LINEZOLID ASSAY USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC-DAD) (REFERENCE – 2014.02.06)

Notice of Assessment

December 2014

1. GENERAL INFORMATION

1.1. Requester: Centre hospitalier universitaire Sainte-Justine

1.2. Application for Review Submitted to MSSS: April 25, 2013

1.3. Application Received by INESSS: July 10, 2014

1.4. Notice Issued: October 31, 2014

Note:

This notice is based on the scientific and commercial information submitted by the requester and on a complementary review of the literature according to the data available at the time that this test was assessed by INESSS.

2. TECHNOLOGY, COMPANY, AND LICENCE(S)

2.1 Name of the Technology

High performance liquid chromatography with diode array detection (HPLC-DAD).

2.2 Brief Description of the Technology, and Clinical and Technical Specifications

High performance liquid chromatography with diode array detection (HPLC-DAD) separates and detects linezolid molecules and generates a specific quantitative chromatographic profile [Belal *et al.*, 2013; Cios *et al.*, 2013].

The technique can be divided into three main steps:

- sample preparation by protein precipitation (PP);
- high performance liquid chromatography (HPLC);
- ultraviolet detection with a diode array (DAD).

Several methods to assay linezolid in serum or plasma following protein removal are described in the literature: HPLC – UV [Baietto *et al.*, 2013; Cios *et al.*, 2013; Helmy, 2013; Cattaneo *et al.*, 2010; Polillo *et al.*, 2010; Baietto *et al.*, 2009; Swoboda *et al.*, 2007]; HPLC – DAD [Cios *et al.*, 2013], LC-MS/MS [Zander *et al.*, 2014]; methods in plasma involve UPLC - PDA or HPLC - PDA [Baietto *et al.*, 2013]; [Fortuna *et al.*, 2013; Cattaneo *et al.*, 2010].

2.3 Company or Developer: In-house method.

2.4 Licence(s): Not applicable.

2.5 Patent, If Any: Not applicable.

2.6 Approval Status (Health Canada, FDA): Not applicable.

2.7 Weighted Value: 72.52

3. CLINICAL INDICATIONS, PRACTICE SETTINGS, AND TESTING PROCEDURES

3.1 Targeted Patient Group

Patients with a mycobacterial infection or a gram-positive bacterial infection resistant to the usual antibiotics, who are treated with linezolid, and are at risk of significant variability in product pharmacokinetics, for example those with cystic fibrosis of the pancreas or invasive infections. These include hospitalized infants (neonatology) [Li et al., 2013; Rasigade et al., 2013] and children and adolescents 18 years of age and under.

3.2 Targeted Disease

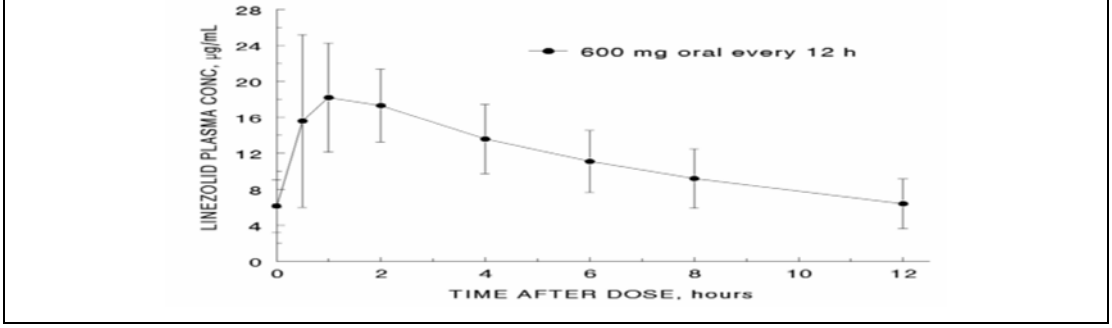
Linezolid is an oxazolidinone antibiotic. It is active against gram-positive bacteria resistant to the usual antibiotics, such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE), and mycobacteria. It is inactive against gram-negative bacteria. It is included in Quebec's list of exceptional medications.

The problem with using this antibiotic, which is prescribed for specific conditions, is highly variable plasma concentrations, which range from ineffective suboptimal levels to levels that are toxic, especially hematologically [Cattaneo et al., 2013b], as a result of 1) concomitant administration of other drugs; 2) the patient's medical condition; or 3) the method of administration.

