

BLOODVIR System: Use of a capture metagenomics for surveillance of viruses in blood

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Das Paul-Ehrlich-Institut ist ein Bundesinstitut im Geschäftsbereich des Bundesministeriums für Gesundheit.

The Paul-Ehrlich-Institut is an Agency of the German Federal Ministry of Health.

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Plasma derived medicinal products

Table 1. Effect of manufacturing scale on risk of exposure. Adapted from Lynch et al. 1996 with permission.

Manufacturing Scale (Number of Donors)	Number of Independent Infusions		
	1	10	100
Prevalence of agent = 5×10^{-5}			
60,000	11%	70%	100%
25,000	2%	18%	86%
1000	0.2%	2%	18%
Prevalence of agent = 5×10^{-4}			
60,000	70%	100%	100%
25,000	39%	99%	100%
1000	2%	18%	86%
Prevalence of agent = 5×10^{-3}			
60,000	100%	100%	100%
25,000	99%	100%	100%
1000	18%	86%	100%

Vaccines

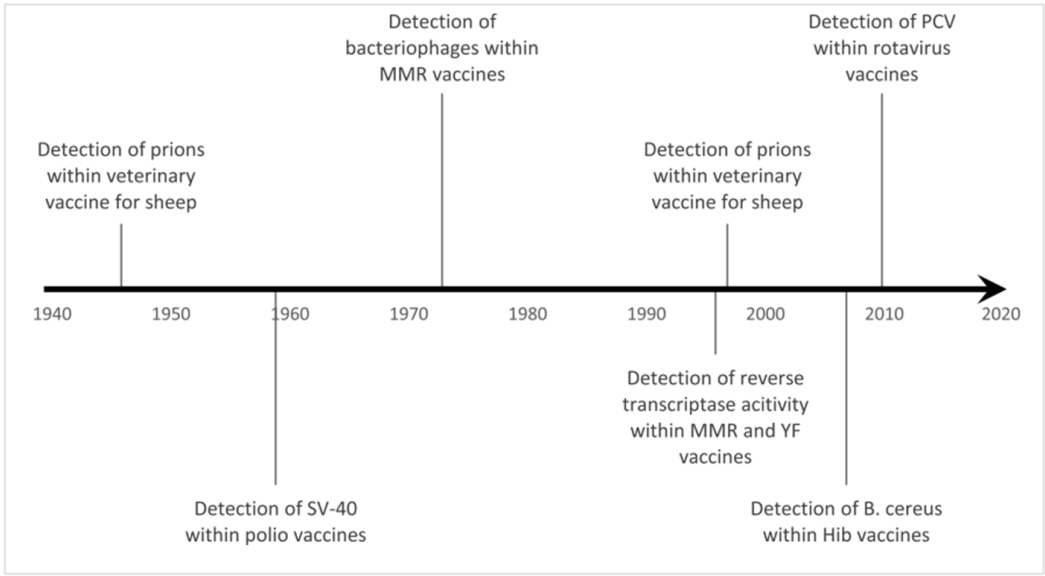
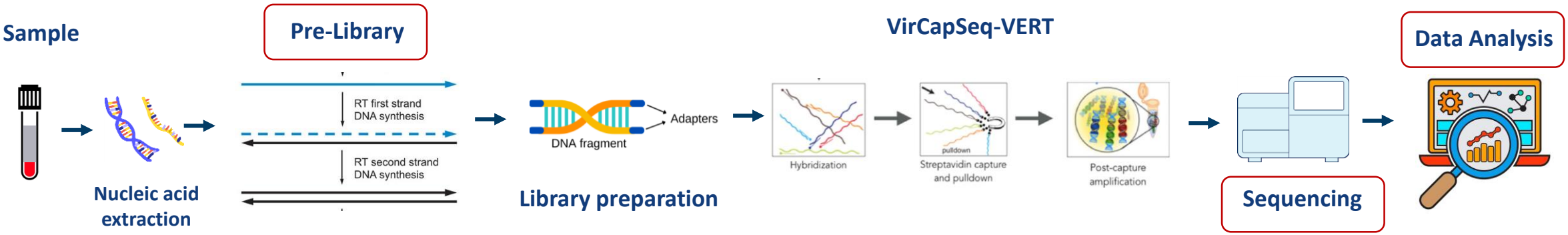


Fig. 1. A selected timeline of adventitious agent contamination
Each of the discussed events of adventitious agent contamination are listed chronologically, with the date in reference to the year the contamination was detected.

