International Journal of Veterinary Sciences and Animal Husbandry 2018; 3(5): 43-49

ISSN: 2456-2912
VET 2018; 3(5): 43-49

www.veterinarypaper.com
Received: 21-07-2018
Accepted: 24-08-2018

Hardik Mistry
Quality Assurance Department Hester Biosciences Limited. Pushpak, Panchvati Cross Road, Ahmedabad, Gujarat, India

Dr. Upendra Jani
Ex. Vice President Quality Assurance Hester Biosciences Limited. Pushpak, Panchvati Cross Road, Ahmedabad, Gujarat, India

Tejas Patel
Regulatory Affairs Department Hester Biosciences Limited. Pushpak, Panchvati Cross Road, Ahmedabad, Gujarat, India

Nupur Dave
Biotechnology Department Mehsana Urban Institute of Science, Ganpat University, Ganpat Vidyanagar, Mehsana-Gozaria Highway, Mehsana, Kherva, Gujarat, India

Correspondence
Hardik Mistry
Quality Assurance Department Hester Biosciences Limited. Pushpak, Panchvati Cross Road, Ahmedabad, Gujarat, India

Adaptation of quality by design concept for the development & manufacturing of the new castle disease virus antibody detection test kit

Hardik Mistry, Dr. Upendra Jani, Tejas Patel and Nupur Dave

Abstract
QbD (Quality by Design) is the recent systematic, scientific, risk-based, holistic, proactive approach & regulatory requirement for designing and developing the product and the manufacturing process to achieve the predefined product quality objectives and to reduce rejection or reprocessing of the batch, with increased cost and regulatory burden.
New Castle Disease is one of the most common causing factors which can affect the high demand of the poultry eggs and meats. Although vaccines are commercially available to have protection against the New Castle Disease infection in poultry, there is need for the sero monitoring and quick detection of the developed antibody in the poultry.
QbD concept is to be adopted for the development and manufacturing of the New Castle Disease (NDV) antibody detection test kit to have the detection kit with identified and required target profile. The results of the adaptation is to be evaluated on the products manufactured after the QbD concept is built into process of development and manufacturing of the NDV antibody detection.

Keywords: Quality by Design (QbD), New Castle Disease (NDV), QTPP, CQA

1. Introduction
New castle disease (ND) is one of the most important diseases affecting chicken throughout the world. NDV antibody test kit is an enzyme linked immunosorbent assay for the detection of antibody against New Castle Disease virus in chicken serum samples in which NDV antigen is coated on the 96 wells.
Statistics show a 2% increase per annum in the agricultural production during the last 30 years, while the growth in the poultry sector has been 12%. The annual average growth shown by the Indian broiler industry is about 10% and has been characterized as steady but volatile.
Statistics of year 2011-12 shows that egg production is around 66.45 billion and poultry meat production is estimated to be about 2.47 million tonnes. Exports of poultry products are at around 531.65 crore for year 2016-17 as per the report of APEDA (Agricultural & processed food products export development authority) [1].
As the new castle disease most impacting disease to the poultry for production of eggs and meats, a quick detection of the new castle disease antibody in poultry can become more beneficial. QbD approach for the new castle disease describes characteristic which are essential to quality from end user's point of view and coverts them into critical quality attributes (CQAs) that product should have [2].
QbD was first defined by the Dr. Joseph M. Juran. Juran indorse that most quality issues and crises arise due to a deficiency of importance allotted to it during product planning. To secure the quality of the manufactured products, QbD is an essential conversion from the traditional quality by testing (QbT) view, which determines the product's quality by conforming it with approved regulatory specifications at the end of the manufacturing process [3].

1.1 Steps for the approach of the life cycle management [4, 5].
Phase 1: Product development stage
Phase 2: Product manufacturing and commercialization stage
Phase 3: Post manufacturing stage

1.1.1 Phase 1: Product Development Stage

When the product is in the stage of the development, Quality by Design is to be followed as a proactive approach. Steps of the quality by design concept are as below

- Quality target product profile (QTPP)
- Critical quality attributes (CQA)
- Critical quality product parameters (CQPP)
- Design space
- Design of experiment
- Risk assessment
- Change management

1.1.2 Phase 2: Product Manufacturing & Commercialization

At the stage of the technology transfer from the lab scale to the commercial scale, risk assessment at the commercial scale is to be performed. Process validation is to be performed and results of the same are to be evaluated before commencing the final commercial manufacturing of the product.