Pharmacokinetics

The mean pharmacokinetic parameters of linezolid in adults after administration of single or multiple oral and intravenous doses are summarized in the ZYVOXAM™ monograph. Average minimum and maximum plasma concentrations at steady state following oral administration of 400 or 600 mg of linezolid every 12 hours are reported in the product monograph (C_{min}: 3.08 et 6.15 µg/mL, respectively, and C_{max}: 11.0 and 21.2 µg/mL; Figure 1). These results indicate that, for those dose regimens, the C_{min} values are near or above the highest minimum inhibitory concentrations (MIC_{90}) (4 µg/mL) established for target microorganisms.

Figure 1: Steady-state plasma concentrations of linezolid in 16 adults following administration of 600 mg every 12 hours (mean plus or minus standard deviation)



Source: Pfizer Canada Inc. ZYVOXAM monograph. Date of revision: December 4, 2013. Available at: http://www.pfizer.ca/en/our_products/products/monograph/143.

3.3 Number of Patients Targeted

The requester estimates that approximately 100 to 200 tests are expected in Quebec over the next three years, but did not specify whether this includes only children. It should be noted that statistics from the Régie de l'assurance maladie du Québec (RAMQ) show that in 5 years (from 2009 to 2013), 28 patients under the age of 17 years took linezolid (73 prescriptions) while covered by the Public Prescription Drug Insurance Plan.

This does not include individuals with private insurance or those in hospital, whether children or adults.

Special Populations (Children and Adolescents[1])

At present there is very little information on the pharmacokinetics of linezolid following administration of multiple doses to children of all ages. There are insufficient data on the safety and efficacy of linezolid in children and adolescents < 18 years. Further studies are needed to establish safe and effective dosage recommendations. Pharmacokinetic studies indicate that, after single and multiple doses in children, linezolid clearance was greater in children than in adults, decreasing with age. In children 1 week to < 12 years, daily administration of 10 mg/kg every 8 hours provided exposure close to that achieved with a dosage of 600 mg twice a day in adults.

In infants up to 1 week of age, systemic clearance of linezolid increases rapidly in the first week of life. Therefore, if 10 mg/kg is administered every 8 hours daily to infants, systemic exposure is greatest the first day after birth. In adolescents (≥ 12 to < 18 years), pharmacokinetic data are similar to that observed in adults following administration of a 600 mg dose.

Other Populations

Therapeutic monitoring of linezolid could apply to other populations. For example, linezolid can be used for diabetic patients with soft tissue infections [Eslam et al., 2014], orthopedic patients with resistant bacterial infections [Joel et al., 2014] and patients with tuberculosis [Garcia-Prats et al., 2014], to name just a few.

3.4 Medical Specialties and Other Professions Involved

Medical biochemistry, pediatrics, hematology, infectious diseases.

3.5 Testing Procedure

According to information submitted by the requester, unless otherwise indicated, blood samples should be collected at steady state, that is, at least 20 to 40 hours after linezolid is started [Cattaneo et al., 2013b]. Turnaround time is 24 to 72 hours (1 to 3 working days). The test is performed based on clinical needs. Plasma concentration can be measured repeatedly over time for the same patient, as determination of the area under the curve (AUC) over 24 hours is the best predictor of linezolid efficacy [Pea et al., 2012; Dryden, 2011; Alffenaar et al., 2010; Andes et al., 2002].

1. Pfizer Canada inc. ZYVOXAM monograph. Date of revision: December 4, 2013. Available at: http://www.pfizer.ca/en/our_products/products/monograph/143.

4. TECHNOLOGY BACKGROUND

4.1 Nature of the Diagnostic Technology

Unique test to monitor antibiotic therapy.

4.2 Brief Description of the Current Technological Context

As previously mentioned, the test measures plasma concentrations of linezolid using high performance liquid chromatography with ultraviolet and diode array detection. Stock solutions are prepared fresh using the commercial injectable form of the drug. The test is based on certified analytical reagents, not reagents in a kit licensed by Health Canada. Results are collected in accordance with a procedure recognized by Accreditation Canada. A similar technique was adapted by Cios et al. [2013] and is used for patients in intensive care to determine linezolid serum concentrations to establish pharmacokinetic profiles.

