510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
DEVICE ONLY TEMPLATE

A. 510(k) Number:
k032199

B. Analyte:
Plasma free metanephrine and free normetanephrine

C. Type of Test:
Quantitative High Performance Liquid Chromatography

D. Applicant:
ESA, Inc.

E. Proprietary and Established Names:
Plasma Free Metanephrine Analysis Kit

F. Regulatory Information:

1. Regulation section:
   21 CFR 862.1165
2. Classification:
   Class I
3. Product Code:
   CHQ
4. Panel:
   75 Clinical Chemistry

G. Intended Use:

1. Intended use:
   The Plasma Free Metanephrine Analysis kit is intended for in vitro diagnostic use in clinical laboratories that hold a CLIA certificate to perform tests of high complexity to measure endogenous free levels of the metanephrines (normetanephrine and metanephrine) in plasma using high performance liquid chromatography with electrochemical detection.
2. Indication(s) for use:
   The analysis of these analytes is used in the differential diagnosis of adult male and female patients with pheochromocytoma.
3. Special condition for use statement(s):
   Prescription Use Only
4. Special instrument Requirements:
   High performance liquid chromatography

H. Device Description:
The ESA Plasma Free Metanephrine Analysis Kit consists of reagents for the extraction of metanephrines (normetanephrine and metanephrine) sufficient for 100 plasma samples. Sample clean-up is achieved with ion-exchange solid phase extraction. The final extracts are evaporated to dryness, reconstituted and analyzed via reversed phase ion-pair high performance liquid chromatography and electrochemical detection using the ESA CoulArray Detector. The multichannel

electrode system oxidizes the metanephrines and the internal standard, 4-Hydroxy-3-methoxybenzylamine (HMBA), followed by reduction at a downstream electrode. Total chromatographic run time is approximately 28 minutes per sample.

I. Substantial Equivalence Information:

1. Predicate device name(s):
   Model 5500 CEAS Urinary Metanephrine and Normetanephrine
2. Predicate K number(s):
   K931148
3. Comparison with predicate:

Similarities		
Item	**Device**	**Predicate**
Intended Use	Quantitative Measurement of Plasma Free Metanephrines	Quantitative Measurement of Urinary Metanephrines
Indications for Use	Differential Diagnosis of Pheochromocytoma	Same
Methodology	High Performance Liquid Chromatography	Same
Differences		
Item	**Device**	**Predicate**
Matrix	Plasma	Urine

J. Standard/Guidance Document Referenced (if applicable):
None referenced

K. Test Principle:
This device is a complete kit for the measurement of plasma metanephrines by high performance liquid chromatography (HPLC) with coulometric electrochemical detection. This method consists of extracting the metanephrines and an internal standard from 1.0 milliliter of venous EDTA plasma with solid phase extraction followed by evaporation, reconstitution and finally injection onto the HPLC system. The solid phase extraction reagents have been optimized to selectively isolate the metanephrines on a cation exchange resin and then elute them at an elevated pH. The extracts are then concentrated by evaporation to provide adequate sensitivity. The HPLC separation uses a very robust reversed phase analytical column and mobile phase combination.

L. Performance Characteristics (if/when applicable):

1. Analytical performance:
   a. *Precision/Reproducibility:*
   With-in run imprecision was assessed by measuring three levels each of metanephrine (MN) and normetanephrine (NMN) in a single run with the following results:

Metanephrine

Mean (pg/mL)	40.0	27.9	327.8
SD	2.9	2.0	20.5
CV (%)	7.3	7.0	6.2
N	11	11	11

Normetanephrine

Mean (pg/mL)	47.8	53.8	613.8
SD	2.7	2.8	46.3
CV (%)	5.6	5.2	7.5
N	11	11	11

Total imprecision was assessed by measuring two levels each of metanephrine (MN) and normetanephrine (NMN). Samples were analyzed in quadruplicate in 20 separate runs over 19 days with the following results:

Metanephrine

Mean (pg/mL)	78.8	252.9
SD	8.5	21.9
CV (%)	10.8	8.7
N	80	80

Normetanephrine

Mean (pg/mL)	100.2	458.7
SD	7.8	38.6
CV (%)	7.8	8.4
N	80	80

b. Linearity/assay reportable range:

