Positive Controls are Now Available!

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DLS Mission

To improve the quality of laboratory practices by:

- providing global leadership
- fostering partnerships/collaborations

As we

- conduct research and surveillance
- disseminate information
- provide training and education
- promote standards and guidelines
- assess technologies

In support of the continuous improvement of the public's health

CDC's Positive Sample Development Work

1999 - CDC Contract #200-98-0011 - DynCorp
2000 - 03 CDC Contract #200-2000-10050 - Duke/Coriell
2000 - 03 CDC Contract #200-2000-10030 - UCLA
2004 - CDC Contract #2004-Q-01403 - Coriell

"CDC/DynCorp Project"
- Needs assessment for QA
- Expert panels
- Positive Controls

"CDC/Duke/Coriell Project"
- Expert panel – targeted diseases/mutations
- Established Cell Lines
- Validated Mutations
- Pilot Tested – "Mock Performance Evaluation/PT"

"CDC/UCLA Project"
- Recombinant Technology
- "Artificial" positive controls"

"CDC/Coriell Project"
- Project Director – Jeanne C. Beck, Ph.D.
- Project Officer – Laurina O. Williams, Ph.D., MPH

CDC/Coriell Project

Re-establishment, Maintenance, Storage, Expansion, and Distribution of Cell Lines and Products Containing Mutations Associated with Diseases of Public Health Importance for Use as Positive Controls for Genetics Testing and Quality Assurance

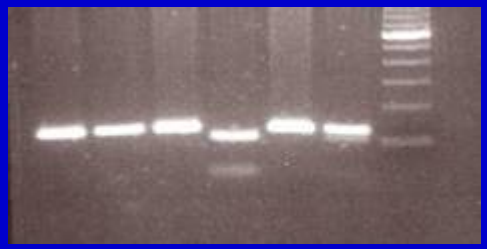
Project Identification:

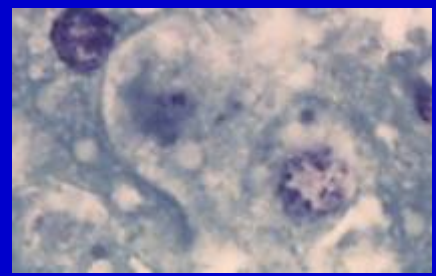
"Project requires the re-establishment, maintenance, storage, expansion, and distribution of cell lines, cells, and products containing mutations associated with genetic diseases of public health importance."

CDC/Coriell Project - Continued

What Materials?

- Cell lines
- Cells
- DNA
- Other products





CDC/Coriell Project - Continued

For What Purposes?

"The cells, cell lines and products derived from them will be distributed for use as positive controls for human genetics testing, for quality assurance purposes, performance evaluation, proficiency testing, and for research and development."

CDC/Coriell Project - Continued

To whom:

- Clinical genetics testing labs
- Research and development
- Proficiency testing/ performance evaluation

"Positive control materials will be distributed at nominal cost to laboratories performing genetic testing, to organizations providing proficiency testing and performance evaluation, to researchers and developers of genetic testing methods, and for any other quality assurance purposes."

CDC/Coriell Project - Continued

Costs:

The cells and products will be offered to clients **according to reasonable pricing structures** established as customary by the contractor and agreed upon by CDC. The costs should be reasonable for laboratories and entities involved with quality assurance.

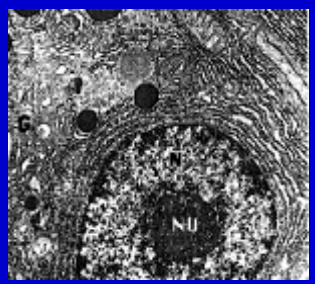
"The contractor is expected to recover the costs of expanding cell lines for distribution, extracting positive control products if necessary, reference testing, packaging and distributing cell lines through the charging the recipients a reasonable fee for cells and products."

CDC/Coriell Project - Continued

What exactly do we have?