Other technologies have been described and will be used to assess clinical and analytical validity. They are as follows:

- liquid chromatography-mass spectrometry (LC-MS/MS) [Szultka et al., 2013];
- ultra performance liquid chromatography-photodiode array detection (UPLC-PDA) [Baietto et al., 2013];
- spectrofluorimetry [Belal et al., 2013];
- capillary electrophoresis [Hernandez et al., 1999]; high performance liquid chromatography (HPLC) with capillary fluorescence was also mentioned [Szultka et al., 2013; Cavazos-Rocha et al., 2007].

4.3 Brief Description of the Advantages Cited for the New Technology

The advantage of using “deproteinization” or “protein precipitation” is that it enables an assay method that is easy to use on a routine basis. Recovery is excellent with this method (close to 100%), and the procedure is simple and rapid [Cios et al., 2013].

One of the advantages of high performance liquid chromatography (HPLC) with UV detection is that this method remains sensitive despite the use of low plasma volumes [Traunmuller et al., 2010], although LC-MS/MS remains the method of choice because of its selectivity [Zander et al., 2014].

4.4 Cost of Technology and Options: Not assessed.

5 EVIDENCE

5.1 Clinical Relevance

5.1.1 Other Tests Replaced: This test does not replace any other tests.

5.1.2 Diagnostic or Prognostic Value: Not applicable.

5.1.3 Therapeutic Value

Therapeutic drug monitoring of linezolid involves assaying linezolid in plasma or serum.

The literature indicates therapeutic efficacy if the ratio of the area under the curve for linezolid plasma concentrations ($AUC_{0\text{-}24h}$) to minimum inhibitory concentration (MIC) is greater than 80, and possible toxicity if it is greater than 300. The therapeutic range is from

1 to 30 µg/mL [Szultka et al., 2013]. Residual concentration greater than 10 µg/mL can be associated with hematological toxicity [Zoller et al., 2014; Pea et al., 2010]. Thrombocytopenia is a common side effect of linezolid [Boak et al., 2014].

Several authors have studied the utility of therapeutic drug monitoring for the antibiotic linezolid using various assays, including HPLC with diode array detection [Baietto et al., 2013; Cios et al., 2013; Fortuna et al., 2013]. Therapeutic drug monitoring is closely related to the drug's pharmacokinetics. Retrospective studies [Dong et al., 2014; Pea et al., 2012; Pea et al., 2010] have documented a significant association between linezolid plasma concentrations and the drug's toxicity. However, a safe upper limit for linezolid has not been determined with certainty.

The retrospective study carried out by Pea et al. in 2010 proposed a fixed 600 mg dose of linezolid every 12 hours to ensure adequate pharmacodynamic exposure in 60% to 70% of cases. For the other 30% to 40% of cases, therapeutic drug monitoring would prevent treatment failure or dose-dependent toxicity.

A prospective observational study was conducted to compare minimum plasma concentrations in patients with and without hematological toxicity [Cattaneo et al., 2013b]. Table 1, taken from this article, shows linezolid plasma concentrations in 50 patients grouped by whether or not they developed hematological toxicity. The nine patients who exhibited a toxic effect all had significantly higher C_{min} values during the first week of treatment and after the end of linezolid therapy.

Table 1: Minimum plasma concentrations (C_{min}) of linezolid measured in patients with and without hematological toxicity

		DAY COLLECTED	LINEZOLID C_{min} mg/L (mean ± standard deviation)
Patients with toxicity (n = 9)	1st evaluation	3	9.0 ± 6.4
	2nd evaluation	9	10.7 ± 5.3
	3rd evaluation	12[a]	10.7 ± 5.8
	4th evaluation (n = 5)[b]	16	4.0 ± 1.4
Patients without toxicity (n = 41)	1st evaluation	3	4.9 ± 3.7
	2nd evaluation	10	4.8 ± 3.3
	3rd evaluation	15	5.0 ± 1.9
	4th evaluation	24	4.9 ± 4.6

Source: Cattaneo et al., 2010.

a. Median duration of linezolid treatment to onset of hematological toxicity.

b. Four patients withdrew from the study after developing side effects, while five received a reduced dosage.

All patients (n = 9) who developed linezolid-related hematological toxicity had higher C_{min} values during the first week of treatment (9.0 +/- 6.4 mg/L versus 4.9 +/- 3.7 mg/L; $P < 0.01$). The association between linezolid plasma concentrations and hematological toxicity indicates that therapeutic drug monitoring can improve safety for patients receiving linezolid therapy.

