New Solutions to Enhance Testing Efficiency and Blood Safety

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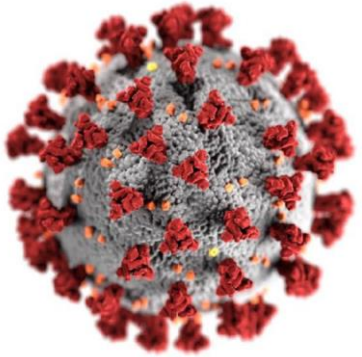
Disclaimers

- Procleix Plasmodium assay and Procleix ArboPlex assay are under development and performance characteristics have not been determined; both assays are not available for commercial sale
- Procleix is a trademark of Grifols Worldwide Operations Limited
- Panther is a trademark of Hologic, Inc.

Topics

- Grifols NAT Research & Development update
- New Assays under development for use on the Procleix Panther system:
  - Procleix Plasmodium assay: new assay for detection of 5 species of *Plasmodium*
  - Procleix ArboPlex assay: new multiplex assay for detection of West Nile, Usutu, chikungunya, dengue and Zika viruses



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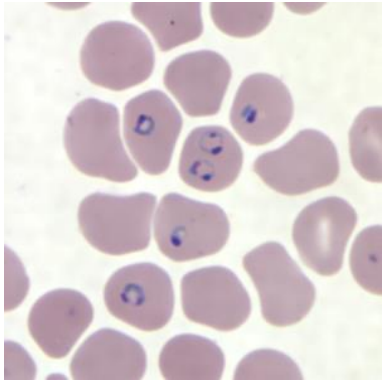
Scott Bauer. (USDA ARS)



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Grifols NAT Research & Development Update

- Procleix UltrioPlex E assay
  - Multiplex assay for detection of 5 viruses: HIV-1, HIV-2, HCV, HBV, and HEV
  - Launched in Japan for nation-wide screening: August 2020
  - CE mark: January 2021
- Procleix Babesia assay
  - Detection of 4 *Babesia* species: *B. microti*, *B. divergens*, *B. duncani*, and *B. venatorum*
  - US FDA approval: January 2019; CE mark: January 2021
- Procleix Panther featuring ART (Automation Ready Technology)
  - Significant hardware & software improvements to decrease “hands-on” time and total labor hours
  - Simplified user management through connection of multiple instruments to track system and control from central dashboard
  - CE mark: October 2019; US FDA approval: April 2020
- Procleix SARS-CoV-2 assay
  - Same technology as blood screening assays (magnetic-based target capture, TMA, and chemiluminescent detection) with modifications for respiratory specimen testing
  - Available commercially in selected EU markets; organ-tissue donor screening in US (under US FDA Emergency Use Authorization)
  - Research studies at Vitalant Research Institute & American Red Cross
  - CE mark: May 2020

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Procleix Plasmodium Assay (Under Development)

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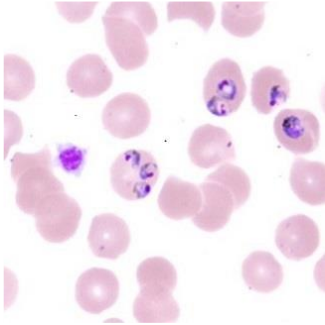
Procleix Plasmodium Assay on Panther System

NAT under development for detection of *Plasmodium* ribosomal RNA



- Requires whole blood sample lysis step performed manually or on automated pipettor (Procleix Xpress System)
  - Whole blood added to Parasite Transport Medium (PTM) to disrupt red blood cells, parasites, and stabilize *Plasmodium* 18s rRNA
  - Sample preparation identical to US FDA licensed and CE-marked Procleix Babesia assay
  - Testing of individual or pooled lysates (16-lysate samples)
- Assay designed to detect five *Plasmodium* species with equivalent sensitivity:
  - *P. falciparum, P. knowlesi, P. malariae, P. ovale, P. vivax*
- RUO version of assay currently used in research study to evaluate donors deferred for malaria risk

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Research Study: Malaria Risk in Deferred Donors

Two Phases using RUO version of Procleix Plasmodium assay

Phase 1

Preliminary Clinical Specificity

- 10,000 – 20,000 unique, unlinked US donations
- Testing at American Red Cross (ARC, Gaithersburg, MD)
- Initially reactive results:
  - Re-tested in duplicate using the same lysate
  - New lysate tested in triplicate
  - Further confirmatory testing with alternative NAT, antigen, and antibody detection
- Confirmed positive samples to be evaluated in lysate pools of 8 and 16

