Generating PSPT coating antigen for DCVMN

Results

Liesbeth Vercruyssen

Project Leader

BioLyo

Activities at BioLyo & Intravacc

BioLyo

Purchase strain *B. pertussis* 18323 form PHE

↓

generate Research Cell Bank (lyophilized)

↓

engineering run inactivation & lyophilization
(testing 7 conditions)

→ **Intravacc**: evaluation & selection of best product

↓

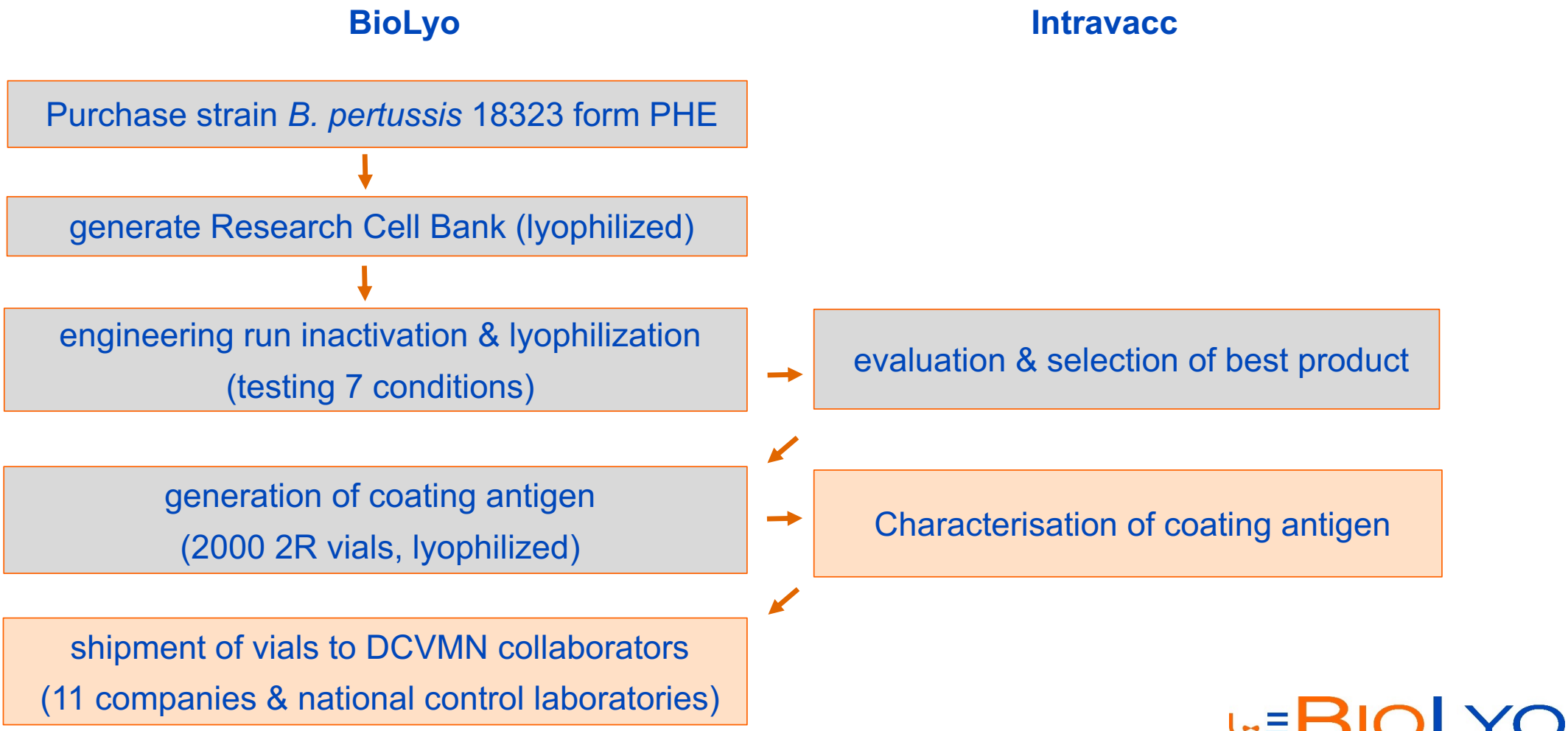
generation of coating antigen
(2000 2R vials, lyophilized)