40 Cell lines:

- **27 from residual samples**
- **6 from NIGMS collection**
- **7 negative cell lines**



Abstract - AMP Poster # G33 - Saturday
(www.amjpathol.org)

The Centers for Disease Control and Prevention and Coriell Institute for Medical Research are making available positive control samples derived from transformed cell lines containing mutations of public health importance. Validated cell lines or products derived from them are available through Coriell Cell Repositories (Camden, NJ, USA; www.coriell.org/ccr) for genetics testing quality assurance. To capture rare mutations associated with genetic diseases, residual blood samples were collected from clinical laboratories. Thirty-three cell lines were established. Associated diseases included: cystic fibrosis (CF), 5' 10' methylenetetrahydrofolate reductase deficiency (MTHFR), hemochromatosis, thrombophilia, Huntington disease (HD), fragile X syndrome, Muenke syndrome, connexin 26-associated deafness, alpha-thalassemia, sickle cell, and chronic hemolytic anemia. Important CF-associated cell lines contain mutations not previously available (1898+1G>A, 2184delA), mutations associated with unique populations (394delTT, S1235R), and combinations of IVS8 poly-thymidine tract variants (5T, 7T, 9T). Three cell lines with homozygous MTHFR-related mutations are available. Hemochromatosis-associated samples include a compound heterozygote (H63D/S35C). Cell lines with intermediate-range triplet repeat regions associated with HD (31 repeats) and fragile X (57 repeats) are available. A compound heterozygote line containing hemoglobin S mutation and hemoglobin C mutations provides a double-positive control. Mutations were confirmed by reference testing and multi-laboratory pilot testing. Control cell lines negative for all mutations were also confirmed and are available. Thus transformed cell lines can be established from residual specimens as a source of positive controls otherwise difficult to obtain. **Laboratories are encouraged to contact Coriell Cell Repositories about obtaining samples or donating samples as candidates for transformation. (http://ccr.coriell.org/cdc)**

Methods

1. Residual blood samples containing targeted mutations were collected from donor laboratories and the mutations was verified.

2. Samples were transformed at Coriell using an EBV transformation procedure.

3. Stability testing was done by verifying mutations at first and fifth cell culture passage (20 population doublings).

4. Cell lines that were part of the original study were reference-tested by at least 5 laboratories using at least 2 different techniques. Other cell lines, collected later, were reference-tested by at least two different laboratories.

5. Cells lines from the original study were pilot-tested by at least 5 laboratories in a mock performance evaluation program. Mutation-negative cell lines were pilot-tested simultaneously.

6. Cell lines were re-established from CDC stock.

Guidelines Developed for Sample Submission

- samples should be no more than 14 days old (preferably no more than 7 days old)
- for samples up to 7 days old, storage should be at ambient temperature or at 4°C
- samples 8-14 days old should be stored at 4°C
- samples should have a minimum volume of 1 ml
- samples collected in ACD or EDTA tubes may be used

Table 1. Validated Samples from CDC Project Available from the Coriell Cell Repositories