Phase 2

Screening Deferred Donors

- 5,000 deferred donors collected in US and Canada
- Testing at American Red Cross (ARC, Gaithersburg, MD USA)
- 3 individual lysates each tested in singlet to increase sensitivity
  - Estimated to allow 97% probability of detecting ~1 parasite/mL, based on a 95% limit of detection of ~3 parasites/mL
- Initially reactive results: re-tested same as Phase 1
- Confirmatory testing: same as Phase 1
- Confirmed positive samples to be evaluated in lysate pools of 8 and 16

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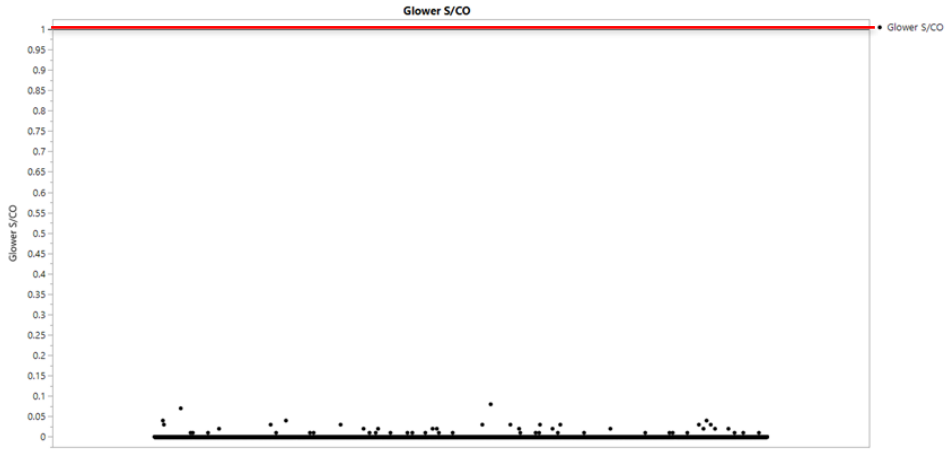
Preliminary Clinical Specificity of Procleix Plasmodium Assay

Phase 1 Results

- 10,751 fresh whole blood donations collected by ARC: June 21, 2019 to January 16, 2020

# Specimens Tested	# Initial Reactive	# Nonreactive	# Confirmed Positive	% Specificity (95% CI)
10,751	0	10,751	0	100 (99.966 –100)

#: number; CI: Confidence Interval



100% specificity observed in normal donors collected in the US

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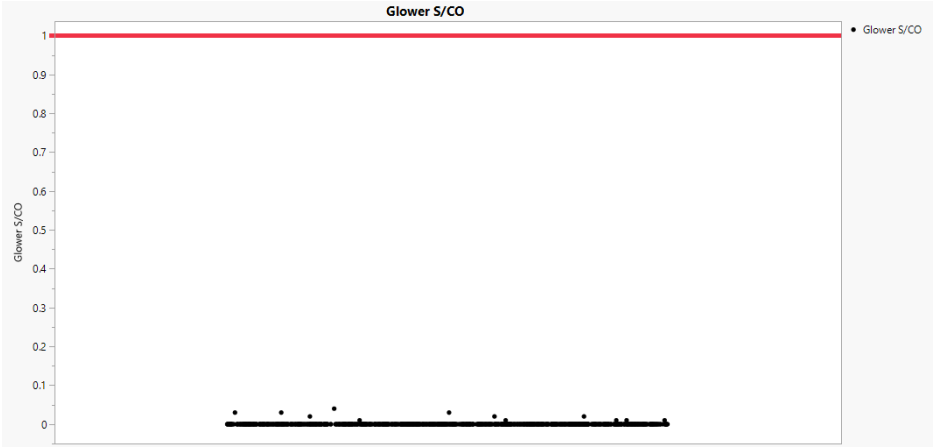
Plasmodium RNA in US Donors Deferred for Malaria Risk

Phase 2 (interim results)

- 315 deferred US donors tested with Procleix Plasmodium RUO Assay (3 individual lysates tested)
  - ~15% born in the US / ~85% born outside of the US (India ~33%, Brazil ~16%, Mexico ~8%, Philippines and China ~4%, Columbia ~3%)
  - ~80% never been in Africa / ~20% lived or traveled to Africa

# Donations Tested	# Initial Reactive	# Nonreactive	# Confirmed Positive
315	0	945	0

#: number; CI: Confidence Interval



No reactive donors were identified among deferred donors tested thus far

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Procleix Plasmodium Assay

Preliminary analytical sensitivity (RNA copies/mL)

