In-house molecular PCR assays applicable for clinical diagnostics

Profitability of integrating our efforts in (high volume) assay design and validation: generating (and sharing) new value

definition of “in-house assay”

PCR assay which was build from component materials (certificates analysis for each component, like sequence of primers/probes/ characterization of enzyme/master mix), with defined upstream NA-isolation protocol, PCR instrument and downstream detection systems

Analytical validation for sensitivity, specificity limits (LoD), inter – intra assay reproducibility, operative window range and

Clinical validation on minimum ~1000+ samples (10% positive rate example) with defined parameters/ threshold ranges for Positive/Negative/Equivocal calling (equivocal less then 0.1%)

Validation on the group/panel of know positive with different genotypes (collection from provincial lab, or hospital or commercial panel)

Pessimistic and optimistic arguments to get actively involved in assay design and validation

Benefits of in-house *versus* commercial assay: fast evolution of medical device

* Fast ``correction`` of errors/defects reported by users: 2011 FDA/ Health Canada approved MRSA, know 6% false positives (dynamical feedback will be applicable to in house assays)
* Ability to introduce 4-12 hours incubation-growth with antibiotic (in media) and detect Staphylococcus aureus specific genes.
* same strategy for Van A false positives by PCR
* (vanA-containing Enterococcus faecium susceptible to vancomycin and teicoplanin because of major nucleotide deletions in Tn1546 by Simon Gagnon1, Simon Le´vesque, Brigitte Lefebvre, Anne-Marie Bourgault, Annie-Claude Labbe´ and Michel Roger J Antimicrob Chemother, doi:10.1093/jac/dkr379

EXAMPLE: LSPQ and Montreal case of H1N1 (2009)

Commercial suppliers compatible with our equipment: Roche and Seegene

Price: DNA isolation (7-10$), Enzyme/Master mix (~1-2$), Detection kit 17$, volume 30-80 per day, projected spending on diagnostics 337 500-540000$ for 90 days.

Hugues Charest and his team from LSPQ designed H1N1 assay (IDT oligos, QIAGEN master mix…, cycling conditions…) and paste it with existing FluA/CDC protocol, validated and **provided protocol to the hospitals.** Public Health Agency of Canada was involved initially, but majority was done locally

We start having ~9$ assay (including NA isolation), generating saving of 16$ per sample (compared to commercial assays). He provided attenuated H1N1 Positive extraction and PCR positive and negative controls.

Bruno Lamontagne (Hôpital Maisonneuve-Rosemont) re-adjusted extraction control and introduced RSV primer/probe combinations. Roche RNase P was generating false positives even when all RNA was destroyed.

With combined effort, we generated qPCR (FLURS) assay presently running in 2 hospitals (and 3 others with minor modifications). Our assay was analytically validated using Health Canada approved RV15 Seegene kit.

Quality and cost effectiveness are on OUR side

High volume assay: 20+ per day (VRE, MRSA, C.diff…) times 3 assays

(20 x 17$ x365days x 5 hospitals (Montreal) =620500$x 3 assay examples of high volume PCR= 1 861 500$ per year per 5 hospitals

(this is minimalist estimation with small volume scenarios, there are hospitals running 100+ samples per day) ; real estimations are in the range of 5 000 000$ per tear per 5 hospitals

There are smaller hospitals also running commercial assays in Montreal, but they are not included

Intra and inter-provincial cooperation on high volume assays?

See next example!

In Canada ?

20+ samples per day
3 assays (PCR)
17$/per assay
365days

284.700$

X hospitals

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*X=100 28. 470.000$

* x=500 , number of assays =100+ $$930.750.000$$

Negative points

In spite of huge potential for savings of public money and higher quality of service, in-house developments are sometimes considered as gray zone diagnostics with questionable performance.

WHY?

Excuse based on unresolved liability issues (MRSA example)

FDA and/or Health Canada 2011 MRSA assay is officially cleared for use in molecular diagnostics.

