Development of Effective Risk Mitigation Strategy for Cell Culture Process of Recombinant Protein Production

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***Abstract*—Microbial contamination, instability in production yields and cell lines are the challenges that must be faced by any pharmaceutical biotechnology industry when conducting cell culture as one of the stages of its production process. In the past, Company X has experienced twice contamination cases during cell culture process for its recombinant protein products. As a result, Company X suffered financial lossess at approximately 3.05 billion rupiah and resulted in disruption of the stock of recombinant protein drug which are the active pharmaceutical ingredient for most of injection products. Therefore, an effective mitigation strategies are needed to manage any potential risks in the cell culture process. This study aims to design a risk mitigation strategy for risk in cell culture process by using House of Risk (HOR) method. There are two HOR matrices used in this study. HOR1 is used to determine which risk agents should be prioritized for prevention. In HOR2, several effective risk management strategies will be chosen. There are 40 risks events and 31 risk agents identified. Based on risk assessment results and applying Pareto Chart analysis there are two risk agents selected. Five proactive actions to mitigate these risks are proposed. They are training and personnel qualification program periodically, changing workflow become more efficient and convenient, more detailed revision of related documents, changing manual process to semi-automatic process gradually and additional person on each team as a verifier for double-checking.**

***Keywords*— HOR1, HOR2, House of Risk, Risk Mitigation.**

I. INTRODUCTION

THE BIOTECHNOLOGY industry has grown significantly in recent years and continues to grow rapidly. Experts predict that new protein therapeutics are starting to enter the marketplace, with the first wave of protein drugs, antibody groups and peptide drugs expected to enter later in the next 10-20 years [1]. This rapid growth in protein therapeutics has been led by a diverse range of products produced in animal cell culture. The increasing demand for therapeutic protein products from animal cell cultures has resulted in the development of animal cell culture processes on a large scale with more efficient and reliable processes [2].

Company X is one of manufacturing company engaged in pharmaceutical biotechnology in Indonesia that produces recombinant protein as a drug subtances using animal cell culture. Some challenges that must be faced by using animal cell culture are controling product quality while maximizing productivity, controlling carbon dioxide concentration in the process, and minimizing the risk of contamination during the manufacturing process as well as from raw materials [1]. Some limitations or deficiencies that exist in the animal cell culture process, such as the need of a reliable and experienced operator in handling cell culture, the amount of target protein produced in the cell culture process is very small when compared to the amount of material used, the cell growth problems also affects the results of the target protein produced, the last is the variability product result from the continuous cell line [3]. Contamination in the cell culture process also one of challenge in the animal cell culture process and it is one of the biggest problems faced by Company X which resulted in a total loss of 3,05 billion rupiah in 2019. This also has an impact on the disruption of the stock of recombinant protein drug subtances in Company X which is an active pharmaceutical ingredient for major injection products. Contamination in the animal cell culture is the most frequently encountered and critical risk. Contamination in the animal cell culture process can caused by microorganisms or viruses. It can arise due to various reasons such as personnel errors, inadequate aseptic protocols, failures in the sterilization process, failure of equipment integrity, as well as the use of new materials that are resistant to inactivation or removal procedures [4].

Based on the statement that has been described, this research raises the problem about development House of Risk (HOR) method in the biopharmaceutical company especially in the animal cell culture of protein recombinant production process. The house of risk (HOR) developed by [5] was chosen to select a set of proactive actions deemed cost-effective in managing risk in Company X. The HOR method is a method that focuses on formulating strategies for preventive, reduction and handling some of risk agents that potentially lead to more than one risk. The HOR method mostly has been used to analyze risks in the supply chain context such as managing risk for Tuna supply chain as reported in [6]. The method also has been used manage risk in new product development process for fashion industry [7] and risk mitigation for project Gempol-Pasuruan highway project [8]. Implementation of risk management is important to control the risks occur which can threaten company business continuity.

This study is aimed to identify and map the risks in the in the animal cell culture of protein recombinant production process. The model of HOR consisting of two steps, the first step (HOR1) was conducted by identifying all risk events and risk agents, then measured the severity of risk event and occurrences of risk agent, determine the relation between risk event and risk agent to calculate the Aggregate Risk Priority (ARP) value. The second step (HOR2) is intended to

Table 1.
House of Risk 1 Model

Process	Risk Event (Ei)	Risk Agent (Aj)					Severity
		A1	A2	A3	A4	Aj	
1st Culture	E1	R1					S1
	E2		R2				S2
	E3			R3			S3
2nd Culture	E4				R4		S4
	E5					R5	S5
	E6				R6		S6
Main Culture	E7			R7			S7
	E8		R8				S8
Harvest and Filtration	E9	R9					S9
	E10		R10				S10
	E11			R11			S11
	Ei					Rj	Si
Occurance		O1	O2	O3	O4	Oj	
ARPj		ARP1	ARP2	ARP3	ARP4	ARPj	
Rank		R1	R2	R3	R4	Rj	