Limit of Detection (LoD) by probit analysis using *in vitro* synthesized transcripts for 5 Plasmodium species in copies/mL

In vitro transcript, N=24	50% LoD in Copies/mL (95% Fiducial Limits)	95% LoD in Copies/mL (95% Fiducial Limits)
P. falciparum	**1.9 (1.0 - 2.7)**	**7.0 (4.6 – 17.4)**
P. knowlesi	**4.9 (3.5 - 6.4)**	**12.5 (9.2 – 21.0)**
P. malariae	**2.3 (1.2 – 3.3)**	**11.6 (7.3 – 29.7)**
P. ovale	**6.7 (4.9 – 8.3)**	**12.3 (10.1 – 18.5)**
P. vivax	**4.5 (3.0 – 6.2)**	**16.3 (11.1 – 32.5)**

- **Similar LoD values for 5 species tested**
- **95% LoD ranged from 7.0 to 16.3 copies/mL (similar to other Procleix assays)**

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Procleix Plasmodium Assay

Preliminary analytical sensitivity (parasitized RBCs/mL)

- Cultured *P. falciparum* infected erythrocytes and naturally infected specimens for 4 species
- Infected erythrocytes quantified by Fluorescence-Activated Cell Sorting (FACS)
- Naturally infected human whole blood quantified with research real-time Plasmodium TMA assay

Sample type, N=16	50% LoD in pRBC/mL (95% Fiducial Limits)	95% LoD in pRBC/mL (95% Fiducial Limits)
***P. falciparum*-infected erythrocytes**	**0.6 (0.3 - 0.9)**	**2.6 (1.7 – 6.3)**
P. falciparum*	**0.4 (0.0 – 0.7)**	**3.2 (1.9 – 11.1)**
P. malariae*	**0.7 (0.3 – 1.1)**	**3.3 (2.2 – 6.9)**
P. ovale*	**1.5 (1.0 – 2.0)**	**5.0 (3.6 – 9.3)**
P. vivax*	**0.9 (0.5 – 1.2)**	**2.8 (2.0 – 5.8)**

Cultured *P. falciparum* infected erythrocytes obtained from New York Blood Center; * Naturally Infected Human Whole Blood obtained from Wadsworth Center, NYSDOH

- **Similar LoD values for 4 species tested**
- **95% LoD ranged from 2.6 to 5.0 pRBC/mL (similar to Procleix Babesia Assay)**

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Procleix Plasmodium Assay

Preliminary analytical sensitivity (IU/mL): 1st WHO International Standard (04-176)

- Freeze-dried whole blood preparation collected from a patient by exchange transfusion
- Resuspended in 0.5 mL of nuclease-free water for 20 min; concentration 1 x 10^9 IU/mL
- Half-log dilutions from 10,000 IU/mL to 3.16 IU/mL

1st WHO IS (04-176), IU/mL	% Reactivity, n = 24
10,000	100
3,160	100
1,000	100
316	100
100	100
31.6	100
10	79
3.16	33
0	0

Note: the Plasmodium 1st WHO IS was developed for nucleic acid tests targeting **DNA**. Procleix Plasmodium assay targets **RNA**; handling of the material is critical

1st WHO IS (04-176)	50% LoD in IU/mL (95% Fiducial Limits)	95% LoD in IU/mL (95% Fiducial Limits)
P. falciparum	5.0 (2.7 – 6.9)	16.6 (11.2 – 44.3)

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Procleix ArboPlex Assay (Under Development)

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Summary of Design Goals for the ArboPlex Assay

Assay under development for detecting 5 arboviruses

DENV | CHIKV
ZIKV | IC
WNV | USUV

- Detect 5 viruses and internal control (IC) in one reaction:
  - Dengue viruses (DENV): types 1-4
  - Chikungunya virus (CHIKV): West African, East/Central/South African, and Asian genotypes
  - Zika virus (ZIKV): African and Asian lineages
  - West Nile virus (WNV): lineages 1 and 2 and Usutu virus (USUV)
- Use existing technology: magnetic-based target capture, TMA, and chemiluminescent detection
  - Compatible with existing Panther instruments (or Panther ART) at current testing sites; no new hardware or major system SW changes needed
- No increase in staffing needs—no increase in turn-around time
- Detection of WNV/USUV from initial reactive result (no discrimination)
- Maintain or improve performance compared to the Procleix Dengue, Zika and WNV assays

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Procleix ArboPlex Assay

Preliminary analytical sensitivity: WNV

- Lineage 1: virus in processed plasma (defibrinated, delipidated citrate sodium plasma)
- Lineage 2: *in vitro* synthesized transcript