→ **Intravacc**: Characterisation of coating antigen

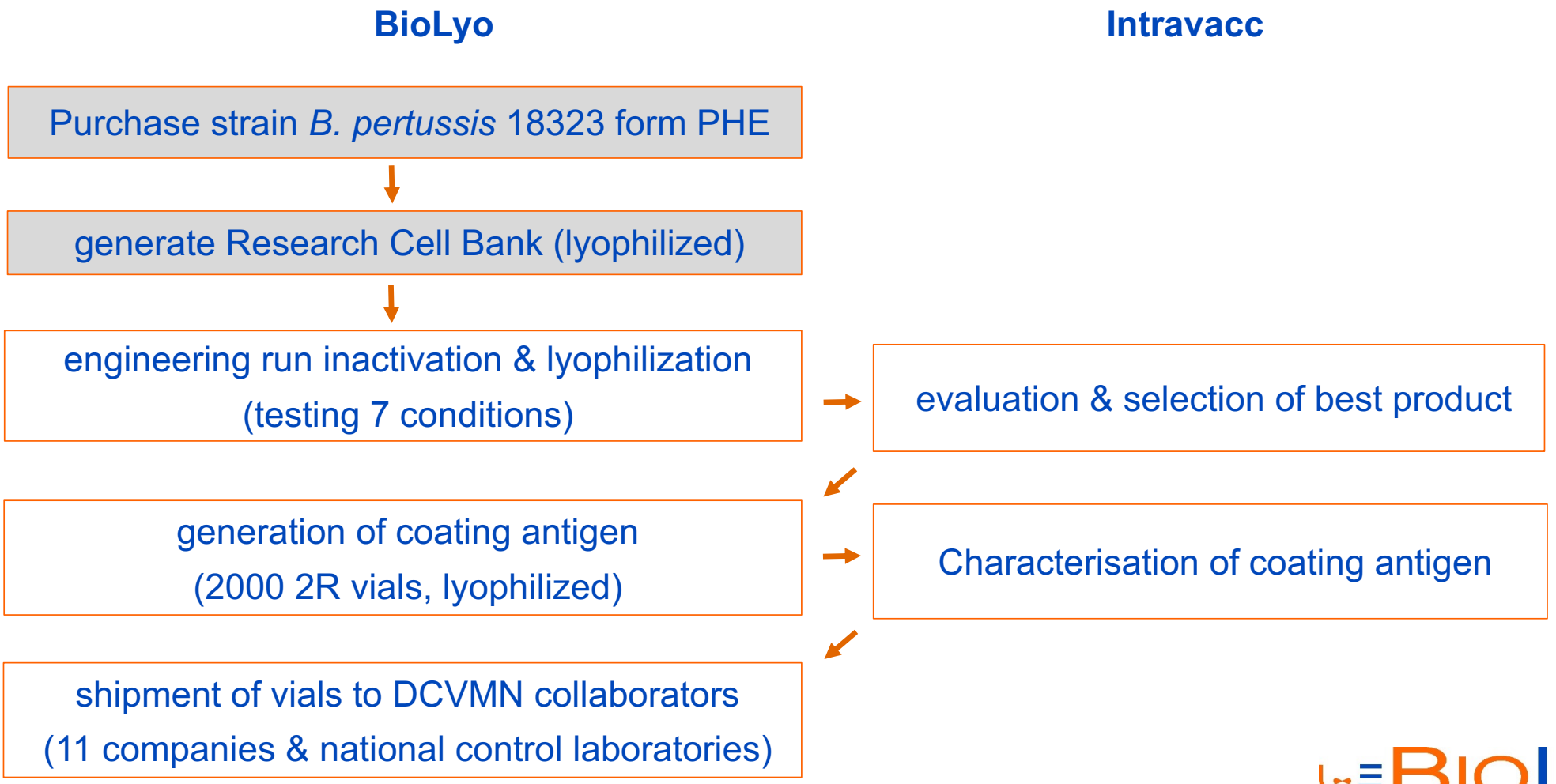
↓

shipment of vials to DCVMN collaborators
(11 companies & national control laboratories)

Overall time schedule



Results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RCB generation – process

- *B. pertussis* strain 18323 (NC10739, ATCC 9797) resuspended in 500 µL THIJS medium
- Plated on Bordet Gengou agar, 7 days at 35°C, used as inoculum

Pre-culture 1 (5 mL)

↓ 23,0 h

Pre-culture 2 (43 mL)

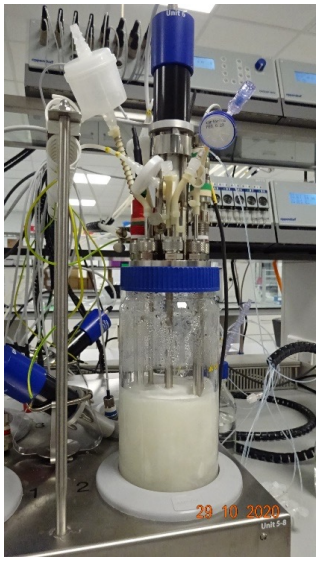
↓ 23,3 h

Pre-culture 3 (90 mL)

↓ 6,6 h

Bioreactor (1,2 L)

19,8 h



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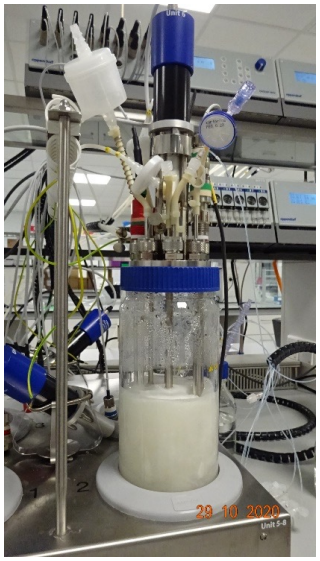
Bioreactor (1,2 L)

19,8 h

OD_{600nm} 1,2



- 240 mL harvested & 1:1 diluted with Lyophilization buffer (100 g/L sucrose)
- Lyophilized 200 2R vials, 2 mL aliquots

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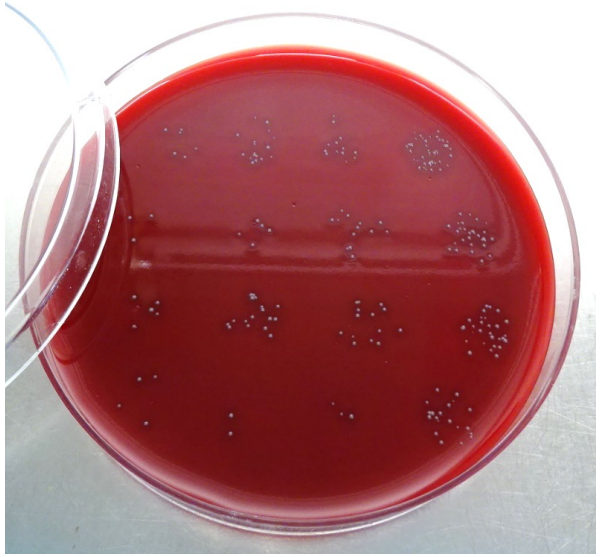
RCB generation – Quality Control