Change management at this stage for the commercial scale is to be done. Changed and revised risk assessment is to be done in case of the changes and deviations. Quality management system elements to be used at this phase including change control, failure analysis and effective Corrective Actions and Preventive Actions (CAPA).

1.1.3 Phase - 3: Post Manufacturing Phase

Once the product manufacturing technology is transferred from R&D scale to commercial scale, and by managing such changes product quality improvement can be done as a phase of the continual improvement and post approval of the regulatory changes. At this phase also quality management system elements are used.

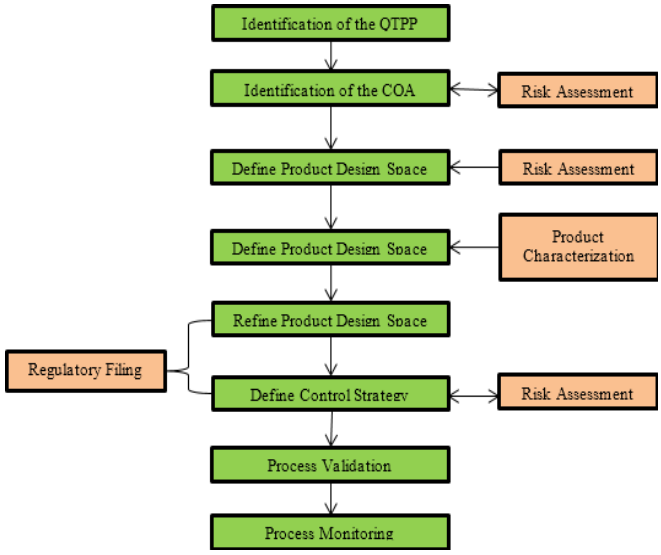

Fig 1: Key steps in implementation of QbD for a Pharmaceutical product [6].

2. Pre experimental: quality by design

2.1 Step 1: Quality Target Product Profiling for the New Castle Disease virus antibody diagnostic kit. (QTPP).

NDV kit must have the following performance characteristics

- Correlation with reference
- % agreement with reference
- Diagnostic sensitivity
- Diagnostic specificity
- Analytic sensitivity
- Analytical specificity
- Reproducibility

Table 1: For the achievement of the QTPP kit components must have the following characteristics

Kit components	Visual appearance	Stability	Sterile status
NDV antigen coated 96 well plate	NA	Stable	NA
Positive control serum	Colourless	Stable	Sterile
Negative control serum	Colourless	Stable	Sterile
Goat anti-chicken peroxidase conjugate	Colourless	Stable	Sterile
Sample diluent	Colourless	Stable	Sterile
TMB (Teramethyl benzidine) substrate	Colourless	Stable	Sterile
Stop solution	Colourless	Stable	Sterile

2.2 Step 2: Identification of the Critical Quality Attributes (CQA)

Table 2: Relation between the identified QTPP & CQA

QTPP	CQA
Correlation with reference	Source of harvest & testing Pelleting of the ND virus Preparation o sucrose gradients
% Agreement with reference	Source of harvest & testing Pelleting of the ND virus Preparation of sucrose gradients
Diagnostic Sensitivity	Preparation of the TNE buffer solution Preparation of the solutions
Diagnostic Specificity	Clarification of the allantoic fluid harvest Preparation of the solutions
Analytical Sensitivity	Preparation of the TNE buffer solution Preparation of the solutions
Analytical Specificity	Clarification of the allantoic fluid harvest Preparation of the solutions
Reproducibility	Preparation of the TNE buffer solution Antigen coating on 96 well plate Blocking and stabilization of antigen coated plates Sealing of antigen coated plates Filling of the reagents in containers