Linearity was assessed by spiking in metanephrine and normetanephrine at concentrations of 50, 100, 200, 500, and 1000 pg/mL and assaying the spiked samples. After correcting for endogenous metanephrine and normetanephrine, the following results were obtained:

Metanephrine

Target	Measured	% Recovery
71	81	114
121	127	105
221	241	109
521	560	107
1021	1056	103

Normetanephrine

Target	Measured	% Recovery
69	80	116
119	119	100
219	233	106
519	540	104
1019	1047	103

c. Traceability (controls, calibrators, or method):
DL-Normetanephrine hydrochloride and DL-Metanephrine hydrochloride are commercially available standards used to prepare the calibrators and controls for this assay.

d. Detection limit:
The functional sensitivity of these analytes was determined by calculating the coefficient of variation at a designated concentration. The acceptance criteria was a CV of 20% or less. The concentrations chosen were 28 pg/mL for metanephrine and 20 pg/mL for normetanephrine.

e. Analytical specificity:
Analytical specificity and cross-reactivity were assessed qualitatively by directly injecting 62 compounds, including several amine-like compounds, onto the chromatographic column. None of the compounds listed below caused an interfering chromatographic peak.

	Mass injected in 100 µL		Mass injected in 100 µL		Mass injected in 100 µL
2,3 benzo pyrole	10 ng	Dopamine	2 ng	Melatonin	5 ng
2,5 Dihydroxybenzoic acid	10 ng	Epinephrine	2 ng	Methionine	25 ng
2-hydroxyphenylacetic acid	20 ng	Gentisic Acid	10 ng	Methoxy-hydroxyphenyl glycol	2 ng
3-nitro tyrosine	10 ng	Glutathione	50 ng	m-tyrosine	2 ng
3-hydroxyanthranilic acid	2 ng	Glutathione (reduced)	50 ng	n-acetyl serotonin	2 ng

3-hydroxykynurenine	2 ng	Guanine	10 ng	Norepinephrine	2 ng
3-indoxyl phosphate	15 ng	Guanosine	20 ng	n-methyl serotonin	2 ng
3-o-methyldopa	2 ng	Homogentisic acid	2 ng	o-tyrosine	20 ng
4-hydroxybenzoic acid	20 ng	Homovanillic acid	10 ng	p-tyrosine	20 ng
4-hydroxyphenylacetic acid	20 ng	Hypoxanthine	10 ng	Salicylic acid	10 ng
4-hydroxyphenyllactic acid	10 ng	Indole-3-acetic acid	15 ng	Serotonin	2 ng
5-hydroxyindoleacetic acid	25 ng	Indole-3-acetyl-alanine	15 ng	Tryptamine	10 ng
5-hydroxytryptophol	2 ng	Indole-3-acetyl-glycine	15 ng	Tryptophan	20 ng
5-hydroxytyptophan	2 ng	Indole-3-acetyl-l-aspartic acid	15 ng	Tryptophol	2 ng
7-methyl guanine	10 ng	Indole-3-butyric acid	15 ng	Tyramine	10 ng
8- hydroxy guanine	10 ng	Indole-3-butyryl-ß-alanine	15 ng	Tyrosine	20 ng
Acetaminophen	10 ng	Indole-3-poprionic acid	2 ng	Uric acid	1 µg
Ascorbate	1 µg	Indole-3-pyruvic acid	2 ng	Vanillylmandelic acid	2 ng
Ascorbic acid	1 µg	Indoxyl acetate	15 ng	Xanthine	20 ng
Cysteine	20 ng	Kynurenine	2 ng	Xanthosine	100 ng
Dihydroxphenylacetic acid	2 ng	L-Dopa	2 ng		

f. Assay cut-off:
N/A

2. Comparison studies:
   a. Method comparison with predicate device:
   A method comparison study was performed comparing the device to an equivalent HPLC method at the National Institutes of Health. For metanephrine, 144 samples were compared. The correlation coefficient was 0.90 and the equation of the regression line was:

   ESA method = 1.06 NIH method + 5 pg/mL

   For normetanephrine, 147 samples were compared. The correlation coefficient was 0.99 and the equation of the regression line was:

   ESA method = 1.04 NIH method + 0.2 pg/mL

   b. Matrix comparison:
   N/A
3. Clinical studies:
   a. Clinical sensitivity:
   Using the sponsor's result interpretation flowchart (see section (c) below), 225 out of 228 known positive samples were correctly identified.

b. Clinical specificity:
Using the sponsor's result interpretation flowchart (see section (c) below), 640 out of 651 known negative samples were correctly identified.