Farrugia, A. The Evolution of the Safety of Plasma Products from Pathogen Transmission— A Continuing Narrative. Pathogens 2023, 12, 318. https://doi.org/10.3390/pathogens12020318

BLOODVIR NGS System

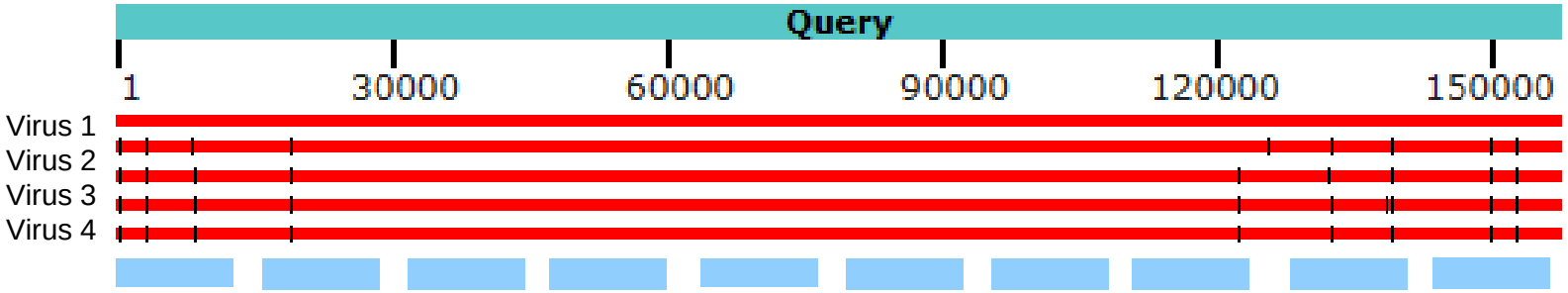


1. Known virus detection
2. Novel virus identification

- Validation using the WHO International Reference Panel for Adventitious Virus Detection in Biological Products by High-throughput Sequencing (HTS) Technologies

Broad capture system design (VirCapSeq-VERT)

- >207 Virus taxa included
- Coding sequences
- Homology grouping >85-96%
- 50-120nt Probe length (70nt-79nt)
- 700.000 Hybridization Probes



Patent Pub . No . : US 2018 / 0265935 A1 Lipkin et al .
Pub . Date : Sep . 20 , 2018

Bioinformatic Analysis and Report

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Sample: 363NR_CDMX_S8

The following table lists the detected virus candidates. Taxa selected by the pipeline *PASSED* reclassification as well as filtering.

TaxID	Species name	Blast hit	Blast species	Read number	Reclassification	Filtering	Consolidation
10368	*Human betaherpesvirus 6*	*Human betaherpesvirus 6*	*Human betaherpesvirus 6*	*2,983*	*PASSED*	*PASSED*	*PASSED*
93465	*Avian endogenous retrovirus EAV-HP*	*Avian endogenous retrovirus EAV-HP*	*Avian endogenous retrovirus EAV-HP*	*774*	*PASSED*	*PASSED*	*PASSED*
68887	*Torque teno virus*	*Torque teno virus*	*Torque teno virus*	*540*	*PASSED*	*PASSED*	*PASSED*
3052608	*Pegivirus hominis*	*GB virus C*	*Pegivirus hominis*	*309*	*PASSED*	*PASSED*	*PASSED*
3050299	*Lymphocryptovirus humangamma4*	*human gammaherpesvirus 4*	*Lymphocryptovirus humangamma4*	*156*	*PASSED*	*PASSED*	*PASSED*
2055263	*Anelloviridae sp.*	*Anelloviridae sp.*	*Anelloviridae sp.*	*400*	*PASSED*	*FAILED*	*PASSED*
463676	*Rhinovirus C*	*rhinovirus C34*	*Rhinovirus C*	*46*	*PASSED*	*FAILED*	*PASSED*
3052610	Pegivirus platyrrhini	GB virus C	Pegivirus hominis	241	PASSED	PASSED	FAILED
3071193	Anellovirus D_HF7_66	Anelloviridae sp.	Anelloviridae sp.	110	FAILED	PASSED	FAILED
136966	SEN virus	Anelloviridae sp.	Anelloviridae sp.	72	FAILED	PASSED	FAILED
3050297	Roseolovirus humanbeta6b	-	-	2,464	PASSED	FAILED	FAILED
3050296	Roseolovirus humanbeta6a	-	-	836	PASSED	FAILED	FAILED
11864	Avian leukosis virus	-	-	303	PASSED	FAILED	FAILED
11885	Fujinami sarcoma virus	-	-	79	PASSED	FAILED	FAILED
11876	Avian sarcoma virus	-	-	52	PASSED	FAILED	FAILED
1862824	Gemycircularvirus HV-GcV1	-	-	38	PASSED	FAILED	FAILED
11886	Rous sarcoma virus	-	-	37	PASSED	FAILED	FAILED
2853118	Torque teno virus AZ1_2	-	-	31	PASSED	FAILED	FAILED
11866	Avian myeloblastosis virus	-	-	29	PASSED	FAILED	FAILED
432261	Torque teno midi virus	-	-	25	PASSED	FAILED	FAILED

