Division of Veterinary Microbiology & Immunology
Faculty of Veterinary Sciences & Animal Husbandry
Shuhama, Srinagar – 190006, Kashmir (J&K)
Phone:0194-2262211 Email: vmifvsc@skuastkashmir.ac.in

DBT

"Development of an aptamer based kit for detection of *Campylobacter* infections in cattle and poultry"

No.: FVS/MICRO/DBT-/2026-27/191
Date: 30-09-2026

Livestock and Animal Biotechnology Division
Department of Biotechnology.
Ministry ofScience & Technology, Government of India,
Room No. - 612; 6th Floor; Block- 2; CGO Complex,
Lodhi Road, New Delhi- 110003

Subject: Request for retaining of the equipment procured under the project sanctioned vide Ref:BT/PR40347/ADV/90/286/2020, Dated: 22/09/2021

Respected Sir/Madam,

With reference to subject cited above it is to submit that, two equipment namely RPA Based Lateral Flow Device, costing Rs7.96000 lakhs and Fluorescence Detection Device priced Rs 9.98000 lakhs procured under capital head budget of Rs. 17.94000 lakhs under the project entitled "**Development of an aptamer-based kit for detection of *Campylobacter* infections in cattle and poultry**" sanctioned vide number BT/PR40347/ADV/90/286/2020, Dated: 22/09/2021.
The project has been benefited from the use of these instruments, which continue to hold significant utility for ongoing and future research activities in the Division. The equipment will also serve as valuable resources for undergraduate and postgraduate teaching, student training, and skill development in advanced molecular diagnostics. The aforesaid equipment has been transferred to the Division on 29.09.2026 and duly entered in the Divisional Stock Register. A photocopy of the relevant stock register entry is enclosed herewith for kind perusal and record.

It is therefore requested that permission may kindly be accorded for retention of the above-mentioned equipment in the Division for continued utilization in research, teaching, and academic activities. The said equipments shall also be displayed on the University website as per the applicable guidelines.

This is for favour of your kind consideration and necessary action please.

Yours faithfully,

Dr.Sabia Qureshi
Principal Investigator
DBT Project

Principal Investigator, DBT Project
"Development of aptamer kit
campylobacter infections

SKUAST-Kashmir
Faculty of Veterinary Sciences &Animal Husbandry
Shuhama, Kashmir, J & K 190 006

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E-mail: qureshi.sabia@gmail.com
Visit us at: www.skuastkashmir.ac.in

Form GFR-22 **S.No. 114**

{Rule 211 (ii) (a)}

REGISTER OF FIXED ASSETS

Name and description of the Fixed Assets --- Fluorescence Detection Device **Code No.** L/FOD/2023-24

Date	Particulars of Asset	Particulars of Supplier		Cost of the Asset	Location of the Asset	Remarks
		Name & address	Bill No. & date			
29/9/2026	Fluorescence Detection Device	Zitam & Company Srinagar	GEM23129-804 Dt. 30-09-2023	998000.00	NAE Lab	2023-24/01

"Certified that the items listed above have been physically verified by the Physical Verification Committee. The report of which is placed in the Physical Verification File for the Year 2025-26"

Store Keeper I/c Store Officer Prof. & Head

Form GFR-22 S.No. **115**

{Rule 211 (ii) (a)}

REGISTER OF FIXED ASSETS

Name and description of the Fixed Assets RPA Based Lateral Flow Device Code No. 2/1/01/2023-24/01

Date	Particulars of Asset	Particulars of Supplier		Cost of the Asset	Location of the Asset	Remarks
		Name & address	Bill No. & date			
29/09/2026	RPA Lateral Flow Device	Zitamb Company Srinagar	GEM23129-561 Dt: 20-09-2023	796000.00	NAE lab	2023-24/01

"Certified that the items listed above have been physically verified by the Physical Verification Committee. The report of which is placed in the Physical Verification File for the Year 2025-26"

Store Keeper I/c Store Officer Prof. & Head

Division of Veterinary Microbiology & Immunology
Faculty of Veterinary Sciences & Animal Husbandry
Shuhama, Srinagar – 190006, Kashmir (J&K)
Phone:0194-2262211 Email: vmifvsc@skuastkashmir.ac.in

DBT

"Development of an aptamer based kit for detection of *Campylobacter* infections in cattle and poultry"

No.: FVS/MICRO/DBT/2026-27/192
Date: 30-09-2026

Livestock and Animal Biotechnology Division
Department of Biotechnology.
Ministry ofScience & Technology, Government of India,
Room No. - 612; 6th Floor; Block- 2; CGO Complex,
Lodhi Road, New Delhi- 110003

Subject: Position of Unspent Balance of Rs. 77,630/-

Sir,

With reference to the subject cited above, it is submit that an amount of **Rs. 77,630/- (Rupees Seventy-Seven Thousand Six Hundred and Thirty only)** under the **Manpower head** of the DBT project entitled **"Development of Aptamer Based Kit for Detection of Campylobacter Infections in Cattle and Poultry"** (BT/PR40347/ADV/90/286/2020, dated 22-09-2021), implemented in the Division of Veterinary Microbiology and Immunology, Faculty of Veterinary Sciences & Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-Kashmir), Shuhama, Srinagar, remained unutilized.

