

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE**

A. 510(k) Number:

k053553

B. Purpose for Submission:

New device

C. Measurand:

Urine Albumin (microalbumin)

D. Type of Test:

Solid phase, sandwich-format, immunometric assay

E. Applicant:

Axis-Shield PoC AS

F. Proprietary and Established Names:

NycoCard® U-Albumin
NycoCard® U-Albumin Control

G. Regulatory Information:

1. Regulation section:

21 CFR §866.5040, Albumin, Antigen, Antiserum, Control
21 CFR §862.1660, Quality control material (assayed and unassayed)

2. Classification:

Class II (Special Controls) - This device is exempt from the premarket notification procedures in subpart E of part 807 of this chapter, but subject to the limitations to the exemption outlined in 866.9(c)(5).

Class I, reserved - Controls

3. Product code:

DCF - assay
JJX -control

4. Panel:

82 (Immunology)
75 (Clinical Chemistry)

H. Intended Use:

1. Intended use(s):

See Indications for use.

2. Indication(s) for use:

The NycoCard U-Albumin is intended to measure by immunochemical techniques the albumin (a plasma protein) in urine. Measurement of albumin aids in the diagnosis of kidney and intestinal diseases.

The NycoCard U-Albumin Control Kit consists of two control materials based on human urine. The controls should be used to confirm the efficacy of the MycoCard U-Albumin reagents and correct performance of the test.

3. Special conditions for use statement(s):

For Prescription use only.

4. Special instrument requirements:

NycoCard READER II. (previously cleared under k993131)

I. Device Description:

The NycoCard U-Albumin test and NycoCard U-Albumin controls consists of the following:

- TD/Test Device: 1 x 24 units – plastic device containing a membrane coated with monoclonal anti-albumin antibodies.
- R1/ Dilution Liquid: 1 x 24 x 1.0 mL - Phosphate buffer (pH 5.6), organic solvent (<10%) and a small amount of yellow pigment.
- R2/Conjugate: 1 x 2.0 mL - Borate buffered solution containing monoclonal anti albumin antibodies labeled with ultra-small gold particles.
- R3/Wash Solution: 1 x 2.0 mL - Phosphate buffered NaCl solution (pH 7.4)
- NycoCard U-Albumin Control Kit – 2 x 1 x 1.0 mL

C+/Control positive	1 x 1.0 ml
C- /Control negative	1 x 1.0 ml

Liquid human urine materials with sodium azide (>0.1%). The albumin concentration is printed on the vial labels.

J. Substantial Equivalence Information:

1. Predicate device name(s):

PolyChem Microalbumin, MIC500

2. Predicate 510(k) number(s):

k002674

3. Comparison with predicate:

Similar colorimetric methods for measuring low levels of albumin in human urine are well established. These test methods are based on other predicate test methods and sample recommendations. The NycoCard® U-Albumin test method for the determination of low levels of albumin in human urine is substantially equivalent to other commercially available testing methods.

Predicate Methodology	Immunoturbidimetric
Test Methodology	Colorimetric
Predicate Reagent Storage	2 – 8° C
Test Reagent Storage	2 – 8° C
Predicate Sample types	Urine
Test Sample types	Urine
Predicate Controls	Recommended
Test Controls	Recommended
Correlation studies were performed comparing the urine Microalbumin results generated on the NycoCard U-Albumin k053553, against the PolyChem Microalbumin, MIC500. k020852/A021	40 urine samples spanned from 9 mg/L to 188 m/L. The regression equation was $y = 1.012x - 0.203$ and $r = 0.9974$.

K. Standard/Guidance Document Referenced (if applicable):

None Referenced.

L. Test Principle:

The NycoCard U-Albumin is a solid phase, sandwich-format, immunometric

assay. The test device contains a membrane coated with immobilized albumin specific monoclonal antibodies. When the diluted sample is applied to the test device, the sample flows through the membrane, and immobilized antibodies on the membrane capture the albumin molecules. Albumin trapped on the membrane will bind the gold-antibody conjugate that is added in a sandwich-type reaction. The paper layer underneath the membrane absorbs excess liquid. Due to the bound gold particles, the membrane appears purple with color intensity proportional to the albumin concentration of the sample. The color intensity is measured quantitatively by using the color densitometer NycoCard READER II.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Intra assay Precision

Intra assay precision (within run) was assessed at three levels. Twenty replicates of the same sample within one analytical run were evaluated at three levels. The mean, standard deviation (SD) and coefficient of variation (CV) in percent were calculated. An intra assay CV of < 5% was determined to be acceptable. The data are presented in the table below.

Intra Assay Precision on NycoCard® READER II.
Results are reported in mg/L.