Repository #	Disease (or Test)	Gene	Allele 1	Allele 2	Intron-8 Poly T	Degree/Validation*
CD00001	Nonsyndromic Deafness	*Connexin 26 (CX26)*	35delG	WT		RP
CD00002	Craniosynostosis/Muenke	*FGFR3*	C749G	WT		RP
CD00003	Cystic Fibrosis	*CFTR*	1078delT	WT	7T/7T	V2
CD00004	Cystic Fibrosis	*CFTR*	1898+1G>A	WT		V2
CD00005	Cystic Fibrosis	*CFTR*	1898+1G>A	WT		V2
CD00006	Cystic Fibrosis	*CFTR*	1898+1G>A	WT	7T/7T	V2
CD00007	Cystic Fibrosis	*CFTR*	1898+1G>A	WT	7T/7T	V2
CD00008	Cystic Fibrosis	*CFTR*	2184delA	WT	7T/9T	V2
CD00009	Cystic Fibrosis	*CFTR*	394delTT	WT		V2
CD00010	Cystic Fibrosis	*CFTR*	I148T		9T/9T	V2
CD00011	Cystic Fibrosis	*CFTR*	I148T	WT		V2
CD00012	Cystic Fibrosis	*CFTR*	I148T	WT	7T/9T	V2
CD00013	Cystic Fibrosis	*CFTR*	S1235R	WT	7T/7T	RP
CD00014	Fragile X	*FMR1*	57 CGG repeats (male)			RP
CD00015	Hemochromatosis	*HFE*	C282Y	WT		RP
CD00016	Hemochromatosis	*HFE*	C282Y	WT		RP
CD00017	Hemochromatosis	*HFE*	H63D	H63D		RP
CD00018	Hemochromatosis	*HFE*	H63D	WT		RP
CD00019	Hemochromatosis	*HFE*	S65C	WT		RP
CD00020	Hemochromatosis	*HFE*	S65C	WT		RP
CD00021	Hemochromatosis	*HFE*	H63D	S65C		RP
CD00022	Huntington Disease	*HD*	31 CAG repeats	18 CAG repeats		RP
CD00023	MTHFR Thermolabile Poly	*MTHFR*	C677T (A222V)	C677T (A222V)		RP
CD00024	MTHFR Thermolabile Poly	*MTHFR*	C677T (A222V)	C677T (A222V)		RP
CD00025	MTHFR Thermolabile Poly	*MTHFR*	C677T (A222V)	C677T (A222V)		V2
CD00026	Alpha-Thalassemia	*HBA1*	Type 1 SEA	WT		RP
CD00027	Alpha-Thalassemia	*HBA1*	Type 1 SEA	WT		V2

RP: reference-tested by ≥5 labs and pilot-tested by 5 labs. At least 2 different techniques used. V2: mutation was verified by at least 2 genetics labs

Table 2. Additional Cell Lines Validated during the CDC Project

Repository Number	Disease (or test)	Targeted Gene	Validated mutations	Additional Mutations *	Validation
GM 07441	Cystic Fibrosis	CFTR	3120+G>A/621+1G>T; 7T/9T	MTHFR C677T/WT*	RP
GM 13591	Cystic Fibrosis	CFTR	DeltaF508/R117H; 5T/9T	HFE H63D/H63D; MTHFR C677T/WT*	RP
GM 16028	MTHFR Thermolabile Polymorphism	MTHFR	MTHFR C677T/WT	Prothrombin G20210A/WT; Factor V Leiden R506Q/WT; HFE S65C/WT*	RP
GM 14641	Thrombophilia; Factor V mutation	Factor V Leiden	(F5) FVL; R506Q/WT	HFE C282Y/H63D	RP
GM 16000	Thrombophilia; Prothrombin mutation	Prothrombin mutation	(F2) Prothrombin; G20210A/G20210A	HFE H63D/WT; MTHFR C677T/WT*	RP
GM 16266	Sickle Cell/Hemoglobin C Disease/Hemoglobin SC	HBB	HbS/HbC	none	RP

* Mutation was tested by at least one laboratory during the project.

RP: All cell lines in this group were validated by at least 5 laboratories and pilot-tested by at least 5 laboratories. At least 2 different techniques were used in sample validation.