Table 2.
House of Risk 2 Model

Risk Agent (Aj)	Proactive Action (PA)					ARPj
	PA1	PA2	PA3	PA4	PAk	
A1	E1					ARP1
A2		E2				ARP2
A3			E3			ARP3
A4				E4		ARP4
A5					Ejk	ARP5
Total Effectiveness (TEk)	TE1	TE2	TE3	TE4	TEk	
Degree of Difficulty (Dk)	D1	D2	D3	D4	Dk	
Effecttiveness to Difficulty (ETDk)	ETD1	ETD2	ETD3	ETD4	ETDk	
Rank of Priority (R)	R1	R2	R3	R4	Rk	

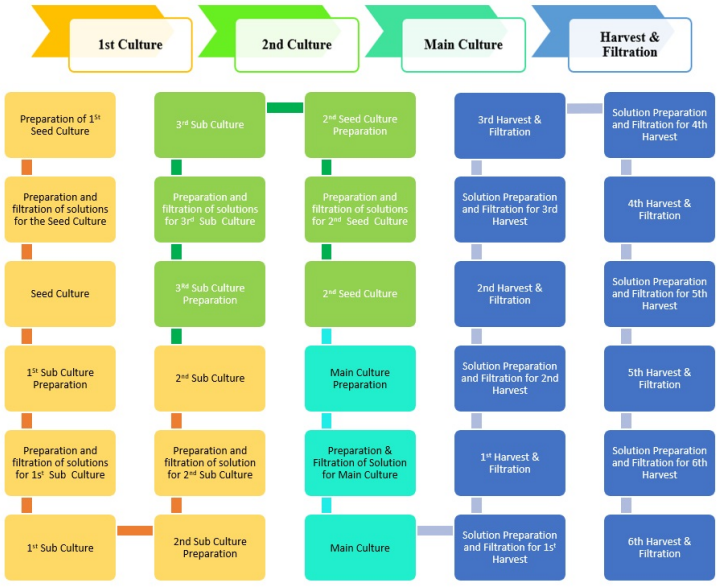

Figure 1. Cell Culture Process Mapping in Company X.

formulate and prioritize the proactive action for risk handling to formulate risk control strategy recomendation for Company X.

II. METHOD

This study examines animal cell culture of protein recombinant production process in the biopharmaceutical company using House of Risk (HOR) method. Originally House of Risk is aimed at managing risk in supply chain processess context, therefore all activities are mapped based on Supply Chain Operations Reference (SCOR) model, which are Plan, Source, Make, Deliver, and Return. But in this study all the cell culture steps of protein recombinant production process are catagorized and mapped based on internal company documents such as batch record. The steps

Table 3.
Risk Event and its Severity level in Cell Culture Process

Code	Risk Event	Severity
E1	Room not Cleaned Yet	1
E2	Room temperature does not meet the requirements.	4
E3	Relative Humidity (RH) room does not meet the requirements.	4
E4	Differential Pressure (DP) room does not meet the requirements.	4
E5	Environmental microbial monitoring does not meet the requirements.	4
E6	Equipment not cleaned yet.	4
E7	Filter integrity test results do not meet the requirements.	4
E8	Failure of container and equipment sterilization.	4
E9	Sterile material exposed to dirty air.	5
E10	Conductivity of water does not meet the requirements.	4
E11	Water endotoxin does not meet the requirements	4
E12	The water microbial limit does not meet the requirements.	5
E13	Incorrect of weighing material amount	4
E14	An error occurred while adjust pH	3
E15	Contaminated material.	5
E16	Personnel microbial monitoring does not meet requirements.	5
E17	Hose connection leaks	3
E18	Many materials are wasted during filtration.	2
E19	Error in setting equipment	2
E20	Equipment failed to operate	3
E21	Equipment temperature does not meet the requirements/not achieved	2
E22	The CO2 levels of equipment do not meet the requirements.	3
E23	The quantity of the prepared material is inadequate.	4
E24	The media is exposed to outside air for a long time.	5
E25	Microbial contamination	5
E26	Media spill during transfer	4
E27	Error in taking WCB (Working Cell Bank)	4
E28	WCB in not good condition	4
E29	WCB thawing time is too long	3
E30	Many cells are dead/wasted.	4
E31	Cell calculation error.	4
E32	Number/growth of cells does not meet requirements.	4
E33	Cell resistance does not meet the requirements.	4
E34	Microbial contamination during incubation process.	4
E35	Number of cells not divided homogen.	3
E36	Equipment speed does not meet the requirements/not achieved.	3
E37	Leak on Flexboy.	4
E38	Auto dispenser hangs.	3
E39	Operator experiences fatigue.	4
E40	Equipment is not cleaned properly.	4