The said unutilized amount has been reflected in the **Final Utilization Certificate** and remains with the **TSA Hibrid Model (BIO-RIDE)**. The limit was not reassigned to the said amount during the financial year **2026–2027**.

A certificate to this effect is enclosed herewith for your kind information and ready reference.

Thank you

Yours faithfully,

Dr.Sabia Qureshi
Principal Investigator

Assistant Comptroller
FVSc and AH, Shuhama

Assistant Comptroller
Faculty of Veterinary Sciences
& A.H. Alusteng Shuhama Srinagar

SKUAST-Kashmir
Faculty of Veterinary Sciences &Animal Husbandry
Shuhama, Kashmir, J & K 190 006

Mob. No. 91-9419094880
E-mail: qureshi.sabia@gmail.com
Visit us at: www.skuastkashmir.ac.in

UTILISATION CERTIFICATE

(From 1st April 2025 to 21st September 2025)

(Financial figures given in this UC are shown in lakhs)

1.	Title of the Project	:	**Development of an aptamer based diagnostic kit for detection of *Campylobacter* infections in cattle and poultry**
2	Name of the Institution	:	**SKUAST-Kashmir**
3	Principal Investigator	:	***Dr. Sabia Qureshi***
4	Sanction Order No. & Date of Sanctioning the Project	:	**BT/PR40347/ADV/90/286/2020 Dated 22.9.2021**
5	Amount brought forward from the previous financial year quoting DBT letter No. & date in which the authority to carry forward the said amount was given **i)** Letter No. **ii)** Amount **iii)** Date	: : :	Nil
6	Amount received/assigned from DBT during the financial year *(also give No. and date(s) of sanction order(s) showing the amount(s) released/assigned by DBT)*	:	Rs.1.14712 **BT/PR40347/ADV/90/286/2020** **Dated:14-05-2025**
7	Details of other receipts/interest earned, if any, on the DBT grants	:	Nil
8	Total amount that was available for expenditure during the financial year (*S. Nos. 5, 6 and 7*)	:	Rs.1.14712
9	Actual expenditure (*excluding commitments*) incurred during the financial year (*statement of expenditure in this regard is enclosed herewith*)	:	Rs.0.37082
10	Unspent balance refunded, if any (*a copy of BharatKosh receipt in this regard is enclosed herewith*)	:	Nil
11	Balance amount available at the end of the financial year		Rs.0.77630
12	Amount allowed to be carried forward to the next financial year vide letter No. & date	:	Rs.0.77630
	i). Certified that the amount of Rs.0.37082 mentioned against col. 9 has been utilised on the project for the purpose for which it was sanctioned and an amount of Rs.Nil has been surrendered to Govt. (vide BharatKosh Receipt No. .....dated .....).		
	ii). Certified that I have satisfied myself that the conditions on which the grants-in-aid was sanctioned have been duly fulfilled / are being fulfilled and that I have exercised the following checks to see that the money was actually utilised for the purpose for which it was sanctioned.		
	Kind of checks exercised: 1.Ledger 2 Stock Register	:	

PROJECT INVESTIGATOR
(Name, Signature and Stamp)

Principal Investigator DBT Project "Development

FINANCE OFFICER OF THE INSTITUTION
(Name, Signature and Stamp)

Assistant Comptroller
Faculty of Veterinary Sciences
& A.H. Alusteng Shuhama Srinagar

HEAD OF THE INSTITUTION
(Name, Signature and Stamp)

DIRECTOR RESEARCH
Sher-e-Kashmir Univ. of Agri. Sc. & Tech
Shalimar, Srinagar-190025

Consolidated Statement of Accounts (*Towards final Settlement of Accounts*)

(For the period from Official Sanctioned Date to Official Completion Date 01/04/2025 to 21/09/2025)

1. Title of the Project : **Development of an aptamer based diagnostic kit for detection of *Campylobacter* infections in cattle and poultry**
2. Name of the Grantee Institution : SKUAST- Kashmir
3. Principal Investigator : Dr. Sabia Qureshi
4. Sanction Order No. & Date of Sanctioning of the Project : **BT/PR40347/ADV/90/286/2020 Dated 22.9.2021**
5. Actual Duration of the Project : Three years

