HPLC-DAD Quantification of Flucytosine (5-Fluorocytosine) (Reference 2014.01.006)

Notice of Assessment

June 2014

1 GENERAL INFORMATION

1.1 Requester: CHU Sainte-Justine

1.2 Application for Review Submitted to MSSS: August 1, 2013

1.3 Application Received by INESSS: March 1, 2014

1.4 Notice Issued: June 30, 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

2.1 Name of the Technology

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

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

Flucytosine (5-fluorocytosine or 4-amino-5-fluoro-2-pyrimidine or 5-FC) is an antifungal agent used primarily to treat invasive fungal infections. It can cause severe adverse effects such as bone marrow depression and hepatotoxicity. Therapeutic drug monitoring is usually performed by assaying plasma and serum samples.

Samples for HPLC quantification of flucytosine are prepared by protein precipitation with trichloroacetic acid [Ng et al., 1996; Hulsewede, 1994; St-Germain et al., 1989; Schwertschlag et al., 1984; Miners et al., 1980; Bury et al., 1979].

High-performance liquid chromatography with diode array detector (HPLC-DAD) enables separation and ultraviolet detection of flucytosine molecules to obtain a unique chromatographic profile distinct from 5-fluorouracil, a closely related metabolite [Torano et al., 2001]. Results obtained are quantitative.

2.3 Company or Developer

The test is performed in accordance with an in-house method. Flucytosine is assayed using plasma or serum from samples collected. The main steps for the procedure are as follows:[1]

- The calibration curve is prepared with naive plasma at concentrations ranging from 6.25 µg/mL to 100 µg/mL. Three levels of quality control are used with concentrations of 12.5 µg/mL, 25 µg/mL and 50 µg/mL. These values are compared with historical analytical values, and the results are compiled in accordance with a procedure recognized by Accreditation Canada.
- The sample preparation protocol (protein precipitation) applies equally to the calibration curve, quality controls and patient samples.

[1] Based on information provided by the requester.

- An internal standard stock solution (5-methylcytosine) and a precipitating agent (trichloroacetic acid) are added and mixed successively into a plasma sample. The tubes are centrifuged, then a 10 µL aliquot is injected into the HPLC-DAD system in isocratic mode with detection at 266 nm.

Threshold values are as follows:

- Ineffectiveness (and risk of secondary resistance): less than 25 µg/mL
- Optimal therapeutic level: 30 to 80 µg/mL
- Risk of toxicity: greater than 100 µg/mL

Other authors report different threshold values.

2.4 Licence: Not applicable.

2.5 Patent, If Any: Not applicable.

2.6 Approval Status (Health Canada, FDA)

Not applicable. The requester uses an in-house method and certified analytical reagents for each batch of reagents used. The requester has also participated in the International Interlaboratory Quality Control Program for Therapeutic Drug Monitoring of Antifungal Drugs since 2009.

2.7 Weighted Value: 93.31

3 CLINICAL INDICATIONS, PRACTICE SETTINGS, AND TESTING PROCEDURES

3.1 Targeted Patient Group

Target patients are those receiving long-term treatment with flucytosine (5-FC) for an invasive infection with *Candida*, *Cryptococcus neoformans* or an infection causing chromoblastomycosis or aspergillosis.[2]

3.2 Targeted Disease

Antifungal and Pharmacokinetics

Opportunistic systemic fungal infections have become a major cause of morbidity[3] and mortality[4] because of the increase in immunocompromised patients[5] treated with immunosuppressants (cancer, organ transplants, HIV) [Perfect et al., 2010; Eloy et al., 1992] and hospitalized high-risk patients, including low-birth-weight and premature infants [Kontoyiannis, 2012; Nailor and Chandrasekar, 2009]. Antifungal agents are often used to treat invasive fungal infections, systemic mycoses and cryptococcal meningitis [Ng et al., 1996]. Flucytosine is used in the systemic treatment of *Candida albicans* [Hulsewede, 1994; Bury et al., 1979], *Cryptococcus neoformans* [Hulsewede, 1994; Bury et al., 1979], *Cladosporium trichoides* [Blair et al., 1975] and *Torulopsis glabrata* [Petersen et al., 1994; Bury et al., 1979], as well as aspergillosis [Goodwin and Drew, 2008] and chromomycosis [Warnock and Turner, 1981].

[2] *Idem*.
[3] In the United States, *Candida* infections are the fourth most common nosocomial infection transmitted through blood (9%) [Drew et al., 2013].
[4] The mortality rate associated with cryptococcal meningitis ranges from 10% to 30% [PHAC, 2010].
[5] Immunocompromised patients are individuals who do not have normal immune responses.