In a therapeutic drug monitoring study by Pea et al. [2012] with and without co-treatment with rifampicin, dosage adjustments were required in 40% of patients (n = 35) in the group receiving linezolid alone after median treatment of 21.5 days. Thrombocytopenia appeared in 51.4% of cases in the group receiving linezolid alone compared with 0% in the group receiving linezolid and rifampicin. The 35 patients in the linezolid group had significantly higher C_{min} and AUC_{24} [medians and interquartile ranges (IQR)] [C_{min}: 3.71 mg/L (1.43-6.38) versus 1.37 mg/L (0.67-2.55); $P < 0.001$] or [AUC_{24}: 212.77 mg/L·h (166.67 – 278.42) versus 123.33 mg/L·h (97.36 – 187.94); $P < 0.001$].

Maintenance over time of C_{min} between 2 and 7 mg/L and/or AUC_{24} between 160 and 300 mg/L·h would improve treatment safety while providing appropriate efficacy in adult patients receiving prolonged linezolid treatment.

These data suggest a cause and effect relationship between high linezolid concentrations and adverse effects.

In 2010, Alffenaar et al. investigated whether a reduction in linezolid dosage resulted in serum concentrations above an AUC_{24}/MIC ratio of > 100. This prospective study evaluated two dosages of linezolid, 300 mg twice daily for 3 consecutive days followed by 600 mg twice daily, in patients resistant to the usual treatments for tuberculosis. Blood samples were taken from eight patients at defined intervals to measure serum linezolid concentrations by LC-MS/MS. This study used the AUC_{24}/MIC ratio as a model to predict efficacy. Adverse effects of linezolid, including peripheral neuropathy, were evaluated clinically and through laboratory assessments.

Median duration of treatment was 56 days. The following table presents different AUC_{24}/MIC ratios for linezolid for doses of 300 and 600 mg of linezolid twice daily.

Table 2: AUC_{24}/MIC ratios for 300 and 600 mg doses of linezolid administered every 12 hours

Patient	MIC_{90} (mg/L)	300 mg			600 mg		
		AUC_{12} (mg/L·h)	AUC_{24}/MIC	T > MIC (%)	AUC_{12} (mg/L·h)	AUC_{24}/MIC	T > MIC (%)
1	0.25	83.4	667	100	155.9	1247	100
2	0.5	41.8	167	100	86.6	347	100
3	0.25	50.2	402	100	94.7	757	100
4	0.25	63.6	509	100	181.0	1448	100
5	0.25	65.9	527	100	131.9	1055	100
6	0.25	27.8	445	100	115.6	1850	100
7	<0.125	28.7	460	100	103.4	1654	100
8	1.0	46.2	92	100	176.0	352	100

Source: Alffenaar et al., 2010.

Abbreviations: AUC_x = area under the curve at time x; MIC = minimum inhibitory concentration; MIC_{90} = minimum inhibitory concentration required to inhibit growth of 90% of organisms; T > MIC = time that concentrations exceed minimum inhibitory concentration.

In this study [Alffenaar et al., 2010], effective serum concentrations were achieved after 3 days of twice daily administration of linezolid 300 mg, and in 7 of 8 patients the AUC_{24} /MIC ratio was ≥ 100. Dosage should be adjusted based on mean steady-state concentration values (300 mg = C_{min} of 1.9 mg/L and C_{max} of 9.5 mg/L, and 600 mg = C_{min} of 5.8 mg/L and C_{max} of 20.4 mg/L) to optimize product efficacy in certain patient populations known to be at risk for significant variability in product pharmacokinetics (e.g., cystic fibrosis of the pancreas), especially with the oral formulation. For example, Stalker et al. [2003] reported that the C_{max}/MIC ratio for linezolid and exposure time above MIC are evidence of efficacy against gram-positive infections. Previously, some in vitro and animal models had shown that the AUC/MIC ratio was the best parameter to measure efficacy in patients treated with fluoroquinolones [Andes and Craig, 2002; Craig, 1998; Drusano et al., 1993; Forrest et al., 1993].

It should be noted that other biochemical and clinical parameters are indicated for individuals with critical conditions such as severely ill patients in intensive care [Zoller et al., 2014] or patients with reduced creatinine clearance [Matsumoto *et al.*, 2014].

In conclusion, although studies are still underway in certain populations [Gostelow et al., 2014], it would be appropriate to initiate therapeutic drug monitoring for patients receiving linezolid [Morata et al., 2013].