* "we will pay more – but we are not liable…"

  * Paradox: we know assay failure examples and we know that we can resolve it

* Clinical Application of Real-Time PCR to Screening Critically Ill and Emergency-Care Surgical Patients for Methicillin-Resistant *Staphylococcus aureus*: a Quantitative Analytical Study, doi: 10.1128/JCM.01332-09

* How to justify spending of 250 000$ per year (our hospital) knowing that :
* (A) there are ~6% MRSA false positives results and (B) in-house assay for target price of 3$, can have better quality (C) price of quality control is so high to be ready to pay 250 000$ per year per assay?

Excuse based on cost of development and validation

Validation of in house assay would cost minimum 6 months of PhD time+ technologist, so we will end up with higher cost. Since we are already short of man power – we would not have incentive to go into this direction.

WRONG: with cooperative approach all can be achieved among partners on 3 months scale without significant extra engagement of human resources (PhD or technologist, only 10% time)

Excuse based on contract duration or issues of ``complex QC`` or destruction of (foreign) industry

*...

I will do it on my own

Better then nothing: however note that multiplex kits are already 3-4$ per pathogens. Contra arguments about 10-100x lower sensitivity are absurd in the context of screening assays done with few hours enrichment or with acute request asking for key pathogen in emergency call (not for precise monitoring of therapeutic response or similar).

I have local $ and support to develop - hospital admin is happy –I am happy-we are happy - we are saving …

* How long is your personal assay going to resist challenging pressure of multiplexing,
* 1-2 years? How much is PhD / Scientific salary per year + separate bodies to perform each tests separately?
* FACT: we are unable to follow dynamics and validation volume of assay development in the context of competing word

REAL REASONS

Traditionally hospital budget operated in deficit. Strategy of presenting bigger deficit to make bigger re-compensation from government agency is driving force in institutional management

Individual benefits/industrial expert consultations, funding from industry to institution: we have to choose collectively: short term benefit, versus long term dependence and deficit

At the beginning, in-house assays can not pay for consultations and validation of assays which are typically re-injected by industry to facilitate contract formation,

There is no enough motivation to introduce extra effort in development, due to the flat salary approach, no venture initiative, closed cycle of passive ignorance (until?)

