Invalid Assay Reduction Initiative for Silver Stained Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis Assays in Quality Control Laboratories

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Abstract *— In this research, the Define, Measure, Analyze, Improve and Control methodology was used to identify and mitigate the common reasons of invalid or unacceptable Silver Stained Polyacrylamide Gel Electrophoresis assays performed in Quality Control Laboratories. The research was focused in the prevention of contaminant bands seen on the gels after staining. It also measured the economic impact to the Quality Control Laboratories operations.*

Key Terms *— Contaminant Bands, Quality Control Laboratories, Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE), Silver Nitrate Stain for SDS-PAGE.*

INTRODUCTION

The Separation of macromolecules in an electric field is called electrophoresis. The Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) uses a polyacrylamide gel as a support medium and sodium dodecyl sulfate (SDS) to denature proteins.

The SDS is an anionic detergent. When dissolved, its molecules have a net negative charge within a wide pH range. A polypeptide (protein) chain binds amounts of SDS in proportion to its relative molecular mass. The negative charges on SDS destroy most of the complex structures of proteins, and are strongly attracted toward the anode (positive-charged electrode) in an electric field.

Polyacrylamide gels restrain larger molecules from migrating as fast as smaller molecules. Because the charge-to-mass ratio is nearly the same among SDS-denatured polypeptides, the final separation of proteins is dependent almost entirely on the differences in relative molecular mass in the polypeptides [1].

After electrophoresis, proteins in the gel are made visible using Silver Nitrate Stain techniques (as shown in figure 1). There rationale of silver staining is quite simple. Proteins bind silver ions, which can be reduced under appropriate conditions to build up a visible image made of finely divided silver metal [2]. Silver stained gels are use to test product identity and purity because its high sensitivity, in the very low nanograms range. To estimate purity, samples are visually compared to known standards or controls. For identity, an approximate molecular weight can be estimated by comparing distances of migration to known products standards and molecular weight markers.

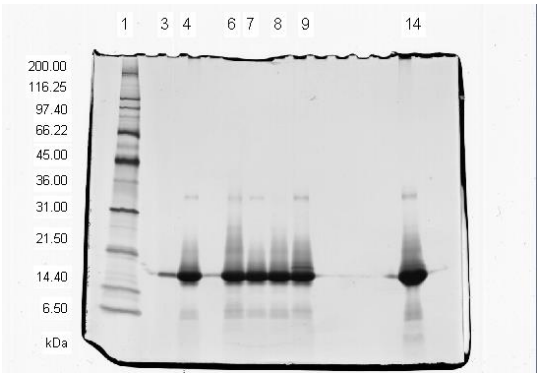


Figure 1
Silver Stained Polyacrylamide Gel Electrophoresis

Lane 1: molecular weight markers; lanes 3, 4 and 14: product standards; lanes 6-9: test samples.

RESEARCH DESCRIPTION

The first step in creating a lean process is to achieve a basic level of process stability. The primary objective in developing stable processes is to reach a consistent level of capability [3]. The initial level of stability is generally defined as the capability to produce consistent results. This is measured based on the outcome and is related to producing the same quantity of products, with the

same amount of resources, time, people and equipment, with a high degree of reliability.

Quality Control Laboratories are being constantly improving its operations with an aggressive operational excellence campaign to comply with corporate goals of being a lean manufacturing corporation. On 2014, Quality Control Laboratories demonstrated that the organization can consistently comply with product release disposition metrics at the lower cost in a safety environment. For this reason, the plant was awarded with the stability certification.

Now for 2016, the new corporate goal is to achieve the flow certification. To obtain flow certification, Quality Control as an organization has several goals. First, Quality Control Laboratories must comply with two-day sample testing. This means that the lead time from sample collection to sample testing must be no more than two days. The second goal is to lower the lot release and lot stability testing from 18 days and 30 days respectively, to 15 days. In addition, the identity testing for shipping samples must be lowered from 8 days to 3 days. The third goal is to reduce the invalid assays and non-conformances due to human error. This last goal is the one selected to be addressed for this project. All these initiatives are part of the global goals to reduce the waste caused by large inventories and rework. All this must be done with cero reportable safety incidents in our Quality Control Laboratories. In this way the company will continuously supply medicines to our customers.

