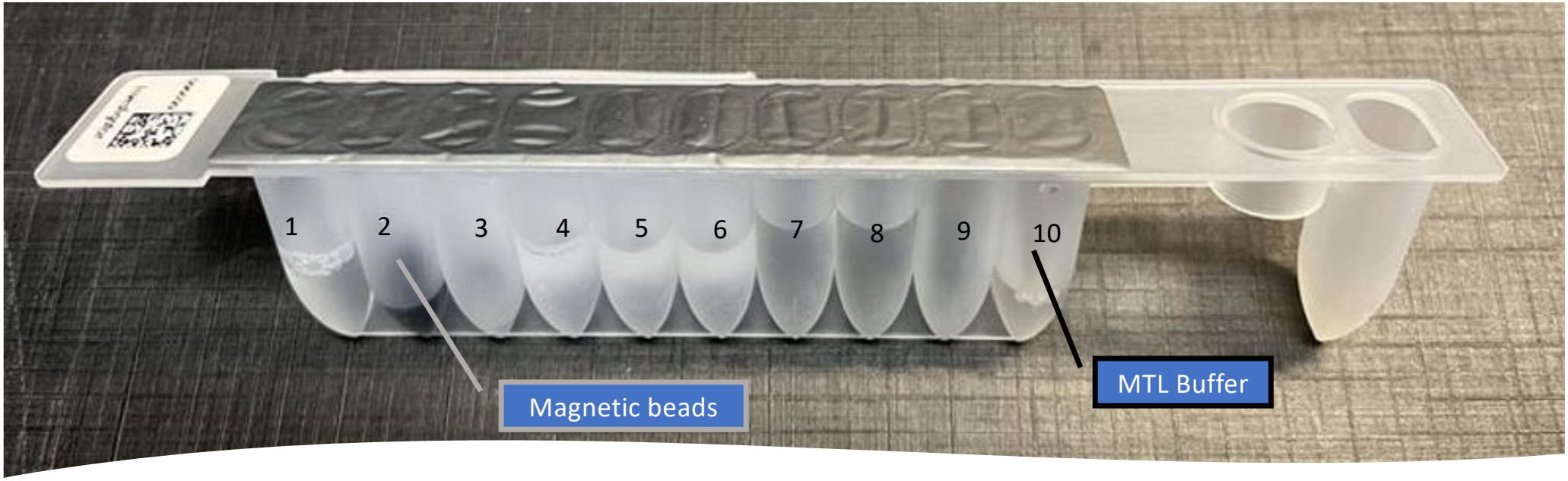
Qiagen EZ1/EZ2 Investigator Kit Issues

Texas Forensic Science Commission Meeting

Peter Stout, CEO, Houston Forensic Science Center, President Texas Association of Crime Lab Directors

Erika Ziemak, TFSC Commissioner

April 11, 2025



- DNA binds to the magnetic beads in the presence of high salt and low pH environment – pH is important
- DNA stays bound to the magnetic bead during the purification process
- Once purified, the DNA is recovered from the magnetic beads in the presence of low salt and high pH environment