Analyzer NycoCard® READER II		Level 1	Level 2	Level 3
n		20	20	20
U-Albumin	Mean	17.9	51.7	152.5
	SD	0.85	2.52	7.54
	%CV	4.8	4.9	4.9

Inter assay Precision

Inter assay (between run) precision was determined by analyzing duplicates of three different samples in each of ten different runs over ten different occasions. An inter assay CV of < 10% was determined to be acceptable. The data are presented in the table below.

Inter Assay Precision on NycoCard® READER II.
Results are reported in mg/L.

Analyzer NycoCard® READER II		Level 1	Level 2	Level 3
Days		5	5	5
	n	20	20	20
U-Albumin	Mean	12.6	51.8	148.8
	SD	1.10	4.61	8.38
	%CV	8.7	8.9	5.6

b. Linearity/assay reportable range:

Linearity studies

The sponsor claims a reportable range for their assay of 6 – 200 mg/L. Linearity of the assay was evaluated using a dilution series that spanned most of the reportable range of the test. Serial dilution sets were prepared using 7% BSA and made up fresh and assayed with each of three calibrated runs. The results at each level of analyte were averaged and the linear fit was assessed.

The NycoCard® U-Albumin was found to be linear from 17.3 to 189.7 mg/L. The results are summarized below.

Summary of the NycoCard® U-Albumin Linearity Study.
Means in mg/L.

Theoretical Level	Mean Actual Level	Standard Deviation	% CV	Deviation from Theoretical	% Deviation	% Recovery
18.9	17.3	0.58	3.3	-1.5	-8.1	91.9
37.7	35.3	1.15	3.3	-2.4	-6.4	93.6
56.6	56.7	0.58	1.0	0.1	0.1	100.1
75.5	77.0	3.46	4.5	1.5	2.0	102.0
94.3	94.3	3.79	4.0	0.0	0.0	100.0
113.2	115.7	2.31	2.0	2.5	2.2	102.2
132.1	134.7	2.89	2.1	2.6	2.0	102.0
150.9	149.0	3.61	2.4	-1.9	-1.3	98.7
169.8	172.7	2.31	1.3	2.9	1.7	101.7
188.7	189.7	0.58	0.3	1.0	0.5	100.5
Linear Fit Parameters						
Slope: 1.018						
Intercept: -1.378						
r ² : 0.9992						

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Calibration

The NycoCard® U-Albumin test was calibrated using an in house reference preparation and an external control preparation. Both preparations were assayed against the IFCC/BCR/CAP Reference Preparation ERM 470.

The in house reference preparation is prepared using urine ultra filtrated to remove any trace of albumin. Ultra-pure human albumin is then added to a final

concentration of 200 mg/l. The other calibrators at 100, 50, 20, and 10 mg/L are prepared by diluting the 200 mg/L reference preparation to the appropriate concentration.

A commercially available human serum protein calibrator traceable to ERM 470, is used as an external control preparation. The commercially available calibrator is also diluted to 100, 50, 20, and 10 mg/l in 0.9% NaCl.

Stability of sample material

Human urine samples were stored under various conditions before analysis using NycoCard® U-Albumin and the NycoCard® READER II. The sponsor defined a significant change in result as $>2 \times CV_{\text{between runs}}$, i.e. >10.2 . See below:

Stability at -20 °C

25 fresh urine samples were analyzed in duplicate and then frozen at -20 °C. After one week storage an aliquot from each sample was thawed, analyzed and frozen again. The same aliquot was thawed, analyzed and frozen again after two weeks and three months storage at -20 °C. After three months another aliquot from the same sample was thawed for the first time and analyzed. The data are presented in the table below.

Stability at 4 and 23 °C

61 fresh urine samples were analyzed in duplicate and stored at 4 and 23 °C. The samples were re-tested after one and two weeks. The data are presented in the table below.

Stability of diluted samples

92 fresh urine samples were diluted in R1/Dilution Liquid according to the test procedure described in the NycoCard® U-Albumin package insert. The samples were analyzed and stored at 4 and 23 °C for two weeks. The data are presented in the table below.

Stability of urine samples at various storage conditions.

Sample storage condition	Samples (N)	Increased Result¹	Decreased result²	Unstable samples (%)
3 months at -20 °C	25	0	0	0
2 weeks at 4 °C	61	1	0	2
2 weeks at 23 °C	61	5	3	13
2 weeks at 4 °C, diluted	92	0	0	0
2 weeks at 23 °C, diluted	92	0	0	0

N: number of samples

1: Increased result was defined as $\text{recovery} > \text{target} \times 1.102$ ($2 \times CV_{\text{between run}}$)

2: Decreased result was defined as $\text{recovery} < \text{target} \times 0.898$ ($2 \times CV_{\text{between run}}$)

No significant change in measured albumin concentration was observed after

three months storage at -20 °C.

One sample showed significantly increased recovery after two weeks of storage at 4 °C, while three samples showed lower recovery and five samples increased recovery after two weeks storage at 23 °C.