Bioinformatic Analysis and Report

FILTERED OUT - Torque teno virus 16 (NC_014091.1, TaxID 687355, 1-level-up TaxID 687331, 2-levels-up TaxID 687329) (3.8Kbp)

```
>  90.00% |  :          ::                          |  Number of reads: 97
>  80.00% |  :          ::                          |
>  70.00% |  :         :::                          |  Covered bases:   541bp
>  60.00% |  ::        :::                          |  Percent covered: 14.17%
>  50.00% |  :::       :::                          |  Mean coverage:   2.39x
>  40.00% |  :::       :::                          |  Mean baseQ:      33.1
>  30.00% |  :::       :::                          |  Mean mapQ:       27.3
>  20.00% |  :::       ::::                         |
>  10.00% |  :::       ::::     :                   |  Histo bin width: 95bp
>   0.00% | ::::       ::::     :                   |  Histo max bin:   100%
          1        950       1.9K      2.9K       3.8K
```

Copy consensus sequence | Open Blast | Blast consensus sequence

IDENTIFIED - Torque teno virus (KT163895.1, TaxID 68887, 1-level-up TaxID 363628, 2-levels-up TaxID 687329) (2.5Kbp)

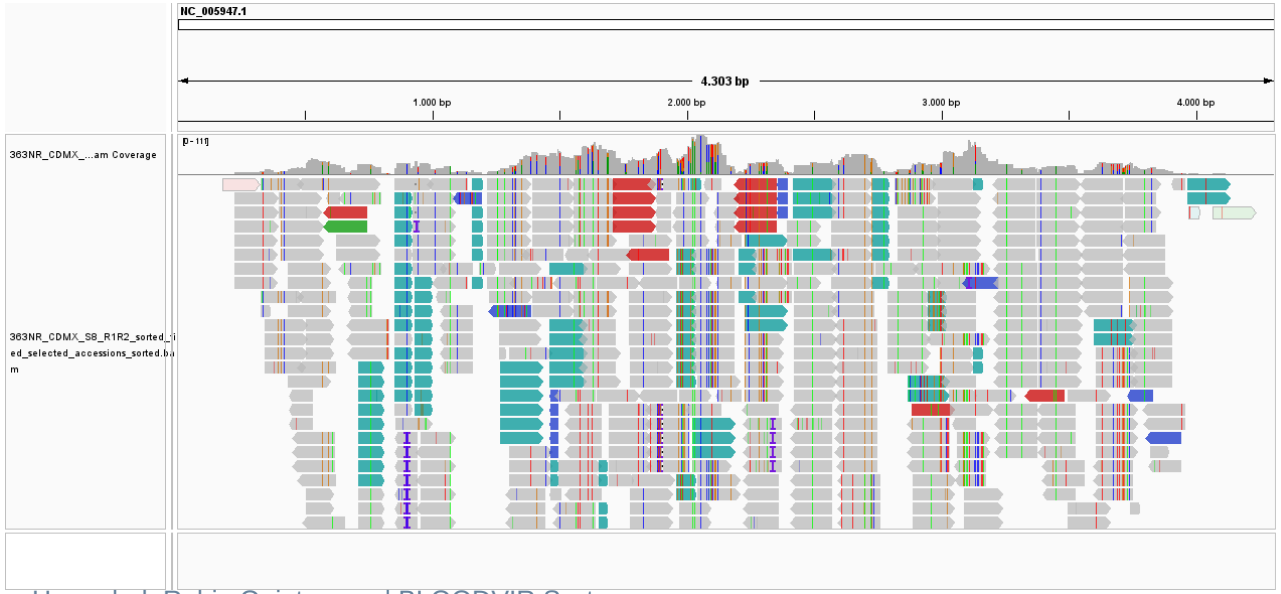
```
>  90.00% |::::::::            ::: ::  :::    ::    |  Number of reads: 130
>  80.00% |::::::::            :::.::  ::::   ::    |      (12 filtered)
>  70.00% |::::::::            ::::::  ::::  :::    |  Covered bases:   1.4Kbp
>  60.00% |::::::::            ::::::  :::: ::::    |  Percent covered: 57.59%
>  50.00% |::::::::.           ::::::  :::: ::::    |  Mean coverage:   6.37x
>  40.00% |:::::::::           ::::::  :::: ::::    |  Mean baseQ:      32.7
>  30.00% |:::::::::          :::::::: :::: :::::   |  Mean mapQ:       59.7
>  20.00% |:::::::::          :::::::.:::: :::::    |
>  10.00% |:::::::::          :::::::::::.:::::     |  Histo bin width: 62bp
>   0.00% |:::::::::          ::::::::::::::::::    |  Histo max bin:   100%
          1        620       1.2K      1.9K       2.5K
```