Qiagen Published Quality Systems

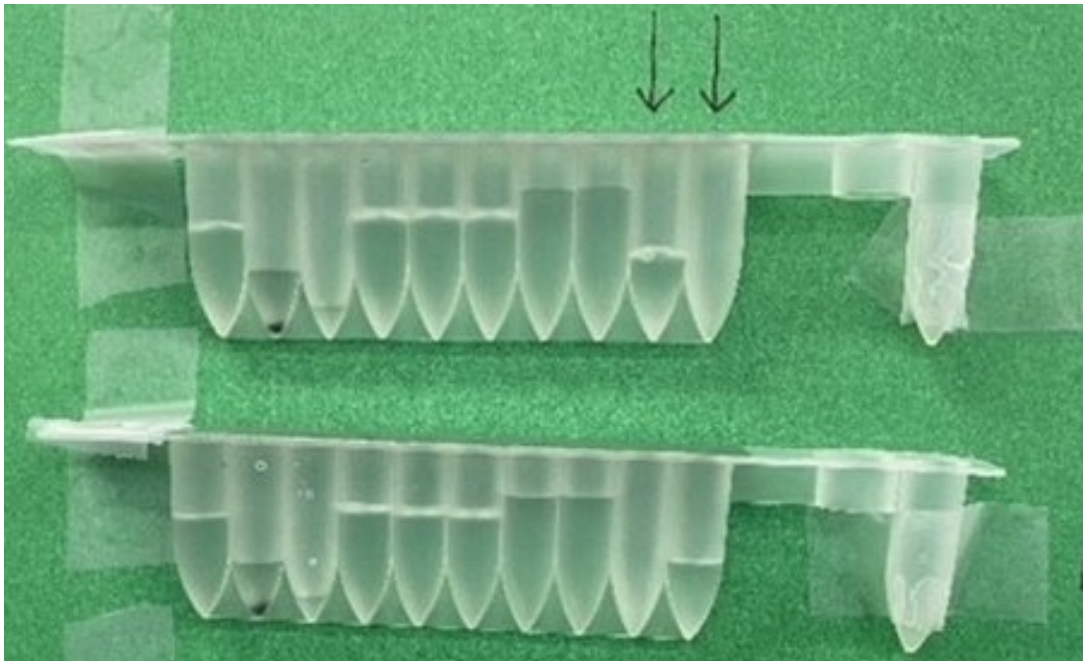
- **Human ID division**
- QIAGEN Forensic DNA Grade Quality
- ISO 18385 Production and supplier quality standard
- Automated manufacturing and EO (ethylene oxide) treatment
- Risk analysis of manufacturing processes
- QIAGEN has maintained a staff DNA profile database managed by an independent custodian from a German forensic institute.