A. Consolidated Budget Statement

(Financial figures given in this CBS are shown in lakhs)

Budgetary Object Heads	Total Sanctioned Grant within each Budget Head *(as per the Sanctioned Order)*	Grants Release Made by DBT					Expenditure Incurred *(This must align with UCs and SoEs submitted yearly)* **					Amount Refunded through Bharat Kosh	Unspent Balance left with the Institution	Remark *(if any)*
		Details to be furnished Financial Year wise					Details to be furnished Financial Year wise							
		2021 - 2022	2022 - 2023	2023 - 2024	2024 - 2025	Total	2021 - 2022	2022 - 2023	2023 - 2024	2024 - 2025	2025 - 2026	Total		
		Year 1	Year 2	Year 3	Year 4...		Year 1	Year 2	Year 3	Year4	Year			
Grants for Creation of Capital Assets	17.94	17.94	0.00			17.94	0.00	0.00	17.94			17.94		0.00	
Grants-in-aid General	34.32286	17.97280	0.00	16.350006	15.74378	34.32286	3.17546	6.25233	9.15129	14.59666	0.37082	33.54656		0.77630	
Interest Earned	0.81519	0.25682	0.55837	0.00	0.00	0.81519	0.00	0.55837	0.25682	0.00		0.81519	0.81519	0.00	
Total	53.07805	36.16962	0.55837	16.350006	0.00	53.07805	3.17546	6.8107	27.34811	14.59666	0.37082	52.30175	0.81519	0.77630	

***Indicate if there was any deviation/revision as against the sanctioned order, and reason there of in remark section*

B. Details of Equipment Procured During the Project Period

(Financial figures given in this equipment procured details are shown in lakhs)

S. No.	Name of Equipment(s)/ Asset(s) Acquired	Sanctioned Cost	Quantity Procured	Actual Cost *(Figures Not to round off)*	Balance to be reimbursed
	1	2	3	4	5
a.	**RPA Based Lateral Flow Device**	7.96	01	7.96	0.00
b.	**Fluorescence Detection Device**	9.98	01	9.98	0.00
c.					
d....					
	Total	17.94	02	17.94	0.00

- *The signatories hereby certify that the expenditure incurred from the released grant was utilized solely for the purpose of implementation of the project under consideration, and incompliance with the sanction order. We also certify that monthly emoluments of engaged human resource in this project were disbursed in accordance with the duly notified norms/guidelines of the Government Department/Ministry/Autonomous Bodies. Detailed expenditure, if asked for, will be submitted for the purpose of Audit.*

- *It is also certified that the Institute has not utilized more than the amount sanctioned under the 'Overhead' component.*

Principal Investigator
DBT Project

Principal Investigator DBT Project
"Development of aptamer kit
campylobacter infections

Assistant Comptroller

Faculty of Veterinary Sciences

Director Research
SKUAST-K, Shalimar

DIRECTOR RESEARCH
S.K. University of Agri. Sc. & Tech.
Shalimar, Srinagar-190025

Statement of Expenditure (SoE) (*Referred to in para 9 of the Utilization Certificate*)

(*Showing grants received from the Department of Biotechnology, and the Expenditure incurred in FY 2025 - 2026. during the period from 01/04/2024 to 21/09/2025*)

(Financial figures given in this SoE are shown in lakhs)

S. No.	Budgetary Object Heads	Unspent Balance Carried Forward from Previous Financial Year	Grant Received/ Assigned from DBT during the Financial Year	Other receipts/ Interest Earned on the DBT Grants	Total Grant Available	Actual Expenditure (*excluding commitments*)*incurred during the financial year*	Balance Grant Available	Remarks (*If any*)
1	2	3	4	5	(3+4+5) = 6	7	(6-7) = 8	9
A.	Grants for Creation of Capital Assets**							
B.	Grants-in-aid General		Rs.1.14712		Rs.1.14712	Rs.0.37082	**Rs.0.77630**	
C.	Details of other receipts/ interest earned on the DBT grants (if any)							
	Grand Total		Rs.1.14712		Rs.1.14712	Rs.0.37082	**Rs.0.77630**	

***This SoE must be substantiated with valid & duly executed Capital Assets Acquired Certificate (CAAC)*

- *The signatories hereby certify that the expenditure incurred from the released grant was utilized solely for the purpose of implementation of the project under consideration, and in compliance with the sanction order. We also certify that monthly emoluments of engaged human resource in this project have been disbursed in accordance with the duly notified norms/guidelines of the Government Department/Ministry/Autonomous Bodies. Detailed expenditure, if asked for, will be submitted for the purpose of Audit.*

