Anti-folate-binding protein antibodies

Folate plays a key role in normal metabolism, DNA synthesis and red blood cell regeneration. Its deficiency is associated with megaloblastic anemia, neural tube defects, inborn errors (1), and determination of folate in human blood is necessary component of regular health check-ups. Folate is not synthesized in human body and could be derived from food or intestinal microbiota (2). Folate deficiency could lead to some health issues and itself could result from increased folate turnover such as during pregnancy, breast feeding, certain skin issues and haematological disorders. There are some indications that reduced folate level is associated with increase homocysteine levels which is considered a risk factor for cardiovascular diseases (1).

MULTIPLE FOLATE FORMS, ONE DETECTION APPROACH

Folic acid (Fig.1) is an essential component of human metabolism. Chemical structure of folic acid (pteroylglutamic acid) allows for binding of methylene groups/reducing equivalents which could be transferred onto various chemical substances leading to creation of new moieties. So, folic acid participates in amino acid and nucleic acid synthesis, one carbon unit transfer, and redox reactions, which are required for normal metabolism and regulation (2).

It has to be noted that since folate is metabolically active substance, in the bloodstream there are several folate forms which could co-exist and need to be measured in similar manner. Among metabolites of folate, the main one is N5-methyl-tetrahydrofolate (5-MTHF) (Fig1. B). Human blood specimen may contain various amounts of folate and its metabolites depending on the time after meal and meal type. Folate and its metabolites exist in the bloodstream in form of a complex with

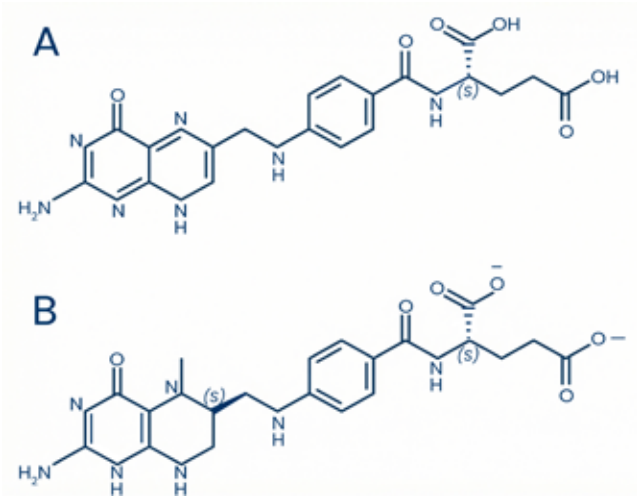

Figure 1.
Chemical formulas of Pteroyl-L-glutamic acid, folic acid (A) and N5-methyl-tetrahydrofolate (B) (from 3, with changes)

folate-binding protein (FBP) which transports it to the tissues. Folate binding protein binds folate and 5-MTHF with different affinity, but at pH 9.3 affinity of binding of these components is equal (1,4).

FBP-BASED FOLATE TESTING

Detection of folate and its derivatives could be accomplished by several methods including microbiological assays and immunochemical assays, the latter being the faster and more

CLINICAL UTILITY

- **Megaloblastic anemia**

reliable way of testing for folate. It is known that FBP binds folate and 5-MTHF with nanomolar affinity (1,4) allowing for use of that protein in folate assays. Majority of immunochemical methods employ Folate-binding protein as an affinity binder. Majority of FBP-based folate assays for folate are built on competitive principle when labeled folate competes with natural one for binding to FBP (5). In heterogeneous immunoassays, FBP is often anchored onto support by means of an antibody which is specific to FBP (6). To perform this function, binding of folate/metabolites of folate to FBP has to be unaffected by interaction of anti-FBP antibody with FBP. Hytest offers a range of monoclonal antibodies (MAbs) which are specifically designed to fulfil this role.

MONOCLONAL ANTIBODIES SPECIFIC TO FBP

Monoclonal antibodies offered by Hytest are capable of fulfilling the role of a linker which fixes the affinity binder – FBP – onto solid support in immunoassay for determination of folate (Fig.2).

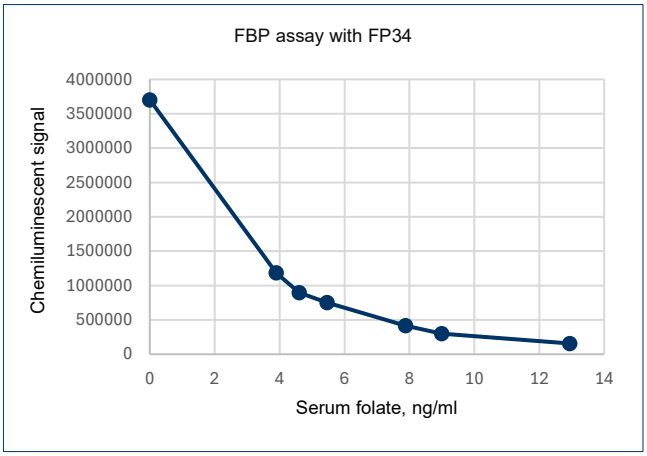

Figure 2.
Calibration curve for serum folate determination in FBP-based folate assay with FP34. Automatic chemiluminescent immunoassay, MAbs were preadsorbed onto paramagnetic beads.