Flucytosine is a synthetic fluorinated analogue of cytosine and was one of the first antifungal compounds developed in 1957 [Loyse et al., 2013; Smith and Andes, 2008; Pasqualotto et al., 2007; Vermes et al., 2000; Gerson, 1987]. Flucytosine has no antifungal activity of its own. Its antimycotic action results from its rapid conversion, in the cytosol of fungal cells, into 5-fluorouracil, an antimetabolite that causes RNA coding errors and inhibits DNA synthesis [Loyse et al., 2013].

Monotherapy with flucytosine is limited because of the risk of microbial resistance, which occurs frequently with this drug [Fraisse et al., 2011; Vermes et al., 2000]. Flucytosine is usually administered in combination with amphotericin B, the gold standard for most systemic fungal infections [Eloy 1992], to treat severe *Candida* and *Cryptococci* infections. Treatment with flucytosine is effective in both adults, and infants and premature babies [Vermes et al., 2000].

Flucytosine is rapidly absorbed in normal individuals, with bioavailability of 76% to 89% after oral administration [Loyse et al., 2013; Vermes et al., 2000]. Flucytosine is a small molecule that is highly water soluble and penetrates well into tissue and cerebrospinal, peritoneal and ocular (vitreous body) fluid, as well as inflammatory joint fluid [Vermes et al., 2000]. Only 2.9% to 4% of the drug binds to protein. Peak flucytosine concentrations are reached 1 to 3 hours after administration and its half-life[6] is 3 to 4 hours [Loyse et al., 2013; Vermes et al., 2000] or 3 to 6 hours [Drew et al., 2013; Smith and Andes, 2008]. It is eliminated primarily through the kidneys [Loyse et al., 2013; Vermes et al., 2000]; between 80% and 95% of flucytosine is excreted unchanged in urine. Plasma clearance of the drug is closely related to creatinine clearance [Loyse et al., 2013; Vermes et al., 2000]. Flucytosine displays wide intrapatient and interpatient pharmacokinetic variability. Renal clearance accounts for this variability [Drew et al., 2013; Andes et al., 2009; Smith and Andes, 2008].

Serious adverse effects of flucytosine are bone marrow depression,[7] including neutropenia, leucocytopenia, thrombocytopenia and pancytopenia, and hepatotoxicity [Torano et al., 2001; Vermes et al., 2000]. Other adverse effects are gastrointestinal and include diarrhea, nausea, vomiting and abdominal pain [Loyse et al., 2013; Soltani et al., 2006; Vermes et al., 2000]. Bone marrow depression and hepatotoxicity are associated with prolonged high serum concentrations of flucytosine (greater than 100 μg/mL) [Loyse et al., 2013]. The risks and benefits of flucytosine use must be carefully considered during pregnancy given its teratogenic potential [Loyse et al., 2013].

Limited Access

Flucytosine[8] was marketed in the United States in 1976 and introduced to the Canadian marketplace in 1977. The product was withdrawn from the market in August 1998 and is therefore no longer available in the Canadian marketplace. It is sometimes approved through Health Canada's Special Access Programme (SAP). SAP provides access to nonmarketed drugs for practitioners treating patients with serious or life-threatening conditions when conventional therapies have failed, are unsuitable, or unavailable.[9]

[6] For patients with advanced kidney failure, half-life can be longer, up to 85 hours [Loyse et al., 2013; Vermes et al., 2000] or more.
[7] Bone marrow depression is the complete or partial disappearance of hematopoietic bone marrow cells [Kernbaum, 2008].
[8] Flucytosine (250 mg and 500 mg capsules), marketed under the name Ancotil (Hoffmann-La Roche), was approved in the United States on September 28, 1976 [May 5, 2014 electronic communication with Health Canada].
[9] May 5, 2014 electronic communication with Health Canada.

Therapeutic Drug Monitoring

According to the requester, flucytosine concentrations should be monitored to ensure an optimal therapeutic concentration: 1) to obtain a maximum effective concentration (as the antifungal effect is time-dependent); 2) to prevent the development of resistance due to subtherapeutic concentrations; and 3) to avoid the onset of myelosuppressive or hepatotoxic adverse effects.

Pharmacology in the pediatric population is very complex. The pharmacokinetics of flucytosine have been studied very little [Jullien, 2011] and are unpredictable [Soltani et al., 2006] in children. Therefore, the proper dosage for different age groups (that is, infants, children and adolescents) is unknown. Individualized adjustments based on plasma concentrations are often required [Jullien, 2011].

As 5-FC has a short half-life (3 to 6 hours), monitoring involves measuring peak concentrations, which should be 30 to 80 µg/mL [Drew et al., 2013; BSAC Working Party, 1991]. The risks of ineffectiveness (and secondary resistance) and toxicity are increased for flucytosine concentrations of < 25 µg/mL and > 100 µg/mL, respectively [Begg et al., 2001].