- *It is also certified that the institute has not utilized more than the amount sanctioned under the "Overhead" component.*

PROJECT INVESTIGATOR
(Name, Signature and Stamp)

Principal Investigator
"Development of aptamer for campylobacter infections

FINANCE OFFICER OF THE INSTITUTION
(Name, Signature and Stamp)

Assistant Controller
Faculty of Veterinary Sciences
A.H. Alusteng Shuhama Srinagar

HEAD OF THE INSTITUTION
(Name, Signature and Stamp)

DIRECTOR RESEARCH
S.K. University of Agri. Sc. & Te
Shalimar, Srinagar-19006

Please also note the following:

1. Grant utilization details for this UC and all other pending UCs of this project need to be compulsorily updated in PFMS website. After that please generate GFR- 19 from PFMS website. Get signed GFR-19 and upload the signed GFR-19 in PFMS website and enclose original copy of the same with financial documents to be submitted to DBT.

2. Please cross check and ensure that all financial documents are complete in all respect and all are in proper order and in proper DBT formats.

3. Please ensure that all financial documents should tally with each other:

    a. Details in Capital Assets Acquired Certificate are matching with equipment expenditure details given in UC &SoE and are in consonance with the DBT approvals/ sanction equipment /Capital assets item list.

    b. Expenditure details in Utilization Certificates are matching with the GFR-19 generated from PFMS website.

4. All financial documents duly signed by PI, Finance & Accounts Officer, and Executive Authority of Institution. In case of NGO/ Private Institutions UC &SE also needs to be Audited by Charted Accountant.

5. All financial documents are required to be submitted by email to the program officer.

6. In case of multicentric project, grant release/assignment will be considered together after receipt of all financial documents in proper order from all participating institutions. Therefore, all project investigators may co-ordinate with the project coordinator for timely submission of project documents to DBT.

Capital Assets Acquired (*wholly or substantially*) Out of Government Grants (*Separate Register to be maintained by Grantee Institution*)

(For the financial year 2025- 2026, for the period from 01/04/2025 to 21/09/2025)

(Financial figures given in this CAAC are shown in lakhs)

1.	Name of the Sanctioning Authority	:	DBT
2.	S. No. of Assets Register	:	02
3.	Name of the Grantee Institution	:	SKUAST-Kashmir
4.	Project No. & Date of sanction order	:	BT/PR40347/ADV/90/286/2020 Dated 22.9.2021
5.	Amount of the sanctioned grant for creation of capital assets	:	17.94000
6.	Whether any condition regarding the right of ownership of Govt. in the property or other assets acquired out of the grant was incorporated in the grant-in-aid sanction order	:	NA
7.	Particulars of assets actually created or acquired *(Details as per enclosed format)*	:	1:RPA Based Lateral Flow Device 2. Fluorescence Detection Device
8.	Value of the assets as on date	:	17.94000
9.	Purpose for which assets utilized at present	:	Lab Work
10.	Encumbered or not	:	NO
11.	Reasons, if encumbered		NA
12.	Disposed of or not	:	NO
13.	Reasons and authority, if any, for disposal	:	NA
14.	Amount realized on disposal *(before initiating such disposal process prior approval of DBT shall be obtained)*	:	NA
15.	Remarks	:	

Details of Capital Assets Acquired During the Period

(Financial figures given in this CAAC are shown in lakhs)

S. No.	Name of Equipmet/ Asset	Sanctiond Cost	Quantity Procured	Actual Cost *(Actual figure, not toroundoff)*	Date of Placing order	Date of Receiving Equipment	Payment Voucher No. withDate *(Copy of payment voucher(s) mayplease be enclosed)*	Balance to be reimbursed *[Provide reason(s) for fluctuation in price (ifany)]*
	1	2	3	4	5	6	7	8
Sanctioned Equipment/Asset								
a.	**RPA Based Lateral Flow Device**	**7.96**	**01**	**7.96**	**24-05-2022**	**20-09-2022**	**AU/FVSC/VMI/DBT/ SABIA/90**	**Nil**
b.	**Fluorescence Detection Device**	**9.98**	**01**	**9.98**	**24-05-2022**	**20-09-2022**	**AU/FVSC/VMI/DBT/ SABIA/91**	**Nil**
c.								
d.								
e.								
f.								
g....								
Equipment/Asset acquired without sanction but for which the prior approval of the DBT was obtained								
a.								
b.								
c....								
Total		**17.94**	**02**	**17.94**	**24-05-2022**	**20-09-2022**	**AU/FVSC/VMI/DBT/ SABIA/90 AU/FVSC/VMI/DBT/ SABIA/91**	**NIL**