RESEARCH OBJECTIVE

The objective of this research is to identify the most commonly invalid assay reasons in Silver Stained Polyacrylamide Gel Electrophoresis testing and to mitigate the root cause in order to reduce invalid assays in this technique.

An invalid assay is defined as an assay that does not met the acceptance criteria defined in the analytical method and thereby the assay results are not representative and must not be considered for reporting results. One assay may contain multiple representative samples from product lots. Invalid assays represent a rework and a waste since invalid assays must be repeated. One of the major offenders in terms of invalid assay rate is the silver stained polyacrylamide gel electrophoresis testing (silver stained SDS-PAGE) performed on gels laboratory of Quality Control Laboratories.

DEFINE PHASE

A project charter was developed on this phase with the purpose of defining the project scope and objective. The project charter is a live document that was signed by the departments involved in the project. The project charter defines the problem statement, goals, business impact and the team members of the project. Table 1 presents the project charter that was developed and approved by the team members and the champion.

Table 1
Project Charter

Project Charter			
Invalid Assay Reduction Initiative for Silver Stained Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis Assays in Quality Control Laboratories			
Project Leader	Luis Negrón, QC SR. Associate	Champion	Eillen Rodriguez Franco Manager QC
Start Date	10/2015	Target End Date	12/2016
Problem Statement	Quality Control Laboratories are seeking to obtain the Corporate Flow Certification. To achieve this the invalid assay rate must lower and maintain upon acceptable limits for the gel electrophoresis technology. One of the assays with high number of invalids is Silver Stained SDS-Gel Electrophoresis.		
Project Goals	Identify the most common causes of invalid assays for Silver Stained SDS-Gel Electrophoresis and lower the invalid rate from 13% as of Q3 2015 to 8%, the acceptable percentage for this technology.		
Team Members	Luis Negrón Vicente, QC Sr. Associate Eillen Rodriguez Franco, Manager QC Maria E. Vega Colón, Specialist QC QC Gels Laboratory Members		
Business Impact	• Decrease invalid rate and rework • Decrease operational cost • Contribute to the continuous improvements initiatives and lean manufacturing initiatives corporate goals • Contribute to obtain Flow Certification for Quality Control Laboratories		