Table 3: Assessment of the quality attributes

Sr. No.	Critical Steps	Acceptance Criteria
1	Source of harvest & testing	
	Quality parameters for allantoic fluid harvest	- All the parameters for the allantoic fluid harvest should meet acceptance criteria specified in harvest specification. Virus Titer Value: Expected EID_{50} > $10^{9.5}$ - Sterility testing: Free from bacteria, fungi and extraneous pathogens
	Container to collect allantoic fluid harvest - type of container - cleaning of the container - sanitization/sterilization of container	- Container should be cleaned using validated cleaning procedure. - It should be using validated sterilization cycle load.
2	Clarification of the allantoic fluid harvest	
	Centrifugation of the allantoic fluid harvest	Heavy duty high cooling centrifuge should be calibrated for its operating range. (Range of high duty cooling centrifuge: 500 RPM to 15000 RPM)
	Time for the centrifugation	Time for the centrifugation should be at least for 30 minutes
	Quantity of the supernant allantoic fluid	Quantity of the supernant allantoic fluid to be recorded for information purpose
3	Preparation of the TNE buffer solution	
	pH of the TNE buffer solution	The pH of the buffer before & After sterilization should be controlled
	Sterilization of the TNE buffer solution	Cycle for the buffer sterilization should be validated.
4	Pelleting of the New Castle Disease virus	
	Centrifugation for pelleting	Type Z36 HK centrifuge machine should be calibrated
	Deep freezing	Deep freezer - 80 ºC should be calibrated
	Volume of the TNE buffer solution taken is to be recorded	Volume of the TNE buffer solution is for information only
	Temperature record for the overnight rehydration in TNE is to be recorded.	Temperature should not be less than 4 ºC
	Time for vortexing	Time for the vortexing is to be recorded & is for information purpose only.
	Volume of recovered rehydrated pellet	Recovered volume of the rehydrated pellet is for information purpose only.
	Temperature for the resuspended virus storage is to be recorded.	Temperature should not be less than - 80 ºC
5	Preparation of sucrose gradients	
	Preparation of 60%, 40% & 30% Sucrose	Preparation of the 60%, 40% & 30% Sucrose solution should be as per SOP
	Ultra-centrifugation	- Ultra-centrifuge should be calibrated - Centrifuge should be for 1 Lac RPM for at least 3 Hours.
	Spectrophotometery	Spectrophotometer should be validated or calibrated.
	Deep freezer	Deep freezer of -80 ºC should be calibrated
6	Preparation of the solutions	
	Buffers, Antigen for coating, Positive control, Negative Control, Substrate, Conjugate, Stop solution	Preparation of all the solutions should be as per SOP and complied to their respective specifications.
7	Antigen coating on 96 well plate	
	Micro pipette	Micro pipette to be used should be calibrated
	Cold Room	Cold room for the 4ºC is to be validated for the 24 hours.
8	Blocking and stabilization of antigen coated plates	
	Micro pipette	Micro pipette used for dispensing of 100 µl virus antigen must be calibrated.
9	Sealing of antigen coated plates	
	Sealing process	- Sealing of the antigen coated plates should be done as per SOP. - Sealing rejection should be nil
10	Filling of the reagents in containers	
	Filling of reagents	- Filling of the reagent should be done with calibrated pipette & volumetric flask. - All the reagent should comply with their respective specifications.
11	Ingredients	
	Ingredients for the buffer & reagent preparation	- All the buffers & ingredient should be received from approved supplier. - All the ingredients used are tested for their specified identity, purity and sterility - Proportion or composition of the ingredients (consumption record) - Check the pH of buffer before and after sterilization
12	Environmental monitoring	
	Environmental monitoring during blending by swabs, plate exposures and finger Dabs test	- Environment of the room is routinely monitored by plate exposure techniques. - Swabs are taken during the activity from the surface of table and LAF for monitoring the microbiological condition. - Finger Dabs of the persons involved in the activity are taken as and tested.
13	Validation status	
	Validation status of LAF and HVAC and cleanroom of the inoculation process by particle count, air velocity, recovery study, DOP or PAO test	- Validation of the LAF, HVAC and cleanroom must be complying with acceptance criteria that in the VMP and validation protocol for them.

2.3 Step 3: Risk assessment to identify the critical quality attributes (CQA)

Assessment of parameters identified for the manufacturing of the NDV antibody diagnostic test kit based on the defined QTPP is to be done.

Risk analysis should be assessed on Occurrence, Severity, and Delectability and GMP criticality. The identified risks are mitigated and tested in process. Procedural controls should be in place (if needed) to identify and manage risks to patient safety, product quality, and data integrity that arise from failure of the function under consideration.

