

Development and Use of Synthetic Controls: Issues and Challenges to Developers, Laboratories, and the Community

Bassem A. Bejjani, MD, FACMG

Co-Director,

Molecular Diagnostics Laboratory,

Sacred Heart Medical Center, Spokane



What is available?

- Coriell cell lines.
- Genomic samples from other laboratories.
- Identified patient samples.
- Incomplete mutation set that cannot be used to check every reagent in every plate.

A QC scheme that is not testing every reagent/reaction in the assay.

Issues and Challenges to Developers

- What kind of controls are needed?
- Do most labs have the same needs and requirements?
- What are our requirements and needs as developers?
- Initial feedback and integration of laboratory recommendations.

What we Expected...

- Genomic DNA is not an ideal control for multiplex testing.
- As the complexity of multiplex testing increases, synthetic substitutes are more likely to become a source of controls.

What we Expected...

- Everyone will want to use these synthetics to answer the question: “Are all of the components of my reaction working in this test run?”
- Everyone will want a control that can test every mutation in a single reaction tube.

What we Expected...

- Laboratories in the U.S. would be willing to pay for such controls.

What we Didn't Quite Expect...

- Laboratories have a wide range of expectations and requirements for reference standards.
- There is a wide range of QC procedures ranging from very complete to not so complete.

What we Didn't Quite Expect...

- There is a lot of resistance to change “set” procedures in a laboratory.
- Some laboratories don't see the need for controls that check all the mutant primers/probes in an assay.

Challenges and Unexpected Hurdles

- We are a clinical laboratory not a product manufacturing facility.
- Costs of development: patent, GMP, ISO, equipment...
- Additional resources, lawyers, marketing, consultants...
- Although we have a high level of flexibility with our controls it is difficult to determine what labs require.

Challenges and Unexpected Hurdles

- Do we make a control that is satisfactory for everyone or do we make several controls that custom fit each platform/user?
- Will we be able to collaborate with kit manufacturers?
- How do we make the transition from clinical testing to product development?

Costs

- R&D time taken from clinical assay development.
- Testing and validation on different platforms.
- Outsourcing GMP production ~\$100,000 - \$150,000/yr.
- In-house GMP production costs?
- No grant support; hospital budget limited.
- Additional costs, legal fees, consultants, marketing...

Approach

- Make one control that covers the highest volume of CF test kits.
 - CF32 for OLA and ASPE
- Use sales from this control to pay for current costs and future development.
- See what different laboratories need and make controls accordingly.
- Identify the various laboratory QC schemes to develop a standardized QC strategy.

Integration of Synthetic Substitutes

- Modification of current QC scheme.
- Mutation specific primers/probes can now be evaluated by integrating a synthetic control into a standard QC scheme.
- A synthetic control can be used in addition to (or in place of) a genomic mutant control.

What do we need?

- Synthetic control for rare mutations or mutations in short supply.
- A QC scheme that evaluates every reagent in the assay.
- A control set that can do this in as few reagent wells as possible.

What do we have?

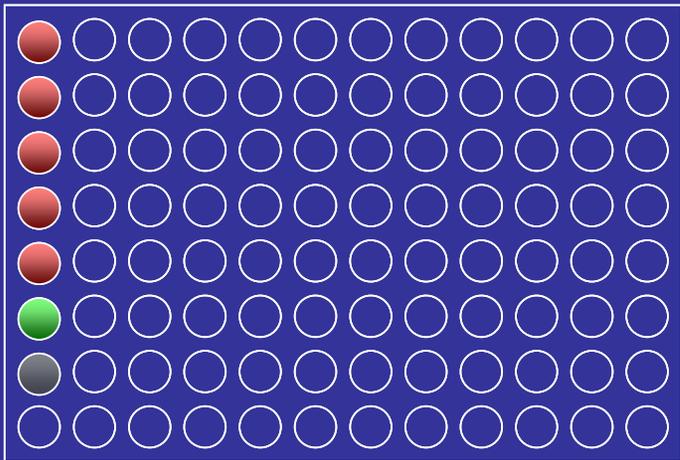
- A synthetic control for every mutation that is tested by our CF assay.
- A comprehensive QC scheme that tests every reagent in the plate using only three (or four) test wells.
- Stockpiled genomic DNA for most of the mutations that are tested by our assay.