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

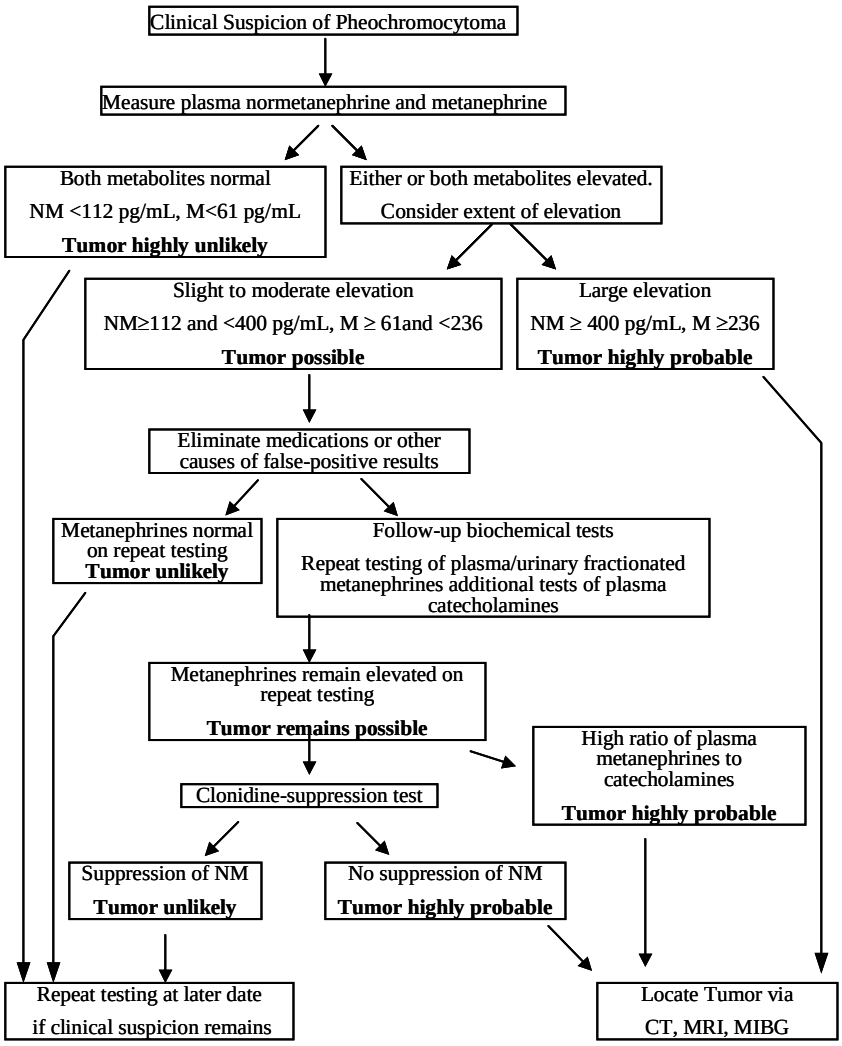
The following are recommendations by the sponsor regarding the proper use and interpretation of the assay.

As a screening tool, any sample in which the concentration of BOTH normetanephrine and metanephrine are less than the upper reference range limit should be considered normal and the presence of pheochromocytoma is highly unlikely.

Any sample where the concentration of EITHER normetanephrine or metanephrine exceeds their respective upper reference range limit should be considered elevated.

Whenever the normetanephrine or metanephrine concentration exceeds the indeterminate range, the presence of pheochromocytoma is highly probable and should be located via imaging techniques.

There are instances where this assay will yield false positive results. Whenever the results of normetanephrine and/or metanephrine are within but neither result exceeds the indeterminate range, follow-up biochemical testing is suggested according to the flow chart shown below adapted from the National Institutes of Health.



4. Clinical cut-off:
   N/A

5. Expected values/Reference range:
   Normetanephrine 18-112 pg/mL
   Metanephrine 12-61 pg/mL

M. Conclusion:

Based upon the information provided for the file, I recommend that the ESA Plasma Free Metanephrine Analysis be found substantially equivalent to the predicate device.