No significant change in measured albumin concentration was observed for the diluted samples stored at 4 °C and 23 °C.

Stability of the test kit and components

The sponsor defined a significant change in stability as a persistent change of assay performance of more than 2xSD (standard deviation) based on the reproducibility between runs data, compared to mean at the start of the stability studies. See below:

NycoCard® U-Albumin test kit, cool storage (4 °C)

Test kits from three validation lots of NycoCard® U-Albumin were stored refrigerated at 4 °C. The test kits were tested regularly using four levels of urine samples (stored at -20 °C) and NycoCard® U-Albumin C+/Control Positive.

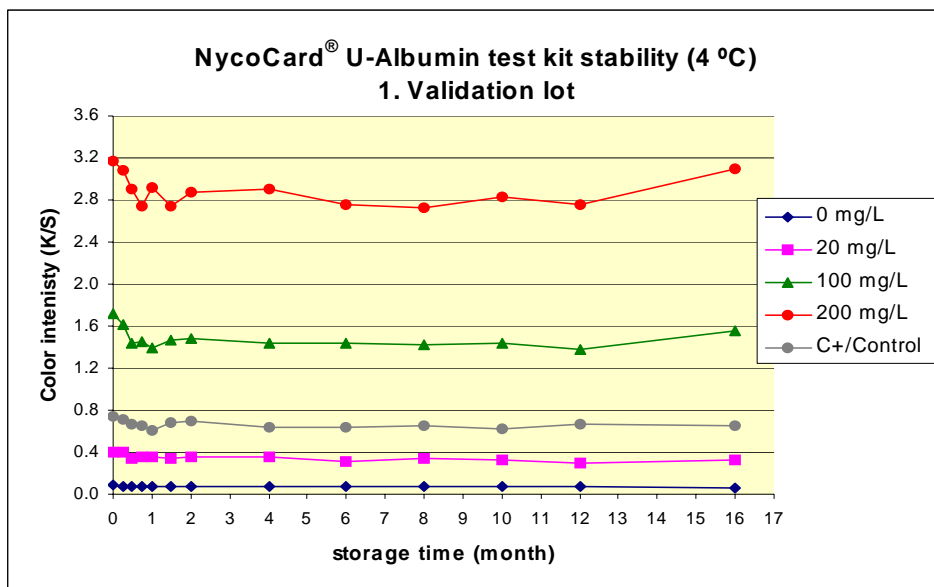
The test result was measured as color intensity (K/S) by the NycoCard® READER II.

$$\text{Color intensity (K/S)} = \frac{(1-R)^2}{2R}$$

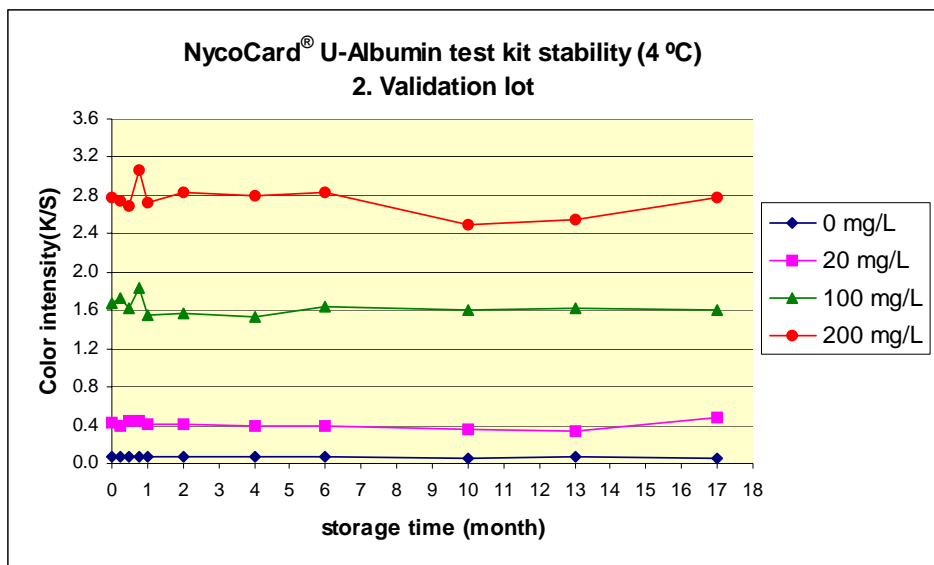
Where K is the light absorbed, S is the light scattered and R is the reflectance.

The data are presented in the graphs below.

