Design Verification

PHOSPHORUS liquirapid

CONTENTS

1. Introduction

The performance characteristics of PHOSPHORUS liquirapid multipurpose reagent have been tested and documented in order to verify the clinical usefulness and compliance with the essential requirements of directive 98/79/EC.

2. Imprecision

The imprecision (within-run and day-to-day) of the PHOSPHORUS liquirapid method was calculated from 6 determinations on 10 consecutive days. For sample material, low, medium and high concentration control sera were used.

Results

No. of test	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
Low control serum										
1	2.90	2.81	2.85	2.90	2.84	2.93	2.93	2.85	2.81	2.85
2	2.89	2.80	2.88	2.84	2.82	2.92	2.86	2.79	2.77	2.80
3	2.88	2.83	2.98	2.92	2.87	3.01	2.88	2.78	2.80	2.86
4	2.88	2.82	2.83	2.87	2.86	2.93	2.87	2.73	2.79	2.83
5	2.92	2.76	2.89	2.87	2.87	2.95	2.86	2.86	2.76	2.88
6	2.85	2.88	2.86	2.88	2.88	2.94	2.92	2.85	2.77	2.83
Medium control serum										
1	4.80	4.71	4.99	4.69	4.81	4.08	4.51	4.69	4.42	4.51
2	4.93	4.80	4.78	4.80	4.91	4.15	4.44	4.67	4.44	4.41
3	4.76	4.63	4.88	4.90	4.54	4.25	4.54	4.70	4.61	4.54
4	4.87	4.72	4.97	4.82	4.87	4.31	4.63	4.68	4.59	4.50
5	4.84	4.67	4.98	4.80	4.75	4.27	4.67	4.66	4.59	4.60
6	4.82	4.67	4.96	4.83	4.67	4.30	4.59	4.60	4.58	4.56
High control serum										
1	10.0	10.3	11.1	10.8	10.6	10.7	9.85	9.94	10.5	10.7
2	9.70	10.4	11.1	10.8	10.6	10.8	10.0	10.3	10.4	10.7
3	9.94	10.4	11.1	10.8	10.6	10.7	9.78	10.1	10.6	10.7
4	9.65	10.3	10.9	10.6	10.5	10.5	9.63	10.3	10.5	10.4
5	10.3	10.6	10.9	10.8	10.3	10.8	10.0	10.3	10.6	10.4
6	10.1	10.4	10.8	10.5	10.7	10.6	10.0	10.3	10.6	10.4

	Intra-assay		Inter-assay	
Mean (mg/dl)	**SD (mg/dl)**	**%CV**	**SD (mg/dl)**	**%CV**
2.86	0.035	1.2	0.057	2.0
4.65	0.081	1.7	0.215	4.6
10.5	0.149	1.4	0.373	3.6

3. Linearity

The linearity of PHOSPHORUS liquirapid multipurpose reagent was tested on an AU 480 analyser. A high concentrated pool serum was diluted successively with physiological saline. The evaluation is performed by calculating the first, second and third derivation of the regression line of the analyzed concentrations ('actual mean', n = 2) and the assessment of the deviation between the 3 regression lines.

Criteria

	Acceptance criteria
Deviation of 2^{nd}-1^{st} and 3^{rd}-1^{st} regression lines	≤ 10,0 %

Used Material

Reagent	Manufacturer	REF	LOT
PHOSPHORUS liquirapid	HUMAN	PHOREA010*	0052

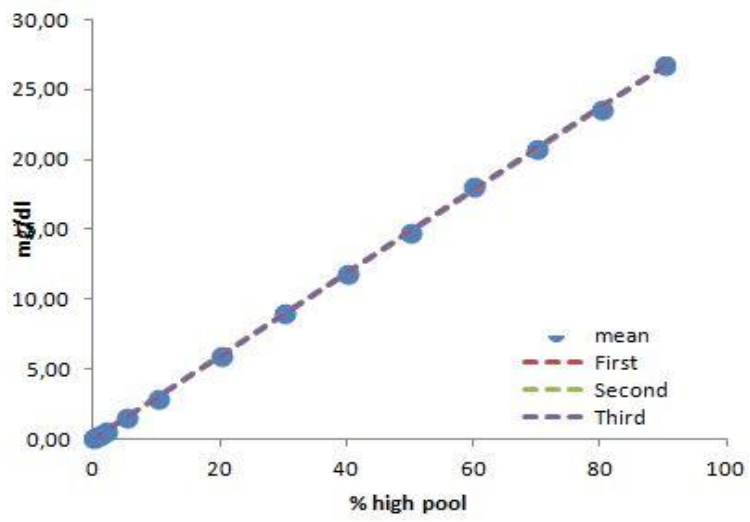
AUTOCAL	HUMAN	13160	H016
Sample	Diluted high concentrated pool serum: BOG303		