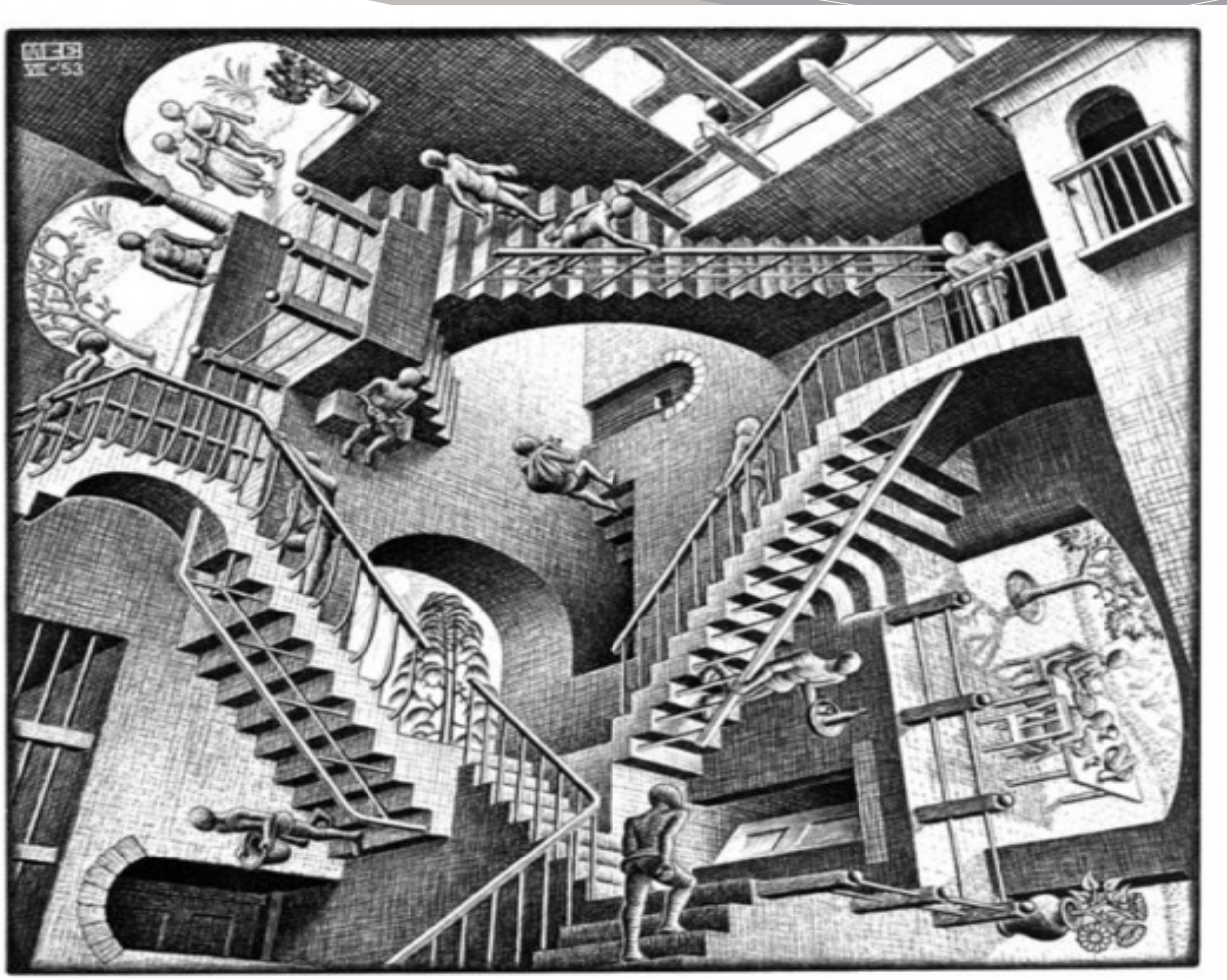
Consequences

Continuation of collapse of public budget and health care quality