Moreover, for all MAbs offered by Hytest calibration curves for folic acid, 5-MTHF and serum folate are in good accordance with each other indicating that FBP-based folate assays built upon these MAbs could be successfully used for natural folate determination in the human serum samples (Fig.3).

Important feature of folate assays is cross-reactivity with folate-like substances which could be used in clinical setting and compromise detection of folate. Testing of Hytest Mab in FBP-based folate assay demonstrated zero cross-reactivity with Methotrexate and Phenytoin and very low cross-reactivity with Aminopterin (Table 1).

FBP-based folate assays built with Hytest anti-FBP Mabs demonstrate good correlation to FBP-based folate assay which has been on the market for long time (Fig.4).

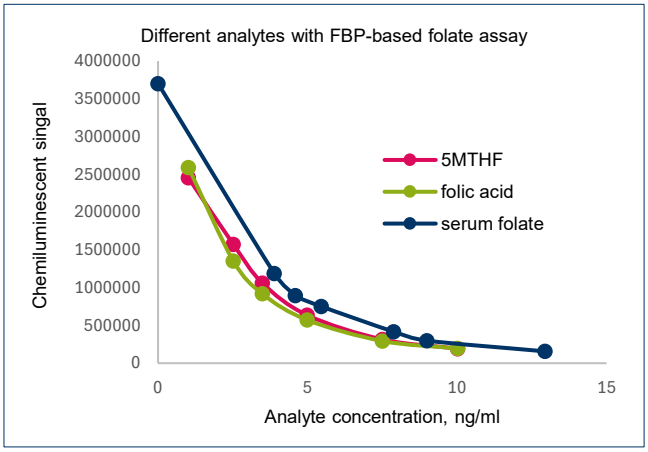

Figure 3.
Representative calibration curves for folic acid (FA), N5-methyltetrahydrofolate (5-MTHF), serum folate (serum) for FBP-based folate assay with FP34. Automatic chemiluminescent assay, MAbs were preadsorbed onto paramagnetic beads.

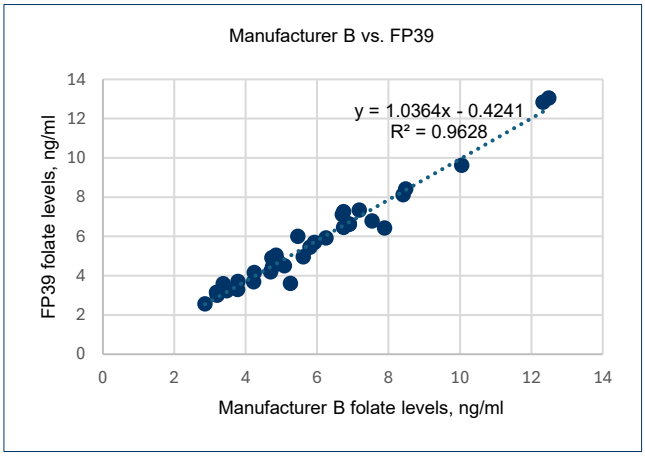

Figure 4.
Correlation study conducted with 36 frozen human serum samples. Manufacturer B FBP-based folate assay was carried out according to manufacturer's instruction, FBP-based folate assay with FP39 Mab was carried out using chemiluminescent automatic analyser with FP39 preadsorbed onto paramagnetic particles.

Table 1.
Cross-reactivity of Hytest MAbs in FBP-based folate assay with folate-related substances utilized in clinical settings.

Hytest Anti-FBP MAbs	Cross-reactant	Cross-reactant concentration, ng/ml	Cross-reactivity rate, %
FP14	Aminopterin	500	0.42
	Methotrexate	100	0
	Phenytoin	100	0
FP17	Aminopterin	500	0.42
	Methotrexate	100	0
	Phenytoin	100	0
FP55	Aminopterin	500	0.37
	Methotrexate	100	0
	Phenytoin	100	0
FP34	Aminopterin	500	0.38
	Methotrexate	100	0
	Phenytoin	100	0
FP39	Aminopterin	500	0.36
	Methotrexate	100	0
	Phenytoin	100	0

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ORDERING INFORMATION

MONOCLONAL ANTIBODIES

Product name	Cat. #	MAb	Subclass	Remarks
Folate-binding protein (FBP)	4FBP3	FP14	IgG1	CLIA, in vitro
		FP17	IgG1	CLIA, in vitro
		FP34	IgG	CLIA, recombinant
		FP39	IgG	CLIA, recombinant
		FP55	IgG1	CLIA, recombinant chimeric

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