All critical to quality (CtQ) parameters shall be identified using comprehensive evaluation plan and identified CtQ settings shall be further evaluated.

Parameter evaluation: The answers to the following questions will be used to aid in determining if a parameter is considered critical to quality or non-critical to quality. An affirmative answer to questions 1 through 7 generally indicates that the parameter is critical.

Table 4: Component Criticality Assessment Criteria

Sr. No	Component Criticality Assessment Criteria
1	The parameter directly impacts acceptance or rejection of the product.
2	The parameter represents control characteristics for a physical, chemical, biological, or microbiological property that affects product quality.
3	Failure of the parameter has a direct effect on product quality.
4	Information from this parameter is recorded as part of the production record, lot release data or other GMP data.
5	The parameter is used as a direct measurement of a drug product or its excipients to ensure product quality.
6	The parameter controls critical process elements that may affect product quality, without independent verification.
7	The parameter is considered an Acceptable Quality Limit (A parameter or tooling used to set an in-process quality limit).

Table 5: CQA Criticality Assessment

No.	Parameter	Description	1	2	3	4	5	6	7	Critical to quality or non-critical to quality
List of assessed parameters (Mark Y for Yes or N for No)										
1.	Quality parameters for allantoic fluid harvest	Virus titer and Sterility	Y	Y	Y	Y	N	Y	Y	Critical to quality
2.	Clarification of the allantoic fluid harvest	Centrifuge & RPM.	N	N	N	N	N	N	N	Non critical to quality
3.	Preparation of the TNE buffer solution	To suspend Virus	N	N	N	N	N	N	N	Non critical to quality
4.	Pelleting of the ND virus	Virus storage– 80 ^{O}C	Y	Y	Y	Y	N	Y	Y	Critical to quality
5.	Preparation of sucrose gradients	For isolation of virus	N	N	N	N	N	N	N	Non critical to quality
6.	Virus purification by ultra-centrifugation	For virus purity	Y	Y	Y	Y	Y	N	Y	Critical to quality
7.	Preparation of the solutions	Buffers Positive control Negative control Substrate Conjugate Stop solution	Y	Y	Y	Y	Y	Y	Y	Critical to quality
8.	Antigen coating on 96 Well Plate	Incubation at 4 ^{0}C	Y	Y	Y	Y	N	Y	Y	Critical to quality
9.	Blocking and stabilization of antigen coated plates	Blocking buffer Washing buffer Stabilizer	Y	Y	Y	Y	N	Y	Y	Critical to quality
10.	Filling of the reagents in containers	Filling process	N	N	N	N	N	N	N	Non critical to quality

2.4 Step 4: Design space

ND antibody test kit can be having specificity for the ND virus antibody among the broad range of the antibody detection for the IBV (Infectious Bronchitis Virus), AE (Avian Influenza), IBD (Infectious Bursal Disease), EDS (Egg Drop Syndrome) and ND (New Castle Disease). The testing of the same is to be done and results are to be evaluated during the experimental work. ND antibody test kit response cannot be altered by the presence of the any other antibody as the antigen-antibody reaction is very specific. Varying concentration of the antigen and process temperature are to be tested and results are to be evaluated.

2.5 Step 5: Design of Experiment

For the Design of the Experiment for the NDV antibody detection test kit, full factorial design is to be used in which identified variable are as below. Among them two Independent variables at the three different levels using the 3^2 full factorial model are to be performed.

Table 6: Variable for the Design of Experiment

Dependant Variable			
Analytical Sensitivity			
Analytical Specificity			
Assay Control			
Independent Variable			
Antigen Concentration		Temperature	
Low Concentration	Antigen virus titre less than $1*10^{9.5}$	Low Temperature	Temperature less than 2 ^{0}C
Middle Concentration	Antigen Virus titre is $1*10^{9.5}$	Middle Temperature	Temperature is at 4 ^{0}C
High Concentration	Antigen Virus titre More than $1*10^{9.5}$	High Temperature	Ambient Temperature

Table 7: 3^2 Factorial design model & experimental result

Trial No	Concentration	Temperature	Result of Kit Performance
Trial – 1	Low	Low	Not Acceptable
Trial – 2	Low	Middle	Not Acceptable
Trial – 3	Low	High	Not Acceptable
Trial – 4	Middle	Low	Not Acceptable
Trial – 5	Middle	Middle	Acceptable
Trial – 6	Middle	High	Not Acceptable
Trial – 7	High	Low	Not Acceptable
Trial – 8	High	Middle	Not Acceptable
Trial - 9	High	High	Not Acceptable

Based on the Design Space and Design of Experiment, set of experiment conducted and optimize result for the diagnostic kit observed for the middle level concentration (Virus Titre $1*10^{9.5}$ EID50) and middle level process temperature (Incubation at the temperature 4 ^{0}C).