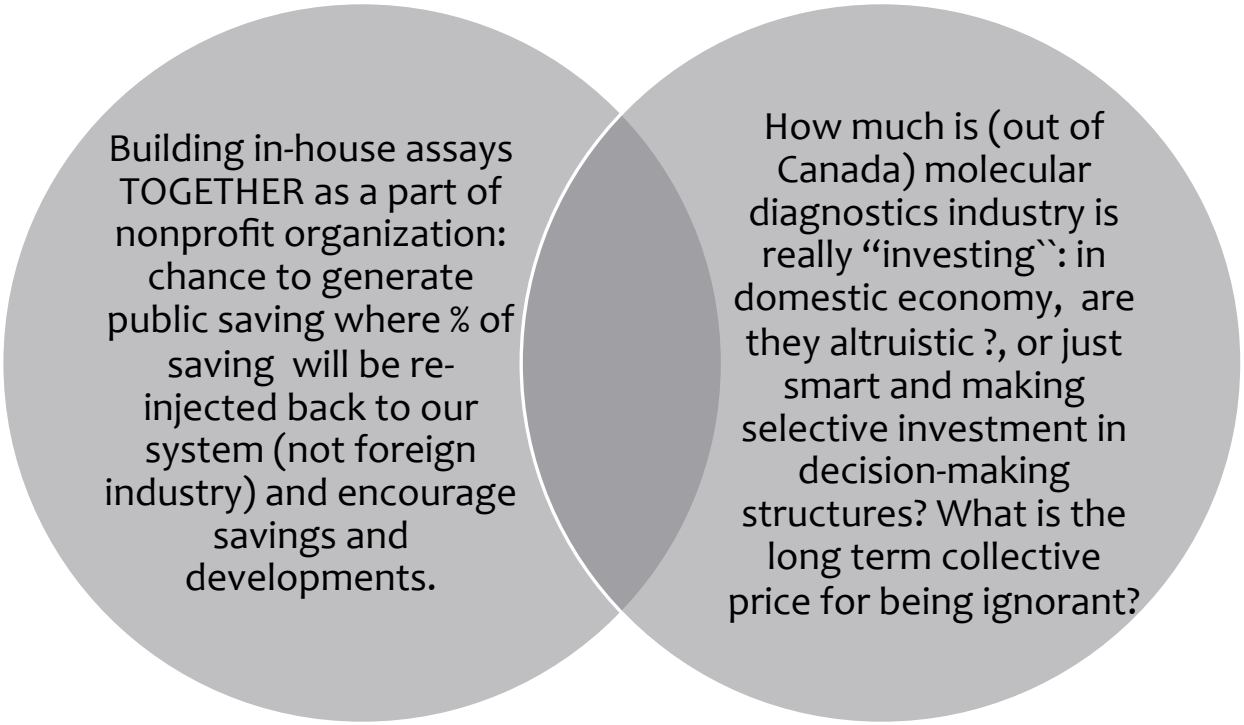
Lost of knowledge, manufacturing know-how, and ability to invent new