Copy consensus sequence | Open Blast | Blast consensus sequence

IDENTIFIED - Avian endogenous retrovirus EAV-HP (NC_005947.1, TaxID 93465, 1-level-up TaxID 206036, 2-levels-up TaxID 35276) (4.3Kbp)

```
>  90.00% |   ::::: ::::::::::::::::::::::::::::::.:  |  Number of reads: 1013
>  80.00% |   ::::: ::::::::::::::::::::::::::::::   |      (22 filtered)
>  70.00% |   ::::::::::::::::::::::::::::::::::::   |  Covered bases:   4.0Kbp
>  60.00% |   ::::::::::::::::::::::::::::::::::::   |  Percent covered: 93.31%
>  50.00% |   ::::::::::::::::::::::::::::::::::::   |  Mean coverage:   28.4x
>  40.00% |   ::::::::::::::::::::::::::::::::::::   |  Mean baseQ:      33
>  30.00% |  .::::::::::::::::::::::::::::::::::::   |  Mean mapQ:       59.2
>  20.00% |  ::::::::::::::::::::::::::::::::::::    |
>  10.00% |  ::::::::::::::::::::::::::::::::::::    |  Histo bin width: 107bp
>   0.00% |  ::::::::::::::::::::::::::::::::::::    |  Histo max bin:   100%
          1        1.1K      2.1K      3.2K       4.3K
```

Copy consensus sequence | Open Blast | Blast consensus sequence

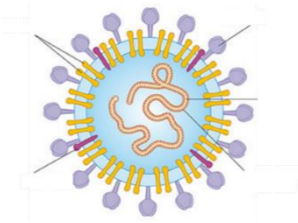


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World Health Organization

EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION
Geneva, 11 to 14 March 2024

A Collaborative Study to Evaluate the Proposed First WHO International Reference Panel for Adventitious Virus Detection in Biological Products by High-throughput Sequencing (HTS) Technologies



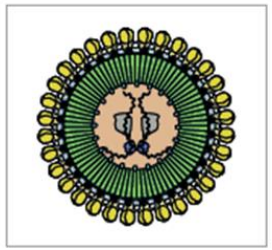
Human coronavirus OC43



Mammalian Orthoreovirus



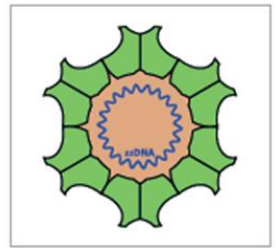
Orthopneumovirus (RSV)



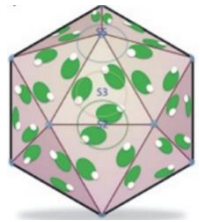
Feline Leukemia Virus



Epstein-Barr-virus / Human gammaherpesvirus 4



Porcine Circovirus (PCV)



Minute Virus of mice

#	Virus	Family	Genus	Nucleic Acid	Segmented	Baltimore	Enveloped
1	Porcine circovirus, type 1	Circoviridae	Circovirus	cssDNA	No	II	No
2	Betacoronavirus, OC43	Orthocoronavirinae	Betacoronavirus	ssRNA+	No	IV	Yes
3	Epstein-Barr Virus (human herpes virus 4), strain B95-8	Orthoherpesviridae	Lymphocryptovirus	dsDNA	No	I	Yes
4	Minute virus of mice, Prototype (p) strain	Parvoviridae	Protoparvovirus	dsDNA	No	I	No
5	Human respiratory syncytial virus, A2	Pneumoviridae	Orthopneumovirus	ssRNA-	No	V	Yes
6	Feline leukemia virus, (Kawakami-) Thielen strain (KT)	Retroviridae	Gammaretrovirus	ssRNA+ RT	No	VI	Yes
7	Mammalian orthoreovirus, Lang strain	Spinareoviridae	Orthoreovirus	dsDNA	Yes	I	No

WHO reference standards - Results by Pipeline

Ebstein Barr Virus							
copies/ml	0,1	1	10	100	1.000	10.000	100.000
copies/rx	0,02	0,23	2,29	22,86	228,57	2.285,71	22.857,14
Replicate 1							
replicate 2							
replicate 3							
replicate 4							
replicate 5							

Mammalian Orthoreovirus Type 1 Lang							
copies/ml	0,1	1	10	100	1.000	10.000	100.000
copies/rx	0,02	0,23	2,29	22,86	228,57	2.285,71	22.857,14
Replicate 1							
replicate 2							
replicate 3							
replicate 4							
replicate 5							

Porcine Circovirus							
copies/ml	0,1	1	10	100	1.000	10.000	100.000
copies/rx	0,02	0,23	2,29	22,86	228,57	2.285,71	22.857,14
Replicate 1							
replicate 2							
replicate 3							
replicate 4							
replicate 5							

Feline Leukemia Virus							
copies/ml	0,1	1	10	100	1.000	10.000	100.000
copies/rx	0,02	0,23	2,29	22,86	228,57	2.285,71	22.857,14
Replicate 1							
replicate 2							
replicate 3							
replicate 4							
replicate 5							

Minute Virus of Mice							
copies/ml	0,1	1	10	100	1.000	10.000	100.000
copies/rx	0,02	0,23	2,29	22,86	228,57	2.285,71	22.857,14
Replicate 1							
replicate 2							
replicate 3							
replicate 4							
replicate 5							

Respiratory Syncytial Virus							
copies/ml	0,1	1	10	100	1.000	10.000	100.000
copies/rx	0,02	0,23	2,29	22,86	228,57	2.285,71	22.857,14
Replicate 1							
replicate 2							
replicate 3							
replicate 4							
replicate 5							