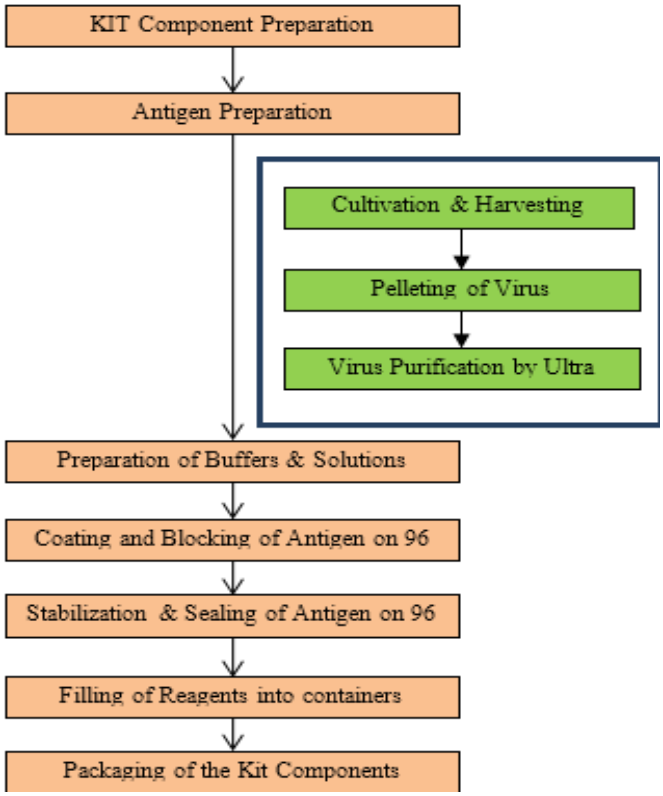

Fig 2: Flow diagram of the manufacturing of the NDV antibody detection kits

3 Experimental materials and methods

3.1 Kit components preparation

Positive control reagents, Negative control reagents, Sample Diluent, Conjugated antibody
TMB Substrate, Stop Solution, 96 well plates

3.2 Antigen Preparation

3.2.1 Cultivation and Harvesting

NDV antigen was prepared using the whole virus grown in chicken embryo. The lasota strain of NDV was used as master seed after quality control check to assure its purity and free of extraneous pathogens. SPF (Specific Pathogen Free) eggs were inoculated at 10 day old embryonic stage by allantoic cavity route following the standard laboratory procedures. Embryos were further incubated in humidified temperature controlled egg incubator. Allantoic fluid harvest was collected on day 4 of inoculation and stored at 2-8 ºC until processed.

3.2.2 Pelleting Of Virus

The allantoic fluid harvest was initially subjected to low speed centrifugation to remove cell debris followed by high speed centrifugation to pellet the virus particles. ND virus is to be pelleted by high speed centrifugation in centrifuge tubes at 2000 RPM for at least 3 hours at 4 °C using the type Z36 HK centrifuge machine. Supernatant is to be discarded. Pellets are to be rehydrated in 300 µl of TNE buffer to each tube and keep it to overnight at 4°C cold room pellets are re-suspended by vortexing. Re-suspended virus is to be pulled and to be stored at 80 °C deep freezer.

3.2.3 Virus purification by ultra-centrifugation

Virus purification is done by overlaying the re-suspended pelleted virus on top of the stabilized sucrose gradient and centrifugation the tubes at 1, 00,000 RPM for 3 hours. The distinct virus band is collected and repurified using the same gradient twice. Finally band was collected and pelleted by centrifugation over 30% sucrose gradient at 1, 00,000 RPM for 1 hour. Final virus pellet is rehydrated in 1ml of TNE buffer overnight at 4 °C and resuspended by vortexing. The virus quantity is determined by protein estimation at A280 by Nan drop spectrophotometer, divided into aliquots of 250 µl, labelled and stored at -80° C deep freezer until further use.