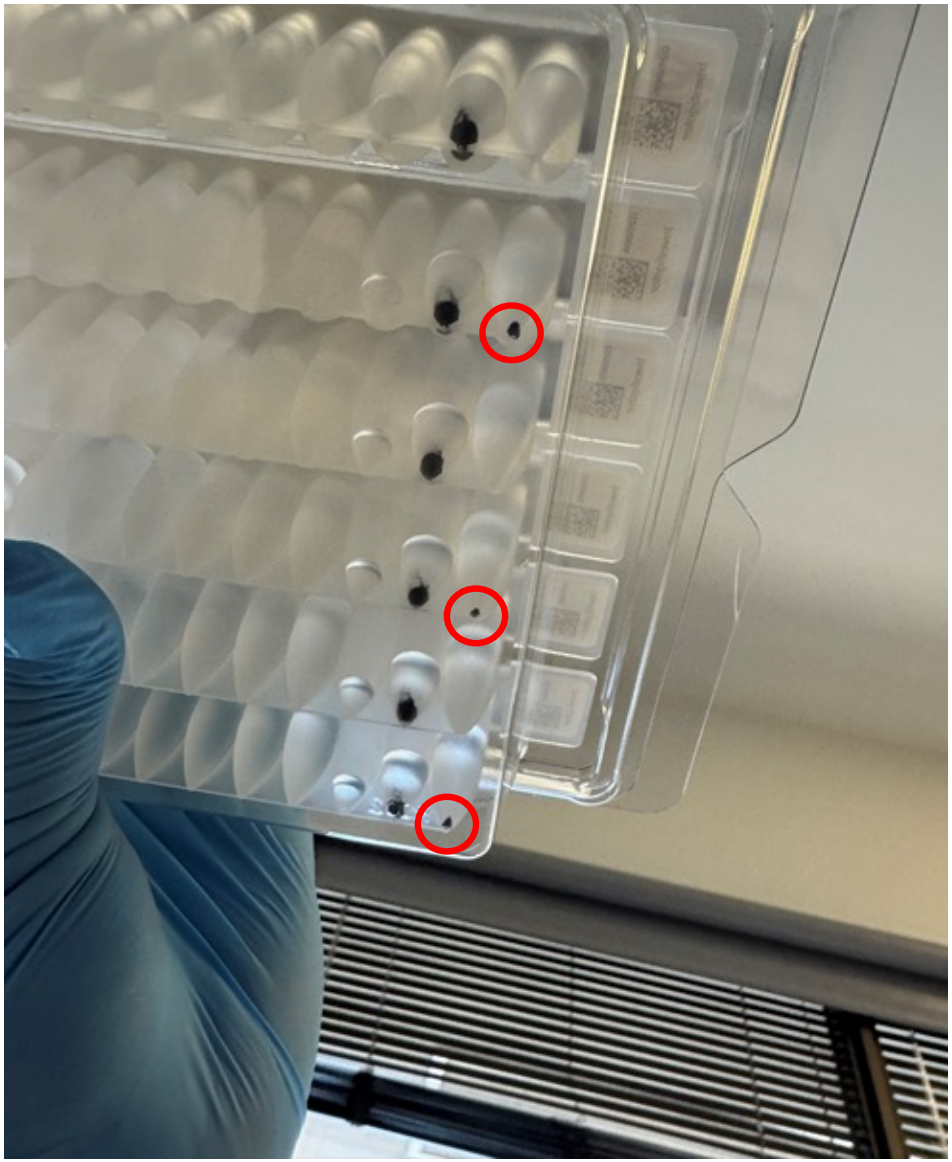
3 Qiagen Manufacturing Issues

- Low or no DNA recovery due to issue with pH in MTL buffer
- MTL buffer misplaced, should be in well 10 but in well 9 for some lots
- Magnetic beads in well 1 but should be in well 2

Issues 2 and 3

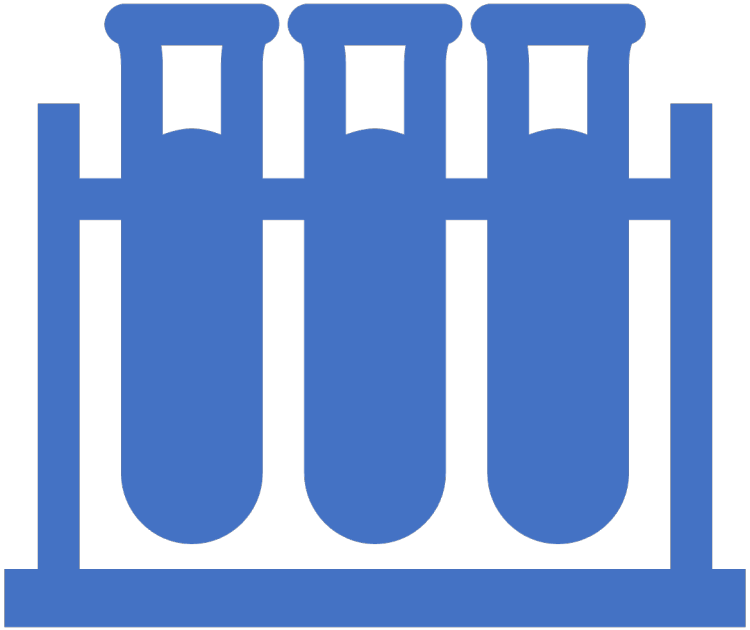
- Visual inspection is part of the QC process
  - MTL Buffer switch from well 10 to well 9
  - Mag beads located in well 1 when they should be in well 2 (from new and improved manufacturing process)





HFSC identified samples with unintuitive results tested at Signature Science

- Uptick in samples presumptive positive for blood at HFSC with no, or low, DNA recovery at SigSci
- All samples extracted at SigSci utilize EZ1/EZ2 investigator kit
- HFSC went back to look at blood samples sent to SigSci to begin troubleshooting
  - Nov 2022-May 2024 247 blood samples sent to SigSci
    - **7/247** samples had unintuitive DNA results **2.8%**
  - June 2024 – Aug 2024 258 blood samples sent to SigSci
    - **37/258** samples had unintuitive DNA results **14.3%**