*production batch, transferred to system reagent bottle

Results

% Dilution	Actual Mean (mg/dl)	% Difference	
		$2^{nd} - 1^{st}$	$3^{rd} - 1^{st}$
0	0.07	-28.6	0
0.5	0.21	-4.8	4.8
1	0.34	-2.9	2.9
2	0.62	-1.6	0
5	1.49	-0.7	-0.7
10	2.93	0	-1
20	5.93	0.2	-0.5
30	8.99	0.2	-0.1
40	11.8	0.3	0.2
50	14.8	0.1	0.2
60	18.1	0.1	0.2
70	20.8	0	0.2
80	23.6	0	0
90	26.7	-0.1	-0.3

Graphic



Linearity range

0.2 – 20.0 mg/dl at least

The linear range of PHOSPHORUS liquirapid multipurpose reagent fulfilled the acceptance criteria.

Conclusion

The linearity range of PHOSPHORUS liquirapid multipurpose reagent was found as follows:
0.2 – 20.0 mg/dl

4. Sensitivity

A 20-fold determination of a 0 mg/dl calibrator (phys. saline) on a Hitachi 717 analyser revealed an absolute mean of -0.01 mg/dl and a SD of 0.02 mg/dl. From the three-fold standard deviation the analytical sensitivity can therefore be calculated to < 0.08 mg/dl phosphorus.

5. Recovery of Control Sera

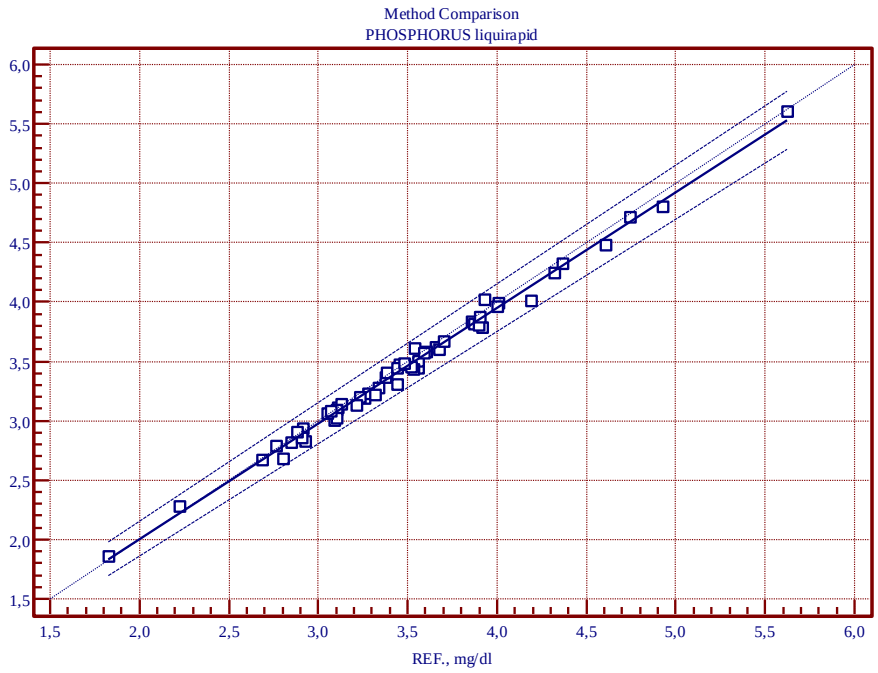
Commercially available control sera have been employed. The control sera have been reconstituted/prepared according to the manufacturer's instructions. Fivefold determinations of each control serum have been performed with the PHOSPHORUS liquirapid method. The means of the fivefold determination have been calculated and compared with the target values.

CONTROL SERUM RECOVERY				HUMAN fresh		Reference		HUMAN 12 d 56 °C	
Control serum	LOT	Target mg/dl	Range mg/dl	Result mg/dl	Deviation (%)	Result mg/dl	Deviation (%)	Result mg/dl	Deviation (%)
HUMATROL N	019	5.5	4.68 – 6.33	5.44	-1.09	5.52	0.36	5.51	0.18
HUMATROL P	017	9.2	7.82 – 10.6	8.96	-2.61	9.14	-0.65	8.94	-2.83
SERODOS	6868	4.9	4.17 – 5.60	4.92	0.41	4.95	1.02	4.87	-0.61
SERODOSplus	6795	10.3	8.76 – 11.85	9.45	-8.25	9.67	-6.12	9.21	-10.58
Precinorm	154916	3.94	3.34 – 4.54	3.58	-9.14	3.59	-8.88	3.51	-10.91
Precipath	199459	6.01	5.11 – 6.91	5.97	-0.67	6.03	0.33	5.88	-2.16
Summary		39.85		38.32		38.9		37.92	