Coronavirus OC43							
copies/ml	0,1	1	10	100	1.000	10.000	100.000
copies/rx	0,02	0,23	2,29	22,86	228,57	2.285,71	22.857,14
Replicate 1							
replicate 2							
replicate 3							
replicate 4							
replicate 5							

WHO reference standards - Coverage Target Analysis

Ebstein Barr Virus							
copies/ml	**0,1**	**1**	**10**	**100**	**1.000**	**10.000**	**100.000**
copies/rx	**0,02**	**0,23**	**2,29**	**22,86**	**228,57**	**2.285,71**	**22.857,14**
Replicate 1	0,19%	0,07%	3,08%	3,54%	36,27%	94,67%	98,80%
replicate 2	not found	0,03%	0,13%	5,72%	39,44%	7377%	98,83%
replicate 3	0,06%	not found	0,15%	20,53%	17,62%	49,14%	99,32%
replicate 4	0,11%	0,17%	0,10%	19,68%	9,87%	76,75%	97,74%
replicate 5	0,07%	0,53%	not found	0,71%	14,89%	77,75%	98,85%

Mammalian Orthoreovirus Type 1 Lang							
copies/ml	**0,1**	**1**	**10**	**100**	**1.000**	**10.000**	**100.000**
copies/rx	**0,02**	**0,23**	**2,29**	**22,86**	**228,57**	**2.285,71**	**22.857,14**
Replicate 1	0/10	2%	1/10	6/10	9/10	10/10	10/10
replicate 2	0/10	20%	0/10	0/10	5/10	10/10	10/10
replicate 3	0/10	0/10	1/10	7/10	5/10	10/10	10/10
replicate 4	0/10	0/10	0/10	7/10	2/10	10/10	10/10
replicate 5	0/10	5%	0/10	3/10	7/10	10/10	10/10

Porcine Circovirus							
copies/ml	**0,1**	**1**	**10**	**100**	**1.000**	**10.000**	**100.000**
copies/rx	**0,02**	**0,23**	**2,29**	**22,86**	**228,57**	**2.285,71**	**22.857,14**
Replicate 1	14,51%	23,89%	16,04%	58,53%	100%	100%	100%
replicate 2	not found	29,58%	16,10%	10,98%	97,50%	100%	100%
replicate 3	53,19%	20,76%	23,15%	54,49%	75,26%	100%	100%
replicate 4	8,30%	43%	not found	31,06%	32,14%	100%	100%
replicate 5	52,73%	not found	12,40%	11,66%	95,90%	100%	100%

Feline Leukemia Virus							
copies/ml	**0,1**	**1**	**10**	**100**	**1.000**	**10.000**	**100.000**
copies/rx	**0,02**	**0,23**	**2,29**	**22,86**	**228,57**	**2.285,71**	**22.857,14**
Replicate 1	not found	not found	not found	2,04%	25,91%	84,85%	99,95%
replicate 2	not found	not found	not found	not found	8,11%	56,32%	94,94%
replicate 3	not found	not found	4,11%	1,34%	1,85%	31,27%	99,89%
replicate 4	not found	not found	not found	not found	not found	55,59%	94,15%
replicate 5	not found	not found	not found	not found	12,88%	77,35%	97,60%

Minute Virus of Mice							
copies/ml	**0,1**	**1**	**10**	**100**	**1.000**	**10.000**	**100.000**
copies/rx	**0,02**	**0,23**	**2,29**	**22,86**	**228,57**	**2.285,71**	**22.857,14**
Replicate 1	not found	not found	7,76%	25,58%	37,11%	91,82%	97,36%
replicate 2	not found	not found	2,81%	4,42%	20,22%	71,92%	97,67%
replicate 3	not found	not found	not found	25,05%	26,98%	54,48%	97,03%
replicate 4	not found	not found	not found	3,63%	5,86%	86,37%	97,55%
replicate 5	not found	not found	not found	not found	30,59%	94,37%	98%

Respiratory Syncytial Virus							
copies/ml	**0,1**	**1**	**10**	**100**	**1.000**	**10.000**	**100.000**
copies/rx	**0,02**	**0,23**	**2,29**	**22,86**	**228,57**	**2.285,71**	**22.857,14**
Replicate 1	not found	not found	not found	not found	5,97%	43,98%	78,45%
replicate 2	not found	not found	not found	not found	0,93%	20,19%	80,78%
replicate 3	not found	not found	0,79%	0,12%	not found	3,09%	91,38%
replicate 4	not found	not found	not found	1,07%	not found	12,90%	62,71%
replicate 5	not found	not found	not found	not found	3,06%	32,29%	91,10%