As the dose related to hepatotoxicity has been reported, rapid determination of drug plasma concentrations in clinical samples is indicated, especially for patients with kidney failure [Hulsewede, 1994; Bury et al., 1979; Diasio et al., 1978; Blair et al., 1975].

According to some reviews of the literature [Lewis, 2010; Andes et al., 2009], therapeutic drug monitoring can be beneficial in clinical practice for an antifungal that meets the following criteria:

- a clear relationship between drug concentrations in the blood and toxicity or treatment efficacy;
- a need to show variable or unpredictable pharmacokinetics (dose-exposure);
- a narrow therapeutic window;
- a clinically defined therapeutic range;
- a validated analytical method for quantification of the drug.

Flucytosine meets all these criteria [Lewis, 2010; Andes et al., 2009].

3.3 Number of Patients Targeted

Ten to 20 tests are expected each year. They may be performed periodically on the same patient.[10]

3.4 Medical Specialties and Other Professions Involved

Medical biochemistry, microbiology and hematology.

3.5 Testing Procedure

Plasma 5-FC should be measured 3 to 5 days after initiation of oral dosing and approximately 2 hours post-dose (peak concentration) [Perfect et al., 2010; Goodwin and Drew, 2008; Begg et al., 2001; BSAC Working Party, 1991]. Serum should be collected 30 minutes after intravenous administration [Begg et al., 2001; BSAC Working Party, 1991].

[10] Information submitted by the requester.

Monitoring should be repeated if levels are sub- or supratherapeutic, if there is hematologic toxicity[11] or if kidney function deteriorates [BSAC Working Party, 1991].

The usual conditions for handling, storing and shipping these types of samples apply. No other special details were noted.

4 TECHNOLOGY BACKGROUND

4.1 Nature of the Diagnostic Technology: Single test.

4.2 Brief Description of the Current Technological Context

The 5-fluorocytosine (5-FC) assay (code 40350) was in the 2011-2012 Index (WV = 63.0), but it was removed from the 2013-2014 version because there had been no tests in the previous two years.[12] The Hôtel-Dieu de Québec[13] was the designated laboratory and provided 5-FC assays using the biological method (measuring the diameter of inhibition zones).[14] At present, the Laboratoire de santé publique du Québec (LSPQ) also offers the test using the same method [LSPQ, 2014].

In contrast to these laboratories, the requester's laboratory offers the test using HPLC-DAD. The number of tests performed[15] in recent years breaks down as follows: 7 assays in 2013, 1 assay in 2012, none in 2011 and 46 assays in 2010.

There are several methods for quantifying 5-FC: the biological method (also called a bioassay) [LSPQ, 2014; Torano et al., 2001; Hulsewede, 1994; Miners et al., 1980; Bury et al., 1979], high-performance liquid chromatography (HPLC) [Torano et al., 2001; Hulsewede, 1994; Petersen et al., 1994], gas chromatography [Petersen et al., 1994; Warnock and Turner, 1981], gas-liquid chromatography [St-Germain et al., 1989; Miners et al., 1980], spectrofluorometry [Miners et al., 1980], fluorometry [Warnock and Turner, 1981; Bury et al., 1979] and the enzymatic method [Torano et al., 2001; Petersen et al., 1994].

The biological method is the conventional method for this type of assay [Bury et al., 1979; Diasio et al., 1978]. Agar cut with wells containing aliquots of serum is seeded with a flucytosine-susceptible strain of *Saccharomyces cerevisiae*. The diameter of the inhibition zone is measured after a 24-hour incubation period. It is proportional to the serum concentration of flucytosine [LSPQ, 2014].

4.3 Brief Description of the Advantages Cited for the New Technology

The HPLC method is rapid and simple to perform [Schwertschlag et al., 1984], so large numbers of samples can be processed, and required results can be provided quickly [Hulsewede, 1994]. It is also precise, as results are obtained from absorption values. It offers traceability, as the results are displayed on a computer, unlike the biological method, which depends on accurate measurement of the diameter of bacterial growth inhibition zones.