Current vs. Proposed

Component	G	G/S	Component	G	G/S	Component	G	G/S	Component	G	G/S
Buffer	●	●	I148 wt	●	●	1717-1 wt	●	●	3120+1 wt	●	●
Taq	●	●	I148T	?	●	1717-1G-A	?	●	3120+1G-A	?	●
Ligase	●	●	621+1 wt	●	●	R560 wt	●	●	R1162 wt	●	●
Primers	●	●	621+1G-T	?	●	R560T	?	●	R1162X	?	●
F508 wt	●	●	711+1 wt	●	●	R553 wt	●	●	3659 wt	●	●
ΔF508	●	●	711+1G-T	?	●	R553X	?	●	3659delC	?	●
I507 wt	●	●	1078 wt	●	●	G551 wt	●	●	3849+10kb wt	●	●
ΔI507	●	●	1078delT	?	●	G551D	?	●	3849+10kbC-T	?	●
G542 wt	●	●	R334 wt	●	●	1898+1 wt	●	●	W1282 wt	●	●
G542X	●	●	R334W	?	●	1898+1G-A	?	●	W1282X	?	●
G85 wt	●	●	R347 wt	●	●	2184 wt	●	●	N1303 wt	●	●
G85E	●	●	R347P	?	●	2184delA	?	●	N1303K	?	●
R117 wt	●	●	A455 wt	●	●	2789+5 wt	●	●	G:Genomic Strategy		
R117H	●	●	A455E	?	●	2789+5G-A	?	●	G/S:Genomic/Synthetic		

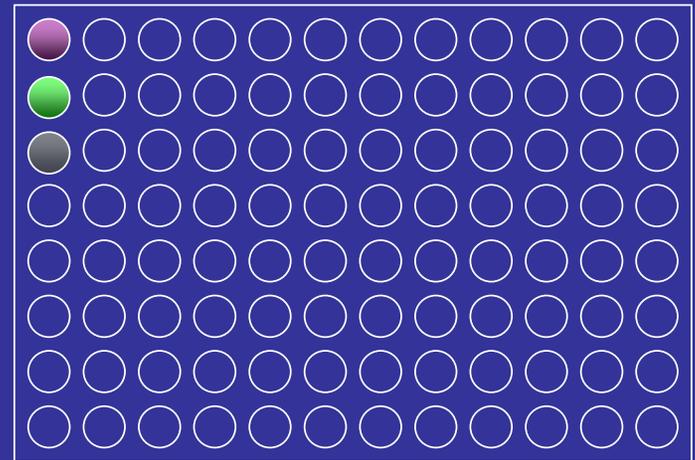
Genomic vs. Genomic/Synthetic (labeling plates and cost savings)

Genomic strategy



Rotate
CF32

Genomic/Synthetic strategy



Comparison between a genomic QC scheme and a genomic/synthetic QC scheme.

Genomic Strategy: uses a wild-type genomic control (green circle), a no template control (grey circle), and 5 mutant genomic controls (red circles).

Genomic/Synthetic strategy: uses a genomic wild type control (green circle), a no template control (grey circle), and 1 synthetic control (purple circle).

Are we satisfied?

- Yes.
- We have been using it for over 2 years.
- We currently run 3 controls per plate providing a comprehensive QC scheme for every plate:
 - Wild-type Genomic
 - No template
 - Synthetic control CF32
- One control mix eliminates the need to obtain, maintain, and rotate multiple mutant control stocks.

Challenges

- Software is not designed to evaluate several mutations within a single sample.
- ASR kits are a “black box”.
- No accurate correlation studies can be performed without knowing the specific reagent parameters of these proprietary ASR kits.

Conclusions

- Our initial QC scheme included the synthetic control and a $\Delta F508$ genomic control.
- Peace of mind in reporting knowing we have evaluated every reagent for every run.
- No additional cost in reagents.
- We can change the well position of our CF32 control to identify and track each specific plate.
- Simplicity and time savings for technologists.

Comparison between QC schemes

- **Traditional Genomic**

- 3-8 controls and reagent wells per setup.
- Many of the mutant probes are not checked.
- Difficult to maintain supply of genomic stocks.
- The more mutant controls tested the higher the reagent cost and fewer patient samples per plate.
- Must maintain large collection of working controls.

- **Genomic/Synthetic**

- 3 controls and reagent wells per setup.
- Every component of the assay is checked.
- Decreased reagent costs and setup time per plate.
- Additional space for patient samples.
- Synthetic source commercially available.