

DEFICIENCY DETECTION

Blood Tests

**Standard blood measurements of detecting folic acid deficiencies are not conclusive. Current established "normal" red cell folate and plasma levels are proving to be too low to reflect early disease states and localized tissue deficiencies (Donnelly JG, 2000, Nilsson K, 1999). Folate enzymes are found in higher concentrations in certain epithelial cells such as those of the cervix, CNS, brain, and gastric mucosa. Due to rapid use and/or an inherently high cell turnover rate, some cells express a deficiency in spite of coexisting "normal" levels (Heimbürger DC, 1992). Even so, lower than normal blood folate levels have been found in association with benign cervical cell changes, with improvement occurring after therapy (Butterworth, 1982, Li X, 1995). Folate deficiency related diseases are more accurately reflected in measurements of total plasma homocysteine (Nilsson K, 2000, Bottiglieri T, 2000, Godfrey GP, 1998), but not always. A series of blood chemistry tests may be required to detect important subtle deficiencies, especially in psychiatric patients (Zittoun J, 2000, Snow CF, 1999). Some of these patients, as well as others with renal impairment and/or cardiovascular disease carry the common folate gene mutation (Kimura H, 2000).*

The Cervical Pap Test, a Built-in Existing Tool

**Folic acid is concentrated within cervical cells. Endogenous as well as exogenous estrogens stimulate the proliferation of these cells and increase the need for folic acid (Harper JM, 1994, Butterworth CE 1993, Heimbürger DC, 1992). It has been known for years that a folic acid/B12 deficiency can be detected on Pap Tests long before it can be detected using blood tests and well before anemia presents itself clinically (DeMay RM, 1996, Butterworth CE, 1992, Whitehead N, 1989, Koss LG 1979, Klaus H, 1971, Van Niekerk W, 1966). Many laboratories used to specifically report folic acid deficiency but now with the universal Bethesda system (TBS) of reporting, it is put in the "I don't know" categories of BCC (benign cellular changes), ASC-US (atypical squamous cells of undetermined significance), or AGC-US (atypical gland cells of undetermined significance), or it is just ignored and signed out "within normal limits." Folic acid deficiency changes may even appear as mild dysplasia (Valente PT, 1998, DeMay RM, 1996, Koss LG 1974). Although overall cytomegaly (enlargement of both cytoplasm and nuclei with maintenance of a normal N/C ratio; see illustration) has been considered the hallmark feature of folic acid deficiency, it may not always be the primary finding. Even though Van Niekerk found this to be true, with cytoplasmic vacuolization and multinucleation (3.5%) the next most common observations, Klaus found nuclear creasing and folding (6%) and nuclear enlargement (4%) to be the most common features. Klaus observed these changes on Pap smears 8-10 weeks before clinical megaloblastic anemia was detectable (Koss LG 1979). In Naib's studies, cytoplasmic vacuolization frequently occurred around the nucleus in association with radiation and chemotherapy, which are known inhibitors of folic acid enzymes (Ames BN, 1999, DeMay RM, 1996). Electron microscopic analysis revealed the cytoplasmic vacuoles were associated with destruction of the golgi apparatus, mitochondria, and endoplasmic reticulum (Naib ZM, 1976). In cervical specimens they present as wide pale perinuclear halos (Meisels A, 1992). Interestingly, these cells also appear similar to HPV koilocytes, although they are less pronounced (see illustration). Macrocytes, especially in association with chemotherapy or radiation, and hyperplastic endocervical cells in association with birth control pills (BCP's) or pregnancy, may also be observed (DeMay RM, 1996, Koss LG 1979). Hypersegmented neutrophils (5-6 lobes) are possible as well (Zittoun J, 1999, Thompson WG, 1989). The above changes are known to reverse after folic acid therapy (DeMay RM, 1996, Koss LG 1979). Women who are folic acid deficient often bear children with birth defects and are more likely to contract HPV and have an abnormal cytology report, especially if on BCP's (Piyathilake CJ, 2004, Hernandez 2003, Harper JM, 1994). BCP's deplete folic acid and their side-effects are often more pronounced among the poor and malnourished (Lacey JV Jr, 1999, Jones MD, 1993, Brinton LA, 1990, Murthy NK, 1980). Studies continue to link both folic acid deficiency and hormone use with cardiovascular disease and reproductive cancers. Folic acid protects the integrity of DNA during cell division and promotes normal differentiation and morphology of cells, and therefore, folic acid deficiency changes on Pap Tests should be reported.*