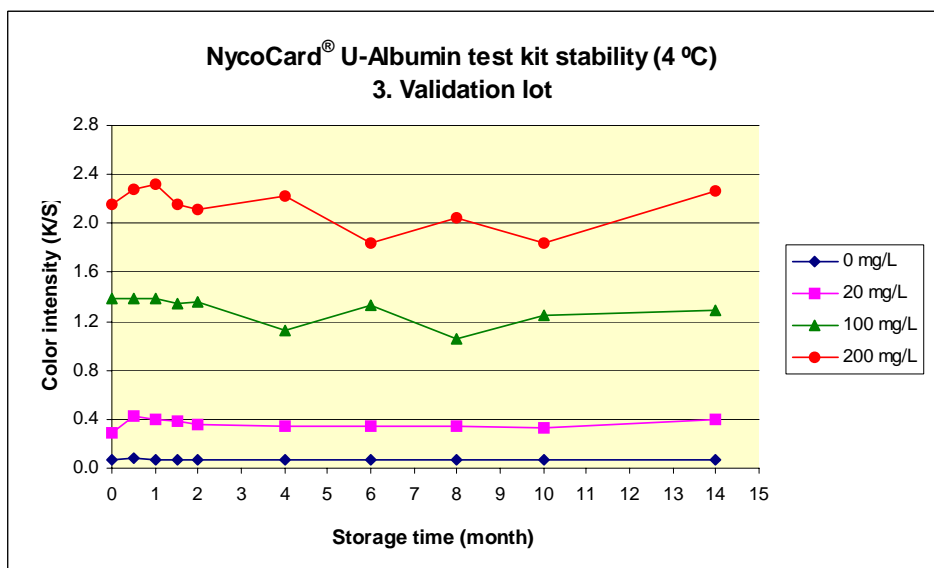
Stability of the NycoCard® U-Albumin test kit, validation lot 1, at 4 °C. Color intensity (K/S) as a function of storage time.



Stability of the NycoCard[®] U-Albumin test kit, validation lot 2, at 4 °C. Color intensity (K/S) as a function of storage time.



Stability of the NycoCard[®] U-Albumin test kit, validation lot 3, at 4 °C. Color intensity (K/S) as a function of storage time.



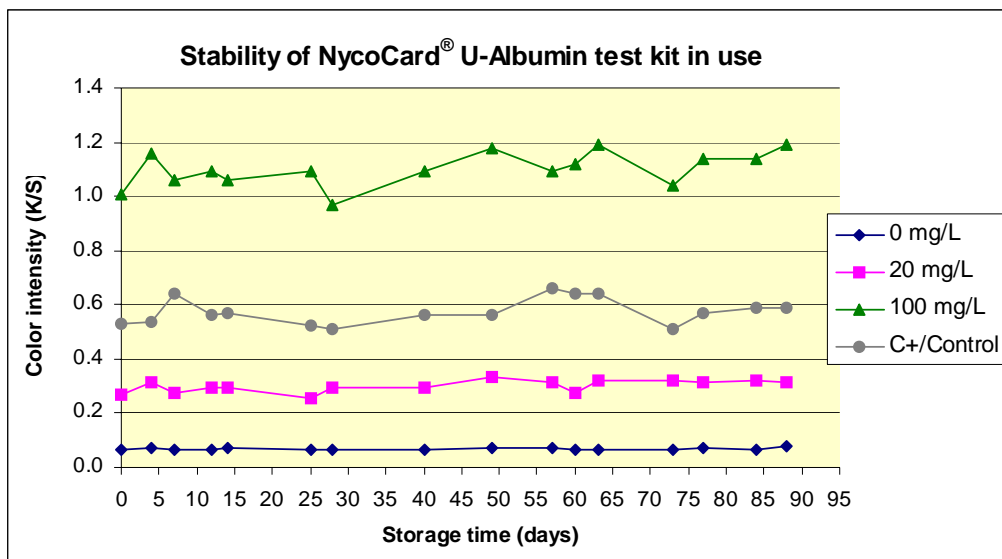
Real time stability test was performed for 16, 17 and 14 months for validation lot 1, 2 and 3, respectively. No significant change in performance was observed over this period of time. The shelf life for NycoCard[®] U-Albumin was set as 12 months.

NycoCard[®] U-Albumin test kit in use

NycoCard[®] U-Albumin test kits were stored according to the recommendations given in the NycoCard[®] U-Albumin Package Insert (Storage and Stability, Opened kits section): the R2/Conjugate vials, TD/Test Device bags and C+/Control Positive vials were stored refrigerated (4 °C), while R1/Dilution liquid microcentrifuge tubes and R3/Washing Solution vials were stored at room temperature (23 °C) protected from light.

The NycoCard[®] U-Albumin test kit components were used in an ordinary manner over a 3 months period. Reference materials in three levels and the control were analyzed in one replicate each day. The data are presented below.

Stability of the NycoCard[®] U-Albumin test kit in use. Color intensity (K/S) as a function of storage time.



The NycoCard[®] U-Albumin test kit (enlarged to last the total test period) was used regularly for 87 days. No significant change in performance was observed during this period of time.