Additionally, HPLC is more specific as there is little interference from a large number of other drugs and their metabolites [Bury et al., 1979], including 5-fluorouracil and amphotericin B [Schwertschlag et al., 1984]. In fact, for the biological method, the microbial

[11] Information submitted by the requester.
[12] April 16, 2012 telephone conversation with MSSS.
[13] Ministère de la Santé et des Services sociaux du Québec (MSSS). Répertoire des procédures suprarégionales de biologie médicale [index of supraregional medical biology procedures] [Web site]. Available at: http://www.msss.gouv.qc.ca/repertoires/biomed/fiche.php?id=40350.
[14] May 6, 2014 telephone converation with professionals at the Hôtel-Dieu de Québec.
[15] Information submitted by the requester. As of May, two assays have been performed in 2014.

strain chosen must be resistant to the various antimicrobial agents that could interfere with the drug being assayed, especially when it is given in combination therapy [Bury et al., 1979]. However, the main advantage of the biological method is that it does not require any special equipment [Hulsewede, 1994].

In short, the advantages of the HPLC method are as follows [Diasio et al., 1978]:

- quantification of flucytosine concentrations in 30 minutes or less;
- accurate quantification of flucytosine concentrations, especially for concentrations less than 25 µg/mL or greater than 100 µg/mL;
- very little interference from other antimicrobial agents, especially amphotericin B;
- easy-to-perform assay that can be automated for routine use.

Furthermore, HPLC-DAD can distinguish 5-fluorouracil from flucytosine with adequate resolution, unlike enzymatic and biological methods as well as other HPLC assays with basic spectrophotometric detection that lack this ability [Torano et al., 2001].

The disadvantages of gas chromatography include poor recovery (30% to 40%) and the need for derivatization[16] of 5-FC [Petersen et al., 1994; Diasio et al., 1978]. One of the main disadvantages of the enzymatic method is interference from lipemia, bilirubinemia and elevated serum creatinine [Petersen et al., 1994].

4.4 Cost of Technology and Options: Not assessed.

5 EVIDENCE

5.1 Clinical Relevance

5.1.1 Other Tests Replaced: None.

5.1.2 Diagnostic or Prognostic Value

Morbidity and Mortality

Optimal drug dosages could prevent the potentially fatal consequences of suboptimal treatment of an invasive fungal infection or the serious adverse effects of antifungal treatment (bone marrow depression and hepatotoxicity). None of the studies found assessed the relationship between flucytosine dosage and mortality prevention.

Adjusting flucytosine doses would also prevent bacterial resistance, which complicates overall clinical patient management. No quantitative data were found on the relationship between flucytosine dosages and morbidity prevention although it is mentioned in numerous publications (systematic reviews, expert opinions).

Data on clinical utility show indirectly that bone marrow depression and hepatotoxicity are associated with flucytosine serum concentrations > 100 µg/mL. A retrospective study shows that only 20.5% of patients had flucytosine blood levels in the therapeutic window[17] [Pasqualotto et al., 2007]. For 40.5% of patients, assays returned a flucytosine concentration below the recommended window, while the concentration was too high for 38.9% of patients. Of these patients, 9.9% were at high risk of toxicity (greater than 100 µg/mL). A

[16] Derivatization is a technique used in chemistry to transform a compound into a product with a similar chemical structure called a "derivative."

[17] For newborns (1 to 30 days old), target flucytosine concentrations are (lower limit) 20 µg/mL to 40 µg/mL to (upper limit) 50 µg/mL to 80 µg/mL; for other age groups, they are (lower limit) 30 µg/mL 40 µg/mL to (upper limit) 70 µg/mL to 80 µg/mL.

second study revealed similar percentages: 35.4% of patients had concentrations less than 30 µg/mL and 30.6% of patients had concentrations greater than 81 µg/mL [Soltani et al., 2006]. These data show that a considerable percentage of patients had flucytosine concentrations approaching the toxicity threshold, supporting the clinical usefulness of flucytosine therapeutic monitoring.

Therapeutic Value

Assaying flucytosine allows for adequate therapeutic drug monitoring to optimize drug dosages for patients in order to achieve maximum treatment response while avoiding drug toxicity and microbial resistance [Begg et al., 2001; Diasio et al., 1978]. However, one review states that assaying serum flucytosine helps predict toxicity but does not ensure efficacy [Nailor and Chandrasekar, 2009].

According to the literature, values for the therapeutic window (peak concentration, approximately 2 hours after oral dosing) vary: 10 µg/mL to 50 µg/mL [Smith and Andes, 2008], 20 µg/mL to 50 µg/mL [Lewis, 2010; Andes et al., 2009], 25 µg/mL to 100 µg/mL [Begg et al., 2001; Petersen et al., 1994; Schäfer-Korting, 1993], 30 µg/mL to 80 µg/mL [Drew et al., 2013; BSAC Working Party, 1991], 25 µg/mL to 120 µg/mL [Miners et al., 1980], 50 µg/mL to 80 µg/mL [Summers et al., 1997], 50 µg/mL to 100 µg/mL [Song and Deresinski, 2005; Schwertschlag et al., 1984]. One review indicates a therapeutic window of 30 µg/mL to 80 µg/mL for cryptococcal infections and 40 µg/mL to 60 µg/mL for *Candida* meningitis [Goodwin and Drew, 2008].