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Atrophy and Mild Atypias

Atrophy-related mild squamous atypias on Pap Tests in postmenopausal women **less often prove to be true lesions on biopsy (Abati A, 1998). **The uniform nuclear enlargement** that is commonly seen in these atypias is a consistent finding with folate/B12 deficiency as observed by Klaus and others. Topical estrogen is often recommended to resolve dilemmas in inflammatory verses true abnormal changes. Estrogen may stimulate maturation of atrophic cells distinguishing them from neoplastic cells, but its effect is only temporary. An antioxidant/B vitamin regimen following, could improve and/or assure optimal cell functioning (division, secretion, and immune response), and help **sustain** appropriate morphology. **Supplementation** would most likely **reduce the number of recurring mild atypias** as well as lower plasma homocysteine levels which are often elevated in menopause. Similar persistent atrophic-like reactive cell changes found on **postpartum** smears, and uniform nuclear enlargement sometimes seen in **pregnancy** may also be resolved with folate/B12 supplementation.*

Pap Test Reporting

****Increased awareness and identification** by cytology professionals of all folic acid deficiency's characteristic cell changes on routine Pap Tests could **greatly benefit clinicians who are trying to avoid the consequences of a deficiency (eg: NTD's in offspring)**. Presently, benign cellular changes depicting classic folic acid deficiency changes are being ignored and reported as normal, or are reported as ASCUS/AGUS with a recommendation to biopsy or repeat the smear in the near future to see if cell changes resolve naturally or continue to progress. It would be helpful and within reason to include the comment in all early mildly atypical cases: "**consider a folate/B12 deficiency,**" especially for those who are **pregnant, postpartum, or on anti-folate drugs** (Lewis DP, 1998, Valente PT, 1998, Liu T, 1995). Supplementation could reverse any megaloblastic cell changes, and **help guard against an abnormal cytology** (Harper JM, 1994). It would also **benefit women's health in general**, providing protection against other folic acid deficiency related conditions, such as **heart disease and stroke** (Yang Q, 2006, Zittoun J, 2000, Perry DJ, 1999).*

A General Micronutrient Recommendation

Folic acid requires many other micronutrients (e.g.: magnesium, zinc, copper, iron) as well as members of its own family (B6, B12, B2, betaine, choline, inositol etc.) to work most effectively in promoting and maintaining normal cell differentiation, replication, and repair. An even better comment accompanying mildly atypical cases would be: "consider a general micronutrient deficiency, including the antioxidant and B-complex vitamins.**" These cases have cells showing visual signs and characteristics of micronutrient depletion (Ames BN, 1999).*

Other Cytology Specimens

Folic acid deficiency changes, including dysplasia, can be seen in other types of cells routinely examined in cytology (bronchial, esophageal, gastric, colon, etc.). **Dysplastic respiratory and gastric epithelium, in particular have been shown to be deficient in folate enzymes sometimes due to folate gene mutations (Lashner BA, 1993, see gastric illustration). **Improvement or complete reversal** after folate therapy has been shown to occur, especially in smokers (Piyathilake CJ, 2000, Mason JB, 1996, Saito M, 1994, Heimburger DC, 1988). A suggestion in cytology reports that these atypical changes have been associated with folate/antioxidant deficiencies, could **aid clinicians in the treatment of high risk populations**, such as smokers, alcoholics, those with GI symptoms or who are on antifolate drugs. A mass screening program would not even be necessary.*