Before Lyophilization

- Plate count on Bordet Gengou agar: 2,1 x10^9 CFU/mL
- Identity & morphology: Silver/grey colonies, hemolytic halo
- pH culture after harvest: 8,2
- Microbial examination by plate count on SDA and TSA: no growth



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RCB generation – Quality Control

After Lyophilization

- Appearance lyophilized cake by visual inspection:

  off white cake, cap tight, no visual cracks

- Appearance upon reconstitution by visual inspection:

  opaque liquid

- pH upon reconstitution: 6,7

- Residual moisture by Karl Fischer: 1,77%

- Plate count on Bordet Gengou agar: 4,2 x10^8 CFU/mL

  Calculated survival after Lyo: 46%

- Identity & morphology: Silver/grey colonies, hemolytic halo

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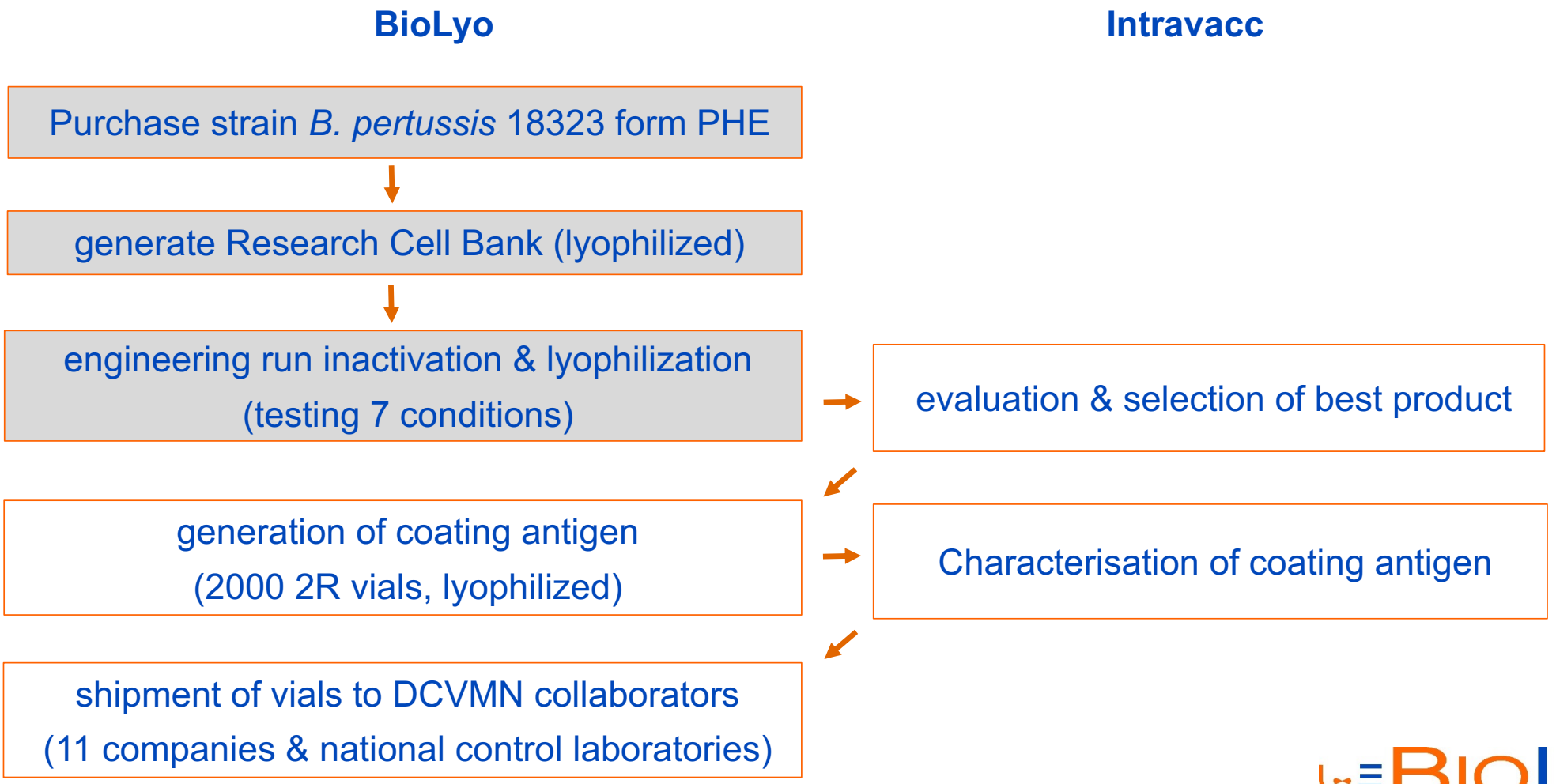
Results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Engineering run conditions

Suspension	Inactivation conditions	Inactivation matrix of 50 IOU (dilute 1:1 with)	Resulting suspension to be lyophilized of 25 IOU (dilute 1:1 with)
100 IOU/mL in saline	30 minutes 56°C	10 g/L casamino acids (dilute 1:1 with 20 g/L casamino acids)	10 g/L casamino acids (dilute 1:1 with 10 g/L casamino acids)
			10 g/L casamino acids & 75 g/L sucrose (dilute with 150 g/L & 10 g/L casamino acids)
	240 minutes 56°C	10 g/L casamino acids (dilute 1:1 with 20 g/L casamino acids)	10 g/L casamino acids (dilute 1:1 with casamino acids)
	30 minutes 56°C in presence of 6 mM formaldehyde	10 g/L casamino acids 6 mM formaldehyde (dilute 1:1 with 20 g/L casamino acids & 12 mM formaldehyde) initial pH = 7.8	10 g/L casamino acids & 75 g/L sucrose (dilute with 150 g/L & 10 g/L casamino acids)
			10 g/L casamino acids (dilute 1:1 with casamino acids)
	30 minutes 56°C in presence of 6 mM formaldehyde	6 mM formaldehyde (dilute 1:1 with 12 mM formaldehyde) initial pH = 7.8	50 g/L sucrose (dilute 1:1 with 100 g/L sucrose)
			75 g/L sucrose (dilute 1:1 with 150 g/L sucrose)