3.3 Preparation of the TNE & Other buffer solution

TNE Buffer, Conjugate Diluent, Blocking buffer, Coating Buffer, Phosphate citrate buffer, 10% BSA, 10% Thiomersal, Sample Diluent, Washing buffer, Antigen stabilizer, PBS solution, TMB in DMSO solution, 10% Sodium Azide and 50% Tween 20 are to be prepared and tested as per specifications.

3.4 Coating, Blocking & Stabilization Of NDV Antigen On 96 Well Plates

Dissolve purified virus at antigen concentration (NDV: 3µg/ml) in coating buffer. Dispense 100 µl of diluted virus antigen to the wells of 96 well plates. Cover plate with lead/aluminium foil and incubate at 4 ºC for overnight. Discard unbound antigen to the appropriate vessel containing decontaminating agent. Add 200 µl of blocking buffer to each well. Incubate for 2 hours at room temperature Wash plate once with 300ul of washing buffer & tap each plate onto absorbent material to remove any residual was fluid. Add 200 µl of antigen stabilizer to each well. Discard stabilizer solution to the appropriate vessel containing decontaminating agent and tap each plate onto absorbent material to remove any residual solution. Air dry the plate in laminar flow.

3.5 Preparation of the final kit

Preservatives, Sample diluent, Positive control, and Negative control reagents contains sodium azide as preservative at a final concentration of 0.01%. Conjugated antibody solution

contains thimerosal as preservative at a final concentration of 0.01%.

4. Post experimental: testing of ndv antibody diagnostic test kit

4.1 Principle of ELISA testing

Solid phase consists of a 96-well polystyrene plate in ELISA technique. Antigens or antibodies in the sample are immobilize as they bind to the solid phase. After incubation, unbound material can be removed by washing. Then conjugate is added to the plate. The plate is then incubated. The conjugate have either an antibody or antigen that has been labelled with an enzyme. The plates are washed and an enzyme substrate is added and then allow the plate to incubate. Colour develops and the OD is taken with an ELISA plate reader. The amount of colour developed which is proportional to the amount of antibody present in the test sample is measured using the microplate reader at 650 nm.

4.2 Requirements (Materials/Equipment)

Coated 96 Well Antigen Plate, Positive and Negative Controls, Sample Diluent, Conjugate, Precision Pipettes and Auto Dispenser, Disposable Pipettes, Stock Solution, Distilled Water, Aspiration Device and Vacuum Source, Plate Reader

4.3 Procedure for testing

4.3.1 Sample Preparation

Thaw all reagents to come to room temperature before use. Mix reagents by gentle inverting or swirling. Do not dilute positive and negative controls. Dilute test serum samples 500 fold by diluting the sample diluent (e.g. Dilute 1 µl of test serum sample with 500 µl of sample diluent). Dilute 10 known positive test sera samples (PS1 to PS10) and 7 known negative test sera samples (NS1 to NS7) as 1:500 with sample diluent.

Dilute NDV monospecific serum sample as per table no. 8. Dilute monospecific sera for NDV, IBV, IBD, AE and EDS as 1:500 with sample diluent.

Samples are prepared and labeled as D1, D2, D4, D8, D16, D32, D64 and D128 for dilution of 1:500, 1:1000, 1:2000, 1:4000, 1:8000, 1:16000, 1:32000 & 1:64000 respectively.

4.3.2 Testing procedure

Obtain antigen coated plate and record the sample position. Dispense 100 µl of undiluted negative control into duplicate wells & Dispense 100 µl of undiluted positive control (PC) into duplicate wells. Dispense 100 µl of diluted test sample into appropriate wells. Incubate for 30 minutes (± 2 Minutes) at room temperature. Tap out liquid from each well into appropriate vessel containing decontaminating agent. Using 8 or 12 channel pipette, wash each well with approximately 300 µl of deionized water 3 times. Avoid plate drying between plate washings and prior to the addition of the next reagents. Tap each plate onto adsorbent material after the final wash to remove any residual wash fluid. Dispense 100 µl of conjugate into each well. Incubate for 30 minutes (± 2 minutes) at room temperature. Repeat step no 6. Dispense 100 µl of TMB substrate into each well. Incubate for 15 minutes (± Minutes) at room temperature. Dispense 100 µl of stop solution into each well.

