FMEA analysis on a “pilot process” to validate aptamers as therapeutic purposes

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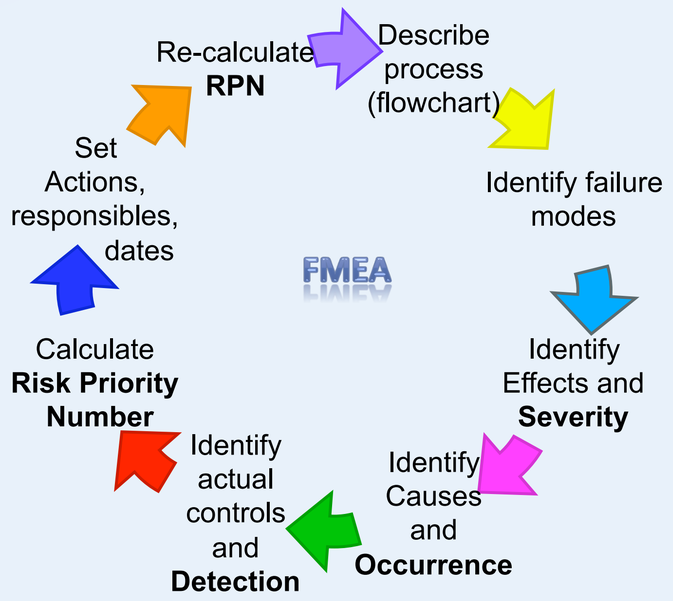
Quality principles and methodologies can strongly support the management of scientific research, in both basic and applied research laboratories, where procedures and results are rapidly changing and can hardly be standardized. The "Quality and Project Management OpenLab" (q-PMO) CNR Research project, aims to identify, develop and test models of quality management that can strongly support the management of scientific research. In this view, Quality methodologies, such as Failure Mode and Effect Analysis - FMEA, was borrowed from the industrial field, where it is widely used in risk control and process optimization procedures, to validate and support research activities and results, to create a standard and controlled workplace, and to support the interaction between research and industrial application. Aptamers represent attractive targets for cancer diagnosis or therapy and therefore are subjected to intensive investigation and interest of technology transfer. We applied FMEA analysis on a “pilot” process, constituted by 3 subprocesses developed for the selection of cell-specific aptamers, in agreement with the needs of companies interested in the development The 3 subprocesses are: 1) Dephosphorylation and purification; 2) Phosphorilation and Purification; 3) Cells binding assay We showed the FMEA analysis of the first subprocess of this methodology.



FAILURE MODE AND EFFECT ANALYSIS (FMEA)

Failure Modes and Effects Analysis (**FMEA**) is a systematic, proactive method for evaluating a process to identify where and how it might fail and to assess the relative impact of different failures, in order to identify the parts of the process that are most in need of change.

•FMEA is used to evaluate processes for possible failures and to prevent them by correcting the processes proactively, rather than reacting to adverse events after failures have occurred. FMEA is particularly useful in evaluating a new process prior to implementation and in assessing the impact of a proposed change to an existing process.

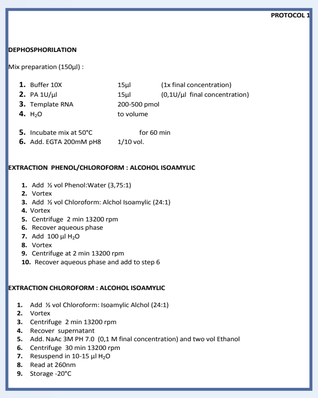
•For each process step, FMEA proceed with the following phases:

- **Failure modes** (What could go wrong?)
- **Failure causes** (Why would the failure happen?)
- **Failure effects** (What would be the consequences of each failure?)

•Three parameters are used:

- **Severity** (S): weights the importance of the effect of failure on the final product and/or user;
- **Occurrence** (O): measures the probability for the failure cause to happen;
- **Detection** (D): identify the control coverage of the process step in the present configuration

•The product of the 3 parameters leads to a summary index, named **Risk Priority Number,** that gives measure of the risk associated with each process step. Comparing RPN of single process steps with a pre-definite risk threshold helps in decide whether setting corrective action to make them more robust.