of cell culture of protein recombinant production process are divided into four steps, which are 1st Culture, 2nd Culture, Main Culture also Harvest & Filtration step. After activities in the process are mapped, next step is identify the risk event and risk agent by doing direct observation and also brainstorming using focus group discussion, the data will be validated by interviewing company internal stakeholder. The data are collected from January 2019 until February 2020. HOR is a combination between FMEA (Failure Mode and Effect Analysis) and HOQ (House of Quality) models. HOR method consists of two matrixes (steps) which are HOR1 and HOR2.

Table 4.
Risk Agents and its Occurence level in Cell Culture Process

Code	Risk Agent	Occurence
A1	Operator disobedience from procedure	4
A2	Problems on AHU (Air Handling Unit)	2
A3	Boiler problems	2
A4	There are rooms that are not closed properly	2
A5	Sampling Errors	3
A6	Inadequate aseptic protocols	3
A7	Room overload	5
A8	Filter leak	2
A9	Clogged filters	2
A10	Pure Steam Generator (PSG) problem	2
A11	Error in setting equipment	2
A12	Overload equipment	3
A13	Leak on the outer packaging material	2
A14	Inadequate procedures	4
A15	Problems in water treatment (WT)	2
A16	Error in reading instrument	2
A17	Analytical error	2
A18	Leak in heat exchanger (HE)	4
A19	There is no crosscheck procedure	3
A20	Unqualified Operator	3
A21	There is no testing for incoming materials	4
A22	Material exposed to dirty air for a long time	3
A23	Error in handling material by operator	4
A24	Filter capacity too large	3
A25	Amount of filtered material is too small	3
A26	Broken / not good condition equipment	2
A27	Difference with ambient temperature too far	2
A28	CO2 Generator / Line problem	2
A29	Inappropriate storage temperature	1
A30	Insufficient of operator	5
A31	Operators work manually and for a long time	5

HOR1 is used to determine which risk agents are to be given priority for preventive actions. The steps as follows :

a. Identify risk events that could happen in cell culture of protein recombinant production process. In HOR1 model shown in Table 1, the risk events are put in the left column, represented as Ei.
b. Assess the impact (severity) of such risk event if happened. We use a 1-5 scale where 5 represents extremely severe or catastrophic impact. The severity of each risk event is put in the right column of Table 1, indicated as Si.\
c. Identify risk agents and assess the likelihood of occurrence of each risk agent. Here, a scale of 1-5 is also applied where 1 means almost never occurred and a value of 5 means almost certain to happen. The risk agents (Aj) are placed on top row of the table and the associated occurrence is on the bottom row, notated as Oj.
d. Develop a relationship matrix, i.e. relationship between each risk agent and each risk event, Rij {0, 1, 3, 9} where 0 represents no relation and 1, 3, and 9 represent, respectively, low, moderate, and high relations.
e. Calculate the Aggregate Risk Potential of agent j (ARPj) which is determined as the product of the likelihood of occurrence of the risk agent j and the aggregate impacts generated by the risk events caused by the risk agent j as in equation (1) above.

Table 5. HOR1 Matrix

Risk Event	Risk Agent															Si
	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13	A14	A15	
E1	9															1
E2		9	9													4
E3		9	9													4
E4		9		3												4
E5		9			3	9	9									4
E6	9															4
E7	1							9	9							4
E8										9	3	1				4
E9													9	1		5
E10					3										9	4
E11					3										9	4
E12					3										9	5
E13														3		4
E14														3		3
E15																5
E16					3	9										5
E17														3		3
E18																2
E19														9		2
E20											3					3
E21											3					2
E22											3					3
E23														9		4
E24						9										5
E25						9							9			5
E26														9		4
E27														9		4
E28														3		4
E29														9		3
E30																4
E31														1		4
E32																4
E33																4
E34						9										4
E35																3
E36											3					3
E37																4
E38											3					3
E39																4
E40	9															4
Oj	4	2	2	2	3	3	5	2	2	2	2	3	2	4	2	
ARP	**340**	**288**	**144**	**24**	**198**	**621**	**180**	**72**	**72**	**72**	**108**	**12**	**180**	**816**	**234**	

$$ARPj = Oj \times \sum SiRij \quad (1)$$

f. Rank risk agents according to their aggregate risk potentials in a descending order.