Measure and record absorbance values at 650 nm. Record the O.D values and analyze using the ELISA software.

5. Data analysis

Analysis of purity: Microbial bioburden count to be done for the Positive Control serum, Negative Control serum, Goat anti-chicken peroxidase Conjugate, Sample diluent, TMB substrate & Stop solution

Analysis of performance: ELISA plate is to be settled using the positive, negative samples and Dilution done as per the table no 8 and titer is to be calculated and recorded.

Titre correlation: Use Excel formula function "CORREL (array1=known, array2=test batch).

%Agreement: (Positive/negative)= (Positive in both + Negative in both)/Total*100

Diagnostics Sensitivity: (TP/TP+FN)*100

Diagnostic Specificity: (TN/FP+TN)*100

TP: Test Positive, TN; Test negative, FN: False negative, FP: False Positive

Validation of assay controls: Positive and negative control O.D is to be found and recorded.

Analytical sensitivity (limit of detection): For the Dilution D1 which is 1:500 to D128 which is 1:64000 positive cut off value is to be found and result recorded.

Analytical specificity: S/P ratio is to be calculated for the NDV, IBV, IBD, AI and EDS nonspecific sera.

6. Finish product testing

Table 8: Results of diagnostic kit performance

Test Name	Test Parameter	Acceptance Criteria	Batch 1
NDV antigen coated 96 well plate	CFU Count	NA	NA
Positive Control Serum	CFU Count	< 10 CFU/ml	< 10 CFU/ml
Negative Control Serum	CFU Count		< 10 CFU/ml
Goat Anti Chicken Peroxidase Conjugate	CFU Count		< 10 CFU/ml
Sample Diluent	CFU Count		< 10 CFU/ml
TMB substrate	CFU Count		< 10 CFU/ml
Kit Performance	Correlation with reference	>0.90%	0.99
	% Agreement with reference	>90%	100
	Diagnostic Sensitivity	>90%	100
	Diagnostic Specificity	>90%	100
	Analytical Sensitivity	>1/8000	1/64000
	Analytical Specificity	NDV>0.2 IBV, IBD, AI, EDS <0.2	Complies
Assay Control	Positive	>0.2, <0.6	Complies
	Negative	<0.2	Complies

7. Conclusion

QTPP for the NDV antibody detection kit was determined based on the literature review and requirement of the customer. Based on the QTPP identified, all possible Quality Attributes for the NDV antibody detection kit were identified. Risk assessment for all identified Quality Attributes was performed. Result of the risk assessment has provided CQAs.

Dependant and independent variables associated with the manufacturing process of the NDV antibody detection kit were identified and among them 3^2 full factorial model was performed for 3 levels and 2 independent factors. Among all 8 Trials, Trial with optimum results was selected for the manufacturing process of NDV antibody detection kit.

By performing the FMEA model for the manufacturing process, all the procedural steps were found under control so no additional control or mitigation plan for risk required. Designed manufacturing process with identified CQA performed and final performance of the NDV antibody detection kit along with the testing of the identified QTPP in the NDV antibody detection kit.

As a result of experimental works reveals, all the QTPP identified and designed obtained in NDV antibody detection kit which provide the performance as defined in identified and designed QTPP.

8. References

1. Report of the India export of agro food products by APEDA. http://agriexchange.apeda.gov.in/indexp/Product_description_32head.aspx?gcode=0406
2. International Conference on Harmonization Tripartite Guideline, Q8 (R2) Pharmaceutical Development, 2009.
3. Lawrence X Yu. Quality by Design: Concept for ANDA's. AAPS J. 2008; 10:268-276
4. Sharmada S. Sinai Kakodkar *et al*. Ijrm. Human, 2015; 1(1):1-19.
5. Sangshetti JN *et al*. Quality by design approach: Regulatory need. Arabian Journal of Chemistry (2014),
6. Sandipan Roy, Int J Biomed. 2012; 3(2):100-108.