Long term dependence on foreign technology and inability to compete and generate value

Virtuous cycle (Echer)



Stopping virtuous cycle



HOW TO DO IT (EXAMPLE) and is it really difficult_1?

* LABs: Send name, coordinates (LAB), full mailing address and emails to (Molconsortium@gmail.com) with subject "in-house PCR assay consortium"
* LABs and Consortium: Registered and incorporate nonprofit organization with our names (or use the existing one), open bank account with access to bank statements to all members.
* LABs: Send email with List of in-house assays offered by the labs (subject : "list of assays, name of the LAB/member"), time 1-2 weeks
* LABs: Send email with assay files (subject: assay name) and attach relevant files like SOP, validation files, Material, references, Quality Control (time line: 3 weeks)
* Consortium: All messages will be deposit in COMMON dropbox.com in original form, using folder (name: email of the sender)
* LABs: Read what WE have TOGETHER and vote on assay priority (cost-effectiveness/quality) and universality, (example we started with high volume VRE, MRSA and C diff). Pick up the assay, order/obtain from paternal laboratory suggested material, Time: 4 weeks

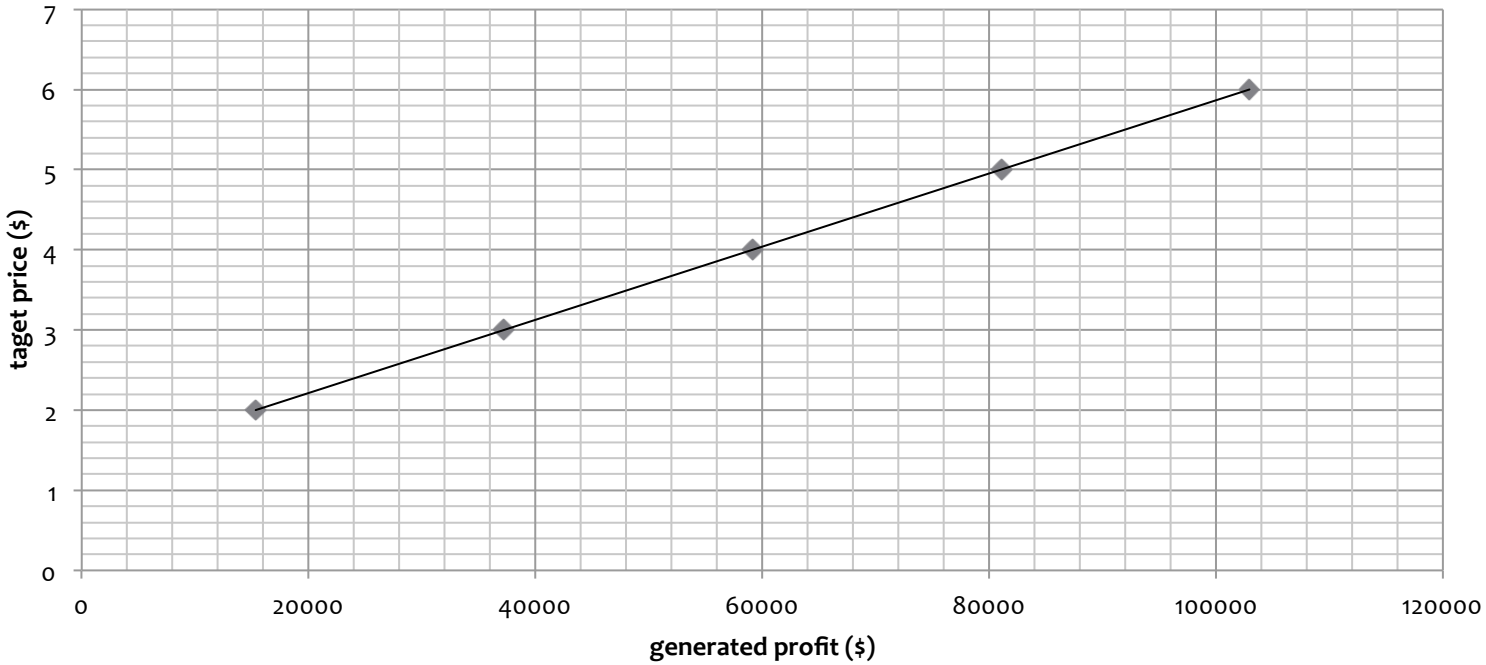
HOW TO DO IT (EXAMPLE) and is it really difficult_2?