5.2 Clinical Validity: No study assessing clinical validity was found.

5.3 Analytical (or Technical) Validity

According to information submitted by the requester, the current test is based on use of a calibration line without an internal standard. Internal quality controls (different dilution) and "patient" controls are also used. Results are also compared with past analytical values. Results are compiled in accordance with a procedure recognized by Accreditation Canada.

There is currently no external control program, but the laboratory has advised KKGT (under the governance of the Dutch Foundation for Quality Assessment in Medical Laboratories) that it is interested in participating in an interlaboratory quality control program on therapeutic monitoring of certain antibiotics, when the program becomes available.

Data on the analytical validity of the HPLC-DAD method are taken from the results of twelve studies, including seven comparative studies using the HPLC-UV method.

PARAMETER	PRESENCE	ABSENCE	NOT APPLICABLE
Repeatability	X		
Reproducibility	X		
Analytical sensitivity	X		
Analytical specificity	X		
Matrix effect		X	
Concordance		X	
Correlation between test and comparator	X		

Repeatability, reproducibility, and accuracy results are shown in Table 3. Intra-assay variation (intraday) was not reported for the HPLC-DAD method. Interday coefficients of variation for HPLC-DAD reproducibility (method used by the requester) are less than 5.5% and bias is less than 11%; values for other methods of chromatography are similar, or generally less than 15%. Despite the increase in spiked concentrations, coefficients of variation remain acceptable.

Table 3: Repeatability, reproducibility, and accuracy of high performance liquid chromatography with diode array detector (HPLC-DAD) and other methods of chromatography

STUDY	NUMBER OF SPECIMENS	NOMINAL CONCENTRATION (µg/mL)	*INTRA-ASSAY*		*INTERDAY*	
			CV (%)	% BIAS*	CV (%)	% BIAS*
HPLC-DAD						
Cios et al., 2013	7 calibration curves on 7 different days	0.5	-	-	5.42	10.85
		2.5	-	-	1.74	3.18
		5	-	-	0.67	1.1
		10	-	-	0.76	1.06
		20	-	-	0.92	1.53
		30	-	-	0.97	0.74
UPLC-PDA or HPLC-PDA						
Baietto et al., 2013	*Intra-assay*: 5 replicates *Interday*: 5 replicates (5 different days)	0.2	7.15	4.41	12.83	4.46
		1	8.75	-0.49	10.91	9.40
		5	2.09	-2.76	10.51	-1.33
		10	7.6	13.96	8.39	-6.69
Fortuna et al., 2013	*Intra-assay*: 5 *Interday*: 17 (5 different days)	1	5.34	- 4.76	8.90	-1.98
	Intra-assay: 5 *Interday*: 17 (5 different days)	5	7.22	- 2.59	5.86	-3.06
	Intra-assay: 5 *Interday*: 17 (5 different days)	10	2.67	5.17	7.49	-0.04
HPLC-UV						
Baietto et al., 2009	5	0.75	1.87	-0.75	14.45	0.40
	5	7.5	5.64	3.31	12.14	6.78
	5	15	2.63	-9.54	4.74	0.56

STUDY	NUMBER OF SPECIMENS	NOMINAL CONCENTRATION (µg/mL)	*INTRA-ASSAY*		*INTERDAY*	
			CV (%)	% BIAS*	CV (%)	% BIAS*
Cattaneo et al., 2010	5	0.2	10.61	1.6	18.80	-1.75
	5	0.6	3.92	-5.71	8.16	-0.33
	5	4.8	3.34	8.18	5.37	-0.37
	5	30	2.83	5.99	5.25	2.65
Cios et al., 2013	7 calibration curves on 7 different days	0.5	-	-	4.69	12.63
		2.5	-	-	3.59	6.65
		5	-	-	2.63	2.40
		10	-	-	3.56	3.04
		20	-	-	2.60	1.68
		30	-	-	3.47	0.97
Helmy, 2013	*Intra-assay*: 3 replicates (calibration curve) *Interday*: 6 calibration curves on 6 different days	0.1	9.1	105†	8.9	100†
		0.5	4.36	97.9†	4.24	97.3†
		2	3.22	106.2†	4.15	104.1†
		10	0.66	101.1†	0.35	107†
Polillo et al., 2010	3 calibration curves on 3 consecutive days	0.7813	-	-	6.19	103.78†
		1.5625	-	-	6.31	105.49†
		3.125	-	-	7.83	107.38†
		6.25	-	-	0.66	105.09†
		12.5	-	-	9.66	104.06†
		25	-	-	2.29	95.54†
		50	-	-	4.53	95.83†
		100	-	-	0.12	101.78†

