Development of Typhoid Conjugate Vaccine in Bio Farma

Quality Data of TCV

Erman Tritama

Bio Farma

9th International Conference on Typhoid and Invasive NTS Disease

Vaccines prequalification priority list 2013 -2014

High priority vaccines	Medium priority vaccines	Low priority vaccines	No priority
bOPV1+3	DTwP-HepB-Hib-IPV	BCG	DTwP based tetra valent combinations
fully liquid DTwP-Hep B-Hib	DTwP-HepB + Hib	DTwP-Hib liquid	DT
Inactivated polio (IPV)	Hepatitis A	DTaP ; DTaP-HepB/IPV	Hib monovalent
DTwP	HPV	DTwP-Hib-IPV ; DTwP-IPV	Hep B monovalent
Measles-Rubella	Influenza H5N1	Hepatitis E	OPV1
Pneumococcal conjugate	Influenza seasonal (south)	Influenza seasonal (north)	Pneumococcal polysach.
Rotavirus	Japanese Encephalitis	Men AC-containing polysac	Influenza H1N1
Trivalen OPV	Measles and MMR	OPV3	Rubella monovalent
Yellow fever	Men A-containing conjugate	Rabies	
	Men W-containing polysach	TT	
	Men ACWY	Typhoid non conjugate	
	OPV2	Varicella	
	Td	DTwP-based tetravalen or pentavalen cont. IPV	
	Typhoid conjugate		

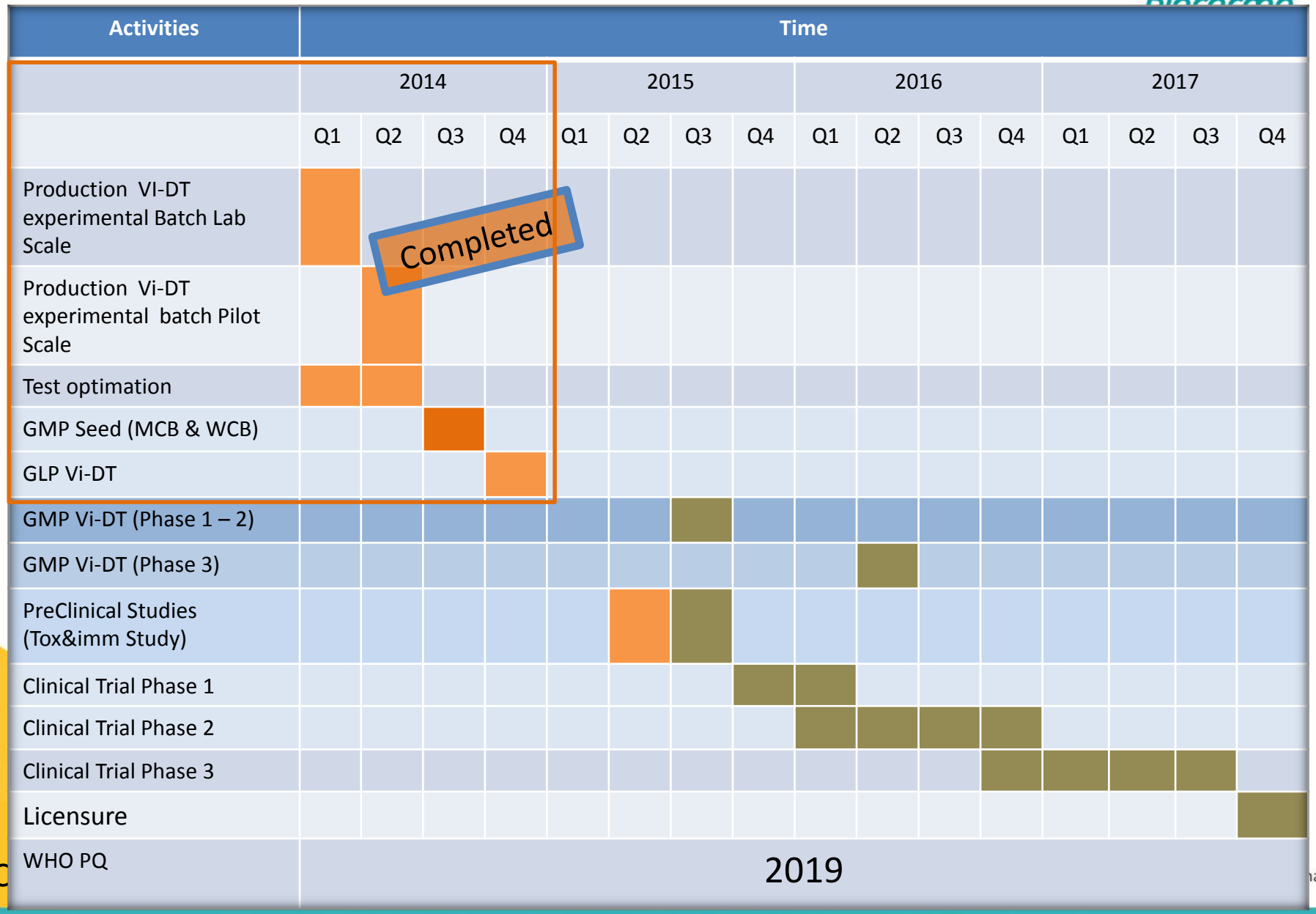
Typhoid Conjugate Vaccine Development Partner

Technology Transfer Agreement	**Q1 2013**
Technology Transfer on Vi-DT Manufacturing	**Q2 – Q3 2013**
Seed Received from IVI	**Q4 2013**
Experimental starting	**Q1 2014**

Targeted Product Profile

	Characteristics
Presentation	Single /multiple dose presentation : -Prefill syringe/Vials
Dose	25 µg
Appearance	Clear, fully liquid Presentation
Storage	2 – 8 ^{0}C
Shelf Life	Minimum 36 months at 2-8 ^{0}C
Age Group	All age groups
	Age group 9 months to 2 years : 2 doses
	Age group > 2 years : 1 dose
Route	Intramuscular
Target Countries	Indonesia; WHO PQ

Progress on Typhoid Conjugate Vaccine Development



Quality Data of TCV

BACTERIAL STRAIN

Typhoid Strain (Parent Seed)

Guidelines

- Bacterial strain :
  - Single
  - Well characterized stock
  - Should capable of stably producing Vi polysaccharide

Typhoid Strain

IVI

- *Salmonella typhi* seed lot C6524
  - The original isolate was obtained from Dr. Nair of NICED Kolkata India
  - Passages performes 5x by IVI

Source : Production and Quality Control of Vi-DT Conjugate Vaccine, IVI.