* LABs: Start validation and potential troubleshooting related to site-dependent differences (instrument…), time 8 weeks. Made final assay estimation with all relevant files, offered to all members (and non-members) by email.
* Consortium and LABs: Get/send ordering volume request by email.
* Consortium: Define price (target price around 5-6$ for standard qPCR), for VRE, MRSA, Cdiff, apply glass beads/CHELEX strategy). Ask for initial investment from hospitals, or make initial ordering to buy requested volume of primers and probes and redistribute to others. Define one of 2 manufacturing places and allocate small manufacturing bonus (our month consumption could be aliquoted / manufactured / labeled in 2h) or pay 3rd part entity to do this
* Consortium: Use generating $ to: pay new material, regulatory/legal issues like registration of assay to Health Canada, preserve QC of compounds and further development
* Consortium: Ask extra government funding for promoting research and development inside the group-consortium

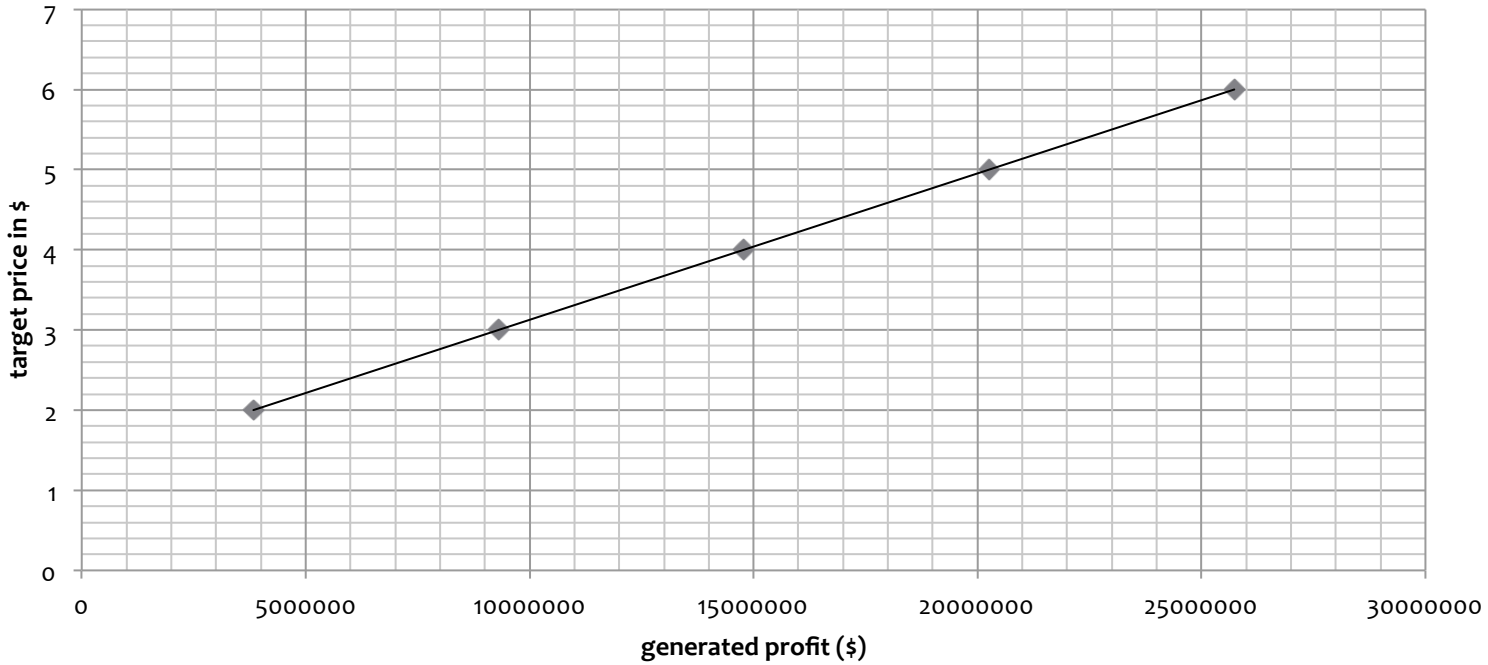
Gain

Oligo price	aliquoting/QC	enzime/MM	NA isolation	TOTAL	profit margin (6$-A2)	volume per year	Vol x porf/assay*3	target price	hospital no	assay no=3
CASE 1: Jewish General Hospital 20 samples per day 3 assays (VRE MRSA, Cdiff)										
0.2	0.2	0.7	0.2	1.3	4.7	7300	102930	6	1	3
0.2	0.2	0.7	0.2	1.3	3.7	7300	81030	5	1	3
0.2	0.2	0.7	0.2	1.3	2.7	7300	59130	4	1	3
0.2	0.2	0.7	0.2	1.3	1.7	7300	37230	3	1	3
0.2	0.2	0.7	0.2	1.3	0.7	7300	15330	2	1	3
5 hospitals in montreal (very minimalist volume?)										
0.2	0.2	0.7	0.2	1.3	4.7	7300	514650	6	5	3
0.2	0.2	0.7	0.2	1.3	3.7	7300	405150	5	5	3
0.2	0.2	0.7	0.2	1.3	2.7	7300	295650	4	5	3
0.2	0.2	0.7	0.2	1.3	1.7	7300	186150	3	5	3
0.2	0.2	0.7	0.2	1.3	0.7	7300	76650	2	5	3
5o hospitals										
0.2	0.2	0.7	0.2	1.3	4.7	7300	5146500	6	50	3
0.2	0.2	0.7	0.2	1.3	3.7	7300	4051500	5	50	3
0.2	0.2	0.7	0.2	1.3	2.7	7300	2956500	4	50	3
0.2	0.2	0.7	0.2	1.3	1.7	7300	1861500	3	50	3
0.2	0.2	0.7	0.2	1.3	0.7	7300	766500	2	50	3
CASE 4: 100 per day volume - 50 hospitals										
0.2	0.2	0.7	0.2	1.3	4.7	36500	25732500	6	50	3
0.2	0.2	0.7	0.2	1.3	3.7	36500	20257500	5	50	3
0.2	0.2	0.7	0.2	1.3	2.7	36500	14782500	4	50	3
0.2	0.2	0.7	0.2	1.3	1.7	36500	9307500	3	50	3
0.2	0.2	0.7	0.2	1.3	0.7	36500	3832500	2	50	3

Example of JGH, 3 assays, 20 per day



Example of 100samples per day, 3 assays, 50 hospitals



SENSE OF INFLUENCE AND CHOICE