Table 3. Evaluation of Cell Lines as Potential Negative Controls

Gene	Mutations Screened	GM00536	GM09820	NS01862	GM03469	GM00130	GM07752	GM06160
Degree/ Validation	P*	P	P	P	P	P	P	P
HFE	C282Y; H63D; S65C	WT/WT	WT/WT	H63D/WT	WT/WT	WT/WT	C282Y/WT	C282Y/WT
CFTR	Numerous**	No mutations detected; 7T/7T	No mutations detected; 7T/7T	No mutations detected; 7T/7T	R170H; 7T/7T	M470V/M470V;4375-36delT; 7T/7T	No mutations detected; 7T/7T	No mutations detected; 7T/7T
HBB	Hb S; Hb C	Negative (Hb A/A)	Negative (Hb A/A)	Negative (Hb A/A)	Negative (Hb A/A)	Negative (Hb A/A)	Negative (Hb A/A)	Negative (Hb A/A)
F2	G20210A	WT/WT	WT/WT	WT/WT	WT/WT	WT/WT	WT/WT	WT/WT
MTHFR	C677T	WT/WT	WT/WT	WT/WT	WT/WT	WT/WT	WT/WT	C677T/WT
FVL	R506Q	WT/WT	WT/WT	WT/WT	WT/WT	WT/WT	WT/WT	WT/WT
HBA1	SEA deletion; Fil deletion	Negative	Negative	Negative	Negative	Negative	Negative	Undetermined abnormality
FMR1	CGG repeat	20 repeats; normal male	36 repeats; normal male	23/31 repeats; normal female	30 repeats; normal male	30 repeats; normal male	20 repeats; normal male	31 repeats; normal male
HD	CAG repeats	20/20; normal	18/17; normal	16/15; normal	18/17; normal	17/17; normal	20/17; normal	18/16; normal
Cx26	35delG/WT	WT/WT	WT/WT	WT/WT	WT/WT	WT/WT	WT/WT	WT/WT
FGFR3	P250R	WT/WT	WT/WT	WT/WT	WT/WT	WT/WT	WT/WT	WT/WT

*P – pilot-tested by 5 or more laboratories

* *The cell lines were tested for all mutations identified by the Linear Array CF-31 from Roche Diagnostics and the ABI Cystic Firbosis Assay, plus I148T and S1235R.

The cell lines highlighted in light blue were negative for all mutations tested and are suitable for use as negative controls.

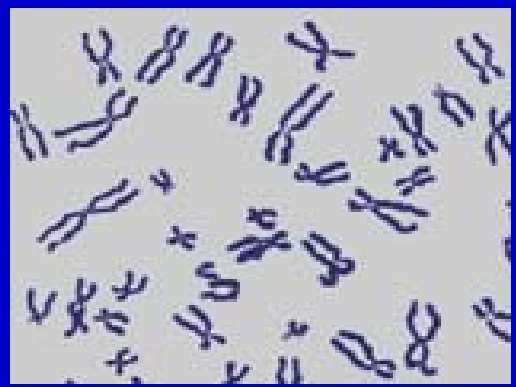
Conclusions

- Establishing stably transformed cell lines from residual clinical specimens is one way to provide high quality positive genetic control materials.
- In the CDC projects, guidelines for collecting residual samples and for validating mutations were developed. As a result, **40 cell lines containing mutations associated with diseases of high public health impact were established and are now available to genetic testing laboratories.**
- Negative control cell lines were also validated and are available.

Conclusions - Continued

- **Many more samples containing mutations associated with other diseases are needed.**

- **CDC and Coriell Cell Repositories are soliciting genetic testing laboratories to contact Coriell if interested in donating potential samples for transformation. (http://ccr.coriell.org/)**



Continuous partnerships and cooperation between the genetic testing community, healthcare organizations, professional organizations, the government, and the private sector are needed to further quality assurance goals and to provide the best genetic testing services possible.

References

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2. Williams, L.O., Cole, E.C. General Recommendations for Quality Assurance Programs for Laboratory Molecular Genetic Tests, Final Monograph. Centers for Disease Control and Prevention, Division of Laboratory Systems, Atlanta, GA., 1999.

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5. Bernacki, S.H., Stankovic, A.K., Williams, L.O., Beck, J.C., Herndon, J.E., Snow-Bailey, K., Prior, T.W., Matteson, K.J., Wasserman, L.M., Cole, E.C., and Stenzel, T.T. Establishment of stably EBV-transformed cell lines from residual clinical blood samples for use in performance evaluation and quality assurance in molecular genetic testing. *J. Mol. Diagn.* 5: 227-230 (2003).

What Next ?

Solicit your help!

- Donating potential samples
- Testing
- Proposing targeted products
- National Coordinator