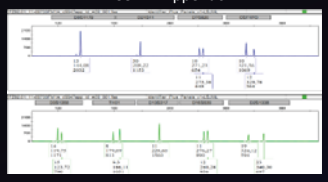
Effect on stored DNA



- Within 24h, an important decrease in DNA amount was observed for the two commercial DNAs (9947A and NIST)
- For the 9947A control, that decrease continued and no DNA was detected after 96h
- LIC did not seem to be affected
- Rinsing the tubes prior to use eliminated the "M" tubes impact

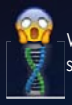
Effect on stored DNA – 24h

9947A Eppendorf



9947A « M » tubes

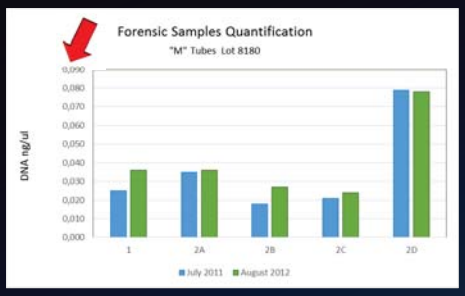
9947A « M » tubes rinsed



Were the casework samples stored in those tubes affected?

Effect on stored forensic samples

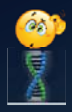
5 low concentration casework samples, stored in tubes from the problematic lot, were re-quantified and re-amplified after 1 year of storage



No obvious difference

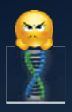
Communications with manufacturer

- All contacts were established through sales representatives and the research & development departement. Never the production line.
- First contacts in November 2011, formal complaint opened July 18th 2012 and answered October 2012
- Contradictory informations:
  - 18th August 2012 « « M » tubes **are sterilised using gamma irradiation** »
  - 1st November 2012 « **The catalog number you are purchasing is not gamma irradiated** »



Communications with manufacturer

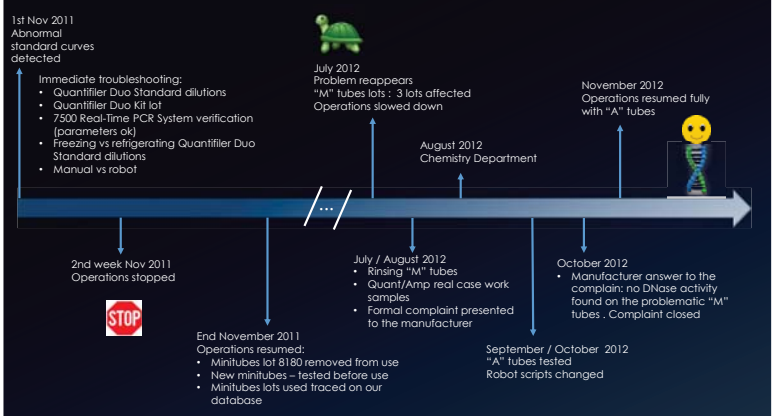
- October 24th 2012 we received the answer to our complaint :
  - Tubes are produced within a controlled manufacturing environment (filtered air, hair bonnet, facemask, frocks and gloves are mandatory)
  - No chemicals used during manufacturing process
  - Tests done on the defective « M » tubes show no DNAse activity
  - Nothing had changed on the production line
  - Complaint closed



Possible solutions

- Rinsing tubes before use
  - Logistics difficult to implement in regular operations
- Change manufacturer
  - Only one other manufacturer available : « A » tubes
  - « A » tubes had been previously used in our lab, replaced because 2D bar code often caused problems
  - « A » tubes dimension and format were different than « M » tubes: all robot scripts needed to be changed, verification of robotic process required

Troubleshooting timeline



1 year / operations affected for 4 months

Conclusions

- Plastic labware (some « M » tubes lots) strongly interfered with DNA controls
- Highly purified and low concentration DNA severely affected (degradation, amplification inhibition?)
- Cause: an unidentified leachable substance ?

Recommandations

- Laboratory quality control/management:
  - Track all plastic labware use
  - Test critical plastic labware prior to use
  - Order early enough so new lots can be tested before the lots in use are finished

Recommandations

- Manufacturer :
  - Troubleshooting should be closer to the production line
  - Improved communications with customer
  - Technical specifications of laboratory consumables should include full disclosure of the additives used in manufacturing process [1]

Thanks to all members of the troubleshooting team:

Sarah Bourgoin, Anita Rogic, Alphonse Ligondé, Laure Delpech, Sara-Kim Rochette, France Mailly

« N'avance rien que tu ne sois capable de prouver »

Dr Wilfrid Derome

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References

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5. L.K. Lewis, M.H. Robson, Y. Vecherkina, C. Ji, G.W. Beall, Interference with spectrophotometric analysis of nucleic acids and proteins by leaching of chemicals from plastic tubes, BioTechniques 48 (2010) 297-302.
6. A. Katsnelson, *Plastic Hampers DNA assay*, Nature news online, April 2010

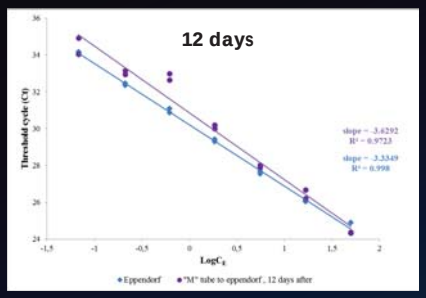
Chemical Testing

- 5 "M" tubes and 5 eppendorf[R] tubes sent to the chemistry department to testing
  - Tert-butyl-alcohol detected in one of the affected "M" tubes.
- Tert-butyl-alcohol dilutions (non diluted, 1/10, 1/100 et 1/1000) added to two DNA controls (9947A and NIST) and quantified after 24h incubation time

✓ No effect

Leachable transfer?

Standard curve from the Duo Standard DNA control when placed into "M" tube for <30 seconds prior to being transferred into a eppendorf



Slope value decreases indicating that DNA interference continues after the solution is transferred into a neutral tube: leachables seem to be present