Stability of NycoCard[®] U-Albumin test kit after transport stress

To simulate transport stress, NycoCard[®] U-Albumin test kits were exposed to the following conditions:

- Continuous storage at 4 °C, followed by
- 2 days on a roller at room temperature (23 °C), followed by
- 2 days refrigerated (4 °C), followed by
- 2 days at high temperature (37 °C), followed by
- 2.5 days at room temperature (23 °C)

Test kits stored refrigerated (4°C) were used as a control in the study. The data are presented in the table below.

Stability of NycoCard® U-Albumin test kits after exposure to transport stress.

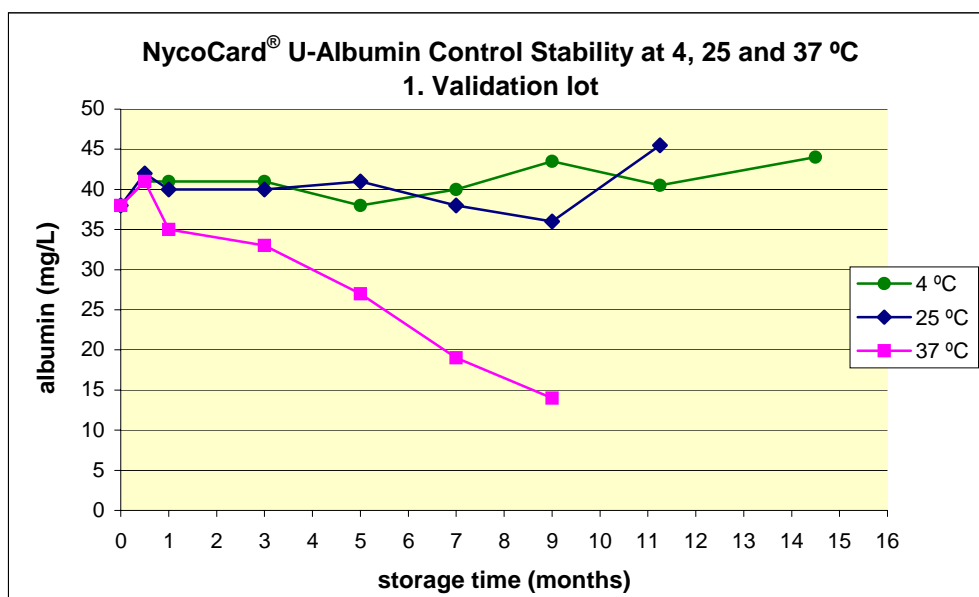
Sample	Albumin (mg/l)	Recovery (albumin mg/l)		Deviation (%) Stressed kit vs. Control
		Stressed test kit	Control	
1	10	14	12	+16
2	20	25	22	+14
3	50	50	51	- 2
4	100	105	117	-10

For sample 1 and 2 a percent change > 10% was observed.

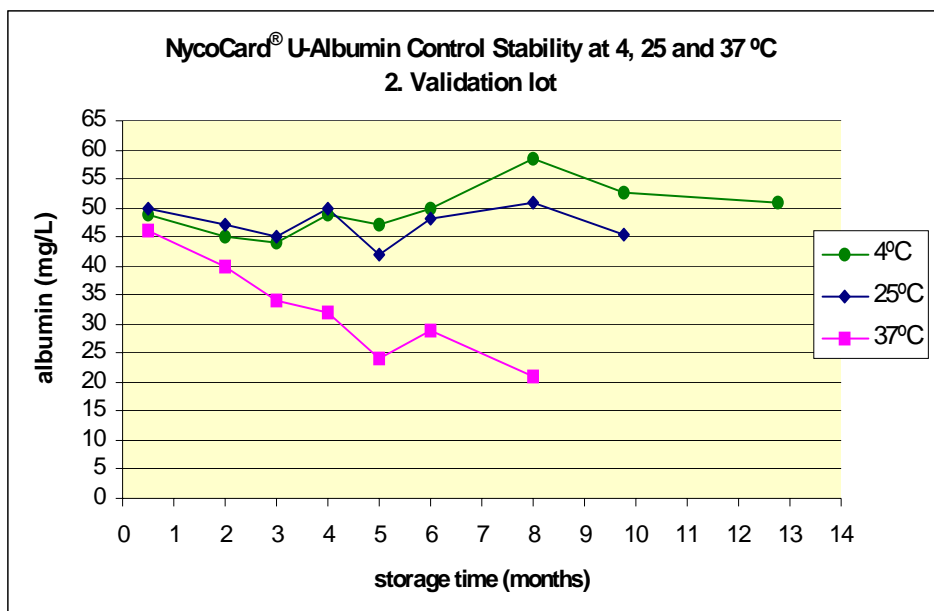
Stability of NycoCard® U-Albumin Control

Two validation lots of NycoCard® U-Albumin Positive Control were stored at 4, 23 and 37 °C and tested regularly in the NycoCard® U-Albumin assay. The data are presented in the graphs below.