Target flucytosine concentrations are chosen arbitrarily, primarily on the basis of expert opinion. Optimal concentrations cannot be derived from the limited available data. Target peak flucytosine serum concentration is based on patient tolerance and an acceptable incidence of toxicity [Pasqualotto et al., 2007; Summers et al., 1997].

5.2 Clinical Validity

No direct assessment of the clinical validity of quantification of flucytosine was found.

5.3 Analytical (or Technical) Validity

Data on the analytical validity of HPLC and HPLC-DAD were taken from the results of eleven method validation studies. They featured primarily plasma and serum samples.

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		

Analytical Validity Data from CHU Sainte-Justine

Technical validation of the method is based on the use of naive human plasma samples. The requester's laboratory has taken part in an international quality control program[18] for therapeutic monitoring of antifungal agents (including flucytosine) since 2009. In this respect, local performances (peer-assessed) of flucytosine assays (target concentrations of 12.7 µg/mL to 147 µg/mL) have accuracy and precision variations of 1.9% and 4.7%, respectively, for 2012 and 2013.

Analytical Sensitivity and Linearity of Calibration Curves

Analytical sensitivity (or limit of detection) for HPLC is between 0.078 µg/mL and 1 µg/mL. Coefficients of linearity for the calibration curves are greater than 0.99 (Table 1).

Repeatability and Reproducibility

Table 2 presents data on repeatability (precision) and reproducibility. HPLC is precise (CV[19] ≤ 5%) and reproducible (CV of 1.2 % to 10%), except in the study by St-Germain et al. [1989] (CV up to 18.8% for a flucytosine concentration of 6.25 µg/mL).

Analytical Specificity, Interference and Recovery

The recovery rate varies from 76% to 113%. Most studies found no interference (Table 3) from several other drugs and antifungal agents (Appendix A).

[18] International Interlaboratory Quality Control Program for Therapeutic Drug Monitoring of Antifungal Drugs, Association for Quality Assessment in TDM and Clinical Toxicology (developed in the Netherlands).
[19] CV: coefficient of variation.

Table 1: Validation studies of HPLC and HPLC-DAD flucytosine quantification

STUDY	TYPE OF SAMPLE	NUMBER OF SPECIMENS	VOLUME (µL)*	HPLC ANALYSIS						
				PREPARATION METHOD	INTERNAL CONTROL	DETECTION (nm)	LOD (µg/mL)	LLOQ (µg/mL)	LINEARITY (µg/mL)	COEFFICIENT OF LINEARITY
Blair et al., 1975	serum, urine	-	5/5	-	-	280	-	-	1-10	Slope: 0.052 Intercept: 0.042
Bury et al., 1979	plasma	-	1,000/100	PP	-	280	1	-	-	-
Diasio et al., 1978	serum	6	1,000/50	PP, ultrafiltration	-	254	1	-	10-200	0.998
Hulsewede, 1994	serum	-	200/30	PP	5-fluorocytosine 4-aminobenzoic acid	280	-	5	10-120	-
Miners et al., 1980	plasma	-	100/10	PP	5-methylcytosine	276	-	-	5-200	0.998
Ng et al., 1996	serum	-	100/10-20	PP	Other antifungal agent	260	0.078	-	-	0.998
Petersen et al., 1994	serum	21	100/5	PP	5-methylcytosine	285 (HPLC-DAD)	0.5	-	5-500	0.9996
Schwertschlag et al., 1984	plasma	-	100/10	PP	5-iodocytosine	254	-	-	10-200	0.9987
St-Germain et al., 1989	serum	-	n. d.	PP	-	276	-	-	6.25-100	1.000
Torano et al., 2001	plasma	6 (calibration curve: 2)	500/50	PP	5-methylcytosine	266 (HPLC-DAD)	-	0.3	4.816-192.6	0.99989
Warnock and Turner, 1981	serum	2	n. d./10	PP	-	254	1	-	6.25-100	0.9996

Abbreviations: LLOQ = lower limit of quantification; LOD = limit of detection; nm = nanometer; PP = protein precipitation; µg/mL = microgram per millilitre.

*Sample volume/aliquot volume injected in the HPLC system.