WNV Lineage 1 $TCID_{50}$/mL	ArboPlex Assay		Procleix WNV Assay	
	# Reactive / # Tested	% Reactivity	# Reactive / # Tested	% Reactivity
0.692	20/20	100%	20/20	100%
0.347	20/20	100%	20/20	100%
0.115	19/20	95%	20/20	100%
0.035	13/20	65%	14/20	70%
0.011	5/20	25%	10/20	50%
0	0/20	0%	0/20	0%

WNV Lineage 2 Copies/mL**	ArboPlex Assay		Procleix WNV Assay	
	# Reactive / # Tested	% Reactivity	# Reactive / # Tested	% Reactivity
60	20/20	100%	20/20	100%
30	20/20	100%	20/20	100%
10	19/20	95%	16/20	80%
3	12/20	60%	5/20	25%
1	1/20	5%	2/20	10%
0	0/20	0%	0/20	0%

** RNA *in vitro* transcript (IVT)

Assay	Lineage (strain)	Detection Probabilities, $TCID_{50}$/mL or Copies/mL	
		50% (95% Fiducial Limit)	95% (95% Fiducial Limit)
ArboPlex	Lineage 1 (NY2001-6263)	0.025 (0.013-0.037)	0.110 (0.069-0.269)
Procleix WNV Assay		0.014 (0.004-0.022)	0.079 (0.047-0.347)
ArboPlex	Lineage 2 (Hungary 2004)	3.13 (2.03 – 4.28)	8.86 (6.30 – 15.86)
Procleix WNV Assay		5.27 (3.49 – 7.35)	16.23 (10.94 – 35.15)

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Procleix ArboPlex Assay

Preliminary Analytical Sensitivity: WNV lineages 1 and 2 and Usutu virus

Lineage (Strain)	$TCID_{50}$/mL or c/mL	% Reactive	
		ArboPlex Assay	Procleix WNV assay
WNV L1 (NY2001-6263)	0.347 $TCID_{50}$/mL	100%	100%
	0.115 $TCID_{50}$/mL	90%	100%
WNV L1* (NY99)	30 c/mL	90%	100%
	10 c/mL	90%	90%
WNV L2* (2014/Hungary)	30 c/mL	90%	100%
	10 c/mL	40%	10%
WNV L2* (Italy/Montova/40.1)	30 c/mL	90%	50%
	10 c/mL	80%	10%
WNV L2 (1986)	0.036 $TCID_{50}$/mL	100%	100%
	0.012 $TCID_{50}$/mL	80%	100%
WNV L2 (B-956 Uganda)	0.479 $TCID_{50}$/mL	100%	100%
	0.162 $TCID_{50}$/mL	100%	80%
USUV Europe 3* (1477)	100 c/mL	100%	NA
	30 c/mL	90%	
USUV African 3* (491)	100 c/mL	100%	
	30 c/mL	70%	

* RNA *in vitro* transcript (IVT); NA = Not applicable due to USUV panel levels being below the limit of detection with Procleix WNV assay

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Procleix ArboPlex Assay

Preliminary analytical sensitivity: WHO International Standard ZIKV (11468/16)

ZIKV PEI Code 11468/16, IU/mL*	ArboPlex Assay		Procleix Zika Virus Assay	
	# Reactive / # Tested	% Reactivity	# Reactive / # Tested	% Reactivity
30	20/20	100%	NT	NT
10	20/20	100%	20/20	100%
3	19/20	95%	20/20	100%
1	14/20	70%	16/20	80%
0.3	4/20	20%	8/20	40%
0	0/20	0%	0/20	0%

*Diluted in processed plasma: defibrinated, delipidated citrate sodium plasma; NT = Not tested

Assay	Detection Probabilities, IU/mL	
	50% (95% Fiducial Limit)	95% (95% Fiducial Limit)
ArboPlex	0.70 (0.38 – 1.01)	2.71 (1.80 – 5.96)
Procleix Zika Virus Assay	0.42 (0.17 – 0.63)	1.69 (1.09 – 5.39)
Procleix Zika Virus Assay Package Insert*	0.64 (0.54 – 0.76)	2.90 (2.22 – 4.18)

* tested in negative plasma

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Procleix ArboPlex Assay

Preliminary analytical sensitivity: Dengue virus types 1 & 4

- DENV-1 and DENV-4 *in vitro* synthesized transcripts tested

DENV-1 Copies/mL	ArboPlex Assay		Procleix Dengue Virus Assay	
	# Reactive / # Tested	% Reactivity	# Reactive / # Tested	% Reactivity
100	20/20	100%	20/20	100%
30	20/20	100%	20/20	100%
10	16/20	80%	15/20	75%
3	9/20	45%	9/20	45%
1	7/20	35%	3/20	15%
0	0/20	0%	0/20	0%