Coronavirus OC43							
copies/ml	**0,1**	**1**	**10**	**100**	**1.000**	**10.000**	**100.000**
copies/rx	**0,02**	**0,23**	**2,29**	**22,86**	**228,57**	**2.285,71**	**22.857,14**
Replicate 1	not found	0,09%	not found	not found	0,08%	0,09%	9,96%
replicate 2	not found	0,56%	not found	not found	not found	not found	6,05%
replicate 3	not found	not found	not found	0,57%	0,13%	not found	7,04%
replicate 4	not found	not found	not found	not found	not found	1,01%	2,02%
replicate 5	not found	not found	not found	not found	0,06%	1%	12,57%

WHO reference standards - Target Analysis (no filter)

Ebstein Barr Virus							
copies/ml	**0,1**	**1**	**10**	**100**	**1.000**	**10.000**	**100.000**
copies/rx	**0,02**	**0,23**	**2,29**	**22,86**	**228,57**	**2.285,71**	**22.857,14**
Replicate 1							
replicate 2							
replicate 3							
replicate 4							
replicate 5							

Mammalian Orthoreovirus Type 1 Lang							
copies/ml	**0,1**	**1**	**10**	**100**	**1.000**	**10.000**	**100.000**
copies/rx	**0,02**	**0,23**	**2,29**	**22,86**	**228,57**	**2.285,71**	**22.857,14**
Replicate 1							
replicate 2							
replicate 3							
replicate 4							
replicate 5							

Porcine Circovirus							
copies/ml	**0,1**	**1**	**10**	**100**	**1.000**	**10.000**	**100.000**
copies/rx	**0,02**	**0,23**	**2,29**	**22,86**	**228,57**	**2.285,71**	**22.857,14**
Replicate 1							
replicate 2							
replicate 3							
replicate 4							
replicate 5							

Feline Leukemia Virus							
copies/ml	**0,1**	**1**	**10**	**100**	**1.000**	**10.000**	**100.000**
copies/rx	**0,02**	**0,23**	**2,29**	**22,86**	**228,57**	**2.285,71**	**22.857,14**
Replicate 1							
replicate 2							
replicate 3							
replicate 4							
replicate 5							

Minute Virus of Mice							
copies/ml	**0,1**	**1**	**10**	**100**	**1.000**	**10.000**	**100.000**
copies/rx	**0,02**	**0,23**	**2,29**	**22,86**	**228,57**	**2.285,71**	**22.857,14**
Replicate 1							
replicate 2							
replicate 3							
replicate 4							
replicate 5							

Respiratory Syncytial Virus							
copies/ml	**0,1**	**1**	**10**	**100**	**1.000**	**10.000**	**100.000**
copies/rx	**0,02**	**0,23**	**2,29**	**22,86**	**228,57**	**2.285,71**	**22.857,14**
Replicate 1							
replicate 2							
replicate 3							
replicate 4							
replicate 5							

Coronavirus OC43							
copies/ml	**0,1**	**1**	**10**	**100**	**1.000**	**10.000**	**100.000**
copies/rx	**0,02**	**0,23**	**2,29**	**22,86**	**228,57**	**2.285,71**	**22.857,14**
Replicate 1							
replicate 2							
replicate 3							
replicate 4							
replicate 5							

Workflow Summary and Cost Comparison (consumables only!)

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Process steps	BLOODVIR	Standard Metagenomic
Nuclease sample pre-treatment	No	Yes
Elution	55ul	
Pre-amplification	No	
Denature temperature	Multiple	One
Retrotranscription	2 hours	1 hour
Second strand synthesis	Nick translation based	
Library preparation Kit	Library Preparation EF 2.0 with Enzymatic Fragmentation and Twist Universal Adapter System	NEBNext Ultra II FS DNA Library Prep Kit for Illumina
PCR amplification	11 Cycles	Variable
Hybridization/ capture	Yes	No
second PCR amplification	Yes	No
Sequencing equipment	Illumina Nextseq 2000	
Sequencing kit	P3	P3
Sequence depth/ sample	17 million reads	100 million reads

	BLOODVIR	Std. Metagenomics
Nucleic Acid Purification	5	5
Nuclease treatment	0	20
cDNA synthesis	15	10
2nd strand synthesis	13	13
Library Preparation	88	64
Hybridization/Capture		0
Sequencing (P3)	64	381
Analysis	50	80
Total	**235**	**573**

Cost per individual sample in pool :