PROJECT INVESTIGATOR
(Name, Signature and Stamp)

FINANCE OFFICER OF THE INSTITUTION
(Name, Signature and Stamp)

HEAD OF THE INSTITUTION
(Name, Signature and Stamp)

Principal Investigator DBT Project
"Development of aptamer kit
campylobacter infections

Assistant Professor
Faculty of Veterinary Sciences
& A.H. Alusteng Shuhama Srinagar

DIRECTOR RESEARCH
S.K. University of Agri. Sciences
Shalimar, Srinagar-190025

Project Name: Development of an aptamer-based kit for detection of *Campylobacter* infections in cattle and poultry
Name of the PI/ Project Coordinator: Dr. Sabia Qureshi
Total Sanctioned Budget: Rs: 55.94 Lakhs; Start of the project date: 22.09.2021 Duration: 04 Years.

No. of workshop/symposia/brainstorming meeting/ trainings organized in financial year: NIL

Quarterly Deliverables

(If multi-centric, separate for each centre)

S.N.	Quarterly Deliverables of the project (As per Sanction Order)	Achievement Status [Achieved/partially achieved/yet to start etc.]	Remarks (*Achievement in detail/other*)
1.	Isolation, biochemical, Molecular characterization and virulence gene profiling and preservation of *Campylobacter* isolates from clinical samples (poultry, cattle and sheep).	**Achieved**	**Isolation and biochemical identification were carried out for various species of Campylobacter** ❖ A total of 1088 samples have been collected and processed from various farms (Poultry and cattle) in and around Kashmir region till date. ❖ A total of 1.10% (n=4/362), 10.49% (n=38/362), 7.73% (n= 28/310) poultry cloacal swab samples were detected positive by multiplex PCR for *C. jejuni*,*C. coli*, and*C. jejuni* +*C. coli* (Co-infection), respectively. Among the 171 sheep vaginal swab samples collected 7 (4.09%) were detected positive for *C.fetus*subsp*fetus*and 13 (7.60%) for *C. jejuni*. Of the 139 sheep rectal swabs, 4 (2.87%) samples were positive for *C. jejuni* and 4 (2.87%) for *C. coli* and one sample (0.72%) was positive both for *C. jejuni* and *C. coli*. One sample (12.5%) of the 8 abomasal content samples of the aborted sheep foetus were positive for *C. fetussubspfetus*. *C. jejuni* was isolated from 1 sample (0.86%) of the 116 cattle vaginal swabs. *C. fetussubspfetus*. Was isolated from18 cattle cervico vaginal mucous samples [01 (5.55%)] and 41 Preputial washing [one (2.43%)]. *C. jejuni* [3 (2.7%)] and *C. coli* [14 (12.7%)] were isolated from 110 poultry meat samples. One (0.98%) of the 102 milk samples was positive for *C. coli*. ❖ The samples were subjected for detection and isolation as per standard protocols. ❖ A total 65 confirmed isolates have been obtained till date. **Molecular and characterization was carried out for all isolates.**

			Virulence Gene profiling has been carried out. ❖ Virulence gene profiling of *C. jejuni*&*C. coli* isolatesrevealed presence of *cdt*B, *cad*F, *fla*A in all the isolates.The virulence gene *wla* N was present in 3 *C.jejuni* isolates from poultry.The representative samples of various virulence genes were sequenced and submitted to Gen Bank. The SVR-*flaA* typing of *Campylobacter* isolates revealed presence of 6, SVR-*flaA* types viz., 79, 72, 44, 62, 33, and 78 circulating in poultry and poultry products from Kashmir. The other *fla*A-SVR gene sequences in this study were OL471369, OL471370, OL471371, OL471372, OL471373, and OL471374. The *Dde*I-RFLP pattern of all isolates in the present study differed from each other ranging from 100-1000bp indicative of strain variation among the *Campylobacter* strains from Poultry in from Kashmir. ❖ **Preservation technique has been developed for long term storage of isolates for more than 03 months.** ❖ **More than 23 Gen bank submission have been done in this Project.*Campylobacter coli*flaA sequence 0L471375 is a new strain with a novel allele type 1675 and peptide sequence 5 and sequence is deposited in PubMLST Campylobacter database.**
2.	Designing and synthesis of primer for isothermal RPA PCR amplification.	**Achieved**	**Design and synthesis of RPA primer and probe was carried out** ❖ RPA primer and probe were designed targeting *Campylobacter* spp specific region as per principle of RPA base amplification assay. ❖ Parameter of each primer (Normal, Fluorescence and biotin) and probe tested (Tm, GC content and Loop formation) to avoid non-specific amplification. ❖ The RPA primers developed in this study were evaluated against *Campylobacter jejuni, S. aureus* and *E. coli* isolates available in the Division. The primer set used in this study showed highest specificity (100%) with no cross reaction. The primer set showed 89% sensitivity for identifying 16srRNA of Campylobacter from clinical samples. In addition, negative results were seen in all PCR negative isolates (100% specificity).
3.	Standardization of isothermal RPA PCR assay platform.	**Achieved**	❖ Amplification condition of RPA based assay carried out using RPA Twist DX Kit (Twist DX).The amplicon was checked in agarose gel as well RT PCR for Realtime monitoring. ❖ The lateral flow strips provided a clear, easy-to-interpret visual readout within 3-5 minutes. Positive samples display a distinct red test line and a control line, while negative samples and no-template controls displayed only the control line. The test line intensity was directly correlated with the initial