Table 2: Repeatability and reproducibility of HPLC and HPLC-DAD for flucytosine quantification

STUDY	METHOD	NUMBER OF SPECIMENS	NOMINAL CONCENTRATION* (µg/mL)	INTRA-ASSAY	INTER-ASSAY	INTRA-DAY	INTER-DAY
				CV (%)	CV (%)	CV (%)	CV (%)
Blair et al., 1975	HPLC	Standard curve	10			2	8
Bury et al., 1979	HPLC	10	40	1.3			
		4 (> 5 week)	12				5.7
		4 (> 5 week)	30				1.2
		4 (> 5 week)	75				1.5
Diasio et al., 1978	HPLC	10	25			0.677†	
		10	100			0.665†	
Hulsewede, 1994	HPLC	6	-	3§			
Miners et al., 1980	HPLC	22 (inter-assay: 12 in 1 month)	10	3.7	6.88		
		22 (inter-assay: 12 in 1 month)	200	1.9	3.18		
Ng et al., 1996	HPLC	-	-	3.97†			
Petersen et al., 1994	HPLC-DAD	10	5				3.0
		10	30				3.0
		10	100				3.7
Schwertschlag et al., 1984	HPLC	10 (inter-day: 10 separate days)	50	1.8			10
		10	150	2.3			
St-Germain et al., 1989	HPLC	6 standard curves (17 different days)	6.25				18.8
			12.5				14.0
			25				8.7
			50				5.8
			75				3.6
			100				2.5

STUDY	METHOD	NUMBER OF SPECIMENS	NOMINAL CONCENTRATION* (µg/mL)	INTRA-ASSAY	INTER-ASSAY	INTRA-DAY	INTER-DAY
				CV (%)	CV (%)	CV (%)	CV (%)
Torano et al., 2001	HPLC-DAD	5 (inter-day: 1 in 5 consecutive days)	0.329	5			-
			0.4816	2			2
			20.22	0.8			1.7
Warnock and Turner, 1981	HPLC	6	50	2.19§			
		6	100	4.57§			

Abbreviations: CV = coefficient of variation; HPLC = high-performance liquid chromatography; HPLC-DAD = high-performance liquid chromatography with diode array detector; inter-day = between days; intra-day = within day.

* Units from the studies are converted for purposes of uniformity.

†Accuracy.

§Relative standard deviation (%).

Table 3: Recovery and interference

STUDY	METHOD	n	NOMINAL CONCENTRATION (µg/mL)	RECOVERY (%)	INTERFERENCE
Bury et al., 1979	HPLC	-	-	103	No interference from 5-fluorouracil or 22 other drugs.
Diasio et al., 1978	HPLC	-	10	76	No interference from seven other drugs.
			40	79	
			60	81	
			100	79	
			150	81	
			200	76	
Hulsewede, 1994	HPLC	-	10-100	≥ 98	None
Miners et al., 1980	HPLC	-	5-150	97.5	Interference from 5-fluorouracil (same retention time). No interference from 21 other drugs.
Ng et al., 1996	HPLC	-	-	96.2	Interference from aztreonam (same retention time). No interference from 23 other drugs.
Petersen et al., 1994	HPLC-DAD	-	10	97.4	No interference from 34 other drugs.
			25	99.5	
			50	99.2	
			100	102.7	
			200	99.4	
Schwertschlag et al., 1984	HPLC	-	-	-	No interference from 40 other drugs.
Torano et al., 2001	HPLC-DAD	5	0.329	98	None
			0.4816	113	
			48.16	110	
			192.6	108	
Warnock and Turner, 1981	HPLC	-	50	100.32	No interference from 34 other drugs.
			100	103.50	

Abbreviations: HPLC = high-performance liquid chromatography; HPLC-DAD = high-performance liquid chromatography with diode array detector; n = number of specimens.

Correlation Between Test and Comparator

Diasio et al. [1978] compared calibration curves from HPLC and the microbiological method. Results from each method were compared with calculated (expected) flucytosine levels. The HPLC coefficient of correlation is $r^2 = 0.998$, and for the microbiological method, it is $r^2 = 0.992$.

Five other studies compared HPLC with the microbiological method or with spectrofluorometry (Table 4). The results show good correlation between HPLC and the other methods, as coefficient of correlation values are greater than 0.92.

Table 4: Correlation Between HPLC and a Comparator Method

STUDY	METHODS	COEFFICIENT OF CORRELATION R	NUMBER OF SAMPLES (TYPE OF SPECIMENS)
Bury et al., 1979	HPLC versus biological method	0.9548	28 (plasma)
Hulsewede, 1994	HPLC versus biological method	0.96	54 (serum)
Miners et al., 1980	HPLC versus spectrofluorometry	0.99	5 (plasma), 5 (cerebrospinal fluid), 13 (prepared control plasma)
St-Germain et al., 1989	HPLC versus biological method with wells	0.946	-
	HPLC versus biological method with cylinders	0.932	-
Warnock and Turner, 1981	HPLC versus biological method	0.9202	18 (serum)

Concordance

In two of the studies, HPLC produced lower values than the biological method at low flucytosine concentrations, and higher values at higher concentrations [St-Germain et al., 1989; Diasio et al., 1978].