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First Engineering Run - Suspension

- 1 vial of RCB *B. pertussis* 18323 resuspended in 2 mL

Pre-culture 1 (42 mL)

↓ 24,6 h

Pre-culture 2 (120 mL)

↓ 16,8 h ↓

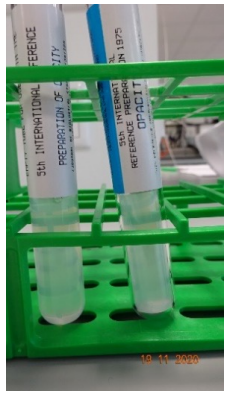
Bioreactor 1 (1,2 L) + Bioreactor 2 (1,2 L) → Culture (2,4 L)

24,8 h OD_{600nm} > 1,4 24,8 h

↓ Concentration 4x
Diafiltration against saline

100 IOU/mL suspension
~ 600 mL

Visual comparison of suspension 10x diluted with WHO 10 IOU standard solution



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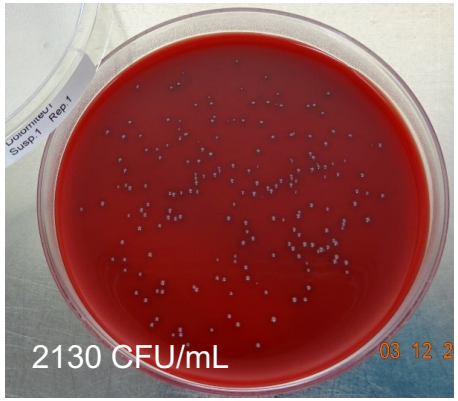
First Engineering Run - Inactivation

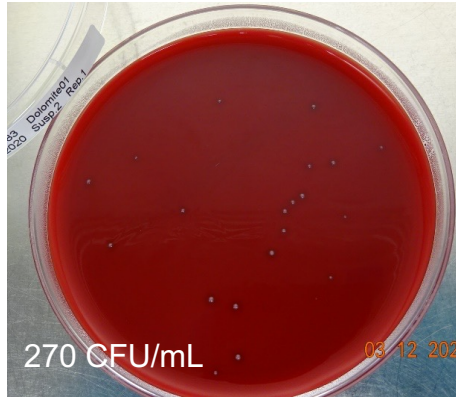
Bio reactor	Inactivation condition	Lyo Buffer
5	30 min 56°C Casamino acids	Casamino acids
		Casamino acids + 75 g/L sucrose
6	240 min 56°C Casamino acids	Casamino acids
7	30 min 56°C Casmino acids 6 mM formaldehyde	Casamino acids + 75 g/L sucrose
		Casamino acids
8	30 min 56°C 6 mM formaldehyde	50 g/L sucrose
		75 g/L sucrose

BIOLYO

First Engineering Run - Inactivation

Bio reactor	Inactivation condition	Lyo Buffer	Complete Inactivation
5	30 min 56°C Casamino acids	Casamino acids	No
		Casamino acids + 75 g/L sucrose	No
6	240 min 56°C Casamino acids	Casamino acids	No
7	30 min 56°C Casmino acids 6 mM formaldehyde	Casamino acids + 75 g/L sucrose	Yes
		Casamino acids	Yes
8	30 min 56°C 6 mM formaldehyde	50 g/L sucrose	Yes
		75 g/L sucrose	Yes





First Engineering Run - Lyophilization

Bio reactor	Inactivation condition	Lyo Buffer	Complete Inactivation	Cake visual appearance	Cake resuspension	Residual moisture
5	30 min 56°C Casamino acids	Casamino acids	No	Off white, flakey	Easy	0,84 %
		Casamino acids + 75 g/L sucrose	No	Yellowish, sticky, boiled	Difficult	5,09 %
6	240 min 56°C Casamino acids	Casamino acids	No	Off white, contains small fragments	Takes longer than usual	1,01 %
7	30 min 56°C Casmino acids 6 mM formaldehyde	Casamino acids + 75 g/L sucrose	Yes	Yellowish, sticky, boiled	Difficult	6,93 %
		Casamino acids	Yes	Off white, powdery	Easy	0,71 %
8	30 min 56°C 6 mM formaldehyde	50 g/L sucrose	Yes	Off white, powdery	Easy	1,22 %
		75 g/L sucrose	Yes	Off white, contains small fragments	Takes longer than usual	1,59 %

BIOLYO

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		Casamino acids	Yes	Off white, powdery	Easy	0,71 %
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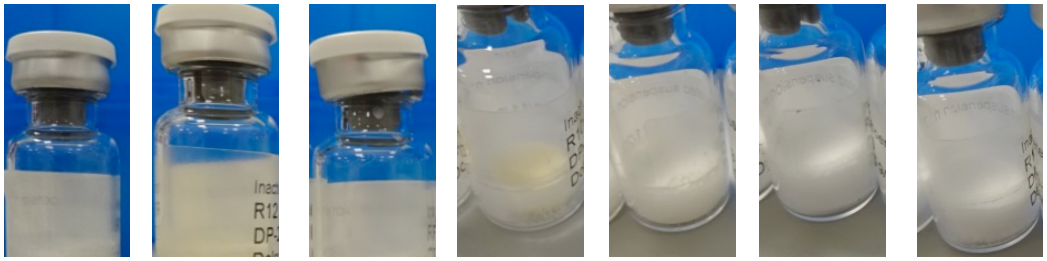
		75 g/L sucrose	Yes	Off white, contains small fragments	Takes longer than usual	1,59 %