Parent Seed

Summary of testing of each passage level and the Master Cell Bank

Passage number	OD_{600}	Gram stain	Colony morphology on LB agar	Agglutination with anti Vi serum	Dilution for passage	Cumulative dilution
1	ND	Gram negative rods	ND	ND	10^{-3}	10^{-3}
2	0.97	Gram negative rods	ND	ND	10^{-3}	10^{-6}
3	0.926	Gram negative rods	ND	ND	10^{-3}	10^{-9}
4	0.984	Gram negative rods	ND	ND	$10^{-2.7}$	$10^{-11.7}$
5	1.036	Gram negative rods	Uniform circular pale cream colonies	Agglutination positive	$10^{-2.7}$	$10^{-14.4}$

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CERTIFICATE OF ANALYSIS

Salmonella enterica serotype Typhi isolate designated as C6524.

The original isolates were cultured on MacConkey agar and incubated for 10 days.
Lactose non fermenting colonies were screened using Kligler's iron agar.
Standard biochemical and serological methods were used to identify *Salmonella* Typhi.

Results at NICED (Kolkata, India) confirm that the isolate C6524 was *Salmonella* Typhi.

PCR performed at IVI confirmed that the isolate C6524 contained the genes (*tviB* at the *via*B locus) for Vi expression.

PCR performed at IVI confirmed that the isolate C6524 contained the gene (*fliC:d*) for flagella antigen d.

Confirming that the isolate C6524 was *Salmonella* Typhi

The Master seed lot of C6524 was tested for the following tests and the test results are included:

Test	Test result
OD_{600}	1.036
Gram stain	Gram negative rods
Colony morphology on LB agar	Uniform circular pale cream colonies
Agglutination with anti Vi serum	Agglutination positive
Cumulative dilution from isolate to seed lot	$10^{-14.4}$

Rodney Carbis
Head Vaccine Development
International Vaccine Institute
Seoul Korea
26 July 2013

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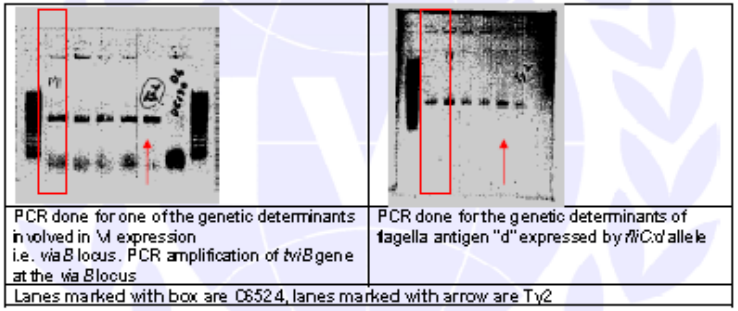
PCR PRIMERS

Vi (X65788)	*fliC:d* (L21912)
>*tviB*-F	>DirF
CGAGTGAAACCGTTGGTACA	GCTTAATGTCCAAGATGCCTAC
>*tviB*-R	>DirR
CAATGATCGCATCGTAGTGG	GAGCAACGCCAGTACCATCTG



PCR done for one of the genetic determinants involved in Vi expression i.e. *viaB* locus. PCR amplification of *tviB* gene at the *via B* locus

PCR done for the genetic determinants of flagella antigen "d" expressed by *fliC:d* allele

Lanes marked with box are C6524, lanes marked with arrow are Ty2

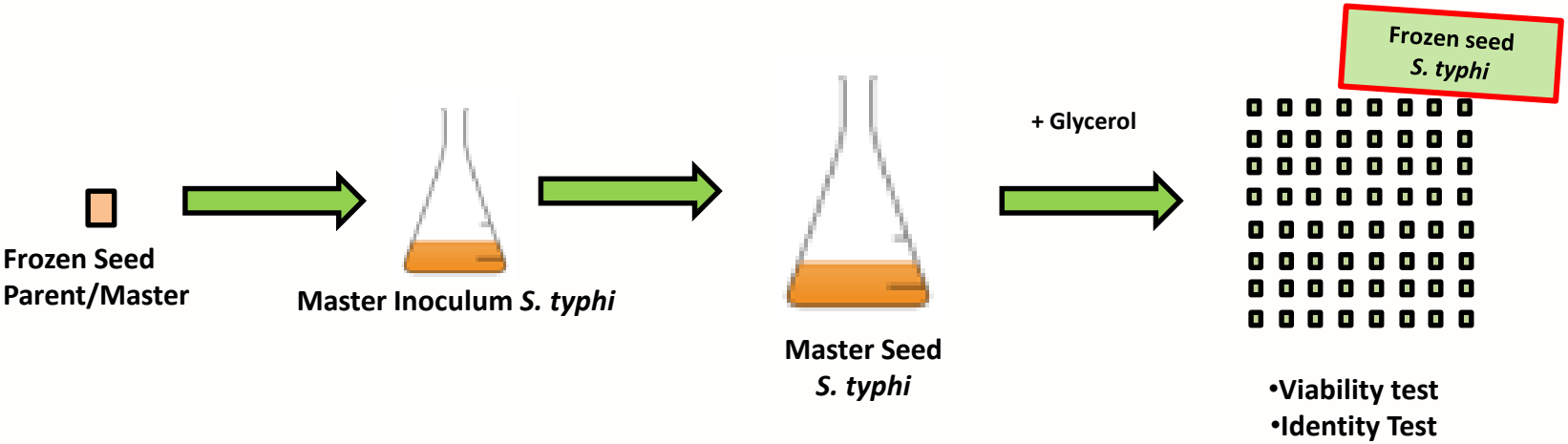
Conclusion: Both C6524 and Ty2 are group D Salmonella.