In the study by St-Germain et al. [1989], linearity was maintained with HPLC, whereas the standard curve was not linear at high flucytosine concentrations (greater than 100 µm/mL) with either biological method.

5.4 Recommendations from Other Organizations

Clinical practice guidelines from the Infectious Diseases Society of America (IDSA) for the management of cryptococcal infections recommend flucytosine quantification in combination with frequent complete blood counts to ensure monitoring for serious adverse events [Perfect et al., 2010]. Serum quantification of flucytosine should be performed after 3 to 5 days of treatment, with a target concentration (2 hours post-dose) of 30 µg/mL to 80 µg/mL. Concentrations greater than 100 µg/mL should be avoided. A preferred assay method was not indicated.

When flucytosine quantification is not available, special attention must be paid to hematologic parameters (white blood cell and platelet counts) and flucytosine doses should be adjusted based on creatinine clearance [Perfect et al., 2010].

Therapeutic drug monitoring of flucytosine is routinely performed in several institutions in the United Kingdom [Pasqualotto et al., 2007] and Netherlands [Torano et al., 2001; Vermes et al., 2000] to avoid toxicity and ensure proper effectiveness.

6 ANTICIPATED OUTCOMES OF INTRODUCING THE TEST

6.1 Impact on Material and Human Resources

The equipment required for HPLC analysis can generally be found in laboratories. HPLC-DAD is available and operational in the requester's lab.

At the requester's institution, a multidisciplinary team, which includes the attending physician, always decides on whether to perform therapeutic monitoring of flucytosine. If therapeutic monitoring is required clinically, the test is performed because the institution has the technology, the clinical team and the skills to conduct individualized therapeutic drug monitoring.[20]

6.2 Economic Consequences of Introducing 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

Flucytosine testing enables adequate therapeutic drug monitoring in order to optimize drug dosages for patients with invasive fungal infections, achieving maximum treatment response while avoiding drug toxicity and microbial resistance.

7.2 Clinical Validity

No direct assessment of the clinical validity of flucytosine quantification was found.

7.3 Analytical Validity

Most method validation studies are old (from 1975 to 1996) and deal with HPLC rather than HPLC-DAD. However, the difference in detection does not affect sample preparation techniques. Results are similar.

HPLC is a sensitive, specific, accurate and reproducible method for quantification of flucytosine in serum and plasma samples.

7.4 Recommendations from Other Organizations

The Infectious Diseases Society of America recommends flucytosine quantification in combination with frequent complete blood counts for monitoring for serious adverse events. However, the assay method is not indicated.

[20] May 2, 2014, electronic communication with the requester.

8 INESSS NOTICE IN BRIEF

HPLC-DAD Quantification of Flucytosine (5-fluorocytosine)

Status of the Diagnostic Technology

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

INESSS Recommendation

- ☒ Keep test in the Index subject to presentation of clinical data (monitoring of test results and connection with clinical results)
- ☐ Remove test from the Index
- ☐ Reassess test

Additional Recommendation

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

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APPENDIX A

Table of Drugs Tested to Determine Interference with Flucytosine

DRUGS TESTED	BURY ET AL., 1979	DIASIO ET AL., 1978	MINERS ET AL., 1980	NG ET AL., 1996	PETERSEN ET AL., 1994*	SCHWERTSCHLAG ET AL., 1984	WARNOCK AND TURNER, 1981
ANTIMICROBIAL AGENTS (ANTIBIOTICS AND ANTIVIRALS)							
Amikacin					30 mmol/L	15 µmol/L	X
Amphotericin B	X	X	X			200 µmol/L	X
Amoxicillin						5 µmol/L	
Ampicillin	X	X		X		200 µmol/L	X
Aztreonam				X			
Benzylpenicillin			X	X			X
Carbenicillin		X		X		200 µmol/L	X
Cefamandole				X			
Cefotaxime				X			
Cefoxitin							X
Ceftazidime				X			
Ceftriaxone				X			
Cefuroxime							X
Cephalexin	X						X
Cephalothin		X				100 µmol/L	
Cephradine			X				
Chloramphenicol					65 µmol/L	30 µmol/L	X
Ciprofloxacin				X			
Clindamycin		X					
Cyclosporine A				X			
Cyclosporine					147 mg/L		
Doxycycline			X				
Eythromycin				X			
Framycetin	X						
Fusidic acid				X			
Ganciclovir				X			
Gentamicin		X			40 mg/L	8 µmol/L	