6. Comparison of Methods

The PHOSPHORUS liquirapid has been compared against a commercially available Phosphorus method. Control sera as well as patient samples have been employed in the comparison (N=55).
The results have been evaluated by a main component analysis. The linear regression obtained could be described as follows:

r =	**0.996**
Y =	**0.973 * X + 0.057**
X_{mean} =	**3.48 mg/dl**
Y_{mean} =	**3.45 mg/dl**



Both methods showed a good agreement and no significant deviation could be observed with any specific sample.
The graph on the following page show the results obtained with a non-parametric regression analysis acc. to Passing-Bablok.

7. Interference

Interference of PHOSPHORUS liquirapid multipurpose reagent was studied by adding known amounts of the potentially interfering substance to a known sample. The deviation in recovery of spiked and unspiked samples was calculated and compared.

Criteria

Check	Acceptance criteria
Recovery spiked samples vs. unspiked sample	90 – 110%

Used Material

Reagent	Manufacturer	REF	LOT
PHOSPHORUS liquirapid	HUMAN	PHOREA010 (10027)	0052
AUTOCAL	HUMAN	13160	H016
Samples	Samples spiked with interfering substance		

Results

Ascorbic acid			Bilirubin			Hemoglobin		
Concentration	Analytical Results		Concentration	Analytical Result		Concentration	Analytical Result	
mg/dl	mg/dl	%	mg/dl	mg/dl	%	mg/dl	mg/dl	%
0	5.84	100	0	5.79	100	0	6.58	100
2	5.84	100.0	4	5.44	94.0	50	5.98	90.9
4	5.88	100.8	8	5.33	92.1	100	5.40	82.1
6	5.86	100.4	12	5.22	90.2	150	4.86	73.8
8	5.87	100.6	16	5.02	86.7	200	4.29	65.2
10	5.94	101.7	20	4.90	84.6	250	3.71	56.3
12	5.88	100.8	24	4.79	82.6	300	3.09	46.9
14	5.90	101.0	28	4.61	79.5	350	2.68	40.7
16	5.88	100.7	32	4.45	76.8	400	1.89	28.6
18	5.86	100.3	36	4.22	72.8	450	1.39	21.1
20	5.92	101.4	40	4.08	70.4	500	0.73	11.1
Glick number		**1**			**3**			**4**

Triglycerides		
Concentration	Analytical Results	
mg/dl	mg/dl	%
0	4.99	100
250	5.15	103.1
500	5.21	104.3
750	5.24	104.9
1000	5.31	106.4
1250	5.46	109.4
1500	5.44	109.0
1750	5.55	111.2
2000	5.54	110.9
2250	5.59	112.0
2500	5.68	113.7
Glick number		**2**

Conclusion

No interference of PHOSPHORUS liquirapid multipurpose reagent was detected up to following concentrations:

Interfering substance	AU 480
Ascorbic acid	up to 20 mg/dl
Bilirubin	up to 12 mg/dl
Hemoglobin	up to 50 mg/dl / strong interference
Triglycerides	up to 1500 mg/dl

Visibly icteric, hemolytic and lipemic samples require a sample blank to correct for potential interferences.

8. Stability

8.1 Linearity

Used Material

Instrument	Manufacturer
AU 400	Beckman Coulter

Reagent	Manufacturer	REF	LOT
PHOSPHORUS liquirapid	HUMAN	10027/ PHOREA010*	0052/24 months*
PHOSPHORUS liquirapid	HUMAN	10027	16003 / 36 months
PHOSPHORUS liquirapid	HUMAN	10027	16004 / 36 months
AUTOCAL	HUMAN	13160	H016; 0017
Sample	Linearity pools: LOT LKU079; BOG303; physiological saline		

*Transfer LOT for production

8.1.1 Linearity – LOT 0052

The linearity of PHOSPHORUS liquirapid multipurpose reagent, REF 10027, was controlled by employing a high concentrated linearity pool successively diluted in different steps with a physiological saline. The analysed concentrations were compared with the theoretical concentrations obtained from a linear regression.
The linearity of the test was evaluated over the difference between best fitting nonlinear polynomial and linear polynomial. In this case difference from the 2nd to 1st order was used. In accordance with the European Society for External Quality Assessment, the specification is based on a deviation of up to 0.37 mg/dl to 2.32 mg/dl phosphate and up to 16% from a concentration of 2.32 mg/dl phosphate.