			template concentration. Amplification was detectable as early as 15 minutes; however, the signal intensity and reproducibility were optimal at 20 minutes ❖ The LFA-RPA assay demonstrated high analytical sensitivity (refer detailed Project Report). The assay consistently detected down to 10 genome copies per reaction in all triplicate experiments. At 5 copies, detection was inconsistent (67%), and at 1 copy, it was unreliable (33%). Therefore, the limit of detection (LOD) was defined as 10 genome copies per reaction. This sensitivity is comparable to conventional PCR.
4.	Evaluation of specificity and sensitivity of the developed assays.	**Achieved**	❖ Specificity evaluation demonstrated that the developed assay did not cross-react with genomic DNA from non-target bacterial ❖ All non-target samples consistently produced only the control line on lateral flow strips, with no visible test line formation. ❖ This confirmed that the primer–probe set was highly specific for the selected Campylobacter target region and did not amplify homologous sequences from related or commonly co-existing foodborne bacteria. ❖ Analytical specificity was calculated as 100% based on the tested panel, demonstrating excellent discriminatory capability of the assay. ❖ The assay has a high specificity, while sensitivity was comparatively lower.
5.	Conversion of isothermal RPA PCR assay to a LFA device.	**Achieved**	❖ Of the 80 field samples tested, PCR identified 20 as positive for *Campylobacter* after primary enrichment in buffered peptone water for 6hrs at 37C. ❖ The LFA- RPA assay identified 17 of these 20 PCR-positive samples after a similar enrichment in buffered peptone water for 6hrs at 37C and identified both tests identified 60 negative samples. ❖ This yielded a diagnostic sensitivity of 82.35%.and a specificity of 98.3%. The positive predictive value (PPV) was 93%and the negative predictive value (NPV) was 95% ❖ The design of RPA based assay carried out as per the opinion of expert committee during annual report presentation. ❖ The LFA-RPA platform developed in this study demonstrates strong potential to meet the ASSURED criteria (Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment free, and Deliverable to end-users) for point-of-care diagnostics. However, further optimization of the assay is required to ensure consistent and reproducible results. Notably, the current assay failed to detect *Campylobacter fetus* isolates derived from sheep and cattle samples. ❖ A plausible explanation is that the *C. fetus* isolates analyzed in this study

			exhibit genetic divergence from globally reported strains, particularly in relation to the sapB2 gene. Consequently, the primer–probe sets and target regions may need to be redesigned to improve detection sensitivity and specificity for this species.
6.	Standardization of a SELEX protocol for selection of high affinity aptamers against whole cell of *Campylobacter* spp.	**Achieved**	❖ Standardization of a SELEX protocol for selection of high affinity aptamers against whole cell of Campylobacter spp. was carried out. ❖ Aptamer library was designed which contain N40 random region and 17 bp and 19 bp primer binding region each side. ❖ Whole cell SELEX was optimized and high affinity aptamer selected for the target's organisms. ❖ Whole cell Selex was carried out for selection of high affinity aptamer against selected whole cell targets. ❖ A total 10 round of SELEX was performed with in between 3 round of counter SELEX (After 3rd, 6th and 9th round). ❖ Final aptamer pool was cloned using TOPO TA cloning kit.Positive clone was selected using colony PCR using M13 F/R primer. ❖ The positive clone was amplified with aptamer specific primer and with fluorescence and biotin labelled primer.High affinity binder was selected and sequence ❖ Secondary structure prediction and dG value was calculated using MfoldSoftware.Kd value was determined. Aptamer with Lower Kd value selected for assay development. ❖ Cloning and sequencing of the final enriched pool (Round 12) yielded 12 distinct sequences, designated CAMPHY1 through CAMPHY12. ❖ Sequence alignment revealed conserved motifs within the randomized region. Based on its frequency of occurrence (6 out of 50 clones) and preliminary binding assays, CAMPHY1referred to as Apt-CJ1, was selected for all subsequent characterization and assay development.
7.	Development of aptamer-quantum dots-based fluorescence biosensor for rapid detection of *Campylobacter Jejuni* and *C fetus*.	**Achieved**	❖ Aptamer Quantum dots-based fluorescence assay developed as proposed in the proposal. ❖ Selected aptamer was labelled with fluorescence (Directly) and quantum dots (Using biotinylated aptamer and streptavidin quantum dots). ❖ Bead labelled Polyclonal/Monoclonal/IgY specific for *Campylobacter* sp was used as a capturing probe for cell from clinical samples and fluorescence labelled aptamer for signal generation.
8.	Development of fluorescence	**Achieved**	❖ Under optimized conditions, the fluorescence signal demonstrated a linear