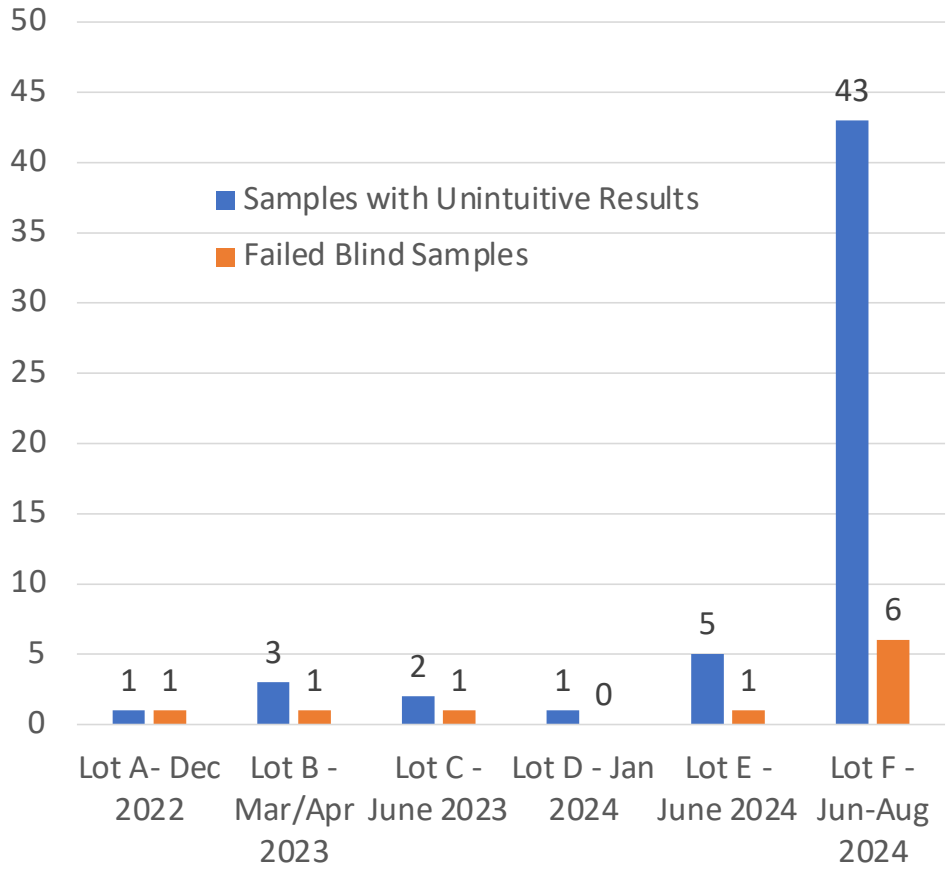


Is it only blood samples or could it be trace DNA samples, too?

- Trace DNA samples are tough to identify because there is no expected level of DNA recover
- Low or no DNA results aren't surprising
- HFSC took a closer look at ***blind quality cases*** containing trace DNA samples sent to SigSci
- ***9 blind quality cases*** identified with no DNA or low DNA where samples were uninterpretable
- HFSC retested the 9 blind quality cases and in all cases a higher amount of ***DNA was detected and all samples were interpretable***

Closer look at all HFSC tested samples at SigSci with unintuitive results to look for trends

6 lots of EZ1/EZ2 Investigator kits were used



*Lot F is cartridge lot 178019172 and kit lot 178020200

Certificate of Analysis for Lot “F”

QIAGEN Certificate of Analysis

Product Name: **EZ1&2 DNA Investigator Kit (48)**
Material Number: **952034**
Lot Number: **178020200**
Exp. Date: **2025/04/14** (YYYY/MM/DD)

Catalog Number: 952034 EZ1&2 DNA Investigator Kit (48)

Characteristic	Unit	Value	Lower Limit	Upper Limit
EZ1&2 DNA Investigator Kit				
Recovery of 4 ng DNA per sample		Passed		
Absence of human genomic DNA		Passed		
MagAttract Suspension B				
Particle size		Passed		
Yield >/= 50 % after WBC count		Passed		
A260 / A280		Passed		
PCR performance		Passed		
Buffer G2				
Conductivity	mS/cm	55,91	53,19	61,24
Proteinase K				
Activity >/= 318 mAU/ml (30°C, 1:6660)		Passed		
Functional absence of RNase		Passed		
Functional absence of DNase		Passed		

This certifies that the product was manufactured and tested in accordance with QIAGEN QC and QA procedures. The product has met all QIAGEN quality requirements.

Storage
Store at room temperature (15-25°C).

Dr. Henning Plücken
Quality Assurance

This Certificate is a computer printout generated after product release and therefore valid without signature.