Pool of 100 – 23.5 EUR
Pool of 1000 – 2.35 EUR

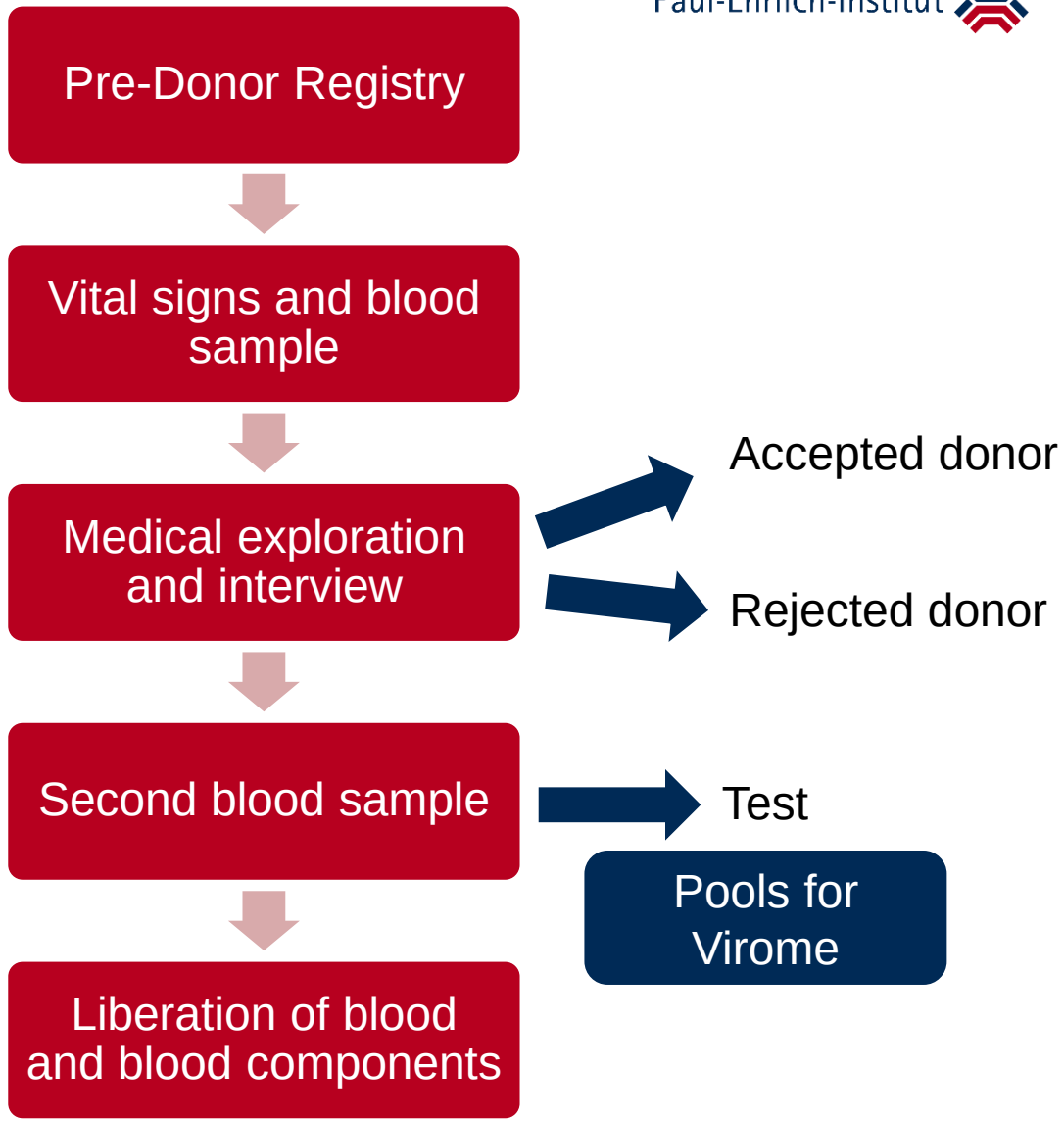
General Panorama of Blood Transfusion in Mexico

- 556 blood banks
  - 86 belongs IMSS

- 1.830.000 blood units per year
- 43% of whole country blood collection
- 99.70% under reposition scheme
- 100% of the units are tested

Test and workflow

Pathogen	Test	Prevalence
HIV	Antigen + Antibody + PCR	0,04%
HBV	Antigen + PCR	0,02%
HCV	Antigen + PCR	0,03%
Syphilis	Antibody	0,48%
Chagas	Antibody	0,23%