Growth chart for serial dilution of Clinical Isolates of Salmonella Typhi

IB#	Genus & species	Original ID No.	Source	LB plate 26 ml	Tube1 6ml	Flask1 100 ml	Tube2 6ml	ELISA Vi	PCR	Tube3 6ml	Flask 2,100 ml
Inoculum size				loop full	Single colony 6µl	100µl	6µl	µg/ml		10µl	200µl
						O.D. at 600nm				O.D. at 600nm	
3688	*S.typhi*	A2884	Kolkata	√	√	0.302	0.939	8.8	positive		
3691	*S.typhi*	A8176	Kolkata	√	√	0.32	0.935	3.6	positive		
3696	*S.typhi*	B6861	Kolkata	√	√	0.231	0.965	7.7	positive		
3706	*S.typhi*	B8287	Kolkata	√	√	0.342	0.968	5.8	positive		
3732	*S.typhi*	C7820	Kolkata	√	√	0.633	0.921	nd	positive		
3738	*S.typhi*	B7076	Kolkata	√	√	1.036	0.936	4.1	positive		
3753	*S.typhi*	C8073	Kolkata	√	√	1.027	0.964	5.8	positive		
3766	*S.typhi*	C6524	Kolkata	√	√	0.97	0.926	20.3	positive	0.984	1.036
3781	*S.typhi*	C6876	Kolkata	√	√	0.743	0.963	8.2	positive		
3792	*S.typhi*	C7087	Kolkata	√	√	1.033	0.964	1.93	positive		
3801	*S.typhi*	C7643	Kolkata	√	√	1.007	0.941	2.5	positive		
3812	*S.typhi*	D8190	Kolkata	√	√	1.032	0.963	4.1	negative		
3825	*S.typhi*	D8884	Kolkata	√	√	1.035	0.968	2.6	doubtful		
3832	*S.typhi*	D7130	Kolkata	√	√	0.968	0.966	3.4	positive		
3850	*S.typhi*	D7638	Kolkata	√	√	1.043	0.968	4.1	positive		
3857	*S.typhi*	E2338	Kolkata	√	√	0.144	0.911	1.3	positive		
3860	*S.typhi*	E8018	Kolkata	√	√	0.345	0.969	3.24	positive		
3896	*S.typhi*	E8333	Kolkata	√	√	0.5	0.962	3.24	positive		
3876	*S.typhi*	G1484	Kolkata	√	√	1	0.964	2.55	negative		
3879	*S.typhi*	G1848	Kolkata	√	√	0.931	1.003	3.73	positive		
	ATCC Ty2	19430	ATCC				1.011	39.4	positive		
Dilution factor					1:1000	1:1000	1:1000			1:500	1:500
Total dilution of C6524				1: 2.5×10^{11}							

Source : Production and Quality Control of Vi-DT Conjugate Vaccine, IVI.

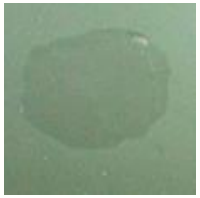
Seed Production Process



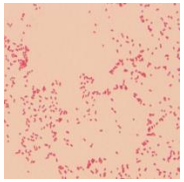
Results

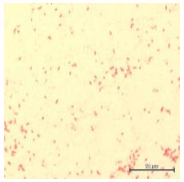
GMP Seed

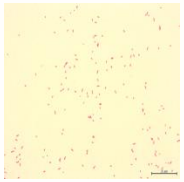
Aglutination

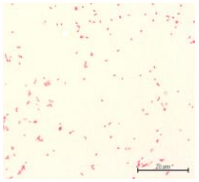


Gram Staining









Identity

API 20E

EXCELLENT IDENTIFICATION	
Strip	API 20 E V4.1
Profile	4 4 0 4 5 4 0
Note	CONFIRM BY SEROL.TESTS/ HIGHLY PATHOGENIC ORGANISM

Significant taxa	% ID	T	Tests against					
Salmonella typhi	99.9	0.82	H2S	8%				

Next taxon	% ID	T	Tests against					
Salmonella choleraesuis ssp choleraesuis	0.1	0.33	ODC	99%	RHA	99%	MEL	20%

Results

Assay	Acceptance Criteria (BF)	WHO TRS 987	Master Seed	Working Seed
OD	> 1	-	2.8	2.68
Gram stain	Gram Negative	Gram Negative	Gram Negative	Gram Negative
Identity/Purity	*Salmonella typhi* (+)	*Salmonella typhi* (+)	*Salmonella typhi* (+)	*Salmonella typhi* (+)
Agglutination	Agglutinated specific anti-Vi serum	Agglutinated specific anti-Vi serum	Agglutinated (+)	Agglutinated (+)
Viability Test	-	-	9.1×10^8 CFU/ml	9.2×10^8 CFU/ml

Quality Data of TCV

POLYSACCHARIDE ANTIGEN PRODUCTION

Process Flow

(Vi polysaccharide)

Fermentation

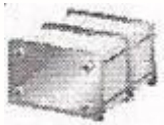
S. typhi Inoculums

Fermentation

Inactivation

Purification

Clarification & Diafiltration



TFF (MF)