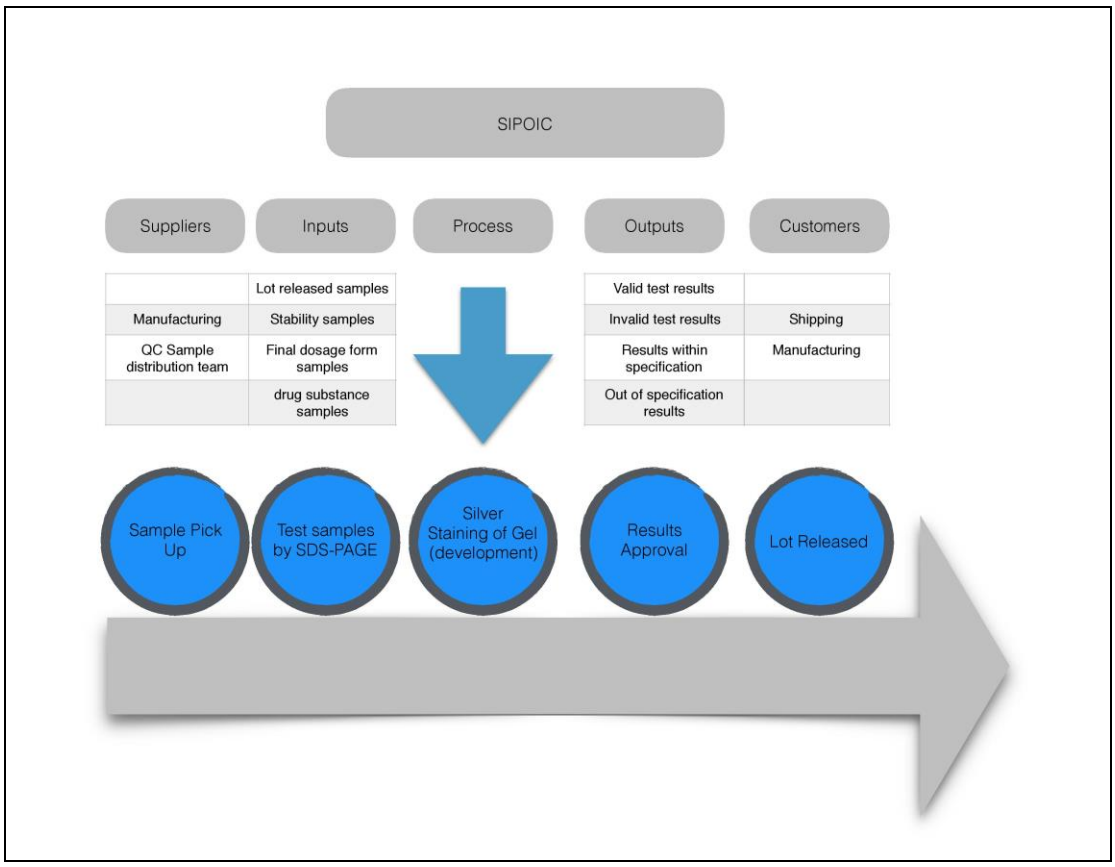


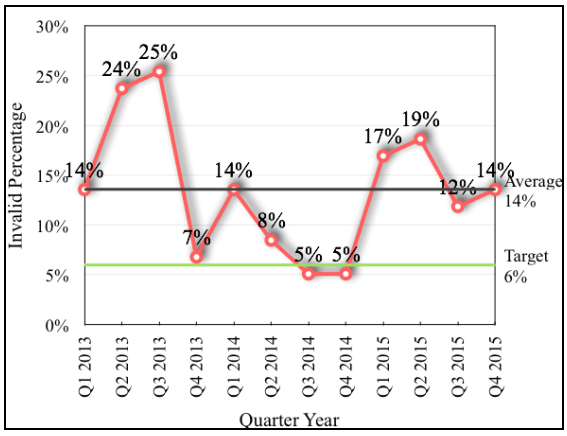
Figure 2
SIPOIC Diagram Silver Stained SDS-PAGE Test

In addition to the project charter, a SIPOC diagram was developed in order to define the suppliers, inputs, outputs and customers of the process related to silver stained polyacrylamide gel electrophoresis test. The SIPOC diagram of actual process is defined in Figure 2.

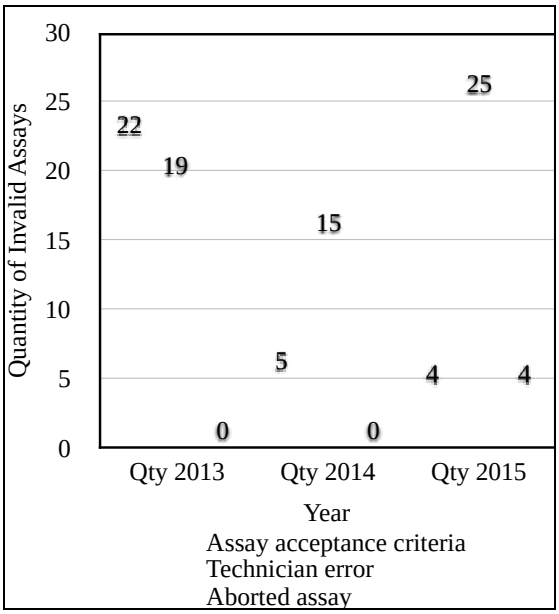
MEASURE PHASE

In Gels laboratory, the average quantity of silver stained SDS-PAGE assay performed per quarter year is 59 assays, or approximately 236 assays per year. The actual average invalid rate for silver stained SDS-PAGE is 14% which represents six percent above the acceptable 8% rate for the gel electrophoresis technology (see graph 1).

There are three major reason codes established by our procedures in which an invalid assay can be categorized: Invalid because assay does not meet assay acceptance criteria stablished on specific analytical method, invalid due to obvious technician error during assay preparation or invalid abort when the assay will be interrupted and will not be continued for any reasonable issue (see graph 2).



Graph 1
Silver Stained SDS-PAGE Invalid Rate 2013-2015



Graph 2
Invalid Assays Reason Codes for Silver Stained SDS-PAGE from 2013-1015

The assay acceptance criteria for Silver Stained SDS-PAGE are: molecular weight markers cover the anticipated range, protein band location of standards and control relative to molecular weight markers and protein loads are appropriate, no artifacts of staining that obscure the visualization of standards or controls protein lanes and/or the stain intensity control must be visible. If any assay acceptance criterion is not met the assay results are invalid. A technician error is defined as any obviously error during control or standards preparation, contamination present in the assay, wrong reagent used to prepare the assay. Under the aborted reason code, it could be an equipment malfunction during assay execution for example.