Criteria Linearity

Range limit		Acceptance criteria max. Deviation from linearity	
From	To	Value	Unit
0	2.32	0.37	mg/dl
2.32	20	16	%

Results

Linearity for LOT 0052 after 24 months							
High pool	Mean	Predicted Polynominal		Difference (3rd – 1st)		Specification	
	mg/dl	1st-order	3rd-order	mg/dl	%	≤	
0	0.03	0.04	0.02	-0.02	-80.0	0.371 mg/dl	✓
0.5	0.14	0.17	0.15	-0.02	-14.3	0.371 mg/dl	✓
1	0.27	0.3	0.28	-0.02	-7.5	0.371 mg/dl	✓
2	0.54	0.56	0.54	-0.02	-3.7	0.371 mg/dl	✓
5	1.31	1.34	1.33	-0.01	-0.8	0.371 mg/dl	✓
10	2.69	2.64	2.64	0	0.0	16 %	✓
20	5.25	5.23	5.25	0.02	0.4	16 %	✓
30	7.83	7.83	7.86	0.03	0.4	16 %	✓
40	10.45	10.42	10.46	0.04	0.4	16 %	✓
50	13.04	13.01	13.05	0.04	0.3	16 %	✓
60	15.77	15.61	15.64	0.03	0.2	16 %	✓
70	18.24	18.2	18.22	0.02	0.1	16 %	✓
80	20.73	20.8	20.8	0	0.0		> high limit

The PHOSPHORUS liquirapid multipurpose reagent on AU 400 multipurpose reagent is linear from 0 - 20.0 mg/dl.

8.1.2 Linearity LOT 16003 and 16004

The linearity of PHOSPHORUS liquirapid multipurpose reagent on AU 400 was determined by employing a high concentrated linearity pool successively diluted in steps of about 10% with physiological saline. The analysed concentrations (n=2) were compared with the calculated concentrations obtained from a linear regression.
The linearity was investigated for 2 different reagent LOTs.

Results

PHOSPHORUS liquirapid, REF 10027, LOT 16003 / 36 months at 15 – 25°C				
High Pool Content	Analytical Data	Regressed Data	Deviation from Regression Line	
(%)	(mg/dl)	(mg/dl)	(mg/dl)	(%)
0	0.03	0.76	-0.73	
10	3.01	3.18	-0.17	-5.4
20	5.46	5.60	-0.14	-2.5
30	8.11	8.02	0.09	1.1
40	10.9	10.4	0.4	4.1
50	13.6	12.9	0.7	5.4
60	16.1	15.3	0.8	5.2
70	18.3	17.7	0.6	3.5
80	19.9	20.1	-0.2	-1.0
90	21.1	22.5	-1.4	-6.2
100	21.9	25.0	-3.0	-12.2

After 36 months storage at 15-25°C, all real-time linearity of PHOSPHORUS liquirapid multipurpose reagent on AU 400 is at least up to 20 mg/dl.

PHOSPHORUS liquirapid, REF 10027, LOT 16004 / 36 months at 15 – 25°C				
High Pool Content	Analytical Data	Regressed Data	Deviation from Regression Line	
(%)	(mg/dl)	(mg/dl)	(mg/dl)	(%)
0	0.03	0.76	-0.73	
10	3.01	3.18	-0.17	-5.4
20	5.46	5.60	-0.14	-2.5
30	8.11	8.02	0.09	1.1
40	10.9	10.4	0.4	4.1
50	13.6	12.9	0.7	5.4
60	16.1	15.3	0.8	5.2
70	18.3	17.7	0.6	3.5
80	19.9	20.1	-0.2	-1.0
90	21.1	22.5	-1.4	-6.2
100	21.9	25.0	-3.0	-12.2

After 36 months storage at 15-25°C, all real-time linearity of PHOSPHORUS liquirapid multipurpose reagent on AU 400 is at least up to 20 mg/dl.

Conclusion

All real-time stability data for linearity for PHOSPHORUS liquirapid multipurpose reagent confirm shelf life of 22 months at 2...25°C.

8.2 Recovery in Control Sera

A number of commercially available control sera have been employed according to the procedure already described in section 4 on an AU 400. The mean values obtained with fresh reagent (=reference) and 1 reagent LOT have been calculated and compared with the fresh means of the respective control sera.