*Lot F is cartridge lot 178019172 and kit lot 178020200

Lot F* clearly seems to be a problem

- 43 impacted samples
- Tested over 3-month time frame June 2024 – July 2024
- Samples extracted on 6 different dates
- Samples extracted by 3 different analysts
- Samples were in 13 different batches
- Samples were extracted on 5 different instruments

This issue does not seem to be a SigSci problem

*Lot F is cartridge lot 178019172 and kit lot 178020200

Signature Science was doing their own investigation

- HFSC paused outsource testing with SigSci
- Determined root cause of extraction problem could be a Qiagen manufacturing issue
- Qiagen worked with SigSci to develop a testing plan evaluate instruments and chemistry
- **340 samples were tested** at SigSci across all their EZ1 and EZ2 instruments
  - Blood and semen samples were tested
  - Dilution sets were also tested to evaluate potential low yield
  - Different lots of DNA Investigator kits were utilized (Old, New, and Bad)
  - 24 samples sent to HFSC to evaluate data from 2 different labs for consistency
- **87 HFSC created/tested blind cases** were sent to SigSci for re-testing
- No issues identified with instruments or staff, results of testing was as expected
- Resume outsource testing with SigSci very soon

Meetings with Qiagen

- March 19, 2025 – Qiagen manufacturing/QA in Germany, HFSC, SigSci
- March 19, 2025 – Qiagen and CHI
- March 25, 2025 – Qiagen manufacturing/QA in Germany, HFSC, SigSci, ANAB, ASCLD, TFSC

Topics discussed and follow up questions:

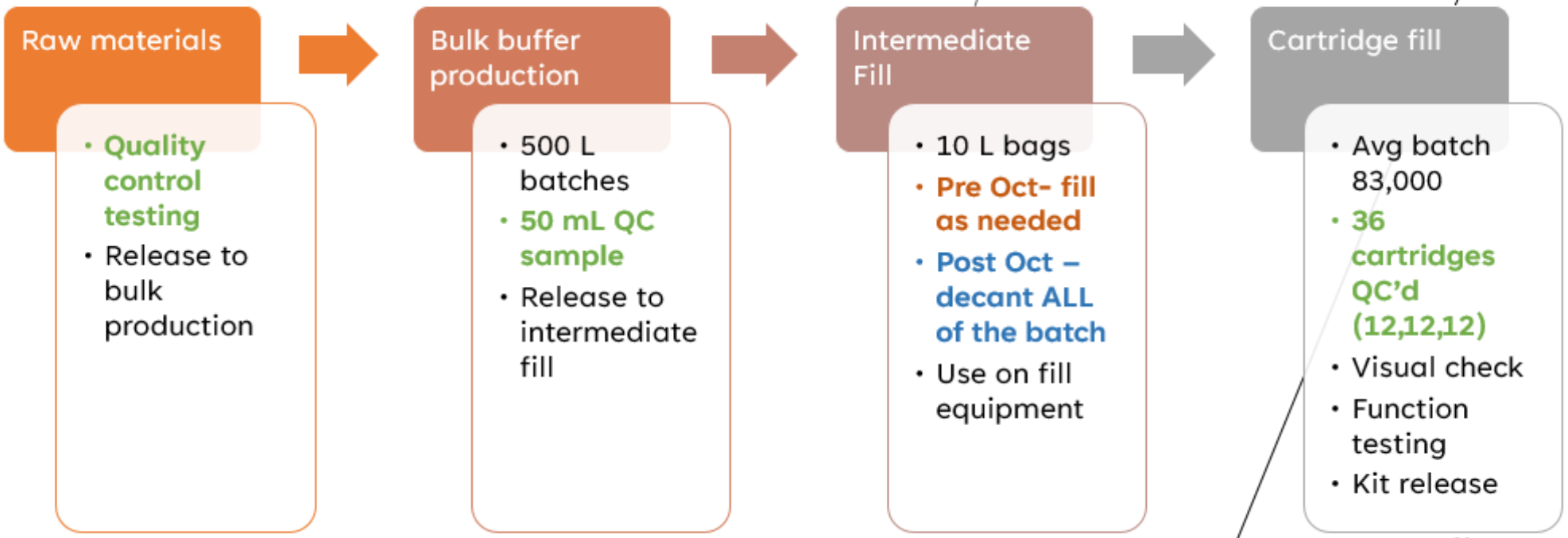
- Covered Qiagen quality incident investigations
- General production, manufacturing and root cause analysis
- Quality assurance testing
- Customer complaint tracking