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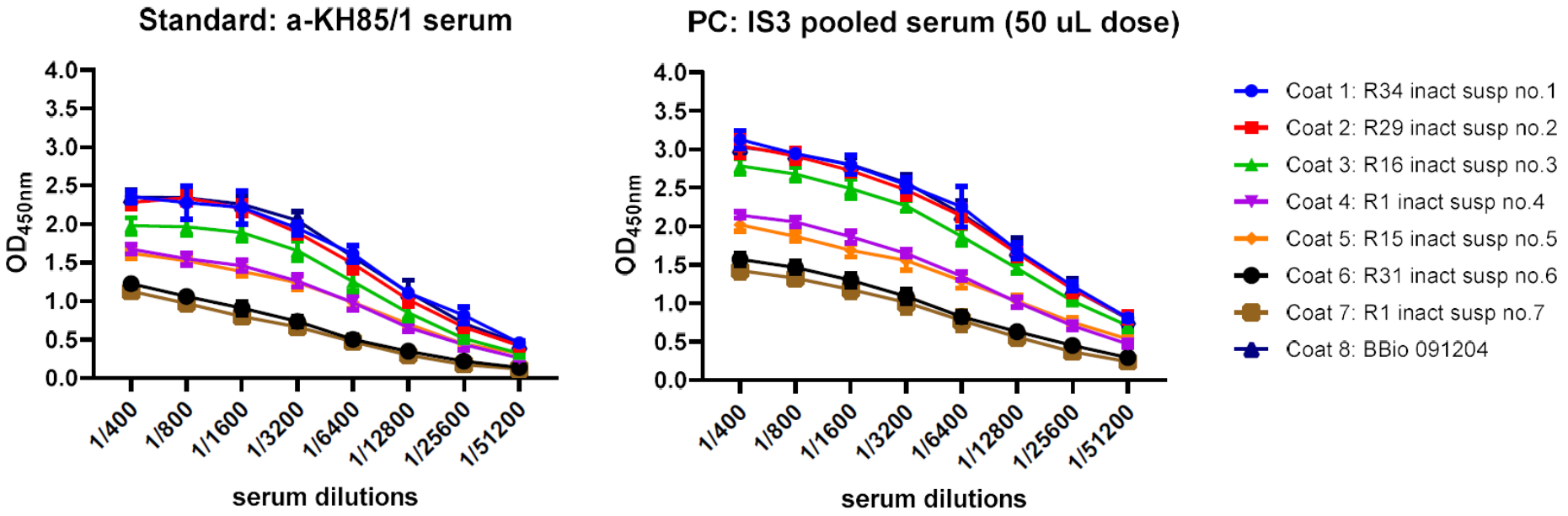
First Engineering Run – INTRAVACC ELISA



Suspensions without formaldehyde showed better response in PSPT ELISA

Cake structure of lyophilized vials: no use of sucrose + casamino acids

Conclusion: use inactivation conditions with casamino acids, for either 30 or 240 minutes

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Results

BioLyo

Intravacc

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generate Research Cell Bank (lyophilized)

↓

engineering run inactivation & lyophilization
(testing 7 conditions)

→ evaluation & selection of best product

↙

generation of coating antigen
(2000 2R vials, lyophilized)

→ Characterisation of coating antigen

↙

shipment of vials to DCVMN collaborators
(11 companies & national control laboratories)

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Second Engineering Run

B. pertussis cannot survive 56^{o}C for 30 minutes: survial is artefact caused by cold spots or aerosol dripping back into the suspension after inactivation.

Approach to achieve full inactivation 3 conditions:

- In bottles: perform inactivation in bottles completely submerged in 56^{o}C waterbath. Test 30 and 240 minutes.
- In bioreactor: replace all bioreactor piping & probes with blind plugs. Test 30 minute inactivation only.
- Inactivation will be carried out 'at scale', i.e. sufficient material to fill 2000 vials after confirming complete inactivation.

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Second Run - Suspension

- 2 vials of RCB *B. pertussis* 18323 each resuspended in 2 mL

Pre-culture 1 (30 mL) | Pre-culture 1 (30 mL)

↓ 23 h

Pre-culture 2 (125 mL) | Pre-culture 2 (125 mL) | Pre-culture 2 (125 mL)

↓ 16,3 h

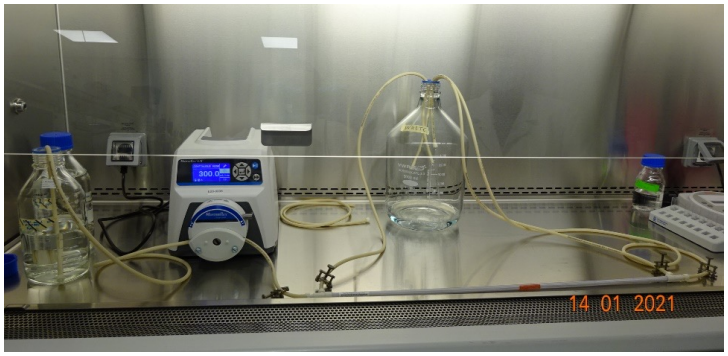
Bioreactor 1 (1,2 L) | Bioreactor 2 (1,2 L) | Bioreactor 3 (1,2 L) | Bioreactor 4 (1,2 L) | Bioreactor 5 (1,2 L) | Bioreactor 6 (1,2 L)

26,1 – 28,5 h OD_{600nm} > 1

→ Culture (7,2 L)