Stability of NycoCard® U-Albumin C+/Positive Control validation lot 1 at 4, 25 and 37 °C as a function of storage time.



Stability of NycoCard® U-Albumin C+/Positive Control validation lot 2 at 4, 25 and 37 °C as a function of storage time.



No significant change in measured results was observed for positive control, validation lot 1, stored at 4 and 23 °C. A 50% reduction in recovery was observed after approximately 7.5 months storage at 37 °C.

No significant change in measured results was observed for positive control, validation lot 2, stored at 4 and 23 °C. A 50% reduction in recovery was observed after approximately 6.5 months storage at 37 °C.

d. Detection limit:

Sensitivity

Functional sensitivity was assessed by diluting a pool to 10 different concentrations below the lower limit of the normal test range for each analyte. Each dilution was assayed in replicates of ten. The mean, standard deviation and percent coefficient of variation were calculated for the ten replicates of each dilution. The functional sensitivity of the test was defined at the value of the dilution where the CV is approximately 20% (taking into consideration that the actual mean was within $\pm 10\%$ of the expected target). The functional sensitivity results are summarized below:

	Sensitivity	%CV
U-Albumin	6.0	13.6

e. Analytical specificity:

A morning urine sample was sterile filtrated and h-albumin was added to final concentrations of approximately 20 and 150 mg/l.

Samples containing added substances were tested in duplicate in the NycoCard[®] U-Albumin assay. Aliquots without substances added were used as controls in the study. The test result was measured as color intensity (K/S) by the NycoCard[®] READER II.

The sponsor defined no interference from an added substance on the assay performance as recovery within mean $\pm 2SD$ of the controls. The control sample data is presented in the table below. The effect of possible interfering substances at various concentrations on the measured albumin value is presented below.

Control samples (urine samples without possible interfering substance) n=24.

Albumin	0 mg/l	20 mg/l	150 mg/l
mean (K/S)	0.145	0.365	1.89
SD	0.008	0.020	0.10
CV	5.4	5.4	5.0
Calculated range of no interference ¹	0.129-0.161	0.325-0.405	1.70-2.08

¹mean recovery $\pm 2SD$

Effect of possible interfering substances on the measured urine albumin value. Results compared with control samples.

Substance	Concentration¹	Range 2xSD	Range 3xSD
Creatinine	5 mmol/l	Within ¹	
	20 mmol/l	Within	
	60 mmol/l	Within	
Glucose	0.5 mmol/l	Within	
	30 mmol/l	Within	
	50 mmol/l	Within	
Sodium chloride	50 mmol/l	Within	
	250 mmol/l	Within	
	500 mmol/l	Within	
Sodium nitrite	0.01 mmol/l	Within	
	0.1 mmol/l	Within	
	10 mmol/l	Within	
Urea	5 g/l	Within	
	80 g/l	Not within ²	Within
	200 g/l	Not within	Within
Acetone	3 mg/l	Within	
	1200 mg/l	Within	
	2400 mg/l	Within	
Bilirubin	0.5 mg/l	Within	
	25 mg/l	Within	
	50 mg/l	Not within	Within

<i>Substance</i>	<i>Concentration¹</i>	<i>Range 2xSD</i>	<i>Range 3xSD</i>
Myoglobin	0.5 mg/l	Within	
	50 mg/l	Within	
	500 mg/l	Within	
β_2 -microglobulin	0.3 mg/l	Within	
	25 mg/l	Within	
	250 mg/l	Within	
IgG	10 mg/l	Within	
	50 mg/l	Within	
	500 mg/l	Within	
IgA	10 mg/l	Within	
	50 mg/l	Within	
	500 mg/l	Within	
Hemoglobin	0.005 mg/l	Within	
	0.5 mg/l	Within	
	50 mg/l	Within	
	500 mg/l	Within	
Whole blood (EDTA)	0.005 mg Hb/l	Within	
	0.5 mg Hb/l	Within	
	50 mg Hb/l	Not within	Not within
	500 mg Hb/l	Not within	Not within
pH	4	Not within	Within
	7	Within	
	9	Within	

SD: Standard Deviation

¹Within: Obtained results are within 2xSD or 3xSD range of the control results.

²Not within: Obtained results are not within 2xSD or 3xSD range of the control results.

Samples that contained added urea or bilirubin, and whose pH varied, obtained single test results that were outside the 2xSD range of the control values. All test results were within 3xSD. The data are presented in detail in the tables below.

The effect of urea on the urine albumin measurement. Mean of duplicate measurements.

Albumin	Normal urine	20 mg/l	150 mg/l
2xSD ¹	0.129-0.161	0.325-0.405	1.70-2.08
5 g urea/l	0.131	0.357	1.81
80 g urea/l	0.134	0.340	1.67
200 g urea/l	0.128	0.331	1.66

¹Range of no interference. 2xSD (Standard Deviation) of the control sample test results.