DENV-4 Copies/mL	ArboPlex Assay		Procleix Dengue Virus Assay	
	# Reactive / # Tested	% Reactivity	# Reactive / # Tested	% Reactivity
100	20/20	100%	20/20	100%
30	19/20	95%	19/20	95%
10	17/20	85%	10/20	50%
3	8/20	40%	6/20	30%
1	4/20	20%	0/20	0%
0	0/20	0%	0/20	0%

Assay	DENV Type	Detection Probabilities, Copies/mL	
		50% (95% Fiducial Limit)	95% (95% Fiducial Limit)
ArboPlex	DENV-1	2.80 (1.28 – 4.46)	20.28 (11.69 – 62.84)
Procleix Dengue Virus Assay		4.16 (2.41 – 6.11)	19.12 (12.06 – 45.83)
ArboPlex	DENV-4	3.81 (2.00 – 5.88)	23.91 (14.54 – 57.73)
Procleix Dengue Virus Assay		8.66 (5.65 – 12.16)	30.95 (20.76 – 62.01)

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Procleix ArboPlex Assay

Preliminary Analytical Sensitivity: Dengue virus types 1-4 (*in vitro* synthesized transcripts)

DENV Type (Strain)	Copy/mL	% Reactive, n = 10	
		ArboPlex Assay	Procleix Dengue Assay
Type 1 (Hawaii)	100	100%	100%
	30	100%	90%
	10	90%	90%
Type 2 (New Guinea C)	100	100%	100%
	30	100%	100%
	10	70%	70%
Type 3 (80-2)	100	100%	100%
	30	100%	100%
	10	90%	80%
Type 4 (H241)	100	100%	100%
	30	90%	100%
	10	80%	80%

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Procleix ArboPlex Assay

Preliminary Analytical Sensitivity: WHO International Standard Chikungunya Virus (11785/16)

CHIKV PEI Code 11785/16, IU/mL*	ArboPlex Assay		Real-Time Triplex Research Assay (RUO)	
	# Reactive / # Tested	% Reactivity	# Reactive / # Tested	% Reactivity
30	20/20	100%	20/20	100%
10	19/20	95%	18/20	90%
3	15/20	75%	9/20	45%
1	6/20	30%	5/20	25%
0.3	4/20	20%	0/20	0%
0	0/20	0%	0/20	0%

*Prepared in processed plasma (defibrinated, delipidated citrate sodium plasma)

Assay	Detection Probabilities, IU/mL	
	50% (95% Fiducial Limit)	95% (95% Fiducial Limit)
ArboPlex Assay	1.55 (0.86 – 2.37)	9.27 (5.60 – 22.83)
Real-Time Triplex Research Assay (RUO)	3.28 (2.13 – 4.66)	12.32 (8.13 – 25.29)

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Procleix ArboPlex Assay

Preliminary Analytical Sensitivity: CHIKV East/Central/South African, West African, Indian Ocean and Asian lineages

CHIKV lineage* (Strain)	Copies/mL	% Reactive, n =10
East/Central/South African (S27)	100	100%
	30	100%
	10	90%
West African (SH2830)	100	100%
	30	100%
	10	60%
Indian Ocean (BNI-CHIKV 899)	100	100%
	30	100%
	10	100%
Asian (99659)	100	100%
	30	100%
	10	80%

**in vitro* synthesized transcripts

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Summary

- Grifols continues to develop multiplex assays to consolidate testing into a single reaction
  - Procleix UltrioPlex E assay (HIV-1, HIV-2, HCV, HBV, and HEV)
  - Procleix ArboPlex assay (WNV, USUV, DENV, ZIKV, and CHIKV)
- Panther ART will allow advances in automation
  - Increase in efficiency possible by decreasing hands-on time and centralized control of multiple instruments
- Procleix Plasmodium assay
  - Demonstrated high specificity and sensitivity (both in RNA copies/mL and parasitized red cells)
  - Sensitive detection of ribosomal RNA *could* change current testing and deferral strategies (possible elimination of antigen or antibody detection assays)
- Procleix ArboPlex assay
  - Preliminary results using a wide range of genetic variants showed similar or improved analytical sensitivity compared to current monoplex assays
  - Multiplex testing will help address the unpredictable nature of arboviral outbreaks

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