↓ Concentration 3,5x
Diafiltration against saline

100 IOU/mL suspension
~ 1,8 L



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Second Run - Inactivation

100 IOU/mL suspension
~ 1,8 L

Bioreactor 300 mL +
Casamino acids 300 mL
30 min 56°C

Bioreactor 300 mL +
Casamino acids 300 mL
30 min 56°C

Water bath 550 mL +
Casamino acids 550 mL
30 min 56°C

Water bath 550 mL +
Casamino acids 550 mL
240 min 56°C

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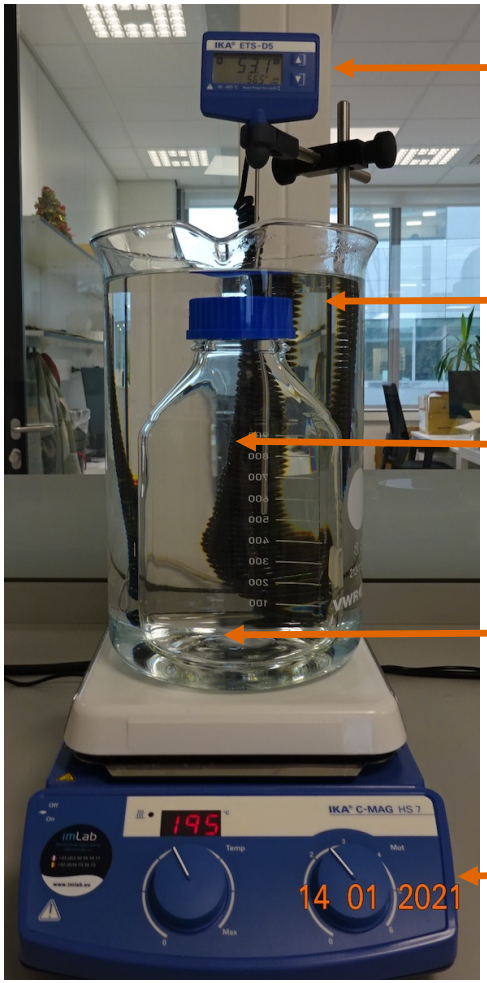
Second run: bottle inactivation set-up



Temperature probe of water in beaker glass

Water in beaker glass heated to 56.5°C

Bottle with suspension to be inactivated

Stirbar in bottle with suspension

Heater & stirrer

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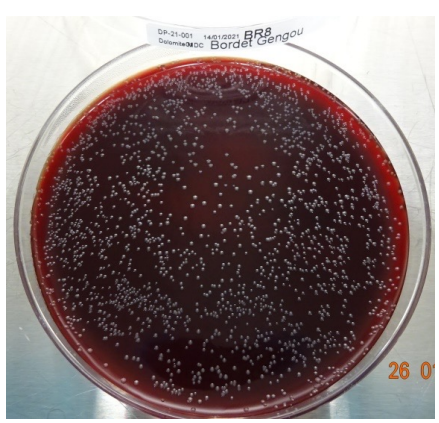
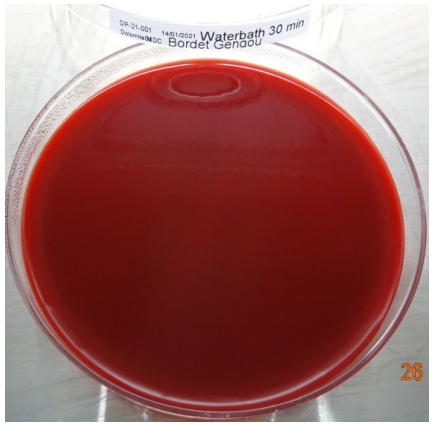
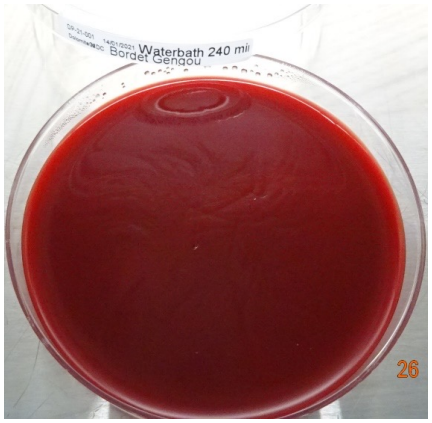
Second Run - Inactivation

100 IOU/mL suspension
~ 1,8 L

Bioreactor 300 mL + Casamino acids 300 mL 30 min 56°C	Bioreactor 300 mL + Casamino acids 300 mL 30 min 56°C	Water bath 550 mL + Casamino acids 550 mL 30 min 56°C	Water bath 550 mL + Casamino acids 550 mL 240 min 56°C







Conclusion: Water bath set-up was successful for inactivation of the culture

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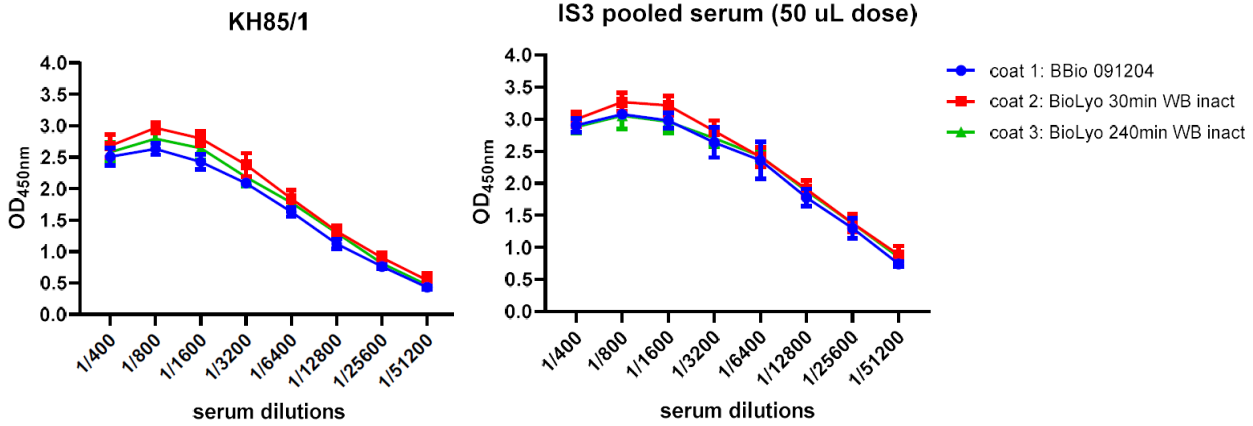
Second Run – INTRAVACC ELISA