Permeate

Concentration & Diafiltration

UF/DF

Retentate

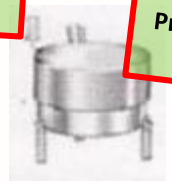
Cetavlon Precipitation

Trap on filter

Wash Et-OH

Dissolve w. Et-OH

Trap on filter and wash precipitate



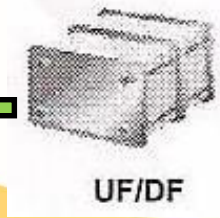
Precipitate in Et-OH

Precipitation

Dissolve precipitate

Impurities removal

Concentration & Diafiltration



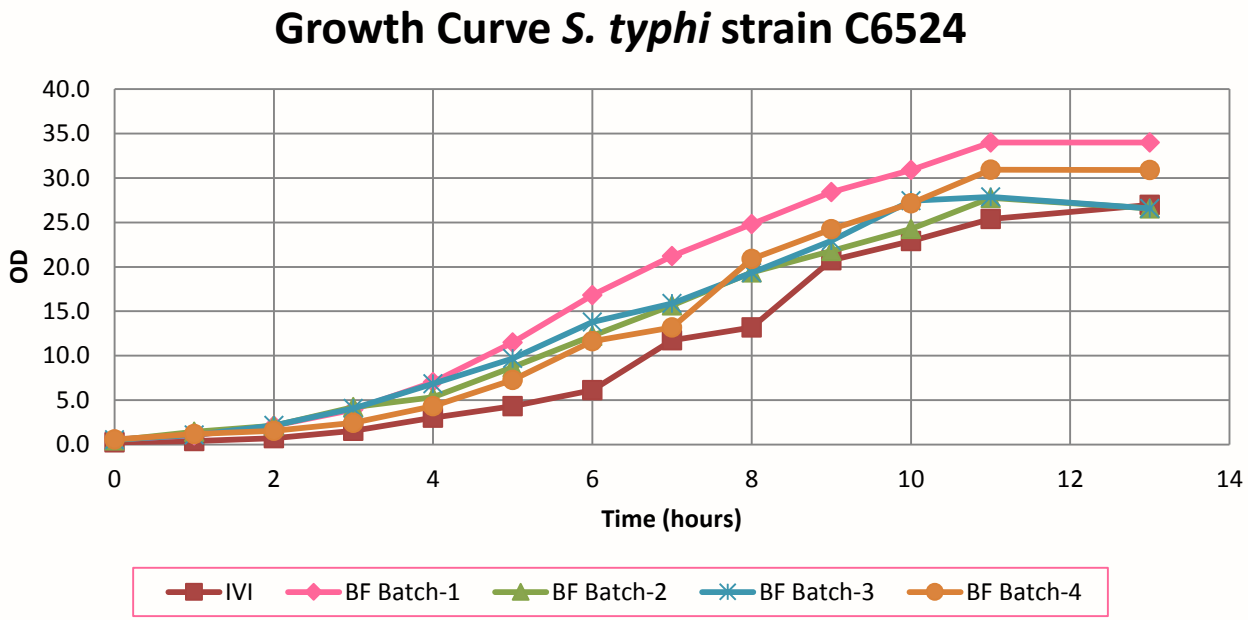
UF/DF

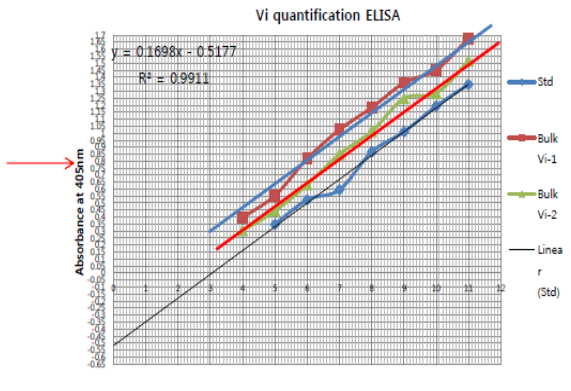
Sterile Filtration

Bulk Vi

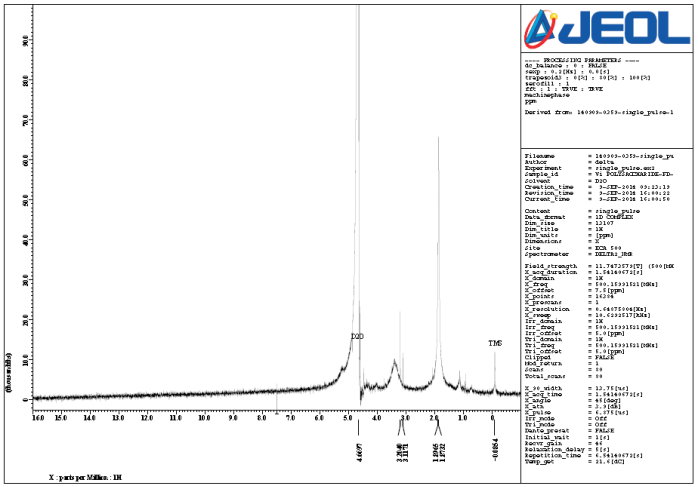
Confidential

Progress
(Fermentation Profile)