HOR2 is used to give priority to those actions considered effective but with reasonable money and resource commitments. The steps as follows :

a. Select a number of risk agents with high-priority rank, possibly using Pareto analysis of the ARPj, to be dealt with in the second HOR. Those selected will be placed in the left side of HOR2 as depicted in Table 2. The corresponding ARPj values are put in the right column.
b. Identify actions considered relevant for preventing the risk agents, one risk agent could be tackled with more than one actions and one action could simultaneously reduce the likelihood of occurrence of more than one risk agent. The actions are put on the top row in Table 2.
c. Determine the relationship between each preventive action and each risk agent. The values could be {0,1,3,9} where 0 represents no relation and 1, 3, and 9 represent respectively, low, moderate and high relations between action k and agent j. This relationship (Ejk) could be considered as the degree of effectiveness of action k in reducing the likelihood of occurrence of risk agent j.
d. Calculate the total effectiveness of each action as follows:
$$TEk = \sum ARPj \times Ejk \forall k \quad (2)$$
e. Assess the degree of difficulties in performing each action represented as Dk, and put those values in a row below the total effectiveness. The degree of difficulties, which can be represented by a scale should reflect the fund and other resources needed in doing the action.
f. Calculate the total effectiveness to difficulty ratio.
$$ETDk = \frac{TEk}{Dk} \quad (3)$$
g. Assign rank of priority to each action (Rk) where Rank 1 is given to the action with the highest ETDk.

III. RESULT AND DISCUSSION

A. Process Activity Mapping

Cell culture process in Company X consists of four main steps which are 1st Culture, 2nd Culture, Main Culture also Harvest & Filtration step as described in Figure 1.

B. Risk Identification and Analysis

Risk identification data was colected from brainstorming using focus group discussion and direct observation by expert team such as QA, QC and production manager, engineering supervisor. The data was validated by interviewing company internal stakeholder such as production manager, culture supervisor and also senior operator from culture part. Risk event and severity described in Table 3. There are 31 risk agents were identified in this step and the occurence was determined in Table 4.

C. Risk Evaluation

The risk analysis consists of identifying risk agents by using ARP scoring through HOR1. ARP assessment in HOR 1 aims to gain risk agent ranking in the process of risk mitigation. ARP score is obtained by multiplying the value of risk severity, likelihood or probability risk events, and the value of relation between risk agent and risk event. Calculation of ARP of each risk agent using HOR1 matrix described in Table 5. Pareto Charts was used to determine priority of risk agent. Result of Pareto Chart described in Figure 2.

From the calculation of ARP and Pareto diagram obtained two priority risk agents that contribute to 80% of the total ARP which are A23 (material handling error by the operator, with an ARP value 1032 and cumulative percentage of 16%), then A14 (inadequate procedure, with an ARP value 816 and cumulative percentage 28%).

D. Risk Mitigation Strategy

The next step is designing risk mitigation strategy for associated risk agents that are selected from HOR1 analysis, then determine of the effectiveness level of each proposed proactive action with its associated risk agent. Then, the degree of difficulty (Dk) of each proactive action is determined. The Table 6 below presents a list of some proactive action, its relation score and its degree of difficulty (Dk).

This study proposes some risk mitigations for two priority risk agent. Then, the most effective proactive actions for the mitigation strategy through HOR2 analysis are selected for recomendation. The Total Effectiveness can be calculated from ARP of each risk agent with its relation. Then ETDk can be calculated from TEk score divided to Dk. The result of calculation of Effectiveness to Difficulty (ETDk) described in Matrix HOR2 Table 7. The result shown from the highest priority rank of proactive action is PA1 and PA3, then followed by PA5, PA4 and PA2.

IV. CONCLUSION

Risk control strategy in cell culture process of recombinant protein production has been performed using the House of Risk method. The results obtained that there are 40 risk events and 31 risk agents. Based on the calculation ARP and Pareto Chart analysis there are two risk agents were selected to be designed for the strategy risk control. There are five proactive actions proposed for the risk mitigation strategy in cell culture process of recombinant protein production. Based on calculation of Effectiveness by Difficulty (ETDk) the priority rank of each proactive action was determined. The result shown from the highest to lowest priority rank of proactive action recomendation for Company X are training and personnel qualification accommodated by QA Department periodically (PA1), changing in workflow become more efficient and convenient, e.g. rotation of job types (PA3) then followed by more detailed revision of related documents (PA5), changing the manual process to semi-automatic process gradually (PA4) and the last is additional person on each team as a verifier for double-checking (PA2). Further research can be done by considering dependency between risk events that may occur in the other process in the biopharmaceutical company.

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