STUDY	NUMBER OF SPECIMENS	NOMINAL CONCENTRATION (µg/mL)	*INTRA-ASSAY*		*INTERDAY*	
			CV (%)	% BIAS*	CV (%)	% BIAS*
Swoboda et al., 2007	6	2	2.63	101.2†	2.08	103.2†
	6	10	4.91	100.6†	1.78	101.7†
	6	40	3.45	101.2†	2.18	102.3†
Traunmuller et al., 2010	*Interday*: 4 (4 replicates) *Interday*: 12 (4 replicates, on 3 different days)	0.05	13.9	-13.6	10.2	-13
		0.5	1.6	-2.7	2.4	-3.8
		1	2.5	9.3	4.9	8.3
		10	1	1.6	1.6	2.3
LC-MS/MS						
Szultka et al., 2013 (MEPS)	3 replicates	1	-	104.8†	-	-
	3 replicates	8	-	99.7†	-	-
	3 replicates	15	-	100.6†	-	-
Szultka et al., 2013 (SPE)	3 replicates	1	-	113†	-	-
	3 replicates	8	-	102.6†	-	-
	3 replicates	15	-	102.2†	-	-
Szultka et al., 2013 (preparation method not indicated)	10	1	6.83	108.3†	-	-
		3	1.66	107.7†	-	-
		5	6.25	105.5†	-	-
		8	4.41	101.4†	-	-
		10	3.10	101.5†	-	-
		15	3.26	99.1†	-	-
		20	2.44	100.4†	-	-
		30	1.51	99.3†	-	-

STUDY	NUMBER OF SPECIMENS	NOMINAL CONCENTRATION (µg/mL)	*INTRA-ASSAY*		*INTERDAY*	
			CV (%)	% BIAS*	CV (%)	% BIAS*
Zander et al., 2014 System 1 (Quattro Micro™)	5	0.38	2.49‡	106†	2.64¥	-
	5	0.5	3.72‡	99†	7.34¥	-
	5	4	2.62‡	103†	2.48¥	-
	5	6	1.65‡	105†	2.7¥	-
	5	16	5.23‡	101†	2.97¥	-
Zander et al., 2014 System 2 (Micromass Quattro LC™)	5	0.38	1.89‡	102†	2.26¥	-
	5	0.5	2.71‡	98†	4.28¥	-
	5	4	3.22‡	101†	2.01¥	-
	5	6	0.96‡	101†	3.57¥	-
	5	16	3.86‡	101†	4.23¥	-

Abbreviations: CV: coefficient of variation; HPLC-DAD: high performance liquid chromatography with diode array detector; µg/mL: microgram per millilitre; wk.: week.

* Refers to the percentage of inaccuracy or relative error; † Accuracy percentage; ‡ Intra-assay and ¥ Interassay.

The requester's proposed method (HPLC-DAD) demonstrates comparable analytical sensitivity (limit of detection or LOD) to that of other techniques identified in the literature [Baietto et al., 2013; Cattaneo et al., 2013a; Cios et al., 2013; Fortuna et al., 2013; Helmy, 2013; Cattaneo et al., 2010; Polillo et al., 2010; Baietto et al., 2009; Swoboda et al., 2007]. The limit of detection is 0.1 µg/mL, comparable to the other techniques used, which range from 0.04 to 0.4 µg/mL [Baietto et al., 2013; Cios et al., 2013; Helmy, 2013; Cattaneo et al., 2010; Polillo et al., 2010; Baietto et al., 2009; Swoboda et al., 2007]. This is also true of linearity (0.5 to 30 µg/mL); the method proposed by the requester has a correlation coefficient that is close to 1 (r = 0.9997), which is comparable to the coefficient of other chromatography methods ([Cios et al., 2013; Baietto et al., 2013; Fortuna et al., 2013; Helmy, 2013; Szultka et al., 2013; Cattaneo et al., 2010; Polillo et al., 2010; Traunmuller et al., 2010; Baietto et al., 2009; Swoboda et al., 2007]).