	biosensor for rapid detection of *Campylobacter Jejuni* and *C fetus*, directly from clinical samples.		correlation ($R^2 = 0.985$) with the logarithm of C. jejuni concentration over a dynamic range of 1.0×10^2 to 1.0×10□ CFU/mL (Figure 4b). The limit of detection (LOD), calculated as the mean blank signal plus three standard deviations, was determined to be 1.5×10^2 CFU/mL. The intra-assay CVs were < 7%, and inter-assay CVs were < 10% for all concentrations tested, indicating high reproducibility. ❖ When tested against a panel of non-target bacteria at 10□ CFU/mL, none produced a fluorescence signal exceeding the LOD threshold (Figure 22). The assay successfully detected both C. jejuni and C. coli but not C. fetus The analytical specificity was therefore calculated as 100% against the tested panel. ❖ The fluorescence biosensor for rapid detection assay exhibited high specificity, while sensitivity was comparatively lower. These findings highlight the promising potential of the tests for early detection of abortions and food contamination. At the same time, further experimental fine-tuning and optimization are required to enhance sensitivity and ensure robustness before these assays can advance toward practical application and eventual market introduction.
9.	Development of aptamer-based disease diagnostic system for *Campylobacteriosis* in cattle and poultry.	**Achieved**	• Of the 80-field food and sheep/cattle vaginal swabs samples tested, the reference PCR method identified 18 as positive for Campylobacter. The developed fluorescence sandwich assay correctly identified 15 of these 18 positive samples and 61 of the 62 PCR-negative samples (Table 4). This resulted in a sensitivity of approximately 84%, a specificity of 98.4%, and a near- perfect agreement with PCR, as indicated by a Cohen's kappa coefficient (κ) of 0.84. The three false negative, and one false positives were near the LOD of both methods, possibly due to low bacterial loads or the presence of non-viable but PCR-detectable cells. • The aptamer-based assay exhibited high specificity, while sensitivity was comparatively lower. These findings highlight the promising potential of the tests for early detection of abortions and food contamination. At the same time, further experimental fine-tuning and optimization are required to

			enhance sensitivity and ensure robustness before these assays can advance toward practical application and eventual market introduction. • Although these results are promising, the assay requires further validation across larger and more diverse sample sets, as well as additional future studies, before it can be considered for routine diagnostic use or market deployment
10.	Projection completion and report writing and left over work if any.	**Achieved**	Final project report presented before the expert committee .All year wisereports, utilization certificates and final utilization certificates submitted to DBT

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Objectives Status

(If multi-centric, separate for each centre)