PROTOCOL 1

DEPHOSPHORILATION

Mix preparation (150µl) :

1. Buffer 10X 15µl (1x final concentration)
2. PA 1U/µl 15µl (0,1U/µl final concentration)
3. Template RNA 200-500 pmol
4. H_2O to volume
5. Incubate mix at 50°C for 60 min
6. Add. EGTA 200mM pH8 1/10 vol.

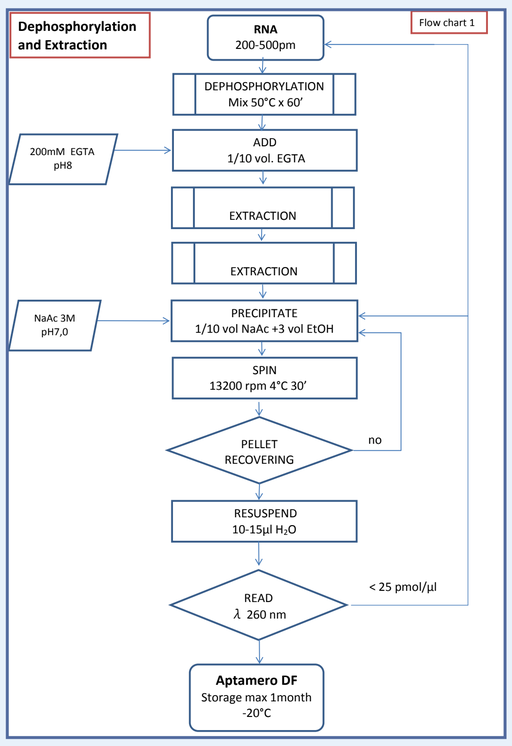
EXTRACTION PHENOL/CHLOROFORM : ALCOHOL ISOAMYLIC

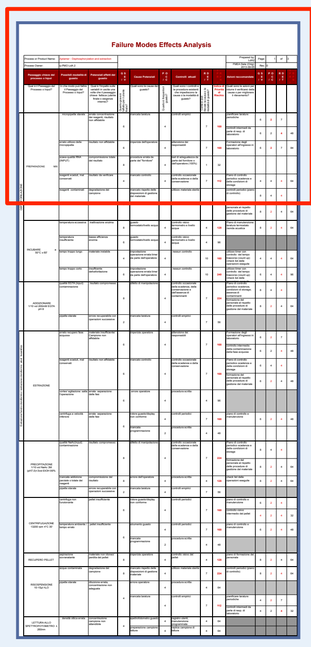
1. Add ½ vol Phenol:Water (3,75:1)
2. Vortex
3. Add ½ vol Chloroform: Alchol Isoamylic (24:1)
4. Vortex
5. Centrifuge 2 min 13200 rpm
6. Recover aqueous phase
7. Add 100 µl H_2O
8. Vortex
9. Centrifuge at 2 min 13200 rpm
10. Recover aqueous phase and add to step 6

EXTRACTION CHLOROFORM : ALCOHOL ISOAMYLIC

1. Add ½ vol Chloroform: Isoamylic Alchol (24:1)
2. Vortex
3. Centrifuge 2 min 13200 rpm
4. Recover supernatant
5. Add. NaAc 3M PH 7.0 (0,1 M final concentration) and two vol Ethanol
6. Centrifuge 30 min 13200 rpm
7. Resuspend in 10-15 µl H_2O
8. Read at 260nm
9. Storage -20°C

A detailed flowchart of the process is a good starting point to identify each process component.

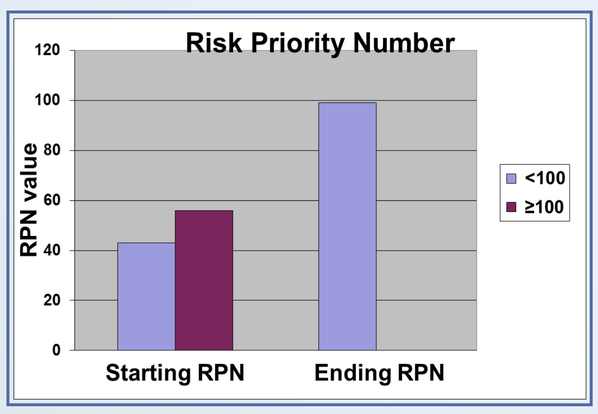




FMEA analysis begins by identifying all of the probable failure modes, cause and effect.