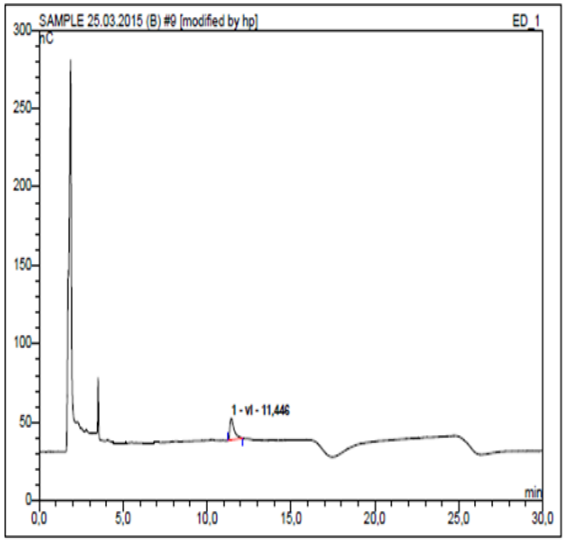
ELISA



NMR

9 Vi 200 ug/ml

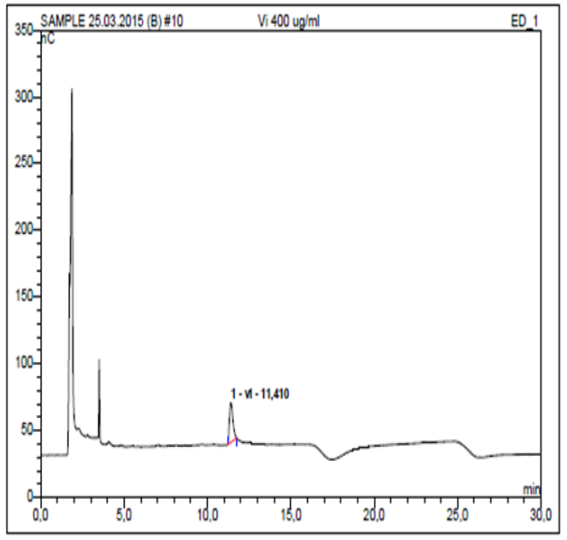
Sample Name:	Vi 200 ug/ml	Injection Volume:	50,0
Vial Number:	27	Channel:	ED_1
Sample Type:	standard	Wavelength:	n.a.
Control Program:	Free VI	Bandwidth:	n.a.
Quantif. Method:	Uji PRP vaksin Hib	Dilution Factor:	1,0000
Recording Time:	26/3/2015 12:02	Sample Weight:	1,0000
Run Time (min):	30,00	Sample Amount:	1,0000



No.	Ret.Time min	Peak Name	Height nC	Area nC*min	Rel.Area %	Amount ug/mL	Type
1	11,45	vi	13,730	4,021	100,00	226,117	BMB*
Total:			13,730	4,021	100,00	226,117	

10 Vi 400 ug/ml

Sample Name:	Vi 400 ug/ml	Injection Volume:	50,0
Vial Number:	28	Channel:	ED_1
Sample Type:	standard	Wavelength:	n.a.
Control Program:	Free VI	Bandwidth:	n.a.
Quantif. Method:	Uji PRP vaksin Hib	Dilution Factor:	1,0000
Recording Time:	26/3/2015 12:33	Sample Weight:	1,0000
Run Time (min):	30,00	Sample Amount:	1,0000



No.	Ret.Time min	Peak Name	Height nC	Area nC*min	Rel.Area %	Amount ug/mL	Type
1	11,41	vi	29,637	7,226	100,00	406,384	BMB
Total:			29,637	7,226	100,00	406,384	

HPAEC-PAD

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Polysaccharide Results

Assay	Acceptance Criteria (BF)	WHO TRS 987	BF Product
After Fermentation*			
Purity	*Salmonella typhi* (+)	*Salmonella typhi* (+)	*Salmonella typhi* (+)
Inactivation	Inactivated	Inactivated	Inactivated
After Purification**			
Identity	Contain Vi Polysaccharide	Contain Vi Polysaccharide	Positive
Protein	< 10 mg protein/gr Vi polysaccharide	NMT 1% weight protein/weight Vi Polysaccharide	4.55 mg
Nucleid Acid	< 20 mg protein/gr Vi polysaccharide	NMT 2% weight protein/weight Vi Polysaccharide	6.26 mg
O acetyl (Vi)	≥ 2.0 mmol/g Vi polysaccharide	≥ 2.0 mmol/g Vi polysaccharide	2.0 mmol/g Vi
Molecular size	information	Information	300 kDa
Vi concentration	information	information	2.6 mg/ml
LPS concentration using LAL test	< 10 EU/µg of Vi polysaccharide	Agreed with NRA	2.39 EU/µg Vi
pH	7 ± 0.5	7 ± 0.5	7
Free Formaldehid Content	≤ 0.02%	Agreed with NRA	0.000011%

Quality Data of TCV

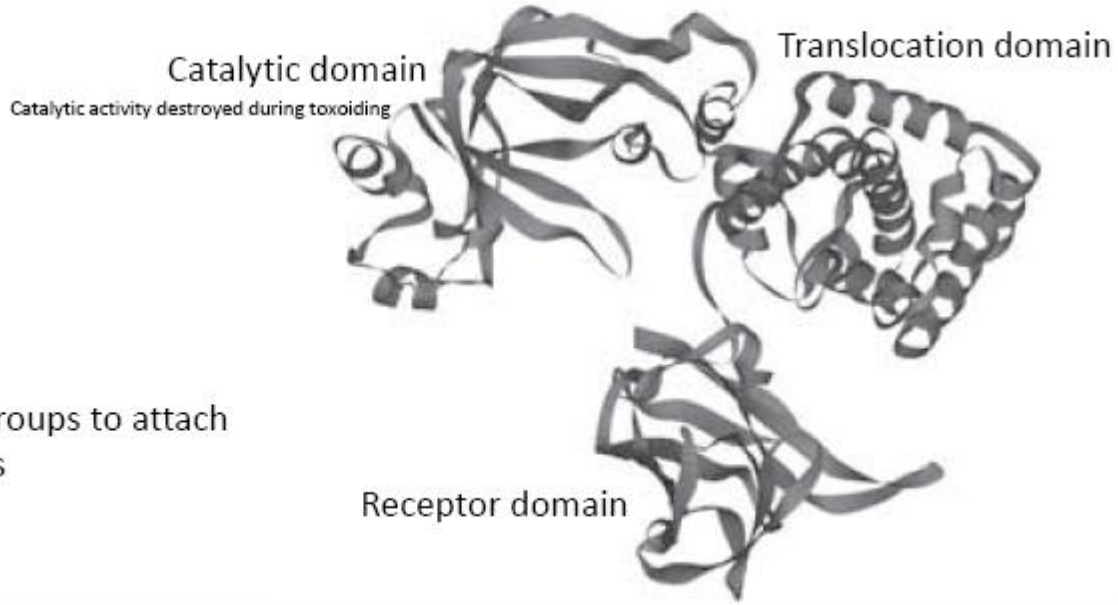
VI-DT CONJUGATION

Why Diphtheria Toxoid?