The addition of 80 g/l urea results in urine albumin measurements slightly below

the 2xSD range of no interference for the sample with 150 mg/l albumin. The addition of 200 g/l urea results in urine albumin measurements slightly below the 2xSD range for the normal sample and the sample with 150 mg/l albumin. The sample with 20 mg/l albumin was not affected.

The effect of bilirubin on the urine albumin measurement. Mean of duplicate measurements.

Albumin	Normal urine	20 mg/l	150 mg/l
2xSD ¹	0.129-0.161	0.325-0.405	1.70-2.08
0.5 mg bilirubin/l	0.130	0.344	1.75
25 mg bilirubin/l	0.126	0.353	1.85
50 mg bilirubin/l	0.137	0.323	1.78

¹Range of no interference. 2xSD (Standard Deviation) of the control sample test results.

The addition of 25 mg/l of bilirubin results in urine albumin measurements slightly below the 2xSD range of no interference for the normal urine sample. Lower or higher bilirubin concentrations do not affect the urine albumin analysis. The addition of 25 mg/l of bilirubin results in urine albumin measurements slightly below the 2xSD range for the sample with 150 mg/l albumin.

The effect of pH on the urine albumin measurement. Mean of duplicate measurements.

Albumin	Normal urine	20 mg/l	150 mg/l
2xSD ¹	0.129-0.161	0.325-0.405	1.70-2.08
pH 5	0.143	0.380	1.90
pH 4	0.135	0.315	1.83
pH 9	0.136	0.375	1.75

¹Range of no interference. 2xSD (Standard Deviation) of the control sample test results.

Adjusting pH to 4 gave urine albumin results slightly below the 2xSD range of no interference for the urine sample with 20 mg/l albumin. Samples with a normal albumin concentration or 150 mg/l albumin were not affected by the low pH.

Addition of whole blood to the urine sample resulted in single test results outside both the 2xSD and 3xSD range of the control values. This is due to biological high content of albumin in human whole blood. The data are presented in detail in below.

The effect of whole blood (measured as Hb content) on the urine albumin measurement. Mean of duplicate measurements.

Albumin	Normal urine	20 mg/l	150 mg/l
2xSD ¹	0.129-0.161	0.325-0.405	1.70-2.08
0.005 mg Hb/l	0.145	0.348	1.88
0.5 mg/l	0.143	0.366	1.79
50 mg/l	0.243	0.444	1.94
500 mg/l	1.13	1.31	2.75

Hb: hemoglobin

¹Range of no interference. 2xSD (Standard Deviation) of the control sample test results.

Whole blood contains large concentrations of albumin. Traces of blood in urine samples will thus influence the measurement of urine albumin. Addition of whole blood up to 0.5 mg Hb/L urine does not affect the urine albumin analysis.

f. Assay cut-off:

Not applicable for this type of device.

2. Comparison studies:

a. Method comparison with predicate device:

Comparison Studies

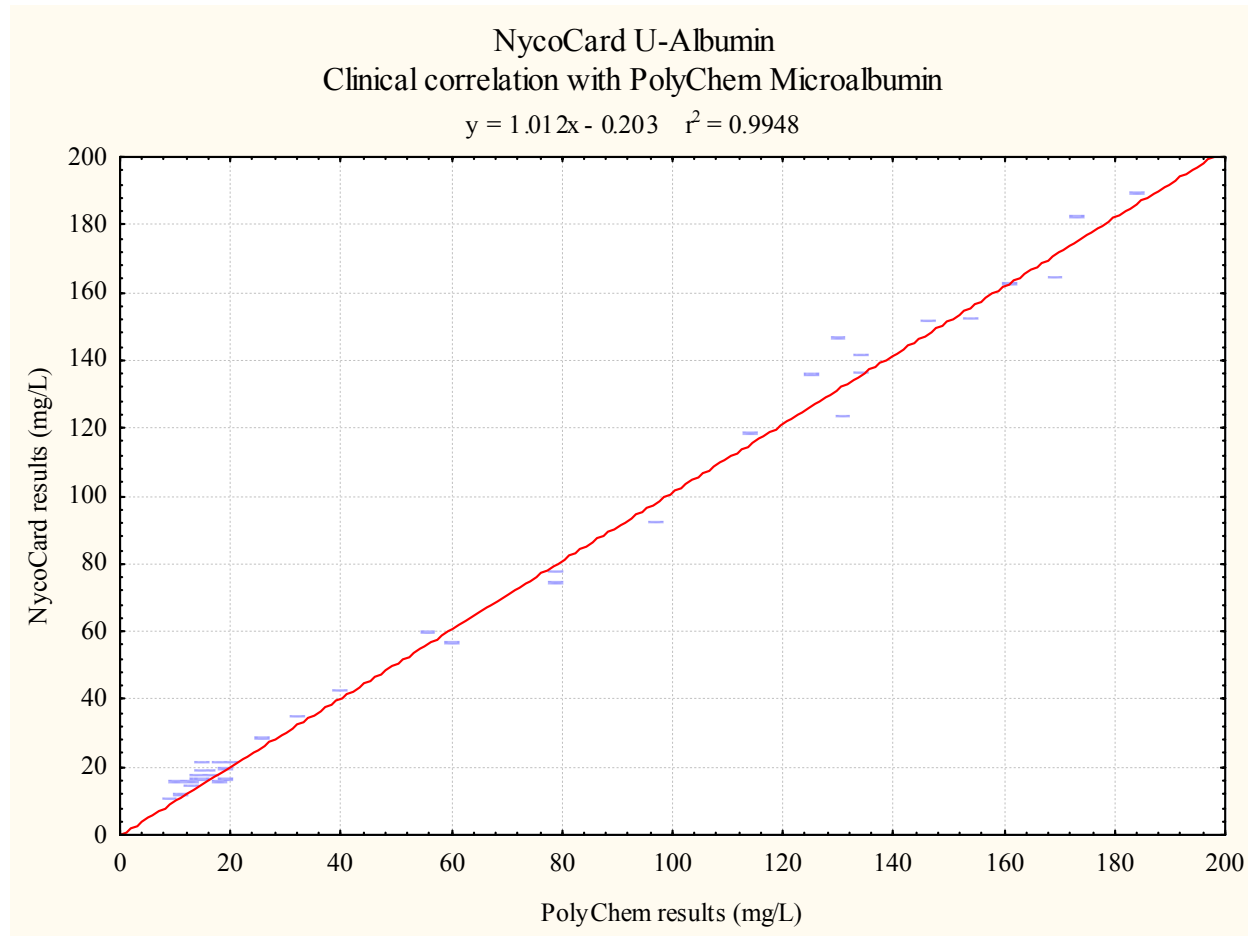
Correlation studies were performed comparing the urine microalbumin results generated on the NycoCard[®] READER II against the results generated from a legally marketed test.

The 40 urine samples spanned from 9 mg/L to 188 mg/l. The regression equation was $y = 1.012x - 0.203$ and $r = 0.9974$. The summary of the correlation data is presented in the table and graph below.

Correlation Data Summary for urine microalbumin the NycoCard[®] READER II and the PolyChem. Results in mg/l.

Variable X,Y	Mean	Std. Dv.	r(X,Y)	r²	t	p	N
PolyChem	63.3	59.61	0.9974	0.9948	84.887	0.000	40
NycoCard [®] READER II	63.8	60.48					

Plot of the Comparison Study Results



b. Matrix comparison:

Not applicable.

3. Clinical studies:

a. Clinical Sensitivity:

Not applicable.

b. Clinical specificity:

Not applicable.

c. Other clinical supportive data (when a. and b. are not applicable):

4. Clinical cut-off:

Not applicable.

5. Expected values/Reference range:

The sponsor has provided the following reference ranges:

Category	24-h collection (mg/24h)	Timed collection (ug/min)	Spot collection (ug/mg creatinine)
Normal	<30 (mg/24h)	<20 (ug/min)	<30 (ug/mg creatinine)
Microalbuminuria	30-299	20-199	30-299
Clinical albuminuria	≥300	≥200	≥300

The sponsor recommends that each laboratory establish its own reference range to reflect the age, sex, diet and geographical location of the population.

Normal Values (<20 mg/L)

Albumin is normally excreted in the urine at the rate 5-20 µg/min (up to 30 mg/24 hours), giving a urine albumin concentration up to 20 mg/L with a normal urine volume.

Increased Values

Microalbuminuria is present when the urine albumin excretion is persistently elevated to 30-300 mg/24 hours, giving a urine albumin concentration of 20-200 mg/L with a normal urine volume⁴

The urine albumin excretion rate (UAER) is given as µg/min when timed urine is used:

$$\frac{C_{\text{albumin}} \times V_{\text{urine}}}{\text{Sampling time, min}} = \mu\text{g/min}$$

$$\begin{aligned} C_{\text{albumin}} &= \text{albumin concentration, mg/L} \\ V_{\text{urine}} &= \text{total urine volume, mL} \end{aligned}$$

The expected range presented in the proposed package insert was established from scientific literature with references listed below:

1. Henry, J.B., Clinical Diagnosis and Management by Laboratory Methods 20th ed. W.B. Saunders Co., Philadelphia (1976).

2. Tietz, N.W. Textbook of Clinical Chemistry 2nd ed. W.B. Saunders Co., Philadelphia (1994).
3. Fielding, B.A., Price, D.A., Houlton, C.A., Clin. Chem. 29(2):355-357 (1983).
4. American Diabetes Association, Diabetes Care, Vol 25 Supplement 1 (2002)

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.