100 IOU/mL suspension
~ 1,8 L

Bioreactor 300 mL + Casamino acids 300 mL 30 min 56°C	Bioreactor 300 mL + Casamino acids 300 mL 30 min 56°C	Water bath 550 mL + Casamino acids 550 mL 30 min 56°C	Water bath 550 mL + Casamino acids 550 mL 240 min 56°C



Conclusion: 30 min at 56° submerged in water batch is sufficient for inactivation

Second Run - Conclusion

Suspension	Inactivation conditions	Inactivation matrix of 50 IOU (dilute 1:1 with)	Resulting suspension to be lyophilized of 25 IOU (dilute 1:1 with)
100 IOU/mL in saline	30 minutes 56°C	10 g/L casamino acids (dilute 1:1 with 20 g/L casamino acids)	10 g/L casamino acids (dilute 1:1 with 10 g/L casamino acids)
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			75 g/L sucrose (dilute 1:1 with 150 g/L sucrose)

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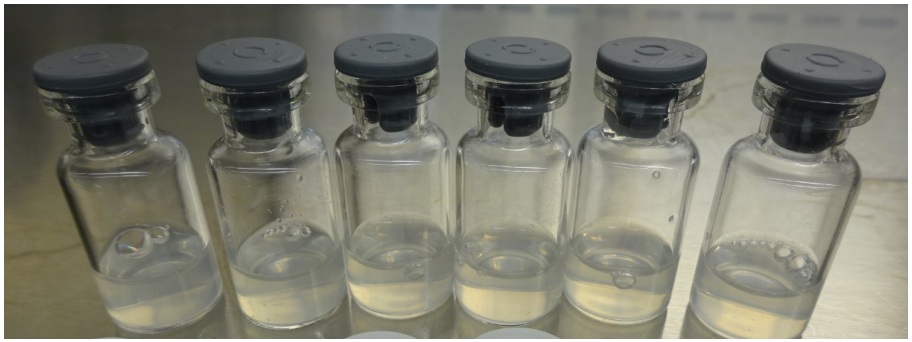
Second Run – Quality Control

After Lyophilization

- Appearance lyophilized cake by visual inspection:
  off white cake, cap tight, no visual cracks
- Appearance upon reconstitution by visual inspection:
  Opalescent
- pH upon reconstitution (5 vials): 6,14



- Residual moisture by Karl Fischer (18 vials): 1,16%
- Plate count on Bordet Gengou agar (6 vials): no growth
- Microbial examination by plate count on SDA and TSA (6 vials): no growth

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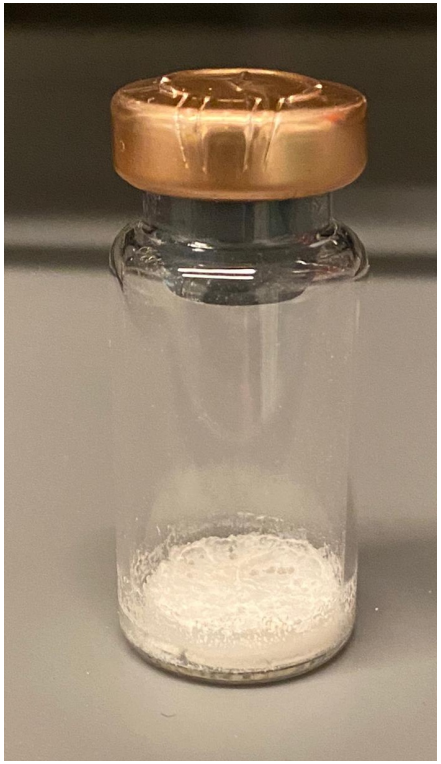
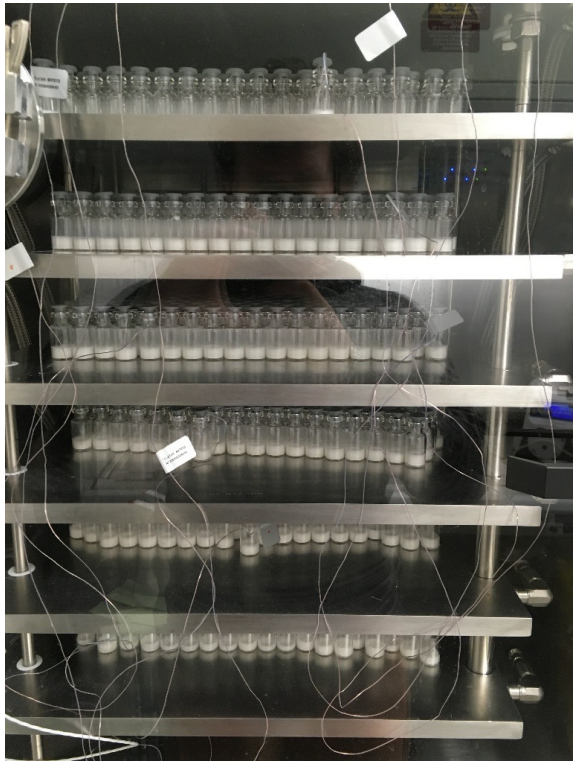
New coating antigen versus existing coating antigen



← Current coating antigen

New coating antigen →



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Second Run – Quality Control

