donated blood and the use of validated viral inactivation and/or removal steps during the manufacture of blood products. Serologic screening procedures have substantially reduced the risk of transmission of blood-borne viruses. However, there are still residual risks despite these measures due to the inclusion of "window period" donations. To reduce the durations of the window period, nucleic acid amplification technology (NAT) is being instituted in Europe, Japan and the United States. Standardization of NAT assay is necessary before the introduction of such an assay for routine screenings of blood and blood products and can be achieved by use of international standard of HBV DNA for NAT assays. Validation characteristics are described as specificity, detection limit and robustness. The specificity was established by studying conditions that might be expected to cause cross-reactivity or interference in the analysis. The conditions that cause cross-reactivity or interference by other relevant blood-borne viruses include human immunodeficiency virus (HIV), hepatitis C virus (HCV), hepatitis A virus (HAV) and parvovirus B19 and other human DNA viruses including human papilloma virus (HPV) 18 & 6b, cytomegalovirus (CMV) and human herpesvirus 1 & 2. In order to validate the specificity of the analytical procedure, at least 100 HBV DNA-negative plasma pools were tested and shown to be non-reactive. To determine the positive cut-off point, a diluted series of the WHO HBV international standard (97/746) were tested under these conditions, and the detection limit was calculated to be approximately 50 IU/mL. To demonstrate robustness, at least 20 HBV DNA negative plasma pools, and spiked with HBV DNA to a final concentration of 3 times the previously determined 95% cut-off value, were tested and found positive.

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Temperature Regulates Melanin Synthesis in Mel–Ab Cells

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Temperature change is one of the major environmental factors to influence human skin. However, the relationship between temperature and melanogenesis has received little attention. In the present study, we investigated the effects of temperature change including heat shock on melanogenesis using a mouse melanocyte cell line, Mel–Ab. Our results demonstrated that cells maintained at 37°C showed maximal melanin synthesis. Cells cultured at low temperatures produced less melanin than cells at 37°C. Heat treatment for 1 h also decreased melanin production. The melanin production is accompanied by tyrosinase activity at each temperature, indicating that the tyrosinase activity is regulated by temperature. To examine how heat shock decreases melanin synthesis, we treated cell with suramin (an inhibitor of growth factor receptors) or N–acetyl–L–cysteine (a free radical scavenger) before heat shock. However, neither suramin nor N–acetyl–L–cysteine restored heat-induced depigmentation. It has been reported that activated ERK is responsible for MITF degradation, leading to decreased melanin synthesis. Our results showed that heat shock induces sustained ERK activation, which may inhibit melanogenesis.

[PA1–74] [ 10/18/2002 (Fri) 09:30 – 12:30 / Hall C ]

Characterization of Human Epidermal Stem Cells and Living Skin Equivalents

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Human epidermal keratinocytes consist of stem cells, transit amplifying cells, and postmitotic differentiating cells. Among them, stem cells play a critical role in cell renewal, wound healing, and neoplasia. However, till now, specific markers of human epidermal keratinocytes are not clearly defined. In the present study, we separated putative stem cells from other cells using fluorescence activated cell sorting (FACS), based on differences in α6-integrin and CD71 expression. We next analyzed keratinocytes obtained from young and old donors. We found that stem cell portion