To measure the cost of a Silver Stained SDS-PAGE analysis, the cost of every reagent was tabulated. Then, the price per quantity was calculated. The quantity needed to prepare the solutions for every assay was calculated to obtain the price per assay value of every working solution. Then, the 12 hours necessary to perform and approve the data were calculated (see tables 2 and 3). The final cost to perform and approved single assay was calculated to be $436.25 to run the electrophoresis procedure plus $130.87 the development with silver nitrate stain for a total cost of $567.12 per assay.

Table 2
Reagents for Silver Stained SDS-PAGE and their Costs

Reagents	Quantity	Units	Price per Units	Price
DTT	5	g	$38.60	$193.00
Bromophenol Blue	10	g	$4.00	$40.00
Glycerol mL	500	mL	$0.32	$157.83
Glycine	1000	g	$0.05	$53.00
10-20% Gels	10	Ea.	$12.90	$129.00
10% SDS	1000	mL	$0.10	$99.00
SDS	100	g	$0.33	$33.00
Tris Base	500	g	$0.15	$73.50
10X Tris/Glycine Buffer	1000	mL	$0.03	$28.00
Micron YM-10 Filter device	100	Ea.	$4.29	$429.00
Microcentrifuge Tubes 1.5mL	500	Ea.	$0.15	$77.45
Purified Water	20000	mL	$0.01	$178.63
Filter system Acrodisc 0.2um	75	Ea.	$4.32	$324.19
Broad Range MWM	0.2	mL	$575.00	$115.00
Pipettes Tips 1uL	10	Ea.	6.84	68.40
Gloves	1	Ea.		$97.75
Kim Wipes	1	Ea.		$26.00
Acetic acid Glaciar	500	mL	$0.13	$63.33
Ethanol 95%	4,000	mL	$0.03	$134.05
Formaldehyde 37%	500	mL	$0.12	$57.74
Methanol Absolute	4,000	mL	$0.04	$179.10
Silver Nitrate	5	g	$4.98	$24.92
Sodium Carbonate	500	g	$0.29	$143.00

Table 3
Solutions Prepared per Assay and their Costs

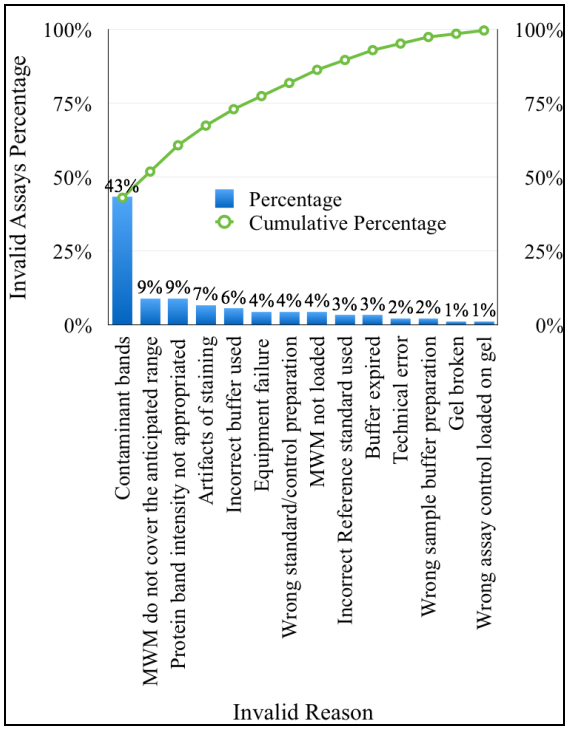
Solution	Quantity	Units	Cost per Units	Cost per Prepara-tion	Cost per Assay
0.1% SDS	100	mL	$0.05	$5.35	$0.08
1X Running Buffer	1000	mL	$0.01	$11.74	$11.74
4X Sample Buffer NR	40	mL	$0.62	$24.73	$0.25
4X Sample Buffer R	40	mL	$2.07	$82.87	$0.83