Table 4: Sensitivity and analytical linearity of various chromatography methods

STUDY	NUMBER AND TYPE OF SAMPLES	VOLUME (µL)*	HPLC ANALYSIS						
			PREPARATION METHOD	INTERNAL CONTROL	DETECTION (nm)	LOD (µg/mL)	LLOQ (µg/mL)	LINEARITY (µg/mL)	COEFFICIENT OF LINEARITY
HPLC-DAD									
Cios et al., 2013	Serum	200/50	PP	Piperacillin	258	0.1	0.5	0.5 – 30	r = 0.9997§
UPLC-PDA or HPLC-PDA									
Baietto et al., 2013	Plasma	-/4	PP	Quinoxaline	254	0.058	0.117	0.117 – 30	$r^2 > 0.999$
Fortuna et al., 2013	Plasma	-/25	SPE	6,7-Dimethyl-2,3-di (2-pyridyl)-quinoxaline	254	-	-	0.025 – 25.6£	R = 0.9997§
HPLC-UV									
Baietto et al., 2009	Plasma	300/40	PP	Quinoxaline	280	0.04	0.31	-	$r^2 > 0.998$†
Cattaneo et al., 2010	10 Plasma	300/20	PP	*p*-toluic acid	254	-	0.2	0.2 – 48	$r^2 = 0.9996$†
Cios et al., 2013	Serum	200/50	PP	Piperacillin	258	0.1	0.25	0.5 – 30	R = 0.9995§
Helmy, 2013	12 Plasma	250/25	PP	Metronidazole	260	0.05	0.1	0.1 – 30	$r^2 = 0.9999$†
Polillo et al., 2010	Plasma	100/-	PP	-	214	0.3775	0.7813	0.7813 - 100	$r^2 = 0.9976$
Swoboda et al., 2007	Plasma	-/100	PP	-	251	0.1	0.3	0.5 – 40	> 0.999
Traunmuller et al., 2010	Plasma	20/12	LLE	Fluconazole	251	-	0.05	0.05 – 40	R ≥ 0.9992

STUDY	NUMBER AND TYPE OF SAMPLES	VOLUME (µL)*	HPLC ANALYSIS						
			PREPARATION METHOD	INTERNAL CONTROL	DETECTION (nm)	LOD (µg/mL)	LLOQ (µg/mL)	LINEARITY (µg/mL)	COEFFICIENT OF LINEARITY
LC-MS/MS									
Szultka et al., 2013	3 Plasma	50/-	MEPS	Gemifloxacin	251 ESI, MRM mode (positive)	0.1407 ng/mL	0.3814 ng/mL	1 – 30	r^2 = 0.9988
		120/15	SPE						r^2 = 0.9889
Zander et al., 2014	Serum	50/10	PP and SPE	Linezolid-d_3	ESI, MRM mode (positive) ‡	-	0.5	0.13 – 32	r^2 > 0.999¥

Abbreviations: ESI: electron spray ionization; HPLC-DAD: high performance liquid chromatography with diode array detector; HPLC-UV: high performance liquid chromatography with ultraviolet detection; LC-MS/MS: liquid chromatography-tandem mass spectrometry; LLE: liquid-liquid extraction; LLOQ: lower limit of quantification; LOD: limit of detection; MEPS: microextraction in packed syringe; µg/mL: microgram per millilitre; MRM: multiple reaction monitoring; nm: nanometre; PP: protein precipitation; SPE: solid phase extraction; UPLC-DAD: ultra performance liquid chromatography with photo diode array detector.

* Sample volume/aliquot volume injected in the HPLC system

† Coefficient of determination

‡ Ionization

§ Coefficient of correlation

¥ Coefficient of correlation of 3 calibration curves (linear regression)

£ Units were converted for consistency.

Recovery was approximately 100% with HPLC-DAD (Cios et al., 2013). Other methods of chromatography obtain similar values, ranging from 95% to 112%, with the exception of a single study using HPLC-UV [Baietto et al., 2013], in which recovery was 75.9%. No interference was observed from endogenous substances, antibiotics or other drugs. When known quantities of linezolid were added, recovery and measurement accuracy were excellent (100.5%). The chromatography technique used by the proposed test indicates that the test is specific.