DRUGS TESTED	BURY ET AL., 1979	DIASIO ET AL., 1978	MINERS ET AL., 1980	NG ET AL., 1996	PETERSEN ET AL., 1994*	SCHWERTSCHLAG ET AL., 1984	WARNOCK AND TURNER, 1981
Imipenem				X			
Ketoconazole						5 µmol/L	
Methicillin		X					
Metronidazole							X
Miconazole						5 µmol/L	
Nitrofurantoin						10 µmol/L	
Nystatin	X						
Ofloxacin				X			
Oxacillin						40 µmol/L	
Penicillin		X					
Penicillin G						30 µmol/L	
Piperacillin				X			
Streptomycin					43 µmol/L		
Sulfamethoxazole	X					50 µmol/L	
Teicoplanin				X			
Tetracycline			X	X			
Tobramycin					9.6 µmol/L	8 µmol/L	
Trimethoprim	X			X			
Trimethoprim-sulfamethoxazole							X
Vancomycin	X			X	25 mg/L		
OTHER DRUGS							
Acetaminophen					370 µmol/L	25 µmol/L	
2-(acetyloxy)benzoic acid	X		140 ppm				
Allopurinol							X
Amitriptyline					829 nmol/L	1 µmol/L	
Amylobarbital			X				
Atropine	X						
Aztreonam				Interference			
Bendroflumethiazide							X

DRUGS TESTED	BURY ET AL., 1979	DIASIO ET AL., 1978	MINERS ET AL., 1980	NG ET AL., 1996	PETERSEN ET AL., 1994*	SCHWERTSCHLAG ET AL., 1984	WARNOCK AND TURNER, 1981
Benzodiazepine			X				
Caffeine			X		11.3 mg/L		
Carbamazepine					43.6 µmol/L	5 µmol/L	
Chloral hydrate	X						
Chlorambucil							X
Chlordiazepoxide						1 µmol/L	X
Chloroquine						10 µmol/L	
Chlorpheniramine						30 µmol/L	X
Chlorpropamide							X
Cimetidine	X					1 µmol/L	
Clonazepam					50 µmol/L		
Codeine	X					1 µmol/L	
Cortisol					535 nmol/L		
Cyclophosphamide							X
Cytarabine							X
Daunorubicin							X
Desipramine					785 nmol/L		
Dextropropoxyphene	X						X
Diazepam					4.9 µmol/L	1 µmol/L	X
Digoxin	X				2 nmol/L		
Dihydrocodeine							X
Disopyramide			X		8.5 µmol/L		
Ephedrine						1 µmol/L	
Estriol					475 nmol/L		
Ethosuximide					538 µmol/L		
5-fluorouracil			Interference			1 µmol/L	
Flurazepam						1 µmol/L	
Furosemide							X

DRUGS TESTED	BURY ET AL., 1979	DIASIO ET AL., 1978	MINERS ET AL., 1980	NG ET AL., 1996	PETERSEN ET AL., 1994*	SCHWERTSCHLAG ET AL., 1984	WARNOCK AND TURNER, 1981
Heparin	X						
Hydrocortisone				X			
Imipramine	X				799 nmol/L	1 µmol/L	
Isoniazid						5 µmol/L	
Lidocaine					17.9 µmol/L		
Lithium					1.5 mmol/L		
Melphalan							X
6-mercaptopurine			X			10 µmol/L	
Methotrexate					1.4 µmol/L		X
Metoclopramide							X
Morphine	X						
N-acetylprocainamide					15.3 µmol/L	4 µmol/L	
Nalidixic acid				X			
Nitrazepam	X						
Nortriptyline					113 nmol/L		
Paracetamol	X		X				X
Pethidine	X						
Phenobarbital			X		102 µmol/L	30 µmol/L	
Phenytoin			X	X	52 µmol/L	20 µmol/L	
Prednisolone	X						X
Prednisone							X
Primidone					42 µmol/L		
Procainamide			X		22 µmol/L	6 µmol/L	
Procarbazine							X
Prochlorperazine							X
Quinidine			X		11.1 µmol/L	5 µmol/L	
Salicylate					1.4 mmol/L	300 µmol/L	

DRUGS TESTED	BURY ET AL., 1979	DIASIO ET AL., 1978	MINERS ET AL., 1980	NG ET AL., 1996	PETERSEN ET AL., 1994*	SCHWERTSCHLAG ET AL., 1984	WARNOCK AND TURNER, 1981
Theobromine			X				
Theophylline			X		83 µmol/L	20 µmol/L	
Thioguanine							X
Thyroxine					175 nmol/L		
Tolbutamide							X
Tricyclic antidepressants			X				
Valproic acid					499 µmol/L		
Warfarin			X				

*HPLC-DAD method.