Molecular Weights	0.1	mL		$5.75	$5.75
10-20% Gels	2	Ea.	$12.90		$25.80
Micron YM-10 Filters	22	Ea.	$4.29		$94.38
Pipette Tips	2	Ea.	$6.81		$13.68
Gel Wash I	1	L	$35.04	0.4L	$14.02
Gel Wash II	1	L	$17.28	0.8L	$13.82
DTT Stock	0.4	mL	$0.06	0.4mL	$0.06
1X Reduced	400	mL	$3.63	400mL	$3.63
Silver Solution	400	mL	$6.96	400mL	$6.96
Developer Solution	500	mL	$9.07	500mL	$9.07
5% Acetic Acid	1	L	$8.57	0.4L	$3.43
Gloves	1	box	$97.75		$97.75
Kim Wipes	1	box	$26.00		$26.00
Man Hours	**12**	**Hrs.**	**$20.00**		**$240.00**
Total Cost per Assay					**$436.25**

ANALYZE PHASE

From the measure phase, it can be concluded that in average, 14% of silver stained SDS-PAGE tests were invalids from 2013 to 2015 and that we are six percent above the 8% target invalid rate expected for this technology. These invalid assays represent a total cost of approximately $17,769.76 per year. Data from our Assay Trending Program database was collected. The team classified invalid assays under the three reason codes established by our analytical methods that are: invalid due to failure to comply with assay acceptance criteria, invalid due to technician error and invalid due to aborted assay. As graph 2 shows, the majority of invalid assay are due to technician errors.

A Pareto analysis was performed to analyze all possible invalid reason for silver stained SDS-PAGE from years 2013 to 2015 (graph 3). The Pareto rule states that 20% of defects cause the 80% of the problems [4]. From the Pareto analysis it was determined that the major offender of the invalid assays was invalid assays due to contaminant bands bands across the gel between 45kDa y 66kDa areas (see figure 3). Invalid assays due to contaminant bands represents 43% of invalid assays for 2013-2015. Therefore, the scope of the research was narrowed to that cause.



Graph 3
Pareto Chart Silver Stained SDS-PAGE Invalid Assays from 2013-2015

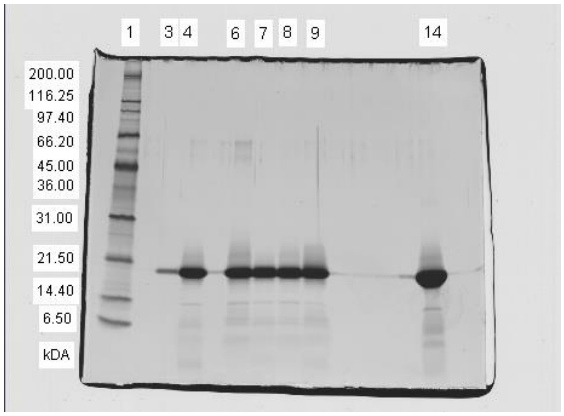


Figure 3
Contaminant Bands between 45kDa and 65kDa

IMPROVE PHASE

From the measure phase, the team identified the major offender as invalid assays due to contaminant bands across the gel between 45kDa and 66kDa area with about 43% of total invalids for 2013-2015. Contaminant bands are classified as technician error since it is mostly related to the

manipulation given by technicians during assay execution.

A special study was performed with silver stained SD-PAGE to try to reproduce the defect of contaminant bands. In this special study, the product reference standard was loaded several times and one preparation was contaminated on purpose with surface dust and skin particles (see figure 4). It is thought that contaminant bands may be caused by contamination of surface dust and/or keratin from human skin.

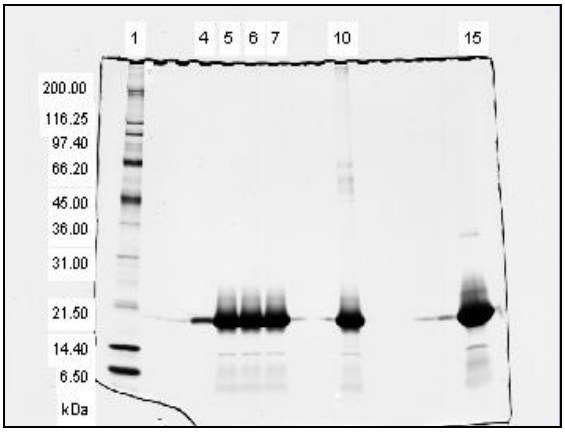
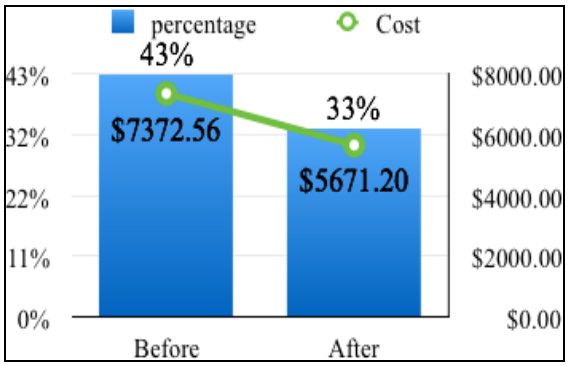