S. No	Objectives	Status (Achieved/partially achieved/yet to start etc.)	Remarks (*Achievement in detail/other*)
1.	Isolation, biochemical and molecular characterization of *Campylobacter jejuni*and *Campylobacter fetus* from clinical samples of poultry(150),Cattle(150), sheep(150), poultry meat (100), milk and milk products (100).	**Achieved**	**Isolation and biochemical identification were carried out for various species of Campylobacter.** ❖ A total of 1088 samples were collected from various farms (Poultry and cattle) in and aroundKashmir region till date. ❖ The samples were subjected for detection and isolation as per standard protocols. ❖ Total 65 confirmed isolates were obtained and use in this study. **Molecular and virulence gene characterization was carried out for all isolates.** **Preservation technique has been developed for long term storage of isolates for more than 03 months.** **More than 23 Gen bank submission have been done in this Project.*Campylobacter coli*flaA sequence 0L471375 is a new strain with a novel allele type 1675 and peptide sequence 5 and sequence is deposited in PubMLST Campylobacter database.**
2.	Development of isothermal RPA PCR based LFA device for detection of *Campylobacter jejuni and C fetus* from poultry and cattle and sheep.	**Achieved (Validation and fine tuning in process)**	❖ RPA primer and probe was design targeting *Campylobacter* spp specific region as per principle of RPA base amplification assay. ❖ Parmeter of each prime (Normal, Fluorescence and biotin) and probe tested (Tm, GC content and Loop formation) to avoid Non-specific amplification. ❖ The RPA-LFA assay has been developed and validated on 80 clinical samples and results have been mentioned in detail in the final project report. ❖ The assay's robustness was checked through its performance in spiked chicken and milk samples, where it detected as few as 10^1-10^2 CFU per sample ❖ The LFA- RPA assay identified 17 of these 20 PCR-positive samples after a similar enrichment in buffered peptone water for 6hrs at 37C and identified both tests identified 60 negative samples. This yielded a diagnostic sensitivity of 82.35%.and a specificity of 98.3%.However, further optimization of the assay is required to ensure consistent and reproducible results

	Selection andcharacterization of high affinity aptamersUsing whole cell of *Campylobacter jejuni*and *C fetus* isolates from cattle,Poultry and sheep	**Achieved**	**Whole cell SELEX was optimized and high affinity aptamer selected for the target's organisms.** ➢ Whole cell Selex was carried out for selection of high affinity aptamer against selected whole cell targets. ➢ A total 10 round of SELEX was performed with in between 3 round of counter SELEX (After 3rd, 6th and 9th round). ➢ Final aptamer pool was cloned using TOPO TA cloning kit. ➢ Positive clone was selected using colony PCR using M13 F/R primer. ➢ The positive clone was amplified with aptamer specific primer and with fluorescence and biotin labelled primer. ➢ High affinity binder was selected and sequence ➢ Sencondary structure prediction and dG value was calculated using Mfold Software. ➢ Kd value was determined. ➢ Aptamer with Lower Kd value selected for assay development.
4.	Development of aptamer-based disease diagnostic system for Campylobacteriosis in cattle and poultry	**Achieved**	• Development of aptamer-based assay exhibited high specificity, while sensitivity was comparatively lower • The developed fluorescence sandwich assay correctly identified 15 of these 18 positive samples and 61 of the 62 PCR-negative samples (Table 4). This resulted in a sensitivity of approximately 84%, a specificity of 98.4%, and a near- perfect agreement with PCR, as indicated by a Cohen's kappa coefficient (κ) of 0.84. • Although these results are promising, the assay requires further validation across larger sample and future studies,

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Details of Publications *(If multi-centric, combined details from all the centre:*

1. **Virulence profiling of Campylobacter spp. Campylobacter jejuni and C. fetussubspfetus abortions rise in sheep farms in Kashmir, India.**M. Hafiz, S. Qureshi*, M. Gulzar, Z. Kashoo, M. Sharief Banday, S. Farooq,M. Altaf Bhat, P. Dar, S.A. Hussain, S.M. Andrabi, M.I. Hussain, G. Badroo, F. U Din:**[Polish Journal of Veterinary Sciences]**
2. **Short Variable Regions flaA Gene (SVR-flaA) Diversity and Virulence Profile of Multidrug–Resistant Campylobacter from Poultry and Poultry Meat in India.**
   Saima Iqbal , Sabia Qureshi* , Muddasir S. Banday , Shaheen Farooq , Zahid A. Kashoo , Maliha Gulzar , M. Altaf Bhat , Arif Pandit , Md. Isfaqul Hussain 1, Pervaiz Dar 1, Gulzar Badroo 1, Mahrukh Hafiz 1, Faheem U Din 1, Junaid Mehraj **1: [Journal of Food Protection].**
3. **Antimicrobial resistance profile of Campylobacter jejuni from sheep and poultry: Insights from Kashmir, India**
   HudzaWali, Ali Khan, Faheem U Din, Sabia Qureshi, Mir Nadeem Hassan, Zahid A Kashoo, Pervaiz Dar, Muddasir S Banday, Fawaz Banday,Md. Isfaqul Hussain, MansoorNabi, GulzarBadroo, Shaheen Farooq, MalihaGulzar, Junaid Mehraj, Mahrukh Hafiz, Kifayat Afzal
   ACTA VET. BRNO 2026, 95: 000–000; https://doi.org/10.2754/avb202695030000 (In press)

Details of Patents (filed/Granted)*(If multi-centric, combined details from all the centre):*

1. **NA**

Details of Technologies Developed/ Transferred/Commercialized *(If multi-centric, combined details from all the centre):*

1. **Nil**

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