Carrier protein

Diphtheria toxoid

- Readily available at many developing country vaccine manufacturers.
- Inexpensive, so helps keep the conjugate prices affordable.
- Stable at pH used for conjugation



Spherical shape
Multiple carboxyl groups to attach to spacer molecules

Process Flow
(Vi-DT Conjugation)

Carrier Protein Preparation

DT Concentration & Diafiltration

UF/DF

DT Derivatization

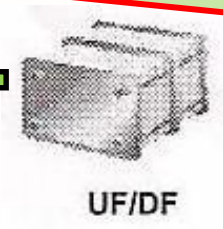
UF/DF

DT-AH

Vi Polysaccharide preparation

Vi-MES

Vi Concentration & Diafiltration



UF/DF

Conjugation

Vi-DT Diafiltration

UF/DF

Sterile Filtration

Bulk Vi-DT

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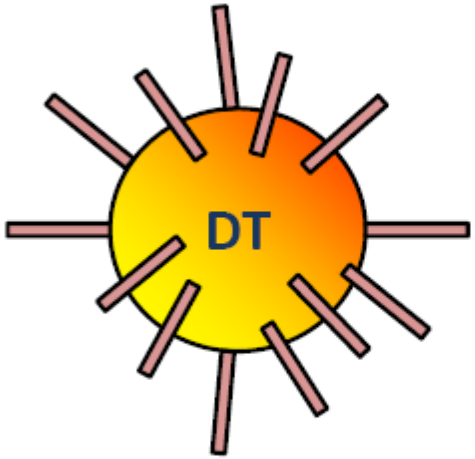
Info Imunisasi @infoimunisasi @biofarmaID

Derivatization of DT



After derivatization about 13 ADH spacer molecules bound to the DT

Control the number of spacer molecules bound by adjusting EDAC concentration.

PROCESS CONTROL

Spacer (ADH):Protein 2.0 to 4.0% (w/w)

7 to 14 spacer molecules per DT

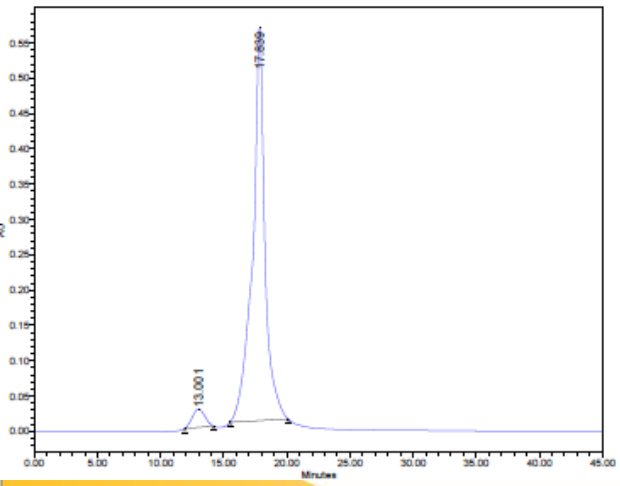
Soure : IVI Presentation

HPLC Profile Vi-DT Conjugate

- HPLC Analysis

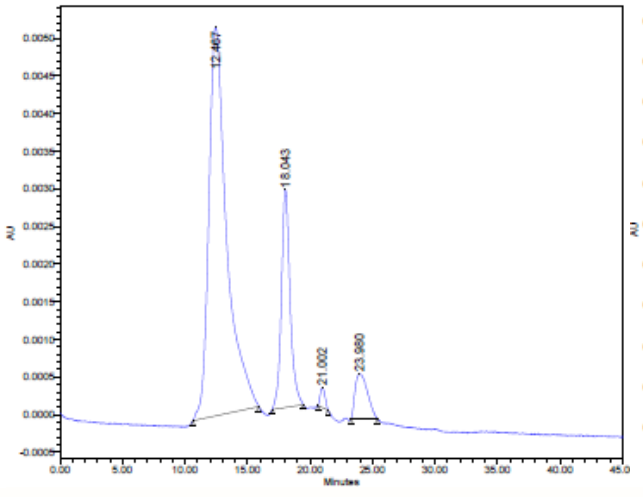
DT-AH

Sample Name : DT - AH
Injection Volume : 50.00 ul
Run Time : 45.0 Minutes
Curent date : 1/5/2014
Wavelength : Channel 2487Channel 2



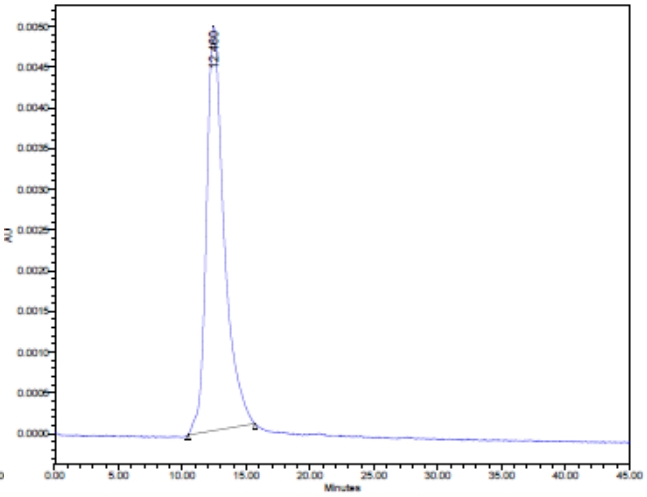
Vi-DT Before Dialysis

Sample Name : Feed Dialisis Vi - DT
Injection Volume : 50.00 ul
Run Time : 45.0 Minutes
Curent date : 1/5/2014
Wavelength : Channel 2487Channel 2