Certificate of Testing

Name	Manufacturing date	BioLyo n°	Storage conditions	Retest date
Bordetella pertussis 18323 coating antigen	15 Feb 2021	DP-21-008	Store 2-8 °C	15 Feb 2026

Test	Method	Result
Appearance lyophilized cake	Visual inspection	Off white cake
Residual moisture	Karl Fisher	1.16 % ± 0.24 %
Appearance upon reconstitution	Visual inspection	Opalescent
Plate count	Bordet Gengou Agar	No growth
pH upon reconstitution	Potentiometric	6.14
Microbial examination	SDA	No growth
Microbial examination	TSA	No growth

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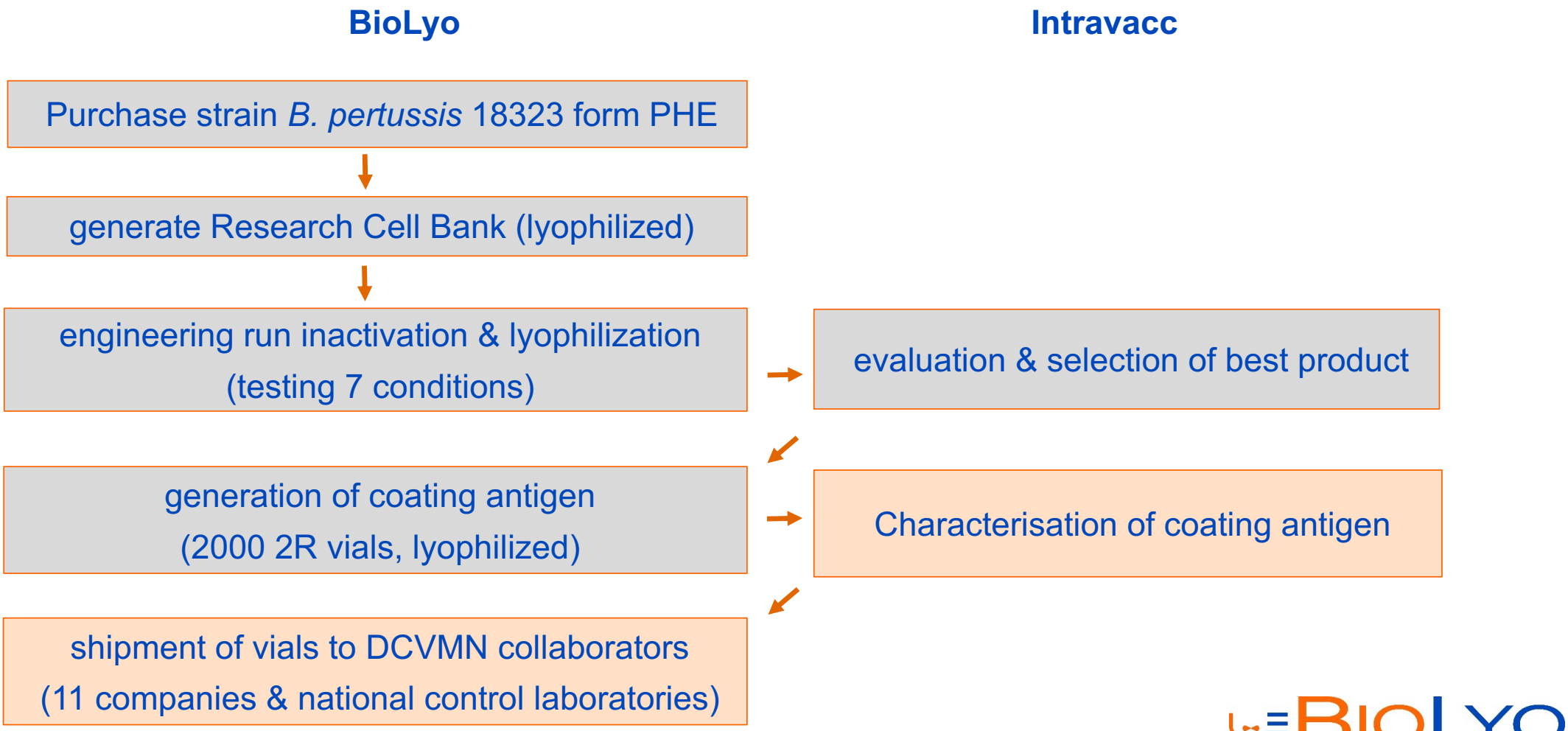
Overall time schedule



Shipment

Participant	Contact person	Country
Biological E. Limited		India
Sanofi	Sreenivasulu Reddy. B	India
BharatBiotech	Mr. Gopal Singh	India
Serum Institute	Dr. (Mrs) Yojana Shinde	India
Bulbio	Ms Saveta Mladenova	Bulgaria
National Quality Control Laboratory of Drug and Food	Elizabeth Ika Prawahju	Indonesia
BioFarma	Dori Ugiyadi	Indonesia
Pancea Biotech	Deepak Mahajan/ Maya Ramdas	India
CDSCO India	Dr. Arun Bhardwaj	India
NCL Thailand	Mr. Apichai Supasansathorn	Thailand
Pasteur Institute	Shri T. Sekar	India

- 1 box with 20 vials
- Dry Ice – Temperature logger included
- Incoterm EXW (Ex Works)

- Import into country of destination:

  Documented approval for import?

  Contact with the government?

  Suggestion for a local transport company to import the goods on dry ice?

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Questions?

Timeline expectations

Activity	Completed by:
Purchase strain *B. pertussis* 18323 form PHE	Done
Generate Research Cell Bank (lyophilized)	Ongoing
Engineering run inactivation & lyophilization	Early November
Evaluation & selection of best product	Early December
Generation of coating antigen	December / January
Characterisation of coating antigen	March
Shipment of vials to DCVMN collaborators	March/April (after signing MTA)

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