Failure Modes and Effects Analysis

Process or Product Name:	Aptamer - Dephosphorylation and extraction								Prepared by: LdA2	Page:	1	of	3		
Process Owner:	q-PMO LdA2								FMEA Date (Orig): 2013-09-05	Rev.	0				
Key Process Step or Input	**Potential Failure Mode**	**Potential Failure Effects**	**G / SmS V**	**Potential Causes**	**P / OcC**	**Current Controls**	**R / DmD T**	**RPN**	**Actions Recommended**	**SmS V**	**OcC**	**DmD T**	**RPN**
What is the Process Step or Input?	In what ways can the Process Step or Input fail?	What is the impact on the Key Output Variables once it fails (customer or internal requirements)?	How **Severe** is the effect to the customer?	What causes the Key Input to go wrong?	How **often** does cause or FM **occur**?	What are the existing **controls** and procedures that prevent either the Cause or the Failure Mode?	How well can you **detect** the Cause or the Failure Mode?		What are the actions for reducing the occurrence of the cause, or improving detection?				
MIX PREPARATION	pipets not calibratated	wrong reagent concentration, unreliable results	6	wrong pipet calibration	4	empirical check	7	168	scheduled calibrations	6	2	7	
									lab supervisors periodic check	6	2	4	48
	wrong use of pipets	unreliable results	6	lack of training of workers	4	control done by group leaders	7	168	training of workers when entering the lab	6	2	7	84
	low RNA quality (INPUT)	untreastable results	8	wrong isolation and storage procedure from the suppliers	4	workers or suppliers perform quality check (100%)	1	32					
	expired reagents, not well stored	results need to be verified	4	missed controls	4	random control of expiration dates and storage conditions	7	112	scheduled control of expiration dates and storage conditions	4	4	4	64
	contaminated reagents	sample degradation	8	lack of regulation for handling reagents	4	use of steril material	7	224	periodic control (plan of scheduled control)	8	4	4	
									training of workers according to rules for handling the reagents	8	2	4	64



Correttive actions reduce RPN

Many actions arising from the FMEA analysis are related to the organization of the laboratory, as you can expect when you apply the principles of quality management to a non-regulated research laboratory. We have identified two main groups relating to:

•***Quality Management of laboratory***: scheduled maintenance and calibrations of instruments, training of staff according to specific procedures, scheduled control of materials (expiration dates and storage conditions) – according to International Quality Standards (es: UNI EN ISO 9001:2008)

•***Specific process under analysis:*** Intermediate control of pellet, intermediate control of labelled cells….

Since the process is not automated, it was not surprising therefore that the starting RPN was over the established threshold of 100 in more than 50% of the operations; all of them were reduced by the application of corrective actions identified (see "Ending RPN”).

SEVERITY Table		
10	Extremely severe	Dead or severe injuries
8	severe	Final product definively damaged
6	Moderate issue	damage is not important and significant dissatisfaction (wrong data)
4	medium	Effect on service parameters
2	Medium slight	Slight effect on parameters
1	slight	undetectable by the end user or not influential on the service
OCCURRENCE Table		
10	High frequency	Once in a month
8	Medium frequency	5 to 10 times in a year
6	occasional	1 to 5 times in a year
4	infrequent	Once in a year
2	Very infrequent	Once in 5 years
1	Extremely rare	Once in 5 to 30 years
DETECTION Table		
10	Severe uncoverage	Error detection in less than 50% of occurrence
7	Slight uncoverage	Error detection in the 50% of occurrence
4	Good coverage	Error detection in the 70% of occurrence
1	Best coverage	Error detection in the 90% of occurrence

The analysis helps to identify risky operations and define corrective actions (for items with RPN above the established threshold)

The outcome of the FMEA is a well-documented record to reduce overall risk to an acceptable level, and can be used as a source for designing a control strategy
This quality approach led to several major advantages. At first, a set of improvement actions was generated covering most lab aspects, such as management of instrumentation or training of personnel involved. Then, FMEA methodology contributed to the definition of good laboratory practice, provided a strong support for the streamlining of protocols and was useful for generating information suitable for knowledge management. The use of a common language oriented towards results is expected to facilitate technology transfer, thus promoting interaction between research and industrial applications.

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