Figure 4
Special Study Performed to Reproduce the Contaminant Bands Defect in Lane 10

In order to decrease the incidence of contaminant bands due to environment, surfaces and/or skin contamination, a Standard Operating Procedure (SOP), was created. The SOP titled "Silver Staining General Guidelines", requires the analysts to identify a low movement working area to perform the assay and to clean working area with alcohol and 7X proteolytic cleaning solution. In the SOP, the correct gowning to perform Silver stained SDS-PAGE analysis is established. New disposable lab coat, hair net, face mask, nitrile gloves and sterile disposable sleeves are required. This standard operating procedure also recommends labeling certain reagents and materials for Silver Stained SDS-PAGE analysis only. In addition, the proper way to manage gel cassettes is established using visual aids throughout the document.

In October 2015 a 5S: sort, straighten, shine, standardize and sustain revamp program was launched at QC Laboratories. With this initiative the laboratory lay out was re-arranged having in mind our issue with contaminant bands in silver stained SDS-PAGE analysis. The team dedicated a laboratory work station for every analysis performed at gels laboratory: Coomassie Stained SDS-PAGE work station, SDS-PAGE Western Blot work station, Isoelectric Focusing work station and of course, the laboratory area with lowest traffic was assigned as Silver Stained SDS-PAGE work station. In each station the area was delimited using a kanban color coding for every type of instrument used during each assay type. Materials and reagents were organized and dedicated for every work station in order to reduce the movement of technician during assay execution and prevent undesirable contaminations. Drawers and cabinets were labeled with the materials and reagent that they should contain and a kanban was established to specify the maximums and minimums of each one.

Since the implementation of the SOP and the 5S exercise, the average invalid assay due to contaminant bands has decreased from 43% to 33% which represents a $1,701.36 of cost avoidance per year (see graph 4).



Graph 4
Invalid Assays Due to Contaminant Bands Percentage before and after SOP and 5S Implementation

CONTROL PHASE

As a corrective action QC laboratories added the Silver Stained General Guidelines Standard Operating Procedure to the training module for Sodium Dodecyl Sulfate Polyacrylamide Gel

Electrophoresis technique. Several learning forums regarding to the best practices stated on this SOP have been given to already trained analysts.

A characterization of environmental contaminants for Silver Stained SDS-PAGE analysis will be performed with Analytical Sciences Laboratories in order to identify the specific environmental contaminants that are causing gel contamination.

Since environmental contaminations were identified as the major offender in terms of Silver Stained Gels a clean bench will be bought and installed in order to isolate the Silver Stained Gels working station and mitigate or eliminate potential environmental contaminants. This improvement was possible based on the cost avoidance obtained due to the reduction of invalid assays caused by contaminant bands.

With the clean bench installation, it is expected to control and reduce even more the incidence of contaminants bands. Clean benches are equipped with HEPA filters. HEPA filters used in clean benches and biosafety cabinets should have a minimum filtration efficiency of 99.99% against airborne particles 0.3 microns in size. Filtration efficiency will be greater than 99.99% on particles that are larger and smaller than 0.3 microns. In a clean bench, HEPA-filtered laminar airflow is delivered in either a vertical or horizontal direction across the work area to provide a virtually particulate-free area for conducting procedures and protecting the product from contamination [5].

Quality Control Laboratories are looking forward to establish and maintain a continuous improvement culture in order to eliminate the 6% of invalid assays due to contaminant bands and reduced the current 14% rate to 8%, which is the acceptable rate for the Silver Stained SDS-PAGE technique.

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