Information from Qiagen

- Meeting information and follow up questions:
  - Indicated manufacturing QC process worked as expected
  - QC sampling beginning, middle, end of manufacturing did not indicate issue
  - Acknowledged some kits contain MTL buffer with a higher than acceptable pH
    - Acceptable pH range 7.4-7.6 while kits were identified as having a pH of 8.5
  - Manufacturing process improvement made October 2024
  - Identified time frame of affected lots as February 28– October 23, 2024
  - Low complaint rate 0.1% of kits shipped
  - No indication of broader systemic issue
  - No evidence kits beyond those reviewed are affected or that test results are unreliable
- Qiagen issues letter to all North American Customers April 3,2025
  - Identified 7 different EZ1/EZ2 cartridge lot numbers impacted

QIAGEN EZ1/2 CARTRIDGE MANUFACTURE SCHEMATIC

Step where Qiagen attributes the pH variability



Hypergeometric Model

For example, if the lot size is 83,000, there is an unknown number of correct items. The tolerable rate of defective items is 1 in 100, and the desired confidence level is 0.95, we would need to inspect a minimum of **235 randomly selected items** from the lot to be able to say that with 95% confidence the number of non-defective items in the lot is at least 82,170, and the number of defective items is no more than 830 items.

Customer Complaint Trending

- Complaint trending is performed for all product groups manufactured by QIAGEN to identify recurring issues in the field and drive continuous improvement.
- Calculated rate of complaints per unit per month. Average of 2,500 kits shipped per month which equates to 120,000 cartridges
- Since January 2022, the complaint rate has been between 0 - 0.1%
- Began investigation in August 2024 when complaint rate increased to ~0.12% (3 complaints)

Opportunities for Improvement

- Provide more information to customers about the complaint process
- Transparency with customer notifications
  - Sharing information across labs could help others be on the lookout for potential issues and report them in a timely manner
- Increase number of tested samples in the manufacturer QC process to provide higher level of statistical confidence to detect variability
- Use a lower amount of DNA input to detect potential issues with low yield to QC

HFSC laboratory changes

- Evaluating other non-cartridge-based extraction platforms
  - Variability concerns with cartridges
  - QC of one cartridge may not be a reflection of all cartridges rec'd in lab
- Change QC process to use lower input of DNA (1:75 dilution) to evaluate performance with low yield samples
- Establish pass/fail range based on quant value during QC
  - This process can also show performance over time from lot to lot is consistent
- More stringent visual inspection of each cartridge needed prior to use

HFSC actions taken

- Despite the Qiagen customer letter there seems to be other impacted lots
  - SigSci Lot F not included in customer letter (nor any of the other 5 lots)
- HFSC nor SigSci received any of the lots included in the Qiagen customer letter
- HFSC disclosed to the Harris County District Attorney's office due to the issues seen at SigSci and the info in the customer letter
- Focus on Qiagen timeframe February – October 2024
- Working to identify potentially impacted cases at HFSC and SigSci
- HFSC will coordinate retesting for potentially impacted cases

HFSC actions taken, con't

- HFSC has used EZ1/EZ2 kits for ~15 years
- For ~15 years of internal quality checks, performance checks, proficiency tests, and blind quality control tests, samples have given expected results
- Internal process to check for unintuitive results
  - No trends identified
- All internal checks balances have worked as expected to suggest reagents used in our laboratory have NOT been impacted by a gross manufacturing issue
- Low or no DNA recovery from a sample may not always be the result of a manufacturing issue; sampling or a rare one-off processing issue could arise
  - HFSC utilizes ~half of the sample for testing in most cases
  - Sample remains for retesting or future testing
  - To limit bias, HFSC has minimal case information and generally does not know the impact of a sample on a case
  - If low or no DNA recovery was the original result; stakeholder should contact HFSC to discuss if additional testing may be an option