Criteria

Check	Acceptance criteria
Recovery	within range
Mean deviation of Real time Test to Reference	≤ 10%

Used Material

Reagent	Manufacturer	REF	LOT
PHOSPHORUS liquirapid	HUMAN	10027	20002/reference
PHOSPHORUS liquirapid	HUMAN	10027 / PHOREA010*	0052/24 months*
PHOSPHORUS liquirapid	HUMAN	10027	16003 / 45 months
PHOSPHORUS liquirapid	HUMAN	10027	16004 / 45 months
AUTOCAL	HUMAN	13160	H017
Sample	HUMAN's and commercial controls (Beckman Coulter, INVICON)		

*Transfer LOT for production

Results

Control recovery				Reference	Real time - Test		
				LOT 20002	LOT 0052		
				fresh	24months		
Name	**LOT**	**Target mg/dl**	**Range mg/dl**	**Result mg/dl**	**Result mg/dl**	**Within range YES/NO**	**%Dev to Reference**
HumaTrol N	0006	4.44	3.64 - 5.24	3.59	3.71	YES	3.49
HumaTrol P	0004	9.18	7.53 - 10.8	8.36	8.67	YES	3.71
SERODOS	0004	3.40	2.79 - 4.01	2.99	3.32	YES	11.0
SERODOS^{plus}	0005	9.03	7.40 - 10.7	8.33	8.50	YES	2.10
Seronorm Human	1806848	3.16	2.87 - 3.44	3.36	3.48*	NO	3.57
Seronorm Human high	1801801	9.51	8.65 - 10.4	9.87	9.62	YES	-2.48
Control serum L1	1043	6.67	5.47 - 7.87	6.77	6.82	YES	0.74
Control serum L1	1044	11.6	9.51 - 13.7	11.3	11.5	YES	2.09
%MeanDev							**3.0**

*out of control range; not excluded from calculation

Control recovery				PHOSPHORUS liquirapid, REF 10027			
				Reference	Test		
				LOT 18006	LOT 16003		
				Fresh	45 months stored at 15-25°C		
Name	**LOT**	**Target mg/dl**	**Range mg/dl**	**Result mg/dl**	**Result mg/dl**	**Within range Y/N**	**Def. to Fresh**
HumaTrol N	0006	4.44	3.64 - 5.24	3.78	3.82	YES	1.2
HumaTrol P	0004	9.18	7.53 - 10.8	8.50	8.35	YES	-1.8
SERODOS	0004	3.40	2.79 - 4.01	3.11	3.26	YES	4.8
SERODOSplus	0005	9.03	7.40 - 10.7	8.47	8.19	YES	-3.3
Seronorm	1512606	3.19	2.90 - 3.48	3.41	3.30	YES	-3.1
Seronorm high	1801801	9.51	8.65 - 10.4	9.79	9.42	YES	-3.8
Control serum L1	1041	6.70	5.49 - 7.91	6.87	6.55	YES	-4.7
Control serum L1	1042	11.3	9.23 - 13.3	11.0	10.4	YES	-5.0
						Mean Dev.%	**-1.9**

Control recovery				PHOSPHORUS liquirapid, REF 10027			
				Reference	Test		
				LOT 18006	LOT 16004		
				Fresh	45 months stored at 15-25°C		
Name	**LOT**	**Target mg/dl**	**Range mg/dl**	**Result mg/dl**	**Result mg/dl**	**Within range Y/N**	**Def. to Fresh**
HumaTrol N	0006	4.44	3.64 - 5.24	3.78	3.81	YES	0.8
HumaTrol P	0004	9.18	7.53 - 10.8	8.50	8.31	YES	-2.3
SERODOS	0004	3.40	2.79 - 4.01	3.11	3.23	YES	3.9
SERODOSplus	0005	9.03	7.40 - 10.7	8.47	8.24	YES	-2.8
Seronorm	1512606	3.19	2.90 - 3.48	3.41	3.33	YES	-2.2
Seronorm high	1801801	9.51	8.65 - 10.4	9.79	9.37	YES	-4.2
Control serum L1	1041	6.70	5.49 - 7.91	6.87	6.53	YES	-5.0
Control serum L1	1042	11.3	9.23 - 13.3	11.0	10.4	YES	-5.2
						Mean Dev.%	**-2.1**

With one exception, the control recovery met the acceptance criteria and confirms the reliability of the product.

Conclusion

Real time stability data for control recovery confirm the shelf life of 22 months at 2 – 25°C for PHOSPHORUS liquirapid multipurpose reagent, REF 10027.

9. Traceability

The internal master standard for PHOSPHORUS liquirapid, against which the working standards are calibrated, was standardised using atom absorption spectrometry (AAS) according to DIN 118855. The standards are therefore traceable to this method.