Table 5: Analytical specificity, recovery, interference, and matrix effects

STUDY	NUMBER OF SPECIMENS	NOMINAL CONCENTRATION (µg/mL)	RECOVERY (%)	INTERFERENCE
HPLC-DAD				
Cios et al., 2013	-	-	100.5	No interference from endogenous substances or 10 other antibiotics
UPLC-PDA or HPLC-PDA				
Baietto et al., 2013	-	-	112.2	No interference from endogenous substances or 11 other antibiotics
Fortuna et al., 2013	-	-	104.4	No matrix effect observed No interference from drugs
HPLC-UV				
Baietto et al., 2009	5	-	75.9	No significant interference from antiretrovirals, antibiotics, antituberculosis drugs, or other drugs
Cattaneo et al., 2010	10 specimens in triplicate	4.8 ng/mL	99.8	No interference from antiretrovirals, antibiotics, or other drugs
Cios et al., 2013	-	-	100.5	No interference from endogenous substances or 10 other antibiotics
Helmy, 2013	3	0.5	100.2	No interference (n = 10 in triplicate)
	3	2	98.44	
	3	10	99.75	
Polillo et al., 2010	-	-	95.4	-
Swoboda et al., 2007	6	2	100.62	No interference from endogenous substances
	6	10	98.13	
	6	40	97.97	
Traunmuller et al., 2010	-	-	-	No interference from 14 other drugs

STUDY	NUMBER OF SPECIMENS	NOMINAL CONCENTRATION (µg/mL)	RECOVERY (%)	INTERFERENCE
LC-MS/MS				
Szultka et al., 2013 (MEPS)	3 replicates	1	14†	No interference from endogenous substances
	3 replicates	8	12†	
	3 replicates	15	14†	
Szultka et al., 2013 (SPE)	3 replicates	1	49†	
	3 replicates	8	47†	
	3 replicates	15	44†	
Zander et al., 2014	3	0.5	102.7*	-
	3	≈ 8.6	101.4*	
	3	16	101.1*	

Abbreviations: µg/mL: microgram per millilitre.

* Recovery at room temperature, after storing for 24 hours.

† Matrix effect (%)

Correlation Between Test and Comparator

In the study by Cios et al. [Cios et al., 2013], the HPLC-DAD and HPLC-UV methods yielded similar results from serum samples (n = 84) with a coefficient of correlation between the two methods of 0.998.

5.1 Recommendations from Other Organizations

We did not find any recommendations from learned societies or organizations regarding measurement of linezolid concentrations during treatment.

6. ANTICIPATED OUTCOMES OF INTRODUCING THE TEST

6.1 Impact on Material and Human Resources

The equipment required for HPLC-UV or HPLC-DAD analysis can usually be found in laboratories. However, the sample preparation process before analysis can vary depending on the protocol concerned. Therefore, material and human resources must be planned accordingly.

6.2 Economic Consequences of Introducing the Test into Quebec's Health Care and Social Services System

Not assessed.

6.3 Main Organizational, Ethical, and Other (Social Legal, Political) Issues

Not assessed.

7. IN BRIEF

7.1 Clinical Relevance

Expert opinion indicates that quantitation of linezolid allows for therapeutic drug monitoring to optimize drug dosage and avoid hematological toxicity.

7.2 Clinical Validity: no data on clinical validity were found.

7.3 Analytical Validity

Analytical validity data show that HPLC-DAD is a reliable method (precise, accurate, and reproducible) for quantifying linezolid in plasma and serum. No significant interference was found from endogenous substances, antiretrovirals, other antibiotics, antituberculosis drugs or other drugs.

7.4 Recommendations from Other Organizations

We did not find any recommendations from learned societies or organizations regarding therapeutic monitoring of linezolid during treatment.

8. INESSS NOTICE IN BRIEF

Linezolid Assay Using High Performance Liquid Chromatography with Diode Array Detection (HPLC-DAD)

Status of the Diagnostic Technology

- ☒ Established
- ☐ Innovative
- ☐ Experimental (for research purposes only)
- ☐ Replacement for technology: _____, which becomes obsolete

INESSS Recommendation

- ☐ Include test in the Index
- ☐ Do not include test in the Index
- ☒ Reassess test when:
  - Reference values have been determined
  - There is stronger support for clinical utility
  - External quality control has been clarified
  - The application includes local validation data

Additional Recommendation

- ☐ Draw connection with listing of drugs, if companion test
- ☐ Produce an optimal use manual
- ☐ Identify indicators, when monitoring is required

NOTE

This test is definitely relevant for individual cases.

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