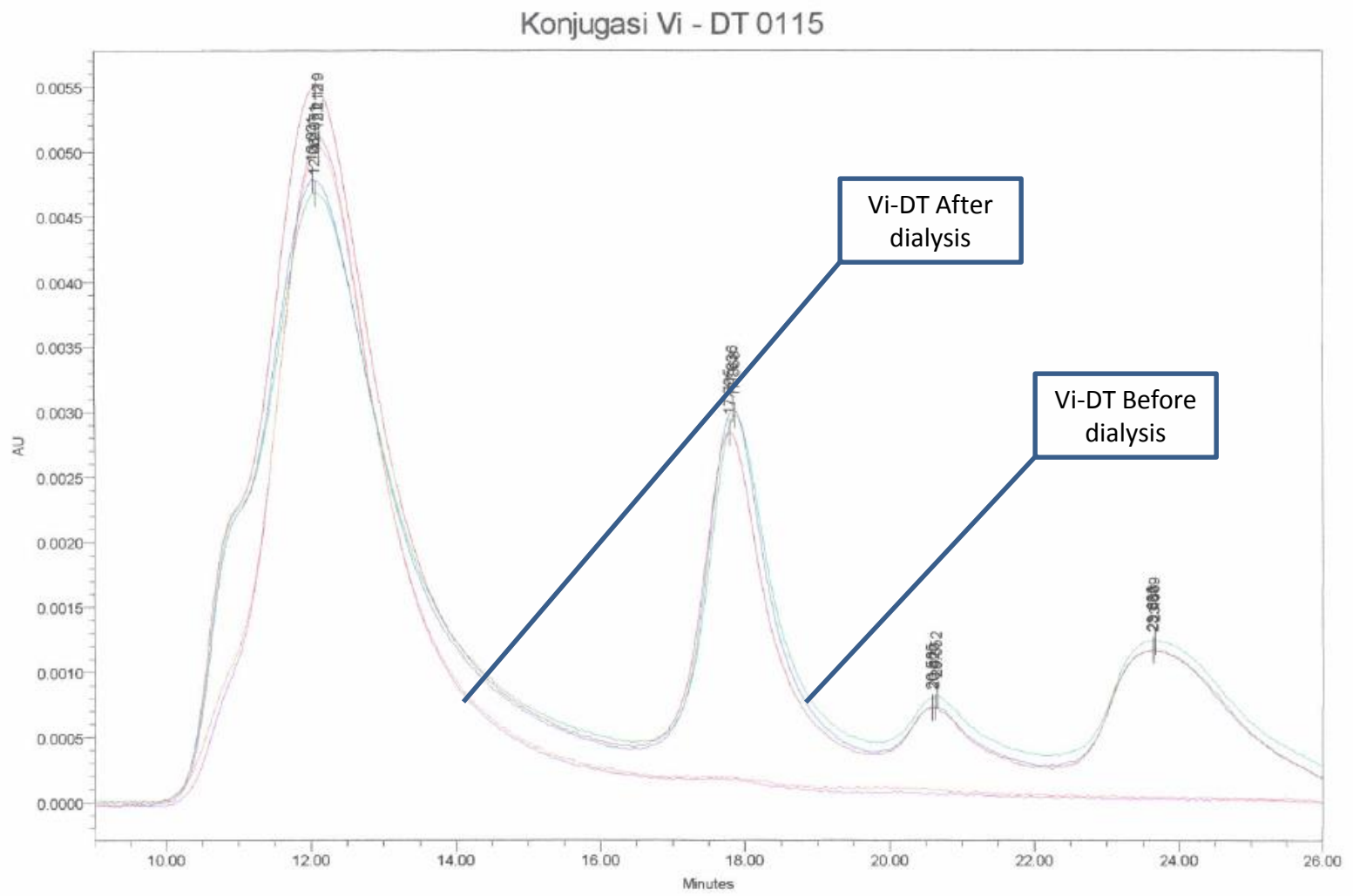
Vi-DT After Dialysis

Sample Name : Konjugat Post Filtrasi Vi - DT
Injection Volume : 50.00 ul
Run Time : 45.0 Minutes
Curent date : 1/5/2014
Wavelength : Channel 2487Channel 2



HPLC Profile Vi-DT conjugate

Research & Development Division



Confi

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Results (1)

Assay	Acceptance Criteria (BF)	WHO TRS 987	BF Product
Appearance	Clear to moderately turbid and colourless to pale yellow	Clear to moderately turbid and colourless to pale yellow	Clear to moderately turbid and colourless to pale yellow
The Amount of Hydrazide	Information (TNBS)	Information	0.5 µg/ml
Identity	Positive Vi polysaccharide	Positive Vi polysaccharide	Positive Vi polysaccharide
Vi content	Information	Information	153 µg/ml
O acetyl (Vi)	Information	Information	237 µg/ml
Protein	Information	Information	171 µg/ml
Vi/Protein Ratio	≥ 0.5, ≤ 1.0	Agreed with NRA	0.89
Molecular size	Information	Information	Vi-DT : 1300 kD
Free Vi *	Information	Information	11%

Results (2)

Assay	Acceptance Criteria (BF)	WHO TRS	BF Product
Free Protein	Information	Information	< 5 %
Endotoxin	< 100 EU/ml	Agreed with NRA	37.8 EU/ml
pH	7.2±0.2	information	7
Residual content (ADH)	Information	Information	1 µg/ml
Sterility	Sterile	Sterile	Sterile
Specific Toxicity	Pass	Pass	Pass

Quality Data of TCV

FINAL BULK AND FINAL PRODUCT

Process Flow

(Final Bulk and Final Product)