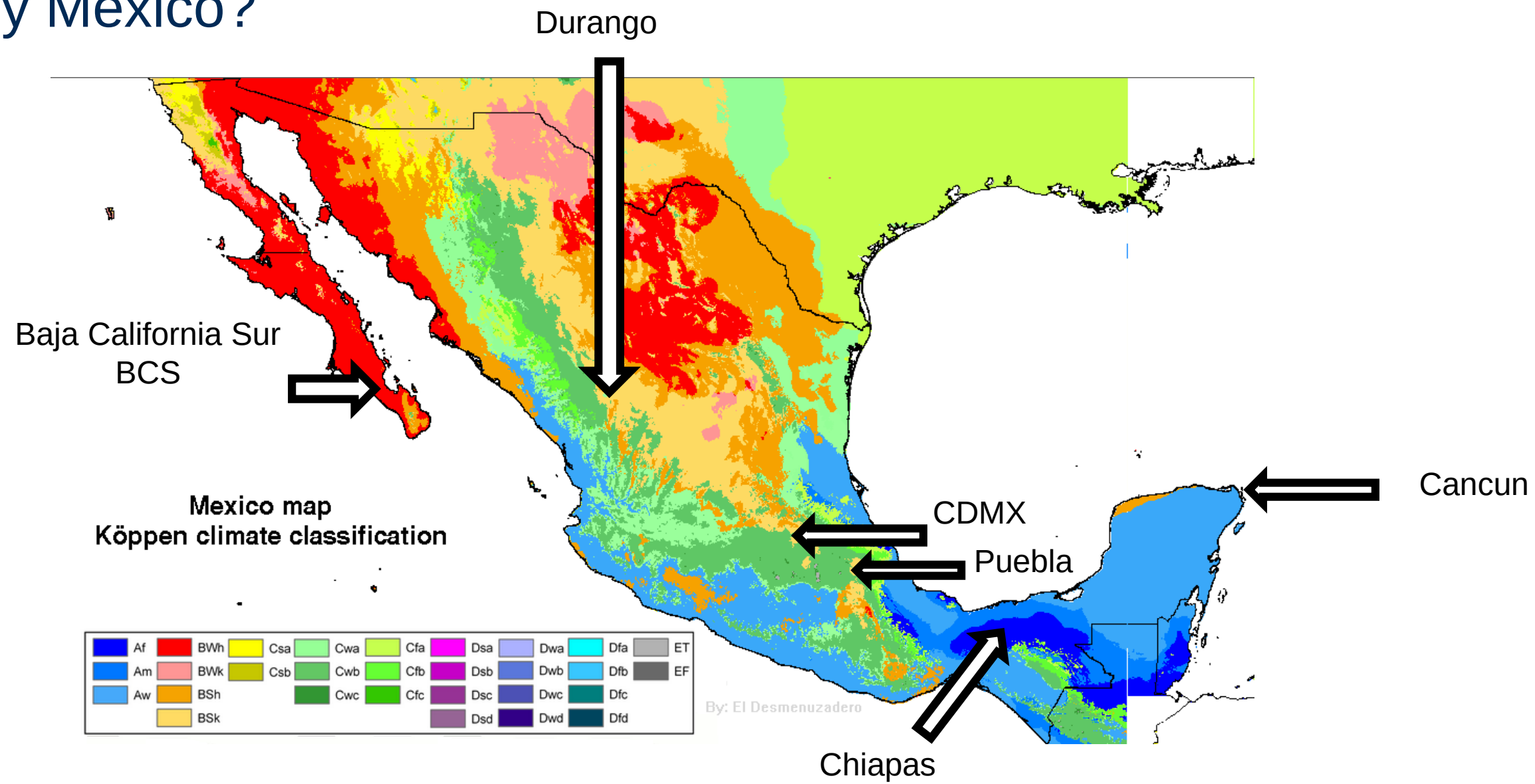
Mejía Domínguez, A. M., Soster-Contreras, M. d. l. Á., Campos-Morales, N., Sánchez-Díaz, M. d. R., Chavira-Trujillo, G., Cruz-Hervert, L. P., & Jiménez-Corona, M. E. (2024). Prevalence of HIV, Syphilis, and Hepatitis B and C Among Blood Donors in a Tertiary Care Hospital in Mexico. Venereology, 3(4), 172-182. https://doi.org/10.3390/venereology3040014

Collection Centers



Center	Average Monthly Donations
CDMX	5000
Puebla	1800
Durango	600
Cancun	1000
Chiapas	700
BCS	700

Why Mexico?


Durango
Baja California Sur
BCS
Mexico map
Köppen climate classification
Af
Am
Aw
BWh
BWk
BSh
BSk
Csa
Csb
Cwa
Cwb
Cwc
Cfa
Cfb
Cfc
Dsa
Dsb
Dsc
Dsd
Dwa
Dwb
Dwc
Dwd
Dfa
Dfb
Dfc
Dfd
ET
EF
By: El Desmenuzadero
CDMX
Puebla
Cancun
Chiapas

Summary

Paul-Ehrlich-Institut

Collected

	Pools (100)		MetaPools (1.000)	
	Acepted Donors	Rejected Donors	Acepted Donors	Rejected Donors
CDMX	496	320	51	33
Puebla	15	0	3	0
Cancun	36	9	6	3
Chiapas	4	0	1	0
Durango	5	0	3	0
BCS	5	5	2	1
Total Pools	**561**	**334**	**66**	**37**
	895		**103**	
Donors	**56.100**	**33.400**		
	89.500			

Sequenced

	Pools (100)		MetaPools (1.000)	
	Acepted Donors	Rejected Donors	Acepted Donors	Rejected Donors
CDMX	351 (70%)	103 (32%)	47 (92%)	23 (70%)
Puebla	10 (66%)	0	2 (66%)	0
Cancun	17 (47%)	6 (66%)	3 (50%)	3 (100%)
Chiapas	4 (100%)	0	1 (100%)	0
Durango	2 (40%)	0	1 (33%)	0
BCS	5 (100%)	5 (100%)	2 (100%)	1 (100%)
Total	**389 (69%)**	**114 (34%)**	**56 (85%)**	**27 (73%)**
	503 (56%)		**83 (80%)**	

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THANK YOU

FOR YOUR ATTENTION