Results

Assay	Acceptance Criteria (BF)	WHO TRS	BF Preclinical Lot
Final Bulk			
Sterility	Sterile	Sterile	Sterile
Vi content	45-55 ug/ml	Information	49 ug/ml
Protein Content	Information	Information	41 ug/ml
Molecular size	Information	Information	1300 kDa
Final Product			
Identity (immunoassay)	Positive Vi polysaccharide	Information	Positive Vi polysaccharide
Vi content	45-55 ug/ml	Agreed with NRA	49 ug/ml
O acetyl (Vi)	Information	Agreed with NRA	122.5 umol/ml
Conjugate size/Molecular size	Information	Agreed with NRA	1300 kDa
Free Vi *	Information	Agreed with NRA	11 %
Endotoxin	< 50 EU/dosage	Agreed with NRA	12.5 EU/dosage
pH	7.2±0.2	7.2±0.2	7.2
Sterility	Sterile	Sterile	Sterile
Safety (Inocuity Test)/Abnormal toxicity	Pass	Agreed with NRA	Pass
Osmolality	Information	Agreed with NRA	287,7 mOsm/kg

Toxicity and Immunogenicity Test

Immunogenicity : WHO TRS 987 and WHO TRS 927

B.3 Nonclinical immunogenicity studies

Immunogenicity studies in animal models should be conducted because they provide valuable proof-of-concept information that can be used to support a clinical development plan. In addition, immunogenicity data derived from appropriate animal models are useful in establishing the immunological characteristics of the Vi polysaccharide conjugate product, and may guide the selection of doses, schedules and routes of administration that will be evaluated in clinical trials. To ensure immunogenicity in nonclinical testing weaning mice (younger than 6 weeks) should receive intramuscularly two injections 2 weeks apart of the conjugate vaccine and Vi should be used for a control group. Anti-Vi IgG should then be measured. The conjugate should induce a response that is at least four times higher than the response induced by Vi, and a booster response should occur after the second dose (*100*). Immunogenicity studies of Vi polysaccharide

Toxicity : WHO TRS 927

In Process and Will be finished on June or July 2015

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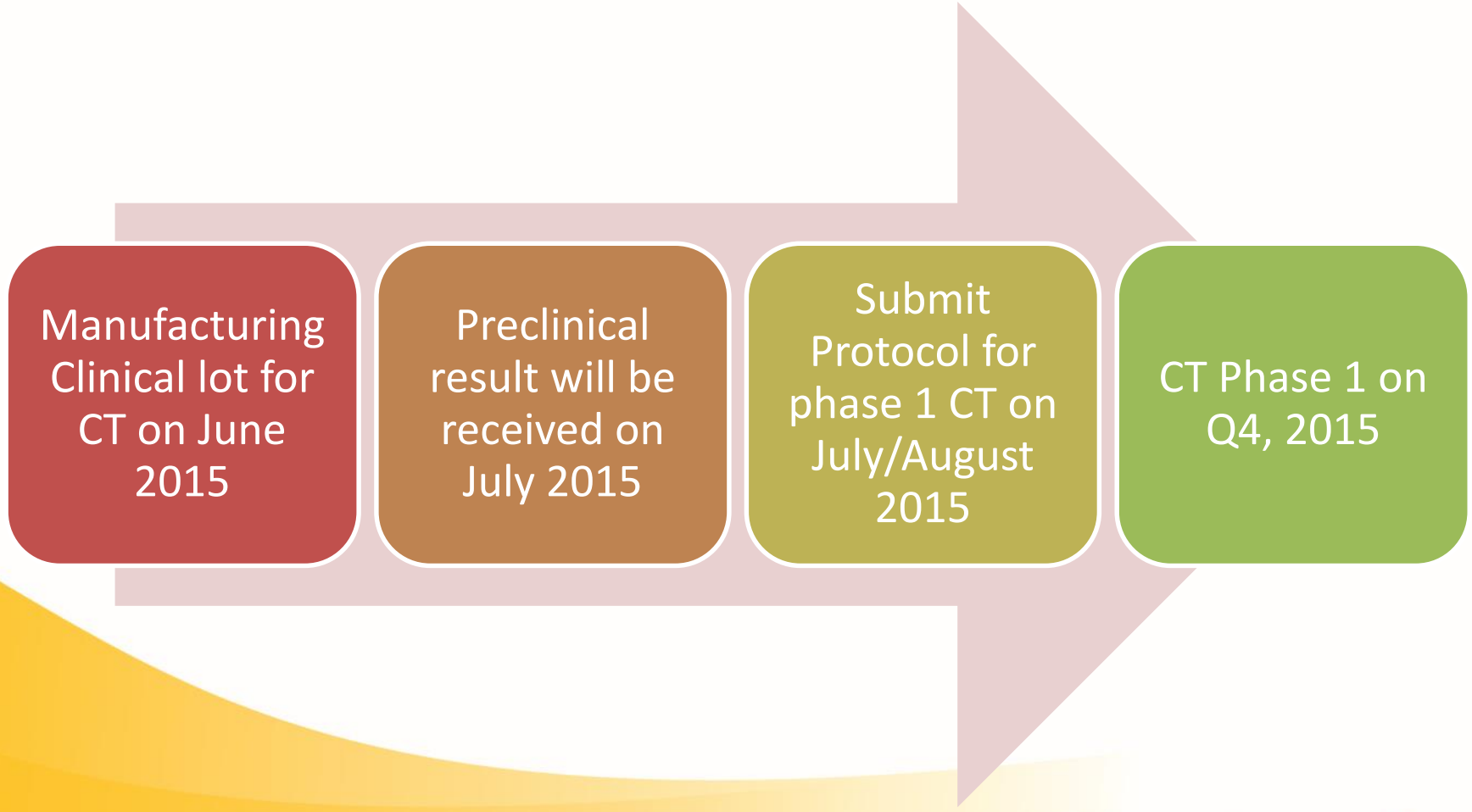
Next Plan



Terima Kasih

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